BRIEF COMMUNICATION

Roles of Deletion of *Arid1a*, a Tumor Suppressor, in Mouse Ovarian Tumorigenesis

Bin Guan, Yohan Suryo Rahmanto, Ren-Chin Wu, Yihong Wang, Zhong Wang, Tian-Li Wang, Ie-Ming Shih

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Correspondence to: Ie-Ming Shih, MD, PhD, Department of Gynecology and Obstetrics, Johns Hopkins Medical Institutions, 1550 Orleans Street, CRB-2 Rm 305, Baltimore, MD 21287 (e-mail: ishih@jhmi.edu) and Tian-Li Wang, PhD, Department of Pathology, Johns Hopkins Medical Institutions, 1550 Orleans Street, CRB-2 Rm 306, Baltimore, MD 21287 (e-mail: tlw@jhmi.edu).

The chromatin remodeling gene, *ARID1A*, has been implied as a tumor suppressor, and its somatic inactivating mutations occur in a wide variety of human cancers, most frequently in ovarian and uterine endometrioid and ovarian clear cell carcinomas. Tumors with *ARID1A* mutations also frequently harbor *PTEN* or *PIK3CA* mutations, suggesting their collaboration in tumorigenesis. Here, we used a conditional knockout mouse model in which *Arid1a* and *Pten* were deleted either individually or in combination in the mouse ovarian surface epithelium. After 6 months, 59.1% of mice with *Arid1a* and *Pten* double knockout developed ovarian endometrioid or undifferentiated carcinoma, whereas the remaining mice showed hyperplasia of ovarian surface epithelium. In contrast, 52 mice with homozygous or heterozygous deletion in either *Arid1a* or *Pten* did not develop ovarian lesions. These results demonstrate that inactivation of *Arid1a* alone is insufficient for tumor initiation but it requires additional genetic alteration(s) such as *Pten* deletion to drive tumorigenesis.

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ARID1A is one of the most commonly mutated chromatin remodeling genes in human cancer, especially in endometrium-related neoplasms, including ovarian clear cell carcinoma, ovarian endometrioid carcinoma, and uterine endometrioid carcinoma (1–5). The great majority of *ARID1A* mutations are frameshift or nonsense mutations that occur throughout the coding region, suggesting that *ARID1A* functions as a tumor suppressor. Indeed, restoring expression of wild-type *ARID1A* in ovarian cancer cells harboring deleterious *ARID1A* mutations suppresses cellular proliferation and growth of tumor xenografts in mice (6). Conversely, silencing *ARID1A* expression in nontransformed epithelial cells enhances cellular proliferation and tumorigenicity (6–9). It has been demonstrated that loss of *ARID1A* expression, presumably due to inactivating mutations, occurs in the precursor stage of ovarian clear cell and ovarian endometrioid carcinomas

(2,10,11), and loss of *ARID1A* immunoreactivity correlates with tumor progression in endometrial carcinomas (12). These studies indicate that *ARID1A* functions as a tumor suppressor and may participate in both tumor initiation and progression of endometrium-related neoplasms. However, it remains unclear whether *ARID1A* inactivation per se is sufficient to initiate tumor development in vivo or whether tumor initiation requires molecular collaboration with aberration in other cancer pathway(s). Here, we sought to determine the role of *Arid1a* in tumor development using *Arid1a* conditional knockout mice.

We first tested whether deletion of *Arid1a* alone in mouse ovarian surface epithelium (MOSE) was sufficient to initiate tumor development. All of the animal procedures were approved by the Johns Hopkins University Animal Care Committee. Detailed information on the mouse strains is given in the [Supplementary](#)

[Methods](#) (available online). We performed intra-ovarian bursa injection of AdCre virus on left ovaries in 29 *Arid1a*^{fllox/fllox} mice (see [Supplementary Methods](#), available online). The right ovaries were not injected and were used as controls. Twelve months after AdCre administration, no gross or microscopic lesions were observed in either AdCre-injected or control ovaries (Table 1), suggesting that additional molecular changes are required to collaborate with *Arid1a* inactivation in transforming MOSE. It has been noted that *ARID1A* mutations frequently co-occur with either *PIK3CA* mutations or *PTEN* mutations in human tumor samples (5,13,14); therefore, in this study, we tested whether *Arid1a* deletion in collaboration with abnormalities in the *PTEN*/*PI3K*/*AKT* pathway facilitated ovarian tumorigenesis. We used conditional *Pten* knockout mice to test the above hypothesis because 84% of uterine endometrioid carcinomas with *ARID1A* mutations contain *PTEN* mutation [based on The Cancer Genome Atlas data (5)] and 49% of ovarian endometrioid carcinomas with *ARID1A* mutations contain *PTEN* mutation (14). *Pten* knockout mice are commercially available (15), and *Pten* deletion in combination with *Apc* or *Kras* mutations induces ovarian cancers after intrabursal administration of AdCre virus (16,17).

Arid1a^{fllox/fllox} mice were crossed with *Pten*^{fllox/fllox} mice to generate *Arid1a* and *Pten* conditional double knockout mice (see [Supplementary Methods](#), available online). The littermates with double knockout were identified by genotyping polymerase chain reaction (see [Supplementary Methods](#) and [Supplementary Figure 1A](#), available online). We observed MOSE hyperplasia as early as 2 months after AdCre administration in the ovaries treated with AdCre virus (Figure 1A; [Supplementary Table 1](#), available online). Six months after AdCre injection, six of 13 (46.2%) *Arid1a*^{fllox/fllox};*Pten*^{fllox/fllox} mice developed ovarian tumors, and the remaining seven mice developed MOSE hyperplasia (Figure 1A; [Supplementary Table 1](#), available online). Eight to 9 months after knockout of both genes, seven of nine (77.8%) mice developed ovarian tumors, and the remaining two developed MOSE hyperplasia (Figure 1A; [Supplementary](#)

Table 1. Summary of tumor phenotypes of ovary in mice with different genotypes

Genotype	Time after AdCre injection	No. of mice with			Total No. of mice	P*
		Tumor	Hyperplasia	No lesion		
<i>Arid1a</i> ^{fllox/fllox} ; <i>Pten</i> ^{fllox/fllox}	≥6 Months	13	9	0	22	NA
<i>Arid1a</i> ^{+fllox} ; <i>Pten</i> ^{fllox/fllox}	12 Months	0	0	5	5	<.001
<i>Arid1a</i> ^{fllox/fllox} ; <i>Pten</i> ^{+fllox}	12 Months	0	0	5	5	<.001
<i>Arid1a</i> ^{+fllox} ; <i>Pten</i> ^{+fllox}	12 Months	0	0	13	13	<.001
<i>Arid1a</i> ^{fllox/fllox} ; <i>Pten</i> ^{+/+}	12 Months	0	0	29	29	<.001

* Statistical analyses were performed using two-tailed Fisher exact test to compare the frequency of ovarian tumor formation after AdCre administration between genotype *Arid1a*^{fllox/fllox}; *Pten*^{fllox/fllox} and other genotypes (*Arid1a*^{+fllox}; *Pten*^{fllox/fllox}, *Arid1a*^{fllox/fllox}; *Pten*^{+fllox}, *Arid1a*^{+fllox}; *Pten*^{+fllox}, and *Arid1a*^{fllox/fllox}; *Pten*^{+/+}). NA = not applicable.

Figure 1. Ovarian lesions in *Arid1a*^{fllox/fllox}; *Pten*^{fllox/fllox} mice. **A)** Schematic of the phenotypes of all 30 *Arid1a* and *Pten* conditional double knockout mice. **B)** Gross and histologic features of an endometrioid carcinoma (left panels) and an undifferentiated carcinoma (right panel). Scale bar = 1 cm. **C)** Immunostaining of markers in a representative undifferentiated carcinoma. Scale bar = 250 μm. **D)** Immunostaining of markers in a representative endometrioid carcinoma. Scale bar = 250 μm. CA = carcinoma; OSE = ovarian surface epithelium; SM = squamous metaplasia.

Table 1, available online). In this study, we did not observe transformation of potential stem cells in the ovarian hilum (data not shown). In contrast, none of the littermates without double knockout developed ovarian neoplasms or hyperplasia (Table 1). In all, by 9 months after AdCre injection, 13 of 22 (59.1%) *Arid1a*/*Pten* double knockout mice

had developed ovarian tumors, whereas none of the 52 mice without double knockout had developed ovarian lesions ($P < .001$, two-tailed Fisher exact test; $P < .05$ was considered statistically significant) (Table 1). These results are consistent with previous reports demonstrating that deletion of *Pten* alone in MOSE is insufficient

to generate neoplasms and at least two molecular genetic alterations are needed for tumorigenesis (16–20). Histologically, five and eight of 13 ovarian neoplasms were endometrioid and undifferentiated types, respectively (Figure 1, A–D). Prominent squamous differentiation, which occurs in up to 50% of

human ovarian endometrioid carcinoma (21), was detected in all mouse endometrioid carcinomas (Figure 1B). Of note, two endometrioid carcinomas also contained undifferentiated carcinoma, and morphological transition from endometrioid to undifferentiated carcinoma was evident (Supplementary Figure 2, available online). All pure endometrioid carcinomas were confined to ovaries, whereas the eight undifferentiated carcinomas and the two endometrioid carcinomas containing undifferentiated carcinoma had disseminated within the peritoneal cavity. In humans, ovarian and uterine undifferentiated carcinomas (22–25) are frequently associated with and clonally derived from low-grade endometrioid carcinoma (26). Unlike endometrioid carcinoma, undifferentiated carcinoma takes a highly aggressive clinical course. Hence, our mouse model appears biologically relevant and may, in fact, recapitulate late ovarian tumor progression.

Immunohistochemically, tumor cells were positive for the epithelial cell marker cytokeratin-8 and phospho-AKT (S473) but negative for ARID1A and PTEN (Figure 2, C and D). Western blot analysis confirmed the immunostaining result (Supplementary Figure 1B, available online). Detailed immunohistochemistry and western blot methods can be found in the Supplementary Methods (available online). For those 16 mice that did not develop ovarian tumors, we observed MOSE hyperplasia by hematoxylin and eosin staining (Figure 1A), which showed the same staining pattern as tumors (Supplementary Figure 3, available online).

Gene expression profiling analysis (see Supplementary Methods, available online) of the mouse *Arid1a*^{flax/flax}; *Pten*^{flax/flax} tumors were performed by referencing to a set of 128 discriminant genes (Supplementary Table 2, available online) selected from the dataset of human ovarian serous, endometrioid, clear cell, and mucinous carcinomas (16). We found that mouse tumors had the overall highest mean probability to be human endometrioid carcinoma as compared with other ovarian tumor subtypes. Specifically, four of the five representative mouse tumors were most likely to be endometrioid type, whereas one was serous type (Supplementary Table 3, available online).

ARID1A mutations are frequently observed not only in endometrioid carcinoma

but also in clear cell carcinoma; however, we did not observe definitive morphological features characteristic of clear cell carcinoma (Figure 1; Supplementary Figure 2, available online). This might be expected because we codeleted *Pten* rather than expressing activating *Pik3ca* together with *Arid1a* deletion in MOSE. *ARID1A* and *PIK3CA* mutations frequently co-occur in human ovarian clear cell carcinomas (1,2), whereas *ARID1A* and *PTEN* mutations frequently co-occur in human endometrioid carcinomas (5,14). Moreover, PTEN has been reported to cooperate with p53 in a lipid phosphatase-independent manner (27). Thus, this AKT-independent mechanism may facilitate the development of endometrioid rather than clear cell carcinomas.

Although this study demonstrates new functional roles of *Arid1a* in mouse ovarian cancer initiation, several questions remain to be answered. For example, it would be of great interest to determine the effects of *Arid1a* deletion with other molecular genetic alterations that are common in human endometrioid carcinomas such as *Pik3ca* and *Ctnnb1* activating mutations in mouse ovaries. Future studies should also focus on using this mouse model to determine the molecular contributors that propel the tumor progression from endometrioid carcinoma to undifferentiated carcinoma.

In summary, our results show that *Arid1a* deletion collaborates with *Pten* deletion in driving tumorigenesis of MOSE, whereas *Arid1a* deletion alone is insufficient to do so. Our results provide biological significance of molecular genetic changes involving *ARID1A* and *PTEN* that have been identified in human ovarian endometrioid carcinomas. This mouse model is potentially useful for future molecular studies of *ARID1A* in cancer and for preclinical studies of molecular targeted therapy for tumors with *ARID1A* mutations.

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Notes

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Affiliations of authors: Department of Pathology, Departments of Gynecology and Obstetrics and Oncology, Johns Hopkins University School of Medicine, Baltimore, MD (BG, YSR, RCW, YW, TLW, IMS); Department of Pathology, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taoyuan, Taiwan (RCW); Department of Surgery, University of Michigan, Ann Arbor, MI (ZW); Genetics Endocrinology Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD (current address for BG).