Inhibition of DNA-Dependent Protein Kinase Induces Accelerated Senescence in Irradiated Human Cancer Cells

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Abstract

DNA-dependent protein kinase (DNA-PK) plays a pivotal role in the repair of DNA double-strand breaks (DSB) and is centrally involved in regulating cellular radiosensitivity. Here, we identify DNA-PK as a key therapeutic target for augmenting accelerated senescence in irradiated human cancer cells. We find that BEZ235, a novel inhibitor of DNA-PK and phosphoinositide 3-kinase (PI3K)/mTOR, abrogates radiation-induced DSB repair resulting in cellular radiosensitization and growth delay of irradiated tumor xenografts. Importantly, radiation enhancement by BEZ235 coincides with a prominent p53-dependent accelerated senescence phenotype characterized by positive β-galactosidase staining, G2–M cell-cycle arrest, enlarged and flattened cellular morphology, and increased p21 expression and senescence-associated cytokine secretion. Because this senescence response to BEZ235 is accompanied by unrepaired DNA DSBs, we examined whether selective targeting of DNA-PK also induces accelerated senescence in irradiated cells. Significantly, we show that specific pharmacologic inhibition of DNA-PK, but not PI3K or mTORC1, delays DSB repair leading to accelerated senescence after radiation. We additionally show that PRKDC knockdown using siRNA promotes a striking accelerated senescence phenotype in irradiated cells. Significantly, we show that specific pharmacologic inhibition of DNA-PK, but not PI3K or mTORC1, delays DSB repair leading to accelerated senescence after radiation. We additionally show that PRKDC knockdown using siRNA promotes a striking accelerated senescence phenotype in irradiated cells.

Introduction

Ionizing radiation is a widely used anticancer modality. However, the high relapse rates following radiotherapy indicate the urgent requirement for novel radiosensitizing strategies. As radiation is a potent inducer of DNA double-strand breaks (DSB; ref. 1), 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 (2), in which DNA-dependent protein kinase (DNA-PK) plays a critical role. Upon recruitment to DSB sites, the catalytic subunit of DNA-PK (DNA-PKcs) phosphorylates key DNA repair proteins and facilitates direct ligation of broken DNA ends (3). Accordingly, DNA-PK–deficient cells have ineffective DSB repair and are exquisitely sensitive to DSB-inducing agents (4). Conversely, upregulation of DNA-PK promotes repair of DSBs leading to tumor radioresistance preclinically (5) and clinically (6–8). Thus, DNA-PK is an important molecular target for inhibiting DSB repair and enhancing the cytotoxicity of radiation.

Another attractive target for potentiating radiation efficacy is the phosphoinositide 3-kinase (PI3K)/mTOR signaling pathway. Aberrant upregulation of this pathway occurs in many human malignancies (9) and is implicated in resistance to radiation preclinically (10, 11) and clinically (12–14). Although radiosensitization was reported with early PI3K inhibitors such as wortmannin and LY294002 (15), these agents had unacceptable pharmacokinetic and toxicity profiles (16) and have now been superseded by novel PI3K inhibitors with superior pharmacologic properties. One of these is NVP-BEZ235 (Novartis Pharma AG), an orally available PI3K/mTOR inhibitor (17) that is currently in phase II clinical testing.

In addition to inhibiting PI3K and mTOR, it has recently been reported that BEZ235 has potent activity against DNA-PK (18). This suggests that treatment of irradiated...
cells with BEZ235 is likely to impair NHEJ resulting in accumulation of un repaired DSBs. In turn, the clonogenic potential of irradiated tumor cells may be compromised by BEZ235 as un repaired DSBs can trigger a range of therapeutically desirable outcomes that include apoptosis, necrosis, mitotic catastrophe, and terminal growth arrest (accelerated senescence; ref. 19). Although the induction of apoptosis underlies the antitumor efficacy of radiation in certain malignancies, solid tumors commonly exhibit resistance to radiation-induced apoptosis (20). Nevertheless, radiation remains an effective modality for the treatment of many tumor types, thus emphasizing a potentially valuable role for nonapoptotic responses to treatment. In keeping with this, emerging evidence indicates that apoptosis is not the sole or even predominant mechanism through which some DNA-damaging agents and PI3K/mTOR pathway inhibitors increase radiosensitivity (20–23). Thus, nonapoptotic mechanisms may be a significant factor in radiosensitization of epithelial cancer cells by BEZ235.

Herein, we examined the cellular and molecular outcomes of inhibiting DNA-PK in irradiated cancer cells. After confirming that BEZ235 potently inhibits DNA-PK, we determined that treatment with BEZ235 attenuates radiation-induced DNA DSB repair leading to p53-dependent accelerated senescence after radiation in vitro and in vivo. Significantly, using complementary genetic and pharmacologic approaches, we also found that selectively targeting DNA-PK reproduces the phenotypic changes observed with BEZ235. These findings collectively identify accelerated senescence as a critical determinant of outcome when DNA-PK blockade is combined with radiation and provide a sound rationale for specific therapeutic targeting of DNA-PK in irradiated tumor cells.

Materials and Methods

Cell culture

All cell lines were obtained from the American Type Culture Collection apart from p53-inducible H1299 cells (24). Cells were incubated at 37°C/5% CO₂ in RPMI-1640 (H460, PC3, SKBR3, and MCF7 cells), Dulbecco’s Modified Eagle’s Medium (DMEM)/F12 (A549 cells), α-MEM (A431 cells), or DMEM (U87-MG and H1299 cells) supplemented with 10% FBS from aseptically handled fetal bovine serum (FBS). In addition, some cell lines were maintained in 1:10,000 plasmocin (InvivoGen) at 1:10,000. Also, although some cell lines were maintained in RPMI-1640 (U87-MG cells), supplemented with insulin (0.2 U/mL) and H1299 cells were supplemented with insulin (0.2 U/mL) and H1299 cells were also supplemented with insulin (0.2 U/mL) and H1299 cells were maintained in plasmocin (InvivoGen) at 1:10,000.

Inhibitors and inhibitor treatment

BEZ235, BKM120, and RAD001 were obtained from Novartis Pharma AG. KU557788 was purchased from SYN-Immuno (Queensland Institute of Medical Research, Herston, Queensland, Australia). BEZ235, BKM120, and RAD001 were dissolved in dimethyl sulfoxide except RAD001, which was dissolved in high-grade ethanol.

Delivery of irradiation

Cell lines were irradiated using a 137Cs source (Gammacell 40, Atomic Energy of Canada) at a dose rate of 0.57 Gy/min. In all in vitro studies, drugs were added to cells 1 hour before irradiation. With the exception of clonogenic survival assays (see later), drugs were maintained in growth medium until time of harvest.

Clonogenic survival assays

Clonogenic survival assays were conducted as previously described (25). Briefly, cells were treated with BEZ235 (50 or 100 nmol/L) or vehicle control and irradiated (1–8 Gy) 1 hour later. BEZ235 was removed 24 hours after irradiation, and cells were incubated in drug-free medium for 10 days before formaldehyde fixation and crystal violet staining. Colonies consisting of 50 or more were counted in 3 replicate plates. Clonogenic survival curves were fit using multistarget single hit (MTSH) regression to determine D0 (radiation dose resulting in 37% surviving fraction).

Senescence-associated β-galactosidase assay

Cells were treated with drug for 48 hours and then fixed in 2% paraformaldehyde/0.2% glutaraldehyde. After addition of staining solution containing 20 mg/mL X-gal (Promega), cells were incubated for 16 to 24 hours at 37°C in a non-CO₂ chamber. Images were obtained with a Leica inverted microscope at 200× magnification using a SPOTLight digital camera.

Flow cytometry

Cell-cycle analysis was conducted using a FACS Calibur flow cytometer (Becton Dickinson) as previously described (25). The percentage of apoptotic cells was quantified by measurement of sub-G₁ levels using FCS Express (De Novo Software).

Cytokine antibody arrays

After treatment for 96 hours, cell supernatant was collected and stored at −20°C. Supernatant volumes were normalized to cell number and then analysed using antibody arrays (R&D Systems; #ARY005) as per the manufacturer’s instructions.

Immunoblotting

Immunoblotting was conducted as previously described (25). The following antibodies were used: phospho-AKTSer273 (#9271; 1:1,000), total AKT (#9272; 1:1,000), phospho-S6Ser245/246 (#2211; 1:2,000), total S6 (#2217; 1:2,000; Cell Signaling Technology), total p53 (sc-6243; 1:500), total p21 (sc-397; 1:2,000; Santa Cruz Biotechnology), and total-DNA-PK (1:1,000; gift from Dr. Kum Kum Khanna, Queensland Institute of Medical Research, Herston, Queensland, Australia).

Immunofluorescence

Cells were cryopreserved on to Superfrost plus slides (Lomb) or grown on 4-well chamber slides (Lab-Tek), fixed in 4% paraformaldehyde for 15 minutes, and blocked for 60 minutes with 10% FBS. The following primary antibodies were incubated for 90 minutes at room temperature: γH2AXSer139 (ab22551; 1:250), phospho-DNA-PKcsThr266 (ab18356; 1:250; Abcam), phospho-ATMSer1981 (#39530; 1:500; Active

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Sections were stained for senescence-associated 

Signaling) using standard techniques in a Dako autostainer formalin, and embedded in paraf/ 

ction and stored at −80°C. Frozen sections (4 μm) were fixed and stained in accordance with in vitro experiments and then counterstained with nuclear fast red.

**Results**

**BEZ235 inhibits DNA-PK and enhances G<sub>2</sub>–M growth arrest after radiation**

The radiosensitizing properties of BEZ235 were tested in H460 and A549 non-small cell lung cancer (NSCLC) cells. These cell lines have constitutive activation of the PI3K/ 

with 100 nmol/L BEZ235 did not increase the sub-G<sub>1</sub> (apoptotic) fraction at 48 hours after radiation in either cell line (Fig. 1C). To provide an additional mechanism for the effect of BEZ235 on the DNA-PKcs, which notably has structural homology with class IA PI3K (17). We found that 100 nmol/L BEZ235 significantly decreased the number of phospho-DNA-PKcs foci per cell after 4 Gy (H460, 19.6 to 8.7; A549, 16.1 to 7.3) and 10 Gy radiation (H460, 31.4 to 9.9; A549, 29.1 to 9.1; Fig. 1A). Having established that BEZ235 is a potent inhibitor of PI3K/mTOR and DNA-PK, we conducted clonogenic survival assays on H460 and A549 cells. Treatment with BEZ235 for 24 hours significantly increased cellular radiosensitivity, as reflected by a dose-dependent reduction in colony formation after 10 days (Fig. 1B). The D0 (radiation dose resulting in 37% surviving fraction) decreased from 2.9 to 2 and 1.4 Gy in H460 cells and from 5 to 2.2 and 1.2 Gy in A549 cells, following treatment with 50 and 100 nmol/L BEZ235, respectively. These results were normalized to account for the effect of BEZ235 alone, which reduced colony numbers by approximately 15% to 30% compared with untreated controls.

To investigate potential mechanisms of radiation enhancement by BEZ235, fluorescence-activated cell-sorting (FACS) analysis was conducted on H460 and A549 cells stained with propidium iodide. We determined that treatment with 100 nmol/L BEZ235 did not increase the sub-G<sub>1</sub> (apoptotic) fraction at 48 hours after radiation in either cell line (Fig. 1C). To provide an additional mechanism for quantification of apoptosis, we conducted the TUNEL assay in H460 and A549 cells. Results from these experiments confirmed the findings from the measurement of sub-G<sub>1</sub> fraction that BEZ235 does not enhance apoptosis in irradiated cells (Supplementary Fig. S2). In contrast to these findings, we found that 100 nmol/L BEZ235 substantially...
Figure 1. BEZ235 inhibits DNA-PK and enhances G2–M growth arrest after radiation. A, representative immunofluorescent images of phospho-DNA-PKcs (Thr2609) foci at 30 minutes after radiation (×60 magnification). Green, phospho-DNA-PKcs foci; blue, DAPI staining. Right, number of phospho-DNA-PKcs foci per cell. Data are mean ± SEM from 3 independent experiments. *, *P < 0.01; **, **P < 0.001. B, clonogenic survival assays of H460 and A549 cells. Data points represent the mean surviving fraction ± SEM of 3 independent experiments. C, cell-cycle profile of H460 and A549 cells stained with propidium iodide after 48 hours treatment. Right, quantitation of sub-G1 fraction. Data are mean ± SEM from 3 independent experiments.
increased G2–M phase cell-cycle arrest at 48 hours following 4 Gy (H460, 10%–30%; A549, 19%–30%) and 10 Gy radiation (H460, 29%–50%; A549, 35% to 47%; Fig. 1C). Consistent with our earlier observation that short-term treatment (24 hours) with BEZ235 leads to a prolonged decrease in postradiation clonogenic survival (Fig. 1B), these data suggest that BEZ235 and radiation induce irreversible growth arrest in H460 and A549 cells.

**BEZ235 induces accelerated senescence after radiation in vitro**

As irreversible cell-cycle arrest is a feature of accelerated senescence, H460 and A549 cells were examined for senescence-associated β-galactosidase (SA-βGal) activity (Fig. 2A). In both cell lines, treatment with 100 nmol/L BEZ235 significantly enhanced SA-βGal staining after 4 Gy (H460, 3%–32%; A549, 9%–34%) and 10 Gy radiation (H460, 7%–61%; A549, 14%–44%; Fig. 2B). Untreated control cells, in contrast, exhibited no SA-βGal staining, thereby confirming the absence of replicative (nonaccelerated) senescence. Treatment with drug alone also did not result in a major senescence response indicating that BEZ235 promotes accelerated senescence in combination with but not independently of radiation. Notably, positive SA-βGal staining induced by BEZ235 in irradiated cells was accompanied by characteristic morphologic features of accelerated senescence.

![Figure 2](https://www.aacrjournals.org/MolCancerRes;2011/OF5)

**Figure 2.** BEZ235 induces accelerated senescence after radiation (IR) in vitro. A, representative images of SA-βGal activity after 48 hours treatment (×200 magnification). B, percentage of SA-βGal positive cells. Data are mean ± SEM from 3 independent experiments. **, P < 0.001; ***, P < 0.01; ***, P < 0.05. C, Western blot showing total p21 levels after 48 hours treatment in H460 and A549 cells. DMSO, dimethyl sulfoxide.
senescence (flattened, enlarged, elongated, and multinucleated cells). Interestingly, although multinucleation is also a feature of mitotic catastrophe, M phase (phospho-histone H3 positive) cells displaying typical changes of abnormal mitoses (multipolar spindles, multiple centrosomes, and microtubule misalignment; ref. 27) were infrequently seen irrespective of treatment (Supplementary Fig. S3).

Senescent cells commonly secrete high levels of proinflammatory cytokines and growth factors, a process known as the senescence-associated secretory phenotype (SASP; ref. 28). Using cytokine antibody arrays to analyze cell culture supernatants, we found that the combination of BEZ235 (100 nmol/L) and 4 Gy radiation noticeably elevated interleukin (IL) 8, macrophage migration inhibitory factor, (MIF) and plasminogen activator inhibitor-1 (PAI-1) levels in H460 cells and MIF levels in A549 cells (Supplementary Fig. S4). Importantly, these cytokines are all recognized as components of the SASP (29, 30). Senescent cells also typically exhibit upregulation of the cyclin-dependent kinase inhibitor (CDKI) p21, which is a key downstream target of p53 (31). Using Western blot analysis, we determined that the addition of BEZ235 increased p21 levels following 4 and 10 Gy radiation (Fig. 2C). Together, these results show that the response of irradiated H460 and A549 cells to BEZ235 is characterized by many of the key features of accelerated senescence.

BEZ235 delays repair of radiation-induced DNA DSBs in vitro

Sustained G2-M phase cell-cycle arrest is recognized as a classical cellular response to DNA damage (32). As previous reports indicate that up to 20 DNA DSBs are required for enforcing the G2-M checkpoint (33), we conducted staining for γH2AX (a marker of DNA DSBs) on H460 and A549 cells. In both cell lines, the addition of 100 nmol/L BEZ235 significantly increased the number of γH2AX foci per cell at 24 hours following 4 Gy (H460, 6.0–22.2; A549, 2.7–15.8) and 10 Gy radiation (H460, 12.7–31.3; A549, 8.0–45.3; Fig. 3). In contrast, treatment with BEZ235 did not increase γH2AX expression at early time points after radiation (Supplementary Fig. S5). Indeed, the rate of appearance of γH2AX foci formation was delayed by BEZ235 in irradiated cells suggesting that BEZ235 causes persistence of radiation-induced γH2AX foci by inhibiting DSB repair rather than augmenting DNA damage at earlier time points.

The major DNA DSB repair pathways in mammalian cells are the NHEJ and homologous recombination pathways, in which DNA-PK and ataxia telangiectasia mutated (ATM) play crucial roles (34). Therefore, having previously established that BEZ235 inhibits DNA-PKcs, we investigated its effects on ATM phosphorylation in irradiated cells. Consistent with the findings of Maira and colleagues (17), we determined that treatment with BEZ235 did not inhibit radiation-induced phosphorylation of ATM at S1981 in either H460 or A549 cells (Supplementary Fig. S6). Thus, DNA-PK, but not ATM, blockade underlies the inhibition of DSB repair by BEZ235.

BEZ235 sustains DNA DSBs and induces accelerated senescence after radiation in vivo

The in vivo activity of BEZ235 and radiation was assessed in H460 xenografts established subcutaneously in athymic...
nude mice. Preliminary experiments revealed that a radiation dose of 8 Gy delivered in 4 consecutive daily 2 Gy fractions elicited measurable antitumor activity without causing permanent tumor eradication (data not shown). We also established that the maximum tolerated dose of BEZ235 was 40 mg/kg administered orally once daily. Using this treatment schedule, we subsequently found that the combination of BEZ235 and radiation substantially increased tumor growth delay compared with either treatment alone (BEZ235 + radiation: 9.5 days vs. radiation: 3.5 days vs. BEZ235: 3.5 days; Fig. 4A).

In a parallel experiment, H460 tumors were harvested from mice 48 and 96 hours after a single 8 Gy radiation fraction and then examined for SA-βGal activity. While negligible staining was shown in tumors treated with BEZ235 alone (40 mg/kg daily on days 1 and 2), there was a striking increase in SA-βGal activity when BEZ235 was combined with radiation (Fig. 4B). This effect was most pronounced 96 hours after radiation and coincided with a marked reduction in cellular proliferation (Ki67). Staining for γH2AX was also conducted on H460 xenografts to assess the impact of combined treatment on the formation of DSBs. Although treatment with BEZ235 alone did not increase staining above that of untreated controls, γH2AX levels at 96 hours were markedly increased in irradiated tumors treated with BEZ235 (Fig. 4B). These data collectively indicate that the enhancement of radiation efficacy by BEZ235 in H460 xenografts involves both the accumulation of unrepaired DNA DSBs and the induction of accelerated senescence.

**Induction of accelerated senescence by BEZ235 in irradiated cells is p53 dependent**

Stimuli that activate the DNA damage response (DDR) primarily drive senescence through the p53–p21 signaling pathway (35). As both H460 and A549 cells have wild-type p53 (36), we investigated whether the senescence phenotype produced by BEZ235 in irradiated cells is p53 dependent. Initially, a panel of cell lines harboring wild-type (MCF-7, U87-MG; ref. 37) and deleted/mutated p53 (HT29, SKBR3, PC3, and A431; ref. 36) was screened for SA-βGal activity. Although BEZ235 strongly induced SA-βGal staining after radiation in p53 wild-type MCF-7 and U87-MG cells, no staining was
observed in the cell lines with mutated or absent p53 (Supplementary Fig. S7A).

Given these findings, SA-βGal staining was conducted on p53-null H1299 NSCLC cells cotransfected with vectors for the ecdysone receptor and inducible p53 (24). Addition of the ecdysone analogue Ponasterone A (Invitrogen) induced p53 and p21 expression in H1299 cells (Supplementary Fig. S7B), which in turn was associated with positive SA-βGal staining and morphologic features of senescence following treatment with BEZ235 and radiation (Supplementary Fig. S7C). In comparison, no SA-βGal activity was seen in H1299 cells in the absence of Ponasterone A. Together, these results confirm that accelerated senescence response following treatment with BEZ235 and radiation is dependent on the p53–p21 signaling axis.

Selective pharmacologic inhibition of DNA-PK leads to accelerated senescence in irradiated cells

To further investigate the significance of DNA-PK inhibition in the accelerated senescence response to radiation, we compared the activity of a selective DNA-PK inhibitor (KU557788) with that of BEZ235 in H460 cells. Following 10 Gy radiation, we observed that KU557788 did not alter sub-G1 levels (radiation: 13% vs. KU57788 + radiation: 11%) and had comparable effects to BEZ235 on γH2AX expression (45 vs. 40 foci per cell), G2–M growth arrest (50% vs. 55%), and SA-βGal staining (65% vs. 67%; Fig. 5A–C). Similar to BEZ235, we also established that p53 is required for KU557788 to induce accelerated senescence in irradiated cells (Fig. 5D). In contrast to these findings, selective PI3K (BKM120) and mTORC1 (RAD001) inhibitors used alone or in combination did not reproduce the effects of BEZ235 or KU557788 on DNA DSBs, G2–M cell-cycle arrest, or SA-βGal staining. Importantly, the activity of KU557788 was not related to inhibition of related kinases as it selectively inhibited DNA-PK (Supplementary Fig. S8), without influencing the activity of AKT, S6, or ATM (data not shown). Likewise, neither BKM120 nor RAD001 inhibited DNA-PK (Supplementary Fig. S8) at doses that were sufficient to suppress phosphorylation of AKT and S6, respectively (data not shown). These data collectively suggest that selective pharmacologic inhibition of DNA-PK leads to p53-dependent accelerated senescence after radiation.

DNA-PK knockdown by siRNA induces accelerated senescence in irradiated cells

On the basis of our findings with KU557788, we proceeded to knockdown DNA-PK in H460 cells using siRNA. Total DNA-PK expression was abolished by PRKDC siRNA in unirradiated cells, whereas a scrambled (control) siRNA sequence had no impact on DNA-PK levels (Fig. 6A). Similarly, phospho-DNA-PK expression was effectively reduced by PRKDC siRNA in irradiated cells (Fig. 6B, top), leading to persistence of γH2AX foci at 24 hours after radiation (Fig. 6B, middle). Significantly, the residual DNA DSBs were accompanied by a dramatic increase in SA-βGal staining (PRKDC siRNA 67% vs. vehicle 5% and scrambled siRNA 4%) and striking morphologic changes typical of accelerated senescence (Fig. 6B, bottom). These results show that selective targeting of DNA-PK is sufficient to induce accelerated senescence in irradiated cancer cells.

Discussion

Radiotherapy is a major treatment modality administered to approximately half of all patients with cancer (1). Unfortunately, a significant number of these patients develop and ultimately die of locally recurrent disease, emphasizing the need to identify novel means of enhancing radiation efficacy. In this study, we examined the radiosensitizing properties of BEZ235 in H460 and A549 NSCLC cells. We found that BEZ235 potentiated the antitumor activity of radiation in vitro and in vivo, consistent with recent reports of radiation enhancement by novel PI3K/mTOR/DNA-PK inhibitors (38, 39). Significantly, BEZ235 did not increase the radiosensitivity of H460 and A549 cells by augmenting radiation-induced apoptosis or mitotic catastrophe. Instead, irradiated cells treated with BEZ235 displayed many of the hallmarks of accelerated senescence (31) including irreversible cell-cycle arrest, positive SA-βGal staining, classical morphologic changes, and increased expression of p21 and a SASP.

Accelerated senescence is increasingly recognized as an important and therapeutically advantageous outcome of treatment with DNA-damaging agents (20, 40, 41). While several studies have previously shown that epithelial cancer cells readily undergo senescence after radiation treatment in vitro (reviewed in ref. 20), a recent report from Efimova and colleagues identified accelerated senescence in breast cancer xenografts treated with radiation and the PARP inhibitor ABT-888 (42). We similarly found that growth delay of irradiated H460 xenografts by BEZ235 was associated with a robust accelerated senescence response. As a result, our data identify BEZ235 as just the second pharmacologic agent to promote accelerated senescence after radiation in vivo.

The induction of accelerated senescence in irradiated cells and tumors treated with BEZ235 was associated with delayed repair of DNA DSBs. These findings mirror those of Efimova and colleagues and support an emerging paradigm that links cellular senescence with a prolonged DDR (33). While transient DNA damage signaling results in a reversible cell-cycle arrest, Rodier and colleagues recently showed that irreversible growth arrest and the acquisition of a SASP are dependent upon persistent activation of the DDR (43). Our data are consistent with this as the addition of BEZ235 to radiation resulted in SASP cytokine secretion and other features of accelerated senescence. In contrast, cells treated with radiation alone exhibited neither persistent DSBs nor a prominent senescence phenotype. Thus, residual DNA DSBs appear to be a crucial determinant of accelerated senescence after treatment with BEZ235 and radiation.
Figure 5. Selective pharmacologic inhibition of DNA-PK leads to accelerated senescence after radiation (IR). A, representative immunofluorescence images of γH2AX (Ser139) foci in H460 cells at 24 hours after 10 Gy radiation (×60 magnification). Cells were treated with vehicle, BEZ235 (250 nmol/L), KU57788 (1 μmol/L), BKM120 (1 μmol/L) or RAD001 (10 nmol/L). Green, γH2AX foci; blue, DAPI staining. Right, number of γH2AX foci per cell. Data are mean ± SEM from 3 independent experiments. **, P < 0.01; ***, P < 0.001. B, cell-cycle profile of H460 cells at 48 hours after 10 Gy radiation. Right, percentage of H460 cells arresting with 4n DNA content. Data are mean ± SEM from 3 independent experiments. **, P < 0.01. C, representative images of SA-βGal activity in H460 cells at 48 hours after 10 Gy radiation (×200 magnification). Right, percentage of SA-βGal-positive cells. Data are mean ± SEM from 3 independent experiments. **, P < 0.01; ***, P < 0.001. D, representative images of SA-βGal activity in p53-inducible H1299 cells at 48 hours after 10 Gy radiation (×200 magnification). PoA, Ponasterone A.
On the basis of these findings, we postulated that DNA-PK inhibition is a critical factor in the senescence response of irradiated cells to BEZ235. This assertion is supported by the observation that a selective DNA-PK inhibitor (KU557788) exerted comparable effects to BEZ235 on DNA repair and senescence following radiation. To confirm that inhibition of DNA-PK is sufficient to induce accelerated senescence after radiation, we then showed that knockdown of PRKDC with siRNA leads to accelerated senescence in irradiated H460 cells. Although previous studies have also reported that DNA-PK inhibitors retard DSB repair after radiation (44–47), the precise cellular mechanisms through which DNA-PK inhibition increases tumor radiosensitivity are currently unknown. Our data provide the first demonstration of a mechanistic link between DNA-PK knockdown and accelerated senescence in irradiated cells and indicate that accelerated senescence induced by DNA-PK blockade is a significant factor in radiosensitization of p53 wild-type tumors.

The results of this study highlight the therapeutic potential of combining DNA-PK blockade with radiation. Our findings are complemented by earlier reports of radiosensitization using selective (44–47) and nonselective (15, 16, 38) DNA-PK inhibitors. Importantly, in addition to attenuating NHEJ, pharmacologic inhibition of DNA-PK has been shown to suppress other major DSB repair pathways including homologous recombination (48) and backup-NHEJ (B-NHEJ; ref. 49). Thus, the pronounced radiosensitization achieved with DNA-PK inhibitors in this study and others is likely to reflect simultaneous activity against multiple DSB repair pathways. We speculate that concurrently inhibiting key molecules in each of these pathways may further compromise radiation-induced DSB repair, as has been previously shown by combining DNA-PK and PARP-1 inhibitors to target NHEJ and B-NHEJ, respectively (50). Moreover, by prolonging the DDR, this approach is likely to offer additional therapeutic benefits arising from an enhanced accelerated senescence response.

In conclusion, we have shown that selective targeting of DNA-PK induces p53-dependent accelerated senescence in irradiated human cancer cells. To the best of our knowledge, DNA-PK inhibitors such as BEZ235 are not currently being combined with radiation in clinical trials. Our data highlight the potential benefits of using DNA-PK blockade to modulate repair of therapeutically induced DSBs and thereby promote radiation-induced accelerated senescence. These findings provide a rationale for further preclinical and clinical evaluation of DNA-PK inhibitors in combination with anticancer agents that induce DSBs or inhibit DSB repair.

Disclosure of Potential Conflicts of Interest

S.-M. Maira is a stockholder and employee of Novartis Pharma AG. W. Hackl is an employee of Novartis Pharma AG. G.A. McArthur and B. Solomon are on an advisory board for Novartis Pharma AG. No potential conflicts of interest were disclosed by other authors.

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