Mutation and Loss of Expression of *ARID1A* in Uterine Low-grade Endometrioid Carcinoma

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Abstract: *ARID1A* is a recently identified tumor suppressor gene that is mutated in approximately 50% of ovarian clear cell and 30% of ovarian endometrioid carcinomas. The mutation is associated with loss of protein expression as assessed by immunohistochemistry. In this study, we evaluated *ARID1A* immunoreactivity in a wide variety of carcinomas to determine the prevalence of *ARID1A* inactivation in carcinomas. Mutational analysis of *ARID1A* was carried out in selected cases. Immunoreactivity was not detected (corresponding to inactivation or mutation of *ARID1A*) in 36 (3.6%) of 995 tumors. Uterine low-grade endometrioid carcinomas showed a relatively high-frequency loss of *ARID1A* expression, as 15 (26%) of 58 cases were negative. The other tumor that had a relatively high-frequency loss of *ARID1A* expression was gastric carcinoma (11%). Mutational analysis showed 10 (40%) of 25 uterine endometrioid carcinomas; none of 12 uterine serous carcinomas and none of 56 ovarian serous and mucinous carcinomas harbored somatic *ARID1A* mutations. All mutations in endometrioid carcinomas were nonsense or insertion/deletion mutations, and tumors with *ARID1A* mutations showed complete loss or clonal loss of *ARID1A* expression. In conclusion, this study is the first large-scale analysis of a wide variety of carcinomas showing that uterine low-grade endometrioid carcinoma is the predominant tumor type harboring *ARID1A* mutations and frequent loss of *ARID1A* expression. These findings suggest that the molecular pathogenesis of low-grade uterine endometrioid carcinoma is similar to that of ovarian low-grade endometrioid and clear cell carcinoma, tumors that have previously been shown to have a high-frequency loss of expression and mutation of *ARID1A*.

Key Words: *ARID1A*, BAF250, uterine carcinoma, ovarian carcinoma

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Acquisition of somatic mutations is a molecular hallmark of neoplasia. Sequence mutations that are acquired during tumor evolution can lead to activation of oncogenes and inactivation of tumor suppressor and DNA repair genes, thereby propelling tumor development and progression.[8] Identification and characterization of somatic mutations are not only fundamental in understanding the molecular pathogenesis of cancer but also can provide the rationale for the development of personalized diagnostic tests and therapy. With the use of whole exome sequencing and transcriptome sequencing, 2 independent studies recently reported *ARID1A* (also known as *BAF250A*) mutations in 43% to 56% of ovarian clear cell carcinomas and 30% of ovarian low-grade endometrioid carcinomas[6,21] but not in matched controls, confirming the somatic nature of the mutations. As both these tumor types are believed to be derived from endometriosis and because 1 of these studies also found *ARID1A* mutations in adjacent atypical endometriosis, it is conceivable that *ARID1A* loss is a relatively specific molecular event in the genesis of these tumors. Many *ARID1A* mutations are insertion/deletion mutations, leading to the generation of premature stop codons by frameshift that result in truncated proteins prone to degradation. It has been previously shown that loss of *ARID1A* expression, as assessed by immunohistochemistry (IHC), correlates closely with *ARID1A* mutations.[11,21]

ARID1A is located in the chromosome 1p36 region, and encodes a large nuclear protein involved in chromatin remodeling. *ARID1A* interacts with several other proteins including the core protein, BRG or BRM with ATPase activity.[4,20] The *ARID1A*-BRG/BRM complex belongs to the SWI/SNF chromatin remodeling complex; remodeling activity is facilitated by ATP hydrolysis of BRG or BRM. In contrast, the noncatalytic subunits of the SWI/SNF complex, such as *ARID1A*, are responsible for modulating the target specificity and activity of the ATPase. The chromatin remodeling activity of SWI/SNF

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has been shown to play an integral role in controlling gene expression[19] and is critical in tissue development, cellular differentiation, and tumor suppression.[3,4,15] *ARID1A* is essential for SWI/SNF complexes to suppress DNA synthesis. Inactivation of *ARID1A* is thought to enhance cell cycle progression by potentially involving c-myc, thereby contributing to uncontrolled cellular proliferation in cancer cells.[5,13,14]

Although *ARID1A* has emerged as a new cancer-associated gene, which is frequently mutated in endometriosis-related ovarian neoplasms, it is not known whether its mutation, such as *FOXL2*[10,17] and *APC*,[9] is detected only in specific types of cancer or mutations, such as *TP53* and *KRAS*, which occurs in a variety of neoplastic diseases. As *ARID1A* mutations are randomly distributed in 20 exons and are the insertion/deletion type of mutations that lead to truncated proteins, we used loss of *ARID1A* immunoreactivity as a surrogate marker for a mutation to screen a variety of carcinomas. Sequence analysis was then carried out in the specimens that showed the highest frequency of loss of *ARID1A* expression.

MATERIALS AND METHODS

Tissue Material

Paraffin-embedded tissue sections of normal and tumor tissues from various organs were obtained from the Department of Pathology of the National Taiwan University Hospital and Johns Hopkins Hospital, from 1994 to 2009. The normal tissues studied by IHC included esophagus, stomach, colon, salivary gland, liver, pancreas, lung, kidney, prostate, adrenal gland, testis, breast, thyroid, tonsil, and placenta. The tumors included 41 hepatocellular carcinomas, 27 bile duct carcinomas, 52 pulmonary carcinomas (42 adenocarcinomas, 10 squamous carcinomas), 73 renal cell carcinomas, 91 breast invasive ductal carcinomas, 272 ovarian tumors (221 high-grade serous carcinomas, 15 low-grade serous carcinomas, and 36 mucinous carcinomas), 58 trophoblastic tumors (35 choriocarcinomas, 6 placental site trophoblastic tumors, 17 epithelioid trophoblastic tumors), 125 cervical carcinomas (114 squamous cell carcinomas, 11 adenocarcinomas), 75 uterine carcinomas (58 conventional low-grade endometrioid carcinomas, 15 serous carcinomas, 2 carcinosarcomas), 35 prostate carcinomas, 49 colon carcinomas, 45 gastric carcinomas, 48 pancreatic carcinomas, and 4 oral squamous cell carcinomas. The use of the archival materials was approved by the internal review board of both institutions.

For mutation analysis, genomic DNA isolated from affinity purified tumor samples was used. Those samples included 25 uterine endometrioid carcinomas (International Federation of Gynecology and Obstetrics grade 1), 12 uterine serous carcinomas, 32 ovarian high-grade serous carcinomas, 19 ovarian low-grade serous carcinomas, and 5 ovarian mucinous carcinomas. As the *ARID1A* mutation status has been previously reported in ovarian clear cell and ovarian low-grade endometrioid carcinomas,[6,11,21] these carcinomas were not included in this study. The methodology for the isolation of tumor cells from fresh carcinoma specimens has been previously described.[6]

IHC

IHC analysis was carried out on tissue microarrays except for 20 uterine endometrioid carcinomas and 33 renal cell carcinomas, which were carried out on whole-tissue sections. Loss of *ARID1A* expression detected in the tissue microarrays was confirmed on whole-tissue sections. A polyclonal rabbit anti-*ARID1A* antibody (Sigma-Aldrich HPA005456) was generated by immunizing a rabbit with the following peptide sequence: PGLGNVAMGPRQHYPYGGPYDRVRTEPGIGPEG NMSTGAPQPNLMPSNP DSGMYSPSRYPPQQQQQQ QQRHDSYGNQFSTQGTPSGSPFPSQQTTMYQQQQ QNYK. The specificity of the antibody was confirmed by Western blotting. Antigen retrieval was performed by placing sections in a citrate buffer (pH 6.0), which were then placed in an autoclave at 120°C for 10 minutes. The sections were incubated with the rabbit antibody overnight at 4°C. A positive reaction was detected by the EnVision + System (Dako, Carpinteria, CA). Tumor stromal cells served as positive internal controls. Only nuclear staining was scored. A previous study showed that loss of nuclear expression correlated with mutation of the gene. Hence, absence of nuclear staining (diffuse or focal) was considered to be positive for gene mutation.

Mutation Analysis

A total of 93 tumor samples were analyzed for somatic *ARID1A* mutations. The normal tissues from the matched cases were also sequenced in parallel. Nucleotide sequences of polymerase chain reaction primers that amplified exon 1 to exon 20 were previously reported.[6] Polymerase chain reaction products were prepared and purified for Sanger sequencing.

Gene Knockdown and Western Blot

The lentivirus-expressing *ARID1A* short hairpin ribonucleic acids (shRNAs) were produced using HEK293FT cells transfected with the pLKO.1-puro lentiviral plasmids (the RNAi consortium) and the second generation packaging system, pSPAX2 (Addgene plasmid 12260) and pMD2.G (Addgene plasmid 12259). The shRNA sequences were: shRNA1, GCCTGATCTATCT GGTTCAAT; shRNA2, CCTCTCTTATACACAGCA GAT; and shRNA3, CCGTTGATGAACTCATTGGTT. HeLa cells were transduced with lentiviral particles, and lysates in Laemmli sample buffer were prepared from the cells 3 days after transduction. For Western blots, SDS-PAGE was used to separate proteins that were then transferred onto PVDF membranes. The rabbit anti-*ARID1A* antibody as used in IHC was used to hybridize the membranes (at a dilution of 1:2000), and anti-GAPDH antibody was also applied to detect the GAPDH, serving as the loading control. After incubating

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at room temperature for 2 hours, the membranes were washed with TBST (0.01% Tween 20 in tris-buffered solution) and were blotted with HRP-conjugated anti-rabbit antibodies (Pierce, Rockford, IL) at a dilution of 1:1000 for 1 hour at room temperature. *ARID1A* and GAPDH bands were shown by chemiluminescence (Arlington Heights, IL).

RESULTS

A total of 995 carcinomas from a variety of tissue origins were studied for *ARID1A* expression using IHC. To confirm the specificity of the anti-*ARID1A* antibody used in this study, we performed a gene knockdown experiment by transducing HeLa cells with 3 different *ARID1A* shRNAs. Western blot analysis showed a significant decrease of *ARID1A* protein in HeLa cells after treatment with *ARID1A*-specific shRNAs, especially the shRNA-2 and shRNA-3 compared with control shRNA, indicating the specificity of the *ARID1A* antibody (Fig. 1). Previous studies have shown that inactivating mutations of *ARID1A* are associated with loss of protein expression.[11,21] Therefore, we focused our attention on those tumors with undetectable *ARID1A* immunoreactivity, and used a scoring system to classify all cases into *ARID1A*-negative (undetectable) and *ARID1A*-positive cases, which showed any levels of *ARID1A* immunoreactivity. As the *ARID1A* mutation status has been previously reported in ovarian clear cell and ovarian endometrioid carcinomas,[6,11,21] these carcinomas were not included in this study.

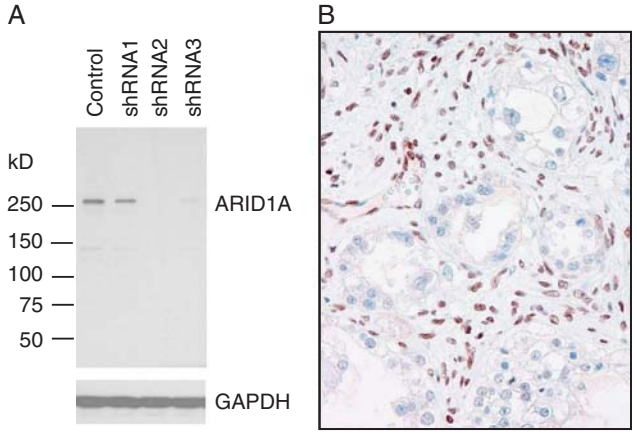

FIGURE 1. Specificity of the antibody in detecting *ARID1A*. Western blot analysis shows a predominant protein band, with a molecular mass corresponding to *ARID1A* protein (approximately 280 kD) in HeLa cell lysate. Protein expression is significantly decreased in shRNA-2-treated and shRNA-3-treated HeLa cells compared with control shRNA-treated cells (A). IHC using this anti-*ARID1A* antibody on an ovarian clear cell carcinoma with known biallelic somatic insertion/deletion mutations of *ARID1A* (B). The tumor cells show undetectable *ARID1A* immunoreactivity, whereas the stromal cells show intense nuclear staining.

TABLE 1. IHC Study of *ARID1A* on 995 Carcinomas

Tumor	Total Case No.	IHC–	Percentage of IHC–
Hepatocellular carcinoma	41	0	0
Bile duct carcinoma	27	2	7.4
Lung			
Adenocarcinoma	42	1	2.4
Squamous carcinoma	10	1	10
Renal cell carcinoma	73	0	0
Breast carcinoma	91	1	1.1
Ovary			
High-grade serous carcinoma	221	0	0
Low-grade serous carcinoma	15	0	0
Mucinous carcinoma	36	0	0
Uterine cervix			
Squamous carcinoma	114	2	1.8
Adenocarcinoma	11	1	9.1
Uterine corpus			
Endometrioid carcinoma	58	15	26
Serous carcinoma/carcinosarcoma	17	0	0
Trophoblastic tumor			
Choriocarcinoma	35	0	0
Placental site trophoblastic tumor	6	0	0
Epithelioid trophoblastic tumor	17	0	0
Prostate carcinoma	35	0	0
Colon carcinoma	49	2	4.1
Gastric carcinoma	45	5	11
Pancreatic carcinoma	48	4	8.3
Oral squamous carcinoma	4	0	0

There was loss of expression of *ARID1A* in 34 (3.4%) of 995 cases. Stromal cells in these cases were positive for *ARID1A* immunoreactivity, indicating that the negative staining in tumor cells was not due to technical artifacts. The immunostaining findings for each tumor type are summarized in Table 1 and representative cases illustrated in Figure 2. Specifically, among 21 types of carcinoma in this study, uterine low-grade endometrioid carcinomas showed loss of expression in 15 (26%) of 58 cases. In contrast, all the normal endometrial tissues examined in this study together with 38 normal endometrial tissues analyzed in our previous study[11] were intensely positive for *ARID1A*. In addition to uterine low-grade endometrioid carcinomas, gastric carcinomas were negative for *ARID1A* expression in 11% of cases. We also observed that some carcinomas, especially uterine low-grade endometrioid carcinomas, exhibited "clonal loss" of *ARID1A* immunoreactivity. This means that in a background of *ARID1A*-positive tumor cells, large groups of tumor cells did not express *ARID1A* (Fig. 3). Although several other tumor types including carcinomas of the bile duct, lung, breast, uterine cervix, colon, and pancreas contained at least 1 case with negative *ARID1A* staining, the frequency of negative cases was very low (< 10% of cases in each tumor type). In contrast, *ARID1A* was expressed in virtually all the epithelial cells in all normal adult and embryonic tissues tested which included breast, prostate, gastrointestinal tract, pancreas, bile duct, mullerian duct including endometrium (both premenopausal and postmenopausal), skin, respiratory tract, urinary tract, and trophoblast. In addition, lymphoblasts

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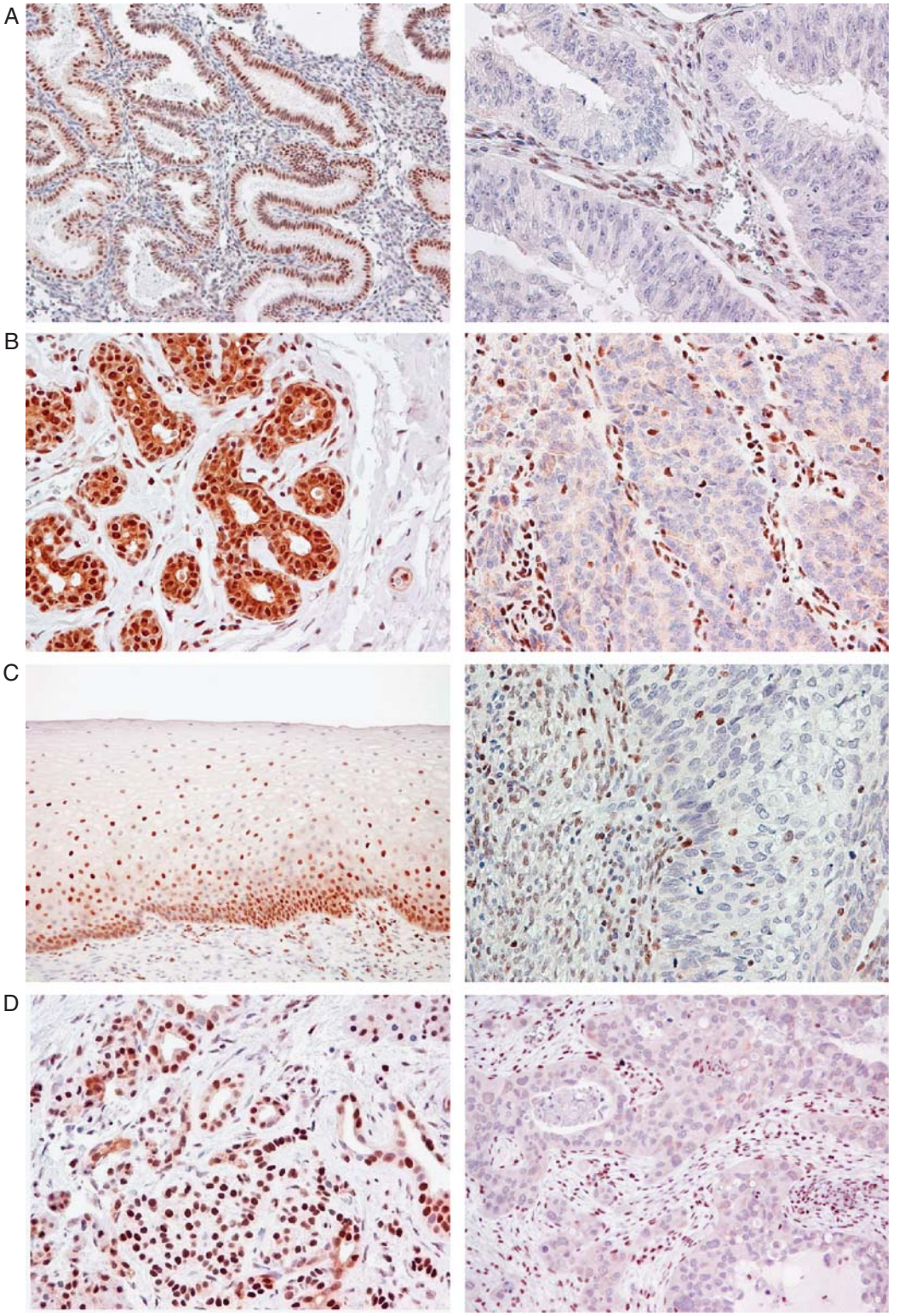

FIGURE 2. *ARID1A* immunoreactivity in representative carcinoma types (right panel) and their normal tissue counterparts (left panel). Negative staining (undetectable level) of *ARID1A* in an International Federation of Gynecology and Obstetrics grade I endometrioid carcinoma (A), an infiltrating ductal carcinoma of the breast (B), a cervical squamous carcinoma (C), and a pancreatic carcinoma (D).

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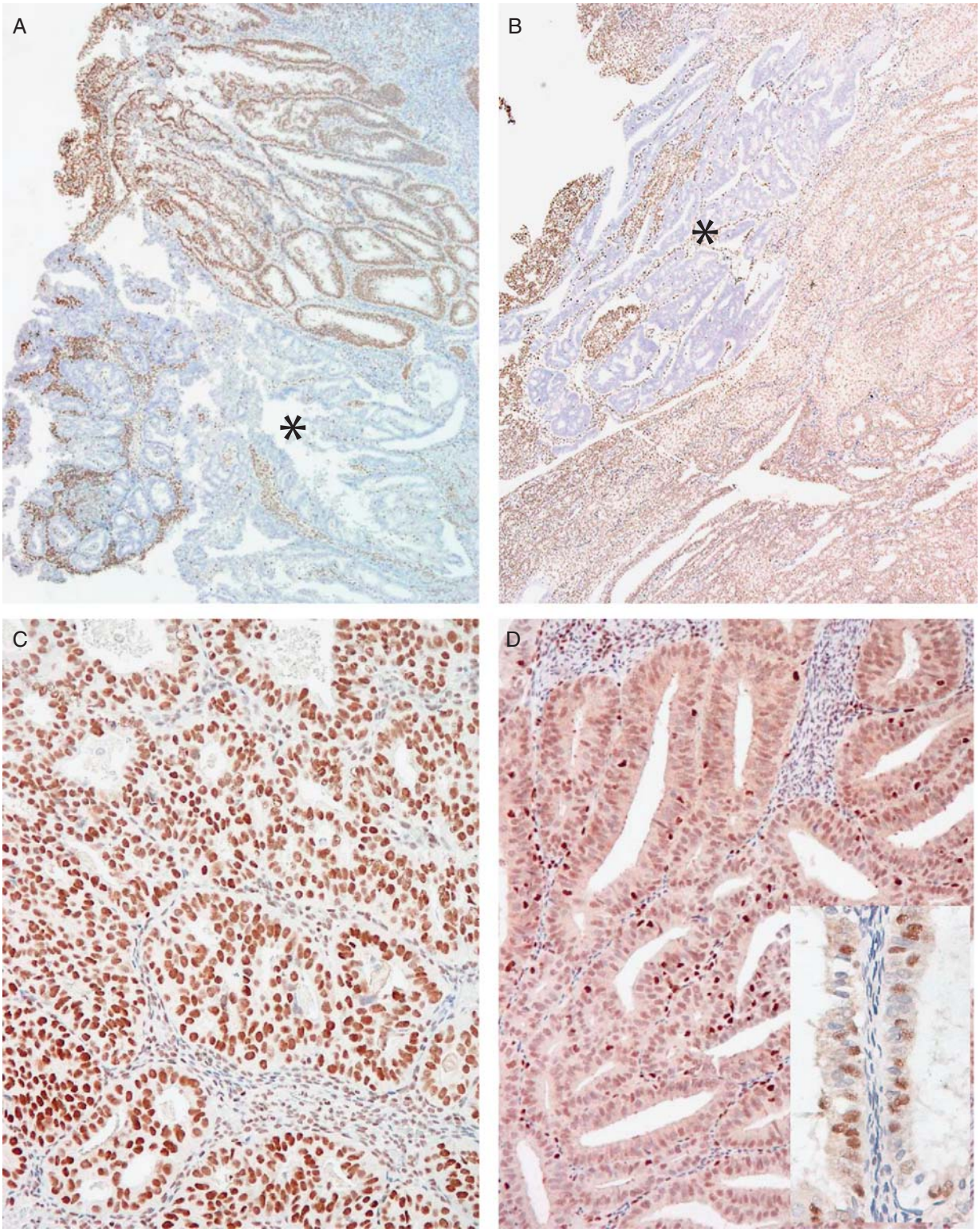

FIGURE 3. Pattern of *ARID1A* immunoreactivity in 4 uterine endometrioid carcinomas. A, The carcinoma (UEM-5) harbors biallelic *ARID1A* mutations (nonsense and deletion mutation) and shows a clonal loss of *ARID1A* expression (asterisk). B, The carcinoma (UEM-3) containing monoallelic nonsense mutation shows a clonal loss of *ARID1A* expression (asterisk). C, The carcinoma with wild-type *ARID1A* exhibits a diffuse and intense pattern of *ARID1A* staining. D, The carcinoma with wild-type *ARID1A* shows diffuse but less intense *ARID1A* immunoreactivity than the tumor in C. Occasionally, patchy staining can be observed (inset).

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TABLE 2. *ARID1A* Somatic Mutations in Uterine and Ovarian Carcinomas

	Uterine Endometrioid	Uterine Serous	Ovarian HG	Ovarian LG	Ovarian Mucinous
Cases with *ARID1A* mutation	10	0	0	0	0
Total cases	25	12	32	19	5

HG indicates high grade; LG, low grade.

in the germinal center, smooth muscle cells, skeletal muscle cells, and endothelial cells were also positive for *ARID1A*.

Mutational analysis was carried out on uterine low-grade endometrioid carcinomas because they showed the highest frequency of loss of *ARID1A* expression. Nineteen ovarian low-grade, 32 ovarian high-grade, 5 ovarian mucinous, and 12 uterine serous carcinomas were also analyzed, none of which showed loss of *ARID1A* expression. As shown in Table 2, somatic *ARID1A* mutation was detected in 10 (40%) of 25 uterine low-grade endometrioid carcinomas. As in ovarian clear cell and ovarian endometrioid carcinomas, the mutations were either insertion/deletion mutations or nonsense mutations that were widely distributed in the *ARID1A* gene (Table 3). Three of these 10 cases (UEM-1, UEM-5, and UEM-8) showed 2 independent *ARID1A* mutations, likely affecting both alleles. Correlation of *ARID1A* mutation status and immunoreactivity was made in 25 uterine endometrioid carcinomas and 51 ovarian serous carcinomas. We found that 5 (50%) of 10 tumors with *ARID1A* mutations did not show any detectable level of *ARID1A* immunoreactivity. Interestingly, 4 *ARID1A*-positive cases with *ARID1A* mutations exhibited a pattern of immunoreactivity in which areas of negative cells were present adjacent to positive areas suggesting that mutations arose in clones within the tumor (Fig. 3). In contrast, only 2 of 15 *ARID1A* wild-type endometrioid carcinomas showed complete loss of *ARID1A* staining, whereas the majority of cases showed diffuse *ARID1A* staining (> 80% of tumor cells being positive). We did not observe any pattern of clonal loss in *ARID1A* wild-type carcinomas as in *ARID1A*-mutated cases. All 19 ovarian low-grade serous carcinomas and 32 ovarian high-grade serous carcinomas showed diffuse *ARID1A* positivity; neither the above-mentioned carcinomas nor the 5 ovarian mucinous carcinomas contained *ARID1A* mutations (Table 2). Thus, the complete loss of *ARID1A* expression significantly correlated with its mutation status ($P = 0.0014$, Fisher exact test). Combining cases that were completely negative with those showing clonal loss and correlating them with *ARID1A* mutation was found to be highly significant ($P < 0.0001$, Fisher exact test).

DISCUSSION

In the past few years, substantial progress has been made in cataloging molecular genetic alterations at a genome-wide scale. One of the main findings has been the identification of somatic mutations of several chromatin-remodeling genes in certain types of human cancer. These genes include *JARID1C* in renal cell carcinoma,[1] *BRG1* (*SMARCA4*) in lung carcinoma,[12,16] and, most recently, *ARID1A* in ovarian clear cell and ovarian low-grade endometrioid carcinoma.[6,21] The findings in this study extend previous observations and provide cogent evidence that epigenetic changes, like genetic alteration, is a "driver" rather than a "passenger" that is directly involved in the tumor development of uterine low-grade endometrioid carcinoma.[7]

In this study, a large number and variety of normal and tumor tissues were evaluated. The main findings were the loss of *ARID1A* immunoreactivity and mutation of *ARDIA* in low-grade uterine endometrioid carcinoma compared with other types of carcinomas. Although loss of *ARID1A* expression also occurs in other tumor types, the frequency of loss of expression is low, indicating that *ARID1A* mutations are associated with specific types of carcinoma. Mutational analysis showed somatic *ARID1A* mutations in 40% of uterine endometrioid carcinoma, a finding that has not been previously reported.

Endometrial carcinomas are divided into 2 broad groups, designated as type I and type II. Type I tumors are composed of endometrioid carcinomas that frequently harbor sequence mutations in *CCNB1*, *PTEN*, and *PIK3CA*, whereas type II tumors are largely serous carcinomas that contain *TP53* mutations in the majority of cases.[2] Patients with type I tumors are usually younger, present at an earlier clinical stage, and have a more indolent clinical course compared with women with type II tumors. Type I tumors arise from endometrial hyperplasia, and type II tumors develop from endometrial intraepithelial carcinoma that is frequently associated with endometrial polyps. Thus, the relatively frequent loss of *ARID1A* expression and *ARID1A* mutations in uterine endometrioid carcinoma but not in uterine serous carcinoma further supports their distinct pathogenesis. Given the well-established roles of the Pten-AKT pathway and the Wnt pathway in the development of low-grade uterine endometrioid carcinoma (type I tumor), it will be important to determine whether the *ARID1A* pathway cross talks with those signaling pathways and to assess how inactivation of *ARID1A* contributes to tumor initiation and progression in this type of carcinoma.

Although *ARID1A* mutations were most often associated with complete loss of its protein expression in low-grade uterine endometrioid carcinoma, we observed several uterine endometrioid carcinomas with *ARID1A* mutations that showed a heterogeneous staining pattern. In these cases, there were significantly large areas

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TABLE 3. Correlation of *ARID1A* Mutations and Immunoreactivity

Case	Tumor Type	Nucleotide Change (Allele 1)	Nucleotide Change (Allele 2)	Amino Acid	Mutation Type	*ARID1A* Immunoreactivity
UEM-1	Uterine EMCA	6393delC	5553insG	Frameshift	Insertion/deletion	Negative
UEM-2	Uterine EMCA	2352_2353insG	Wild type	Frameshift	Insertion/deletion	Diffusely positive
UEM-3	Uterine EMCA	5701G > T	Wild type	1901G > X	Nonsense	Clonal loss; 20% negative
UEM-4	Uterine EMCA	1010delG	Wild type	Frameshift	Insertion/deletion	Negative
UEM-5	Uterine EMCA	6298C > T	1996_2000delATTTC	2100Q > X	Nonsense	Clonal loss; 50% negative
UEM-6	Uterine EMCA	3211delA	Wild type	Frameshift	Insertion/deletion	Clonal and mixed; 50% negative
UEM-7	Uterine EMCA	4683_4684insC	Wild type	Frameshift	Insertion/deletion	Clonal; 20% negative
UEM-8	Uterine EMCA	6446_6447insA	6446_6447insA	Frameshift	Insertion/deletion	Negative
UEM-9	Uterine EMCA	2023_2032delAATCCAGCTC	Wild type	Frameshift	Insertion/deletion	Negative
UEM-10	Uterine EMCA	2368C > T	Wild type	790Q > X	Nonsense	Negative
UEM-11	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	Mixed; 10% negative
UEM-12	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	Diffusely but weakly positive
UEM-13	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	Mixed; 10% negative
UEM-14	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	Negative
UEM-15	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	Diffusely positive
UEM-16	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	Negative
UEM-17	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	Diffusely but weakly positive
UEM-18	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	Diffusely positive
UEM-19	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	Mixed; 20% negative
UEM-20	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	Diffusely positive
UEM-21	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	Mixed; 20% negative
UEM-22	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	Diffusely positive
UEM-23	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	NA
UEM-24	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	NA
UEM-25	Uterine EMCA	Wild type	Wild type	Wild type	Wild type	NA
OVEM	Ovarian EMCA	Exon20 5′ intron1G > T (homo)	Exon20 5′ intron1G > T (homo)	Frameshift	Splicing	Negative
OVCC	Ovarian CC	4247insCAGC	6026_6052del	Frameshift	Insertion/deletion	Negative

CC indicates clear cell carcinoma; EMCA, endometrioid carcinoma; NA, not applicable.

that were negative in what were otherwise positive cases. This geographic distribution of loss of *ARID1A* expression strongly suggests that *ARID1A* mutation occurred in clones of cells within the tumor. This type of clonal loss of *ARID1A* immunoreactivity was only detected in uterine endometrioid carcinomas with *ARID1A* mutations but not in those without mutations. It is likely that *ARID1A* mutations occur after tumor initiation in endometrioid carcinoma, creating tumor subclones during evolution of the carcinoma. It is of great interest to study *ARID1A*

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immunostaining patterns in endometrial hyperplasia to determine how early *ARID1A* protein is lost during tumor progression of endometrioid carcinoma.

Similar to endometrial carcinoma, ovarian epithelial carcinomas have been divided into type I and type II categories based on their distinctive clinicopathologic and molecular features.[18] Ovarian clear cell carcinoma and low-grade ovarian endometrioid carcinoma comprise the majority of type I ovarian tumors and are frequently associated with endometriosis. The results from this study along with our previous reports[6,11,21] strongly suggest that loss of *ARID1A* expression and/or its mutations are largely confined to ovarian clear cell and ovarian endometrioid carcinomas, because the high-grade and low-grade serous carcinomas and mucinous carcinomas did not show *ARID1A* mutations or loss of expression. It is, therefore, conceivable that *ARID1A* mutation plays an important role in the development of ovarian tumors derived from endometriosis. As it is generally thought that endometriosis develops from retrograde menstruation, the underlying critical molecular event for the development of uterine low-grade endometrioid, ovarian endometrioid, and clear cell carcinomas in some cases is mutation of *ARIDIA* in the endometrial tissue. Although we did not detect *ARID1A* mutation in ovarian mucinous carcinomas, the small number of mucinous carcinomas analyzed in this study precludes a definitive conclusion regarding them.

In conclusion, based on *ARID1A* IHC and mutational analysis, we found that *ARID1A* inactivation, either by somatic mutations or by loss of expression, frequently occurs in uterine low-grade endometrioid carcinomas in addition to ovarian low-grade endometrioid and ovarian clear cell carcinomas. Thus, it seems that *ARID1A* inactivation is mainly confined to certain types of gynecologic cancers that arise from endometrial tissue, either from the uterine cavity or from an ectopic site, that is, endometriosis. This therefore leads to the conclusion that these endometrium-related tumors share a similar molecular pathogenesis. Our findings also support the use of complete loss or clonal loss of *ARID1A* immunoreactivity as a surrogate marker to detect *ARID1A* mutations in tissues. However, further studies are necessary to determine the role of *ARID1A* in the differential diagnosis. It would be also interesting to determine the mutation status and immunoreactivity of *ARID1A* in uterine high-grade endometrioid carcinoma and clear cell carcinoma.

REFERENCES

1. Dalgliesh GL, Furge K, Greenman C, et al. Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. *Nature*. 2010;463:360–363.
2. Di Cristofano A, Ellenson LH. Endometrial carcinoma. *Annu Rev Pathol*. 2007;2:57–85.
3. Gao X, Tate P, Hu P, et al. ES cell pluripotency and germ-layer formation require the SWI/SNF chromatin remodeling component BAF250a. *Proc Natl Acad Sci USA*. 2008;105:6656–6661.
4. Ho L, Crabtree GR. Chromatin remodelling during development. *Nature*. 2010;463:474–484.
5. Huang J, Zhao YL, Li Y, et al. Genomic and functional evidence for an *ARID1A* tumor suppressor role. *Genes Chromosomes Cancer*. 2007;46:745–750.
6. Jones S, Wang TL, Shih IM, et al. Frequent mutations of chromatin remodeling gene *ARID1A* in ovarian clear cell carcinoma. *Science*. 2010;330:228–231.
7. Kaiser J. Genes link epigenetics and cancer. *Science*. 2010;330:577.
8. Kinzler KW, Vogelstein B. Cancer-susceptibility genes. Gatekeepers and caretakers [news; comment]. *Nature*. 1997;386:761, 763.
9. Kinzler KW, Nilbert MC, Vogelstein B, et al. Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. *Science*. 1991;251:1366–1370.
10. Kobel M, Gilks CB, Huntsman DG. Adult-type granulosa cell tumors and FOXL2 mutation. *Cancer Res*. 2009;69:9160–9162.
11. Maeda D, Mao T-L, Fukayama M, et al. Clinicopathological Significance of Loss of *ARID1A* Immunoreactivity in Ovarian Clear Cell Carcinoma. *Int J Mol Sci*. 2010;11:5120–5128.
12. Medina PP, Romero OA, Kohno T, et al. Frequent BRG1/SMARCA4-inactivating mutations in human lung cancer cell lines. *Hum Mutat*. 2008;29:617–622.
13. Nagl NG Jr, Patsialou A, Haines DS, et al. The p270 (*ARID1A*/SMARCF1) subunit of mammalian SWI/SNF-related complexes is essential for normal cell cycle arrest. *Cancer Res*. 2005;65:9236–9244.
14. Nagl NG Jr, Zweitzig DR, Thimmapaya B, et al. The c-myc gene is a direct target of mammalian SWI/SNF-related complexes during differentiation-associated cell cycle arrest. *Cancer Res*. 2006;66:1289–1293.
15. Reisman D, Glaros S, Thompson EA. The SWI/SNF complex and cancer. *Oncogene*. 2009;28:1653–1668.
16. Rodriguez-Nieto S, Canada A, Pros E, et al. Massive parallel DNA pyrosequencing analysis of the tumor suppressor BRG1/SMARCA4 in lung primary tumors. *Hum Mutat*. 2010;32:E1999–2017.
17. Schrader KA, Gorbatcheva B, Senz J, et al. The specificity of the FOXL2 c.402C > G somatic mutation: a survey of solid tumors. *PLoS One*. 2009;4:e7988.
18. Shih I-M, Kurman RJ. Ovarian tumorigenesis- a proposed model based on morphological and molecular genetic analysis. *Am J Pathol*. 2004;164:1511–1518.
19. Van Rechem C, Boulay G, Leprince D. HIC1 interacts with a specific subunit of SWI/SNF complexes, *ARID1A*/BAF250A. *Biochem Biophys Res Commun*. 2009;385:586–590.
20. Wang X, Nagl NG, Wilsker D, et al. Two related ARID family proteins are alternative subunits of human SWI/SNF complexes. *Biochem J*. 2004;383:319–325.
21. Wiegand KC, Shah SP, Al-Agha OM, et al. *ARID1A* mutations in endometriosis-associated ovarian carcinomas. *N Engl J Med*. 2010;363:1532–1543.

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