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AZD5438, an Inhibitor of Cdk1, 2, and 9, Enhances the Radiosensitivity of Non-Small Cell Lung Carcinoma Cells

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Abstract

Purpose—Radiation therapy (RT) is one of the primary modalities for treatment of non-small cell lung cancer (NSCLC). However, due to the intrinsic radiation resistance of these tumors, many patients experience RT failure, which leads to considerable tumor progression including regional lymph node and distant metastasis. This preclinical study evaluated the efficacy of a new-generation cyclin-dependent kinase (Cdk) inhibitor, AZD5438, as a radiosensitizer in several NSCLC models that are specifically resistant to conventional fractionated RT.

Methods and Materials—The combined effect of ionizing radiation and AZD5438, a highly specific inhibitor of Cdk1, 2, and 9, was determined in vitro by surviving fraction, cell cycle distribution, apoptosis, DNA double-strand break (DSB) repair, and homologous recombination (HR) assays in 3 NSCLC cell lines (A549, H1299, and H460). For in vivo studies, human xenograft animal models in athymic nude mice were used.

Results—Treatment of NSCLC cells with AZD5438 significantly augmented cellular radiosensitivity (dose enhancement ratio ranging from 1.4 to 1.75). The degree of radiosensitization by AZD5438 was greater in radioresistant cell lines (A549 and H1299). Radiosensitivity was enhanced specifically through inhibition of Cdk1, prolonged G₂-M arrest, inhibition of HR, delayed DNA DSB repair, and increased apoptosis. Combined treatment with AZD5438 and irradiation also enhanced tumor growth delay, with an enhancement factor ranging from 1.2–1.7.

Conclusions—This study supports the evaluation of newer generation Cdk inhibitors, such as AZD5438, as potent radiosensitizers in NSCLC models, especially in tumors that demonstrate variable intrinsic radiation responses.

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Supplementary material for this article can be found at www.redjournal.org.

Introduction

Non-small cell lung cancer (NSCLC) is both the most prevalent type of lung cancer and the leading cause of cancer death worldwide. Up to 40% of NSCLC patients present with locally advanced and mostly inoperable disease (1). For patients who present with advanced disease, concurrent chemoradiation therapy remains the only effective treatment; combined therapy results in 2-year survival rates of between 8% and 43% (2). Poor overall survival rates in NSCLC patients may be attributed to the intrinsic radiation resistance of many tumors. Solid tumors, including NSCLC, are heterogeneous and contain subpopulations of cells with divergent levels of sensitivity to established cancer therapy including radiation therapy (RT). Perturbation of cell cycle regulation is a key factor in the development of most cancers (3). The regulatory proteins that control cell cycle progression are the cyclins, cyclin-dependent kinases (Cdks), and their substrate proteins Cdk inhibitors, tumor suppressor gene products, p53 and pRb. Several Cdk inhibitors including flavopiridol, indisulam, AZD5438, P276-00, EM-1421, seliciclib, PD0332991, and SCH727965 have entered clinical trials (4, 5) and have demonstrated promising outcomes especially in combination with other chemotherapeutic agents (4). Cdk inhibitors preferentially target proliferating cells, but these inhibitors can also induce cell death in noncycling radioresistant tumor subpopulations (6–8).

In this study, we tested the efficacy of AZD5438 (9), a new-generation inhibitor of Cdk 1, 2, and 9 in combination with fractionated RT in NSCLC cell lines (A549, H1299, and H460) and in animal models. AZD5438 significantly enhanced the effect of radiation in NSCLC cells. This enhanced radiosensitivity was due mostly to Cdk1 inhibition and was partially attributed to persistent DNA double-strand breaks (DSB) and the inhibition of DNA homologous recombination (HR) repair.

Methods and Materials

Cell culture and reagents

The human NSCLC cell lines H460, A549, and H1299 were kindly provided by Dr John D. Minna at University of Texas Southwestern Medical Center, Dallas, TX, and maintained in RPMI 1640 medium with 10% fetal bovine serum and 50 units/mL penicillin and 50 µg/mL streptomycin in 5% CO₂ at 37°C. AZD5438 (molecular weight, 471.36) was obtained from AstraZeneca (London, UK). Cells were irradiated using a ¹³⁷Cs source (Mark 1–68 irradiator; JL Shepherd and Associates, San Fernando, CA) at a dose rate of 3.47 Gy/min (8).

Clonogenic survival assay

Cells were treated with AZD5438 for 24 h and then treated with increasing doses of IR (0, 2, 4, 6, and 8 Gy). Colony formation assay (CFA) and determination of dose enhancement ratio (DER) were performed as described previously (7, 8). CFA was also performed using short interfering RNA (siRNAs) against Cdk1 and Cdk9 (Life technologies Grand Island, NY) and Cdk2 (Dharmacon, Inc Chicago, IL). Cells were transiently transfected with either individual siRNAs or scrambled siRNAs. After 48 h, cells were plated for CFA and Western blot analysis.

Western blot assay

Cell lysates were prepared from each sample, and total protein (20 µg) was subjected to immunoblot analysis and probed with antibodies as indicated. β-Actin was used for loading control.

Double-strand break repair assay

DSB repair assay was performed as described previously (10). The number of phospho- γ H2AX foci (green) was determined at each time point (average of 50 nuclei), and the percentage of foci remaining was plotted against time to obtain DSB repair kinetics (10). Data is represented as mean $\pm$ SEM.

Direct repeat-green fluorescent protein HR assay

The direct repeat-green fluorescent protein (DR-GFP) assay was performed as described by Chan et al (11). Transient expression of the I-SceI endonuclease generates a DNA DSB at the integrated GFP gene sequences and stimulates HR. For each experiment, 50,000 cells were scored per treatment group, and the frequency of recombination events was calculated from the number of GFP-positive cells divided by the number of cells analyzed after correction for transfection efficiency.

Cell cycle analysis

Cells cycle assays were performed with propidium iodide (PI, 100 μ g/mL) as previously described (10, 12). At least 20,000 cells were counted, and the proportions of cells at different phases were gated and calculated using Flowjo version 8.7.1 software (Tree Star, Inc).

Tumor growth delay

Female athymic nude mice (nu/nu, 5–6 weeks old) were injected (1×10^6 cells in 100 μ L) subcutaneously into the right posterior flanks. Tumors were treated when they reached 2–3 mm in diameter. Treatment groups (5 animals per group) included untreated control (treated with 0.9% saline), those treated with AZD5438 (25 mg/kg/day for 5 days, by mouth [po]), with radiation (2 Gy/day, 5 days), and those that received combined treatment with AZD5438 and IR. AZD5438 was administered 1 h before radiation. Tumor growth delay and the dose enhancement factor were then determined (7). All experiments were conducted under Institutional Animal Care and Use Committee of UTSW approved guidelines for animal welfare.

Statistical analysis

Statistical analysis of DSB repair and HR assays were done using 1-sided unpaired *t*-tests. Clonogenic survival curves were modeled with the linear quadratic equation ($S = e^{-(\alpha D + \beta D^2)}$) for radiation treatment and a four-parameter variable slope regression for drug toxicity.

Results

Specificity of AZD5438 in NSCLC cells

AZD5438 inhibits Cdk 1, 2, and 9, therefore, the levels of Cdk protein in 3 NSCLC cell lines were determined. The relative expression levels of these 3 proteins were similar in all 3 cell lines (Fig. 1A). Next, the toxicity of AZD5438 in these cells was analyzed by CFA. H1299 cells (96.3 nM) and A549 cells (208 nM) cells were highly sensitive, while H460 was the most resistant (435.8 nM) to AZD5438 (Fig. 1B). AZD5438 specifically inhibited the phosphorylation of Rb (pSer₇₈₀) by inhibiting Cdk2 activity (Fig. 1C), whereas the activity levels of other cell cycle regulatory proteins such as Chk1 (pSer₁₃₇) and Chk2 (pThr₆₈) were not perturbed. These results show that AZD5438 is highly specific to Cdks and demonstrates differential sensitivity in NSCLC cells.

AZD5438 increases the sensitivity of NSCLC cells to IR in vitro and in vivo

Radiation caused a dose-dependent reduction in clonogenic survival in all NSCLC lines, and we found significant variation in intrinsic radiosensitivity (Fig. 2A-2C, left panels). The SF (surviving Fraction) at 2 Gy (SF₂) of the A549, H460, and H1299 cells were 0.84, 0.44, and 0.72, respectively. However, these SF₂ values were significantly decreased to 0.44, 0.23, and 0.36 for the A549, H460, and H1299 cells, respectively, upon treatment with AZD5438 and IR. The DER at 10% survival was 1.5 for A549, 1.3 for H460, and 1.3 for H1299 cells. SF at different IR doses and the corresponding DERs are listed in Supplementary Table ES1. To further analyze the efficacy of AZD5438 as a radiosensitizer in vivo, tumor growth delay assays were performed in athymic nude mice (Fig. 2A-2C, right panels). Tumors were treated with AZD5438 (po) 1 h prior to IR. For A549 xenografts (Fig. 2A), a tumor volume of 800 mm³ was reached in 41, 43, 46, and 55 days for the control, AZD5438 alone, IR alone, and IR plus AZD5438 groups, respectively, which resulted in a dose enhancement factor of 2.4. Whereas the dose enhancement factors for H1299 and H460 xenografts were 1.8 and 0.6, respectively (Fig. 2C and 2D, right panel). These results demonstrated that A549 and H1299 xenografts are highly responsive to the combined treatment of AZD5438 plus IR.

Cdk1 siRNA significantly enhanced IR sensitization

Because AZD5438 inhibits Cdk 1, 2, and 9, further analysis of the role of each individual Cdk in IR sensitization was performed using siRNAs. A significant decrease in the expression level of targeted Cdk was achieved in all cell lines (Fig. 3A-3E, inset). Cdk1 inhibition particularly caused IR sensitization in A549 (Fig. 3A) and H1299 cells (Fig. 3E). Modest sensitization was observed in A549 cells when Cdk2 was knocked down (Fig. 3B), whereas no effect was observed when Cdk9 was completely ablated in A549 cells (Fig. 3C). SF and DER values upon Cdk1 inhibition are listed in Supplementary Table ES2. Interestingly, Cdk1 knockdown did not cause IR sensitization in H460 cells (Fig. 3D). Cdk2 and Cdk9 inhibition did not cause IR enhancement in H1299 and H460 cells (data not shown). These results indicate that the inhibition of the Cdk1 pathway by AZD5438 may be associated with IR sensitization in A549 and H1299 cells.

AZD5438 inhibits HR repair

Several reports describe the role of Cdk1 in DNA HR repair processes (13, 14), and a recent study with a dual kinase inhibitor, NU6027 (ATR: ATM and Rad3-related and Cdk2), inhibited Rad51 foci formation and blocked HR repair (15). The effect of AZD5438 on HR repair was then studied using H1299 cells containing an integrated DR-GFP HR reporter in which functional GFP can only be restored by HR repair (Fig. 4A) (11). H1299 cells were transfected with vector encoding I-SceI endonuclease to generate a DSB, with pGFP for transfection efficiency control, and with phCMV-1 I-SceI negative control in the presence or absence of AZD5438 (75 nM). Results clearly showed that AZD5438 reduced the frequency of HR by almost 50% (Fig. 4B and 4C). There was also a noticeable decrease in Rad51 expression after treatment with AZD5438 (Fig. 4C, right panel).

IR sensitization by AZD5438 is associated with delayed DSB repair

To measure the induction and repair of IR-induced DSBs, the 3 NSCLC cells were exposed to AZD5438 for 24 h, followed by IR (Fig. 5A-5C). The effect of AZD5438 treatment on γ H2AX foci (a surrogate for DNA DSB repair) formation is shown in Fig. 5A, indicating no induction of foci formation. In all 3 cell lines, almost complete (>95%) repair occurred within 8 h post-IR (Fig. 5B and 5C) but nearly 30%–40% of foci were retained at 8 h when treated with IR plus AZD5438. At 24 h, the foci count returned to baseline in H460 cells,

while in A549 and H1299 cells, a small number of foci (5%–10%) remained. These results indicate that AZD5438 modulates the repair kinetics of radiation-induced γ H2AX foci.

AZD5438 treatment selectively induced G₂-M checkpoint arrest in NSCLC cells

The NSCLC cells showed G₂ arrest at 8 h post-IR (2 Gy). Results shown in Figure 6 indicate that approximately 50% of A549 cells were blocked at G₂-M after 24-h treatment of AZD5438, whereas 32% of H1299 and 11% of H460 cells were arrested at the same time. When these cells were treated with AZD5438 (after 24 h) and IR (at 8 h), 68% of A549 and 49% of H1299 cells were blocked at the G₂-M checkpoint compared to only 25% of H460 cells under similar conditions. In an additional study, apoptosis was measured at 24 and 48 h after treatment with AZD5438 and radiation. Combined treatment enhanced apoptosis specifically in A549 (~5-fold) and H1299 (~3-fold) cells at 48 h after treatment (see Supplemental Table ES3). Taken together, these results clearly indicate that the combined treatment of IR plus AZD5438 causes significant delay in DSB repair, prolonged G₂-M blockage, and enhanced apoptosis, which may contribute to less survival.

Discussion

Many lung cancers, especially NSCLC, display intrinsic radiation resistance. Previous studies with older generation Cdk inhibitors have shown increased radiation sensitivity (7, 8). SF and DER of the three NSCLC cell lines used in this study changed significantly when combined with AZD5438 treatment. Several mechanisms could contribute to AZD5438-mediated radiosensitizing effects in NSCLC cells. Cdk2, it has been suggested, compensates for lack of Cdk1 (14); however, an important finding of this study (Fig. 3) indicates that AZD5438-mediated radiosensitivity is induced mostly through the inhibition of Cdk1 alone and only modestly through the Cdk2 pathway. These results are also in agreement with the important physiological roles of Cdk1 such as (1) promotion of mitotic entry, (2) inhibition that causes G₂ arrest, and (3) involvement in DNA repair through HR (5, 13). Results shown in Figure 4 indicate that AZD5438 causes significant reduction (50%) in HR repair in H1299 cells. Thus, the blockage of Cdk1 may be the mechanism by which NSCLC cells are sensitized to IR.

Generally, cells show significant variation in their radiation sensitivity based on the cell cycle, with G₂-M being the most radiation-sensitive phase (16). Radiation-induced arrest at G₂-M is critical in preventing cell death. This study shows that in combination with radiation, AZD5438 treatment causes a greater number of cells to be arrested at G₂-M, at least in the A549 and H1299 cell populations, which implies a larger proportion of cancer cells are disrupted by combination therapy.

Cell cycle inhibitors, such as flavopiridol, have been shown to induce apoptosis in a variety of cell lines (17). AZD5438 alone enhances apoptosis nearly 3.0-fold in A549 cells, whereas AZD5438 combined with IR increases the number of apoptotic cells approximately fivefold in A549 cells and threefold in H1299 cells after 48 h. The smaller population of cells undergoing apoptosis after combined treatment with AZD5438 plus IR may indicate that other modes of cell death are involved; the exact mechanism is currently under investigation. There are reports demonstrating that Cdk1 is involved in several modes of cell death including apoptosis and mitotic catastrophe (5, 18).

Although all 3 cell lines tested in this study had similar levels of Cdk1, -2, and -9 as well as similar levels of all the other cell cycle regulatory proteins (see Supplementary Table ES4), they displayed divergent levels of sensitivity to AZD5438. In addition, all 3 cell lines carried a variety of mutations (see Supplementary Table ES5), but none was exclusive to the responding cell lines or explained this divergence. This is also an important finding of this

study, indicating that the patient population with the H460 phenotype (metastatic large-cell carcinoma) will not receive significant therapeutic advantage from treatment with combined radiation and this class of cell cycle inhibitor. While Cdk inhibition may be an effective strategy for inducing radiation sensitivity, it is clear that the differential patterns of tumor growth and response to therapy (radiation, drug, and both) lie in the genetic background of each cell line, which ultimately regulates therapeutic outcome. Therefore, patient selection is extremely important in ensuring the highest efficacy is received when combined treatments of radiation and Cdk inhibitors are administered.

Conclusions

In conclusion, AZD5438 enhances radiation-induced cell death by blocking Cdk1 in A549 and H1299 cells. This enhanced radio-sensitivity is associated with inhibition of DNA DSB repair processes through HR repair. While clinical development of AZD5438 has been discontinued due to low tolerability in phase II studies (19), several other Cdk inhibitors such as SCH727965, P276-00, and EM-1421, which belong to the same new generation of inhibitors, are currently under investigation in phase I/II trials for treatment of both solid tumors and chronic lymphocytic leukemia (5). This preclinical study confirms that Cdk inhibitors are potent radiation-sensitizing agents and are promising candidates for clinical evaluation as part of a combined regimen.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. *CA Cancer J Clin.* 2008; 58:71–96. [PubMed: 18287387]
2. Jassem J. The role of radiotherapy in lung cancer: where is the evidence? *Radiother Oncol.* 2007; 83:203–213. [PubMed: 17482301]
3. Sherr CJ. G1 phase progression: cycling on cue. *Cell.* 1994; 79:551–555. [PubMed: 7954821]
4. Dickson MA, Schwartz GK. Development of cell-cycle inhibitors for cancer therapy. *Curr Oncol.* 2009; 16:36–43. [PubMed: 19370178]
5. Manchado E, Guillaumot M, Malumbres M. Killing cells by targeting mitosis. *Cell Death Differ.* 2012; 19:369–377. [PubMed: 22223105]
6. Bible KC, Kaufmann SH. Flavopiridol: a cytotoxic flavone that induces cell death in noncycling A549 human lung carcinoma cells. *Cancer Res.* 1996; 56:4856–4861. [PubMed: 8895733]
7. Kim JC, Saha D, Cao Q, et al. Enhancement of radiation effects by combined docetaxel and flavopiridol treatment in lung cancer cells. *Radiother Oncol.* 2004; 71:213–221. [PubMed: 15110456]
8. Kodym E, Kodym R, Reis AE, et al. The small-molecule Cdk inhibitor, SNS-032, enhances cellular radiosensitivity in quiescent and hypoxic non-small-cell lung cancer cells. *Lung Cancer.* 2009; 37:47. [PubMed: 19193471]
9. Byth KF, Thomas A, Hughes G, et al. AZD5438, a potent oral inhibitor of cyclin-dependent kinases 1, 2, and 9, leads to pharmacodynamic changes and potent antitumor effects in human tumor xenografts. *Mol Cancer Ther.* 2009; 8:1856–1866. [PubMed: 19509270]

10. Kong Z, Xie D, Boike T, et al. Downregulation of human DAB2IP gene expression in prostate cancer cells results in resistance to ionizing radiation. *Cancer Res.* 2010; 70:2829–2839. [PubMed: 20332235]
11. Chan N, Koritzinsky M, Zhao H, et al. Chronic hypoxia decreases synthesis of homologous recombination proteins to offset chemoresistance and radioresistance. *Cancer Res.* 2008; 68:605–614. [PubMed: 18199558]
12. Kong Z, Raghavan P, Xie D, et al. Etoposide B confers radiation dose enhancement in DAB2IP gene knock-down radioresistant prostate cancer cells. *Int J Radiat Oncol Biol Phys.* 2010; 78:1210–1218. [PubMed: 20970033]
13. Ira G, Pellicioli A, Balijja A, et al. DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. *Nature.* 2004; 431:1011–1017. [PubMed: 15496928]
14. Johnson N, Li YC, Walton ZE, et al. Compromised CDK1 activity sensitizes BRCA-proficient cancers to PARP inhibition. *Nat Med.* 2011; 17:875–882. [PubMed: 21706030]
15. Peasland A, Wang LZ, Rowling E, et al. Identification and evaluation of a potent novel ATR inhibitor, NU6027, in breast and ovarian cancer cell lines. *Br J Cancer.* 2011; 105:372–381. [PubMed: 21730979]
16. Gorski DH, Beckett MA, Jaskowiak NT, et al. Blockage of the vascular endothelial growth factor stress response increases the antitumor effects of ionizing radiation. *Cancer Res.* 1999; 59:3374–3378. [PubMed: 10416597]
17. Melillo G, Sausville EA, Cloud K, et al. Flavopiridol, a protein kinase inhibitor, down-regulates hypoxic induction of vascular endothelial growth factor expression in human monocytes. *Cancer Res.* 1999; 59:5433–5437. [PubMed: 10554012]
18. Castedo M, Perfettini JL, Roumier T, et al. Cyclin-dependent kinase-1: linking apoptosis to cell cycle and mitotic catastrophe. *Cell Death Differ.* 2002; 9:1287–1293. [PubMed: 12478465]
19. Boss DS, Schwartz GK, Middleton MR, et al. Safety, tolerability, pharmacokinetics and pharmacodynamics of the oral cyclin-dependent kinase inhibitor AZD5438 when administered at intermittent and continuous dosing schedules in patients with advanced solid tumours. *Ann Oncol.* 2010; 21:884–894. [PubMed: 19825886]

Summary

This preclinical study evaluated the efficacy of new therapies in the treatment of lung cancer. The combination of a new generation Cdk inhibitor, AZD5438, and conventional radiation therapy was tested in several non-small-cell lung cancer (NSCLC) models. Treatment with AZD5438 significantly enhanced the response of NSCLC cells to radiation, both in vitro and in vivo. These findings indicate that Cdk inhibitors are promising candidates for clinical evaluation as adjuvant therapy for NSCLC.

Fig. 1. Specificity of AZD5438 in human NSCLC cells. (A) Immunoblot analysis of Cdk1, Cdk2, and Cdk9 in NSCLC cells. (B) Clonogenic survival assay for drug sensitivity determination. NSCLC cells were plated with or without AZD5438 (5 nM, 50 nM, 500 nM, 5 μ M, and 50 μ M). Colonies were counted and percentage of survival was plotted against log [Dose]. (C) AZD5438 prevents Cdk2 activity. H460 cells were treated with nocodazole (50 ng/mL) for 16 h to synchronize cells in M phase. Cells were washed and treated with AZD5438 (435 nM) for 4 h, followed by immunoblot analysis of pRb (Ser₇₈₀). Total Rb protein was used for loading control. (D) AZD5438 does not abrogate Chk1/2 activation. Both A549 (250 nM) and H460 (435 nM) cells were treated with AZD5438 for 24 h and treated with IR (5

Gy) for 30 min. Immunoblot analysis using pChk1(Ser₃₁₇) and pChk2 (T₆₈). Total Chk1 and Chk2 proteins and actin were used for loading control.

Fig. 2.

AZD5438 increased the sensitivity of NSCLC cells to IR. (A-C, left panels) Clonogenic survival of NSCLC cells with or without AZD5438. A549 (75 nM), H1299 (50 nM), and H460 (200 nM) cells were treated with AZD5438 for 24 h and treated with IR as indicated. Cells were trypsinized immediately and colony formation was counted. (A-C, right panels) AZD5438 in combination with IR enhanced the tumor growth delay in NSCLC cells: subcutaneous tumors were treated, as indicated, with IR (2 Gy per day, 5 days), AZD5438 (25 mg/kg/day for 5 days), and IR plus AZD5438 (25 mg/kg/day plus 2 Gy per day for 5 days). AZD5438 was administered po 1 h before IR. Tumor volume (mm³) was measured twice per week and was plotted against days.

Fig. 3.

IR sensitization of NSCLC cells transfected with Cdk siRNAs. All 3 NSCLC cells were transfected with either Cdk-specific siRNAs (200 pmol) (A-E) or scrambled siRNAs (200 pmol) using Lipofectamine 2000. After 48 h, cells were trypsinized, counted, and plated for CFA. Remaining cells were lysed and subjected to immunoblot assay for detecting Cdk (insets). Three hours after plating, cells were irradiated, and colonies were counted after 10 days. (A) A549 cells with Cdk1 siRNA; (B) A549 cells with Cdk2 siRNA; (C) A549 cells with Cdk9 siRNA; (D) H460 cells with Cdk1 siRNA; (E) H1299 cells with Cdk1 siRNA.

Fig. 4.

AZD5438 inhibits HR repair. (A) DR-GFP construct schematic for HR repair assay. (B) H1299 cells containing a DR-GFP construct were transfected with pCMV-1-I-SceI (negative control), pCMV3xnl-I-SceI (functional endonuclease), or pGFP (transfection efficiency control), and 75 nM of AZD5438 was added as indicated. GFP signal was assayed at 3 days post-transfection with a FACSCalibur flow cytometer. A total of 50,000 cells were scored per treatment group. (C, left and middle panels) The frequency of recombination events was calculated from the number of GFP-positive cells divided by the number of cells analyzed following correction for transfection efficiency. (Right panel) Immunoblot analysis of Rad51 expression in H1299 cells treated with AZD5438 (75 nM) for 72 h, lysed for immunoblotting.

Fig. 5.

IR sensitization by AZD5438 was associated with delayed DSB repair in NSCLC cells. (A) Effect of AZD5438 on DNA DSB in 3 NSCLC cell lines. (B) DNA DSB repair kinetics in NSCLC cell lines. Cells were incubated with or without AZD5438 for 24 h (210 nM, 90 nM, and 435 nM for A549, H1299 and H460, respectively), irradiated (2 Gy), immunostained for γ H2AX foci, and counted for each time point (average, 50 nuclei). (C) Repair kinetics of NSCLC cells was obtained by plotting the percentage of remaining foci against time; D, AZD5438; R, radiation.

Fig. 6.

AZD5438 exhibited G₂-M checkpoint arrest in NSCLC cells. Cells were treated with AZD5438 followed by IR (2 Gy) and collected 8 h post-IR. (A) Cells were stained with PI to detect distribution of cell cycle after the treatments. (B) Quantitation of the percentage of cells at different phases of the cell cycle in response to drug, IR, and IR plus drug.