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Loss of ARID1A expression in endometrial samplings is associated with the risk of endometrial carcinoma

Ting-Tai Yen [a,1], Tsutomu Miyamoto [b,c,1], Shiho Asaka [d,e,f], M. Herman Chui [c], Yeh Wang [c,d], Shiou-Fu Lin [c,d], Rebecca L. Stone [a], Amanda N. Fader [a], Ryoichi Asaka [b,c], Hiroyasu Kashima [b], Tanri Shiozawa [b], Tian-Li Wang [c,d], Ie-Ming Shih [c,d,*], Edward J. Tanner III [a,*]

[a] Kelly Gynecologic Oncology Service, Department of Gynecology and Obstetrics, Johns Hopkins School of Medicine, Baltimore, MD, United States of America
[b] Department of Obstetrics and Gynecology, Shinshu University School of Medicine, Matsumoto, Japan
[c] Gynecologic Pathology Laboratroy, Department of Gynecology and Obstetrics, Johns Hopkins Medical Institutions, Baltimore, MD, United States of America
[d] Departments of Oncology and Pathology, Johns Hopkins Medical institutions, Baltimore, MD, United States of America
[e] Department of Diagnostic Pathology, Shinshu University Hospital, Matsumoto, Japan
[f] Department of Laboratory Medicine, Shinshu University School of Medicine, Matsumoto, Japan

HIGHLIGHTS

- Inactivating *ARID1A* somatic mutations are common in endometrial cancer but rare in complex atypical hyperplasia.
- Loss of ARID1A immunoreactivity in endometrial sampling specimens predicts concurrent carcinoma in subsequent hysterectomy.
- Loss of ARID1A immunoreactivity may play a role in tumor progression and serve as a predictive cancer biomarker.

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ABSTRACT

Objectives. Inactivating somatic mutations of *ARID1A*, a chromatin remodeling gene, are common in endometrioid endometrial carcinoma (EEC) but rare in complex atypical hyperplasia (CAH). Our objectives were to determine the clinical significance of ARID1A loss during tumor progression from CAH to EEC and to assess its role as a predictive cancer biomarker.

Methods. In cohort A, ARID1A immunoreactivity was evaluated in endometrial sampling (biopsy/curettage) specimens showing CAH to determine whether ARID1A expression correlates with the presence of EEC at subsequent hysterectomy. In cohort B, ARID1A immunoreactivity was evaluated in the hysterectomy specimens with concurrent CAH and EEC to assess for the concordance of ARID1A expression in both components.

Results. In cohort A, loss of ARID1A immunoreactivity was identified in the endometrial sampling specimen of 31% of patients undergoing hysterectomy for a preoperative diagnosis of CAH. EEC was identified in the hysterectomy specimen of 94% of patients with loss of ARID1A in the endometrial sampling specimen while only 15% of patients with retained ARID1A expression ($P < 0.0001$). No association was observed between ARID1A expression and demographic characteristics. In cohort B, 14 (31%) of 45 patients with concurrent CAH/EEC in their hysterectomy specimens had complete loss of ARID1A expression in the EEC components. Among these 14 patients, 50% also had loss of ARID1A immunoreactivity in the CAH component.

Conclusions. ARID1A immunostaining may correlate with malignant transformation and the presence of concurrent EEC in patients with CAH identified at pre-hysterectomy endometrial sampling. Further investigation to determine the potential utility of ARID1A expression as a tissue biomarker is warranted.

1. Introduction

Endometrioid endometrial cancer (EEC) is the most common gynecologic cancer and its incidence is rising worldwide [1]. Although the role of complex atypical endometrial hyperplasia (CAH, also known as endometrial intraepithelial neoplasia or atypical hyperplasia) as a

* Corresponding authors at: Department of Gynecology and Obstetrics, Johns Hopkins School of Medicine, 1550 Orleans Street, CRB-2, Room: 305, Baltimore, MA 21231, United States of America.
E-mail address: ishih@jhmi.edu (I.-M. Shih).
[1] Both authors contributed equally.

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precursor lesion of EEC has been well documented, the molecular pathogenesis underlying this progression remains largely unclear. Endometrial sampling, either by biopsy or curettage, is the preferred method of diagnosing endometrial lesions. Approximately 40% of patients with CAH identified during endometrial sampling will be diagnosed with EEC at a subsequent hysterectomy [2, 3]. Although hysterectomy is the most effective procedure to treat CAH, conservative management may also be considered if patients are poor surgical candidates or desire to retain fertility [3, 4]. Conservative treatment options include placement of a local-acting levonorgestrel-containing intrauterine device (LNG-IUD) or oral administration of progestins such as medroxyprogesterone acetate (MPA) and megestrol acetate (MA) [4, 5]. Currently, there are no biomarkers to predict which women with CAH in their endometrial sampling specimens will ultimately be diagnosed with concurrent EEC at the time of hysterectomy. Such a biomarker would be useful, as patients at higher risk for EEC could be directed to a gynecologic oncologist for surgery while patients at little or now risk for EEC could potentially be managed by a general gynecologist.

Somatic mutations of ARID1A, a tumor suppressor gene, are frequently identified in EEC and endometriosis-related neoplasms [6–9]. ARID1A encodes a nuclear protein, BAF250, which participates in forming the SWI/SNF chromatin remodeling complex. The protein is involved with critical cellular functions including transcription modulation, DNA damage repair, DNA synthesis and DNA methylation [6, 10, 11]. Inactivating mutations of ARID1A result in loss of ARID1A protein expression and negative ARID1A immunoreactivity is a surrogate of its mutation [9, 12]. Loss of ARID1A expression is common in EEC but rare in CAH in the absence of concurrent carcinoma [9, 13]. Previous studies have suggested a correlation between loss of ARID1A expression and tumor progression. In conjunction with other markers such as p53, loss of ARID1A expression may portend inferior survival in some subtypes of endometrial cancer [13, 14]. These previous studies did not assess tumorogenesis from CAH to EEC in the same individual, nor did they compare ARID1A patterns between concurrent EEC and adjacent CAH from the same patient.

Our objective was to determine whether loss of ARID1A expression in endometrial sampling prior to hysterectomy is associated with concurrent EEC in subsequent hysterectomy specimens. We also compared ARID1A immunostaining patterns between concurrent CAH and EEC from the same hysterectomy specimens in a separate cohort, to better understand the timing of ARID1A loss in the tumorigenesis of EEC.

2. Material and method

2.1. Tissue material selection

A two-site study was conducted by retrospectively enrolling women in two separate cohorts. Cohort A included women who were diagnosed with CAH in endometrial sampling specimens (biopsy or curettage) and subsequently underwent a hysterectomy at the Johns Hopkins Hospital from 2005 to 2017. Endometrial sampling methods included endometrial biopsy performed in the office or hysteroscopic-guided curettage in the operation room. All women underwent subsequent hysterectomy. Women who received hormonal therapy prior to hysterectomy were excluded. Matched clinical information including age, race, body mass index, obstetric and gynecologic history, other medical history, hysterectomy pathology result (carcinoma versus non-cancer), tumor type, surgical stage, means of endometrial sampling, and the days from biopsy to hysterectomy were collected. Patients were evaluated for a correlation between ARID1A expression in the endometrial sampling specimen and whether or not EEC was subsequently identified in the hysterectomy specimen. Patients in cohort A were sungrouped into two groups based on the expression status of ARID1A.

In cohort B, patients whose hysterectomy specimens contained concurrent CAH and EEC were selected from the Shinshu University Hospital in Japan (n = 26, 2012 to 2016) and the Johns Hopkins Hospital (n = 19, 2015 to 2016). Tumoral characteristics including FIGO grade and stage, depth of myometrial invasion and the presence of lymphvascular space invasion. The diagnoses for both cohorts were confirmed by a panel of four expert gynecologic pathologists (SA, MHC, SFL and IMS). ARID1A expression was examined in both the CAH and EEC components of each specimen to explore the timing of ARID1A loss. This study was approved by the institutional review boards of both institutions.

2.2. Immunohistochemistry

Loss of ARID1A protein expression has been established as a surrogate for inactivating somatic mutations in EEC [9, 11, 13]. Immunohistochemistry (IHC) of ARID1A was performed in formalin-fixed paraffin-embedded tissue sections of all cases from both cohort A and cohort B. The methodology has been described in previous studies with some modifications [9, 13]. Briefly, unstained sections were deparaffinized and subjected to antigen retrieval by incubating slides in Trilogy™ at 88 °C for 30 min. Tissue sections were then incubated with the primary antibody (ARID1A: Sigma-Aldrich HPA005456, polyclonal, 1:2000) at 4 °C overnight. The specificity of the ARID1A antibody has been previously demonstrated [9]. Immunoreactivity was detected by using EnVision+ System (Dako, Carpinteria, CA).

Nuclear ARID1A immunoreactivity was considered definitive for positive expression. For the ARID1A negative (loss of expression) cases, stromal cells served as a positive internal control. Loss of ARID1A expression in the nuclei was defined as either a complete loss or a heterogeneous loss. The definition of complete loss was the absence of ARID1A immunoreactivity in virtually all tumor cells examined, whereas heterogeneous loss was defined as decreased immunointensity or patchy, focal or clonal

Table 1
Demographics of patients enrolled in cohort A.

Clinical Information	ARID1A loss[a] N = 16	ARID1A retain N = 36	*P*	95% CI
Age, mean (SD)	58.9 (11.6)	57.2 (10.9)	0.61[b]	−5.00–8.37
BMI, mean (SD)	37.9 (12.4)	37.2 (11.1)	0.84[b]	−6.23 - 7.64
Race				
White	14 (88%)	20 (56%)	0.07[c]	
Black	1 (6%)	13 (36%)		
Other	1 (6%)	3 (8%)		
Parity			0.94[c]	
Nulliparous	4 (25%)	10 (28%)		
Multiparous	11 (69%)	23 (64%)		
Unknown	1 (6%)	3 (8%)		
Post-menopause	12 (75%)	23 (64%)	0.53[d]	
Endometrial thickness, mm (SD)[e]	15.7 (10.5)	12.3 (6.3)	0.25[b]	−2.49 - 9.18
Smoking[f]			1.00[d]	
Not current smoke	16 (100%)	33 (92%)		
Current smoke	0 (0%)	2 (6%)		
Endometrial sampling method			0.07[d]	
Biopsy	12 (75%)	16 (44%)		
D&C	4 (25%)	20 (56%)		
Time to hysterectomy, days (SD)	51.4 (38.3)	88.5 (72.3)	0.06[b]	−75.75 - 1.55
Subsequent TH showing carcinoma	15 (93.8%)	5 (13.9%)	<0.0001[d]	
Subsequent TH showing myometrium invasion	10 (62.5%)	1 (2.8%)	<0.0001[d]	

D&C, dilation and curettage; TH, total hysterectomy.
Data are mean (SD), N (%) unless otherwise specified.
[a] ARID1A loss includes complete and heterogeneous loss of ARID1A.
[b] *t*-Test.
[c] Chi-square test.
[d] Fisher's exact test.
[e] Six missing data in ARID1A loss group and 11 in ARID1A retain group.
[f] One missing data in ARID1A retain group.

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