

SWI/SNF Factors Required for Cellular Resistance to DNA Damage Include ARID1A and ARID1B and Show Interdependent Protein Stability

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Abstract

The SWI/SNF chromatin-remodeling family contains various protein complexes, which regulate gene expression during cellular development and influence DNA damage response in an ATP- and complex-dependent manner, of which details remain elusive. Recent human genome sequencing of various cancer cells revealed frequent mutations in SWI/SNF factors, especially ARID1A, a variant subunit in the BRG1-associated factor (BAF) complex of the SWI/SNF family. We combined live-cell analysis and gene-suppression experiments to show that suppression of either *ARID1A* or its paralog *ARID1B* led to reduced nonhomologous end joining activity of DNA double-strand breaks (DSB), decreased accumulation of KU70/KU80 proteins at DSB, and sensitivity to ionizing radiation, as well as to cisplatin and UV. Thus, in contrast to transcriptional regulation, both ARID1 proteins are required for cellular resistance to various types of DNA damage, including DSB. The suppression of other SWI/SNF factors, namely SNF5, BAF60a, BAF60c, BAF155, or BAF170, exhibits a similar phenotype. Of these factors, ARID1A, ARID1B, SNF5, and BAF60c are necessary for the immediate recruitment of the ATPase subunit of the SWI/SNF complex to DSB, arguing that both ARID1 proteins facilitate the damage response of the complex. Finally, we found interdependent protein stability among the SWI/SNF factors, suggesting their direct interaction within the complex and the reason why multiple factors are frequently lost in parallel in cancer cells. Taken together, we show that cancer cells lacking in the expression of certain SWI/SNF factors, including ARID1A, are deficient in DNA repair and potentially vulnerable to DNA damage. *Cancer Res*; 74(9): 2465–75. ©2014 AACR.

Introduction

Various types of DNA damage are continually inflicted on cells by environmental agents and metabolic products, leading to genomic mutation and cell killing, eventually, cancer. DNA damage is, however, generally repaired by cellular enzymatic DNA repair systems that maintain genome integrity and cellular survival. Because of the cell killing effect of DNA damage, physical or chemical agents producing DNA damage are often used for cancer therapy. Because DNA repair interferes in the killing effect of DNA damage, DNA repair defect in cancer cell may be a useful target for cancer therapy.

DNA double-strand breaks (DSB) are one of the most serious types of DNA damage induced by ionizing radiation or chemical agents. In mammalian cells, two pathways conserved in evolution, nonhomologous end joining (NHEJ) and homologous recombination, contribute to repair of DSBs and sustain genome integrity. Homologous recombination seeks and uses genetic information from an undamaged sister chromatid or homologous chromosome to repair DSBs, whereas NHEJ directly joins two broken DNA ends without significant homologous sequences. NHEJ contributes greatly to cellular survival soon after DSBs are introduced but not much to genome integrity, because of its error-prone nature in the repair process. In NHEJ, the DSB ends are recognized and bound by the KU70 and KU80 heterodimer (1). The KU complex at a DSB recruits the DNA-dependent protein kinases, DNA-PKcs, and stabilizes KU binding to DNA (2), followed by the rejoining of DSBs with XRCC4-LIGASE4 (3, 4).

Chromatin remodeling facilitates transcriptional regulation either by posttranslational modification of histones or by ATP-dependent chromatin remodeling (ATP-CR). Although chromatin remodeling has been characterized in transcriptional regulation processes, it has recently been established that chromatin remodeling propagates DNA damage signals within cells and facilitates access of DNA repair proteins to DNA damage in chromatin (5–8). Factors of ATP-CR are divided into four families, SWI/SNF, CHD, ISWI, and INO80, each of which

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contains a family-specific ATPase subunit and various complexes for each regulation purpose. Although several ATP-CR complexes have been reported to be involved in DNA damage response and repair especially in yeast (8, 9), factors and mechanisms contributing to DNA repair remain elusive.

The complexes belonging to the SWI/SNF family in human cells are further divided into various BRG1-associated factor (BAF) complexes based on their functions and each complex consists of around 10 subunits. The chromatin remodeling complexes bind to a fixed position on DNA, break contact between histone and DNA, and translocate DNA through the ATPase activity, and generate a space for DNA binding factors for transcription (10). The major complex in SWI/SNF is either BAF or polybromo-associated BRG1-associated factor (PBAF) complex, which contains an ATPase, either BRM or BRG1, and a set of highly conserved core subunits such as SNF5, BAF155, and BAF170 (11) with other variant subunits, which determine the target of the chromatin remodeling. *ARID1A* and *ARID1B* encode variant subunits in BAF complexes, large nuclear proteins, BAF250a and BAF250b, respectively, and are responsible for directing the SWI/SNF complex to target promoters and regulate the transcription of certain genes by altering the chromatin structure around those genes (12). By way of caution, *ARID1A* and *ARID1B* are used as protein names, not BAF250a and BAF250b. *ARID1A* was reported to contribute to cell-cycle arrest (13), to interact with p53 and BRG1/BRM (14), and with topoisomerase II α (15), suggesting that *ARID1A* is important as a tumor suppressor. *ARID1B* shows approximately 60% amino acid sequence identity with *ARID1A* across their entire lengths. Mutations in the *ARID1B* gene cause Coffin-Siris syndrome, exhibiting developmental defects, and haploinsufficiency of *ARID1B* is a frequent cause of intellectual disability (16–19). Recently, however, mutations in the *ARID1B* gene have been reported in breast cancer (20). Despite their high similarity in amino acid sequence, the patterns of expression of *ARID1A* and *ARID1B* are different during early development in mouse embryos (21) and SWI/SNF complexes containing *ARID1A* have an antiproliferative function, whereas the SWI/SNF complex harboring *ARID1B* showed a pro-proliferative function (22), indicating that *ARID1A* and *ARID1B* have different functions in development and cell-cycle control. Their functions in relation to DNA damage response and repair have not yet been studied.

Although yeast SWI/SNF complex has been linked to DSB repair especially in homologous recombination (8, 9, 23), how the mammalian SWI/SNF complex is involved in DSB repair remains elusive. Although BRM and its alternative, BRG1, contribute to DNA repair of DSBs and UV damage in human cells (24–26) and involvement of SWI/SNF-interacting BRIT1/MCPH1 in DNA damage response were reported (27), details about the involvement of other factors in DNA damage response and repair are not known. Importantly, the difference in chromatin remodeling factors between transcription regulation and DNA damage response is not known yet. Recent cancer genome sequencing efforts revealed that *ARID1A* is one of the most frequently mutated genes across all cancer types, especially in ovarian, breast, gastric, and lung cancer (28, 29). Furthermore, in primary cancer cells of various tissues, expres-

sion of *ARID1A* is frequently and significantly reduced or absent (29). Various mutations in SWI/SNF family members were identified by genome sequencing of various cancer tissues (10, 30, 31), and these prompted us to analyze whether and which SWI/SNF factors are involved in DNA repair, especially in the response to DSB, using live cell analysis.

Materials and Methods

Cell culture

All the cell lines used in this article were obtained from National Cancer Center Research Institute, Tokyo, Japan, for this research purpose. Cell types of the cancer cell lines are shown in Supplementary Table S1. All the cell lines used in this article were obtained from National Cancer Center Research Institute, Tokyo, Japan, for this research purpose. Cell types of the lung cancer cell lines are shown in Supplementary Table S1. Cell lines used in this article were not authenticated by cell bank, but thoroughly tested for the cell line-specific expression of various SWI/SNF factors, which was shown in this article. U2OS cells were maintained in Dulbecco's Modified Eagle's Medium (Wako), H1299, H1299dA3-1 cells, and lung cancer cell lines were maintained in RPMI-1640 (Wako) supplemented with 10% FBS (Gibco), L-glutamine (Wako), and penicillin-streptomycin (Nacalai Tesque) and cultured at 37°C with 5% CO₂. Puromycin (Sigma-Aldrich), 2 μ g/mL final, was added to maintain H1299dA3-1 cells.

NHEJ assay

As reported previously (26), the I-SceI expression plasmid, pCMV-3xNLS-I-SceI (a generous gift of Dr. M. Jasin), was introduced by transfection with Lipofectamine 2000 reagent (Invitrogen) into H1299dA3-1 cells harboring two I-SceI sites located 1.3 kb apart between the promoter and GFP. The cells were pretransfected with siRNA for 48 hours using Lipofectamine RNAiMAX (Invitrogen) into 0.1×10^5 H1299dA3-1 cells in 24-well plates. Cells were harvested by trypsinization 48 hours after I-SceI transfection, washed with PBS, and applied to the CYTOMICS FC500 (Beckman Coulter). The percentage of GFP-positive cells in 1×10^4 cells were counted using CXP software (Beckman Coulter).

Laser micro-irradiation

Laser micro-irradiation was performed using the FV500 confocal scanning laser microscopy system (Olympus). Cells in glass-bottomed dishes were treated with 0.1 μ mol/L 8-methoxypsoralen (Sigma-Aldrich) for 3 hours before irradiation and micro-irradiated with a 405-nm pulse laser (Olympus). The irradiation dose was fixed at 25 scans after 8-MOP treatment. Although with this wavelength of light, cross-linking of 8-MOP to DNA hardly occurs, at least 500 DSBs are produced per 25 scans on the irradiated track determined by the number of γ H2AX, which corresponds to that in the same cell irradiated with ionizing radiation (IR) of 50 Gy. To examine the accumulation of DSB repair proteins at laser irradiated sites, H1299 cells were transfected with GFP-KU70 and GFP-KU80, GFP-XRCC4, or GFP-BRM 48 hours before micro-irradiation. At least 10 cells were irradiated in every experiment, and representative data were shown. Fluorescence images were obtained at 90 seconds after micro-irradiation.

For statistical analysis, protein accumulation at laser-irradiated site was quantitated by intensity of GFP or dsRed fluorescence using FluoView software (Olympus), and the mean fluorescence intensity at the irradiated region was subtracted from that of nonirradiated nuclear region for each cell. The value of quantitated fluorescence intensity at control siRNA-treated cell is defined as 100% and relative percentage of the fluorescent intensity in cells treated with each siRNA is shown in the graphs. The mean values of three independent transfection experiments of each siRNA are given. To exhibit statistical significance, at least 10 cells per transfection ($n > 10$) are analyzed, and two-sided and homoscedastic Student *t* test method was used to determine *P*-value.

Plasmids

GFP-tagged genes were constructed by introducing full-length cDNA into the pEGFP-C1 vector (Clontech) behind GFP in frame, resulting in expression vectors pEGFP-ARID1A, pEGFP-KU70, pEGFP-KU80, and pEGFP-XRCC4. An N-terminally Halo-tagged ARID1B (pHalo-ARID1B) was obtained from KAZUSA DNA Research Institute.

siRNA

To avoid off-target effects by siRNA and increase the reliability of gene suppression experiment, at least two sets of siRNA (set 1 and set 2) or 3'-UTR-ARID1A and 3'-UTR-ARID1B were used for each experiment in this work (see the sequences of siRNA in Supplementary Table S2). Most of the siRNA of set 1 consist of three different siRNAs targeted for each gene purchased from Qiagen and most of the set 2 consist of four different siRNAs from Dharmacon. None of the siRNA sequences used in this article overlaps with each other. U2OS cell line was used for cellular survival experiments. Because of cross-reaction found among set 1 siRNAs in U2OS cells, only set 2 siRNA was used for cell survival experiments. This cross-reaction was not observed in the H1299 cell line. Absence of off-target effect in set 2 was confirmed except siBRG1, which was not used for survival experiment. For siRNA transfections, Lipofectamine RNAiMAX (Invitrogen) was used, and Lipofectamine 2000 (Invitrogen) was used for cotransfection of siRNA and plasmids according to the manufacturer's instructions. Expression of the target proteins was checked by Western blots in each of the siRNA treatments.

Western blots and antibodies

Western blots were performed with anti-ARID1A (Abcam), anti-ARID1B (ABGENT), anti-KU80 (Santa Cruz Biotechnology), anti-KU70 (Neomarkers), anti-BRG1 (Santa Cruz Biotechnology), anti-BRM (Abcam), anti-SNF5 (GeneTex), anti-BAF53a (Abcam), anti-BAF57 (Abgent), anti-BAF60a (Abgent), anti-BAF60c (Abgent), anti-BAF155 (Abgent), and anti-BAF170 (Santa Cruz Biotechnology). The secondary antibodies were horseradish peroxidase-linked antibodies raised against rabbit or mouse immunoglobulin G (Santa Cruz Biotechnology).

Survival assays

Three days after transfection with siRNA, about 400 U2OS cells were plated on 6-cm dishes. Cells were irradiated with IR

or cisplatin was added to medium at indicated dose 6 hours after plating. Eight to 10 days later, cells were fixed with ethanol and stained with 0.3% crystal violet, and the numbers of colonies were counted. Each experiment was performed three times and standard errors were calculated.

Results and Discussion

Tagged ARID1A and ARID1B accumulate at DNA damage site and suppression of either expression reduces NHEJ activity

We first wanted to know whether ARID1A and ARID1B respond to DNA damage. As shown in Fig. 1A, GFP-tagged ARID1A and Halo-tagged ARID1B accumulated at the site of DNA damage produced by laser micro-irradiation in the presence of a photosensitizer 8-MOP, which produces efficiently DSBs as determined by the accumulation of KU proteins with the same irradiation condition in H1299 cells (Fig. 1A, left). Both ARID fusion proteins did not accumulate at the site irradiated with lower light dose or without photosensitizer. Although available antibodies are not good enough to show the response of endogenous ARID1 proteins, these data suggest that both GFP- or Halo-tagged ARID1 proteins have responded to DSB.

We next tested their involvement in DSB repair. First, we suppressed the expression of either ARID1A and/or ARID1B using two independent sets of siRNA specific for each gene and analyzed their effects on NHEJ activity (see Supplementary Table S2 for sequences of siRNA used in this article). We confirmed that both siRNA sets warrant specific suppression effects on either ARID1A or ARID1B in H1299 (Fig. 1B, top three panels) and the suppression of ARID1A or ARID1B expression did not influence the expression of NHEJ proteins (Fig. 1B, bottom). We then determined the NHEJ activity in H1299dA3-1 cells, a derivative cell line of H1299, in which NHEJ activity was determined by the percentage of GFP-positive cells after the induction of two DSBs by *I-SceI* transfection, removal of TK gene fragment, and successful NHEJ repair leading to the expression of GFP gene (see Supplementary Fig. S1; ref. 26). Suppression of either ARID1A or ARID1B expression significantly reduced the efficiency of the repair, but double knock-down of both genes did not further reduce the NHEJ activity obtained with single siRNA treatment (Fig. 1C). Set 1 siRNAs for ARID1A or ARID1B more effectively reduced NHEJ activity than set 2, which may be explained by an off-target effect of set 1 siRNA for ARID1A and ARID1B. These data indicate that both ARID1A and ARID1B were required for the NHEJ pathway.

Suppression of ARID1A or ARID1B expression reduces the accumulation of repair proteins involved in the NHEJ pathway at DSB

Having found that suppression of ARID1 proteins reduces NHEJ activity within cells, we wanted to know where ARID1 proteins are needed in NHEJ. We applied laser micro-irradiation and analyzed whether the suppression influences accumulation of KU70 and KU80 at laser-irradiated sites in H1299 and U2OS cells. Although the coexpressed GFP-KU70 and GFP-KU80 accumulate at laser-induced DSBs very effectively, suppression of either ARID1A or ARID1B significantly reduced the

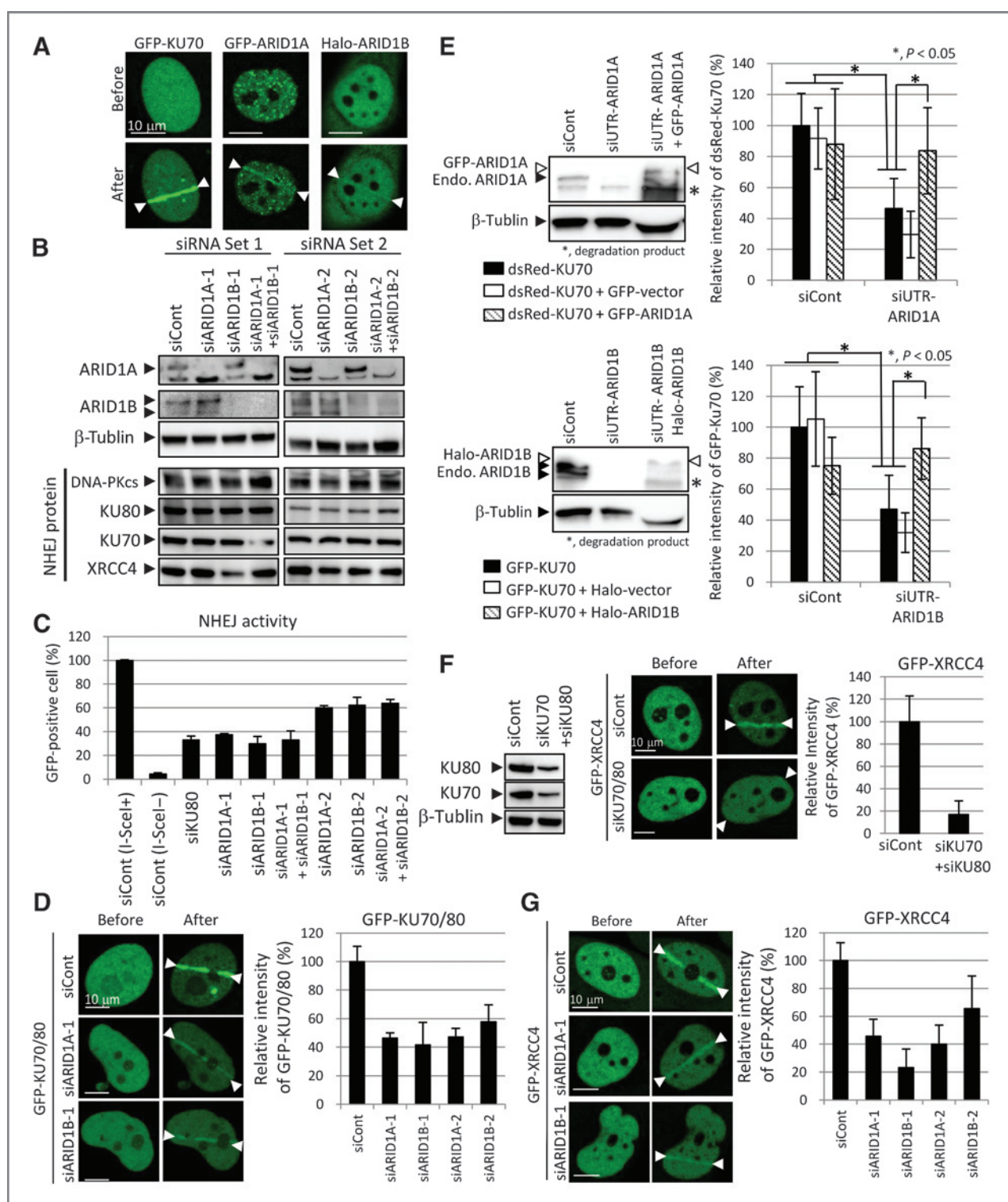


Figure 1. ARID1A and ARID1B are required for NHEJ and for accumulation of KU proteins at DSBs in H1299 cells. **A**, accumulation of GFP-tagged KU70 and ARID1A and Halo-tagged ARID1B at laser irradiated DSB site. **B**, suppression of expression of ARID1A and/or ARID1B by 2 sets of siRNA for each gene. Lack of influence of the gene suppression on the expression of NHEJ proteins is shown below. **C**, NHEJ activity was decreased in cells depleted with ARID1A and/or ARID1B expression. **D**, influence of ARID1A or ARID1B suppression on the accumulation of coexpressed GFP-KU70 and GFP-KU80 at laser-irradiated sites. Examples of accumulation (left) and decreased GFP-KU accumulation in ARID1-depleted cells (graph) are shown. **E**, complementation of dsRed-KU70 or GFP-KU70 accumulation at laser-irradiated sites in cells suppressed with siRNA targeted at 3'-UTR of ARID1A (top) or 3'-UTR of ARID1B (bottom) by expression of GFP-ARID1A or Halo-ARID1B, respectively. GFP-vector (top) or Halo-vector (bottom) was expressed as control shown in the middle of each set. **F**, accumulation of GFP-XRCC4 at laser-irradiated sites depends on the presence of KU protein within cells. **G**, accumulation of GFP-XRCC4 at laser-irradiated sites is decreased by suppression of ARID1A or ARID1B.

accumulation of coexpressed GFP-KU70 and GFP-KU80 in H1299 cell (Fig. 1D) and in U2OS cell (Supplementary Fig. S2B), suggesting that both ARID1A and ARID1B are required for the recruitment of GFP-tagged KU70 and KU80 proteins at DSBs. In contrast to Fig. 1C, there was no difference in the suppression effects on KU accumulation between set 1 and set 2 siRNAs. Fig. 1E shows rescue experiments, in which we expressed the GFP-ARID1A or Halo-ARID1B (indicated with open triangle in blots) in cells where endogenous ARID1A or ARID1B expression was suppressed, respectively, using siRNA targeted at 3'-UTR sequences of either gene. Correspondingly, the accumulation of dsRed-KU70 (top graph) or GFP-KU70 (bottom graph) at laser-irradiated site was recovered in the cells expressing both red and green fluorescent proteins. Although the transfection efficiency of Halo-tagged ARID1B was low as shown in the Western blot analysis (bottom blot right), those cells expressing Halo-tagged ARID1B were identified and analyzed under microscope. We obtained the identical data by using U2OS cells instead of H1299 (Supplementary Fig. S2C).

Accumulation of XRCC4 at DSBs is dependent on the presence of KU proteins at DSBs (3). In laser micro-irradiation experiments, accumulation of GFP-tagged XRCC4 at laser-irradiated sites also depends on the presence of endogenous KU protein (Fig. 1F). Therefore, it is reasonable to suppose that accumulation of GFP-XRCC4 at laser-irradiated site reflects the amount of endogenous KU proteins accumulated at DSBs. Fig. 1G shows that the suppression of either ARID1A or ARID1B by either set of siRNA significantly reduced the accumulation of GFP-XRCC4 at laser-irradiated sites in H1299 cell, indicating that ARID1A and ARID1B are necessary for the accumulation of endogenous KU proteins at DSBs. We obtained identical results using U2OS cell line (Supplementary Fig. S2D and S2E). Taken together, both ARID1A and ARID1B are involved in the NHEJ pathway at an early stage of the process. Because both ARID1 proteins are variant subunits in the BAF complex, these data indicate that the BAF complex of the SWI/SNF family is involved in DNA DSB repair. However, which subunits in the BAF complex are involved in the damage response process remains elusive.

Determination of SWI/SNF subunits required for NHEJ

Having established the requirement of ARID1A and ARID1B in NHEJ, we wanted to extend our knowledge to which subunits in the SWI/SNF complex are required for NHEJ repair of DSBs. We performed the three experiments used for ARID1 proteins, namely, NHEJ activity and accumulation of GFP-KU70/GFP-KU80 and GFP-XRCC4 at laser-irradiated sites using two sets of siRNA specific to nine well-known SWI/SNF subunits, BRM, BRG1, SNF5, BAF53a, BAF57, BAF60a, BAF60c, BAF155, and BAF170 in H1299 cells, in which NHEJ assay system was established and all the tested SWI/SNF factors except BRG1 are well expressed. Therefore, siBRG1 was used as a negative control in gene suppression experiments. The suppression effects with set 1 as well as set 2 siRNA were very effective on every genes tested as shown in the Western blots in Fig. 2A and B. No influence on the expression of NHEJ proteins by set 1 and set 2 siRNA treatments was confirmed (Fig. 2C and D,

respectively). Fig. 2E and F show the results of three different experiments obtained by set 1 and set 2 siRNA, respectively, together with those for ARID1 proteins obtained in Fig. 1. Results of three different experiments correspond very well within each suppressed gene by either set of siRNA treatment. Suppression of BAF53a and BAF57, like the negative control of BRG1, decreased neither NHEJ activity, nor KU accumulation of GFP-KU and GFP-XRCC4 after treatment with either set of siRNA, whereas suppression of other factors significantly and similarly decreased both NHEJ activity and GFP tagged and endogenous KU accumulation at DSBs. As apparent from the comparison of the skylines of the graphs in Fig. 2E and F, the results obtained with set 1 siRNAs are the same as those obtained with set 2 siRNAs. It should be noted that siBRG1 of set 2 suppressed NHEJ activity but not the accumulation of GFP-KU and GFP-XRCC4, suggesting that this siBRG1 has an off-target effect on a factor required for accomplishment of NHEJ but not for the accumulation of KU at DSB. Thus, besides the ATPase factor, SNF5, BAF60a, BAF60c, BAF155, BAF170, and two ARID1 proteins are necessary for NHEJ.

We used H1299 or its derivative cell line, in which one of the ATPase core subunit BRG1 is not expressed but BRM is expressed. We suppressed BRM expression and obtained the accumulation of GFP-KU by expression of BRG1 (Supplementary Fig. S3), indicating that either ATPase subunit, BRM or BRG1, is required and enough for KU accumulation at DSB.

Suppression of ARID1A and ARID1B increased cellular sensitivity to IR in U2OS cell

The above results suggest the involvement of ARID1A, ARID1B, and some other SWI/SNF subunits in NHEJ repair. We next examined the effects of SWI/SNF factor's suppression on colony-forming ability in U2OS cells exposed to IR. U2OS cell was chosen for analysis of cellular effect of unrepaired DNA damage. In U2OS cell, all the SWI/SNF factors tested here, including both BRM and BRG1, are expressed (Supplementary Fig. S4). We used set 2 siRNA because of cross reactivity of set 1 siRNA for ARID1B against ARID1A (Supplementary Fig. S2A, left top blot). In Fig. 3A (blots) suppression effects on ARID1A and/or ARID1B are shown together with those on KU70 and KU80 (siKU70/80) in U2OS cells. If the expression of either ARID1A or ARID1B gene was suppressed, cells were mildly sensitive to IR. Importantly, double suppression of both ARID1 genes did not further increase the sensitivity to IR, as was the case in the previous NHEJ assays, suggesting that both ARID1A and ARID1B contribute to the same pathway of cellular resistance. Simultaneous suppression of KU70 and KU80 provided cells with higher sensitivity to IR than the ARID1A and ARID1B suppression, suggesting that KU may be able to contribute to IR resistance even in the absence of the ARID1 proteins. Because the cells suppressed with KU70/80 and ARID1A, or KU70/80 and ARID1B expression showed sensitivity at the level of KU depleted cells, indicating that KU and ARID1 proteins function in the same pathway. Cells with simultaneous suppression of KU70/80 and ARID1 showing slightly resistant than KU70/80-depleted cells might be because of less effective knockdown of KU proteins after triple suppression (Fig. 3A, left bottom blots).

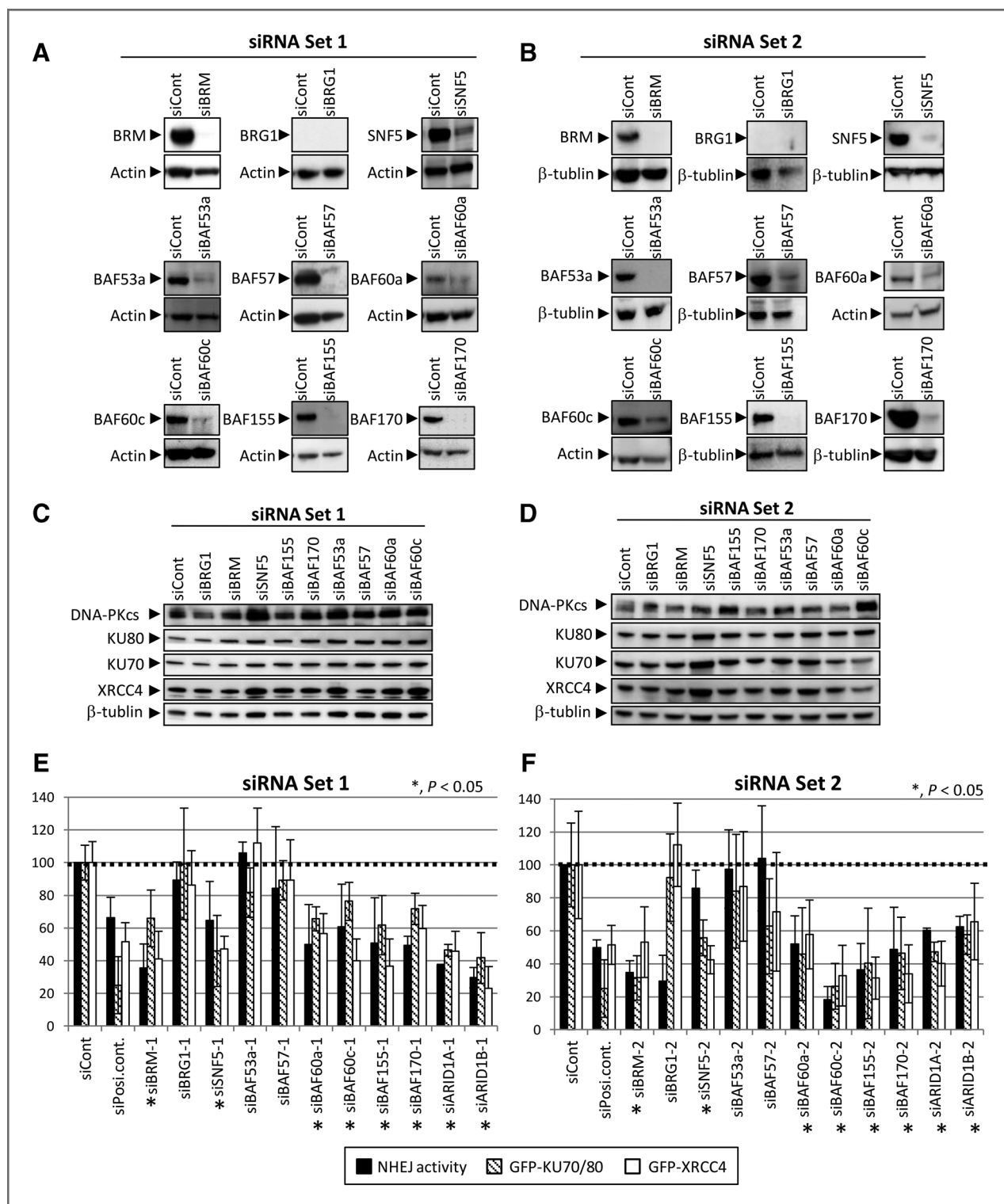


Figure 2. Influence of suppression of SWI/SNF factors on NHEJ activity, accumulation of GFP-KU70/KU80, and GFP-XRCC4 at laser-irradiated site in H1299 cells. Western blotting of SWI/SNF factor in cells suppressed with siRNA set 1 (A) and with set 2 (B) for each factor. H1299 cell does not express BRG1. No influence of suppression with siRNA set 1 (C) and set 2 (D) on NHEJ protein expression. E, influence of suppression with siRNA set 1 for each factor on NHEJ activity, accumulation of GFP-KU70/KU80, and GFP-XRCC4 at laser-irradiated sites. Positive control of a reduction of NHEJ frequency by gene suppression was siKU80. Positive control of reduced accumulation of GFP-KU70 and GFP-KU80 as well as of GFP-XRCC4 by gene suppression was siACF1, as previously reported (40). F, influence of suppression with siRNA set 2 for each factor on NHEJ activity, accumulation of GFP-KU70/KU80, and GFP-XRCC4 at laser-irradiated sites.

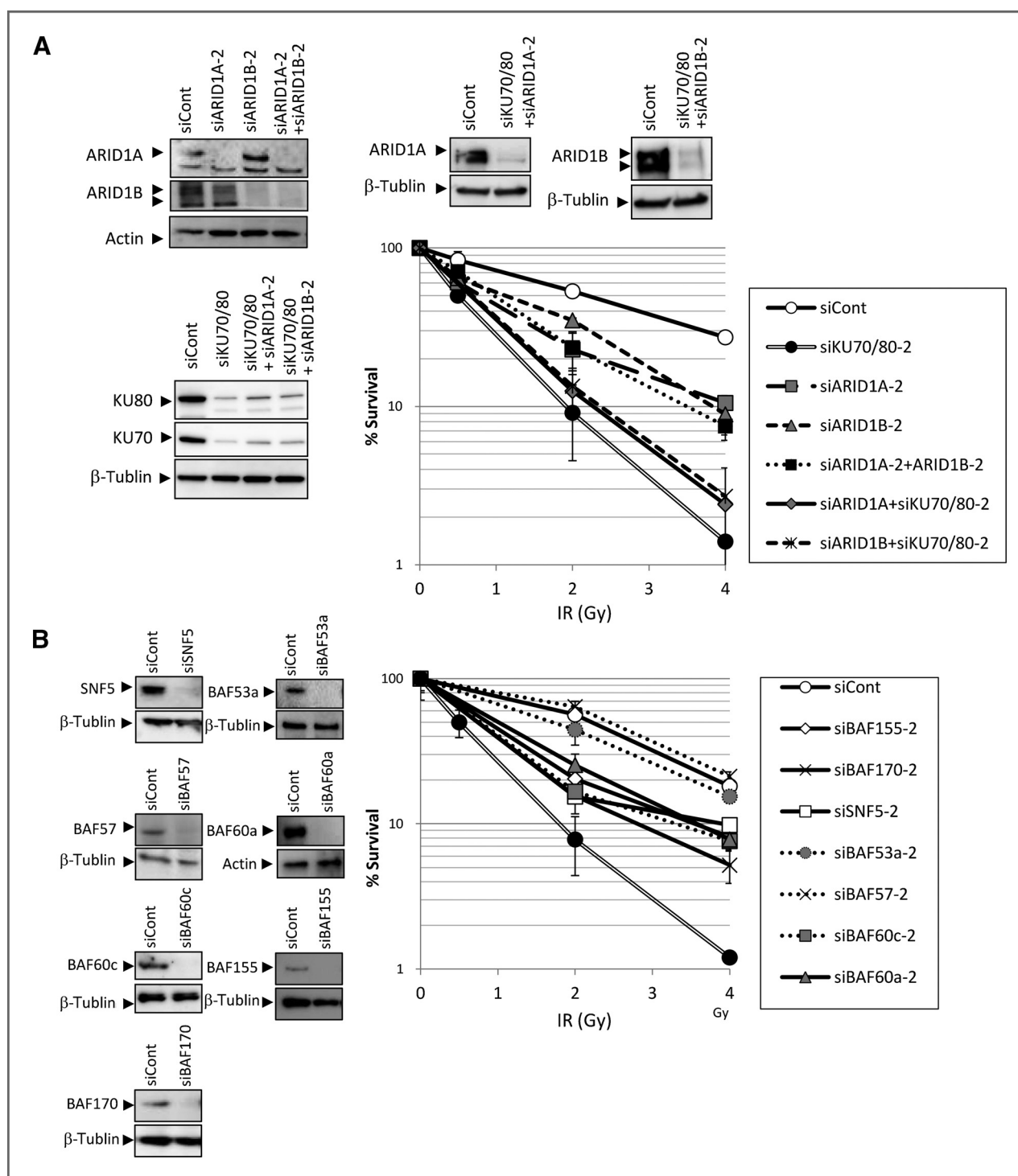


Figure 3. Influence of suppression of SWI/SNF factor expression on colony-forming ability of U2OS cells after IR treatment. A, Western blotting of cell extracts from cells suppressed with ARID1A and/or ARID1B (left top blots), KU70 and KU80 (left bottom blot, second lane from left), KU70, KU80, and ARID1A or ARID1B (third or rightmost lane), KU70, KU80, and ARID1A or ARID1B (top middle or right blot) siRNA in U2OS cells. Colony-forming ability of cells suppressed with either of the siRNA combination after IR. B, Western blotting of cell extracts from cells suppressed with siRNA for each SWI/SNF factor. Colony-forming ability of cells suppressed with siRNA for each SWI/SNF factor after IR.

SWI/SNF factors required for cellular resistance to IR

We investigated further the influence of other SWI/SNF subunits tested in Fig. 2 on cellular resistance to IR. Successful

suppression of each subunit protein expression was obtained in U2OS cells (Fig. 3B). Survival curves show the sensitivity to IR after suppression of each SWI/SNF subunits. The suppression

of the SWI/SNF subunits, SNF5, BAF155, BAF170, BAF60a, and BAF60c, which were shown to be required for efficient NHEJ in the previous assays, increased cellular sensitivity to IR, which was as sensitive as those of ARID1 suppression, whereas BAF53a and BAF57, suppression of which did not influence NHEJ in the previous assays, were not necessary for IR resistance. Thus, the results of NHEJ assays are consistent with the sensitivity to IR of cells.

Suppression of SWI/SNF factors sensitizes cells to cisplatin

In order to know the influence of drug used for cancer therapy on the SWI/SNF-depleted cells, we applied cisplatin, a DNA intra- and interstrand crosslinks (ICL)-inducing agent. DNA damage induced by cisplatin is repaired by several pathways, including nucleotide excision repair (NER), homologous recombination, and translesion synthesis (32). Suppression of ARID1A as well as ARID1B sensitized cells to cisplatin more significantly than IR but double knockdown of both ARID1 proteins did not increase the cellular sensitivity of single suppression (Fig. 4A). Suppression of SNF5, BAF155, BAF170, BAF60a, and BAF60c sensitized cells at the same level as ARID1A and ARID1B suppression, whereas suppression of BAF53a or BAF57 expression did not influence to cellular resistance to cisplatin (Fig. 4B). These data suggest that the SWI/SNF factors required for NHEJ and cellular resistance to IR are important for the cisplatin resistance as well. Correspondingly, we found that ARID1A- and/or ARID1B-depleted cells are sensitive to UV irradiation (Fig. 4C). Previously, the SWI/SNF complex in yeast and mammalian SNF5 have been reported to contribute to NER of UV damage in human cells (25, 33), suggesting that the SWI/SNF complex, including ARID1A and ARID1B, responds to various types of DNA damage and contributes at least to NHEJ and NER. Further analysis is required for understanding the role of the SWI/SNF factors in the repair processes.

Interdependent protein stability of the SWI/SNF factors required for cellular resistance to DNA damage

Because expression of SWI/SNF factors can be influenced by transcriptional regulation of other factors and protein stability is often acquired by protein-protein interactions, we analyzed the effects of suppression of each of the SWI/SNF factors on the expression of the others in H1299. Fig. 5A depicts the effects of suppression of ARID1A or/and ARID1B on the expression of the other SWI/SNF factors in the H1299 cell line using 2 sets of siRNA. H1299 cells were used for this purpose, because most of the SWI/SNF factors examined are expressed properly except BRG1 (Fig. 5A). Western blots showed that suppression of ARID1A obtained with either set of siRNA mildly reduced the level of BAF155 as well as that of SNF5 more mildly. However, suppression of ARID1B had no apparent influence on the expression of the BAF proteins examined. We further analyzed the interrelationships of the expression of other factors used in the experiments of this article (Fig. 5B). Because off-target effect of the suppression cannot be excluded, only when the results of 2 sets of suppression experiments were consistent,

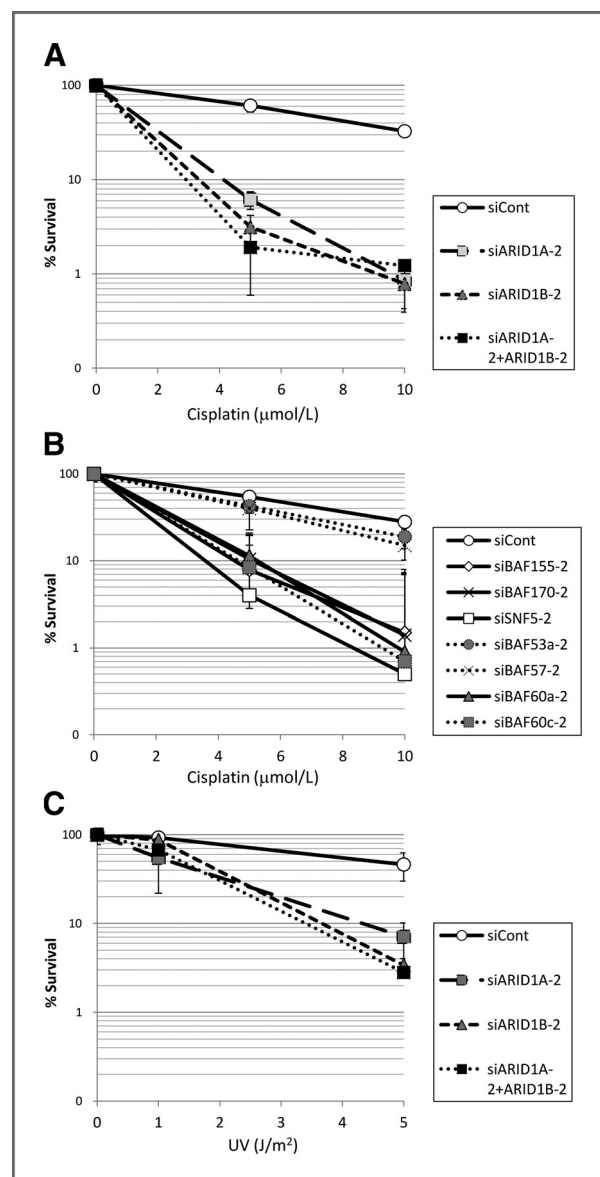


Figure 4. Influence of suppression of SWI/SNF factor expression on colony-forming ability of U2OS cells after cisplatin or UV treatment. A, ARID1A or ARID1B suppression sensitizes cells to cisplatin more than to IR, but no increase in the effect, even if both ARID1 proteins are suppressed simultaneously. B, suppression of other SWI/SNF factors required for IR resistance provided cells with cisplatin sensitivity, which is comparable with that obtained with ARID1 suppression. C, suppression of ARID1A and/or ARID1B expression sensitizes cells to UV irradiation.

we defined the relation as interactive. Fig. 5C summarized the results shown in Fig. 5B, in which a decrease of stability of one factor by suppression of other factor is indicated with a coming arrow. Suppression of SNF5 significantly decreased the level of BAF170 and BAF155 as well as ARID1B, but not ARID1A, whereas suppression of either BAF170 or BAF155 decreases the expression of SNF5. Suppression of ARID1A strongly and mildly decreased the expression of BAF155 and SNF5, respectively (Fig. 5A), suggesting that this decrease of SNF5

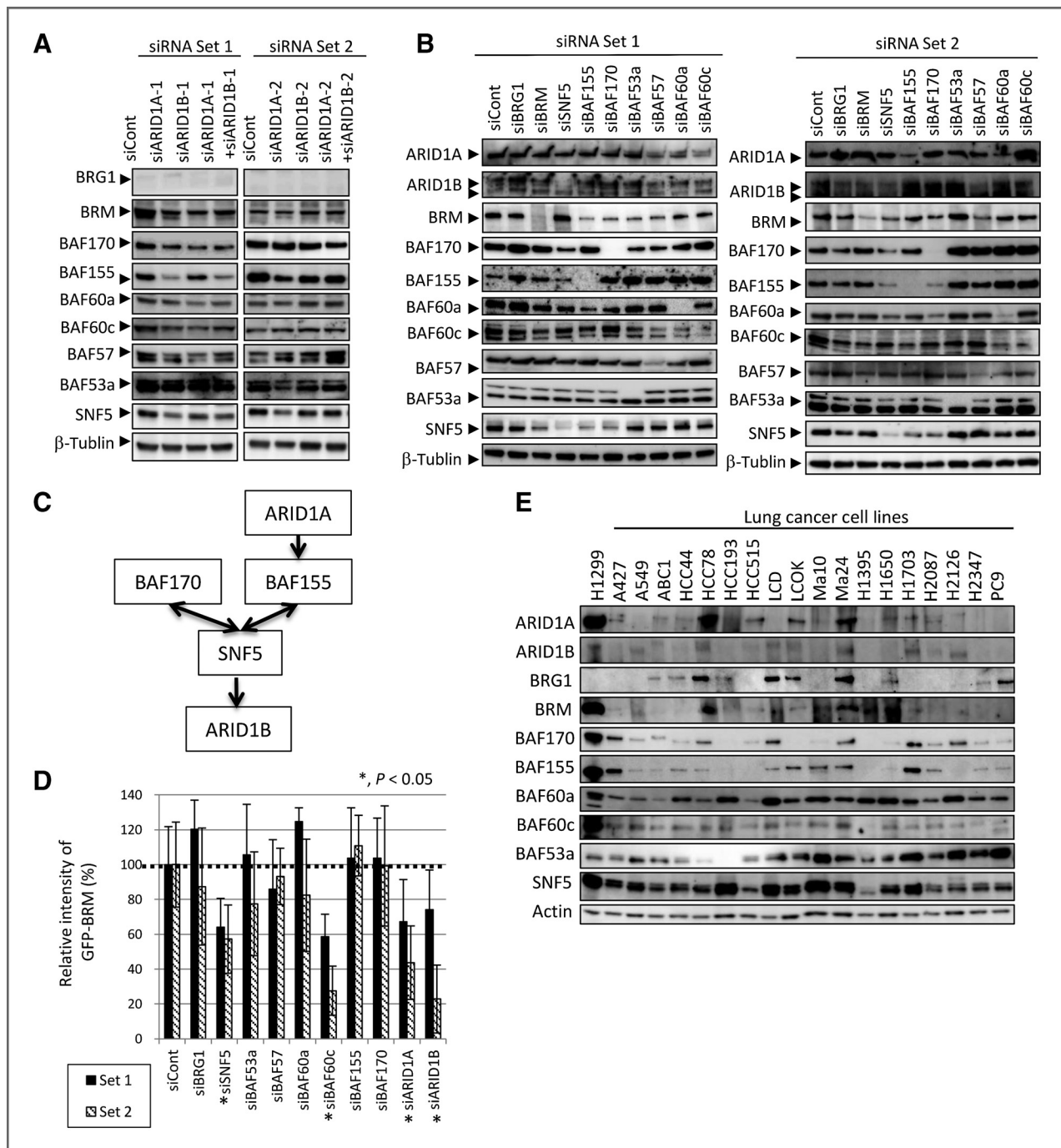


Figure 5. Expression of SWI/SNF factors. A, expression of SWI/SNF factors in H1299 cells depleted with ARID1A and/or ARID1B with set 1 (left) or set 2 (right) siRNA. B, expression of SWI/SNF factors in H1299 cells depleted with set 1 (left) or set 2 (right) siRNA. C, direction of suppression of SWI/SNF factor expression by suppression of other factor; decrease of expression of one factor influenced by suppression of another factor is indicated with an arrow. D, influence of gene suppression with set 1 and set 2 siRNA on the accumulation of GFP-BRM at laser-irradiated sites. E, Western blotting of total cell extracts from various lung cancer cell lines using antibody against the SWI/SNF factors used in this article. Antibody against BAF53a was derived from different lots of the same manufacturers and reacted differently, but recognized BAF53.

expression may be caused via BAF155, but ARID1B, a protein further downstream, is not influenced by the suppression of ARID1A. Suppression of BAF60a may influence the expression of BAF60c (Fig. 5B). These relationships of expression among

SWI/SNF factors possibly reflect the protein stability obtained by direct protein–protein interaction within the complex. Indeed, physical interactions between ARID1A and BAF155 (34), SNF5 and BAF155 (35), SNF5 and BAF170 (36), and SNF5

and ARID1B (37), have been reported by immunoprecipitation method.

SWI/SNF factors required for the accumulation of GFP-BRM at laser-irradiated site

An ATPase core subunit of the SWI/SNF complex is essential for the remodeling of chromatin structure. We, therefore, asked which SWI/SNF subunits are necessary for the recruitment of the ATPase subunit, BRM, to DSB sites. GFP-BRM and GFP-BRG1 have been reported to accumulate at DSBs in response to laser-micro irradiation (26). H1299 cells were treated with either set 1 or set 2 siRNA for SWI/SNF factors and the accumulation of GFP-BRM at laser-irradiated DNA damage sites was evaluated (Fig. 5D). Results of either siRNA set coincide very well and suppression of ARID1A, ARID1B, SNF5, and BAF60c, but not the other factors, reduced GFP-BRM accumulation at laser-irradiated sites. It suggests that the above four (but not other) subunits are necessary for the recruitment of BRM to DSB sites and both ARID1 proteins cooperate in this process.

Expression of SWI/SNF factors in lung cancer cell lines

To examine expression of SWI/SNF subunits in cancer cells, we performed Western blot analysis of the ATPases (BRG1 and BRM) and other subunits (SNF5, BAF53a, BAF60a, BAF60c, BAF155, BAF170, ARID1A, and ARID1B) in the total extracts of various lung cancer cell lines (see Supplementary Table S1 for their cell types; Fig. 5E). Blots were overexposed to clarify the absence of expression of each factor in the cell lines. Expression of various SWI/SNF factors examined in this article is much less in most cancer cells than in H1299 cell line, which is shown at the most left lane in the blot. Significant reduction or loss in the expression of ARID1A or ARID1B was observed very frequently (27.7% or 33.3%, respectively) in the cancer cell lines tested and that of ATPase subunits, BRG1 and BRM, was also quite frequent (44.4% and 33.3%, respectively), whereas alterations in the expression of BAF60a, BAF60c, BAF53a, and SNF5 were rare but present. A similar result has been reported in breast cancer cell lines (38), suggesting that loss of expression of SWI/SNF factors is a common feature in cancer cells. Reduction in gene expression of SWI/SNF factors may be explained either by genomic mutation-like ARID1A or by epigenetic silencing. Suppression of chromatin remodeling function required for cellular development and gene regulation may be advantageous for proliferation of cancer cell. Multiple silencing of the expression of SWI/SNF factors in cancer cells may be achieved by loss of interaction partner as shown above. These results suggest that cancer cells tend to

suppress chromatin remodeling for rapid proliferation and ignore its function in DNA damage response and repair.

In summary, we have examined 10 well-known SWI/SNF factors and found that the BAF complex, including both ARID1A and ARID1B, contributes to NHEJ and cellular resistance to IR and cisplatin. The results show a difference in chromatin remodeling factors required for transcription regulation and DNA damage response. Importantly, although ARID1A and ARID1B are mutually exclusive variant subunits in the SWI/SNF complex with respect to transcription regulation (39), both ARID1 proteins play essential roles in DNA damage response and are required for recruiting the ATPase subunit of the complex to DNA damage site. Among the BAF factors, we found interdependent protein stability, which may explain very frequent silencing of expression of the factors in various types of primary and established cancer cells (Fig. 5E). Although cancer cells become more resistant to DNA damage with mutated p53, cells lacking the expression of SWI/SNF factors identified here are DNA repair deficient, and that is a fundamental vulnerability of the cells and may be useful for cancer therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. Yasui

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Kohno

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Watanabe, S.-I. Kanno, A. Yasui

Writing, review, and/or revision of the manuscript: R. Watanabe, H. Ogiwara, T. Nagase, A. Yasui

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Ui, H. Ogiwara, T. Nagase

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