FACULTY OF BIOCHEMISTRY, BIOPHYSICS AND BIOTECHNOLOGY

50th FBBB Winter School

MOLECULES ACTING IN CANCER BIOLOGY AND THERAPY

Kraków, February 22–24, 2023

Contents

Organisers's foreword

The 50th Winter School entitled "Molecules acting in biology and cancer therapy" is a continuation of the series of scientific meetings organized since 1970, first for the community of the Institute of Molecular Biology and then for the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University. In the past, these meetings have usually been held at a location outside Kraków, but, this time due to the geopolitical situation, the Winter School will be held from February 22 to 24 in Kraków.

The theme of this year's school focuses on cancers considered at the molecular level, though the poster session will also present results of research in other thematic areas. We have invited both experienced scientists from the Faculty, as well as from other research and medical institutions in Poland and the USA, to deliver the lectures.

We are very happy that PhD students and young scientists have also applied to participate in the School, where they will have the opportunity to share the results of their research in short oral presentations.

The poster sessions promise to be very interesting. They will be divided into two thematic groups: Cancer biology and therapy; and Progress in biochemistry, biophysics and biotechnology. Within each of these groups, a specially appointed committee will award the most interesting posters with distinctions.

Finally, in reference to one of the main guiding principles of former Schools in the series, namely, the integration of the academic community, laboratories recently established at the Faculty will be presented as part of the "New Horizons" session.

We believe that the 50th Winter School will prove to be a forum for inspiring discussion and exchange of experiences and an opportunity to establish fruitful co-operations. We wish you all an enjoyable time and stimulating conference.

Hanna Rokita and Irena Horwacik

Organisers

Organising Committee

Irena Horwacik – head
Hanna Rokita
Magdalena Tworzydło
Małgorzata Durbas
Anna Gontarska

IT support

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Patronage

The 50th FBBB Winter School is organised under the honorary patronage of the Dean of the Faculty of Biochemistry, Biophysics and Biotechnology, **Professor Jolanta Jura**

Acknowledgements/Sponsors

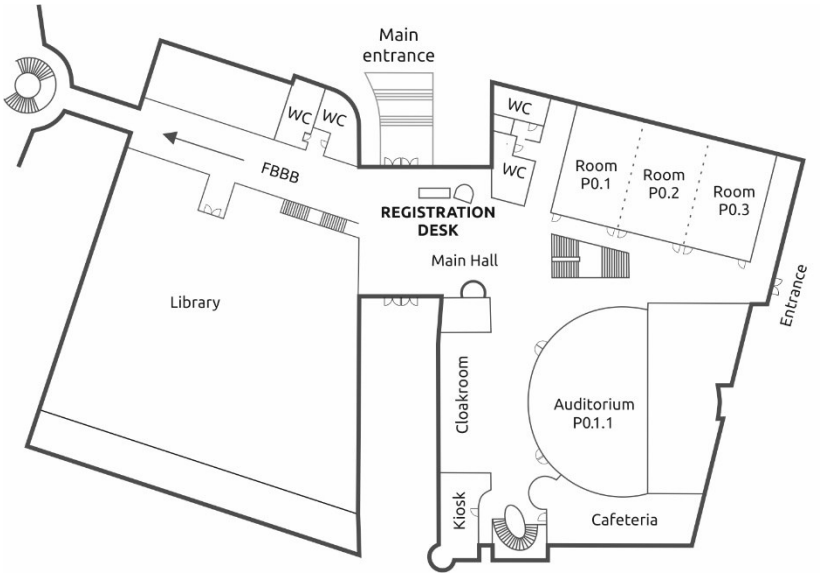
The organization of the conference was possible thanks to the funding granted by the Faculty of Biochemistry, Biophysics and Biotechnology and the support from sponsors.

Our great Patrons are Lab-JOT, LKB Biotech, IRtech (owner of ICLab), Eppendorf and EPRO.

Floor plans

Venue

Campus of the 600th Anniversary of the Jagiellonian University Revival, Educational and Library Complex, Gronostajowa 7, Kraków.



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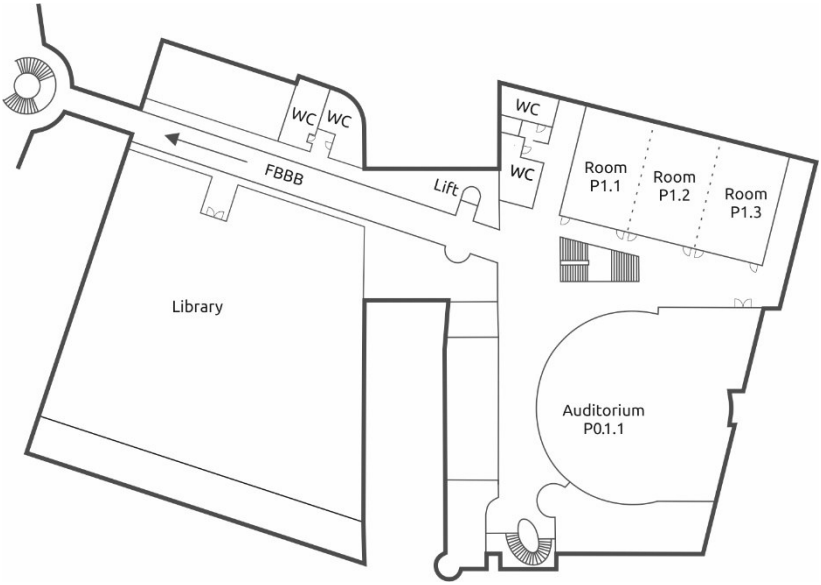
WC
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FBBB
Lift
Room
P1.1
Room
P1.2
Room
P1.3
Library
Auditorium
P0.1.1

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Programme

Programme at a glance

WEDNESDAY

12.00–14.00 REGISTRATION

14.00–14.30 OPENING OF CONFERENCE

14.30–16.00 SESSION 1
T. J. Sarnowski
A. Wieczorek
H. Rokita

16.00–17.00 BUFFET

THURSDAY

9.00–10.30 SESSION 2
M. Elas
K. Miękus
A. Piechota-Polańczyk
M. Bzowska

10.30–11.00 COFFEE BREAK

11.00–12.00 YOUNG SCIENTISTS SESSION 1
K. Pogoda-Mieszczak
A. Andrzejczak
J. Sopel
M. Rąpała

12.00–13.15 LONG BREAK

13.15–14.45 SESSION 3
J. Dulak
A. Słomiński
D. Ścieglińska

14.45–15.00 COFFEE BREAK

15.00–16.30 POSTER SESSION PART 1

19.00–21.00 GUIDED TOUR

FRIDAY

9.00–10.30 POSTER SESSION PART 2

10.30–10.45 NEW TECHNOLOGIES SESSION
P. Keša

10.45–11.30 YOUNG SCIENTISTS SESSION 2
A. Lichawska-Cieślar
Z. Głowacka-Grzyb
M. Figiel

11.30–12.00 COFFEE BREAK

12.00–13.00 SESSION 4
A. Mackiewicz
K. Lisowska

13.00–14.15 LONG BREAK

14.15–15.00 NEW HORIZONS SESSION
G. Ylla
K. Szade

15.00–15.30 CLOSING OF CONFERENCE

19.00–22.00 BANQUET

WEDNESDAY, February 22, 2023

12.00–14.00 REGISTRATION

14.00–14.30 OPENING OF CONFERENCE, room P0.1.1

Speech by the Dean of FBBB, **Jolanta Jura**
A word of introduction from the Head of Organising Committee, **Irena Horwacik**

14.30–16.00 SESSION 1, room P0.1.1, chairperson: Jolanta Jura

14.30–15.00 **Tomasz J. Sarnowski** (Institute of Biochemistry and Biophysics, PAS, Warsaw)
Control of tumor suppressors by various epigenetic mechanisms as the potential black horse in the treatment of clear cell renal cell carcinoma

15.00–15.30 **Aleksandra Wieczorek** (Institute of Pediatrics, JU Medical College, Kraków)
Bench to bed-side: translating pre-clinical research into clinical trials in neuroblastoma

15.30–16.00 **Hanna Rokita** (FBBB JU, Kraków)
Molecular mechanisms of neuroblastoma cell death induced by GD2 ganglioside-recognizing therapeutic antibodies

16.00–17.00 BUFFET

THURSDAY, February 23, 2023

9.00–10.30 SESSION 2, room P1.1, chairperson: Irena Horwacik

9.00–9.30 **Martyna Elas** (FBBB JU, Kraków)
Tumor microenvironment in preclinical cancer studies – hypoxia, vasculature and redox state

9.30–9.50 **Katarzyna Miękus** (FBBB JU, Kraków)
Cellular and molecular mechanisms responsible for renal cancer progression and resistance to therapy – the role of MCPIP1

9.50–10.10 **Aleksandra Piechota-Polańczyk** (FBBB JU, Kraków)
The usefulness of intestinal organoids in in vitro studies

10.10–10.30 **Monika Bzowska** (FBBB JU, Kraków)
Intracellular antibody immunity – how can we exploit it to target undruggable intracellular proteins involved in tumorigenesis?

10.30–11.00 COFFEE BREAK

11.00–12.00 YOUNG SCIENTISTS SESSION 1, room P1.1, chairperson: Katarzyna Miękus

11.00–11.15 **Kinga Pogoda-Mieszczak** (Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice; Silesian University of Technology)
Effect of oncolytic myxoma virus knock-out construct on apoptosis of glioma cells

11.15–11.30 **Anna Andrzejczak** (Hirszfeld Institute of Immunology and Experimental Therapy, PAS, Wrocław)
Association between HVEM, CD160, TIM-3, LGALS9 gene variants and ccRCC risk

11.30–11.45 **Justyna Sopel** (FBBB JU, Kraków)
The effect of quisinostat on the development of uveal melanoma tumors in the chorioallantoic membrane model (CAM)

11.45–12.00 **Michał Rąpała** (FBBB JU, Kraków)
Cytotoxic effect of manganese porphyrins MnTPPS and MnF2Met and sodium ascorbate to cancer cell line in in vitro condition

12.00–13.15 LONG BREAK

13.15–14.45 SESSION 3, room P1.1, chairperson: Martyna Elas

13.15–13.45 **Józef Dulak** (FBBB JU, Kraków)
Stem cells for disease modelling, drug research and therapy: possibilities, hopes and hypes

13.45–14.15 **Andrzej Słomiński** (University of Alabama at Birmingham)
Photo-endocrinology: the central role of the skin

14.15–14.45 **Dorota Ścieglińska** (Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice)
Normal but not malignant epithelial cells are dependent on the activity of the Heat Shock Protein A2 (HSPA2)

14.45–15.00 COFFEE BREAK

15.00–16.30 POSTER SESSION PART 1, rooms P0.1 and P0.2

19.00–21.00 GUIDED TOUR, meeting point: St. Mary Magdalene Square

Walking tour of the University City of Kraków by night

FRIDAY, February 24, 2023

9.00–10.30 POSTER SESSION PART 2 (WITH MORNING COFFEE AND BUN), rooms P0.1 and P0.2

10.30–10.45 NEW TECHNOLOGIES SESSION, room P1.1, chairperson: Martyna Elas

10.30–10.45 **Peter Keša** (FUJIFILM VisualSonics, Inc., Amsterdam)
High frequency ultrasound and photoacoustic imaging: Tumor microenvironment characterization

10.45–11.30 YOUNG SCIENTISTS SESSION 2, room P1.1, chairperson: Monika Bzowska

10.45–11.00 **Agata Lichawska-Cieślar** (FBBB JU, Kraków)
MCPIP family members as modulators of tumor-related processes in cutaneous squamous-cell carcinoma

11.00–11.15 **Zuzanna Głowacka-Grzyb** (FBBB JU, Kraków)
Bacteriocins as potential therapeutic strategy against cancer

11.15–11.30 **Małgorzata Figiel** (FBBB JU, Kraków)
The role of transcription factor YY1 in pathogenesis of cancer

11.30–12.00 COFFEE BREAK

12.00–13.00 SESSION 4, room P1.1, chairperson: Hanna Rokita

12.00–12.30 **Andrzej Mackiewicz** (Maria Skłodowska–Curie Greater Poland Cancer Centre, Poznań)
Molecular mechanisms of human melanoma immunotherapy

12.30–13.00 **Katarzyna Marta Lisowska** (Maria Skłodowska–Curie National Research Institute of Oncology, Gliwice)
Negative prognostic signature in ovarian cancer

13.00–14.15 LONG BREAK

14.15–15.00 NEW HORIZONS SESSION, room P0.1.1, chairperson: Artur Osyczka

Guillem Ylla (FBBB JU, Kraków)
The Laboratory of Bioinformatics and Genome Biology: computational approaches to study genes, genomes, and gene regulatory networks

Krzysztof Szade (FBBB JU, Kraków)
Laboratory of Stem Cell Biology and Single Cell Biology Research Core

15.00–15.30 CONFERENCE CLOSING, room P0.1.1, chairperson: Jolanta Jura

Awarding diplomas for the best posters. Commemorative photo.

19.00–22.00 BANQUET IN THE CITY CENTER

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Od lat skupiamy się na współpracy z jednostkami naukowymi
i dokładamy wszelkich starań, aby podążać za wymaganiami Klientów.

Oferujemy szeroki wybór najwyższej jakości odczynników
oraz innowacyjnych rozwiązań do badań naukowych.

Zapewniamy profesjonalną obsługę, szybką realizację zamówień,
a także wsparcie techniczne i wszelką pomoc merytoryczną.

Jesteśmy przedstawicielem czołowych firm działających na globalnym rynku Life Science:
Cell Signaling Technology, New England BioLabs, ABclonal Technology
oraz **Norgen Biotek**.

- przeciwciała pierwszo i drugorzędowe oraz odczynniki do analiz WB, IP, IF, IHC, ChIP
- zestawy ELISA
- testy komórkowe i biochemiczne
- zestawy do izolacji i oczyszczania DNA
- zestawy do izolacji i oczyszczania RNA
- zestawy do pobierania i przechowywania materiału biologicznego
- odczynniki do przygotowania bibliotek NGS
- produkty do Real-Time PCR
- polimerazy DNA i RNA
- odwrotne transkryptazy
- zestawy do PCR i RT-PCR
- enzymy restrykcyjne
- ligazy
- enzymy modyfikujące RNA/DNA
- zestawy odczynników do klonowania, mutagenezy i transformacji
- komórki kompetentne
- systemy do edycji genów CRISPR/Cas-9 oraz odczynniki do transfekcji
- drabinki i markery wielkości DNA, RNA i białek
- odczynniki do badań epigenetycznych i glikobiologii
- inhibitory, aktywatory, cytokiny, czynniki wzrostu, hormony, białka rekombinowane
- bufory, roztwory barwiące i inne odczynniki pomocnicze
- wiele innych odczynników niezbędnych w biologii molekularnej

Posters overview

	CANCER BIOLOGY AND THERAPY
C1	Aleksandra **Bienia** *Normoxic and hypoxic spheroids of pancreatic ductal adenocarcinoma (PDAC) – environment oxygenation as a driven factor of invasiveness and proliferation* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C2	Aleksandra **Bienia** *Multi-module treatment against pancreatic cancer – synergetic effects of nanogold, gemcitabine, and hyperthermia on cancer cells* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C3	Aleksandra **Bienia** *Architecture of three-dimensional cancer cell cultures as a factor determining invasiveness at the in vitro level* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C4	Kinga **Chlebicka** *Bacteria in carcinogenesis* Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C5	Agnieszka **Drzał** *Characterization of ectopic glioma model by noninvasive oximetric imaging with the use of LiNc-BuO-based microspheres* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C6	Małgorzata **Durbas** *Anti-GD2 ganglioside ch14.18/CHO antibodies and aurora A kinase inhibitors lead to apoptosis in human neuroblastoma cells* Laboratory of Molecular Genetics and Virology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C7	Gabriela **Dziurman** *Optimization of CW and Pulse EPR measurements of OxyChip probesto noninvasive measurements of tissue hypoxia* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków

C8	Justyna **Gałuszka** *Analysis of EZH2 methyltransferase expression in lung cancer cells and its influence on the sensitivity of these cells to the antiproliferative activity of vitamin D* Laboratory of Experimental Anticancer Therapy, Department of Experimental Oncology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław
C9	Maja **Kudrycka** *In search for possible targets of therapy of neuroblastoma – cellular studies and bioinformatic analysis of clinical data* Laboratory of Molecular Genetics and Virology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C10	Oliwia **Kwapisz** *MCPIP1 proteinfunction in HCC development* Department of General Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C11	Paulina **Marona** *MCPIP1 protein in the treatment of hepatocellular carcinoma* Department of General Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C12	Renata **Mężyk-Kopeć** *mTOR-dependent expression and activation of TGFβ. Impact on proliferation, migration and chemokine expression in TSC2-deficient cells* Department of Cell Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C13	Aleksandra **Murzyn** *Biodistribution of a gold nanoparticle-based construct in a mouse model of pancreatic ductal adenocarcinoma (PDAC)* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C14	Bartłomiej **Olajossy** *Downregulation of RIPK4 decreases the levels of ABCG2 but not ABCB1 and ABCC1 in melanoma cells* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C15	Maciej **Pudełek** *Redox homeostasis in the adaptation of glioblastoma cells to chemotherapeutic stress* Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków

C16	Maksym **Pudełek** *L-Glutamine metabolism in glioblastoma homeostasis and chemotherapeutic stress response* Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C17	Dominik **Robak** *Development of a Convolutional Neural Network (CNN) model for automated cell counting and classification in transmitted light microscopy images of cancer cell lines* Department of Cell Biophysics/Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C18	Aleksandra **Solecka** *ZC3H12B regulates the expression of matrix metalloproteinase 2 (MMP2)* Department of Cell Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C19	Justyna **Sopel** *Knockout of PMEL17 potentially related to murine melanoma tumour development* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C20	Weronika **Sowińska** *Regnase-2 controls the expression of Regnase-1 and inhibits cell proliferation* Department of Cell Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C21	Piotr **Świerzewski** *Simulating liver niche in uveal melanoma spheroids* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C22	Dariusz **Szczygieł** *Biological meaning of nitrosohemoglobin in tumors* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C23	Małgorzata **Szczygieł** *Heterogeneity of the uveal melanoma cell population derived from a single metastasis to the liver* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków

C24	Małgorzata **Szczygieł** *Tracking of human uveal melanoma metastases growth in animal model by photoacoustic imaging* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C25	Weronika **Szukała** *Loss of myeloid Mcpip1 suppresses the development of squamous cell carcinoma of the skin* Department of General Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C26	Klaudia **Wiecha** *Developing a new model for testing the differential impact of HSPA1 and HSPA2 chaperone proteins on the phenotype of human bronchial epithelial cells* Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice
C27	Olga **Wiecheć-Cudak** *Combined chemotherapy with hyperthermia and calcitriol in immunocompetent and orthotopic pancreatic tumor mouse models* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C28	Norbert **Wroński** *Lack of RIPK4 impairs Wnt/β-catenin signaling in melanoma cells* Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
C29	Honorata **Zachary** *The effect of calcitriol and tacalcitol treatment on OPN receptors expression and Th17/Treg cells subsets in various mammary gland cancer models* Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław

PROGRESS IN BIOCHEMISTRY, BIOPHYCICS AND BIOTECHNOLOGY	
P1	Danuta **Bryzek** *The effect of citrullination on the inflammatory potential of vimentin* Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków

P2	Joanna **Budziaszek** *DNases as a virulence factor in Streptococcus anginosus* Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P3	Patryk **Chudy** *The role of heme oxygenase 1 in the regulation of the cell cycle* Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P4	Izabela **Ciastoń** *Proteolytic activity-independent activation of the immune response by gingipains from Porphyromonas gingivalis* Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P5	Ewelina **Dobosz** *Kgp affects TLR3 signaling pathway leading to impairment of anti-viral response* Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P6	Dominik **Dreszer** *Uncovering nanotoxicity of a water-soluble and red-fluorescent [70] fullerene nanomaterial* Institute of Chemistry, University of Silesia, Katowice
P7	Patrycja **Dudek** *Immortalized and primary adipose tissue-derived mesenchymal stem/ stromal cells as a source of extracellular vesicles – comparative study* Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P8	Anna **Gąsiorek** *Establishment of the organotypic model of gingiva to study bacterial and viral infections* Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P9	Adrian **Kania** *Matrix representations in biological sequence analysis* Department of Computational Biophysics and Bioinformatics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P10	Angelika **Kapinos** *The effect of FGF-2 on the TGF-β1-induced myofibroblastic transitions of human lung fibroblasts* Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków

P11	Andrzej **Kubiak** *Measuring adhesion between bone marrow mesenchymal stromal cells and extracellular matrix* Laboratory of Stem Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P12	Aleksandra **Lazar** *Resequencing Tannerella forsythia strain ATCC 43037 using Nanopore technology* Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P13	Svitlana **Levchenko** *FLIM to sense all-protein concentration in DNA damage repair foci* Department of Cell Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P14	Agnieszka **Łoboda** *Proteome profiling of mouse dystrophic diaphragm reveals decreased expression of H2S-generating enzymes* Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P15	Katarzyna **Mikruta** *Characterization of a new secretion system in Porphyromonas gingivalis* Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P16	Krystian **Mokrzyński** *Light-induced toxicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list* Department of Biophysics/Department of Biophysics and Cancer Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
P17	Krzysztof **Mrowiec** *DYRK1A inhibitors as potential therapeutics in treatment of diabetes* Kinase Inhibition and Nanotechnology for Diabetes Research Group, Małopolska Centre of Biotechnology, Jagiellonian University, Kraków
P18	Małgorzata **Myszka** *Hydrogen sulfide exerts protective effects in the mouse model of Duchenne muscular dystrophy* Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków

P19	Małgorzata **Myszka** *Slow-releasing hydrogen sulfide donors GYY4137 and AP39 attenuate dystrophic phenotype in a murine model* Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P20	Sylwia **Noga** *The impact of graphene-based substrates on human mesenchymal stem cells potential in tissue repair – in vitro and in vivo studies* Malopolska Centre of Biotechnology, Jagiellonian University, Kraków; Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P21	Witold N. **Nowak** *Heme in preimplantation embryo – one ring to rule them all?* Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P22	Monika **Orpel** *Functional characterization of iPSC-derived endothelial cells as an in vitro model for studying myocardial tissue repair* Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P23	Paweł **Piłat** *N4BP1 is a novel component of P-bodies* Department of General Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P24	Piotr **Rybczyński** *Clozapine regulates cholesterol metabolism at the level of cell nucleus* Department of Physical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P25	Izabella **Skulimowska** *Deletion of netrin-1 in endothelial cells decreases survival after hematopoietic stem cells transplantation and impairs hematopoiesis during aging* Department of Medical Biotechnology/Laboratory of Stem Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P26	Agata **Szade** *Mobilization of cells from the bone marrow to blood using cobalt protoporphyrin IX* Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków

P27	Katarzyna **Szczęśniak** *Anhydrotetracycline as an inducer in the Porphyromonas gingivalis inducible gene expression system* Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P28	Szymon **Szrajer** *Computational pipeline to identify genes of interest in unannotated genomes based on the domain architecture* Laboratory of Bioinformatics and Genome Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P29	Aureliusz **Schuster** *Periodontic bacterium Fusobacterium nucleatum amplifies interferon-γ-induced activation of gingival fibroblasts during infection* Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P30	Mateusz **Szwalec** *High resolution cryo-EM structures of cytochrome b6f imply a one-way traffic of quinones for efficient photosynthesis* Department of Molecular Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P31	Katarzyna **Trzos** *Results of pharmacological treatment of mice that develop primary biliary cholangitisdue to the lack of the Mcpip1 protein in the liver* Department of General Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków
P32	Monika **Tuleja** *Anticancer fullerenes in callus culture of medical plant Lilium martagon L.* Department of Plant Cytology and Embryology, Faculty of Biology, Jagiellonian University, Kraków
P33	Marta **Wadowska** *MCPIP1 is a regulator of IFNγ signalling* Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków

Lecture abstracts

Lecture abstracts are presented in the same order
as in the conference programme

Control of tumor suppressors by various epigenetic mechanisms as the potential black horse in the treatment of clear cell renal cell carcinoma

Magdalena Wilga[1], Anna Maassen[1], Joanna Kosior[1], Joanna Szarkowska[2], Natalia Rusetska[2], Janusz A. Siedlecki[2], Elżbieta Sarnowska[2], Tomasz J. Sarnowski[1]

[1]Laboratory of Gene Expression Regulation, Institute of Biochemistry and Biophysics, Polish, Academy of Sciences, Warsaw, Poland; [2]Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland

About 40% of clear cell renal cell carcinoma (ccRCC) cases exibit mutation in *PBRM1* gene encoding non-core subunit of SWI/SNF chromatin remodeling complexes (CRCs).

Here we identified alterations in expression level of genes encoding various subunits of SWI/SNF CRCs in ccRCC despite the lack of mutations in their *loci* and correlated them with methylation and histone modification changes. We additionally found unusual subcellular translocation of some SWI/SNF subunits which may serve as predictory factor for the local reccurence of ccRCC. Collectively, we postulate that the epigenetic-based alterations of SWI/SNF CRC may be important not only for ccRCC development but also for its progression.

ACKNOWLEDGEMENTS
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Bench to bed-side: translating pre-clinical research into clinical trials in neuroblastoma

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Neuroblastoma (NBL) is the most common solid tumor in children. The course of disease depends on many clinical and biological prognostic factors, and in high-risk group the prognosis is unsatisfactory despite intensive combined therapy. Implementation of new treatment methods, based on immunotherapy (anti-GD2 antibodies) and targeted therapy (inhibitors of ALK) allowed for marked improvement of treatment results. New markers that can be used as treatment targets or prognostic markers are still investigated and as immunotherapy is one of the most important treatment methods, new models letting for evaluation of immune system function are needed, as well as establishing of new models based on the tumor cells taken both at diagnosis and disease relapse. Close cooperation between clinics and laboratory is crucial for further improvement of treatment results.

Molecular mechanisms of neuroblastoma cell death induced by GD2 ganglioside-recognizing therapeutic antibodies

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Antibodies binding to gangliosides on tumor cells can induce direct cytotoxic effects. Our studies show that anti-GD2 ganglioside (GD2) antibodies affect pivotal signaling routes that influence the neuroblastoma cell fate. Additionally, we elucidated structural basis of recognition of GD2 and its peptide mimics by monoclonal antibody 14G2a. Moreover, gene expression profiling helped us to identify *PHLDA1* (pleckstrin-homology-like domain family A member 1) as the most upregulated gene in the studied IMR-32 human neuroblastoma cells treated with the antibody and contribution of PHLDA1 to response of neuroblastoma cells to the experimental treatment is currently being characterized. Recently, mass spectrometry-based proteomic analyses were applied to define PHLDA1 binding partners and better characterize a role of *PHLDA1* gene silencing in the IMR-32 cells.

ACKNOWLEDGEMENTS
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Tumor microenvironment in preclinical cancer studies – hypoxia, vasculature and redox state

Agnieszka Drzał, Aleksandra Bienia, Aleksandra Murzyn, Ewa Kowolik, Justyna Sopel, Gabriela Dziurman, Piotr Świerzewski, Dariusz Szczygieł, Anna Kozińska, Małgorzata Szczygieł, Martyna Krzykawska-Serda, Przemysław M. Płonka, Martyna Elas

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The tumor microenvironment (TME) is recognized as a major factor in cancer development, progression, and response to therapies. In our laboratory we use a wide range of cellular and animal models to study TME influence on tumor growth and dissemination. Noninvasive imaging of hypoxia, vasculature structure and function, and redox state allow for characteristics of TME. Oximetry in orthotopic tumors of breast, pancreas, and brain, as well as redox and metabolic studies in metastatic models will be presented as examples of EPR spectroscopy and imaging in living animals.

ACKNOWLEDGEMENTS
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Cellular and molecular mechanisms responsible for renal cancer progression and resistance to therapy – the role of MCPIP1

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The ability of tumor cells to metastasis and acquired resistance to antitumor treatment is regulated by the plasticity of cells in the tumor and the acquisition of mesenchymal phenotype during the epithelial to mesenchymal transition (EMT) process. One of the EMT inducers during cancer progression is inflammation regulated by MCPIP1 (Monocyte Chemotactic Protein-1 Induced Protein).

MCPIP1 plays a role during the process of tumorigenesis regulating viability, proliferation and apoptosis. We have already documented that MCPIP1 decreases during clear cell renal cell carcinoma (ccRCC) progression and low MCPIP1 protein level correlates with better tumor vascularity and metastasis into lungs and livers. Our last data showed that MCPIP1 regulates EMT influencing Wnt/β-catenin dependent pathway and plays a role in the acquisition of resistance to antiangiogenic drugs used in ccRCC therapy.

The usefulness of intestinal organoids in in vitro studies

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Cultured organoids can be used to study different conditions, such as inflammation or colorectal cancer. We aimed to verify if intestinal organoids collected from wild-type and Nrf2 knockout mice proliferate, differentiate and respond to inflammatory stimuli in a similar way.

We showed that the lack of Nrf2 transcriptional activity in mice changes the morphology of colon organoids, the kinetics of proliferation, and the tendency to differentiate into enteroendocrine cells. These organoids also respond differently to inflammatory factors.

Therefore, organoids may be a useful model for studying how genetic modifications in mice can determine the growth and response of intestinal organoids to external stimulation, but most likely, after the introduction of mutations, they may be used to study the molecular background of different diseases including cancer.

Intracellular antibody immunity – how can we exploit it to target undruggable intracellular proteins involved in tumorigenesis?

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Antibodies (Ab) act not only extracellularly, eliminating pathogens by complement or antibody-dependent cellular toxicity. Ab attached to the virus infecting the cells are intracellularly bound by cytosolic Ab receptor – TRIM21 (tripartite motif-containing 21), a ubiquitin ligase. TRIM21 performs then auto-polyubiquitination and undergoes proteasomal degradation together with Ab and the bound target. Therefore, Ab "have second life" inside cells participating in the intracellular arm of adaptive immunity. Since TRIM21 targets for degradation of any antibody-bound protein, it can be employed to deplete intracellular antigens after their recognition by Ab delivered to the cytosol. Methods for Ab delivery into the cells could be of critical importance for TRIM21-directed depletion of oncogenes or other proteins involved in cancer progression.

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Stem cells for disease modelling, drug research and therapy: possibilities, hopes and hypes

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Anti-cancer treatments are life-saving but chemotherapeutics can exert also long-term unwanted effects. Drug-induced cardiotoxicity is the serious problem limiting efficacy of therapy and exposing patients to difficult to control consequences. Induced pluripotent stem cells (iPSC) allow to determine the effectiveness, safety and the risks of drugs. iPSCs are also indispensable for modelling mechanisms of diseases affecting difficult to reach cells, such as neurons or cardiomyocytes. Stem cells are also commonly considered as panacea for numerous conditions what is unfortunately linked with unjustified treatment with "stem cells" offered by physicians not understanding the complexity of real stem cells. In this lecture the potential of iPSCs for disease modelling and drug research will be presented together with the brief overview of "stem cells" hype.

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Normal but not malignant epithelial cells are dependent on the activity of the Heat Shock Protein A2 (HSPA2)

Damian Sojka[1], Agnieszka Gogler[1], Małgorzata Adamiec-Organiściok[1], Daria Kania[1], Klaudia Wiecha[1], Natalia Matysiak[2], Agata Wilk[3], Alexander Cortez[3], Dorota Ścieglińska[1]

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The HSPA (HSP70) family groups molecular chaperones that control the protein folding. HSPAs are overproduced in various cancers to provide stress resistance and sustain cell growth.

In this study we focused on HSPA2, one of least known members of the family. This male fertility-related factor, beside the testis is expressed also in stratified epithelia (e.g. epidermis) and many cancers. We used loss-of-function and overexpression approaches to find the processes relying on HSPA2 in cancer cells and keratinocytes.
We found that the phenotype of cancer cells is not dependent on HSPA2. In contrast, HSPA2 turned out to be a factor related to keratinocyte differentiation and formation of stratified tissue in the reconstructed human epidermis model.

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Negative prognostic signature in ovarian cancer

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In our previous microarray study we identified the 96-gene signature significantly related to the worse survival of patients with high-grade serous ovarian cancer [1]. Top differentially expressed genes were e.g. POSTN, COL11A1, SFRP2, MFAP5, ITGBL1, LOX, FN1. Similar mesenchymal signature with negative impact on survival, has been observed also by others. However, it has been regarded rather as a specific feature of cancer associated fibroblasts, while not epithelial cells.

We postulate that these genes can be also expressed by cancer cells themselves and affect their phenotype. Here, we will present results of our studies on the role of selected genes from the negative prognostic signature. We analyzed both, biological role of these genes in ovarian cancer cells, and their significance as potential prognostic biomarkers [2-4].

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The Laboratory of Bioinformatics and Genome Biology: computational approaches to study genes, genomes, and gene regulatory networks

Guillem Ylla

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The overarching goal of the Laboratory of Bioinformatics and Genome Biology is to develop and use computational tools to study genes, genomes, and gene regulatory networks. Keeping always an evolutionary perspective, we aim to understand how genomes produce different phenotypes, and especially, how phenotypic innovations have emerged and evolved. With that goal in mind, we investigate the functions of different gene regulatory elements, and the role they played in shaping animal evolution.

In this talk, I will give an overview of the main scientific achievements of the laboratory in its first year of existence. This will include an overview of some of our most exciting manuscripts, examples of current collaborations at Jagiellonian University and beyond, and future research plans in the context of funded projects.

Laboratory of Stem Cell Biology and Single Cell Biology Research Core

Krzysztof Szade

Laboratory of Stem Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland,

The new Laboratory of Stem Cell Biology at the Faculty of Biochemistry, Biophysics and Biotechnology aims to understand the biology of adult stem cells with focus on hematopoietic stem cells. The long-term goal of our research is to translate the basic knowledge about self-renewal and differentiation of adult stem cells toward new clinical strategies.

Our studies base on prospective identification and isolation of adult stem cells, and experiments performed on single cell level. Here, we present our methodology, that include single cell RNA sequencing, single cell targeted DNA sequencing, single cell sorting and clonal functional assays. We would like to share our expertise and new research infrastructure within Single Cell Biology Research Core.

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Short oral presentation abstracts

Short oral presentation abstracts are presented in the same order as in the conference programme

Effect of oncolytic myxoma virus knock-out construct on apoptosis of glioma cells

Kinga Pogoda-Mieszczak[1,2], Aleksander Sochanik[1], Masmudur M. Rahman[3], Grant McFadden[3], Joanna Jazowiecka-Rakus[1]

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vMyx-M011L-KO, an oncolytic myxoma virus construct devoid of Bcl-2 homologue gene was designed to promote apoptosis of infected brain tumor initiating cells, largely responsible for glioma recurrency.
We envisaged systemic glioma therapy strategy based on systemically delivering this oncolytic construct with adipose-derived mesenchymal stem cells (ADSCs) to avoid adverse response of the host immune system. Preliminary results showed cultured glioma cells to be permissive to this myxoma virus, whereas ADSCs less so making possible to use them as viral carrier. Demonstrated expression of apoptotic proteins by infected glioma cells paves the way to the planned systemic delivery of the therapeutic system to glioma foci *via* intracarotid microsurgery.

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Association between *HVEM*, *CD160*, *TIM-3*, *LGALS9* gene variants and ccRCC risk

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Numerous data showed associations between variants in genes encoding immune checkpoints (ICs) - crucial immune response modulators, with cancer risk and overall survival (OS). We aimed to study the influence of HVEM, CD160, TIM-3, and LGALS9 variants on the risk of clear cell renal cell carcinoma (ccRCC) and patient OS.

We found that rs1886730 A (*HVEM*) and rs2234167 A alleles (*HVEM*) increased, while rs10057302 A (*TIM-3*) and rs4794976 T (*LGALS9*) alleles decreased ccRCC risk. Subgroup analysis showed an association of ICs gene variants with clinical features of the disease. In addition, ICs genes haplotype analysis revealed that particular haplotypes increased risk of ccRCC. Moreover, rs1036199 (*TIM-3*) and rs1886730 (*HVEM*) significantly influenced OS.

Our results indicate that *HVEM*, *TIM-3*, and *LGALS9* variants might modulate ccRCC risk and OS.

The effect of quisinostat on the development of uveal melanoma tumors in the chorioallantoic membrane model (CAM)

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Uveal melanoma is a rare cancer, usually metastasizing to the liver. The most commonly used therapies are surgical methods and radiotherapy. In our research, we have used quisinostat – a histone deacetylase inhibitor as an anticancer therapy [1].

Three uveal melanoma cell lines obtained from patients were treated with a solution of quisinostat. Then, untreated and treated cells were implanted on the CAM of the chicken embryo. Tumor growth was monitored for 6 days on CAM. The tumors were then isolated, weighed and analyzed by histology and Western Blot.

After treatment we observed inhibition of tumor growth on the CAM membrane by approximately 25% .The obtained data show that the CAM model can be used for research on uveal melanoma and chemotherapeutic agents.

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*These authors contributed equally

Cytotoxic effect of manganese porphyrins MnTPPS and MnF2Met and sodium ascorbate to cancer cell line in *in vitro* condition

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The combination of manganese porphyrins and sodium ascorbate have cytotoxic impact to cancer cells. These properties depend on the *invitro* conditions, such as the composition of the culture medium, the pH of the reaction, the number of cells and the concentration of porphyrins and sodium ascorbate [1]. The combination of MnTPPS and sodium ascorbate reduces the viability of cells of various lines. We observed cell membranes damages and changes in migration activity of AT-2, MCF-7 and T98 cells. The exact reaction mechanism is unknown. Manganese porphyrins exhibit catalytic properties, which can oxidize sodium ascorbate. This reaction can produce reactive oxygen species that can damage cancer cells and lead to cell death [2].

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High Frequency Ultrasound and Photoacoustic Imaging: Tumor microenvironment characterization

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Multimodal high-frequency ultrasound and photoacoustic imaging offer non-invasive, repeatable, and low-cost approach to monitor tumor growth and its microenvironment. High spatial resolution of ultrasound imaging can reveal early tumor stadium or detect metastasis within the whole animal body. Moreover, functional Doppler imaging of the tumor vasculature or tumor perfusion studies often correlates with tumor aggressiveness because relates to the oxygen supply of the tumor [1].

The tumor oxygenation can be assessed at the same time by photoacoustic imaging based on the different optical properties of both oxygenated and deoxygenated hemoglobin. The newest Whole-body imaging setup enables to do a biodistribution studies of new drugs or assess the anticancer treatment impact obtaining the anatomical and molecular information at the same time.

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MCPIP family members as modulators of tumor-related processes in cutaneous squamous-cell carcinoma

Agata Lichawska-Cieslar[1], Weronika Szukala[1], Maria Kulecka[2], Izabela Rumieńczyk[2], Michał Mikula[2], Iwona Chlebicka[3], Jacek C Szepietowski[3], Jolanta Jura[1]

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Monocyte chemoattractant protein-induced protein (MCPIP) family comprises four members, which share conserved PIN domain that confers their ribonucleic activity. MCPIP1 is the most extensively studied member of this family and has been described to possess tumor suppressive properties in many types of cancer. We previously showed that in the squamous cell carcinoma of the skin (SCC), expression of MCPIP1 is reduced.

In this study, we investigated the activity of another MCPIP member, MCPIP3, in cutaneous SCC. In comparison to MCPIP1, expression of MCPIP3 turn out to be very high within malignant tissue. We next developed several *in vitro* models of malignant keratinocytes, in which the activity of MCPIP1 and MCPIP3 was modulated. In all, our work imply existence of both common and unique mechanisms dependent on MCPIP1 and MCPIP3 activity in the course of skin cancer.

Bacteriocins as potential therapeutic strategy against cancer

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The conventional cancer treatments are imperfect since they lack target specificity and arise severe side effects. Bacteriocins are bacterial competition strategy, also proven to be effective approach against cancer *in vitro* and *in vivo*.

Bacteriocins' characteristics allow them to preferentially target malignant cells without causing harm to healthy tissues, simultaneously being effective in tumor elimination with no risk of resistance development.

Huge diversity of bacteriocins mirror their vast mechanisms of action against cancer cells, i.e., apoptosis and necrosis induction, destabilization and permeabilization of cell membrane or cell cycle arrest.

Bacteriocins display some disadvantages, mainly immunogenicity and difficulties regarding stability, half-life, and clearance in the body. However, those issues can be resolved with genetic modifications.

The role of transcription factor YY1 in pathogenesis of cancer

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Transcription factor YY1 regulates many processes associated with cancer progression: cell proliferation, metastasis and metabolic reprogramming. It controls the expression of numerous oncogenes by recruiting co-activators and mediating long-range interactions between enhancers and promoters. DNA motifs bound by YY1 can differ in normal and transformed cells, as exemplified by a recurrent T372R mutation in yy1 gene, observed in 30% of insulinomas. YY1 is also upregulated in large number of cancers and is considered a prognostic marker. On the other hand, its ortholog YY2 acts as a tumor suppressor. The mechanism underlying this intriguing functional antagonism of the two structurally similar proteins is the subject of our current studies.

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Poster abstracts:
cancer biology and therapy

Poster abstracts are listed in alphabetical order
of the presenting authors' last names

Normoxic and hypoxic spheroids of pancreatic ductal adenocarcinoma (PDAC) – environment oxygenation as a driven factor of invasiveness and proliferation

Aleksandra Bienia[1], Aleksandra Murzyn[1], Sylwia Drabik[2], Dominik Robak[3], Martyna Krzykawska-Serda[1]

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One of the disadvantages of 2D cell culture is loss of contact with the extracellular matrix. Spheroids (3D models) better represent the physiological conditions in a tumor, which is of great importance in better understanding basic cancer processes and testing anti-cancer therapies [1,2].

The research aims to determine the optimal formation conditions and characterization of spheroids under different oxygen concentrations. For this purpose, cells (PAN_02 and PANC-1) were cultured using the hanging drop method. Spheroids were stained by immunohistochemistry and imaged under a microscope, and biochemical analysis was performed by Western blot and ELISA.

The results indicate that the oxygen environment during spheroids formation leads to different morphological properties and affects invasiveness potential.

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Multi-module treatment against pancreatic cancer – synergetic effects of nanogold, gemcitabine, and hyperthermia on cancer cells

Aleksandra Bienia[1], Aleksandra Murzyn[1], Małgorzata Szygieł[1], Dariusz Szczygieł[1], Olga Wiecheć-Cudak[1], Dominik Robak[2], Rafał Sitko[3], Maciej Zubko[4], Mateusz Dulski[4], Maciej Serda[3], Martyna Krzykawska-Serda[1]

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Pancreatic cancer is one of the most difficult-to-treat cancers [1]. The proposed strategy uses multi-module therapy: mild hyperthermia and chemotherapy, together with the nanogold construct. In the outcome, better selectivity and fewer side effects are expected.

The goals were to design and characterize (by mass spectroscopy, Z-sizer, and HPLC) a new theragnostic compound and verify the mechanism of action *in vitro*. The intense absorbance spectrum for the nanogold construct was observed at 828 nm. The solubility, stability and toxicity was studied *in vitro*. MTT and long-term microscopic (JuliStage®) cell survival tests were performed in PANC-1, AsPc-1, PAN_02.

New nanoconstruct can perform gemcitabine-based chemotherapy with gold nanorods-based hyperthermia treatment and infrared light. The synergetic effects were observed.

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Architecture of three-dimensional cancer cell cultures as a factor determining invasiveness at the *in vitro* level

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Spheroids as a three-dimensional model better reflect the growth conditions and the microenvironment of tumor than the monolayer cells. The aim of this research was to determine the degree of invasiveness, migration and the level of adhesion proteins in several cell lines of glioma, skin and uveal melanoma and also pancreatic cancer growing as spheroids.

An important aspect of our study was extending the time window of the experiment by transferring spheroids from hanging drop to 2.5% polyHEMA covered plates.

As it turned out, spheroids differed substantially in their morphology and architecture and could form tight, compact or loose structures, probably reflecting the adhesive properties of cells [1].

The results obtained will allow us to correlate the structure of spheroids with their aggressive and invasive properties.

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Bacteria in carcinogenesis

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The cancer incidence in population is affected by genetic inheritance and unhealthy lifestyle. A lesser-known cause of cancer are infections. Many bacteria are commensals but under some condition may cause infections and consequently lead to the formation of a malignant tumor. Lung, breast, colon, prostate and gastric cancer can be caused by bacterial mechanisms. Bacteria like *Helicobacter pylori, Chlamydia trahomatis, Fusobacterium nucleum* may promote formation and progression of cancer via secretion potential carcinogens: DNA damage toxins, cell signaling disrupting toxins and metabolites like nitrosamines, acetaldehyde, bile acid degradation products as well as interaction with host immune system and promote release cytokines. The composition of the microbiome changes during carcinogenesis, therefore bacteria may be predictors of cancer prognosis.

Characterization of ectopic glioma model by noninvasive oximetric imaging with the use of LiNc-BuO-based microspheres

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Tumor oxygenation has strong prognostic significance in the outcome of chemotherapy and radiotherapy [1]. The aim of this study was to characterize temporal changes in the microenvironment of the GL261 model ectopic glioma by Doppler ultrasonography and noninvasive oximetric imaging using EPR and LiNc-BuO-based microspheres implanted together with cancer cells. Moreover, the impact of LiNc-BuO-based microspheres on cell morphology and migration was analyzed *in vitro*.

In vitro studies did not show any impact of LiNc-BuO-based microspheres on cells, which makes them perfect for *in vivo* use. After ectopic inoculation in the interscapular fat pad, tumor growth was rapid, highly heterogeneous, and led to hypoxic characteristics of a tumor from the second week of its growth. The mice were sacrificed around week four due to the size approaching the ethical limit.

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Anti-GD2 ganglioside ch14.18/CHO antibodies and aurora A kinase inhibitors lead to apoptosis in human neuroblastoma cells

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Our previous findings showed that an anti-GD2 ganglioside mouse 14G2a monoclonal antibody combined with MK-5108 aurora A inhibitor significantly increased cytotoxicity against human neuroblastoma cells, as compared to monotherapy [1].

In this study we measured the level of ATP, apoptotic markers, and the activity of caspase 3/7 following the addition of an anti-GD2 chimeric ch14.18/CHO and two aurora A inhibitors (MK-5108 and MK-8745). ch14.18/CHO treatment of IMR-32 cells induced caspase 3 cleavage, which indicated the induction of apoptosis. Most importantly, the effects of the combination of ch14.18/CHO and aurora A kinase inhibitors were shown to enhance apoptosis in IMR-32 cells compared to when used individually [2]. These studies revealed the mechanism of neuroblastoma cell death induced by ch14.18/CHO and aurora A kinase inhibitors *in vitro*.

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Optimization of CW and Pulse EPR measurements of OxyChip probes to noninvasive measurements of tissue hypoxia

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Low oxygenation in tumors favors an aggressive tumor phenotype and adversely affects anticancer therapies. Measurements of the oxygen partial pressure (pO_2), especially the ability to study pO2 changes in real-time in defined cancer areas, could significantly impact clinical and preclinical research.

LiNc-BuO crystals (OxyChip), are a paramagnetic probe that enables noninvasive, fast, precise, and repeatable results. Molecular oxygen interaction with OxyChip changes the spin probe relaxation times, which is also reflected in changes in OxyChip EPR-signal linewidth. Our aim was to analyze how OxyChip relaxation times depend on oxygen concentration and to correlate them with the peak-to-peak line width of the EPR signal. The research was carried out by introducing Oxychip either into water or tumor tissue in the PDAC mouse model and measuring EPR signal both in CW and pulse mode.

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Analysis of EZH2 methyltransferase expression in lung cancer cells and its influence on the sensitivity of these cells to the antiproliferative activity of vitamin D

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After discovery of the anticancer properties of calcitriol, many studies were aimed at understanding the mechanism of this activity. Calcitriol has been shown indeed to have anti-proliferative activity against different types of cancer, but with different effectiveness [1].

The antiproliferative activity of calcitriol and EPZ6438 EZH2 inhibitor alone and in combination was tested in lung cancer cells. Next, the expression levels of EZH2, VDR and few other proteins was analyzed in these cells. Inhibition of EZH2 can improve antiproliferative activity of vitamin D in some types of lung cancer cells. Additionally, a higher VDR level may be one of the factors responsible for greater sensitivity of lung cancer cells to antiproliferative activity of calcitriol.

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In search for possible targets of therapy of neuroblastoma – cellular studies and bioinformatic analysis of clinical data

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Downregulation of *PHLDA1* (Pleckstrin homology-like domain, family A, member 1) gene is a predictor of poor prognosis in some types of cancer. Recently, we searched for proteins affected by silencing of *PHLDA1* in a human neuroblastoma cell line. Proteins isolated from *PHLDA1*-silenced IMR-32 cells and mock cells were subjected to mass spectrometry in order to reveal changes in the proteome. The protein showing the most spectacular increase was ABCB1 (ATP Binding Cassette Subfamily B Member 1) encoding an efflux pump. QRT-PCR and western blot followed by densitometric analyses were applied to confirm the increase. Additionally, a group of proteins that are down-regulated in *PHLDA1*-silenced cells and their expression negatively correlated with survival of neuroblastoma patients, was selected. The proteins that were found to be regulated in PHLDA1-silenced cells could be used as potential new targets in therapy of neuroblastoma.

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MCPIP1 protein function in HCC development

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Recent studies showed that MCPIP1 level decreases during the progression of breast cancer, neuroblastoma and clear cell renal cell carcinoma. A fall-off in MCPIP1 protein level leading to faster tumor growth and metastatic progression, but its role in the development of hepatocellular carcinoma (HCC) has not been described. In our study, after induction of HCC in mice lacking MCPIP1 protein in the liver, we observed larger and more numerous tumors. The results obtained so far indicate the initiation and progression of cancer according to different pathways for example β-catenin, STAT and NFκB. In addition, in mice lacking the MCPIP1 protein, we observed reduced infiltration of certain groups of immune cells.

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MCPIP1 protein in the treatment of hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is highly malignant and most common type of liver cancer. For patients with advanced HCC, the first line treatment is sorafenib, a multikinase inhibitor. However, after an initial period of improvement, most patients acquire a resistance to this drug followed by relapse. Our aim was to determine the role of MCPIP1 in HCC resistance to sorafenib.

We found a decrease in the level of MCPIP1 in HCC patients with tumor progression and lower level of MCPIP1 in tumor tissues compared to healthy tissue. Moreover, resistant HCC cell lines also expressed lower level of MCPIP1 than sorafenib sensitive cells. We found that 7 days stimulation with sorafenib leads to increased expression of c-Met, STAT3, ZEB2 and Vimentin. In addition we found a slight decrease in the level of MCPIP1 after sorafenib stimulation.

Our data indicates that MCPIP1 may affects the process of acquiring resistance to sorafenib treatment.

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mTOR-dependent expression and activation of TGFβ. Impact on proliferation, migration and chemokine expression in TSC2-deficient cells

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Limfangioleiomiomatosis (LAM) is a low-grade neoplasm characterized by the uncontrolled proliferation of TSC2-deficient cells within the lung parenchyma which causes damage to lung tissue and respiratory failure.

Here we show that TGFβ, a pleiotropic cytokine involved in many cellular processes, plays a vital role in the biology of LAM cells. We found that in LAM cells three isoforms of TGFβ are expressed. We also showed that LAM cells express proteins that tether TGFβ to the cell membrane and are involved in its activation. Although the inhibition of TGFβ did not affect the proliferation of LAM cells, it diminished their migration. It also affected the level of MCP-1 and IL-8 expression.

Together, our results indicate that TGFβ expressed and activated in LAM cells is involved in processes crucial for LAM progression and thus is an attractive potential target for LAM therapy.

Biodistribution of a gold nanoparticle-based construct in a mouse model of pancreatic ductal adenocarcinoma (PDAC)

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Nanoparticle conjugates are often associated with the improved efficacy of chemotherapeutic drugs due to enhanced stability, changes in biodistribution and pharmacokinetic profiles, and lower side effects. Properties such as size, shape, and surface charge determine their pharmacokinetics and biodistribution.

The study aimed to verify the biodistribution and pharmacokinetics of the gold nanorods constructs combined with gemcitabine in mice bearing orthotopic pancreatic cancer.

The nanoparticles were injected intravenously into mice with PDAC tumors, and their biodistribution in tumor and vital organs was compared. Photoacoustic imaging, computational tomography, and mass spectroscopy were used to determine construct concentrations.

Described results are essential to defining therapeutic effects and, as a result, toxicity against PDAC tumors.

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Downregulation of RIPK4 decreases the levels of ABCG2 but not ABCB1 and ABCC1 in melanoma cells

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Cisplatin, widely used in the chemotherapy of cancers, may have reduced effectiveness, because of the presence of multidrug resistance proteins (MRP). We have recently shown that downregulation of RIPK4, a member of the threonine-serine kinase group of proteins, increases the sensitivity of melanoma cells to cisplatin treatment. Since the mechanism of this process is unknown, we investigated whether downregulation of RIPK4 affects MRP levels in melanoma. For this purpose, the gene encoding RIPK4 was silenced in A375 and WM266.4 cells using the CRISPR/CAS9 method. NGS analysis showed changes in MRP expression levels between control and cells with downregulation of RIPK4. Western blot analysis shows that deletion of RIPK4 reduces the level of ABCG2 and does not affect ABCB1 and ABCC1.

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Redox homeostasis in the adaptation of glioblastoma cells to chemotherapeutic stress

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Chemoresistance of glioblastoma is one of the biggest challenges for the contemporary neurooncology. Redox balance is considered as an effector crucial for the maintenance of cellular homeostasis under chemotherapeutic stress. To trace this parameter in T98G cells undergone a pulse doxorubicin treatment, we combined the analyses of (i) mitochondrial ROS levels, (ii) GSH content and (iii) lipid oxidation status with (iv) the mass spectrometry (MS)-assisted analysis of redox homeostasis proteins. Our data show that chemotherapy-induced microevolution of glioma cells is strongly related to enhanced cellular antioxidant defense. Thus, the redox balance of glioma cells is crucial for their ability to survive under chemotherapeutic stress.

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L-Glutamine metabolism in glioblastoma homeostasis and chemotherapeutic stress response

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Metabolic plasticity of glioblastoma cells is crucial for their adaptation to microenvironmental constrictions. Metabolic reprogramming compensates an increased energetic demand under chemotherapeutic stress. It is often associated with the modulation of L-glutamine (Gln) metabolism that supports the oxidative metabolism in drug-stressed cells.

Here, we show that increased Gln bioavailability stimulates the proliferation and motile activity of T98G cells. Concomitantly, mass spectrometry (MS)-assisted analyses revealed profound effects of doxorubicin on Gln metabolism-related proteins. We interpret these observations in the context of literature data on the possible consequences of L-glutamine metabolism for T98G adaptation to doxorubicin-induced stress.

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Development of a Convolutional Neural Network (CNN) model for automated cell counting and classification in transmitted light microscopy images of cancer cell lines

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Automated assessment of cell culture state based on images of unstained cells will greatly improve the assessment of the effectiveness of anticancer drugs.

The goal is to develop a deep learning model to determine the number, positions, and state of non-stained cells in transmitted light microscopy images. A CNN model was created and trained on a prepared dataset of microscopy images of a mouse pancreatic cancer cell line, PAN_02. This type of deep learning neural network was chosen for its potential in automatically counting and classifying cells, as it can retain fine details in image segmentation [1][2]. The model was evaluated on a test dataset of images and showed promise in the accuracy of assessing cell areas and their state.

The application of this model should improve the evaluation of treatment efficacy on the culture cells model in cancer therapy research.

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ZC3H12B regulates the expression of matrix metalloproteinase 2 (MMP2)

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The ZC3H12/MCPIP family of proteins consists of 4 members, called ZC3H12A-D/MCPIP1-4. All of them have a highly conservative NYN/PIN domain and a CCCH zinc finger in their structure. All members of the family function as endonucleases that control the half-life of mRNAs and microRNAs. ZC3H12B is the most enigmatic member of this family since today there are only two studies about this protein. In our laboratory, we try to discover its secrets and understand its functions in cells.

In this study, we focused on the influence of investigated protein on the expression of metalloproteinase 2 (MMP2). Our results indicate that *MMP2* mRNA level is downregulated when ZC3H12B is overexpressed. Using gelatin zymography, we also showed a decrease in the activity of this metalloproteinase. Our study is an introduction to examining the effect of ZC3H12B on the invasiveness of cancer cells.

Knockout of PMEL17 potentially related to murine melanoma tumor development

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PMEL17 is the major component of the melanosome fibrils and participates in the polymerization of melanin, which makes it an important protein in melanogenesis [1]. We have checked how the lack of Pmel17 affects the growth and migration properties of B16F10 melanoma cells.

Knockout PMEL17 B16F10 cells were obtained by CRISPR/Cas9-mediated genome editing. The viability test did not show significant differences between the wild-type (WT) and knockout PMEL17 (KO) sublines. The cell cycle in PMEL17 KO cells is disrupted and there is a higher level of ROS than in WT cells. The PMEL17 KO cells show faster wound healing. PMEL17 KO tumors grew more quickly in chorioallantoic membrane model *in vivo*. Lowering the PMEL17 level and disturbing melanogenesis may increase the aggressiveness of B16F10 cells.

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Regnase-2 controls the expression of Regnase-1 and inhibits cell proliferation

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Regnase-2 (Reg-2/ZC3H12B/MCPIP2) has the most enigmatic physiological function among the proteins of the MCPIP family. It is highly expressed in the healthy brain, however, its expression is reduced during neuroinflammation and glioblastoma progression. At the same time, Regnase-1 expression is elevated. Overexpression of Reg-2 in glioblastoma cell lines is accompanied by the downregulation of Reg-1 in a NYN/PIN domain-dependent manner. Interestingly, low levels of Reg-2 and high levels of Reg-1 correlate with poor glioblastoma patients' prognoses. What's more, Reg-2 inhibits the proliferation of both human and mouse glioma cells. These data suggest that Reg-2 may play an important role in the regulation of brain homeostasis.

Simulating liver niche in uveal melanoma spheroids

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Uveal melanoma is a type of cancer that develops in the uvea of the eye. Despite the very effective management of primary UM in the eye, approximately 50% of patients develop metastases mainly in the liver. Moreover, within a year, 90% of metastatic patients die.

The purpose of the study is to simulate the liver niche environment by using spheroids containing three cell types: metastatic human uveal melanoma, human microvascular endothelial wells (HMEC-1) and hepatic stellate cells. This will allow to check the role of the metastatic tumor microenvironment cells in the growth of UM cells. We also aim to compare the migration of UM cells in normoxia and hypoxia using wound healing assay.

The results show that spheroids containing more than UM cells were more stable. Hypoxia was slowing down the wound healing. Mixed-cell type spheroids are a simplified, but efficient model to study tumor microenvironment.

Biological meaning of nitrosohemoglobin in tumors

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In search of factors related to tumor development the level of nitrosylhemoglobin (HbNO) may be considered. We compared the intensities of electron paramagnetic resonance (EPR) signals of various iron-nitrosyl complexes detectable in three different types of murine tumors. The results were analyzed in the context of tumor blood supply. Strong HbNO EPR signals were found in the tumor with a hemorrhagic necrosis, whereas strong signals of NO complexes with iron and DETC could be induced in poorly vascularized tumors. HbNO EPR signals emerge during active destruction of well-vascularized tumor tissue as a result of hemorrhagic necrotization which in turn facilitates NO generation in a positive feedback. EPR signals of various iron-nitrosyl complexes can defines the quality and quantity of complicated tumor-host interactions.

Heterogeneity of the uveal melanoma cell population derived from a single metastasis to the liver

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Tumor heterogeneity is one of the major problems in the effective treatment of cancer. The present work demonstrates the complexity and heterogeneity in an individual case of uveal melanoma (UM). Searching for new models of UM, we have established two cell lines of spindle and epithelioid character, derived from the same patient biopsy of a UM metastasis. The two lines have very distinct phenotypes, differing in morphology, proliferation, pigmentation. These features are retained in vivo in several model environments. Spindle UM cells can create heterogeneous experimental models, and reflect what is really going on in a tumor growing in situ.

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Tracking of human uveal melanoma metastases growth in animal model by photoacoustic imaging

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There is a need to develop and characterize an in vivo metastatic model of uveal melanoma to test new therapeutic strategies against distant metastases. To monitor of disseminated tumor progression it is necessary to use a rapid, sensitive and non-invasive technique.
Photoacoustic imaging (PAI), which combines optical excitation and ultrasound detection, can detect melanin in tissues with high resolution and sensitivity, also with high penetration depth.

The aim of this study was to find out how PAI will prove useful in the localization of pigmented human melanoma metastases in nude mice.
As it turned out, accurate positioning of melanoma metastases in distant tissues was achieved, demonstrating the applicability of PAI as a useful tool in preclinical research.

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Loss of myeloid Mcpip1 suppresses the development of squamous cell carcinoma of the skin

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Immune cells are key modulators in skin cancer. MCPIP1 is an RNase that acts as a negative regulator of inflammation. We showed that mice deficient of keratinocyte Mcpip1 developed chemically-induced squamous cell carcinoma (SCC) tumors faster and with more aggressive morphology.

Here, we investigated the role of myeloid Mcpip1 deficiency ($Mcpip1^{MKO}$) in the DMBA/TPA-induced skin carcinogenesis. We observed that none of the $Mcpip1^{MKO}$ mice developed SCC-like tumors, but all of them obtained multiple melanocytic nevi. Control mice developed SCC-like tumors, as expected. RNA-sequencing analysis revealed upregulated inflammation-related processes in the skin of $Mcpip1^{MKO}$, such as response to interferon-gamma and T cell mediated cytotoxicity. In contrary, cell cycle and Wnt-signaling pathways were downregulated.

In all, our results showed an unique role of myeloid Mcpip1 in skin cancer.

Developing a new model for testing the differential impact of HSPA1 and HSPA2 chaperone proteins on the phenotype of human bronchial epithelial cells

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The HSPA1 and HSPA2 proteins belong to the HSP70 family of molecular chaperones. HSPA1 is a cytoprotective protein encoded by stress-inducible genes, and HSPA2 is encoded by the stress-non-inducible gene expressed in certain tissues (e. g. bronchial epithelium). We have previously found that HSPA2 regulates keratinocytes differentiation. We hypothesize that the activity of HSPA2, but not HSPA1, is necessary for proper differentiation of the bronchial epithelium.

HSPA1 and HSPA2 genes were targeted by the CRISPR/Cas9 double nickase system in immortalized human bronchial epithelial cells. We established cell lines with the knockout of these genes. We examine how this affects the cell's growth, viability, sensitivity to stress. The cells will be used in the 3D Air-liquid Interface model to test how modifications affect cell's ability to form differentiated epithelium.

ACKNOWLEDGEMENTS
The study was financed by the National Science Center from the grant with a registration number: 2020/39/O/NZ4/02616

Combined chemotherapy with hyperthermia and calcitriol in immunocompetent and orthotopic pancreatic tumor mouse models

Olga Wiecheć-Cudak[1*], Aleksandra Murzyn[1*], Dariusz Szczygieł[1], Iga Łakoma[1], Mariia Oliinyk[1], Martyna Krzykawska-Serda[1]

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Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal and aggressive malignancy[1]. Satisfactory treatment still needs to be discovered, so new models and therapies are required. An appealing option seems to be the combination of gemcitabine (a standard chemotherapeutic) with mild hyperthermia (<42°C) and calcitriol (the active form of vitamin D3). *In vitro* studies showed the synergetic effects of combinatory treatment against pancreatic and breast cancer.

The aim was to optimize an orthotopic murine model of pancreatic cancer in mice (Pan_O2 in C57BL/6J mice) and determine the effectiveness of combined chemotherapy with gemcitabine and calcitriol with hyperthermia *in vivo*.

Combinatory therapy can lead to better anti-cancer effects. The new model showed >80% acceptability and presented to response to treatment. Each step of the combinatory treatment was successfully optimized.

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ACKNOWLEDGEMENTS

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Lack of RIPK4 impairs Wnt/β-catenin signaling in melanoma cells

Norbert Wroński[1], Ewelina Madej[1], Maja Grabacka[2], Agnieszka Wolnicka-Głubisz[1]

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Receptor-interacting protein kinase (RIPK4) targets multiple signaling pathways including Wnt/β-catenin. Although we have recently demonstrated the oncogenic nature of RIPK4 in melanoma, the effect of this kinase on the regulation of the Wnt pathway in melanoma is not well understood.

Since many studies indicate the involvement of WNT signaling in oncogenesis and melanoma drug resistance, in this study we determined the effect of genetic manipulation of RIPK4 in human malignant melanoma cells: A375 and WM266.4 on WNT pathway signal transduction using Western blot and TopFlash reporter system.

Our study showed that the expression of β-catenin and RIPK4 correlates in melanoma cell lines and knockout of RIPK4 reduces the expression of pGSK3β, Axin1 and β-catenin. In addition, we observed that RIPK4 impairs Wnt3a-induced activation of LPR5/6 and β-catenin.

ACKNOWLEDGEMENT
This research was funded by the National Science Centre, Poland, grant number UMO-2018/31/B/NZ5/01423.

The effect of calcitriol and tacalcitol treatment on OPN receptors expression and Th17/Treg cells subsets in various mammary gland cancer models

Honorata Zachary, Aleksandra Strzykalska, Beata Filip-Psurska, Mateusz Psurski, Joanna Wietrzyk

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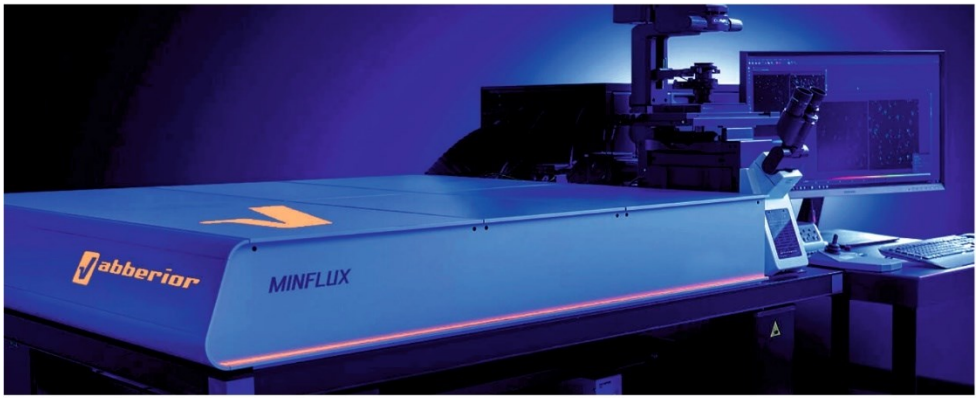
Vitamin D affects organisms physiology in many areas, among others hormonal and calcium balance not to mention immune system. Such a wide influence does not remain without impact on pathophysiology, especially tumorgenesis.

The aim of the following studies was to analyze the direct effect of vitamin D analogues on its nuclear receptors in Th17 cells and the consequent changes in IL-17 production. Additionally, it's interaction with osteopontin (OPN) was tested as the example of indirect influence on Th17 cells population in mouse mammary gland cancer models.



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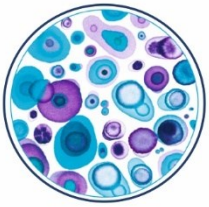

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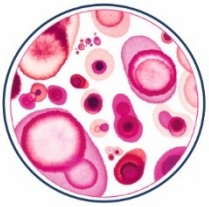

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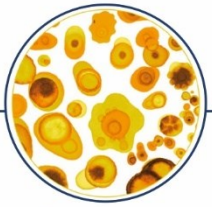

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Poster abstracts: progress in biochemistry, biophysics and biotechnology

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The effect of citrullination on the inflammatory potential of vimentin

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Vimentin has been considered for many years as an intracellular intermediate filament protein that plays a structural role in the cell. However, recent data indicate that vimentin can also occur on the cell surface and is secreted as extracellular protein. The rich source of vimentin are neutrophils extracellular traps (NETs). These structures play a bactericidal and immunomodulatory role. The aim of our project was to examine the role of vimentin anchored to the structure of NETs in the inflammatory response. We found that activity of vimentin strongly depends on post-translational modification, including citrullination, which impairs its anti-inflammatory potential.

ACKNOWLEDGEMENTS
Our study was financed by the National Science Center, Poland 2016/22/E/NZ6/00336

DNases as a virulence factor in *Streptococcus anginosus*

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Streptococcus anginosus is a Gramme-positive bacteria, which belongs to the *Streptococcus anginosus* group. *S. anginosus* has been classified as commensal bacteria of the colon, oral cavity, and vagina, however, nowadays it is considered as opportunistic pathogen leading to brain or liver abscesses. Despite the increased number of clinical reports, the molecular mechanisms of *S. anginosus* pathogenesis remain unknown. In the presented study, we examined the role of DNases expression by *S. anginosus* on the evasion of immune system. We focused on neutrophil extracellular traps finding their inactivation by bacterial nucleases. Obtained data revealed for the first time the pivotal role of DNAses as the virulence factors of *S. anginosus*.

ACKNOWLEDGEMENTS
Supported by National Science Centre, Poland 2018/29/B/NZ6/00624

The role of heme oxygenase 1 in the regulation of the cell cycle

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Heme oxygenase 1 (Hmox1) degrades free heme, decreasing hemolytic and oxidative stress. Our recent data show that Hmox1 protects cells also from replication stress. Thus, it could play a role in regulation of cell cycle. Our aim was to verify this supposition.

Experiments were done in iPS and HEK293T cells i) devoid of endogenous heme oxygenases, with or without constitutively active nuclear or cytoplasmic Hmox1 transgene ii) wild-type or Hmox1 deficient cells. In the absence of Hmox1, cell proliferation was decreased, and replication forks were more often stalled, possibly due to formation of G-quadruplexes. RNA-seq analyses identified cell cycle-related genes deregulated in Hmox1-deficient cells and sensitive to heme. Hmox1 affected also PARP1 interactome and activity. Our results suggest that Hmox1 regulates cell cycle by targeting p53 and PARP1-dependent pathways.

Immune Response by Gingipains from *Porphyromonas gingivalis*

Izabela Ciastoń[1], Joanna Budziaszek[1], Dorota Satała[2], Barbara Potempa[3], Andrew Fuchs[3], Maria Rąpała-Kozik[2], Danuta Mizgalska[1], Ewelina Dobosz[1], Richard J. Lamont[3], Jan Potempa[1,3], Joanna Kozieł[1]

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Gingipains are the key virulence factors of *Porphyromonas gingivalis*, significantly influencing functions of immune system. Efficient diffusion beyond the bacterial biofilm associated with an increased oxygen level, significantly reduces their proteolytic activity. Here, we show that proteolytically inactive RgpA is bound to TIGK cells in region of lipid rafts and interacts with EGFR inducing an expression of proinflammatory cytokines. This response was mediated via the EGFR-PI3K-AKT signaling pathway, which when activated in the gingival tissue rich in dendritic cells in the proximity of the alveolar bone, may contribute to bone resorption and progression of periodontitis. Collectively, our findings presented the new biological role of gingipains, acting as proinflammatory factors in the gingiva, creating a favorable milieu for growth of inflammophilic pathobionts.

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ACKNOWLEDGEMENTS
The study was funded by National Science Center, Poland grant UMO-2018/29/B/NZ6/ 01622 to J.K.

Kgp affects TLR3 signaling pathway leading to impairment of anti-viral response

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Periodontitis (PD) is a chronic inflammatory disease of the gingiva, with a high prevalence. Among pathogens, considered to play a crucial role in the development of PD, is *Porphyromonas gingivalis* (*P.g*.). However, viruses were also reported as etiological factors of periodontal disease, with an emphasis on the Herpesviridae family. Recent clinical data revealed the increased prevalence of viral infection of the oral cavity and lungs in PD patients. Our data indicated that proteolytic enzyme Kgp gingipain, which is virulence factor of *P.g.* efficiently modifies TLR3 signaling pathway affecting TRIF, TRAF3, TBK1 and IRF3 leading to impairment of antiviral response and improvement of HSV-1 proliferation. Obtained results broaden our basic knowledge about the mechanism of mixed infections and may serve as a premise, for considering PD as a gate of viral infection.

ACKNOWLEDGEMENTS
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Uncovering nanotoxicity of a water-soluble and red-fluorescent [70]fullerene nanomaterial

Dominik Dreszer[1], Grzegorz Szewczyk[2], Magdalena Szubka[3], Anna Maroń[1], Anna Urbisz[4], Karol Małota[4], Justyna Sznajder[4], Magdalena Rost-Roszkowska[4], Robert Musioł[1], Maciej Serda[1]

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Engineered fullerene materials have attracted the attention of researchers in the biomedical sciences, especially when their synthetic methodology is developed to endow them with significant levels of water-solubility and bioavailability. In this study, we characterized and synthesized a water-soluble fluorescent C70 nanomaterial. Biophysical measurements confirmed the formation of reactive oxygen species, namely singlet oxygen, with a weak signal for superoxide anion radicals in cells, while toxicological studies performed on Drosophila melanogaster showed that the formed C70 derivative had better bioavailability than pure C70. We believe that this work offers a new strategy for the synthesis of water-soluble and fluorescent fullerene nanomaterials with potential applications in bioimaging and photodynamic therapies.

Immortalized and primary adipose tissue-derived mesenchymal stem/stromal cells as a source of extracellular vesicles – comparative study

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Progressive senescence of primary mesenchymal stem/stromal cells (MSCs) is one of the major obstacles in harvesting sufficient amounts of their extracellular vesicles (EVs) |for therapeutic applications. Thus, immortalized MSCs (iMSCs) may be considered as an alternative source of EVs (MSCs-EVs) comparing to primary cells.

Therefore, in this study we compared selected biological properties of human adipose tissue primary MSCs and iMSCs. Our results showed higher proliferation and no evidence of senescence of iMSCs during prolonged culture, comparing to primary cells, which allows a long-term expansion required for abundant EV collection. Importantly, we demonstrated a significantly higher yeld of EVs from medium conditioned by iMSCs when compared to primary MSCs. Our results suggest that the immortalized MSCs may be a preferred EV source for further applications.

ACKNOWLEDGEMENTS
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Establishment of the organotypic model of gingiva to study bacterial and viral infections

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Recently, the use of three-dimensional (3D) tissue models is gaining popularity, perfectly bridging the gap between conventional two-dimensional (2D) cell cultures and testing in vivo. Gingival organotypic culture has shown its potential in the field of oral irritation, screening of oral care products, response to tobacco, drug testing, and most importantly infections with pathogens implicated in gum diseases. In our work we establish a normal stratified gingiva epithelium composed of TIGKs and immortalized human gingival fibroblasts-hTERT and use this model for infection with pathogens implicated in periodontitis – *Porphyromonas gingivalis* and Herpes simplex virus 1. The presented model will allow to investigate pathogen-host interactions, which will lead to the development of novel antibacterial and antiviral therapies.

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Matrix representations in biological sequence analysis

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Chaos game representation (CGR) has been successfully applied to bioinformatics for over 30 years. This transformation provides the recipe for the conversion of an amino or nucleotide sequence into a series of numbers. CGR provides a convenient way to characterise the sequence's composition. We analysed the usefulness of CGR in phylogenetic tree reconstruction and pattern searching. In the first case, we considered CGR and DFT combined and showed they provide reliable trees very quickly compared to traditional alignment-based methods. The second case study embraced the miRNAs energy analysis. We presented some interesting miRNA patterns using this matrix depending on the binding energy between these molecules and corresponding transcripts.

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The effect of FGF-2 on the TGF-β1-induced myofibroblastic transitions of human lung fibroblasts

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Subepithelial fibrosis is a part of airway remodeling observed in asthmatics in which human lung fibroblasts undergo phenotypic shifts into myofibroblasts (FMT). This phenotypic changes appears in response to inflammatory cytokines such as TGF-β1. Myofibroblasts are characterized by the enhanced expression of α-Smooth Muscle Actin (α-SMA) and collagens. Several studies have reported that fibroblast growth factor-2 (FGF-2) can inhibit FMT. The aim of this study was to examine the effect of FGF-2 on the TGF-β1-induced FMT of diseased human lung fibroblasts (DHLF). Non-cytotoxic concentrations of FGF-2 were determined by crystal violet and MTT assay. Our results indicate that FGF-2 decrease level of α-SMA and collagen1 in TGF-β1-treated DHLFs (in-cell ELISA). These results justify further studies on the FGF-2 involvement on FMT inhibition during pulmonary fibrosis.

Measuring adhesion between bone marrow mesenchymal stromal cells and extracellular matrix

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Mesenchymal stromal cells (MSC) create a specialized bone marrow (BM) niche and regulates hematopoiesis [1], [2]. While the interaction of MSC to extracellular matrix (ECM) is crucial for formation of BM niche, we measured adhesion of BM-MSC to ECM proteins: fibronectin and collagen IV.

We applied single-cell force spectroscopy [3] and established a method to precisely characterize adhesion between MSC and ECM.

We showed that the both force and work of MSC adhesion is significantly higher for fibronectin than collagen IV (534,2 pN vs 80,93 pN and $1{,}34*10^{-15}$ vs $5{,}99*10^{-17}$ J respectively). In presence of RGD peptide the mean adhesion force for fibronectin drop significantly from 534,2 pN to 71,2 pN, indicating that adhesion to fibronectin is dependent on integrins.

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Resequencing *Tannerella forsythia* strain ATCC 43037 using Nanopore technology

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Tannerella forsythia, a gram-negative bacteria, is a key player in the red complex of periodontal pathogens and is considered a primary cause of human periodontitis. However, the genome sequence of the ATCC 43037 *T. forsythia* strain had inaccuracies and incompleteness, leading to confusion with other strains. To address this issue, we decided to re-sequence the genome using Nanopore technology, a cost-effective and efficient method. The data obtained was then supplemented with Illumina technology, and compared with the latest published genome sequence[1]. Our efforts aim to eliminate inaccuracies and aid in the discovery of new therapeutic targets for *T. forsythia*-induced periodontitis. Further, this will also help to understand the pathogenesis of this bacteria, which will be crucial for the development of new treatments and preventions for periodontal disease.

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FLIM to sense all-protein concentration in DNA damage repair foci

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The role of molecular crowding in the DNA repair has been actively studied in recent years. It has been suggested that recruitment of repair proteins and a resulting high concentration of such proteins around DNA lesions creates protein-enriched crowded local micro-environment, which is required for repair processes to proceed. The currently available microscopy detection methods do not allow all types of the recruited proteins to be visualized at the same time. In this regard, FLIM based approach [1] offers an opportunity to use a fluorescent tag attached to one recruited repair protein as a probe for monitoring the concentration of all proteins in the repair focus.

Our data revealed that DNA damage induces dense packing of nuclear proteins in repair focus. Moreover, the 53BP1-EGFP lifetime distribution map revealed the internal architecture of individual DSB repair foci, with the most protein-dense center and a surrounding less dense region.

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Proteome profiling of mouse dystrophic diaphragm reveals decreased expression of H_2S-generating enzymes

Olga Mucha[1], Małgorzata Myszka[1], Paulina Podkalicka[1], Bianka Świderska[2], Agata Malinowska[2], Józef Dulak[1], Agnieszka Łoboda[1]

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Duchenne muscular dystrophy (DMD) is characterized by irreversible impairment of muscle functions with the diaphragm being affected earlier and more severely than other skeletal muscles.

The thorough tandem mass tag (TMT)-based proteomic analysis revealed 953 significantly changed proteins with 867 upregulated and 86 downregulated in the diaphragm of *mdx* mouse DMD model. Consequently, several dysregulated processes were demonstrated, including the immune response, fibrosis, translation, and programmed cell death. Interestingly, in the dystrophic diaphragm we found a significant decrease in the expression of enzymes generating H_2S suggesting that this gaseous mediator might be an important regulator of DMD progression.

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Characterization of a new secretion system in *Porphyromonas gingivalis*

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Porphyromonas gingivalis is a pathogenic bacterium involved in the development of periodontitis, an inflammatory disease of tooth-supporting tissues. It produces various virulence factors, including fimbriae – long fibers extending from the outer membrane [1], composed of subunits secreted to the OM as lipoproteins [2] via a thus far undescribed mechanism. Here, we identified and characterized proteins involved in lipoprotein secretion in *P. gingivalis*. To this aim, we prepared a series of deletion mutants of putative proteins of the secretion system and determined their importance for fimbriae formation by Western blotting. We also purified complexes of those proteins which co-purified with various surface lipoproteins as confirmed by mass spectrometry. Finally, using cryo-EM, we obtained preliminary structural data showing molecular architecture of the complex.

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Light-induced toxicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list

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The process of combustion of fossil fuels is always incomplete, resulting in the production of polycyclic aromatic hydrocarbons (PAHs), widely spread mutagenic and tumorigenic environmental contaminants. Human contact with PAHs is inevitable and occurs mainly by skin absorption, respiration, or food consumption. Due to multiple aromatic ring systems, PAHs can absorb light in the UVA and visible range, resulting in the formation of reactive species. Although PAHs have been previously reported to be more toxic when exposed to light, the photoreactive properties of many of these compounds have not yet been studied[1]. Here, light-induced photoproduction of free radicals and singlet oxygen was analyzed for 16 PAHs included in the US EPA priority pollutants list. Phototoxicity of selected PAHs induced by solar-simulated light was examined using HaCaT cells as an *in vitro* skin model.

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DYRK1A inhibitors as potential therapeutics in treatment of diabetes

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Diabetes is characterized by the progressive destruction of pancreaticβ-cells, which leads to insulin deficiency, hyperglycemia, and metabolic collapse. Recent studies identify DYRK1A kinase as one of the best targets for therapies aimed at stimulating pancreaticβ-cells regeneration1,2.Here, we present our set of DYRK1A small-molecules inhibitors that potentially enhance proliferation and insulin secretion in MIN6 and INS-1E model cells. To evaluate the efficiency of our compounds, we used MTT assay to determine cytotoxicity and flow cytometry as well as Ki67 staining to show inhibitors-induced increase incell proliferation and insulin secretion compared to control, which lends a considerable promise for novel diabetes treatment.

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Hydrogen sulfide exerts protective effects in the mouse model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is an incurable genetic disorder leading to muscle degeneration and premature death. We checked the therapeutic properties of intraperitoneal administration of hydrogen sulfide donor, sodium hydrosulfide (NaHS), on the state of the skeletal muscles in the *mdx* mouse model of DMD.

NaHS reduced oxidative stress and inflammation through modulation of the GSH/GSSG ratio, increase in the level of cytoprotective heme oxygenase-1 (HO-1), and down-regulation of the NF-κB pathway. Furthermore, we showed a decrease in DMD biomarkers as well as pro-angiogenic and anti-fibrotic properties of NaHS. However, NaHS treatment did not improve muscle strength of *mdx* animals. Overall, NaHS exerted cytoprotective effects in a mouse model of DMD.

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Slow-releasing hydrogen sulfide donors GYY4137 and AP39 attenuate dystrophic phenotype in a murine model

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Duchenne muscular dystrophy (DMD) is a severe type of muscular dystrophy. We previously demonstrated the cytoprotective properties of the fast releasing hydrogen sulfide donor, NaHS, on DMD progression; therefore, in the present study we investigated the effect of new slow-releasing donors: GYY4137 and AP39.

In vivo study on the *mdx* mouse model of DMD showed improved exercise capacity and muscle strength of the treated animals followed by decreased inflammation and muscle degeneration as assessed by histological analyses of the gastrocnemius muscle. Moreover, new donors reduced oxidative stress by up-regulating antioxidant proteins and CTH, the H_2S generating enzyme. Furthermore, we showed autophagy regulation by affecting the AMPK level. These promising findings revealed the potential of new H_2S donors in attenuating the dystrophic phenotype.

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This work was supported by grant #2019/35/B/NZ3/02817 (to AŁ) from the National Science Centre.

The impact of graphene-based substrates on human mesenchymal stem cells potential in tissue repair – *in vitro* and *in vivo* studies

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The main goal of this study was to investigate the potential of graphene oxide (GO) and reduced graphene oxide (rGO) substrates to promote angiogenic differentiation of human mesenchymal stem cells (MSCs). The impact of various GO and rGO scaffolds on biological properties of umbilical cord MSCs such as viability, proliferation and adhesion were investigated. The most biocompatible graphene-based substrates were used to differentiate MSCs in to endothelial cells *in vitro*. Gene expression and capillary tube formation were analysed. Moreover, angiogenic potential of MSCs cultured on rGO scaffold was evaluated in murine model of limb ischemia *in vivo*. The results showed that selected rGO surfaces may promote angiogenic differentiation of MSCs both *in vitro* and *in vivo*. Thus, the graphene surfaces may be used for enhancing MSC angiogenic activity prior their applications.

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This study was funded by Maestro 11 NCN grant: 2019/34/A/NZ3/00134, Symfonia 3 NCN grant: UMO2015/16/W/NZ4/00071, and STRATEGMED3 NCBR grant: STRATEGMED3/303570/7/NCBR/2017.

Heme in preimplantation embryo – one ring to rule them all?

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Recently, it has become more apparent that cellular metabolism and its regulation may be a deciding factor governing proper preimplantation embryonic development and oocyte maturation. Changes in cellular metabolism are precisely timed and depend on the embryo development stage. Heme is crucial for healthy mitochondria, but its synthesis consumes glycine and succinyl-CoA. Although levels of labile heme in oocytes and embryos may play an essential role in their biology, the role of heme in developmental biology has been largely neglected so far.

Our data show that δ-aminolevulinate (δ-ALA), a heme precursor, stimulates the maturation of mouse oocytes but inhibits preimplantation embryonic development. Mouse embryos cultured with 350 µmol/L δ-ALA are photosensitized and show inhibited cleavages when kept in the dark. The effects of δ-ALA on embryo development are reversed with succinylacetone, which inhibits δ-aminolevulinate dehydrogenase. Preimplantation embryos can take up hemin and N-methylmesoporphiryn and up-regulate *Hmox1* in response to hemin or δ-ALA. Finally, stimulation with heme accelerates embryonic development. Based on our data and available literature, we hypothesize that labile heme is a significant regulator of oocyte and embryonic metabolism and the embryonic cell cycle.

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Functional characterization of iPSC-derived endothelial cells as an *in vitro* model for studying myocardial tissue repair

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Human induced pluripotent stem cells (hiPSCs) are an important source of cells for disease modeling, including cardiac ischemia.
As endothelialcells (ECs) play a pivotal role in heart repair, hiPSC-derived ECs (hiPSC-ECs) are important model candidates mimicking ECs present in the heart. However, the functional response of hiPSC-ECs may potentially vary from primary ECs. Thus, in this study, we compared biological characteristics of hiPSC-EC sand primary cardiac ECs *in vitro*. Our results indicate similar phenotype, but differentia properties of both cell types including their response to cytokine stimulation.

This study may contribute to the development of optimal ECs models for studying mechanisms accompanying myocardial tissue repair.

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N4BP1 is a novel component of P-bodies

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Nedd4 Binding Protein 1 (N4BP1) is a ribonuclease originally found in cell nucleus. It is proved that N4BP1 is an important factor in modulation of immunological response as well as signal transduction to NFκB factor and degradation of viral mRNA. The aim of this research was to validate the interactions between N4BP1 protein and its binding partners identified previously by mass spectrometry analysis. Western blot experiments confirmed our mass spectrometry results showing interactions between N4BP1 and several key components of P-Bodies. Furthermore all of these components co-localize with N4BP1 in N4BP1-GFP expressing cells after immunofluorescence staining. In conclusion, our results prove that N4BP1 is a novel ribonuclease in P-Bodies.

Clozapine regulates cholesterol metabolism at the level of cell nucleus

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30% of schizophrenia patients are resistant to treatment. Clozapine (CLO) is the only drug registered specifically for treatment-resistant schizophrenia and it is considered the most efficacious antipsychotic drug (APD), but it usage is limited due to severe side effects. Huge efforts have been made to develop a safer drug with similar efficacy but so far all attempts have failed. The mechanism of molecular action of CLO is unclear. An unbiased proteomic study of a set of more than 7,000 proteins of human neurons and astrocytes succeeded in determining the changes exerted by CLO and risperidone (one of the most commonly prescribed APDs). CLO alters the level of many enzymes and regulatory nuclear proteins involved in cholesterol metabolism.

Deletion of netrin-1 in endothelial cells decreases survival after hematopoietic stem cells transplantation and impairs hematopoiesis during aging

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Hematopoietic stem cells (HSCs) produce all blood cells throughout the lifetime, but the regenerative potential of HSCs during aging declines. For proper function HSCs require specialized bone marrow (BM) niche formed mainly by endothelial cells (ECs). However, the role of the BM niche in this phenomenon is still poorly understood.

We identified netrin-1 as a potential factor that mediates the interaction between HSCs and the niche and may regulate aging of HSCs. We used mice with conditional deletion of Ntn-1 in ECs and demonstrated that deletion of Ntn-1 in ECs disturbs the balance between myeloid and lymphoid lineage during aging and decreases survival in mice subjected to irradiation and HSCs transplantation. Our results indicate that netrin-1 regulates HSCs' function both at steady state and after transplantation.

Mobilization of cells from the bone marrow to blood using cobalt protoporphyrin IX

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Treatment with cobalt protoporphyrin IX (CoPP) increases endogenous granulocyte colony-stimulating factor (G-CSF) and induces the mobilization of granulocytes and hematopoietic stem cells (HSC) from the bone marrow to the blood. Our aim was to characterize the phenotype and clinically relevant functional properties of the cells mobilized by CoPP and compare them to the cells mobilized by recombinant G-CSF. Using flow cytometry, we observed different kinetics of G-CSF- and CoPP-induced granulocyte mobilization. CoPP mobilized more HSC than recombinant G-CSF. Moreover, we showed the effective mobilization in two immunodeficient mouse strains. CoPP could be a potential mobilizing agent for the treatment of blood disorders.

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Anhydrotetracycline as an inducer in the *Porphyromonas gingivalis* inducible gene expression system

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Porphyromonas gingivalis (*Pg*) a dysbiotic component of the oral microflora is an etiological factor of chronic periodontitis. Because of asaccharolytic metabolism, it is impossible to use conventional glucose-based induction systems to investigate the biological role of essential genes. Therefore, we employed the inducible Tet operon with anhydrotetracycline (aTC), as molecule inducing gene expression. As the proof-of-concept, we engineered a *Pg* strain with the aTC-inducible expression of Kgp gingipain by inserting of the aTC promoter and the TetR repressor protein-coding sequence into the genome upstream of the *kgp* gene. The obtained strain DM105 was characterized, and this novel inducible expression system was optimized. The generated molecular tool can be used to study the function of other *Pg* genes and search for new therapeutic targets.

Computational pipeline to identify genes of interest in unannotated genomes based on the domain architecture

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Despite a large amount of available genomic assemblies, only a small fraction have gene annotations given the difficulty and cost of obtaining them. This lack of annotated genomes makes it difficult to study the evolution of gene families across species. To overcome this limitation, we develop a bioinformatics pipeline to scan genomes for given protein domain combination that are unique to our gene family of interest.

Given two or more protein domains that are unique to our gene family of interest and a set of unannotated genomes, our pipeline can identify how many genes belonging to the gene family are present in each genome. We tested our pipeline with the identification of Argonaute genes, which have known domain architecture in insect genomes. Results of the tests determined that the pipeline is not only able to identify known Argonaute genes, but to discover novel ones.

Periodontic bacterium *Fusobacterium nucleatum* amplifies interferon-γ-induced activation of gingival fibroblasts during infection

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Fusobacterium nucleatum (*Fn*), one of the bacteria responsible for periodontitis, plays an important part in overstimulation of the immune system. Gingival fibroblasts (GFs) aid the immune response by producing inflammatory mediators after exposure to bacterial cells, products, |and virulence factors, as well as cytokines produced by other cells – including interferon-γ (IFN-γ).

Here, we show the impact of Fn on inflammatory activation of GFs stimulated with IFN-γ. GF stimulation with IFN-γ in presence of *Fn* has shown amplified STAT1/3/5 phosphorylation, and expression of CXCL9, CXCL10, CXCL11 and CCL20. CXCL9/10 production was amplified when stimulated with IFN-γ/*Fn* or its lipopolysaccharide. The amplification of IFN-γ-induced GF responses by Fn was partly dependent on NFκB and MAP kinase signaling pathways and required protein secretion, suggesting an autocrine mechanism.

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High resolution cryo-EM structures of cytochrome b_6f imply a one-way traffic of quinones for efficient photosynthesis

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The oxidation of plastoquinol and reduction of plastocyanin (PC) by cytochrome b_6f (cytb_6f) is one of the key steps of photosynthesis. Still the details of catalytic mechanism of cytb_6f remain elusive[1]. Here we show two high resolution cryo-EM structures of spinach cytb_6f (at 2.1 Å and 2.7 Å) with endogenous PQs and in complex with PC[2]. The structures revealed that three PQs line up one after another head-to-tail near the oxidation site suggesting existence of a channel which allows for continuous flow of PQs through the protein. We thus propose a one-way traffic model to explain efficient PQH_2 oxidation in photosynthesis.

In addition, our structures reveal thylakoid soluble phosphoprotein (TSP9) as a novel partner binding to cytb_6f.

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Results of pharmacological treatment of mice that develop primary biliary cholangitis due to the lack of the Mcpip1 protein in the liver

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Primary biliary cholangitis (PBC) is a chronic autoimmune disease of the liver. Progression of PBC leads to the development of fibrosis, cholestasis, and cirrhosis. We found, that Mcpip1fl/flAlbCre mice, which are characterized by a deletion of the Zc3h12a gene (encoding the Mcpip1 protein) in liver cells, develop a number of typical PBC symptoms. Mcpip1fl/flAlbCre knockout mice and Mcpip1fl/fl control mice at 6 weeks of age were randomized into five groups for drug treatment (control, UDCA, Lakcid, UDCA+Lakcid, UDCA+OCA). After 6 weeks of treatment, the mice were sacrificed and the collected material analysed. Mcpip1fl/flAlbCre treated with Lakcid had reduced amount of total bile acids in the serum and decreased proliferation of cholangiocytes. That is why, they were selected for further analysis (next generation sequencing, mass spectrometry). We hope, that analysis of Mcpip1fl/flAlbCre may shed new light on the pathology of PBC development.

Anticancer fullerenes in callus culture of medical plant *Lilium martagon* L.

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The effects of [60]fullerenes are studied in cancer human therapies [1]. However, little is known about fullerenes impact on plant tissue. *Lilium martagon* L. callus was cultured in standard conditions [2] and in the presence of fullerenes (F19 and F33).

These nanoparticles did not show a toxic effect on lily callus culture and photosynthetic activities were not detected. The highest relative increase in calli weight was noted after two weeks and the differences between control, and F33 and between F19 and F33 were statistically significant, in favor of F33. Histological observations showed higher content of starch, wall polysaccharides, protein granules in fullerenes treated callus. Both fullerenes decreased protein content and have no significant effect on carotenoids concentration in callus of lily culture.

Our results point to application potential of fullerenes.

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MCPIP1 is a regulator of IFNγ signalling

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MCPIP1 is a potent regulator of TLR4 and IFN type I signalling pathways (1, 2). We aimed to investigate its role in IFN type II signalling pathway. Using myeloid specific k/o of MCPIP1(LysMcre+) we found it as a regulator of STAT1 mediated pathway. Deficiency of MCPIP1 leads to upregulation of STAT1 and expression of genes dependent on this transcription factor. Our observation could explain the symptoms of extramedullary hematopoiesis in MCPIP1 deficient mice.

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