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Biology Contribution

Co-targeting Deoxyribonucleic Acid—Dependent Protein Kinase and Poly(Adenosine Diphosphate-Ribose) Polymerase-1 Promotes Accelerated Senescence of Irradiated Cancer Cells

Arun Azad, PhD,[*,‡] Patricia Bukczynska, MSc,[*] Susan Jackson,[*] Ygal Haput, PhD,[*,‖] Carleen Cullinane, PhD,[*,‖] Grant A. McArthur, PhD,[*,†,§,‖] and Benjamin Solomon, PhD[*,†,§,‖]

[*]Division of Cancer Research and [†]Division of Cancer Medicine, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia; Departments of [‡]Pathology and [§]Medicine, St. Vincent's Hospital, University of Melbourne, Parkville, Victoria, Australia; and [‖]Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria, Australia

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Summary

Accelerated senescence (terminal growth arrest) is a therapeutically beneficial cellular response to sustained DNA double-strand breaks (DSBs). Because DNA-dependent protein kinase (DNA-PK) and poly(adenosine diphosphate-ribose) polymerase-1 (PARP-1) have critical roles in DSB repair, we hypothesized that combined DNA-PK and PARP-1 blockade would promote accelerated senescence in irradiated cancer cells. We show that

Purpose: To examine the effects of combined blockade of DNA-dependent protein kinase (DNA-PK) and poly(adenosine diphosphate-ribose) polymerase-1 (PARP-1) on accelerated senescence in irradiated H460 and A549 non-small cell lung cancer cells.

Methods and Materials: The effects of KU5788 and AG014699 (inhibitors of DNA-PK and PARP-1, respectively) on clonogenic survival, DNA double-strand breaks (DSBs), apoptosis, mitotic catastrophe, and accelerated senescence in irradiated cells were examined in vitro. For in vivo experiments, H460 xenografts established in athymic nude mice were treated with BEZ235 (a DNA-PK, ATM, and phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor) and AG014699 to determine effects on proliferation, DNA DSBs, and accelerated senescence after radiation.

Results: Compared with either inhibitor alone, combination treatment with KU57788 and AG014699 reduced postradiation clonogenic survival and significantly increased persistence of Gamma-H2AX (γH2AX) foci in irradiated H460 and A549 cells. Notably, these effects coincided with the induction of accelerated senescence in irradiated cells as reflected by positive β-galactosidase staining, G2-M cell-cycle arrest, enlarged and flattened cellular morphology, increased p21 expression, and senescence-associated cytokine secretion. In irradiated H460 xenografts, concurrent therapy with BEZ235 and AG014699 resulted in sustained Gamma-H2AX (γH2AX) staining and prominent β-galactosidase activity.

Conclusion: Combined DNA-PK and PARP-1 blockade increased tumor cell radiosensitivity and enhanced the prosenescent properties of ionizing radiation in vitro and in vivo. These data

Reprint requests to: Arun Azad, PhD, i.e., Peter MacCallum Cancer Centre, Locked Bag 1, A'Beckett St, Melbourne 3002, Victoria, Australia. Tel: (+61) 3-9656-1517; E-mail: arun.azad@bccancer.bc.ca

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co-targeting DNA-PK and PARP-1 results in persistence of radiation-induced DSBs, leading to enhanced radiation efficacy and accelerated senescence in vitro and in vivo.

provide a rationale for further preclinical and clinical testing of this therapeutic combination.

Introduction

Deoxyribonucleic acid double-strand breaks (DSBs) are highly genotoxic lesions generated by ionizing radiation (1). Left unrepaired, DSBs severely compromise the reproductive capacity of cells through the induction of apoptosis, mitotic catastrophe, or accelerated senescence (terminal growth arrest) (2). As a result, targeting signaling networks involved in DSB repair is a promising approach for enhancing cellular radiosensitivity. In mammalian cells the primary repair mechanism of radiation-induced DSBs is the nonhomologous end-joining (NHEJ) pathway (3), in which DNA-dependent protein kinase (DNA-PK) plays a critical role (4). We previously demonstrated that radiation enhancement by DNA-PK blockade coincides with a prominent p53-dependent accelerated senescence phenotype in vitro and in vivo (5). Our data established accelerated senescence as a novel mechanism of radiosensitization induced by DNA-PK blockade and illustrate the possible therapeutic benefits of targeting DNA-PK in irradiated cells.

Another key target for enhancing radiation efficacy is the poly(adenosine diphosphate [ADP]-ribose) polymerase (PARP) enzyme family. Although PARP-1 and PARP-2 have a well-established role in base excision repair of single-strand DNA breaks (6), these enzymes also participate in DSB repair (7-9). Poly(ADP-ribose) polymerase 1 contributes to DSB repair by regulating the stress-inducible transcription factor nuclear factor κB and is a key molecular driver in the DSB repair pathway backup NHEJ (B-NHEJ) (10-13). Significantly, it is now recognized that B-NHEJ promotes DSB repair in cells that are deficient in DNA-PK-dependent NHEJ (D-NHEJ) (11). Therefore, we hypothesized that co-targeting DNA-PK and PARP-1 would effectively suppress radiation-induced DSB repair, leading to the induction of accelerated senescence and radiosensitization. Using KU57788 and AG014699, selective pharmacologic inhibitors of DNA-PK (14) and PARP-1/2 (15), respectively, we examined the impact of concurrently targeting these enzymes on clonogenic survival, DSBs, and accelerated senescence in irradiated cells.

Methods and Materials

Cell culture

All cell lines were obtained from the American Type Culture Collection (Manassas, VA), apart from p53-inducible H1299 cells (16). Cells were incubated at 37°C/5% CO_2 in Roswell Park Memorial Institute 1640 medium (H460, H2228, H23, and H358 cells), Dulbecco's modified Eagle medium/F12 (A549 cells), or Dulbecco's modified Eagle medium (U87-MG and H1299 cells) supplemented with 10% fetal bovine serum.

Inhibitors and inhibitor treatment

BEZ235 was obtained from Novartis (Basel, Switzerland). AG014699, KU57788, and Pifithrin-alpha (PFT-α) were purchased from Selleck Chemicals (China), SYNthesis Selleck Chemicals (Shanghai, China), and Sigma (St. Louis, MO; P4359), respectively.

Delivery of irradiation

Cell lines were irradiated using a ^{137}Cs source (Gamacell 40; Atomic Energy of Canada, Chalk River, ON, Canada). Drugs were added to cells 1 hour before irradiation and maintained in growth medium until time of harvest (with the exception of clonogenic survival assays, in which AG014699 and KU57788 were washed off after 24 hours and PFTα after 48 hours).

Clonogenic survival assays

Clonogenic survival assays were performed as previously described (5). Dose enhancement ratio (DER) was calculated as the surviving fraction (SF) at a given dose of radiation alone divided by the SF at a given dose of radiation plus drug (normalized for the effects of drug alone). Colonies of >50 cells were counted 10 days after radiation.

Senescence-associated β-galactosidase assay

Senescence-associated β-galactosidase (SA-βGal) staining was performed as previously described at neutral pH (6.0) (5). Images were obtained with a Leica inverted microscope (Bannockburn, IL) using a SPOTlight digital camera (SPOT imaging, Sterling Heights, MI, USA).

Flow cytometry

Cell cycle analysis was performed using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) as previously described (17). Apoptosis was quantified by measurement of sub-G1 levels using FCS Express (De Novo Software, Los Angeles, CA).

Immunoblotting

Immunoblotting was performed as previously described (17). Primary antibodies were total p53 (sc-6243; 1:500) and total p21 (sc-397; 1:2000) (both Santa Cruz Biotechnology, Santa Cruz, CA).

Immunofluorescence

Immunofluorescence staining and image capture and analysis were performed as previously described (5). Primary antibodies were Gamma-H2AX (γH2AX)^{Ser139} (ab22551; 1:250) (Abcam,

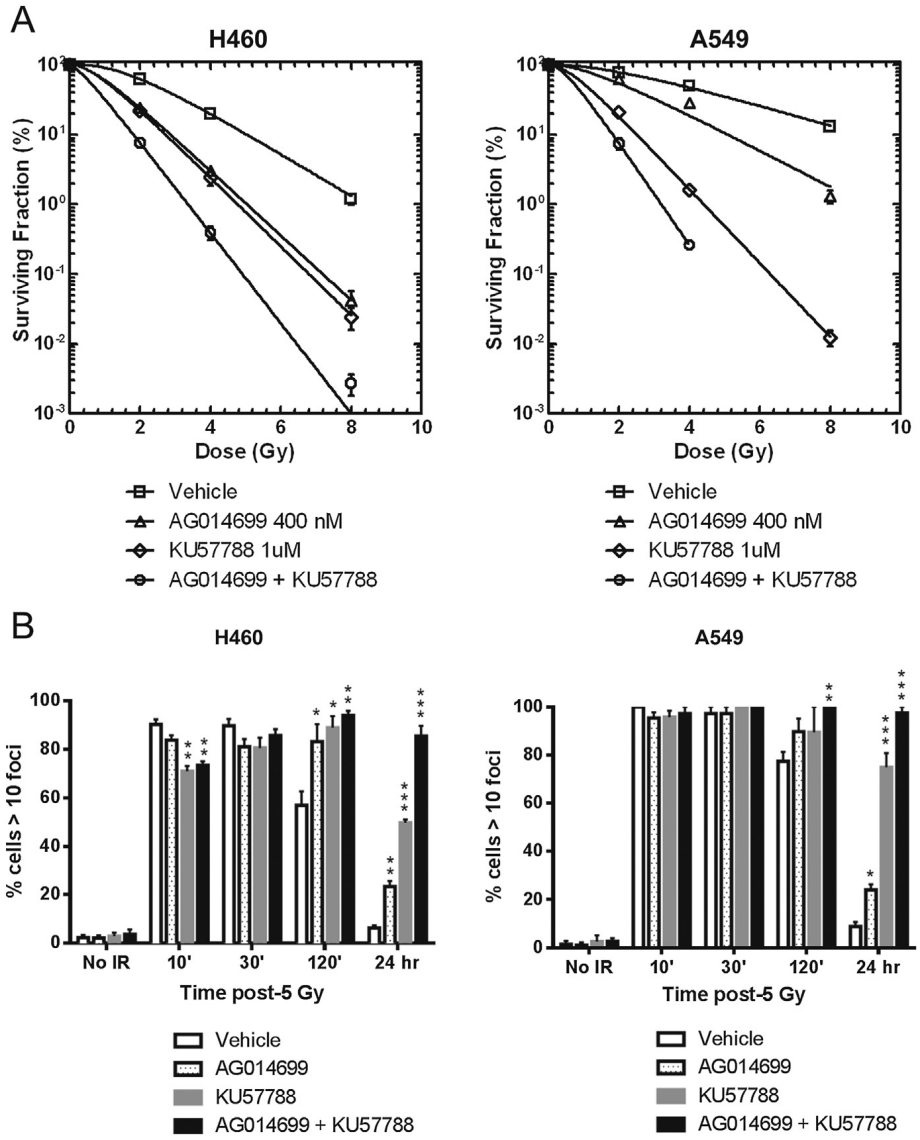

Fig. 1. Co-targeting DNA-dependent protein kinase and poly(adenosine diphosphate-ribose) polymerase-1 increases cellular radiosensitivity and persistence of radiation-induced double-strand breaks. (A) Clonogenic survival of H460 and A549 cells after indicated treatment. (B) Quantification of percentage of H460 and A549 cells exhibiting >10 γH2AX (Ser139) foci at indicated time points after 5 Gy radiation. At least 100 cells were analyzed per time point. Data are mean ± SEM from 3 independent experiments. ***$P<.001$, **$P<.01$, *$P<.05$.

Cambridge, UK), phospho-histone H3 ^{Ser10} (06-570; 1:1000) (Upstate, Lake Placid, NY), and β-tubulin (E7, 1:100) (Developmental Studies Hybridoma Bank, Iowa City, IA). Secondary antibodies were anti-mouse Alexa Fluor 488, 1:500 and anti-rabbit Alexa Fluor 568, 1:500 (Molecular Probes, Eugene, OR).

Small interfering RNA

H460 cells were seeded at 20% confluency and transfected 24 hours later with 10 nM small interfering RNA (siRNA) targeting p53 (Dharmacon, Lafayette, CO, USA, M-003329-03). Twenty-four hours after transfection, cells were plated for clonogenic assays, with inhibitors added 6 hours later. Clonogenic assays were then performed as previously described (5).

Animal studies

H460 cell xenografts were established subcutaneously in the right hind limb of athymic nude mice (ARC, Canning Vale, Australia) as previously described (17) and treated as followed once tumors reached approximately 100 mm^3: vehicle control (N-methyl-2-pyrrolidone (NMP)/Polyethylene glycol (PEG) 300, day 1), BEZ235 (40 mg/kg/d oral, day 1), AG014699 (15 mg/kg/d oral, day 1), and BEZ235 plus AG014699 (40 mg/kg/d oral and 15 mg/kg/d oral, respectively, day 1). Radiation was delivered 2 hours before drug treatment using a linear accelerator (Varian Medical Systems, Palo Alto, CA) under the supervision of a medical physicist.

Immunohistochemical studies

Staining for Ki67 and γH2AX was performed on H460 xenografts as previously described (5).

Cytokine antibody arrays

Cytokine antibody arrays were performed as previously described, according to the manufacturer's instructions (R&D Systems, Minneapolis, MN; #ARY005).

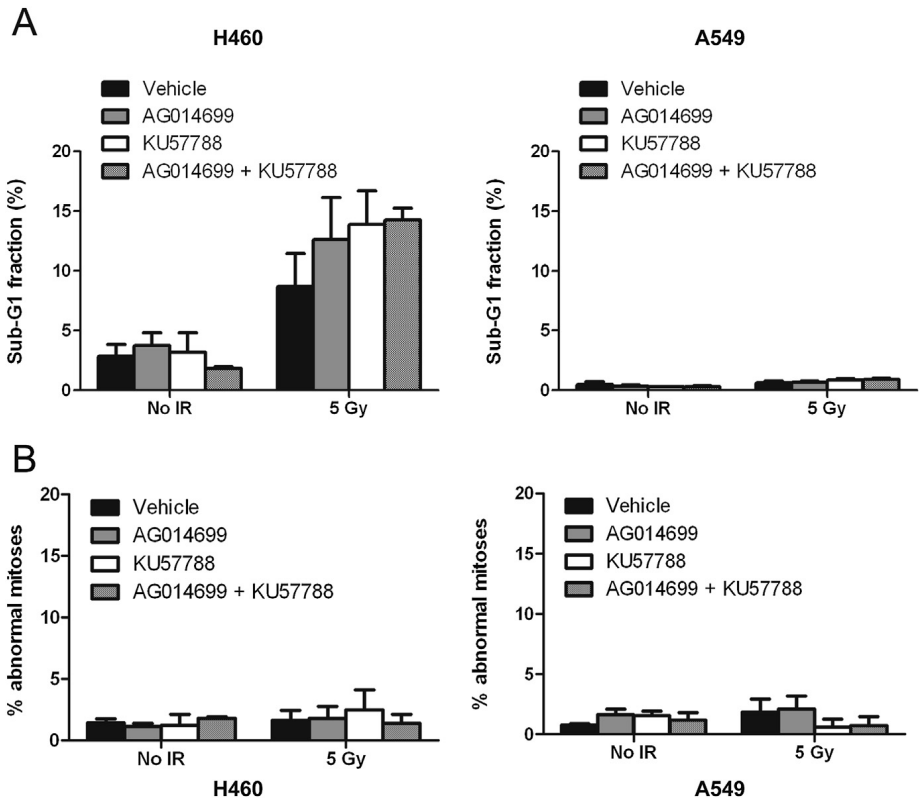

Fig. 2. Co-targeting DNA-dependent protein kinase and poly(adenosine diphosphate-ribose) polymerase-1 does not promote apoptosis or mitotic catastrophe after radiation. (A) Quantification of sub-G1 population at 48 hours after indicated treatment. Data are mean ± SEM from 3 independent experiments. (B) Number of abnormal mitoses at 48 hours after indicated treatment. An average of 80 cells per group was counted to determine mean ± SEM.

SA βGal assay in vivo

Senescence-associated β-galactosidase staining was performed as previously described at neutral pH (6.0) (5).

Statistical analysis

Statistical significance was determined using Student *t* test calculated with GraphPad Prism software (GraphPad Software, San Diego, CA).

Results

Co-targeting DNA-PK and PARP-1 increases cellular radiosensitivity and causes sustained radiation-induced DSBs

Initial experiments sought to determine the radiosensitizing effects of combined DNA-PK and PARP-1 inhibition using KU57788 and AG014699, respectively, in H460 and A549 non-small cell lung cancer (NSCLC) cells. KU57788 and AG014699 were used at 1 μM and 400 nM, respectively, concentrations sufficient to inhibit DNA-PK and PARP-1 and increase cellular radiosensitivity (5, 18). In H460 cells, KU57788 and AG014699 both reduced D0 (radiation dose resulting in 37% SF) from 3.2 Gy to 1.6 Gy (Fig. 1A). Combined treatment with KU57788 and AG014699 further decreased the D0 to 0.9 Gy. At 2 Gy, the DER for AG014699, KU57788, and AG014699 + KU57788 was calculated as 2.6, 2.8, and 8.2, respectively. In A549 cells, KU57788 and AG014699 decreased D0 from 5.1 Gy to 1.5 Gy and 2.9 Gy, respectively (Fig. 1A). Co-therapy with KU57788 and AG014699 resulted in further radiosensitization, as evidenced by a D0 of 1 Gy. At 2 Gy, the DER for AG014699, KU57788, and AG014699 + KU57788 was calculated as 1.3, 3.6, and 10.8, respectively. Thus, we conclude that combined DNA-PK and PARP-1 inhibition has greater than additive effects on post-radiation clonogenic survival of both H460 and A549 cells.

γH2AX immunofluorescence staining was next performed to assess the impact of treatment on radiation-induced DSBs. The proportion of cells with more than 10 γH2AX foci at various time points (10 minutes, 30 minutes, 120 minutes, and 24 hours) after a single 5-Gy fraction was quantified (Fig. 1B, Supplementary Table e1 [available online]). At 10 minutes and 30 minutes a high proportion of H460 and A549 cells contained more than 10 γH2AX foci, irrespective of inhibitor treatment. However, compared with radiation alone, concurrent AG014699 and KU57788 therapy significantly increased the percentage of irradiated H460 and A549 cells with >10 γH2AX foci at 120 minutes ($P = .0036$ and $P = .0042$, respectively) and 24 hours ($P < .0001$ for both cell lines). At 24 hours, notably, combined AG014699 and KU57788 treatment also significantly increased the number of residual γH2AX foci in comparison with monotherapy with either AG014699 (H460: $P = .002$; A549: $P < .0001$) or KU57788 (H460: $P = .0014$; A549: $P = .0245$).

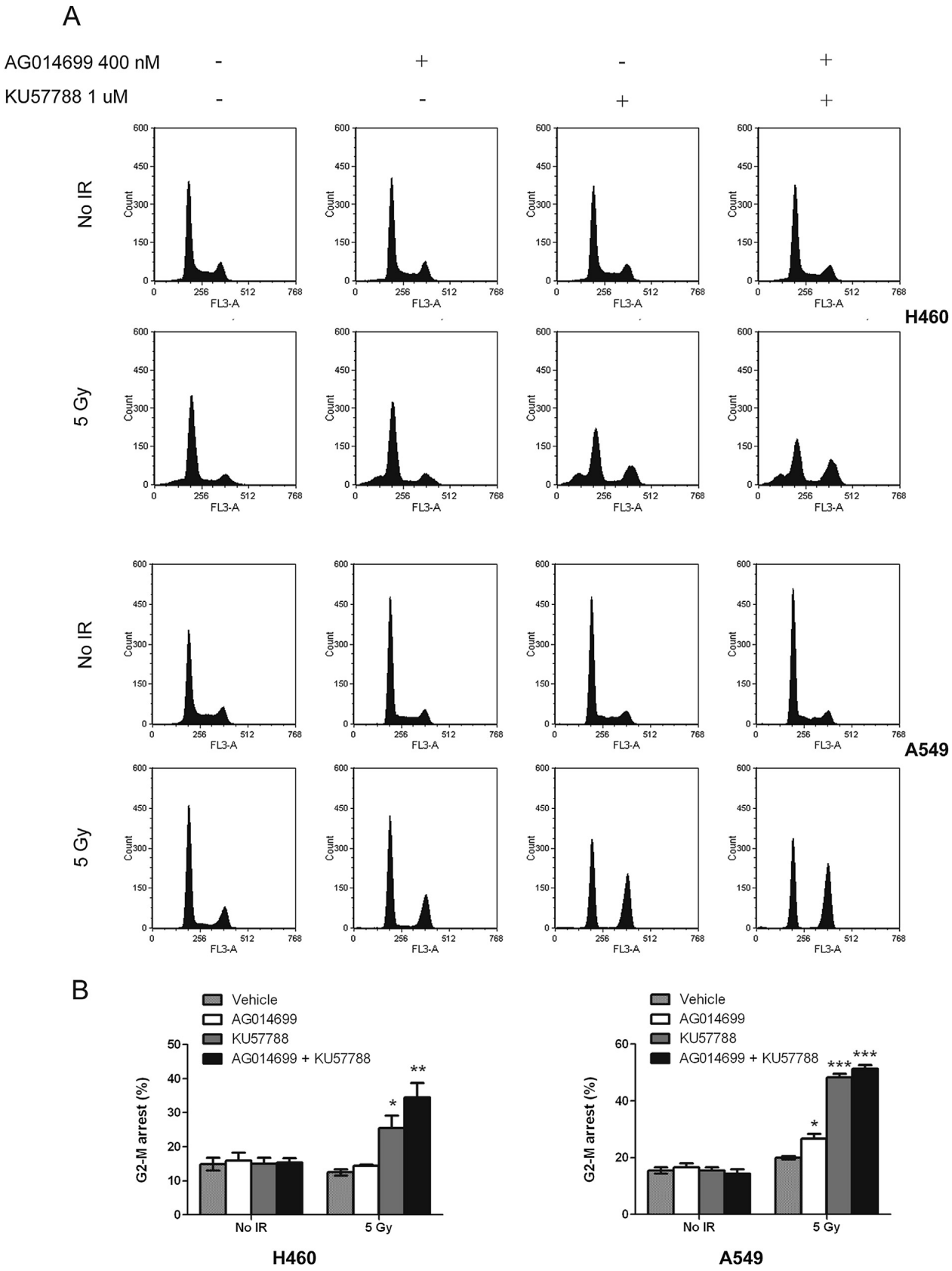

Fig. 3. Co-targeting DNA-dependent protein kinase and poly(adenosine diphosphate-ribose) polymerase-1 promotes G2-M cell cycle arrest after radiation. (A) Cell cycle profile of H460 and A549 cells after 48 hours of indicated treatment. (B) Quantification of proportion of cells arresting at G2-M. Data are mean ± SEM from 3 independent experiments. ***$P<.001$, **$P<.01$, *$P<.05$.

Co-targeting DNA-PK and PARP-1 promotes G2-M cell cycle arrest after radiation

We next sought to investigate potential mechanisms of radiation enhancement by KU57788 and AG014699. Using fluorescence-activated cell sorting measurement of the sub-G1 cell cycle fraction, we found that treatment with KU57788 and AG014699 did not significantly increase apoptosis after radiation in either H460 or A549 cells (Fig. 2A). Similarly, using an immunofluorescence protocol for mitotic catastrophe (5), we noted that abnormal mitoses occurred infrequently in both cell lines, irrespective of treatment (Fig. 2B).

Because sustained G2-M phase growth arrest is a classic cellular response to DNA damage (19), we also examined the impact of treatment on cell cycle (Fig. 3A, B). Notably, compared with radiation alone, co-treatment with KU57788 and AG014699 increased G2-M arrest in irradiated H460 (12% to 35%, $P<.01$) and A549 cells (20% to 48%, $P<.001$). However, combination therapy did not result in a statistically significant increase in G2-M arrest after radiation compared with KU57788 alone in either cell line. Nevertheless, because we had already established that short-term treatment (24 hours) with KU57788 and AG014699 leads to a sustained decrease in postradiation clonogenic survival at 10 days (Fig. 1A), our data suggest that combined KU57788 and AG014699 therapy can induce irreversible growth arrest in irradiated H460 and A549 cells.

KU57788 and AG014699 induce accelerated senescence after radiation in vitro

Because irreversible cell-cycle arrest is a feature of accelerated senescence, H460 and A549 cells were examined for SA-βGal activity (Fig. 4A). Combined therapy with KU57788 and AG014699 significantly increased the proportion of SA-βGal-positive cells over that induced by KU57788 alone in H460 (81% vs 47%; $P<.05$) and A549 (83% vs 55%; $P<.05$) cells at 48 hours after 5 Gy radiation (Fig. 4B). In both cell lines, typical morphologic features of senescence, including cell enlargement, flattening, and elongation, accompanied SA-βGal staining. Drug

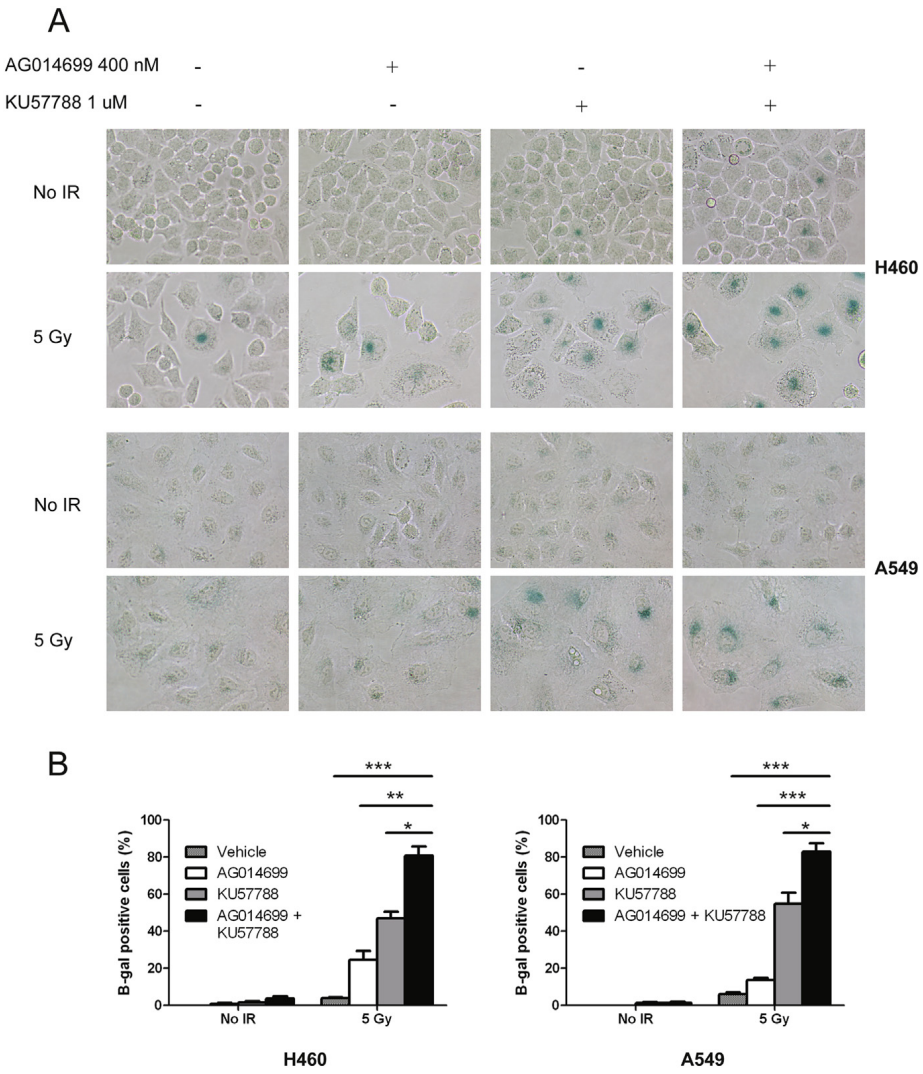

Fig. 4. KU57788 and AG014699 induce accelerated senescence after radiation in vitro. (A) Representative images of senescence-associated β-galactosidase (β-Gal) activity after 48 hours of indicated treatment (×200 magnification). (B) Percentage of Senescence-associated β-galactosidase-positive cells. Data are mean ± SEM from 3 independent experiments. ***$P<.001$, **$P<.01$, *$P<.05$.

treatment alone in the absence of radiation did not induce SA-βGal staining, signifying that the prosenescence response to combined DNA-PK and PARP blockade occurs in the context of concurrent radiation therapy.

Because DNA-damaging agents primarily drive senescence through the p53-p21 signaling pathway (20), effects of treatment on p21 expression were next analyzed. In both cell lines, p21 expression was clearly up-regulated at 48 hours after 5 Gy radiation and further enhanced by KU57788 in irradiated cells (Supplementary Fig. e1). In irradiated A549 cells, combining KU57788 and AG014699 led to a small additional increase in p21 levels. Using cytokine antibody arrays, the impact of treatment on senescence-associated secretory phenotype cytokine secretion was also examined at 96 hours after 5 Gy radiation. Co-treatment with KU57788 and AG014699 elevated interleukin-1α, Macrophage migration inhibitory factor (MIF), and Regulated upon activation, normal T-cell expressed and secreted levels in H460 cells and MIF and Growth-regulated oncogene-α levels in A549 cells (Supplementary Fig. e2). In irradiated H460 cells, combined therapy increased interleukin-1α expression over KU57788 alone.

Collectively, these results demonstrate that co-targeting DNA-PK and PARP in irradiated H460 and A549 cells leads to a senescent phenotype characterized by G2-M cell cycle arrest, SA-βGal staining, morphologic features of senescence, increased p21 levels, and senescence-associated secretory phenotype cytokine secretion.

p53 is required for induction of accelerated senescence but not radiosensitization in irradiated cells treated with KU57788 and AG014699

Because H460 and A549 cells have wild-type p53, we examined whether the prosenescence response of irradiated cells to KU57788 and AG014699 is p53 dependent. To do this, SA-βGal staining was performed on p53-null H1299 NSCLC cells co-transfected with vectors for the ecdysone receptor and inducible p53 (16). In the absence of the ecdysone analogue ponasterone A, SA-βGal activity was not observed in any treatment group (Fig. 5). In contrast, after the addition of ponasterone A and consequent induction of p53, prominent SA-βGal staining was observed in irradiated cells treated with AG014699 and KU57788. In keeping with these results, no SA-βGal staining was observed in 3 other p53-deficient NSCLC cell lines (H23, H2228, and H358) (Supplementary Fig. e3).

To determine whether p53 is required for radiosensitization after treatment with KU57788 and AG014699, we used siRNA to knock down p53 (Supplementary Fig. e4A) and PFTα to inhibit p53-mediated transcriptional activity (Supplementary Fig. e4B) in H460 cells. Postradiation clonogenic survival after transfection with p53 siRNA was nonsignificantly higher in cells treated with KU57788 (SF: 2.0% vs 0.9% in untransfected cells; $P = .19$), AG014699 (SF: 3.7% vs 2.2%; $P = .40$), and KU57788 + AG014699 (SF: 0.98% vs 0.32%; $P = .40$) (Supplementary Fig. e4C). Similarly, postradiation clonogenic survival was not increased in the presence of PFTα after treatment with KU57788 (SF: 1.0% vs 1.7% with DMSO control; $P = .65$), AG014699 (SF: 5.3% vs 6.4%; $P = .77$), and KU57788 + AG014699 (0.14% vs 0.22%; $P = .5613$) (Supplementary Fig. e4D).

BEZ235 and AG014699 induce accelerated senescence in irradiated H460 xenografts

To examine the effects of combined DNA-PK and PARP blockade in vivo, H460 xenografts were established in nude mice. For these experiments the multikinase DNA-PK inhibitor BEZ235 was used instead of KU57788, whose solubility and oral bioavailability is unsuitable for in vivo delivery (14). Minimal SA-βGal activity was observed in unirradiated xenografts or at 96 hours after 5 Gy radiation in xenografts treated with vehicle or AG014699. However, SA-βGal staining in irradiated xenografts was elevated after treatment with BEZ235 and further increased by combination

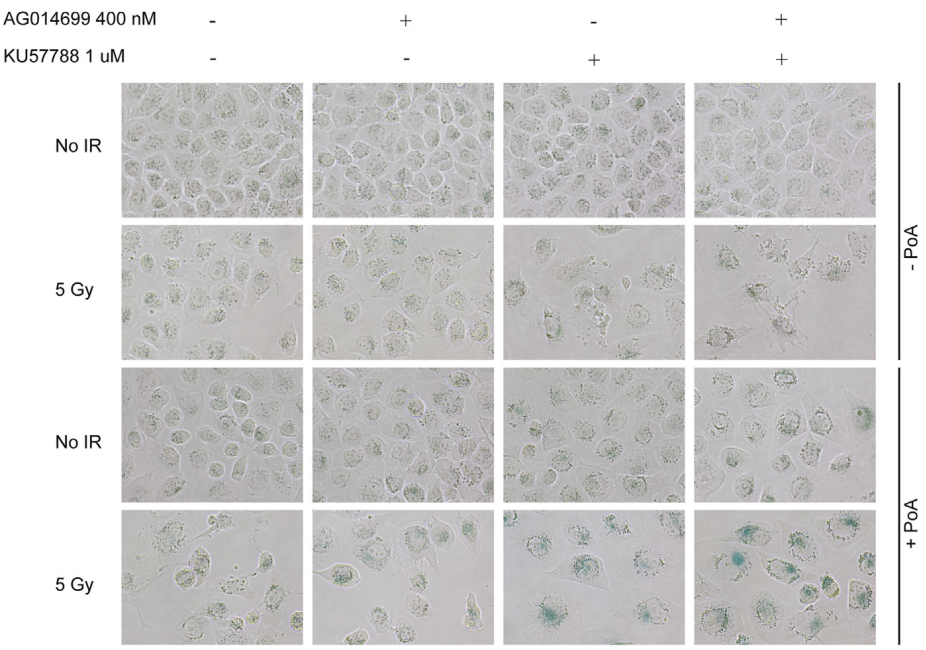

Fig. 5. KU57788 and AG014699 induce p53-dependent accelerated senescence in irradiated cells. Representative images of senescence-associated β-galactosidase activity in p53-null H1299 cells co-transfected with vectors for the ecdysone receptor and inducible p53. PoA = ponasterone A.

treatment with AG014699 and BEZ235 (Fig. 6A). In keeping with these findings, co-therapy with BEZ235 and AG014699 decreased Ki67 staining (Fig. 6B) and led to persistent γH2AX staining in irradiated xenografts (Fig. 6C). Together, these findings illustrate that co-targeting DNA-PK and PARP-1 in irradiated H460 xenografts results in the accumulation of DNA DSBs, decreased tumor cell proliferation, and the induction of accelerated senescence.

Discussion

In this study, we found that pharmacologic inhibition of PARP-1 using AG014699 enhances the radiosensitizing properties of KU57788, a selective DNA-PK inhibitor, in H460 and A549 NSCLC cells. Co-targeting DNA-PK and PARP-1 also increased the number of persistent radiation-induced DSBs over that seen with either KU57788 or AG014699 alone. Significantly, these effects were accompanied by the induction of p53-dependent accelerated senescence in vitro and in vivo, thereby providing a molecular mechanism for enhancement of radiation efficacy by combined DNA-PK and PARP-1 blockade.

Several studies have previously examined the impact of co-targeting DNA-PK and PARP in the context of ionizing radiation therapy. Initial studies showed additive effects of combined DNA-PK and PARP inhibition on postradiation DSB repair (21-23), leading to radiosensitization of Chinese hamster ovary (CHO) cells and mouse embryonic fibroblasts (21, 22). Significantly, these studies used supralethal radiation doses (≥75 Gy) for DSB repair assays. In contrast, 2 more-recent studies used a dose of 2 Gy radiation to demonstrate that DNA-PK and PARP-1 have nonadditive effects on the rapid phase of radiation-induced DSB repair in mouse embryonic fibroblasts and CHO cells (24, 25). Although radiation dosing may account for the discrepancies in DSB repair seen between these studies, it is notable that

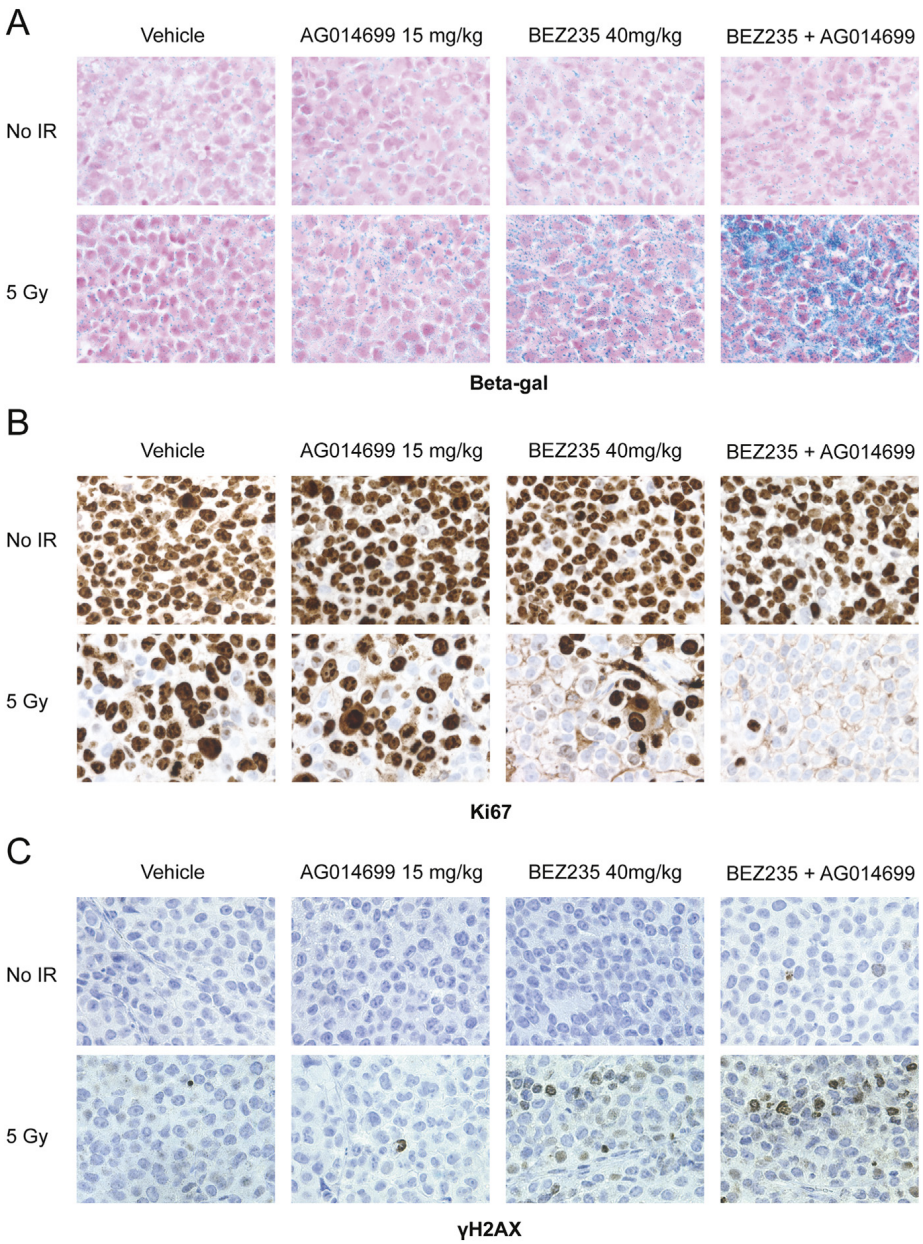

Fig. 6. BEZ235 and AG014699 induce accelerated senescence in H460 xenografts 96 hours after radiation. Representative images (40× magnification) are shown of (A) senescence-associated β-galactosidase activity, (B) Ki67 staining, and (C) γH2AX staining.

concurrent DNA-PK and PARP-1 blockade has additive effects on clonogenic survival of CHO cells after 2 Gy radiation (21, 22). Our results are in keeping with these findings: KU57788 and AG014699 had greater than additive effects at 2 Gy on clonogenic survival of H460 and A549 cells.

Significantly, none of the aforementioned studies examined the outcome of targeting DNA-PK and PARP-1 in human tumor-derived cell lines. Hence, this study is the first to confirm that concurrent administration of selective DNA-PK and PARP inhibitors results in radiosensitization of human cancer cells. With this in mind, we eagerly await the development of specific DNA-PK inhibitors with suitable properties for in vivo use, because this would enable an examination of the therapeutic efficacy of selective DNA-PK and PARP-1 inhibition in irradiated human tumors. In the absence of a selective DNA-PK inhibitor that can be delivered in vivo, we used the nonselective DNA-PK inhibitor BEZ235. Although BEZ235 also inhibits phosphatidylinositol 3-kinase/mammalian target of rapamycin (26), we have previously established that genetic and selective pharmacologic inhibition of DNA-PK mirrors the impact of BEZ235 on accelerated senescence in irradiated H460 and A549 cells (5). Thus, inhibition of DNA-PK seems to be a critical factor underpinning the prosenescent properties of BEZ235 in irradiated cells, supporting its use in our animal experiments.

Consistent with the essential roles of DNA-PK and PARP-1 in D-NHEJ and B-NHEJ, respectively, we found that combined therapy with KU57788 and AG014699 led to persistence of radiation-induced DSBs. These results are consistent with a failure to complete DSB repair, an outcome that has deleterious consequences for the clonogenic potential of cancer cells because unrepaired DSBs can trigger apoptosis, necrosis, mitotic catastrophe, and terminal growth arrest (accelerated senescence) (2). Interestingly, we found that co-targeting DNA-PK and PARP-1 did not up-regulate apoptosis or mitotic catastrophe in irradiated H460 and A549 cells. Instead, treatment resulted in the induction of accelerated senescence, which is increasingly recognized as a therapeutically beneficial response of cancer cells to DNA-damaging agents such as ionizing radiation (2).

Two recent studies have shown that PARP-1 inhibition enhances radiation-induced accelerated senescence in breast cancer and prostate cancer models in vitro and in vivo (27, 28). We have also recently demonstrated that targeting DNA-PK using selective and nonselective pharmacologic inhibitors as well as siRNA results in accelerated senescence in irradiated cancer cells and tumors (5). Following on from these studies, the data presented here indicate that co-targeting DNA-PK and PARP-1 is a promising approach for enhancing the prosenescent properties of ionizing radiation in p53 wild-type tumor cells in vitro and in vivo. Interestingly, we found that genetic and pharmacologic inhibition of p53 did not abrogate radiosensitization of H460 cells by KU57788 and AG014699 (or by either inhibitor alone). These findings are in contrast to a recent study that found that p53 knockdown or inhibition attenuated radiation-induced senescence and radiosensitization of H460 cells (29). However, our data are in keeping with previous studies that have shown that selective PARP-1 and DNA-PK inhibitors increase the radiosensitivity of p53-deficient tumor cells (14, 27, 28, 30). Future studies evaluating the mechanisms through which p53-deficient tumors are radiosensitized by combined DNA-PK and PARP-1 blockade would be highly valuable.

In summary, we have illustrated the potential benefits of concurrently targeting DNA repair pathways to modulate repair of therapeutically induced DSBs and thereby promote radiation-induced accelerated senescence. These findings provide additional evidence that accelerated senescence is a key outcome of DSB-inducing agents and support further preclinical and clinical evaluation of combined targeting of DNA-PK and PARP-1 in combination with ionizing radiation.

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