42nd Annual Scientific Meeting of the

ISEH – Society for Hematology and Stem Cells

Thursday 22 August – Sunday 25 August 2013

The Imperial Riding School Renaissance Hotel

Vienna, Austria

Program and Abstracts

Experimental Hematology

Journal for Hematology and Stem Cell Biology and Transplantation

Official Publication of the ISEH - Society for Hematology and Stem Cells

Aim and Scope

Experimental Hematology publishes original research reports (regular and fast-track submissions), reviews, perspectives, letters to the editor, and abstracts of the annual meeting of ISEH - Society for Hematology and Stem Cells. We welcome manuscripts describing basic in vitro and in vivo research centered on normal and malignant hematopoiesis as well as non-malignant hematologic diseases. Submissions focused on non-hematopoietic stem cells (e.g. mesenchymal stem cells, embryonic stem cells and induced pluripotent stems) with potential relevance to hematopoiesis are also welcome, as are studies involving experimental or early phase clinical cell transplantation. Studies employing genomic and systems biology approaches to the study of normal and malignant hematopoiesis are strongly encouraged, as are those employing model organisms.

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Welcome Message

On behalf of ISEH – the International Society for Experimental Hematology and Stem Cells— it is a pleasure to welcome you to the 42nd Annual ISEH Scientific Meeting in Vienna, Austria. The Scientific Program Committee has organized an outstanding schedule of educational sessions that include presentations by researchers in basic, translational and clinical hematology. This year's scientific program features 12 plenary and two concurrent sessions, as well as events for young investigators, poster sessions and social activities.

Our hope is that this meeting provides you with many opportunities to connect with scientists who share your passion, to interact with the brightest minds in our field, and to explore new product offerings from the industry's leading vendors.

Vienna's historic center is considered one of the most beautiful city landmarks in Europe. Known for its music, architecture and cafes the city has undergone many transitions and adaptations over the ages. Like Vienna, the ISEH is transitioning to keep pace with the rapid changes in hematology and stem cell research by offering more opportunities to educate and learn from our colleagues. New initiatives of the ISEH this year are:

- The launching of social media groups on Facebook and Linked-in and the start of a Twitter feed.
- The recording of several key sessions from this year's meeting
- Webinars for online dissemination of scientific research knowledge.

We sincerely hope that through these new initiatives and the 42nd Annual ISEH Scientific Meeting you will leave with memories of remarkable science, enjoyable social events and renewed interactions with your colleagues.

Sincerely,

Elaine Dzierzak, PhD
ISEH President 2012 – 2013

Margaret Goodell, PhD
ISEH Scientific Program Committee Chair 2013

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2007 R. Keith Humphries
2008 Mervin C. Yoder
2009 Thalia Papayannopoulou
2010 Toshio Suda
2011 David Scadden
2012 Gerald de Haan
2013 Elaine Dzierzak (Current)

Meeting Committees

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Hitoshi Takizawa
China

Keiyo Takubo
Japan

Samir Taoudi
Australia

Wei Tong
USA

David Traver
USA

Marcel van den Brink
USA

2013 Local Organizing Committee

Peter Valent
Austria

Meinrad Busslinger
Austria

Awards

ISEH Donald Metcalf Lecture Award

Leonard Zon, MD
Boston Children's Hospital/Howard Hughes Medical Institute, Boston, MA, USA

ISEH McCulloch and Till Lecture Award

Hanna Mikkola, PhD
University of California Los Angeles, Los Angeles, CA, USA

ISEH New Investigator Awards

Each year the Society takes great pride in supporting the work of New Investigators by awarding prizes in recognition of their outstanding promise and excellent work. Awards are divided into two groups: students and postdoctoral, with two prizes award in each category. Awards will be determined onsite after the New Investigator Session. Members of Program Committee act as judges for the New Investigator Awards.

Student

1st Prize: $1,000 (USD)	Dirk van Bekkum Award
2nd Prize: $500 (USD)	T. Ray Bradley Award
3rd Prize: $250 (USD)	Greg Johnson Award

Postdoctoral Fellow

1st Prize: $1,000 (USD)	Eugene Cronkite Award
2nd Prize: $500 (USD)	George Brecher Award
3rd Prize: $250 (USD)	Christa Muller-Sieburg Award

ISEH Travel Grants

Anna Beaudin
USA

Kelli Carroll
USA

Sara Charmsaz
Australia

Phyllis Chong
Singapore

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USA

Emma de Pater
Netherlands

Serena De Vita
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Canada

Shalin Naik
Australia

Thao Nguyen
Australia

Justyna Rak
Sweden

Pauline Rimmele
USA

Jessica Salmon
Australia

Peter van Galen
Canada

Joanna Wegrzyn Woltosz
Canada

General Meeting Information

Meeting Venue

The Imperial Riding School Renaissance Hotel • Ungargasse 60, Vienna, 1030 Austria

The Imperial Riding School Renaissance Hotel offers complimentary guest room and meeting space internet access (included in the ISEH room rate) for delegates staying in the hotel. For non-resident delegates there is a charge of €16,90 per 24 hour period in the meeting space or anywhere in the hotel.

Registration

Registration Hours

Thursday 22 August	13:00 – 18:00
Friday 23 August	08:00 – 18:00
Saturday 24 August	08:00 – 16:30
Sunday 25 August	08:00 – 17:00

A name badge will be provided onsite with your registration materials. Name badges must be worn at all times to allow access into the ISEH sessions, exhibits and social events. Registration will be located in the Grosses Foyer.

Official Language

The official language of the ISEH Annual Scientific Meeting is English.

Certificate of Attendance

Certificate of attendance can be obtained at the registration desk. There are no Continuing Medical Education credits given for this meeting. If you require additional information, please contact ISEH at info@iseh.org.

Cellular Phones, Pagers, Filming, Taking of Photos and Recording of Sessions

Attendees are reminded that mobile phones and pagers should be switched off during sessions and that recording sessions and taking film and photographs during sessions and of posters is strictly forbidden.

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General Meeting Information

Food and Beverage

ISEH will be providing welcome coffee, coffee during breaks and a buffet lunch. This is included in the registration fee for the meeting.

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About Vienna

Vienna, described as Europe's cultural capital, is a metropolis with unique charm, vibrancy and flair. It boasts outstanding infrastructure, is clean and safe, and has all the inspiration that you could wish for in order to discover this wonderful part of Europe.

Known as a romantically imperial city, for its classical music, medieval alleyways and historical architecture the city boasts being home to music masters such as Mozart, Beethoven, Schubert and Johann Strauss. Today, you can indulge your senses by attending the Wiener Philharmoniker or Vienna Boy's choir tours.

Set on the banks of the majestic Danube River, Vienna is surrounded by natural beauty that offers opportunities to explore the Vienna woods or it's numerous parks. Or you may enjoy a stroll through history as you visit the gothic St. Stephen's Cathedral to the Imperial Palace to the Art Nouveau splendor of the Secession, from the magnificent baroque palace Schönbrunn to the Museum of Fine Arts to modern architecture at the Museums Quartier. Vienna also lays claim to a record breaking 27 castles and more than 150 palaces. No matter how long you stay, there is sure to be something exciting for you to discover.

With over 2 million inhabitants and an influx of visitors from all over the world, Vienna is the most popular urban tourist destination in Austria.

To find out more about Vienna and things to do around the area visit: http://www.wien.info/en.

Special Events

Social Event

Saturday 24 August 2013 19:45 – 24:00

Join ISEH for an evening steeped in Viennese Heurigen culture at Mayer Am Pfarrplatz. The traditional winery has been producing the best of Viennese wine since 1683. This landmark-protected house, located in the district of Heiligenstadt-Nussdorf, is where Beethoven lived in 1817. During his time here, the composer worked on his greatest opus, the 9th Symphony. The fee includes buffet dinner, beverages, transportation to and from the event and a great venue for socializing. Buses will board at 19:45 from the lobby of the Imperial Riding School Renaissance Hotel.

To purchase a ticket for $75 USD visit the Registration desk. If you already purchased a ticket, it can be found in your registration materials.

ISEH General Membership Meeting

Sunday 25 August 2013 15:00

Agenda

- ISEH Activities in 2012
- 2012 Financial Status
- ISEH Future Activities

Exhibitor Hours

Friday 23 August	10:30 – 20:00
Saturday 24 August	10:30 – 19:30

Poster Hours

Thursday 22 August

Poster Set-up Begins (Odd # Abstracts Only)	14:00

Friday 23 August

Poster Viewing and Discussion (Odd # Abstracts)	18:00 – 20:00
Poster Take-down (Odd # Abstracts)	20:00 – 21:00

Saturday 24 August

Poster Set-up (Even # Abstracts Only)	07:30 – 10:00
Poster Viewing and Discussion (Even # Abstracts)	18:00 – 19:30

Sunday 25 August

Poster Take-down (Even # Abstracts)	13:15 – 15:30

New Investigator Events

As with the past years, ISEH is focusing on our young researchers with several events that have been organized by the New Investigator Committee (NIC). These events provide forums for new investigators to meet established professors from around the world that are experts in many aspects of stem cell biology and hematopoiesis, discuss career paths, and socialize with other junior investigators who will be their colleagues for years to come. The NIC believes that these sessions would be of interest to PhD-students and postdocs, as well as for new principal investigators who are in the process of starting up their own groups. Everyone is welcome!

Special Events

Career Panel Discussion #1: Effective Networking

Friday 23 August 12:15 – 13:30

Speakers include:
- Louise Purton, St. Vincent's Institute, Stem Cell Regulation Unit, Australia
- Toshio Kitamura, University of Tokyo, Tokyo, Japan
- Leonard Zon, Howard Hughs Medical Institute, Children's Hospital Medicine Department, Boston, MA, USA

Moderator:
- Sofie Singbrant Söderberg, Lund University, Lund, Sweden

In this session we will discuss how to best make use of the time at a conference to meet new contacts and potential collaborators, but also how to overcome possible cultural differences in networking. After getting a quick lesson in how to pitch your science, we will end with a fun "speed networking exercise" where you'll get the opportunity to network with each other, as well as some of our invited speakers and ISEH leadership. Don't miss out!

Meet-the-Expert Lunch

Saturday 24 August 12:30 – 14:00

This is your opportunity to meet with prominent stem cell and hematopoiesis researchers in a small informal setting. Tables will consist of one professor and up to nine junior investigators.

Registration: Limited seating and pre-registration required

Career Panel Discussion #2: Effectively Presenting Yourself and Your Science

Sunday 25 August 12:15 – 13:15

Speakers include:
- Margaret Goodell, Baylor College of Medicine, Houston, TX, USA
- Ross Levine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA
- Peter van Galen, Ontario Cancer Institute, Toronto, ON, Canada
- Eugenia Flores-Figueroa, Fondo de Investigacion en Salud, Mexico City, Mexico

Moderator:
- Peter van Galen, Ontario Cancer Institute, Toronto, ON, Canada

In this session you will receive advice on how to turn your data into a great presentation, including how to construct effective and aesthetically pleasing power point slides. We will also discuss how to manage your digital image in social media like LinkedIn, Twitter and Facebook, and how to use social media to broaden your scientific network.

New Investigator Networking "Midsummer Night" Mixer

Friday 23 August 20:00 – 21:00

Join other new investigators from around the world for an evening of mingling, drinks and light snacks under the stars at the Imperial Riding School Renaissance Hotel.

NOTES

Schedule at a Glance

Thursday 22 August 2013

Time	Event
13:00 – 18:00	Registration open *Grosses Foyer*
14:00	Odd numbered poster set-up begins
15:00 – 16:00	Session 1: Donald Metcalf Lecture *Pirouette*
16:00 – 16:30	Break *Pirouette and Grosses Foyers*
16:30 – 18:00	Session 2: Lineage Specification I *Pirouette*
18:00 – 20:00	Welcome Reception *City Garden*

Thursday 22 August 2013

13:00 – 18:00 **Grosses Foyer**

REGISTRATION OPEN

14:00

ODD NUMBERED POSTER SET-UP BEGINS

15:00 – 16:00 **Pirouette**

SESSION 1: DONALD METCALF LECTURE

Session Chair: Margaret Goodell

Leonard Zon
Boston Children's Hospital/Howard Hughes Medical Institute, USA
Making and Improving the Function of Adult Blood Stem Cells (S1001)

16:00 – 16:30 **Pirouette & Grosses Foyers**

BREAK

16:30 – 18:00 **Pirouette**

SESSION 2: LINEAGE SPECIFICATION I

Session Chairs: Elaine Dzierzak and Margaret Goodell

Meinrad Busslinger
Research Institute of Molecular Pathology, Austria
Transcriptional Control of B Cell Commitment and Development (S1002)

Camilla Forsberg
University of California Santa Cruz, USA
Hematopoietic Stem Cell Fate Decisions (S1003)

Alan Warren
Medical Research Council Laboratory of Molecular Biology, UK
Recycling of eIF6 Couples Ribosome Surveillance to Growth During Development (S1004)

18:00 – 20:00 **City Garden**

WELCOME RECEPTION

Schedule at a Glance

Friday 23 August 2013

Time	Event
8:00 – 18:00	Registration open *Grosses Foyer*
8:30 – 10:30	Session 3: Stem Cells I *Linke Pirouette*
8:30 – 10:30	Session 4: Hematopoietic Development I *Rechte Pirouette*
10:30 – 20:00	Exhibits Open
10:30 – 11:00	Break *Pirouette & Grosses Foyers*
11:00 – 12:15	Session 5: Lineage Specification II *Linke Pirouette*
11:00 – 12:15	Session 6: Hematopoietic Development II *Rechte Pirouette*
12:15 – 13:30	Lunch *Borromaeus Restaurant*
12:15 – 13:30	New Investigator Session 1: Effective Networking *Grosse Reitschule*
13:30 – 15:30	Session 7: Stem Cells II *Pirouette*
15:30 – 16:00	Break *Pirouette & Grosses Foyers*
16:00 – 18:00	Session 8: Leukemia I *Pirouette*
18:00 – 20:00	Poster Viewing & Discussion (Odd numbered posters) *Haute Ecole & Grosses Foyers*
20:00 – 21:00	New Investigator Networking Event *Schanigarden*

Friday 23 August 2013

8:00 – 18:00 **Grosses Foyer**

REGISTRATION OPEN

8:30 – 10:30 **Linke Pirouette**

SESSION 3: STEM CELLS I

Session Chairs: Camilla Forsberg and Grant Challen

Keiichi Nakayama
Kyushu University, Japan
Cell Cycle and Cancer Stem Cells: Abrogation of Quiescence by Fbw7 Ablation Eliminates Leukemia Stem Cells (S1005)

David Bryder
Lund University, Sweden
Probing Cellular Hierarchies as Tools to Understand Normal and Aberrant Hematopoiesis

Abstract Presentations

Takaharu Kimura
IMS, University of Tokyo, Japan
Human Hematopoietic Stem/Progenitor Cells Hibernate in Vascular Niche Harboring GFAP-Expressing Cells (O1001)

Peter van Galen
Campbell Family Institute for Cancer Research, Ontario Cancer Institute, Princess Margaret Cancer Centre, University Health Network, Netherlands Cancer Institute, Canada
Human Hematopoietic Stem Cell Integrity is Guarded by the Unfolded Protein Response (O1002)

Claudia Waskow
Technische Universitaet Dresden, Germany
Stable Human Hematopoietic Stem Cell Engraftment Supports Continuous de novo Generation of Mature Human Blood Cells in Mice (O1003)

Justyna Rak
Lund University, Sweden
RNAi Screen Identifies Cytohesin1 as a Mediator of Adhesion in Human Hematopoietic Stem/Progenitor Cells (O1042)

8:30 – 10:30 **Rechte Pirouette**

SESSION 4: HEMATOPOIETIC DEVELOPMENT I

Session Chairs: Nancy Speck and Kateri Moore

Manuela Tavian
Institut National de la Santé et de la Recherche Médicale, France
Renin-Angiotensin System is Involved in Hematopoietic Emergence (S1007)

Katrin Ottersbach
University of Cambridge, UK
Characterizing the Niche of Emerging Hematopoietic Stem Cells (S1008)

Abstract Presentations

James Palis
University of Rochester, USA
Megakaryopoiesis in the Mammalian Embryo is Associated with Rapid LineageMmaturation and Synthesis of Platelets with Altered Activation (O1004)

Ayaku Nakamura-Ishizu
Keio University School of Medicine, Japan
Megakaryocytes Produce a Distinctive HSC Niche Through CLEC-2 Guided Secretion of TPO (O1005)

Ingmar Bruns
Albert Einstein College of Medicine, USA
Peri-vascular Megakaryocytes Restrain Hematopoietic Stem Cell Proliferation (O1006)

Kelli Carroll
Beth Israel Deaconess Medical Center, USA
17Beta-Estradiol has a Biphasic Effect on the Formation of Hematopoietic Stem Cells (O1007)

10:30 – 11:00 **Pirouette & Grosses Foyers**

BREAK

11:00 – 12:15 **Linke Pirouette**

SESSION 5: LINEAGE SPECIFICATION II

Session Chairs: Annarita Migliaccio and Louise Purton

Benjamin Kile
The Walter and Eliza Hall Institute of Medical Research, Australia
The Intrinsic Apoptosis Caspase Cascade Regulates Hematopoietic Stem Cell Homeostasis and Function (S1009)

Friday 23 August 2013

Abstract Presentations

Shalin Naik
WEHI, NKI, Australia
Diverse and Heritable Lineage Imprinting of Early Hematopoietic Progenitors (O1008)

Daniel Klimmeck
Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM), Deutsches Krebsforschungszentrum (DKFZ), European Molecular Biology Laboratory (EMBL), Germany
Global Landscape of Hematopoietic Stem Cells and Multipotent Progenitors (O1009)

Charlotta Böiers
Lund Stem Cell Center, Lund Strategic Center for Stem Cell Biology, Sweden
Establishment of Lympho-myeloid Restricted Progenitors Prior to the Emergence of Definitive Hematopoietic Stem Cells (O1010)

Ashwini Hinge
Cincinnati Children's Research Foundation, USA
Rho GTPase Activating Protein p190-B Regulates Hematopoietic Stem Cell Self-renewal Decisions (O1011)

11:00 – 12:15 **Rechte Pirouette**

SESSION 6: HEMATOPOIETIC DEVELOPMENT II

Session Chairs: James Palis and Andrew Elefanty

Cristina Lo Celso
Imperial College London, UK
In Vivo Imaging of Quiescent and Physiologically Activated Hematopoietic Stem Cells (S1010)

Abstract Presentations

Andrés García
National Center for Cardiovascular Research (CNIC), Spain
Circadian Parasympathetic Regulation of Hematopoietic Stem Cell Traffic (O1013)

Felicia Ciuculescu
Boston Children's Hospital, Harvard Medical School and Harvard Stem Cell Institute, USA
Deletion of Murine Rac GTPases in the Perivascular Niche Disrupts Trabecular Bone Formation, Medullary Blood Vessels and Hematopoiesis (O1014)

Late Breaking Abstract Presentations

12:15 – 13:30 **Borromaeus Restaurant**

LUNCH

12:15 – 13:30 **Grosse Reitschule**

NEW INVESTIGATOR SESSION 1: EFFECTIVE NETWORKING

Louise Purton
St. Vincent's Institute, Stem Cell Regulation Unit, Australia

Toshio Kitamura
University of Tokyo, Tokyo, Japan

Leonard Zon
Boston Children's Hospital/Howard Hughes Medical Institute, Boston, MA, USA

13:30 – 15:30 **Pirouette**

SESSION 7: STEM CELLS II

Session Chairs: Paul Frenette and Sofie Singbrant Soderberg

Jürgen Knoblich
Institute of Molecular Biotechnology, Austria
Asymmetric Cell Division and Spindle Orientation in Neural Stem Cells - from Drosophila to Humans (S1011)

Samir Taoudi
Walter and Eliza Hall Institute, Australia
Haematopoiesis During Embryonic Development (S1012)

Gerald de Haan
University of Groningen, Netherlands
Epigenetic Control of Hematopoietic Stem Cells (S1013)

Abstract Presentations

Grant Challen
Washington University in St. Louis, USA
de novo DNA Methylation is Required for Maintaining Genomic and Transcriptional Integrity in Hematopoietic Stem Cells (O1015)

Catherine Forristal
Mater Research, Australia
Hypoxia Inducible Factor (HIF)-2α Enhances Proliferation of Malignant Haematopoietic Cells in the Hypoxic Malignant Bone Marrow (O1016)

Friday 23 August 2013

David Grote
IRIC/Université de Montréal, Canada
The Bmi1-interacting Protein E4f1 is Vital for Hematopoietic Stem/Progenitor Cell Function (O1017)

15:30 – 16:00 **Pirouette & Grosses Foyers**

BREAK

16:00 – 18:00 **Pirouette**

SESSION 8: LEUKEMIA I

Session Chairs: Patricia Ernst and Ulrich Steidl

Wei Tong
Children's Hospital of Philadelphia and University of Pennsylvania, USA
Investigation of Novel Signaling Pathways in Normal and Malignant Hematopoiesis (S1014)

Peter Valent
University of Vienna, Austria
Biology, Phenotype and Target Expression Profiles of Neoplastic Stem Cells in Myeloid Leukemias (S1015)

Christoph Klein
Hannover Medical School, Germany
Hematopoietic Stem Cell Gene Therapy for Wiskott-Aldrich-Syndrome - vg ck (S1016)

Abstract Presentations

Juergen Fink
Cambridge Institute for Medical Research, University of Cambridge, UK
Homozygous JAK2V617F drives Rapid Hematopoietic Stem Cell Proliferation and Differentiation at the Expense of Self-renewal (O1018)

Serena De Vita
Boston Children's Hospital, USA
Cooperation of KITD816V and TET2 Loss of Function in the Pathogenesis of Systemic Mastocytosis and Associated Hematological Malignancies (O1019)

Hirotaka Matsui
Hiroshima University, Japan
Haploinsufficiency of Samd9L, Encoding an Endosome Fusion Facilitator, develops Myeloid Malignancies in Mice Mimicking Human Diseases with Monosomy 7 (O1020)

18:00 – 20:00 **Haute Ecole & Grosses Foyers**

POSTER VIEWING & DISCUSSION (ODD NUMBERED POSTERS)

20:00 – 21:00 **Schanigarden**

NEW INVESTIGATOR NETWORKING EVENT

Schedule at a Glance

Saturday 24 August 2013

Time	Event
8:00 – 16:30	Registration open *Grosses Foyer*
8:30 – 10:30	Even numbered poster set-up
8:30 – 10:30	Session 9: Leukemia II *Pirouette*
10:30 – 19:30	Exhibits Open
10:30 – 11:00	Break *Pirouette & Grosses Foyers*
11:00 – 12:30	Session 10: Transcriptional Regulation I *Pirouette*
12:30 – 14:00	Lunch *Borromaeus Restaurant*
12:30 – 14:00	New Investigator: Meet the Expert Lunch *Grosse Reitschule*
14:00 – 16:00	Session 11: New Investigator Session *Pirouette*
16:00 – 16:30	Break *Pirouette & Grosses Foyers*
16:30 – 18:00	Session 12: Presidential Symposium *Pirouette*
18:00 – 19:30	Poster Viewing & Discussion (Even numbered posters) *Haute Ecole & Grosses Foyers*
19:45 – 24:00	Social Event *Mayer Am Pfarrplatz*

Saturday 24 August 2013

8:00 – 16:30 **Grosses Foyer**

REGISTRATION OPEN

8:30 – 10:30

EVEN NUMBERED POSTER SET-UP

8:30 – 10:30 **Pirouette**

SESSION 9: LEUKEMIA II

Session Chairs: Koichi Akashi and Vivienne Rebel

Jan Cools
VIB Center for Biology of Disease, Belgium
Molecular Genetics of T-cell Acute Lymphoblastic Leukemia (S1017)

Yueying Wang
Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine, China
Genetic and Epigenetic Changes Contributing to Leukemogenesis and Prognostic Impact in Acute Myeloid Leukemia (S1018)

Veronica Sexl
Institute of Pharmacology and Toxicology, Austria
STAT5 – a Central Player in Bcr/Abl+ leukemia (S1019)

Abstract Presentations

Ross Dickins
Walter and Eliza Hall Institute, Australia
Pax5 Loss Imposes a Reversible Differentiation Block in B-progenitor Acute Lymphoblastic Leukemia (O1021)

Stephen Chung
Memorial Sloan-Kettering Cancer Center, USA
CD99 Identifies Disease Stem Cells in Acute Myeloid Leukemia (AML) and the Myelodysplastic Syndromes (MDS) (O1022)

Constanze Bonifer
University of Birmingham, UK
Identification of a Dynamic Core Transcriptional Regulatory Network for t(8;21) AML (O1023)

10:30 – 11:00 **Pirouette & Grosses Foyers**

BREAK

11:00 – 12:30 **Pirouette**

SESSION 10: TRANSCRIPTIONAL REGULATION I

Session Chairs: Frank Rosenbaur and Berthold Göttgens

Daniel Tenen
Cancer Science Institute of Singapore, Singapore
Regulation of Myeloid Transcription Factors and DNA Methylation by Noncoding RNAs (S1020)

Ulrich Steidl
Albert Einstein College of Medicine, USA
Novel Non-Clustered Homeobox Genes as Key Regulators in Normal and Malignant Hematopoiesis (S1021)

Abstract Presentations

Dan Duan
UCLA, USA
Scl represses Cardiogenesis via Distant Enhancers During Hemogenic Endothelium Specification (O1024)

John Pimanda
University of New South Wales, Australia
Genome-wide Analysis of Transcriptional Regulators in Human HSCs Reveals a Densely Interconnected Network of Coding and Non-coding Genes (O1025)

Joanna Wegrzyn Woltosz
BC Cancer Agency, Canada
miR-146a Regulates Hematopoietic Stem Cell Maintenance and Cell Cycle Entry (O1026)

12:30 – 14:00 **Borromaeus Restaurant**

LUNCH

12:30 – 14:00 **Grosse Reitschule**

NEW INVESTIGATOR: MEET THE EXPERT LUNCH

Pre-registration required.

14:00 – 16:00 **Pirouette**

SESSION 11: NEW INVESTIGATOR SESSION

Session Chair: Teresa Bowman

Ross Levine
Memorial Sloan-Kettering Cancer Center, USA
Role of Mutations in Epigenetic Regulators in Pathogenesis of Myeloid Malignancies (S1022)

Saturday 24 August 2013

Session Chairs: Gerald de Haan and Jean-Pierre Levesque

Student Abstracts

Karin Klauke
European Research Insitute for the Biology of Ageing (ERIBA), University Medical Centre Groningen (UMCG), Netherlands
Clonal evolution of Leukemic Stem Cells in a Cbx7-induced Leukemia Mouse Model (O1027)

Kran Suknuntha
University of Wisconsin, USA
Induced Pluripotent Stem Cell Model of Chronic Myeloid Leukemia Revealed Olfactomedin 4 as a Novel Therapeutic Target in Leukemia Stem Cells (O1028)

Daichi Inoue
The Institute of Medical Science, The University of Tokyo, Japan
C-terminal-truncating ASXL1 Mutations induce MDS via Repression of PRC2 Function (O1029)

Post-Doc Abstracts

Isao Kobayashi
University of California, San Diego, USA
Cell-to-cell Interaction Between Endothelial-hematopoietic Precursors and Somites Regulates Developmental Specification of Hematopoietic Stem Cells through Notch Signaling (O1030)

Anna Beaudin
UC Santa Cruz, USA
Flk2 Lineage Tracing Reveals a Novel, Developmentally Restricted Hematopoietic Stem Cell (01031)

Emma de Pater
Erasmus MC, Netherlands
Gata2 is Required for HSC Generation and Survival (O1032)

16:00 – 16:30 **Pirouette & Grosses Foyers**

BREAK

16:30 – 18:00 **Pirouette**

SESSION 12: PRESIDENTIAL SYMPOSIUM

Session Chair: Elaine Dzierzak

Nancy Speck
University of Pennsylvania, USA
A Role for Inflammatory Signaling in Lymphoid Progenitor Formation in the Fetus (S1023)

Ann Dean
National Institutes of Health, USA
Functional Organization of β-Globin Locus Chromatin Loop Over Long Distances (S1024)

Ellen Rothenberg
California Institute of Technology, USA
Transcriptional and Epigenetic Thresholds for Entry to the T-cell Developmental Program (S1025)

18:00 – 19:30 **Haute Ecole & Grosses Foyers**

POSTER VIEWING & DISCUSSION (EVEN NUMBERED POSTERS)

19:45 – 24:00 **Mayer Am Pfarrplatz**

SOCIAL EVENT

Refer to page x for more details on this event and how to purchase your ticket.

Schedule at a Glance

Sunday 25 August 2013

Time	Event
8:00 – 17:00	Registration open *Grosses Foyer*
8:30 – 10:30	Session 13: Transcriptional Regulation II *Pirouette*
10:30 – 11:00	Break *Pirouette & Grosses Foyers*
11:00 – 12:00	Session 14: Mechanisms of Transformation I *Pirouette*
12:00 – 13:15	Lunch *Borromaeus Restaurant*
12:15 – 13:15	New Investigator Session 2: Effectively Presenting Yourself and Your Science *Grosse Reitschule*
13:15 – 15:00	Session 15: Mechanisms of Transformation II *Pirouette*
15:00 – 15:30	Break *Pirouette & Grosses Foyers*
15:30 – 16:45	Annual General Meeting Session 16: McCulloch & Till Lecture *Pirouette*
16:45	Closing Remarks *Pirouette*

Sunday 25 August 2013

8:00 – 17:00 **Grosses Foyer**

REGISTRATION OPEN

8:30 – 10:30 **Pirouette**

SESSION 13: TRANSCRIPTIONAL REGULATION II

Session Chairs: Daniel Tenen and Jonas Larsson

Koichi Akashi
Kyushu University, Japan
Epigenetic Landscape of Hematopoietic Lineage Commitment Can Be Visualized by Analysis of Incorporated H3.3 Variant (S1026)

Frank Rosenbauer
University of Munster, Germany
Control of PU.1 Expression by Three-Dimensional Chromatin Architecture in Hematopoiesis and Leukemia (S1027)

Emery Bresnick
University of Wisconsin School of Medicine and Public Health, USA
Genetic Basis for Hematopoietic Stem Cell Generation in the Mammalian Embryo (S1028)

Abstract Presentations

Marnie Blewitt
Walter and Eliza Hall Institute of Medical Research, Australia
Function of PRC2 Accessory Factors in Haematopoietic Stem Cells (O1033)

Alessandra Pasquarella
Adolf Butenandt Institut (LMU), Germany
Deletion of the Histone Methyltransferase Setdb1 during Hematopoiesis Results in Hematopoietic Stem Cell Failure and Abrogates B cell Development (O1034)

Nicola Wilson
CIMR, Wellcome Trust/MRC Building, University of Cambridge, Wellcome Trust - Medical Research Council, University of Cambridge, UK
Interrogating the Relationship between Transcription Factor Complex Binding and Transcriptional Activation (O1035)

10:30 – 11:00 **Pirouette & Grosses Foyers**

BREAK

11:00 – 12:00 **Pirouette**

SESSION 14: MECHANISMS OF TRANSFORMATION I

Session Chairs: Emery Bresnick and Constanze Bonifer

Guilio Superti-Fuerga
CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Austria
Elucidating the Molecular Mechanism of Action of Cancer Drugs in the Second Decade of the New Millennium (S1029)

Abstract Presentations

Patricia Ernst
Geisel School of Medicine at Dartmouth, USA
MLL1-dependent Pathways and Mechanisms that Maintain Hematopoiesis (O1036)

Florian Grebien
CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Austria
Recruitment of the MLL Complex via Specific Interaction of the p30 Variant of C/EBPα with Wdr5 is Essential for Development of Acute Myeloid Leukemia (O1037)

12:00 – 13:15 **Borromaeus Restaurant**

LUNCH

12:15 – 13:15 **Grosse Reitschule**

NEW INVESTIGATOR SESSION 2: EFFECTIVELY PRESENTING YOURSELF AND YOUR SCIENCE

Margaret Goodell
Baylor College of Medicine, Houston, TX, USA

Ross Levine
Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Peter van Galen
Ontario Cancer Institute, Toronto, ON, Canada

Eugenia Flores-Figueroa
Fondo de Investigacion en Salud, Mexico City, Mexico

Sunday 25 August 2013

13:15 – 15:00 **Pirouette**

SESSION 15: MECHANISMS OF TRANSFORMATION II

Session Chairs: Wei Tong and Ihor Lemishka

Andreas Strasser
The Walter and Eliza Hall Institute, Australia
The Role of the Bcl-2 Protein Family in Tumorigenesis and Cancer Therapy (S1030)

Joanna Loizou
CeMM Research Center for Molecular Medicine, Austria
Regulation of the DNA Damage Kinase ATM in the Immune System (S1031)

Abstract Presentations

Eva Schmidt
University Hospital Münster, Germany
Lats 1 is a Putative Tumor Suppressor in HoxA9 / Meis Induced Leukemia (O1038)

Michael Milyavsky
Tel Aviv University, University Health Network, Israel
Genome-wide shRNA Screening Approach Towards Identification and Characterization of Therapy Resistance Determinants In Leukemia (O1039)

David Kent
Cambridge Institute for Medical Research, University of Cambridge, UK
Order Matters: Sequence of Mutation Acquisition Influences Human Disease Pathogenesis (O1040)

Catherine Carmichael
Walter and Eliza Hall Institute, University of Melbourne, Australia
Overexpression of the ETS-family Transcription Factor ERG and Dysregulated Cytokine Signaling Drive Erythroid Leukemia Development in Mice (O1041)

15:00 – 15:30 **Pirouette & Grosses Foyers**

BREAK

15:30 – 16:45 **Pirouette**

ANNUAL GENERAL MEETING SESSION 16: MCCULLOCH & TILL LECTURE

Session Chairs: Elaine Dzierzak and Margaret Goodell

Hanna Mikkola
University of California Los Angeles, USA
Uncovering the Roadmap to Hematopoietic Stem Cells (S1032)

16:45 **Pirouette**

CLOSING REMARKS

NOTES

NOTES

A B S T R A C T S

SPEAKER ABSTRACTS

Numbers beginning with "S" refer to Invited Speaker Abstracts
Numbers beginning with "O" refer to Oral Short Talk Presentations
Numbers beginning with "P" refer to Poster Presentations

ELSEVIER

Experimental Hematology 41 (2013) S2–S9

Experimental Hematology

Invited Speakers

S1001 - MAKING AND IMPROVING THE FUNCTION OF ADULT BLOOD STEM CELLS

Leonard Zon[1,2,3]

[1]Stem Cell Program and Hematology/Oncology, HHMI/Boston Children's Hospital and Dana-Farber Cancer Institute, Boston, Massachusetts, USA; [2]Harvard Stem Cell Institute, Boston, Massachusetts, USA; [3]Harvard Medical School, Boston, Massachusetts, USA

An embryo knows how to make blood stem cells. The process initiates in the aorto-gonads-mesonephros (AGM) region of the embryo, and the stem cells migrate to subsequent sites of hematopoiesis. We have used the zebrafish to uncover the molecular steps to making a stem cell, and improving engraftment. Our prior studies have demonstrated that the notch pathway and blood flow is required stem cell production. A chemical screen demonstrated that PGE2 is capable of expanding HSCs in the AGM, and can also enhance engraftment of mouse bone marrow. A clinical trial of PGE2 treated cord blood for patients with leukemia suggested enhanced engraftment. Recent transgenic fish with GFP coupled to a mouse runx1 enhancer demonstrated expression in HSC, and it was possible to visualize stem cell production and engraftment in subsequent sites. The process of engraftment can be separates in stages: attachment, transmigration through the endothelial cells, migration to the abluminal space, endothelial cuddling, and cell division. A chemical screen showed that distinct pathways regulate each step. The zebrafish is also an excellent transplantation model, and a recent chemical screen found that other inflammatory lipids similar to PGE2 can stimulate stem cell migration. Our studies have uncovered a number of pathways important for stem cell production and engraftment, and produced some candidate molecules for study in humans.

S1002 - TRANSCRIPTIONAL CONTROL OF B CELL COMMITMENT AND DEVELOPMENT

Meinrad Busslinger, *Tanja Schwickert, Hiromi Tagoh, Jasna Medvedovic, and Anja Ebert*

Research Institute of Molecular Pathology, Vienna, Austria

Transcription factors, chromatin regulators and cell signaling are critically involved in cell fate decisions during development. B lymphopoiesis is an ideal system to study the interplay of these processes in the context of lineage commitment. The transcription factors PU.1 and Ikaros are essential for the differentiation of hematopoietic stem cells to lymphoid-primed multipotent progenitors and common lymphoid progenitors (CLPs). Entry of the CLPs into the B cell lineage depends on the transcription factors E2A and EBF1, which function as B cell specification factors by activating B-lymphoid genes including Pax5. Pax5 in turn restricts the developmental potential of lymphoid progenitors to the B cell pathway by repressing B-lineage-inappropriate genes and activating B-cell-specific genes at the onset of pro-B cell development. A major task of committed pro-B cells is to functionally rearrange the immunoglobulin heavy-chain (Igh) locus, which consists of a 0.25 Mb-long proximal domain containing the DH, JH and CH gene segments at the 3' end of the Igh locus and of a large cluster of 200 upstream VH genes spread over a 2.5-Mb region. DH-JH recombination occurs within the proximal domain already in uncommitted lymphoid progenitors. In contrast, VH genes participate in V(D)J recombination only in committed pro-B cells, where the Igh locus undergoes contraction by looping, which juxtaposes VH genes next to the rearranged proximal DJH domain, thus facilitating VH-DJH rearrangements. Expression of a functionally rearranged Igμ chain subsequently leads to the appearance of the pre-B cell receptor that promotes the developmental transition from pro-B cells to pre-B cells undergoing immunoglobulin light-chain rearrangements. We have studied the role of cis-regulatory elements and trans-acting factors involved in the control of long-range interactions at the Igh locus and investigated the function of Ikaros in regulating the pro-B to pre-B cell transition. I will discuss recent results of these experiments in my presentation.

S1003 - HEMATOPOIETIC STEM CELL FATE DECISIONS

E. Camilla Forsberg

Biomolecular Engineering, University of California Santa Cruz, Santa Cruz, California, USA

A fundamental question in stem cell biology is how stem cells balance the need for self-renewal with generation of the appropriate numbers and types of differentiated cells. We focus on understanding the cell fate decisions of hematopoietic stem cells (HSC) and their non-self-renewing progeny. Even though mouse hematopoieisis is one of the most intensely investigated stem cell models, lineage maps of HSC differentiation are incomplete and controversial. Our goal is to understand hematopoietic fate decisions under physiologically relevant conditions and we have therefore developed strategies to enable highly sensitive and quantitative assessment of lineage potential in vivo. These approaches include transplantation and lineage tracing models to assess the relative contribution to mature cells by stem and progenitor populations, and manipulation of specific genes to understand the molecular regulation of cell fate decisions. Collectively, our results are consistent with HSC giving rise to multipotent progenitors that contribute to all hematopoietic lineages under a variety of physiological conditions.

S1004 - RECYCLING OF ELF6 COUPLES RIBOSOME SURVEILLANCE TO GROWTH DURING DEVELOPMENT

Alan Warren

Medical Research Council Laboratory of Molecular Biology, UK

Ribosome assembly is an essential, highly conserved process that is tightly coupled to cell growth and proliferation. However, the machinery that monitors the fidelity of ribosome assembly and couples this to growth and development is poorly defined. We aim to better understand this process to shed light on the pathogenesis of the inherited leukaemia predisposition disorder Shwachman-Diamond syndrome (SDS) that is caused by deficiency of the SBDS protein. We have recently identified SDS as a "ribosomopathy" caused by defective maturation of the large ribosomal subunit. Defective ribosome biogenesis is thought to activate p53 through ribosomal protein-mediated suppression of Mdm2 E3 ligase activity. Challenging the generality of this model, we have identified a p53-dependent ribosome surveillance pathway in Drosophila, which lack an encoded Mdm2 orthologue. Using SBDS-deficient flies, we found that p53 deletion rescued the apoptosis and larval lethality triggered by defective eIF6 recycling from nascent 60S ribosomes. Two parallel signalling pathways involving the ATM-CHK1 and p38 MAP kinases mediate this p53-dependent ribosome assembly checkpoint. We propose that the release and intracellular recycling of the anti-association factor eIF6 from pre-60S ribosomal subunits acts as a rheostat for coupling ribosome assembly to growth. Our Drosophila animal model may find utility in the development of mechanism-based therapeutics for SDS.

S1005 - CELL CYCLE AND CANCER STEM CELLS: ABROGATION OF QUIESCENCE BY FBW7 ABLATION ELIMINATES LEUKEMIA STEM CELLS

Keiichi Nakayama
Kyushu University, Fukuoka, Japan

Hematopoietic stem cells (HSCs) are characterized by pluripotentiality and self-renewal ability. To maintain a supply of mature blood cells and to avoid HSC exhaustion during the life span of an organism, most HSCs remain quiescent (G0 phase), with only a limited number entering the cell cycle. The proto-oncoprotein c-Myc determines the state of cellular proliferation or quiescence, and its abundance is controlled by Fbw7, the F-box protein subunit of an SCF-type ubiquitin ligase. The Fbw7-dependent degradation of c-Myc is essential for maintenance of both quiescence and the "stemness" of normal HSCs. Given that leukemia stem cells (LSCs) share many properties with HSCs, the Fbw7-c-Myc axis is a promising target for leukemia therapy. Here we show that Fbw7 plays an essential role in the maintenance of LSCs in a mouse model of chronic myeloid leukemia (CML). Deletion of the Fbw7 gene resulted in the accumulation of c-Myc in LSCs, leading to transient activation of the cell cycle followed by p53-dependent apoptosis. Serial transplantation experiments revealed that the ability of LSCs to cause disease was markedly reduced by Fbw7 ablation. Furthermore, abrogation of quiescence in LSCs by Fbw7 ablation increased their sensitivity to imatinib, and the combination of Fbw7 ablation with imatinib treatment resulted in a greater depletion of LSCs than of normal hematopoietic stem cells in mice. Our results suggest that purging of cancer stem cells from their quiescence by targeting Fbw7 may provide the basis for a new approach to the treatment of cancer.

S1006 - PROBING CELLULAR HIERARCHIES AS TOOLS TO UNDERSTAND NORMAL AND ABERRANT HEMATOPOIESIS

David Bryder
Section for Immunology, Institution for Experimental Medical Science, Lund University, Lund, Sweden

The ability of hematopoietic stem cells (HSCs) to produce a wide range of diverse terminally differentiated cells represents a paradigm system for cellular development in the adult. In this stepwise process, defining HSC traits such as self-renewal and multilineage potential are gradually lost, with downstream progeny successively gaining increasing rates of monolineage commitment. Although HSCs and progenitor cell subsets are quite infrequent, the hierarchical structure of hematopoiesis coupled with their large potential for cellular expansion makes it obvious that perturbations at intermediate progenitor cell levels can have a significant effect for the individual. Examples for this include normal biological situations such as aging, where alterations in progenitor cell function/potential underlie a gradual change in several hematopoietic parameters. However, the consequences of deregulated progenitor cell function are perhaps even more evident in classical hematopoietic progenitor diseases such as acute leukemia, although less is known about the normal target cells of these diseases. To understand the pathways of hematopoietic differentiation from HSCs, we have in previous work elucidated many of the intermediate precursor cell types between HSCs and the terminally differentiated progeny. This has provided tools for the direct dissection of cellular and molecular events at defined stages of hematopoietic development. In my talk, I will discuss approaches currently taken in my laboratory that take advantage of the high resolution of progenitors that can be obtained within the hematopoietic system. This includes strategies to both identify and functionally characterize regulators of stem and progenitor fates. Finally, I will present data on a novel mouse model of acute myeloid leukemia; with a particular focus on the approaches we have taken to gain insight into the normal target cells of disease.

S1007 - RENIN-ANGIOTENSIN SYSTEM IS INVOLVED IN HEMATOPOIETIC EMERGENCE

***Manuela Tavian**, Kathia Biasch, Lidia Sinka, and Emmanuelle Julien*
UMR-S949, INSERM/University of Strasbourg/EFS, Strasbourg, France

The continuous generation of blood cells throughout life relies on the existence of hematopoietic stem cells (HSC) generated during embryogenesis. Adult-type HSC are first generated in the aorta-gonad-mesonephros (AGM) region between days 27 and 40 of human embryonic development, but an elusive blood forming potential is present earlier in the underlying splanchnopleura (Sp) (1). The origin of blood cells has been the subject of an intense scientific debate during the last decade. Although it is relatively well accepted that the first HSCs are generated in the AGM through a hemogenic endothelium, the direct precursor of this cell type in the embryo, either a mesenchyme or a hemangioblast, remains to be clearly identified. Recently, we have shown that the angiotensin-converting enzyme (ACE) is a cell surface marker of human HSC (2). Furthermore, ACE identifies inside the human splanchnopleura rare CD45-CD34- cells that are responsible for the hematopoietic activity detected in the early embryo, suggesting that this enzyme is a marker of mesodermal pre-hematopoietic precursors in the human embryo (3). ACE is a key component of the renin-angiotensin system (RAS) as it catalyzes the production of angiotensin II (Ang II), well known for its effect in the control of blood pressure, cardiovascular regulation and fluid homeostasis through AT1 and AT2 receptors (4). We report here that the key components of this system - namely the Ang II-receptors, the angiotensinogen and the renin - are present in the same region of the embryo which expresses ACE, meaning that a local RAS exists in the embryo. Functional in vitro analyses in the mouse model show a stimulatory effect of Ang II in the emergence of hematopoietic precursors, an effect inhibited by the addition of a specific AT1 antagonist in the culture. These observations suggest for the first time that RAS is functionally active in this embryonic region and has a direct role in the emergence of first HSC during ontogeny.

S1008 - CHARACTERISING THE NICHE OF EMERGING HAEMATOPOIETIC STEM CELLS

***Katrin Ottersbach**, Simon Fitch, Bahar Mirshekar-Syahkal, Maria Mascarenhas, and Chrysa Kapeni*
Haematology, University of Cambridge, Cambridge, UK

Adult-repopulating haematopoietic stem cells (HSCs) emerge in the aorta-gonads-mesonephros (AGM) region from the aortic endothelium starting from embryonic day 10.5 of mouse development. While there has been recent progress in the identification of the cell-intrinsic factors that regulate this transition, very little is currently known about the microenvironment in which this process occurs. We previously carried out microarray expression screens to identify novel regulators of HSC emergence in the AGM. This resulted in the identification of several niche factors that revealed that the AGM haematopoietic microenvironment is composed of several different components. One of the identified regulators is the transcription factor Gata3. Within the AGM, we found it to be expressed in isolated endothelial cells, sub-aortic mesenchymal cells, mesonephric cells and sympathoadrenal cells of the developing sympathetic nervous system. The analysis of the knockout mice revealed an important function for Gata3 in HSC development, the deficiency of which could be ameliorated via a rescue of the sympathetic nervous system defect in these mice. Analysis of mice null for tyrosine hydroxylase, the enzyme that catalyses catecholamine synthesis, further confirmed that mediators secreted from the developing sympathetic nervous system are key players in HSC development. In addition to sympathoadrenal cells, we also discovered that sub-endothelial mesenchymal cells form part of the AGM haematopoietic microenvironment. Some of these cells express the transmembrane protein Dlk1 downstream of the transcription factor Runx1. Interestingly, Dlk1 was found to have a negative effect on haematopoietic stem and progenitor cell production in the AGM, thus pointing to the possible existence of a negative feedback loop. We have also more recently started investigating the role that cytokine signalling pathways play in the production of haematopoietic stem and progenitor cells in the AGM. These results will be discussed and put into context within the existing network of niche regulators.

S1009 - THE INTRINSIC APOPTOSIS CASPASE CASCADE REGULATES HEMATOPOIETIC STEM CELL HOMEOSTASIS AND FUNCTION

Benjamin Kile
Cancer and Hematology Division, The Walter and Eliza Hall Institute, Parkville, Victoria, Australia

Since the demonstration that overexpression of pro-survival Bcl-2 causes an expansion of hematopoietic stem cell (HSC) number (Domen et al. 2000 J Exp Med), it has become accepted that apoptotic death is a common fate for HSCs. Apoptosis is believed to be critical for regulating the size of the HSC pool. Consistent with this, mice lacking Caspase-3 were reported to have increased numbers of HSCs (Janzen et al. 2008 Cell Stem Cell). This, however, was ascribed to perturbations in cytokine signaling, rather than impaired HSC apoptosis. To examine the role of the intrinsic apoptosis pathway in HSCs in more detail, we generated bone marrow chimeras lacking the pro-apoptotic proteins Bak/Bax, Apaf-1, Caspase-9 or Caspase-3/7. Surprisingly, loss of Bak and Bax - the essential mediators of the intrinsic pathway - had little impact on HSC number. In contrast (but consistent with Janzen et al.) bone marrow chimeras lacking the downstream effectors Apaf-1, Caspase-9 or Caspase-3/7 exhibited a 10-fold expansion of the HSC compartment. Casp9-/- bone marrow exhibited an impaired ability to reconstitute irradiated recipients. Thus, apoptosis mediated by Bak and Bax is dispensable for HSC homeostasis, whereas, the function of the apoptotic caspase cascade is not. This raised the questions 1) Is the HSC expansion in Caspase-9-deficient mice intrinsic to HSCs? 2) Is the function of the apoptotic caspase cascade in HSCs to promote cell death? To answer them, we firstly generated bone marrow chimeras containing 50% wild-type and 50% Casp9-/- cells. In these animals, wild-type HSCs exhibited the same expansion and proliferation as Casp9-/- HSCs. Thus, the defect in Caspase-9 deficient mice is the result of HSC extrinsic factors. We then tested the relationship between Caspase-9-deficient HSC expansion and Bak/Bax-mediated apoptosis by generating Bak-/- Bax-/- Casp9-/- mice. Deletion of Bak/Bax rescued the HSC phenotype in Caspase-9 deficient mice. Together, these data suggest that the intrinsic caspase cascade is essential for normal Bak/Bax-mediated cell death in the hematopoietic system, and in its absence, aberrant cell death feeds back to drive HSC expansion and dysfunction. In support of this notion, we found evidence of up-regulated type I interferon signaling in the HSC compartment. Our study demonstrates that Bak/Bax-mediated cell death of HSCs is dispensable for the maintenance of the HSC pool at steady state. This raises the question of whether HSCs die via alternative cell death pathways, or, whether their death is less frequent than previously believed.

S1010 - IN VIVO IMAGING OF QUIESCENT AND PHYSIOLOGICALLY ACTIVATED HAEMATOPOIETIC STEM CELLS

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Understanding the mechanisms linking stem cell-niche interaction and stem cell fate is critical for developing regenerative medicine approaches. The nature of such interactions between hematopoietic stem cells (HSC) and the bone marrow (BM) microenvironment has long been elusive due to the difficulty of penetrating bones for direct observation and the fluid nature of the hematopoietic tissue itself. Several functional studies based on ablating or over-expressing specific genes in the hematopoietic or distinct BM stroma compartments have highlighted the presence of an intricate and dynamic network of regulatory signals responsible for the crosstalk between HSC and the BM microenvironment. The question, however, remains open as to whether multiple, molecularly and functionally distinct HSC niches exist within the bone marrow and whether HSC trafficking between them may be necessary to switch fate between quiescence and proliferation, self-renewal and differentiation. To address this question, we developed an imaging technique combining two photon and confocal microscopy that allows in-vivo imaging of live transplanted hematopoietic stem and progenitor cells (HSPC) in mouse BM with single cell resolution. Using this technique we showed that engrafting long-term repopulating HSC (LT-HSC) localize near osteoblastic cells, while their progeny are more distal. Our results also highlight that localization of LT-HSC and their progeny near osteoblasts correlates with improved engraftment outcomes. Studies based on single time-point observations demonstrated that asynchronous HSPC proliferation initiates BM reconstitution, however did not provide information about long-term interactions between HSC and their BM niche (or niches), which are responsible for maintenance of balanced haematopoiesis. We therefore developed a new in vivo imaging experimental protocol, allowing time-lapse imaging of HSPC, leading us to uncover their differential abilities to engage with the BM microenvironment over time. Moreover, using a physiological model of HSC activation, we observed that changes in the nature of the interactions between stem cells and the BM microenvironment accompany switches in fate choice.

S1011 - ASYMMETRIC CELL DIVISION AND SPINDLE ORIENTATION IN NEURAL STEM CELLS - FROM DROSOPHILA TO HUMANS

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When we think of mitosis, we commonly have a process in mind where a cell gives rise to two identical daughter cells. In whole organisms, however, many cell divisions are actually asymmetric and give rise to two daughter cells of different size, shape or developmental fate. Asymmetric cell divisions are particularly important in stem cells, as they allow those cells to generate both self-renewing and differentiating daughter cells, an ability that is common to all stem cells. We therefore use stem cells in the developing brain of both fruitflies and mice as a model to understand the principle mechanisms that regulate and orient asymmetric cell divisions. More recently, we have extended our efforts to mammalian model systems, where mutations in regulators of basic cell biological processes like the orientation of the mitotic spindle are known to cause strong brain malformations resulting in severe mental retardation. As recent experiments have shown striking differences between human and mouse brain development, we have made an effort to establish experimental strategies where those regulators and their effects on brain development can be studied in a human setting.

S1012 - HAEMATOPOIESIS DURING EMBRYONIC DEVELOPMENT

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Mature blood cells have multiple functions ranging from transporting oxygen around the body to protecting us from infection. The ability to continue producing enough blood cells throughout life is critical to our health. This process is known as haematopoiesis. At the foundation of blood cell production is the haematopoietic progenitor/stem cell (HSCs); these cell types are not specialised for any function except to ensure that when necessary large mature blood cells can be readily generated. In the adult, the ability to continuously generate mature blood whilst preserving sufficient numbers of progenitor/stem cells is ensured by a process known as self-renewal - a question that remains unanswered is how these cells are produced in the first place. Haematopoietic development in the embryo occurs in a sequential process, during which primitive erythropoiesis and progenitor formation occurs in the yolk sac. Once this first wave of haematopoiesis is established HSCs are then formed; this is a complex multi-site process involving the AGM region, yolk sac and placenta. During foetal life the majority of HSCs reside within the liver, where HSCs continue to expand and initiate definitive haematopoiesis (a sustained haematopoietic system driven by a self-renewing HSC). The embryo provides an invaluable resource for tackling the problems central to haematopoiesis research, particularly the complex problem of how concomitant HSC self-renewal and hematopoietic differentiation can be achieved. I will be discussing how our laboratory is teasing apart these processes, with particular focus on identifying when specification of early hematopoietic lineages is first detectable. Biography (250 word limit): In 2002 Samir joined the laboratory of Professor Alexander Medvinsky at the Institute for Stem Cell Research (Edinburgh), initially as a PhD student and then as a post-doctoral researcher. During this time Samir investigated the process of haematopoietic stem cell formation during mouse embryogenesis

S1013 - EPIGENETIC CONTROL OF HEMATOPOIETIC STEM

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The balance between self-renewal and differentiation of adult stem cells is essential for tissue homeostasis. Here we show that in the haematopoietic system this process is governed by polycomb chromobox (Cb×) proteins. Cb×7 is specifically expressed in haematopoietic stem cells (HSCs), and its overexpression enhances self-renewal and induces leukaemia. This effect is dependent on integration into polycomb repressive complex-1 (PRC1) and requires H3K27me3 binding. In contrast, overexpression of Cb×2, Cb×4 or Cb×8 results in differentiation and exhaustion of HSCs. ChIP-sequencing analysis shows that Cb×7 and Cbx8 share most of their targets; we identified approximately 200 differential targets. Whereas genes targeted by Cbx8 are highly expressed in HSCs and become repressed in progenitors, Cb×7 targets show the opposite expression pattern. Thus, Cb×7 preserves HSC self-renewal by repressing progenitor-specific genes. Taken together, the presence of distinct Cbx proteins confers target selectivity to PRC1 and provides a molecular balance between self-renewal and differentiation of HSCs.

S1014 - INVESTIGATION OF NOVEL SIGNALING PATHWAYS IN NORMAL AND MALIGNANT HEMATOPOIESIS

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Hematopoietic stem cell (HSC) expansion is regulated by complex signaling network, and dysregulation of cytokine signaling leads to hematologic malignancies. The adaptor protein Lnk is a key negative regulator of JAK2 signaling and HSC expansion, mediated by thrombopoieticn (Tpo). Lnk-/- mice have a 10-fold expansion in HSCs with superior self-renewal. Lnk deficiency promotes myeloproliferative neoplasm (MPN) development in mice, and Lnk loss-of-function mutations are found in human MPNs and acute leukemias, emphasizing its pivotal role in normal and malignant HSPCs. Molecular mechanisms by which Lnk regulates cytokine/JAK2 signaling and HSC homeostasis, however, remain enigmatic. I will discuss our progress on identifying novel Lnk interacting proteins and elucidating their roles in HSC homeostasis under normal and stress conditions. Furthermore, I will discuss our recent findings on Lnk functions in a wide range of hematologic malignancies.

S1015 - BIOLOGY, PHENOTYPE AND TARGET EXPRESSION PROFILES OF NEOPLASTIC STEM CELLS IN MYELOID LEUKEMIAS

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Since their description and identification in leukemias and solid tumors, cancer stem cells (CSC) have been the subject of intensive research in translational oncology. Important issues in CSC research are the characterization of CSC-related markers and CSC-augmenting oncogenic pathways, the identification of molecular targets in CSC, and the preclinical and clinical evaluation of the CSC-eradicating potential of various targeted drugs. However, although diverse CSC-markers, targets, and target pathways have been identified, several questions remain, such as the origin and evolution of CSC, resistance against conventional or/and targeted drugs, and the mechanisms underlying stroma-CSC interactions in the so-called 'stem cell niche'. Additional aspects that have to be taken into account when considering CSC-elimination as primary treatment goal, are the heterogeneity, plasticity, and subclone formation of CSC. Notably, various cell fractions with different combinations of molecular aberrations may display CSC function in a given neoplasm, and subclone-formation and CSC plasticity are also considered to contribute to acquired drug resistance. In the current lecture, we will discuss new developments in the field of CSC research and address the question as to whether these new concepts can be translated and can be exploited in clinical practice in the foreseeable future.

S1016 - HEMATOPOIETIC STEM CELL GENE THERAPY FOR WISKOTT-ALDRICH-SYNDROME - VG CK

Chrisoph Klein
Hannover Medical School, Germany

See Program Supplement for abstract details.

S1017 - MOLECULAR GENETICS OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive leukemia resulting from the malignant transformation of T cell progenitors. T-ALL accounts for 15% of pediatric and 25% of adult ALL cases. Treatment strategies have dramatically improved over the past 50 years, and long-term survival for pediatric T-ALL patients is now over 70%. However, as few as 40% of adults reach long-term remission, and patients who relapse have much worse prognosis for survival. In addition, current treatment is based on combination chemotherapy, which is associated with many short term and long term toxic side effects. Therefore, there is a strong need to improve therapy in the older patients and reduce the toxicity in the younger patients. Understanding the biology of T-ALL may identify new targets for therapy and pave the way for the development of new drugs. To get further insight in the complex genetics of T-ALL, we used exome sequencing and RNA-sequencing. Exome sequencing of 67 T-ALL cases revealed the presence of an average of 8 protein-altering mutations in pediatric T-ALL cases, and an average of 21 protein-altering mutations in adult patients. As expected we detected many of the known driver mutations in T-ALL such as mutations in NOTCH1, WT1, BCL11B, PHF6, FBXW7. In addition, we also detected 7 new predicted oncogenic drivers, including mutations in TET1, CNOT3, RPL5, and RPL10. Mutations in CNOT3 are likely to cause inactivation of one allele of CNOT3, which is in agreement with our observation that CNOT3 is a tumor suppressor gene in a fly cancer model. Mutations in the ribosomal protein RPL10 are clustered at one single amino acid and lead to an impaired ribosomal biogenesis. In addition to the identification of these mutations, RNA-sequencing of 31 T-ALL cases led to the identification of several novel fusion transcripts including in-frame fusions involving the FER and JAK2 tyrosine kinases. Our data identify new drivers genes of T-ALL, and demonstrate genetic differences between pediatric and adult T-ALL.

S1018 - GENETIC AND EPIGENETIC CHANGES CONTRIBUTING TO LEUKEMOGENESIS AND PROGNOSTIC IMPACT IN ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is a group of heterogeneous diseases. Abnormalities in genes that encode transcription factors and tyrosine kinases represent two classes of the most frequently detected genetic events in human AMLs. Recently, a new category of gene mutations associated with epigenetic regulation have also been proposed. Recurrent somatic mutations of DNMT3A were identified in 23 of 112 (20.5%) cases using exome sequencing technique. The DNMT3A mutants showed reduced enzymatic activity or aberrant affinity to histone H3 in vitro. Gene expression profiles and DNA methylation patterns showed some differences between patients with and without DNMT3A mutations. Notably, we found that members of HOXB genes were extensively upregulated with mutations of DNMT3A, and hypomethylation of certain CpG islands in the HOXB locus. DNMT3A mutations enabled 32D cells to acquire growth and survival advantage with and without IL3. Leukemias with DNMT3A mutations constituted a group of poor prognosis with elderly disease onset. These data suggest a contribution of aberrant DNA methyltransferase activity to the pathogenesis of acute monocytic leukemia and provides a useful new biomarker for relevant cases. We performed the study to systemically investigate the frequencies and the prognostic relevance of previously known genetic events and newly established molecular markers in a large series of 1,185 adult AML patients. In particular, we intent to stratify the AML patients who lacked cytogenetic prognostic markers into appropriate prognostic groups. Multivariate analysis identified DNMT3A and MLL mutations as independent factors predicting inferior overall survival (OS) and event free survival (EFS), while bi-allelic CEBPA mutations or NPM1 mutations without DNMT3A mutations conferred a better OS and EFS in whole group and younger patients aged less than 60 years. Further studies will be focused on using different treatment strategies according to AML genotypes.

S1019 - STAT5 - A CENTRAL PLAYER IN BCR/ABL+ LEUKEMIA

Veronika Sexl[1], *Wolfgang Warsch*[1], *Eva Grundschober*[1], *Angelika Berger*[1], *Lukas Höfling*[1], *Andrea Hölbl-Kovacic*[1], *and Richard Moriggl*[2]
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It is beyond doubt that the transcription factor STAT5 has an important and unique role in BCR-ABL- driven neoplasias. STAT5 is an essential component in the signaling network that maintains the survival and growth of BCR-ABL1-transformed cells. The transcriptional activity of STAT5 is required to allow proliferation and survival of the cells. Furthermore, we and others have shown that STAT5 is up-regulated in leukemic cells of CML patients during disease progression; we propose a selection process resulting in cells with high STAT5 expression levels which cause reduced responsiveness towards tyrosine kinase inhibitors such as imatinib even in the absence of BCR-ABL1 mutation. Remarkably, we also found a clear correlation between STAT5 expression levels and the presence of BCR-ABL1 mutations. Our data indicate that STAT5 triggers ROS production accounts for the enhanced mutation rate detected in human patient samples harboring elevated STAT5 levels. Accordingly, STAT5 qualifies as a potential therapeutic target; one possibility to inhibit its transcriptional activity is to hinder post-translational modifications. For full transcriptional activity STAT5 requires tyrosine phosphorylation in the C-terminal domain which is controlled by JAK kinases in untransformed hematopoietic cells. Whether JAK2 functions as an upstream kinase of STAT5 in CML is still under debate. Although there is widespread agreement that JAK2 is part of the signaling network downstream of BCR-ABL1, it is unclear whether and under what circumstances JAK2 inhibitors may be beneficial for CML patients. Our studies in murine models have cast doubt on the importance of JAK2 in CML maintenance. Strikingly, in BCR-ABL1-transformed cells signaling is rewired and the BCR-ABL1 oncoprotein itself accounts for STAT5 phosphorylation - independent of JAK kinases. Additionally, STAT5 is phosphorylated on highly conserved serine residues present in the transactivation domain of the protein. Novel insights in the role of serine phosphorylation for BCR-ABL1-driven leukemogenesis will be provided.

S1020 - REGULATION OF MYELOID TRANSCRIPTION FACTORS AND DNA METHYLATION BY NONCODING RNAS

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Tight regulation of myeloid transcription factors PU.1 and C/EBPa is necessary for proper hematopoietic stem cell function and granulopoiesis, and dysregulation of these genes can lead to development of leukemia. We have focused on regulation of these two genes by noncoding RNAs (ncRNAs). In the case of PU.1, we previously described a long antisense RNA that is initiated from a discrete promoter in intron 3 and extends past the transcription start site. This transcript is expressed at highest levels in T cells, in which PU.1 is not expressed, and can inhibit PU.1 protein. siRNAs which target this antisense transcript can increase PU.1 mRNA and protein, and induce differentiation of leukemic cells. There is a second long noncoding RNA in the PU.1 locus, which is initiated in the upstream regulatory element (URE), and extends for greater than 10 kb toward the transcription start site. This transcript is entirely nuclear, and its function is unknown. We are in the process of testing the function of both transcripts by using BAC transgenics in which transcription terminators have been used to abrogate expression of the noncoding RNA. In the case of C/EBP alpha, we identified a ncRNA extending beyond the polyadenylation signal of the C/EBPa gene. In contrast to PU.1, this extracoding transcript correlates positively with C/EBP alpha mRNA, and siRNA knockdown of the ncRNA leads to a decrease in C/EBP alpha mRNA and increase in methylation of the locus. Overexpression of this ncRNA leads to an increase in expression of C/EBP alpha in a cell line (K562) in which C/EBP alpha is methylated and not expressed. In summary, we have initiated studies of long noncoding RNAs in both the PU.1 and C/EBP alpha genes. The function of these RNAs appears to be completely different. In the case of PU.1, an antisense noncoding RNA downregulates PU.1 expression. In the case of C/EBP alpha, an extracoding RNA inhibits methylation of the locus and increases mRNA levels.

S1021 - NOVEL NON-CLUSTERED HOMEOBOX GENES AS KEY REGULATORS IN NORMAL AND MALIGNANT HEMATOPOIESIS

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Utilizing transcriptional analysis of highly purified hematopoietic stem cells (HSC) from mouse and human model systems, we have recently identified two non-clustered homeodomain-containing genes, H2.0-like homeobox (HLX) and special AT-rich sequence-binding protein 1 (SATB1), as novel key regulators of stem and progenitor cell function in normal hematopoiesis and in leukemia. We found that HLX is overexpressed in the majority of patients with acute myeloid leukemia and that high HLX expression levels independently correlate with inferior overall survival. Elevated HLX levels lead to formation of aberrant progenitors with unlimited serial clonogenicity and a block in differentiation. Decrease of HLX inhibits leukemic cell growth in vitro, overcomes the myeloid differentiation block, and prolongs survival in vivo. HLX regulates a key set of genes which mediate its leukemia-promoting function and may be used for targeted therapy. We found SATB1 expressed at high levels in HSC. Functional studies revealed Satb1 as a critical regulator of HSC fate. HSC lacking SATB1 have defective self-renewal, are less quiescent and show accelerated lineage commitment, which results in progressive depletion of functional HSC. The enhanced commitment is caused by decreased symmetric self-renewal and more symmetric differentiation divisions of Satb1-deficient HSC. SATB1 simultaneously represses sets of genes involved in HSC activation and cellular polarity, including Numb and Myc. Thus, SATB1 is a new regulator that promotes HSC quiescence and represses lineage commitment. In summary, non-clustered (class II) homeobox genes are emerging as new transcriptional key regulators in hematopoiesis and leukemia.

S1022 - ROLE OF MUTATIONS IN EPIGENETIC REGULATORS IN PATHOGENESIS OF MYELOID MALIGNANCIES

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Clinical, cytogenetic, and gene-based studies have been used to inform biology and improve prognostication for patients with acute myeloid leukemia (AML), myelodysplasia (MDS), and myeloroliferative neoplasms (MPN). Most recently, a series of candidate gene and whole genome studies have identified recurrent somatic mutations in AML, MDS, and MPN patients including TET2, ASXL1, DNMT3A, and EZH2, mutations. We and others have shown these mutations are of prognostic relevance, and can be used to improve risk stratification in AML, MDS, and MPN. Of biologic importance, the TET family of proteins have been shown to place a hydroxyl mark on methylated DNA and lead to DNA demethylation. We and others have found that TET2 mutations leads to loss of DNA hydroxymethylation and a hypermethylation phenotype in leukemia patients. In addition, in vitro and in vivo studies show that TET2 loss leads to impaired hematopoietic differentiation, increased stem cell self-renewal, and myeloid transformation in vivo. Most recently studies have revealed a role of mutations in chromatin modifying enzymes in hematopoietic transformation, including mutations in the enhancer of trithorax and polycomb (ETP) gene ASXL1. We have elucidated the effects of ASXL1 mutations on chromatin state, gene expression, and hematopoietic function, and identified a specific role for ASXL1 in regulating H3K27 trimethylation and PRC2 function at specific loci in hematopoietic cells including at the HoxA cluster. We have also shown that hematopoietic specific loss of ASXL1 loss leads to myeloid transformation in vivo. These data demonstrate that novel mutations coopt the epigenetic state of hematopoietic stem/progenitor cells in order to contribute to transformation.

S1023 - A ROLE FOR INFLAMMATORY SIGNALING IN LYMPHOID PROGENITOR FORMATION IN THE FETUS

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Hematopoietic progenitors, including erythroid/myeloid and lymphoid progenitors, and hematopoietic stem cells (HSCs) differentiate from hemogenic cells in the embryo. Hemogenic endothelium is not homogeneous, as erythroid/myeloid progenitors (EMPs) and HSCs differentiate from distinct populations of hemogenic endothelial cells: EMPs differentiate from hemogenic endothelium in the yolk sac that does not express a Ly6a-GFP transgene, while HSCs differentiate from Ly6a-GFP expressing cells. It was previous shown that all HSCs in embryonic day (E) 11.5 Ly6a-GFP transgenic embryos are in the GFP+ population of cells from the aorta/gonad/mesonephros (AGM) region and placenta. Lymphoid progenitors in E11.5 embryos are also in the GFP+ population of hematopoietic cells in the AGM region, vitelline and umbilical arteries, while EMPs are predominately in the GFP- populations of CD45+ fetal liver and yolk sac cells. To identify signaling pathways involved in lymphoid progenitor and HSC formation, Ly6a-GFP+ and Ly6a-GFP- endothelium and hematopoietic cells were FACS sorted from AGM regions, vitelline and umbilical arteries of E10.5 Ly6a-GFP transgenic embryos, and their gene expression profiles compared. The results show that genes in cytokine, chemokine, and immune response pathways were upregulated in both Ly6a-GFP+ endothelial and hematopoietic cells. Moreover, after exposure to inflammatory cytokines, Ly6a-GFP expression was enhanced in the endothelium and hematopoietic clusters of explanted E9.5 embryos. Interferon gamma and alpha4 were particularly potent inducers of Ly6a-GFP expression, and interferon gamma deficient embryos have decreased numbers of lymphoid progenitors. Together these data indicate that inflammatory signaling regulates lymphoid progenitor numbers in the embryo.

S1024 - FUNCTIONAL ORGANIZATION OF β-GLOBIN LOCUS CHROMATIN LOOP OVER LONG DISTANCES

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Chromatin loop formation to achieve proximity between distant enhancers and target genes is a well established mechanism underlying enhancer function. Loop formation appears to be closely linked to transcription activation. When proteins that are functionally involved in long range interactions are reduced using RNAi both the looping and transcription diminish. How chromatin loops initially form and the relationship between loop formation and transcription remain important open questions. In the β-globin locus, reduction of the erythroid factors GATA-1, FOG1 and EKLF (KLF1) or the more widely expressed LIM-domain 1 (Ldb1) protein showed that they are required for β-globin activation and for looping between the gene and the β-globin locus control region (LCR) enhancer. Ldb1 is a non-DNA binding protein with a conserved, 200 amino acid N-terminal domain through which it can dimerize or multimerize in vitro and that is required for rescue of Ldb1 in vivo functions in the developing nervous system of flies and vertebrates. Ldb1 is required for both primitive and definitive erythropoiesis in the mouse. In erythroid cells, the C-terminal LIM-interacting domain of Ldb1 binds to LMO2 which, in turn, provides association of Ldb1 with chromatin through DNA-binding partners GATA-1 and TAL1. We expressed mutated or fused versions of Ldb1 in the background of Ldb1-depleted erythroid cells and investigated their ability to rescue β-globin/LCR proximity and β-globin expression. Deletion analysis revealed a small conserved region of the Ldb1 dimerization domain that was dispensable for dimerization and chromatin looping but necessary for co-activator recruitment, promoter remodeling, RNA polymerase II recruitment and transcription activation. The results provide mechanistic insights into the role of Ldb1 and associated proteins in mediating chromatin looping and indicate that looping and transcription activation can be separated.

S1025 - TRANSCRIPTIONAL AND EPIGENETIC THRESHOLDS FOR ENTRY TO THE T-CELL DEVELOPMENTAL PROGRAM

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The T-cell development program is induced in hematopoietic progenitors by encounter with Notch ligands in the thymus. It is known that T-lineage transcription factors, TCF-1 (encoded by Tcf7) and GATA-3, are activated during the early stages of the response. With the activation of GATA-3, the cells lose access to the B-cell fate alternative. However, the cells still maintain a program of progenitor-associated gene expression including Tal1, Sfpi1, Hhex, Bcl11a, Gfi1b, Lyl1, and others. The cells expressing these progenitor-associated genes still appear to be uncommitted to the T-cell fate and include many that retain conditional access to nonlymphoid alternatives including dendritic cell and Gr-1+ Mac-1+ myeloid cell differentiation. The cells only become committed as they turn on another wave of T-cell genes, including the lineage-specific transcription factor Bcl11b. The individual steps in this process are slow, requiring multiple cell cycles per transition. Transcriptional perturbation, genomic analysis, and live-cell imaging experiments shed light on the staging of individual transitions in the process as well as the rate-limiting steps that yield the slow overall pace. We will present evidence for three specific regulatory thresholds that the cells must overcome: an initial PU.1-restricted threshold for the activation of definitive T-cell genes by Notch signals; a separate threshold, partly dependent on GATA-3, for the silencing of PU.1; and yet another, epigenetically controlled threshold for the activation of Bcl11b. The separation of these steps permits the system to adapt to radically different kinetic regimes in fetal and adult T-cell generation.

S1026 - EPIGENETIC LANDSCAPE OF HEMATOPOIETIC LINEAGE COMMITMENT CAN BE VISUALIZED BY ANALYSIS OF INCORPORATED H3.3 VARIANT

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Cell differentiation is achieved by sequential gene expression. Late differentiation marker genes are regulated at the chromatin level prior to cell differentiation. Histone H3 molecule, the basic component of chromatin, has at least 3 variants: H3.1, H3.2, and H3.3. It has been shown that H3.3 was localized in the euchromatin region, whereas H3.1 and H3.2 are within heterochromatin region, suggesting that H3.3 incorporation is requirement for the open chromatin state. Thus, the histone H3.3 is the target of methylation to activate or inactivate chromatin, thereby allowing or blocking transcription factors and other functional proteins to access the DNA. Here we report that the deposition of histone variant H3.3 around the hematopoiesis-related genes specifically occurs in hematopoietic stem/progenitor cells, prior to differentiation. To visualize genome-wide endogenous H3.3 deposition, we first generated monoclonal antibodies that specifically recognize the H3.3 tail that has only 1 amino-acid differences between those of other H3 variants, and performed ChIPSeq assays for purified hematopoietic stem cells (HSCs), common myeloid progenitors (CMPs), granulocyte/monocyte progenitors (GMPs), and megakaryocyte/erythroid progenitors (MEPs). The high-resolution genome-wide analysis of histone incorporation revealed that more than a half of H3.3 exists not only in the gene body and proximal promoter regions, but rather in the inter-genic regions around hematopoietic genes. Interestingly, H3.3 marks the region including ∼70kbp of upstream and downstream of hematopoietic lineage-related genes such as transcription factors, prior to differentiation, and therefore the existence of the H3.3 variant appeared to predict the transcribed region in subsequent stages of hematopoiesis. For example, in HSCs, the H3.3 marking was observed broadly in genes related to myeloerythroid and lymphoid genes, whereas in downstream progenitor populations, it was restricted to genes of myeloid, erythroid, and/or lymphoid lineages. This hierarchical H3.3 deposition was partially disrupted in human acute myelogenous leukemia. The present findings suggest that the incorporation of H3.3 is the earliest epigenetic event involved in determining hematopoietic cell fate, which is necessary for the maintenance of normal hematopoiesis.

S1027 - CONTROL OF PU.1 EXPRESSION BY THREE-DIMENSIONAL CHROMATIN ARCHITECTURE IN HEMATOPOIESIS AND LEUKEMIA

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Acute myeloid leukemia (AML) is a heterogeneous disease for which numerous subtype-defining mutations and epigenetic aberrations have been identified. However, disease-associated changes in higher-order chromatin structure have not been described in AML or any other leukemia. Using a genome-wide screen, we captured distal chromatin regions spatially associated with the promoter of the gene encoding the myeloid tumor suppressor PU.1. This strategy revealed that PU.1 expression is frequently silenced across all human AML subtypes by CTCFdependent reprogramming of spatial chromosomal conformation. CTCF induces a looping architecture in AML that mimics the structure that physiologically represses PU.1 in T cells. Thus, genetically heterogeneous leukemias can share common disease-linked three-dimensional chromatin alterations, which contribute to tumor suppressor silencing. Targeting of such repressive chromosomal loops may provide novel opportunities for therapeutic intervention in leukemias.

S1028 - GENETIC BASIS FOR HEMATOPOIETIC STEM CELL GENERATION IN THE MAMMALIAN EMBRYO

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The generation of hematopoietic stem cells (HSCs) from hemogenic endothelium within the aorta gonad mesonephros (AGM) region of the mammalian embryo is a crucial step in the development of the adult hematopoietic system. Mechanisms underlying this stem cell-generating process are poorly understood. We described a deletion of a Gata2 locus cis-regulatory element (+9.5) that depletes fetal liver HSCs, is lethal at days 13-14 of embryogenesis, and is mutated in a human immunodeficiency that progresses to myelodysplasia and acute myeloid leukemia. As AGM-derived HSCs populate the fetal liver, we tested whether defective HSC generation within the AGM underlies the fetal liver HSC deficiency. I will discuss the role of the +9.5 element in Gata2 expression, HSC generation, and establishment of a complex genetic network, consisting of an ensemble of known and novel regulators of hemogenic endothelium and HSCs. These studies defined a crucial genetic mechanism that controls the emergence of HSCs from hemogenic endothelium to establish the adult hematopoietic system.

S1029 - ELUCIDATING THE MOLECULAR MECHANISM OF ACTION OF CANCER DRUGS IN THE SECOND DECADE OF THE NEW MILLENNIUM

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Biologically active chemical compounds produce complex molecular responses already at the cellular levels. The majority of compounds bind to several proteins: proteins of the cognate target class, those for which the compound was designed, but often also proteins bearing completely different folds. These proteins affect different pathways and cellular processes. While we usually monitor the net outcome of all these interactions in terms of selected biological readouts we are mostly oblivious of the intricacies that occur at the molecular level. We have investigated the mechanism of action of several compounds in clinical use against cancer, mostly of hematological origin. We used a number of approaches, ideally in parallel: 1) chemical proteomics (affinity purifications using immobilized drug matrices followed by mass spectrometry), 2) chemical genetics (random mutagenesis of genome of near-haploid CML cells), 3) functional proteomics (affinity purification / mass spectrometry), 4) transcriptional profiling, 5) phosphoproteomics (where appropriate), 6) computational network analysis and modeling (protein-protein, drug-protein, protein-disease a.s.o.), and 7) validation by focused gene inactivation (RNAi and genome editing). We try to obtain a detailed picture of the actual molecular events and requirements of the drugs under investigation. Using this integrated approach we have identified: 1) new targets for known drugs, 2) previously unknown mechanisms of drug resistance, 3) "effector" genes for the compounds (genes required for the drug to exert its action), 4) mechanisms of synergy between compounds and in a few cases 5) new medical use of existing drugs. We hope that systematic adoption of this more rigorous and "systems-level" characterization of chemical entities will help understanding the biology of drug action better and allow the development of improved drugs. We also believe it should help the community in rationalizing patient stratification, thus increasing the efficacy of clinical trials and reduce unwanted side effects, but also contribute to the employment of mechanism-based combination therapy with existing drugs.

S1030 - THE ROLE OF THE BCL-2 PROTEIN FAMILY IN TUMORIGENESIS AND CANCER THERAPY

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Impaired apoptosis is considered one of the prerequisites for the development of most, if not all, cancers, but the mechanisms that guarantee the sustained survival of most cancer cells remain unknown. Members of the Bcl-2 family are key regulators of apoptosis and include proteins essential for cell survival and those required to initiate cell death. Studies with transgenic mice have shown that over-expression of Bcl-2 or related pro-survival family proteins, such as Bcl-xL or Mcl-1, can promote tumorigenesis, particularly in conjunction with mutations that deregulate cell cycle control, such as deregulated c-myc expression. However, chromosomal translocations or other mutations that promote Bcl-2 over-expression only account for a small proportion of cancers, such as follicular center B lymphoma. As most tumors do not harbor such cytogenetic abnormalities, it is likely that expression of endogenous Bcl-2-like anti-apoptotic proteins, driven by pathways activated by oncogenic lesions, is required to maintain the survival of cells undergoing neoplastic transformation. Using Eμ-myc transgenic mice, a well-characterized model of human Burkitt's lymphoma, and Bcl-2-deficient mice we investigated the role of endogenous Bcl-2 in lymphoma development. Bcl-2 was found to be largely dispensable for the development and survival of pro-B, pre-B and immature B cells expressing the transgene but reduced the numbers of mature Eμ-myc B lymphocytes by ∼10-fold. Despite the resulting marked deficit in pre-malignant B cells, the absence of bcl-2 neither delayed the onset nor reduced the incidence of Eμ-myc B lymphoma. In contrast, loss of Bcl-xL, which also reduced the numbers of pre-leukaemic Eμ-myc B lymphoid cells by ∼10-fold, completely abrogated Eμ-myc induced lymphoma development. These results demonstrate that Bcl-xL but not Bcl-2 is critical to sustain the survival of cells undergoing neoplastic transformation in the Eμ-myc mouse lymphoma model. We are currently investigating whether Bcl-xL is also critical for the development of other types of cancers and whether it is essential for sustaining the growth of already established tumours.

S1031 - REGULATION OF THE DNA DAMAGE KINASE ATM IN THE IMMUNE SYSTEM

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ATM is the protein kinase that is mutated in the hereditary autosomal recessive disease ataxia telangiectasia (A-T). A-T patients display immune deficiencies, cancer predisposition, neuronal degeneration and radiosensitivity. The molecular role of ATM is to respond to DNA double-strand breaks and alterations in chromatin structure by phosphorylating its substrates, thereby promoting repair of damage or arresting the cell cycle. ATM is activated by two known co-factors in a stimulus dependent manner. Following the induction of double-strand breaks by ionising radiation, NBS1 (mutated in Nijmegen breakage syndrome) is required for activation of ATM. ATM can also be activated in the absence of DNA damage by agents that induce changes in chromatin structure. We have recently described ATMIN, for ATM INteractor, to be required for ATM activation under these conditions. How ATM is regulated within the context of the immune system is unclear. We are using genetic mouse models to dissect the contribution of the ATM co-factors, ATMIN and NBS, to its function within the immune system. We will present data on how ATM is regulated within lymphocytes and hematopoietic stem cells in vivo, in order to maintain a functional immune system and suppress tumorigenesis.

S1032 - UNCOVERING THE ROADMAP TO HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cells (HSC) emerge from hemogenic endothelium during development and then become responsible for sustaining lifelong hematopoiesis. To succeed in generating HSCs from pluripotent stem cells, it is critical to define the intrinsic and extrinsic mechanisms that establish hemogenic endothelium and direct these precursors to multipotent, self-renewing HSCs rather than transient embryonic progenitors. Hematopoietic stem/progenitor cells (HS/PC) are generated in multiple anatomical locations, including the yolk sac, the AGM and the placenta. The bHLH factor Scl/tal1 dictates the specification of embryonic endothelium to hematopoietic fate; in the absence of Scl, the prospective hemogenic endothelium becomes mis-specified to cardiac fate. Scl binds to poised enhancers in lateral plate mesoderm and both activates a broad gene regulatory network required for HS/PC development and represses regulators of cardiogenesis. Surprisingly, Scl partners Gata 1 and 2 are dispensable for hematopoietic vs. cardiac specification although they enable Scl to activate transcription factors required for HS/PC emergence from hemogenic endothelium. Several signaling pathways promote the generation of HS/PCs form hemogenic endothelium. Our work suggests that the establishment of lymphoid potential in hemogenic endothelium is sensitive to the dosage of VEGF. Although VEGF haploinsufficient embryos can generate primitive erythroid and transient erythro-myeloid progenitors in the yolk sac, the generation of multipotent HS/PCs with lymphoid potential becomes severely impaired in all hemogenic tissues. Modulation of VEGF levels may thus have an impact on the types of hematopoietic cells that are generated from pluripotent cells. Work by us and others indicate that human ES cells can generate myeloerythroid and lymphoid (at least T) cells in culture, evidencing establishment of multipotent hemogenic endothelium. However, these progenitors lack self-renewal ability and lose proliferative potential prematurely. Our data suggest that the inability to induce critical self-renewal regulators such as the HOXA cluster genes during HS/PC differentiation from hESCs compromises developmental maturation and self-renewal of these HS/PCs. Studies focusing on the mechanisms that establish HSC self-renewal during development will be critical for succeeding in the generation of self-renewing HSCs from pluripotent cells for therapeutic applications.

A B S T R A C T S

ORAL SHORT TALK ABSTRACTS

Numbers beginning with "S" refer to Invited Speaker Abstracts
Numbers beginning with "O" refer to Oral Short Talk Presentations
Numbers beginning with "P" refer to Poster Presentations

Oral Short Talk Presentations

O1001 - HUMAN HEMATOPOIETIC STEM/PROGENITOR CELLS HIBERNATE IN VASCULAR NICHE HARBORING GFAP-EXPRESSING CELLS

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Hematopoietic stem cells (HSCs) reside in specialized bone marrow (BM) microenvironments "niches". Osteoblasts, endothelial cells, CXCL12-abundant reticular cells, nestin+ mesenchymal stem cells, and SCF+ endothelial and perivascular stromal cells are niche constituents. In addition, BM non-myelinating Schwann cells activate TGF-β and induce HSC hibernation in the mouse BM niche (Yamazaki et al., Cell. 2011). Little is known about the HSC niche in human BM. We assessed niche constituents and the role of TGF-β in BM of humanized mice and in clinical human BM specimens. Humanized mice were generated by transplantation of human CD34+ cells into immunodeficient mice. Because these mice sustain human hematopoiesis, human HSCs are likely maintained in the mouse HSC niche. First, we examined lipid raft clustering on the cell surface of Lin-CD34+CD38+ hematopoietic progenitor cells (HPCs) and Lin-CD34+CD38- hematopoietic stem/progenitor cells (HSPCs) using anti-c-kit antibody. In both mouse and human, c-kit molecules cluster on the surfaces of most HPCs, whereas HSPCs do not bear clustered c-kit. As with mouse HPCs and HSPCs, then, most human HSPCs appear postnatally to exist in a hibernating state without lipid raft clustering. In addition, we found that expression of p57 and nuclear localization of FOXO3a are more frequent in HSPCs than in HPCs. Furthermore, in humanized mouse BM, some human HSPCs exist in contact with non-myelinating Schwann cells. These results suggest that hibernation is maintained in human HSPCs by mechanisms like those in mouse HSCs. In human BM specimens, GFAP+ cells immediately adjoining vascular endothelial cells marked on immunostaining with antibodies against active TGF-β. We also found CXCL12-abundant vascular endothelial cells among putative niche cells in human BM specimens. However, unlike in mouse BM, CXCL12-abundant cells were distinct from SCF+ cells. Since these SCF+ cells also expressed smooth muscle actin, we infer them to represent vascular smooth muscle cells. Thus, besides GFAP+ cells, CXCL12-abundant cells, SCF+ cells, and mesenchymal stem cells accompany vessels in human BM. Whilst these results indicate that mechanisms of BM niche hibernation in human HSPCs resemble those in mouse HSCs, the constituents of the human BM niche remain elusive.

O1002 - HUMAN HEMATOPOIETIC STEM CELL INTEGRITY IS GUARDED BY THE UNFOLDED PROTEIN RESPONSE

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Hematopoietic stem cells (HSCs) sustain the entire blood system over the lifetime of an organism. Their long-term potential to produce all mature blood cell types necessitates the maintenance of intact HSCs to prevent the propagation of damaged cells. However, the specific mechanisms responsible for protecting HSC integrity remain poorly understood. We developed a high-throughput method to assess candidate stem cell regulatory genes in vivo using the xenograft assay. Genes were selected based on enriched expression in primitive cord blood and leukemia cell populations compared to less primitive populations. A pooled lentiviral over expression screening approach was used to test the effect of 96 genes on the long-term repopulation capacity of human cord blood cells. We discovered 4 genes that enhanced HSC output: ID2, CD48, DNAJB9 and TNFSF4. Over expression of DNAJB9 resulted in the most significant competitive repopulation advantage and also reduced exhaustion of human HSCs in vitro. DNAJB9 is a co-chaperone that is upregulated upon ER-stress through the Unfolded Protein Response (UPR). We also found differential expression of a number of members of the UPR signaling pathway that are expressed at higher levels in HSCs compared to committed progenitor cells. Functional experiments showed that this pathway predisposed HSCs to ER-stress induced apoptosis. Thus, by applying an in vivo high-throughput screen to primary human cord blood cells, we have uncovered new regulators of human HSC function, including the ER-stress inducible gene DNAJB9. Overall, our work has uncovered a novel pathway that HSCs use to sense and respond to ER-stress in order to protect the life-long integrity of the stem cell pool.

O1003 - STABLE HUMAN HEMATOPOIETIC STEM CELL ENGRAFTMENT SUPPORTS CONTINUOUS DE NOVO GENERATION OF MATURE HUMAN BLOOD CELLS IN MICE

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To determine molecular control mechanisms regulating human hematopoietic stem cell function a surrogate environment that supports stem cell maintenance is necessary. We generated a novel mouse strain, Rag2-/- Il2rg-/- KitWv/Wv mice that carry a defective Kit receptor rendering endogenous mouse HSCs functionally impaired. We find that the mutant Kit receptor opens up the stem cell niches across species barriers and allows for robust and sustained engraftment of human HSCs after transfer into adult mice without the necessity for irradiation conditioning prior transplantation. Following stable engraftment in the mouse bone marrow niches, human HSCs give rise to lymphoid cells and to robust numbers of erythroid and myeloid lineage cells over long periods of time in primary and secondary recipient mice. Therefore, Kit-signaling regulates the competition between mouse hematopoietic stem and progenitor cells by a xenogenic blood stem cell graft.

O1004 - MEGAKARYOPOIESIS IN THE MAMMALIAN EMBRYO IS ASSOCIATED WITH RAPID LINEAGE MATURATION AND SYNTHESIS OF PLATELETS WITH ALTERED ACTIVATION

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We have previously determined that megakaryocyte (MK) progenitors first emerge in the yolk sac of E7.5 mouse embryos and are subsequently found in the E10.5 liver, concomitant with initiation of platelet release into the bloodstream (Tober, Blood 2007). While megakaryopoiesis is established prior to hematopoietic stem cell emergence, little is known regarding the differentiation and function of the MK lineage during embryogenesis. We have now compared E12.5 fetal liver versus the adult marrow and find that primary embryonic MKs have lower mean ploidy than adult MKs. Unlike adult MKs, low-ploidy embryonic MKs reveal evidence of maturation, including mature cell surface marker expression and the presence of a punctate pattern of α-granule components and demarcation membranes in the cytoplasm. These differences between embryonic and adult MKs appear to be cell intrinsic, since MKs derived from the in vitro culture of E9.5 yolk sac versus adult marrow have lower ploidy and late-stage cell surface marker expression. While we had previously shown that embryonic platelets are larger, more reticulated, with small α-granules than adult platelets, our recent histochemical studies reveal similar distribution patterns of the α-granule components VEGF and endostatin. To begin to determine if there are functional differences between embryonic and adult platelets, we first compared expression of transcripts associated with signaling cascades regulating platelet activation. While the transcripts for many components are similar, embryonic platelets express much lower levels of ADP-receptors (P2Y12, P2Y1) and P-selectin. Consistent with these results, E12.5 platelet activation failed to increase P-selectin on the platelet surface. Taken together, our findings indicate that embryonic MKs are characterized by low ploidy and rapid maturation leading to the generation of platelets with marked differences in size, structure and function compared to adult platelets. Hypothesizing that differentiating ES cells recapitulate early embryonic events, we find that MKs generated from ES cells have the low ploidy and rapid maturation characteristics of primary embryonic MKs. A better understanding of embryonic megakaryopoiesis will lay a foundation for the development of clinically useful platelet therapies from ES/iPS cell sources.

O1005 - MEGAKARYOCYTES PRODUCE A DISTINCTIVE HSC NICHE THROUGH CLEC-2 GUIDED SECRETION OF TPO

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The bone marrow (BM) microenvironment (i.e. the niche) governs the integrity of hematopoietic stem cells (HSCs) through the production of various niche factors. Thrombopoietin (TPO) functions as a secretory niche factor to maintain HSCs in a quiescent state, yet regulatory mechanisms of TPO production in the BM remains elusive. While non-hematopoietic cells mainly constitute the niche, we focused on mature hematopoietic cells, megakaryocytes (Mgk), and their influence within the niche for HSCs. We previously reported that specific deletion of platelet activation receptor C-type lectin like receptor-2 (CLEC-2) on Mgk lineages (PF4Cre:CLEC-2floxed/floxed; Clec2PltΔ/Δ) confined the function of BM HSCs. Taken that long term (LT-) HSCs frequently reside near Mgks, we attempted genetic and neutralizing antibody oriented ablation specific to Mgks. This resulted in a drastic loss in the number, a reduction in quiescence and a lower repopulation potential of BM LT-HSCs. Although Clec2PltΔ/Δ mice exhibit a modest decrease in mature Mgks in the metaphyseal region of the BM, Clec2PltΔ/Δ Mgk progenitors matured without arrest in normal ploidy. Yet when co-cultured with HSCs, Clec2PltΔ/Δ Mgks failed to maintain LT-HSC populations in vitro. Genetic expressions of Clec2PltΔ/Δ Mgks revealed a decrease in various niche factors including TPO, TGF-β, KitL and osteopontin. Furthermore, cultured Mgks increased the gene expression of similar niche factors when treated with a stimulatory CLEC-2 antibody. Despite presenting thrombocytopenia in the peripheral blood, Clec2PltΔ/Δ mice exhibited remarkably low concentrations of TPO in the serum, BM and spleen, which signified a defective feedback regulation on systemic as well as intra-BM TPO levels in the absence of platelet and Mgk CLEC-2. Taken together, CLEC-2 expressed on Mgks possibly promotes juxtacrine secretion of niche factors including TPO for the development of a BM niche that maintains HSCs. We identify Mgks as a novel entity of the HSC niche along with an eminent delineation of systemic TPO regulation through CLEC-2.

O1006 - PERI-VASCULAR MEGAKARYOCYTES RESTRAIN HEMATOPOIETIC STEM CELL PROLIFERATION

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Cell cycle quiescence is a critical feature maintaining hematopoietic stem cells (HSCs). However, the mechanisms that regulate HSC behavior are incompletely understood. Mature megakaryocytes (Mk) reside in sinusoidal bone marrow (BM) vasculature where they shed platelets into the circulation. We recently developed a whole-mount confocal immunofluorescence imaging technique combined with computational modeling to assess the 3D relationships of HSCs with stromal and vascular structures in the BM. In the course of these studies, we noted that ∼ 50% of Lin- CD41-CD48-CD150+ HSCs are immediately adjacent to Mk. The overall average distance of HSCs to Mk was 15.4μm which differed significantly from the distance simulated as random distribution (29μm;P=6x10-8). To assess the functional role of Mk in HSC regulation in vivo, we generated PF4-cre; iDTR mice (cre recombination under the Mk-specific platelet factor 4 promoter induces expression of the diphtheria toxin receptor) to specifically deplete BM Mk and circulating platelets upon DT injection. Strikingly, Mk depletion increased 11.5-fold ($P<0.001$) the number of Lin-c-kit+Sca-1+CD105+CD150+ HSCs due to enhanced proliferation (by 5.5-fold, as per BrdU incorporation; $P<0.001$). Injection of neuraminidase, which depleted platelets but not BM Mk, failed to trigger HSC expansion. These results suggest that Mk negatively regulate HSC pool size. In search of mechanisms through which Mk maintain HSC quiescence, we are currently investigating Mk-derived factors that are capable of limiting proliferation. Although TGFβ1 has been reported to be expressed at high levels in Mk and to regulate HSC quiescence, deletion of TGFβ1 in Mk using PF4-cre; TGFβ1fl/fl mice did not alter HSC numbers, proliferation and engraftment potential compared to control animals. In contrast, our results suggest that the chemokine PF4 itself may play an important role. Indeed, recombinant PF4 reduced HSC proliferation in vitro (∼4 fold; $P<0.04$), and PF4 injections into wild-type mice reduced HSC proliferation and numbers in the BM ($P<0.006$ and $P<0.05$, respectively). These studies support the idea of a feedback loop wherein an HSC progeny, the Mk, can directly regulate HSC pool size and proliferation.

O1007 - 17BETA-ESTRADIOL HAS A BIPHASIC EFFECT ON THE FORMATION OF HEMATOPOIETIC STEM CELLS

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While the mechanisms regulating hematopoietic stem cells (HSCs) are increasingly understood, less is known about the impact of environmental factors on this process. Estrogenic compounds were isolated as modifiers of runx1 expression in a zebrafish chemical screen. Exposure to 17beta-estradiol (E2) significantly decreased runx1+ HSCs in the AGM compared to controls by in situ hybridization and qPCR ($p<0.01$) which was confirmed by CD41 FACS ($p<0.0001$). E2 treatment from 12-24 hours post-fertilization disrupted vessel formation and angiogenic sprouting, as indicated by flk1 (kdrl) and altered the assignment of ephrinb2+ arterial identity. Similarly, E2 decreased activity in a Notch pathway-reporter line and blocked arterial expression of deltaC and notch5 as well as VEGFAa expression, upstream of the notch cascade. Hyperactivation of either the VEGF or Notch pathways rescued E2 mediated hematopoietic defects confirming changes in this transcriptional network underlie observed phenotypes. In contrast, when exposure to E2 occurred after arterial establishment and initiation of blood flow, from 27-34hpf, E2 enhanced HSCs by in situ and qPCR (runx1; $p<0.05$). qPCR revealed elevated levels of cell cycle markers cyclinD1 ($p<0.01$) and c-myc ($p<0.001$) and AGM phospho-histone H3 increased ($p<0.01$), suggesting E2 enhances cell cycling. This effect was not dependent on Notch or VEGF signaling. Interestingly, E2 also increased expression of gata1 by in situ and FACS. However, expression of globin was repressed, suggesting that while estrogen enhances self-renewal of progenitors, it may impair their differentiation into mature lineages. To analyze conservation of effect, we examined hematopoietic development in embryos with 5-alpha reductase deficiency and increased estrogen levels. Homozygotes show fewer phenotypic HSCs by FACS; heterozygotes display increased erythryocyte progenitors but no increase in mature erythrocytes, suggesting that the role of estrogen in HSC induction and erythropoiesis is conserved in a mammalian system. Together, these data indicate a novel role for estrogen in the development of the hematopoietic niche and identify estrogen as an enhancer of HSC proliferation.

O1008 - DIVERSE AND HERITABLE LINEAGE IMPRINTING OF EARLY HEMATOPOIETIC PROGENITORS

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Hematopoietic stem cells (HSCs) and their subsequent progenitors produce blood cells, but the precise nature and kinetics of this production is contentious. In one model, lymphoid and myeloid production split after the lymphoid-primed multipotent progenitor (LMPP), with both branches subsequently producing dendritic cells (DCs). However, this model is largely based on in vitro clonal assays and population-based tracking in vivo, which could miss in vivo single cell complexity. Here we avoid these pitfalls using a new quantitative version of "cellular barcoding", to trace the in vivo fate of hundreds of LMPPs and HSCs at the single cell level. These data demonstrate that LMPPs are highly heterogeneous in the cell types they produce, separating into combinations of lymphoid-, myeloid-, and DC-biased producers. Conversely, while we also observe a known lineage bias of HSCs, most cellular output is derived from a small number of HSCs that each generates all cell types. Crucially, in vivo analysis of the output of sibling cells derived from single LMPPs demonstrates that they often share a similar fate, suggesting that the fate of these progenitors was imprinted. Furthermore, as this imprinting is also observed for DC-biased LMPPs, DCs may be considered a distinct lineage on the principle of separate ancestry. These data suggest a 'graded commitment' model of hematopoiesis, in which heritable and diverse lineage imprinting occurs earlier than previously appreciated.

O1009 - GLOBAL LANDSCAPE OF HEMATOPOIETIC STEM CELLS AND MULTIPOTENT PROGENITORS

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In the hematopoietic system, hematopoietic stem cells (HSC) harbor the highest self-renewal activity and generate a series of multipotent progenitors (MPP) that differentiate into lineage-committed progenitors. To explore essential HSC features such as self-renewal and quiescence, we performed an extensive global analysis combining the latest generation of quantitative proteome and transcriptome (RNA-seq) analyses. Therefore, we ex vivo-isolated and FACS-sorted HSC (Lin-Sca1+cKit+ (LSK) CD34- Flt3- CD150+ CD48-), MPP1 (LSK CD34+ Flt3- CD150+ CD48-), MPP2 (LSK CD34+ Flt3- CD150+ CD48+), MPP3 (LSK CD34+ Flt3- CD150- CD48+) and MPP4 (LSK CD34+ Flt3+ CD150+ CD48+), as previously described in our lab (Wilson et al.). By employing stable isotope dimethyl labeling and high-resolution tandem mass spectrometry, more than 7,000 proteins were identified. Expression profiling highlights energy metabolism, immune response, cell cycle and DNA repair to be modulated along differentiation. To our knowledge, these data represent the first global protein signature of HSC defined at this level. Furthermore, using in-depth RNA-seq we achieved more than 11-fold coverage of the genome enabling for robust identification of over 22,000 genes. We describe specific expression clusters of cell adhesion molecules and TFs and present a comprehensive landscape of polyA-long non-coding RNAs (lncRNA) in HSC or MPP. Finally, to address differentiation potential of MPP2, 3 and 4 we complement our OMICs approach with functional transplant experiments. In summary, the gene and protein signatures for stemness and multipotency defined in this study represent a novel unique resource for the scientific community and will significantly extend the current understanding of HSC biology.

O1010 - ESTABLISHMENT OF LYMPHO-MYELOID RESTRICTED PROGENITORS PRIOR TO THE EMERGENCE OF DEFINITIVE HEMATOPOIETIC STEM CELLS

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In jawed vertebrates, development of an adaptive immune-system is essential for protection of the born organism against otherwise life-threatening pathogens. Myeloid cells of the evolutionary older innate immune-system are formed early in development, while lymphopoiesis has been suggested to initiate much later, following emergence of definitive hematopoietic stem cells (HSCs). Herein, we prospectively identified a lympho-myeloid restricted embryonic progenitor as early as E11.5 that expresses IL7Rα and that sustains combined lymphoid and GM transcriptional lineage priming and lineage potentials at the single cell level but no MkE potential. Using Rag1-GFP knock-in mice, we tracked the establishment of this lympho-myeloid restricted progenitor back to as early as E9.5, preceding both hematopoietic colonization of the FL and the establishment of definitive HSCs. Moreover, through in vivo fate mapping we confirmed the inability of Rag1 expressing early embryonic progenitors to contribute to the MkE lineage, while unequivocally and robustly contributing to the myeloid innate as well as lymphoid adaptive immune systems of the mammalian embryo. These findings identify the developmentally earliest immune-restricted progenitor and establish the lympho-myeloid restriction step as a physiologically important lineage commitment step in embryonic mammalian hematopoiesis, preceding the emergence of definitive HSCs.

O1011 - RHO GTPASE ACTIVATING PROTEIN P190-B REGULATES HEMATOPOIETIC STEM CELL SELF-RENEWAL DECISIONS

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Hematopoietic stem cells (HSC) can self-renew or commit to differentiate into all blood cells. The signaling network that regulates these choices is poorly understood. We previously showed that p190-B loss, a negative regulator of Rho activity, enhanced long-term engraftment without altering HSC proliferation or survival or lineage development (Xu, Blood 2009), suggesting an effect on HSC fate decisions. Here, we compared single HSC divisions ex vivo by analyzing multilineage potential of the paired-daughter cells. Non-transplanted (NT) HSCs generated two multipotent daughter cells (self-renewing divisions). However, after secondary transplantation, wild-type (WT) HSCs generated one multipotent and one committed daughter cells (differentiating divisions). p190-B-/- HSCs maintained self-renewal divisions, indicating p190-B loss mediates higher probability of 'stemness' inheritance through divisions to prevent HSC depletion following transplantation. Unexpectedly, p190-B loss does this by decreasing autocrine TGF-β. Active TGF-β protein levels, TGF-β target genes increased in WT but not in p190-B-/- HSCs upon transplantation relative to NT HSC. TGF-β signaling inhibitors restored self-renewal divisions of transplanted WT HSC as seen in vitro and in engraftment in vivo. TGF-β inhibitor treatment of transplanted mice significantly increased HSC frequency - without changing their proliferation - that yielded higher chimera in tertiary transplant compared to DMSO treated mice. Conversely, recombinant TGF-β1 changed non-transplanted HSC choice to differentiating divisions in vitro. Finally, p38MAPK activity mediated TGF-β effect on HSC fate decisions. Hence, HSC decisions to self-renew or differentiate are specified by a p190-B/TGF-β signaling pathway during HSC regeneration. This study uncovers a novel role for autocrine TGF-β in HSC fate decisions uncoupled from its role on HSC quiescence that may have important implications for regenerative medicine.

O1013 - CIRCADIAN PARASYMPATHETIC REGULATION OF HEMATOPOIETIC STEM CELL TRAFFIC

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Hematopoietic stem cell (HSC) traffic follows circadian oscillations. HSCs are preferentially released into blood during the resting phase following rhythmic noradrenaline release in the bone marrow (BM), activation of β3-adrenergic receptors in nestin+ mesenchymal stem cells (MSCs) and Cxcl12 downregulation (Méndez-Ferrer et al., Nature 2008 and 2010). Here we studied the role of the parasympathetic nervous system (PNS) in circadian HSC regulation. PNS activity is defective in mice lacking the GDNF family receptor alpha-2 (Gfra2-/-). We found that Gfra2-/- mice show circadian-specific deregulation of HSC traffic. During the darkness phase, circulating leukocytes, hematopoietic progenitors (HSPCs) and HSCs were 2-3-fold higher in Gfra2-/- mice than in control Gfra2+/- littermates. However, the number of BM HSPCs was not affected, indicating that PNS dysfunction specifically affects HSC traffic during the activity phase. Reciprocal BM transplantation showed a hematopoietic non-cell autonomous defect. While the number of BM nestin+ MSCs was normal, a higher number of circulating HSCs inversely correlated in Gfra2-/- mice during darkness phase with reduced expression of HSC maintenance genes (Cxcl12, Kitl) in BM nestin+ MSCs. Strikingly, despite the increased number of circulating HSCs in Gfra2-/- mice, Lin- Sca-1+ c-kit+ cells exhibited increased homing to the BM of non-irradiated Gfra2-/- mice during this phase. This suggested impaired sympathetic-PNS feedback loops in Gfra2-/- mice. Indeed, nocturnal urine norepinephrine was higher in Gfra2-/- mice. In addition, while treatment with a β3-adrenergic receptor antagonist increased BM Cxcl12 expression and reduced circulating HSPCs in control mice during the darkness phase, this treatment was inefficient in Gfra2-/- mice. Finally, administration of blocking antibodies for VCAM-1, E- and P-selectins, which induce HSC mobilization from and prevent homing to BM, abolished dysfunctional HSC traffic in Gfra2-/- mice. These results uncover a previously unrecognized role for the PNS in the regulation of a stem cell niche. They also demonstrate that both branches of the autonomic nervous system cooperate for fine-tuned circadian regulation of HSCs

O1014 - DELETION OF MURINE RAC GTPASES IN THE PERIVASCULAR NICHE DISRUPTS TRABECULAR BONE FORMATION, MEDULLARY BLOOD VESSELS AND HEMATOPOIESIS

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The relative importance of the osteoblastic and perivascular (PV) niches in hematopoiesis remains unclear. Rac signaling is important for non-hematopoietic cell shape, adhesion, migration and survival. We hypothesized that deletion of Rac in the PV niche, which is made up of cells derived from Nestin+ mesenchymal stem cells (Nes+MSCs), would interfere with cell-cell interactions in the microenvironment, affecting hematopoiesis. We conditionally deleted Rac1 from the PV niche in a Rac3-null genetic background to generate Rac-deficient PV cells in vivo. Analysis of Rac1/3 PV-deficient mice showed ~80% deletion of Rac1 sequences from isolated PV cells. We observed that deletion of Rac in the PV space led to abnormal trabecular bone formation and vascular organization in the medullary cavity. These mice also showed altered hematopoietic function including a significant decrease in bone marrow (BM) cellularity (17.9±1.61x107cells/4 bones vs 24.5±3.48x107/4 bones, Rac PV-deficient vs Rac WT, N=7, $p<.05$) and ~30% reduction in number of HSCs (Lin-Sca-1+c-kit+: 2.00±0.50x103/mouse vs 3.36±0.80x103/mouse, N=7, $p<.01$) and progenitors (BM CFUs: 72±8.16 vs 105±12.05/105 cells, N=7, $p<.01$). In addition, there was a 35% decrease in circulating peripheral blood (PB) progenitors CFUs (6.1±1.48 vs 9.4±2.16/ml, N=7, $p<.01$). In the PB there was also an overall 2.3-fold decrease in CD3+ T cells (1.48±0.55x103/µl vs 3.3±0.54/x103/µl, N=7, $p<.01$), but no decrease in B220+ B cells or Gr-1+ granulocytes. Previous studies suggest a role for the PV niche in homing of HSCs/progenitors. We observed a 2.7-fold decrease in homing of DiD-labeled WT LDBM cells to the Rac PV-deficient BM (0.7±0.25 vs 1.8±0.38%, N=16, $p<.001$) and a >50% reduction in retention of WT after 48hrs in Rac PV-deleted mice (1.0±0.21% vs 2.5±0.71% of injected cells, N=10, $p<.001$). These data suggest that Rac GTPases play an important role in the integrity of the PV space and Rac deletion in Nestin+ cells adversely affects trabecular bone formation and hematopoiesis leading to reduced number of HSCs and progenitors in the BM and reduced homing of transplanted HSC.

O1015 - DE NOVO DNA METHYLATION IS REQUIRED FOR MAINTAINING GENOMIC AND TRANSCRIPTIONAL INTEGRITY IN HEMATOPOIETIC STEM CELLS

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DNA methylation is catalyzed by the DNA methyltransferase enzymes Dnmt1, Dnmt3a, and Dnmt3b. We have shown that Dnmt3a is essential for hematopoietic stem cell (HSC) differentiation. Conditional knockout of Dnmt3a (Dnmt3a-KO) resulted in HSCs that could not sustain blood generation after serial transplantation, while mutant HSCs accumulated in the bone marrow. As Dnmt3b is also highly expressed in HSCs, we reasoned it may also have a specific role in HSC function. We performed conditional deletion of Dnmt3b in HSCs, as well as Dnmt3a and Dnmt3b simultaneously, using the Mx1-cre system. Unlike the Dnmt3a-KO HSCs, loss of Dnmt3b had a minimal impact on blood production. However, the Dnmt3ab-dKO (double knock-out) peripheral blood contribution was rapidly diminished, accompanied by a dramatic accumulation of Dnmt3ab-dKO HSCs in the bone marrow (>50-fold), paralleling the phenotype of the Dnmt3a-KO HSC, but more extreme. Using Whole Genome Bisulfite Sequencing (WGBS), loss of Dnmt3a led to both increases and decreases of DNA methylation at distinct genomic regions. Ablation of both enzymes primarily resulted in loss of DNA methylation that was much more extensive. RNA-SEQ of mutant HSCs revealed loss of transcriptional integrity including increased expression of repetitive elements, inappropriate splicing, and premature truncation of 3'UTRs. These data show that Dnmt3a and -3b act synergistically to regulate HSC differentiation. The accumulation of mutant HSCs cannot be attributed to altered proliferation or apoptosis, but rather an imbalance between self-renewal and differentiation. The methylation targets of Dnmt3a and -3b may serve as candidates for therapeutic intervention in malignancies caused by defective DNA methyltransferases. This work highlights the importance of DNA methylation in HSC cell fate decisions and further contributes to understanding the epigenetic regulation of hematopoiesis.

O1016 - HYPOXIA INDUCIBLE FACTOR (HIF)-2α ENHANCES PROLIFERATION OF MALIGNANT HAEMATOPOIETIC CELLS IN THE HYPOXIC MALIGNANT BONE MARROW

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In this study, we investigated the role of hypoxia and HIF2α in leukaemia. In normal bone marrow (BM), HIF2α mRNA was mainly expressed in non-hematopoietic stromal cells, with low to undetectable levels in hematopoietic cells. However, HIF2α mRNA and protein were detected in the BM of moribund NOD/SCID mice engrafted with 3 different primary human ALL, and in cultured human ALL and AML cell lines, suggesting that HIF2α is abnormally expressed in leukemic cells. To investigate the potential roles of HIF-2α in leukemic cells, we cloned human HIF2α cDNA in the MXIE retroviral vector. In a 1st model, human HIF2α or empty Mxie vector was retrovirally transduced into HSC from vavBcl2 transgenic mice and transplanted into lethally irradiated wild-type recipients. Transduction of vavBcl2 HSC with HIF2α enabled dominance of HIF2α expressing B cells, not observed in recipients of vavBcl2 HSC transduced with empty vector. Consequently a significantly higher proportion of recipients of HIF2α transduced vavBcl2 HSC succumbed to spontaneous lymphoma compared to controls (P=0.036 log rank). In a 2nd model, HIF2α was transduced into GM-CSF-dependent mouse pre-leukemic cell line FDCP1, which does not express HIF2α. HIF2α provided a significant proliferative advantage to FDCP1 cells in hypoxic or normoxic cultures and reduced GM-CSF dependency. We next transplanted retrovirally transduced FDCP1 cells into non-irradiated syngeneic DBA/2 mice. All recipients of FDCP1 transduced with HIF2α MXIE vector succumbed to leukemia by week 28 post-transplant whilst leukaemia penetrance in recipients of FDCP1 transduced with empty MXIE vector, was just 15% by week 45 (P=0.0001 log rank). Finally, HIF2α was lentivirally knocked down in human AML cell lines U937 and HL60. While in vivo studies are ongoing, HIF2α knock-down resulted in a 2-fold decrease in proliferation in vitro. Together this data suggests that expression of HIF2α in malignant haematopoietic cells provides a proliferative advantage enabling them to keep proliferating in the hypoxic leukemic BM while the proliferation of normal HSC, which do not express HIF2α, is blocked.

O1017 - THE BMI1-INTERACTING PROTEIN E4F1 IS VITAL FOR HEMATOPOIETIC STEM/PROGENITOR CELL FUNCTION

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Stem cell maintenance critically depends on a delicate balance between cell self-renewal and differentiation. The polycomb protein Bmi1 was shown to be essential for stem cell function in normal and cancerous stem cells in various tissues. The molecular program regulated by Bmi1 remains, however, still poorly understood. In an attempt to identify novel Bmi1-mediators, we isolated the Zn-finger protein E4f1 in a yeast-2-hybrid screen. Interestingly, downregulation of E4f1-levels was sufficient to partially rescue Bmi1-mutant hematopoietic stem cell activity (Chagraoui J. et al., Genes & Dev., 2006), thereby pointing towards a role of E4f1 in regulating stem cell maintenance. The subsequent analysis of E4f1 knockout mice revealed pivotal cellular functions for E4f1 in the hematopoietic system. Mutant mice succumbed quickly due to systemic bone marrow failure, which was caused by the rapid depletion of progenitor cell populations. Unexpectedly, immature stem/progenitor cells appeared to be more tolerant towards E4f1 loss than more committed progenitor cells. At the single cell level, E4f1-deletion resulted in chromosomal segregation errors and massive DNA damage. Detailed cell cycle and DNA combing analysis further confined the onset of the E4f1-phenotype to the G1/S phase transition. These results were in strong agreement with the observation of E4f1-deficient cells being incapable of properly inducing the S-phase regulatory program. In conclusion, E4f1 is indispensible in stem/progenitor cell populations by regulating S-phase entry and progression.

O1018 - HOMOZYGOUS JAK2V617F DRIVES RAPID HEMATOPOIETIC STEM CELL PROLIFERATION AND DIFFERENTIATION AT THE EXPENSE OF SELF-RENEWAL

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Human myeloproliferative neoplasms (MPNs) are clonal disorders characterized by increased proliferation of the myelo-erythroid lineage and derive from a mutation, or series of mutations, in a hematopoietic stem cell (HSC). The JAK2V617F mutation is present in the majority of MPN patients and many patients bear two copies, implicating gene dosage as a potential regulator of distinct disease subtypes. To investigate whether JAK2V617F dosage affected HSC proliferation, differentiation, and self-renewal, we used knock-in mouse models where human JAK2V617F is expressed heterozygously (JAK2 Het) or homozygously (JAK2 Hom) under the control of the endogenous Jak2 promoter. Previously we showed that JAK2 Het mice have a stem cell defect in HSCs $>$6 months of age. Here we show that, despite having a more robust phenotype including a marked erythocytosis, JAK2 Hom mice have a more severe stem cell defect. At 8-12 weeks of age, there are already reduced HSC numbers in JAK2 Hom mice and whole bone marrow transplantation experiments reveal reduced repopulating ability compared to heterozygous and wild-type littermate controls. Highly purified JAK2 Hom E-SLAM HSCs (CD45+EPCR+CD150+CD48-) enter the first cell cycle more rapidly and display a pronounced proliferative advantage in short-term cultures. Moreover, clones derived from JAK2 Hom HSCs were comprised of proportionally fewer stem and progenitor cells. Transplantation of E-SLAM cells reveals that JAK2 Hom HSCs have a reduced per cell repopulation capacity. This may be the result of JAK2 Hom stem and progenitor cells localizing to a distinct hematopoietic stem cell niche as in vivo homing assays using live imaging of osteoblast-restricted collagen 1α promoter (Col2.3-GFP) reporter mice show that JAK2 Hom Lin-CD48-CD150+ are located significantly further from osteoblasts within 2 days post-transplantation. Taken together these results indicate that homozygous expression of JAK2V617F compromises HSC self-renewal by increasing proliferation and differentiation and by affecting niche-selection upon homing, leading to HSC exhaustion amidst expansion of terminally differentiated cells.

O1019 - COOPERATION OF KITD816V AND TET2 LOSS OF FUNCTION IN THE PATHOGENESIS OF SYSTEMIC MASTOCYTOSIS AND ASSOCIATED HEMATOLOGICAL MALIGNANCIES

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Systemic mastocytosis (SM) is a clonal disease of the hematopoietic stem cell compartment, frequently associated with the *KITD816V* activating mutation. This mutation alone cannot account for the variable clinical phenotypes of this disease, ranging from indolent forms to mast cell leukemia (MCL). Loss of function mutations in *TET2* have been described in various myeloproliferative neoplasms including SM, where their coexistence with the *KITD816V* mutation at a frequency as high as 50% has an uncertain biological significance. In a human *KITD816V* positive MCL cell line (HMC1.2), we found that knock-down of TET2 enhanced proliferation, indicating that loss of TET2 in the mast cell lineage contributes to a more aggressive disease phenotype. To further study the cooperation between the two genetic lesions, we bred conditional transgenic *KitD814V* (the mouse homolog of *KITD816V*) to *Tet2* conditional KO animals. Expression of KitD814V in the hematopoietic compartment leads to mastocytosis in all animals, and causes Acute Lymphoblastic Leukemia (ALL) in about 40% of the mice. Simultaneous expression of KitD814V and deletion of one or both copies of Tet2 in the hematopoietic compartment caused increased proliferation and slightly impaired differentiation of mast cells without altering the incidence of ALL across genotypes in primary animals, or the latency of disease in leukemia transplants. We conclude that the cooperation between the two hits is mast cell-specific, and that Tet2 is not required for mastocytosis-associated ALL. *KITD816V* positive patients are resistant to targeted therapy with imatinib, and variable clinical responses have been obtained with other tyrosine kinase inhibitors, such as dasatinib. Since TET2 regulates gene expression through an epigenetic mechanism, we treated HMC1.2 (dasatinib-sensitive) with a priming dose of epigenetic modifiers followed by dasatinib in the presence or absence of a TET2 KD. We observed enhanced cytotoxicity of dasatinib in pre-treated TET2 KD cells, suggesting a novel therapeutic avenue in SM associated with KITD816V and loss of function of TET2.

O1020 - HAPLOINSUFFICIENCY OF *SAMD9L*, ENCODING AN ENDOSOME FUSION FACILITATOR, DEVELOPS MYELOID MALIGNANCIES IN MICE MIMICKING HUMAN DISEASES WITH MONOSOMY 7

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Monosomy 7 is a common chromosomal abnormality found frequently in MDS and AML. We previously identified a common microdeletion cluster in 7q21.3 in JMML patients. This cluster contains three poorly characterized genes: *sterile alpha motif (SAM) domain-9 (samd9)* which is absent in mice, *samd9-like (samd9L)* and *miki (LOC253012)*. Although *samd9L*-deficient mice were grown normally, half of both *samd9L* (+/-) and (-/-) mice naturally developed myeloid neoplasia (mainly MDS) after the age of 18 months. Moreover, when these mice were infected with Mol4070a retrovirus, almost all mice developed myeloid malignancies mainly AML with a wide variety of subtype. This mimics an association of -7/7q- with secondary AML in patients with predisposing leukemia syndromes or therapy-related leukemia. By detecting virus integration sites, we identified co-operative genes, *evi1* and *fbxl10* (encoding a histone H3K4 demethylase), that accelerate leukemia development by aberrant over-expression. Immunofluorescence staining revealed localization of Samd9L as vesicular patterns that partially overlapped with Early endosome antigen 1 (EEA1). In *samd9-L*(-/-) fibroblasts, while rapid internalization of PDGF-receptor by PDGF stimulation occurred in a time-course similar to that in *samd9L*(+/+) cells, homotypic fusion of endosomes containing PDGFR delayed. Inhibition of endosome fusion in *samd9L*(-/-) cells lead to the accumulation of PDGFR that were remained to be phosphorylated in early endosome, resulting in the prolonged activation of cytokine signals. Accumulation of cytokine receptors in early endosome and persistent cytokine signals were also found in hematopoietic progenitors derived from *samd9L*-deficient mice. Bone marrow cells obtained from *samd9L*-deficient mice showed sustained colony formation activity. In addition, in vivo repopulation ability of *samd9L*-deficient early progenitors was superior to wild type progenitors in the competitive repopulation assay. These data suggest that the enhancement of cytokine signals by haploinsufficiency of *samd9L* is a critical mechanism through which loss of chromosome 7 contributes to myeloid malignancies.

O1021 - PAX5 LOSS IMPOSES A REVERSIBLE DIFFERENTIATION BLOCK IN B-PROGENITOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Acute lymphoblastic leukemia (ALL) is characterised by an accumulation of transformed lymphoid progenitor cells. Hypomorphic mutations of the essential B lineage transcription factor PAX5 occur in one third of B-progenitor ALL cases, however their functional consequences remain undefined. We have developed transgenic mice allowing reversible, shRNA-mediated knockdown of Pax5 in vivo, and show that this co-operates with activated STAT5 to induce fully penetrant pre-B ALL. Remarkably, restoring endogenous Pax5 expression in these leukemias promotes cell cycle exit and surface expression of the mature B cell markers IgM/IgD, indicating release of the pre-B stage differentiation block. Acute Pax5 restoration induces global gene expression changes closely resembling the normal developmental progression of large proliferative to small resting pre-B cells, including marked repression of the proto-oncogene Myc. Pax5 restoration in leukemia cells in vivo disables their tumor-initiating capacity and results in tumor clearance, significantly increasing host survival. These results are the first to directly demonstrate that reduced Pax5 activity locks ALL cells into a self-renewing state by impairing a maturation program that normally promotes cellular quiescence. Moreover, by showing that transformed B-ALL cells retain the ability to differentiate despite harboring multiple oncogenic lesions, our results suggest that induction of differentiation represents a rational therapeutic strategy for B-progenitor ALL.

O1022 - CD99 IDENTIFIES DISEASE STEM CELLS IN ACUTE MYELOID LEUKEMIA (AML) AND THE MYELODYSPLASTIC SYNDROMES (MDS)

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MDS and AML are initiated by self-renewing stem cells. To identify markers that may identify these cells, we analyzed transcriptome data from hematopoietic stem cells (HSC) from MDS patients and identified 25 dysregulated transcripts encoding surface proteins. We validated their expression on MDS HSCs by flow cytometry using bone marrow (BM) from MDS patients (n=26), finding CD99 to be the most frequently increased (85%). We also assessed 78 paired diagnosis/relapse AMLs and determined that CD99 is also overexpressed frequently in AML, both at diagnosis (81%) and relapse (83%). To test if CD99 is a leukemic stem cell (LSC) marker, we separated CD99 high and low cells from the CD34+CD38- fraction of three AML BM specimens. CD99 low cells generated more myeloid colonies in methylcellulose than CD99 high cells, and previously identified AML-associated molecular abnormalities (e.g. Flt3-ITD) were absent in CD99 low derived colonies. Transplantation of CD34+CD38-CD99 low cells from a Flt3-ITD+ AML into NOD/SCID/IL2-R null mice (NSG) led to lymphomyeloid engraftment at 12 weeks. Flt3-ITD was absent in engrafted cells, indicating that CD99 expression distinguishes LSCs from normal HSCs. To determine the function of CD99 in AML, we transduced the AML cell line MOLM13 with a CD99 shRNA (8.0-fold knockdown) and xenografted it into NSG mice. Animals transplanted with these cells showed improved survival compared to vector controls (58d vs. 34d, p=0.02). In primary AML samples, CD99 expression was higher on PB blasts as compared with BM blasts (p=0.03). Overexpression of CD99 in AML cell lines promoted transendothelial migration in transwell assays. Together, these findings suggest that CD99 promotes AML disease aggressiveness by enhancing transendothelial migration and mobilization. Unexpectedly, CD99 transcript expression in the ECOG 1900 AML patient cohort (n=308) positively correlated with survival (p=0.001). These findings suggest that CD99 may improve survival in the context of chemotherapy by promoting mobilization and thus chemosensitivity of AML cells, with CD99 lo AML cells exhibiting increased retention in chemoprotective BM niches. In line with this hypothesis, the poor prognosis of low CD99 expression appeared to be mitigated by intensification of chemotherapy.

O1023 - IDENTIFICATION OF A DYNAMIC CORE TRANSCRIPTIONAL REGULATORY NETWORK FOR T(8;21) AML

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The t(8;21) translocation fuses the DNA binding domain of the hematopoietic master regulator RUNX1 to the ETO protein. The resultant RUNX1/ETO fusion protein is a leukemia-initiating transcription factor that interferes with RUNX1 function. The result of this interference is a block in differentiation and, after the acquisition of additional mutations, the development of acute myeloid leukemia (AML). We have previously determined genome-wide binding sites of RUNX1/ETO and RUNX1. We also measured open chromatin regions, histone acetylation and RNA-Polymerase II binding as well as global gene expression. Our work demonstrates that selective removal of RUNX1/ETO leads to a widespread reversal of epigenetic reprogramming, including alterations in histone modification patterns and RNA-Polymerase II occupancy as well as inhibition of self-renewal and the induction of myeloid differentiation (Ptasinska et al., 2012, Leukemia). To obtain further insights into the transcriptional network defining t(8;21) AML we have now determined the genome-wide binding sites for the transcription factors RUNX1, LMO2, PU.1 and C/EBPalpha before and after the removal of RUNX1/ETO. We demonstrate that depletion of RUNX1/ETO causes a global re-distribution of RUNX1, LMO2, PU.1 and C/EBPalpha binding. Our studies give detailed insights of the extent of how a single aberrant transcription factor reshapes the epigenetic landscape of normal blood cells into a cancer epigenome. Moreover, the detailed analysis of genome wide data from our perturbation experiments identified a t(8;21)-specific core transcriptional network dynamically responding to the presence or absence of RUNX1/ETO and highlights multiple pathways relevant for therapeutic intervention.

O1024 - SCL REPRESSES CARDIOGENESIS VIA DISTANT ENHANCERS DURING HEMOGENIC ENDOTHELIUM SPECIFICATION

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Understanding the mechanisms directing mesoderm specification holds a great potential to advance the development of cell-based therapies for blood and cardiovascular disorders. The bHLH transcription factor Scl is known as the master regulator for establishing hemogenic endothelium during hematopoietic stem/progenitor cell (HS/PC) development. We recently discovered that Scl is also required to repress cardiomyogenesis in endothelium in hematopoietic tissues and endocardium in the heart (Van Handel, Montel-Hagen et al. Cell 2012). However, the mechanisms for the cardiac repression remained unknown. Combining ChIP-sequencing and microarray analysis of Flk+ mesoderm differentiated from mouse ES cells, we show that Scl both directly activates a broad gene regulatory network required for HS/PC development (e.g. Runx1, cMyb, Lyl1) and directly represses transcriptional regulators for cardiogenesis (e.g. Gata4, Gata6, Myocd) and mesoderm development (e.g. Eomes, Mixl1, Etv2). Repression of cardiac and mesodermal programs occurs during a brief developmental window in Flk1+ mesoderm through Scl binding to distant enhancers that are marked by H3K4me1 at this stage. In contrast, Scl binding to hematopoietic regulators extends throughout HS/PC and red blood cell development and encompasses both distant and proximal binding sites. Surprisingly, Scl complex partners Gata 1 and 2 are dispensable for hematopoietic vs. cardiac specification and Scl binding to majority of its target genes. Nevertheless, Gata factors co-operate with Scl to activate selected transcription factors (e.g Runx1, Gfi1b, cMyb and PU.1) that facilitate HS/PC emergence from hemogenic endothelium. These results denote Scl as a true master regulator of hematopoietic vs. cardiac fate choice and suggest a mechanism by which lineage-specific bHLH factors direct the divergence of competing fates.

O1025 - GENOME-WIDE ANALYSIS OF TRANSCRIPTIONAL REGULATORS IN HUMAN HSCS REVEALS A DENSELY INTERCONNECTED NETWORK OF CODING AND NON-CODING GENES

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Combinatorial transcription factor (TF) interactions regulate hematopoietic stem cell (HSC) formation, maintenance and differentiation, and are recognized as drivers of stem cell signatures in cancer. However, genome-wide combinatorial binding patterns for key regulators have not been reported for primary human hematopoietic stem/ progenitor cells (HSPCs) and have constrained analysis of the global architecture of the molecular circuits controlling these cells. Here we provide high-resolution genome-wide binding maps for a heptad of key TFs (FLI1, ERG, GATA2, RUNX1, SCL, LYL1 and LMO2) in human CD34+ HSPCs together with quantitative RNA and microRNA expression profiles. We catalogue binding of TFs at coding genes and microRNA promoters and report that combinatorial binding of all seven TFs is favored and is associated with differential expression of genes and microRNA in HSPCs. We also uncover a hitherto unrecognized association between FLI1 and RUNX1 pairing in HSPCs, establish a correlation between the density of histone modifications, which mark active enhancers and the number of overlapping TFs at a peak and identify complex relationships between specific miRNAs and coding genes regulated by the heptad. Taken together, these data reveal the power of integrating multifactor ChIP-seq with coding and non-coding gene expression to identify regulatory circuits controlling cell identity.

O1026 - MIR-146A REGULATES HEMATOPOIETIC STEM CELL MAINTENANCE AND CELL CYCLE ENTRY

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Maintenance of blood homeostasis depends on the balance between self-renewal of hematopoietic stem cells (HSCs) and their differentiation into blood cell progenitors. A variety of different intrinsic or extrinsic regulators, including multiple microRNA (miRNA) species, have been described to play a role in the regulation of these processes. Disruption of any of these regulators could lead to stem cell exhaustion or increased risk of leukemogenesis. Given recent reports of the role of miR-146a in malignant hematopoiesis, we evaluated its role in hematopoietic stem progenitor cell (HSPC) function. We show that miR-146a is highly expressed in HSCs and its expression decreases in committed progenitors. miR-146a- deficient HSCs had dramatically reduced self-renewal capacity as measured by serial competitive bone marrow transplantation assays. The lower self-renewal capacity was accompanied by decreased quiescence in miR-146a-deficient cells, as revealed by decreased proportion of miR-146a-/- HSPCs (Lin- Sca-1+ c-kit-, LSK) in G0 of the cell cycle (Ki-67- negative), and their increased proliferation, measured by BrdU incorporation. We further showed that increased proliferation of HSPCs is cell intrinsic. By sorting EPCR+ CD48- CD150+ (ESLAM) HSCs and examining cell division kinetics at the single cell level, we found that miR-146a-/- HSC undergo cell division earlier and differentiate more rapidly than wild-type HSCs, thereby producing larger colonies containing more differentiated (Lin+) cells. Our data provide evidence that miR-146a loss attenuates HSC quiescence and impairs their self-renewal ability, leading to hyperproliferation of progenitor cells. The phenotype seen is cell autonomous and the findings suggest that miR-146a plays a critical role in maintaining long term HSC function.

O1027 - CLONAL EVOLUTION OF LEUKEMIC STEM CELLS IN A CBX7-INDUCED LEUKEMIA MOUSE MODEL

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Sequential acquisition of mutations was long thought to drive leukemogenesis. However, the use of advanced genomic techniques now suggests that tumor cells are often organized in a non-linear branching hierarchy. Understanding leukemic progression implies determination of the nature and number of leukemic stem cells (LSCs) and their clonal offspring within a cancer. We recently developed a method in which cells and their offspring are marked by the viral introduction of a unique, heritable mark ("barcode") which can be detected by DNA sequencing (Gerrits et al. Blood 2010). We also have shown that overexpression of Polycomb Cbx7 causes a spectrum of distinct leukemic types (immature, lymphoid, erythroid) (Klauke et al, Nat. Cell Biol., 2013). By implementation of a barcoded retroviral Cbx7 vector, we have now generated a mouse model in which Cbx7 overexpression serves as the initial leukemic 'hit' and every pre-LSC is uniquely labelled. Quantifying barcodes by deep sequencing thus allows for the identification of LSC-derived clones. Our analysis revealed three general patterns of clonal evolution of leukemias. First, some leukemic clones are highly stable and dominant. These clones rapidly and strongly dominate the hematopoietic system of the primary donor, and upon serial transplantation cause a similar leukemia type. Second, minor clones in primary donors can become activated and highly dominant after serial transplantation. Leukemias in these recipients are thus of different clonal origin than the donor disease-causing clone. As a consequence, the leukemia type (immature, lymphoid, erythroid) in the recipients can be similar but also different as observed in the donor. Third, we have observed evolution of leukemic clones from immature to the lymphoid lineage. In these cases, leukemias in the donor and the recipients are of the same clonal origin, but in the recipients the leukemia manifests as a different type. Overall, we conclude that the clonal organisation of leukemia is more complex than previously anticipated. We provide direct evidence of the quiescent nature of LSCs. Therefore, therapies should focus on eliminating those critical LSCs as well.

O1028 - INDUCED PLURIPOTENT STEM CELL MODEL OF CHRONIC MYELOID LEUKEMIA REVEALED OLFACTOMEDIN 4 AS A NOVEL THERAPEUTIC TARGET IN LEUKEMIA STEM CELLS

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The definitive cure of leukemia requires identification of novel therapeutic targets to eradicate leukemia stem cells (LSCs). However, rarity of LSCs within the pool of malignant cells remains major limiting factor for their study in humans. Reprogramming somatic cells to pluripotency allows generation of induced pluripotent stem cells (iPSCs) that are capable of self-renewal and differentiation toward derivatives of all three germ layers, including blood. Because iPSCs capture the entire genome of diseased cell, they have been already used successfully to model human genetic diseases. Recently, we generated transgene-free iPSCs from bone marrow mononuclear cells from the patient in chronic phase of chronic myeloid leukemia (CML). iPSCs generated from CML patient captured the entire genome of neoplastic myeloid cells in this patient, including the unique 4-way translocation between chromosomes 1, 9, 22, and 11. Following differentiation on OP9, CML-iPSCs generated lin-CD34+CD45+ primitive hematopoietic cells with HSC phenotype. These cells displayed many unique features of CML LSCs, including expression of Bcr/Abl, high ALDH and rhodamine efflux activity, LTC-IC potential, adhesion defect, rapid cytokine independent proliferation, and imatinib resistance. Following differentiation in vitro these cells loss CD34 expression and became sensitive to imatinib. Comparative analysis of gene expression in lin-CD34+CD45+ cells obtained from normal bone marrow- and CML-iPSCs treated and not treated with imatinib identified olfactomedin 4 (OLFM4) as the top-ranking gene induced by imatinib. OLFM4 knockdown using siRNA decreased CFCs and increased apoptosis in CML-iPSCs-derived lin-CD34+CD45+, as well as parental somatic LSCs and LSCs from other unrelated CML patients. Overall this study successfully demonstrates for the first time the validity of iPSC model for CML LSC studies and identification of novel targets for drugs in CML LSCs.

O1029 - C-TERMINAL-TRUNCATING ASXL1 MUTATIONS INDUCE MDS VIA REPRESSION OF PRC2 FUNCTION

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Although recurrent mutations in ASXL1 are found in various myeloid malignancies and are associated with poor prognosis, the mechanism underlying myeloid transformation largely remains to be elucidated. Most ASXL1 mutations are heterozygous and thought to result in stable truncation of ASXL1. Here we demonstrate that expression of C-terminal truncating ASXL1 mutations (ASXL1-MT) inhibits myeloid differentiation, and induces myelodysplastic syndromes (MDS)-like disease in mice. In addition, ASXL1-MT collaborated with N-Ras-G12V in inducing progression of N-Ras-G12V-induced MPN to AML, suggesting that ASXL1-MTs played class II-like roles. Of note, gene set enrichment analysis (GSEA) indicated that ASXL1-MT induced an expression profile which inversely correlated with known polycomb repressive complex (PRC) target genes. ASXL1-MT derepressed expression of Hoxa9 and miR-125a through interrupting the binding of ASXL1-WT to EZH2 followed by loss of EZH2-mediated H3K27 trimethylation around the transcription start sites. In addition, miR-125a targets 3'UTR of Clec5a, which is the surface receptor and turned out to be indispensable for myeloid differentiation. Moreover, HOXA9 and CLEC5A expression were shown to be high and low, respectively, in MDS patients with ASXL1-MT. Our data provide evidence for a novel axis in MDS pathogenesis and implicate both mutant forms of ASXL1 and miR-125a as therapeutic targets in MDS.

O1030 - CELL-TO-CELL INTERACTION BETWEEN ENDOTHELIAL-HEMATOPOIETIC PRECURSORS AND SOMITES REGULATES DEVELOPMENTAL SPECIFICATION OF HEMATOPOIETIC STEM CELLS THROUGH NOTCH SIGNALING

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Notch is a juxtacrine signal that requires a direct contact between two cells, and plays a role in the specification of cell fates. Our previous study showed that the somitic wnt16/Notch signaling pathway is essential for hematopoietic stem cell (HSC) specification in zebrafish. It is still unclear, however, how somitic Notch signaling contributes to the process of HSC specification. Here, we show that zebrafish orthologues of junctional adhesion molecule (Jam) regulate interaction of endothelial-hematopoietic precursors with somites and activate Notch signaling in these precursors. Zebrafish *jam1a* was expressed in posterior lateral plate mesodermal (PLM) cells, which give rise to both endothelial and hematopoietic lineages. PLM cells migrate to the midline along the ventral side of somites expressing *jam2a* whose protein product potentially binds to jam1a. Morpholino knockdown of either *jam1a* or *jam2a* resulted in the delay of PLM cell migration and the reduction of *runx1* expression in the dorsal aorta. The activation of a Notch reporter line, *TP1:GFP*, was also reduced in the dorsal aorta, whereas the expressions of aortic Notch receptor genes (*notch1b* and *notch3*) and somitic Notch ligand genes (*dlc* and *dld*) were unaffected in both morphants. Overexpression of Notch intracellular domain in either *jam1a* or *jam2a* morphants rescued the expression of *runx1*. These results suggest that jam1a-jam2a heterophilic interaction mediates a direct contact between PLM cells and somites, and that this interaction is required for the Notch signal transmission between these.

O1031 - FLK2 LINEAGE TRACING REVEALS A NOVEL, DEVELOPMENTALLY RESTRICTED HEMATOPOIETIC STEM CELL

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We aim to understand the cellular and molecular mechanisms of hematopoietic stem cell (HSC) specification during development. We recently established a lineage tracing mouse model wherein differentiation stage-specific expression of Cre recombinase driven by expression of Flk2 (Flt3) results in the irreversible switching of a ubiquitous dual-color reporter from Tomato to GFP expression. Using this model, we have demonstrated that, in adult mice, all hematopoietic lineages differentiate through a Flk2-positive stage; that Flk2+ progenitors contribute equally to granulocyte/monocyte and megakaryocyte/erythroid lineages in vivo; and that Flk2+ cells do not dedifferentiate into HSC. In addition, we have demonstrated conclusively that all functional, adult HSC remain Tomato+ and therefore that all developmental precursors of adult HSC lack a history of Flk2 expression. Surprisingly, we observed the presence of GFP+ HSC that coexist with Tomato+ HSC during fetal development. These GFP+ HSC support long-term, multilineage hematopoiesis when transplanted into adult recipients, and like Tomato+ adult and fetal HSC, the GFP+ HSC retain long-term reconstitution potential in serial transplantation assays. However, GFP+ HSC display differential lineage bias and give rise to distinct subsets of mature immune cells, suggesting that they represent transiently existing HSC responsible for differential immune cell composition during development. Thus, we have identified a novel, functionally-distinct fetal population of HSC that are capable of reconstituting adult recipients, but do not persist in adulthood or give rise to adult HSC. This finding has important implications for developmental hematopoietic disorders, pediatric leukemias, and derivation of engraftable HSC from pluripotent precursors. Additionally, this finding challenges the dogma that HSC exhibiting long-term multilineage reconstitution necessarily contribute to adult hematopoiesis.

O1032 - GATA2 IS REQUIRED FOR HSC GENERATION AND SURVIVAL

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Knowledge of the key transcription factors that drive hematopoietic stem cell (HSC) generation is of particular importance for current hematopoietic regenerative approaches and reprogramming strategies. Whereas GATA2 has long been implicated as a hematopoietic transcription factor and its dysregulated expression is associated with human immunodeficiency syndromes and vascular integrity, it is as yet unknown if GATA2 functions in the generation of HSCs. The permanent adult hematopoietic system initiates with the formation of HSC in clusters lining the major embryonic vasculature (aorta, vitelline and umbilical arteries) in a process called endothelial-to-hematopoietic transition (EHT) beginning at mouse embryonic day (E)10.5. Since germline Gata2-/- embryos die at E10, we took a conditional knockout approach to analyze the function of GATA2 in EHT and HSC generation - Gata2f/f in combination with VE-Cadherin-Cre (Cre expression in endothelial cells) and VAV-Cre transgenes (Cre expression in HSCs and their progeny). We found that VE-Cadherin-Cre:Gata2f/f AGMs do not contain any HSCs, and the number of hematopoietic progenitors and hematopoietic clusters are severely reduced. These results indicate that GATA2 functions in endothelial cells to form hematopoietic clusters, progenitors and HSCs. Interestingly, not all progenitors are absent in VE-Cadherin-Cre:Gata2f/f AGMs, indicating that some progenitors are GATA2 independent. Similarly, VAV-Cre:Gata2f/f E11.5 AGMs do not contain any transplantable HSCs. However, unlike VE-Cadherin-Cre:Gata2f/f embryos, E14 VAV-Cre:Gata2f/f fetal livers are not anemic. Phenotypic HSCs are detected, but they are severely reduced and are apoptotic, thus showing that GATA2 is required for HSC survival after their generation. Our results demonstrate for the first time that GATA2 is required for HSC generation in the AGM, and unlike RUNX1, it continues to be required for HSC survival.

O1033 - FUNCTION OF PRC2 ACCESSORY FACTORS IN HAEMATOPOIETIC STEM CELLS

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We are interested in the epigenetic mechanisms that control gene expression in haematopoietic stem cells (HSCs). Our focus has been on Polycomb Repressive Complex 2 (PRC2), whose enzymatic component Ezh2 or Ezh1 establishes H3K27me3 at hundreds of genomic loci. The role of PRC2 in stem cells has predominantly been studied in embryonic stem (ES) cells, where it represses key developmental regulators. In addition to the core PRC2 members Ezh2/Ezh1, Suz12 and Eed, accessory factors appear to modulate PRC2 activity and direct its binding to specific targets in ES cells. We have previously shown that reduction in the levels of core PRC2 members Suz12 and Ezh2 results in enhanced HSC repopulating capacity, but the role of PRC2 accessory factors in HSCs has not been described. Using shRNA-mediated knockdown, we examined the function of six known PRC2 accessory factors in HSPCs by testing the capacity of transduced murine foetal liver cells to competitively reconstitute irradiated recipients. Foetal liver cells depleted of the enzymatically inactive histone methyltransferase Jarid2 show enhanced contribution to all blood cell lineages compared to cells containing non-silencing control constructs, similar to the phenotype observed upon Ezh2 or Suz12 depletion. Our data suggest that the enhanced activity of Jarid2 depleted cells is due to an increase in haematopoietic stem and progenitor cell (HSPC) number post Jarid2 knockdown, but that this role for Jarid2 is restricted to foetal HSPCs. Interestingly, mutations have been identified in PRC2 core components in haematopoietic malignancy, and recently mutations and deletions in JARID2 have also been reported. In addition to molecular studies of the Jarid2 depleted HSPCs, we are now using our bespoke shRNA library targeting over 250 enzymatic epigenetic modifiers to perform an in vivo screen designed to identify additional factors that, similar to PRC2, restrict HSC function and may cooperate with PRC2 to regulate HSC activity.

O1034 - DELETION OF THE HISTONE METHYLTRANSFERASE SETDB1 DURING HEMATOPOIESIS RESULTS IN HEMATOPOIETIC STEM CELL FAILURE AND ABROGATES B CELL DEVELOPMENT

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Hematopoietic stem cells (HSCs) give rise to all blood lineages. The fact that transcription factors (TFs) determine gene expression profiles during hematopoiesis has been broadly demonstrated. However, we still lack a detailed understanding about how the chromatin context influences the ability of TFs in activating or repressing genes. Because chromatin modifications might play significant roles for TF binding and function, we investigate the importance of H3K9me3, a repressive chromatin mark established by the histone methyltransferase Setdb1. We generated mice where Setdb1 conditional knockout alleles are deleted in all hematopoietic lineages using the Vavcre recombinase (Setdb1Vav). Setdb1Vav mice are underdeveloped, lack lymphocytes and show increased numbers of myeloid cells. These defects prompted us to investigate hematopoietic progenitors. Setdb1Vav common lymphoid progenitors (CLPs) are unaltered, while granulocyte macrophage progenitors (GMPs) are expanded. Furthermore, we examined if HSCs were compromised. Interestingly, while Setdb1Vav HSCs are increased 2-fold in foetal liver and 2-3 weeks old bone marrow; they completely drop after the 4th week. To find out whether Setdb1 is directly involved in B cell development, we also generated Setdb1Mb-1 mice, where we use Mb-1Cre to delete exclusively in the B cell lineage. These mice lack mature B cells because of a developmental arrest in the Pro B cell stage. Notably, RNA-Seq performed in sorted Pro B cells shows up-regulation of lineage inappropriate transcripts which might interfere with the establishment of the B cell program. In addition we observe that VDJ recombination, an essential process that mediates Pro B to Pre B transition, is impaired in Setdb1Mb-1 Pro B cells. In summary, our results show that Setdb1 is crucial for central processes during hematopoiesis such as, stem cell maintenance, lineage decision and Pro B to Pre B cell maturation; demonstrating that epigenetic modifications are essential for differentiation.

O1035 - INTERROGATING THE RELATIONSHIP BETWEEN TRANSCRIPTION FACTOR COMPLEX BINDING AND TRANSCRIPTIONAL ACTIVATION

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We recently reported the genome-wide binding patterns of 10 important haematopoietic transcription factors (TFs) in a blood stem/progenitor cell line (Wilson et al., 2010). Further analysis of this dataset highlighted the presence of multiple combinatorial binding events, including the observation that multiple TFs can be seen bound to regions within non-expressed genes. One such binding event was observed within the Fms-like tyrosine kinase 1 (Flt1 or VEGF-R1) gene locus. Flt1 is important for the development of embryonic vasculature and maintenance of endothelial cells (Breier et al., 1992; Kearney et al., 2002; Peters et al., 1993). The Flt1 +67kb region was bound by 9 TFs, yet showed no evidence of active histone marks. To further characterise the +67kb element, we show that the Flt1 +67kb element is not active in blood progenitor cell lines but is highly active in an endothelial cell line. Moreover, when assayed in transgenic mouse embryos, we observed clear endothelial activity at E11.5. Despite expression at anatomical sites of blood cell development, staining was restricted to endothelial cells with no activity in haematopoietic cells. Given the binding of multiple TFs in blood cells coupled with the lack of haematopoietic activity of the +67 kb element, we next wanted to identify potential negative regulators that might override potential activating functions of the multiple haematopoietic TFs bound to the element. To this end, we used a recently described affinity purification technique based on stable isotope labelling by amino acids in cell culture (SILAC) (Ong et al., 2002). This analysis highlighted the TFs Eto2 and Ikaros, which prompted us to perform ChIP-Seq analysis for both factors to identify their genome-wide distribution. Integrated analysis of these datasets is currently underway, and should allow us to investigate potential co-operative interactions between our previously mapped 10 TFs with Eto2 and Ikaros.

O1036 - MLL1-DEPENDENT PATHWAYS AND MECHANISMS THAT MAINTAIN HEMATOPOIESIS

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Epigenetic regulation of gene expression plays a central role in normal hematopoietic stem cell (HSC) maintenance and leukemogenesis. The Trithorax and Polycomb-group proteins, first described in Drosophila, are paradigmatic epigenetic regulators that impart heritable gene expression patterns. The histone methyltransferase and Trithorax homolog, MLL1, is essential for the maintenance of HSCs and is a common target of chromosomal translocations that result in acute leukemia. Using conditional knockout models, we identified genes that were deregulated upon Mll1 deletion in the HSC-enriched LSK/CD48neg population. Mini-ChIP experiments identified a series of transcriptional regulators that were direct MLL1 target genes including Mecom, Pbx1, and Prdm16, which are known to control HSC self-renewal and quiescence. Interestingly, not all identified MLL1 target genes required Menin, a cofactor that directs MLL1 to particular genomic loci in vivo, and not all targets were Mll1-dependent in non-hematopoietic tissues. Functional studies suggest that the identified genes act within a series of parallel pathways downstream of MLL1. To determine the mechanisms by which MLL1 regulates its target genes, we analyzed hematopoietic cells lacking the MLL1 histone methyltransferase domain as well as cells after deletion of Mll1 to identify chromatin alterations at HSC-specific target genes. These genome-wide studies revealed mechanisms by which MLL1 maintains its target genes in this cell type, and illustrated that the histone H3K4 methylation activity is not required for this function. These data underscore recent findings illustrating the complexity of gene regulation by Trithorax homologs. Furthermore, by elucidating the normal Mll1-dependent transcriptional network within HSCs, we show that this pathway is overlapping but distinguishable from the leukemogenic pathway characteristic of cells transformed with MLL-fusion proteins, demonstrating the feasibility of targeting the aberrant actions of the fusion proteins without harming HSCs.

O1037 - RECRUITMENT OF THE MLL COMPLEX VIA SPECIFIC INTERACTION OF THE P30 VARIANT OF C/EBPα WITH WDR5 IS ESSENTIAL FOR DEVELOPMENT OF ACUTE MYELOID LEUKEMIA

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The transcription factor C/EBPα is a master regulator of mature myeloid gene expression programs. In 10% of acute myeloid leukemia (AML) patients, the structure and function of the C/EBPα protein is altered due to somatic mutations in the CEPBA gene. The majority of such genetic aberrations represents frameshift mutations in the N-terminal part of the gene, causing the expression of an N-terminally truncated variant of C/EBPα, termed p30. Although differences between C/EBPα wild-type (p42) and p30 isoforms have been extensively studied, the molecular mechanisms underlying p30-dependent leukemogenesis have remained unclear. Using an affinity purification-mass spectrometry approach to map interaction partners of p42 versus p30 C/EBPα-isoforms in an isogenic cell system, we found that Wdr5, a critical component of the MLL methyltransferase complex, was exclusively associated with p30, but not p42. shRNA-mediated silencing of Wdr5 restored terminal granulocytic differentiation potential in p30-expressing cell lines and interfered with enhanced, pre-leukemic proliferation of cells from p30/p30 knock-in mice, while having little effect on wild-type cells or on cells over-expressing p42. In line, p30/p30 cells displayed increased sensitivity towards pharmacological targeting of the MLL complex. ChIP-seq studies showed that p30-bound genomic regions featured a greater enrichment for the MLL-dependent H3K4me3 mark than p42-bound regions. Our data show that p30, but not p42, selectively recruits the MLL complex to its target genes via interaction with Wdr5, indicating that C/EBPα p30 could represent a gain-of function mutation. Targeting of the MLL complex may therefore represent an attractive alternative to interfere with C/EBPα-mutated AML.

O1038 - LATS 1 IS A PUTATIVE TUMOR SUPPRESSOR IN HOXA9 / MEIS INDUCED LEUKEMIA

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Deregulation of Hox genes and their cofactors such as Meis1 are hallmarks of leukemogenesis. Studies in drosophila showed that hth/MEIS directly interacts with YKI, a transcription co-factor and component of the Hippo signaling network. The Hippo signaling network has been proposed to play a tumor suppressive role in carcinoma development but little is known about its function in hematopoiesis and leukemia. Expression of the core Hippo pathway constituents in different subpopulations of primitive hematopoietic cells could be detected by quantitative RT-PCR. The core components of the Hippo pathway are also expressed in the Hoxa9+Meis1-induced leukemia named FLA2 in which approximately 70% of cells represent leukemia stem cells (LSC) (Wilhelm B et al., 2011). Freshly isolated FLA2 cells were retrovirally infected with shRNA targeting Hippo signaling network core components and transplanted into recipient mice. The proportions of shRNA transduced (GFP+) cells were determined at the time of transplantation (day 0), and at the time of sacrifice. During this period, the proportions of shTaz(GFP+) cells to the leukemic cell populations decreased to 10-20% of the initial day 0 values. Conversely, the Lats1 knockdown leads to $>$ 50% increase over the initial proportion of the GFP+ cells ($p < 0.05$, Mann-Whitney-Test). To exclude the possibility that this effect is limited to FLA2 leukemia we isolated the CD150+CD48- Lin- stem/progenitor cells from BM, co-infected them first with Hoxa9 and Meis1 cDNA carrying retroviruses, and then knocked down Yap or Lats1. Similar to observations in FLA2 leukemia model, AML development in mice induced by overexpression of HoxA9 and Meis is accelerated with additional Lats 1 loss of function. The Median survival of transplanted mice is 46 days (range 45-50 days) compared to 53 days of the shLuciferase control (range 52-56 days). Yap loss of function prolongs the latency of leukemia onset significantly ($p < 0.05$, Mantel Cox Test) to $>$ 70 days. Our observations suggest that LATS1 acts as a negative modulator of Hox/Meis-induced leukemia and indicates a possibility for a specific targeting of the Hox/Meis-activated cellular pathways.

O1039 - GENOME-WIDE SHRNA SCREENING APPROACH TOWARDS IDENTIFICATION AND CHARACTERIZATION OF THERAPY RESISTANCE DETERMINANTS IN LEUKEMIA

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Acute Myeloid Leukemia (AML) cure remains challenging due to resistance mechanisms operating in the minority of leukemia cells with high regenerative potential (Leukemia Stem cells, LSC). Currently, molecular determinants governing escape of LSC from DNA damaging therapy remain largely undefined. We devised a genome-wide shRNA screen using a library of 80,000 lentiviral shRNA vectors targeting $>$16,000 human genes to isolate genes responsible for inducing human leukemia cell survival and regeneration following serial treatment of transduced cells with irradiation as the DNA damaging agent. We isolated numerous shRNA clones that drastically increased leukemia cell regeneration after genotoxic injury. Some of the screen candidates are known DNA damage response players (e.g. p53 and CHEK2), but the majority of the high confidence hits remain uncharacterized in terms of their involvement in stress responses. Here, we report studies that further characterize a small subset of hits implicated in epigenetic regulation (e.g. CTCF) and provide their on-target validation and initial insights into protective mechanisms. Furthermore, using bioinformatics approaches, we attempted to integrate our functional genomics screen hits with the transcriptional signatures of human AML samples that differ in their resistance toward standard chemotherapy drugs. The role of CTCF in mediating LSC chemotherapy resistance in the xenotransplantation model of human AML is currently under investigation. We believe that our integrated experimental platform will identify crucial determinants of LSC resistance that could be used as prognostic markers and, most importantly, will provide a foundation for their therapeutic targeting.

O1040 - ORDER MATTERS: SEQUENCE OF MUTATION ACQUISITION INFLUENCES HUMAN DISEASE PATHOGENESIS

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Recent descriptions of stem cell and cancer heterogeneity emphasize the need to understand how single stem cells are subverted to form tumors. Human myeloproliferative neoplasms (MPNs) arise from a transformed hematopoietic stem cell (HSC) and are a paradigm for the early stages of tumor growth. Most MPNs harbor an acquired V617F mutation in JAK2 and many have collaborating mutations, including in TET2 which occurs in ∼10% of cases. We screened 335 MPN patients for JAK2 and TET2 mutations by genotyping over 8000 colonies to determine the order of mutation acquisition and persistence of distinct clones. In most patients, multiple clones stably persist over time resulting in longterm competition between clones with distinct genetic backgrounds. The order of acquisition was determined in 24 patients and TET2 mutations preceded JAK2 in 50% of cases. Interestingly, patients who acquired TET2 first had a mean age of presentation that was 12.3 years later than patients who acquired JAK2 first. Furthermore, the proportion of JAK2 homozygous erythroid colonies was significantly lower in TET2-first patients and the relative proportion of hematopoietic progenitors was shifted, with JAK2-first patients having more megakaryocyte/erythrocyte progenitors and TET2-first patients having more common myeloid progenitors. Genotyping of single HSC-derived clones revealed that JAK2 single mutant clones were less prevalent than double mutant clones in the HSC compartment. By contrast, TET2 single mutant clones were dominant over double mutant clones. Moreover, secondary colony forming ability was significantly reduced in double mutant HSCs compared to those from HSCs with a TET2 mutation alone.These data demonstrate that the order with which mutations are acquired influences tumor phenotype. Our results are consistent with at least two mechanistic explanations which are not mutually exclusive: 1) Intracellular competition such that the extent to which a double mutant clone can expand depends on its competitiveness relative to its single mutant ancestor and 2) The epigenetic program that a second mutation can enact is restricted by fixed epigenetic changes imposed by the first mutation, resulting in shifted cell fate outcomes.

O1041 - OVEREXPRESSION OF THE ETS-FAMILY TRANSCRIPTION FACTOR ERG AND DYSREGULATED CYTOKINE SIGNALING DRIVE ERYTHROID LEUKEMIA DEVELOPMENT IN MICE

Catherine Carmichael[1,2], *Jian Zhong Tang*[1,2], *Donald Metcalf*[1,2], *Wei Shi*[1,2], *Katya Henley*[1], *Ashley Ng*[1,2], *Craig Hyland*[1], *Ladina Di Rago*[1], *Sandra Mifsud*[1], *Nancy Jenkins*[3], *Neal Copeland*[3], *Viive Howell*[4], *Zhizhuang Joe Zhao*[5], *Warren Alexander*[1,2], *and Benjamin Kile*[1,2]
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ETS-related gene, ERG, is a regulator of hematopoietic stem cell function, and a potent oncogene that is strongly implicated in leukemia development. We have recently shown that overexpression of Erg or its oncogenic fusion protein, TLS-ERG, rapidly induces a potent and uniform erythroid leukemia (EL) in mice (1,2). In humans, acute erythroid leukemia (AEL) is a poorly treated and rapidly morbid disease that accounts for 1-5% of all myeloid leukemias diagnosed. The genetic aberrations that drive disease pathogenesis in AEL remain largely unknown. To begin to understand the relationship between Erg overexpression and EL, we performed a transposon-mediated insertional mutagenesis screen. Transposition of the "Sleeping Beauty" (SB) transposon array in hematopoietic cells overexpressing Erg resulted in a significant acceleration of Erg-induced EL. Deep sequencing of these ELs identified the Jak2 cytokine receptor locus as a common transposon insertion site. Conversely, when we activated SB transposition in a mouse transgenic for the activating V617F mutation in Jak2, we found a predominance of EL, and identified transposon insertions at the Erg locus in nearly 90% of these tumours (2). These data strongly suggest that cooperation between Erg overexpression and activated Jak2 cytokine signalling is a key mechanism in EL development in mice. We are now investigating whether this mechanism, as well as other potentially novel erythroid leukemia driver genes identified in these two screens, may also play important roles in human AEL. 1.Carmichael CL et al., PNAS 2012 2.Tang JZ et al., PNAS 2013

O1042 - RNAI SCREEN IDENTIFIES CYTOHESIN1 AS A MEDIATOR OF ADHESION IN HUMAN HEMATOPOIETIC STEM/PROGENITOR CELLS

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To understand mechanisms of homing and retention of hematopoietic stem and progenitor cells (HSPCs) to their niche in the bone marrow (BM), we developed a functional screen for regulators of human HSPC adhesion. We used the firm adherence between cord blood CD34+ cells and BM derived mesenchymal stroma cells (MSCs) as a surrogate niche model in vitro to screen for modifiers (shRNAs) that disrupt adhesion. CD34+ cells were transduced with a pooled lentiviral shRNA library targeting 366 adhesion-associated genes (5 shRNAs/gene) and plated on MSCs. Gravity force was used to retrieve non-adherent cells. Distribution of integrated shRNAs in the adherent vs non-adherent cell fractions was determined by deep sequencing. Genes, for which two or more independent shRNAs showed a strong enrichment among non-adherent cells, were selected for further validation. We found multiple shRNAs against CD29 (beta1 integrin), consistent with the well-established role of CD29 in HSPC adhesion. Among the novel candidate genes we focused on the GTPase cytohesin1 (CYTH1). Knockdown (KD) of CYTH1 from four independent shRNAs significantly increased the fraction of non-adherent cells in the stroma adhesion assay. Moreover, CYTH1 KD specifically inhibited adherence of CD34+ cells to fibronectin and ICAM1. In vivo, shRNA-mediated KD of CYTH1 significantly decreased both short-term and long-term engraftment of CD34+ cells in NSG mice, indicating a role for CYTH1 in mediating homing and engraftment of HSPCs. Preliminary results, from homing assays using intravital microscopy show increased mobility of CD34+CD38- cells with CYTH1 KD coupled with a tendency to localize at a further distance from the bone surface and endothelium compared to control cells. In summary, we identified CYTH1 as a novel regulator of cell adhesion in human HSPCs both in vitro and in vivo. We are currently using interference reflection microscopy (IRM) and total internal reflection (TIRF) microscopy to understand in more details how lack of CYTH1 alters the adhesive properties of HSCPs.

ABSTRACTS

POSTER PRESENTATION ABSTRACTS

Numbers beginning with "S" refer to Invited Speaker Abstracts
Numbers beginning with "O" refer to Oral Short Talk Presentations
Numbers beginning with "P" refer to Poster Presentations

Poster Presentations

P1001 - BMI1 MAINTAINS THE SELF-REPLENISHING ABILITY OF B-1A CELLS IN POSTNATAL MICE

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Mouse B-1 cells are innate immune cells that secrete natural antibodies and play important roles in the fist line of defense to bacterial infection. B-1 cells display an IgM^{hi}Ig-$D^{lo}$$CD11b^{+}$ phenotype, and preferentially localize within the peritoneal cavity. $CD5^{+}$B-1a cells are considered to be of fetal origin since adult BM HSCs fail to reconstitute B-1a cells while fetal liver cells reconstitute B-1a cells in irradiated recipient mice. We have recently demonstrated that YS cells at E9.5 (at the time prior to HSC emergence in the mouse embryo) are the earliest origin of B-1a cells. The mechanisms that permit B-1a cells to undergo self-replenishment in vivo in the absence of ongoing stem cell input remain unclear. Bmi1 is a polycomb group protein that is known to be critical for self-renewal of adult BM HSCs and neural stem cells. In $Bmi1^{-/-}$ postnatal BM, the number of HSCs was markedly reduced and long-term reconstituting ability was lost, while enforced expression of Bmi1 in HSCs promoted their self-renewal ability. We hypothesized that Bmi1 is critical for B-1a cell self-replenishment and examined B-1 cell populations in $Bmi1^{-/-}$ embryonic and adult mice. Bmi1 mRNA expression was higher in B-1a cells than other lymphoid subsets in wild type mice. In $Bmi1^{-/-}$mice, the B-1a cell population was significantly ($p < 0.05$) decreased in the percentage (4 fold less than WT) and total cell number (50 fold less than WT). On the contrary, the percentage and number of B-1 specific progenitors ($lin^{-}AA4.1^{+}CD19^{+}B220^{dim}$ cells) was increased in the E14-15 fetal liver of $Bmi1^{-/-}$ embryos. When the peritoneal cells from adult $Bmi1^{-/-}$ mice were transferred into the peritoneal cavity of sublethally irradiated NOG neonates, cells only transiently engrafted, and no long-term reconstitution was observed. Wild type peritoneal cells reconstituted B-1a cells for 4-9 months as previously reported. When $Bmi1^{-/-}$ fetal liver cells were rescued for Bmi1 expression by retroviral gene transfer and transplanted, B-1a cells were recovered in the recipient mice > 4 months after transplantation at numbers equivalent to wild type transplanted cells. Thus, Bmi1 plays an important role in the self-replenishing ability of B-1a cells in the postnatal mouse.

P1002 - EFFECTS OF GROWTH FACTORS GDNF, BMP4 AND RA ON PROLIFERATION AND SELF RENEWAL OF SPERMATOGONIAL STEM CELLS

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Introduction: The effect of this study was to evaluation of growth factors GDNF, BMP4 and RA in self renewal and colony formation which is the criteria of stem cell activity of spermatogonial stem cells (SSC). **Material and Methods:** Testis cells were collected from adult mouse via a two-step mechanical and enzymatic digestion. The spermatogonia cells were cultured in two groups: The first group which received GDNF and BMP4 and the second group which received RA. The colony formation was monitored during 1 month in culture. To identification of the colony, they were stained with GCNA using immunostaining. **Results:** In the presence of GDNF and BMP4, cells proliferated rapidly and many compact clumps were appeared. In addition after each subculture cells divided and reformed clumps again whereas after exposure to RA cells formed small clumps, that disappeared after subsequent subculture. Cells colonization was observed in the presence of GDNF and BMP4, but no colony was formed in cultures exposure with RA. Also the colonies were immunopositive with GCNA marker. **Conclusion:** Our results proved the growth factors GDNF and BMP4 are essential for self renewal of SSC and colony formation that confirm the stem cells activity of these cells but RA inhibits stem cell activity of SSC. **Key words:** Spermatogonial stem cells, BMP4, GDNF, RA.

P1003 - ANALYSIS OF TIM MOLECULES IN FETAL HEMATOPOIESIS REVEALS THAT TIM-4 IS DIFFERENTIALLY EXPRESSED IN MYELOID PROGENITOR CELLS

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TIM molecules are known regulators of immune response whose function in hematopoiesis is unknown. Earlier, we found that tim-1 and tim-4 are expressed by CD45+ cells in para-aortic region of chicken embryo. Since the para-aortic region is a known site for HSC and HPC differentiation and expansion, we further investigated the expression and role of TIM molecules in embryonic hematopoiesis. We studied mRNA and protein expression of TIM-1, TIM-3 and TIM-4 in chicken and mouse embryonic tissues and particularly in mouse fetal liver CD45+ cells. Additionally, we examined the hematopoietic potential of TIM-4+ mouse fetal liver cells by colony-forming assay. tim-1 gene expression was detected in chicken and mouse embryos in the AGM-region at the time of HSC emergence. tim-3 mRNA was widely expressed in different tissues, and in fetal liver, by CD45+F4/80+ cells. tim-4 expression was restricted to fetal liver CD45+F4/80+ cells. Moreover, two TIM-4+ populations: CD45+F4/80loTIM-4lo and CD45+F4/80hiTIM-4hi were distinguished. Majority of CD45+F4/80loTIM-4lo cells were c-kit+, Sca-1+ and FCγRlo, had primitive morphology and multilineage colony-forming ability. Instead, CD45+F4/80hiTIM-4hi cells were mostly FCγRhi, had no hematopoietic potential and had morphology similar to mature macrophages. In conclusion, TIM molecules are expressed already during embryonic development. Here we show that tim-1 is expressed in AGM, and its role in early hematopoiesis needs further investigation. TIM-3 instead is expressed broadly in the embryo. TIM-4 expression is limited to two separate populations in fetal liver. TIM-4lo cells are suggested to be multi-potential myeloid progenitors and TIM-4hi cells yolk sac-derived macrophages.

P1004 - IDENTIFICATION OF THE TRANSCRIPTIONAL PROGRAMME CONTROLLED BY RUNX1 IN THE HAEMOGENIC ENDOTHELIUM BY UTILISING DAMID-SEQ

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The RUNX1 transcription factor is indispensible for the establishment of the haematopoietic system. RUNX1 mediates the production of blood cells from endothelial cells which have haemogenic potential (haemogenic endothelium or HE) through a process termed endothelial to haematopoietic transition (EHT). Several studies have provided insights into the transcriptional programme governed by RUNX1 during EHT, suggesting that RUNX1 plays a role both in the activation of haematopoietic genes and inactivation of endothelial genes. However, due to the scarcity of cells and their transient nature, very little is known about whether RUNX1 influences the development of the HE itself. To this end, we adapted a technology, called DamID, to identify the very early genome-wide targets of RUNX1 in the HE. DamID is a methylation-based tagging technique where the Dam methylase (Dam) is fused to DNA binding proteins and stably marks their DNA binding sites. We generated RUNX1::Dam and control Dam expressing ES cell lines and purified cells at the onset of haematopoiesis and applied DamID coupled with high-throughput sequencing. To analyse the results we also developed a sequencing analysis pipeline specific for DamID data. We next correlated the RUNX1 binding sites with RNA-Seq expression data of the same cell populations. Our findings suggest, surprisingly, that at this very early stage of haematopoietic development RUNX1 positively regulates the endothelial gene expression programme. This up-regulation correlates with the formation of endothelial cell clusters, which precedes the generation of blood progenitors and corresponds to the development of HE. Overall, our results suggest a novel and unexpected role for RUNX1 in the establishment of HE that precedes its more established function in the EHT process.

P1005 - JAGGED1 GOVERNS THE LOSS OF ENDOTHELIAL IDENTITY OF THE AORTIC ENDOTHELIUM DURING HSC SPECIFICATION

Leonor Gama-Norton[1], *Jordi Guiu*[1], *Abul Islam*[2], *Cristina Ruiz-Herguido*[1], *Eva Ferrando*[1], *Lluis Espinosa*[1], *and Anna Bigas*[1]
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Specialization of the aortic hemogenic endothelium and the subsequent formation of intra-aortic hematopoietic clusters precede the generation of Hematopoietic Stem Cells (HSCs). This endothelial-to-hematopoietic transition (EHT) is governed by a complex genetic network that is still poorly understood. Notch signalling plays different roles in the dorsal aorta during organogenesis. While Notch1/Dll4 axis is essential for the specification of arterial identity, Notch1/Jag1 axis is indispensable for hematopoietic determination. Specific downstream targets of Notch/Jag1 such as Gata2 and Hes1 are known to be required for HSC emergence, however the global gene expression pattern associated with the acquisition of the hematopoietic fate remains to be defined. To better understand the process of HSC generation, we analysed the transcriptional program of specific AGM-derived subpopulations before the acquisition of the CD45 hematopoietic fate using microarrays. Our results revealed that acquisition of hematopoietic c-kit marker involves the loss of endothelial identity. Interestingly, incubation of endothelial c-kit- cells on OP9-Jag1 did not result in the direct activation of hematopoietic-related genes, but rather imposed a significant decrease of the endothelial-specific signature, thus resembling the CD31+c-kit+CD45- population. These results suggest that down-regulation of the endothelial program is the driving force that governs the initial steps of EHT. This research is funded by by Ministerio Ciencia e Innovación (PLE2009-0111, SAF2010-15450, RD12/0036/0054), AGAUR (2009SGR-23 and CONES2010-0006) and MC-IEF-PIEF-GA-2011-302226.

P1006 - HEMOGENIC ENDOTHELIUM GENERATES MESOANGIOBLASTS THAT CONTRIBUTE TO SEVERAL MESODERMAL LINEAGES IN VIVO

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The embryonic endothelium is a known source of hematopoietic stem cells. Moreover, vessel-associated stem cells, endowed with multilineage mesodermal differentiation potential, such as the embryonic mesoangioblasts, originate in vitro from the endothelium. Using a genetic lineage tracing approach, we show that early extraembryonic endothelium generates, in a narrow time-window, and prior to the hemogenic endothelium in the dorsal aorta and major embryonic arteries, hematopoietic cells that migrate to the embryo proper, and are subsequently found within the mesenchyme. A subpopulation of these cells, distinct from embryonic macrophages, expresses mesenchymal markers. We also found that hemogenic endothelium derived cells contribute to skeletal and smooth muscle and to other mesodermal cells in vivo, and specifically display features of embryonic mesoangioblasts in vitro. Therefore, we provide new insights on the distinctive characteristics of the extraembryonic and embryonic hemogenic endothelium and we identify the in vivo counterpart of embryonic mesoangioblasts, suggesting their identity and developmental ontogeny.

P1007 - DECLINED PRESENTATION
CONDITIONAL REQUIREMENT OF SMAD3 IN EMBRYONIC HEMATOPOIESIS

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Transforming growth factor-β (TGF-β) plays pleiotropic and context-dependent roles in hematopoietic ontogeny and homeostasis. Targeted disruption of Smad3, a specific intra-cellular transducer of TGF-β signaling, results in impaired mucosal immunity and extra-medullary hematopoiesis. However, little is known about the physiological function of Smad3 during hematopoietic development. In the present study, we firstly performed in vitro differentiation of embryonic stem cells, known to recapitulating early blood-forming events. The enhanced generation of hematopoietic embryoid bodies was observed in Smad3-deficient embryonic stem cells, accompanied by an up-regulated expression of Gata1 and adult-type hemoglobin in the mutant embryoid bodies. In mid-gestational embryos, Smad3 knockout resulted in an increase in hematopoietic progenitors by CFU-C assay, with a concomitant increase in Gata1 and beta-major hemoglobin transcripts in the mutant embryonic tissue. In contrast, we found that Smad3 null fetal livers at embryonic day 14.5 contained similar numbers of phenotypic and functional hematopoietic stem progenitor cells, although Smad3-deficient hematopoietic colonies were resistant to TGF-β-mediated inhibition. In conclusion, the data collectively strengthen the notion that Smad3-mediated TGF-β signaling is required for restraining the over-expansion of early-generated hematopoietic progenitors, but the role is ultimately dispensable along development.

P1008 - IDENTIFICATION OF NOTCH/RBPJ-TARGET GENES INVOLVED IN HEMATOPOIETIC STEM CELL GENERATION

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Notch/RBPj signals are required for Hematopoietic Stem Cells (HSCs) specification during embryonic development, however the mechanisms downstream of Notch activation are not understood. With the aim of identifying novel Notch transcriptional targets involved in the HSC emergence, we performed ChIP-on-chip with an anti-RBPj antibody using chromatin from dissected Aorta-Gonad-Mesonephros (AGM) from E11.5 mouse embryos. With this strategy, we have obtained several candidate genesthat recruit RBPj to their promoters and contain RBPj DNA binding consensus. We have selected some of the genes for their expression pattern in the midgestation aorta and further restrict the list of candidates by the presence of Notch1 in the promoters as tested by ChIP assay, to exclude inactive RBPj targets. We will present our data on the characterization of a novel Notch target gene, named Cdca7, which is expressed in the aortic endothelium and hematopoietic clusters of the AGM. Cdca7 is also expressed in pre-hematopoetic and hematopoietic cell populations obtained from human ES cell differentiation. The function of Cdca7 is presently unknown but it has previously been associated with transformation of several cell types. Our preliminary functional data indicates that Cdca7 is important in maintaining the undifferentiated state of hematopoietic progenitors from the AGM. This research is funded by by Ministerio Ciencia e Innovación (PLE2009-0111, SAF2010-15450, RD12/0036/0054), AGAUR (2009SGR-23 and CONES2010-0006).

P1009 - HISTOLOGICAL STUDY OF THE MID-GESTATIONAL MURINE PLACENTA WITH EMPHASIS ON ITS HEMATOPOIETIC POTENTIAL

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Some studies have demonstrated the hematopoietic potential of the placenta, showing the presence of self-renewal and multipotent progenitors. We approached this issue by morphological in situ analysis of murine placentas at the mid-gestational period, evaluating the effective participation of this organ in the hematopoiesis ontogeny. For that, we made an histological analysis of Swiss Webster mice placentas between 9,5 and 11,5 days post-coitum (dpc) through serial sections and the use of conventional and specific stainings. In the fetal circulation of the labyrinth region, large erythroid cells were observed with more spherical shape than the other immature erythroid cells, probably seeded by the yolk sac. Such cells seemed to be attached to each other and in some areas they did not appear to be involved by endothelium, as was reinforced by endothelial labeling by FITC-conjugated PNA lectin. Budding of immature cells was also observed, showing adhesion to endothelium or adjacent trophoblast. In some areas next to trophoblast giant cells and spongiotrophoblast, erythroid cells with trophoblastic morphology were observed forming agglomerates within the trophoblastic plate, with cells attached and presenting different levels of hemoglobinization. Hematopoietic clusters similar to those of HSC in the embryos large vessels were also found close to the fetus. Immunostaining revealed presence of erythropoietin and hepcidin-25 in decidual cells, which can then have an important role in this erythropoiesis. Also, von Willebrand factor was found inside cells protruded into the fetal circulation and some cells with throphoblastic aspect into the blood vessels were Sca-1 positive. In conclusion, our results suggest that the early functional placenta plays an important role in hematopoietic ontogeny in mice, specially in erythropoiesis, producing blood cells from three different mechanisms: trophoblast migration, "plate-like" differentiation inside trophoblast mass, and hemogenic endothelium. The expression of erythropoietin and hepcidin-25 would provide supportive conditions for the erythropoiesis occurrence.

P1010 - DECLINED PRESENTATION
MITOCHONDRIAL ATPIF1 REGULATES HEME SYNTHESIS IN DEVELOPING ERYTHROBLASTS

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Defects in the availability of heme substrates or the catalytic activity of the terminal enzyme in heme biosynthesis, ferrochelatase (Fech), impair heme synthesis, and thus cause human congenital anemias. The inter-dependent functions of regulators of mitochondrial homeostasis and enzymes responsible for heme synthesis are largely unknown. To uncover this unmet need, we utilized zebrafish genetic screens and cloned mitochondrial ATPase inhibitory factor 1 (atpif1) from a zebrafish mutant with profound anemia, pinotage (pnt). Morpholino-induced loss of function, cRNA over-expression, quantitative RT-PCR, and mutational analysis show that atpif1 is the gene disrupted in the pnt locus. Here we report a direct mechanism establishing that Atpif1 regulates the catalytic efficiency of vertebrate Fech to synthesize heme. The loss of Atpif1 impairs hemoglobin synthesis in zebrafish, mouse, and human hematopoietic models as a consequence of diminished Fech activity, and elevated mitochondrial pH. To understand the relationship among mitochondrial pH, redox potential, [2Fe-2S] clusters, and Fech activity, we used (1) genetic complementation studies of Fech constructs with or without [2Fe-2S] clusters in pnt, and (2) pharmacological agents modulating mitochondrial pH and redox potential. The presence of [2Fe-2S] cluster renders vertebrate Fech vulnerable to Atpif1-regulated mitochondrial pH and redox potential perturbations. Therefore, Atpif1 deficiency reduces the efficiency of vertebrate Fech to synthesize heme, resulting in anemia. The novel mechanism of Atpif1 as a regulator of heme synthesis advances the understanding of mitochondrial heme homeostasis and red blood cell development. A deficiency of Atpif1 may contribute to important human diseases, such as congenital hypochromic and sideroblastic anemias and mitochondriopathies.

P1011 - THE ROLE OF INKA1B IN ZEBRAFISH PRIMITIVE HEMATOPOIESIS

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Objective: Inka1b gene was shown up-regulated in the intermediate cell mass (ICM) of the zebrafish chordin morphant but its role in embryonic hematopoiesis is unknown. In this study, we characterized the spatio-temporal expression pattern and hematopoietic functions of for inka1b in zebrafish embryos. **Methods:** Spatio-temporal expression of zebrafish inka1b gene was examined by reverse transcription polymerase chain reaction (RT-PCR) and whole-mount in situ hybridization (WISH). Inka1b was knocked-down by anti-sense morpholino (MO) (herein inka1bMO embryos) and the hematopoietic phenotype was analyzed by WISH, real-time RT-PCR, O-dianisidine, Sudan Black staining and quantitatively by flow cytometry in Tg(mpo:EGFP) embryos. **Results:** INKA is present in vertebrates and relatively conserved from human to zebrafish. In zebrafish embryos, inka1b is expressed since the zygotic stage, indicating the presence of maternal transcript. At 18 and 24 hpf, inka1b can be detected in the ICM in wild-type and chordin morhants. Erythropoiesis was reduced in inka1bMO embryos, as shown by reduction of gata1 and alpha- and beta-embryonic hemoglobin expression as well as O-dianisidine staining at 48 hpf and the hematopoietic defects could be rescued by co-injection with wild-type inka1b mRNA. Differentiation was not affected and cytological examination of erythrocytes in inka1bMO embryos showed no difference from those in un-injected control. Inka1b knock-down also resulted in modest up-regulated expression of myelomonocytic markers pu.1 and l-plastin but reduced granulopoiesis as confirmed by RT-PCR, WISH and flow cytometric analysis in Tg(mpo:EGFP) embryos. Definitive hematopoiesis and angiogenesis were not affected. **Conclusion:** Zebrafish inka1b is involved in the maintenance of erythropoiesis and macrophage in primitive hematopoiesis. Its roles in murine embryonic stem cells (ESC) are being evaluated.

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P1012 - DIFFERENTIATION OF NUCLEATED ERYTHROCYTES USING GATA1 REPORTER MEDAKA FISH

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Non-mammalian mature erythrocytes in the circulation retain nuclear unlike in mammalian. We generated transgenic medaka fish (*Oryzias latipes*) expressing DsRed under the control of gata1 regulatory regions. We then characterized the differentiation of erythrocytes linage of the gata1-DsRed medaka. Most DsRed-positive cells in the spleen were mature erythrocytes; though immature erythrocytes were found in the kidney. Likewise we observed DsRed-positive mature erythrocytes mostly in the circulation. We then attempted to define the development of erythrocytes by flow-cytometry. DsRed-positive cells were plotted as forward scatter (FSC) and DsRed, and these three sub-populations were clarified. Based on morphologic characteristics, respective sub-population broadly corresponded to proerythroblast in the $FSC^{high}DsRed^{med}$ (ProE); later stage, basophilic and polychromatophilic erythroblasts in the $FSC^{low}DsRed^{med}$ (LateE); and mature erythrocytes in the $DsRed^{high}$ (RBC). We further examined the gene expression profiles by qRT-PCR analysis. Erythroid specific genes (epor, gata1, β-globin and alas2) were most highly expressed in LateE, followed by RBC and ProE fraction. Therefore we hypothesized that erythropoietin (EPO) stimulation exerted cellular functions toward cells at later stage of erythrocytes and mature erythrocytes. To test in vitro cellular response of erythrocytes under EPO stimulation, peripheral erythrocytes were cultured with or without recombinant medaka EPO. Most erythrocytes were impaired by day 4 without EPO; while, more than 60% of erythrocytes were maintained and viable under EPO stimulation. This result demonstrated that EPO in medaka fish exerts ability of maintaining viability of circulating erythrocytes, rather that of cell proliferation.

P1013 - TOLL-LIKE RECEPTOR 2 TRACKS THE EMERGENCE OF EARLIEST MYELOID PROGENITORS IN PRECIRCULATION EMBRYO

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Toll like receptors (TLRs) are critically important in the pathogen recognition and regulation of the innate and adaptive immune responses. In addition, a direct pathogen sensing of bone marrow hematopoietic stem cells and hematopoietic progenitors via TLRs seem to play a crucial role in directing the hematopoietic cell fates under inflammatory conditions. So far the expression of TLRs during early stages of embryonic development has not been addressed. Using a transgenic model to trace cells of embryonic origin we show that TLRs are expressed on embryonic myeloid cells as well as hematopoietic precursors committed to the myeloid lineage. Together with the prototypic marker of hematopoietic progenitors, c-kit, TLR2 is specifically expressed on the surface of hematopoietic precursors in early gastrulation embryos (E6.5-E7.0). E7.5-E8.5 TLR2+ c-kit+ cells express CD45 mRNA and gradually differentiate through an intermediate c-kit+ CD45+ stage to c-kit- CD45+ myeloid cells. Upon TLR2 ligand recognition, E8.5 TLR2+ c-kit+ cells proliferate and differentiate to CD45+ CD11b+ myeloid cells in a MyD88 dependent manner. Precirculation (E7.5) TLR2+ c-kit+ progenitors are predominantly detected in the yolk sac (YS) region. Nevertheless the scarce TLR2+ c-kit+ cells with a distinct surface expression profile can be also found in the embryo proper (EP). This opens the question whether the emergence of hematopoietic precursors in EP is dependent or not on YS primitive hematopoiesis. Our results indicate that TLR2 could be used as one of the earliest markers of embryonic hematopoietic progenitors and suggest a functional link between embryonic hematopoiesis and inflammation.

P1014 - ENDOTHELIAL-HEMATOPOIETIC TRANSITION: FROM THE DORSAL AORTA TO THE DISH

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Hematopoietic stem cells (HSCs) arise from a specialized population of endothelial cells, termed hemogenic endothelium, in the dorsal aorta (DA) at embryonic day (E) 10.5 during embryonic development. This process is known as endothelial to hematopoietic transition (EHT). We aim to determine genes crucial for the development of embryonic HSCs. We are using a transgenic mouse model, from Dr. Motomi Osato, which expresses GFP tagged to an intronic enhancer of the hemogenic endothelial gene *Runx1*, to sort specialized endothelial cell (EC) populations from the DA of E10.5 embryos. Whole-transcriptome data will be generated from these sorted E10.5 cell populations, and additionally similar cell populations isolated from E9.5 embryos will be cultured to define an *ex vivo* assay system that supports differentiation of pre-hemogenic ECs to HSCs. The *ex vivo* assay system is modeled after existing OP9 based techniques used for embryonic stem cell and dorsal aorta culture. This assay will allow us to functionally validate potentially important genes, identified using bioinformatic analysis of our whole-transcriptome sequencing data, in the process of EHT. These genes of interest will be manipulated using shRNA-mediated knockdown. Additionally the effect of blocking Notch, thought to be important in EHT, with a gamma secretase inhibiting compound will be used as another variable for modulating cells in this assay. This project contributes further to the understanding of genes important in EHT, while potentially defining transcriptional networks involved.

P1015 - IFN STIMULATION IMPAIRS SELF-RENEWAL AND PROMOTES DIFFERENTIATION OF STEM CELLS

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Interferons (IFNs) are known regulators of immune responses. However, limited information is available on whether IFNs influence early stem cell behaviour. In this study we investigated how early during development the receptors for IFN α and γ were expressed and whether these levels altered during embryonic haemopoiesis. Our data clearly demonstrates that pluripotent embryonic stem (ES) cells express the IFN receptors and respond to these cytokines. Following stimulation IFN α and γ activated the Jak-STAT pathway and rapidly induced IFN response genes (*IRF, IFIT, OASL, GBP,* family members). This was accompanied by ES cells losing self-renewal potential as measured by several pluripotency markers (Alkaline phosphatase, SSEA 1 & 3 and TRA 1-181). Differentiation was also reflected at the transcription level with altered levels of genes involved in maintaining ES cell pluripotency and promoting differentiation (*Oct4, Lefty 1 & 2, Sox17, Cdx2, ICAM1, PECAM, Vav1, DKK1 & CEBPE*). Analysis of embryonic haemopoietic tissue samples revealed IFN receptors were expressed in the yolk sac (E7.5) and foetal liver (E15) with higher levels detected in the more mature liver, spleen, and bone marrow samples (Day 14). Short-term IFN stimulation of foetal liver cells and ES derived haemangioblasts increased cell proliferation and elicited a strong IFN response. Whereas during long-term cultures, in conditions to sustain multipotent progenitors, IFN stimulation resulted in enhanced differentiation, with a $>20\%$ reduction in the long-term HSC (Sca1^{Hi} c-Kit^{Low}) population. Differentiation was also biased, with IFN stimulation enhancing granulocyte-macrophage progenitor and suppressing megakaryocyte-erythroid progenitor formation. This study clearly demonstrates that IFN α and γ are capable of modulating early stem cell behaviour and have the potential to influence developmental haemopoiesis.

P1016 - THE DARK SIDE OF HEMATOPOIETIC STEM CELL EXPANSION - IN VITRO CULTURE ENTAILS SPECIFIC DNA-HYPERMETHYLATION WHICH SEEMS TO BE RELEVANT FOR LOSS OF STEM CELL FUNCTION

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Hematopoietic stem and progenitor cells (HPCs) can be maintained in vitro, but the vast majority of their progeny loses stemness during culture. In this study, we analyzed DNA-methylation (DNAm) profiles of either freshly isolated or expanded CD34+ cells cultured with or without mesenchymal stromal cells (MSCs) using HumanMethylation450 BeadChip technology. Expansion of CD34+ cells either with or without MSCs has relatively little impact on DNAm - although proliferation is greatly increased by stromal support. Notably, all cultured HPCs (even those which remained CD34+) acquired extensive DNA-hypermethylation within seven days in vitro, particularly in up-stream promoter regions, shore-regions of CpG islands and binding sites for PU.1, HOXA5 and RUNX1. The vast majority of these DNAm changes were not related to senescence-associated DNAm changes but they were rather located in relevant developmental genes. Furthermore, DNAm changes were associated with differential expression of hematopoietic genes and aberrant splicing of DNMT3A. Low concentrations of demethylating agents (such as zebularine or epigallocatechin-3-O-gallate) slightly increased the frequency of colony-forming unit initiating cells indicating that inhibition of DNAm may support expansion of progenitor cells. Taken together, our results demonstrate that culture expanded HPCs - even those which maintain a primitive immunophenotype - acquire significant DNAm changes. These epigenetic modifications reflect the inefficient self-renewal under in culture conditions even with stromal support. Control of epigenetic modifications during culture expansion may prevent loss of stemmness.

P1017 - DECLINED PRESENTATION

G9A IS REQUIRED FOR THE DEVELOPMENT AND FUNCTION OF GROUP 2 INNATE LYMPHOID CELLS

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Group 2 innate lymphoid cells (ILC2) are a recently identified cell type found in mucosal tissues capable of producing high amounts of the Th2-type cytokines interleukin (IL)-5 and IL-13 in response to the epithelial cell-derived cytokines IL-33 and thymic stromal lymphopoietin (TSLP). ILC2s have been shown to play a role in protease-induced type 2 immune responses in the lung. ILC2s are derived from bone marrow-resident precursor cells with the cell surface phenotype Lin-Sca1+cKit-CD127+CD25+IL-33R+. The molecular mechanisms that regulate ILC2 ontogeny remain unclear. Here we identify a role for the epigenetic modifier G9a in the regulation of ILC2 development and function. G9a is a histone lysine methyltransferase that has been associated with transcriptional repression via dimethylation of histone 3 lysine 9 (H3K9me2). G9a-dependent H3K9me2-mediated repression is essential for early embryogenesis and is involved in the transcriptional repression of developmental genes. In addition, G9a also has been shown to promote gene expression and is required for efficient CD4+ T-helper 2 (Th2) cell responses, including the production of IL-5 and IL-13. Using mice with a hematopoietic cell-specific deletion of G9a (VavG9a-/- mice), we found a significant reduction in the frequency of bone marrow ILC2 progenitors as well as mature lung ILC2s in the absence of G9a. Following stimulation of ILC2s with TSLP and IL-33, ILC2s isolated from the lungs of VavG9a-/- mice were defective in their ability to produce IL-5 and IL-13 compared to control G9afl/fl mice. Consistent with a role for G9a in ILC2 function, intranasal administration of VavG9a-/- mice with papain resulted in decreased expression of Il5 and Il13 mRNA in the lung and reduced eosinophil influx into the brochoalveolar lavage. Further, VavG9a-/- mice also display heightened resistance to house dust mite antigen-induced allergic lung inflammation. Taken together, our data demonstrate a role for G9a in ILC2 ontogeny and function during allergic lung inflammation.

P1018 - GENOME-WIDE MAPPING OF HISTONE MODIFICATIONS MEDIATED BY THE POLYCOMB-GROUP COMPLEXES IN FETAL LIVER AND ADULT BONE MARROW HEMATOPOIETIC PROGENITOR CELLS

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Epigenetic regulation of transcription is one of the major determinants of the developmental stage-specific characteristics of hematopoietic stem/progenitor cells. We previously showed that the dependency on the polycomb group (PcG) protein Ezh2 distinguishes fetal from adult hematopoietic stem cells. This finding suggests that the epigenetic regulation of hematopoiesis in fetal liver (FL) and adult bone marrow (BM) is different. However, the details of this regulation remain unknown. To address this, we purified Lin-c-Kit+Sca1- (LK) cells from mouse E14.5 FL and adult BM and performed ChIP-sequence assays for H3K27me3 and H2AK119Ub1, H3K4me3, H3K9me3 and H3K36me3. We also performed microarray analysis in LK and Lin-Sca1+c-Kit+ (LSK) cells from FL and BM hematopoietic progenitor cells (HPCs). More genes were marked by the PRC2 mark H3K27me3 in FL HPCs than in BM HPCs (1922 vs. 622 genes). In contrast, more genes were marked by the PRC1 mark H2AK119Ub1 in BM (871 genes) compared to FL (178 genes). H2AK119Ub1 closely coincided with H3K27me3, suggesting that the PRC2-mediated gene silencing tends to be reinforced by PRC1 during the transition from FL to BM hematopoiesis. Among bivalent genes marked with both H3K4me3 and H3K27me3 in BM, half of the genes were also marked with H2AK119Ub1 (250/491 genes). Of note, 242 genes with H3K4me3 mark had H2AK119Ub1 marks but little H3K27me3. Nonetheless, these genes were transcriptionally repressed like canonical bivalent genes. Recently, a novel mechanism of gene silencing has been proposed in which PHF19 binds H3K36me3 and recruits PRC2, followed by removal of H3K36me3 and addition of H3K27me3. The majority of H3K36me3-marked genes had H3K4me3 marks in both FL (454/547) and BM (431/660). Although a significant portion of H3K27me3 and H2AK119Ub1 marks highly overlapped with H3K4me3, PcG marks and H3K36me3 appeared to be mutually exclusive, as expected. Our findings imply unique epigenetic regulation of FL and adult BM hematopoietic stem/progenitor cells.

P1019 - COMPARISON OF PROMOTER DNA METHYLATION AND EXPRESSION LEVELS OF GENES ENCODING CCAAT/ENHANCER BINDING PROTEINS IN AML PATIENTS

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The family of CCAAT/enhancer binding proteins (CEBPs) are transcriptions factors indispensible for myeloid differentiation. Aberrations in individual CEBPs genes are found in acute myeloid leukemia (AML) patients. The aim of the study was to compare promoter methylation and expression levels of CEBPA, CEBPD, CEBPE and CEBPZ in a group of 78 AML patients and controls: normal bone marrow (BM) samples and FACS-sorted hematopoietic precursor cells. DNA from Ficoll-isolated mononuclear cells was bisulfite converted and DNA methylation level of regulatory regions was determined by quantitative methylation specific PCR (qMSP). Relative expression level was measured by qRT-PCR. CEBPA, CEBPD and CEBPE promoters methylation were elevated in 37%, 35,5% and 56,7% of AML patients, respectively when compared to control samples. No CEBPZ promoter methylation was observed, except normal hematopoietic progenitors. An inverse correlation of DNA methylation and mRNA levels was found in AML patients for CEBPA and in the group of methylation positive patients for CEBPD. Favorable cytogenetic risk patients shared common methylation profile in hierarchical clustering analysis. They revealed substantially elevated methylation level of CEBPA and lack of CEBPD methylation. Significantly different CEBPE methylation levels were observed between patients with inv16 and those with t(15:17) or t(8:21). CEBPD and CEBPE expression levels were relevantly decreased in intermediate risk patients (mainly cytogenetically normal). Patients carrying distinct favorable aberrations significantly differed in CEBPs expression that was reflected in clustering analysis of a good cytogenetic prognostic group. AML cytogenetic risk groups and patients with particular translocation are characterized by distinct methylation/expression profile of CEBPs encoding genes.

P1020 - RED BLOOD CELL GENERATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS OF DIFFERENT DONOR CELL TYPE ORIGIN

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Manufacturing red blood cells (RBC) from human induced pluripotent stem cells (iPS cells) offers the potential to produce large quantities of patients' specific RBC for transfusions purposes. Epigenetic memory in iPS cells in regard to their donor cell type of origin might lead to variations in their differentiation capacities. We have generated iPS cells from human cord blood CD34+ hematopoietic stem cells (HSC) (CD34-iPS) and neural stem cells (NSC-iPS) and evaluated their differentiation potential into hematopoietic precursor and mature RBC. For hematopoietic induction, iPS cells were allowed to form embryoid bodies (EBs) under cytokine stimulation for 21 days. Thereafter, dissociated single cells were applied to a three-step protocol for human erythropoiesis for additional 18-25 days. We have found a similar hematopoietic induction potential among our cell lines. After EB dissociation on day 21, hematopoietic commitment, measured by CD43 expression, was about 20% for all cell lines. Colony-forming unit assays demonstrate a similar distribution of myeloid (CFU-M/CFU-GM), erythroid (BFU-E/CFU-E) and mixed (CFU-GEMM) colonies. Hematopoietic cells further developed into erythroid precursors as determined by $>90\%$ expression of glycophorin A, followed by maturation into normoblasts and partially enucleated RBC. All human iPS derived erythrocytes predominantly present fetal hemoglobin ($>85\%$), some embryonic and only a minor amount of adult hemoglobin. In summary, we were able to recapitulate the development of RBC from all human iPS cell lines evaluated. In addition, our data hint at a similar erythrocyte induction potential of iPS cells, independent of their donor cell type origin.

P1021 - DECLINED PRESENTATION
NOVEL FUNCTIONAL ROLES FOR TEN-ELEVEN-TRANSLOCATION 2 (TET2) IN NORMAL AND LEUKEMIC GROWTH OF MAST CELLS

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Mast cells play a vital role in innate and adaptive immunity. KIT receptor signaling is essential for mast cell growth and survival. Gain-of-function mutations in KIT receptor have been identified in humans in 90% of systemic mastocytosis (SM) patients. KITD816V mutation found in SM is imatinib-resistant and current anti-KIT therapies for KITD816V+ SM are ineffective. Our recent clinical studies have identified the presence of Tet2 mutations in 23% of mastocytosis patients and are associated with poor overall prognosis. However, the physiologic role(s) of Tet2 in normal mast cell development and cooperation between Tet2 and KITD816V mutations in SM are not well studied. In this study, we have determined the functional role of Tet2 in normal mast cell development and molecular mechanisms of cooperation between Tet2 and KITD816V mutations in SM. We found significantly increased number of immature mast cells in the peritoneal cavity of Tet2-/- mice compared to WT mice in vivo. Bone marrow-derived mast cells (BMMCs) from Tet2-/- mice show significant reduction in 5-hydroxymethyl cytosine levels compared to WT BMMCs. Tet2-/- BMMCs show significant reduction in mast cell differentiation, reduced expression of mast cell-specific genes MCP-5, MCP-6 and CPA, and altered expression of key transcription factors Mitf, Gata-2 and PU.1 compared to WT BMMCs. In addition, Tet2-/- BMMCs show enhanced cytokine-induced growth and survival. Furthermore, Tet2-/- murine or knocked down human cells bearing KIT mutation show significantly enhanced growth compared to cells bearing KIT mutation alone. Hyperproliferation of Tet2-/- BMMCs in the presence and absence of KIT mutation is associated with reduced expression of Pten and enhanced activation of the PI3Kinase/AKT pathway. Treatment with demethylating agent 5-azacytidine or PI3Kinase inhibitor GDC-0941 (p110α/δ-specific), but not TGX221 (p110β-specific) or IC87114 (p110δ-specific), significantly reduced the hyperproliferation of Tet2-/- BMMCs and cell lines as well as primary BM blasts derived from mastocytosis patients bearing KITD816V mutation. Thus, combinatorial therapy involving both demethylating agent and PI3Kinase inhibitor is likely to be ideal for treating SM patients with Tet2 and KIT mutations.

P1022 - OPTIMIZATION OF X-LINKED CHRONIC GRANULOMATOUS DISEASE MODELIZATION BY USING PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) are reprogrammed somatic cells with embryonic stem cell (ESC)-like characteristics generated by the introduction of combinations of specific transcription factors. Patient-specific iPSCs can be used to recapitulate disease-specific phenotypes for the screening of new therapies. Chronic granulomatous disease (CGD), a rare inherited immunodeficiency, is characterized by recurrent and severe infections in childhood. The most frequent form is the X-linked CGD (X-CGD) due to mutations in CYBB leading to the absence of Nox2 of the phagocytic NADPH oxidase complex, responsible for the production of microbicidal reactive oxygen species. Our objective was to optimize the generation of phagocytes reproducing the X-CGD physiopathology in order to test a new therapeutic approach based on protein therapy. X-CGD and control iPSC lines were reprogrammed from human fibroblasts with OSKM retroviral vectors. iPSC lines expressing pluripotency markers were able to form embryoid bodies (EBs) with the three embryonic germ layers. Hematopoietic differentiation induced after coculture of iPSCs or EBs with OP9 mouse bone marrow stromal cell line, was characterized by a fast decrease of pluripotency markers with a progressive appearance of CD34+ progenitors. Terminal differentiation into mature neutrophils or macrophages (5.10^5 cells) was obtained in 10-14 days by using specific hematopoietic cytokines. No significant difference between both coculture types (iPSC or EB) was noticed. Freezing and thawing of the CD34+ progenitors did not modify the progenitor ability. X-CGD neutrophils and macrophages derived from X-CGD iPSCs showed no Nox2 expression and NADPH oxidase activity unlike control cells. In conclusion, we succeeded to generate a cellular model of X-CGD ready to test new therapeutic approaches.

P1023 - CONTROLLED GENERATION OF HEMATOPOIETIC PROGENITOR CELLS FROM PLURIPOTENT STEM CELLS USING MICROENVIRONMENTAL CUES

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Human pluripotent stem cells (hPSCs) have great potential for regenerative medicine due to their ability to develop into any of the somatic cells including blood cells. However, in vitro protocols for generating hPSC-derived hematopoietic progenitor cells (HPCs) utilize xenogenic products, display low yields and typically generate primitive blood phenotypes. Current evidence suggests a hemogenic endothelial (HE) population giving rise to definitive blood cells. Obtaining this population from hPSCs has been difficult since definitive hematopoiesis during embryogenesis is a dynamic and tightly controlled process. We hypothesize that hPSC-derived endothelial cells can be obtained using defined and controlled conditions and can be micropatterned into artificial niches to mimic blood forming HE. The AggreWellTM technology was used to generate variable aggregate sizes to culture PSCs towards HPCs in serum-free defined media. Expression of CD45 peaked by day 20 (35%) and colony forming cells from PSC-derived cells were highest on day 13 at 200 colonies/105 seeded cells. HE expression was assessed by tracking CD34+VECAD+CD43- which was highest on day 8. Thereafter, day 8 cells were sorted into CD34+VECAD+ and were positive for von willebrand factor and uptake of low density lipoprotein confirming its endothelial nature. To assess if these cells can give rise to definitive-like blood cells, day 8 CD34+CD43- cells (29% expression pre-sort) were magnetically sorted and placed on non-irradiated OP9-DL1 cells for co-culture. By day 21 of co-culture, cells were 11% positive for CD5+CD7+ indicative of pro-T lymphoid cells thereby implicating their definitive potential. We have previously shown that precise control of colony size allows for the manipulation of hESC fate. Building on this, day 8 HE cells are currently being patterned as 200 μm diameter spots with a 500 μm pitch (distance b/w spots). These cells will be supplemented with factors involved in embryonic definitive hematopoiesis to enhance blood induction compared to our co-culture experiments. All in all, these studies demonstrate the use of tools that allow us to recapitulate aspects of the embryonic microenvironment to guide PSCs towards hematopoietic cells in an efficient and scalable fashion.

P1024 - IN VITRO GENERATION OF FUNCTIONAL T-CELLS STARTING FROM GENE-CORRECTED WISKOTT-ALDRICH SYNDROME HUMAN IPSC

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Introduction: Wiskott-Aldrich syndrome (WAS) is an X-linked primary immunodeficiency disease characterized by thrombocytopenia, recurrent infections and increased autoimmunity. This disease is caused by mutations in the WAS protein(-WASp) gene which is exclusively expressed in hematopoietic cells and results in functional abnormalities in T- and B-cells, hematopoietic progenitors, platelets, etc. We investigated restoration of T-cell functionality using the zinc finger nuclease targeted gene-correction strategy. **Results:** We generated iPSC from a WAS patient carrying an insertional mutation. Using ZFN-technology, a WAS-2A-eGFP transgene was targeted at the endogenous chromosomal location, under native gene transcriptional control. eGFP expression during hematopoietic differentiation was evaluated by flow cytometry and qPCR. We confirmed hematopoietic specificity of eGFP+ cells and showed that only eGFP+ cells are expressing the corrected WAS sequence. Next, we generated CD3+T-cells using an OP9DLL1-coculture system. Briefly, iPSC colony fragments were cocultured with OP9 cells to generate CD34+ and CD43+ cells. These cells were then recultured into a secondary coculture with OP9DLL1 cells. After 14 days, up to 13% of CD5+CD7+ lymphoid committed progenitors were present and nearly 100% of hematopoietic cells were eGFP+. We observed 12% of CD4+CD8+ cells at day 28 of coculture. After 37 days, 1.5% of our culture contained CD3+T-cells which were all eGFP+ and contained both TCRαβ+CD3+ and TCRδγ+CD3+ cells. **Conclusion:** Targeted integration of the WAS gene in WAS-iPSC results in restoration of WAS expression during in vitro hematopoiesis. We generated CD3+ T-cells from these iPSC assaying expression throughout lymphoid development. It is now necessary to confirm that restoration of WASp expression results in restoration of WASp-dependent T-cell function.

P1025 - RESISTANCE TO ZINC FINGER NUCLEASE (ZFN)-MEDIATED FANCA DISRUPTION IN HUMAN EMBRYONIC STEM CELLS (HESC)

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Fanconi anemia (FA) is characterized by progressive bone marrow failure (BMF), which leads to acute myeloid leukemia (AML) in 30% of cases. The high cancer predisposition and hypersensitivity to cross-linking agents reflect the genomic instability in FA patients and illustrate the link with DNA repair processes. To investigate the mechanism underlying the hematopoietic defects seen in FA, we aimed to create a human pluripotent stem cell model by knocking-out the FANCA gene in hESC via ZFN-mediated homologous recombination (HR). After targeting FANCA by inserting a Puromycin (Puro) selection cassette, we found one clone where southern blot (SB) suggested a higher abundance of transgenic (TG) alleles than wild-type (WT). Given that hESC are split as clumps, we hypothesized that this clone might represent a mixture of heterozygously (+/-) and homozygously (-/-) targeted cells. Disappearance of the band corresponding to the WT allele upon reselection with a higher dose of Puro confirmed this hypothesis. However, when the same clone was assessed by SB a few passages after Puro selection, the WT allele was again detected, suggesting a selective growth advantage of +/- cells, which can escape or withstand selection pressure, over -/- cells. We performed HR with a Hygromycin (Hygro) cassette in the WT allele of a clone that was heterozygous for the Puro insertion. Some HygroR clones, in which the absence of the WT allele (as a confirmation of bi-allelic targeting) was demonstrated, underwent growth arrest and could not be maintained in culture. The WT allele however reappeared one passage later and remained present ever after despite extensive reselection. The presence of WT FANCA in spite of clear demonstration of bi-allelic targeting was further substantiated by Western blot and immunofluorescence data. Together these data point towards a severe growth disadvantage of FANCA KO hESC. Whether stem cells may invoke alternative mechanisms to circumvent the loss of important DNA repair genes is currently under investigation.

P1026 - REGULATION OF MOUSE EMBRYONIC STEM CELL FATE BY RIBOSOMAL PROTEINS

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Embryonic stem cells (ESC) have the capacity to differentiate into all cell types of the organism (pluripotency). Regulation of self-renewal and differentiation is critical in order to maintain this property. The possibility that some key ESC fate determinants are not identified yet prompted us to create a collection of ESC clones that carry nested chromosomal deletions. We used this library of more than 1000 clones to screen for embryoid body (EB) formation determinants, followed by complementation experiments of the phenotypic groups in order to identify new genomic elements regulating ESC fate. This strategy allowed us to identify the ribosomal protein S14 (Rps14) as essential for ESC differentiation. Since a number of genes from this family were deleted in individual library clones, we decided to evaluate the impact of ribosomal protein (RP) on ESC behavior. GO Term analysis of our EB formation screen showed that deletions of RP coding genes were likely to produce an abnormal differentiation phenotype. Complementation experiments also showed that ESC are tolerant to RP imbalance in order to self-renew, but that their differentiation potential is affected upon such deletions. Moreover, while p53 signaling proved to be important in the context of several ribosomal biogenesis disorders, we identified a potential p53-independent regulation of ESC fate upon some specific RP gene deletions. Our ongoing studies on ESC fate regulation by RP are likely to shed light on important mechanisms used to restrict ESC differentiation potential.

P1028 - NOREPINEPHRINE IMPROVES THE GENERATION OF HEMATOPOIETIC CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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The possibility of differentiating human pluripotent stem cells (hPSCs) to hematopoietic stem cells (HSCs) could provide an unlimited source of donor cells for the treatment of hematological disorders and malignancies where HSC transplantation is required. Recently, signaling from the developing peripheral nervous system (PNS) has been implicated in the generation of HSCs in the aorta gonad mesonephros (AGM) of mouse embryos. Our own observations of explants of AGM and urogenital ridge (UR) explants from 6 weeks old human embryos show neurogenic potential. Since the AGM and UR are active sites of hematopoietic emergence, we hypothesize that migrating neural crest (NC) cells, precursors of the PNS, might have an active role in HSC generation. Given that, at the time of HSC emergence, NC cells express enzymes required for catecholamine production, we added norepinephrine (NE) to our optimized hPSC differentiation system and assessed for hematopoietic progenitor cell output. We observed a 2 fold increase of cells with an HSC phenotype (CD43+CD34+CD38-CD90+CD45RA-) compared to control settings. We identified this phenotype as a cell with highest lymphoid and myeloid differentiation potential. Importantly, the increase was specific to this most immature fraction, since the proportion of progenitors (CD43+CD34+CD38- cells) and of total blood (CD43+) did not display a significant increase in the presence of NE. Continuing the differentiation culture for 5 additional days did not show additional increase in the number of HSCs. This indicates that higher proportion of HSCs in the presence of NE is due to increased emergence from hemogenic endothelium rather than to better maintenance of the cells in culture. Improved generation of immature progenitors was also consistent with an increase of colonies in the colony forming unit assay. The increased output of primitive hematopoietc progenitors was reversed when the Adrenergic Receptor β2 specific inhibitor ICI 118,551 was added together with NE, showing that the effect of NE is mediated by the activation of this receptor. Currently we are in the process of transplanting these cells in immunocompromised mice, in order to determine whether NE is the factor needed to achieve engraftment of hPSC-derived HSCs.

P1029 - PROFILING OF MICRORNA EXPRESSION INVOLVED IN IPSC GENERATION, MAINTENANCE AND DIFFERENTIATION USING A HIGH THROUGHPUT, LNA™-ENHANCED QPCR METHOD

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Stem cells have received increasing attention in recent years due to their notable potential in self-renewal and the ability to differentiate into different cell types. The creation of induced pluripotent stem cells (iPSC) further demonstrates their broad applications in drug discovery and cell-based therapeutic development by circumventing the ethical dilemma of using human embryonic stem cells (hESC). MicroRNAs are small (∼22 nt), single stranded non-coding RNAs that play critical roles in post-transcriptional regulation of gene expression. Recently, it has become evident that microRNAs are key players in the regulation of stem cell differentiation and homeostasis. Thus, characterizing stem cell populations by microRNA profiling at various stages of differentiation may improve our understanding of stem cell development. We have applied LNA™-enhanced microRNA qPCR to study microRNAs expression in differentiating pluripotent stem cells. The Exiqon Stem Cell Focus microRNA PCR panel offers a fast and highly sensitive method for microRNAs quantification with accurate and linear detection over several orders of magnitude, which is imperative when detecting small, but significant changes in microRNA expression levels. The current study examines how miRNAs, as known regulators of pluripotency and lineage development, may be involved in differentiation efficiency of iPSC, a major determinant in stem cell-based therapeutic development. The goal is to determine distinct microRNA signatures from iPSC of different stages of differentiation that can reveal relevant signaling pathways that control the downstream fate or therapeutic potential, depending on various factors such as cellular origins or methods of reprogramming, etc. The Stem Cell Focus microRNA PCR panel thus offers an efficient tool to characterize iPSCs for use in research, drug discovery and the manufacture of cell-based therapies.

P1030 - HOXB4 COOPERATES WITH RUNX1 TO PROMOTE THE DEVELOPMENT OF HEMATOPOIETIC CELLS AT THE HEMOGENIC ENDOTHELIUM STAGE, *IN VITRO*

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Hematopoietic differentiation of pluripotent stem cells and expansion of emerging stem and progenitor cells (HSPCs), *in vitro*, are strongly enhanced by ectopic expression of the homeodomain transcription factor HOXB4. However, the underlying mechanisms are still ill-defined. Therefore, we investigated its influence on major fate decisions during different stages of embryonic stem (ES-) cell differentiation, *in vitro*. For this purpose, we retrovirally expressed HOXB4 or a Tamoxifen-inducible HOXB4-ERT2 fusion in different reporter ESC-lines. These lines were differentiated as embryoid bodies (EBs) for 6 days and, after dissociation, cultured on OP9 stroma cells for different periods of time. During cocultivation on OP9 cells, HOXB4 mediated a significant increase in the number of circular, sheet-like structures which expressed Endothelial Protein C Receptor (CD201), VE-Cadherin (CD144), PECAM (CD31) and were capable of Dil-ac-LDL uptake. When using ESCs containing a Venus reporter gene integrated into one Runx1 allele (Runx1/Venus^{+}; S. Nishikawa, Kobe), we noted that the emerging Runx1/Venus^{+} CD41^{+} hematopoietic cells were associated with such sheet-structures, thus presumably representing hemogenic endothelium layers. In this reporter line, HOXB4 did not induce or alter initial Runx1 expression during EB-development. However, during the subsequent OP9-cocultivation phase it promoted the appearance of a subpopulation expressing increased levels of Runx1/Venus, which contained all arising CD45^{+} cells. Furthermore, use of a Runx1$^{-/-}$ ESC-line allowing for Doxycycline-inducible Runx1 expression (iRunx1; G. Lacaud, Manchester) and additionally expressing Tamoxifen-inducible HOXB4 uncovered that both transcription factors cooperate in hemogenic endothelium cells. Whilst HOXB4 alone strongly promoted the formation of hemogenic endothelium structures, CD45^{+} cells were only generated after additional induction of Runx1 expression. So far, our results suggest that HOXB4 and Runx1 may interdepend during the transition of the hemogenic endothelium to definitive hematopoietic progenitor cells, at least during ESC differentiation, *in vitro*.

P1031 - IDENTIFICATION OF PLURIPOTENT AREA DURING IN VITRO DIFFERENTIATION OF EMBRYOID BODIES

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Introduction: The aim of this study was to determined pluripotent area following favorable condition medium and its time period on differentiation Primordial Germ Cells (PGCs) from mouse Embryonic Stem Cells (mESCs) in vitro. **Methods and Matherials:** Embryoid Bodies (EBs) from mES cells were cultured in combination of 10 ng/ml BMP4 and 2μM Retinoic Acid at the different time interval 2,4,6,8 and 10 days (D). The pluripotent marker Oct-4 screened by Immunocytochemistry. To confirm early germ cells, double staining was performed with post-migratory germ cell marker Mvh. **Results:** Based on Oct4 positve cells (OPCs), EBs were classified to 5 types. In D2 and D4 OPCs were observed in EBs in small clusters in center (type I) and peripheral part (type II) of EBs respectively. However, in D6, single cells expressing Oct4 were found in EBs (type III). The greatest variation in expressing of Oct4 was observed in D6 and D8 (types I-IV, type IV; no reaction). In D10, only the single cells expressing Oct4 were detected in the surface part of EBs (type V). The most EBs that co- expression Oct4 / Mvh were detected only D8 and especially D10. The putative germ cells still express pluripotency marker Oct4 that detected in the nucleus of putative of PGCs. That seems to be expressed in the areas where chromatin is not condensed. Another marker is Mvh, which was presented in the cytoplasm has also bigger nucleus thus distinguishable from other cell types in EBs. **Conclusions:** The OPCs in D2-D4 like germ cells arise at the base of allantois following in D6 single cells expressing Oct4 indicate migratory germ cells (type III). The single cells expressing Oct4 in D10 are just pluripotent germ cell with characteristic of early germ cells.

Key words Primordial Germ Cells, Mvh, Embryoid Bodies, Pluripotente Cells.

P1032 - QUANTIFICATION OF STEM CELL / NICHE INTERACTIONS BY COUPLING IN VIVO IMAGING AND IN SILICO SIMULATION

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For decades the bone marrow has been recognized as the home of hematopoietic stem cells (HSCs). Their regulation has been studied extensively, in particular regarding single molecules and pathways, but a comprehensive and dynamic picture is still elusive. This holds especially true for spatial organization. Recent advances in imaging techniques now allow acquisition of data on position and migration of HSCs in vivo. However, those techniques are limited by a relatively small observation volume and time span due to experimental limitations. Here, we complement in vivo imaging of HSCs in a mouse model by a mathematical model of spatio-temporal HSC dynamics and exploit its potential to identify basic principles of stem cell / niche organization. The computer model allows us to test a number of general mechanisms of HSC / niche interaction independent of molecular details. Specifically, we investigate various modes of cell migration and niche geometries with respect to their influence on HSC organization. The in silico model demonstrates that spatial distribution of HSCs crucially depends on different mechanisms, such as motility of cells, attraction forces of HSCs towards niche sites, but potentially also repulsion of HSCs from already occupied niches. The simulation results are compared to in vivo experiments, which demonstrate that the distances of transplanted hematopoietic cells to osteoblasts and bone surface varies between different cell types. Interestingly, simulated distance distributions of various hematopoietic cell types to osteoblasts turned out to be most similar to experimental data for random motion and not for an explicit niche-mediated attraction.

P1033 - DECLINED PRESENTATION
CD34 FAMILY PROTEINS ARE KEY REGULATORS OF HEMATOPOIETIC CELL MIGRATION AND VASCULAR PERMEABILITY

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CD34 is a widely used marker of hematopoietic progenitors and vascular endothelia yet, its function remains obscure. It is the founding member of a family of three proteins (CD34, podocalyxin and endoglycan) with overlapping expression patterns on progenitors and endothelia, but with distinct expression patterns on other tissues. For example, CD34 is uniquely expressed by mast cells, eosinophils and dendritic cell precursors, and we find that mice lacking CD34 are remarkably resistant to a variety of mucosal inflammatory diseases including allergic asthma, hypersensitivity pneumonitis and colitis. This resistance reflects defective inflammatory cell trafficking due to enhanced adhesion and impaired chemotaxis, which is reversed through ectopic expression of a human CD34 transgene. Podocalyxin, in contrast, is uniquely expressed by a variety of embryonic tissues and inactivation of the Podxl gene leads to perinatal lethality due to a failure of tissues to undergo appropriate morphogenesis. Furthermore, selective inactivation of Podxl in adult endothelial cells results in increased vascular leakage and development of an emphysema-like phenotype due to poor vessel patency in the lung. This suggests that podocalyxin plays an important function in maintaining homeostasis in the adult vasculature. Finally, these proteins also play key roles in hematopoietic precursor homing; podocalyxin, in particular, facilitates CXCR4-dependent chemotaxis, likely by stabilizing polarized signaling through CXCR4. Intriguingly, despite the impaired homing of Podxl-/- progenitors, they exhibit a competitive advantage in engraftment of transplanted mice that is highly reminiscent of CXCR4-/- progenitors. In summary, our data suggest that CD34-type proteins play critical roles in a variety of normal developmental processes, hematopoiesis and immunity. Mechanistically, they act as key regulators of adhesion, chemotaxis, cell morphogenesis and, potentially, stem cell expansion.

P1034 - IDENTIFICATION OF NOVEL REGULATORY GENES OF HUMAN POST-EMBRYONIC HEMATOPOIESIS

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Hematopoietic stem cell (HSC) fate is controlled by intrinsic and extrinsic factors regulating proliferation, self-renewal, apoptosis or differentiation thereby adapting blood cell production to the need of the organism. We and others have shown that genetic manipulation of hematopoiesis using retroviral vectors (RV) can trigger clonal expansion and malignancy by the integration of the therapeutic vector into specific gene regions. In a total of 16 human gene therapy trials we detected genetic loci which are highly overrepresented, indicating for a selective advantage of clones carrying these common vector integration sites (CIS) in vivo. We therefore hypothesize that CIS significantly enriched in genetically modified blood cells point to a regulatory function of nearby genes in post-embryonic hematopoiesis. To identify potential stem cell regulatory genes we systematically analyzed the insertion site (IS) repertoire of gene corrected blood cells within a cohort of 10 gene therapy patients with Wiskott-Aldrich-Syndrome. Using highly sensitive linear amplification mediated PCR combined with high-throughput 454 sequencing a total of 12.887 unique IS in the vicinity of 3.268 genes were identified. Next, we selected all genes with at least 10 different IS within a 200 kb window around the gene (n=588). To enrich for genes with increased probability of transcriptional activation we then chose those genes with at least 10 IS within a 50kb window around the transcriptional start site (n=424). After stringent exclusion of all genes located within gene clusters 32 candidate genes were identified. To evaluate the hematopoietic activity of gene corrected cell clones with CIS we monitored their contribution to blood formation post transplantation. IS close to candidate genes were detectable 15 to 93 times in a total of 102 analyzed patient samples during a time period of 4 years, demonstrating long term activity of HSC clones carrying IS near candidates . Interestingly, 12 of 32 identified genes have not been linked to hematopoiesis so far. Of these, 4 genes are known proto-oncogenes and 3 are highly expressed in HSC. The highest ranked genes EVI1, CCND2 and LMO2 are known as hematopoietic regulators and key factors in malignant transformation, thereby validating our candidate selection strategy. These data demonstrate that RV IS datasets derived from gene therapy patients can be used to identify regulatory genes of hematopoiesis. Systematic identification of novel regulatory genes in meta datasets derived from a larger number of gene therapy studies and subsequent validation in vitro and in vivo will allow new insights into the biology of post-embryonic hematopoiesis.

P1035 - DECLINED PRESENTATION INFLUENCE OF GRANULOCYTIC COLONY-STIMULATING FACTOR ON PERIPHERAL LYMPHOID SUBSETS IN PATIENTS WITH ARTERIOSCLEROSIS OBLITERANS

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Peripheral hematopoietic stem cells (PHSC) are used in treatment of patients with broad spectrum of diseases. Nevertheless, there are few data about the influence of granulocytic colony-stimulating factor (G-CSF) on immune system of patients who received G-CSF for the harvesting of PHSC.
Aim: To evaluate the influence of G-CSF on parameters of peripheral lymphoid subsets in patients with critical ischaemia of lower extremities due to arteriosclerosis obliterans (AO), who received G-CSF before autologous PHSC transplantation. 14 patients with AO received G-CSF (filgrastim, 10 mg/kg) for 3 days followed by apheresis of PSC. The number of CD34+ cell was measured by standard flow cytometry protocol. PHSC were introduced by multiple intramuscular injections under ultrasound control. Expression of CD3, CD4, CD8, CD19, DR, CD16/CD56 on peripheral blood lymphoid cells was evaluated using 5-color flow cytometry at 3 points: 1) before the 1st G-CSF injection, 2) at the next day after the last injection and 3) 1 month after autologous PHSC transplantation. Control group included 52 patients with AO on standard therapy. Initial values of lymphoid subsets in patients with AO did not differ significantly neither from normal values obtained from healthy volunteers, nor from control group. There were no significant changes in relative numbers of T-cells (CD3+) and their main subsets - CD3+CD4+ and CD3+CD8+, as well as B-cells (CD19+ DR+) and NK-cells (CD16/56+) during G-CSF therapy.
Conclusion: We did not establish any significant shifts in main lymphoid subsets in peripheral blood of patients with AO, who received G-CSF in standard doses for the harvesting of PHSC. Our data could be considered during CD34+ cell therapy for some other cohorts, particularly, immunocompromised patients.

P1036 - HEMATOPOIETIC STEM CELLS FROM TS65DN MOUSE BONE MARROW ARE DEFICIENT IN THE REPAIR OF DNA DOUBLE-STRAND BREAKS

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Down syndrome (DS) is the most common chromosomal abnormality in humans caused by trisomy 21. Pediatric patients with DS display various hematologic disorders and are at increased risk of leukemia. Ts65Dn mice are trisomic for 104 orthologs of the genes on human chromosome 21 and are one of the most widely used mouse models for DS research. It has been shown that aneuploidy can impair DNA damage repair and induce genomic instability. Therefore, we investigated whether hematopoietic stem cells (HSCs) from Ts65Dn mice are deficient in the repair of DNA double-strand breaks (DSBs), because the deficiency in the repair of DSBs in HSCs can potentially contribute to DS-associated hematological abnormalities and malignancies by impairing HSC self-renewal and inducing hematopoietic genetic instability. Our results showed that Ts65Dn mice had significantly less bone marrow HSCs than wild-type (WT) littermate controls, whereas the levels of bone marrow hematopoietic progenitor cells (HPCs) were similar. The lower level of HSCs in Ts65Dn mice was associated with a significantly higher level of DSBs and lower level of clonogenic activity when compared to the cells from WT controls. Although levels of DSBs in HSCs were similar at 1 hr after exposure to γ-irradiation in vitro, HSCs from Ts65Dn mice had significantly higher levels of unrepaired DSBs than the cells from WT mice at 3 and 6 hr after irradiation. Moreover, after exposure to γ-irradiation in vitro, the reduction in clonogenic function was significantly greater in Ts65Dn HSCs than WT HSCs. In contrast, no significant differences in these parameters were observed in HPCs from Ts65Dn and WT mice with or without irradiation. These findings suggest that an additional copy of genes on human chromosome 21 can selectively impair the ability of HSCs to repair DSBs, which may contribute to DS-associated hematological abnormalities and malignancies. The mechanisms by which HSC repair of DSBs is regulated by gene dosage remain to be elucidated.

P1037 - CD133 IS A MODIFIER OF HEMATOPOIETIC PROGENITOR FREQUENCIES BUT IS DISPENSABLE FOR THE MAINTENANCE OF MOUSE HEMATOPOIETIC STEM CELLS

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Pentatransmembrane glycoprotein prominin-1 (CD133) is expressed at the cell surface of multiple somatic stem cells, and it is widely used as a cell surface marker for the isolation and characterization of human hematopoietic stem cells (HSCs) and cancer stem cells. CD133 has been linked on a cell biological basis to stem cell-fate decisions in human HSCs and emerges as an important physiological regulator of stem cell maintenance and expansion. Its expression and physiological relevance in the murine hematopoietic system is nevertheless elusive. We show here that CD133 is expressed by bone marrow-resident murine HSCs and myeloid precursor cells with the developmental propensity to give rise to granulocytes and monocytes. However, CD133 is dispensable for the pool size and function of HSCs during steady-state hematopoiesis and after transplantation, demonstrating a substantial species difference between mouse and man. Blood cell numbers in the periphery are normal; however, CD133 appears to be a modifier for the development of growth-factor responsive myeloerythroid precursor cells in the bone marrow under steady state and mature red blood cells after hematopoietic stress. Taken together, these studies show that CD133 is not a critical regulator of hematopoietic stem cell function in mouse but that it modifies frequencies of growth-factor responsive hematopoietic progenitor cells during steady state and after myelotoxic stress in vivo.

P1038 - THE BALANCE BETWEEN SELF-RENEWAL AND DIFFERENTIATION PROBABILITIES DETERMINES LONGEVITY OF HEMATOPOIETIC STEM CELLS

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Heterogeneity in hematopoietic stem cells (HSCs) is the focus of our attention. After transplantation with single HSCs, the hematopoietic system is reconstituted for varying degrees and periods of time. There are supposedly at least three distinct classes of HSCs such as long-term (LT, >12 months), intermediate-term (IT, <12 months), and short-term (ST, <6 months) HSCs. It is suggested that longevity remarkably differs among HSCs. In this study we attempted to understand what determines the lifespan in HSCs. We addressed this issue by mathematical modeling. The ordinal differential equation was used to simulate reconstitution dynamics after single-cell transplantation. The cell cycle interval (I) was defined as G0 time. The division which produces two HSCs was called symmetric self-renewal. The division which produces two progenitors was called symmetric differentiation. The division which produces one HSC and one progenitor was called asymmetric self-renewal. The fate probability (φ) was defined as (the probability of symmetric self-renewal) minus (the probability of symmetric differentiation). These two parameters (I and φ) were changed to distinguish a sigmoid reconstitution curve given by LT-HSC from a ballistic reconstitution curve given by ST-HSC. We found that the longevity of HSCs is determined by φ at the maintenance phase after transplantation. Asymmetric division seemed to play a minor role in regulation of HSC number, but should be useful for stable maintenance of the HSC pool. The number of HSCs produced via self-renewal was regulated by both I and φ at the initial phase after transplantation, but appeared to be irrelevant to the longevity in HSCs. The fate probability is likely to be predetermined in each class of HSCs. It is extremely interesting in stem cell biology to clarify how the fate probability is regulated at the molecular level.

P1039 - CMYB EXPRESSION DURING HUMAN IN VITRO HEMATOPOIESIS

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Hematopoietic cells are generated during two spatio-temporally separated waves. In the primitive wave, nucleated red blood- and myeloid cells emerge in the Yolk Sac. During the definitive wave, hematopoietic progenitor- and hematopoietic stem cells are generated in the AGM region. One of the key factors in hematopoiesis and stem cell maintenance is cMYB with a fundamental role in the regulation of differentiation of multiple lineages. We herein sought to study this crucial factor during in vitro human embryonic stem cell (hESC) differentiation. To study cMYB expression, we generated a cMYBeGFP reporter line. Using recombineering, we targeted the first start-codon of a BAC-cMYB clone. The construct was then nucleofected in single-cell adapted hESC. After selection, the selection cassette was removed by Cre transfection. FISH and arrayCGH confirmed random integration of a single BAC on Chr11q, containing the full cMYB gene and approx. 75kb upstream-sequence, thus harnessing all known regulatory elements. We next induced hESC hematopoietic differentiation by generating spin-embryoid bodies. eGFP+ cells became apparent during differentiation and were confirmed to express cMYB. To follow the expression dynamics of cMYB, spin-EB's were differentiated up to 21 days and analyzed with 2-3day intervals. From d11 of differentiation, eGFP+ blood cells appeared. Interestingly eGFP+ cells were also present in the CD34+ progenitor population. We recultured d11 CD43+ cells under myeloid, erythroid and lymphoid conditions to map lineage specific expression. Lineage and differentiation-stage specific differences were clearly apparent, depicting multiple hallmark stages in hematopoietic differentiation. We conclude that cMYB is expressed in hematopoietic stem cell populations generated from hESC, suggesting definitive hematopoiesis is induced. This model will help to further elucidate the complex role of cMYB in HSC regulation and differentiation.

P1040 - GENE ARRAY ANALYSIS OF HUMAN HEMATOPOIETIC STEM CELLS REVEALS ASSOCIATION BETWEEN AGE, ALTERED SIGNALING AND LOSS OF LYMPHOCYTE POTENTIAL

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Aging of the hematopoietic system has widespread effects on all cellular components, including hematopoietic stem cells (HSCs) and lymphoid progenitors. Age-related defects in the HSC compartment can culminate in clinically relevant consequences, such as decreased function of adaptive immunity and increased incidence of myeloid diseases, including leukemia. In addition, difficulties associated with allogeneic hematopoietic cell transplantation for the treatment of malignant and non-malignant hematopoietic diseases may be compounded, since the risk of life-threatening complications is increased with prolonged periods of T-cell deficiency. The ability to promote T-cell generation from mobilized adult peripheral blood (PB) could provide improved immune reconstitution following transplantation, and as a result, improved patient outcomes. In the present study we examined the gene expression profiles of young and aged human HSCs from cord blood and adult mobilized PB, respectively, and found that Wnt signaling genes are differentially expressed in young and aged human HSCs, with less activation of Wnt signaling in aged HSCs. Utilizing OP9-DL1 co-culture to promote T-cell development under stable Notch signaling conditions, we found Wnt signaling is also important for T-lineage differentiation. Examination of Wnt signaling components and target gene activation in young and aged HSCs during T-differentiation revealed a correlation between reduced Wnt signal transduction, increasing age, and impaired or delayed T-cell differentiation. The defect in Wnt signal activation of aged HSCs appeared to occur in the early T-progenitor subset derived during in vitro differentiation. Our results reveal that reduced Wnt signaling activity may play a role in the age-related intrinsic defects of aged HSCs and early hematopoietic progenitors and suggest that manipulation of this pathway could contribute to improving T-cell generation and immune reconstitution.

P1041 - RADIATION AND SENESCENCE: CELL CYCLE OF PRIMITIVE HEMATOPOIETIC PROGENITOR CELLS (CFU-S13) ACCELERATED IN 2-GY WHOLE-BODY IRRADIATED SENESCENT MICE, WHICH ORIGINALLY DECELERATED DURING AGING IN THE STEADY STATE

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Aging is a functional retardation of continuous xenobiotic responses after developmental period; thus, the effects of ionizing radiation over a lifetime are formulated by a function of simultaneous equations representing the effects of "lifetime" and "dose of radiation". This study was conducted to evaluate possible additive/synergic effects of radiation during aging by determining various cell-cycling parameters, such as the percentage of cycling fraction at time zero (CF0), generation doubling time (GDt), and whole cycling fraction (CFw), by bromodeoxyuridine-ultraviolet (BUUV) assay, which enables determination of cycling capacity of hematopoietic stem/progenitor cells (HSCs/PGCs) in vivo (Hirabayashi et al. 2002). Accordingly, these parameters of PGCs [mature PGCs (CFU-GM); less mature PGCs (CFU-S9); and immature PGCs (CFU-S13)] from young mice (6 weeks of age) and old mice (21 months of age) with or without 2-Gy whole-body irradiation and allowed a 4-week recovery period were measured and compared. As a result, the cell cycling parameters in young mice without irradiation showed changes along with differentiation/maturation of PGCs from CFU-S13 to CFU-GM: from low to high percentage of CF0; from short to long GDt; and from small to large CFw. These parameters in old mice without irradiation were essentially identical to those of young mice except for the CFw of CFU-S13, which was significantly smaller (54%) than that of young mice. After irradiation, the percentage of CF0 and GDt of all PGCs were higher and decelerated, respectively, in both groups except CFU-S13 in old mice, which were oppositely lower and accelerated, respectively. In conclusion, changes in the effects of aging and radiation exposure on PGCs were significant only in the immature PGCs, in which the cell cycle was suppressed along with aging and contrarily accelerated reactively after irradiation.

P1042 - ROLE FOR NOTCH/RBPJ IN PRESERVING HEMATOPOIETIC STEM CELL ACTIVITY

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Notch and Wnt signals are required for Hematopoietic Stem Cells (HSCs) generation, however, their putative role for maintenance of HSC in physiological conditions remains unclear. For example specific deletion of RBPj or β-catenin in the hematopoietic system does not compromise the viability of the mice and HSC maintain some repopulation capacity. However, limiting dilution demonstrated that β-catenin-deficient HSCs have decreased self-renewal capacity. In contrast, no evidence for Notch signalling on HSC function has been described. We here analyzed the reconstitution capacity of vav-cre;RBPjlox bone marrow cells by competitive transplant assays into irradiated syngenic mice. We found a significant decrease in the capacity of RBP mutant cells to engraft in primary recipients when compared to wildtype cells, similar to the β-catenin-deficient phenotype. Since both pathways function in an intimate collaboration in other tissues, we generated double RBPj and β-catenin deletion mutants of the hematopoietic system (by using vav1-cre). Interestingly, double knockout animals were viable and born at Mendelian ratios. We will here describe and discuss the phenotype of these double mutants including a detailed analysis of HSC potential and differentiation of the different hematopoietic lineages. Our preliminary data reveals a new role for Notch in maintaining the functionality of adult HSCs, which may involve Wnt/β-catenin. This research is funded by by Ministerio Ciencia e Innovación (PLE2009-0111, SAF2010-15450, RD12/0036/0054), AGAUR (2009SGR-23 and CONES2010-0006).

P1043 - HYPOXIC INDUCTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR REGULATES ERYTHROPOIESIS BUT NOT HEMATOPOIETIC STEM CELL FUNCTION IN THE FETAL LIVER

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Hypoxia is an important factor in the hematopoietic stem cell (HSC) niche in the bone marrow (BM) but whether it also plays a role in the regulation of fetal liver (FL) HSCs is unclear. Definitive HSCs first arise in the AGM region and the placenta during development and later migrate to the fetal liver (FL). FL HSCs undergo extensive proliferation in the liver and migrate to the bone marrow (BM) shortly before birth, where they become quiescent. The molecular mechanisms behind this switch in proliferation remain elusive. While many components of the BM stem cell niche have been identified, relatively little is known about the FL HSC niche. Vascular endothelial growth factor A (VEGFA) is essential for adult HSC survival and hypoxic induction of Vegfa in adult HSC is required for proper function. Mice with defective hypoxia-induced Vegfa expression (Vegfaδ/δ) show a partial embryonic lethality and increases the numbers of phenotypically defined hematopoietic stem and progenitor cells in the FL, but whether stem cell function is affected in FL HSCs has not been examined. To investigate whether hypoxia-induced Vegfa is involved in regulation of fetal HSC function and to identify the reason for the embryonic lethality we have examined FL hematopoiesis in the Vegfaδ/δ model. We show that fetal erythropoiesis is severely impaired when hypoxic induction of Vegfa is lacking. FL HSCs deficient for hypoxia-induced Vegfa expression have normal HSC function arguing against a hypoxic FL HSC niche. However, after adaptation of FL HSCs to the BM microenvironment, FL HSCs lose their function as measured by serial transplantation.

P1044 - VISUALIZATION AND FUNCTIONAL DISSECTION OF GEMININ, A MOLECULE GOVERNING CELLULAR PROLIFERATION AND DIFFERENTIATION IN HEMATOPOIETIC STEM CELLS

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Polycomb-group (PcG) complex 1 (also designated as Polycomb repressive complex 1) and Hoxb4/Hoxa9 are well-known cell-intrinsic factors regulating the activity of hematopoietic stem cells (HSCs). Although these factors act as transcriptional regulators, we previously uncovered that each of these factors also acts as an E3 ubiquitin ligase for Geminin to regulate expression level of the protein. Geminin negatively regulates cellular proliferation and differentiation through the direct inhibition of a DNA replication licensing factor, Cdt1, and chromatin remodeling. Geminin protein accumulation gave rise to HSC defect in mice deficient in Rae28, a member of PcG complex 1, and that was compensated by retroviral transduction of Hoxb4. So Geminin is presumed to act as a central regulator sustaining the HSC activity. Geminin expression is higher in HSCs to maintain the undifferentiated states, while that is down-regulated in the progeny to provide proliferative potential and cellular differentiation. Knock-down of Geminin by retroviral transduction of the shRNA augmented the peripheral blood production, while that reduced the number of HSCs, confirming the functional significance of the higher expression of Geminin in HSCs. Recently we found that Geminin stability at the M phase is regulated by Aurora kinase A, which phosphorylates threonine in the destruction box to prevent the anaphase promoting complex/cyclosome-mediated ubiquitination (Nature commun. in press). Aurora kinase A-mediated stabilization of Geminin secures DNA replication in the next round of the cell cycle by stabilizing Cdt1. Thus Geminin not only inhibits but also stabilizes Cdt1. It is curious from a viewpoint of stem cell biology that Geminin provides cells with a proliferation potential in the next round of the cell cycle. We then generated Geminin-EYFP knock-in mice for the purpose of tracing expression of Geminin in vivo by the visualization. Currently we aim to dissect a molecular mechanism for switching self-renewal and differentiation of HSCs, focusing on Geminin. In this meeting we argue for a molecular role for Geminin in sustaining the activity of HSCs.

P1045 - IDENTIFICATION OF "TRUE" OLIGOPOTENT GMPS AND LINEAGE-RESTRICTED GRANULOCYTE PROGENITORS (GPS) AND MONOCYTE PROGENITORS (MPS) IN MOUSE BONE MARROW

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Common myeloid progenitors (CMPs) give rise to granulocytes, monocytes/macrophages and dendritic cells (DCs) via the production of lineage-restricted progenitors. The restricted progenitors for conventional and plasmacytoid DCs have recently been identified, but the restricted granulocyte progenitors (GPs) and monocyte progenitors (MPs) remain elusive. Granulocyte-monocyte progenitors (GMPs), which have the potential to produce both granulocytic and monocytic cells, are contained in the Lin^- $c\text{-}Kit^+$ $Sca\text{-}1^-$ (LKS^-) $CD34^+$ $Fc\gamma R^{hi}$ subset of mouse bone marrow, but not all LKS^- $CD34^+$ $Fc\gamma R^{hi}$ cells have the potential to produce both granulocytes and monocytes. Under permissive conditions in methycellulose, only about 40% of colony-forming LKS^- $CD34^+$ $Fc\gamma R^{hi}$ cells produce mixed GM colonies; the remainder form either G colonies or M colonies. Although several transcription factors have been implicated in the regulation of myelopoiesis, the inability of researchers to accurately isolate pure populations of oligopotent GMPs and lineage-restricted GPs and MPs has hampered efforts to precisely define the molecular mechanisms underlying myeloid fate choice e.g. in response to infection or during leukemogenesis. We isolated two major sub-populations of LKS^- $CD34^+$ $Fc\gamma R^{hi}$ cells and defined their lineage potential. Liquid cultures revealed that both fractions were capable of producing neutrophils and monocytes/macrophages, but with different kinetics, and that one sub-population was produced by the other. Methylcellulose assays confirmed that one sub-population represents "true" oligopotent GMPs, while the other comprises a mixture of GPs and MPs. We were also able to further sort the mixed progenitor population to enrich for either GPs or MPs, which have not previously been identified. This now permits us to more precisely study the molecular mechanisms that control granulocyte versus monocyte cell fate choice during homeostasis and upon infection, as well as during the development of myeloid leukemia.

P1046 - POLYMORPHIC SIRPA IS THE GENETIC DETERMINANT FOR NOD-BASED MOUSE LINES TO ACHIEVE EFFICIENT HUMAN CELL ENGRAFTMENT

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To evaluate stem cell potential of human cells in xenotransplant models, a variety of immunodeficient mouse lines have been developed. Depletion of lymphoid cells including T, B and NK cells by introducing with Il2rgnull, and SCID or RAGnull mutations is necessary to avoid rejection of human cells in these models. Interestingly, in mice having these immunodeficiencies, the NOD strain shows even better engraftment of human cells as compared to C57BL/6 or Balb/c strains. Recently, we found that in xenograft rejection, the innate phagocytic reaction of mouse macrophages could occur because murine signal regulatory protein alpha (mSIRPA) on macrophages cannot bind to human CD47 (hCD47). However, NOD-specific polymorphism of mSIRPA allows NOD-type SIRPA to bind hCD47, resulting in inhibition of phagocytic reaction against human cells in this strain at least in vitro. Here, we have established a new immunodeficient BRGS mouse line, C57BL/6.Rag2nullIl2rgnull mice with NOD-type SIRPA. To test the reconstitution activity of human hematopoiesis in vivo, we transplanted CD34+CD38- human cord blood cells intrafemorally into C57BL/6.Rag2nullIl2rgnull (C57BL/6-RG), BRGS or NOD.Rag1nullIl2rgnull (NOD-RG) mice. After transplantation, human CD45+ cells were not detectable in C57BL/6-RG mice. Both BRGS and NOD-RG showed successful reconstitution, and their frequency of human CD45+ cells in the bone marrow was 59.9 % and 55.8% in average, respectively. These data show that replacement of the C57BL/6-Sirpa with the NOD-Sirpa is sufficient for the C57BL/6-RG strain to gain the human cell engraftment ability equal to the NOD-RG strain. In addition, BRGS mice are free from other NOD-related abnormalities, and for example have normalized C5 function that enables to evaluate complement-dependent cytotoxicity of antibodies against human grafts in the humanized mouse model. Thus, this study formally proves that the excellent transplantability of human grafts in the NOD strain is explained simply by a single gene mutation, NOD-specific polymorphism of SIRPA, and that the BRGS strain should be very useful in future xenotransplant experiments of human stem cells.

P1047 - MOLECULAR MECHANISM OF HEMATOPOIETIC LINEAGE CHOICE INSTRUCTION

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Stem and progenitor cell fate decisions are regulated by the timed integration of extracellular signals and intracellular molecular states. Through continuous single cell observations we could recently provide evidence for the long disputed instructive effect of the hematopoietic cytokines M- and G-CSF on granulocyte-macrophage progenitor (GMP) lineage choice. M-CSF activates a multitude of signalling pathways which can differentially affect different cell fates. However, which of these pathways are involved in mediating lineage choice instruction is yet unknown. Combining M-CSF receptor loss of function studies with novel bioimaging technologies allowing long-term quantification of single GMP behaviour, we are investigating the molecular mechanism orchestrating M-CSF mediated lineage choice instruction.

P1048 - RETROVIRAL INSERTIONAL MUTAGENESIS AS A PLATFORM TO STUDY PROTO-ONCOGENES AND THEIR COLLABORATING PARTNERS

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Retroviral insertional mutagenesis (RIM) is a well known adverse effect in gene therapy trials which can lead to leukemia or preleukemic conditions. However, it can also serve as a powerful tool to study cancer and stem cell biology by studying genes adjacent to insertion sites. This study is to demonstrate the capacity of RIM to study proto-oncogenes in stem cell regulation and their potential collaborating partners in leukemogenesis. By recording retroviral insertion sites from hematopoietic dominant clones in murine bone marrow transplantation (BMT) model, our group has developed the Insertional Dominance Database (IDDb). Bcl2l1, an apoptosis regulator, is one of the top listed common insertion sites in the IDDb, with multiple roles in normal and malignant hematopoiesis. Here, we focus on the effect of Bcl2l1 overexpression in Lin- Sca1+ Ckit+ cells (LSK) using murine BMT model and perform insertional analysis to identify potential collaborating partners of Bcl2l1 in causing malignancy. 18 weeks after BMT, expression of eGFP was detected at high percentage in the blood, bone marrow (BM) and spleen of the Bcl2l1 group but limited in control group. Spleen enlargement was observed in the Bcl2l1 group without further sign of malignancy. In addition a higher LSK number was observed in the Bcl2l1 transplanted animals. Progeny output per transplanted LSK showed no difference between the reconstitution ability of eGFP and Bcl2l1 LSK in short term (6 weeks), but clear advantage of Bcl2l1 input cells in long term (12 weeks). With all the animals exhibiting normal phenotype up to 18 weeks, homeostatic regulations must have occurred between transgene expressing and non-expressing LSK in balancing their progeny output. Preliminary clonality studies supported by insertional analysis showed oligo- or polyclonal contribution to the blood, BM and spleen of recipients. In conclusion, overexpression of Bcl2l1 supports long term hematopoiesis without establishing a strong bias for clonal selection. Either a longer latency is needed for clonal outgrowth, or multiple collaborating partners contribute to a common pathway are present in these samples. Sequencing the insertion sites and identifying adjacent genes will reveal further details in this regard.

P1049 - SIRT1 DEACETYLASE IS ESSENTIAL FOR HEMATOPOIETIC STEM CELL ACTIVITY

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SIRT1 NAD-dependent sirtuin deacetylase has critical functions in cellular metabolism, cancer and aging. SIRT1 has recently been implicated in the pathogenesis of chronic myeloid leukemia (CML). As inhibition of SIRT1 appears to be an effective treatment for CML and several groups using germline deleted SIRT1 mice found SIRT1 to be dispensable for normal adult hematopoietic stem cell (HSC), SIRT1 inhibitors are proposed to be used in the treatment of CML. We have shown using an adult-tamoxifen inducible SIRT1 knockout mouse model circumventing the potential developmental adaptation of germ-line deleted SIRT1 mice (only 10% of mice reach adulthood), that loss of SIRT1 compromises severely the CD48-CD150+LSK long-term repopulating HSC frequency and function. Importantly, loss of SIRT1 in young adult mice leads to anemia, significant increase in peripheral blood neutrophils, monocytes and eosinophils and significant decrease overtime of lymphocytes. Interestingly, these abnormalities are associated with a specific and significant increase in granulocyte-monocyte progenitors (GMP), a known hallmark of aging in both mouse and human, and a significant decline overtime in common lymphoid progenitors (CLP) altogether suggesting that SIRT1-deficient HSC recapitulates prematurely key features of aging HSC and hematopoiesis. In agreement with an aging phenotype, we show an increase in reactive oxygen species (ROS) and an accumulation of DNA damage overtime in SIRT1-/- HSC. Importantly, we find that SIRT1 expression is decreased in aged HSC (16 months old) and identify Foxo3 transcription factor that is essential for the HSC maintenance, as a key target of SIRT1 in HSC. Our findings contrast with previously published data obtained from germline deleted SIRT1 mice, and suggest a critical function for SIRT1-Foxo3 in the control of HSC aging.

P1050 - A NOVEL HIGH-CONTENT SCREEN LEADS TO THE IDENTIFICATION OF PROMISING NEW COMPOUNDS FOR HEMATOPOIETIC STEM AND PROGENITOR CELL EXPANSION

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Despite significant advances in the hematopoietic research field in the last two decades, hematopoietic stem cell (HSC) transplantation still suffers by the limitation to expand these cells ex vivo. A high-content screen allows a systematic search for new compounds and may uncover new mechanisms that mediate expansion, survival or other effects on HSCs. The main challenge in the development of cell-based screens is the establishment of robust read-outs using suitable formats. In order to minimize the false-positive and even more important the true-negative rate, we combined cell-count, phenotype and functional analysis in the screening protocol. Therefore, we integrated a flow cytometer in a liquid handling system. With this system we were able to establish an automated multiplexed high-content analysis enabling enumeration and detection of surface proteins of interest (e.g. CD34, CD133, CD45RA, CD90, CD38). For the functional analysis the colony forming cell (CFC) standard assay was translated into a screening suitable 96-well format. In this regard, we also invented a new analysis algorithm for the enumeration of colonies. We found a clear correlation of CD34 and CFC counts proving the robustness of both assays. To address our purpose to find new conditions for HSC expansion we prepared a cytokine library counting 103 cytokines. Additionally to our own cytokine library we got the commercially available chemical libraries from Merck (Stem Select and Inhibitor Select) and Micro Source (Spectrum Collection). Finally, we screened in total 3195 compounds based on CD34 peripheral blood derived cells and identified several promising compounds for HSC expansion which we are currently validating. This unique combination of enumeration, phenotyping and functional assay performance already in the screen ensures the identification of highly qualified compounds. Additionally, the risk to lose active compounds is mitigated by obtaining true-negative results. Furthermore, the proposed high-content screening is not only suitable for the analysis of HSC expansion, but will also help to address many further questions.

P1051 - HIF-1α STABILIZATION IN PHD1/3-/- MICE RESULTS IN INCREASE IN HEMATOPOIETIC STEM CELL NUMBER AND ENHANCED HSC MAINTENANCE IN BM NICHE

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It is believed that hypoxic nature of BM niche plays an important role in HSC function. Prolyl hydroxylase domain enzymes (PHDs) sense oxygen levels and regulate the levels of hypoxia-inducing factors (HIFs). Recently, Forristal et al., found that stabilization of HIF-1α by inhibiting PHD function resulted in an increased number of quiescent HSCs in the BM, which supported improved hematopoietic recovery following sub-lethal irradiation. We here used Phd1/3-/- mice to evaluate the effect of stabilization of HIF-1α on murine hematopoiesis. We confirmed total absence of expression of Phd1/3 in BM cells, while we did not find any significant change in the expression of Phd2. The frequency of primitive HSCs (CD150+CD48-KLS cells) in Phd1/3-/- bone marrow (BM) was increased. This was also reflected in LTC-IC assays as well as their competitive repopulation ability. Phd1/3-/- mice showed faster hematopoietic recovery from sub-lethal radiation. Significantly better survival of lethally radiated Phd1/3-/- mice compared with WT mice was also observed when limited number of BM cells was transplanted. As expected, increased HIF-1α stabilization was observed in Phd1/3 deficient primitive HSCs. Also, primitive HSCs expressed higher levels of various HIF-1α targets such as Itga4, Itga5 and Cxcr4. In addition, osteoblasts in Phd1/3-/- mice are activated and expressed higher transcript levels of Cxcl12, one of the major chemo-attractants for HSCs, resulting in higher levels of CXCL12 in Phd1/3-/- BM plasma. Consistent with increased expression of Itga4, Itga5 and Cxcr4 in Phd1/3-/- HSCs, increased migration towards CXCL12 and attachment to fibronectin was found. As we did not observe any change in Phd2 expression in Phd1/3-/- mice, it was concluded that HIF-1α stabilization and the subsequent effects were solely due to PHD1/3 deficiency. Thus, PHD1/3 mediated stabilization of HIF-1α, and this in the absence of Phd2 silencing, results in enhanced HSC function, in part due to HSC-intrinsic as well as HSC-niche based mechanisms. Additional studies to assess HSC homing as well as proliferation are ongoing.

P1052 - HEMATOPOIESIS AND INFLAMMATION: THE ROLE OF TIMP-1 IN HUMAN HSPCS

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Hematopoietic Stem and Progenitors Cells (HSPCs) reside within the bone marrow, a nurturing environment shielding HSPCs from external insults. However, recent findings showed that danger signals and inflammatory cytokines actively affect HSPCs. The Tissue Inhibitor of MetalloProteinases-1 (TIMP-1) is a member of the inflammatory network: first described as a mere inhibitor of MMPs, TIMP-1 also displays cytokine-like functions. We recently found that TIMP-1-/- mice have decreased BM cellularity and impaired engraftment capabilities due to cell-cycle defects. Here, as a contribution to dissecting the molecular pathways underlying the cross-talk between hematopoiesis and inflammation, we investigated the role of TIMP-1 in human HSPCs. In vitro, rhTIMP-1 significantly increased the clonogenic potential of cord blood-derived CD34+ HSPCs. In addition, rhTIMP-1 modulated the expansion of more immature LTC-ICs in long-term cultures: interestingly, while single stimulations induced cell proliferation but had no effect on LTC-IC numbers, repeated treatments contributed to expand LTC-ICs even at the lowest tested concentrations. In vivo, ongoing studies are expected to elucidate how TIMP-1 affects the engraftment of human CD34+ cells in NOD/Shi-scid/IL-2Rγnull mice. Mechanistically, TIMP-1's cytokine-like effect in HSPCs proliferation emerged to be independent on MMP-inhibition, as demonstrated by synthetic MMP-inhibitors, which failed to recapitulate TIMP-1 effects in CFU-C assays. TIMP-1's role in HSPC proliferation was confirmed by the increase in CCND1 levels after treatment and apoptosis assays indicate that TIMP-1 may also contribute to HSPC survival in vitro. Although the membrane receptor igniting TIMP-1 signaling in HSPCs is yet to be identified, preliminary data indicate that TIMP-1's activity requires the activation of PI3K/pAkt cascade. In summary, our study indicate that TIMP-1 modulates human HSPCs proliferation and may represent a new key player in the hematopoiesis-inflammation cross-talk.

P1053 - THPO/MPL SIGNALING SIGNATURE IN HEMATOPOIETIC STEM CELLS

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Thrombopoietin signals via its receptor Mpl and mediates megakaryopoiesis and HSC maintenance. Furthermore, Mpl signaling is essential for the post-transplant expansion of hematopoietic stem cells (HSC). MPL deficiency in human patients causes thrombocytopenia and aplastic anemia. Mpl deficiency (in Mpl-/- mice) can be corrected by lentiviral overexpression of Mpl. Expression of a signaling defective Mpl receptor in wildtype mice exerts dominant-negative (dn) effects, resulting in thrombocytopenia and HSC defects. dnMpl mice had 4-fold reduced lineage negative, Sca-1 and c-Kit positive (LSK) cell numbers and bone marrow (BM) cells did not engraft in secondary recipients. Moreover, LSK cells of dnMpl mice were more cycling. A second wildtype BM transplant could engraft in dnMpl mice without further conditioning and led to longterm reconstitution of the BM ($\sim$15-79% chimerism, 0.2-1.1% in controls). To better understand the molecular changes in defective HSC that had impaired Mpl signaling, we performed transcriptome analysis of dnMpl expressing wildtype LSK cells and compared expression to LSK cells of wildtype mice and Mpl-/- mice which were transplanted with Mpl gene corrected BM cells. We found the expression profile of dnMpl to negatively correlate with known HSC stemness signatures, while these pathways were positively enriched in Mpl corrected Mpl-/- LSK cells. Wnt signaling, Jak/Stat and PI3K/Akt signaling were negatively enriched in dnMpl LSK cells while expression of genes involved in cell cycle progression were positively correlated. We confirmed differential expression of the receptors for angiopoietin 1 (Tie2) and activated protein C (EPCR), and the cell adhesion molecule Esam1, all known to mark the stem cell population. Expression of all three marker in dnMpl mice was significantly reduced (**$P<0.005$) compared to control mice and similar to expression in Mpl-/- and Thpo-/- mice.

P1054 - TLR9 LIGATION PROMOTES NK CELL DIFFERENTIATION FROM ADULT, BUT NOT NEONATAL, HUMAN LYMPHOID PROGENITORS

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During biological contingencies, strengthening production of specific hematopoietic lineages may be crucial for the clearance of noxious agents and the initiation of both innate and adaptive immune responses. We have previously shown that pathogen products influence early cell fate decisions of progenitor cells in mouse, contributing to the development of innate cells through a Toll-like receptor (TLR)-dependent mechanism. A number of recent investigations indicate that TLR are also expressed by human hematopoietic stem/progenitor cells, and that blood cell production is biased towards myeloid lineages as a result of their stimulation. Using controlled stromal cell co-culture systems, our new findings demonstrate that TLR9 ligation in adult bone marrow (ABM) Lin-CD34+CD38-CD45RA+ multi-lymphoid progenitors (MLP) and in more differentiated Lin-CD34+CD38+CD45RA+ B/NK cell progenitors, accelerates the production of functional natural killer (NK) cells. This phenomenon involved up-regulation of IL15-Rβ (CD122) on NK cell precursors and their positive selection after 10 days of culture. In contrast, no changes were recorded in NK cell differentiation potentials or CD122 expression from umbilical cord blood (UCB) lymphoid progenitors upon TLR9 stimulation when compared to mock conditions. Interestingly, the same was true when inactivated Herpes virus was used as a source of TLR9 ligands, suggesting that in response to TLR agonists, MLP increase the NK cell production, and that neonatal primitive cell responses differ from that of adult cells.

P1055 - DECLINED PRESENTATION
MULTIPLE ROLES OF NOTCH SIGNALING IN HEMATOPOIETIC STEM CELL SPECIFICATION

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Notch signaling is required cell-autonomously in cells fated to become HSCs, as shown by failure of Notch1 deleted cells to contribute to the adult hematopoietic system of chimeric mice. Determining the precise way in which Notch signaling functions to generate HSCs is greatly complicated by the fact that Notch is also required for vascular patterning, which occur in close temporal and spatial proximity to the initiation of the hematopoietic program through expression of Runx1. For example, Notch signaling is required for arterio-venous specification. Because HSCs emerge from the dorsal aorta and not the vein, it is conceptually possible that the requirement for Notch in HSC specification in fact reflects its requirement in arterio-venous specification, and that without normal arterial programming, HSCs cannot be specified. Our laboratory demonstrated that before dorsal aorta specification there is an additional environmental requirement for Notch signaling downstream of Wnt16. Interestingly, Wnt16 morphants failed to specify the sclerotome subcompartment of the somite, suggesting that the sclerotome may be involved in HSC specification. I hypothesized that multiple requirements for Notch signaling in HSC specification are regulated by specific Notch receptors. My findings show that notch1a, notch1b, and notch3 but not notch2 are required for HSC specification. Interestingly, defects in dorsal aorta specification were the most severe in notch1a morphants, while defects in sclerotome were unique to notch3 morphants. These data suggest that each Notch receptor is utilized non-interchangeably for specific requirements in HSC specification.

P1056 - DECLINED PRESENTATION
PODOCALYXIN REGULATES CXCR4-DEPENDENT HEMATOPOIETIC STEM CELL TRAFFICKING AND ENGRAFTMENT

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Hematopoietic stem and progenitor cells (HSPCs) are maintained in specialized "niches" (primarily in the bone marrow (BM) in adult mammals) that allow for both survival and quiescence of hematopoietic stem cells (HSCs) and appropriate expansion and differentiation of progenitors. The CXCR4-CXCL12 axis is a key regulator of HSPC niche-homing and long-term maintenance: HSPCs deficient in CXCR4 fail to migrate efficiently to the niche and loss of CXCR4 leads to an expansion of proliferating HSPCs in the bone marrow (BM). Recently, we have found that deletion of the CD34-family sialomucin, podocalyxin ($Podxl^{-/-}$) leads to impaired chemotactic migration of HSPCs in response to CXCL12, indicating a role for podocalyxin in the regulation of CXCR4 signalling. Furthermore, in competitive HSC transplant experiments, we find that $Podxl^{-/-}$ HSPCs exhibit a dramatically enhanced ability to reconstitute hematopoiesis in sub-lethally irradiated recipients. These data, together with strikingly similar defects (including perinatal lethality) observed in *Podxl*- and *CXCR4*–deficient transgenic mice suggest these molecules are on the same genetic pathways that govern HSPC migration, survival, expansion and quiescence.

P1057 - IDENTIFICATION OF QUANTITATIVE GENETIC VARIATIONS REGULATING HEMATOPOIETIC REGENERATION

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Slow hematopoietic recovery following irradiation can result in adverse complications for many patients, but the genetic basis of recovery is ill defined. Using a zebrafish model of irradiation-induced hematopoietic regeneration, we defined differences in recovery between two commonly used wild type strains (AB and Wik) and mapped quantitative trait loci (QTL) that govern these differences. Regeneration was assayed by measuring the frequency of multilineage precursors, which are the earliest hematopoietic population detectable following irradiation injury in the zebrafish. Wik fish had a higher precursor frequency (mean-35) at 7 days post irradiation (dpi) than AB fish (mean-30) indicating Wik regenerate faster. To establish the genetic determinant underlying the difference in hematopoietic regeneration, AB and Wik fish were crossed to generate F2 ABXWik hybrid animals for mapping. The F2 animals displayed a range of precursor frequencies from 4.5 to 52.9 at 7dpi. Interval mapping was performed on the fish with the 10% highest and lowest precursor frequency. Initial mapping performed with over 200 single nucleotide polymorphisms distributed across the genome indicated significant linkage on Chromosome 3 (LOD-3.258), which was then confirmed by analyses in F3 progeny. Whole genome sequencing was employed to further refine the genetic interval to a 20 Mbp region. The genes within this QTL (136) were then compared to 132 genes found in a mouse QTL (Scp2) on Chromosome 11 associated with differences between HSC proliferation in C57/Bl6 and DBA.2 mice. Four out of the 12 common genes tested displayed differential gene expression between AB and Wik fish, suggesting that variable expression in multiple genes likely contribute to fluctuations in regeneration. This study is one of the first to use zebrafish genetics to define factors that control natural fluctuations in regenerative potential and can provide targets for therapies to boost hematopoietic output during times of stress.

P1058 - SHELTERIN COMPONENT PROTECTION OF TELOMERES 1 (POT1) PLAYS A CRITICAL ROLE IN THE SELF-RENEWAL OF HSCS

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Appropriate control of hematopoietic stem cell (HSC) self-renewal is critical for the maintenance of life-long hematopoiesis. Aging or long-term repeated cell division induces the accumulation of DNA damage, restricts differentiation capacity, and decreases the bone marrow (BM) repopulation ability of HSCs. Pot1 is a component of the shelterin complex, which plays a critical role in the protection of telomere from DNA damage signaling. We recently demonstrated that the expression of Pot1a in HSCs significantly decreased with age and in vitro culture. Moreover, we found that knockdown (KD) of Pot1a in HSCs significantly increased the level of DNA damage response (DDR) after in vitro culture. In addition, KD of Pot1a significantly reduced long-term repopulation (LTR) activity of HSCs. We hypothesized that Pot1a plays a critical role in the maintenance of HSC integrity during in vitro culture or stem cell aging. Here, we examined the function of exogenous Pot1a in the control of DDR signaling at telomere in HSCs. We prepared a recombinant mouse Pot1a (MTM-mPot1a) protein that had a membrane-translocating motif (MTM). Treatment of LT-HSCs with MTM-mPot1a decreased the telomere dysfunction-induced foci, which is a marker of DDR at telomeres, in LT-HSCs during in vitro culture and in the aged LT-HSC fraction. MTM-mPot1a maintained the colony formation activity of LT-HSCs after long-term culture. We next evaluated BM repopulation capacity of HSCs after in vitro culture with or without MTM-mPot1a. Notably, MTM-mPot1a increased the number of long-term HSCs and induced the higher repopulation capacity of LT-HSCs. Furthermore, the limiting-dilution competitive BM transplantation assay showed that MTM-mPot1a increased the frequency of functional HSCs compared to the control, indicating that MTM-mPot1a may induce the self-renewal of HSCs. Altogether, these data suggest a new insight for the strategy of ex vivo expansion of HSCs by inhibiting the accumulation of DDR in the telomeric region.

P1059 - CHROMATIN REMODELING FACTOR SMARCA2 CONTRIBUTES TO MAINTAIN HEMATOPOIETIC STEM CELL QUIESCENCE

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Hematopoietic stem cells (HSCs) are maintained in quiescent status by fine-tuned transcriptional regulation of stem cell factors. During transcriptional activation, it is needed to open chromatin by ATP-dependent chromatin remodeling complexes, which involve Polycomb and Trithorax group proteins. Although these proteins are also known as stem cell factors, the roles of chromatin remodeling in HSC regulation are still unclear. So we focus on SmarcA4 and SmarcA2, which is the center player of chromatin remodeling SWI-SNF complex. We found that SmarcA2 is specifically enriched in hematopoietic stem and progenitor cells, especially in immature long-term HSCs, whereas SmarcA4 is highly expressed not only in HSCs but in some of the progenitors and differentiated cells. These results suggest that SmarcA2 has important role in HSC regulation. However, the ratios and quantities of each fraction of bone marrow hematopoietic cells in SmarcA2 deficient mice are comparable to that in wild-type mice. So, to reveal the roles of SmarcA2 in the maintenance of HSCs, we performed competitive bone marrow reconstitution assays. The percentages of donor cells in peripheral blood show no significant difference between that of SmarcA2-null HSCs and wild-type HSCs, except for higher reconstitution by SmarcA2-null HSCs at early point of bone marrow transplantation (BMT). Surprisingly, bone marrow analysis four months after transplantation reveals that SmarcA2-null HSCs can reconstitute dramatically fewer HSCs and hematopoietic progenitors than wild type HSCs. At the same time, reconstituted SmarcA2-null HSCs exhibit more activated cell cycle status than wild type HSCs do. Moreover, when subjected to 5-FU administration in vivo, SmarcA2-null HSCs show earlier hematopoietic recovery than wild-type HSCs. Taken together, our results suggest that when cell cycle of HSCs are activated, SmarcA2 keep HSCs quiescent, which in turn it contributes to maintain HSCs.

P1060 - DECIPHERING THE ROLE OF MICRORNAS IN EARLY STAGES OF HAEMATOPOIESIS

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Early steps of development are crucial to the emergence and expansion of haematopoieitic stem cells (HSC) as these cells are responsible for the formation of all blood cells throughout life. This research project focuses on the identification of miRNAs that can act as modulators of HSC emergence and expansion during embryonic life. MiRNAs are small non-coding RNAs that modulate the expression of many transcripts simultaneously and their biological role can be cell-type specific. First, we generated a miRNA expression profile of tissues that are involved in HSC emergence and expansion (E11 AGM/Aorta-Gonad-Mesonephros, E11 Yolk Sac, E12 Placenta, E14 Fetal Liver), using Illumina deep sequencing technology. In parallel, we also captured the gene expression profile of these same developmental stages by mRNA microarray to identify a miRNA target signature. Then, for all stages, we used TaqMan® Low Density Arrays to generate the miRNA expression profile of their HSC-enriched populations (ckit+ CD34+) and all remaining haematopoietic cells (CD45+ CD34-). Both methods led to the identification of a subset of miRNAs that display differential expression between each developmental stage and/or cell population. This was correlated with the transcriptome of each haematopoietic tissue. Then, we selected a subset of miRNAs that are highly expressed at one or more developmental stage and separated them into three groups: higher expression in HSC compared to more mature haematopoietic cells, high expression in both cell populations and no expression in both cell populations. MiRNA in situ hybridization was used to further define the expression pattern of selected miRNAs. Functional validation is underway to assess the role of these miRNAs in HSC emergence and expansion. Using transduction of primary murine cells at all development stages, we are performing colony-forming cell assays and transplantation studies to measure the effect on the differentiation and self-renewal potential of HSCs exerted by these miRNAs. This study represents a starting point to understanding the role of miRNAs during early stages of HSC development and is expected to lead to the identification of novel modulators of haematopoiesis.

P1062 - THE VALUE OF SORTED MURINE HEMATOPOIETIC STEM CELLS IN SERIAL TRANSPLANTATION EXPERIMENTS - A TRANSGENIC MOUSE MODEL EXAMPLE

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Balanced regulation of hematopoietic stem cell (HSC) numbers and performance is a requisite for life-long blood cell replenishment. Our knowledge of the factors that regulate HSC activity, largely derived from experimental mouse models, remains incomplete. In such experimental models optimal control of experimental variables is crucial. Serial transplantation experiments, considered a "golden standard" to evaluate HSC performance, are however not performed uniformly and could potentially lead to misinterpretation of experimental data with regard to HSC activity. In this study we therefore compared HSC reconstitution behavior using either unfractionated bone marrow (uBM) cells or sorted HSCs. Following both primary and secondary transplantations in wild type C57BL/6 mice, we found different donor HSC frequencies within separate bones (tibias, femurs, and hips) of individual recipients. Also, we observed a lymphoid-biased output in secondary recipients competitively transplanted with uBM cells compared to sorted HSCs, possibly caused by the co-transplantation of long-lived progenitor cells. Based on these observations we argue that competitive serial transplantation of sorted HSCs is preferred over serial transplantation of uBM when evaluating long-term HSC function. In this line of experimentation, we studied HSC activity in mice deficient for the caveolae-component Cavin-1, a protein highly expressed in HSCs. Following a competitive secondary transplantation with sorted HSCs we observed balanced lymphoid versus myeloid reconstitution derived from Cavin-1 deficient cells at similar levels when compared to wild type HSCs. Hence, we were unable to establish a role for Cavin-1 in the regulation of HSC activity as concluded from secondary transplantation experiments using sorted HSCs.

P1063 - HETEROGENEITY OF YOUNG AND AGED MURINE HEMATOPOIETIC STEM CELLS REVEALED BY QUANTITATIVE CLONAL ANALYSIS USING CELLULAR BARCODING

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The number of hematopoietic stem cells (HSCs) that contributes to blood formation and the dynamics of their clonal contribution is a matter of on-going discussion. Here we used cellular barcoding combined with multiplex high-throughput sequencing to provide a quantitative and sensitive analysis of clonal behavior of hundreds of young and old HSCs. Purified lineage-negative Sca1+ c-Kit+ CD48- CD150+ (LSK48-150+) cells from young and aged donors were transduced with barcoded vector library and transplanted into irradiated recipients. Clonal composition of different blood populations was analyzed up to 15 months post-transplant and upon secondary transplantation. The majority of transplanted clones steadily contributed to hematopoiesis in the long-term, although clonal output in granulocytes, T cells and B cells was substantially different. Contributions of individual clones to blood were dynamically changing; most of the clones either expanded or declined with time. Once the animals were sacrificed, the clonal make-up of bone marrow cells was analyzed at different stages of hematopoietic differentiation (LSK48-150+ cells, myeloid progenitors and mature cells). We demonstrate that the pool of old HSCs is composed of multiple small clones while the young HSC pool is dominated by fewer, but larger, clones. Finally, we observed that both young and old HSCs were asymmetrically distributed throughout the mouse skeleton, indicating similar migration behavior after transplantation.

P1064 - THE FUNCTIONAL CHARACTERIZATION OF MIR-125 FAMILY IN HEMATOPOIESIS

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MicroRNAs (miRs) are small, non-coding RNAs playing an essential role in the control of hematopoiesis. MiRs tune the expression of certain genes by extensive pairing of the seed sequence with the complementary sequence in 3‘ UTR of the target mRNAs. Previously, we have shown miR-125a as an important regulator of hematopoietic cells (HSCs) (Gerrits et al., 2012). MiR-125a is a close homolog of miR-125b1 and miR-125b2 and share over 85% sequence similarity. However, there have been reports suggesting that miR-125 family members may have distinct effects on hematopoiesis. To functionally compare the phenotypes of all miR-125 in HSPCs, we overexpressed them individually in bone marrow cells and functionally assessed in a range of in vitro and in vivo assays. The colony forming unit-granulocyte monocyte assay showed that cells overexpressing miRs-125 family members generate more colonies and have increased self-renewal potential as measured by replating activity. We also observed increased and prolonged activity of miR-125-overpressing cells in cobblestone area forming cell assay. In vivo transplantation experiments in which miR-125 overexpressing cells were transplanted in conditioned recipients showed decreased levels of apoptosis in the lineage negative cell population and a vast proliferative advantage of these cells. We observed increased numbers of progenitors and a differentiation biased towards the myeloid lineage. However, the frequency of phenotypically defined HSCs (Lin-Sca+c-Kit+CD48-150+) was significantly diminished already 10 weeks post transplantation. To functionally analyze this phenomenon, we have serially transplanted bone marrow cells and assessed blood chimerism 20 weeks after transplantation. This revealed loss of the initial proliferative advantage, suggesting exhaustion of HSCs overexpressing miR-125 family members. Combined, our data show that all miR-125 family members do indeed affect HSPCs characteristics in a similar way, enhancing differentiation into myeloid lineage, causing exhaustion of the stem cell pool and expansion of progenitors, while at the same time decreasing the frequency of apoptotic cells in lineage negative compartment.

P1065 - UMG-LENTI: NOVEL DUAL-PROMOTER LENTIVIRAL VECTORS FOR EFFICIENT TRANSGENE- AND REPORTER PROTEIN EXPRESSION IN PRIMITIVE HAEMATOPOIETIC PROGENITORS

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Lentiviral vectors are widely used to investigate the biological effects of regulatory proteins and candidate oncogenes by stably enforcing their expression in diverse target cells. In these studies it is essential to be able to monitor and/or isolate the cell subpopulation(s) infected by the virus, and hence expressing the relevant transgene. This can be achieved through the insertion in the vectors of sequences encoding selectable markers or reporter proteins whose expression can be revealed by flow cytometry. To ensure simultaneous expression of transgene and selectable/ reporter gene, intra-ribosomal entry site (IRES) sequences are often inserted between the two cDNAs to generate bi-cistronic mRNAs whose transcription is directed by a single promoter. However, depending on the different transgenes and on the target cell types, the expression of the cDNA located downstream of the IRES sequences (typically encoding the reporter protein) is frequently inconsistent and this makes the recognition of the infected, transgene-expressing cells, problematic. To circumvent this problem, we have designed novel lentiviral vectors (named UMG-LV5 and -LV6) where the expression of the transgene is driven by the UbiC promoter and that of the reporter protein, EGFP, by the 170-bp-long minimal promoter of the WASP gene. These vectors, harbouring two distinct transgenes (ZNF521 or MSI2), were tested in a variety of human haematopoietic cell lines as well as in primary human CFD34+ cells in comparison with the FUIGW vector that contains the expression cassette UbiC-transgene-IRES-EGFP. In all cells studied, infection with UMG-LV5 and -LV6 resulted in a transgene expression slightly lower than that of FUIGW, but a considerably stronger expression of EGFP, that allowed to easily distinguish the transduced cells from the non-transduced counterpart. These vectors are therefore valuable new tools for gene transfer-based studies in haematopoietic stem and progenitor cells. Supported by: AIRC, PhD Programme in Molecular Oncology and POR Calabria FSE 2007/2013 (HEMMAS project).

P1066 - ROLE OF MATRILIN-4 IN STRESS-INDUCED HSC ACTIVATION AND HOMEOSTASIS

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The life-long maintenance of the blood system is accomplished by a pool of self-renewing and pluripotent hematopoietic stem cells (HSCs). Adult HSCs are found in a dormant state for most of their lifetime, entering cell cycle only to maintain homeostatic blood supply. This balance between dormancy and activation is controlled by external factors such as chemokines and cytokines as well as the interaction of HSCs with cells of the stem cell niche. Under stress conditions such as chemotherapy or bleeding, HSCs are stimulated to proliferate and give rise to various specialized blood and immune cells. The molecular mechanisms underlying the processes of activation of HSCs are still largely unknown. Our group has previously shown that HSCs proliferation can be induced by in vivo IFNα treatment of mice. This response is dependent on signaling via the IFNα receptor (IFNAR), STAT1 and the cell-surface marker Sca-1 leading to downstream induction of IFNα target gene expression (Essers et al., 2009). To gain insight into the mechanism of activation, we compared the transcriptional response of HSCs of C57Bl/6 wildtype treated with PBS or IFNα by microarray expression analysis. Interestingly, we discovered several cell cycle inhibitors, such as p57 to be downregulated upon IFNα treatment, enabling cells to exit G0 and start cycling. Even more striking is the downregulation of the extracellular matrix protein Matrilin-4. Matrilin-4 is a member of the von Willebrand factor A-containing family of extracellular adapter proteins, which form filamentous structures outside of cells. We found that Matrilin-4 is highly expressed in long-term HSCs compared to short-term HSCs or committed progenitors during homeostasis and it is almost completely depleted upon in vivo treatment with IFNα or other inflammatory cytokines. It is therefore possible that Matrilin-4 plays an essential role in niche remodeling following stress. Here we present our data on the investigation of the function of Matrilin-4 in HSC activation and homeostasis using KO mice and in vivo retroviral overexpression.

P1067 - DECLINED PRESENTATION
ROLE OF P70S6 KINASE IN REGULATION OF NORMAL AND STRESS HEMATOPOIESIS

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Hematopoietic stem cells' (HSCs) ability to reconstitute the hematopoietic system following myeloablative stress depends on its self-renewal property. The role of different components of the phosphatidylinositol 3-kinase (PI3K)/Akt/ mammalian target of rapamycin complex1 (mTORC1) pathway in HSC self-renewal and function have been studied extensively. Previous studies have shown that both hyperactivation as well as deficiency of PI3K/Akt/mTORC1 pathway results in defective hematopoiesis. However, the role of downstream effectors of this pathway are unknown. Activation of mTORC1 results in phosphorylation and subsequent activation of p70S6 kinase (S6K1). We used a genetic model of S6K1 knockout mice (S6K1-/-) to determine the role of S6K1 in HSC development and function under homeostasis as well as myelosuppressive stress. Although S6K1-/- mice have reduced bone marrow cellularity under steady-state conditions, there is no significant difference in peripheral blood counts relative to controls. The absolute number of bone marrow HSCs was found to be decreased and frequency of granulocyte-macrophage progenitor (GMP) population was found to be higher in S6K1-/- mice relative to controls. Moreover, hematopoietic cells from S6K1 -/- mice showed increased colony forming ability in response to cytokines which was associated with hyperactivation of Akt and ERK MAP kinase. Importantly, S6K1 deficient HSCs show reduced engraftment when transplanted into lethally irradiated primary and secondary recipients. Interestingly, overexpression of S6K1 in WT HSCs also results in reduced engraftment in transplant recipients. In response to genotoxic stress, S6K1 deficiency results in increased frequency of HSCs in bone marrow despite significant reduction in bone marrow cellularity. Additionally, S6K1-/- mice are more sensitive to repeated genotoxic stress. Our studies indicate that S6K1 plays a critical role in the development of normal HSCs and in engraftment of HSCs following transplant as well as in the regeneration of HSCs following myeloablative stress.

P1068 - LOSS OF SPARC PROTECTS HEMATOPOIETIC STEM CELLS FROM THE TOXIC EFFECTS OF REPEATED CYCLES OF CHEMOTHERAPY BY ACCELERATING THEIR RETURN TO QUIESCENCE

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We have identified the matricellular protein SPARC in a microarray screen to be highly expressed in hematopoietic stem cells (HSCs) that have newly colonized the bone marrow of newborn mice. Follow-up experiments showed that SPARC does not seem to have a cell-autonomous function in HSCs, however, we uncovered that loss of SPARC expression by niche cells protects HSCs from the toxic effects of repeated cycles of chemotherapy. Intriguingly, we demonstrate that SPARC-deficient mice have a reduced sensitivity to serial treatment with the chemotherapeutic agent 5-fluorouracil (5-FU) in 10-day intervals. Using straight and reverse chimeras, we could further show that this is due to a role of SPARC in the BM niche. While the speed of recovery of the blood system is normal, HSCs in a SPARC-deficient niche show an accelerated return to quiescence, which seems to protect them from the lethal effects of serial 5-FU treatment. Our findings are of clinical relevance, as future work along theses lines could reduce side effects of chemotherapy and even allow dose escalation above current limits. Moreover, SPARC is down-regulated or deleted in certain disorders of the hematopoietic system, implying a relevance of our work for an improved understanding and treatment of these malignancies.

P1069 - SINGLE CELL ANALYSIS OF CYTOKINE-DEPENDENT TRANSCRIPTION FACTOR DYNAMICS IN HEMATOPOIETIC PROGENITORS

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Transcription factor networks act as master regulators of hematopoietic lineage commitment. However, their regulation remains poorly understood. In particular, and despite the importance of cytokines in influencing hematopoietic cell fates, evidence for direct regulation of core hematopoietic transcription factors by these cell extrinsic regulators is still missing. We therefore screened for cytokines directly regulating PU.1 expression. Using time-lapse imaging we quantified the expression dynamics of PU.1 protein continuously and live in large numbers of individual hematopoietic stem and progenitor cells with highest temporal resolution. All cell types investigated showed a cell-cycle dependent increase of PU.1 levels, illustrating the absolute requirement for continuous single cell quantification. We were able to identify several cytokines increasing PU.1 protein expression. PU.1 induction was detectable within less than 3h after cytokine challenge in all cell types tested, and peaked prior to the first cell division. Notably this effect was observed already in CD150+CD34-CD48- KSL hematopoietic stem cells. Through small molecule inhibitors of signaling pathways, we could further identify specific requirements of different signaling pathways for the PU.1-inducing effect of different cytokines in different cell types. These observations have important implications for the understanding of the regulation of the transcription factor network controlling hematopoietic lineage choice.

P1070 - REGULATION OF HEMATOPOIETIC STEM/PROGENITOR CELL DIFFERENTIATION AND ENGRAFTMENT BY ANGIOTENSIN II

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Angiotensin II (Ang II) is a key product in the renin angiotensin system that has been recognized for cardio-vascular effects. Studies have shown that Ang II also has multiple effects in tissue regeneration and acts as a mitogen for smooth muscle cells, fibroblasts, endothelial cells and hematopoietic cells. Despite limited in vitro evidence that Ang II induces proliferation of hematopoietic stem/progenitor cells (HSPCs), the mechanism of its action at the level of bone marrow remains unclear. In the present study, we aimed to understand how Ang II regulates HSPC population in vivo. We observed a 31% decrease of Sca-1+, c-Kit+, Lin- (SKL) HSPCs and 46% increase of myeloid/monocytic cells in the bone marrow of mice chronic infused with of Ang II using osmotic minipumps (1500ng/kg/min). In addition, we observed increased number of CX3CR1+/Ly6C+ inflammatory monocyte in the peripheral blood and spleen. Interestingly, the effect of Ang II on HSPC was completely reversed by treatment of Ang II receptor antagonist, losartan. Next, we aimed to investigate how the Ang II level affects HSPC homing and engraftment, which are critical steps in bone marrow transplantation. We observed a significant delay of the homing GFP+ SKL cells in Ang II infused and lethally irradiated recipient mice. In addition, the SKL cells exposed to Ang II failed to efficiently engraft to the osteoblastic niche. Consistent with this observation, colony formation unit-Spleen (CFU-S) in the Ang II infused recipients was 65% compared to control mice. These findings indicate that Ang II accelerates HSC differentiation to myeloid lineage and that Ang II acts as an important regulator for homing and engraftment of HSC.

P1071 - TWO DISCRETE STAGES OF LONG TERM HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cell (HSC) hierarchy based on transplantation assays defines long-term (LT), intermediate term and short-term stem cells. Recent studies showed that the LT-HSC pool is functionally heterogeneous and that HSCs vary in their differentiation potential and length of reconstitution but display unpredictable differences on the magnitude of repopulation. Transplanting single LT-HSCs into into non-conditioned recipient mice allowed for the identification of two distinct functional stem cell stages that robustly repopulate primary recipient mice but that differ in the magnitude of engraftment. Stage I LT-HSCs are quiescent cells that express genes mapping to cellular adhesion pathways and that have excessive clonal expansion capacity after transplantation whereas stage II LT-HSCs express genes mapping to metabolic pathways and display limited proliferative capacity. Transplantation of prospectively separated stem cell subtypes revealed that stage I cells precede stage II LT-HSCs during differentiation, suggesting that initiation of differentiation is marked by the transit from stage I to stage II LT-HSCs.

P1072 - EXTRINSICALLY REGULATED GADD45γ BALANCES HEMATOPOIETIC STEM CELL SELF-RENEWAL AND DIFFERENTIATION

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Hematopoietic stem cells (HSCs) provide the life-long blood regeneration by maintaining a strictly controlled balance of self-renewal and differentiation. Further investigation is needed for detailed understanding of the molecular orchestration of these HSC fate choices. We identified the transcriptional repressor and tumor suppressor Growth arrest and DNA damage-inducible gene 45 gamma (Gadd45γ) as an important mediator of early HSC differentiation control. In contrast to previous studies with cancer cell lines, where Gadd45γ functioned as growth suppressor and activator of apoptosis, we neither observed growth arrest nor enhanced cell death in Gadd45γ expressing LT-HSCs. FACS-purified LT-HSCs transduced with Gadd45γ showed an instant and accelerated differentiation mainly into the myelomonocytic lineage with the expense of megakaryocyte and erythrocyte cell fate. In transplantations in vivo, Gadd45γ expressing LT-HSCs, although homing to the bone marrow after transplantation, immediately start their differentiation program and rapidly lose their self-renewal capacity. Concordantly, LT-HSCs from Gadd45γ-knock-out mice showed an increased ability to long-term repopulate secondary recipients demonstrating enhanced self-renewal of Gadd45γ deficient HSCs. We further demonstrate that Gadd45γ expression is rapidly activated by differentiation promoting cytokines via STAT5 signal transduction linking extrinsic cytokine signaling with HSC self-renewal and differentiation control. A single amino acid exchange in Gadd45γ, that inhibits its dimerization, abolishes Gadd45γ function. Dimeric Gadd45γ induces differentiation by activating MAP3K4 that leads to selective p38 phosphorylation with absent JNK activation. Ongoing videomicroscopy-based single cell tracking will uncover the mechanism of myeloid lineage choice mediated by Gadd45γ. Here we demonstrate a new extrinsically mediated differentiation induction by Gadd45γ expression in HSCs influencing the balance of self-renewal and differentiation.

P1073 - DORMANT AND ACTIVATED HEMATOPOIETIC STEM CELLS DURING HOMEOSTASIS

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We have investigated the homeostatic behavior of Hematopoietic Stem Progenitor Cells (HSPCs) using a "tet-off" double transgenic system where HSPCs specifically label with Histone 2BGFP until Doxycycline (Dox) is given. Cells that continue to divide after Dox progressively dilute H2BGFP by one half with each cell division. The amount of label retained is a reflection of cell division over time and allows the isolation of viable HSPCs with varying GFP levels. Our studies show that there are dormant (rarely dividing) and active (routinely dividing) populations of HSC in the BM during homeostasis. As homeostatic HSCs traverse a GFP dilution cascade over time they lose repopulating potential with greater divisional history within very few cell divisions. In addition, divisional history overrides both phenotype and immediate quiescence in determining functional activity. As LSKCD150+CD48- cells divide over time their ability to return to G0 parallels their loss of GFP. In addition, the functional activity of G0 LSKCD150+CD48- cells is lost in parallel to divisional history. Perhaps most surprisingly, CD34+ high GFP label retaining cells (LRCs) that are also LSKFlk2-CD48-CD150+, are capable of both long-term repopulation and self-renewal in secondary mice at levels similar to CD34- cells that are also LSKFlk2-CD48-CD150+ and GFP high LRCs. We show that dormant HSCs are heterogeneous in both phenotype and function; LSKCD48-CD150- dormant cells can repopulate primary mice but they are compromised in their self-renewal ability. As such, not all dormant cells are self-renewing. The loss of CD150 expression parallels repopulation and gene expression in the dormant fraction. A gene expression signature has been developed from HSCs with varying levels of GFP that provides insights into homeostatic HSCs as they divide in situ. Most evident from our expression profiles is that although dormant HSCs are repressed transcriptionally and are metabolically inactive there is a constant dialogue between dormant HSCs and their microenvironment that changes dramatically once activated. These studies suggest that once HSCs become activated during homeostasis without stress they initiate a divisional cascade and are slated for extinction by differentiation. As such, this may provide an important control mechanism to maintain the genomic integrity of the HSC pool throughout life.

P1074 - IN VIVO MUTAGENESIS ANALYSIS OF HEMATOPOIETIC STEM AND PROGENITOR CELLS IN RELATION TO AGE, DIFFERENTIATION AND DISEASE STATE

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It has been shown that hematopoietic stem cells (HSCs) accrue DNA damage with age. However, it is unknown if this causes an increase in permanent DNA mutations, thereby increasing the potential for disease development, such as myelodysplastic syndrome (MDS) and leukemia. To begin to address this question, we used mice transgenic for a mutation reporter gene, LacI. The LacI gene can be isolated from any cell type of interest and allows one to measure the frequency of DNA mutations therein. LacI mice were crossed to mice heterozygous for CREB binding protein (Crebbp); these mice have demonstrated DNA repair abnormalities and develop MDS as they age. We isolated various hematopoietic cell populations from LacI+/-;Crebbp+/+ (wild-type) mice and LacI+/-;Crebbp+/- mice at different ages (E14.5, 6 and 12 months) and determined the frequency and type of DNA mutations. Comparative analysis of wild-type samples shows an increase in spontaneous mutation frequency from E14.5 to 6 months of age, but no further increase at 12 months. A more in-depth analysis of 6 month-old bone marrow subpopulations revealed that the spontaneous mutation frequency decreases between the Lin-;Sca-1+;c-Kit++ (LSK) stem cell compartment and the common myeloid progenitor population (CMP). Interestingly and contrary to expectations, we found that the spontaneous mutant frequency in 6 month-old Crebbp+/- blood cells (no MDS developed yet) is similar to that observed in control mice, except in LSKs, where it is significantly lower. However, at 12 months of age, when MDS is fully developed, all Crebbp+/- populations, including LSKs, show an increased mutant frequency compared to controls. These data suggest that during normal hematopoiesis, the CMP stage may serve as a checkpoint at which immature cells with too much DNA damage are actively eliminated from the pool. However, in Crebbp+/- mice without symptoms of MDS, this elimination seems to occur at an earlier stage, while it is ineffective in the clinical stages of MDS.

P1075 - THE PI3K/AKT1 PATHWAY ENHANCES STEADY STATE LEVELS OF FANCL

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Fanconi anemia hematopoietic stem cells display poor self-renewal capacity when subjected to a variety of cellular stress. This phenotype raises the question whether the Fanconi anemia proteins are stabilized or recruited as part of a stress response and protect against stem cell loss. Here we provide evidence that FANCL, the E3 ubiquitin ligase of the Fanconi anemia pathway, is constitutively targeted for degradation by the proteosome. We confirm biochemically that FANCL is poly-ubiquitinated with lysine-48 linked chains. Evaluation of a series of N-terminal deletion mutants revealed that FANCL's E2-like fold may direct ubiquitination. Additionally, our studies revealed that FANCL is stabilized in a complex with Axin1 when glycogen synthase kinase-3β is overexpressed. This result led us to investigate the potential regulation of FANCL by upstream signaling pathways known to regulate glycogen synthase kinase-3β. We report that constitutively active, myristoylated-Akt increases FANCL protein level by reducing poly-ubiquitination of FANCL. 2D-PAGE analysis revealed that acidic forms of FANCL, some of which are phospho-FANCL, are not subject to poly-ubiquitination. These results indicate that a signal transduction pathway involved in self-renewal and survival of hematopoietic stem cells also functions to stabilize FANCL and suggests that FANCL participate directly in support of stem cell function.

P1076 - REDUCTION IN ENDOPLASMIC RETICULUM (ER) STRESS ENABLES MAINTENANCE OF FUNCTIONAL HEMATOPOIETIC STEM CELLS IN VITRO

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Despite recent advances in hematopoietic stem cell (HSC) research, it is still problematic to expand or activate HSC effectively in vitro. The reasons may be explained by the differences between the HSC niche environment and the in vitro culture, as the HSC niche largely maintains HSC by inducing a dormant state and a low proportion of HSC are actively dividing under physiological conditions. To study the regulation of dividing stem cells, we designed a screening strategy to compare gene expression profiles between undifferentiated and differentiated ES cells. Using this approach, we identified a novel HSC regulator, Dppa5 (developmental pluripotency associated 5). Expression levels of Dppa5 directly correlate with HSC function, as Dppa5 overexpression robustly increases the reconstitution level following transplantation. Conversely, down-regulation of Dppa5 significantly reduces long-term reconstitution capacity demonstrating a physiological role for Dppa5 in the regulation of HSC (Miharada et al, ISEH 2012). Overexpression of Dppa5 reduced the levels of glycolytic enzymes and endoplasmic reticulum (ER) stress chaperones, whereas amino acid/protein processing proteins and a redox protein were increased. Consistent with this, the metabolic flow in Dppa5 overexpressing cells was switched from glycolysis to increased oxidative phosphorylation. Furthermore, cellular reactive oxygen species (ROS) were kept at low levels. Importantly, when the ER stress inducer, Tunicamycin, was used to treat HSC in vitro, the competitive advantage of Dppa5 was abolished. Next, we asked whether in vitro cell culture increased ER stress levels in HSC. Cultivation of c-kit+ BM cells for 3 days clearly up-regulated ER stress response proteins. Interestingly, an ER stress inhibitor, Tauroursodexicholate acid (TUDCA), maintained functional HSC for 2 weeks in vitro as TUDCA treated CD34-KSL cells exhibited robustly increased long-term repopulation capacity. Since TUDCA treatment simultaneously inhibited glycolysis and mitochondrial activity, its method of action cannot be explained by a metabolic switch from glycolysis to oxidative phosphorylation, e.g. through PKM2 (a rate-limiting glycolysis enzyme). These findings strongly indicate that reduced ER stress and improved protein folding in vitro may improve HSC function and activity.

P1077 - INDUCIBLE DEPLETION OF HEMATOPOIETIC STEM CELLS IN VIVO CHALLENGES NICHE AVAILABILITY AS THE CRITICAL DETERMINANT FOR BONE MARROW ENGRAFTMENT

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Pre-conditioning for bone marrow (BM) or hematopoietic stem cell (HSC) transplantation is accomplished by irradiation or cytoreductive drug treatment of the recipient, resulting in loss of hematopoietic stem and progenitor cells (HSC/P). Current believe is that pre-conditioning empties HSC niches and thereby allows engraftment of donor HSC. Here we report a mouse model allowing inducible Cre/lox*P*-mediated depletion of primitive HSCs in vivo. A strain expressing tamoxifen-inducible HSC-specific Cre (HSC-scl-CreERT) was mated to Rosa26-lox*P*-STOP-lox*P*-Diptheriatoxin A (R-DTA) mice, in which diphtheria toxin (DT) is expressed upon Cre-mediated deletion of the loxP-flanked stop element. Tamoxifen induction resulted in 100-fold reduction of primitive HSC ($Kit^+Sca\text{-}1^+lin^-CD48^-CD150^+CD135^-CD34^-$) but only a mild reduction of more mature HSC/P in the BM of Cre^+R-DTA^+ mice animals. Hematopoietic niche cells including mesenchymal stem/progenitor cells, CXCL12 abundant reticular cells and osteoblasts were not directly altered by the depletion procedure. Prolonged maintenance of these mice on tamoxifen allowed for efficient suppression of HSC numbers for at least ten weeks. Competitive transplantation of HSC depleted and control BM into irradiated recipients confirmed the reduction of functional HSC. Thus, most HSC niches should be empty in induced Cre^+R-DTA^+ mice. Contrary to expectation, congenic BM transfer into (un-irradiated) HSC-depleted Cre^+R-DTA^+ mice recipients did not result in improved engraftment of donor HSC. Even long-term tamoxifen-treatment of Cre^+R-DTA^+ mice recipients (before and after donor BM transfer) did not result in engraftment. These findings demonstrate that vacating HSC niches is not sufficient to allow engraftment of donor HSC and stimulate research into additional factors determining the success of HSC engraftment.

P1078 - HEB GENE ESTABLISHES AN INTRINSIC EXPANSION LIMIT IN HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cells (HSCs) are uniquely endowed with sustained self-renewal capacity and multipotent differentiation potential. In homeostasis, most of adult HSCs are in quiescence and genetic disruption of HSC quiescence always correlates with decreased long term self-renewal capacity. The objective of this study is to evaluate the importance of three hematopoietic transcription factors of the basic helix-loop-helix (bHLH) family, Scl, E2a and Heb for HSC functions and self-renewal capacities. To measure the functional importance of these genes for the control of self-renewal in HSCs, we first performed serial transplantation assays under limiting conditions using cells from wild-type (Wt), Scl+/-, E2a+/- and Heb+/- mice. Our results indicate that Scl is haploinsufficient for stem cell functions, whereas E2a and Heb are haplosufficient, probably due to their functional redundancies. Second, we decided to impose a more extensive proliferative stress to HSCs using a 5-fluorouracil (5-FU) based protocol. Two treatments of 5-FU caused a 100-fold decrease in the numbers of phenotypic HSCs. Nonetheless, the remaining HSCs proliferated extensively to re-establish the HSC pool size, which was twice larger than that of untreated mice after 1 month. At this point, most HSCs have exited the cell cycle and were back to quiescence except in E2a+/- HSC which were still in cycle, suggesting that E2a facilitates cell cycle exit. Despite a normal stem cell pool size, HSCs from Wt, Scl+/- and E2a+/- mice treated with 5-FU cannot compete against untreated cells to regenerate the host in transplantation assays, indicating that stem cell activity is severely impaired by extensive proliferation. We next showed that this was due to impaired expansion and differentiation per individual HSCs, as measured by the mean activity of stem cell (MAS). In contrast, the competitive potential was maintained in Heb+/- HSCs, associated with a normal MAS, after 5-FU induced proliferative stress. Based on these results, we conclude that Heb establishes an intrinsic expansion limit in HSCs, beyond which their regenerative potential is impaired. We surmise that quiescence preserves HSC integrity by preventing cells from reaching this intrinsic expansion limit.

P1079 - DECLINED PRESENTATION
DECREASED PU.1 AND ENHANCED CITED2 COOPERATE TO MAINTAIN SELF-RENEWAL

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CITED2 has a conserved role in maintaining HSCs. We have shown before that CD34+ cells from a subset of acute myeloid leukemia (AML) patients display enhanced CITED2 expression and interfering with this expression is detrimental for leukemia maintenance. Ectopic expression of CITED2 in normal CD34+ stem and progenitor cells (HSPCs) results in increased proliferation and skewed myelo-erythroid differentiation. Microarray studies indicated that 1648 genes were differentially expressed in HSPCs upon expression of CITED2. GSEA analysis indicated that CITED2 modulates hypoxia and p53 signaling. This is consistent with an increased percentage of HSCs, which demonstrated decreased levels of apoptosis. Colony forming cells assays indicated that these cells have increased CFC forming capacity, but harvesting and replating of these colonies demonstrated no secondary colony forming activity. This demonstrates that enhanced CITED2 expression cannot maintain the self-renewal characteristics of HSCs, suggesting that additional genetic changes contribute to self-renewal of leukemic stem cells. We recently identified PU.1 as a strong negative regulator of CITED2 and enhanced CITED2 expression in AML samples correlates with low PU.1 expression. Combined lentiviral downregulation of PU.1 with overexpression of CITED2 gave a huge proliferative advantage to transduced CD34+ cells. Also in CFC assays a large increase of progenitor numbers was observed. To stringently assess whether self-renewal was maintained, cells were first cultured for 4 weeks on MS5 and subsequently plated into CFC assays, followed by secondary and tertiary replating experiments. The only cells that after 10 weeks of culture still generated colonies were the ones that demonstrated low PU.1 and increased CITED2 expression, indicating that this combination preserves self-renewal. Together, these data suggest that in AML with low PU.1, CITED2 not only functions to increase proliferation and skew myeloid differentiation, but that together, low PU.1 and high CITED2 are necessary to maintain leukemic stem cell self-renewal.

P1080 - MIR-29A MAINTAINS HEMATOPOIETIC STEM CELL SELF-RENEWAL AND IS REQUIRED FOR LEUKEMIC TRANSFORMATION

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Hematopoietic stem cells (HSCs) are unique among blood cells in their ability to indefinitely self-renew in addition to their ability to generate all cell types in the blood system. We previously demonstrated that miR-29a is highly expressed in mouse and human HSCs, as well as in human acute myeloid leukemia (AML) stem cells, and that over-expression of miR-29a in immature mouse hematopoietic cells induces a myeloproliferative disorder that progresses to AML. We have now extended our studies to investigate the function of miR-29a in HSCs in mice lacking the miR-29a/b bicistron. Homozygous deletion of miR-29a/b resulted in reduced bone marrow cellularity and reduction of colony forming capacity in hematopoietic stem and progenitor cells (HSPCs). The phenotype is mediated specifically by miR-29a, since miR-29b expression is not significantly altered in HSCs. In addition, reconstitution oif miR-29a/b null HSPCs with miR-29a, but not miR-29b, rescued colony formation defects in vitro. Defects in self-renewal were observed in in miR-29a deficient HSCs in both competitive and non-competitive transplantation assays, and these deficits were associated with increased cell cycling and apoptosis. Transcriptomal studies demonstrated widespread gene expression alterations in HSCs, revealing that miR-29a deficient HSCs were more similar to wild-type myeloid progenitor cells than to wild-type HSCs. In addition, a number of miR-29a target genes were up-regulated in miR-29a deficient HSCs including DNA methylation enzymes (Dnmt3a, -3b) and cell cycle regulators (e.g. Cdk6, Tcl1, Hbp1, Pten). Knockdown of one of these targets, Dnmt3a, in miR-29a deficient HSCs resulted in partial restoration of colony formation, providing functional validation that Dnmt3a mediates part of miR-29a null HSPCs functional defects. miR-29a loss also abrogated leukemogenesis in the MLL-AF9 retroviral leukemia model. Together, these studies demonstrate that miR-29a contributes to HSC self-renewal and myeloid leukemogenesis.

P1081 - LOW C-KIT EXPRESSION IDENTIFIES HEMATOPOIETIC STEM CELLS WITH ENHANCED SELF-RENEWAL POTENTIAL

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A growing body of evidence supports the view that hematopoietic stem cells (HSCs) are heterogeneous with respect to their self-renewal, lineage, and reconstitution capacities. However, the molecular mechanisms underlying these differences are not well-understood. Because gain- and loss-of-function c-Kit mutants exhibit significant alterations in HSC function, we hypothesized that even small changes in c-Kit signaling would result in profound effects on HSC function, and thus, we investigated whether HSCs (Lin-c-Kit+Sca-1+SLAMf150+CD34-) expressing differing levels of c-Kit may exhibit distinct phenotypes. Our studies demonstrate that HSCs expressing low levels of c-Kit (lowest 30% of expressors; c-Kitlo) exhibit enhanced self-renewal and long-term reconstitution potential compared to HSCs expressing high levels of c-Kit (highest 30% of expressors; c-Kithi) as demonstrated through non-competitive and competitive transplantation assays. c-Kithi HSCs also exhibit a strong megakaryocytic lineage bias, as demonstrated through in vitro culturing and in vivo transplantation studies, and this bias was confirmed by transcriptomal analysis of c-Kithi HSCs, which exhibited increased expression of megakaryocyte associated genes such as Gata-1 and reduced expression of self-renewal genes such as HoxB4. c-Kitlo HSCs readily give rise to c-Kitlo and c-Kithi HSCs both in vitro and in vivo, but c-Kithi HSCs have little to no capacity to give rise to c-Kitlo HSCs, consistent with a hierarchical relationship between these two populations. These qualitative differences between c-Kithi and c-Kitlo HSCs are preserved even in situations of physiologic stress in which c-Kit expression is altered, notably following 5-FU treatment. Overall, these data indicate that c-Kitlo HSCs are enriched for long-term reconstituting HSCs. These findings have important implications for investigations of HSC function as they suggest that most currently used immunophenotypic definitions of HSCs identify cell populations significantly contaminated by non-self-renewing and lineage-biased cells.

P1082 - A GENOME-WIDE RNAI SCREEN IDENTIFIES JARID2 AS A CRITICAL REGULATOR OF HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS

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To gain insights into the mechanisms underlying renewal and differentiation of human hematopoietic stem and progenitor cells (HSPCs), we developed a lentiviral shRNA screening strategy. Umbilical cord blood derived CD34 positive cells were transduced with libraries of pooled shRNA targeting 15 000 genes. We used the limited persistence of HSPCs under ex vivo culture conditions as a basis for functional selection of shRNAs conferring prolonged maintenance or expansion of undifferentiated HSPCs. The distribution of shRNAs before and after selection was determined by next generation sequencing of proviral inserts and a strong enrichment of independent gene-specific shRNAs used as the main scoring criteria to rank candidate genes. Notably, known cancer related genes (in particular tumor suppressors) were highly enriched among the top-scoring genes. We initially focused on transcriptional regulators and DNA binding factors and found that the Jumonji and ARID-domain containing protein, JARID2, scored highest in this category. JARID2 is a known interacting partner to the polycomb repressive complex 2 (PRC2) and critically mediates the binding of PRC2 to its target genes. Very recently several groups have reported inactivating mutations of JARID2 in myeloid neoplasms but a direct functional role of JARID2 in hematopoietic cells has not been described yet. We found that CD34 cells transduced with several independent shRNAs against JARID2 showed an overall increase in cell numbers and maintained CD34 expression better than control cells. When transplanted to primary and secondary NSG mice shJARID2 transduced cells showed a significant increase in human reconstitution especially in the bone marrow (primary mice: 11.4±4.7% vs 49.7±4.0%, secondary mice: 0.07±0.03% vs 10.3±2.0%) indicating an increased expansion of HSCPs in vivo upon knockdown of JARID2. These findings identify JARID2 as an important regulator of human HSPCs, and together with recent reports of recurrent deletions of JARID2 in myeloid neoplasms, highlight a possible direct role of JARID2 deficiency in development of hematopoietic malignancies. We are currently performing molecular profiling studies to further elucidate the mechanisms by which JARID2 regulates HSCPs.

P1083 - ROLE OF HMGA2 IN HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Hematopoietic stem cells maintain life long blood generation during homeostasis and stress. Factors controlling the key characteristics of these cells such as self-renewal, quiescence and the potential to form various blood lineages are poorly understood. We reasoned that genes found to be located near common sites of vector insertion in clinical gene therapy studies might have important functions in this regard. The DNA binding protein, high mobility Group AT hook 2 (HMGA2) is one such factor. HMGA2 activation coupled with a myeloid biased clonal dominance of stem/progenitor cells was observed in a clinical gene therapy trial for β-thalassemia. Here, we used gain-of-function and loss-of-function approaches to study the role of HMGA2 in human hematopoietic stem and progenitor cells (HSPCs). Umbilical cord blood derived CD34+ cells were transduced with HMGA2-overexpressing or control lentiviruses and cultured in vitro. HMGA2 overexpression provided a growth and survival advantage to the cells as measured by proliferation kinetics of GFP positive cells and colony-forming cell (CFC) numbers. To evaluate the effect of HMGA2 in more primitive cells we transplanted transduced cells to NSG mice. Unlike control cells, the frequency of GFP positive HMGA2 expressing cells progressively increased between 6 and 14 weeks post-transplantation (26.53 ± 2.633% N=8 vs 39.33 ± 3.738% N=7), suggesting that HMGA2 promotes proliferation of HSCPs in vivo. Bone marrow analysis showed that HMGA2 overexpressing cells were significantly skewed toward the myeloid lineage and had a higher frequency of the primitive, CD34+CD38-, cell fraction (HMGA2 1.285 ± 0.1666 % N=7 vs control 0.5630 ± 0.1063 % N=6). We detected no signs of leukemia in primary transplanted mice and are currently monitoring mice from secondary transplantations. To further understand the role of HMGA2 in HSPCs we performed knockdown studies using lentiviral shRNA. Knockdown of HMGA2 by independent shRNAs in CD34 cells severely inhibited cell proliferation ex vivo. Cell cycle analysis showed an accumulation of shHMGA2 cells in the G0/G1 phase. In addition, the impaired proliferation kinetics was associated with a higher ROS content in shHMGA2 cells. Taken together our finding indicates a key role of HMGA2 in regulation of human HSPCs. Current efforts are aimed at defining the upstream and downstream mediators of HMGA2 activity in hematopoietic cells.

P1084 - FUBP1 IS AN ESSENTIAL REGULATOR OF HEMATOPOIETIC STEM CELL SELF-RENEWAL

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Analyzing the regulatory mechanisms that control self-renewal and differentiation of hematopoietic stem cells (HSCs) is important to understand the pathophysiology of certain hematopoietic diseases and to succeed in expanding multipotent HSCs for therapy ex vivo. In a gene trap mouse model, the functional impairment of the transcriptional regulator Far Upstream Element Binding Protein 1 (FUBP1) led to embryonic lethality at day E15.5. Homozygous Fubp1-/- embryos showed largely reduced numbers of long-term repopulating (LT-) HSCs in the fetal liver, and lower engraftment of Fubp1-/- fetal liver cells was observed in competitive transplantation experiments. However, all hematopoietic lineages were produced in the absence of FUBP1, indicating an HSC-specific function. Eventually, all Fubp1-/- LT-HSCs were exhausted in the transplanted animals, suggesting an intrinsic self-renewal defect in these cells. Similarly, the transplantation of adult Fubp1 knockdown (kd) LT-HSCs resulted in low engraftment and their eventual loss. Fubp1 kd in ex vivo cultured LT-HSCs led to reduced cell expansion, and single cell tracking following time-lapse microscopy revealed prolonged generation times and increased apoptosis upon Fubp1 kd, confirming the essential role of FUBP1 in fetal and adult HSC self-renewal and expansion. Furthermore, our groups aim at the identification of HSC-relevant FUBP1 target genes, as well as transcription factors (TFs) involved in the upstream regulation of Fubp1 expression. Transcriptome profiling of Fubp1 kd HSCs revealed potential FUBP1 target genes that might account for its pro-proliferative and anti-apoptotic function. In silico analyses and genome-wide ChIP-sequencing data predicted a putative binding of TAL1/SCL, an important TF in early hematopoiesis, within the Fubp1 promoter region. This could be confirmed by ChIP experiments using murine bone marrow mononuclear cells. Further analyses will show whether TAL1 is indeed involved in the regulation of Fubp1 expression in early hematopoiesis. Our studies have demonstrated the requirement of FUBP1 for HSC self-renewal, and the ongoing experiments will further our understanding of how FUBP1 is implicated in the regulatory network that controls the development and maintenance of the hematopoietic system.

P1085 - TRANSCRIPTIONAL CONTROL OF HEMATOPOIESIS DURING EMBRYONIC DEVELOPMENT

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Transcription factors are important players in regulating hematopoietic development including hematopoietic stem cell generation (HSC). HSCs originate from hemogenic endothelial cells through a process called endothelial to hematopoietic transition (EHT). To date, a system in which the expression of pivotal transcription factors in EHT (or most other lineage differentiation systems) can be observed in real-time is lacking. As expected from knockout studies revealing the absence of HSCs, the Runx1 and Gata2 transcription factors are involved in HSC generation. However, nothing is known of the dynamics of their expression in vivo - whether they are expressed simultaneously in the same cell or same cell pedigree during development. Marker mice for these factors have been made using strategies that either affect the levels of transcription factor per cell or produce a fusion protein. Since HSC generation is profoundly affected by transcription factor dose, we generated a novel Gata2Venus knock in mouse in which the expression of the reporter fluorochrome does not affect the transcription factor expression levels or the proteins conformation. This was achieved by introducing the fluorochrome coding gene into the 3'UTR of the transcription factor gene. Venus expression is observed in aortic endothelial cells and hematopoietic clusters paralleling Gata2 expression. An extensive characterization of Gata2-Venus expressing cells and their role in hematopoietic development is ongoing. A surprising aspect of the Gata2 expression was revealed when we performed in vivo real-time imaging of Gata2Venus embryos. We found that Gata2 expression is remarkably dynamic and fluctuating - some cells turn Gata2 on and some turn it off during the 12 hour imaging session. Moreover, Gata2 expression levels seem to vary between cells and oscillate in individual cells. Whether similar oscillation patterns can be observed for the other major hematopoietic regulators (such as Runx1) and whether these oscillations correlate with their function will be a focus of our future studies. A Runx1 mKate knock in mouse, using a similar strategy, is in the process of generation.

P1086 - TWO DEFINITIVE ADULT-TYPE HSC SUBSETS COEXISTENT IN MOUSE

Mihaela Crisan[1], *Chris Vink*[1], *Tomoko Yamada-Inagawa*[1], *Alex Neagu*[1], *Caterina Purini*[1], *Karine Bollerot*[1], *Sofia Karkanpouna*[2], *Reinir van der Linden*[1], *Alvin Chan*[1], *and Elaine Dzierzak*[1]
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Adult-type hematopoietic stem cells (HSCs) emerge in the aorta-gonad-mesonephros (AGM) region in the mid-gestation mouse embryo. Thereafter, HSCs migrate to the fetal liver (FL) and at birth, colonize the adult bone marrow. The molecular signaling cascades controlling HSCs in the AGM region is not yet known. Exogenous Hedgehog (Hh) added to AGM explants prior to HSC generation induces AGM HSCs and BMP pathway inhibition abolishes AGM HSC activity. In the FL, the BMP signaling pathway is not required for hematopoiesis. A role for the MAPK signaling pathway has been demonstrated in the adult hematopoiesis, but whether this pathway also controls AGM HSCs is not known. We hypothesize that Hh and BMP control the induction/expansion of AGM HSCs and cooperate with the MAPK pathway to control their maintenance and differentiation. To test whether HSCs are directly activated by BMP, we used transgenic BMP-response element (BRE)-gfp mice, in which GFP expression is an indicator of BMP pathway activation. When BRE-gfp E11 AGM cells were sorted for GFP expression and injected into adult irradiated mice, all long term repopulating HSCs were found in the BMP activated (GFP+) fraction. We next asked whether HSCs in the FL are activated by BMP. Surprisingly, we found high level, multilineage, self-renewing HSCs in both GFP+ and GFP- fractions. This suggests that subsets of HSCs shift from an exclusively BMP-activated state, to a state of non-activation. This shift may be involved in the expansion of HSCs in the FL. Since HSCs expand in explant cultures of E11 AGMs, we examined whether after explant, AGM HSCs remain BMP-activated. Interestingly, after explant, both GFP+ and GFP- fractions contained multilineage, self-renewing HSCs. In such explants when Hh or MAPK pathways are inhibited, only the non-BMP-activated HSC activity is abolished. The Hh inhibition effect on AGM HSCs could be rescued by exogeneous VEGF addition, but VEGF could not rescue the HSC defects caused by MAPK inhibition. These results suggest that VEGF acts downstream of Hh and further activates the MAPK pathway to control AGM HSCs. In conclusion, our data provide clear evidence of an interactive molecular cascade involving the BMP, Hh and MAPK signaling pathways in the control of AGM HSC development.

P1087 - TWO ADULT-TYPE HSC SUBSETS COEXISTENT IN MOUSE

Mihaela Crisan[1], *Chris Vink*[1], *Tomoko Yamada-Inagawa*[1], *Alex Neagu*[1], *Caterina Purini*[1], *Sofia Karkanpouna*[2], *Reinir van der Linden*[1], *and Elaine Dzierzak*[1]
[1]Cell Biology, Erasmus MC Stem Cell Institute, Rotterdam, Netherlands; [2]Dept. of Molecular and Cell Biology, Centre of Biomedical Genetics, Leiden, Netherlands

Adult-type hematopoietic stem cells (HSCs) emerge in the aorta-gonad-mesonephros (AGM) region in the mid-gestation mouse embryo. Thereafter, HSCs migrate to the fetal liver (FL) and at birth, colonize the adult bone marrow. The molecular signaling cascades controlling HSCs in the AGM region is not yet known. Exogenous Hedgehog (Hh) added to AGM explants prior to HSC generation induces AGM HSCs and BMP pathway inhibition abolishes AGM HSC activity. In the FL, the BMP signaling pathway is not required for hematopoiesis. A role for the MAPK signaling pathway has been demonstrated in the adult hematopoiesis, but whether this pathway also controls AGM HSCs is not known. We hypothesize that Hh and BMP control the induction/expansion of AGM HSCs and cooperate with the MAPK pathway to control their maintenance and differentiation. To test whether HSCs are directly activated by BMP, we used transgenic BMP-response element (BRE)-gfp mice, in which GFP expression is an indicator of BMP pathway activation. When BRE-gfp E11 AGM cells were sorted for GFP expression and injected into adult irradiated mice, all long term repopulating HSCs were found in the BMP activated (GFP+) fraction. We next asked whether HSCs in the FL are activated by BMP. Surprisingly, we found high level, multilineage, self renewing HSCs in both GFP+ and GFP- fractions. This suggests that subsets of HSCs shift from an exclusively BMP-activated state, to a state of non-activation. This shift may be involved in the expansion of HSCs in the FL. Since HSCs expand in explant cultures of E11 AGMs, we examined whether after explant, AGM HSCs remain BMP-activated. Interestingly, after explant, both GFP+ and GFP- fractions contained multilineage, self-renewing HSCs. In such explants when Hh or MAPK pathways are inhibited, only the non-BMP-activated HSC activity is abolished. The Hh inhibition effect on AGM HSCs could be rescued by exogeneous VEGF addition, but VEGF could not rescue the HSC defects caused by MAPK inhibition. These results suggest that VEGF acts downstream of Hh and further activates the MAPK pathway to control AGM HSCs. In conclusion, our data provide clear evidence of an interactive molecular cascade involving the BMP, Hh and MAPK signaling pathways in the control of AGM HSC development.

P1088 - THE CYTOKINE-INDUCED MICRORNA193B MODULATES CKIT EXPRESSION AND STAT5 SIGNALING

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Normal hematopoiesis requires the right composition of microRNAs (miRNA) controlled in a developmental stage and cell type-dependent fashion. MiRNAs are small, non-coding RNAs with the ability to simultaneously target multiple mRNAs for the orchestration of complex gene expression networks in development, regeneration and disease. In a screening for STAT5A/B -induced miRNAs in highly purified murine LT-HSCs, we found miR193b to be upregulated by the cytokine Thrombopoietin. Constitutive activation of STAT5A/B enhances HSC self-renewal and thereby can contribute to myeloproliferative disease. Videomicroscopy-based single cell tracking revealed that miR193b expression in HSCs leads to increased cell death and slowed proliferation in vitro, and a loss of functional HSCs in vivo, both caused by a targeted downregulation of ckit surface expression. To determine the physiological function of miR193b in hematopoiesis, we generated a knock-out mouse model. In miR193b-/- mice, peripheral white blood cell numbers of all lineages are increased, hematopoietic progenitors show accelerated proliferation capacity, and aged animals gain an enlarged HSC pool. During the stress response to 5-FU treatment, miR193b-/- mice show an altered cytokine profile and an upregulated platelet production. The potential molecular mechanism explaining these observations is the increased basal activation of STAT5A/B, which we observed in miR193b-/- bone marrow and spleen cells. RNA sequencing of defined stem and progenitor cells before and after cytokine stimulation will shed light on the regulatory network governed by miR193b. The role of miR193b in contributing to feedback regulation of cytokine responsiveness and STAT5 signaling warrants further investigation and may reveal new therapeutic strategies for hematologic diseases.

P1089 - MODEL-BASED PREDICTION OF RESIDUAL LEUKEMIC STEM CELL DYNAMICS IN TYROSINE KINASE INHIBITOR-TREATED CHRONIC MYELOID LEUKEMIA (CML)

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Molecular response to imatinib (IM) in CML is characterized by a biphasic decline of BCR-ABL transcript levels. Although qualitatively similar for most patients, there is a considerable patient-specific heterogeneity in treatment response. For the decision whether or not treatment stop is a save option for a particular patient, the number of residual leukemic stem cells (LSC) is one of the most important parameters. Because this number is clinically unobservable, we apply mathematical modelling and computer simulation to predict the stem cell dynamics, providing estimates for the individual risk of molecular relapse upon treatment cessation. To account for inter-individual heterogeneity, we extended our previously developed CML model. To estimate the model parameters we used 7-year follow-up data from a clinical trial. The obtained values have been validated on the basis of independent trial data. Our model predicts that a subset of patients (14%) can achieve complete eradication of LSCs within less than 15 years of IM treatment. Furthermore, the model prognosticates that 31% of the patients will remain in deep molecular remission following treatment stop after a fixed period of two years in MR(5.0), while 69% are expected to relapse. We propose to use model-predicted residual LSC number to assess the patient-specific risk of molecular relapse upon treatment discontinuation. Application of the suggested rule for deciding about the time point of treatment cessation is predicted to result in a significant reduction in rate of molecular relapse. The proposed model-based procedure allows for a better appraisal of individual prognosis of CML patients with direct impact on clinical decision-making. Because the mechanisms of action of second generation tyrosine kinase inhibitors are similar to IM, the described approach can also be applied in an analogous way to these treatment options.

P1090 - GLOBAL DISCOVERY OF DYSREGULATED PROTEIN EXPRESSION AND PHOSPHORYLATION NETWORKS IDENTIFIES LEO1 AS A NOVEL TARGET OF PRL-3 PHOSPHATASE (PTP4A3) IN LEUKEMOGENESIS

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Identification of novel oncogenic alterations and pathways are the keys to the understanding and discovery of therapeutic targets in the improved management of AML. PRL-3 is a small 20kDa prenylated dual-specificity phosphatase that is over-expressed in 50% of AML and associated with poor survival, but the mechanisms regulating the associated malignancy remains unknown. Our data demonstrates that ectopic PRL-3 expression in the factor-dependent TF1 AML cells confers cytokine-independent growth, induces colony-forming ability and tumorigenesis in vivo. To characterize novel substrates of PRL-3, unbiased large-scale SILAC-based MS was performed between the parental TF1 and isogenic TF1-PRL3 cells to discover critical differences in signaling networks. We obtained quantitative measurements on 803 proteins, where 331 were significantly up-regulated (>1.5-fold) and 67 were under-expressed (<0.6-fold). Importantly, PRL-3 alters the phosphorylation status of 192 proteins. Our proteomics profiling and in vitro validation revealed that Leo1, component of the Polymerase II-associating factor 1 (PAF) complex, is upregulated and dephosphorylated by PRL-3. Mechanistically, PRL-3 upregulates Leo1 by relieving the repressive H3K9me3 mark on Leo1 promoter through direct binding of JMJD2C histone demethylase, promoting a permissive chromatin state for Leo1 transcription. Conversely, abrogation of PRL-3 using RNAi reduced Leo1 levels and destabilizes the PAF complex, consequently leading to the downregulation of PAF-regulated pluripotency target genes like Sox2, Sox4 and Tbx3. Our functional analyses corroborated the contribution of Leo1 towards PRL-3 oncogenicity, whereby inhibition of Leo1 in PRL-3 cells impeded cell proliferation, induced significant apoptosis and abolished colony formation. Finally, we relate these data to clinical relevance where 46% of the human AML patient samples ($n=24$) showed PRL3+Leo1+ expression, with a statistically significant association of $P<0.01$. In conclusion, our work allows the identification of bona fide PRL-3 targets, which established starting points for a complete enumeration of PRL-3 function in AML signaling and provides valuable leads for designing future therapies targeting PRL-3 in leukemia.

P1091 - JAK2 MUTATION-NEGATIVE SECONDARY ERYTHROCYTOSIS IN MULTIPLE MYELOMA: A CASE REPORT FOLLOW UP

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The association between erythrocytosis and MM has been rarely described. We report a case of MM with coincidental JAK2 V617F and exon 12 mutation-negative secondary erythrocytosis. A 55-year-old male was presented with an increased Hb level. He stopped smoking 7 years ago. On examination, no abnormality except a reddish face was found. Initial CBC showed Hb level of 19.5 g/dL, a platelet count of 183,000/uL and a WBC count of 3,800/uL. The biochemical profile showed protein/albumin 8.6/3.9 g/dL and creatinine 1.1 mg/dL. His arterial blood oxygen saturation was 95.8%. On abdominal sonography, no mass in kidney, adrenal gland or liver was found. The serum erythropoietin level was elevated. JAK2 V617F mutation was absent and JAK2 exon 12 showed no mutation. Bone marrow study showed 14.6% of all nucleated cells were plasma cells. Serum protein EP/IEP showed IgA/lambda M-protein of 2.6 g/dL. He was diagnosed as MM with secondary erythrocytosis. IgA increased to 3.9 g/dL after 1 month (mo). He moved to Asan Medical Center and found bone lesion on left humerus, which was treated with 21 Gy of radiotherapy. He received induction therapy with 2 cycles of high-dose dexamethasone and reached to PR. Serum erythropoietin level decreased to 26.1 mIU/ml, which was in the reference range, and Hb level returned to normal. Two cycles of VAD chemotherapy was added before autologous peripheral blood stem cell transplantation (autoPBSCT) with Mel200 conditioning. He had been in CR for 8 mo but relapsed with M-protein of 0.5 g/dL. He received 2nd autoPBSCT with same conditioning and got VGPR, but disease progressed 6 mo later, with increasing M-protein and bone lesion. He was treated with bortezomib+dexamethasone and got PR, but progressed 6 mo later. Treatment was changed to melphalan prednisolone thalidomide combination therapy. During the course since his first chemotherapy, his Hb level did not increased and kept around 13 g/dL without any treatment for erythrocytosis. (The case was reported previously, before chemotherapy, as Brief Communication in Acta Haematologica 2011; 126:169-171)

P1092 - ROLE OF PAX5 FUSION PROTEINS IN B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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PAX5 is one of the most frequently mutated genes in B-cell precursor acute lymphoblastic leukemia (B-ALL), as it is altered in over 30% of all cases analyzed. In addition, ~2.5% of all B-ALL cases contain specific chromosomal translocations generating PAX5 fusion proteins. These novel transcription factors are generated by linking the N-terminal part of PAX5 with the C-terminal sequences of different fusion partner proteins, such as the transcriptional regulators ETV6 and FOXP1. All PAX5 fusion proteins contain the N-terminal DNA-binding paired domain, but lack the potent C-terminal transactivation region of PAX5. As all leukemia cells still contain a wild-type PAX5 allele in addition to the PAX5 translocation, the PAX5 fusion proteins are thought to contribute to B-ALL formation by interfering with the normal function of PAX5. To date, little is however known about the oncogenic role of PAX5 fusion proteins in B-ALL. We have investigated the oncogenic function of two PAX5 translocations in B-ALL by generating knock-in mice expressing the PAX5-ETV6 and PAX5-FOXP1 fusion proteins from the endogenous Pax5 locus. These knock-in mice provide the unique opportunity to investigate the effect of these two fusion proteins on B cell development, leukemogenesis and the interference with wild-type Pax5 function. B cell development was severely impaired in Pax5(Etv6/+) and Pax5(Foxp1/+) mice, whereas it was normal in Pax5(Prd/+) knock-in mice expressing only the DNA-binding paired domain (Prd) of Pax5. Hence, the impaired B lymphopoiesis in these mice is caused by the sequences of the fusion partners ETV6 and FOXP1. Importantly, Pax5(Etv6/+) and Pax5(Foxp1/+) mice, which were crossed with additional gain- or loss-of-function mutations occurring in B-ALL, rapidly developed tumors, indicating that the PAX5-ETV6 and PAX5-FOXP1 fusion proteins cooperate with other genetic lesions in B-ALL development. To gain insight into the molecular function of the two PAX5 fusion proteins, we currently analyze the gene expression profile of leukemic cells by RNA-sequencing and identify direct target genes of PAX5 fusion proteins by ChIP-sequencing to elucidate their molecular mechanism of action in tumor development.

P1093 - DECLINED PRESENTATION
A PU.1 SUPPRESSIVE TARGET GENE, METALLOTHIONEIN-1G, DISTURBS RETINOIC ACID-INDUCED NB4 CELL DIFFERENTIATION AND ACCELERATES G1/S TRANSITION

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We recently demonstrated that PU.1 directly represses metallothionein (MT)-1s gene promoter (Imoto et al., JBC 2010)(Suzuki et al., BBRC 2013). MT-1s play a role in metal chelation and cell growth but the roles in myelopoiesis are largely unknown. To clarify the functions of MT-1G (a major form of MT-1s) in myeloid cells, we established MT-1G-overexpressing NB4 (NB4MTOE) cells. First, we analyzed the cell cycle profiles of these cells and found that the proportions of S-phase cells were significantly increased in serum-stimulated NB4MTOE cells. Next, we investigated whether MT-1G functionally contributes to myeloid cell differentiation. Nitroblue tetrazolium (NBT) reduction assays revealed that the proportions of NBT-positive cells were decreased in NB4MTOE cells in the presence of all-trans retinoic acid (ATRA). Real-time PCR analyses demonstrated that the inductions of CD11b and CD11c and reductions in myeloperoxidase and c-myc by ATRA were attenuated in NB4MTOE cells. Since G1 arrest is a hallmark of ATRA-induced NB4 cell differentiation, we observed a decrease in G1 accumulation, as well as decreases in p53 target genes, p21 and cyclin D1 inductions, by ATRA in NB4MTOE cells. p53 is a zinc finger transcription factor and depletion of intracellular zinc result in the loss of its DNA-binding capacity. EMSA and ChIP assays revealed that the DNA-binding activity of p53 tended to be attenuated in ATRA-treated NB4MTOE cells. Microarray analyses showed that, in addition to the genes already confirmed in the differentiation assays, the changes in expression of several myeloid differentiation-related genes (GATA-2, azurocidin 1, pyrroline-5-carboxylate reductase 1, defensin α4, C-X3-C motif receptor 3, matrix metallopeptidase -8, S100 calcium-binding protein A12, neutrophil cytosolic factor 2 and oncostatin M etc.) induced by ATRA were disturbed in NB4MTOE cells. Collectively, overexpression of MT-1G disturbs the proper proliferation and differentiation of myeloid cells. The present study provides evidence that expression analysis of MT-1G in APL patients may be a good prediction marker to estimate the efficacy of ATRA.

P1094 - ROLE OF HIF-1α IN LEUKEMIA INITIATING CELLS IN ACUTE MYELOID LEUKEMIA

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The microenvironment in which hematopoietic stem cells (HSCs) reside provides them with conditions that maintain quiescence and prevent differentiation into committed progenitors. Quiescent HSCs are located in the endosteal region of the bone marrow (BM) niche, where both biological and physical mechanisms participate in their regulation. One important factor is low oxygen tension, the hypoxic environment switching HSCs metabolism from oxydative phosphorylation to glycolysis. This prevents HSCs from producing reactive oxygen species (ROS) responsible for DNA damage. Mainly in this way, hypoxia signaling contributes to keeping HSCs free from potentially disruptive mutations. Leukemia initiating cells (LICs) share many properties with HSCs, including maintenance of quiescence, self-renewal and differentiation properties, all essential for the development of disease. In addition, hypoxia has recently been identified as an important factor for LICs in a number of hematological malignancies. Our aim is to study the role of hypoxia signaling in the initiation and maintenance of Acute Myeloid Leukemia (AML), using two different approaches: (A) Transduction of MLL-ENL expressing cells with a hypoxia reporter, in which the expression of GFP is driven by the hypoxia response element (HRE), to localise LICs in vivo according to their GFP expression. This allows us to identify and isolate LICs residing in hypoxic or normoxic conditions and test their respective potential to recreate the disease in secondary recipients. (B) Transduction of c-Kit+ BM cells from a conditional knock-out mouse model for HIF-1α (Hypoxia inducible factor-1α, the main transcription factor involved in hypoxia signaling in HSCs) with fusion gene AML-ETO9a and transplantation into recipient mice to generate AML in vivo. This allows the investigation of the role of HIF-1α in disease initiation in primary and secondary recipients.

P1095 - OPAL1: FROM B CELL ALL MARKER TO E3 UBIQUITIN LIGASE ADAPTOR

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Ubiquitination as a system for orchestrated degradation of proteins has been recognized for a long time. It is involved in the regulation of a number of cellular processes, including protein turnover and trafficking, as well as cellular signal transduction. Ubiquitination is a multi-step process in which the final step, the conjugation of ubiquitin to the target protein, requires the activity of a member of E3 ubiquitin ligase family. Activity of E3 ubiquitin ligases and their interactions with various substrates are regulated in many cases by adaptor proteins. We describe an identification of a novel adaptor protein for NEDD4 family E3 ubiquitin ligases, Outcome Predictor of Acute Leukemia 1 (OPAL1). OPAL1 interacts with several members of NEDD4 family and this interaction enhances their activity. By recruiting ITCH or another NEDD4 family member OPAL1 is involved in the regulation of chemokine receptor CXCR4 in leukocytes, likely by mediating ubiquitination of CXCR4 followed by lysosomal degradation of this receptor. ShRNA-mediated downregulation of OPAL1 expression resulted in significant increase in CXCR4 signaling after stimulation with CXCR4 ligand SDF-1α in murine macrophage progenitors, in 32D cell line (murine myeloblast-like), as well as in REH cell line (human pre B ALL, TEL-AML1+). This phenotype was also associated with increased chemotaxis towards SDF-1α. Since expression of OPAL1 was allegedly associated with better prognosis of TEL-AML+ B cell ALL patients, its involvement in CXCR4 regulation could, at least partially, explain potentially better outcome of the treatment and the disease recurrence. Altogether, our data suggest the role of OPAL1 in the regulation of CXCR4 expression and/or activity via the recruitment of NEDD4-family ubiquitin ligases.

P1096 - IDENTIFICATION OF JAK2 DEPENDENT TRANSCRIPTIONAL REGULATORS IN CML

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Chronic myeloid leukaemia (CML) is characterised by the t(9;22)(q34;q11) chromosomal translocation which gives rise to the active tyrosine kinase fusion protein BCR-ABL, responsible for driving the disease from the haemopoietic stem cell through to mature myeloid cells. The JAK2 pathway is activated in CML through several mechanisms and has effects on numerous transcription factors and cell cycle regulators, making it an attractive additional target for further treatment in CML. Following bioinformatics analysis of microarray data obtained from the literature, we identified a transcriptional regulatory network downstream from JAK2 and generated a linked gene interaction map. The key genes identified control multiple cell functions which could be affected by deregulation of JAK2 signalling. Using Fluidigm technology this network was analysed in CML patient peripheral blood mononuclear cells (PB-MNC) and compared to normal PB-MNC by real time PCR. Data was obtained on 29 transcriptional regulation genes, of these 45% were up-regulated including regulators of cell cycle (*CDKN1B, CDK1, FOXM1*), differentiation and proliferation (*TFDP1, EZH2*), 10% were down-regulated including regulators of apoptosis (*TNFSF10*), and 45% were unchanged including *HSP90* and *PI3K*. Treatment of K562 cells using concentrations of JAK2 inhibitors based on IC50 experiments showed concentration dependent reversal in the up-regulated genes (31% for AT9283, 46% for TG101209 and 46% for ruxolitinib treated cells), and in the down-regulated genes (33% for AT9283, 67% for TG101209 and 67% for ruxolitinib treated cells). Preliminary data in CML CD34+ patient samples treated with single agent nilotinib and ruxolitinib or combination followed a similar pattern, but requires further validation. Overall this study has identified an important transcriptional network downstream of activated JAK2 which is altered in CML and can be corrected by JAK2 inhibition.

P1097 - DECLINED PRESENTATION
STAT5 - A CENTRAL PLAYER IN BCR/ABL+ LEUKEMIA

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It is beyond doubt that the transcription factor STAT5 has an important and unique role in BCR-ABL- driven neoplasias. STAT5 is an essential component in the signaling network that maintains the survival and growth of BCR-ABL1-transformed cells. The transcriptional activity of STAT5 is required to allow proliferation and survival of the cells. Furthermore, we and others have shown that STAT5 is up-regulated in leukemic cells of CML patients during disease progression; we propose a selection process resulting in cells with high STAT5 expression levels which cause reduced responsiveness towards tyrosine kinase inhibitors such as imatinib even in the absence of BCR-ABL1 mutation. Remarkably, we also found a clear correlation between STAT5 expression levels and the presence of BCR-ABL1 mutations. Our data indicate that STAT5 triggers ROS production accounts for the enhanced mutation rate detected in human patient samples harboring elevated STAT5 levels. Accordingly, STAT5 qualifies as a potential therapeutic target; one possibility to inhibit its transcriptional activity is to hinder post-translational modifications. For full transcriptional activity STAT5 requires tyrosine phosphorylation in the C-terminal domain which is controlled by JAK kinases in untransformed hematopoietic cells. Whether JAK2 functions as an upstream kinase of STAT5 in CML is still under debate. Although there is widespread agreement that JAK2 is part of the signaling network downstream of BCR-ABL1, it is unclear whether and under what circumstances JAK2 inhibitors may be beneficial for CML patients. Our studies in murine models have cast doubt on the importance of JAK2 in CML maintenance. Strikingly, in BCR-ABL1-transformed cells signaling is rewired and the BCR-ABL1 oncoprotein itself accounts for STAT5 phosphorylation - independent of JAK kinases. Additionally, STAT5 is phosphorylated on highly conserved serine residues present in the transactivation domain of the protein. Novel insights in the role of serine phosphorylation for BCR-ABL1-driven leukemogenesis will be provided.

P1098 - A ZEBRAFISH MODEL OF FMS-LIKE TYROSINE KINASE 3 (FLT3) IN DEFINITIVE HEMATOPOIESIS AND HUMAN ACUTE MYELOID LEUKEMIA

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FMS-like tyrosine kinase 3 (FLT3) is expressed in human hematopoietic stem and progenitor cells but its role during embryogenesis is unclear. In acute myeloid leukemia (AML), internal tandem duplication (ITD) of FLT3 at the juxtamembrane (JMD) and tyrosine kinase (TKD) domains (FLT3-ITD+) occurs in up to 30% patients and is associated with inferior clinical prognosis. Mutations at the TKD (FLT3-TKD+) occur in about 5% cases. We made use of zebrafish to examine the role of flt3 (zflt3) in developmental hematopoiesis and model human FLT3-ITD+ and FLT3-TKD+ AML. zflt3 JMD and TKD were remarkably similar to its mammalian orthologs. Morpholino knock-down significantly reduced the expression of l-plastin (monocyte/macrophage), c-myb (definitive HSC), lck (T-lymphoid) and rag1 (immature T and B-lymphoid). Expressing human FLT3-ITD in zebrafish embryos resulted in expansion and clustering of myeloid cells (pu.1+, mpo+, and cebpα+) which were ameliorated by AC220 and associated with stat5, erk1/2, and akt phosphorylation. Expressing human FLT3-TKD (D835Y) induced significant, albeit modest, myeloid expansion resistant to AC220. This study provides novel insight to the role of flt3 during normal hematopoiesis and establishes a zebrafish model of FLT3-ITD+ and FLT3-TKD+ AML that may facilitate high throughput screening of novel and personalized therapeutic agents for this disease.

P1099 - CLONAL OR INFLAMMATORY INTERLEUKIN-6 LEVELS IN ESSENTIAL THROMBOCYTHEMIA

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The essential thrombocythemia (ET) is characterized by a state of chronic inflammation that may be an initiator and a driver of clonal myeloproliferation. It is reported that the JAK2V617F-positive cells are more sensible to chronic inflammation that the JAK2V617F-negative cells. Interleukin-6 (IL.-6) is a prooncogenic inflammatory cytokine elated to myeloproliferation. Therefore, we evaluated platelets, red blood cell (RBC), haemoglobin (Hb) concentration, hematocrit (HCT) and white blood cell (WBC), as myeloproliferative markers, fibrinogen (Fg), as inflammatory indicator, JAK2V617F mutation and IL-6. We recruited 82 patients with ET who fulfilled WHO criteria. Their mean duration of disease was 10 years (range, 3-20 years). Of 82 ET patients, 45 were JAK2V17F mutated (15 males and 30 females, mean age 65 years) and 37 were JAK2V617F WT (17 males and 20 females, mean age 62 years). All patients were on aspirin. Eighty-seven healthy subjects served as controls. Platelets, RBC, Hb, HCT and WBC were measured by automated analyzer. Fg and IL-6 were measured by Clauss and ELISA assay, respectively. The JAK2 mutated patients had higher platelets, RBC, Hb, HCT and WBC (920 ± 252x109/L, 5.64 ± 0.88x106/L, 14.8 ± 1.6 g/dl, 45 ± 4%, 10 ± 2.8x109/L) than JAK2 WT patients (7802 ± 204x109/L, 4.48 ± 0.93x106/L, 13 ± 1.8 g/dl, 40 ± 4%, 7 ± 2.7x109/L) ($p = 0.031$, $p < .0.0001$, $p < .0001$, $p < .0001$, $p < .0001$), whereas the JAK2 WT patients had higher Fg (411 ± 98 mg/dl) than JAK2 mutated patients (285 ± 61 mg/dl) ($p < .0001$). The JAK2 mutated patients and JAK2 WT patients had elevated IL-6 (80 ± 28 pg/ml and 83 ± 43 pg/ml, respectively, vs 10.5 ± 1 pg/ml) ($p < .0001$). These results suggest that IL-6 may facilitate clonal expansion in JAK2 mutated ET patients and represent an unfavourable prognostic indicator.

P1100 - TOWARDS AN UNDERSTANDING OF THE BIOLOGICAL SIGNIFICANCE OF INCREASED IL-3Rα EXPRESSION IN ACUTE MYELOID LEUKAEMIA STEM CELLS

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The interleukin-3 receptor α chain (IL-3Rα) is a biomarker for leukemia stem cells (LSC) in patients with acute myeloid leukemia (AML) due to its enhanced expression on AML LSC compared to normal haematopoietic stem cells. Given the survival and proliferation promoting activities of IL-3, we hypothesise that increased IL-3Rα confers a biological advantage to AML LSC. Initial studies focused on a model, in which the leukaemic cell line TF-1 was used to create additional lines expressing increased IL-3Rα. Results demonstrate that cells with high IL-3Rα have increased proliferative capacity, survival properties and signaling in response to low dose IL-3. Most strikingly, when we co-cultured cells with different levels of IL-3Rα, and determined the proportion of each over time, IL-3Rα high cells had a clear competitive advantage in IL-3 but not GM-CSF. To determine the advantage of high IL-3Rα *in vivo* we are conducting competitive repopulation assays in mice. To understand the underlying mechanism through which IL-3Rα provides a biological advantage we used mutagenesis to identify critical sites within IL-3Rα and identified a proline rich motif which presents as a potential PI3-Kinase interaction site. By creating proline to alanine substitutions, we demonstrated a novel association between PI3-Kinase and IL-3Rα, which was disrupted when the prolines were mutated. Using IL-3 response assays we have shown functionally that this site is required for normal IL-3 signaling. Preliminary evidence suggests the signal from these pathways may be important in cell metabolism. Our data reveals the proliferative, survival and growth advantages of increased IL-3Rα expression and provides evidence for IL-3Rα eliciting a signal from IL-3 stimulation. These studies, supported by *in vivo* cell growth and leukemia models, may define the biological role of enhanced IL-3Rα expression and its contribution to leukaemogenesis.

P1101 - DECLINED PRESENTATION
KRASG12D INITIATES ACUTE T-CELL LYMPHOBLASTIC LEUKEMIA IN COOPERATION WITH LOSS OF THE WILD-TYPE KRAS ALLELE

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Activating mutations in RAS are frequently found in patients with both myeloid and lymphoid leukemia. Activation of KRAS in hematopoietic malignancy has been studied using the Mx1-Cre;KrasLSL-G12D mouse model, where expression of oncogenic KrasG12D is induced in the bone marrow and other interferon responsive tissues. Activation leads to rapid development of myeloproliferative disease (MPD). Surprisingly, when bone marrow from these MPD-mice is transplanted to wild-type recipients this induces acute T-cell lymphoblastic leukemia (T-ALL). The cause for this lineage switch is not known. Activation in the primary mice leads to expression of KrasG12D also in some non-hematopoietic tissues which may affect the malignant phenotype. Alternatively, during transplantation, KrasG12D tumor initiating cells of myeloid and T-lymphoid lineage might home differently. To determine the cause of this lineage switch and to investigate possible differential effects of Kras-activation in myeloid and T-lymphoid cells we have performed cross bone marrow transplantations between Mx1-Cre;KrasLSL-G12D and wild-type mice. Our results show that KrasG12D drives proliferation and differentiation in myeloid cells which leads to induction of MPD in the primary mice. In parallel, expression of KrasG12D expands immature T-cell populations leading to a pre-leukemic state in T-cells. Also, pIpC injected Mx1-Cre;K-rasLSL-G12D cannot be rescued by transplanting wild-type bone marrow meaning that the cause of the rapid death in the primary mice is unrelated to their MPD. Transplanting the KrasG12D bone-marrow extends the lifetime of the tumor, creating time for additional mutations to occur that transforms the pre-leukemic state into T-ALL. One of these additional mutations is loss of the wild-type Kras allele. In conclusion, Kras-activation increases proliferation and differentiation which induces MPD without the ability to transform into AML. In T-lymphoid cells Kras-activation leads to a pre-leukemic state that with time and additional mutations transforms into T-ALL. Also, loss of the wild-type Kras allele in the T-ALL tumors indicates a tumor-suppressive role for wild-type KRAS in T-cell tumorigenesis.

P1102 - ECTOPIC EXPRESSION OF TIRAP IN MURINE MARROW RESULTS IN MARROW FAILURE MEDIATED BY INTERFERON-γ

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The Myelodysplastic Syndromes (MDS) are characterized by ineffective hematopoiesis in one or more lineage of the bone marrow, resulting in peripheral cytopenias and the propensity to develop into either acute myeloid leukemia (AML) or bone marrow failure (BMF). The molecular and cellular events involved in MDS development are yet to be elucidated. Previous work from our lab has shown that haploinsufficiency of miR-145 and miR146a recapitulates some of the characteristics associated with del(5q) MDS. Pathway analysis predicts that innate immune signaling is among the most highly deregulated pathways following loss of these two miRNAs. The innate immune signaling moleculesTRAF6 and TIRAP are targets of miR-146a and miR-145 respectively. While the role of TRAF6 in del(5q) MDS has been investigated, little is known about the role of TIRAP in MDS pathogenesis. To investigate the role of TIRAP in aberrant hematopoiesis, we transplanted lethally irradiated mice with marrow cells expressing TIRAP or vector control. Similar to low risk MDS patients, the marrows of TIRAP transplanted mice are characterized by increased apoptosis as measured by Annezin V staining. Furthermore, they develop BMF characterized by pancytopenia as early as 4 weeks post-transplant (unlike TRAF6 transplanted mice which succumb to BMF or AML approximately 4 months post-transplant). In MDS, normal hematopoiesis is blocked in the normal fraction of the marrow by autoimmunity associated cytokines. We performed expression profiling to identify factors responsible for suppression of normal hematopoiesis in our BMF model. Q-RT-PCR showed increased expression of both IL-10 and IFNγ in TIRAP expressing marrow compared to vector control. This increase in cytokine expression occurs in a TRAF6 independent manner suggesting the involvement of TIRAP in a non-canonical signaling pathway. Interestingly, IFNγ and IL-10 have been implicated in MDS and other BMF conditions. Using IL-10 and IFNγ KO mice, we found that loss of IFNγ but not IL-10 rescues the pancytopenia phenotype and prevents early death due to BMF. Interestingly, mice transplanted with TIRAP expressing IFNγ KO bone marrow succumb to a myeloproliferative disorder at later time points, suggesting that TIRAP activates both myelosuppressive pathways mediated by IFNγ as well as myeloproliferative pathways.

P1103 - TARGETING SPHINGOSINE KINASE 2 SUPPRESSES MYC EXPRESSION AND KILLS ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

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Sphingosine kinases, of which there are two isoforms, catalyze the synthesis of sphingosine 1-phosphate (S1P) from sphingosine. While sphingosine kinase 1 has indisputable tumor-promoting properties, the role of sphingosine kinase 2 in cancer is less clear. We show that sphingosine kinase 2 plays an oncogenic role in acute lymphoblastic leukemia by influencing the expression of the oncogene MYC. Sphingosine kinase 2 protein was over-expressed in leukemic cell lines and patient samples, although there was no change in gene expression. Genetic loss of sphingosine kinase 2 impaired leukemia development in a murine model, while pharmacological inhibition significantly extended survival in mouse xenograft models of human disease. Inhibition or genetic deletion of sphingosine kinase 2 reduced expression of MYC in leukemic cells. This was associated with reduced association of acetylated histone H3 with the MYC gene and lower expression of the Myc proto-oncogene protein (c-Myc) and c-Myc regulated genes. Direct inhibition of c-Myc resulted in the death of acute lymphoblastic leukemia cells, supporting a direct role for c-Myc in the demise of leukemic cells exposed to sphingosine kinase 2 inhibitors. This demonstrates that sphingosine kinase 2 can regulate MYC, which plays an important role in hematological malignancies, and therefore this pathway may prove a useful therapeutic target in this disease. The sphingosine kinase 2 inhibitor, ABC294640, is in clinical trial for solid tumors making the potential translation of this approach feasible.

P1104 - DEVELOPMENT OF TARGETED THERAPIES FOR ACUTE MYELOID LEUKEMIAS

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Acute myeloid leukaemia (AML) accounts for approximately 25% of all leukemias. The current standard of treatment for AML involves initiation and consolidation chemotherapy followed by stem cell transplantation. Responses to this therapy vary however and, even if effective, most patients will relapse within 2-3 years. The need to develop a more targeted approach to treat this vastly heterogenous disease is of high significance. We have established several pre-clinical mouse models of AML that are caused by rearrangements of either of the two core binding factor (CBF) genes: AML1 (t(8;21)); or CBFβ (Inv16/t(16;16)), or the mixed lineage leukemia gene (MLL) on chromosome 11. These rearrangements are analagous in that they result in the formation of oncogenic fusion proteins that are able to abberantly recruit epigenetic modifiers. This epigenetic alteration results in the transcriptional dysregulation of the myeloid lineage, which is critical to the pathogenesis of the disease. The development of individualised therapies that target molecular events that underpin leukemogenic transformation in AML will likely result in improved patient response. These models provide us with the opportunity to validate such therapeutic strategies. Deacetylase inhibitors (DACi) target the activity of histone deacetylases (HDACs) altering the acetylation of histones and other proteins. Treatment with the DACi panobinostat (LBH-589) resulted in a clear survival benefit in mice harboring tumors possessing CBF rearrangements compared with MLL, highlighting the need for HDACs in CBF leukemias. The effects that we observe include degradation of the fusion protein and the transcriptional reactivation of mediators associated with cell cycle arrest and terminal myeloid differentiation. These studies indicate DACi may be used as rational therapeutics for the treatment of CBF AML and offers a more tailored approach to the treatment of these leukemias.

P1105 - E2F1 POSITIVELY REGULATES TRIB2 PSEUDOKINASE EXPRESSION AND PROLIFERATION IN ACUTE LEUKAEMIA

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Deregulation of the transcription factor E2F1 occurs in AML, and it has been shown to induce both cell cycle progression and apoptosis. In normal granulopoiesis, proliferation arrest and differentiation mediated by C/EBPalpha involves repression of E2F1 target genes. Elevated Trib2 expression has been linked with a subset of human AML and dysregulated C/EBPalpha. Using promoter assays, mutational analyses and chromatin immunoprecipitation experiments, we show that E2F1 (and E2F2, E2F3, but not E2F4 or E2F5) are bound directly to the DNA on site-specific regions on the Trib2 promoter in leukaemic cells. Trib2 expression is decreased following siRNA-mediated knockdown of E2F1, and in E2F1 knockout cells as compared to wild type cells. The reintroduction of E2F1 rescued Trib2 expression showing that E2F1 is regulating the expression of endogenous Trib2. Further analyses revealed that this activation of Trib2 by E2F1 is repressed by wild type C/EBPalpha consistent with C/EBPalpha having a negative regulatory role on E2F1, and in normal GMP cells we detect C/EBPalpha bound to the Trib2 promoter. Conversely there was synergistic activation upon coexpression of the oncogenic C/EBPalpha truncated mutant, and Trib2 expression levels were elevated in GMPs from preleukaemic mutant C/EBPalpha mice compared to wild type GMPs. Indeed a positive correlation between Trib2 and E2F1 expression in AML datasets support these findings. Finally, inhibition of the cell cycle pathway in leukaemia cells expressing high endogenous levels of Trib2 protein resulted in G1 arrest with a reduction in E2F1 levels and Trib2 protein levels. Our work indicates that the cell cycle regulator E2F1 plays a key role in the control of Trib2 expression important for the control of cell proliferation and may have important implications for normal and malignant haematopoiesis.

P1106 - CBFB-MYH11/RUNX1 TOGETHER WITH A COMPENDIUM OF HEMATOPOIETIC REGULATORS, CHROMATIN MODIFIERS AND BASAL TRANSCRIPTION FACTORS OCCUPIES SELF-RENEWAL GENES IN INV(16) ACUTE MYELOID LEUKEMIA

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The inversion of chromosome 16, inv(16)(p13q22), is associated with 5% of acute myeloid leukemia's. This inversion generates a chimeric gene CBFB-MYH11, which encodes a fusion protein between CBFβ and smooth muscle myosin heavy chain (SMMHC/MYH11). Since its discovery, many different mechanisms for CBFB-MYH11 function in inv(16) AML have been proposed, most of them associated with transcriptional repression of RUNX1 target genes. These mechanisms include tethering of RUNX1 outside the nucleus, interference with transcription factor complex assembly and recruitment of histone deacetylases. In this study we use ChIP-seq, RNA-seq and quantitative interaction proteomics to analyze these molecular roles at the genome-wide level. We find that CBFB-MYH11 localizes to RUNX1 occupied promoters where it interacts with TBP associated factors (TAFs) in the context of a variety of hematopoietic transcription factors as well as epigenetic coregulators including TAL1, FLI1, ERG, GATA2, PU.1/SPI1, EP300 and HDAC1. shRNA mediated CBFB-MYH11 knock down in combination with RNA-seq analysis reveals that a small subset of the fusion protein target genes is involved in transcriptional repression, while the majority of CBFB-MYH11 target genes, including genes implicated in hematopoietic stem cell (HSC) self-renewal such as ID1, LMO1 and JAG1, is involved in transcriptional activation. Together these results suggest an essential role for CBFB-MYH11 in regulating expression of genes involved in maintaining a stem cell phenotype.

P1107 - CELLULAR ASPECTS OF MYELOFIBROSIS DEVELOPMENT ACCESSED IN VITRO WITH HUMAN PLATELET LYSATE

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Myelofibrosis remains to be a general problem, which cannot be solved easily The purpose of our study is to characterize human platelet lysate (HPL) from patients with primary myelofibrosis (PMF) and post-polycythaemia vera myelofibrosis (post-PV MF)and to explore its influence on physiology of mesenchymal stromal cells (MSC).
Methods: HPL was produced from the pooled platelet concentrate of healthy donors and platelet-rich plasma of patients with PMF and post-PV MF; patients' HPL was normalized according to protein concentration. MSC from bone marrow of healthy donors were cultured in presence of varying concentrations of HPL (5 - 20%) or 10% fetal bovine serum (FBS) as negative control. Proliferation, protein concentration, osteogenic and adipogenic differentiation of MSC were measured, as well as concentration of VEGF, bFGF, TGFβ and HGF in HPL from 17 patiets. Results We observed a significant increase in VEGF and bFGF concentration (2,5 and 2,4 fold, respectively $p < 0.01$) in HPL from patients with myelofibrosis, compared to age-matched healthy donors. Average level of TGFβ and HGF was also higher in patients group, but the difference was statistically insignificant ($p = 0{,}2$). In case of culturing MSC with addition of HPL from patients with myelofibrosis, we found that cells maintained their proliferative activity compared to the HPL from age-matched healthy controls (optical density - 0,155 and 0,145, accordingly, $p = 0{,}17$), did not reach significant difference. In the experiments with donor's HPL we found, that amount of extracellular matrix was highest, using 15% HPL. Osteogenic differentiation did not differ in HPL, but adipogenic lineage differentiation decreased. **Conclusions:** IWe made a system that reproduces bone marrow stromal cells changes in myelofibrosis using HPL, including HPL from patients with myelofibrosis. Our data demonstrate that it can be used as a substrate for culturing of mesenchymal stromal cells to study the mechanisms of myelofibrosis, as it contains increased concentration of growth factors and induces profibrotic changes in MSC.

P1108 - ABSENCE OF THE SHB ADAPTOR PROTEIN ACCELERATES LEUKEMIA PROGRESSION BY CONTROL OF CYTOKINE RESPONSES

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Leukemic progression is highly dependent on cytokine secretion and the cytokine responses within the leukemic cell population. Increased production of hematopoietic cytokines such as G-CSF and IL-6 has for instance been demonstrated as a key element promoting the development of leukemia. Shb is a widely expressed, SH2-domain containing, adaptor protein downstream of active tyrosine kinase receptors that assembles signaling complexes consisting of numerous signaling components of relevance to hematopoietic cell function, of which FAK stands out as particularly important. Absence of Shb was recently shown to reduce hematopoietic stem cell (HSC) proliferation and thus we sought to investigate Shb's role in the progression of leukemia. After transformation of wild type and Shb null bone marrow (BM) cells with a retroviral BRC-Abl construct and subsequent transplantation to wild type recipients it was noted that the Shb knockout BCR-Abl transformed cells produced a more aggressive disease with death occurring at an earlier time point. These mice exhibited an elevated white blood cell count at the time of death and this coincided with an increased relative number of mature neutrophils. In the BM of mice that received the Shb knockout BCR-Abl transformed cells, the c-Kit+Lin-BCR-Abl+ population of cells displays significantly increased proliferation and reduced apoptosis as determined by FACS analysis after staining for Ki-67 and cleaved caspase 3. Cytokine hyper-responsiveness is a characteristic of aggressive leukemias; the Shb knockout BM response to a gradient of GM-CSF was significantly elevated compared to the wild type at lower cytokine concentrations. Moreover, significantly elevated G-CSF mRNA was observed in c-Kit+ cells of the Shb knockout BM after BCR-Abl transformation. Intracellular signaling events were also altered in these cells as demonstrated by elevated phosphorylation of STAT3 and FAK. Further studies are required to determine Shb's exact position in the network that regulates hematopoietic cytokine expression and signaling, but the data implicates Shb as an important modulator of leukemic disease progression with particular relevance to neutrophil dysplasia.

P1109 - IDENTIFICATION OF MEIS-PBX INHIBITORS AS POTENTIAL ANTI-LEUKEMIC AGENTS USING A HIGH-THROUGHPUT BRET-BASED ASSAY

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The deregulated expression of HOX genes and their MEIS1/PBX co-factors represents one of the most frequent molecular anomalies in human acute myeloid leukemia (AML), most prominently in those with chromosomal translocation in the MLL gene. In mouse genetic models, over-expression of MEIS1 significantly accelerates the onset of HOX-induced AML, a unique function that requires heterodimerization with its PBX cofactor. Importantly the expression of MEIS1 is critical for MLL-fusion-induced leukemogenesis, suggesting that MEIS1 inhibition represents a good therapeutic target for AML treatment.
We propose to identify chemical compounds that disrupt the MEIS1-PBX1 interaction using a Bioluminescence Resonance Energy Transfer (BRET)-based high-throughput assay. The BRET technique monitors protein-protein interaction by employing energy transfer from a bioluminescent enzyme (e.g. luciferase) to a fluorescent protein (e.g. GFP) in close proximity, yielding an interaction-specific light emission signal.
Transient expression of LUC-MEIS1 and GFP-PBX1 in HEK293 cells resulted in a specific BRET signal of 0.348 ± 0.015 (A.U.), whereas expression of an interaction-deficient MEIS1 mutant resulted in a non-specific BRET signal of 0.170 ± 0.007 (A.U.). The assay, with a Z-factor of 0.81, is robust enough for HTS and the library of 100,000 compounds available at IRIC is currently being tested. A pilot "run" already processed 2880 compounds, of which 11 reduced the BRET ratio without perturbing the individual LUC or GFP signal.
The potential inhibitors will then be tested for their ability to prevent co-IP of MEIS1-PBX1 using a modified Luciferase ImmunoPrecipitation System (LIPS) assay. The validated hits will then go through a secondary screen monitoring cell proliferation of human AML samples expressing various levels of HOX/MEIS/PBX. In all, this screen will initiate mechanistic-based preclinical studies that could lead to therapeutic interventions targeting the activity of this complex.

P1110 - STARVATION PROMOTES DECREASE OF SURVIVIN GENE EXPRESSION IN HUMAN LYMPHOMA CELLS LINES

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Background: The survivin gene is a member of the IAP family, which is commonly overexpressed in lymphoma cell lines. This phenomenon has been correlated with resistance to radiotherapy-induced apoptosis and chemotherapy. Recently survivin gene expression was identified as an independent prognostic factor of poor clinical outcome. **Objective:** In vitro evaluation of survivin gene expression from four different human lymphoma cell lines cultured in the absence of FBS for up to 72 h **Methods:** Molt-4 (acute lymphoblastic leukemia, ATCC CRL-1582), K562 (chronic myelogenous leukemia, ATCC CCL-243), Raji (Burkitt's lymphoma, ATCC CCL-86) and Jurkat (T-cell leukemia, ATCC TIB-154) were cultivated in triplicate in the absence of FBS for up to 72h. RNA was extracted by Trizol method. cDNA was synthesized using a High Capacity cDNA Reverse Transcription kit and qPCR was performed using TaqMan system. Gusb expression was used as reference control. For quantification of the gene expression a relative curve was performed using cDNA from Lucena cell line (K562/VCR). Data were evaluated by ANOVA using the Prism 5.0 software (GraphPad, San Diego, CA) **Results:** The basal expression of the survivin was: 181.88 relative units (UR) in Raji cell line; 176.47UR in Molt-4 cell line; 136.92UR in Jurkat cell line and 85.33UR in K562 cell line. The absence of FBS decreased survivin expression in the Raji, Jukart and K562 cells in a time-dependent way while expression in Molt-4 was not affected. However, decreased expression was different among cells. In the Jukart cells the decrease was detected after 48h and remained low after 72h. In the Raji and K562 cells the expression reduction was observed after 24h with a further decrease after 72h. **Conclusion:** Starvation promotes decrease of survivin gene expression in human lymphoma cells lines. Supported by CAPES, INCT and FINEP

P1111 - TRK-SIGNALING, NOTCH MUTATIONS AND PTEN LOSS CONVERGE ON MTOR IN T-ALL AND CAUSE ADDICTION TO CAP DEPENDENT MRNA TRANSLATION

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High activation of the mTOR pathway is characteristic for T-cell acute lymphoblastic leukemia (T-ALL) and has been linked to drug resistance and relapse. The activity of the master regulator of this pathway, PTEN, is often impaired in T-ALL. However, even in the absence of PTEN, the PI3-Kinases which link receptor tyrosine kinases and PIP3 signaling, are critical for T-ALL formation in vivo. We recently demonstrated the expression of Neurotrophin receptor tyrosine kinases (TRK) and their respective ligands in T-ALL. To investigate the contribution of TRK-signaling to T-ALL pathogenesis, we expressed constitutively active TRK receptors in murine hematopoietic stem cells. Transplantation of TRK-modified cells efficiently elicited transplantable T-ALL. We show that activating Notch mutations and PTEN loss are cooperating events arising during clonal evolution of TRK-induced T-ALL. All three events independently converged on the PI3K-AKT-mTOR pathway and caused a profound shift in the signaling network towards mTORC2-AKT. The molecular differences between T-ALL blasts displaying hyperactive mTOR and normal thymocytes included the upregulated expression of mRNAs encoding proteins important for transcription, translation and mitochondrial metabolism. We found that TRK+ PTEN- Notch+ T-ALL were highly susceptible to 4EGI-1, a recently developed inhibitor of cap dependent translation. T-ALL clones displaying low mTOR activation as well as hematopoietic progenitors and freshly isolated thymocytes were far less susceptible. Polysomal profiling revealed that mRNAs that were strongly upregulated in T-ALL compared to normal thymocytes as well as D-Cyclins, Bcl-2 and c-myc were strongly shifted out of the actively translated transcriptome by 4EGI-1. In vivo administration of 4EGI-1 abrogated c-myc expression completely and caused apoptosis of tumor cells with minimal side effects. Since c-myc is difficult to target and has been implied in maintenance of T-ALL initiating cells, we demonstrate that cap-dependent translation is a promising target in T-ALL.

P1112 - DECLINED PRESENTATION UNRAVELLING A NOVEL MECHANISM OF STAT5 REGULATION IN ONCOGENIC FLT3 INDUCED ACUTE MYELOID LEUKEMIA

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Among various hematological malignancies, treatment strategies for oncogenic Flt3 (Flt3ITD) induced acute myeloid leukemia (AML) remain inefficient due to patients relapsing with acquired resistant mutations on Flt3ITD. Thus targeting novel signaling molecules in Flt3ITD pathways remain a viable therapeutic option. Among various pathways downstream of Flt3ITD, the role of Stat5 has been extensively studied. However, the mechanism behind the translocation of active Stat5 into the nucleus of cells bearing Flt3ITD mutation to induce transformation remains unclear. Here we provide in vitro and in vivo pharmacological, biochemical and genetic evidence to demonstrate that a FAK/Tiam1-Rac1/PAK1 signaling axis plays an essential role in Flt3ITD induced AML. We observed hyperactivation of FAK in Flt3ITD expressing leukemic cells and human AML patient samples, and also activation of Rac1 downstream of FAK that was downregulated upon treatment with FAK inhibitors. We next ascertained the underlying mechanism of FAK mediated activation of Rac1 in Flt3ITD expressing cells and observed RacGEF Tiam1 to be hyperactive in Flt3ITD bearing cells, which was downregulated upon pharmacological inhibition of FAK. More importantly, expression of Flt3ITD in Rac1-/- or FAK-/- deficient bone marrow cells, showed inhibition of Stat5 activation and its failure to translocate into the nucleus. Similar results were also observed upon shRNA mediated knockdown of Tiam1. To determine a downstream effector from Rac1, we observed an essential role of p21-activated kinase 1 (PAK1), where shRNA mediated knockdown of PAK1 not only inhibited the nuclear translocation of Stat5, it also significantly delayed the onset of AML in vivo, while inhibition of PAK2 showed no such effect. Finally, inhibition of FAK, Tiam1 and PAK1 significantly delayed the onset of AML in the in vivo mouse models along with repression of Stat5 responsive genes involved in survival of leukemic cells. Overall our study indicate an essential role of FAK/Tiam1-Rac1/PAK1 signaling axis in Flt3ITD mediated proliferation, survival and leukemogenesis; and also demonstrates a novel mechanistic role of FAK, Tiam1 and PAK1 in translocating active Stat5 into the nucleus to induce leukemogenic transformation.

P1113 - EFFECTIVELY TARGETING TREATMENT-NAïVE CML STEM/PROGENITOR CELLS FROM IMATINIB-NONRESPONDERS WITH COMBINATION TREATMENTS OF NEW JAK2 AND ABL INHIBITORS IN VITRO AND IN VIVO

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Introduction of Imatinib Mesylate (IM) and other tyrosine kinase inhibitor (TKI) therapies has had a major impact on treatment of chronic phase CML, but early relapses and persistence of leukemic stem cells remain problematic. We recently identified a new AHI-1-BCR-ABL-JAK2 protein complex that contributes to the transforming activity of BCR-ABL and IM-resistance of CML stem/progenitor cells. We have therefore hypothesized that combined suppression of BCR-ABL and JAK2 activities might be more effective in eliminating primitive CML cells in vitro and in vivo. We have now examined the biological effects of an orally bioavailable, selective JAK2 inhibitor (BMS-911543) in combination with TKIs, including IM, dasatinib (DA) and nilotinib, on treatment-naïve CML IM-nonresponder cells. Intracellular staining analyses showed that combined exposure of CD34+ CML cells to BMS-911543 and a TKI produced a deeper, more prolonged suppression of pSTAT5 activity than single agents (2.5 fold, $p<0.05$). Interestingly, combination treatment resulted in greater inhibition in colony growth compared to single agents (74-86% vs. 40-50%, $p<0.05$). Long-term culture-initiating cell assays showed that the more primitive cells were also more significantly eliminated by combination treatments (2-3 fold, $p<0.05$). Importantly, BMS-911543 is less toxic to normal bone marrow CD34+ cells (n=4) than CML samples (n=7, 2-3 fold, $p<0.05$). Finally, oral admission of BMS-911543 and TKIs significantly eliminated primitive CML cells and enhanced survival of leukemic mice compared to mice treated with single agents (median survival of IM + BMS-911543 vs IM: 70 days vs 53 days, $p<0.05$; DA + BMS-911543 vs DA: 85 days vs 78 days). This study indicates that simultaneously targeting BCR-ABL and JAK2 activities in CML stem/progenitor cells may improve outcomes in patients, especially those destined to develop TKI resistance.

P1114 - THE ROLE OF BIN1 TUMOR SUPPRESSOR ISOFORMS IN REGULATION OF PROLIFERATION AND APOPTOSIS OF HUMAN CUTANOUS T-CELL LYMPHOMA CELLS

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The two main types of cutaneous T-cell lymphomas (CTCLs) are mycosis fungoides (MF) and its leukemic variant Sezary Syndrome (SS). We recently demonstrated the importance of AHI-1 oncogene in tumorigenic activity of Sezary cells (Hut78) using in vitro and in vivo assays. BIN1 tumor suppressor was identified through microarray analysis as one of the genes that may be involved in AHI-1-mediated leukemic transformation in CTCL cells. BIN1 is a nucleocytosolic adaptor protein with more than ten isoforms; some isoforms, including the BIN1 isoform (+10, +13), act as tumor suppressors, but the BIN1 (+12A) isoform behaved as a cancer-related isoform in solid tumor models. However, the role of BIN1 in normal hematopoiesis and lymphomagenesis remains unknown. We have recently demonstrated that transcript levels of BIN1 isoforms are significantly lower in patients with MF or SS compared to controls. Four isoforms of BIN1 have been identified in Hut78 and primary CD4+CD7- Sezary cells. To investigate the role of BIN1 in CTCL, the BIN1 isoforms (+10, +13) and BIN1 (+12A) lentiviral constructs were transduced into Hut78 and HH cells. Overexpression of BIN1 isoforms led to a significant reduction in cell proliferation, as assessed by colony forming cell and 3H-Thymidine uptake assays (2-3 ford, $p<0.05$). Furthermore, a significant increase in spontaneous and specific apoptosis was observed in BIN1-transduced cells, with and without exogenous FAS-ligand (2-3 ford, $p<0.05$). Interestingly, a significant reduction in protein expression of c-FLIP (inhibitor of the FAS-mediated apoptosis pathway) and upregulation of downstream cleaved caspase-8 and caspase-3 was demonstrated in BIN1-transduced cells, suggesting that BIN1 isoforms induce apoptosis by downregulating the expression of c-FLIP, which leads to activation of the FAS-mediated apoptosis pathway. In addition, subcellular fractionation and confocal microscopy further shown that BIN1 (+10, +13) is localized in both the cytoplasm and nucleus, whereas BIN1 (+12A) is mostly located in the nucleus. These findings indicate anti-proliferative and pro-apoptotic roles for BIN1 isoforms in human CTCL cells.

P1115 - EPHA3 IS A THERAPY TARGET FOR LEUKAEMIA AND OTHER CANCERS

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Eph receptor tyrosine kinases (RTKs) interact with cell-surface ephrin ligands to direct cell migration and orchestrate developmental patterning during embryogenesis by modulating cell shape and adhesion. High expression in a number of cancers has been linked to progression through facilitation of invasiveness and metastatic spread. EphA3 protein, which was originally described in leukaemia, is also reported to be expressed in sarcomas, lung cancer, melanoma and glioblastoma. The EphA3-specific monoclonal antibody, IIIA4, binds and activates the human and mouse EphA3 with similar affinities. Binding is followed by internalization of receptor-antibody complexes. In a mouse xenograft model of leukaemia, LK63, administration of the IIIA4 antibody leads to inhibition of tumour growth and spread from bone marrow to the spleen and other organs and increases the latency of the disease. A more impressive anti-tumour effect is delivered by targeting of a bound radio-active isotope, illustrated by analpha particle emitting bismuth isotope-linked to IIIA4 in leukaemia or a beta particle-emmitting lutetium isotope linked to IIIA4 in glioma xenograft models. Importantly, little toxicity to normal tissues was observed as EphA3 is expressed at very low/no levels on normal tissues. A high-affinity recombinant human antibody, derived by Antibody Humaneering from the α-EphA3 mouse monoclonal antibody IIIA4 has been developed and shows enhanced Antibody-dependent cellular cytotoxicity (ADCC) against leukaemic targets. This antibody is now in a phase I trial in subjects with haematological tumours.

P1116 - MN1 REGULATES SELF-RENEWAL AND DIFFERENTIATION THROUGH DISTINCT DOMAINS

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Meningioma 1 (MN1) is an independent prognostic marker for normal karyotype acute myeloid leukemia, with high expression linked to all-trans retinoic acid (ATRA) resistance and poor overall and relapse-free survival. MN1 is also a potent and sufficient oncogene in murine leukemia, able to block myeloid differentiation, promote leukemic stem cell self-renewal and transform cells to the common myeloid progenitor level. Yet despite its established leukemic role, little is known about its mechanism of action. To elucidate the role(s) of each portion of MN1, we divided the gene into 7 regions of 200 amino acids. We cloned 16 mutants as distinct region deletions or cumulative deletions from the N- or C-termini. Mouse bone marrow was retrovirally transduced and assayed for (1) proliferation/self-renewal by CFU colony formation and *in vivo* engraftment; (2) erythro-megakaryocyte differentiation by red blood cell (RBC) engraftment and CFU-Mk colony formation; (3) myeloid/lymphoid differentiation by immunophenotypic analysis of transplanted mice; and (4) *in vitro* ATRA resistance. We show that the MN1 N-terminus is required for proliferation and self-renewal, as the N-terminus deletion (MN1Δ1) showed decreasing in vivo engraftment and did not induce leukemia after 181 days. MN1Δ1 cells contained a 1.9-fold higher fraction of GFP^+ cells in RBCs over white blood cells, compared to equivalent ratios in control and MN1 cells (n=5, $P<.05$), and preferentially formed CFU-Mk colonies *in vitro*, suggesting the N-terminus also blocks erythro-megakaryocyte differentiation. MN1 C-terminal regions are crucial to the myeloid differentiation block, as C-terminal deletions showed increased Gr1 and CD11b expression in GFP+ peripheral blood, delayed disease onset, and reversal of ATRA resistance to control levels (n=6, $P<.05$). Gene expression profiling showed C-terminally deleted MN1 cells clustering with $Gr1^+CD11b^+$ cells, underscoring the importance of the C-terminus in blocking myeloid differentiation. This study

demonstrates that the dual abilities of MN1 to promote self-renewal/proliferation and inhibit differentiation can be attributed to specific regions, providing insight into how the structure of MN1 affects its leukemia phenotype.

P1117 - CYCLIN-DEPENDENT KINASE INHIBITOR, DINACICLIB INDUCES ANTI-TUMOUR ACTIVITY OF MLL-AF9 IN VIVO MOUSE MODELS

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Translocations of the mixed lineage leukaemia (MLL) gene occur in 60-80% of all infant acute leukaemia's identified with poor prognosis. MLL-AF9 constitutes 85% of all clinical cases that induce ectopic expression of epigenetic target genes in hematopoietic precursor cells resulting in leukemic transformation. MLL fusion proteins recruit epigenetic regulatory proteins including histone deacetylases (HDACs), histone methytransferases (HMTs), bromodomain-containing proteins (eg Brd4) and/or transcription elongation factors (pTEFb). We hypothesize that dysregulated epigenetic activity underpins the onset and progression of AML driven by oncogenic fusion proteins such as MLL-AF9. Therefore targeting the epigenetic enzymes that drive this process or proteins that important for transcriptional function will result in therapeutic benefit. In order to identify key molecular targets for the treatment of this aggressive disease we conducted a small molecule inhibitor candidate screen to test the efficacy of pharmacological agents targeting HDACs (panobinostat), HMT Dot1L (EPZ470007), BRD4 (JQ1) and the pTEFb component, CDK9 (Dinaciclib) on MLL-AF9 +/- transduced mouse models. Dinaciclib identified itself to be the most potent inducer of apoptosis in MLL-AF9-driven tumour cells in vitro in contrast to conventional chemotherapies and recently published targeted therapies. Dinaciclib is a pan cyclin-dependent kinase inhibitor with a high affinity for CDK9 that drives transcriptional elongation via phosphorylation of RNA polymerase II (RNA Pol II). On-target specificity of Dinaciclib was confirmed by western blot showing a reduction in Ser-2 phosphorylation of RNA Pol II. Importantly, the growth inhibitory and pro-apoptotic effects in vitro of Dinaciclib corresponded with its predicted potency for CDK9 inhibition. Dinaciclib was well tolerated in vivo where it delayed the growth of MLL-AF9 tumours and significantly prolonged the survival of tumour-bearing mice. We propose that Dinaciclib will have potent and selective activity as a clinical candidate in poor-prognosis MLL-AF9 patient settings.

P1118 - DECLINED PRESENTATION
SOX7 WAS HIGHLY EXPRESSED IN CD34+ CELLS AND PROMOTED THE LEUKEMOGENESIS OF ALL

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Introduction: SOX (Sry-related HMG box) genes belong to a family of transcription factors containing a High-Mobility-Group box domain. In an initial screen of SOX genes in human leukemias, SOX7 is uniquely down-regulated in acute myeloid leukemia, myelodysplastic syndrome and chronic myelogenous leukemia but up-regulated in most cases of acute lymphoblastic leukemia. The observation led us to examine the expression and function of SOX7 in acute lymphoblastic leukemia (ALL). **Methods:** Mononuclear cells (MNCs) were isolated from ALL and fractionated by immunomagnetic selection and fluorescence activated cell sorting (FACS). SOX7 expression in different leukemia subsets was evaluated by RT-PCR. To examine its function, SOX7 siRNA was nucleofected to Nalm20 cells and gene knock-down was confirmed by RT-PCR and Western Blot. Apoptosis and cell cycle were analyzed by Annexin V/7AAD assay and flow cytometry. To examine the function of SOX7 in ALL, SOX7 was knocked down with shRNA, and Nalm20 cells transduced with shRNA were injected into NOD/SCID mice. 15 to 18 weeks later, the mice were euthanized and bone marrow cells from the mice were analyzed. **Results:** SOX7 was preferentially expressed in CD34+ cells ($P=0.02$) but not CD34+CD38- cells which were considered stem/progenitor cells in normal hematopoiesis. SOX7 knockdown in Nalm20 cells with siRNA could reduce the cell proliferation at day 7($p=0.03$), induce the apoptosis ($p=0.04$), but had no effect on the cell cycle. Engraftment of transduced Nalm20 cells in NOD/SCID mice were significantly reduced 15 to18 weeks after transplantation (scramble: 51%; SOX7: 14%, $p=0.03$). SOX7 sequencing in ALL patients revealed point mutations in 5 out of 6 patients, and four mutations could affect the protein function of SOX7. These mutations were not found in normal Han-origined individuals. **Conclusion:** SOX7 is preferentially expressed in human CD34+ cells of ALL. SOX7 knock-down reduced cellular proliferation by increasing the apoptosis of the ALL cells. SOX7 knock-down could also impair the repopulating ability of ALL in NOD/SCID mice, indicating SOX7 was involved in the regulation of leukemogenesis in ALL. Its mechanism of action would have to be further evaluated.

P1119 - THE MONOCYTIC LEUKAEMIA ZINC FINGER (MOZ) PROTEIN IS A REPRESSOR OF CELLULAR SENESCENCE, AND HAPLOINSUFFICIENCY FOR MOZ INCREASES SURVIVAL 3-FOLD IN THE Eμ-MYC LYMPHOMA MODEL

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MOZ (KAT6A, MYST3) is a MYST-family histone acetyltransferase, and has been shown to acetylate H3K9 at Hox and Tbx loci during embryonic development. MOZ plays a specific role in the generation of haematopoietic stem cells, in body segment identity specification, and in cardiac development. Furthermore, recurrent translocations involving MOZ have been identified in cases of acute myeloid leukaemia. One of the hallmarks of cancer is the ability of malignant cells to escape cellular senescence. The role of wild type MOZ in regulating cellular proliferation, apoptosis, and senescence is not well understood. Utilising primary mouse embryonic fibroblasts (MEFs), we show that Moz-/- MEFs undergo premature senescence, and express senescence markers including high β-galactosidase activity, and high levels of P16INK4A and P19ARF. Furthermore, Moz-/- MEFs showed a reduced rate of proliferation compared to wild type MEFs, while apoptosis was unaffected. To determine whether this defect in senescence affected cancer onset and progression, we compared the survival of wild type and Moz+/- mice in the Eμ-Myc lymphoma model, which models human Burkitt's lymphoma. Haploinsufficiency for Moz greatly extended the lifespan of Eμ-Myc transgenic mice, with median survival of 380 days, compared to wild type Eμ-Myc transgenic mice, which had a median survival of 110 days. Consistently, the numbers of Pre-B and Immature B cells were halved in Moz+/- mice, while other haematopoietic lineages were largely unaffected. Furthermore, both Moz+/- Pro-B and Pre-B cells showed decreased proliferation when cultured in vitro with IL-7, which became worse over multiple passages. Altogether, this work establishes MOZ as an important regulator of cellular senescence, and suggests that therapeutic inhibition of MOZ may result in retarding lymphoma progression.

P1120 - DELETION OF THE POLYCOMB-GROUP GENE EZH2 CAUSES MYELOPROLEFERATIVE NEOPLASM IN MICE

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Polycomb-group (PcG) proteins form the polycomb repressive complexes (PRC) 1 and 2, functioning as transcriptional repressors through histone modifications. They have been implicated in the maintenance of both hematopoietic and leukemic stem cells, by repressing the transcription of tumor suppressor genes, namely Ink4a and Arf, and thus have been characterized as oncogenes. Of great interest, inactivating mutations of EZH2 have been identified in patients with myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPN), suggesting that PcG genes also have a tumor suppressor function. However the mechanism by which PcG genes exert their tumor suppressor function remains to be clarified in myeloid malignancies. To understand the pathologic role of inactivating EZH2 mutations in myeloid malignancies, we transplanted Ezh2-deficient bone marrow (BM) cells into wild-type recipient mice. Of note, deletion of Ezh2 recapitulated the features of MDS/MPN in recipient mice after a long latency of about 1 year. Deletion of Ezh2 in adult BM, did not significantly compromise hematopoiesis except lymphopoiesis. Nonetheless, at 1 year after deletion, all mice showed myeloproliferative features, such as extramedullary hematopoiesis in the spleen, a significant increase in hematopoietic stem and progenitor cells (HSPCs) in the BM and the spleen and a mild but significant myelofibrosis. Some of the mice developed lethal anemia. Upon examining the gene expression of aged Ezh2-deficient HSPCs, it was found that a group of genes related to the myc became activated, suggesting the acquisition of a growth advantage in HSPCs. Collectively, our findings establish the tumor repressor function in myeloid malignancies. Ezh2-deficient mice will serve as a useful model for MPN and for understanding the pathologic role of inactivating EZH2 mutations.

P1121 - TARGETING SPHINGOSINE KINASE AS A THERAPY FOR ACUTE MYELOID LEUKAEMIA

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Acute myeloid leukaemia (AML) is one of the most common forms of leukaemia in adults, presenting as an accumulation of immature myeloid cells in the bone marrow and peripheral blood. Despite improvements in conventional chemotherapy and bone marrow transplantation, overall survival remains poor; 5 year survival rates of 20-30%. Our research focus is to develop effective therapy for AML and in particular, to better target the leukaemic stem and progenitor cell (LSPC) subpopulation which is notoriously refractory to chemotherapy regimes. Therapeutic strategies that target LSPC are essential to improve patient outcome. Sphingosine kinase 1 (SK1) has emerged as a potential therapeutic target in leukaemia. (Pitson et al 2011) For example, SK1 expression is elevated in leukaemic cell lines and correlates strongly with enhanced cell survival and chemotherapeutic resistance. SK1 is a signalling enzyme with established roles in solid tumour initiation, progression and chemotherapeutic resistance. SK1 catalyses the formation of sphingosine 1-phosphate (S1P), a bioactive lipid that regulates a diverse range of cellular processes, including cell proliferation, survival, and differentiation. We have shown for the first time that SK1 is constitutively activated in primary AML blasts, and that high SK1 expression may correlate with poor patient outcome. Furthermore, we have developed a novel first-in-class SK1 inhibitor that is structurally and mechanistically distinct from other commercially available SK1 inhibitors. We have demonstrated that this agent induces cell death in both AML blasts and LSPCs, sensitises the normally refractory LSPCs to killing by chemotherapeutics and reduces tumour burden in xeno-transplantation models of AML. These results validate SK1 as a therapeutic target for the treatment of AML. Pitson SM, Powell JA and Bonder CS. Regulation of sphingosine kinase in hematological malignancies and other cancers. Anti-Cancer Agents in Medicinal Chemistry.2011;11 799-809

P1122 - DECLINED PRESENTATION
EXPRESSION OF ZEBRAFISH IDH1R146H CAUSES EXPANDED MYELOPOIESIS IN ZEBRAFISH

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Introduction: Isocitrate dehydrogenase (IDH), an enzyme in Krebs cycle, has recently been found mutated in many tumors, with a novel enzymatic activity of 2-hydroxyglutarate (2HG) production from alpha-ketoglutarate. However, the role of IDHs wild-type or mutation in embryonic hematopoiesis is still unknown. In this study, we clarify the sequence homology, gene expression pattern and hematopoietic functions by using zebrafish model. **Method:** Multiple alignments and phylogenetic tree of the full amino acid sequences of the IDH proteins in different species were generated. Spatio-temporal expression of zebrafish idh1/2 gene was examined by reverse transcription polymerase chain reaction (RT-PCR) and whole-mount in situ hybridization (WISH). Idh1/2 was knocked-down by anti-sense morpholino (MO). Site-directed mutagenesis was performed to generate zebrafish idh1R146H, idh2R138Q and idh2R172K mutants, corresponding to the human IDH1/2 mutations commonly seen in AML. The hematopoietic phenotype of knock-down and over-expression was analysed. **Results:** Zebrafish IDH1/2 share more than 80% amnio acid sequence identity with the human ortholog, respectively. In zebrafish emrbyos, idh1/2 is expressed ubiquitously during early development and later, they are more restricted in intestine, liver or pectoral fin. Idh1/2 knock-down resulted in disturbance in myeloid but not erythroid development during primitive hematopoiesis, which was characterized by increased expression of myeloid progenitor marker (spi1) and decrease of macrophage marker (l-plastin) and neutrophil marker (mpo). The effect could be efficiently rescued by co-injection of wild-type idh1/2 mRNA. The definitive hematopoiesis and angiogenesis were not affected. Over-expression of zebrafish idh1R146H but not the wild-type idh1, resulted in overt expansion of myeloid compartments by spi1, cebp-α and mpo. **Conclusion:** Idh1/2 is involved in myeloid lineage development in zebrafish primitive hematopoiesis. Our study also underscored the relevance of zebrafish model in the study of human AML. The role of zebrafish idh2R138Q and R170K mutations are being evaluated. **Acknowledgements:** The study was supported by the Innovative Collaborative Research Fund from the LKS Faculty of Medicine and the University Postgraduate Fellowship from HKU.

P1123 - NPMC COOPERATES WITH MUTANT IDH2 TO INDUCE ACUTE MYELOID LEUKEMIA

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Nucleophosmin mutation (NPMc) is frequently observed in acute myeloid leukemia (AML). We have previously found that the expression of NPMc alone is sufficient to transform mouse bone marrow cells ex vivo but not sufficient to induce AML in vivo. Recent studies have shown frequent co-occurrence of IDH1/2, Dnmt3a and Flt3 mutations with NPMc, which implies that accumulation of these mutations is necessary for the induction of AML. Here we show that AML is induced in all the mice transplanted with bone marrow progenitor cells co-infected with 4 mutant genes (NPMc, IDH2mt, Dnmt3amt and Flt3-ITD). The delayed onset and reduced penetrance of AML were observed without one of the 4 genes. Analysis of the AML cells indicated that NPMc-expressing cells always expressed IDH2mt in mice, and that the NPMc-expressing cells did not remain in mice without transduction of IDH2mt. Furthermore, NPMc-expressing cells were observed in the presence of IDH2mt only. These data indicate that IDH2mt is necessary and sufficient for the engraftment of NPMc+ cells in mice. Expression levels of Hoxa9 and Meis1 were elevated in the 4 genes-induced mice AML models as in the human NPMc+ AML samples. Forced expression of NPMc and IDH2mt respectively upregulated HoxA9 and Meis1 in mouse bone marrow progenitor cells in vitro. These data indicate that NPMc and IDH2mt cooperatively activate Hoxa9/Meis1 pathways for the induction of AML.

P1124 - TARGETING CD47-SIRPA FOR THE CONTROLLING MALIGNANT EFFUSION IN PRIMARY EFFUSION LYMPHOMA

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Background: Recently, the critical roles of CD47 on the surface of resistant cancer cells and its ligand, signal regulatory protein alpha (SIRPA), have been proposed in their evasion of immunosurveillance. Primary effusion lymphoma (PEL) is a subtype of aggressive non-Hodgkin lymphoma that shows serous lymphomatous effusion in body cavities especially in advanced AIDS. PEL is resistant to conventional chemotherapy and has a poor prognosis. In this study, we evaluated the effect targeting CD47-SIRPA signal on PEL using xenograft mouse model. Methods and **Results:** Surface CD47 of PEL cell lines was highly expressed compared with that of peripheral blood mononuclear cells by flow cytometry. Treatment with CD47 monoclonal antibody (mAb) upregulated phagocytic activities of mouse peritoneal macrophage in vitro. In xenograft mouse model, primary PEL cells were injected intraperitoneally into NOD/Rag-2null/Jak3nullmice. Treatment with CD47 mAb inhibited the ascites formation completely in vivo. Moreover, we compared the phagocytic activities of peritoneal macrophages against PEL among Rag-2/Jak3 double-deficient mice with NOD and non-NOD (Balb/c and C57/BL6) genetic backgrounds. The engraftment of PEL cells in NOD mice was superior to those in non-NOD mice. NOD SIRPA polymorphism is considered to contribute to the development of PEL. **Conclusion:** CD47 and SIRPA play the pivotal role in the immune evasion of PEL. CD47-SIRPA signal could be a molecular target for the immunotherapy of PEL.

P1125 - COMPUTER-AIDED SCREENING IDENTIFIED A NOVEL SMALL INHIBITOR THAT DISPLAYS POTENT ANTI-MYELOMA ACTIVITY BY SUPPRESSING CONSTITUTIVE AND INTERLEUKIN-6-TRIGGERED JAK2-STAT3 ACTIVATION

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The JAK2-STAT3 signaling pathway is associated with poor prognosis and chemoresistance in MM patients. In contrast, suppression of STAT3 activity could lead to MM cell apoptosis. Therefore, the STAT3 signaling pathway has been developed as a promising target for MM therapy. In the present study, we performed a computer-aided screening and subsequent structural-activity optimization against JAK2 and found one compound kifocitanib (KIF). KIF was well docked into the JAK2 activity pocket. Subsequent studies revealed that KIF inhibited JAK2 as well as STAT3 activation in a time- and concentration-dependent manner. In addition, KIF prevented STAT3 nuclear export in both resting and stimulated myeloma cells. More importantly, KIF inhibited STAT3 activity in the presence of IL-6, a critical stimulator of the STAT3 signaling pathway and responsible for MM cell proliferation. KIF also suppressed expression of STAT3 regulated genes, including Bcl-2, Mcl-1, VEGF, and D-cyclins. By interfering with the JAK2 and STAT3 signal transduction, kifocitanib inhibited proliferation and induced apoptosis of myeloma cells. Notably, KIF had no significant effects in colony formation of normal blood stem cells. In two myeloma xenograft models developed from MM cell lines OPM2 and JJN3, orally administrated KIF delayed MM tumor grow within 7 days at a dose of 30 mg/kg. Significant decrease was observed within 2 weeks, but KIF presented minimal toxicity. In the analysis of the MM tumor species from mice models, KIF suppressed the activation of JAK2 and STAT3, accompanied by the decrease of cyclin D3, one of the targets of STAT3 signaling pathway. Therefore, via virtual screening, structural activity optimization, cell-based and animal studies, we identified a novel inhibitor of JAK2-STAT3 signaling pathway that displayed great potential for myeloma therapy.

P1126 - THE ROLE OF EPHA2 IN HAEMATOPOIEIS AND LEUKAEMIA

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The Eph receptor tyrosine kinases and their membrane bound ephrin ligands are cell surface molecules important in many biological functions and cell behaviors. Eph/ephrin interactions are involved in regulating cell interactions, migration and positioning during development and have ongoing roles in normal tissue homeostasis and in responses to pathological situations after development. Eph and ephrin proteins show altered expression on many tumors including leukaemia, prostate, breast and colorectal carcinomas and gliomas. Members of Eph/ephrin family of protein are also expressed on hematopoietic cells, reports describing expression of EphA2 on CD34+ cells. Flow cytometry and real time PCR analysis also shows expression of EphA2 along with other members of EphA receptors on the hematopoietic stem cell population. Using an EphA2 knock out mouse, we investigated the effects of EphA2 gene on haematopoiesis and haematopoietic stem cells in vivo. Chimeric transplant recipients were generated with purified primary and secondary stem cell enriched populations (lin-Kit+Sca1+) and these were studied for 16-24 weeks. These studies show that mice lacking the EphA2 gene have normal haematopoiesis and bone marrows derived from these mice have normal repopulating potential both in primary and secondary mouse transplant recipients, perhaps indicating that other Eph receptors can compensate for the absence of EphA2. In contrast, in a syngeneic retroviral model of MLL-AF9 induced acute myeloid leukemia (AML), there appeared to be a role for EphA2. Wild type mice transfected with MLL-AF9 shows over expression of EphA2 on leukaemic blast cells, which indicates that this gene may have an extrinsic or intrinsic role in leukaemogenesis.

P1127 - LEUKEMOGENESIS INDUCED BY C-TERMINAL MUTATIONS IN THE BASIC LEUCINE ZIPPER DOMAIN OF C/EBPα

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Two types of mutations of CCATT/enhancer binding protein α (C/EBPα) transcription factor are found in leukemic cells of 5%-14% of acute myeloid leukemia (AML) patients without chromosomal translocations: N-terminal mutations mainly expressing dominant negative p30 (C/EBPα-Nm) and C-terminal mutations in the basic leucine zipper domain of C/EBPα (C/EBPα-Cm). Interestingly, most AML patients with C/EBPα mutations have C- and N-terminal mutations at different alleles. We previously demonstrated by using a mouse bone marrow transplantation (BMT) model that C/EBPα-Cm alone induces AML and collaborates with C/EBPα-Nm in the efficient induction of AML. In this study, we investigate the molecular mechanism underlying the leukemogenesis induced by C/EBPα-Cm. For this purpose, several C/EBPα-Cm derived from AML patients were prepared: S299_L304 dup, K313dup, N321D, and 304_323dup. Retroviral transduction of any C/EBPα-Cm inhibited G-CSF-induced differentiation of 32Dcl3 cells into mature neutrophils and immortalized BM hematopoietic cells. On the other hand, C/EBPα(N321D) or (304_323dup) showed a stronger leukemogenic potential than C/EBPα(S299_L304 dup) or (K313dup) in a mouse BMT model: the former and the latter induced AML within 3 months and 4 to 12 months after transplantation, respectively. To investigate the target molecule of C/EBPα(N321D), analysis of gene expression profiles of C/EBPα(N321D)-induced leukemic cells is currently underway.

P1128 - DECLINED PRESENTATION
P21-ACTIVATED KINASE AND GUANINE EXCHANGE FACTORS FOR RAC GTPASES IN ONCOGENIC KIT INDUCED SYSTEMIC MASTOCYTOSIS AND ACUTE MYELOID LEUKEMIA

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A gain-of-function mutation at codon 816 in the KIT receptor tyrosine kinase is associated with poor prognosis in patients with systemic mastocytosis (SM) and acute myeloid leukemia (AML). This mutation changes the conformation of the KIT receptor resulting in altered substrate recognition and constitutive tyrosine autophosphorylation and ligand independent growth. As there are currently no efficacious therapies against this mutation, we sought to define novel therapeutic targets that contribute to aberrant signaling downstream from KITD816V and promote transformation of hematopoietic cells in myeloproliferative neoplasms (MPN). We show that murine and human activating KIT bearing leukemic cells exhibit constitutive activation of p21-activated kinase (PAK), Rac GTPases, and guanine exchange factor (GEF) Vav1. Treatment of active KIT-bearing leukemic cells with an allosteric inhibitor of PAK or expression of a dominant negative form of PAK (K299R) in KITD814V bearing cells profoundly inhibited their growth. Upstream of PAK, we show that suppression of Rac GTPases by a dominant negative form of Rac (RacN17) abrogates activating KIT-induced hyperproliferation and PAK activity. Although both Rac1 and Rac2 are constitutively activated via GEF Vav1, loss of Rac1 or Rac2 alone moderately corrects the growth of KIT bearing leukemic cells whereas combined loss results in 75% growth repression. To assess the role of Rac-GEFs in oncogene induced transformation, we utilized a novel inhibitor of Rac, EHop-016, which is a derivative of NSC23766. While NSC23766 targets GEFs Tiam1 and Trio, EHop-016 targets Vav and is significantly more efficient in inhibiting the growth of KITD816V positive mastocytosis-derived patient samples, and suppresses activation of Rac1, Rac2, and PAK. Treatment of KITD814V bearing mice with EHop-016 significantly delayed onset of MPN, while NSC23677 had only modest effects. Furthermore, in vivo loss of either Vav1 or Rac1 and Rac2 completely rescues leukemogenic transform via KITD814V. In this study we provide a mechanism of KITD814V induced transformation and provide potential novel therapeutic targets for treating oncogenic KIT bearing neoplasms.

P1130 - BIOLOGICAL, FUNCTIONAL AND GENETIC CHARACTERIZATION OF BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS ISOLATED FROM PEDIATRIC PATIENTS AFFECTED BY ACUTE LYMPHOBLASTIC LEUKEMIA

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Objective: Alterations in hematopoietic microenvironment of acute lymphoblastic leukemia (ALL) patients have been reported, but little is known about the components of marrow stroma in these patients. In this study, we characterized mesenchymal stromal cells isolated from pediatric patients with acute lymphoblastic leukemia (ALL-MSCs). **Methods:** Mesenchymal stromal cells were expanded *ex vivo* from bone marrow of 45 ALL patients (mean age: 5 years; range: 1-17), at diagnosis and at subsequent time-points (days: +15;+33;+78) according to the Italian Association for Pediatric Hematology and Oncology (AIEOP) treatment protocol. Morphology, proliferative capacity, immunophenotype, differentiation potential, immunomodulatory properties and genetic profile of ALL-MSCs at any time-point were analysed and compared with those of MSCs isolated from 41 healthy donors (HD-MSCs; mean age: 21 years; range: 5-34). **Results:** Morphology, immunophenotype, differentiation potential and *in vitro* life-span did not differ between ALL-MSCs, at any time-point considered, and HD-MSCs. ALL-MSCs showed a reduced proliferative capacity ($p<0.001$), as compared with HD-MSCs, in terms of population doublings. The immunomodulatory properties of MSCs were evaluated in an allogeneic setting (ALL-MSCs/HD-PBMCs) by measuring PHA-induced T cell proliferation. As for ALL-MSCs isolated at diagnosis, they were able to reduce T cell proliferation up to 62% (MSCs/PBMCs ratio 1:2) and 30% (ratio 1:10) and as for HD-MSCs up to 65% (ratio 1:2) and 40% (ratio 1:10) (p=N.S.). Comparable results were obtained with MSCs isolated at subsequent time-points. ALL-MSCs showed neither the typical translocations carried by the leukemic clone (when

present), nor other genetic abnormalities acquired during *ex vivo* culture. **Conclusions:** Our results demonstrate that, despite a reduced proliferative capacity, ALL-MSCs, irrespective of the time-point considered (at diagnosis or during chemotherapy treatment), mantain the typical characteristics of HD-MSCs.

P1131 - DNA METHYLATION CHANGES INDUCED BY MEIS1 IN A HOXA9/MEIS1 ACUTE LEUKEMIA MODEL

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Elevated expression of Meis1 and Hoxa9 is commonly seen in human leukemia and often indicates a poor prognosis. It has also been shown that overexpression of Meis1 together with Hoxa9 cause acute myeloid leukemia (AML) in mice. However, the molecular mechanisms responsible for the collaboration between Meis1 and Hoxa9 in transformation to AML are not fully understood. DNA methylation is a highly conserved mechanism for the regulation of vital biological processes. Changes in DNA methylation are a common feature of human AML suggesting a role in leukemia development. Furthermore, it has been shown that Meis1 can disrupt DNA methylation and the genomic imprint together with HOX-fusion genes, thus, warranting further investigation. To investigate the effect of overexpression of Meis1 together with Hoxa9 on DNA methylation in bone marrow cells we have established a cell culturing system based on retroviral transduction of murine bone marrow cells. These cells are fully transplantable even after extended time in culture. The cells were cultured for 3 weeks before genomic DNA was isolated. Methylated DNA was enriched (MethylMiner®,Invitrogen) and subjected, in biological duplicates, to a whole genome promoter array (2.1M Mouse Promoter Array, NimbleGen). Data analysis showed high consistency between the duplicate samples. There were approximately 38,000 hypermethylated regions identified in both Hoxa9/Meis1 and Hoxa9 overexpressing cells, indicating no obvious difference in overall DNA methylation between Hoxa9/Meis1 or Hoxa9 cells. However, there was a distinct difference in DNA methylation pattern and approximately 1500 regions were found differentially hypermethylated in Hoxa9/Meis1 cells compared to Hoxa9 cells. These results show that overexpression of Meis1 together with Hoxa9 lead to gain or loss of DNA methylation in distinct regions in the genome but do not seem to lead to a change in the overall degree of methylation. Further investigations of regions are underway and will reveal new insight in Hoxa9/Meis1 induced leukemogenesis.

P1132 - CLASSICAL COMPLEMENT CASCADE DEFICIENCIES IN CLL IMPACTS ON RESPONSE TO OFATUMUMAB TREATMENT

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CLL patients with progressive disease often become refractory to standard first line therapies, underpinning the need for novel drugs. In this study we compared the ability of the anti-CD20 MABs ofatumumab (OFA) and rituximab (RIT), to induce complement dependent cytotoxicity (CDC) and determined whether complement defects are an issue in CLL MAB treatment regimes. Following CDC, flow cytometric analysis (PI staining) revealed that OFA induced significantly higher CDC in CLL primary cells than RIT. Analysis of CLL serum sample using CH100 assay, immunonephelometry and radial immunodiffusion indicated that 41% of patients were either deficient, or had reduced expression of, one or more complement components, which impacted on their ability to elicit a CDC response to OFA-bound CLL cells. A large proportion of patients with complement deficiencies initially induced a high level of CDC, however on secondary challenge CDC activity in sera was significantly reduced when compared to normal human serum (NHS; $p= <0.0001$; $n=29$). Importantly, supplementing CLL serum with individual complement components especially C2 was sufficient to protect against exhaustion, restoring activity back to NHS levels, ($p= <0.0001$; $n=11$). Furthermore, high CLL cell burden also resulted in more rapid complement exhaustion. Preliminary in vivo studies indicate that 24 hours post RIT treatment complement components exhaust, with recovery time exceeding 7 days. Our studies have important implications for CLL patients receiving MAB therapy, indicating that due to the high frequency of complement deficiencies within CLL serum, supplementing MAB treatment with fresh frozen plasma may help maintain normal CDC levels, particularly in patients with a high white blood cell count.

P1133 - EXAGGERATED RESPONSE TO TOLL-LIKE RECEPTOR AGONIST CONTRIBUTES TO EXCESSIVE TNF PRODUCTION IN MYELOPROLIFERATIVE NEOPLASM

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Tumor Necrosis Factor alpha (TNF) is elevated in myeloproliferative neoplasm (MPN) and plays a key role in the expansion of the JAK2V617F neoplastic clone. JAK2V617F endows upon hematopoietic stem cells TNF resistance, providing them with a selective advantage over their TNF sensitive counterparts. Decreasing excessive TNF production therapeutically would neutralize the selective pressure driving the persistence of the JAK2V617F neoplastic clone. TNF is classically produced by monocytes after stimulation through Toll-like receptors (TLR), crucial pattern recognition receptors for microbial products. We quantified TLR responses in normal and MPN monocytes, reasoning that JAK2V617F might directly or indirectly influence the activation state of TLR modulatory factors and found evidence for an exaggerated response to the TLR 7/8 agonist R848 in MPN patient monocytes. After stimulation with R848 for 24 hours, CD14+ monocytes from MPN patients ($n=18$) produced increased amounts of TNF as compared to normal controls ($n=10$) at all concentrations tested (0.5, 1, 3, 5μM, $p<0.05$). Pre-treatment with the JAK inhibitor CYT387 (2μM) completely abrogated TNF production in response to R848 similarly in MPN patients and normal controls, demonstrating that R848-mediated TNF production is dependent upon JAK signaling. The percentage of TNF+ CD14+ monocytes after stimulation with R848 by intracellular flow cytometry is similar in MPN ($n=16$) and normal controls ($n=8$). Without stimulation, however, MPN patients have an increased percentage of TNF+ CD14+ monocytes as compared to normal controls (4.6% vs 1.6% respectively, $p<0.05$), suggestive of a hyper-inflammatory state at baseline. Next, we used phosflow to detect whether MPN patients have an exaggerated activation of downstream signaling molecules following stimulation with R848. At early time points (15min) following stimulation with R848 (5μM), MPN patients ($n=6$) similarly activated pp38 and pERK1/2 as normal controls ($n=6$), but at later time points (2hrs) MPN patients maintained the activated status of pp38 and pERK1/2 distinct from normal controls ($p<0.05$). These data suggest that the excessive production of TNF in MPN patients may be due to persistent activation of signaling following stimulation.

P1134 - RESTORATION OF REDOX ENZYMES IS CORRELATED WITH REDUCTION OF BCR-ABL PROTEIN IN PHILADELPHIA POSITIVE CELLS DURING IMATINIB THERAPY

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Background: There are several lines of evidences reactive oxygen species (ROS) appear to play a major role in leukemogensis. BCR/ABL oncogenic tyrosine kinase is known to induce high levels of intracellular ROS which may further induce genomic instability with malignant transformation and even imatinib (IM) resistance. Disrupting of intracellular ROS levels and redox signaling could inhibit tumor cell growth. In this study, we investigated the changes of peroxiredoxin (PRX) and ROS levels during IM therapy in BCR-ABL positive leukemia cells. **Methods:** BCR-ABL1 positive cell lines were cultured using 20% IMDM medium. We evaluated cell growth by MTT assay, and also evaluated BCR/ABL expression by western blot analysis. At the same time, we evaluated changes of intracellular ROS level using 2, 7- DCFH-DA and antioxidant enzymes, PRX 1, 2, 3 using immunoblot assay. We also had analyzed the impact of N-acetyl-L-cysteine and NAD(P)H oxidase inhibitor, DPI on changes of ROS and redox enzymes. We also analyzed the expression of NOX1,2,3,4-based NADPH oxidase using immunoblot assay. **Results:** Both BCR-ABL1 positive cells (K562 and SUP-B15 cells) showed significant decrease of cell viability during IM treatment. Intracellular ROS level and Nox-4 based NADPH oxidase were also decreased during IM treatment as well. These finding were well correlated with eradication of BCR/ABL oncogene by western blot results, and were correlated with restoration of PRX2. The degree of ROS decrease and restoration of Prx2 were well correlated with amount of reduction of BCR/ABL oncogene during IM treatment. But there were significantly differential changes in k562 and in SUP-B15 during IM treatment. **Conclusion:** Significant change of ROS and Prx2 levels according to IM treatment are closely correlated with bcr/abl kinase level in both BCR-ABL1 positive cell lines. This finding was correlated with restoration of the level of PRX2. Understanding the molecular mechanisms of changes on ROS and redox enzymes may be potential tools to develop more potent new drugs in Ph+ CML or Ph+ ALL patients especially for IM resistant.

P1135 - THE ROLE OF CDX2 IN HUMAN LEUKEMOGENESIS

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Introduction: The caudal-type homeobox (Cdx) gene family has been mainly studied during early development for its role in axial elongation and antero-posterior patterning. More recently, Cdx genes were shown to regulate embryonic hematopoiesis via modulation of downstream Hox genes. The role of Cdx genes in adult hematopoiesis is poorly understood and almost no data exists on human cells. In the mouse, healthy bone marrow (BM) derived hematopoietic progenitors express low levels of Cdx1 and Cdx4 but lack Cdx2 expression. Ectopic induction of Cdx2 expression induces myeloid leukemia. Consistently, Cdx2 expression is found in most cases of human acute myeloid (AML) and lymphoid leukemia (ALL). Here we modulate Cdx2 expression in healthy and malignant human blood cells to explore its role during human leukemogenesis. **Methods:** Human BM and mobilized peripheral blood CD34+ cells as well as the leukemic cells lines SKM-1, NOMO-1, EOL-1 and Nalm16 were exposed to lentiviruses containing Cdx2 overexpression, shCdx2 or control constructs. Efficient modulation of Cdx2 expression was confirmed on gene expression level by qRT-PCR and on protein level by immunoblot analysis. CDX2-modified and control cells were subjected to growth, colony forming (CFU), cell cycle, flow cytometry and qRT-PCR assays and analyzed in vivo upon xenotransplantation in NOD/SCID/IL2Rγnull (NSG) mice. **Results:** In both healthy CD34+ and leukemic cells, overexpression of Cdx2 resulted in a G0/G1 cell cycle arrest, reducing in vitro growth and CFU formation. Cdx2 overexpressing cells were smaller in size and showed less granularity. Moreover, gene expression analyses demonstrated induction of Hox genes and Wnt pathway-associated molecules in both CD34+ progenitors and leukemic cells, although some differences were noted between healthy and malignant cells. Cdx2 overexpressing and control CD34+ human stem and progenitor cells were able to repopulate NSG mice and are currently under observation. Until week 12 post-transplantation, no signs of leukemia induction were observed in the xenotransplanted mice in contrast to the data reported in mice. **Conclusion:** Our data suggest that Cdx2 expression promotes quiescence in human hematopoietic cells by inducing Hox and Wnt-pathway related molecules. Other than in mice, Cdx2 may require another cooperating genetic event to induce human leukemia.

P1136 - IL7R RECEPTOR MUTANTS INITIATE LEUKEMIA FROM MULTIPOTENT PRIMITIVE THYMOCYTES IN A NOVEL MOUSE MODEL OF ETP LEUKEMIA

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Early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) is a recently described hematopoietic malignancy in children and adults and that exhibits lymphoid, myeloid and stem cell features and carries a poor clinical prognosis. Whole genome sequencing of human ETP-ALL cases has identified a number of characteristic mutations in signaling, histone modification and hematopoietic development pathways but it is unclear which of these are sufficient to initiate leukemia. Recurrent mutations in the interleukin 7 receptor are characterized by point substitutions in the alpha chain that confer ligand-independent signaling and proliferation. When either of two of these IL7r mutants were introduced into double negative (DN) thymocytes from p19Arf null mice, T cell development was arrested at the DN2 stage; a stage previously shown to retain significant myeloid developmental potential. The IL7r-mutants phenocopy the developmental block seen with enforced LMO2 expression. The arrested DN2 thymocytes were shown to have significantly enhanced myeloid developmental potential in vitro in CFU-C assays and in vivo when transplanted. When either LMO2-transduced or IL7r mutant-transduced DN2 thymocytes were transplanted into irradiated recipients, the transplanted mice developed a disease that highly resembled human ETP leukemia. The blast cells displayed various myeloid and lymphoid features including expression of myeloperoxidase, Gr1, Mac1, and CD71. Despite these myeloid features, all of the leukemias had monoclonal rearrangements of the TCR-B chain of the T-cell receptor indicating that they were of thymocyte origin. High levels of LMO2 protein expression were seen in IL7r-mut tumors. Furthermore, analysis of RNA samples from 12 cases of human pediatric ETP showed that all but one case was associated with high levels of LMO2 mRNA. Altogether, this new works shows that activating mutations in the Interleukin 7 receptor or enforced expression of LMO2 are sufficient to generate ETP-ALL in mice by blocking thymocyte differentiation at a stage in which myeloid lineage potential is preserved. Our current work is focused on defining the leukemia stem cell population in this model and in using this model to develop new therapeutic strategies for this poor prognosis leukemia.

P1137 - HYPOXIA ENHANCES THE RADIO-RESISTANCE OF MOUSE MESENCHYMAL STROMAL CELLS

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Haematopoietic re-constitution by donor haematopoietic stem cells (HSCs) following total body irradiation is supported by host-derived radio-resistant mesenchymal stromal cells (MSCs). MSCs are also an integral part of tumours where they promote tumour cell growth and inhibit tumour-specific immune responses. The DNA Damage Response (DDR) represents a network of signalling pathways enabling cells to respond to genotoxic damage. We have previously shown that the execution of DDR pathways, including repair of DNA double strand breaks (DNA DSBs), promotes MSC survival post irradiation (IR) [1]. MSCs reside in a hypoxic (2-5% O2) microenvironment in the bone marrow. Hypoxia is known to enhance the radio-resistance of cancer cells by altering their response to IR-induced DNA damage. However, whether hypoxia affects the radio-resistance of MSCs is currently unknown. We have studied the DDR of γ-irradiated mouse MSC lines, MS5 and ST2, cultured in normoxia (21% O2) and hypoxia (5% O2). Hypoxia increased MSC growth rate and enhanced long-term survival post irradiation. Cell cycle analysis by flow cytometry demonstrated that MSC recovery from IR (10 Gy) -induced cell cycle arrest was improved under hypoxic conditions. In MSCs, hypoxia accelerated the resolution of γ-H2AX expression (a marker of DNA DSBs) and the disappearance of γ-H2AX foci. Hypoxia also induced increased expression of DNA DSB repair proteins, including DNA-PKcs and DNA ligase IV in MSCs. Our results demonstrate, for the first time, that hypoxia enhances MSC radio-resistance in vitro most likely by up-regulating DNA DSB repair mechanisms, leading to alterations in the DDR to IR-induced DNA DSBs and thereby enhancing MSC survival. Our results have important implications for our understanding of the roles of MSCs in haematopoietic reconstitution and in the tumour microenvironment. 1. Sugrue T, Brown JAL, Lowndes NF & Ceredig Rh. (2013) Multiple facets of the DNA Damage Response contribute to the radio-resistance of mouse mesenchymal stromal cell lines. Stem Cells 31: 137-145.

P1138 - MOBILISING DOSES OF G-CSF STOP MEDULLARY ERYTHROPOIESIS BY DEPLETING CD169+ MACROPHAGES

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We have previously reported that G-CSF mobilises HSCs by suppressing a subset of HSC niche supportive macrophages. As macrophages are the central component of erythropoietic islands, we examined G-CSF effect on erythropoiesis. Mobilising doses of G-CSF caused a marked whitening of the bone marrow (BM), a 15 fold decrease in the number of phenotypic erythroblasts, a 2 fold decrease in polychromatic erythroblasts, a 1.4 fold decrease in orthochromatic erythoblasts and 5 fold decrease in reticulocytes. Conversely, pro-erythroblasts increased 4 fold. Concomitantly, numbers of CD169+ macrophages and erythropoietic island ER-HR3+ macrophages were decreased in mobilised BM. In contrast with the BM, G-CSF did not deplete macrophages in the spleen. As a result, splenic erythropoiesis was up-regulated to compensate for the loss of medullary erythropoiesis (5-8 fold increase in pro-erythroblast and erythroblast populations). This suggests that mobilising doses of G-CSF block the pro-erythroblast to erythroblast transition specifically in the BM (but not the spleen) by affecting central macrophages in erythropoietic island. Next, macrophages were depleted by injecting clodronate loaded liposomes. This caused a arrest in erythropoiesis and macrophage depletion in both the BM and the spleen. As subsets of macrophages depleted from the BM and spleen following G-CSF or clodronate liposome administration were CD169+, we selectively depleted CD169+ macrophages in CD169DTR/+ mice. Injection of diphtheria toxin caused a loss of CD169+ and ER-HR3+ macrophages in both BM and spleen and also a blockage in erythropoiesis at the proerythroblast-erythroblast transition similar to G-CSF and clodronate liposomes in wild-type mice. In conclusion, we propose that 1) CD169+ macrophages include nursing macrophages at the centre of erythropoietic islands and are essential for the transition of proerythoblasts to erythroblasts and 2) mobilising doses of G-CSF stop medullary erythropoiesis by depleting CD169+ macrophages in erythropoietic islands in the BM, but not the spleen.

P1139 - DECLINED PRESENTATION
ALTERATIONS IN HIERARCHY OF MULTIPOTENT MESENCHYMAL STROMAL CELLS POPULATION OCCURRING WITH CULTIVATION

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Mesenchymal multipotent stromal cells (MMSC) are the fibroblast-like cells adherent to plastic and able to differentiate along main stromal lineages. The population of MMSC is heterogeneous. The aim of this study was to investigate the hierarchical alterations occurring in MMSC with cultivation. MMSC were isolated from the bone marrow of 50 donors and cultivated under standard conditions for 5 passages. The cloning efficiency and differentiation abilities were analyzed on 1-2nd and 5th passages. Relative expression levels (REL) of morphogenetic and differentiation marker genes were evaluated by real time PCR. Cloning efficiency of MMSC differed between the 2nd and 5th passages. It was associated with the REL of FGFR2 ($R=0.75$, $p=0.02$), which shows the dependence of MMSC proliferation capacity on the ability to respond to FGF2. A strong negative relationship between cloning efficiency and the REL of SPP1 was revealed at the 5th passage ($R=-0.73$, $p=0.03$). Thus, proliferative potential reduced during maturation. So, MMSC populations changed with passages, and the portion of cells committed to differentiation became more pronounced. Most cells among MMSC did not proliferate. MMSC proliferative potential did not change significantly during cultivation, and more than half of all dividing cells were clones that were unable to perform more than 14 mitosis. When MMSC were exposed to osteogenic inducers at the 1st passage, no significant alterations in the REL of both SPP1 and BGLAP were revealed. Further MMSC cultivation up to the 5th passage made these cells more sensitive to differentiation stimuli. The increase in the REL of SPP1 was moderate, while the expression of BGLAP was up-regulated by 6-fold. Thus, cultivation increased the proportion of cells committed to differentiation. Prolonged MMSC cultivation had no effect on the response to adipogenic inducers. So, one could conclude that MMSC differentiation capacities did not change with cultivation, while in the population itself the proportion of cells committed to differentiation increased. Summarizing the data, the proportion of cells in different hierarchical subpopulations altered with MMSC cultivation. The aptitude to differentiation increased among the MMSC population.

P1140 - DECLINED PRESENTATION
BONE MARROW DONOR'S AGE RELATED ALTERATIONS IN HUMAN STROMAL PRECURSOR CELLS

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Multipotent mesenchymal stromal cells (MMSC) are utilized in many therapeutic approaches.

The aim of the study was to characterize stromal precursor cells (MMSC and colony-forming unit-fibroblasts - CFU-F), from the bone marrow of 50 healthy donors.

The donors were divided in 2 groups (younger and older than 32.5) on the base of median age. The growth characteristics of MMSC and CFU-F, their proliferative potential, cloning efficiency and differentiation abilities were analyzed. MMSC were cultivated for 5 passages. For proliferative potential studies, MMSC from 2 and 5 passages were cloned from 1 cell. The differentiation capacity was estimated at passages 1 and 5 by standard stimulation. Relative expression levels (REL) of genes were investigated in MMSC by real-time quantitative PCR. Significant increase in CFU-F concentration was revealed in young donors ($p<0.02$). Cumulative MMSC production was significantly decreased in older donors ($p< 0.01$). REL of FGF2 and FGFR2 in MMSC decreased with donor age ($R=-0.44$, $p=0.001$) and this was also true for the VEGF gene ($R=-0.31$, $p=0.03$). The REL of the adipogenic differentiation marker PPARG increased, and the osteogenic one SPP1 decreased with age. The chondrogenic marker SOX9 did not depend on donor age. REL were analyzed in MMSC cultivated without differentiation inducers. REL of differentiation markers can reflect the decreasing with age capacity of mesenchymal cells for osteogenesis and osteoporosis in elderly persons. According cloning capacity MMSC were divided into 3 groups: MMSC with cloning efficiencies that increased from the 2 to the 5 passage, decreased and remained stable. The average donor age was 24.7 ± 1.5 years in the 1 group, 34.6 ± 4.7 years in the 2 group and 46 ± 6.8 years in the 3 group. This means that MMSC populations of young donors were enriched for cells with high proliferative potential. In the group of older donors, the MMSC proliferative potential notably decreased with cultivation, and in the eldest group of donors cloning capacity was decreased for all period of cultivation.

The data are worth to take into account when choosing MMSC for gene therapy, as MMSC are now top candidates for many therapeutic approaches. Thus, donor age is very important for the stable function of MMSC.

P1141 - DECLINED PRESENTATION MULTIPOTENT MESENCHYMAL STROMAL CELLS EXPOSED TO IL-1B DEMONSTRATE THE INCREASED PROLIFERATIVE POTENTIAL AND ENHANCED ABILITY TO MAINTAIN HEMATOPOIETIC PRECURSOR CELLS

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Nestin positive mesenchymal stem cells (MSC) are the main cells that maintain hematopoietic stem cells (HSC) in vascular niches in the bone marrow (BM). Multipotent mesenchymal stromal cells (MMSC) are considered to be the analogues of MSC *in vitro*. Because of clinical use of MMSC their basic characteristics and sensitivity to different factors have been intensively studied. It was shown that interleukin-1 beta (IL1b) induces growth of stromal microenvironment *in vivo* in mice. So, the aim of this study was to evaluate the effect of IL1b on proliferative potential of MMSC and their ability to maintain early and late hematopoietic progenitors - cobblestone area forming cells (CAFC) - 35 days and 8 days respectively. MMSC and hematopoietic cells were obtained from donors of BM for allogeneic HCS transplantation after informed consent. MMSC from 11 donors were cultured in standard conditions for 5 passages (P). 4 pg/ml IL1b was added to MMSC at each passage. MMSC were cloned from 1 cell, cloning efficiency was measured by Poisson statistics. The concentration of IL1b in MMSC lysates was measured by ELISA. 1000 MMSC per well of 96-well plate were seeded 3 days before BM cells plating. CAFC analysis was performed by limiting dilution assay. Addition of IL1b to MMSC culture substantially increased cumulative cell production (164$\pm$20 % of control, p=0.006), and cloning efficiency (150$\pm$25 % of control at P2 and 213$\pm$40 %, p=0.01 at P5). In MMSC from one donor with extremely high cumulative cell production (385% of control) after IL1b exposure accumulation of IL-1b in cells was found. The amount of IL1b was more than 73 pg / $5x10^5$ cells and it was 7-fold increase compared to control. MMSC were used as stromal layers for CAFC assay. MMSC cultivated with IL1b showed the increased capability to maintain hematopoietic precursor cells. The number of CAFC 35 increased 1.5 fold per 10^5 BM cells and CAFC 8 doubled on IL1b treated MMSC. The data show that IL-1b stimulates MMSC proliferation, their cloning efficiency and ability to maintain early and late hematopoietic progenitors. Thus IL1b demonstrates similar effects on BM stromal precursor cells *in vivo* and *in vitro*.

P1142 - LITHIUM INDUCED NOTCH LIGANDS IN STROMA CELLS MEDIATE EXPANSION OF MURINE HEMATOPOIETIC STEM CELLS

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For many years the effects of lithium, a simple cation and known inhibitor of GSK3-β, on hematopoiesis have been appreciated. However, the mechanisms how lithium affects hematopoietic stem cells (HSCs) are poorly understood. Here, we show that in vivo hematopoietic repopulation can be augmented by transplantation of unfractionated bone marrow cells derived from mice treated with lithium. In contrast, treatment of mice with lithium did not affect the quality of LSK cells as measured by CAFC on day 35. In vitro, the expansion of primitive cells was significantly enhanced by lithium in post 5-FU BM cultures, but only modestly in isolated LSK cells, suggesting an accessory cell population is essential in mediating the effects of lithium on HSCs. Additional experiments revealed that lithium treatment activates Wnt-signaling in stromal cells. Lithium added to a surrogate stem cell assay in vitro, the cobblestone area forming cell assay, showed outgrowth of more cells from isolated LSK and HSC. Interestingly, expression of the notch ligands Jagged-1 and Delta-1 increased in stromal cells upon lithium treatment. In addition, the in vitro effects of lithium on HSCs could be prevented by inhibiting Notch signaling by gamma-secretase inhibitors. In conclusion, our observations suggest that lithium stimulates Wnt signaling in stromal cells resulting in up-regulation of Notch ligands in these cells, which subsequently activate Notch signaling in HSCs resulting in augmentation of HSCs.

P1143 - EFFECTS OF IN-VIVO G-CSF STIMULATION ON CYTOKINE LEVELS IN BONE MARROW PLASMA SAMPLES FROM HEALTHY DONORS

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Introduction: Hematopoietic stem cell (HSC) transplantation has become a standart treatment modality for many malignant and non-malignant hematopoietic and metabolic diseases. Granulocyte-colony stimulating factor (G-CSF) is commonly used in the clinics to mobilize hematopoietic stem cells (HSCs) from bone marrow (BM). G-CSF mediated induction of proteases results in cleavage of adhesion molecules, decrease in Stromal Derived Factor 1 (SDF-1), produced by local mesenchymal stromal cells (MSCs) and release of the HSC from the BM. Here we aimed to assess the effects of G-CSF treatment on cytokine levels in the bone marrow plasma of healthy donors. **Materials and Methods:** Bone marrow plasma was collected from 4 day G-CSF (10 µg/kg/d) treated (n=10) and untreated (n=10) healthy pediatric and adolescent donors, ages ranging from 2-17 yrs, and analysed using a multiplex cytokine array for 48 different cytokines and read using the Bioplex MAGPIX (Bio-rad). CFU-GM and BFU-E were assessed using MethoCult® GF H84435 (Stem Cell Technologies) and MSC content was assessed using the CFU-F assay. **Results:** After G-CSF treatment, Interleukin (IL)-1b ($p<0.01$), IL-3 ($p<0.05$), IL-8 ($p<0.05$) and IL-18 ($p<0.01$) were found to be significantly increased in BM plasma. In contrast, Cutaneous T-cell-attracting chemokine (CTACK, CCL27) ($p<0.05$), Eotaxin ($p<0.05$) and Regulated on Activation, Normal T cell Expressed and Secreted (RANTES, CCL5) ($p<0.01$) were found to be significantly decreased after G-CSF treatment. As expected CFU-GM and BFU-E colonies were significantly increased ($p<0.01$), but also the number of MSC colonies (CFU-F) was significantly higher after G-CSF treatment ($p<0.0001$). **Conclusion:** G-CSF treatment has profound effects on a broad range of cytokine levels and affects both HSC and MSC progenitor cell count in human bone marrow plasma samples from healthy pediatric and adolescent donors.

P1144 - ASSESSMENT OF HUMAN BONE MARROW PLASMA SAMPLES SUGGESTS A DIFFERENT ROLE FOR MESENCHYMAL STROMAL CELLS IN DISTINCT AREAS MEDIATED BY PDGF-BB/PDGF-R

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Introduction: The bone marrow environment fosters hematopoietic stem/progenitor cells and maintains hematopoiesis. Mesenchymal stromal cells (MSCs) play an important role in regulating HSC "stemness" by local production of hematopoietic growth factors and cytokines, and by direct interaction through expression of cell-surface and adhesion molecules. Here we assess the differences in characteristics of MSCs and levels of cytokines in the human endosteal and vascular niches. **Materials and Methods:** Human bone marrow (BM) samples were collected from endosteal and vascular regions. A 27-plex cytokine array was performed on BM plasma samples using a Bioplex MAGPIXTM (Bio-Rad). Phenotypic analysis of MSCs/ passage 3 was performed using a panel of 20 antibodies and measured using a FACSARIA (Becton Dickinson). Differentiation assays of MSCs towards osteogenic and adipogenic lineage were performed and quantitatively analyzed. **Results:** Platelet Derived Growth Factor-BB (PDGF-BB) levels were found to be significantly higher in plasma from vascular regions ($p < 0.05$). In contrast, the PDGF-receptor beta (PDGF-Rb, CD140b) was found to be expressed at higher levels by MSCs from the endosteal region ($p < 0.05$). Endosteal MSCs differentiated more readily in osteogenic direction, whereas adipogenic differentiation potential was increased with vascular MSCs . **Conclusion:** PDGF-B signaling has been suggested to play a key role in protection of HSCs from premature differentiation. Our preliminary results indicate that MSCs from the endosteal region of the BM express higher levels of PDGF-R and possess increased potential for osteogenic differentiation providing a suitable microenvironment for HSC maintenance. In contrast, lower levels of PDGF-R in the vascular region may have led to reduced PDGF-B signaling (in spite of increased plasma levels), suitable for HSC differentiation.

P1145 - DIFFERENTIAL CYTOKINE EXPRESSION PROFILE OF HEALTHY HUMAN BONE MARROW PLASMA SAMPLES FROM THE ENDOSTEAL AND VASCULAR REGION

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Introduction: The hematopoietic stem cell niche contains all elements required for housing the hematopoietic stem cell (HSC) and maintaining steady state hematopoiesis. Mesenchymal stromal cells (MSCs), hematopoietic growth factors, cytokines/ chemokines, and interactions with cell-surface and adhesion molecules play an important role in regulating HSC stemness, cycling and behavior in response to exogenous stress signals. Here we assess differences in expression of cytokines in the endosteal and vascular regions of the bone marrow (BM). **Materials and Methods:** Human BM aspirations were collected from the endosteal and vascular regions. Analysis of 48 cytokines present in plasma samples from these aspirations was performed using a Bioplex MAGPIXTM (Bio-Rad). Separate ELISAs were performed for SDF-1a, IL-7 and soluble APO1/FAS (sFas). **Results:** Levels of Regulated And Normal T cell Expressed and Secreted (RANTES) and Platelet-derived Growth Factor-BB (PDGF-BB) were found to be significantly higher in the vascular regions of the bone marrow (both $p < 0.05$), whereas expression of Interleukin (IL)-1a, Stromal Derived Factor (SDF)-1a, IL-7 and sFas were significantly increased in the endosteal regions of the BM, with $p < 0.05$, $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively. **Discussion:** Homing of HSC is regulated by the chemokine SDF1a (CXCL12). In agreement with its role in attracting HSC, levels of SDF1a were found increased in the endosteal regions of the BM. Increased levels of IL-1a, IL-7 and sFas in the endosteal region suggested an active role of the BM endosteum in immune regulation of B and T-cells and immune privilege. Increased levels of the chemokine RANTES (CCL5) in the vascular region of the BM suggested a role in migration and proliferation.

P1146 - DIFFERENTIAL MIRNA EXPRESSION PROFILE OF BONE MARROW DERIVED MESENCHYMAL STEM CELLS IN FANCONI ANEMIA PATIENTS AND HEALTHY DONORS

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Fanconi anemia (FA) is an important disease model having a high incidence of cancer predisposition, hematopoietic defects and developmental dysmorphisms. In this study, miRNA expression profile of mesenchymal stem/stromal cells (MSCs) derived from the bone marrow under the FA environment were compared with those from healthy donor bone marrow (BM). BM-derived MSCs from donor ($n=12$; 6 FA donor and 5 independent donor) and FA patient samples ($n=13$; 10 pre-bone marrow transplantation-BMT- and 3 post-BMT) were expanded in vitro and characterized for their cell surface markers and for their differentiation capacity to adipocytes and osteoblasts. The miRNA expression profile was performed using Affymetrix GeneChip 2.0 Array. Following RMA normalization, data analysis were carried out in Partek Genomics Suite software package (Partek Incorporated). Principal Component Analysis and hierachical clustering were performed. One-way ANOVA was used to determine whether miRNA expressions were different among FA donor, pre-BMT, post-BMT and independent donor groups followed by FDR test. Statistical significance was considered as $P < 0.05$. Finally, venn diagrams were contructed to identify miRNAs intersecting among different groups. There was a statistically significant difference in expression of fourteen non-coding RNA among four groups ($P < 0.05$). Comparison of FA donors and pre-BMT showed fourteen differentially expressed miRNAs while there were eleven differentially expressed miRNAs between independent donors and pre-BMT. Three of them were shared between these two comparisons in Venn diagrams. This Project is supported by The Scientific and Technological Research Council of Turkey (TUBITAK; project no: 110S021) in conjunction with EU COST action BM0805.

P1147 - IRON OXIDE NANOPARTICLE LABELING OF MESENCHYMAL STROMAL CELLS

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Introduction: Mesenchymal stromal cells (MSC) are promising cells for use in regenerative medicine, hematopoietic stem cell transplantation and autoimmunity. Labeling of MSCs for cell tracking in tissues is an useful approach. Here, we used iron nanoparticles to label human bone marrow MSCs in vitro and studied their multilineage differentiation capacity, immunomodulatory function and immunophenotype. **Materials and Methods:** Mononuclear cells were isolated from human bone marrow and cultured in DMEM-LG/MCDB-201, 10% FCS, 2 mM L-Glutamine and 1% pen/strep. Confluent cells were labeled with iron oxide particles (Endorem®) at concentrations of 25-250 ug/mL in presence of poly-l-Lysine and stained with Prussian blue to visualize iron particles. MSCs were stained using monoclonal antibodies against CD29, CD44, CD105, CD166, CD73, HLA-DR-FITC, CD146, CD45, CD14, CD3, HLA-ABC and CD34 and analysed using a FACSAria (Becton Dickinson). For induction of differentiation towards adipocytes and osteoblasts, MSCs were maintained in specific differentiation media. Peripheral Blood Lymphocytes (PBL) were stained with 10 uM CFSE in PBS /2% FCS and 3%, 10% or 30% CFSE-labeled PBLs were added to 25 Gy irradiated control or iron-labeled MSCs and maintained in presence of 5 ug/mL PHA-L + 5 ng/mL IL-2. At day 4, cells were used for FACS analysis. **Results:** Iron-labeling of MSCs did not affect their osteogenic or adipocytic differentiation ability, viability, proliferative capacity and surface marker expression. In vitro iron-labeled MSCs and control MSCs displayed no differences in immunologic characteristics, with similar levels of activated T-cells (CD3/CD28/CD95+ cells) and comparable levels of FoxP3 regulatory T-cells (Treg). **Conclusions:** In vitro experiments demonstrated that MSCs labeled with iron particles preserve their viability, immunological properties, and ability to differentiate towards osteogenic and adipocytic lineage. Thus, MSCs can be stained effectively with iron particles in vitro without loss of their characteristics and can be used for in vivo tracking using MRI. Iron-labeled cells did not induce evident negative effects.

P1148 - POLYMERIC SCAFFOLDS WITH DIFFERENT ALIGNMENT AND THEIR INTERACTIONS WITH BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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Introduction: The development of bone tissue engineering is directly related to changes in materials technology. Both material characteristics and clinical requirements are critical when engineering a clinically relevant bone substitute. In this study, we aimed to investigate the interaction of mesenchymal stromal cells (MSCs) with randomly or aligned nanofiber PHB membranes prepared to mimic the physical structure of bone tissue and spinal axonal design, respectively. **Materials and Methods:** PHB membrane with its two different kinds of surface features as randomly arranged nanoscale fibers and nanoscale fibers oriented in one direction was used as a tissue scaffold. Human bone marrow MSCs were expanded on two different tissue scaffolds with different surface morphology and evaluated in terms of their orientation on tissue scaffolds by confocal and scanning electrone microscopes (SEM). Cells were also assessed for their adhesion characteristics and viability (MTT Assay, real time cell analysis, population doubling methods and Annexin V Assay); differentiation (adipogenic and osteogenic) and surface antigen properties. **Results:** The results of analysis showed that MSCs conserve their stem cell properties, adhesiveness and viability when they were expanded on both types of tissue scaffolds. While adhesiveness of MSCs that were expanded on oriented tissue scaffolds was more than knitted ones, proliferation rates was lower. Although, MSCs on oriented PHB tissue scaffolds showed spindle shape morphology and they were positioned in the direction of spindles, whereas MSCs on knitted nPHB tissue scaffolds distributed homogenously by conserving their poligonal morphology that mimicked bone marrow microenvironment. **Conclusion:** In the present study, it was found that MSCs cultivated on nanofiber PHB tissue scaffolds conserved their multpotentiality and attained morphology compatible with physical alignment of the scaffold; therefore they may provide effective tissue engineering approach by repairing cellular microenvironment in bone tissue engineering.

P1149 - LACTIC ACID BACTERIA CAN REDUCE TLR-4 EXPRESSION ON INFLAMMATED GINGIVAL FIBROBLASTS AND MESENCHYMAL STEM CELLS IN VITRO

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Introduction: Lactic acid bacteria (LAB) have been studied for their health-promoting and immunomodulatory effects. The main field of research has been in gastrointestinal tract. In the past few years LAB have also been investigated in the oral health perspective. We studied competitive adhesion of Lactobacillus strains with Porpyhromonas gingivalis on human gingival fibrolasts (GF) and bone marrow derived mesenchymal stem cells (MSCs). P. gingivalis, an important periodontal pathogen, is an effective colonizer of oral tissues and accounted for periodontitis. It has been shown that bacterial ligands stimulate Toll-like receptor (TLR) expression on a variety of cells. However it is unknown whether whole bacteria can induce TLR expression on GF and MSCs. . In this study, we evaluated the effect of LAB on cellular adhesion and TLR 4 and 2 expression induced by interferon (IFN)-γ. **Material and Methods:** Adhesion assay was conducted (Lactobacillus rhamnosus ATCC 9595, L. brevis OML 28, P. gingivalis ATCC 33277) and competition of LAB and P. gingivalis on GF and MSCs was studied. To determine the suppressive effect of Lactobacillus strains on IFN-γ- and P. gingivalis-based inflammation, TLR 4 and 2 expression by flow cytometer was investigated. **Results:** Adhesion of bacteria on GF and MSCs was significantly different. LAB prevented adhesion of P. gingivalis as shown by light and fluoresence microscopy. In addition,LAB also reduced TLR 4 expression on GF and MSCs, in in-vitro assays with IFN-γ and P. gingivalis. **Conclusion:** Adhesion of probiotic bacteria to oral soft tissues promotes their beneficial effect to the host. Obtained data suggested that cells from different tissues may achieve specific functional characteristics according to the microenvironmental needs. Further data showed differential responses on TLR expression of GF vs MSCs suggesting participation of GFs on immune response at oral cavity by TLRs. On the other hand, lower TLR expression on bone marrow derived MSCs may partially be attributed to the immunprivileged state of the bone marrow niche.

P1150 - LINEAGE SPECIFIC METABOLIC ACTIVITY IN HAEMATOPOIESIS

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Erythroid and neutrophil cells have widely different metabolic requirements and tend to be produced in distinct regions of the bone marrow associated with arterioles or sinusoids respectively. This is suggestive of metabolic compartmentalisation of haematopoietic marrow, and raises questions concerning the partitioning of metabolism in solid tissues, the interdependence of metabolic and signalling pathways in the control of normal and leukemic haematopoiesis and the achievement of high density in vitro culture conditions. A key issue is whether the early progenitor cells of each lineage employ distinct metabolic strategies to satisfy the biosynthetic demands of proliferation. To address this question we have purified mitochondria from large scale cultures of FDCP-Mix cells at various stages of erythroid or granulocyte/macrophage differentiation and subjected them to proteomic analysis using 8-plex iTRAQ liquid chromatography tandem mass spectrometry (LC-MS/MS). In a complementary approach, the uptake and release of key metabolites at various stages of differentiation was determined by mass spectrometry and biochemical assay of conditioned media. We find lineage specific changes in the balance of TCA cycle enzymes and of components of the electron transport chain, as well as in proteins controlling apoptosis and reactive oxygen species. Further changes in co-purified cytoplasmic enzymes suggest differential use of available substrates that is confirmed by lineage specific differences in metabolite uptake. Our results suggests that early neutrophil and early erythroid progenitors do indeed adopt different metabolic strategies to biosynthesis that optimise metabolite usage in bone marrow.

P1151 - DISTINCT LEUKEMIA-INITIATING SUBSETS ARE MAINTAINED BY IL-2/CD25 AXIS IN CHRONIC MYELOID LEUKEMIA NICHE

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Chronic myeloid leukemia (CML) is a clonal hematopoietic disorder originated from hematopoietic stem cells (HSCs) with the Philadelphia chromosome which produces BCR-ABL fusion protein. Targeting tyrosine kinase activity of BCR-ABL by tyrosine kinase inhibitors can deplete most of the differentiated CML cells but not CML leukemia initiating cells (LICs). Therefore it is mandatory to develop the eradication strategy of CML LICs. Here we show that CML LICs show a severely hypoxic phenotype then the normal HSCs in the niche. HIF-1alpha, a master regulator of cellular hypoxia response, plays a critical role for maintaining HSC in the hypoxic niche. HIF-1alpha-deficient p210BCR-ABL retrovirus transduced Lineage marker- Sca-1+ c-Kit+ (LSK) cells are defective in establishing LIC population in vivo. Under normoxic conditions, HIF-1alpha is recognized by an E3 ubiquitin ligase VHL. Although VHL deficient HSCs lose transplantation capacity, they show an accelerated formation of CML LIC after p210BCR-ABL retrovirus transduction. To elucidate the putative LIC markers in the hypoxic niche, we have screened various surface markers using p210BCR-ABL retrovirus-induced CML-like myeloproliferative disease model. Among them, we demonstrate that CML LICs are divided into CD25+FcεRIα- Lineage marker (Lin)- Sca-1+ c-Kit+ (F-LSK) cells and CD25-F-LSK cells. We show that CD25+F-LSK cells have multipotent differentiation capacity with preference to cytokine-producing mast cell commitment. Although both CD25-F-LSK and CD25+F-LSK cells differentiate into one another, CD25+F-LSK cells show higher LIC capacity than CD25-F-LSK cells. High expression of CD25 in CD34+CD38- fraction of human CML indicate that CD25+LICs constitute "LIC-derived niche" that can be a preferential therapeutic target for CML LICs.

P1152 - MULTIPLE MICROENVIRONMENTS IN THE FETAL LIVER WITH DISTINCT FUNCTIONS

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Hematopoiesis takes place during development in successive anatomical sites, each corresponding to different phases of hematopoiesis development. Hematopoietic Stem Cells (HSC) expansion and maintenance is controlled by a specific microenvironment termed niche. The niche during time and through the successive anatomical locations differs not only in terms of cellular and molecular composition, but also in terms of its function. First, it supports the emergence of HSC, then their amplification and finally in the adult, their maintenance. The fetal liver is the site of a major expansion of the HSC pool. Therefore we hypothesized that the fetal liver niche was the source of specific determinants capable of supporting a large expansion of HSC while maintaining their self-renewing potential. The present study focuses on defining the cellular components of the HSC niche. We first determined, using a combination of cell surface markers, the cell populations present in the mouse fetal liver at day 13.5. We identified cell populations that can produce adherent layers and will be referred to as stromal cells. We then tested in serum free conditions, their capacity to support the maintenance and expansion of HSC during a 2 week co-culture. Maintenance of serially transplantable HSC was observed only in cocultures with stromal layers derived from CD45-TER119-CD31-CD51+VCAM-1-PDGFRa+ cells. We concluded that this stromal cell population was an essential component of the fetal HSC niche. In the cocultures with stromal layers derived from CD45-TER119-CD31-CD51+VCAM-1+PDGFRa- cells, we observed an expansion of megakaryocytes, and erythroid progenitors and a loss of serially transplantable HSC. This suggests that this stromal cell population recapitulates a progenitor microenvironment.
Further characterization of these stromal cells and the factors they produce will guide the development of new methods for ex-vivo expansion of HSC or to direct their differentiation toward specific lineages.

P1153 - VARIABLE EFFECTS OF TUMOR SECRETED FACTORS ON HUMAN MESENCHYMAL STEM CELL

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Objective: Recent years has witnessed huge interest in studying the tumor microenvironment, given its apparent role in driving tumor progression and metastasis. Of particular interest, mesenchymal stem cells (MSCs) have been the focus of many research groups as the exact role of MSCs in driving cancer progression remains controversial. Herein, we investigated the effects of tumor secreted factors from a panel of human cancer cell lines (breast (MCF7 and MDA-MB-231); prostate (PC-3); lung (NCI-H522); and head & neck (FaDu)) on MSCs. **Methods:** Morphological changes were assesses using fluorescent microscopy. Changes in gene expression were assessed using Agilent microarray and qRT-PCR. Cell migration was assessed using transwell migration system. **Results:** Morphologically, MSCs exposed to secreted factor from FaDu, MDA-MB-231, PC-3, and NCI-H522, but not from MCF7, exhibited a spindle-shaped morphology, and the cells were more elongated with bipolar processes, compared to control MSCs which were larger and more flattened with multiple processes. Integrated analysis of gene expression and bioinformatics revealed a proinflammatory response of MSCs when exposed to conditioned media (CM) from all cell lines, but not MCF7. Nonetheless, MSCs exhibited significant tropism toward secreted factors from the aforementioned tumor cell lines. **Conclusions:** Thus our data suggest that MSCs might drive tumorigenicity through induction of inflammation.

P1154 - EXPOSURE TO IONIZING RADIATIONS AND STARVATION CULTURE DOES NOT MODIFY PHENOTYPE, FUNCTIONS AND GENETIC PROFILE OF MESENCHYMAL STROMAL CELLS ISOLATED FROM BONE MARROW OF HEALTHY DONORS

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Objective: Mesenchymal stromal cells (MSCs) are multipotent cells located in different human tissues, including bone marrow (BM), where they sustain the creation of the hematopoietic stem cell niche by regulating its differentiation and functions. Many studies have demonstrated that BM exposure to ionizing radiations induces the rapid depletion of hematopoietic precursors, however radiation effects on MSCs have been poorly investigated. Moreover, little is known about MSCs behavior in starvation culture conditions. In this study, we examined morphology, proliferative capacity (in terms of population doublings), immunophenotype, differentiation potential, immunomodulatory properties (PHA-induced T cell proliferation assay) and genetic profile (array-CGH and caryotype) of MSCs isolated from BM of healthy donors and exposed to ionizing radiations and starvation culture. **Methods:** MSCs were isolated from 10 healthy donors (median age: 16 years; range: 5-32) and expanded in culture medium supplemented with 5% platelet lysate up to passage 2. Thereafter MSCs were exposed both to escalating doses of ionizing radiations (3000, 10000 and 20000 rad) and to starvation culture conditions (culture medium supplemented with 1% platelet lysate instead of 5%). **Results:** With escalating doses of ionizing radiations, MSCs lose their typical spindle-shaped morphology, their boundaries are less regular and their growth rate slows down (at 3000 rad) or even stops (at 10000 and 20000 rad). Nonetheless, in the presence of 1% platelet lysate, although showing a slower growth rate as compared with non irradiated MSCs, the effects on morphology are less evident, thus suggesting that the lack of growing factors can slow down the senescent process induced by ionizing radiations. Furthermore, irradiated and starved MSCs maintain the same immunophenotype, differentiation potential, immunomodulatory properties and genetic profile of normal MSCs. **Conclusions:** Our data indicate that irradiated and starved MSCs, although presenting altered morphology and growth rate, maintain the typical characteristics of normal MSCs.

P1155 - SELECTIVE RADIORESISTANCE OF BONE MARROW HEMATOPOIETIC PROGENITOR CELLS COMPARED TO STROMAL CELLS FROM FANCONI ANEMIA (FANCD2-/-) MICE

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The DNA repair defect in FancD2 -/- mice causes sensitivity to total body irradiation. We determined whether cell phenotype specific differences in radiosensitivity, DNA repair, and irradiation induced gene expression were detectable with FancD2 -/- bone marrow stromal cells compared to IL3 dependent hematopoietic progenitor cells. Radiation survival curves were performed with cell lines derived from C57BL/6 FancD2 +/+, FancD2 +/- and FancD2 -/- mice. Cells were irradiated over a range of 0 to 8 Gy, and day 7 colonies of 50 cells counted. Repair of DNA strand breaks was determined by Comet Assay. Gene transcripts were measured by Real Time Polymerase Chain Reaction (RT-PCR) 24 hr after 5 Gy. FancD2-/- marrow stromal cells were radiosensitive (Do = 1.43 + 0.06 Gy, ñ = 4.98 ± 0.65) compared to FancD2 +/+ cells ((Do = 1.70 ± 0.09 Gy and ñ = 8.33 ± 0.72), p = 0.0395 and 0.0040, respectively) or FancD2 +/- cells (Do = 1.67 ± 0.08 Gy and ñ = 3.63 ± 0.44, p = 0.0348 and 0.1365, respectively). In contrast, IL3 dependent FancD2 -/- hematopoietic progenitor cells were radioresistant (Do = 1.65 ± 0.09 Gy and ñ = 2.47 ± 0.37) compared to FancD2+/+ or FancD2+/- hematopoietic progenitor cells (Do = 1.39 ± 0.09 Gy (p = 0.043) and ñ = 2.31± 0.85), and (Do = 1.64 ± 0.08 Gy and ñ = 4.40 ± 0.99 (p=0.004)). Comet assay demonstrated that IL3 dependent FancD2-/- hematopoietic cells repaired DNA strand breaks faster than FancD2 -/- stromal cells. There was an increase in p53 gene transcripts in FancD2 -/- IL-3 dependent cells after 5, compared to all three stromal cell lines or FancD2 +/+ or FancD2 +/- hematopoietic cells. Genes involved in cell cycle regulation including p21 and CHK-1 showed no relative increase in FancD2 -/- hematopoietic cells while p21 was increased in FancD2 -/- stromal cells. Levels of NFkB, Nrf2 and MnSOD remained unchanged after irradiation of FancD2-/- hematopoietic cells while FancD2 -/- stromal cells as well as FancD2 +/- and +/+ hematopoietic and stromal cells all showed increases. Absence of the FancD2 gene confers radiosensitivity to bone marrow stromal cells; however, hematopoietic progenitor cells from the same mice are radioresistant.

P1156 - CHARACTERIZATION OF THE IMMUNOMODULATORY EFFECT IN VITRO OF MICROVESICLES ISOLATED FROM MESENCHYMAL STROMAL CELLS ON IMMUNE CELLS

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Objective: Mesenchymal stromal cells (MSCs) are multipotent cells that exert immunomodulatory effects on immune cells, however the mechanisms underlying these effects and whether the modulation is mediated by cell-to-cell contact or paracrine effects have not been completely clarified. Aim of this study was to compare in vitro the immunomodulatory properties of MSCs with those of microvesicles (MVs) released in supernatants of the same MSCs. **Methods:** MSCs were expanded *ex vivo* from bone marrow of 12 healthy donors and MVs were isolated from their supernatant by serial ultracentrifugation. Both MSCs and MVs were characterized by flow cytometry and co-cultured with PBMCs of 12 healthy donors and stimulated with PHA and CpG to induce T and B cells proliferation, respectively. The release of growth factors and cytokines was quantified by ELISA. **Results:** MVs were identified as 1μm particles positive for CMFDA, CD107 and CD13 (suggesting a mechanism of membrane budding from MSCs). MSCs inhibited T-cell proliferation up to 80% and 60% (MSC:PBMC ratio 1:2 and 1:10, respectively), whereas MVs reduced it up to 30% (with a MVs'dilution of 1:2 in co-culture final volume). Moreover, MSCs could reduce B-cell proliferation up to 70% and plasma cells activation up to 50% (MSC:PBMC ratio 1:10), whereas MV-induced inhibition was up to 60% and 30%, respectively (same diluting conditions). In both T- and B-cell cultures, MSCs induced an increase of IL-6, IL-10, TGFβ and a decrease of IL-2 and IFNγ, whereas MVs produced a decrease of IL-10 and TGFβ and an increase of IFNγ. **Conclusions:** Our data, obtained in a defined *in vitro* system, indicate a lower immunomodulatory effect of MVs than that of their cellular counterpart. Despite the important role played by the soluble factors released by MSCs and contained in MVs, cell-to-cell contact seems to be essential for MSC-induced immunomodulatory effects.

P1157 - INTERACTION OF TUMOR CELLS WITH THE HEMATOPOIETIC STEM AND PROGENITOR CELL NICHE

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The bone marrow microenvironment is known to regulate self renewal and differentiation of hematopoietic stem and progenitor cells (HSPC). The microenvironment becomes site of choice for HSPCs because of chemoattractants,growth factors and cell adhesion signalling network with mesenchymal stromal cells (MSC) as major cellular component. The supportive functions of the niche could also be involved in its neoplastic transformation. Dissemination of breast and prostate cancer cells into the marrow has been described even in early stages of the disease. The study focuses on influence of breast carcinomas on mesenchymal and hematopoietic progenitor cells in the bone marrow. The breast cancer cell lines -MCF-7 and MDA-MB231 caused a significant reduction in HSC adhesion to the MSCs (54% by MCF-7 cells; $p < 0.001$ and 68% by MDA-MB231 cells; $p < 0.0001$). No significant reduction in HSC adhesion was observed upon incubation with conditioned medium from MCF-7 and MDA-MB231 cells.Competitive adhesion thus seems dependent upon cell-cell contact. MCF-7 and MDA-MB231 cells express ICAM-1, which has been shown to promote breast cancer metastasis (Rosette et al, 2005). ICAM-1 and selectins shall be further studied to understand mechanisms appropriated by breast cancer cells to reduce HSC adhesion to MSCs and implications on HSC homing to the niche. A cytokine array was performed to probe if MCF-7 and MDA-MB231 cells affect cytokine profile of MSCs. The array showed altered expression of growth factors and inflammatory molecules-bFGF, PDGF-BB (2.2 fold upregulation and 0.5 fold downregulation in tumor cocultures respectively), IL-17and TNF-alpha (2 fold upregulation and 0.5 fold downregulation in tumor cocultures respecitvely).Based on the array, a bFGF mediated synergistic increase in proliferation of MSCs and breast cancer cells in coculture was observed. Interestingly the breast cancer cells caused a reduction in osteoblastic differentiation of MSCs, indicating a reduced support for HSCs in the neoplastic niche.These findings indicate a perturbed HSC niche upon tumor invasion. The possible role of altered cytokine expression, downstream signalling in niche activation and bone turnover shall be further studied to recapitulate tumor micrometastases to the HSC niche.

P1158 - THE ROLE OF THE ANGIOPOIETIN/TIE SYSTEM IN REGULATING HEMATOPOIETIC STEM CELL MAINTENANCE AND RECRUITMENT

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The Angiopoietin/Tie system controls angiogenesis and vessel maturation. However, signalling through the receptor tyrosine kinase Tie2 and its ligand Angiopoietin-1 (Ang-1) has recently been identified to play a crucial role in controlling quiescence and maintenance of hematopoietic stem cells in the bone marrow niche. Pericytes, smooth muscle cells and fibroblasts express Ang-1 in a paracrine manner. Recently, bone marrow osteoblasts have also been identified to express Ang-1. The receptor tyrosine kinases Tie1 and Tie2 are both expressed by hematopoietic stem cells. In contrast, Ang-2 is secreted exclusively by endothelial cells, where it is stored in Weibel-Palade bodies and released upon endothelial cell activation to act as autocrine antagonist regulator of constitutive Ang-1/Tie2 signaling. Until now, very little is known about the role of the dynamic modulators Ang-2 and Tie1 within the hematopoietic niche. Preliminary data from our laboratory established a role of Ang-2 in controlling the hematopoietic stem cell niche. Furthermore, the Tie1 intracellular domain acts as a Tie2 enhancing co-receptor. Here, we study the expression profile and functional roles of Ang-2 and Tie1 in controlling the maintenance and egress of hematopoietic stem cells in the bone marrow niche under steady-state conditions and upon mobilization. Understanding the crosstalk of the signaling molecules and tightly regulated mechanisms that balance stem cell quiescence versus proliferation in the hematopoietic niche is of major relevance to improve chemotherapy and adult stem cell transplantation.
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P1159 - DECLINED PRESENTATION
EFFECTS OF IONIZING RADIATION ON MESENCHYMAL STEM CELLS OF BONE MARROW HEMATOPOIETIC NICHE

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Background: Total body irradiation followed by bone marrow transplantation is the cornerstone of modern medicine for the treatment of hematological cancers. While transplantation provides donor-derived healthy hematopoietic stem cells, the hematopoietic niche[1] remains patient-derived and undergoes severe physiological stress upon radiation treatment. Therefore, we set out to investigate the effects of ionizing radiation on functional changes of the bone marrow hematopoietic niche that may contribute to the recurrence and/or survival of cancer stem cells. **Methods:** Using X-ray radiation and severe hypoxia as the models of bone marrow injury we looked at the functional changes in the major cellular component of the hematopoietic niche - mesenchymal stem cells (MSCs)[2]. Capacity of human bone marrow derived MSCs for adhesion to fibronectin, colony formation (CFU-F) and tri-lineage differentiation (osteoblastic, chondrocytic and adipocytic) in normoxia (21% O_2) vs. hypoxia (1.5% O_2) and following 0.1 Gy of X-ray radiation were investigated. Additionally, quantitative polymerase chain reaction (qPCR) was employed to measure the expression of genes involved in hematopoiesis and stress response. **Results:** Our studies have demonstrated that radiation did not significantly affect adhesion or colony formation of MSCs. However, hypoxia dramatically decreased the ability of mesenchymal cells to adhere to fibronectin treated surfaces. Similarly, no effect of radiation was noted on the capacity of MSCs to differentiate, while hypoxia resulted in decreased adipocytic differentiation, complete abrogation of osteogenic differentiation and increased chondrocyte formation. Finally, four genes under investigation (p53, angiopoietin 1, stem cell factor and interleukin-7) exhibited a similar trend of expression with small changes upon radiation treatment and dramatically decreased expression in hypoxia. **Conclusions:** Mesenchymal cells exhibited radio-resistant properties and extreme sensitivity to hypoxia. This suggests that radiation-induced vascular changes that perturb oxygen level in the perivascular space of the bone marrow may be of crucial importance in the maintenance of normal hematopoiesis following radiation treatment. **References:** 1) P. Bianco. *Blood.* 2011;117(20):5281. 2) S. Mendez-Ferrer et al. *Nature.* 2010;466(7308):829.

P1160 - CHITOSAN/DNA NANOPARTICLES CHARACTERISTICS DETERMINE THE TRANSFECTION EFFICACY OF GENE DELIVERY TO HUMAN MESENCHYMAL STEM CELLS

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Purpose: The objective of this study was to evaluate the potential of prepared chitosan -plasmid DNA nanoparticles in transferring the exogenous gene into human bone marrow-derived mesenchymal stem cells. **Methods:** Chitosan/pDNA nanoparticles were synthesized through the complex coacervation method using 18, 50 and 136 KD chitosan and pTracer-CMV2 plasmid containing Green Fluorescent Protein (GFP) gene. In this regard several concentrations of chitosan solutions (0.05%, 0.1%, 0.5% and 1%) were used. To examine the complexation, samples were run through an agarose gel. The size and zeta potential of nanoparticles were measured by Nanosizer. Scanning electron microscopy (SEM) imaging was used to observe the morphology of nanoparticles. MSCs were prepared from human bone marrow and transfected with chitosan/pDNA nanoparticles. The cultures transfected by lipofectamine2000 was taken as the control. Cell viability and transfection efficiency were determined by MTT assay and flow cytometric analysis respectively. **RESULTS:** The smallest size of complexes was obtained using 50KD chitosan (about 50nm) and the highest zeta potential was with 136 KD chitosan (29.61mV). The best transfection rate (18.43%) was achieved with 0.1% concentration of 18KD chitosan nanoparticles. commercial lipofectamine transfected 40.57% of cells. MTT assay indicated an average of 95.5% cell viability for 0.1% concentration of 18KD (chitosan compared with about 60% of Lipofectamine2000. **Conclusions:** Nanoparticles produced by 18KD chitosan at 0.1% concentration and pDNA would be a promising gene delivery system to human marrow derived MSCs. Although transfection efficiency of such nanoparticles is lower than that of Lipofectamine2000 but they comparatively possess less cytotoxic effects.

Keywords: Chitosan, pDNA, Nanoparticle, Mesenchymal stem cell, Transfection

P1161 - PLATELET HARVEST, CRYOPRESERVATION AND INFUSION; RE-EXPANDING THE RESOURCES OF THE BMT LAB

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A 53 year old multiparous woman with a hepatic mass was found to have AML. She proceeded to induction chemotherapy resulting in complete remission by day 28. Between cytarabine consolidation cycles, she received 3 TACE procedures for presumed hepatocellular carcinoma. During first cycle, she developed platelet refractoriness with no platelet increment one hour post transfusion. Testing confirmed the presence of an HLA antibody. HLA matched platelet transfusions did not give a satisfactory platelet increment. The decision was made to harvest and cryopreserve her platelets, then reinfuse post chemotherapy.

The patient was scheduled to have plateletpheresis with a goal of 10 x 10^{11}platelets to be harvested and cryopreserved, allowing for 4 infusions as support over the nadir post-chemotherapy. This was to be repeated to support the final round of chemotherapy.

Collections: Once platelet count reached > 150, 4 collections were performed over 2.5 months yielding between 3.1 to 5.5 x 10^{11} platelets per harvest, irradiated at 25Gy, and cryopreserved in 5% DMSO using a controlled rate freezer. Studies on her post-thaw platelets using PRP impedance indicated a 50% reduction in functionality compared with fresh platelets. **Infusions:** Four infusions were performed 11-20 days post consolidation 2 chemotherapy and three infusions 11-19 days post consolidation 3. No adverse reactions were reported. Platelet counts increased typically from 11-29 (1.6 to 2.6 fold) within 24 hours of infusion. HLA matched platelets gave no increase (pre-infusion count of 8, to post of 9, within 24 hours). There were no clinically significant bleeding complications and she was able to finish her scheduled rounds of chemotherapy. **Conclusions:** We have presented a case study requiring harvest, cryopreservation and infusion of autologous platelets. Incorporating archived platelet protocols into current practices was easily and safely achieved by the BMT laboratory. The methodology, equipment and processes involved required little change from the traditional role of progenitor cell work and this can safely extend the services offered by BMT laboratories with minimal additional costs for equipment or consumables.

P1162 - BONE FORMATION AND THE DEVELOPMENT OF BONE MARROW

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Background: Lifelong haematopoiesis is mediated by haematopoietic stem cells (HSCs) that give rise to all blood lineages. Long-term quiescent HSCs localise to the interface between bone and bone marrow (B-BM). This anatomical juxtaposition belies a functional relationship between bone metabolism and haematopoiesis. The mature B-BM interface is a complex structure composed of intricate networks connecting cells, extracellular matrix, and growth factors. This complexity renders investigation of the component parts of the B-BM interface extremely difficult. **Aim:** This study uses a unique model to investigate the early spatial and temporal events leading to B-BM formation. **Methods:** Rat acellular demineralised bone matrix (DBM) was implanted intermuscularly into the hind-limbs of nude mice, and explanted between days 2-28. The resultant ectopic nodules were subjected to macroscopic, histological and morphometric analyses (microCT scanning), gene expression studies and colony-forming unit (CFU) assays. **Results:** The progressive increase in vascularity and hardness of the nodules was reflected in their corresponding histological analyses. Neovascularisation was an early event and appears to be essential for cellular repopulation of the DBM. MicroCT imaging of explanted nodules first detected X-ray dense areas by day 10. This preceded the appearance of haematopoietic progenitors evident first on day 14 (CFU assays). The same types of CFUs were cultured from the corresponding long-bone marrow. DBM remodelling resulted in mature nodules consisting of newly formed bone (with the presence of osteoblasts, lining cells, osteocytes and osteoclasts) encompassing a centralised marrow cavity; which was confirmed histologically. **Conclusion:** Collectively, these findings suggest that bone is integral to, and precedes the development of marrow. On-going studies will determine critical changes in gene expression between days 7-14 that may be associated with bone marrow development. The use of this unique ectopic bone formation model to study the genesis of haematopoiesis may give us specific insights into critical parameters that can influence microenvironmental cues for HSCs.

P1163 - P18INK4C IS AN UNIQUE TARGET FOR HEMATOPOIETIC STEM CELL EXPANSION WITH SMALL MOLECULE INHIBITORS

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The G1-phase of cell cycle is a critical window in which stem cell self-renewal may be balanced with other fate choices. The cyclin-dependent kinase inhibitors (CKIs), including p15, p16, p18, p19, p21, p27 and p57, have been reported to be involved in the regulation of stem cells especially the hematopoietic stem cells (HSCs). Among all the CKIs examined in HSCs, p18 and p27 were shown to have inhibitory effects on HSC self-renewal or repopulation in mice. In this study, we show that p18 is a more potent inhibitor for HSC self-renewal than p27 in vivo. Absence of p18 allowed HSCs to be expanded and maintained in a long-term culture system for more than 15 weeks. Surprisingly, overall cycling of hematopoietic cells was not increased in the absence of p18. Instead, a favored outcome of self-renewal after HSC division was indicated by single cell analyses. To target p18 pharmacologically, we have designed a number of p18 small molecule inhibitors via in silico-screening and validated them by functional assays. Importantly, the identified lead compounds were able to specifically expand HSCs in vitro. Thus, these novel p18 small molecule inhibitors offer valuable chemical probes for dissecting the signaling pathways of self-renewal of tissue stem cells, and serve as important lead compounds to further develop more effective agents for therapeutic stem cell expansion.

P1164 - CHARACTERIZING FUNCTIONAL HETEROGENEITY IN HEMATOPOIETIC PROGENITOR CELL CULTURES: A COMBINED EXPERIMENTAL AND MODELING APPROACH

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Cultures of primary HSCs and hematopoietic cell lines are often characterized by heterogeneous proliferation rates, a non-negligible level of background differentiation and cell death and the maintenance of self-renewal ability within a small subpopulation of cells only. The multipotent murine progenitor cell line FDCP-Mix demonstrates these features during continuous passaging in high IL-3. The mechanisms by which a small fraction of self-renewing cells is maintained in a continuously expanding, intrinsically heterogeneous population are unknown, but are likely to be relevant to similar homeostatic processes in vivo.
We investigated the heterogeneity and maintenance of FDCP-Mix populations using a combined experimental and mathematical modeling approach. The turnover of FDCP-Mix cells was studied at both the population and single cell levels to define the functional heterogeneity. By adapting an established single-cell based model of hematopoiesis to accommodate variable probabilities of commitment to two lineages as well as lineage-specific cell cycle times and death rates we provided a modeling framework on which to interpret the experimental results. We found that measurements at the population level could be accounted for by a wide variety of model scenarios. However, long-term cell tracking of single cell fates and extraction of cellular genealogies permitted correlation analysis of cell death events and cell cycle times, and thereby the exclusion of certain scenarios and a progressive model refinement. We find the observed heterogeneity to be best explained by a combination of differential regulation of cellular turnover and apoptosis during background differentiation along different lineages, with cells that commit to the granulocyte lineage appearing to cycle faster and to have a higher apoptosis rate than those that commit to the monocyte lineage.

P1165 - SMALL MOLECULE INHIBITORS OF PHAGOCYTOSIS FOR TREATMENT OF IMMUNE CYTOPENIAS

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Immune cytopenias are caused by endogenous production of auto- or alloantibodies to blood cells resulting in the destruction of autologous, transfused or neonatal red cells, platelets or neutrophils. These conditions arise primarily due to extravascular destruction of the blood cells by mononuclear phagocytes present in the liver and/or spleen through specific Fc receptor (FcR) recognition of the Fc portion of the antibody opsonizing the blood cells. Although there are a number of treatments available, they require invasive splenectomy or immunoglobulin therapy; or drugs such as rituximab and thrombopoietin receptor agonists; or corticosteroids that take hours to be effective. All of the current therapies have potential side effects that can result in significant morbidity and none of the current treatments are 100% effective. We have investigated a new class of drugs, disulfide-containing pyrazole compounds, which have been designed to inhibit Fc-mediated phagocytosis. A broad screen indicated that 4-methyl-1-phenyl-pyrazole derivative could inhibit the phagocytosis of opsonized blood cells with weak potency. However, discovery of the polysulfide products and additional synthesis of various 1-phenyl-pyrazole derivatives have led to compounds that have increased efficacy to inhibit phagocytosis in vitro and in vivo, using a mouse model of experimental ITP. Current lead candidate compounds have been shown to have low to negligible toxicity and an IC50 of 100 nM for inhibition of phagocytosis using an in vitro assay system with human mononuclear phagocytes while the same compounds showed a significant increase in platelet numbers and, thus, able to partially ameliorate passive antibody-induced ITP in mice. Further studies using additional derivatives designed to increase solubility and potency may provide acceptable toxicity and efficacy that used alone or in combination with currently available treatments may prove beneficial in future therapies for immune cytopenias.

P1166 - THE HUNT FOR A REPLENISHABLE MSC SOURCE TO CREATE (GENETICALLY MANIPULATABLE) ECTOPIC HUMAN HEMATOPOIETIC BONE MARROW NICHES

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Human bone marrow (BM) mesenchymal stem cells (BM-MSCs) are adult stem cells that can differentiate into multiple cell lineages in vitro. We recently showed that upon subcutaneous transplantation into immune-deficient mice, they form human bone, which supports the engraftment/outgrowth of normal and malignant hematopoietic progenitor cells. However, since BM-MSCs progressively lose their in vivo osteoblastic differentiation potential upon prolonged culture, this impedes their potential use as a source to create (genetically manipulatable) ectopic human BM niches. Therefore, we set out to test the in vitro/in vivo differentiation properties of MSCs from less definite sources. For this purpose, we generated telomerase reverse transcriptase (TERT)-immortalized bmMSCs (TERT-MSCs), MSCs derived from human embryonic stem cells (ES-MSCs) as well as from induced pluripotent stem cells (IPS-MSCs) and compared their morphological, immune-phenotypical, and in vitro/in vivo multi-lineage differentiation characteristics. TERT-MSCs, generated by lentiviral transduction of BM-MSCs with human TERT, as well as ES-MSCs and IPS-MSCs, generated by culturing ES and iPS cells for > 4 weeks in MSC medium, morphologically resembled BM-MSCs and for the most part expressed the MSC-defining markers CD73, CD90 and CD105. In vitro, all these MSC types could differentiate towards the osteoblastic lineage and we are currently testing their chondrocytic/adipocytic differentiation capacities. Interestingly, whereas BM-MSCs form an ectopic BM niche containing bone, ES-MSCs formed abundant cartilage tissue under the same in vivo conditions. Together, these results describe the successful generation of TERT, ES- and IPS-MSCs with MSC characteristics. Additional in vivo testing should demonstrate their potential as an inexhaustible MSC source for generating hematopoietic-supporting ectopic BM niches. Furthermore, generation of ipsMSCs derived from patients with bone/cartilage diseases may allow for the development of models to study these diseases.

P1167 - DECLINED PRESENTATION ISOLATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS USING GLYCEROL DENSITY GRADIENT

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Isolation of peripheral blood mononuclear cells (PBMCs) is fraught with challenges including but not limited to the cost of limited gradients available for isolation of (PBMCs). We used a glycerol gradient (1.077g/ml) to isolate PBMCs from adult peripheral blood. The viability and differentiation potential of the isolated cells was assessed by culturing the cells in MEM at 37OC in 5% CO2. The results revealed that the isolated cells could proliferate into committed linages of the erythroid progeny. Glycerol was not cytotoxic to the isolated cells at the final formed gradient as revealed by LDH assay. The use of glycerol density as an alternative might be of significance in cell culture experiments.

P1168 - DEVELOPMENT OF LIGAND-IMMOBILISED SURFACES FOR EX VIVO EXPANSION OF HAEMOPOIETIC STEM CELLS

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Haemopoiesis is regulated by various growth factors and cytokines that are produced by cells residing in the bone marrow (BM). These factors are cell surface-bound or secreted molecules. The Notch ligand, stem cell factor (SCF) and fms-related tyrosine kinase 3 (Flt3) ligand are examples of cell-bound cytokines in the BM and their signalling is essential for the development of haemopoietic stem cells (HSCs). To mimic the cell surface-bound form of these ligands for HSC culture, we have developed a molecularly well-defined surface which installs biological ligands with controlled density and uniform orientation. The self-assembled monolayers (SAMs) of oxyamine- and poly(ethylene glycol)-terminated thiol on a gold surface was used and this mixed monolayer surface can immobilise aldehyde-functionalised ligand via oxime formation. A thrombopoietin (TPO) mimetic peptide RILL and aldehyde-tagged SCF were immobilised on SAMs. Factor-dependent cell lines that die rapidly in the absence of corresponding cytokine were used to validate the stability of immobilised cytokines. We found that the ligand immobilised surface supports higher ex vivo expansion of primitive HSCs compared to the standard culture with soluble counterparts. Cord blood CD34+ cell culture in serum-free medium supplemented with SCF, Flt3, IL-6 and TPO supported a 10-fold increase in CD34+CD15-CD41a- cells in 7 days while the culture on RILL-immobilised SAMs instead of adding soluble TPO resulted in 15-fold expansion. Ligand density on the surfaces can be controlled by changing the ratio of oxyamine-terminated thiol and the quantity of the ligands has been assessed by X-ray photoelectron spectroscopy and electrochemistry. This ligand presenting surface could be beneficial for long term and large scale culture in a bioreactor by delivering cytokines to HSCs in a non-diffusible and non-endocytosible manner.

P1170 - DECLINED PRESENTATION
EFFECT OF PERFLUOROCARBON EMULSION ON HUMAN LYMPHOCYTE ANTIGEN EXPRESSION IN VITRO

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Perfluorocarbon emulsions (PFCE) are the basis of an infusion medium capable of transporting gases to blood. In our Institute we have developed monodisperse PFCE with nanoparticle diameter $\sim$100 nm. Its stability and physicochemical characteristics satisfied medical and physiological requirements imposed on dispersed PFCE media intended as a drug model (pH $\sim$7.4, relative viscosity 1.24-1.6, osmolarity $\sim$240-250 MOcm). Previously we have shown that effect of this emulsion on blood cells depends on blood/emulsion ratio and particles of PFC do not exert any cytotoxic effect on neutrophils, RBC and platelets if amount of emulsion in blood does not exceed 20% by volume. **Aim:** to evaluate effect of PFCE in physiological concentration range on expression of surface antigens of human peripheral lymphocytes in vitro. **Methods:** Ficoll-separated mononuclear cells obtained from healthy volunteers (n=10) were incubated for 1 hour at +37 C together with PFCE added at 4% (1) and 15% (2) of incubation mixture volume, and without PFCE (control). After one washing procedure expression of surface CD3, CD4, CD8, CD19, DR, CD16/56, CD4/25 and CD16/56/3 antigens was studied using 5-color flow cytometry (Beckman Coulter FC 500). **Results:** We have revealed that PFCE at studied doses did not exert any significant effect on relative number of main lymphoid subsets in peripheral blood and on degree of expression of antigens on cell surface. There have been some insignificant increases or decreases of the same parameters in different samples varying from 5 to 25% from baseline, with the exception of NKT-cells (CD16/56+/3+) which relative number had a tendency to increase dose-dependently from 5,4% (control) to 6,5% (1) and 6,7% (2). Thus, PFCE developed in our Institution does not exert any negative influence on surface structures of normal human lymphocytes at physiolocical concentration range. A revealed tendency to dose-dependent increase of percent amount of NKT-cells known to play a key role in regulation of immune response in autoimmune and malignant processes needs to be further studied.

P1171 - BIOETHICAL TRENDS IN TRANSITIONING FROM BASIC HEMATOPOIETIC STEM CELL RESEARCH THRU THE TRANSLATIONAL RESEARCH CYCLE (T1 - T4)

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Bioethical issues in hematopoietic stem cell research vary substantively and by degree, based on the role of the individual participating in the research (recipient, related donor or unrelated donor), the phase(s) of research in which the individual is participating in, and a variety of neuro-cognitive and socio-cultural factors. A literature review was conducted to determine current bioethical issues in hematopoietic stem cell research. Current bioethical implications specific to the use of human subjects (both recipients and donors) from basic hematopoietic stem cell transplantation research through clinical trials and translational applications to the general population were reviewed. Bioethical issues related to Translational phases T1 - T4 were overlaid and diagrammed. Considerations for increasing levels of participation (at the individual and population levels) and decreasing health disparities based on race and ethnicity are addressed, as well as implications for anthropological variables that affect bioethical issues on a global level.

P1172 - DISTINCT PATTERNS OF LUNG GENE TRANSCRIPT INDUCTION IN THORACIC IRRADIATED FIBROSIS-PRONE C57BL/6NHSD COMPARED TO FIBROSIS-RESISTANT C3H/HEN MICE

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A major complication of total body irradiation for marrow transplant is lung fibrosis, the mechanism of which is unknown. Pulmonary endothelial cells have been suggested as the target tissue. After 20 Gy thoracic irradiation C57BL/6NHsd (BLK6) mice display acute lung inflammation (1-21 days), a latent period where the lung appears normal (21-125 days), and late fibrosis stage (125-200 days). In contrast, 14.5 or 16 Gy thoracic irradiated C3Hf/Kam (C3H) mice display no late fibrosis. We examined irradiation induced pulmonary gene transcripts in each strain following 20 Gy thoracic irradiation. Lungs were removed at serial time points, RNA extracted, and RT-PCR used to quantitate expression of transcripts related to genes involved in the irradiation stress-response (NFKB, Sp1, AP1, Nrf2, TGF-B), radioprotection (SOD2, LOX), endothelial cells (vWF, VEGFa) and fibrosis (FGF-1, CTGF). No BLK6 mice died within the first 7 days after irradiation, while 20% of C3H mice died from acute pneumonitis. Pulmonary gene expression in fibrosis prone BLK6 revealed two distinct patterns:1) robust acute expression of NFKB, Nrf2, SOD2, TGF-B, SP1 and AP1, followed by latent period decreased expression, then 2) elevation of the same gene transcripts during late fibrosis. C3H mice showed elevated expression of NFKB but less Nrf2, SOD2, TGF-B, SP1, and AP1, and decreased subsequent expression of NFKB, Nrf2, AP1, and Sp1. In contrast C3H mice had persistent elevation of TGF-B and SOD2 with no return to baseline and no latent phase. Blk6 mice showed persistent elevated expression of endothelial gene transcripts for: vWF, VEGFa over 200 days while C3H mice showed no increased expression of vWF, and lower VEGFa expression. C3H lung histopathology at 2 days after 20 Gy irradiation showed greater inflammation. Profound differences in lung acute irradiation dose tolerance, and chronic gene transcript elevation (in endothelial cell and inflammation related genes) were observed between thoracic irradiated C3H and BLK6 mice. These distinct mouse genotype related differences in lung irradiation mediated gene transcripts should lead to identification of new mechanistic targets for preventing irradiation pulmonary fibrosis.

P1173 - RECONSTRUCTING A CORE REGULATORY NETWORK MODEL FOR BLOOD STEM/PROGENITOR CELLS

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Modelling of haematopoietic gene regulatory networks based on experimentally informed cis-regulatory interactions has so far been restricted to at most three interacting transcription factors (TFs). Here we report a newly generated network model using detailed functional data connecting 10 haematopoietic stem cell TFs (Erg, Fli1, Gata2, Gfi1b, Lmo2, Lyl1, Meis1, PU.1, Runx1 and Scl). Analysis of ChIP-Seq data for the 10 TFs across their gene loci identified 54 candidate regulatory elements showing elevated H3K27 acetylation and binding of at least three of the 10 TFs. 41 of these elements have so far been tested in transgenic mice which allowed us to validate 26 enhancers to possess haematopoietic activity in E11.5 transgenic mouse embryos; 11 of these were previously unknown. To investigate the importance of specific TF binding sites, luciferase reporter assays were performed with wild-type and mutant enhancer constructs, which revealed a plethora of both positive and negative inputs into haematopoietic regulatory elements, where interestingly the same TFs can activate or repress different elements. To identify TF complexes that contribute to combinatorial TF interactions, a comprehensive literature survey has been complemented with co-immunoprecipitation studies (Co-IPs), and revealed several previously unknown interactions between the 10 TFs. The ChIP-Seq and transgenic data as well as luciferase reporter assays, Co-IPs and protein degradation data were then used to develop a stochastic modelling approach to quantitatively model gene expression over time in individual blood stem/progenitor cells. This analysis revealed a stable state where all 10 TFs were expressed simultaneously, thus suggesting that our experimentally informed wiring diagram can be used to generate network models that can reproduce expected HSC expression states. Modelling allowed rapid simulation of perturbation studies and therefore provides a rich source for hypothesis generation; the experimental validation of which is the focus of my current research.

P1174 - NFIX EXPRESSION CRITICALLY MODULATES EARLY B LYMPHOPOIESIS AND MYELOPOIESIS

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The commitment of stem and progenitor cells toward specific haematopoietic lineages is tightly controlled by a number of transcription factors that regulate a differentiation program via the expression of lineage restricting genes. Nuclear factor one (NFI, also known as NF-I, CTF (CAAT box transcription factor), Nuclear factor 1, NF-1) family of transcription factors (NFIA, NFIB, NFIC, and NFIX) act as transcriptional activators and/or repressors of cellular and viral genes and are documented to play a functional role in haematopoiesis, with NFIA being the most studied in this system. NFI protein family members act as homo- and heterodimers and bind promoters of genes expressed in different organs, including brain, lung, liver, intestine, muscle, connective tissue, skeletal elements and haematopoiesis. Data indicates that NFI proteins may act as tumor suppressor or oncogenes and putative drivers of lineage specification and perturbation. There has not been a described role for NFIX in lineage specificity in the haematopoietic system and we report here for the first time an important role of NFIX in B- and myeloid lineage differentiation. We investigated the function of NFIX during haematopoietic differentiation and demonstrate that NFIX represses the differentiation of pro-B cells and favors myeloid cell differentiation; thus it acts as a regulator of lineage specification in the haematopoietic system. NFIX is normally transcriptionally downregulated as B cells differentiate from Pro-B cells into immature and mature B cells and we show that NFIX overexpression in vivo blocks B cell development at the pro-B cell stage, and this correlates with transcriptional changes. Using in vitro differentiation assays NFIX was able to drive myeloid differentiation even under B cell specific culture conditions from the HSC but not from committed pro-B cells. These data highlight a novel role for NFIX transcription factor in haematopoiesis and in lineage specification.

P1175 - DECLINED PRESENTATION
THE RNA-BINDING PROTEIN ATAXIN-2 REGULATES THE ONSET OF MEGAKARYOCYTIC DIFFERENTIATION

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The transcriptional changes leading to the differentiation of hematopoietic stem and progenitor cells (HSPC) towards mature megakaryocytes have been well studied whereas little is known about the contribution of translational regulation to this process. Here we report that the translational regulator Ataxin-2 (ATXN-2) controls the onset of terminal differentiation in megakaryopoiesis. We identified ATXN-2 as a target of MEIS1, a megakaryocyte-specific transcription factor. ATXN-2 is expressed in human CD34+ HSPC's, 3-fold upregulated in CD34+/CD41+ megakaryoblasts, and decreases by 90% in mature megakaryocytes. To unravel its role in megakaryopoiesis, lentiviral ATXN-2 knockdown was performed in CD34+ cells. When transduced CD34+ cells were differentiated towards megakaryocytes with TPO and IL-1b, ATXN-2 knockdown resulted in 50% decrease in CD34+ cells accompanied by a 2-fold increase in CD34+/CD41+ cells compared to short hairpin control indicating premature differentiation. In semisolid media promoting megakaryocytic colony formation, ATXN-2 knockdown resulted in a 2-fold colony-increase compared to short hairpin control. Differentiation of transduced cells towards an erythroid or granulocytic fate was not affected underlining a specific role for ATXN-2 in megakaryopoiesis. Immunoprecipitations revealed interactions of ATXN-2 with PABP and DDX6, proteins regulating translation. Furthermore, polysome profiling showed that ATXN-2 does not bind actively translated mRNA. When we analyzed the hematopoietic compartment of Atxn-2 deficient mice, platelet aggregation in response to specific agonists was diminished by 2-fold while all other peripheral blood cell parameters where unchanged confirming a specific role of ATXN-2 in platelet function. Taken together, our results indicate that ATXN-2 has a specific role in timing terminal megakaryocytic differentiation, most likely by inhibiting translation of target mRNAs.

P1176 - GENOME SCALE TRANSCRIPTIONAL CONTROL OF HAEMATOPOIETIC CELL TYPE IDENTITY

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Transcription factors (TFs) have long been recognised as powerful regulators of cell type identity. The underlying mechanisms however have remained largely obscure, despite major advances in the generation of genome-wide binding maps. Moreover, how so-called master regulators can contribute to the functionality of multiple distinct cell types is even less-well understood. Here we have compared global binding patterns for 10 key haematopoietic TFs in both mast cells and blood progenitors, revealing largely cell-type specific binding profiles. We argue that this cell type specific binding is not opportunistic or simply driven by open chromatin, but instead the source of meaningful contributions to cell-type specific transcriptional control, because (1) mathematical modelling demonstrates that differential binding of shared TFs can explain differential gene expression, (2) motif analysis demonstrates that cell-type specific binding is largely mediated through consensus binding sites, and (3) knock-down of key blood stem cell regulators Gata2, Erg and Runx1 reveals mast cell specific genes as direct targets. Finally we show that the known mast cell regulators Mitf and c-Fos likely contribute to the global reorganisation of TF binding profiles. Taken together therefore, our study elucidates how key regulatory TFs contribute to transcriptional programmes in distinct mammalian cell types.

P1177 - MYELOID LINEAGE CHOICE IS NOT CONTROLLED BY THE PU.1 - GATA1 SWITCH

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Fluctuating transcription factor (TF) networks are commonly assumed to be responsible for stochastic hematopoietic lineage choice. The PU.1 and Gata1 TF pair, driving granulocytic-monocytic (GM) versus megakaryocytic-erythroid (MegE) lineage choice, respectively, is the paradigmatic TF pair underlying this hypothesis. Lineage priming, reprogramming experiments, positive autoregulation and mutual cross-antagonism of PU.1 and Gata1 have led to the hypothesis that random 'noisy' fluctuations in PU.1 and Gata1 expression will allow one factor 'winning' over the other and thus to inducing lineage choice. However, this hypothesis is based on limited expression data. So far, TF expression mostly was measured at the mRNA level in discontinuous snapshot analysis. For a comprehensive understanding, quantification of protein levels before, during and after lineage choice would be required. We therefore developed novel knock-in mouse lines simultaneously expressing PU.1eYFP and Gata1mCHERRY fusion proteins from their endogenous genomic loci. FACS analysis of those phenotypically normal mice allows identification and prospective isolation of several new myeloid hematopoietic subpopulations. Long-term single cell tracking and the development of novel software tools enables us to estimate endogenous molecule numbers and to quantify TF expression kinetics and dynamics with high resolution. This approach provides the quantitative continuous data in living hematopoietic stem and progenitors cells required for testing the above mentioned hypothesis. It demonstrates that fluctuating PU.1 and Gata1 levels are not responsible for GM-MegE lineage decision making.

P1178 - DEFINING THE MOLECULAR SIGNALS OF EARLY LYMPHOID PRIMING IN HUMAN BONE MARROW

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Lymphoid commitment occurs through gradual loss of lineage potential in a process regulated by complex extrinsic and intrinsic signals which activate or repress lineage-specific signaling and transcription. These molecular cues are poorly understood in human lymphopoiesis, particularly in the steady state bone marrow (BM), and studies in this system are essential for meaningful approaches to clinical translation. We recently identified a novel hematopoietic progenitor in the human BM, defined by the immunophenotype CD34+Lin-CD10-CD45RA+CD62Lhi (Kohn et al, Nature Immunology 2012). This CD10-CD62Lhi population (aka lymphoid-primed multipotent progenitors or LMPPs) exhibited T, B, NK, and monocytic potential, but lacked erythroid potential (similar to the murine LMPP). Of note, this population could give rise to the CD34-Lin-CD10+ common lymphoid progenitor (CLP) phenotype in vitro, and microarray analysis showed a global gene expression profile intermediate between HSC and CLP, suggesting it represents the earliest known stage of lymphoid differentiation in the human BM. In order to obtain a comprehensive picture of the lineage programs present at this earliest stage of lymphoid differentiation, whole transcriptome deep sequencing (RNA-seq) of BM HSCs, LMPPs, and CLP populations were done. The data showed down-regulation of pluripotency and self-renewal genes (TAL1,PRDM16,HOXA9,& MEIS1) and the receptor MPL in the LMPP population. However KIT expression persisted and, similar to the murine LMPP population, FLT3 was significantly up regulated. Despite up regulation of key myeloid genes (CSF1R,MPO,MPEG1), lymphoid priming was clearly evident in the increased expression of key lymphoid transcription factors (TCF3,EBF1,IKZF1,GFI1,IRF4,LEF1,PRDM1), as well as expression of IL7R, IL2RG, and CRLF2. Interestingly, expression of RAG2 and DNTT were also significantly increased in LMPP compared to HSC, as were genes associated with both early B cell (VPREB1,VPREB,BLNK) and T cell (CD3E,LCK,ZAP70) receptor signaling. Collectively, these data illustrate coexistence of multiple competing lineage-specific transcriptional and novel signaling programs during lymphoid commitment not previously identified in human BM.

P1179 - MEGAKARYOCYTIC DIFFERENTIATION OF LEUKEMIA CELLS AND HEMATOPOIETIC STEM CELLS INDUCED BY (R)-TEMOSPHO

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2-(Trimethylammonium)ethyl (R)-3-methoxy-3-oxo-2-stearamidopropyl phosphate ((R)-TEMOSPho), a derivative of an organic chemical identified from a natural product library promotes megakaryocytic differentiation of myeloid leukemia cells and primary human CD34+ haematopoietic stem cells [1].
We performed RNA-seq based gene expression profiling in order to characterize genes that may play an important role in megakaryocytic differentiation induced by (R)-TEMOSPho. 1089 differentially expressed genes including 15 lncRNAs during megakaryopoesis were identified ($p < 0.01$). Expression profiles of selected 15 coding genes were confirmed by quantitative RT-PCR and tsc22d1, a transcriptional repressor was prioritized for further functional analysis. As tsc22d1 was up-regulated during megakaryocytic differentiation, functional analysis of target gene can be performed by loss of function study. We provide RNAinterference combined with LZRS retrovirus system as a helpful tool to study the functions of target genes in primary cd34+ cells during megakaryopoiesis. 1. Limb, J.-K., et al., 2-(Trimethylammonium)ethyl (R)-3-methoxy-3-oxo-2-stearamidopropyl phosphate promotes megakaryocytic differentiation of myeloid leukaemia cells and primary human CD34(+) haematopoietic stem cells. Journal of tissue engineering and regenerative medicine, 2012.

P1180 - MECHANISM OF DEREGULATION OF HEMATOPOIESIS BY MUTANT FORMS OF RUNX1

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During embryonic hematopoiesis, hematopoietic stem cells (HSCs) arise from endothelial cells (ECs) lining the dorsal aorta. Some ECs change their endothelial fate and gain hematopoietic potential and the ability to differentiate into blood cells. Endothelial cells with hematopoietic potential are called hemogenic endothelium (HE) and they give rise to intra-aortic clusters of CD45+ HSCs. This progression of HE to HSCs is known as endothelial-to-hematopoietic transition (EHT) and absolutely requires the transcription factor RUNX1. RUNX1 expression starts at low levels in the ECs and is highly upregulated in HE and HSCs. The transition of HE to HSCs depends on the levels of RUNX1, a reduction alters HSC development. Mutations in RUNX1 lead to abnormal hematopoiesis and leukaemia. Here we studied two mutant RUNX1 proteins, RUNX1/ETO (t8:21 translocation) and RUNX1-R203X (point mutation), both associated with acute myeloid leukaemia, to understand how these proteins interfere at different stages of hematopietic differentiation. Although many studies have addressed the block in differentiation by RUNX1/ETO, not much is known about how they affect initial stages of hematopoiesis. Towards this end we generated ES cell lines with inducible expression of either RUNX1/ETO or RUNX1-R203X in a RUNX1 wild-type background. The changes in hematopoiesis caused by these mutant proteins were studied by in vitro differentiation of these ES cell lines into blood precursor cells. Our studies indicate that expression of RUNX1/ETO severely inhibits the progression of ECs to HE and precursors. The RUNX1-R203X mutant showed altered kinetics of hematopoietic differentiation with rapid appearance of CD41+ precursor cells. Experiments are being carried out to get mechanistic insights into how these mutants alter the endothelial and hematopoietic gene expression programs and the results will be presented.

P1181 - PROGRESSIVE DEREGULATION OF GENE EXPRESSION DURING EARLY HAEMOPOIETIC LINEAGE COMMITMENT FROM SP1-/- ES CELLS

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Sp1 is a ubiquitously expressed transcription factor which regulates expression of a variety of genes involved in diverse cellular processes including differentiation, proliferation and chromatin remodelling. Sp1-/- mice die by E11 of multiple heterogeneous developmental defects, however Sp1-/- embryonic stem cells grow normally. Currently the reason for this discrepancy is not understood. To gain insight into how the lack of Sp1 influences one specific developmental pathway, we have used these ES cells to study early haemopoietic development. Sp1 deficient ES cells show a reduced capacity for macrophage production by embryoid bodies grown in methylcellulose. Haemopoietic colony assays show that Sp1-/- myeloid precursors are produced at a lower frequency and are functionally deficient as evidenced by lower colony forming capacity. A more detailed analysis of the block in differentiation using a blast culture differentiation system revealed that Sp1-/- cells can form haemopoietic progenitors from the haemogenic endothelium (HE), but further development of these progenitors to macrophages is impaired. We next performed gene expression microarrays using RNA isolated from purified cell populations from wild type and Sp1 deficient cells representing the Flk+ve haemangioblast, HE1, HE2 and progenitor stages of haemopoietic development. Analysis of this data demonstrates increasing deregulation of multiple genes essential for normal cellular function in Sp1-/- cells as differentiation progresses, providing a molecular explanation for why differentiation is impaired. Gene ontology analysis revealed that a wide range of cellular differentiation and metabolic processes are affected by Sp1 deficiency, consistent with a global role for Sp1 in the expression of house-keeping genes. These expression studies were complemented by ChIP-seq analysis of Sp1 binding in wild type cell populations. In summary, our data shows that while Sp1 is dispensable for mES cell growth and self-renewal, it is essential to maintain a normal program of haemopoietic differentiation.

P1182 - JAK/ STAT SIGNALLING DURING NORMAL AND PATHOLOGICAL MYELOPOIESIS WITH FOCUS ON ERYTHROPOIESIS AND MEGAKARYOPOIESIS

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The JAK/ STAT signalling pathway plays a crucial role in normal haematopoiesis transducing signals from critical cytokines such as erythropoietin (EPO) and thrombopoietin (TPO) from the cell surface to target gene promoters in the nucleus. Dysregulation of JAK/ STAT pathway can lead to an array of haematological malignancies. The single acquired somatic mutation JAK2 V617F contributes to a number of phenotypically diverse myeloproliferative neoplasms (MPNs) characterised by an overproduction of mature myeloid cells with a tendency to transform to acute leukaemia. This includes 97 % polycythaemia vera (PV) and 57 % essential thrombocythaemia (ET) cases where individuals present with increased erythropoiesis and megakaryopoiesis respectively (Baxter el al., 2005). We are currently characterising the transcriptional programmes downstream of wild type JAK2 in EPO-treated erythroblasts (>90% CD71+/CD235a+) and TPO-treated megakaryocytes (>60 % CD41+). Using Chromatin Immunoprecipitation (ChIP) and sequencing of native regulatory elements bound by ChIP-Seq grade STAT antibodies, we aim to identify and present differential STAT binding sites and novel STAT-regulated genes in these two primary human myeloid cell types. Microarray analysis of RNA isolated from both cell types, before and after cytokine treatment, will aid the differentiation of cytokine-dependent and independent target genes. By defining the STAT target genes in normal erythroblasts and megakaryocytes we intend to provide a wild type framework in which to compare subverted STAT signalling as a consequence of mutant JAK2. We have cultured primary human JAK2 V617F positive erythroblasts and will assimilate and present ChIP-Seq data from these cells alongside analyses from normal erythroblasts. Together these findings will create a more comprehensive understanding of wild type JAK/ STAT signalling and may provide new insights into MPN pathogenesis.

P1183 - A NOVEL EMBRYONIC STEM CELL BASED ASSAY PROVIDES SINGLE CELL QUANTITATIVE ANALYSIS OF ENHANCER ACTIVITY DURING HAEMATOPOIETIC SPECIFICATION AND ALLOWS DISSECTION OF REGULATORY INPUTS

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The transcription factor Scl/Tal1 is a critical regulator of haematopoiesis and its precise spatiotemporal expression is necessary for proper developmental patterning in the mouse embryo. Several transcriptional cis-regulatory elements have been identified that determine the haematopoietic expression of Scl including the +19, -4 and +40 enhancers. Investigation of such regulatory elements allows determination of upstream regulators and assembly of TF cascades and networks.Assays currently used to assess putative haematopoietic enhancers include expression analysis of randomly integrated enhancer-linked reporter genes in transient F0 transgenic embryos and luciferase assays. However, transgenic embryos only provide qualitative data while luciferase assays are limited to population level analysis in non-developmental cell types. These assays therefore provide an incomplete picture of enhancer activity. The Scl+19 enhancer has been well characterised by these methods and others; it is sufficient to drive Scl expression during developmental haematopoiesis and contains a highly conserved Ets/Ets/Gata motif necessary for its activity. However, the cell type specificity and the quantitative effects of motif mutation in the Scl+19 during haematopoietic specification cannot be determined by current methods.We have developed a haematopoietic enhancer assay that utilises embryonic stem (ES) cell gene targeting for single copy integration of an enhancer-linked YFP reporter gene. Embryoid body differentiation of targeted ES cell lines provides an accurate model of haematopoietic specification and abrogates the need of transgenic mice. Flow cytometry of YFP and cell surface marker expression captures quantitative single cell data of enhancer activity and specificity. Gene targeting and analysis of mutated enhancers allows further dissection of their regulatory inputs.We will present use of this novel enhancer assay to define Scl+19 activity

during haematopoietic specification and previously unidentified separable roles of the Ets and Gata motifs during this process.

P1184 - PERTURBING HAEMATOPOIETIC TRANSCRIPTION FACTOR NETWORKS AND CELL FATE DECISIONS USING TRANSCRIPTIONAL ACTIVATOR-LIKE EFFECTORS

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Transcription factors are key determinants of cell identity and fate. Numerous TFs are known to play critical roles in haematopoiesis, including Scl/Tal1, PU.1 and Gata1. Our laboratory, along with others, has identified many transcriptional cis-regulatory elements within gene loci of key haematopoietic TFs, often distal to gene promoters. Haematopoietic TFs interact via these regulatory elements to form TF circuits and interconnected networks that act to antagonise or reinforce cell fate decisions. Transcriptional Activator-Like Effector (TALE) proteins have modular and predictable DNA binding domains, a feature that allows their de novo assembly as synthetic sequence-specific TFs. Fusion of transcriptional activator (VP64) or repressor (KRAB) domains to TALEs targeting promoters has recently been shown to modulate gene expression. We have designed TALE activators and repressors to target several haematopoietic TF regulatory elements, including the Scl+40kb, PU.1-14kb and Gata1-3.7kb enhancers. Initial testing in haematopoietic cell lines confirmed their ability to modulate target gene expression from these distal regulatory elements. Interestingly, expression of neighbouring genes can also affected suggesting their co-regulation. We have further applied TALEs to haematopoietic programming from embryonic stem cells and analysed their perturbation of TF networks in haematopoietic progenitor cell fate decisions. We will present validation of TALE activators and repressors targeting distal regulatory elements as an efficient method of perturbing haematopoietic TF networks and the novel insights into cell fate decisions gained.

P1185 - THE CKROX TRANSCRIPTION FACTOR AFFECTS THE DEVELOPMENT AND FUNCTION OF MULTIPLE T CELL TYPES

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The mature T cell population is divided into two main lineages that are defined by the expression of CD4 and CD8 surface molecules. CD8+ T cells are restricted by MHC I molecules and possess cytotoxic activity by virtue of expression of molecules such as perforin and granzyme. By contrast, CD4+ T cells are restricted by MHC II molecules and either provide help or suppress other immune cells through either cytokine secretion and/or expression of specific cell surface molecules. During T cell development the strength and duration of TCR signaling play key roles in CD4/CD8 lineage choice. In addition to TCR signaling, a number of transcription factors have recently been shown to play important roles in CD4/CD8 lineage choice. The zinc finger transcription factor cKrox plays a crucial role in CD4 T cell development and CD4/CD8 lineage decision. In cKrox-deficient mice, developing T cells expressing MHC class II-restricted TCRs are redirected into the CD8 T cell lineage. In this study, we investigated whether the cKrox transgene affected the development and function of two types of regulatory T cells, namely self-specific CD8 and CD4+FoxP3+ T regulatory cells. Self-specific CD8 T cells are characterized by high expression of CD44, CD122, Ly6C, 1B11 and proliferation in response to either IL-2 or IL-15. The cKrox transgene converted these self-specific CD8 T cells into CD4 T cells. The converted CD4+ T cells are no longer self-reactive, lost the characteristics of self-specific CD8 T cells, acquire the properties of conventional CD4 T cells and survive poorly in peripheral lymphoid organs. By contrast, the cKrox transgene promoted the development of CD4+FoxP3+ regulatory T cells resulting in an increase recovery of CD4+FoxP3+ regulatory T cells that expressed higher TGF-β-dependent suppressor activity. These studies indicate that the cKrox transgene differentially affect the development and function of self-specific CD8 T cells and CD4+FoxP3+ regulatory T cells.

P1186 - DOES RIBOSOMAL PROTEIN-MDM2 INTERACTION MEDIATE THE P53-DEPENDENT HEMATOPOIETIC DEFECT IN DIAMOND-BLACKFAN ANEMIA?

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Diamond-Blackfan anemia (DBA) is a congenital erythroid hypoplasia. Mutations in genes encoding ribosomal proteins (RPs) have been identified in 60-70 % of DBA patients. Among these genes, RPS19 is the most common DBA gene. We and others have demonstrated that the defective ribosome biogenesis in DBA results in the activation of p53, which is largely responsible for the hematopoietic symptoms. However, despite its therapeutic potential, direct interference with p53 pathway raises concerns given its role as tumor suppressor. Therefore, identification of disease-specific mechanisms upstream of p53 could reveal more promising therapeutic targets. In vitro studies have shown that p53 response upon ribosomal stress is due to the nuclear accumulation of free RPL5 and RPL11, which bind and inhibit Mdm2, the main negative regulator of p53. However, the physiological relevance of the RP-Mdm2-p53 pathway in DBA is unclear. We have recently generated a mouse model for RPS19-deficient DBA containing Rps19-targeting shRNA under a doxycycline-responsive promoter, allowing inducible and graded downregulation of Rps19 (Jaako, Blood 2011). Mice with severe Rps19 deficiency develop lethal bone marrow failure that is rescued in p53-deficient background. To assess whether the RP-Mdm2-p53 pathway mediates the activation of p53 in Rps19-deficient mice, we have crossed these mice with the Mdm2 C305F knockin mice. The C305F mutation prevents the binding of RPL5 and RPL11 to Mdm2, and subsequent p53 response to perturbed ribosome biogenesis. Our in vitro studies show that the presence of the C305F mutation decreases the expression of p53 target genes in dose-dependent manner improving the proliferation defect of Rps19-deficient hematopoietic progenitors. However, even the homozygosity for C305F does not completely abolish p53 response suggesting that additional pathways may contribute to p53 activation. Consistently, our preliminary in vivo results suggest that in contrast to p53-deficient background, the homozygosity for C305F is not sufficient to rescue the lethal bone marrow failure caused by severe Rps19 deficiency. We are currently studying whether the impact of C305F depends on the level of Rps19 downregulation and whether additional pathways operate upstream of p53.

P1187 - TRACKING ES CELL HEMATOPOIETIC DIFFERENTIATION WITH MULTIPLE HSC REPORTERS

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Hematopoietic stem cells (HSCs) are the basis of the blood cell hierarchy and are the key cell type in blood regenerative medicine. However, the shortage of HSCs for treatment and difficulties in their ex vivo expansion point to the need for de novo production of HSCs from non-hematopoietic cells. In the mouse embryo HSCs are generated between E10 and E12 from hemogenic endothelial cells lining the major vasculature. This process, involving endothelial to hematopoietic cell transition (EHT), is tightly controlled by a combination of several transcription and extrinsic factors. The Gata2 transcription factor has a pivotal role in hematopoiesis. Gata2 deletion results in lethality at E10.5 and severe hematopoietic defects (Tsai, 1994). Differentiation of Gata2-/- embryonic stem (ES) cells into embryoid bodies (EBs) suggests that Gata2 is necessary for the proliferation/survival of early hematopoietic cells (Tsai, 1997). Our goal is to isolate and examine Gata2 expressing cells during EHT to understand what processes this pivotal transcription factor affects in the generation of HSCs. In this study we recapitulate early hematopoietic development by exploiting the ES cell/EB differentiation system using a novel Gata2-Venus:-Ly6A-Gfp ES cell line derived in our lab. Transgenic mouse models show that all HSCs are Ly6A-Gfp (de Bruijn, 2002) and Gata2-Venus (unpublished) expressing. The double transgenic ES cell model allows us to define the dynamics of Gata2 in Ly6A-Gfp expressing cells at the onset of hematopoiesis. Gata2+Ly6A+ and single positive cells are tested in in vitro methylcellulose assays and in vivo transplantations to elucidate which fractions are enriched for hematopoietic progenitors and HSCs. Because Gata2 is thought to function in the same cells (HSCs) with Runx1 transcription factor (Wilson, 2010; North, 2002) and Runx1 is required for EHT (Chen, 2009) we will test whether there is an overlap between Gata2+,Ly6A+,Runx1+ populations in a novel Gata2-Venus:Ly6A-Gfp:Runx1-mKate ES cell reporter line.

P1188 - ENRICHING HEMANGIOBLAST AND HEMOGENIC ENDOTHELIAL CELLS USING REGULATORY ELEMENTS OF THE ENDOGLIN GENE

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Endoglin (CD105) is an accessory receptor for TGF-beta signaling and is required for normal hemangioblast, early hematopoietic and vascular development. We have previously shown that an upstream enhancer, Eng-8, together with the promoter region, mediates robust endothelial expression in developing embryos and when coupled with two intronic enhancers, Eng +7 and Eng +9 also targets blood clusters in the dorsal aorta, fetal liver and placenta. The hemato-endothelial activity of this composite regulatory module was also shown to be dependent on Gata and Ets binding motifs. This current study was motivated by our desire to first, establish whether these enhancer modules cooperate to target the earliest blood precursors, i.e. hemangioblasts and hemogenic endothelial cells (HE) and second, to determine whether these regulatory elements could be used to enrich haemangioblasts and HE cells. To this end, we targeted various combinations of Eng promoter and wild-type and mutant enhancers with lacZ reporters into the HPRT loci of HM1 ES cells and evaluated the potential of Endoglin regulatory elements to target blast colony forming cells (BL-CFCs) and HEl cells. Our results showed that the -8/promoter/+7/+9 combination preferentially targeted BL-CFCs within the Flk1+ cell compartment in day 3 embryoid bodies in a Gata2 dependent manner. By contrast, the -8/P construct targeted cells with hematopoietic potential within Tie2 +, c-Kit + HE cells in day 2 liquid cultures. Importantly, these combinations of regulatory elements enrich these cell fractions when surface Endoglin alone proves inadequate. Utilising these elements as a gauge of the transcriptional milieu of hemangioblast and HE cell commitment, we have sorted cell fractions by flowcytometry and performed transcriptomic analysis to identify signaling pathways that are differentially active in these cells and identified and validated markers in vivo. Taken together, our results show that when different types of cells share expression of a gene but utilise distinct combinations of regulatory elements to express that gene, regulatory elements can be used to isolate and enrich cells when the gene itself lacks sufficient discriminative value.

P1189 - EFFECTS OF UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS TRANSDUCED WITH ADENOVIRUS CONTAINING HUMAN HEPATOCYTE GROWTH FACTOR ON HEPATIC FIBROSIS IN RATS

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Objective: Hepatic fibrosis is a common and refractory disease. Our aim is to provide a new method to treat it. **Methods:** HGF was obtained from templet plasmid pBluescript-HGF by PCR amplification. Amplified product was cloned into pYr-ad-shuttle-2 containing eGFP by enzyme digestion-ligation. Recombinant of the pYr-ad-shuttle-2-HGF with adenovirus backbone plasmid pAD/BL-DEST was performed by adenovirus packaging. Linear recombinant adenovirus DNA was transduced into 293T cells by liposome. Adenovirus containing hHGF (ad-HGF) was obtained. Cells from Wharton's jelly of umbilical cord was cultured and induced to MSCs (HUC-MSCs). Passage 3 HUC-MSCs were examined by morphology, phenotype and multiple differentiation ability and used for ad-HGF plus HUC-MSC group. We established rat liver fibrosis model by CCl4 for 8 weeks. 30 rats were divided into five groups: normal group, model group, ad-HGF group, HUC-MSC group and ad-HGF/HUC-MSC group. Normal group was injected with saline, Model group was untreated; ad-HGF group was injected with ad-HGF; HUC-MSC group was injected with HUC-MSC and ad-HGF/HUC-MSC group was injected with HUC-MSC. All rats of treated groups were injected by tail vein from week 4 and 2 times per week after CCl4 injection. HE, Masson's trichrome and reticulated fiber stained liver tissues were examined under light microscope and the serum levers of HA, LN, PCIII and CIV were tested. **Results:** Treatment of HGF/UC-MSCs could significantly decrease the liver fibrogenesis induced by CCl4. **Conclusion:** Transfusion of HGF/UC-MSC should decrease the hepatic fibrosis in rats and UC-MSCs is better than BM. My previous experiments by microarray analysis showed that UC-MSCs were more favorable to tissue repairing than BM. Expansion of 293T cells in serum-free medium may increase the safety for clinical application.

P1190 - DECLINED PRESENTATION
INDUCTION OF FETAL HEMOGLOBIN SYNTHESIS IN ERYTHROID PROGENITOR STEM CELLS MEDIATED BY ELAIDIC ACID ISOLATED FROM TERMINALIA CATAPPA

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Current novel therapeutic agents for the treatment of sickle cell anemia (SCA) focus on increasing foetal haemoglobin (HbF) levels in SCA patients. Unfortunately, the only approved HbF inducing agent; hydroxyurea (HU) has long term unpredictable side effects. Studies have shown the potential of plants to modulate fetal hemoglobin synthesis in primary erythroid progenitor stem cells. We isolated a novel fetal hemoglobin inducing compound from Terminalia catappa leaves. Studies of the isolated compound showed that it induced early differentiation of the progenitor stem cells and relatively higher fetal hemoglobin synthesis of 6.8 and 9.2 fold increases in both EPO-dependent and EPO-independent progenitor stem cell cultures. The compound was not cytotoxic as revealed by lactate dehydrogenase (LDH) release from the cells. Surprisingly the compound induced modest increase of Caspase 3 activity in primary erythroid progenitor cells. Hence elaidic acid may offer a novel fetal hemoglobin inducing activity with minimal cytotoxic effect.

P1191 - PHARMACOLOGICAL STABILIZATION OF HYPOXIA-INDUCIBLE FACTOR-1α (HIF-1α) ENHANCES HEMATOPOIETIC STEM CELL MOBILIZATION IN RESPONSE TO G-CSF AND PLERIXAFOR

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HIF1α controls HSC proliferation and self-renewal in hypoxic bone marrow niches where quiescent HSC with highest self-renewal potential reside. When O2 concentration is above 2% in the cell microenvironment, HIF1α protein is rapidly degraded in an O2-dependent manner initiated by hydroxylation of specific proline residues by prolyl hydroxylases domain (PHD) enzymes. We have investigated the effect of pharmacological stabilization of HIF1α protein on HSC mobilization in mice using the HIF-PHD inhibitor FG-4497. FG-4497 synergized with G-CSF and Plerixafor to enhance HSC mobilization. C57BL/6 mice treated with G-CSF + FG-4497 mobilized CFC into the blood 2.5-fold and phenotypic Lin-Sca1+ Kit+ CD48- CD150+ HSC 3-fold more than mice mobilized with G-CSF alone ($p < 0.005$), whereas FG-4497 alone had a poor mobilizing effect. Most interestingly, addition of FG-4497 boosted 2.4-fold and 5.7-fold mobilization of CFC and phenotypic HSC in response to G-CSF+Plerixafor ($p < 0.005$). This was confirmed in competitive repopulation assays where a combination of G-CSF+Plerixafor+FG-4497 mobilized competitive repopulating HSC 20-fold more than G-CSF+Plerixafor ($p < 0.005$), the best mobilizing cocktail used in the clinic currently. We next examined the role of Hif1α gene in HSC mobilization. Tamoxifen-inducible deletion of the Hif1a gene specifically in HSCs (SclCreER Hif1aflox/flox mice) resulted in a 12-fold decrease of phenotypic HSC mobilisation into the blood in response to G-CSF. Furthermore, when Hif1α was deleted from osteoprogenitors (osxCre Hif1aflox/flox mice) mobilization of CFC was reduced 2.5-fold at day 2 and 3 of G-CSF treatment. This suggests that deletion of HIF-1α in HSCs impairs their mobilisation and in the osteoblastic lineage is necessary for optimal mobilization in response to G-CSF. In conclusion, our data highlight the critical importance of HIF-1α in HSC mobilization and provide a novel therapeutic strategy for increasing HSC mobilization above levels obtained with combinations of G-CSF plus Plerixafor. Thus PHD inhibitors could be useful agents in patients who still fail to mobilize in response to G-CSF and Plerixafor.

P1192 - EPHB AND EPHRIN-B INTERACTIONS MEDIATE HUMAN MESENCHYMAL STEM CELL SUPPRESSION OF ACTIVATED T-CELLS

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Human bone marrow derived multipotent mesenchymal stromal/stem cells (MSC) exhibit immunomodulatory properties. It is known that MSC express the contact dependent erythropoietin-producing hepatocellular (Eph) receptor tyrosine kinase family and their cognate ephrin ligands, which regulate thymocyte maturation, selection, T cell migration, activation, co-stimulation and proliferation. However, the contribution of Eph/ephrin interactions in mediating human MSC suppression of activated T cells remains to be determined. In this study, we showed that EphB2 and ephrin-B2 are expressed by MSC, while the corresponding ligands, ephrin B1 and EphB4 respectively, are expressed by T-cells. To examine EphB2/ephrin-B1 and ephrin-B2/EphB4 interactions in T-cell proliferation, we employed the mixed lymphocyte reaction (MLR) assay, where irradiated allogeneic lymphocytes were used to stimulate T-cell proliferation. We demonstrated that EphB2-Fc and ephrin-B2-Fc could suppress T-cell proliferation in the MLR compared with human IgG treated controls. Whilst the addition of a third party MSC population demonstrated dramatic suppression of T-cell proliferation responses in the MLR, blocking the function of EphB2 or EphB4 receptors, using inhibitor binding peptides, significantly increased T-cell proliferation. Consistent with these observations, shRNA MSC knockdown lines for EphB2 or ephrin-B2 expression reduced their ability to inhibit T-cell proliferation. Moreover, the expression of immunosuppressive factors, IDO and TGF-β1, expressed by MSC, was induced following stimulation with EphB4 or ephrin-B1. Conversely, key factors involved in T-cell proliferation, IL 2, INF-γ, TNF-α and IL-17, were down-regulated by T-cells treated with EphB2 or ephrin-B2. Our data suggest that inhibition of T-cell proliferation by MSC is partly mediated through EphB2-induced ephrin-B1 reverse signalling or ephrin-B2-mediated EphB4 forward signalling. This work contributes to the current understanding of how MSC exert their immunosuppressive effects on activated T-cells, and may offer a unique therapeutic drug target to facilitate the regulation of T cell populations in immune related conditions.

P1193 - 2-HIT MODEL FOR HUMAN CHRONIC GRAFT-VERSUS-HOST DISEASE

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Chronic graft-versus-host disease (cGVHD) continues to be a major cause of morbidity following allogeneic hematopoietic stem cell transplantation (HSCT). Originally, cGVHD was believed to be due to the cellular processes that caused acute GVHD. Recent clinical and laboratory research has indicated that the pathophysiology of cGVHD is distinct from that of acute GVHD. In a cross-sectional study, pediatric HSCT recipients were divided into four groups: 1) no history of acute or chronic GVHD, 2) a history of only acute GVHD, 3) the presence of active chronic GVHD, and 4) resolved chronic GVHD. HSCT recipients were assayed for the frequency of resting regulatory T lymphocytes (rTreg) using the Miyara classification, which permits the identification of rTreg lymphocytes independent of activated conventional T lymphocytes (Immunity 30:899-911, 2009). HSCT recipients were also assayed for the presence of CCR7 positive, CD45RA expressing effector memory (EMRA) CD4 T lymphocytes, which may contain the effector T lymphocytes that initiate chronic GVHD. All HSCT groups, except Group 2 (only acute GVHD), had increased frequencies of CCR7 positive, EMRA CD4 T lymphocytes. All HSCT groups had fewer ($p < .05$) rTreg lymphocytes than normal controls. However, Group 1 (no history of acute or chronic GVHD) and Group 4 (resolved chronic GVHD) had significantly more rTreg lymphocytes than Group 2 (only acute GVHD) or Group 3 (active chronic GVHD). Thus, active chronic GVHD clinically occurred only in HSCT recipients who had an elevated frequency of CCR7 positive EMRA CD4 T lymphocytes in the context of immune dysregulation. The presence of an elevated frequency of CCR7 positive EMRA T lymphocytes alone did not result in active chronic GVHD if adequate numbers of rTreg lymphocytes (Groups 1 and 4) were present. These results suggest a 2-hit model for chronic GVHD: an increased frequency of CCR7 positive EMRA CD4 T lymphocytes in the context of a decreased frequency of rTreg lymphocytes.

P1194 - DIRECT REPROGRAMMING OF FIBROBLASTS INTO HEMATOPOIETIC CELLS BY DEFINED TRANSCRIPTION FACTORS

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Direct lineage reprogramming provides an alternative tool to ES and iPS cells for regenerative medicine. Recent reports have shown that somatic cells, under appropriate culture conditions, could be directly reprogrammed to cardiac, hepatic or neuronal phenotype by lineage specific transcription factors. In this study, we found that murine embryonic fibroblasts as well as adult fibroblasts can be directly reprogrammed to blood cells by a defined set of hematopoietic transcription factors without transition through a pluripotent state. Cell surface marker, transcriptome and clonogenic potential analyses established the hematopoietic nature of the reprogrammed cells and their different blood lineage potentials. In vivo repopulation potential of the reprogrammed cells is currently under investigation. In addition to hematopoietic transcription factors, we found that small molecule modulators of signaling pathways and the genetic status of the initial cells influence the reprogramming efficiency. In conclusion our results provide an impetus to further evaluate direct reprogramming as an alternate approach to generate blood cell populations for autologous cell replacement therapies.

P1195 - DERIVATION OF OLIGODENDROCYTE LIKE CELLS FROM SPERMATOGONIA STEM CELLS AND TRANSPLANTATION THEM TO A DEMYELINATION MODEL

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Introduction: Embryonic Stem like cells (ES like cells) derived culturing of spermatogonia cells make them as a new and unlimitted source for cell therapy. In present study spermatogonia cells differentiated to oligodendrocyte like cells and they transplanted to demyelination model in rats and their role in remyelination was studied. **Methods and Materials:** The spermatogonia cells were cultured in vitro and ES like cells colonies was appeared within 3 weeks. ES like cells were differentiated to neuroprogenitor cells and oligodendrocyte like cells and were transplanted to demyelination model rats. Cell integration and demyelination extension and intensity changes were evaluated using histological studies and immunocytochemistry. **Results:** Investigation of neural and glial markrs by immunocytochemical and real time PCR studies indicated the differentiation of ES like cells to neuroprogenitor and oligodendrocyte like cells. Histological findings showed a significant decrease in demyelination extension and a significant increase in myelination intensity in cell transplanted groups. Also differentiation of transplanted cells was confirmed to myelinogenic cells on the base of PLP expression. **Conclusion:** ES like cells derived from spermatogonia cells can differentiated to neuroprogenitor and oligodendrocyte like cells that can form myelin after transplantation into the demyelination model in rat. **Key words:** ES like cells, Oligodendrocyte, Transplantation, Demyelination.

P1196 - FETAL ORGAN-DERIVED MESENCHYMAL PROGENITOR CELLS HAVE ABILITY TO SUPPORT IN VITRO AND IN VIVO HEMATOPOIESIS

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Mesenchymal stem cells (MSCs) derived from human fetal appendages, such as placenta, umbilical cord, amniotic membrane, and umbilical cord blood, have in vitro and/or in vivo hematopoiesis-supportive abilities. However, it remains to elucidate whether mesenchyaml progenitor cells (MPCs) that have only restricted differentiation potentials can support hematopoietic cells as well as MSCs. In this study, we assessed in vitro and in vivo hematopoiesis-related abilities of MPCs derived from human amniotic membrane (HAM-MPCs) and human umbilical cord (HUC-MPCs). While co-culture of human cord blood CD34+ cells with HUC-MPCs caused the increase of CD45+ hematopoietic cells more efficiently and rapidly than co-culture with HAM-MPCs, the numbers of colony-forming cells after long-term co-culture with HAM-MPCs were significantly higher than those of co-culture with HUC-MPCs. Quantification analysis of cytokine production revealed that stem cell growth factor-beta (SCGF-beta), interleukin-6 (IL-6), IL-8, and vascular endothelial growth factor (VEGF) were commonly produced from both of cell populations at equal level whereas granulocyte-colony stimulating factor (G-CSF) and growth-related oncogene α (GRO-α) were only detected in co-culture of HUC-MPCs. Chimerisms of human hematopoietic cells in immunodeficient mice were significantly higher when HAM-MPCs or HUC-MPCs were co-transplanted with CD34+ cells than CD34+ cells were transplanted alone. In addition, in vivo hematopoiesis supportive function of HAM-MPCs is more effective when smaller number of CD34+ cells are transplanted than when larger number of CD34+ cells are transplanted. This result suggested that co-transplantation of HAM-MPCs or HUC-MPCs may improve successful engraftment of cord blood CD34+ cells into adult patients. Here we showed that mesenchymal cells derived from HAM and HUC can support in vitro and in vivo hematopoiesis even if they have only restricted differentiation potentials.

P1197 - DEVELOPMENT AND CHARACTERISATION OF A NOVEL BONE-BONE MARROW ORGANOID

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Background: Bone and bone marrow have often been thought of as distinct compartments. However, the link between them is both anatomical and functional, in that a perturbation in one affects the other. Our previous studies using acellular demineralised bone matrix (DBM) demonstrated the development of both an ectopic bone and bone marrow nodule with ultimate characteristics reflecting mature adult long bone. As an extension of this model, we ask the question; can it function as a single organ? **Aims:** Develop and characterise a novel vascularised bone-bone marrow (B-BM) organoid by assessing its response to a) growth factor stimulation; b) stress (blood loss) and c) transplantation potential. **Methods:** B-BM organoid: Male Sprague-Dawley (SD) rats were implanted with a chamber containing a vascular pedicle and filled with DBM in a) the presence or absence of BMP-2; b) SD rats containing the fully developed B-BM organoid were bled (approx. 15% total blood vol.); c) fully formed organoid from male SD rats were transplanted to irradiated female recipients. Blood components were monitored for 4-weeks post transplantation. Histological and morphometric analyses (micro CT), and haematopoietic CFU assays were performed. **Results:** We have successfully created a vascularised B-BM organoid using acellular DBM. BMP-2 significantly augmented the total B-BM volume and the total number of haematopoietic CFUs compared to control. The B-BM organoid's sensitivity to stress was evidenced by the significantly elevated total CFUs four and seven days post-bleeding, compared to non-bled controls. Successful organoid transplantation was demonstrated by patency of blood vessels for at least 28 days and evidence of earlier haematopoietic recovery compared with irradiated controls. **Conclusion:** The organoid as a single operational unit detects and responds to growth factors and stress by enhancing production of haematopoietic progenitors. Furthermore, its viability, functionality, and contribution to bone marrow recovery following irradiation highlight a potential for it to be exploited in a clinical setting.

P1198 - HYDROGEN PEROXIDE INHIBITS PROLIFERATION AND ENDOTHELIAL DIFFERENTIATION OF BONE MARROW STEM CELLS INDEPENDENT OF REACTIVE OXYGEN SPECIES GENERATION

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Aims: Cell therapy with bone marrow mesenchymal stem cells (MSCs) remains a viable option for tissue repair and regeneration. One of the major challenges for cell-based therapy is the limited survival of the cells after in vivo administration. Although the exact mechanism(s) for impaired in vivo cell survival remains to be defined, oxidative stress is considered to be involved in the process of in vivo cell damage. The present study was to investigate the effect of hydrogen peroxide (H2O2) on bone marrow stem cells and their endothelial differentiation and the underlying mechanisms in vitro. Methods and **Results:** Rat bone marrow multipotent adult progenitor cells (MAPCs) were treated with H2O2 (with the final concentration from 0 to 50 uM) with or without the antioxidant N-acetylcysteine (NAC, 1 mM). H2O2 generated a significant amount of reactive oxygen species (ROS) in the culture system as measured with electron paramagnetic resonance spectroscopy, substantially inhibited the proliferation, Oct-4 expression, and endothelial differentiation of MAPCs, and induced the apoptosis of MAPCs in a dose-dependent manner. The phosphorylation levels of p38 and p53 were significantly increased in the cells treated with H2O2, while no significant changes in the expression and activation of Akt and ERK1/2 were observed. ROS production from H2O2 in the culture system was completely blocked by NAC (1 mM). However, NAC treatment didn't prevent H2O2-induced apoptosis, and inhibition of cell proliferation and endothelial differentiation of MAPCs. **Conclusion:** H2O2 exposure increased the apoptosis of MAPCs and inhibited their proliferation, Oct-4 expression, and endothelial differentiation with a mechanism independent of ROS generation in vitro. The effects of H2O2 on rat MAPCs might be mediated through p38 and/or p53-related signaling pathway(s).

P1199 - CONDITIONED MEDIUM FROM HUMAN MESENCHYMAL STEM CELLS (MSC) FROM DIFFERENT SOURCES INDUCE PROLIFERATION OR CELL DEATH IN HEPG-2 AND JURKAT CANCER CELLS DEPENDING ON ITS ORIGIN

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The use of MSCs after cancer treatment present some thoughtful concerns, as their interaction with tumors can enhance tumor cell growth or inhibit its proliferation, depending on the tumor type.
To further explore this issue, we analyzed the effect of conditioned media (CM) obtained from cultured MSCs from 3 different sources (adipose tissue (AT), amniotic liquid (AL) and Wharton's jelly (WJ)) on two cancer cell lines, HepG-2 (hepatocellular carcinoma) and Jurkat (T-cell leukemia) cells.
The CMs were collected after 24hs of incubation of sub-confluent MSCs with α-MEM containing 20% of FBS. CMs were centrifuged, and passed through 0,22 μm filters and stored at -20 C. CMs from HepG-2, Jurkat cells and MRC-5 normal fibroblast were used as control. Effects of CMs on tumour cell proliferation were tested by MTT assay, in several concentrations after 24 h incubation. Cell cycle of HepG-2 and Jurkat cells treated with 25%, 50% or 75% CM was analysed by cytometry (IP staining) using Modfit LT software. Gene expression of bcl-2, bcl-6, ccnd1, ccnd2 and foxp-1 was assayed by q-PCR of Jurkat. We found that CM from AT enhances HepG-2 and Jurkat cell proliferation. CM from AL led to Jurkat cell proliferation with no effect in HepG2 cells. CM from WJ induced cell dead in Jurkat cells, while increased HepG-2 cell proliferation. MRC-5 CM has no effect on proliferation rate or death of HepG-2 and Jurkat cells. CM from WJ induced increase of ccnd2 and fox-p1 expression in Jurkat cells but not of bcl-2, bcl-6 and ccnd1 expression. CM from AT and AL had no effect. It seems that younger cells, as MSCs from Wharton's jelly, can secrete factors that act on differential pathways of death in Jurkat cells. In conclusions, CMs from hMSC led to proliferation or cell death in Jurkat and HepG-2 cell lines depending on the origin of the stem cells.
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P1200 - A DIFFERENT BALANCE IN WNT-SIGNALING IN ADULT AND FETAL BONE MARROW-DERIVED MSC

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Although human adult bone marrow-derived MSC (ABMSC) are the most frequently applied cells in the clinic, fetal bone marrow-derived MSC (FBMSC) share the immune suppressive and regenerative capacities with adult MSC and have been transplanted into patients as well. There are pivotal differences in differentiation and proliferation capacity between adult and fetal derived cells. Unraveling the underlying molecular mechanism governing those aforementioned processes in FBMSCs may enable us to improve clinical expansion protocols for adult MSCs. Therefore, we explored the differential gene expression between ABMSCs and FBMSCs in a genome wide Affymetrix array. Among 687 differentially expressed genes, 16 were Wnt-related. Therefore we investigate the role of Wnt signaling in ABMSC and FBMSC. We exposed ABMSCs and FBMSCs to Wnt3a protein which led to concentration-dependent upregulation of canonical Wnt-target gene expression. ABMSCs responded faster, and to lower concentrations of Wnt3a compared to FBMSC. Media refreshment was sufficient to increase TCF/LEF expression in ABMSC, but not in FBMSC, indicating that ABMSC produce a soluble Wnt-inhibitor that suppresses canonical signaling. Next, MSC were exposed to the Inhibitor of Wnt Production 2 (IWP2) to interfere with autocrine Wnt-production in 48-hour stimulatory assays. In contrast to FBMSC, ABMSC remained responsive to Wnt3a after abrogation of endogenous Wnt production. Addition of IWP-2 to long-term cultures inhibited proliferation of both FBMSCs and ABMSCs with a more pronounced inhibitory effect observed in FBMSCs. The effect of IWP-2 in ABMSC cultures was partially overcome by providing exogenous Wnt3a and Wnt5a while in FBMSCs, this IWP-2 effect was not rescued. In conclusion, our data demonstrate that ABMSC and FBMSC have a different Wnt signature leading to the secretion of distinct quantities of Wnt-inhibitors and/or binding of endogenous Wnt-proteins to different combinations of Frizzled or co-receptor complexes.

P1201 - DECLINED PRESENTATION
COMPARATIVE EXPRESSION ANALYSIS OF EMBRYONIC STEM CELL MARKER GENES IN MESENCHYMAL STEM CELLS DERIVED FROM HUMAN BONE MARROW AND CHORION

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The ability of stem cells to differentiate into multiple cell lineages would be used to regenerate and repair damaged tissues and organs. Due to immunomodulatory and regenerative potential of MSCs, they have shown promising results in preclinical and clinical studies. Bone marrow (BM) was the first source reported to contain MSCs. However, for clinical use, BM may be detrimental due to the highly invasive donation procedure and the decline in MSC number with increasing age. More recently, stem cells isolated from fetal membrane of human placenta tissue has been described as a potential source of stem cells. The chorion contain stromal cells that display characteristics similar to adult BM derived stem cells. The aim of this study is to compare expression level of stemness marker genes include Oct4, Nanog, Sox2, Nestin, klf4 and c-myc in adult mesenchymal stem cell populations derived from bone marrow and chorion. Chorionic plate was isolated from patients undergoing cesarean and subjected to the enzymatic digestion using collagenase IV. Released cells were then plated and cultured. Passaged-3 cells were characterized in terms of surface antigenic phenotype as well as multilineage differentiation potential. The stemness properties of human chorion derived stem cells(hCDSC) and BM mesenchymal stem cells were calculated quantitatively by RT-PCR technique and compared. Immunophenotyping of MSCs from bone marrow and chorion demonstrated that the majority of these cells were positive for mesenchymal markers including CD105, CD44, CD90, and CD73. Hematopoietic cell lineage markers such as CD34 and CD45 were expressed on small percentages of the cells. Quantitative characterization of the stemness markers through real-time PCR indicated the preservation of the multipotential ability of hCDSC and BM MSCs. The results revealed the expression of all 6 before-mentioned stemness marker genes in BM and hCDSC. The proliferation rate and expression profile of them did not show any significant difference($p < 0.1$). Results of our study demonstrate such cells would be of interest and make them a suitable source for therapeutic indicatations.

P1202 - INHIBITORY CHIMERIC ANTIGEN RECEPTORS (ICARS) LIMIT UNDESIRABLE SIDE EFFECTS OF T-CELL THERAPIES

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T-cell therapies have curative potential against cancer. However, major treatment-related complications stem from unintended T-cell reactivity, such as in graft-versus-host disease following donor lymphocyte infusion or "on-target, off-tumor reactivity" reported in engineered T-cell therapies. Non-specific immunosuppression and T-cell elimination are currently the only means to control undesirable T-cell responses, at the cost of abrogating therapeutic benefit and/or causing serious secondary complications. Strategies to prevent rather than treat the consequences of inadequate T-cell specificity are acutely needed. Using the paradigm of immune-inhibitory receptors involved in physiological T-cell regulation, we designed inhibitory chimeric antigen receptors (iCARs) possessing a surface antigen recognition domain combined with intracellular inhibitory signaling domains based on CTLA-4, PD1, LAG-3, 2B4, and BTLA, to limit T-cell responses despite concurrent engagement of an activating receptor. Here we show that iCARs expressed in human T-cells were able to restrict in vitro cytokine secretion, cytotoxicity, and proliferation driven either by endogenous TCRs or by the clinically relevant CD19-specific receptor 19-28z. We developed a novel in vivo induced pluripotent stem cell allogeneic response model, and established that iCAR-expressing T cells can spare human cells expressing the iCAR antigen despite potent TCR-mediated allogenicity. Furthermore, we showed in vivo that iCAR-expressing T cells can restrict 19-28z expressing T-cells to eliminate on-target, CD19 positive tumor cells, but not off-target, CD19 and iCAR antigen dual-positive cells. Crucially, we found that iCARs exert their influence in a reversible and stoichiometric manner, thus enabling T-cells to function normally in sequential encounters with targets. Additionally, we present an antigen strategy for iCARs that can protect multiple normal human tissues. In conclusion, this proof of concept study demonstrates the potential for using iCARs as a novel strategy to regulate T-cell function at off-target sites while preserving therapeutic efficacy in autologous and allogeneic T-cell therapies.

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