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Loss of ARID1A Expression Correlates with Stages of Tumor Progression in Uterine Endometrioid Carcinoma

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Abstract

ARID1A is a recently identified tumor suppressor that functions in chromatin remodeling. Inactivating mutations of ARID1A and loss of its expression most frequently occur in ovarian clear cell carcinoma, ovarian endometrioid carcinoma, and uterine endometrioid carcinoma. In this study, we performed a detailed immunostaining analysis of ARID1A in 246 cases including benign endometrium and endometrioid carcinoma at different stages of progression. Special attention was paid to recording intratumoral heterogeneity of clonal loss of ARID1A immunoreactivity. All normal endometria (n= 51) and endometrial polyps (n= 14) retained ARID1A expression. Among complex atypical hyperplasia (n= 38), 16% exhibited clonal loss of ARID1A, but none showed complete loss. Among low-grade endometrioid carcinomas (n= 88), 25% exhibited complete loss and 24% exhibited clonal loss. In contrast, 44% of high-grade endometrioid carcinomas (n= 55) showed complete loss of ARID1A and 9% exhibited clonal loss. We found that 19 high-grade carcinomas also contained concurrent low-grade carcinomas. In the high-grade areas, 63% exhibited complete loss and 11% exhibited clonal loss, whereas in the low-grade areas, 37% exhibited complete loss and 42% clonal loss. In 5 of these 19 cases, progressive loss of ARID1A from retention or clonal loss to complete loss was observed between the low-grade and high-grade areas. Overall, the percentage of complete ARID1A loss increased from 0% in complex atypical hyperplasia, to 25% in low-grade endometrioid carcinoma, to 44% in high-grade endometrioid carcinoma. These findings suggest that loss of ARID1A expression, presumably due to mutation, plays an important role in tumor progression of uterine endometrioid carcinoma.

Introduction

Access of nuclear factors and enzymes to nucleosome-free DNA is required for a plethora of nuclear activities involving transcription, DNA replication, methylation, and damage repair. This is made possible by chromatin remodeling complexes that facilitate moving and displacing histones along the DNA strand to create nucleosome-free chromosomal regions where those processes can occur. Cancer cells exploit aberrations in chromatin remodeling to drive tumor initiation and progression by a process in which somatic mutations inactivate genes encoding proteins that participate in SWI/SNF (SWItch/Sucose NonFermentable)

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chromatin remodeling. These genes include ARID1A (BAF250A) in endometrium-related carcinoma¹⁻³, ARID2 in hepatocellular carcinoma⁴, PBRM1 (BAF180) in renal cell carcinoma⁵, and BRG1 (SMARCA4) in lung cancer^{6, 7}.

Based on whole exome sequencing, we have previously reported frequent somatic mutations of *ARID1A* in ovarian clear cell carcinomas². Subsequent studies further showed that ARID1A mutations are present in several types of cancer including ovarian endometrioid carcinoma, uterine endometrioid carcinoma, and several other types of carcinoma⁸⁻¹⁰. We found that *ARID1A* mutations occur in 40% of FIGO grade 1 uterine endometrioid carcinomas¹, and this result was validated by a recent study using whole exome sequencing¹¹. Thus, it appears that ARID1A somatic mutations occur most frequently in endometriosis-related ovarian carcinoma¹² and uterine endometrioid carcinoma.

Expression of ARID1A has also been studied in uterine endometrioid carcinomas, and loss of ARID1A expression correlated with ARID1A mutation and was found in 26% of grade 1 endometrioid carcinoma¹. In a comparison of low-grade (FIGO grade 1 and 2) endometrioid carcinoma and high-grade (FIGO grade 3) endometrioid carcinoma, Wiegand et al. found a higher percentage of ARID1A loss in high-grade (39%) than in low-grade endometrioid carcinoma (29%)¹³. However, ARID1A expression in normal endometrium and benign lesions such as endometrial polyps and hyperplasia was not fully assessed, and a direct comparison of concurrent low-grade and high-grade endometrioid carcinomas has not been reported. In order to extend these previous reports of ARID1A expression in uterine endometrioid carcinoma, we conducted a comprehensive study analyzing ARID1A staining at different stages of tumor progression from normal endometrium, hyperplasia, low-grade endometrioid carcinoma, and high-grade endometrioid carcinoma. We have paid special attention to reporting complete versus clonal loss in these cases.

Material and Method

Tissue Material

Normal endometrial tissues included proliferative endometrium (n= 29), secretory endometrium (n=16), atrophic endometrium (n= 4), and gestational endometrium (n= 2). In addition, endometrial polyps (n=14) and complex atypical hyperplasia (CAH) (n=38) were also studied. The specimens were acquired by either curettage or hysterectomy, and were obtained from the National Taiwan University Hospital. Of note, 10 of the 29 proliferative endometrium specimens showed features of disordered proliferative endometrium. Among 38 cases of CAH, 35 were from endometrial curettage, and subsequent hysterectomy was performed in 19 of them. A total of 88 low-grade (FIGO 1 and 2) and 55 high-grade (FIGO 3) endometrioid carcinomas from total hysterectomies were retrieved from the National Taiwan University Hospital and the Johns Hopkins Hospital, Baltimore, MD. Hematoxylin-and-eosin stained slides were re-reviewed (by TLM and IMS) to confirm the diagnoses. Fifty-eight of the low-grade endometrioid carcinomas were from our previous study¹. In high-grade carcinoma, a concurrent low-grade component (usually FIGO1) was recorded when there was a discrete area exhibiting an abrupt change in tumor grade without gradual transition. The age of patients with high-grade endometrioid carcinoma ranged from 33 to 83 years old with a mean of 56 years. FIGO staging was known in 49 carcinomas, and among them, 25 were stage I, 6 were stage II, 14 were stage III, and 4 were stage IV.

Immunohistochemistry

Eighty eight low-grade endometrioid carcinomas and 55 high-grade endometrioid carcinomas including 36 pure high-grade carcinomas and 19 mixed high-grade and low-grade carcinomas were evaluated for ARID1A immunoreactivity. Immunohistochemical staining of ARID1A was performed on at least one and as many as 10 blocks per case,

depending on their availability. For high-grade (grade 3) carcinomas with a concurrent low-grade component, staining was performed on both components. When there was discordant staining between the low- and high-grade components, the rest of the available blocks were further stained. Collection of specimens and immunohistochemical analysis were conducted under approval by the Institutional Review Board at the National Taiwan University Hospital.

Immunohistochemistry was carried out first by autoclaving tissue sections at 120°C for 10 minutes in citrate buffer (pH 6.0). Tissue sections were then incubated with the primary antibody (ARID1A: Sigma-Aldrich HPA005456, polyclonal, 1:250) at 4°C overnight. The specificity of the ARID1A antibody has been previously demonstrated¹. Immunoreactivity was detected using EnVision+System (Dako, Carpinteria, CA). Only nuclear staining was considered positive. Stromal cells within each section served as a positive internal control. Complete loss was defined as the absence of ARID1A immunoreactivity in virtually all tumor cells, whereas clonal loss was defined as the lack of staining in a discrete tumor area(s) against a background of ARID1A positive tumor cells. When there was clonal loss, the percentage of the area with clonal loss was estimated based on the staining result of the available blocks. Sporadic single gland loss was defined as loss of staining involving one or two individual glands while the rest of the glands were positive.

Statistical analysis

Differences in the patterns of ARID1A expression among different groups were assessed using Fisher's exact test; $P < 0.05$ was considered significant.

Results

ARID1A staining patterns in normal and benign endometrium

All normal endometrium specimens and benign endometrial polyps at different phases of hormonal activity demonstrated diffuse and intense ARID1A immunoreactivity, and none of them exhibited either complete or clonal loss of ARID1A staining (Table 1). Sporadic loss of ARID1A staining was observed in 4 cases, but the loss only involved one or two individual glands (Fig. 1A). Those four specimens included two cases of proliferative endometrium, one disordered proliferative endometrium, and one endometrial polyp with proliferative activity (Table 1). Staining on deeper sections of the same tissue blocks did not reveal additional areas showing ARID1A loss in the endometrial glands.

ARID1A staining patterns in complex atypical hyperplasia

Next, we examined ARID1A staining in complex atypical hyperplasia (CAH), which is associated with increased risk in developing endometrioid carcinoma¹⁴ of the uterus. Among the 38 cases of CAH, 27 had retention of ARID1A, 5 cases exhibited sporadic loss in single glands (Fig. 1B) and 6 exhibited focal clonal loss (Fig. 1C). Further staining on the deeper sections of the 5 cases with sporadic loss in single glands did not reveal additional clonal loss. None of the CAH cases demonstrated complete loss of ARID1A. Follow-up data were available in 4 cases with clonal loss. Among them, one patient developed grade 1 endometrioid carcinoma 4 years after the curettage diagnosis of CAH. Colon cancer was also found in this patient 2 years before the diagnosis of endometrioid carcinoma. Of note, there was one CAH showing decreased ARID1A immunostaining intensity as compared with adjacent normal-appearing glands (Fig. 2A)

ARID1A staining patterns in high-grade uterine endometrioid carcinoma

As compared with normal, benign, and hyperplastic endometrium, high-grade endometrioid carcinomas exhibited several different patterns of ARID1A staining. First, among 55 high-

grade endometrioid carcinomas, complete loss of ARID1A was detected in 24 (44%), and clonal loss was detected in 5 (9%) while ARID1A retention was found in 26 (47%). Among the 5 tumors with clonal loss, two had concurrent low-grade components. The areas with clonal loss comprised from less than 10% to as much as 50% of the high-grade component. One tumor without a concurrent low-grade component exhibited two patterns of ARID1A—expression approximately half of the tumor contained diffuse and intense ARID1A staining while the other half had completely lost ARID1A positivity (Fig. 2B). Among the 26 ARID1A positive carcinomas, 24 exhibited diffuse and intense ARID1A staining, whereas two tumors had much weaker ARID1A staining. In contrast to some normal and hyperplastic endometrium specimens, none of the 26 ARID1A positive cases exhibited focal sporadic loss. There were 19 high-grade endometrioid carcinomas containing concurrent low-grade endometrioid carcinomas. Among these, complete loss of ARID1A was found in 12 (63%) high-grade components and 7 (37%) low-grade components. Clonal loss was observed in 2 (11%) high-grade components and 8 (42%) low-grade components (Table 2, Fig. 3). By comparing the high-grade and low-grade components from individual patients, we observed a progressive loss of ARID1A expression in 5 of the 19 tumors with concurrent low-grade components: four tumors had ARID1A clonal loss and one tumor had ARID1A retention in the low-grade component, while the high-grade component of all five tumors had complete loss. The ARID1A staining pattern from one of these cases is shown in Fig. 3B. Among the remaining 14 high-grade endometrioid carcinomas containing concurrent low-grade endometrioid carcinomas, 12 exhibited concordant expression patterns of ARID1A, but the remaining 2 exhibited an opposite pattern in which the high-grade component retained ARID1A staining and the low-grade component exhibited clonal loss.

The pattern of loss of ARID1A immunoreactivity in different stages of tumor progression in endometrioid carcinoma

Since we have applied the same ARID1A antibody and method of immunohistochemistry in assessing ARID1A expression in 58 low-grade (FIGO grade 1 and 2) ¹ and an additional 30 low-grade endometrioid carcinomas in this study, we combined the previous data with the current results in order to generate an overview of changes of ARID1A staining patterns in normal and abnormal endometrium. Three staining patterns were used: complete loss, clonal loss, and no loss of ARID1A immunoreactivity. Complete and clonal loss was found in 22 (25%) and 21 (24%) of 88 low-grade endometrioid carcinomas. We observed a progressive decrease in the percentage of cases with “no loss” as we moved from normal endometrium (including benign endometrial polyps) to high-grade endometrioid carcinoma. A significant progressive increase in the percentage of cases showing “complete loss” was observed as we moved from normal or hyperplastic endometrium to low-grade and then to high-grade endometrioid carcinoma (Fig. 4). Clonal loss of ARID1A expression was present in complex atypical hyperplasia as well as low-grade and high-grade endometrioid carcinoma, but the percentage of cases exhibiting clonal loss was lower in high-grade endometrioid carcinoma than in complex atypical hyperplasia and low-grade endometrioid carcinoma. Complete loss of ARID1A was not observed in benign endometrium and complex atypical hyperplasia.

Discussion

In this study, we compared the ARID1A staining patterns among different progression stages of uterine endometrioid carcinoma from normal endometrium, to hyperplastic endometrium, to low-grade endometrioid carcinoma, to high-grade endometrioid carcinoma. We found that there was a significant increase in the percentage of cases demonstrating complete loss of ARID1A expression accompanied by a gradual decrease in the percentages of cases showing no loss of ARID1A immunoreactivity during tumor progression. As ARID1A is a tumor suppressor, and loss of its expression has been demonstrated to result in

increased cellular proliferation¹⁵, our results suggest an important role of ARID1A inactivation in the pathogenesis of uterine endometrioid carcinoma, and should have several biological and translational implications.

Loss of ARID1A expression appears as early as the stage of complex atypical hyperplasia, presenting as a form of “clonal loss,” in which negative ARID1A immunoreactivity, presumably due to an inactivating mutation, is only present in a small cluster of individual glands. It is plausible that these emerging ARID1A-negative clones eventually outgrow the surrounding ARID1A-positive tumor cells, because loss of ARID1A expression confers an increased proliferative activity compared to tumor cells without loss of ARID1A expression¹⁵. As a result of clonal expansion, ARID1A negative glands prevail in the tumor, resulting in complete loss of ARID1A as time goes by. Interestingly, we found ARID1A loss in a single gland in benign endometrium (an example shown in Fig. 1A). As each endometrial gland develops clonally¹⁶, our result suggests that loss of ARID1A expression, presumably due to inactivating mutations, occurs in the endometrial stem cells that subsequently populate the whole gland. This finding is similar to the observation of PTEN loss in normal endometrium¹⁷ and that molecular changes affecting expression of tumor suppressors, ARID1A and PTEN, precedes morphological changes in endometrial epithelial cells. It can be envisioned that the time window from clonal loss to complete loss of ARID1A may be small, as suggested by the observation that all endometrioid carcinomas with ARID1A loss exhibited either clonal or complete loss, with the exception of one case, which exhibited ARID1A loss in half of the tumor (Fig 2B). It has been demonstrated that loss of ARID1A expression occurs in endometriotic cyst epithelium adjacent to an ovarian endometrioid or clear cell carcinoma¹⁸. Similarly, decreased expression of ARID1A was also found in atypical ductal hyperplasia of the breast as compared with normal breast tissue⁹. Thus, it appears that loss of ARID1A expression represents an early molecular event during tumor development in several types of female reproductive cancer.

The finding of a higher percentage of complete ARID1A loss in high-grade endometrioid carcinoma as compared to low-grade endometrioid carcinoma is consistent with a recent report studying on 214 endometrial endometrioid carcinomas¹³. However, it should be noted that the previous report¹³ was performed in tissue microarrays while the current study used the whole tissue sections to detect clonal loss which is otherwise difficult to evaluate in tissue microarrays. Nevertheless, both studies suggest that ARID1A loss is not only important during tumor initiation but also plays a role in tumor progression. In addition to inactivating mutations in *ARID1A*, molecular genetic alterations found in endometrioid carcinoma include mutations in *PTEN*, *PIK3CA*, *KRAS*, *CTNNB1*, and microsatellite instability¹⁹. Mutations of *PTEN* have also been identified in endometrial hyperplasia, suggesting that *PTEN* mutation is an early event in endometrioid carcinoma²⁰. It has become clear that *PTEN* deletion plays a critical role in tumor initiation, while *TP53* mutation, *ERBB2* overexpression or amplification, and *CNKN2A* inactivation likely represent late events, and occur predominantly in grade 3 tumors²¹. High-grade endometrioid carcinomas have an increased frequency of mutations in several genes including *PTEN* and *TP53* compared to low-grade endometrioid carcinomas²². High-grade endometrioid carcinoma can also arise de novo through *TP53* mutation and HER2/neu amplification without progressing through a low-grade stage²³. Indeed, based on clinicopathological and immunohistochemical studies, some high-grade endometrioid carcinomas exhibit overlapping features of low-grade endometrioid carcinoma and uterine serous carcinomas²⁴. Several studies have investigated the relationship of ARID1A with other tumor suppressor genes in gynecological cancers. In ovarian clear cell carcinoma, loss of ARID1A frequently coexists with *PIK3CA* mutation, and occurs in the early stage of tumor development^{25, 26}. In ovarian clear cell carcinoma and uterine endometrioid carcinoma, mutations in ARID1A occur exclusively in tumors with wild type *TP53*¹⁵. Our

preliminary data based on immunohistochemistry of the high-grade endometrioid carcinomas from the cohort in this study also showed a higher incidence of ARID1A loss in tumors without aberrant p53 expression (data not shown).

Although ARID1A staining pattern in endometrial lesions is either diffusely positive or completely negative in most cases, we observed a weak staining of ARID1A immunoreactivity (as compared to normal-appearing endometrial tissues) in a few complex atypical hyperplasia and endometrioid carcinoma cases. This finding may suggest that reduced expression of ARID1A probably due to inactivation in one of the *ARID1A* alleles is sufficient to contribute to tumor development. Future experiments are needed to determine if *ARID1A*, like *PTEN*, is characterized by haplo-insufficiency in its tumor suppressor functions.

This study also provided a unique opportunity to compare ARID1A expression between low-grade (grade 1 and 2) and high-grade (grade 3) endometrioid carcinomas from the same patient. Most concurrent low-grade and high-grade endometrioid carcinomas exhibited concordant expression pattern of ARID1A (complete loss, clonal loss, or no loss in both low-grade and high-grade tumor components), while approximately one fourth demonstrated a progression of loss (from retention or clonal loss to complete loss). These findings suggest that in at least some cases inactivation of the ARID1A pathway may participate in late progression from low-grade to high-grade uterine endometrioid carcinoma. The 2 cases showing an opposite pattern (clonal loss in low grade, retention in high grade) may reflect a sampling bias resulting from intratumoral heterogeneity of ARID1A inactivation.

In summary, we have performed a comprehensive analysis of ARID1A immunostaining in different stages of progression of uterine endometrioid carcinoma. The findings, together with our previous study, demonstrate a significant progressive increase in ARID1A loss moving from normal endometrium, to complex atypical hyperplasia, to low-grade endometrioid carcinoma, to high-grade endometrioid carcinoma. Our data suggest that loss of ARID1A expression (presumably due to a mutation) plays an important role in tumor progression in endometrioid carcinoma of the endometrium. Thus, it will be important to determine if loss of ARID1A expression provides a molecular marker to predict tumor recurrence, treatment response, and overall survival in endometrioid carcinoma.

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Figure 1. Hematoxylin and eosin staining (upper panels) and ARID1A staining (bottom panels). A. Sporadic loss of ARID1A in proliferative endometrium. B. Sporadic loss of ARID1A in complex atypical hyperplasia. C. Clonal loss in complex atypical hyperplasia.

Figure 2.

ARID1A staining pattern in 2 representative cases. Hematoxylin and eosin staining (Upper panels), ARID1A staining (lower panels). A. Complex atypical hyperplasia showing reduced immunostaining intensity as compared to the normal endometrium (basal layer). Left, low magnification. Right, higher magnification. B. High-grade endometrioid carcinoma showing diffuse and intense ARID1A staining (left lower panel) and complete loss of ARID1A positivity (right lower panel).

Figure 3.

Expression of ARID1A in three representative tumors with concurrent high-grade (upper panels) and low-grade (bottom panels) components. A. Complete loss in both high-grade and low-grade components (case 1); B. Complete loss in high-grade component and clonal loss in low-grade component (case 14); C. Weak staining in high-grade component; low-grade component shows weak staining (left half) and clonal loss (right half). Note that the normal gland (arrow) shows strong nuclear staining (case 8).

Figure 4.

Percent of cases exhibiting ARID1A loss in different stages of tumor progression. Increasing percentage of complete loss of ARID1A from normal endometrium, to CAH, to low-grade endometrioid carcinoma, to high-grade endometrioid carcinoma.

Table 1

Expression of ARID1A in normal endometrium, endometrial polyp and complex atypical hyperplasia.

Endometrium	Total cases	Sporadic single gland loss	Clonal loss
Proliferative	19	2	0
Disordered proliferative	10	1	0
Secretory	16	0	0
Gestational	2	0	0
Atrophy	4	0	0
Endometrial polyp	14	1	0
Complex atypical hyperplasia	38	5	6

Table 2

Expression of ARID1A in tumors with concurrent high-grade and low-grade endometrioid carcinomas.

Case	Age	Stage	Follow-up	ARID1A expression	
				High-grade	Low-grade
1	63	IB	alive	loss	loss
2	42	II	alive	loss	clonal loss
3	54	IA	alive	clonal loss*	clonal loss*
4	55	IVB	na	loss	clonal loss
5	42	II	alive	retained	clonal loss
6	50	IA	alive	retained*	clonal loss
7	66	IB	dead	loss	loss
8	56	IA	alive	loss	loss
9	67	IB	na	loss	loss
10	47	IB	alive	retained	retained
11	55	IIIC1	alive	loss	clonal loss
12	58	IIIC2	alive	loss	loss
13	58	IIIC1	alive	loss	loss
14	40	IB	alive	clonal loss	clonal loss
15	55	II	alive	retained	retained
16	83	IIIC2	na	retained	retained
17	55	IVB	na	loss	clonal loss
18	71	IB	alive	loss	loss
19	50	II	alive	loss	retained

* weak staining in the positive area; na: not available