ARID1A, a Factor That Promotes Formation of SWI/SNF-Mediated Chromatin Remodeling, Is a Tumor Suppressor in Gynecologic Cancers

Bin Guan1, Tian-Li Wang2, and Ie-Ming Shih1,2

Abstract

ARID1A (BAF250A) promotes the formation of SWI/SNF chromatin remodeling complexes containing BRG1 or BRM. It has emerged as a candidate tumor suppressor based on its frequent mutations in ovarian clear cell and endometrioid cancers and in uterine endometrioid carcinomas. Here, we report that restoring wild-type ARID1A expression in ovarian cancer cells that harbor ARID1A mutations is sufficient to suppress cell proliferation and tumor growth in mice, whereas RNA interference–mediated silencing of ARID1A in non-transformed epithelial cells is sufficient to enhance cellular proliferation and tumorigenicity. Gene expression analysis identified several downstream targets of ARID1A including CDKN1A and SMAD3, which are well-known p53 target genes. In support of the likelihood that p53 mediates the effects of ARID1A on these genes, we showed that p53 was required and sufficient for their regulation by ARID1A. Furthermore, we showed that CDKN1A (encoding p21) acted in part to mediate growth suppression by ARID1A. Finally, we obtained evidence that the ARID1A/BRG1 complex interacted directly with p53 and that mutations in the ARID1A and TP53 genes were mutually exclusive in tumor specimens examined. Our results provide functional evidence in support of the hypothesis that ARID1A is a bona fide tumor suppressor that collaborates with p53 to regulate CDKN1A and SMAD3 transcription and tumor growth in gynecologic cancers. Cancer Res; 71(21); 6718–27. ©2011 AACR.

Introduction

It has been well established that chromatin remodeling is critical for essentially all aspects of nuclear activities including transcription, DNA replication, and DNA damage repair (1–3), and molecular genetic changes in chromatin remodeling genes have emerged as a new mechanism in cancer pathogenesis. Amplification of Rsf1, a gene participating in ISWI chromatin remodeling, has been shown to promote chromosomal instability, propel tumor progression, and contribute to disease aggressiveness in ovarian and oral cancer (4–8). Also, somatic inactivating mutations have been detected in several SWI/SNF chromatin remodeling genes including PBRM1 (BAF180; ref. 9) in renal cell carcinoma, BRG1 (SMARCA4) in lung carcinoma (10, 11), and ARID1A (BAF250A) in endometrium-related carcinomas including uterine endometrioid carcinoma, ovarian clear cell carcinoma, and ovarian endometrioid carcinoma (12–14). In previous studies (12–14), we have found that 46% to 57% of ovarian clear cell carcinomas, 40% of uterine endometrioid carcinomas, and 30% of ovarian endometrioid carcinomas harbor somatic sequence mutations in ARID1A. ARID1A, a homologue of yeast SWI1, encodes a large nuclear protein, p270 (also known as BAF250a), which participates in forming a SWI/SNF chromatin remodeling complex (15). Coordinate activity of the proteins of the SWI/SNF complex is responsible for altering chromatin structure that is required to facilitate several cellular functions including transcription, DNA synthesis, and DNA damage repair (16–21). ARID1A binds to DNA nonselectively in vitro and recruits other components to the complex, a process that may confer specificity of the SWI/SNF (22, 23). The core protein and the ATPases, BRG1 or BRM, are responsible for moving or dispersing nucleosomes surrounding specific chromosomal regions such as transcription initiation sites (17, 24). It has been proposed that SWI/SNF gene members play an important role in embryonic development, tissue regeneration, cell senescence, apoptosis, and oncogenesis.

Because the majority of somatic mutations involving ARID1A in human cancers are insertions/deletions causing frameshift or nonsense mutations, ARID1A was thought to behave as a tumor suppressor gene, but direct evidence of its tumor suppressor activity has not been shown. In the present study, we examined the effects of ARID1A expression or loss of expression on carcinoma cells and nontransformed cells, respectively. In addition, we identified ARID1A-regulated genes as a primary step toward unveiling the mechanisms...
by which ARID1A inhibits tumor growth. Demonstration of tumor suppressor functions of ARID1A should be fundamental for future studies aimed at elucidating the biological roles of ARID1A and SWI/SNF complexes in endometrium-related tumorigenesis and should help clarify how aberrant chromatin remodeling activity participates in cancer development.

Materials and Methods

Plasmid construction and lentivirus production

The full-length cDNA of ARID1A (coding sequence of NM_006015) was subcloned from plasmids CMV-T7-hOsa1 (Addgene plasmid 17986; ref. (25) and pC1-neo-BAF250 (2) into pCDNA6-V5/His.b (Invitrogen) and pLenti-puro, with V5/His tags at the C-terminus. The tetracycline-inducible lentiviral vector pLenti-puro was constructed with pLenti4/TO/V5-DEST vector (Invitrogen) as a backbone by removing the Gateway elements between the 2 EcoRV sites and by replacing the Zeocin resistance gene with a puromycin resistance gene. The tetracycline repressor was introduced into cells using virus produced from pLent6/TR (Invitrogen). The control plasmid pLenti-puro-LacZ was constructed by cloning LacZ from pLent6/V5-GW/LacZ (Invitrogen) into the pLenti-puro vector. The short hairpin (shRNA) lentiviral plasmids were obtained from the RNA interference (RNAi) consortium. The shRNA sequences for ARID1A were as follows: sh1 (TRCN0000059090), CCTCTCTTATACACAGCAGAT; and sh2 (TRCN0000059091), CGGTGATGAACCTACTGGTT. The shRNA sequences for p21 were as follows: sh1 (TRCN0000040123), CGCTCTACATCTTCTGCCTTA, and sh2 (TRCN0000040126), GACAGATTTCTACACAGAT. The shRNA sequence for p53 was (TRCN0000003754) TCAGACC-TATGGAAAACCTT. Lentivirus was produced using HEK293FT cells (Invitrogen) with the second-generation packaging system pSPAX2 (Addgene plasmid 12260) and pMD2.G (Addgene plasmid 12259). Lentiviral titer was determined by a real-time qPCR method by measuring viral RNA content in viral supernatant as described (26). The firefly luciferase plasmids for the p21 promoter, WWP-Luc (27), and for SMAD3 (28) have been reported previously. The STR profiles of OSE4 and IOSE-80PC cell lines have not been catalogued by the Fragment Analysis Facility at the Johns Hopkins University, using the short tandem repeat (STR) DNA profiling (PowerPlex 1.2 System, Promega). The STR profiles of HEC-1-A and OVISE were matched to their original profiles in the cell line database at the Japanese Collection of Research Biorources (JCRB, http://cellbank.nibio.go.jp/cellbank_e.html). The STR profiles of OSE4 and IOSE-80PC cell lines have not yet been deposited but they were distinct from any cell line reported in the current STR Profile Databases maintained by JCRB and ATCC (http://www.atcc.org). Mycoplasma was tested negative in all cell lines (PlasmoTest; InvivoGen).

Tumorigenicity in immunocompromised mice

OSE4 cells were transduced by lentivirus expressing shRNA targeting ARID1A (sh1) or green fluorescent protein (GFP), and 2 days later, the transduced cells were injected into the subcutaneous tissue on the right and left flanks, respectively, of 4- to 6-week-old nu/nu mice. For each injection site, 4 × 10^6 cells mixed 1:1 (v/v) with Matrigel (BD Biosciences) were injected. The tumors were excised and weighted 6 weeks after inoculation. All tumors were confirmed by histopathologic and Western blot analysis (Supplementary Fig. S1), we selected the HEC-1-A uterine endometrioid carcinoma cell line and the OVISE ovarian clear cell carcinoma cell line as the models. Both cell lines contained homoygous ARID1A mutations and did not express detectable ARID1A protein. A Tet-On tetracycline-inducible system was established to control ARID1A expression in both cell lines. Expression of ARID1A in

Chromatin immunoprecipitation

Cells were cross-linked with 1% formaldehyde (Sigma) diluted in culture media at room temperature for 10 minutes followed by quenching with 125 mmol/L glycine for 5 minutes. The chromatin was extracted with chromatin immunoprecipitation (ChIP) lysis buffer (1% SDS, 10 mmol/L EDTA, and 50 mmol/L Tris, pH 7.5). After one freeze-thaw cycle, the DNA was fragmented twice for 10 minutes with a Bioruptor (Diagenode) sonicator at 30 second on/off cycles set at the highest intensity. The chromatin was precipitated using 2 μg antibody preabsorbed onto 20 μL Protein A/G (1:1) Dynal magnetic beads (Invitrogen). After reverse cross-linking, the DNA was purified with a PCR purification kit (Qiagen). Binding to promoter regions was analyzed by qPCR and reported as the percentage of input. In ChIP-reChIP, immunocomplex from the first ChIP was eluted with 10 mmol/L dithiothreitol in Tris-EDTA buffer at 37°C. The eluates were then diluted with 10 volumes of ChIP lysis buffer and used for the second round of ChIP.

Cell lines

The nature of ovarian surface epithelial cells OSE4 (29) and IOSE-80PC (30), as well as the ovarian clear cell carcinoma cell line OVISE (31), were previously described. All the cell lines were cultured in RPMI-1640 supplemented with 10% FBS. HEC-1-A cells were obtained from ATCC. These 4 cell lines exhibited distinct morphologic features and were also authenticated by the Fragment Analysis Facility at the Johns Hopkins Medical Institutions.

Results

Restoring ARID1A expression suppresses tumor growth

To determine the tumor-suppressive function of ARID1A, we restored the expression of wild-type ARID1A in cancer cells harboring ARID1A mutations. On the basis of mutation information and Western blot analysis (Supplementary Fig. S1), we selected the HEC-1-A uterine endometrioid carcinoma cell line and the OVISE ovarian clear cell carcinoma cell line as the models. Both cell lines contained homoygous ARID1A mutations and did not express detectable ARID1A protein. A Tet-On tetracycline-inducible system was established to control ARID1A expression in both cell lines. Expression of ARID1A in
those cells was induced by treating cells with doxycycline, a derivative of tetracycline (Supplementary Fig. S2). As a control, we also established Tet-On–inducible LacZ expression in both cell lines (Supplementary Fig. S2). Cellular proliferation and the percentage of cells in the S-phase of cell cycle were markedly decreased in ARID1A-induced cells as compared with the control groups lacking ARID1A expression (Fig. 1A and B). In contrast, induced expression of ARID1A in the ovarian clear cell line JHOC5, which has wild-type ARID1A and abundantly expressed the protein, had minimal effects on cellular proliferation and cell-cycle progression (Supplementary Fig. S3).

To extrapolate the aforementioned in vitro findings to an in vivo setting, we established Tet-On–inducible HEC-1-A tumor xenografts in athymic nu/nu mice. In the experimental group, ARID1A expression was induced 10 days after tumor cell inoculation when small subcutaneous tumors were palpable. We found that expression of wild-type ARID1A significantly...
reduced tumor weights as compared with the control group without restoration of wild-type ARID1A (*P* = 0.025; Fig. 1C). As expected, tumor growth in mice was not affected by induction of the control gene *LacZ* (Fig. 1C). Immunohistochemistry done on the excised tumors showed ARID1A nuclear immunoreactivity in ARID1A-induced tumors (+Tet) but not in mock-induced tumors (−Tet), confirming tetracycline regulation of ARID1A expression in the tumor xenografts (Supplementary Fig. S4). On the basis of immunostaining, the level of ARID1A induction in tumor cells, although varying in intensity, was comparable with mouse stromal cells and endometrium, indicating a physiologic level of ARID1A expression in our Tet-On--inducible system (Supplementary Fig. S4).

**Silencing of ARID1A expression enhances cellular proliferation and tumorigenicity**

Our previous studies (14, 32) together with the Western blot analysis (Supplementary Fig. S1) show that *ARID1A* mutations correlate with loss of protein expression in human endometrium-related cancers. Thus, to simulate the effect of *ARID1A* mutations, we knocked down *ARID1A* in 2 epithelial cell lines, IOSE-80PC and OSE4, which were derived from normal ovarian surface epithelium. It has been shown in an engineered mouse model that ovarian surface epithelium may undergo Mullerian metaplasia to become endometrium tissue and develop ovarian endometrioid carcinoma after genetic inactivation of Wnt/β-catenin and PI3K/Pten pathways (33). Furthermore, ovarian clear cell carcinoma has long been thought to derive from endometriosis. Because normal epithelial cell lines established from uterine endometrium have not been available, ovarian surface epithelial cell lines may represent the relevant models currently available to prove the principle whether loss of *ARID1A* expression promotes oncogenesis.

As compared with cells transduced with control virus carrying GFP-targeting shRNA, cells transduced with ARID1A shRNA virus showed an elevated proliferation rate and an increased cell population in S-phase (Fig. 1D and E). To determine whether ARID1A knockdown affected tumorigenicity in vivo, OSE4 cells, in which ARID1A expression had been knocked down by shRNA, were injected into athymic *nu/nu* mice. Although OSE4 cells are poorly tumorigenic, they became highly tumorigenic when ARID1A expression was reduced by shRNA (Fig. 1F).

**ARID1A regulates p53-controlled genes**

The aforementioned results indicate that ARID1A controls cell-cycle progression and downregulation of ARID1A expression leads to increased cellular proliferation. To determine the possible mechanisms, we compared the transcriptomes of IOSE-80PC and OSE4 cells between ARID1A knockdown and control groups using Illumina BeadChip arrays. We identified a total of 104 genes in which transcript levels were up- or downregulated by at least 2 folds in both cell lines (Supplementary Table S1 and Fig. 2A). Those genes downregulated by ARID1A shRNAs included *CDKN1A, SMAD3, SMAD5, Nagl* (of TGF-β superfAMILY), *TRIM8*, and *SMARCD1* (BAF60A). Pathway analysis of these AIRD1A-regulated genes showed an enrichment of pathways in cell-cycle regulation (Supplementary Table S2), and, in fact, the top 2 pathways involved cell-cycle regulation. Interestingly, even though the expression levels of p53 remained stable, several p53-related genes were downregulated by ARID1A knockdown (Fig. 2A). Among them, 2 prominent molecular hubs, *CDKN1A*, encoding *p21* (Cip1 or WAF1), and *SMAD3*, were identified. Because of the known biological roles of *CDKN1A* and *SMAD3* in pathways related to cell cycle and p53, we selected both for further characterization in this study. Downregulation of expression of *CDKN1A* and *SMAD3* by *ARID1A* knockdown was validated by quantitative RT-PCR or Western blot analyses (Supplementary Fig. S5A and Fig. 2B). In addition, induction of ARID1A expression led to upregulation of p21 protein in HEC-1-A and OVISE Tet-On–cells (Fig. 2C) and to an increase of SMAD3 mRNA levels in OVISE Tet-On–cells (Supplementary Fig. S6A).

To further determine whether p21 mediated the growth-suppressive effects of *ARID1A*, we knocked down *CDKN1A* in the OVISE cell line carrying Tet-inducible ARID1A. As expected, ARID1A induction resulted in growth suppression and p21 overexpression, and more importantly, 2 different p21 shRNAs significantly increased cellular proliferation in cells with ARID1A induction as compared with control (GFP) shRNA-treated cells (Fig. 2E), indicating that p21 was responsible, at least in part, for growth suppression following ARID1A induction.

**Binding of ARID1A/BRG1/p53 complex to the promoters of CDKN1A and SMAD3**

It has been established that ARID1A interacts with BRG1 ATPase to form a SWI/SNF chromatin remodeling protein complex (2, 22). It has also been reported that p53 interacts with SWI/SNF subunits BAF60A, *BRG1*, and *BAF47* (34–36). To determine whether p53 bound to ARID1A/BRG1 complex in our experimental system, we carried out coimmunoprecipitation in OSE4 cells by pulling down endogenous p53 and then blotted with either ARID1A or BRG1 antibodies. As shown in Fig. 3A, both ARID1A and BRG1 coimmunoprecipitated with p53. We further showed that the C-terminus (amino acids 1,759–2,285) but not the N-terminus (amino acids 1–1,758) of ARID1A mediates the interaction with p53 (Fig. 3B). Next, we addressed whether ARID1A and p53 could directly interact with each other. Recombinant GST–p53 (both wild-type p53 and R175H mutant) and the C-terminus of ARID1A (amino acids 1,759–2,4285 with C-terminal V5-tag) were purified from *Escherichia coli* and used in immunoprecipitation assay (Fig. 3C). Recombinant p53 but not glutathione S-transferase (GST) pulled down the C-terminus of ARID1A. Moreover, the ARID1A/BRG1 SWI/SNF complex and p53 occupied the same promoter regions of *CDKN1A* and *SMAD3*, as shown by sequential and reciprocal ChIP–reChIP using p53 and BRG1 antibodies, respectively (Fig. 3D). The aforementioned results provide new evidence that p53 is recruited to the ARID1A/BRG1 complex that binds to *CDKN1A* and *SMAD3* promoters.

To further delineate the role of ARID1A/BRG1/p53 complex in regulating transcription of *CDKN1A* and *SMAD3*, we carried out ChIP using antibodies reacting to BRG1. Antibody against
p53 was used in the ChIP assay as a control. As shown in Fig. 3E, both BRG1 and p53 bound to the promoter regions of CDKN1A and SMAD3, and, more importantly, knockdown of ARID1A significantly reduced BRG1 binding to both promoter sequences. In contrast, interaction of p53 with CDKN1A and SMAD3 promoter regions was unaffected by reduced expression of ARID1A. Besides, promoter reporter (luciferase) analysis showed that the transcriptional activity of p21 and SMAD3 was decreased by ARID1A knockdown (Supplementary Fig. S5B).

**ARID1A regulates p21 in a p53-dependent fashion**

Because p21 is a well-established downstream target of p53 and ARID1A interacts with p53, we asked whether ARID1A-regulated p21 transcription depended on the p53 pathway in 3 cell models. First, 2 different TP53-knockout cell lines, HCT116TP53–/– and MCF10ATP53–/–, were used. These cells, as well as their parental wild-type counterparts, were transfected with a plasmid expressing ARID1A-V5. We observed that ectopic expression of ARID1A-V5 was associated with increased mRNA levels of p21 in both HEC-1-A (left) and OVISE (right) cell lines. Next, we knocked down p53 levels with shRNA in HEC-1-A ARID1A–inducible cells and measured its effect on the transcription level of CDKN1A. The results showed that induction of p21 by ARID1A was significantly reduced by p53-specific shRNA as compared with control (GFP) shRNA-treated ARID1A-induced HEC-1-A cells (P < 0.01).
IgG ically, all 34 tumors with ARID1A showed a mutually exclusive pattern of the mutational status of CDKN1A type, and all 6 carcinomas harboring TP53 were known to have cell carcinomas and uterine endometrioid carcinomas that retained wild-type ARID1A.

Correlation of ARID1A mutation and TP53 mutation in tumor tissues

The aforementioned results suggest that inactivating mutations in either ARID1A or TP53 result in loss of transcriptional regulation of CDKN1A and SMAD3. To this end, we analyzed the mutational status of ARID1A and TP53 in 77 ovarian clear cell carcinomas and uterine endometrioid carcinomas that were known to have ARID1A and TP53 mutations. Our data showed a mutually exclusive pattern of ARID1A and TP53 mutations (P = 0.031, the Fisher exact test; Table 1). Specifically, all 34 tumors with ARID1A mutations were TP53 wild type, and all 6 carcinomas harboring TP53 mutations contained wild-type ARID1A.

Discussion

In this study, we showed the tumor-suppressive role of ARID1A and proposed the possible mechanisms involved. The evidence for tumor suppressor function of ARID1A comes from its ability to inhibit cellular proliferation and tumor growth when expressed in cancer cells harboring mutated ARID1A and to enhance cellular proliferation and tumorigenecity when its expression is silenced in epithelial cells with wild-type ARID1A. Although ARID1A mutations seem to predominate in endometrium-related carcinomas, inactivation of the ARID1A-containing BAF complex, as a consequence of genetic or epigenetic alterations, may be common in development of other human neoplastic diseases including carcinomas arising from kidney, breast, lung, and stomach (14, 37, 38). For example, deletion of the chromosome 1p35 region harboring...
ARID1A has been found in more than half of pancreatic carcinomas (39).

It has been established that SWI/SNF chromatin remodeling genes are required for several nuclear activities including transcription. It remains unclear, however, whether they act globally to facilitate transcriptional activity in transcriptionally active domains or whether they locally target a set of selected genes that work in concert for specific cellular functions. The data presented in this study, together with a recent report (40), favor the latter view, as evidenced by downregulation of specific genes upon ARID1A silencing. Analysis of ARID1A downstream genes reveals that the tumor suppressor role of ARID1A mainly involves negative regulation of cell-cycle progression and identifies 2 p53-regulated genes, CDKN1A and SMAD3, which may serve as the major target genes to mediate its tumor suppressor functions. The induction of p21 by ARID1A and negative regulation of ARID1A on cell-cycle progression in our cell models are in agreement with previous reports showing that ARID1A is required for cell-cycle arrest after induced differentiation in mouse osteoblastic cells (41, 42).

The regulation of p53-related genes by ARID1A raises the possibility whether ARID1A molecularly cooperates with p53 to inhibit tumor growth. In fact, this view is supported by several pieces of evidence reported in this study. Most importantly, both protein coimmunoprecipitation and ChIP analyses show that p53 interacts with ARID1A/BRG1 and the ARID1A/BRG1/p53 complex binds to the promoter regions of CDKN1A and SMAD3. By employing a TP53-knockout cell system and RNAi strategy, we observed that...
ARID1A-induced p21 and SMAD3 expression required the presence of p53. Therefore, it is possible that in nontransformed cells, ARID1A and p53 collaborate as a pair of gatekeepers that prevent tumorigenesis by transcriptional activation of tumor-inhibiting downstream genes such as CDKN1A and SMAD3, as the tumor suppressor role of CDKN1A that encodes for p21 has been well established (43) and deficiency of SMAD3 has been reported to propel tumor progression in a mouse model (44). Besides, we found that all tumors with mutated ARID1A contained wild-type TP53 and tumors with mutated TP53 harbored wild-type ARID1A. Because both ARID1A and TP53 seem to be essential for tumor suppression of endometrium-related cancer, concurrent mutations in both genes are thus not required for tumorigenesis. In other words, mutation in either ARID1A or TP53 is sufficient to inactivate the ARID1A/BRG1/p53 complex and silence transcription of CDKN1A and SMAD3.

Our coimmunoprecipitation study using recombinant p53 and ARID1A indicates that ARID1A directly binds to p53 proteins and thus may recruit p53 to the BAF complex for transcriptional regulation of its downstream targets. In this way, p53 may target the SWI/SNF complex to specific promoters with p53-binding sites, whereas the SWI/SNF complex alters the local chromatin structure by displacing nucleosomes and creating naked DNA sites to be occupied by transcriptional machinery including RNA polymerase II during transcription (Fig. 4C). The mutually exclusive pattern of ARID1A and TP53 mutations supports our view that these tumors do not require concurrent mutations in both genes because as shown in this study mutation of either gene is sufficient to turn off tumor suppressor activity by transcriptionally inactivating the set of genes coregulated by ARID1A and TP53. It has been established that p53 mutant such as R175H failed to induce p21 expression and cell-cycle arrest (27, 45). One of the functions of mutant p53 is to decrease wild-type p53 binding to its target genes including p21 in a dominant-negative manner (46). Recently, it has been reported that mutant p53 protein may bind to a set of unique genes that are not the conventional p53-regulated genes such as GRO1 (47). Thus, the observation that mutant p53 can bind to ARID1A raises the possibility that p53/ARID1A/BRG1 complex might regulate a different set of genes depending on the mutation status of TP53.

Table 1. Mutation status of ARID1A and TP53 in 77 ovarian clear cell and uterine endometrioid carcinomas

<table>
<thead>
<tr>
<th>ARID1A mutation</th>
<th>TP53 mutation</th>
<th>TP53 wild-type</th>
<th>Total</th>
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<tbody>
<tr>
<td>0</td>
<td>34</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>43</td>
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</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>71</td>
<td>77</td>
</tr>
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</table>

NOTE: P = 0.031.

It should be noted that OSE4 and IOSE-80PC were immortalized by SV40 large T antigen (29, 48), and one of the functions of T antigen (Tag) is to interact with p53 and inactivate its transcriptional activity (49). Interestingly, it has also been shown that the p53-Tag complex purified from human cells but not from mouse cells still retains p53 transcriptional activity (50), and p53 is required for the transcription of p53-responsive genes including the insulin-like growth factor I (IGF-I) and p21 in SV40 Tag–immortalized cells (51). Therefore, it warrants further study to determine whether ARID1A binds to p53 alone and/or p53-Tag complex in OSE4 and IOSE-80PC cells. Nevertheless, our data show that ARID1A is required for the expression of several p53-responsive genes and the interaction between ARID1A and p53 can be further supported by our experiments carried out in 4 cell lines (OVISE, HEC-1-A, HCT116, and MCF10A) that do not express Tag.

Similar to any newly identified cancer genes, there are several important questions remaining to be addressed to fully understand their molecular functions and before translational applications in cancer can be proposed. For example, future studies are required to determine the roles of ARID1A mutations in the context of pathway networks, including PI3K/Pten and canonical Wnt pathways, in which alterations have been identified in endometrium-related cancers (52). It will also be important to determine differences in response to therapy, risk for disease recurrence, and overall prognosis between tumors with and without ARID1A mutations. Such information should be valuable in assisting clinical management of patients who suffer from endometrium-related cancer.

In summary, the aforementioned data suggest that ARID1A is a negative cell-cycle regulator and a tumor suppressor gene. One of the mechanisms involving tumor suppressor function of ARID1A is through the molecular collaboration between ARID1A and p53, as the complex formation is required and sufficient to transcriptionally regulate several p53-related genes, including those with known tumor suppressor functions. We show that in the presence of wild-type TP53, tumor cells may take advantage of the disruption of the ARID1A/BRG1/p53 complex caused by ARID1A mutations to down-regulate p53-regulated genes including p21, SMAD3, and perhaps other genes with tumor suppressor functions. The findings presented in this study suggest a close collaboration between genetic and epigenetic alterations in cancer pathogenesis and provide a new molecular mechanism that contributes to tumor suppression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

Correction: ARID1A, a Factor That Promotes Formation of SWI/SNF-Mediated Chromatin Remodeling, Is a Tumor Suppressor in Gynecologic Cancers

In this article (Cancer Res 2011;71:6718–27), which appeared in the November 1, 2011, issue of Cancer Research (1), the authors did not include an accession number for the arrays used in the study. The GEO accession number is GSE37684. The authors regret this error.

Reference


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