

MINI REVIEW

ARID1A Mutations in Cancer: Another Epigenetic Tumor Suppressor?

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ABSTRACT

Although disordered chromatin organization has long been recognized as a feature of cancer, the molecular underpinnings of chromatin structure, epigenetic regulation, and their relationships to transcription are only beginning to be understood. Cancer genome sequencing studies have revealed a novel theme: frequent mutation of epigenetic regulators. Among these, the ARID1A/BAF250A subunit of the SWI/SNF (BRG1-associated factors) chromatin remodeling complex has emerged as recurrently mutated in a broad array of tumor types. We review the genomic and functional data supporting classification of ARID1A as a tumor suppressor.

Significance: Mutations in chromatin remodeling complex genes are increasingly recognized in many cancer types. However, the mechanisms by which chromatin remodeling complexes contribute to gene expression and the cancer phenotype are poorly understood. Understanding how mutation of chromatin remodelers facilitates transformation may offer the potential for development and implementation of novel therapies for cancer. *Cancer Discov*; 3(1); 35–43. ©2012 AACR.

INTRODUCTION

Epigenetic regulators impose upon the genetic code a chromatin structure characterized by chromatin accessibility, nucleosome position, and histone modifications. These features of chromatin structure modulate gene expression, thereby affecting both the identity and function of cells. Sequencing of cancer genomes has revealed frequent mutations in epigenetic regulators, particularly chromatin remodelers and histone modifiers, and disordered chromatin regulation has emerged as a distinct mechanism contributing to tumor development.

The canonical example of the links between chromatin remodeling and cancer may well be SMARCB1/SNF5/INI1/BAF47, a core component of the SWI/SNF chromatin remodeling complexes, of which ARID1A is also a member. In the late 1990s, specific biallelic inactivating mutations of *SMARCB1* were identified in the vast majority of rhabdoid tumors, highly aggressive cancers arising in kidney, brain, and soft tissues in young children (1, 2). Analogous to retinoblastoma and the *Rb* gene product, children having germline heterozygous inactivation of *SMARCB1* are predisposed to cancer, having a high

risk of developing malignant rhabdoid tumors at an especially young age.

Subsequent to these discoveries, implementation of next-generation sequencing technologies have revealed frequent and recurrent mutations in a wide variety of epigenetic modulators, including mediators of DNA methylation (e.g., *DNMT3a*) and covalent modifiers of histones (e.g., *MLL*, *MLL3*, *KDM5A*, *KDM5C*, *KDM6A*). Furthermore, genes encoding subunits of ATP-dependent chromatin remodelers, especially subunits of the SWI/SNF complex, are frequently mutated in a broad array of cancer types (3).

Recently, recurrent inactivating mutations in *ARID1A* have been identified in a wide variety of cancers, suggesting that it too functions as a tumor suppressor in many different cell types. Here, we review the spectrum of *ARID1A* mutations in cancer and what is known about ARID1A structure and function, as well as discuss potential mechanisms of tumor suppression and clinical implications.

ARID1A MUTATIONS IN CANCER

In late 2010, next-generation sequencing revealed that *ARID1A* mutations are present at high frequency in subtypes of ovarian (4, 5), endometrial (6), and uterine cancers (7). Ovarian clear-cell carcinomas (OCC) are an uncommon but aggressive type of ovarian cancer. More than 50% of OCCs carry *ARID1A* mutations, as do 30% of ovarian endometrioid carcinomas (refs. 4, 5; Table 1). These mutations are specific to this ovarian cancer subtype, as no serous ovarian carcinomas contained *ARID1A* mutations. A substantial proportion of uterine endometrioid carcinomas (29%), uterine clear-cell carcinomas (26%),

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Table 1. Recurrent loss or mutation of ARID1A in primary human cancers

Primary cancers	ARID1A mutation (%)	Homozygous (%)	Decreased transcript (%)	Decreased/absent protein (%)	Reference
Ovarian clear-cell carcinoma	55/119 (46%)	12/55 (22%)	N/A	27/37 (73%)	5
	24/42 (57%)	11/24 (46%)	N/A	N/A	4
Gastric cancer	32/109 (29%)	14/32 (44%)	N/A	24/32 (75%)	8
	9/110 (8%)	2/9 (22%)	N/A	6/8 (75%)	9
	10/100 (10%)	N/A	N/A	N/A	15
	N/A	N/A	N/A	26/180 (14%)	6
	N/A	N/A	N/A	5/45 (11%)	7
	N/A	N/A	N/A	95/657 (14%)	10
Hepatocellular carcinoma	21/125 (17%)	1/21 (5%)	N/A	N/A	11
	14/110 (13%)	N/A	N/A	N/A	13
	12/120 (10%)	N/A	N/A	N/A	12
	N/A	N/A	N/A	0/41 (0%)	4
Breast cancer	90/256 (35%) ^a	5/90 (6%)	Deletions associated with decreased expression	N/A	59
	4/114 (4%)	N/A	N/A	N/A	15
	11/82 (13%) ^a	0/11 (0%)	N/A	240/376 (64%)	14
	N/A	N/A	22/40 (55%)	63/112 (56%)	24
	N/A	N/A	N/A	11/315 (3%)	6
	N/A	N/A	N/A	1/91 (1%)	7
	N/A	N/A	5/50 (10%)	N/A	20
Uterine endometrioid carcinoma	N/A	N/A	N/A	90/309 (29%)	6
	N/A	N/A	N/A	15/58 (26%)	7
Uterine clear-cell carcinoma	N/A	N/A	N/A	6/23 (26%)	6
Pancreatic cancer	33/70 (47%) ^a , tumor + cell lines	2/24 (8%)	N/A	N/A	16
	10/119 (8%)	N/A	N/A	N/A	15
	14/39 (36%)	1/14 (7%)	N/A	N/A	17
	N/A	N/A	N/A	5/85 (6%)	6
	N/A	N/A	N/A	4/48 (8%)	7
Transitional-cell carcinoma of bladder	13/97 (13%)	N/A	N/A	N/A	18
Waldenström macroglobulinemia	5/30 (17%)	N/A	N/A	N/A	19
Anaplastic thyroid cancer	N/A	N/A	N/A	5/35 (14%)	6
Renal cancer	N/A	N/A	6/20 (30%)	N/A	20
	N/A	N/A	N/A	1/58 (2%)	6
	N/A	N/A	N/A	0/73 (0%)	7
Colon cancer	12/119 (10%)	N/A	N/A	N/A	15
	N/A	N/A	2/30 (7%)	N/A	20
	N/A	N/A	N/A	2/49 (4%)	7
	N/A	N/A	N/A	1/130 (0.8%)	6
Lung cancer	2/36 (6%)	N/A	N/A	N/A	15
	N/A	N/A	1/21 (5%)	N/A	20
	N/A	N/A	N/A	2/52 (4%)	7
	N/A	N/A	N/A	12/523 (2%)	6
Cervical adenocarcinoma	N/A	N/A	N/A	1/11 (9%)	7
Bile duct cancer	N/A	N/A	N/A	2/27 (7%)	7
Prostate cancer	2/23 (8%)	N/A	N/A	N/A	15
Medulloblastoma	3/125 (2%)	N/A	N/A	N/A	15

Abbreviation: N/A, not applicable.

^aARID1A loss via chromosomal deletion.

KEY CONCEPTS

- Epigenetic dysregulation is a common characteristic of cancer.
- Inactivating mutations of *ARID1A*, a member of the SWI/SNF chromatin-remodeling complex, have been identified in a variety of cancers.
- Mutational and functional data suggest *ARID1A* is a *bona fide* tumor suppressor.
- *ARID1A* may contribute to tumor suppression via effects on the SWI/SNF complex, control of cell proliferation and differentiation, and/or effects on histone ubiquitylation.

uterine serous carcinomas (18%), and uterine carcinosarcoma (14%) also have loss of *ARID1A* protein (also known as BAF250A) by immunohistochemistry (IHC; ref. 6). In addition, loss of *ARID1A* has been shown in patient samples having atypical endometriotic lesions (thought to be precursors to OCC development) contiguous to OCC, raising the possibility that *ARID1A* loss may be an early cancer-promoting event in endometriosis leading to OCC (5).

Genome sequencing and comparative genomic hybridization (CGH) studies have detected *ARID1A* mutations or deletions in a substantial fraction of additional cancer subtypes (Table 1), including gastric cancer (29%; refs. 8–10), hepatocellular carcinoma (10%–17%; refs. 11–13), breast cancer (4%–13%; refs. 14, 15), pancreatic cancer (33%–45%; refs. 16, 17), transitional cell carcinoma of the bladder (13%; ref. 18), and Waldenström macroglobulinemia (17%; ref. 19).

Four cancer survey studies (which excluded OCC), have examined *ARID1A* protein expression, mutations, and transcript level in a variety of tumor types (Table 1; refs. 6, 7, 15, 20). The earliest screen of *ARID1A* expression identified decreased transcript level in 6% of 236 tumors, noting a large proportion of renal tumors (30%) having decreased expression (20). In the largest survey to date, complete loss of *ARID1A* protein was reported in 7% of more than 3,000 tumors examined, adding anaplastic thyroid carcinomas to the list of cancers having frequent *ARID1A* loss (14%; ref. 6). A similar IHC-based study found *ARID1A* loss in 3% of 995 carcinomas, distinctively including 9% of cervical adenocarcinomas, and 7% of bile duct carcinomas (7). Finally, a survey of *ARID1A* in both primary cancers and cell lines used sequencing to identify truncating mutations in 6% of the 759 samples examined, adding prostate cancers (8%) and medulloblastomas (2%) to the cancers associated with inactivating mutations in *ARID1A* (15). With the exception of Waldenström macroglobulinemia, few lymphoma samples (<1%) showed loss of *ARID1A* protein expression (6). No cases having *ARID1A* loss were detected in oral cancer, glioblastoma, or leukemia (6, 7, 15). To date, we are not aware of *ARID1A* mutations having been detected in sarcomas. Thus, *ARID1A* loss occurs in a wide spectrum of cancer subtypes with up to 50% frequency (Table 1).

Nature of the Somatic Mutations—A Haploinsufficient Effect?

The vast majority of cancer-associated mutations in *ARID1A* (>97%) were inactivating, with nonsense or frameshift (rather than silent or missense) mutations detected throughout the gene. However, in only 30% of the OCCs with *ARID1A* mutations were both alleles affected (4, 5). By IHC, 73% of the *ARID1A*-heterozygous tumors lacked protein expression, as did 5% of tumors not found to have coding mutations (5). Several mechanisms may be postulated to explain this interesting finding—loss of *ARID1A* protein expression in the setting of heterozygous mutation without loss of heterozygosity. Mutations affecting *ARID1A* expression may occur in noncoding regions of the genome not assayed by exome sequencing techniques. Alternatively, epigenetic silencing might contribute. One group has postulated that posttranscriptional and/or posttranslational mechanisms account for loss of *ARID1A* protein in OCCs harboring heterozygous mutations, based on the finding that RNA sequencing detects both wild-type and mutant alleles in a small number of cases (5).

Conversely, 27% of the *ARID1A*-heterozygous OCCs retain detectable protein expression. A similar situation occurs in gastric cancer, in which *ARID1A* mutations were biallelic in only 44% of *ARID1A*-mutant samples. Again, 25% of samples harboring heterozygous mutations retained *ARID1A* expression by IHC (9). Array CGH analysis of primary breast tumors showed loss of chromosomal material encoding one copy of *ARID1A* in 13% of samples, without identifying any *ARID1A* coding mutations in the remaining allele (14). At the protein level, 2 surveys of *ARID1A* found complete loss of expression characterized only 1% to 3% of breast cancers (6, 7). Finally, in hepatocellular carcinomas, nearly all of the *ARID1A* mutations were found to be heterozygous (11) and, in a separate study, protein expression was detected in all samples (7). Collectively, observations that *ARID1A* is recurrently and specifically mutated on one allele but expressed from the other allele, have raised the possibility that reduced levels of *ARID1A* may mediate a haploinsufficient effect in promoting cancer.

In vitro studies provide support for a haploinsufficient tumor suppressor role for *ARID1A*. Knockdown studies in a variety of cell types, which achieved only partial depletion of *ARID1A*, showed increased cell proliferation and colony formation (9, 13, 14, 21), impaired differentiation (21, 22), as well as decreased apoptosis (23). Heterozygosity for *Arid1a* in mice results in embryonic lethality, suggesting that biologically relevant haploinsufficient effects are caused by loss of a single allele (22). Furthermore, 2 studies have found that haploinsufficiency and decreased transcript levels of *ARID1A* are associated with high-risk, poor-prognosis breast cancers (14, 24). Collectively, these findings suggest that, much like *p53* and *PTEN*, haploinsufficiency for *ARID1A* is capable of promoting tumor formation in some cancers. Given the diversity of the lineages involved, it is also likely that the effects of *ARID1A* mutation may vary by cell type such that haploinsufficiency may promote transformation in some lineages, whereas homozygous inactivation is required in others (25, 26). Close attention to tissue-specific effects of *ARID1A* mutations and incorporation of mouse models of disease will be critical to testing these hypotheses.

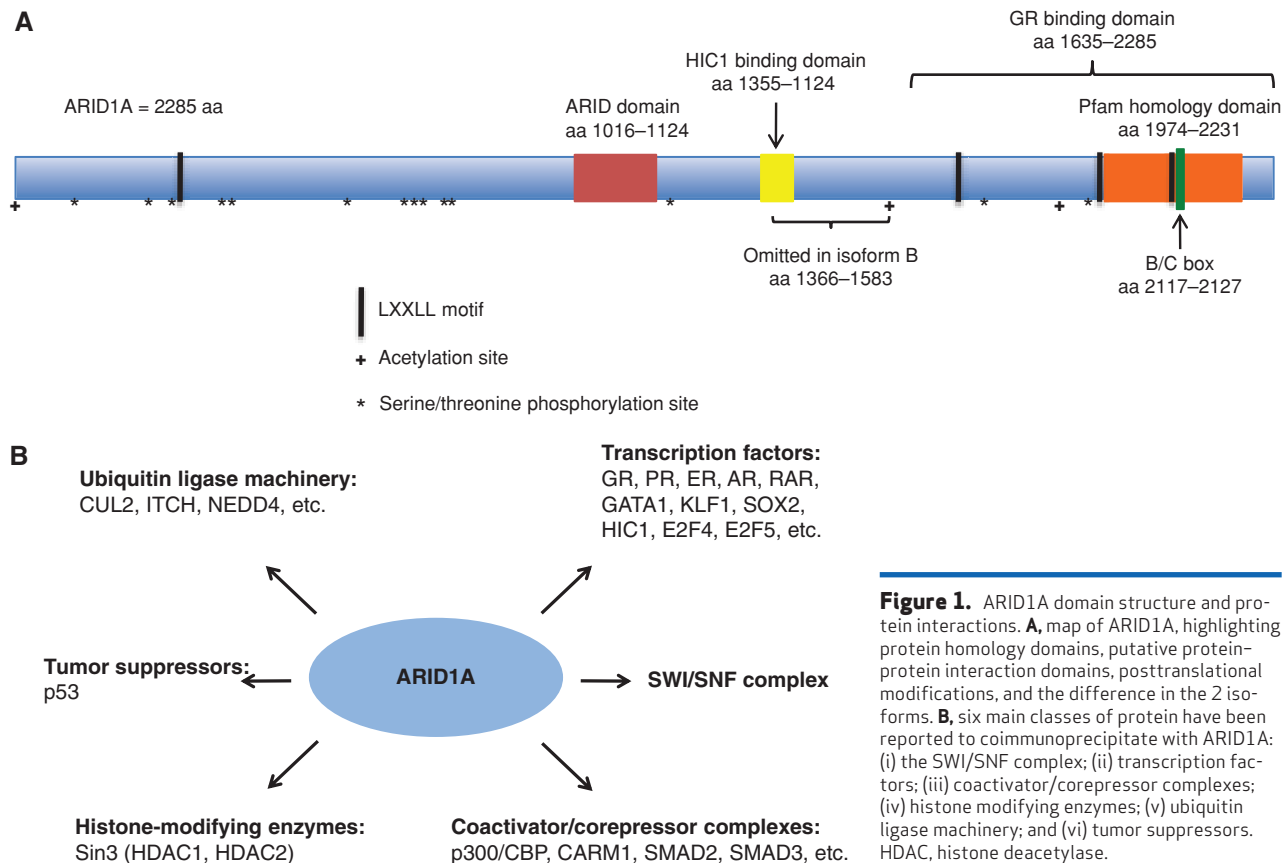


Figure 1. ARID1A domain structure and protein interactions. **A**, map of ARID1A, highlighting protein homology domains, putative protein-protein interaction domains, posttranslational modifications, and the difference in the 2 isoforms. **B**, six main classes of protein have been reported to coimmunoprecipitate with ARID1A: (i) the SWI/SNF complex; (ii) transcription factors; (iii) coactivator/corepressor complexes; (iv) histone modifying enzymes; (v) ubiquitin ligase machinery; and (vi) tumor suppressors. HDAC, histone deacetylase.

ARID1A Structure and Expression

ARID1A, “AT-rich interacting domain containing protein 1A,” has also been called *B120*, *BAF250*, *BAF250a*, *BM029*, *C1orf4*, *ELD*, *held*, *bOSA1*, *MRD14*, *OSA1*, *P270*, and *SMARCF1*. ARID1A belongs to a family of 15 proteins in humans that all contain a characteristic 100-amino acid DNA-binding ARID domain. The ARID domain of ARID1A does not show sequence-specific DNA binding (27), and the only other protein homology domain, located within the C-terminus, has unknown function (Fig. 1A). Seven ARID subfamilies have since been classified, based both on degree of homology within the ARID domain as well as similarity between highly variable non-ARID domain structures (28). The ARID1 subfamily contains 2 members, ARID1A and ARID1B, which share approximately 80% amino acid homology within their ARID domains and 50% homology throughout. The proteins are highly evolutionarily conserved, present as the single gene *Swi1* in *Saccharomyces cerevisiae*, and *Osa* in *Drosophila melanogaster*, and purify as members of SWI/SNF chromatin remodeling complexes.

ARID1A is located on chromosome 1p, migrates at approximately 250 kD, is widely expressed, and is present primarily (perhaps exclusively) in the nucleus. Expression varies during the cell cycle, being highest during G₀-G₁ and significantly diminished in S- and G₂-M phases (29). ARID1A encodes 2 isoforms (2285 and 2086 amino acids) although the relative expression and functional significance of the 2 isoforms are unclear (RefSeq NM_006015.4 and NM_139135.2). ARID1A is posttranslationally modified, including lysine acetylation

and serine/threonine phosphorylation, potentially regulating protein expression or protein-protein interactions (30).

ARID1A and SWI/SNF Complexes

ARID1A has been implicated in numerous protein-protein interactions (Fig. 1B). Among these interactions, the most widely known and studied are those which make ARID1A a part of SWI/SNF chromatin remodeling complexes. SWI/SNF complexes are multisubunit protein complexes that use the energy of ATP hydrolysis to remodel chromatin structure and are capable of sliding nucleosomes along a DNA template *in vitro* (31). The nucleosome remodeling activity is derived from the catalytic ATPase subunit (either SMARCA4/BRG1 or SMARCA2/BRM) and is enhanced by the noncatalytic subunits SMARCB1/SNF5, BAF155, and BAF170 (32). SWI/SNF complexes have been described as having 2 main variants, BRG1-associated factors (BAF) and polybromo BRG1-associated factors (PBRAF; ref. 33). However, many SWI/SNF subunits have multiple isoforms and/or belong to highly homologous multigene families (34). For example, in addition to the 2 variant ATPase subunits, 4 different genes encode the BAF45 subunit, 3 different genes encode the BAF60 subunit, and 2 different genes encode the BAF53 subunit. Consequently, it has been proposed that several hundred variants of the complex may exist (35) and that different complex variants may play distinct lineage-, transcription factor-, and chromatin state-specific roles. Several activities have been ascribed to the complex that may contribute to transcriptional regulation,

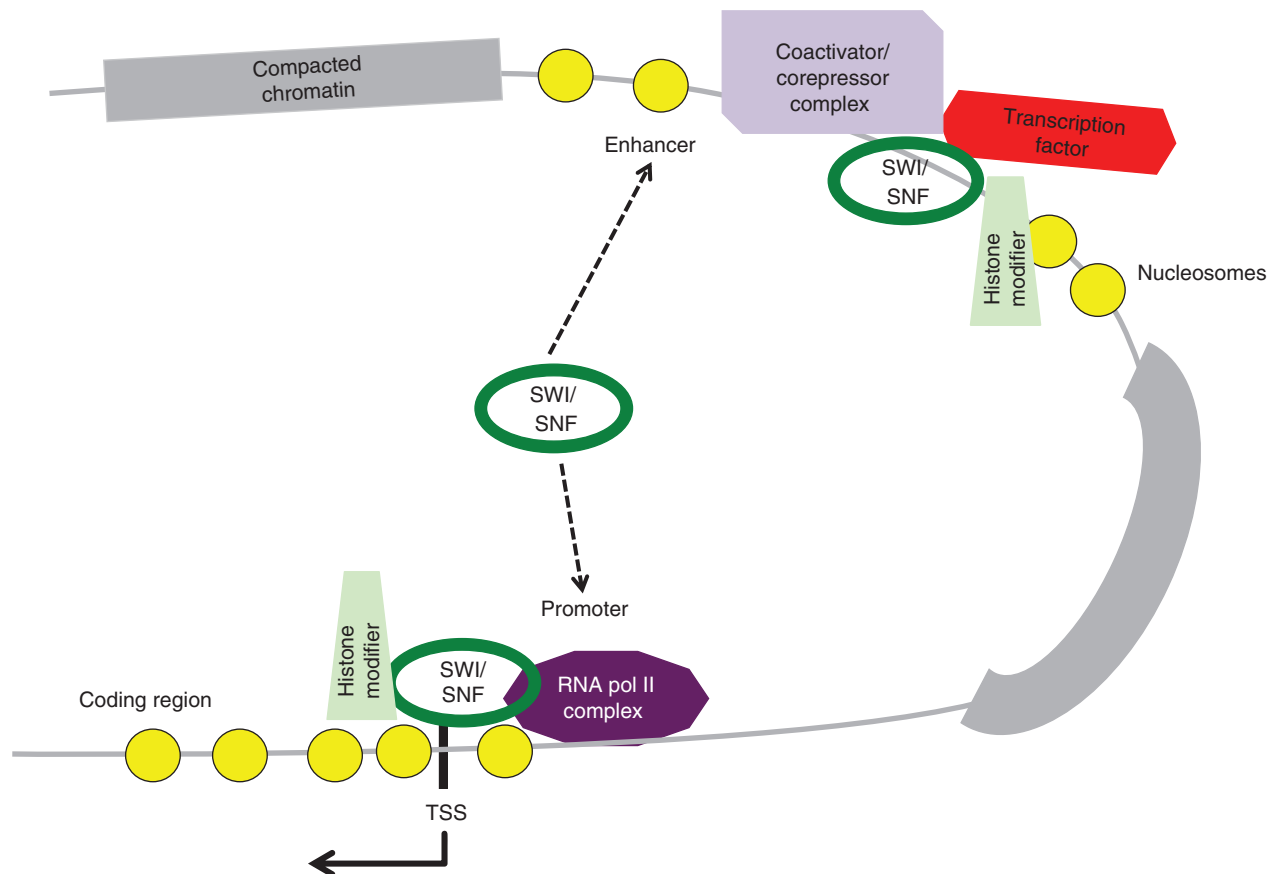


Figure 2. Contributions of the SWI/SNF complex to chromatin structure and transcriptional regulation. Several functions have been ascribed to the SWI/SNF complex, including (i) mobilizing nucleosomes at promoters, enhancers, and/or gene bodies; (ii) facilitating the binding of transcription factors; (iii) recruiting coactivator/corepressor complexes; (iv) recruiting histone modifying enzymes; and (v) facilitating chromatin looping to facilitate enhancer and promoter interaction.

including modulation of chromatin accessibility and transcription factor binding, recruitment of coactivator/corepressor complexes that have histone-modifying activity, and facilitation of the chromatin looping required to approximate promoters with distal enhancers (Fig. 2; ref. 3).

As a member of SWI/SNF complexes, ARID1A is thought to contribute to specific recruitment of its chromatin remodeling activity by binding transcription factors and transcriptional coactivator/corepressor complexes (36–38). Several studies have linked SWI/SNF and ARID1A to transcriptional regulation, particularly nuclear hormone-induced transcription and expression of cell-cycle regulators (21, 37–43). Reexpression of ARID1A in an ARID1A-deficient breast cancer cell line augmented transcriptional activation of reporter plasmids by glucocorticoid, estrogen, and androgen receptors (40). Interaction with these nuclear hormone receptors, particularly the glucocorticoid receptor, has been shown to depend on a broadly defined C-terminal region that contains several putative nuclear hormone receptor-binding sites (LXXLL motifs; ref. 37). It is unknown whether roles for ARID1A in the regulation of hormone signaling contribute to tumor suppression, but it is noteworthy that ARID1A mutations are frequently seen in cancers occurring in hormone-responsive tissues (e.g., breast and ovarian).

Mechanisms of Tumor Suppression

Within the context of functional models of cancer development (44), how might the consequences of ARID1A mutation contribute? Studies have suggested roles for ARID1A in 3 processes relevant to tumor suppression—proliferation, differentiation, and apoptosis—with mixed results. For both breast and gastric cancer cell lines, knockdown of wild-type ARID1A enhanced cellular proliferation, whereas reexpression of ARID1A in mutant cell lines dampened cell proliferation (9). Knockdown of ARID1A also enhanced proliferation of normal ovarian surface epithelial cells (45) and inhibited cell-cycle arrest in murine preosteoblasts (21, 41). In contrast, *Arid1a* knockout in embryonic stem (ES) cells resulted in loss of self-renewal properties. With respect to differentiation, ARID1A knockdown disrupted differentiation of cultured osteoblasts, whereas knockout forced ES cell differentiation into primitive endoderm and permitted *in vitro* development of neurons and skeletal muscle, while preventing that of cardiomyocytes and adipocytes (22). A single study has examined apoptosis following ARID1A knockdown, finding that Fas-mediated cell death is inhibited in Jurkat leukemia cells (23). Taken together, these results raise the possibility that ARID1A loss affects 3 canonical tumor suppressor functions, with

tumor-promoting effects particularly seen in lineages associated with ARID1A cancer mutations.

Given the large number of SWI/SNF subunits that are mutated in cancer, it seems highly likely that the tumor-suppressing activities of ARID1A are related to its role within this complex. In addition to *ARID1A* and *SMARCB1*, at least 5 other SWI/SNF subunits have been found to be recurrently mutated in cancer, including *PBRM1* (40% of renal carcinomas; ref. 46), *ARID2* (18% of hepatitis C-associated liver cancer; ref. 47), *ARID1B* (liver, breast, and pancreatic cancers; refs. 12, 16, 48), *BRD7* (breast cancer; ref. 49), and *SMARCA4* (lung cancer and medulloblastoma; refs. 50, 51). Also noteworthy is the fact that mutations in individual subunits of the SWI/SNF complex have largely distinct cancer spectra (3). Consequently, loss of individual subunits may have distinct effects upon SWI/SNF function and its tumor suppressor roles.

Loss of ARID1A may have many effects on SWI/SNF complexes that lead to transcriptional dysfunction, including disruption of nucleosome sliding activity, assembly of variant SWI/SNF complexes, targeting to specific genomic loci, and/or recruitment of coactivator/corepressor activities. With respect to chromatin remodeling, ARID1A is thought to be dispensable for the *in vitro* nucleosome remodeling activity of SWI/SNF, as measured by DNase hypersensitivity patterns of reconstituted nucleosomal arrays (32). However, contributions of SWI/SNF to the complex states of chromatin that exist *in vivo* are poorly understood and thus contributions from ARID1A to such activities cannot be excluded.

Loss of ARID1A may affect expression or stability of other SWI/SNF subunits. Although one study has shown that several components of SWI/SNF (including BAF155, BAF170, SNF5, and BRG1) remain normally expressed and coassociated upon knockdown of ARID1A (21), another has shown that knock-out of one or both alleles of *Arid1a* in mouse ES cells alters protein levels of SNF5, BAF155, BAF170, and BAF57 (22). This latter observation suggests *ARID1A* mutation may affect the composition or assembly of several SWI/SNF complex variants, not just those that usually incorporate this subunit. Targeting of SWI/SNF to chromatin, as measured by binding of SNF5, BAF155, BAF170, and BRG1 to the *Myc* promoter and a small number of E2F targets, is not affected by knockdown of ARID1A (42). However, as the SWI/SNF complex binds to thousands of loci, genome-wide analyses will be necessary to determine global effects of ARID1A on SWI/SNF targeting. Finally, ARID1A-containing complexes have been shown to be associated with the Sin3 corepressor complex [including histone deacetylase (HDAC) 1 and HDAC2], suggesting that SWI/SNF recruitment of additional transcription modulators and histone modifiers may be impaired by ARID1A loss (21).

Another question of interest is the extent to which ARID1A and ARID1B have complementary or distinct roles in cell function. ARID1A and ARID1B have been characterized as mutually exclusive members of BAF variant SWI/SNF complexes, meaning the 2 proteins do not coimmunoprecipitate (31). Although both are expressed in many tissues, one or the other is present in any single instance of the SWI/SNF complex. Following induction of differentiation in a non-transformed osteoblast model, ARID1A is required for the induction of cell-cycle arrest, whereas knockdown of ARID1B had no effect (41). Similarly, ARID1A and ARID1B have

opposing effects on cell-cycle arrest caused by serum deprivation—knockdown of ARID1A delayed arrest, whereas ARID1B knockdown had no effect. Conversely, cell-cycle reentry following deprivation was modestly accelerated by ARID1A knockdown but markedly delayed by ARID1B knockdown. As noted above, ARID1A is associated with HDAC1/2-containing complexes, whereas ARID1B coimmunoprecipitates with HDAC3 and is associated with both histone deacetylase and acetylase activity (21). Furthermore, ARID1A and ARID1B were found to have differential interactions with E2F family members (21). Collectively, such findings could suggest a tumor suppressor model by which the unopposed actions of ARID1B-containing SWI/SNF complexes disrupt cell-cycle control and predispose to transformation. However, it is also worthy of note that another variant of SWI/SNF incorporates a third ARID protein, known as ARID2. Interestingly, recurrent mutations of both *ARID1B* and *ARID2* have been reported in some of the same types of cancers that contain frequent *ARID1A* mutations, including hepatocellular carcinomas (12, 13, 47), and recurrent *ARID1B* mutations are seen in breast cancers (16), pancreatic cancers (16), and gastric cancers (8). This raises the possibility that ARID1B and ARID2 may also have tumor suppressor activity. Whether these ARID gene mutations are mutually exclusive or have some degree of overlap has not been systematically reported. Consequently, it remains unclear the extent to which complementary or opposing functions of ARID-family proteins contribute to cancer.

A further intriguing question is whether ARID1A may contribute to enzymatic activity distinct from ATP-dependent chromatin remodeling. Studies conducted on ARID1B show that it has E3 ubiquitin ligase activity, thought likely to extend to ARID1A based on structural homology (52). ARID1B has been shown to facilitate monoubiquitination of lysine-120 of histone H2B—a modification that is thought to be a precursor to acquisition of trimethylated H3K4 at promoters and enhancers of actively transcribed genes. The E3 ubiquitin ligase function has been mapped to a putative C-terminal B/C box. However, it remains to be determined whether this ubiquitylation activity is associated with SWI/SNF complexes and whether it relates to the putative tumor suppressor role of ARID1A.

Cooperating Pathways

In addition to identifying frequent *ARID1A* mutations, the studies above have shown several associations with other oncogene and tumor-suppressor pathways. In OCC, one study identified a significant correlation between ARID1A loss and the presence of activating mutations in *PIK3CA* as 46% of ARID1A-deficient tumors contained *PIK3CA* mutations, whereas only 17% of ARID1A-expressing tumors contained *PIK3CA* mutations (53). There was also a strong concordance between *PIK3CA* and ARID1A status in gastric cancer, collectively suggesting potential cooperating effects of these mutations in oncogenesis (9). In breast, gastric, and ovarian cancers, mutation or loss of ARID1A is significantly more common in tumors having wild-type *p53* (8, 9, 24, 45), and *p53* has been shown to directly associate with the C-terminus of ARID1A in coprecipitation experiments (45). In gastric cancer, 2 additional correlations were noted: rates of

ARID1A mutation or loss were significantly higher in tumors having microsatellite instability (i.e., mismatch repair defects) and those associated with Epstein–Barr virus infection (9). In hepatocellular carcinoma, *ARID1A* mutations were correlated with mutations of β -catenin (11). The SWI/SNF complex has also been reported to physically associate and functionally cooperate with the RB protein and pathway, respectively (54, 55). However, no relationship between SWI/SNF mutation and *RB* mutation has yet been reported in the cancer genome sequencing studies described herein. Ultimately, these data reveal potential cooperating interactions between *ARID1A* mutation and other tumor-promoting pathways.

Clinical Implications

Several studies have attempted to analyze the prognostic significance of *ARID1A* mutations, transcript levels, or protein loss in a variety of cancer subtypes—OCCs, gastric cancer, breast cancer, and bladder cancer (8, 10, 14, 18, 24, 53, 56–59). Even within a single cancer subtype, no consistent relationship has emerged between *ARID1A* mutation or expression and prognosis. Ultimately, larger prospective studies, ideally assessing not only *ARID1A* sequence but also loss of heterozygosity and protein expression, will be required to adequately address the prognostic significance of *ARID1A* mutations.

A handful of candidate therapeutic targets, having striking concordance with those identified for SMARCB1-deficient cancers, have emerged from functional studies, including cyclins A, B2, and C (41); MYC (42); and the Polycomb complexes (16). A key question going forward is the degree to which therapeutic dependencies will be similar, or distinct, among cancers characterized by mutations of different SWI/SNF subunits. Furthermore, it will be of interest to determine whether inactivation of chromatin remodelers such as *ARID1A* can be therapeutically exploited by targeting downstream and potentially reversible epigenetic consequences of remodeler mutation (60).

CONCLUSIONS

Ultimately, several lines of evidence support classification of *ARID1A* as a *bona fide* tumor suppressor gene. Somatic mutations are found in significant subsets of several cancer types and are not detected in other specific classes of cancer. Studies show a characteristic pattern of inactivating mutations occurring throughout the gene body. Finally, early functional studies provide evidence that *ARID1A* affects several canonical tumor-suppressor pathways.

The case of *ARID1A* exemplifies the challenges present in assigning mechanistic import to the many mutations being identified through cancer genome sequencing. Haploinsufficient tumor suppressor effects have ample precedent, and their identification and interpretation require synthesis of human sequencing data, as well as cell culture and animal modeling systems. Different mutation patterns among cancer subtypes imply tissue-specific mutational effects, which may limit the extent to which observations in one cancer type or cell line may be applied to other model systems.

Chromatin remodelers, and *ARID1A* in particular, are emerging as a novel class of genes associated with a variety of

cancers. Although functional pathways that promote transformation are beginning to be identified, much remains to be elucidated about the mechanistic basis by which *ARID1A* alters chromatin structure, contributes to SWI/SNF activity, modulates transcription, and ultimately suppresses cancer formation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J.N. Wu, C.W.M. Roberts

Writing, review, and/or revision of the manuscript: J.N. Wu, C.W.M. Roberts

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