ARID1A Deficiency Impairs the DNA Damage Checkpoint and Sensitizes Cells to PARP Inhibitors

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ABSTRACT

ARID1A, SWI/SNF chromatin remodeling complex subunit, is a recently identified tumor suppressor that is mutated in a broad spectrum of human cancers. Thus, it is of fundamental clinical importance to understand its molecular functions and determine whether ARID1A deficiency can be exploited therapeutically. In this article, we report a key function of ARID1A in regulating the DNA damage checkpoint. ARID1A is recruited to DNA double-strand breaks (DSB) via its interaction with the upstream DNA damage checkpoint kinase ATR. At the molecular level, ARID1A facilitates efficient processing of DSB to single-strand ends and sustains DNA damage signaling. Importantly, ARID1A deficiency sensitizes cancer cells to PARP inhibitors in vitro and in vivo, providing a potential therapeutic strategy for patients with ARID1A-mutant tumors.

SIGNIFICANCE: ARID1A has been identified as one of the most frequently mutated genes across human cancers. Our data suggest that clinical utility of PARP inhibitors might be extended beyond patients with BRCA mutations to a larger group of patients with ARID1A-mutant tumors, which may exhibit therapeutic vulnerability to PARP inhibitors. Cancer Discov; 5(7); 752–67. © 2015 AACR.

INTRODUCTION

ARID1A (AT-rich interactive domain 1A) has been identified as one of the most frequently mutated genes in human cancers by multiple next-generation genomic sequencing studies (1–3). ARID1A mutation rates ranging from 10% to 57% have been identified across multiple tumor lineages, including ovarian clear cell carcinoma, uterine endometrioid carcinoma, gastric cancer, hepatocellular carcinoma, esophageal adenocarcinoma, breast cancer, pancreatic cancer, transitional cell carcinoma of the bladder, renal cancer, Waldenström macroglobulinemia, pediatric Burkitt lymphoma, and cholangiocarcinoma (1–3). ARID1A, also known as BAF250A, is a subunit of the evolutionarily conserved SWI/SNF chromatin remodeling complex (4, 5). The SWI/SNF complex repositions, ejects, or exchanges nucleosomes, which serves to modulate DNA accessibility to cellular processes involved in chromatin structure, such as transcription, DNA replication, and DNA repair (6–8). However, how ARID1A deficiency contributes to cancer development and approaches to exploit ARID1A deficiency therapeutically are not known.

Ataxia telangiectasia and RAD3-related protein (ATR) is a member of the phosphatidylinositol 3-kinase-like kinase family. Along with another kinase, ataxia telangiectasia-mutated (ATM), ATR functions as a central regulator controlling cellular responses to DNA damage (9–11). In general, ATM is activated by double-strand DNA breaks (DSB), whereas ATR responds to single-strand DNA breaks (SSB; ref. 12). However, the ATM- and ATR-activating DNA lesions are interconvertible: DSBs activate ATM but can also activate ATR as a consequence of DSB end resection, which generates a single-stranded region (13–15). Unlike ATM, ATR is essential for cell survival (16), supporting the functional importance of ATR for genome maintenance programs. For example, in S phase, ATR regulates replication initiation, repsosome stability, and replication fork restart (17). In G2 phase, ATR prevents premature mitotic entry in the presence of damaged DNA via the G2 checkpoint (18, 19). Thus, a key question remains unanswered: how is ATR signaling regulated, allowing it to perform versatile roles in the DNA damage response (DDR)? One possibility is that ATR-interacting proteins fine-tune the temporal and spatial functions of ATR in DDR. Therefore, we conducted a proteomic analysis to systematically identify ATR-interacting proteins. In addition to many known ATR-binding proteins, such as ATRIP, we identified ARID1A as an unexpected interacting partner of ATR. Human cancers result in large part from the accumulation of multiple genetic alterations, including mutations, deletions, translocations, and amplifications (20). Thus, our proteomic result raised the intriguing question of whether ARID1A, through its interaction with ATR, plays a role in maintaining genomic integrity that could be exploited as a therapeutic liability.

In this study, we found that ARID1A is recruited to DSBs via its interaction with ATR. In response to DNA damage, ARID1A facilitates DNA DSB end processing to generate Replication Protein A (RPA)–coated single-strand DNA (ssDNA) and sustains ATR activation in response to DSBs. Loss of ARID1A leads to impaired checkpoint activation and repair of DNA DSBs, which sensitizes cells to DSB-inducing...
treatments, such as radiation and PARP inhibitors. Thus, our results provide biologic insights into the function of ARID1A as a tumor suppressor in human cancers and a mechanistic basis for targeting ARID1A-deficient tumors.

RESULTS

ARID1A Is Recruited to DNA Breaks via Its Interaction with ATR

To explore the mechanisms regulating the functions of ATR in DDR, we conducted an immunoprecipitation (IP) assay to enrich ATR-associated protein complexes which were then subjected to silver staining and mass spectrometry (Fig. 1A). In addition to known ATR-binding proteins, such as ATRIP, we identified ARID1A as a binding partner of ATR (Fig. 1A and Supplementary Fig. S1). Notably, in addition to ARID1A, multiple subunits of the SWI/SNF complex, including BRG1, BAF57, BAF60, BAF170, and SNF5, were also identified by the mass spectrometry analysis, suggesting that ATR interacts broadly with the SWI/SNF complex. To confirm the interaction between ARID1A and ATR, we performed reciprocal IP with V5-tagged ARID1A (Fig. 1B) and endogenous IP analyses (Fig. 1C and Supplementary Fig. S2), which confirmed that ARID1A interacts with ATR. Given the important role of ATR in DDR, we next tested whether ARID1A is recruited to DNA breaks. We used chromatin IP (ChIP) assay to examine whether ARID1A was recruited to the proximity of a single site-specific...
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I-SceI–induced DSB (Fig. 1D and Supplementary Fig. S3A), as previously described (21, 22). Interestingly, we found that ARID1A was enriched at the chromatin region close to this DSB (Fig. 1D and Supplementary Fig. S3B and S3C). To facilitate visualization of the recruitment of ARID1A to DNA lesions, we used a light activation system (KillerRed System; ref. 23) to determine whether ARID1A localized at sites of DNA damage. Briefly, KillerRed (KR) is a light-stimulated reactive oxygen species inducer fused to a tet-repressor (tetR) or transcription activator (TA; tetR+VP16), which binds to a TRE cassette (∼90 kb) integrated at a defined genomic locus in U2OS cells (U2OS TRE cell line; ref. 24). KR facilitates the formation of oxygen radicals and superoxide through the excited chromophore (25, 26) to induce DNA damage, including SSBs and DSBs. Targeting the expression of KR to one specific genome site allows visualization of the recruitment of specific proteins to a site of DNA damage. As shown in Fig. 1E and Supplementary Fig. S4, EGFP-tagged ARID1A predominantly localized in the nucleus. Upon activation of KillerRed, ARID1A showed a specific enrichment at the DNA damage site, which colocalized with TA-mcherry, indicating that the recruitment of ARID1A at TA-KR is specific to DNA damage. These findings revealed that ARID1A interacts with ATR and is recruited to DSBs.

We next set out to determine whether the recruitment of ARID1A to DSBs is dependent on its interaction with ATR. First, we transiently knocked down ATR and examined the recruitment of ARID1A to DSBs via I-SceI–based ChIP. As shown in Fig. 1F, in ATR-knockdown cells, recruitment of ARID1A to DSBs was significantly reduced. This suggested that ATR is required for recruiting ARID1A to DNA lesions. Second, we examined whether the DNA damage signaling induced by ATM and ATR is required for recruitment of ARID1A to DSBs. We treated cells with chemical inhibitors of ATM or ATR. These inhibitors effectively blocked DNA damage signaling, as shown by their impact on phosphorylation of ATM and CHK1 (Fig. 1G). The ATM inhibitor markedly decreased the recruitment of ARID1A to DSBs (Fig. 1G). The ATR inhibitor also decreased recruitment of ARID1A to DSBs, but to a lesser extent, whereas ATR knockdown significantly reduced the recruitment of ARID1A to DSBs (Fig. 1F and G). Consistent with previous findings that ATR recruitment to DSBs requires ATM and not the converse (13–15), these data suggested that interaction between ARID1A and ATR, with ATR acting as
a scaffold, and DNA damage signaling initiated by ATM are required for efficient ARID1A recruitment to DSBs. To understand the molecular details of the ARID1A-ATR interaction and the recruitment of ARID1A to DSBs, we first used two deletion constructs to test whether ARID1A binds to ATR through its N-terminal or C-terminal half. As shown in Fig. 1H, binding of ATR to the C-terminal of ARID1A was readily detectable, whereas N-terminal ARID1A did not pull down ATR even though the N-terminal ARID1A expressed at levels similar to the full-length protein. This result suggested that the domains required for ATR interaction are located in the C-terminal half of ARID1A. Next, we generated deletion mutants of ARID1A to further map the regions mediating its interaction with ATR (Fig. 1I–K). Using these constructs, we found that regions from 1800 to 1900 amino acids (aa) and 2100 to 2200 aa at the C-terminal half of ARID1A were essential for its interaction with ATR (Fig. 1I and J). We further examined the recruitment of deletion mutants to DSBs. Indeed, we found that deletion of ARID1A-interacting domain suppressed the recruitment of ARID1A to DSBs (Fig. 1L and Supplementary Fig. S5).

Collectively, these data showed that ARID1A interacts with ATR via its C-terminal region, which mediates its recruitment to DSBs.

**ARID1A Is Required for a Proper G2-M DNA Damage Checkpoint**

Next, we tested whether ARID1A deficiency impairs the cellular response to DNA damage. As our model system, we used isogenic HCT116 cell lines with wild-type ARID1A and a knock-in mutant ARID1A (Q456*/Q456*) that abolishes ARID1A expression because of an early stop codon. We first examined cell cycle distribution at different time points after exposure to ionizing radiation (IR). As shown in Fig. 2A, 1 hour after irradiation, control cells started to accumulate at the G2-M checkpoint. At 4 and 8 hours after irradiation, there was a significant increase in cells at the G2-M phase, suggesting that ARID1A-depleted cells had weakened G2-M checkpoint activation. Sixteen hours after irradiation, control cells still had a large proportion of cells arrested at the G2-M checkpoint, whereas ARID1A-depleted cells had reached a markedly reduced proportion of cells at the G2-M checkpoint and a significant increase of cells in G1 phase (Fig. 2A). These results indicated that ARID1A deficiency leads to impaired G2-M checkpoint initiation and maintenance. To confirm these results, we used phospho-Histone H3 staining to measure the fraction of mitotic cells after exposure to IR in ARID1A-depleted cells. Without IR treatment, there was no apparent difference in the percentage of mitotic cells between control and ARID1A-depleted cells (Fig. 2B). After exposure to IR, in control cells the percentage of mitotic cells was significantly reduced and started to recover 16 hours after IR (Fig. 2B). In contrast, ARID1A-deficient cells showed a slower decrease in numbers of mitotic cells at early time points after IR exposure and a significantly increased percentage of cells reentering mitosis at 16 hours after IR, suggesting defective G2-M checkpoint initiation and maintenance (Fig. 2B). In accord with this finding, as shown in Fig. 2C, ARID1A depletion led to a marked increase in cumulative mitotic reentry after IR exposure revealed by paclitaxel treatment, which blocks mitotic exit. Defective G2-M checkpoint maintenance was not due to a differential response to paclitaxel between control and ARID1A-null cells, because we observed a similar block in mitotic accumulation in cells not treated with IR (Fig. 2C). ARID1A expression was effectively depleted in ARID1A-null cells (Fig. 2D). These data are consistent with ARID1A deficiency significantly impairing G2-M checkpoint initiation and maintenance.

As ARID1A is a subunit of the SWI/SNF complex, we next asked whether the chromatin remodeling activity of SWI/SNF is required for G2-M checkpoint response. We knocked down the core catalytic subunits BRG1 or BRM in U2OS cells (Supplementary Fig. S6) and found that BRG1 deficiency led to an increase of mitotic cells 16 hours after exposure to IR, similar to the increase observed in ARID1A-null cells (Fig. 2E). This result suggested that ARID1A-associated BRG1-containing SWI/SNF complexes are required for maintaining G2-M cell-cycle arrest after induction of DSBs.

**ARID1A Deficiency Impairs DSB-Induced ATR Activation**

Having observed a defective DNA damage checkpoint in ARID1A-deficient cells, we further examined whether ARID1A deficiency impairs the DNA damage checkpoint signaling pathway. We treated cells with IR and examined the activation of CHK1, a key G2-M checkpoint regulator. In ARID1A-depleted cells, we found reduced CHK1 (S317) phosphorylation in response to IR, particularly at 8 hours after IR (Fig. 3A). In response to DSBs, CHK1 (S317) can be phosphorylated by either ATM or ATR. Thus, we examined whether ARID1A deficiency affected ATM and/or ATR activation. In response to DNA damage, ATM (S1981) and ATR (T1989) undergo autophosphorylation, which provides a surrogate marker for kinase activation (13, 27–29). ARID1A depletion remarkably reduced ATR activation [phosphorylation of ATR (T1989)] in response to IR (Fig. 3B), but did not alter ATM activation [phosphorylation of ATM (S1981)] or recruitment of ATM to DNA damage sites (Fig. 3C and Supplementary Fig. S7). Previous studies showed that the G2-M checkpoint is impaired in the absence of ATR (16). Thus, our data are consistent with ARID1A depletion impairing ATR activation in response to DSBs and thereby altering checkpoint signaling.

In general, DSB ends are the preferred substrate for ATM binding, which activates first ATM and then ATR to sustain ATM-initiated signaling (11). It has been shown that phosphorylation of H2AX in response to IR is mediated by both ATM and ATR (30, 31). Therefore, we examined the effect of ARID1A deficiency on the dynamics of H2AX phosphorylation. As we expected, chromatin binding of γ-H2AX was significantly reduced in ARID1A-deficient cells at 8 hours compared with 4 hours after IR, indicating impairment of sustained γ-H2AX foci formation (Fig. 3D). To confirm this result, we tested whether ARID1A deficiency reduced γ-H2AX foci formation, which directly reflects the accumulation of γ-H2AX at DSBs. As shown in Fig. 3E, γ-H2AX foci formation was significantly reduced at later time points after IR. In addition, we examined the foci formation by DNA damage-responsive protein 53BP1, a key adaptor protein in checkpoint response whose recruitment to DSBs is dependent on the protein platform assembled by γ-H2AX formation (32). Consistent with the reduced γ-H2AX foci formation at 8 hours after exposure to IR, 53BP1 foci formation was remarkably reduced in ARID1A-deficient cells (Fig. 3F). As shown in Supplementary Fig. S8A, we used a comet assay to determine
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**ARID1A Deficiency Impairs DSB End Resection and Thereby Impairs DSB-Induced ATR Signaling**

Next, we sought to determine the molecular mechanism underlying the defective DSB-induced ATR activation in ARID1A-deficient cells. In response to DSBs, ATM is directly activated by the MRE11–RAD50–NBS1 (MRN) complex, which is required to recruit ATM to DSBs (33–35), whereas
ATR activation and recruitment to DSBs require the formation of RPA-coated SSBs, which arise from 5'-3' resection of DSB ends (18, 36). Therefore, we asked whether ARID1A depletion affects the process of DSB end resection, leading to reduced efficiency of ATR activation.

First, we examined phosphorylation of the ssDNA-binding protein RPA in ARID1A-depleted cells as an indicator of DSB end resection efficiency (37). As shown by Western blotting (Fig. 4A), IR-induced phosphorylation of the RPA2 subunit (Ser4 and Ser8) was significantly reduced after ARID1A depletion. In contrast, ARID1A deficiency did not affect RPA phosphorylation in response to replication stress stimuli hydroxyuridine (HU) and ultraviolet light (UV) even though both HU and UV induce much stronger RPA phosphorylation than IR (Fig. 4B). This result suggested that ARID1A specifically affects DSB-induced formation of RPA-coated SSBs.
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**Figure 4.** ARID1A promotes DSB end resection. A and B, Western blot analysis of RPA phosphorylation [pRPA(S4/S8)] at the indicated time points after exposure to IR (A) or replication stress stimuli (HU 2 mmol/L and UV 50 J/m²; B) in control (+/+) and ARID1A-depleted (−/−) HCT116 cells. Ctrl, control. Densitometry analyses of indicated protein values (phosphorylated protein normalized against total protein for each lane) were shown at the bottom of Western blots (A and B). The control lane was set as 1. Each value represents the mean ± SD of three independent experiments (*, P ≤ 0.05). C, control (+/+) and ARID1A-depleted (−/−) HCT116 cells were exposed to IR and immunostained with pRPAS4/S8. Left, representative images. Scale bar, 2 μm. Right, quantitative results represent the mean ± SD of three independent experiments. *, P < 0.01. Representative images of multiple cells are shown in Supplementary Fig. S8B. D, DR-GFP-U2OS cells were transfected with control siRNA or ARID1A siRNA (SMARTpool). Forty-eight hours later, cells were transfected with I-SceI plasmid. ChIP assay was conducted 8 hours after I-SceI transfection, and qPCR analyses were used to detect the enrichment of H3 relative to the IgG control (mean ± SEM; n = 3; *, P < 0.01). Western blot analyses to demonstrate the effective ARID1A knockdown are shown next to the graph. Ctrl, control. E and F, defective HR repair (E) and single-strand annealing (SSA) repair (F) in ARID1A-deficient cells upon DSB induction by I-SceI. Left, representative flow cytometry profile. Right, each value is relative to the percentage of GFP-positive cells without siRNA transfection, which was set to 1 and represents the mean ± SD of three independent experiments. *, P < 0.01. Western blot analyses to demonstrate the effective ARID1A knockdown are shown next to the graph. Ctrl, control.
of ssDNA. To confirm this observation, we used immuno-
fluorescent staining to detect RPA phosphorylation (p) at
DNA damage sites. Notably, ARID1A depletion markedly
reduced pRPA (Ser4/Ser8) foci formation, consistent with
decreased RPA accumulation and impaired ssDNA forma-
tion at DSBs (Fig. 4C and Supplementary Fig. S8B). We
also tested the effect of ARID1A loss on the chromatin
environment around DSBs by examining histone H3 occu-
pancy at I-SceI–induced DSBs by ChIP assay. We found that
H3 deposition was not altered in ARID1A-depleted cells
before DNA damage (Fig. 4D). However, H3 occupancy
was much higher in ARID1A-depleted than control cells
after induction of DSBs by I-SceI. These data supported
impaired DSB end resection due to loss of ARID1A, sug-
gest that recruitment of ARID1A to DSB is required
to create a favorable chromatin environment for effi cient
DSB end resection. Next, we determined whether ARID1A
depletion affects DSB repair via homologous recombin-
ation (HR) or single-strand annealing (SSA), which are the
repair mechanisms requiring DSB end resection (Supple-
mentary Fig. S9). In line with our finding that ARID1A
is required for efficient DSB end resection, we found that
ARID1A knockout impaired both HR repair and SSA repair
efficiency (Fig. 4E and F). In addition, to exclude an indirect
effect of ARID1A depletion on gene transcription regula-
tion, we examined the key molecules involved in DDR and
DSB end resection in ARID1A-depleted cells and observed
no apparent reduction in protein levels (Supplementary
Figs. S9 and S10). Together, these data suggested that
initial DSB end resection resulting from ATM–MRN com-
plex–dependent signaling recruits ATR in complex with
ARID1A to promote DSB end resection and ATR activation
and thereby augment DSB-induced DNA damage signaling.

ARID1A Deficiency Sensitizes Cells to PARP Inhibitors

Inhibitors of PARP1, an enzyme involved in repairing DNA
SSBs (38, 39), are now in clinical trials and showing promis-
ing activity. PARP inhibitor treatment causes failure of SSB
repair, which can lead to DSBs when DNA replication forks
stall and collapse at persistent SSB lesions (38, 39). Further-
more, PARP inhibitors trap PARP on DNA, eventually leading
to DSBs. Therefore, PARP inhibitors are selectively lethal

Figure 5. ARID1A deficiency sensitizes cells to PARP inhibitors. A–C, stable ARID1A-knockdown nontransformed MCF10A normal breast epithelial
cells (A) and HMECs (B) and ARID1A-knockout HCT116 cells (C) were treated with the indicated PARP inhibitors, each at a concentration of 10 μmol/L.
Clonogenic assay was performed. Left, representative images. Right, quantitative results represent the mean ± SD of three independent experiments.
* P < 0.01. Western blot analyses to demonstrate the effective ARID1A knockdown are shown next to the graphs. Ctrl, control. D, Western blot analyses
show the effective ARID1A knockdown in MDA-MB-231 cells. (continued on following page)
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Inefficient DSB repair (Fig. 2). In addition, ARID1A deficiency in DSBs, which may lead to insufficient cell-cycle arrest to allow repair deficiency (38, 39). We found that ARID1A-depleted cells exhibited a significant G2-M checkpoint defect in response to normal cells and less activity in cancer cells without DSB break repair pathway, but exhibit minimal toxic effects on repairing DSBs, or lacking other components of the DSB repair mechanism(s) (41). Strikingly, ARID1A-knockdown cells showed impaired DSB repair through both HR and SSA mechanisms (Fig. 4). On the basis of these observations, we reasoned that ARID1A deficiency may render cells vulnerable to DSBs induced by PARP inhibitors. We tested this hypothesis in a variety of isogenic models with multiple PARP inhibitors that are currently used in clinical trials.

First, we knocked down ARID1A in two nontransformed breast epithelial cell lines, MCF10A and HMEC, and then treated the cells with the PARP inhibitors olaparib, rucaparib, and veliparib. As shown in Fig. 5A and B, PARP inhibitors selectively decreased the survival of cells with decreased ARID1A expression. Next, we tested whether ARID1A depletion sensitized HCT116 colon cancer cells and MDA-MB-231 breast cancer cells to PARP inhibitors. We treated the cells with olaparib and BMN673, a potent PARP inhibitor (40, 41). Strikingly, ARID1A-knockdown cells showed remarkably reduced colony formation in the presence of PARP inhibitors (Fig. 5C and Supplementary Fig. S11A). As we observed the reduced colony formation in ARID1A-deficient HCT116 cells compared with control cells, we further examined the growth rate of these cells. As shown in Supplementary Fig. S11B, ARID1A knockdown reduced cell growth. However, we did not observe cytotoxic effects of ARID1A knockdown alone. It is noteworthy that not only the number of colonies but also the average colony size was lower with BMN673 than with olaparib in ARID1A-knockout cells, suggesting that BMN673 strongly inhibits cancer cell survival. In line with these findings, significantly enhanced apoptosis after exposure to PARP inhibitors was observed in ARID1A-depleted MDA-MB-231 cells (Fig. 5D and E), HCT116 cells (Fig. 5F), and HOC8 ovarian cancer cells (Supplementary Fig. S12). To confirm that ARID1A deficiency sensitizes cells

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**Figure 5.** (Continued) E and F, stable ARID1A-knockdown MDA-MB-231 cells (E) and ARID1A-knockout HCT116 cells (F) were treated with the indicated PARP inhibitors for 72 hours, and apoptosis was determined by annexin V staining. Quantitative results represent the mean ± SD of three independent experiments. *P < 0.01. Western blot analyses to demonstrate the activation of caspase 3 in ARID1A-knockout HCT116 cells are shown next to the graphs. G, ARID1A-knockout HCT116 cells were reconstituted with wild-type or mutant ARID1A transiently and exposed to BMN673 for 48 hours. Apoptosis was determined by annexin V staining. Representative results from three independent experiments are shown (mean ± SD).
PARP Inhibitor BMN673 Selectively Inhibits ARID1A-Deficient Tumors in Xenograft Models

We treated nude mice bearing ARID1A-deficient and parental MDA-MB-231 breast cancer tumors and ARID1A-depleted and parental HCT116 colon cancer tumors in the opposite flanks with and without oral 0.33 mg/kg BMN673 daily. Because of the significant tumor burden in the untreated group, HCT116 xenograft mice were only treated with BMN673 for 16 days. After 1 week of treatment, a selective antitumor efficacy of BMN673 was observed in ARID1A-depleted HCT116 cells, and this antitumor effect was more marked at treatment day 16 (Fig. 6A and B). In contrast, BMN673 was without effect in mice bearing parental HCT116 cells (Fig. 6C). MDA-MB-231 xenografts, which grew more slowly, were treated with BMN673 for 30 days. Once again BMN673 significantly inhibited tumor growth in ARID1A-deficient cancer cells but not wild-type cells (Fig. 6A, D, and E). Thus in both xenograft models, growth of ARID1A-deficient xenografts was suppressed by BMN673 compared with vehicle (Fig. 6F), but growth of ARID1A-wild-type xenografts was not (Fig. 6F).

We analyzed the expression of the DDR marker phosphorylated CHK1 and the apoptosis marker cleaved caspase 3 in xenograft tumor tissues. As expected, in response to BMN673, ARID1A-deficient cancer cells exhibited much lower expression of pCHK1 than did control cells with wild-type ARID1A (Fig. 6G), consistent with loss of ARID1A decreasing the DDR induced by BMN673. Furthermore, ARID1A-deficient tumor cells exhibited enhanced apoptosis after BMN673 treatment (Fig. 6G). Taken together, our data suggest that targeting the defective DDR that occurs in ARID1A-deficient cells with PARP inhibitors could be beneficial for cancer patients with ARID1A-deficient tumors.

DISCUSSION

As shown in the proposed model (Supplementary Fig. S16), our study indicates that ARID1A interacts with ATR, is recruited to sites of DNA damage in an ATR-dependent manner, and facilitates and/or accelerates effective DNA DSB end resection. This ARID1A-mediated chromatin remodeling, by promoting efficient DSB end resection, is required for sustaining ATR-dependent signaling from DSBs and repair of DSBs through HR pathways. Our in vitro and in vivo data further show that PARP inhibitors demonstrate selective activity against ARID1A-deficient cells. Collectively, our results provide mechanistic insights into how ARID1A suppresses tumorigenesis and how therapeutic liabilities engendered by ARID1A deficiency could be exploited clinically.

In the DNA damage signaling network, similar regulatory mechanisms are used for promoting/sustaining cellular responses to DNA damage. For example, when DSBs occur, ATM and/or ATR phosphorylate the histone H2A variant H2AX, which can spread thousands of base pairs around DSB sites (42, 43). The presence of phosphorylated H2AX (γ-H2AX) provides docking sites to recruit DNA damage-responsive sensors, such as NBS1 and MDC1, and recruitment of these sensor proteins further activates or maintains ATM kinase activity (phosphorylation of H2AX) and amplifies ATM signaling (44–46). Processing of DSB ends to single-strand ends is required...
**Figure 6.** BMN673 selectively inhibits ARID1A-deficient xenograft tumor growth. **A,** representative images of MDA-MB-231 and HCT116 xenografts treated with vehicle control and BMN673 at the end point of scheduled treatment. (left side blue circle: control cells; right side green circle: ARID1A-deficient cells). **B–E,** control and ARID1A-depleted HCT116 cells (B and C) and MDA-MB-231 cells (D and E) were inoculated subcutaneously in athymic nu/nu mice. Mice were randomized to vehicle control and BMN673 treatment groups. Average tumor volume was plotted against days of treatment (n = 6 for each group; *, *P < 0.01). **F,** average tumor volume of each group was determined at the end of the scheduled treatment (n = 6 for each group; *, *P < 0.01). **G,** representative examples of the immunohistochemistry analyses of xenograft tumors with anti-pCHK1 (S317) and anti-activated caspase-3 antibodies. Scale bar, 50 μm. Quantification of anti-pCHK1 (S317) and anti-activated caspase-3-positive cells of three individual tumors. (, *P < 0.01.
for ATR activation. The initial activation of ATR, which is dependent on MRN complex–ATM signaling, may not require ARID1A. Consistent with this recruitment–amplification/sustaining model, our data showed that ARID1A is recruited to DSBs via its interaction with ATR and thereby helps sustain ATR activation via promoting resection. It is worth noting that our data showed that ARID1A deficiency reduced ATR activation in response to IR-induced DSBs. However, no significant change of ATR activation in response to HU was observed in ARID1A-deficient cells as examined in our current experimental condition. There are two possible explanations for this observation. First, the different structures of DSBs caused by IR and HU may be associated with distinct mechanisms of ATR activation. Whereas IR induces DSBs with blunt ends, HU induces replication stress–associated DSBs in S phase (47). These types of DSBs occur at stalled/collapsed replication forks, which normally are one-ended and associated with replication fork structure. We speculate that in response to IR-induced DSBs, chromatin remodeling activity mediated by ARID1A is required to promote efficient DSB end resection to sustain ATR signaling. However, in response to HU-induced replication stress–associated, one-end) DSBs, ARID1A-mediated chromatin remodeling may not be required to promote ATR activation given the relatively open chromatin structure of replication forks during DNA replication in S phase. If the chromatin remodeling activity is required, it is likely that a different chromatin remodeling mechanism independent of ARID1A is involved in HU-induced one-end DSBs. Second, although HU induces replication stress–associated DSBs, HU primarily induces extensive formation of single-strand DNA coated with RPA. This DNA structure provides a molecular basis for activating ATR through ATRIP and TOPBP1 in S phase, which does not require DNA end resection. Thus, on the basis of our data, we speculate that ARID1A does not play a determinative role in regulating HU-induced ATR activation.

Multiple studies have indicated tumor-suppressor roles for ARID1A in transcriptionally regulating cell-cycle progression (48–50). Interestingly, our study shows that ARID1A directly regulates the DNA damage checkpoint independent of its roles in transcriptionally regulating cell-cycle progression. Consistent with this caretaker role of ARID1A, previous studies found that knockdown of SWI/SNF core component BRG1 or BRM could affect DNA repair pathways and proper mitosis segregation (1, 51). Consistent with this contention, a recent study reported that SWI/SNF factors are required for cellular resistance to DNA damage (52). To understand detailed mechanisms of how ARID1A regulates ATR activation in response to DSBs, two additional questions are of critical importance. First, is the role of ARID1A in regulating the function of the SWI/SNF complex related to its effects on ATR function? Our data suggest that the SWI/SNF complex is required for maintaining checkpoint activation. It is likely that ARID1A is required for recruiting or stabilizing the SWI/SNF complex at DSBs. Whether there are functionally restricted ARID1-containing SWI/SNF complexes independently involved in DNA damage repair or transcriptional regulation or whether the same protein complexes can mediate both processes depending on the cellular context will be important to ascertain. Second, how do patient-derived mutations of ARID1A affect its function in the DNA damage response? A variety of mutations are found in ARID1A in human cancers. Many of the mutations result in mutation-mediated RNA decay and unstable protein, but a number of the mutant ARID1A proteins are expressed. Thus to functionalize patient-derived mutations is not only important for understanding the molecular mechanisms of ARID1A in the DNA damage response, but also important for stratifying patients for clinical trials of targeted therapy with PARP inhibitors.

Our results showed that ARID1A deficiency impairs DSB repair, which normally should delay the progression of the cell cycle in order to allow resolution of the DSBs. However, we observed that ARID1A-deficient cells progress more quickly through the cell cycle, suggesting that in the ATR-mediated DNA damage checkpoint, ARID1A plays a dominant role in determining cell-cycle progression after DNA damage. It is likely that impaired DSB repair and cell-cycle checkpoint activity may allow ARID1A-deficient cells to transmit damaged (unrepaired) DNA to the next cell cycle, which could potentially lead to genomic instability. As suggested by a recent study in gastric cancer, ARID1A mutations are associated with increased microsatellite instability (MSI), and it is of interest to investigate whether ARID1A deficiency promotes the mutational process in tumorigenesis or whether ARID1A is a target for MSI.

PARP inhibitor monotherapy is well tolerated in patients but has minimal therapeutic effects in the absence of specific genetic defects, such as BRCA1/BRCA2 mutations (53). Thus, our study provides a mechanistic rationale for testing the efficacy of PARP inhibitors in ARID1A-deficient tumors either alone or in combination. However, as noted above, not all ARID1A mutations are demonstrated to compromise the DNA damage response. Therefore, it is imperative to select ARID1A-mutant tumors for PARP inhibitor treatment on the basis of the biologic significance of their specific mutations.

Specific inactivating mutations in several other SWI/SNF subunits, including PBRM1, ARID2, ARID1B, BRG1, SNF5, and BRD7, have also been frequently found in human cancers (1). However, mutations in different SWI/SNF subunits lead to distinct cancer spectrums (1). In addition, mouse models with genetic knockout of different SWI/SNF subunits exhibit distinct phenotypes (1, 54). These findings suggest that individual subunits may have distinct tumor-suppressor roles in specific tissue contexts. Given the diverse combinations and interactions of subunits in a particular SWI/SNF complex, it is possible that ARID1A has distinct effects on tumorigenesis in different tissues. Therefore, the mechanisms by which ARID1A exerts its tumor-suppressor function may be tissue dependent. The answer to this question will be instrumental to guide therapy with DNA damage–inducing agents such as PARP inhibitors. In the current study, we used well-established cancer cell models such as HOC8, HCT116, and MDA-MB-231 and normal diploid epithelial cell models such as MCF10A and HMEC as representative model systems to test our hypothesis. Thus, it is necessary to confirm our findings in additional cancer models, such as ovarian clear-cell carcinoma models, which exhibit the highest ARID1A mutation rate among all cancer types. It has been found that ARID1A mutations cooperate with activation of the PI3K–AKT pathway in promoting tumorigenesis (2, 3). A recent study showed that depletion of ARID1B reduces survival of ARID1A-mutant cancer cells (55). Thus, PARP inhibitors in combination with...
Targeting ARID1A Deficiency with PARP Inhibitors

**METHODS**

**Cell Culture and Plasmids**

HCT116 parental and ARID1A-knockout (Q456*/Q456*) cell lines were purchased from Horizon Discovery Ltd. and maintained according to the manufacturer’s instructions. U2OS, MCF10A, and HMEC cells and breast cancer cell line MDA-MB-231 were purchased from the American Type Culture Collection (ATCC). Cell lines were validated by short tandem repeat (STR) DNA fingerprinting using the AmpF_STR Identifier Kit according to the manufacturer’s instructions (Applied Biosystems; cat. 4322288). The STR profiles matched known ATCC fingerprints, to the Cell Line Integrated Molecular Authentication database (CLIMA) version 0.1.200080 (Nucleic Acids Research 37:D925-D932 PMC2: PMC2685626), and to the MD Anderson fingerprint database. The STR profiles matched known DNA fingerprints or were unique. The U2OS cell lines were obtained from the ATCC in 2013. The authentication of the breast cancer cell lines and ovarian cancer cell lines was performed in the MD Anderson Cancer Center (MDACC) Chartered Cell Line Core facility in 2012 and 2013. HCT116 and ARID1A-knockout cell lines were obtained from Horizon Discovery, Inc., at the end of 2012 and subsequently, through a contract with the company.

U2OS cells were maintained in McCoy’s 5A medium (Cellgro) supplemented with 10% FBS with glutamine, penicillin, and streptomycin. MDA-MB-231 breast cancer cells were grown in RPMI-1640 medium supplemented with 10% FBS. HMEC cells were grown in HuMEC medium with growth supplement. MCF10A cells were maintained in mammary epithelial growth medium (Clonetics), a 1640 medium supplemented with 10% FBS. HMEC cells and breast cancer cell line MDA-MB-231 were purchased from the American Type Culture Collection (ATCC), a 1640 medium supplemented with 10% FBS. HMEC cells and breast cancer cell line MDA-MB-231 were purchased from the American Type Culture Collection (ATCC). Cell lines were validated by short tandem repeat (STR) DNA fingerprinting using the AmpF_STR Identifier Kit according to the manufacturer’s instructions (Applied Biosystems; cat. 4322288). The STR profiles matched known ATCC fingerprints, to the Cell Line Integrated Molecular Authentication database (CLIMA) version 0.1.200080 (Nucleic Acids Research 37:D925-D932 PMC2: PMC2685626), and to the MD Anderson fingerprint database. The STR profiles matched known DNA fingerprints or were unique. The U2OS cell lines were obtained from the ATCC in 2013. The authentication of the breast cancer cell lines and ovarian cancer cell lines was performed in the MD Anderson Cancer Center (MDACC) Chartered Cell Line Core facility in 2012 and 2013. HCT116 and ARID1A-knockout cell lines were obtained from Horizon Discovery, Inc., at the end of 2012 and automatically replaced with a new batch of the cell lines every year since then, through a contract with the company.

HR and SSA Repair Assays

SSA assay cells were kindly provided by Dr. Jeremy Stark (Beckman Research Institute of the City of Hope). The HR and SSA repair assays were performed as described previously (21, 56).

Immunofluorescent Staining for Foci Formation

For detection of DNA damage-induced foci of γH2AX, 53BP1, and pRPA32, immunofluorescent staining was performed essentially as described previously (21, 56). After treatment, cells were first subjected to cytoskeleton extraction/stranding (cytoskeleton buffer: 10 mmol/L PIPES, pH 6.8; 100 mmol/L NaCl; 300 mmol/L sucrose; 3 mmol/L MgCl2; 1 mol/L EGTA; 0.5% Triton-X 100; stripping buffer: 10 mmol/L Tris-HCl, pH 7.4; 100 mmol/L NaCl; 3 mmol/L MgCl2; 1% Tween 20; 0.25% sodium deoxycholate) to remove the unbound proteins and, second, fixed in PBS-buffered 4% paraformaldehyde. Primary antibodies were incubated at 4°C overnight, and secondary antibody Alexa 488–conjugated goat anti-rabbit IgG was incubated overnight and visualized by confocal microscopy. At least 50 cells per sample were scored, and foci were counted. Although currently available ARID1A antibodies are suitable for Western blot analysis and general fluorescence staining, they failed to detect formation of IR-induced foci of ARID1A at DNA breaks.

ChIP Assay

DSBs were induced in cells transfected with control siRNA or ATR siRNA by I-Scsl expression. At indicated time points, cells were cross-linked with formaldehyde, and ChIP assays were conducted using an EZ ChIP kit (Upstate) according to the manufacturer’s instructions. Cellular lysates were subjected to five sets of sonication on wet ice with a 60 Sonic Dismembrator (Fisher Scientific). Each set consisted of 8 seconds of sonication separated by 1-minute intervals on ice. ARID1A and V5 antibodies (5 μL/reaction) were used for IP. The ChIP primers used to analyze proteins binding at DSBs were primer 1, 5′-TACG GCAAGCGTACCTGAA-3′ (sense) and 5′-GCCATATATGGAGT
TCCGC-3′ (antisense); primer 2, 5′-GCCCATATGGAGTTCGCCG-3′ (sense); and 5′-GCCCATATGGAGTTCGCCG-3′ (antisense); primer 3, 5′-GGCCACCTCTGCTGACTCCA-3′ (sense) and 5′-GGCTATTGGCTATAAGTGGCC-3′ (antisense); primer 4, 5′-GAGGACACCTGGAGCTGAG-3′ (sense) and 5′-GCTGAACCTTGTGCGCCGTATTA-3′ (antisense); and primer 5, 5′-GAGAAGGTTGCGGAAGAACG-3′ (sense) and 5′-GATA GCAGATTGGGAGTG-3′ (antisense).

**Tumor Growth in nude Mice**

Male athymic nu/nu mice (6–8 weeks old) were used for all in vivo xenograft studies. Mice were quarantined for at least 1 week before experiments. All animal studies were conducted in compliance with protocols approved by the MDACC Institutional Animal Care and Use Committee. Exponentially growing MDA-MB-231 (1 × 10⁶) or HCT116 (2 × 10⁶) cells were implanted subcutaneously at the flank of nude mice (left: control cells; right: ARID1A-deficient cells). Mice were treated with vehicle or BMN673 (0.33 mg/kg) once daily by oral gavage. Tumors were measured every 2 days by caliper to determine tumor volume using the formula [length/2] × [width]². Each cell line was tested in six different animals. For immunohistochemistry, tumor tissue samples were fixed in 4% buffered parafomaldehyde and processed for histopathologic evaluation by paraffin embedding and antibody staining.

Methods for the following assays are available in the supplementary information: KillerRed System, Flow Cytometric Analyses, Comet assay, Colony-forming assay, and Tet-On expression of ARID1A.

**Statistical Analysis**

All statistical analyses were performed with a two-tailed Student t test.

**Disclosure of Potential Conflicts of Interest**

G.B. Mills has received PI sponsored research clinical trial support from AstraZeneca and clinical trial support from BioMarin, has licensed a patent to Myriad Genetics, and is an advisory board member for AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

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