The roles of ARID1A in gynecologic cancer

Tsui-Lien Mao¹, Ie-Ming Shih²
¹Department of Pathology, National Taiwan University College of Medicine, Taipei, Taiwan; ²Departments of Gynecology and Obstetrics, Pathology and Oncology, Johns Hopkins Medical Institutions, Baltimore, MD, USA

One of the exciting findings in recent cancer genome studies is the discovery of somatic mutations in several chromatin remodeling genes. These studies not only illuminate the emerging roles of chromatin remodeling in the pathogenesis of human cancer but also provide molecular genetic basis of aberrant epigenomic regulation as one of the key mechanisms driving cancer development. This is because chromatin remodeling influences a variety of DNA activities such as replication, transcription, repair, methylation, and recombination. Among the mutated chromatin remodeling genes reported, ARID1A is frequently mutated in a variety of human cancers, especially in endometrium-related neoplasms including ovarian clear cell carcinoma, ovarian endometrioid carcinomas, and uterine endometrioid carcinomas, all of which arise from endometrial epithelium. This review will summarize the recent advances in studying the roles of ARID1A mutations in gynecologic cancers with special emphasis on how this new knowledge will further extend our understanding of the pathogenesis of endometrium-related carcinomas.

Keywords: ARID1A, BAF250a, Chromatin remodeling, Endometriosis, Ovarian cancer

INTRODUCTION

The AT-rich interactive domain 1A (SWI-like) gene (ARID1A) encodes BAF250a which is a member of the SWI/SNF adenosine triphosphate-dependent chromatin-remodeling complexes [1]. The SWI/SNF complex has been shown to play an essential role in controlling gene expression [2] and is also involved in tissue development and cellular differentiation [3,4]. Inactivation of several components of this complex had been found to be associated with certain type of cancer. For example, INI1 (also called BAF47, SNF5) is frequently deleted in rhabdoid tumor and atypical teratoid/rhabdoid tumor of the central nervous system [5-7]. The ATPase subunit genes including BRG1 and BRM have been found to be lost in about 15%-20% of primary non-small cell lung cancers, and loss of BRG1/BRM are associated with poor prognosis [8,9]. ARID1A, containing a DNA-binding motif (ARID), is located at Ch1p36.11, a region frequently deleted in human cancers [10]. Functional genomics analysis demonstrates homozygous deletion involving the 5’ end of ARID1A in a lung adenocarcinoma cell line, suggesting ARID1A has a tumor suppressor role [11]. Deficient expression of ARID1A had been found in carcinomas from several organs, and most frequent in carcinomas of the breast and kidney [12]. Recently, using whole exome sequencing and transcriptome sequencing, mutation of ARID1A was found in 43%-57% of ovarian clear cell carcinomas [13,14] and 30% of ovarian endometrioid carcinomas [14].

MUTATION PROFILES OF ARID1A IN MALIGNANCIES

Following the discovery of inactivating mutations of ARID1A in gynecologic tumors, mutations of ARID1A had been found in many human malignancies based on next generation...
The roles of ARID1A in gynecologic cancer

BIOLOGY OF ARID1A AS A TUMOR SUPPRESSOR

The majority of ARID1A mutations are frame shift or nonsense mutations, suggesting that ARID1A is a tumor suppressor. It has been reported that restoring the expression of wild-type ARID1A in ovarian cancer cells harboring deleterious ARID1A genes was sufficient to suppress cellular proliferation and tumor growth in mice [26]. Conversely, silencing ARID1A expression in non-transformed epithelial cells enhanced cellular proliferation and tumorigenicity in a mouse tumor xenograft model [26]. Similarly, in an esophageal cancer model, knockdown of ARID1A in cell lines enhanced cellular proliferation, and re-expression of ARID1A in ARID1A-deleted cell lines significantly suppressed proliferation [18]. At the molecular level, ARID1A/BRG1 complex has been shown to directly interact and collaborate with p53 to transcriptionally regulate several downstream effectors including those encoded by CDKN1A (p21) and SMAD3 [26]. Using biochemistry approaches, investigators have found that ARID1A acts as a nucleocytoplasmic protein whose stability depends on its subcellular localization [27]. Nuclear ARID1A is unstable as compared to cytoplasmic ARID1A because the protein is rapidly degraded by the ubiquitin-proteasome system in the nucleus. In-frame deletions that disrupt the consensus nuclear export signal are associated with a reduced steady-state protein level of ARID1A due to its retention in the nucleus and subsequent degradation [27]. These findings delineate the basic biological mechanism regulating ARID1A subcellular distribution and protein stability [27]. In summary, recently published studies provided new evidence that ARID1A functions as a tumor suppressor and may participate in both tumor initiation and progression of in human cancer including endometrium-related neoplasms.

ARID1A EXPRESSION AND MUTATION IN GYNECOLOGIC NEOPLASMS

1. Alteration of ARID1A in ovarian tumors

As previously discussed, among human tumors studied so far, gynecologic tumors have the highest frequency of mutation of ARID1A, in which ovarian clear cell carcinoma is most frequently mutated (43%–57%) [13,14]. Mutation of ARID1A correlates with loss of protein expression [14,28], since most mutations are frameshift or nonsense mutations, leading to loss of protein expression. Ovarian endometrioid carcinoma has a mutation rate of 30%, while none of the high-grade serous carcinomas and mucinous carcinomas has mutation of ARID1A [14,29]. Ovarian clear cell carcinoma can be devided into cystic and adenofibromatous type based on the growth pattern of the tumor [30]. Cystic type clear cell carcinomas are more frequently associated with endometriosis, present at early stage, and tend to have favorable outcome. Yamamoto et al. [31] compared endometriosis and adenofibroma—associated ovarian clear cell carcinomas, and found that loss of ARID1A was more frequent in endometriosis—associated carcinomas than in adenofibroma-associated carcinomas (61% vs. 43%), although the difference was not statistically significant. The endometriotic cyst epithelium of non-atypical endometriosis, atypical endometriosis, benign and borderline clear cell adenofibroma adjacent to carcinomas with deficient ARID1A expression, showed ARID1A loss in 86% to 100% of the cases, reflecting that inactivation of ARID1A occurs in the early stages of tumor development. In contrast to the endometriotic lesions adjacent to carcinomas, endometriosis distant from ARID1A-deficient carcinomas and solitary endometriosis had retained ARID1A expression. Similarly, Ahyan et al. [32] studied the expression of ARID1A in ovarian clear cell and endometrioid carcinomas arising from endometrioma. Concurrent loss of ARID1A was found in carcinomas and the contiguous endometriotic epithelium in all tumors with deficient ARID1A expression, while the cystic epithelium distant from the tumors had retained ARID1A expression. PIK3CA, another gene frequently mutated in ovarian clear cell carcinoma (33%–46%) [33,34], was also found to be frequently mutated in the endometriotic epithelium adjacent to clear cell carcinomas [34]. The same authors further found that mutation of PIK3CA frequently co-exists with loss of ARID1A expression in ovarian clear cell carcinoma [31,35].
In borderline ovarian tumors, loss of ARID1A immunoreactivity was found in 33% of endocervical-type mucinous (seromucinous) borderline tumors, 1 (13%) of borderline endometrioid tumor, and none of the borderline serous tumor and gastrointestinal-type borderline mucinous tumors [36]. Endocervical-type mucinous borderline tumors, although also named seromucinous tumors due to endocervical and serous morphology, frequently contain features of endometrioid tumors, including ciliated cells, endometrial-type cells with abundant eosinophilic cytoplasm, and hobnail-shaped cells. In conjunction with the frequent association of endometriosis and mutation and/or loss of ARID1A, they are more closely related to endometrial-type proliferative lesions such as ovarian clear cell carcinoma and endometrioid carcinoma. Collectively, these tumors are called “endometriosis-related ovarian neoplasms” [37]. How endometriosis leads to the development of these neoplasms is unclear. It is postulated that repeated epithelial damage and repair in an endometriotic microenvironment rich in iron-induced free radicals contribute to the neoplastic transformation of the endometriotic epithelial cells [38,39].

2. Alteration of ARID1A in uterine carcinomas

Similar to the ovarian tumors, endometrial endometrioid carcinomas have high frequency of alteration of ARID1A. Mutation was found in 40% of low-grade endometrioid carcinoma and loss of expression was found in 26%–29% of low-grade endometrioid carcinoma and up to 39% of high-grade endometrioid carcinoma [29,40]. Interestingly, ARID1A mutations frequently co-occur with mutations of PTEN and PIK3CA, as well as overall PI3K pathway aberration. Furthermore, PI3K pathway activity is regulated by ARID1A through phosphorylation of AKT [41]. In the dualistic pathogenetic pathway of endometrial carcinoma, endometrioid carcinoma belongs to type I carcinoma which shows frequent mutations of KRAS, PTEN, PIK3CA, CTNNB1, and microsatellite instability [42]. Alteration of PI3K pathway was found in >80% of endometrial endometrioid carcinoma [43]. Regulation of PI3K pathway by ARID1A further suggests that ARID1A plays an important role in the pathogenesis of type I endometrial carcinomas. McConechy et al. [44] used a nine-gene mutation profile to subclassify endometrial carcinomas. The nine genes included ARID1A, PPP2R1A, PTEN, PIK3CA, KRAS, CTNNB1, TP53, BRAF, and PPP2R5C. By target enrichment sequencing, each endometrial carcinoma subtype exhibits a distinct mutation profile. Mutation of ARID1A was detected in 46.7% low-grade endometrioid carcinoma, 60% high-grade endometrioid carcinoma, 10.8% serous carcinoma, and 23.8% carcinosarcoma. The frequency of mutation of ARID1A is significantly different between endometrioid carcinoma and serous carcinoma.

Fig. 1. Immunohistochemical study of an endometrial endometrioid carcinoma with coexisting high-grade (A) and low-grade (B, C) components. The high-grade component is negative for ARID1A (D) while the low-grade component has areas with retained ARID1A staining (E) and areas with clonal loss of ARID1A (F) (A–F, ×200).
Interestingly, the molecular profile of carcinosarcomas showed either endometrioid type (mutation of *PTEN* and *ARID1A*) or serous type (mutation of *TP53* and *PPP2R1A*). The relationship between loss of ARID1A and MSI in endometrial endometrioid carcinoma has been recently demonstrated [45,46]. In one report [45], loss of ARID1A is significantly more frequent in sporadic MSI than in Lynch syndrome. The authors concluded that *ARID1A* is a causative gene instead of a target gene of MSI by the role in epigenetic silencing of the *MLH1* gene in endometrial carcinoma. In another report [46], loss of ARID1A is associated with mismatch repair deficiency. *In vitro* functional study showed that ARID1A controls tumor growth by collaboration with p53 and regulates p53 downstream targets [26]. By examining tumor samples, mutations of *ARID1A* and *TPS3* were mutually exclusive in ovarian clear cell carcinomas, and uterine carcinomas [46] endometrial endometrioid carcinomas. Immunohistochemically, loss of ARID1A and mutant-like p53 expression was found to be nearly mutually exclusive in endometrial carcinomas [45,46].

Besides genomic study, immunohistochemistry provided an opportunity for the evaluation of heterogeneity in endometrioid carcinomas. Clonal loss of ARID1A immunoreactivity in a discrete tumor area against a background of ARID1A positive tumor cells was observed in some tumors with *ARID1A* mutation, suggesting mutations arising from subclones within these tumors. On the other hand, tumors with wild-type ARID1A did not exhibit any pattern of clonal loss [29]. This phenomenon was further elucidated in a recent study, showing clonal loss of ARID1A immunoreactivity in 16% complex atypical hyperplasia, 24% of low-grade endometrioid carcinoma, and 10% of high-grade endometrioid carcinoma, while complete loss of ARID1A was found in 0% of complex atypical hyperplasia, 25% of low-grade endometrioid carcinoma, and 43% of high-grade endometrioid carcinoma [Fig. 1] [47]. Thus, ARID1A plays important role not only in tumor initiation, but also in tumor progression.

### 3. Alteration of ARID1A and tumor behavior in gynecologic cancers

Several studies had investigated the behavior of tumors showing ARID1A alterations. Maeda el al. [28] found no significant difference between ARID1A negative and positive cases in terms of histopathologic features, age, clinical stage, or overall survival in 149 ovarian clear cell carcinomas. Similarly, Yamamoto et al. [34] also found no correlation of ARID1A immunoreactivity with any clinicopathological parameters except the higher association with adjacent endometriosis in 90 ovarian clear cell carcinomas. Lowery et al. [48] examined 212 ovarian clear cell and endometrioid carcinomas and found no relationship between loss of ARID1A and stage, grade, survival or epidemiological variables. In endometrials, Fadare et al. [49] showed that loss of ARID1A was found in 22.7% of endometrial clear cell carcinomas, and cases with loss of ARID1A were significantly high in late stages, but later on they found no association with reduced overall or progression-free survival [50]. On the other hand, loss of ARID1A was reported to be associated with reduced progression-free survival and chemoresistance in ovarian clear cell carcinoma [51] and deep myometrial invasion in endometrial carcinoma [52]. The differences of the impact on prognosis may be due to different antibodies used, sample size variation, and probably other cofactors that affect the behavior of the tumors.

### CONCLUSION

Disorganized chromatin structure is known to be associated with the appearance of various abnormal phenotypes, including cancer [53,54]. ARID1A, a gene participated in chromatin remodeling, is an emerging tumor suppressor gene. Accumulating evidence has reported somatic inactivating mutations of *ARID1A* and loss of its expression in many types of human cancers, especially in endometrium-derived tumors, including ovarian clear cell carcinomas, ovarian endometrioid carcinomas and uterine endometrioid carcinomas. The high prevalence of somatic mutations in those ovarian and endometrial cancers indicates a pivotal role of ARID1A in their development. Understanding the roles of ARID1A in the pathogenesis of endometrium-derived tumors is fundamental for future translational studies aimed at designing new diagnostic tests for early detection and identifying critical molecular targets for new therapeutic interventions.

### CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

### ACKNOWLEDGMENTS

The study was supported by Taiwan National Science Council (NSC) 100-2320-B-002-081 and NIH/NCI CA165807.
Somatic mutations in the chromatin remodeling gene ARID1A

Jones S, Li M, Parsons DW, Zhang X, Wesseling J, Kristel P, et al. Oncogene 2012;31:4255-6.

DJ, et al. Mutations and deletions of ARID1A in breast tumors.

Cornen S, Adelaide J, Bertucci F, Finetti P, Guille A, Birnbaum Wiegand KC, Shah SP, Al-Agha OM, Zhao Y, Tse K, Zeng T, et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science 2010;330:228-31.

Frequent mutations of chromatin remodeling gene ARID1A in cancer. J Pathol 2003;199:313-21.

Chromatin remodeling factors and BRM/BRG1 expression as prognostic indicators in non-small cell lung cancer. Clin Cancer Res 2007;13:4838-46.

Huang J, Deng Q, Wang Q, Li KY, Dai JH, Li N, et al. Exome sequencing of hepatitis B virus-associated hepatocellular carcinoma. Nat Genet 2012;44:1177-8.

Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, et al. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet 2012;44:760-4.

Sausen M, Leary RJ, Jones S, Wu J, Reynolds CP, Liu X, et al. Integrated genomic analyses identify ARID1A and ARID1B alterations in the childhood cancer neuroblastoma. Nat Genet 2013;45:12-7.

Giulino-Roth L, Wang K, MacDonald TY, Mathew S, Tam Y, Cronin MT, et al. Targeted genomic sequencing of pediatric Burkitt lymphoma identifies recurrent alterations in antiapoptotic and chromatin-remodeling genes. Blood 2012;120:5181-4.

Guo B, Wang TL, Shih IeM. ARID1A, a factor that promotes formation of SWI/SNF-mediated chromatin remodelling, is a tumor suppressor in gynecologic cancers. Cancer Res 2011;71:6718-27.

Shain AH, Giacomini CP, Matsukuma K, Karkkainen MI, Bashyam MD, Hidalgo M, et al. Convergent structural alterations define Switch/Sucrose NonFermentable (SWI/SNF) chromatin remodeler as a central tumor suppressive complex in pancreatic cancer. Proc Natl Acad Sci U S A 2012;109:E252-9.

Huang J, Deng Q, Wang Q, Li KY, Dai JH, Li N, et al. Exome sequencing of hepatitis B virus-associated hepatocellular carcinoma. Nat Genet 2012;44:1177-8.

Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, et al. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet 2012;44:760-4.

Sausen M, Leary RJ, Jones S, Wu J, Reynolds CP, Liu X, et al. Integrated genomic analyses identify ARID1A and ARID1B alterations in the childhood cancer neuroblastoma. Nat Genet 2013;45:12-7.

Giulino-Roth L, Wang K, MacDonald TY, Mathew S, Tam Y, Cronin MT, et al. Targeted genomic sequencing of pediatric Burkitt lymphoma identifies recurrent alterations in antiapoptotic and chromatin-remodeling genes. Blood 2012;120:5181-4.

Guo B, Wang TL, Shih IeM. ARID1A, a factor that promotes formation of SWI/SNF-mediated chromatin remodelling, is a tumor suppressor in gynecologic cancers. Cancer Res 2011;71:6718-27.

Shain AH, Giacomini CP, Matsukuma K, Karkkainen MI, Bashyam MD, Hidalgo M, et al. Convergent structural alterations define Switch/Sucrose NonFermentable (SWI/SNF) chromatin remodeler as a central tumor suppressive complex in pancreatic cancer. Proc Natl Acad Sci U S A 2012;109:E252-9.
The roles of ARID1A in gynecologic cancer

31. Yamamoto S, Tsuda H, Takano M, Tamai S, Matsubara O. Loss of ARID1A protein expression occurs as an early event in ovarian clear-cell carcinoma development and frequently coexists with PIK3CA mutations. Mod Pathol 2012;25:615-24.

32. Ayhan A, Mao TL, Seckin T, Wu CH, Guan B, Ogawa H, et al. Loss of ARID1A expression is an early molecular event in tumor progression from ovarian endometriotic cyst to clear cell and endometrioid carcinoma. Int J Gynecol Cancer 2012;22:1310-5.

33. Kuo KT, Mao TL, Jones S, Veras E, Ayhan A, Wang TL, et al. Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma. Am J Pathol 2009;174:1597-601.

34. Yamamoto S, Tsuda H, Takano M, Iwaya K, Tamai S, Matsubara O. PIK3CA mutation is an early event in the development of endometriosis-associated ovarian clear cell adenocarcinoma. J Pathol 2011;225:189-94.

35. Yamamoto S, Tsuda H, Takano M, Tamai S, Matsubara O. PIK3CA mutations and loss of ARID1A protein expression are early events in the development of cystic ovarian clear cell adenocarcinoma. Virchows Arch 2012;460:77-87.

36. Wu CH, Mao TL, Vang R, Ayhan A, Wang TL, Kurman RJ, et al. Endocervical-type mucinous borderline tumors are related to endometrioid tumors based on mutation and loss of expression of ARID1A. Int J Gynecol Pathol 2012;31:297-303.

37. Maeda D, Shih IeM. Pathogenesis and the role of ARID1A mutation in endometriosis-related ovarian neoplasms. Adv Anat Pathol 2013;20:45-52.

38. Vercellini P, Crosignani P, Somigliana E, Vigano P, Buggio L, Bolis G, et al. The ‘incessant menstruation’ hypothesis: a mechanistic ovarian cancer model with implications for prevention. Hum Reprod 2011;26:2262-73.

39. Yamaguchi K, Mandai M, Toyokuni S, Hamanishi J, Higuchi T, Takakura K, et al. Contents of endometriotic cysts, especially the high concentration of free iron, are a possible cause of carcinogenesis in the cysts through the iron-induced persistent oxidative stress. Clin Cancer Res 2008;14:32-40.

40. Wiegand KC, Lee AF, Al-Agha OM, Chow C, Kalloger SE, Scott DW, et al. Loss of BAF250a (ARID1A) is frequent in high-grade endometrial carcinomas. J Pathol 2011;224:328-33.

41. Liang H, Cheung LW, Li J, Ju Z, Yu S, Stemke-Hale K, et al. Whole-exome sequencing combined with functional genomics reveals novel candidate driver cancer genes in endometrial cancer. Genome Res 2012;22:2120-9.

42. Llobet D, Pallares J, Yeramin A, Santacana M, Eritja N, Velasco A, et al. Molecular pathology of endometrial carcinoma: practical aspects from the diagnostic and therapeutic viewpoints. J Clin Pathol 2009;62:777-85.

43. Cheung LW, Hennessy BT, Li J, Yu S, Myers AP, Djordjevic B, et al. High frequency of PIK3R1 and PIK3R2 mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability. Cancer Discov 2011;1:170-85.

44. McConney MK, Ding J, Cheang MC, Wiegand KC, Senz J, Tone AA, et al. Use of mutation profiles to refine the classification of endometrial carcinomas. J Pathol 2012;228:20-30.

45. Bosse T, Ter Haar NT, Seeber LM, Diest PJ, Hes FJ, Vasen HF, et al. Loss of ARID1A expression and its relationship with PI3K-Akt pathway alterations, TP53 and microsatellite instability in endometrial cancer. Mod Pathol 2013 May 24 [Epub]. http://dx.doi.org/10.1038/modpathol.2013.96.

46. Allo G, Bernardini M, Wu RC, Shih IM, Kalloger S, Pollett A, et al. ARID1A loss correlates with mismatch repair deficiency and intact p53 expression in high-grade endometrial carcinomas. Mod Pathol 2013 Jul 26 [Epub]. http://dx.doi.org/10.1038/modpathol.2013.144.

47. Mao TL, Ardighieri L, Ayhan A, Kuo KT, Wu CH, Wang TL, et al. Loss of ARID1A expression correlates with stages of tumor progression in uterine endometrioid carcinoma. Am J Surg Pathol 2013;37:1342-8.

48. Lowery WJ, Schildkraut JM, Akushevich L, Bentley R, Marks JR, Huntsman D, et al. Loss of ARID1A-associated protein expression is a frequent event in clear cell and endometrioid ovarian cancers. Int J Gynecol Cancer 2012;22:9-14.

49. Fadare O, Renshaw II, Liang SX. Does the loss of ARID1A (BAF-250a) expression in endometrial clear cell carcinomas have any clinicopathologic significance? A pilot assessment. J Cancer 2012;3:129-36.

50. Fadare O, Gwin K, Desouki MM, Crispens MA, Jones HW 3rd, Khabele D, et al. The clinicopathologic significance of p53 and ARID1A loss correlates with mismatch repair deficiency and intact p53 expression in high-grade endometrial carcinomas. Mod Pathol 2013;26:1101-10.

51. Katagiri A, Nakayama K, Rahman MT, Rahman M, Katagiri H, Nakayama N, et al. Loss of ARID1A expression is related to shorter progression-free survival and chemoresistance in ovarian clear cell carcinoma. Mod Pathol 2012;25:282-8.

52. Werner HM, Berg A, Wik E, Birkeland C, Krakstad C, Kusonmano K, et al. ARID1A loss is prevalent in endometrioid hyperplasia with atypia and low-grade endometrioid carcinomas. Mod Pathol 2013;26:428-34.

53. Wu JN, Roberts CW. ARID1A mutations in cancer: another epigenetic tumor suppressor? Cancer Discov 2013;3:35-43.

54. Papamichos-Chronakis M, Peterson CL. Chromatin and the genome integrity network. Nat Rev Genet 2013;14:62-75.