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Anticancer effect of AZD2461 PARP inhibitor against colon cancer cells carrying wt or dysfunctional p53

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ABSTRACT

Colon cancer is one of the most common cancers, currently treated with traditional chemotherapies or alternative therapies. However, these treatments are still not enough effective and induce several side effects, so that the search of new therapeutic strategies is needed. The use of Poly-(ADP-ribose)-polymerase (PARP) inhibitors, although originally approved against BRCA-1 or BRCA-2 mutated cancers, has been extended, particularly in combination with other treatments, to cure cancers that do not display defects in DNA repair signaling pathways. The role of p53 oncosuppressor in the regulating the outcome of PARP inhibitor treatment remains an open issue. In this study, we addressed this topic by using a well-tolerated PARP 1/2/3 inhibitor, namely AZD2461, against colon cancer cell lines with different p53 status. We found that AZD2461 reduced cell proliferation in wtp53 and p53−/− cancer cells by increasing ROS and DNA damage, while R273H mutant (mut) p53 counteracted these effects. Moreover, AZD2461 improved the reduction of cell proliferation by low dose radiation (IR) in wtp53 cancer cells, in which a down-regulation of BRCA-1 occurred. AZD2461 did not affect cell proliferation of mutp53 colon cancer cells also in combination with low dose radiation, suggesting that only wt p53 or p53 null colon cancer cells could benefit AZD2461 treatment.

1. Introduction

Colon cancer represents the 4th most common human malignancy and its incidence is in continuous growth in industrialized countries, in which the population is rather old and the lifestyle is characterized by high fat diet and little exercise. Surgery remains the best option to treat this cancer. However, if disease is no longer localized at the site of origin, chemotherapies and radiotherapy become necessary. Despite the efforts so far made to improve the treatment of this cancer, its prognosis in the advanced state remains poor and therefore the search for new and more efficacious therapeutic strategies is needed [1].

Poly-(ADP-ribose)-polymerase (PARP) is a proteins family that mediates the repair of DNA single-strand breaks and contributes to non-homologous end joining (NHJR) and homologous recombination (HR) that repair DNA double-strand brakes. Following DNA damage, PARPs induce the PARylation of histones and several molecules involved in DNA repair including itself, leading to chromatin de-condensation that allows the access to the enzymes devolved to repair DNA damage [2].

Besides this, PARPs regulate several processes such as cell differentiation, autophagy and inflammation, reason why the interest towards this molecule is constantly growing [3]. Several inhibitors of PARPs have been developed and successfully used against cancers, so that many of them have been introduced in clinical trials. However, the best outcome has been obtained against cancers displaying mutations in BRCA-1 or BRCA-2, genes involved in HR, or with defects in other DDR (DNA damage response) genes [4]. More recently, it is emerging that PARP inhibitors may be successfully used in combination with drugs able to alter the expression or function of DDR molecules or with DNA

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damage agents [5], thus extending their use to the treatment of cancers that do not carry mutations in DNA repair genes. One debated issue is whether PARP inhibitors can be used to treat cancers with different p53 status, as single agents or in combination of DNA damaging agents. Interestingly, wtp53 as BRCA-1, is phosphorylated by ATM, kinase activated in response to DNA double strand brakes and involved in HR [6,7]. Previous studies have shown that the activation of wtp53 following exposure to DNA damaging agents may have an inhibitory effect on BRCA-1, which may render cancers carrying wtp53 more sensitive to PARP inhibitors compare to those p53-defective [8,9].

Regarding mutp53, there are different reports on the use of PARP inhibitors showing opposite results. It has been shown that mutp53 cancer cells display a similar sensitivity to those carrying wtp53 to the treatment with PARP inhibitors in combination with DNA damaging agents [10]. Other studies have reported that mutp53 cancers are even more susceptible to PARP inhibitors in combination with ionizing radiation, due to the induction of a higher ROS increase [11]. The effects PARP inhibitors against colon cancer cells lacking 53 or carrying wt or mutp53 remain even less clarified [12]. In this regard, in this study, we used AZD2461, a new discovered PARP-1,2,3 inhibitor displaying high anti-cancer efficacy and a lower toxicity compared to most used PARP inhibitor Olaparib [13], to treat colon cancer cells with different p53 status, as single agent or in combination with low dose radiation. The outcome on cell proliferation and the underlying molecular mechanisms were assessed.

2. Materials and methods

2.1. Cell cultures, treatments and irradiation

HCT116 (human colon cancer cell line, wtp53) and HCT116 p53−/− (human colon cancer cell line, p53 K/O) were a kind gift from B. Vogelstein, (Johns Hopkins University, Baltimore, MD).

While HT-29 (human colon cancer cell line, carrying R273H mutp53) were kindly provided by N Merendino (Tuscia University, Viterbo Italy). Cells were maintained in DMEM 1640 (Thermo Fisher Scientific) supplemented with 10% Fetal Bovine Serum (FBS) (Corning), L-glutamine, streptomycin (100 μg/ml) (Corning), and penicillin (100 U/ml) (Corning) (complete medium) in 5% CO_2 at 37 °C. Cells were always detached using Trypsin-EDTA solution (Biological Industries, Cromwell, CT, USA). Cells were plated in 6-well plates at a density of 2 × 10^5 cells/well in 2 ml and, the day after, treated with AZD2461 (30 μM) (Sigma Aldrich) for 24 h. In some experiment cells were plated in 6-well plates as above reported and the day after pre-treated with AZD2461 (30 μM) (Sigma Aldrich) for 5 h, then irradiated with 1 Grey (1Gy) radiation and cultured for the next 24 h. Untreated cells were used as control. Irradiation was carried out using an ONCOR Impression Linear Accelerator (Siemens Medical Solutions USA, Inc, Concord, CA) at a dose rate of 1 Gy (95 UM/min).

2.2. Mutp53 transfection

HCT116 p53−/− were plated in 6-well plates at a density of 2 × 10^5 cells/well in 2 ml and, the day after, transfected with empty vector or pcDNA3-p53R273H vector for mutant p53 expression, by using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. After 24 h of transfection, cells were pre-treated with AZD2461 (30 μM) (Sigma Aldrich) for 5 h, then irradiated with 1 Grey (1Gy) radiation and cultured for the next 24 h. Untreated cells were used as control.

2.3. Cell proliferation

Cell proliferation was evaluated by MTT assay (Sigma Aldrich). 5 × 10^3 cells/well were plated in 96-well plates in 100 μL of complete medium. The day after, cells were treated with different doses of AZD2461 (30 μM, 60 μM, 120 μM and 240 μM) for 72 h. In some experiments 5 × 10^3 were plated in 96-well plates in 100 μL of complete medium and transfected as previously described or left un-transfected. After 24 h cells were pre-treated with AZD2461 (30 μM) (Sigma Aldrich) for 5 h, then irradiated with 1 Grey (1Gy) radiation and cultured for the next 72 h. Untreated cells were used as control. MTT assay was performed following manufacturer's instruction. The plates were analyzed by VICTOR Multilabel Plate Reader (PerkinElmer). The experiments were performed in triplicate and repeated three times.

2.4. Cell cycle analysis

For cell cycle analysis, the DNA content of untreated or treated HCT116 wtp53, HCT116 p53−/− and HT-29 R273H mutp53 cell lines, was measured by Propidium Iodide (PI, Sigma Aldrich) staining and FACS analysis. After 24 and 60 h, cell lines treated as above reported, were washed with cold 1 × PBS and fixed in 70% ethanol on ice for at least 1 h. After centrifugation, cell pellet was washed with cold 1 × PBS, stained with 50 μg/ml PI and RNase for 15 min at 37 °C and then analyzed by FACSCalibur (BD Biosciences). Data are representative of at least three independent experiments.

2.5. Indirect immunofluorescence assay (IFA)

To evaluate γH2AX foci formation, HCT116 wtp53, HCT 116 p53−/− and HT-29 mutp53 cells were grown on slides, washed with PBS and air dried. Cells were then incubated with 2% paraformaldehyde (Electron Microscopy Science) for 30 min and permeabilized with 0.1% Triton X-100 (Sigma Aldrich) for 5 min. After 3 washes, cells were incubated with 1% glycine, 3% BSA for a further 30 min. Then cells were incubated with the primary monoclonal antibody against p-H2AX (Ser 139) (1:100) (Santa Cruz Biotechnology Inc., sc-517348) for 1 h at room temperature. Slides were then washed 3 times with PBS and cells were further incubated with a polyclonal conjugated-Cy3 sheep anti-mouse antibody (1:2000) (Jackson ImmunoResearch) for 30 min at room temperature. After 3 washes in PBS, cells were stained with DAPI (1:5000) (SIGMA) for 1 min at room temperature. Slides were further washed in PBS, mounted with glycerol:PBS (1:1) and analyzed with an Apotome Axio Observer Z1 inverted microscope (Zeiss) equipped with an AxioCam MRM Rev.3 at 40 magnification. Foci amount has been counted by Image J software.

2.6. Measurement of intracellular reactive oxygen species production

ROS were measured according to previous studies [14]. Briefly to measure reactive oxygen species production, 2,7-dichlorofluorescein diacetate (DCFDA; Sigma-Aldrich) 10 μM was added to cell cultures for 15 min and live cells, gated according to their forward scatter (FSC) and side scatter (SSC) properties, were analyzed by FACScalibur flow cytometer (BD Transduction Laboratories), using CELLQuest Pro software (version 6.0, BD Biosciences). For each analysis 10000 events were recorded.

2.7. Western blot analysis

Following transfections and treatments, cells were washed in 1X PBS, lysed in RIPA buffer (150 mM NaCl, 1% NP-40, 50 mM Tris-HCl (pH 8), 0.5% deoxycholic acid, 0.1% SDS, protease and phosphatase inhibitors) and centrifuged at 14000 rpm for 20 min at 4 °C. The protein concentration was measured by using the Bio-Rad Protein Assay (BIO-RAD laboratories GmbH) and 15 μg of protein was subjected to electrophoresis on 4–12% NuPage Bis-Tris gels (Life Technologies) according to the manufacturer's instruction. The gels were transferred to nitrocellulose membranes (Biorad, Hercules) for 2 h in Tris-Glycine buffer and the membranes were blocked in 1 X PBS-0.1% Tween20 solution containing 3% of BSA (Serva), probed with specific antibodies and developed using

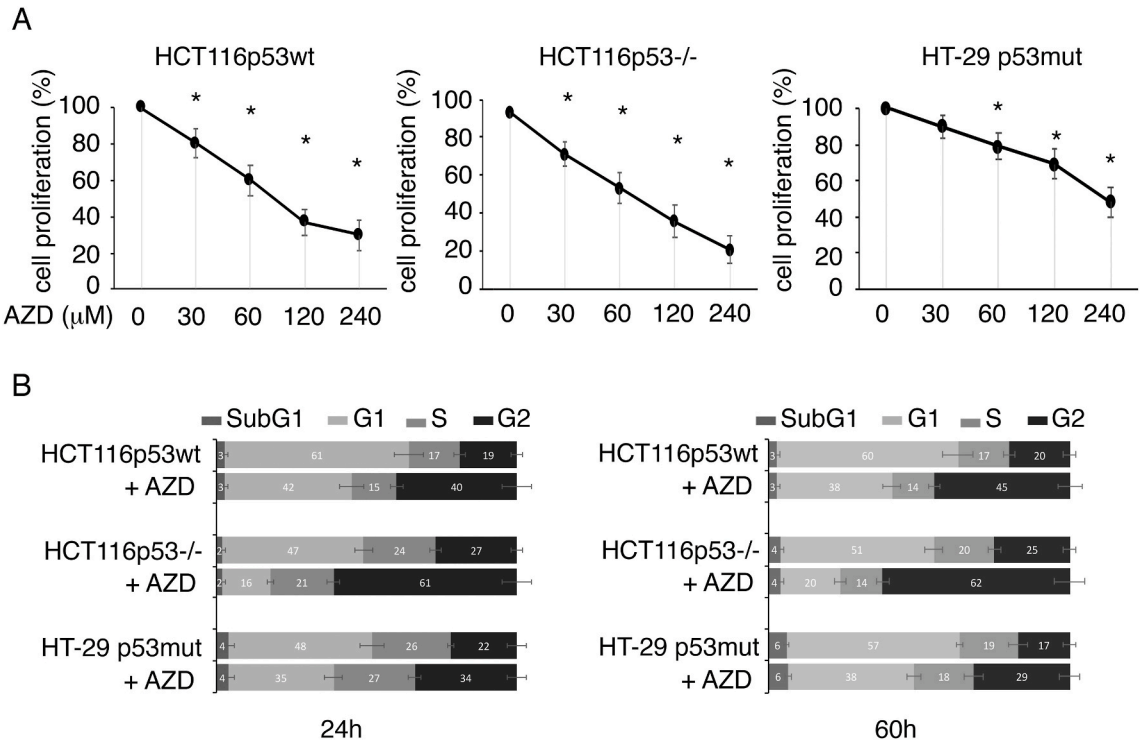

Fig. 1. Dose-response inhibition of proliferation and G2 cell cycle arrest in AZD2461-treated wtp53, p53−/− HCT116 and HT-29 colon cancer cells. (A) Cell proliferation following treatment with different doses of AZD2461 (AZD) (30, 60, 120 and 240 μM) or control, was measured by MTT assay after 72 h; *p-value < 0.05. (B) Cell cycle analysis of AZD (30 μM) treated and untreated HCT116 wtp53 HCT116 p53−/− and HT-29 cells as evaluated after 24 and 60 h of treatment by FACS analysis, following staining with PI. One representative experiment out of three e is shown. The bars represent the mean of the percentage of cells in each phase of cell cycle (subG 1, G1, S and G2) plus S.D of three experiments.

ECL Blotting Substrate (Advansta).

2.8. Antibodies

To evaluate protein expression on Western blot membranes the following antibody were used: mouse monoclonal *anti*-pH2AX (Ser 139) (1:100) (Santa Cruz Biotechnology Inc., sc-517348), mouse monoclonal *anti*-BRCA1 (1:500) (EMD Millipore, OP92), mouse monoclonal *anti*-p53 (1:100) (clone DO-1, Santa Cruz Biotechnology Inc., sc-126) and rabbit polyclonal *anti*-p21 (1:200) (clone C-19, Santa Cruz Biotechnology Inc., sc-397). Mouse monoclonal *anti*-β-actin (1:10000) (Sigma Aldrich) was used as loading control. The goat anti-mouse IgG-HRP (1:30000) (Bethyl Laboratories, A90-116P), goat anti-rabbit IgG-HRP (1:30000) (Bethyl Laboratories, A120-101P) were used as secondary antibodies. All the primary and secondary antibodies were diluted in PBS-0.1% Tween20 solution containing 3% of BSA (SERVA).

2.9. Colony forming assays

After 24 h that HCT116 wtp53, HCT116 p53−/− and HT-29 cells were pre-treated with AZD2461 for 5 h and irradiated with 1 Grey (1 Gy) radiation, cells were detached and plated at low density in 60 mm Petri dishes and grown for twelve days. Surviving colonies were fixed and stained with Cristal Violet (0.5% in methanol) (Sigma-Aldrich), air-dried, and analyzed with Image J. Colony formation capacity in AZD- and/or IR-treated cells was calculated in comparison to untreated control samples, arbitrarily set to 100. The results were plotted as means ± SD of three separate experiments.

2.10. Densitometric analysis

The quantification of proteins bands was performed by densitometric analysis using the Image J software (1.47 version, NIH, Bethesda, MD, USA), which was downloaded from NIH website (http://imagej.nih.gov).

2.11. Statistical analysis

Results are represented by the mean ± standard deviation (SD) of at least three independent experiments and a two-tailed Student's t-test was used to demonstrate statistical significance. Difference was considered as statistically significant when p-value was at least<0.05.

3. Results

3.1. Mutp53 prevents G2 cell cycle arrest induction by AZD2461 in colon cancer cells

We investigated the effect of PARP1/2/3 inhibitor AZD2461 on cell proliferation of colon cancer cells with different p53 status. At this aim, HCT116-wtp53, HCT116-p53−/− and HT-29 (carrying R273H mutp53) were treated with different doses of AZD2461 and cell proliferation was evaluated by MTT assay, after 72 h of treatment. The results shown in Fig. 1A indicate that AZD2461 (AZD) reduced cell proliferation in a dose-dependent fashion in HCT116 wtp53 and p53 −/− while the presence of mutp53 inhibited this effect in HT-29 cells. These findings were corroborated by FACS analysis that, as shown in Fig. 1B, indicated that AZD2461 induced a G2 arrest in HCT116 wtp53 and HCT116 p53−/− cells but not in HT-29 cells. Of note, the finding that AZD2461 exerted a strong cytotoxic effect also against HCT116 p53−/− cells suggests that some other mechanisms other than the ones dictated by wtp53, were involved in response to AZD2461.

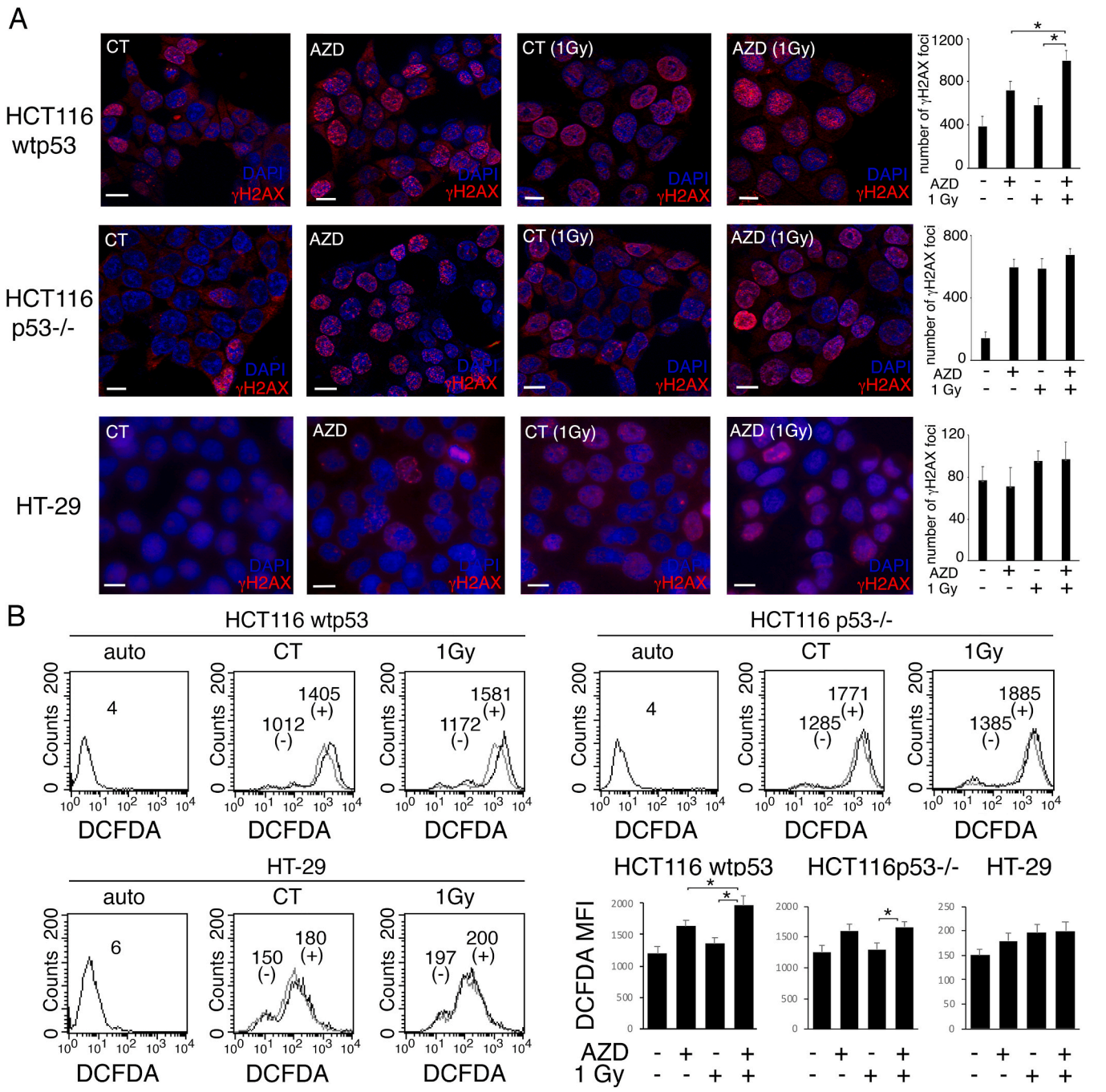

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Fig. 2. The treatment with AZD2461 increases DNA damage and intracellular ROS in colon cancer cell lines wtp53 and p53−/− but not in mutp53 cells and enhances the effects of low dose radiation only in wtp53 HCT116. (A) γH2AX foci (red) were assessed by IFA, in AZD-treated and untreated HCT116 wtp53, HCT116 p53−/− and HT29 cell lines and in the same cells pre-treated with AZD and then irradiated with 1 Grey radiation (1 Gy) (IR) as single or in combined treatments. DAPI (blue) was used for nuclear staining. Images were captured under ApoTome microscope at × 40 magnification. Bars = 10 μm. The histograms represent the mean plus SD of the number of foci/cells from three independent experiments. *p-value < 0.05 (B) Intracellular ROS level was measured by FACS analysis using DCFDA as staining. Mean of fluorescence intensity (MFI) is indicated. One representative experiment out of three is reported. Auto means autofluorescence; (−) indicates control cells and (+) indicates AZD-treatment. The bars in the histogram represent the means of MFI of three independent experiments. *p-value < 0.05. (C) Cell proliferation was measured by MTT assay after 72 h. The bars in the histograms represent the means of three independent experiments. *p-value < 0.05.

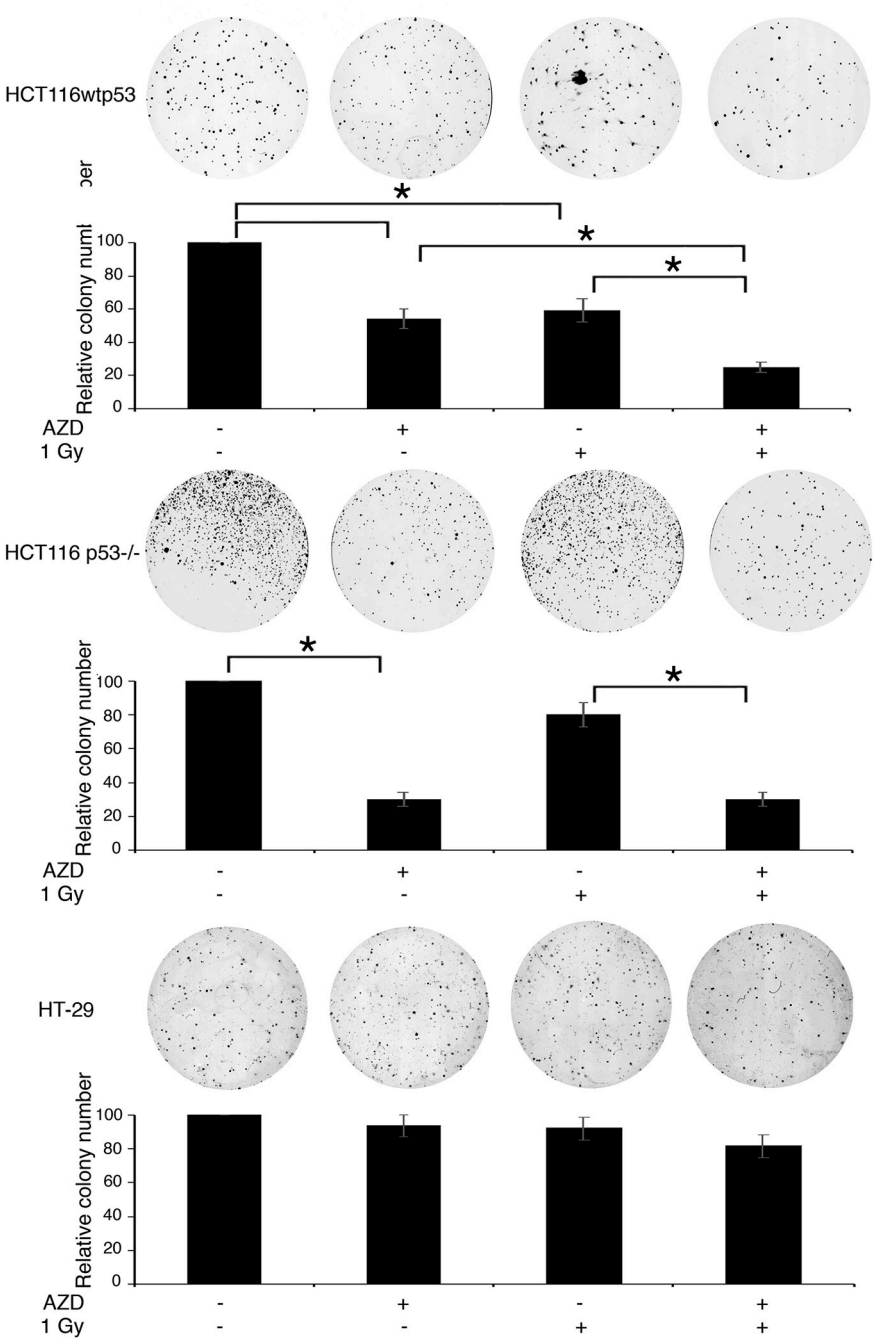

Fig. 3. AZD2461 radiosensitizes HCT116 wtp53 but not HCT116 p53−/− and HT-29 cells. Representative pictures of HCT116wtp53, HCT116 p53−/− and HT-29 colonies stained with crystal violet and histograms of quantitative analyses of colony formation are shown. The numbers of untreated colonies in HCT116wtp53, HCT116 p53−/− and HT-29 cells were set to 100, and AZD, IR and AZD + IR treated cel1s were presented as mean ± SD of percent. *p-value < 0.05.

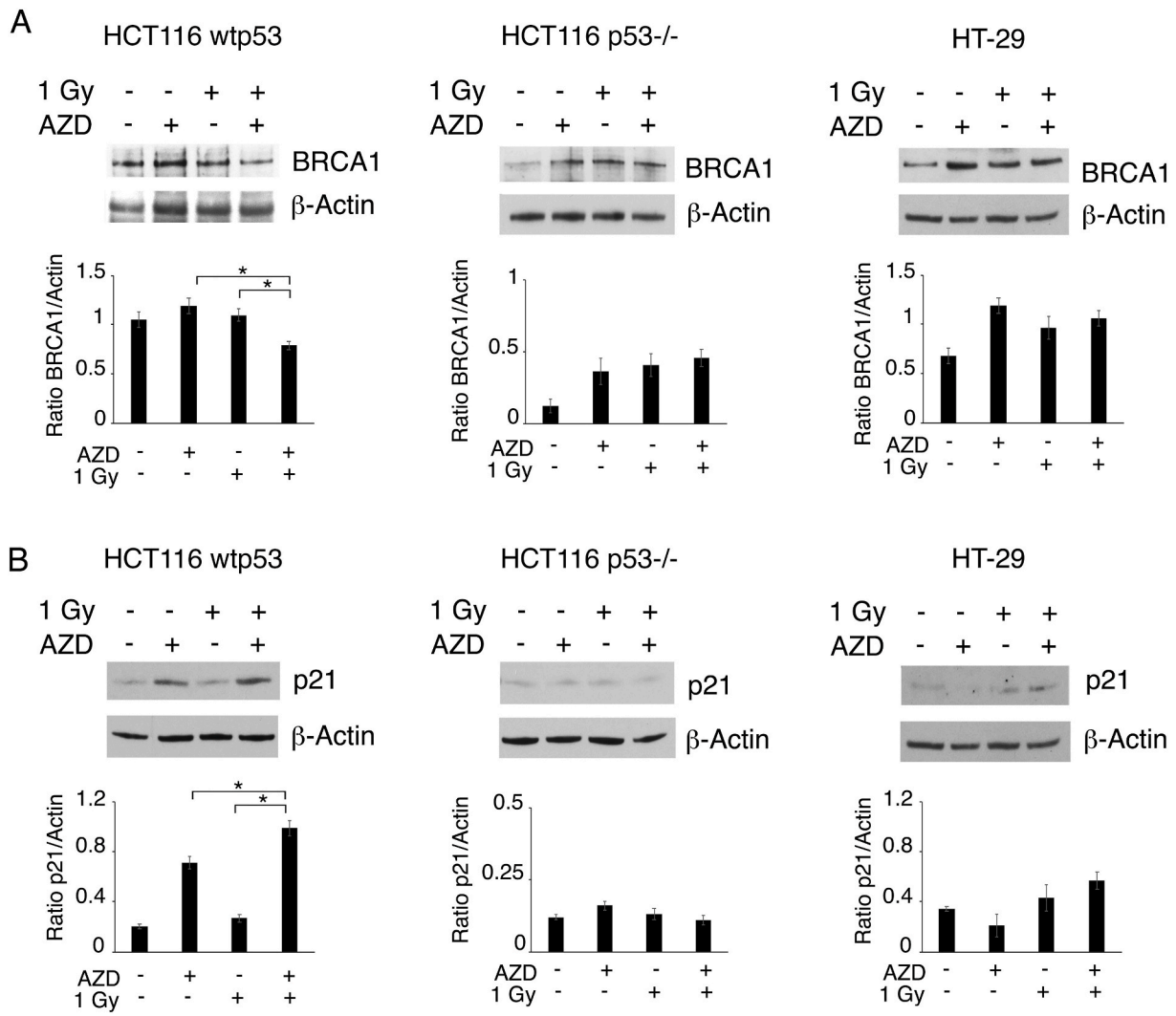

Fig. 4. The treatment with AZD2461/IR combination induces BRCA-1 down-regulation only in HCT116 wtp53 cells. Protein expression of (A) BRCA-1 and (B) p21 was evaluated by Western blot analysis in HCT116 wtp53, HCT116 p53−/− and HT-29 pre-treated with AZD (30 μM) and then irradiated with 1 Gy radiation. Untreated cells were used as control. β -Actin was used as loading control. The histograms represent the mean plus S.D. of the densitometric analysis of the ratio between the protein and the appropriate control. *p-value < 0.05.

3.2. AZD2461 increases DNA damage and intracellular ROS in p53 −/− and wtp53, but not in mutp53 colon cancer cells and potentiates the effects of low dose radiation only in wtp53 cells

Considering that PARP activity strongly contributes to repair DNA breaks [15], frequently occurring in highly proliferating cancer cells, we then investigated the impact of AZD2461 on DNA damage. To this aim, the appearance of γH2AX foci was evaluated, as readout of DNA damage. Indeed, the phosphorylation of H2AX variant histone (γH2AX) mediated by ATM, ATR and DNA-PK kinases, in response to DNA damage, gives rise to foci formation. As shown in Fig. 2A, the treatment with AZD2461 enhanced the number of γH2AX foci/cell in wtp53 and p53−/− cell lines but not in mutp53 HT-29 cells, as evaluated by IFA. DNA damage may be induced also by exposure to radiation (IR) [16], and accordingly here we found that the exposure to low dose ionizing radiation (1 Gy) (IR) enhanced γH2AX foci in wt and p53 null cells and further increased their formation in combination with AZD2461 in wtp53 cells (Fig. 2A). In HT-29 mutp53 cells, the foci formation was slightly affected also by IR and AZD/IR combination treatments (Fig. 2A). It has been previously reported that PARP-1 inhibition [17] and radiation [18] could enhance intracellular ROS. Therefore, we next evaluated ROS level following AZD2461, IR and AZD/IR treatment, by performing DCFDA staining and FACS analysis. The results shown in Fig. 2B, indicate that AZD2461 increased intracellular ROS in HCT116 wtp53 and HCT116 p53 −/− cells and again such effect was slightly induced in HT-29 mutp53 cells. The treatment with AZD2461/IR combination further increased ROS in comparison to single treatments, only in wtp53 (Fig. 2B), mirroring the effect observed on γH2AX foci formation. According to effect induced on foci formation and ROS modulation, we then found that cell proliferation was further reduced by AZD2461/IR combination compare to the single treatments only in wtp53 but not in p53 null or mutp53 cells (Fig. 2C), suggesting that AZD might preferentially radio-sensitize wtp53-carrying colon cancer cells.

3.3. AZD2461, in combination with IR, reduces the clonogenic capacity only in wtp53 HCT116 cells

To further assess the radio-sensitizing property of AZD2461, an *in vitro* clonogenic assay was performed, according to previous studies [19]. Fig. 3 shows that AZD2461 as single treatment impaired colony formation in wtp53 and p53−/− cells and not in mutp53 cells and that this effect was potentiated by AZD2461/IR combination only in wtp53. According to the results obtained on foci formation and ROS modulation, neither AZD2461 or IR or AZD2561/IR combination treatments

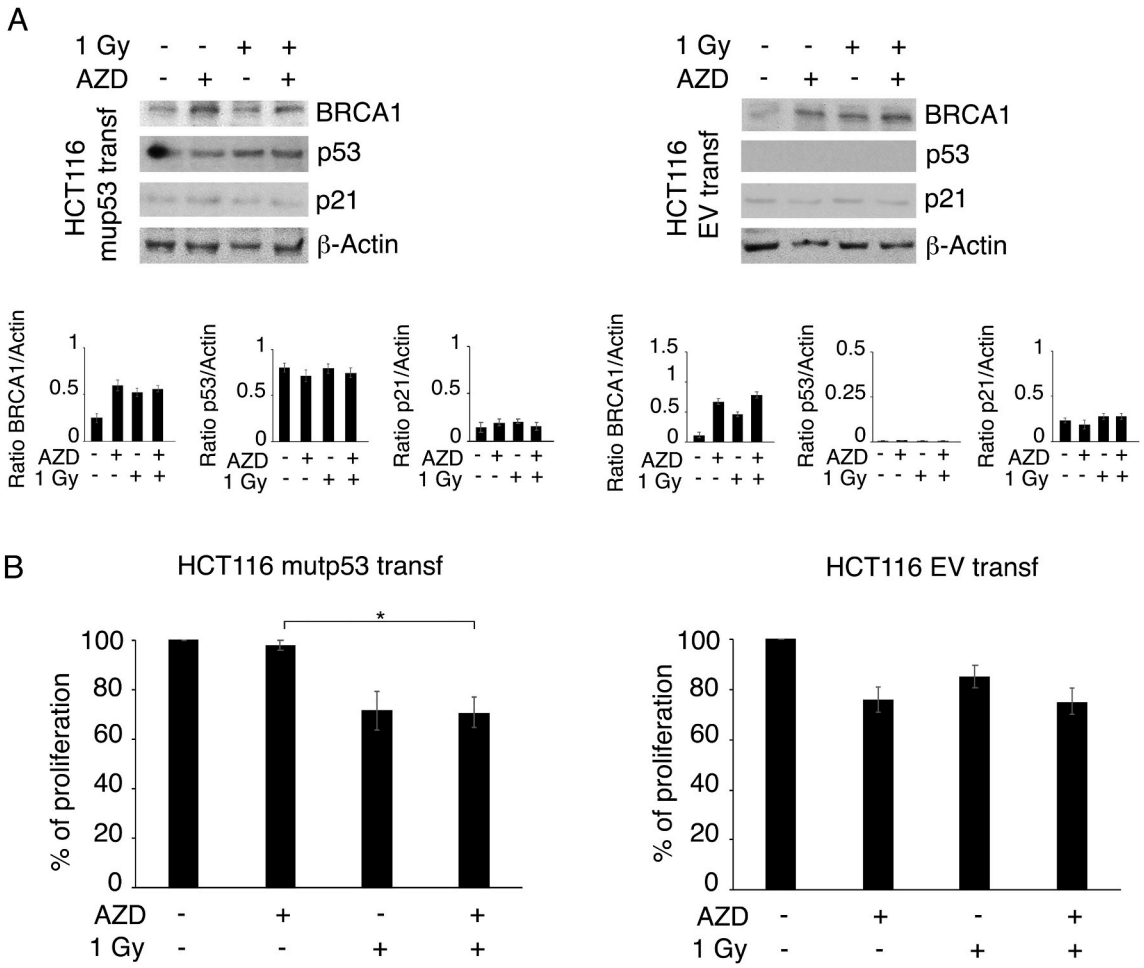

Fig. 5. AZD2461 does not potentiate the cytostatic effect of IR in HCT116 p53−/− transfected with empty vector or with mutp53 (R273H) vector. (A) The expression of BRCA-1, p53 and p21 was evaluated by Western blot analysis in HCT116 p53−/− cells transfected with mutp53 or with empty vector pre-treated with AZD (30 μM) and then irradiated with 1 Gy radiation. Un-treated cells were used as control. β-Actin was used as loading control. The histograms represent the mean plus S.D. of the densitometric analysis of the ratio between the protein and the appropriate control. *p-value < 0.05. B) Cell proliferation was evaluated by MTT assay in HCT116 p53−/− cells transfected with mutp53 or empty vector pre-treated with AZD (30 μM) and then irradiated with 1 Gy radiation for 72 h. Cells un-treated were used as control. The bars in the histograms represent the means of three independent experiments. *p-value < 0.05.

reduced colony formation in HT-29 mutp53 cells (Fig. 3).

3.4. AZD2461/IR combination down-regulates BRCA-1 only in HCT116 wtp53 cells

Searching for the molecular mechanism/s leading to the increased cytostatic effect induced by AZD2461/IR combination in HCT116 wtp53 cells, we analyzed the expression level of BRCA-1. Indeed, it is known that BRCA-1-deficient cancer cells are more susceptible to the treatment with PARP inhibitors [20] and drugs able to reduce its expression/function may sensitize cancer cells to PARP inhibitors [21]. The results shown in Fig. 4A, indicate that the expression level of BRCA-1 was reduced following the combined AZD2461/IR treatment in wtp53 HCT116 cells, compared to the single treatments, while BRCA-1 levels increased rather than decrease in HCT116 p53−/− or HT-29 mutp53 cells, according the lack of radio-sensitization by AZD2461 observed in these cells. The expression level of p21 which suggests p53 activation, was slightly up-regulated in wtp53 HCT116 cells by AZD2461 and strongly increased following AZD2461/IR co-treatment (Fig. 4B), concomitantly to the down-regulation of BRCA-1 (Fig. 4A). As expected, p21 expression did not increase following all these treatments in p53−/− and mutp53 cells (Fig. 4B), in agreement with previous studies showing that activation of wtp53 may downregulate or delocalize BRCA-1 [8,9,22]. To further assess the role of mutp53 on BRCA-1 expression in the HCT116 cellular context, HCT116 p53−/− were transfected with R273H mutp53 expression vector or control vector and then exposed to AZD2461/IR treatment. We found that BRCA-1 expression level increased by AZD2461/IR treatment in mutp53-or empty vector-transfected HCT116 cells (Fig. 5A), similarly to what observed in HCT116 p53 null or HT-29 cells. AZD2461 did not potentiate the reduction of cell proliferation induced by IR in control vector- and mutp53-transfected HCT116, as assessed by MTT assay (Fig. 5B). These results confirmed that only in wtp53-carrying colon cancer cells BRCA-1 could be downregulated following AZD/IR-treatment, effect correlated with the radiosensitization of these cells.

4. Discussion

This study suggests that the well tolerated PARP-1/2/3 inhibitor AZD2461 was able to reduce cell proliferation in colon cancer cells carrying wtp53 and p53−/− and potentiated the effect of low dose radiation in wtp53 HCT116 cells. Differently from those cells, R273H mutp53-transfected HCT116 cells or HT-29 naturally carrying such p53 mutation, displayed a low sensitivity to AZD2461, as single treatment and also in combination with IR. Due to p53 mutations, particularly in the DNA binding domain, such as in the case of R273H, cancer cells may become more resistant to anti-cancer treatments. Indeed, it is known

that, besides losing wtp53 tumor suppressor function, mutp53 may acquire pro-tumorigenic properties [23,24]. It may establish positive feed-back loops with several oncogenic pathways in which the release of pro-inflammatory cytokines may play an important role [25,26]. Regarding the relationship of mutp53 with DDR, it has been shown that mutp53 stimulates chromatin association and nuclear activity of PARP1, resulting in increased poly-ADP-ribosylated targets in breast cancer [27]. Moreover, mtp53 R273H and R248W directly associated with replicating DNA and a positive association between mtp53 R273H and PARP1 was found in breast cancer cells as well as in breast cancer patient-derived xenograft (PDX) samples, tissue microarrays and TCGA database [28]. Mutp53 has been also shown to stabilize replication forks, facilitating the proliferation of cells with genomic abnormalities [29] and block the activation of the apical stress-sensor kinase ATM [26]. Regarding wtp53, this protein has been shown to interact and down-regulate BRCA-1 in cancer cells exposed to DNA damaging agents [8]. Previous studies have also indicated that IR sensitized to PARP inhibitors breast and glioma cancer cells by activating wtp53 that induced BRCA-1 cytoplasmic sequestration, thus impairing its function [9]. In this study, we found that AZD2461-mediated radiosensitization of wtp53 HCT116 cells correlated with the downregulation of BRCA-1, occurring concomitantly to the up-regulation of p21. BRCA-1, is a caretaker protein playing a key role in homologous DNA damage repair [28,30]. Its function is essential for HR and becomes even more essential when DNA damage occurs in cells treated with PARP inhibitors that mainly impair the base excision repair and the *non-homologous* end-joining (NHEJ) DNA repair pathway. A recent study has shown that PARP inhibitors could induce a stronger cytotoxic either effect, alone or in combination with IR, against in cancer cells wtp53-deficient compare to those wtp53-proficient, due to a higher increase of intracellular ROS induced by PARP inhibitors in the former cells [11]. Moreover, mutp53 by promoting the association of PARP1 with replicating DNA has been shown render mutp53 carrying breast cancer cells more susceptible to the treatment with PARP1 inhibitors [28]. These findings suggest that DNA replication and repair may be controlled by both wt and mutp53 and the contradictory results regarding the possibility to use PARP inhibitors to potentiate the cytotoxicity of DNA damaging agents in cancer cells with different p53 status could be due the different in cancer types, p53 mutations, treatments or even the modalities with which they were performed. The results obtained in this study contribute to shed more light in this complex field by showing that a different sensitivity to PARP inhibitor AZD2461 of colon cancer cells with a different p53 status, alone or in combination with low dose radiation. Our data suggest that AZD2461 may offer the opportunity to improve the treatment of colon cancer cells if they do not carry p53 mutations.

Credit author statement

M.A.R.: investigation, visualization, methodology, formal analysis. **M.S.G.M.**: data curation, formal analysis, investigation, validation, writing—review and editing. **R.B.**: software, investigation, formal analysis. **A.A.**: visualization, formal analysis, methodology. **M.M.**: methodology. **E.B.**: investigation. **R.C.**: investigation. **G.D.**: conceptualization, writing—review and editing. **M.C.**: conceptualization, resources, data curation, formal analysis, funding acquisition, validation, project administration, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare no conflict of interest.

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