Loss of PPP2R2A Inhibits Homologous Recombination DNA Repair and Predicts Tumor Sensitivity to PARP Inhibition

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Abstract

Reversible phosphorylation plays a critical role in DNA repair. Here, we report the results of a loss-of-function screen that identifies the PP2A heterotrimERIC serine/threonine phosphatases PPP2R2A, PPP2R2D, PPP2R5A, and PPP2R3C in double-strand break (DSB) repair. In particular, we found that PPP2R2A-containing complexes directly dephosphorylated ATM at S367, S1893, and S1981 to regulate its retention at DSB sites. Increased ATM phosphorylation triggered by PPP2R2A attenuation dramatically upregulated the activity of the downstream effector kinase CHK2, resulting in G1 to S-phase cell-cycle arrest and downregulation of BRCA1 and RAD51. In tumor cells, blocking PPP2R2A thereby impaired the high-fidelity homologous recombination repair pathway and sensitized cells to small-molecule inhibitors of PARP. We found that PPP2R2A was commonly downregulated in non–small cell lung carcinomas, suggesting that PPP2R2A status may serve as a marker to predict therapeutic efficacy to PARP inhibition. In summary, our results deepen understanding of the role of PP2A family phosphatases in DNA repair and suggest PPP2R2A as a marker for PARP inhibitor responses in clinic.

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Introduction

Genome stability is essential for the prevention of undue cellular death and cancer development. To maintain genome integrity, cells have evolved multiple DNA repair pathways (1). One of the most powerful activators of the DNA repair response is double-strand breaks (DSB).

Initiation of DSB repair is controlled by the phosphoinositide 3-kinase–like kinase (PIKK) family. A wave of phosphorylation events radiating from PIKKs is amplified to convey the signals to a large number of substrates. Although this cascade has been studied in a great detail, the biologic relevance of many of these phosphorylation events and the mechanisms that control their downregulation remain unknown (2). It is conceivable that Ser/Thr protein phosphatases could be responsible for keeping proteins involved in DNA repair response in an inactive state under normal conditions or for inactivating the signaling once DNA has been repaired. However, phosphorylation of a number of PIKKs, including ataxia telangiectasia mutated (ATM), ATR, and CHK2, oscillates during the DNA repair process (3, 4), suggesting that phosphatases may serve not only as negative regulators but also as active modulators of DNA damage response.

Protein phosphatase 2A (PP2A) refers to a large family of heterotrimERIC Ser/Thr phosphatases. The PP2A core enzyme consists of a catalytic C subunit and a structural A subunit. In mammals, 2 distinct genes encode closely related versions of both the PP2A A (Aα/PPP2R1A and Aβ/PPP2R1B) and C (Cα/PPP2CA and Cβ/PPP2CB) subunits. The AC dimer recruits a third regulatory B subunit, which is responsible for the substrate specificity and function of the PP2A heterotrimERIC complex. Four unrelated families of B subunits have been identified to date: B/B55/PPP2R2A, B'/B56/PR61/PPP2R5, B'/PR72/PPP2R3, and Striatin/STRN. Approximately 100 distinct complexes can be formed through combinatorial associatiON of these subunits, and it is believed that specific PP2A complexes mediate particular physiologic functions (5, 6).

PP2A has been directly implicated in the negative regulation of DSB DNA repair proteins, including γ-H2AX, ATM, CHK1, and CHK2 (2). However, consistent with the idea that protein phosphatases are not just negative regulators of DNA repair signaling, selective inhibition of PP2A activity impairs DNA repair (7–9). PP2A function is also essential for activation of cell-cycle checkpoints in response to irradiation (10, 11). One potential explanation for this apparent discrepancy is that several distinct PP2A complexes may modulate different steps of the DNA repair process.

Here, we assessed the role of specific PP2A complexes in control of DSB repair and identify PPP2R2A as a critical effector of the homologous recombination (HR) repair through modulation of ATM phosphorylation. Critically, defects of HR DNA repair in PPP2R2A-depleted cells dramatically increased...
their sensitivity to PARP enzyme inhibition. Finally, we show that PPP2R2A is commonly downregulated in non–small cell lung carcinomas (NSCLC) and therefore propose PPP2R2A status as a predictive marker for sensitivity to PARP inhibition.

Materials and Methods

Plasmids and cell lines

Lentiviral pLA CMV N-Flag vector was used to generate Flag-tagged PPP2R2A. The pLKO.1-puro shGF, pLKO.1-puro shLuc, and pLKO.1-puro vectors containing short hairpin RNA (shRNA) targeting specific PP2A subunits were provided by the RNAi Consortium (12). The RNAi experiments were carried out using ON-TARGETplus SMARTpools against PPP2R2A and PPP2CA (Thermo Scientific).

Cells were cultured in Dulbecco’s Modified Eagle Medium or RPMI (GIBCO) supplemented with 10% FBS and penicillin/streptomycin. Lentiviral infections were carried out as described previously (12). To induce DSBs, cells were treated with bleomycin (Sigma-Aldrich) or irradiated using the linear accelerator (6 MV photons, Varian Medical Systems).

Cell-cycle analysis

Cells were washed with PBS, fixed with 70% ethanol, treated with 100 µg/mL RNase A, and stained with 50 µg/mL propidium iodide (PI), 5-Bromo-2′-deoxyuridine (BrdUrd) incorporation was analyzed using the In Situ Cell Proliferation Kit FLUOS (Roche) according to the manufacturer’s protocol. Cell-cycle distribution and BrdUrd immunostaining were assessed by FACSCanto (Becton-Dickinson).

Immunoblotting and immunoprecipitation

Cells were re-suspended in 0.5% Triton X-100, 100 mmol/L NaCl, 3 mmol/L MgCl2, 300 mmol/L sucrose, 1 mmol/L EGTA, 10 mmol/L PIPES, pH 6.8, containing protease inhibitor and phosphatase inhibitor cocktails (Roche). The cell lysates were incubated on ice for 10 minutes and then centrifuged at 500 × g for 5 minutes at 4°C. The supernatant and pellet were designated as cytoplasmic and nuclear fractions, respectively. The nuclear fraction was re-suspended in 10 mmol/L NaCl, 5 mmol/L MgCl2, 250 mmol/L sucrose, 1 mmol/L EGTA, 10 mmol/L Tris-HCl, pH 7.6, containing protease and phosphatase inhibitor cocktails and treated with RNase-free DNase I (80 µg/mL; Roche) for 30 minutes at 37°C.

Immunoprecipitations were conducted 24 hours after transfection in lysis buffer containing 50 mmol/L Tris, pH 7.4, 1% NP-40, 250 mmol/L NaCl, and proteinase inhibitor cocktail (Roche). The protein lysates were precleared by incubation with sepharose-A beads and incubated with anti-Flag M2 beads (Sigma-Aldrich) or with anti-ATM antibody (Novus Biologicals) and sepharose-protein A beads (GE Healthcare) overnight at 4°C. Flag complexes were eluted using 3×Flag peptide (Sigma-Aldrich).

The following antibodies were used: goat polyclonal anti-ATM, (Novus Biologicals), mouse monoclonal anti-PP2AC (BD Biosciences, clone 46), and mouse monoclonal anti-BRCA1 (Santa Cruz, clone D-9). The following antibodies were purchased from Cell Signaling: rabbit polyclonal anti-Flag, rabbit polyclonal anti-phospho-CHK1 (S296), rabbit polyclonal anti-phospho-CHK2 (T68), rabbit polyclonal anti-phospho BRCA1 (S1524), rabbit polyclonal anti-CDC25A, rabbit polyclonal anti-PP2R2A, mouse monoclonal anti-phospho-ATM (S981; clone 10H11.E12), mouse monoclonal anti-CHK2 (clone 1C12), mouse monoclonal anti-PP2A A (clone 4G7), rabbit polyclonal anti-phospho-CHK1 (S317), and rabbit monoclonal anti-phospho-CHK1 (S345; clone I33D3).

Neutral comet assay

Microscope slides were precoated with a thin layer of normal melting point agarose. Cell suspensions were placed into a tube containing low melting point agarose and transferred to a glass microscope slide. Slides were placed in 2 mol/L NaCl, 30 mmol/L Na2EDTA, 10 mmol/L Tris-HCl, 1% Triton X-100, and 10% dimethyl sulfoxide (DMSO), pH 8.0, for 1 hour at 4°C. After lysis, the slides were rinsed for 20 minutes in solution 0.3 mol/L NaOH, 1 mmol/L EDTA and were subjected to electrophoresis at 0.7 V/cm and 50 mA for 20 minutes. The slides were then rinsed with 1 mol/L Tris-HCl, pH 7.5 and dried with prechilled absolute ethanol and stained with PI (2 µg/mL). Comet images were examined at ×200 magnification with Nikon ECLIPSE Ti microscope. The tail moment values were quantified by CometScore software v1.5.

Immunofluorescent microscopy

Cells were plated on cover slips or μClear-96 well plates (Greiner Bio-One) and fixed with 4% paraformaldehyde for 10 minutes at room temperature and permeabilized with ice-cold methanol for 5 minutes at ~20°C. The cells were incubated in blocking buffer 3% bovine serum albumin (BSA) in PBS for 30 minutes at room temperature and then with primary antibodies. After 3 washes with 0.3% BSA in PBS, the secondary antibody was added. The following antibodies were used: mouse monoclonal anti-γ-H2AX (S139; Millipore, clone JW301) and mouse monoclonal anti-RAD51 (Abcam, clone 14B4).

Automated Image analysis was conducted using IN Cell Analyzer 2000 (GE Healthcare; Supplementary Figs. S1 and S2).

Quantitative real-time reverse transcription PCR

Total RNA was isolated using RNeasy (Qiagen), and cDNA was synthesized using the Advantage RT-PCR Kit (Clontech). A list of primers used for real-time quantitative PCR is presented in Supplementary Fig. S3A. Real-time PCRs were carried out in a Roche LightCycler-480-96 (Roche), using SYBR Green PCR Master Mix (Roche).

Quantitative real-time reverse transcription PCR (qRT-PCR) analysis of The Lung Cancer CDNA and Cancer Survey cDNA arrays (OriGene) was conducted using an ABI 7500 (Applied Biosystems).

NHEJ and HR DNA repair assays

Nonhomologous end joining (NHEJ) repair efficiency was analyzed using H1299dA3-1 cells, which contain a chromosomally integrated copy of the pIRES-TK-EGFP DNA construct (a generous gift of Dr. Kohno, National Cancer Center Research Institute, Tokyo, Japan; ref. 13). To assess HR efficiency, we...
used 293 DR-GFP cells, which contain a single integrated copy of the transgenic reporter DR-GFP. H1299dA3-1 and 293 DR-GFP cells were transfected with shRNAs against luciferase or PPP2R2A. Following puromycin selection, cells were transfected with I-Scel expression vector. The number of GFP-positive cells was measured 48 hours later by flow cytometric analysis using a FACSCanto (Becton-Dickinson).

In vitro phosphatase assay
293T cells were transfected with Flag-tagged PPP2R2A, Wip1, and a kinase-dead Wip1-D314A mutant. FLAG-tagged proteins were immunoprecipitated in anti-Flag (M2)-coated 96-well plates (Sigma-Aldrich). Immunoprecipitated complexes were incubated with phosphopeptides for 30 minutes at 37°C. Release of free phosphate was measured 20 minutes after addition of Malachite Green solution using a VictortmX3 Multilabel plate reader (Perkin Elmer).

Clonogenic survival assay and cell viability
About 10^5 cells per 10-cm dish were plated in triplicate and then irradiated or treated with ABT-888 (Enzo Life Sciences). After 12 days, colonies were stained with crystal violet (Sigma-Aldrich) and counted using ImageJ software. Cell viability was analyzed with a Vi-Cell XR counter.

Human xenograft assays
Xenografts were established in female NMRI-nu (nu/nu)/6-week mice (Janvier) with A549 cell line by s.c. injection (2.0 × 10^6 cells per 0.2 mL/mouse). Tumors were staged to a pre-conducted according to the manufacturer’s protocol. Situ Death detection kit, counterstained with hematoxylin. For apoptosis detection In R.T.U. Vectastain kit (Vector laboratories). Sections were Ki67 antibody (Dako). Immunodetection was conducted with phosphoproteptides for 30 minutes at 37°C. Release of free phosphate was measured 20 minutes after addition of Malachite Green solution using a VictortmX3 Multilabel plate reader (Perkin Elmer).

Results
Identification of PPP2A specific complexes involved in DSB repair
Although previous studies showed that PPP2A negatively regulates PIKK-induced signaling, more recent reports have shown that PPP2A inhibition impairs DNA repair (7, 8). To further investigate this apparent conundrum, we silenced the more abundant PPP2A catalytic subunit, PPP2CA. Because cells expressing very low levels of PPP2CA proliferate poorly (14, 15), we used shRNAs that only partially suppress PPP2CA expression (Fig. 1A). Consistent with a previous report (7), both immunoblot analysis of γ-H2AX and automatic image analysis of γ-H2AX immunostaining revealed that partial depletion of PPP2CA resulted in inefficient DNA repair (Fig. 1A and B).

To systematically identify PPP2A complexes that mediate DSB repair, we conducted a loss-of-function screen using shRNA library that targets each of the known PP2A B regulatory subunits (16). qRT-PCR analysis confirmed the knock-down (KD) in each of the shRNA-expressing cell lines (Fig. 1C; Supplementary Fig. S3B). Whenever specific antibodies were available, we also confirmed silencing of the PPP2A subunits by immunoblotting (Supplementary Fig. S3C).

In addition to PPP2CA, we found that suppression of 4 PPP2A regulatory subunits, PPP2R2A, PPP2R2D, PPP2R5A, and PPP2R3C, resulted in a statistically significant (P < 0.05 by Student t test) increase of γ-H2AX compared with cells expressing shGFP (Fig. 1C). For PPP2R5B and STRN3 subunits, only expression of 1 of 2 shRNAs resulted in γ-H2AX increase, most probably due to off-target effects (Fig. 1C). These results indicate that PPP2A regulatory subunits, PPP2R2A, PPP2R2D, PPP2R5A, and PPP2R3C, could be involved in control of DSB repair.

PPP2R2A is commonly downregulated in NSCLCs
Inefficient DNA repair mediated by suppression of specific PPP2A B subunits suggests the contribution of these PPP2A subunits to tumor suppression. In consonance with this idea, recent analysis of outlying expression coincident with extreme copy number alterations has identified PPP2R2A, one of our hits, as a putative tumor suppressor gene in breast cancer (17). Moreover, high-resolution analyses of somatic copy number alterations of large-scale datasets revealed statistically significant loss-of-heterozygosity (LOH) of the PPP2R2A-containing region in human cancers (18, 19). Reduced PPP2R2A mRNA expression was also found in prostate adenocarcinoma samples (20, 21).

Our qRT-PCR analysis of PPP2R2A mRNA expression in 245 samples covering 14 cancer types confirmed that PPP2R2A was downregulated in lung and thyroid carcinomas (P < 0.05; Fig. 2A; Supplementary Fig. S4A). Further analysis of PPP2R2A mRNA expression in a panel of 21 NSCLC samples with matched normal tissues revealed at least a 2-fold decrease in PPP2R2A mRNA in 8 of 21 NSCLC samples (Fig. 2B).

A significant correlation (P = 0.0683) between the frequency of PPP2R2A LOH and the percentage of samples with downregulated PPP2R2A expression across different cancer types (Supplementary Fig. S4B) suggests that decreased PPP2R2A expression in human cancers is due to LOH of the PPP2R2A-containing region. To test this idea, we analyzed PPP2R2A expression in a set of lung carcinoma cell lines with known status of PPP2R2A LOH (Supplementary Fig. S4C). In fact, immunoblot analysis of PPP2R2A expression revealed lower levels of PPP2R2A in cell lines harboring PPP2R2A LOH (Fig. 2C). A common downregulation of PPP2R2A in NSCLCs due to LOH of PPP2R2A-containing region further supports the idea that PPP2R2A functions as a tumor suppressor. Thus, our further studies were focused on PPP2R2A.

PPP2R2A inhibits the HR repair by affecting cell-cycle progression in response to DNA damage
We validated the results of the loss-of-function screen by analyzing γ-H2AX after PPP2R2A suppression at different time points following irradiation or bleomycin treatment (Fig. 3A and B). Moreover, overexpression of shRNA resistant form of PPP2R2A in PPP2R2A-KD cells restored γ-H2AX levels (Fig.
indicating that inefficient DSB repair is triggered specifically by loss of PPP2R2A. Single-cell gel electrophoresis also revealed that unresolved DNA damage induced by camptothecin was significantly higher in PPP2R2A-KD cells (Fig. 3D).

Taken together, our observations strongly support the idea that PPP2R2A plays a key role in DSB DNA repair.

To assess a possible mechanism by which PPP2R2A modulates DSB repair, we elucidated the effect of PPP2R2A suppression on 2 major pathways of DSB repair, NHEJ and HR (22, 23). We found that although suppression of PPP2R2A facilitated the NHEJ repair, the HR pathway was dramatically inhibited in PPP2R2A-KD cells (Fig. 4A and B). Consistently, we found a decreased Rad51 foci formation in cells expressing shPPP2R2A (Fig. 4C; Supplementary Fig. S2). Moreover, 8 hours after irradiation, expression of both BRCA1 and RAD51 was significantly decreased in PPP2R2A-KD cells (Fig. 4D). Together, these results indicate that loss of PPP2R2A impairs the HR mechanism.

Because the balance between NHEJ and HR repair depends on the phase of cell cycle (22, 24), we examined the effect of PPP2R2A on irradiation-induced cell-cycle checkpoints. We found that 8 hours after irradiation, HeLa cells expressing shGFP accumulated in G2 to S-phase, whereas cells with suppressed PPP2R2A were mostly in the G1 phase of the cell cycle (Fig. 4E and F). In addition, BrdUrd incorporation after irradiation was dramatically decreased in PPP2R2A-KD cells (Fig. 4G). We also observed that I-Sce1-induced DSBs resulted in accumulation of 293DR-GFP-shPPP2R2A cells in the G1 phase of the cell cycle (Fig. 4H). In concordance with these results, we found that in response to irradiation expression of the cell-cycle–promoting phosphatase, CDC25A (25) was significantly decreased in PPP2R2A-depleted cells (Fig. 4I). Taken together, these data indicate that PPP2R2A modulates the HR repair through regulation of G1–S cell-cycle checkpoint.

Accelerated Cdc25A turnover after DNA damage is regulated by the CHK1 and CHK2 kinases (26, 27). Therefore, we analyzed the effect of PPP2R2A on CHK1 or CHK2 activation. Although inhibition of PPP2R2A did not affect CHK1 phosphorylation at S296, S217, and S345, we observed a significant accumulation of CHK2 phosphorylated at T68 in PPP2R2A-KD cells (Fig. 4I).
suggesting that G1–S checkpoint induced by loss of PPP2R2A is mediated by prolonged activation of the CHK2 kinase.

**PPP2R2A directly regulates ATM phosphorylation**

CHK2 is a direct downstream target of ATM, whereas ATM has been shown to be directly regulated by PP2A. In particular, a previous report (28) reveals that a specific PP2A inhibitor, okadaic acid, induces ATM autophosphorylation at S1981. Moreover, the authors showed interaction between ATM and PP2A A and C subunits. However, specific PP2A regulatory subunit(s) involved in ATM dephosphorylation has not been identified, whereas our data suggest that PPP2R2A may affect CHK2 activity by regulating ATM dephosphorylation.

Indeed, we found that suppression of PPP2R2A resulted in a significant increase of ATM phosphorylated at S1981 (Fig. 5A), whereas restoration of PPP2R2A expression by overexpression of shRNA-resistant silent mutant of PPP2R2A rescued this increase (Fig. 4B). Although ATM phosphorylation is considered as a sign of ATM activation, there are contradictory data how it affects ATM functions. Studies of ATM-S1987A (mouse homologue of human S1981) mice showed normal ATM-dependent phosphorylation of ATM substrates, activation of cell-cycle checkpoints, and localization of ATM to DSBs (29, 30). In vitro studies using human recombinant ATM proteins revealed that phosphorylation does not affect its kinase activity (31–33). In human cells, ATM phosphorylation at S1981 is also dispensable for the ability of ATM to localize to DSBs, but it is required for ATM retention at DSBs (34). Consistently with this report, immunofluorescent analysis of ATM revealed an increase of the size of ATM foci in PPP2R2A-KD cells (Fig. 5C), further confirming the idea that phosphorylation regulates ATM retention at the sites of DSBs (34).

These findings suggest that PPP2R2A controls DNA repair through regulation of the ATM signaling. To test this hypothesis, we analyzed whether PPP2R2A affects DNA repair in HeLa ATM SilenciX (ATM KD) cells. Indeed, analysis of γ-H2AX levels in ATM-deficient cells revealed that suppression of PPP2R2A in these cells did not affect the kinetics of DNA repair (Fig. 5D).

To determine whether PPP2R2A directly regulates ATM dephosphorylation, we conducted a set of reciprocal immunoprecipitations. We found PPP2R2A together with PP2A A and C subunits in complex with ATM (Fig. 6A). Using in vitro phosphatase assays, we also found that phosphopeptides corresponding to human ATM pS367, pS1893, and pS1981, but not pT1885 and pS2996, were efficiently dephosphorylated by PPP2R2A-specific complexes (Fig. 6B). Given that we observed
an interaction between PPP2R2A heterotrimeric complexes and ATM, increased ATM phosphorylation in PPP2R2A-depleted cells, and in vitro dephosphorylation of 3 ATM autophosphorylation sites (S367, S1893, and S1981) by PPP2R2A-specific complexes, it seems likely that ATM is a direct target for PPP2R2A-specific PP2A phosphatase in vivo.

Wip1 phosphatase has been also dephosphorylate ATM at S1981 and S367, but not at S1893 (refs. 35, 36; Fig. 6B), indicating that PPP2R2A and Wip1 may differentially regulate ATM dephosphorylation at different sites. Moreover, in contrast to PP2A subunit PPP2R2A, Wip1 expression is extremely low in undamaged cells (Fig. 6C), and depletion of Wip1 does not affect the level of phosphorylated ATM under normal conditions (35). Taken together, these data suggest that PPP2R2A-containing PP2A holoenzyme is the major ATM phosphatase in the absence of DNA damage.

Irradiation triggered transient dissociation of the ATM–PPP2R2A complex, allowing accumulation of phosphorylated ATM (Fig. 6D). This observation is consistent with a previous report (28) that revealed a significant reduction in the amount of ATM associated with PP2A A and C subunits after irradiation. Interestingly, ATM mediates the irradiation-induced dissociation of the PPP2R2A subunit from the PP2A AC core dimer (37), implicating ATM in the disruption of functional PPP2R2A-containing PP2A complexes. Consistently with this report, 15 minutes after irradiation, PPP2R2A-associated phosphatase activity was decreased. Thirty minutes after irradiation PPP2R2A was found again in complex with ATM, which is also correlated with an increase of PPP2R2A-specific phosphatase activity toward ATM phosphopeptides (Fig. 6D and E). This kinetics is consistent with the kinetics of ATM recruitment/dissociation to the sites of DSBs (34). These data also suggest the existence of a negative feedback loop between PPP2R2A and ATM.

**PPP2R2A downregulation increases sensitivity to PARP inhibitors**

Inhibition of the HR repair in PPP2R2A-depleted cells suggests that these cells are highly sensitive to DNA damage. Indeed, colony formation assay revealed that inhibition

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**Figure 3.** Loss of PPP2R2A impairs DSB repair. A, automated image analysis of γ-H2AX immunostaining of HeLa cells expressing shGFP or shPPP2R2A at different time points after 2 Gy of IR. Suppression of PPP2R2A was confirmed by immunoblotting. B, analysis of γ-H2AX immunostaining in HeLa cells transfected with ON-TARGETplus SMART pools targeting GFP, PPP2CA, or PPP2R2A at different time points after treatment with bleomycin (10 μmol/L) for 1 hour. Suppression of PPP2A C and PPP2R2A subunits was confirmed by immunoblotting. C, the effect of PPP2R2A expression on γ-H2AX as detected by automated image analysis. shPPP2R2A-resistant (rPPP2R2A) form of PPP2R2A was overexpressed in HeLa-shPPP2R2A-2 cells. V, an empty vector. D, comet assay of HEK 293 cells expressing shGFP or shPPP2R2A after treatment with 2 μmol/L of camptothecin (CPT) for 1 hour. The comet tail moment from 75 cells was quantified with Cometscore software. *P = 0.05; **P = 0.01, as determined by the Student t test.
The Role of PPP2R2A in DNA Repair

Figure 4. Loss of PPP2R2A impairs the HR repair by triggering G1–S cell-cycling arrest. A, the efficiency of I-SceI-induced NHEJ repair in H1299dA3-1 cells expressing shRNAs specific to luciferase or PPP2R2A. B, the efficiency of I-SceI-induced HR repair in 293 DR-GFP cells expressing the indicated shRNAs. C, automated image analysis of Rad51 foci formation of HeLa cells expressing shRNAs targeting GFP or PPP2R2A after 2 Gy of IR. D, the proportion of Rad51 foci in G1 phase of HeLa cells expressing shGFP or shPPP2R2A after 2 Gy of IR. E and F, cell-cycle distribution of PI-stained HeLa cells expressing the indicated shRNAs 8 hours after 5 Gy of IR. G, analysis of BrdUrd incorporation by HeLa cells expressing shRNAs against GFP or PPP2R2A at different time points after 2 Gy of IR. H, the proportion of PPP2R2A sensitized cells to IR (Fig. 7A). Moreover, HR deficiency predicts hypersensitivity to inhibition of PARP (38, 39). In fact, analysis of cell survival after treatment with ABT-888 (veliparib), a potent inhibitor of PARP1 and PARP2, revealed that PPP2R2A-KD cells are more sensitive to ABT-888 than cell lines with lower PPP2R2A expression (Figs. 2C and 7C).

ABT-888 treatment also inhibited growth of PPP2R2A-KD A549 xenografts (Fig. 7D). Moreover, we observed significantly reduced proliferation rate and increased apoptosis in PPP2R2A-KD tumors compared with shGFP-expressing tumors (Fig. 7E and F). These results strongly indicate that both PPP2R2A expression levels and LOH in the PPP2R2A-containing region can be used to predict tumor sensitivity to treatment with PARP inhibitors.
Discussion

PP2A is a ubiquitously expressed family of Ser/Thr protein phosphatases, and the diversity of PP2A functions suggests that particular PP2A complexes may contribute independently to complex phenotypes, such as the DNA damage response (5, 6). Indeed, we found that suppression of 4 different PP2A regulatory B subunits (PPP2R2A, PPP2R2D, PPP2R5A, and PPP2R3C) impairs the efficiency of DNA repair, suggesting that these specific PP2A complexes are involved in control of DNA repair. In our prior study, we conducted a loss-of-function screen using a similar shPP2A library to identify PP2A subunits involved in human cell transformation (16). This screen implicates 3 PP2A regulatory subunits, PPP2R5A, PPP2R5C, and PPP2R3A, in control of cell transformation. Our present results suggest that in addition to modulating c-Myc and Wnt activity (16), PPP2R5A could also contribute to the tumorigenic phenotype by impairing DNA repair efficiency. Consistent with this idea, it has been shown that PPP2R5A could directly dephosphorylate both c-Myc (40) and CHK2 kinase (41). However, the exact mechanism(s) by which PPP2R2D, PPP2R5A, and PPP2R3C contribute to DNA response needs to be further investigated.

In summary, our study revealed that dephosphorylation of ATM at S367, S1893, and S1981 by PPP2R2A-specific complexes regulates retention of ATM foci. Dysregulation of ATM triggered by loss of PPP2R2A induces G1→S checkpoint and inhibits HR repair. These observations further challenge the view that protein phosphatases serve only as negative regulators of DNA repair. Instead, our data suggest that tight regulation of phosphorylation events during DNA repair by specific protein phosphatases is required for timely DSB DNA repair.

Analysis of PPP2R2A expression in human cancers revealed that PPP2R2A mRNA expression is commonly downregulated in NSCLCs. PPP2R2A gene is located at 8p21 chromosome region, which is frequently deleted in a wide range of epithelial cancers (42). However, a homozygous deletion of PPP2R2A-containing locus is a more rare event in human cancers. As an example, a recent analysis of 141 clinical prostate tumors revealed PPP2R2A deletions in 61.7% of tumor samples with only 3 recurrent homozygous deletions (2.1%; ref. 43). These results are consistent with our observation that PPP2R2A expression is downregulated in NSCLC cell lines but never completely lost. As far as no potentially important somatic mutations of PPP2R2A have been reported, these data implicate PPP2R2A as a haploinsufficient tumor suppressor gene in epithelial cancers.

PARP inhibitors are emerging as a promising class of anticancer agents particularly effective against tumors with loss of functional BRCA1 and BRCA2 (38). However, BRCA1 and BRCA2 mutation carriers comprise less than 5% of breast cancer cases, and in lung cancer, BRCA mutations are even much less common. Therefore, there is a pressing need to identify novel biomarkers that would predict...
responsiveness to PARP inhibitors. Here, we show that PARP inhibition suppresses growth of tumors with decreased PPP2R2A expression. Together with the finding that LOH of PPP2R2A-containing region or decreased PPP2R2A expression is observed in about 40% of lung carcinomas, these data implicate PPP2R2A as a novel predictive marker in BRCA-proficient NSCLCs for the efficiency of treatment with PARP inhibitors.

Figure 6. PPP2R2A-containing complexes directly dephosphorylates ATM at S367, S1893, and S1981. A, reciprocal immunoprecipitations of ATM or Flag-tagged PPP2R2A expressed in HEK TE cells followed by immunoblotting using antibodies specific for Flag, ATM, PP2A C, and PP2A A. NS, nonspecific band. B, in vitro phosphatase activity of PPP2R2A-specific complexes and Wip1 toward ATM phosphopeptides. ND, not detected. C, immunoblot analysis of the indicated proteins at different time points after 2 Gy of IR. D, immunoprecipitations of PPP2R2A-containing complexes at different time points after 2 Gy of IR followed by immunoblotting with antibodies specific for Flag, ATM, and PP2A C. E, PPP2R2A-associated phosphatase activity of at different time points after 2 Gy of IR toward the indicated ATM phosphopeptides.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

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