

ARID1A loss correlates with mismatch repair deficiency and intact p53 expression in high-grade endometrial carcinomas

Ghassan Allo^{1,2}, Marcus Q Bernardini^{3,4}, Ren-Chin Wu⁵, Ie-Ming Shih⁵, Steve Kalloger⁶, Aaron Pollett^{1,7}, C Blake Gilks^{6,8} and Blaise A Clarke^{1,2}

¹Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada;

²Department of Pathology, Toronto General Hospital, University Health Network, Toronto, ON, Canada;

³Department of Obstetrics and Gynecology, University of Toronto, Toronto, ON, Canada; ⁴Department of Gynecologic Oncology, Princess Margaret Hospital, University Health Network, Toronto, ON, Canada;

⁵Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD, USA; ⁶Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; ⁷Department of Pathology, Mount Sinai Hospital, Toronto, ON, Canada and ⁸Department of Pathology and Laboratory Medicine, Vancouver General Hospital, Vancouver, BC, Canada

BAF250a (ARID1A) loss is a frequent event in high-grade endometrial cancers. It has been proposed that ARID1A is a driver gene, with ARID1A mutations occurring secondary to deregulated mismatch repair mechanism in gastric cancers, representing an alternative oncogenic pathway to p53 alteration. The prognostic significance of ARID1A loss is controversial. In this study, we investigated the frequency of BAF250a immunohistochemical loss in a cohort of high-grade endometrial cancers ($n=190$) and correlated it with mismatch repair (hMLH1, hMSH2, hMSH6, and hPMS2) and p53 protein expression. The 190 cases consisted of 82 high-grade endometrioid, 88 serous, 10 clear cell, and 10 mixed (carcinosarcomas and mixed histology). There was BAF250a loss in 55/190 (29%) cancers, most commonly in high-grade endometrioid carcinomas (46 vs 9% in serous carcinomas, $P<0.0001$). Loss of any mismatch repair proteins was observed in 63/190 (33%) cancers, most commonly in high-grade endometrioid carcinomas (57 vs 10% in serous carcinomas, $P<0.0001$). Aberrant p53 expression was found in 86/190 (45%) cancers, more commonly in serous carcinomas (77 vs 18% in high-grade endometrioid carcinomas, $P<0.0001$). BAF250a loss was associated with mismatch repair loss ($P<0.0001$) and normal p53 expression ($P<0.0001$). These associations were maintained in the subset analysis within the high-grade endometrioid ($P=0.026$ and $P=0.0083$, respectively) and serous carcinoma cases ($P=0.0031$ and $P<0.0001$, respectively). Survival analysis revealed a superior progression-free survival ($P=0.017$) for patients with BAF250a loss within the entire cohort but not within the high-grade endometrioid and serous subtypes. Additionally, data from The Cancer Genome Atlas were extracted to correlate mutations in ARID1A, TP53, and MMR genes; we found that ARID1A mutations were negatively associated with TP53 mutations but were unrelated to mismatch repair gene mutations. In conclusion, BAF250a loss is more common in high-grade endometrioid carcinomas than in other high-grade endometrial cancers and is associated with mismatch repair deficiency and normal p53 expression.

Modern Pathology (2014) 27, 255–261; doi:10.1038/modpathol.2013.144; published online 26 July 2013

Keywords: ARID1A; BAF250a; endometrioid carcinoma; endometrium; mismatch repair; p53; serous carcinoma

The AT-rich interactive domain 1A (ARID1A) gene encodes the protein BAF250a, which is ubiquitous

in eukaryotes and is a key component of the multi-protein SWI/SNF chromatin remodeling complex.¹ BAF250a (ARID1A) alterations are frequent in high-grade endometrial cancers, with loss of expression reported in 39% of grade 3 endometrioid carcinomas, 18% of serous cancers and 26% of clear cell carcinomas,² and mutations in 60 and 10% of high-grade endometrioid and serous carcinomas, respectively.³

Correspondence: Dr BA Clarke, MBBCh, Department of Anatomical Pathology, Toronto General Hospital, 200 Elizabeth Street, Room 11E-404, Toronto, ON M5G 2C4, Canada.

E-mail: blaise.clarke@uhn.on.ca

Received 9 May 2013; revised 26 June 2013; accepted 27 June 2013; published online 26 July 2013

ARID1A alterations have been associated with microsatellite instability (MSI) status. In gastric carcinomas, Wang *et al*⁴ demonstrated higher frequency of *ARID1A* alterations (inactivating mutations and/or protein deficiency) in carcinomas with MSI than microsatellite stable (MSS) EBV-negative gastric carcinomas (83 vs 11%, respectively, $P=0.001$). The type of *ARID1A* mutations in MSI-associated carcinomas was recurrent, being mostly indels involving short mononucleotide tracts, occurring at a higher rate than the global background rate of indels in similar mononucleotide tracts.⁴ A similar *ARID1A* mutation spectrum was noted by Jones *et al*⁵ in 12 MSI-high tumors (6 colon, 5 gastric and 1 prostate). Wang *et al*⁴ cited the significantly higher *ARID1A* mutation rate in MSI-associated gastric cancers as being a result of clonal selection of a mutant driver gene that had been mutated as a result of disrupted DNA mismatch repair. Recognizing the similar spectrum of *ARID1A* mutations in gastric cancer and ovarian clear cell carcinoma, which may also be associated with MMR deficiency, they postulated that *ARID1A* mutations may be prevalent in MSI-associated tumors of diverse sites. In addition, it has been shown that *ARID1A* mutations in gastric and ovarian carcinomas are negatively associated with mutations in *TP53* ($P=0.002$ and 0.031 , respectively), suggesting an alternate pathway of cell-cycle deregulation, independent of *TP53*.^{4,6}

The prognostic significance of *ARID1A* loss is unclear. In gastric cancers, it is controversial; certain authors showed a trend to prolonged recurrence-free survival in gastric cancers with *ARID1A* alterations ($P=0.058$),⁴ while other studies demonstrated poor prognosis with *ARID1A* loss.^{7,8} However, in the subset analysis, among the EBV-negative and MSS group, *ARID1A* loss was an independent poor prognostic marker.⁷ Other studies confirmed the association between BAF250A/*ARID1A* expression loss and poor prognosis in cervical ($P=0.047$)⁹ and renal cell carcinomas ($P=0.003$).¹⁰ Among endometrial malignancies, a recent report shows that *ARID1A* alterations did not affect overall survival or progression-free survival.¹¹

As *ARID1A* has been implicated in high-grade endometrial cancers, and MSI occurs in about 30% of endometrial cancers, we hypothesized that BAF250A/*ARID1A* loss was associated with mismatch protein deficiency in high-grade endometrial cancers and explored its prognostic significance.

Materials and methods

Patients and Tissue Samples

After approval of the University Health Network Research Ethics Board, consecutive cases of high-grade endometrial cancers (high-grade endometrioid FIGO III/III, serous, clear cell, and carcinosarcoma) from 2001–2009 were retrospectively reviewed

by a gynecologic pathologist (BC), and classified, according to the standard morphological criteria as defined by the World Health Organization classification of tumors,¹² into serous, high-grade endometrioid, clear cell, or mixed carcinoma or carcinosarcoma. Relevant clinical data were obtained. Duplicate core tissue microarrays were constructed as previously described.¹³

Immunohistochemistry

Immunohistochemical staining was performed on 4- μ m-thick paraffin sections of the tissue microarrays using anti-*ARID1A* (Sigma, clone: BAF250a, dilution: 1/75), hMLH1 (1:150, ES05, Novocastra, Vector Labs, Burlington, ON), hMSH2 (1:100, 25D12, Novocastra, Vector Labs), hMSH6 (1:600, PU29, Novocastra, Vector Labs), hPMS2 (1:150, MOR4G, Novocastra, Vector Labs), and p53 (Leica, clone: D07, dilution: 1/1000) antibodies, with appropriate positive and negative controls, according to the manufacturers' standardized staining protocols.

For BAF250a and MMR (hMLH1, hMSH2, hMSH6, hPMS2) immunohistochemistry, tumors were considered aberrant if tumor cells showed complete absence of nuclear staining with positive non-neoplastic internal control (Figure 1a), and intact if tumor cells showed nuclear positivity (Figure 1b), as previously described.^{14–16} The status of cases, in which tumor cores were negative or equivocal for any of the MMR proteins, was confirmed by full-section staining. Tumors were considered MMR deficient if there was loss of expression of at least one MMR protein. For p53, tumors were scored as follows: zero for complete loss of expression (Figure 1c), one for focal expression (1–50% of cells) (Figure 1d), or two for overexpression (defined as >50% of tumor cells showing strongly positive nuclear staining) (Figure 1e), and then binarized to aberrant (score of zero and two) and normal (score of one) as previously reported.¹⁷

Mutation Analysis

Somatic mutation data of 186 uterine corpus endometrioid carcinomas from The Cancer Genome Atlas (TCGA) were downloaded via cBioPortal (<http://www.cbioportal.org/public-portal/>, case_list_id = ucec_tcga_endo_core), including 103 non-hypermutated samples (case_list_id = ucec_tcga_endo_nonhypermut) and 83 hypermutated (case_list_id = ucec_tcga_endo_hypermut & ucec_tcga_endo_ultramut). These cases were selected from those prepared for TCGA publication as of 9 April 2013.

Statistical Analysis

The association of categorical clinico-pathological characteristics and differential biomarker expression and the association between *ARID1A* mutation

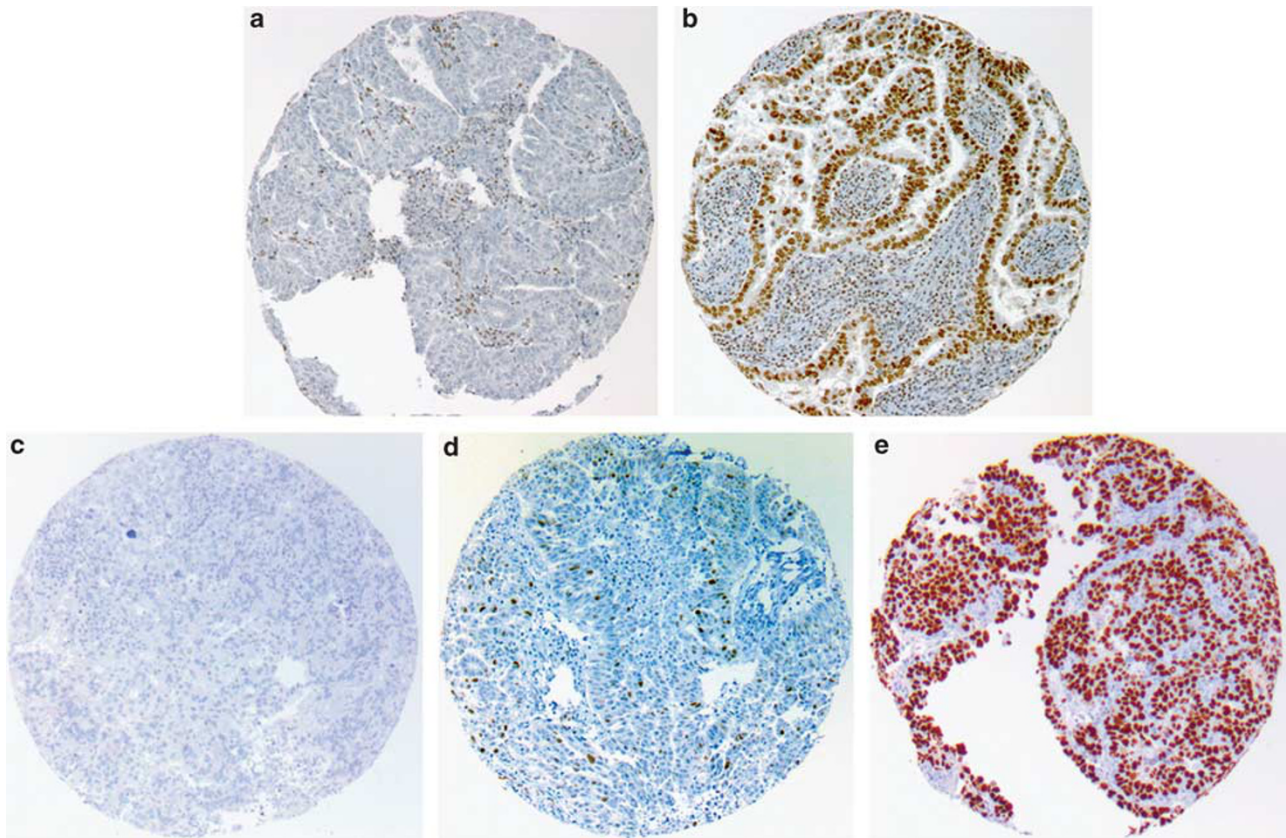


Figure 1 Scoring of immunohistochemistry antibodies. BAF250a is scored either as immunohistochemistry aberrant (a) or intact (b). Mismatch repair markers are scored similarly. p53 is given scores of zero—negative (c), one—focally positive (d), and two—diffusely positive (e).

and *TP53* mutation, MMR genes mutation, and hypermutated phenotype were evaluated by Fisher's exact test for two-by-two comparisons and by Pearson's χ^2 test for comparisons that exceeded the two-by-two criterion. Associations with continuous clinico-pathological characteristics and differential biomarker expression were quantified using the Welch's ANOVA. Univariable overall and progression-free survival were assessed by the generation of Kaplan–Meier curves and quantified with the log-rank statistic. Multivariable survival analysis was carried out using the Cox proportional hazards model. Analyses were not corrected for multiple comparisons, and *p*-values <0.05 were considered statistically significant. All analyses were computed with JMP v10.0, Cary, NC, USA.

Results

Study Cases

The age, stage, and the histological subtypes of this cohort of high-grade EC cases are summarized in Table 1. The median duration of follow-up was 1.32 (0.12–9.32) years. There was no significant association between the histological subtype and the age of diagnosis (*P*=0.25). Serous carcinomas presented at

Table 1 Demographics of study cases

	n	%	Age mean (Years)	P	Stage		P
					Low (I-II)	High (III-IV)	
<i>Histological type</i>							
Endometrioid	82	43%	66	0.25	62 (76%)	20 (24%)	0.004
Serous	88	47%	68		45 (51%)	43 (49%)	
Clear cell	10	5%	71		5 (50%)	5 (50%)	
Mixed	10	5%	65		8 (80%)	2 (20%)	
Total	190	100%	68		120 (63%)	70 (37%)	

a higher stage than endometrioid (*P*=0.0014), and were associated with worse progression-free survival (*P*=0.0061).

Association of BAF250a, MMR, and p53 Expression with Clinico-Pathological Characteristics

BAF250a loss was observed in 29% of the study cases, was more common in younger patients (*P*=0.0008), and was significantly associated with non-serous histology (*P*<0.0001) (Table 2).

Aberrations in MMR protein expression were identified in 33% of all high-grade endometrial

Table 2 Prevalence of BAF250a (*ARID1A*), MMR and p53 immunohistochemical expression and in relation to age and histological subtype

	BAF250a+		BAF250a-		P	MMR retained		MMR lost		P	p53 normal		p53 aberrant		P
Age (years), mean	68.9		62.8		0.0003	67.6		66.4		0.471	66.2		68.3		0.201
Histological type					<0.0001					<0.0001					<0.0001
Endometrioid	44	54%	38	46%		35	43%	47	57%		67	82%	15	18%	
Serous	80	91%	8	9%		79	90%	9	10%		20	22%	68	78%	
Clear cell	6	60%	4	40%		9	90%	1	10%		9	90%	1	10%	
Mixed	5	50%	5	50%		4	40%	6	60%		8	80%	2	20%	
Total	135	71%	55	29%		127	67%	63	33%		104	55%	86	45%	

Table 3 Immunohistochemical loss of the four mismatch repair proteins among various histological subtypes

	Endometrioid	Serous	Clear cell	Mixed	P
hMLH1 loss	34 (59%)	6 (7%)	0 (0%)	6 (60%)	<0.0001
hMSH2 loss	6 (7%)	2 (2%)	1 (10%)	1 (10%)	0.8
hMSH6 loss	11 (13%)	3 (3%)	0 (0%)	1 (10%)	0.13
hPMS2 loss	36 (44%)	6 (7%)	0 (0%)	4 (40%)	<0.0001
Total, <i>n</i>	82	88	10	10	

cancers, the majority affecting MLH1 and PMS2 (Table 3). Across the entire cohort, loss of MMR proteins was significantly associated with endometrioid and mixed histologies rather than the serous or clear cell subtypes ($P < 0.0001$). Aberrant p53 expression was discovered in 45% of cases and was significantly associated with serous subtype ($P = 0.0001$; Table 2).

BAF250a Expression and Status of MMR Protein Immunohistochemistry

In the entire cohort, loss of BAF250a was significantly associated with MMR loss (65% of BAF250a negative are MMR deficient, and 57% of MMR-deficient endometrial cancers are BAF250a negative, $P < 0.0001$; Table 4). This association was maintained within the endometrioid and serous carcinoma subtype ($P = 0.026$ and $P = 0.0031$, respectively; Table 4). In addition, of the 10 clear cell carcinomas, the single case with abnormal MMR status had BAF250a loss and 4 of the 6 mixed carcinomas (67%) with abnormal MMR had BAF250a loss.

BAF250a and p53 Expression Status

BAF250a expression loss was negatively associated with aberrant p53 expression (95% of BAF250a-negative endometrial cancers showed normal p53 expression, and 97% of carcinomas with aberrant p53 expression were positive for BAF250a, $P < 0.0001$; Table 4). This association was also observed among the endometrioid and serous histotypes ($P \leq 0.0083$, and $P < 0.0001$, respectively;

Table 4 Correlation between immunohistochemical expression of BAF250a (*ARID1A*), mismatch repair proteins, and p53 within the entire cohort and within the endometrioid and serous subtypes

	BAF250a+		BAF250a-		P
	n	%	n	%	
<i>Entire cohort</i>					
MMR intact	108	85%	19	15%	<0.0001
MMR lost	27	43%	36	57%	
p53 normal	52	50%	52	50%	<0.0001
p53 aberrant	83	96%	3	4%	
<i>Endometrioid carcinomas</i>					
MMR intact	24	69%	11	31%	0.026
MMR lost	20	42%	27	58%	
p53 normal	31	46%	36	54%	0.0083
p53 aberrant	13	87%	2	13%	
<i>Serous carcinomas</i>					
MMR intact	75	95%	4	5%	0.0031
MMR lost	5	56%	4	44%	
p53 normal	13	65%	7	35%	<0.0001
p53 aberrant	67	98%	1	2%	

Table 4). In addition, only a single case of clear cell carcinoma (10%) had aberrant p53 expression, and this case had retained expression of BAF250a. Among the remaining nine clear cell carcinomas with normal p53, four (44%) were BAF250a negative. Moreover, 2 of the 10 (20%) mixed endometrial cancer subtype had aberrant p53, both of which were BAF250a positive, while 5 of the 8 (62%) mixed cases with normal p53 were BAF250a negative.

Prognostic Significance of BAF250a, MMR, and p53 Expression

Examination of the entire cohort using univariable survival analysis revealed that loss of BAF250a did not affect overall survival but was associated with improved progression-free survival (log-rank $P = 0.27$, and 0.0107 , respectively; Table 5). Although MMR loss and normal p53 expression were associated with improved overall survival (log rank $P = 0.0315$, and 0.0418 , respectively) and

Table 5 Univariable analysis of the prognostic significance of BAF250a, MMR, and p53

	Overall survival		Progression-free survival	
	Hazard ratio	P	Hazard ratio	P
<i>Entire cohort (n = 190)</i>				
BAF250a loss	0.67	0.27	0.25	0.011
MMR deficiency	0.5	0.0315	0.43	0.023
p53 aberrant	1.67	0.0418	1.75	0.055
<i>Endometrioid (n = 82)</i>				
BAF250a loss	0.94	0.917	0.42	0.28
MMR deficiency	0.7	0.483	2.5	0.231
p53 aberrant	1.32	0.612	0.61	0.554
<i>Serous (n = 89)</i>				
BAF250a loss	0.48	0.234	0.34	0.268
MMR deficiency	0.54	0.251	0.23	0.117
p53 aberrant	2.34	0.0528	1.64	0.305

Table 6 Multivariable analysis of the prognostic significance of BAF250a, MMR, and p53

	Overall survival		Progression-free survival	
	Hazard ratio	P	Hazard ratio	P
<i>Entire cohort (n = 190)</i>				
BAF250a loss	1.09	0.098	3.63	0.0062
MMR deficiency	2.12	0.033	1.48	0.34
p53 aberrant	0.52	0.039	0.76	0.48

progression-free survival (log rank $P=0.023$, and 0.055 , respectively) within each of the histological subtypes, BAF250a did not carry a prognostic significance (Table 5).

On multivariable analysis (Table 6), age at diagnosis, stage, and histological subtype were considered, revealing that BAF250a immunonegativity was associated with longer progression-free survival ($P=0.0062$) but was not associated with overall survival ($P=0.098$). Loss of any MMR proteins ($P=0.033$) and normal p53 immunohistochemistry ($P=0.039$) were associated with a better overall survival. There was no significant correlation between MMR and p53 immunohistochemistry with progression-free survival ($P=0.34$ and 0.48 , respectively).

Association Between *ARID1A* Mutation and Mutation in *TP53* and MMR Genes

There is a significant negative correlation between *ARID1A* mutation and *TP53* mutation in endometrioid carcinoma according to pathology reports,

(odds ratio = 0.2284, $P=0.0051$, Fisher's exact test, two tailed). No correlation is detected between *ARID1A* mutation and hypermutated phenotype ($P=0.2269$, Fisher's exact test, 2 tailed). No correlation is observed between *ARID1A* mutation and mutations in any of the MMR genes, including PMS1, PMS2, MSH2, MSH3, MSH6, MLH1, and MLH3 ($P=0.1032$, Fisher's exact test, two tailed).

Discussion

Our data show that BAF250a negativity, present in 29% of high-grade endometrial carcinomas, is associated with loss of MMR protein expression and inversely correlated with aberrant p53 expression. Although BAF250a negativity is associated with progression-free survival in the whole cohort, histotype-specific analyses failed to demonstrate an association with prognosis.

In this study, the rate of BAF250a loss is concordant with previous studies showing mutations in *ARID1A* gene in 29% (32/109) of cases³ and loss of the expression of its protein BAF250a in 24% (85/358).² BAF250a negativity is more common in endometrioid than in serous carcinomas (46 and 9%, respectively, $P<0.0001$), similar to previous studies showing a higher rate of *ARID1A* mutations in the endometrioid subtype than serous endometrial carcinomas (60 vs 11%, $P=2.4E-5^3$) and more frequent loss of BAF250a expression (39 vs 18%, $P=0.001^2$). The limited number of clear cell carcinomas in this study precludes making an inference with regards to the frequency of BAF250a negativity in clear cell carcinomas. The significant differential expression of BAF250a in our study suggests that, in the context of high-grade endometrioid and serous uterine carcinomas, loss of BAF250a expression is mostly but not exclusively an endometrioid phenomenon. The higher frequency of BAF250a loss is similar to a recent report demonstrating that the percentage of complete *ARID1A* loss increased from 0% in complex atypical hyperplasia to 25% in low-grade endometrioid carcinoma and to 44% in high-grade endometrioid carcinoma.¹⁸ As neither mutation of *ARID1A* or loss of BAF250a expression was observed in morphologically pure uterine serous carcinomas,¹⁹ it is interesting to note that a handful of serous carcinomas exhibited BAF250a negativity. These cases demonstrated an unusual immunophenotype, with the majority (7/8, 88%) showing normal p53 expression and half showing MMR deficiency. Cognizant of the poor reproducibility of cell type in the high-grade endometrial spectrum,²⁰ this suggests that this small subset of BAF250a-negative serous carcinomas may indeed be endometrioid carcinomas mimicking the serous histomorphology.

Wang *et al*⁴ demonstrated collusion between MSI and *ARID1A* mutations or protein deficiency in

gastric cancer. They suggested that this was applicable across various tumor sites. In this study, we confirmed that BAF250a negativity is significantly associated with MMR protein defects in high-grade endometrial carcinomas ($P < 0.0001$). However, the biological significance of this correlation is not clear; Wang *et al*⁴ reported that the rate of indels in *ARID1A* in gastric carcinomas with MSI, which is a manifestation of MMR defects, was higher than that in MSS carcinomas. Furthermore, with MSI gastric cancers, the indel rate is significantly higher in *ARID1A* than similar sized mononucleotide tracts. Based on this, they postulated that this frequent recurrent mutational mechanisms might represent targeting of a tumor-driver gene.⁴ In addition, indels and frameshift mutations, which can occur frequently in the context of MMR deficiency, were found to be the most common types of *ARID1A* mutations over a variety of cancer types, including gynecological malignancies (overall, 67%; gynecological tumors, 64%).²¹ These studies suggest a causal relationship between MMR deficiency and *ARID1A* mutations. It is worth noting that 34% (19/56) of BAF250a-negative high-grade endometrial carcinomas in this study displayed intact MMR profile and normal p53 expression, suggesting an independent pathway of MMR loss in a subset of cases. Further studies are needed to investigate whether BAF250a/*ARID1A* alterations directly result from defective MMR mechanisms in endometrial carcinomas and to discover other pathways that may result in inactivation of *ARID1A*.

Furthermore, we demonstrate an inverse relationship between BAF250a expression loss and p53 aberrant expression in high-grade endometrial carcinomas ($P < 0.0001$). This inverse correlation has been reported in gastric carcinomas ($P = 0.002$),¹⁵ esophageal adenocarcinomas ($P = 0.028$),²² and a cohort of ovarian clear cell and uterine endometrioid carcinomas ($P = 0.031$).⁶ Guan *et al*⁶ have studied the relationship and interaction between *ARID1A* and *TP53* pathways and their tumor-suppressor function. They found that some downstream targets of *ARID1A* include *CDKN1A*/p21 and *SMAD3*, which are well-known p53 downstream target genes, and that *ARID1A*/BRG1 complex of SWI/SNF proteins interacts with the same promoter regions of *CDKN1A* and *SMAD3* as p53. Knocking down *ARID1A* significantly reduced BRG1 binding to these regions, reducing the transcriptional activity of p21 and *SMAD3*, but it did not affect p53 binding to these regions.⁶ These findings suggest that inactivating mutations in either *ARID1A* or *TP53* result in loss of transcriptional regulation of *CDKN1A* and *SMAD3*⁶ and support the interpretation that there is some functional equivalence to loss of function of either protein, accounting for the uncommon occurrence of mutations in both the genes in the same tumor. This has been described previously for other genes impacting on

the same signaling pathway, eg, *HER2* amplification and *KRAS* mutation in mucinous carcinoma of the ovary.²³

Different studies have reported inconsistent results on the prognostic significance of *ARID1A* alterations. In gastric carcinomas, these alterations have been reported to be associated with a trend towards better prognosis ($P = 0.058$).⁴ However, other authors described poor survival in gastric cancers with *ARID1A*/BAF250a expression loss.⁸ Furthermore, other reports showed no prognostic significance for *ARID1A* alterations;⁷ however, on subset analysis, in EBV-negative MLH1-preserved gastric carcinomas, BAF250A expression loss was significantly associated with worse overall survival ($P = 0.027$) and progression-free survival ($P = 0.016$).⁷ Similar variability have been reported in ovarian clear cell and endometrioid carcinomas, where Wiegand *et al*¹⁴ showed no association between survival and *ARID1A* mutations in ovarian carcinomas of either subtype, and Maeda *et al*²⁴ also demonstrated no association between loss of BAF250a expression and clinical outcome of ovarian clear cell carcinoma, while Katagiri *et al*²⁵ discovered worse progression-free survival ($P < 0.01$) and resistance to platinum-based chemotherapy ($P = 0.04$) in patients with ovarian clear cell carcinoma. Moreover, *ARID1A*/BAF250a loss was significantly associated with worse overall survival in cervical adenocarcinoma⁹ and worse overall survival ($P = 0.003$) and progression-free survival ($P = 0.01$) in renal clear cell carcinomas. In this study, while there was no association between BAF250a expression status and age at presentation and tumor stage, BAF250a-negative high-grade endometrial carcinomas exhibited a better progression-free survival. The prognostic significance did not hold up within the endometrioid and the serous subtypes. This lack of significance within subtypes may be due to underpowered analysis because of lower number of subjects in the subset analysis, or it might represent a subtype bias with *ARID1A*/BAF250a loss identifying endometrioid subtypes, and the favorable association between progression-free survival and *ARID1A*/BAF250a loss may only reflect a preponderance of the less aggressive endometrioid cases.

The recent deposition of TCGA data on uterine corpus endometrial carcinoma allows us to validate the immunohistochemistry-based findings from this study in a large cohort of cases. Based on analyzing a total of 186 endometrioid carcinomas, we found negative correlation between *ARID1A* and *TP53* mutations, consistent with the inverse association between BAF250a expression loss and p53 abnormal expression. However, the lack of correlation of *ARID1A* mutations with either hypermutation phenotype (characterized by hundreds of somatic mutations) or MMR gene mutation is in contrast to our immunostaining findings. The most likely explanation is that many cases with MMR

dysfunction (lost expression) are caused by promoter methylation rather than mutation, supported by the fact that the majority of the losses in MMR proteins occurred in hMLH1 and hPMS2 (Table 3).^{26,27}

In summary, BAF250a/*ARID1A* abnormalities occur in approximately 30% of high-grade endometrial carcinomas and are significantly associated with MMR protein deficiency and normal p53 expression. The constellation of BAF250a/*ARID1A* loss, MMR deficiency, and normal p53 expression is highly characteristic of the endometrioid subtype, and further studies of the rare cases showing this immunophenotype and serous morphological features are necessary to determine whether such cases are better classified as endometrioid, in light of their underlying molecular abnormalities.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

- Wilson BG, Roberts CW. SWI/SNF nucleosome remodellers and cancer. *Nat Rev Cancer* 2011;11:481–492.
- Wiegand KC, Lee AF, Al-Agha OM, *et al*. Loss of BAF250a (*ARID1A*) is frequent in high-grade endometrial carcinomas. *J Pathol* 2011;224:328–333.
- McConechy MK, Ding J, Cheang MC, *et al*. Use of mutation profiles to refine the classification of endometrial carcinomas. *J Pathol* 2012;228:20–30.
- Wang K, Kan J, Yuen ST, *et al*. Exome sequencing identifies frequent mutation of *ARID1A* in molecular subtypes of gastric cancer. *Nat Genet* 2011;43:1219–1223.
- Jones S, Li M, Parsons DW, *et al*. Somatic mutations in the chromatin remodeling gene *ARID1A* occur in several tumor types. *Hum Mutat* 2012;33:100–103.
- Guan B, Wang TL, Shih IeM. *ARID1A*, a factor that promotes formation of SWI/SNF-mediated chromatin remodeling, is a tumor suppressor in gynecologic cancers. *Cancer Res* 2011;71:6718–6727.
- Abe H, Maeda D, Hino R, *et al*. *ARID1A* expression loss in gastric cancer: pathway-dependent roles with and without Epstein–Barr virus infection and microsatellite instability. *Virchows Arch* 2012;461:367–377.
- Wang DD, Chen YB, Pan K, *et al*. Decreased expression of the *ARID1A* gene is associated with poor prognosis in primary gastric cancer. *PLoS ONE [Electronic Resource]* 2012;7:e40364.
- Cho H, Kim JS, Chung H, *et al*. Loss of *ARID1A*/*BAF250a* expression is linked to tumor progression and adverse prognosis in cervical cancer. *Hum Pathol* 2013;44:1365–1374.
- Lichner Z, Scorilas A, White NM, *et al*. The chromatin remodeling gene *ARID1A* is a new prognostic marker in clear cell renal cell carcinoma. *Am J Pathol* 2013;182:1163–1170.
- Rahman M, Nakayama K, Rahman MT, *et al*. Clinicopathologic analysis of loss of AT-rich interactive domain 1A expression in endometrial cancer. *Hum Pathol* 2013;44:103–109.
- Tavassoli FA, Devilee P(eds). World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Breast and Female Genital Organs. IARC Press: Lyon, France, 2003.
- Alkushi A, Clarke BA, Akbari M, *et al*. Identification of prognostically relevant and reproducible subsets of endometrial adenocarcinoma based on clustering analysis of immunostaining data. *Mod Pathol* 2007;20:1156–1165.
- Wiegand KC, Shah SP, Al-Agha OM, *et al*. *ARID1A* mutations in endometriosis-associated ovarian carcinomas. *N Engl J Med* 2010;363:1532–1543.
- Lu FI, Gilks CB, Mulligan AM, *et al*. Prevalence of loss of expression of DNA mismatch repair proteins in primary epithelial ovarian tumors. *Int J Gynecol Pathol* 2012;31:524–531.
- Alkushi A, Kobel M, Kalloger SE, *et al*. High-grade endometrial carcinoma: serous and grade 3 endometrioid carcinomas have different immunophenotypes and outcomes. *Int J Gynecol Pathol* 2010;29:343–350.
- Kobel M, Reuss A, Bois A, *et al*. The biological and clinical value of p53 expression in pelvic high-grade serous carcinomas. *J Pathol* 2010;222:191–198.
- Mao TL, Ardighieri L, Ayhan A, *et al*. Loss of *ARID1A* expression correlates with stages of tumor progression in uterine endometrioid carcinoma. *Am J Surg Pathol* 2013 (in press).
- Guan B, Mao TL, Panuganti PK, *et al*. Mutation and loss of expression of *ARID1A* in uterine low-grade endometrioid carcinoma. *Am J Surg Pathol* 2011;35:625–632.
- Gilks CB, Oliva E, Soslow RA. Poor inter-observer reproducibility in the diagnosis of high-grade endometrial carcinoma. *Am J Surg Pathol* 2013;37:874–881.
- Guan B, Gao M, Wu CH, *et al*. Functional analysis of in-frame indel *ARID1A* mutations reveals new regulatory mechanisms of its tumor suppressor functions. *Neoplasia* 2012;14:986–993.
- Streppel MM, Lata S, Delabastide M, *et al*. Next-generation sequencing of endoscopic biopsies identifies *ARID1A* as a tumor-suppressor gene in Barrett's esophagus. *Oncogene* 2013 (in press).
- Anglesio MS, Kommos S, Tolcher MC, *et al*. Molecular characterization of mucinous ovarian tumours supports a stratified treatment approach with HER2 targeting in 19% of carcinomas. *J Pathol* 2013;229:111–120.
- Maeda D, Mao TL, Fukayama M, *et al*. Clinicopathological significance of loss of *ARID1A* immunoreactivity in ovarian clear cell carcinoma. *Int J Mol Sci* 2010;11:5120–5128.
- Katagiri A, Nakayama K, Rahman MT, *et al*. Loss of *ARID1A* expression is related to shorter progression-free survival and chemoresistance in ovarian clear cell carcinoma. *Mod Pathol* 2012;25:282–288.
- Kane MF, Loda M, Gaida GM, *et al*. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* 1997;57:808–811.
- Esteller M, Levine R, Baylin SB, *et al*. MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. *Oncogene* 1998;17:2413–2417.