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The clinicopathologic significance of p53 and BAF-250a (*ARID1A*) expression in clear cell carcinoma of the endometrium

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***TP53* mutation (and associated p53 protein overexpression) is probably a negative prognostic marker in endometrial cancers, but its relevance in the rarer histologic subtypes, including clear cell carcinomas, has not been delineated. Preclinical studies suggest functional interactions between p53 and the BAF250a protein, the product of a tumor suppressor gene *ARID1A* (adenine-thymine (AT)-rich interactive domain containing protein 1A) that is frequently mutated in ovarian clear cell carcinoma. In this study, we evaluated the significance of p53 and BAF250a expression, as assessed by immunohistochemistry, in a group of 50 endometrial clear cell carcinomas. Of 50 cases, 17 (34%) were p53+, and the remaining 33 cases had a p53 wild-type (p53-wt) immunophenotype. Of the 11 relapses/recurrences in the entire data set, 73% were in the p53+ group ($P=0.008$). On univariate analyses, the median overall survival for the p53-wt patients (83 months) was longer than the p53+ patients (63 months) ($P=0.07$), and the median progression-free survival for the p53-wt group (88 months) was significantly longer than the p53+ group (56 months) ($P=0.01$). On multivariate analyses, p53 expression was not associated with reduced overall or progression-free survival. In addition, p53 status was not significantly associated with pathologic stage or morphologic patterns. Of the 50 cases, 10 (20%) showed a complete loss of BAF250a expression. There was no significant correlation between p53 and BAF250a expression. The p53+/BAF250a−, p53+/BAF250a+, p53-wt/BAF250a+ and p53-wt/BAF250a− composite immunophenotypes were identified in 8%, 26%, 54% and 12% of cases, respectively, and neither loss of BAF250a expression nor composite p53/BAF250a expression patterns were associated with reduced overall or progression-free survival. In conclusion, a significant subset of CCC express p53, and these cases are apparently not definable by their morphologic features. P53 expression may be a negative prognostic factor in this histotype, and warrants additional studies. Loss of BAF250a expression has no prognostic significance in endometrial clear cell carcinomas.**

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Endometrial clear cell carcinoma is an uncommon histotype that has been under-represented in prospective therapeutic trials.[1–4] This tumor is less responsive to standard therapeutic regimens[4] and may be amenable to targeted therapeutic approaches. However, the molecular pathogenesis

of clear cell carcinoma, on which any such approaches would be based, is unclear.[5] There have been two major analyses of the molecular features of clear cell carcinoma, and both groups independently concluded that this carcinoma exhibits molecular heterogeneity, a spectrum of molecular changes and/or may arise through multiple pathways.[6,7] Although the degree to which the aforementioned heterogeneity can be attributed to variations in the pathologic diagnosis of clear cell carcinoma[8] is unclear, these studies do suggest that there may be molecular subsets of the histotype that are worthy of evaluation for their possible clinicopathologic significance.

The SWI/SNF (mating type switching/sucrose non-fermenting) complex is an evolutionarily conserved multiunit complex of factors that utilize the energy of ATP hydrolysis to remodel nucleosomes and thereby affect gene transcription.[9–13] Eukaryotic SWI/SNF complexes are comprised of two catalytic core subunits, and up to 10 non-catalytic subunits, one of which is BAF250a (the protein product of the *ARID1A* (adenine-thymine (AT)-rich interactive domain containing protein 1A) gene).[9,10] Inactivating somatic mutations of *ARID1A* have been identified in 46–57% of ovarian clear cell carcinomas,[14,15] and they appear to have a tumor suppressor role in this and some other gynecologic cancers.[14–16] Loss of BAF250a expression is not uncommon in endometrial cancers,[17–20] and has been reported in 22.7–26% of endometrial clear cell carcinomas.[18,20] In a previously reported pilot study of 22 clear cell carcinomas, loss of BAF250a expression was found to correlate with advanced stage at diagnosis on univariate analyses;[20] Comparable studies are conflicting on the question of its prognostic significance in ovarian clear cell carcinoma,[21–24] whereas recent studies suggest that loss of BAF250a may represent a negative prognostic factor in gastric and breast cancers.[25,26]

The tumor suppressor gene *TP53* (protein product p53) is the most commonly altered gene in human cancers.[27,28] Emerging lines of evidence indicate that the SWI/SNF complex and its subunits, including the *ARID1A* gene product BAF250a, are key regulators and targets of p53 function, and that *ARID1A* functions as a tumor suppressor by modulating the transcriptional activity of *TP53*-regulated genes.[16,29–35] In preliminary analyses, we have noticed that a small subset of BAF250a − clear cell carcinomas also show p53 overexpression,[5] but it was unclear if the coexistence of these phenotypes was entirely fortuitous.

In this study, we systematically assess the clinicopathologic significance of p53 and BAF250a expression in a group of rigorously classified clear cell carcinomas of the endometrium, with the ultimate goal of determining if there are any singular or composite p53/BAF250a expression profiles that define biologically distinct subsets of this tumor.

Materials and methods

Selection and Review of Cases

This study was approved by the institutional review board at Vanderbilt University (IRB no. 12606), and was based on an analysis of archived material from the authors' institutions. The contributors, who are all gynecologic pathologists, searched their respective files for cases diagnosed as endometrial clear cell carcinoma, reviewed the slides and retrieved cases whose morphologic features they considered to be unequivocally diagnostic of this histotype. A total of 62 cases were generated. These 62 cases were then reviewed by three authors (OF, VP and JH) independently. Each reviewer classified the 62 cases into two groups: endometrial clear cell carcinoma and a histotype other than clear cell carcinoma. Cases of mixed carcinoma with a clear cell component were classified as the latter. The sole prerequisite for inclusion of a case into the final data set was that a diagnosis of clear cell carcinoma was rendered for that case by at least two of the three reviewers. Upon completion of the review, there was excellent diagnostic agreement between the three reviewers, with a κ-value of 0.846. In 53 of 62 cases, the three reviewers had identical classifications (85% agreement rate). In all, 12 (19%) of the original 62 cases were ultimately classified as a histotype other than clear cell carcinoma after the review, leaving a final data set of 50 cases (Figure 1).

Immunohistochemistry

Whole tissue sections from 5 biopsies and 45 hysterectomies were used for immunohistochemical analyses, which were performed on the Leica Bond Max immunohistochemical autostainer (Leica Microsystems, Buffalo Grove, IL, USA). Heat-induced antigen retrieval was performed on the Bond Max using the Leica Epitope Retrieval 2 solution for

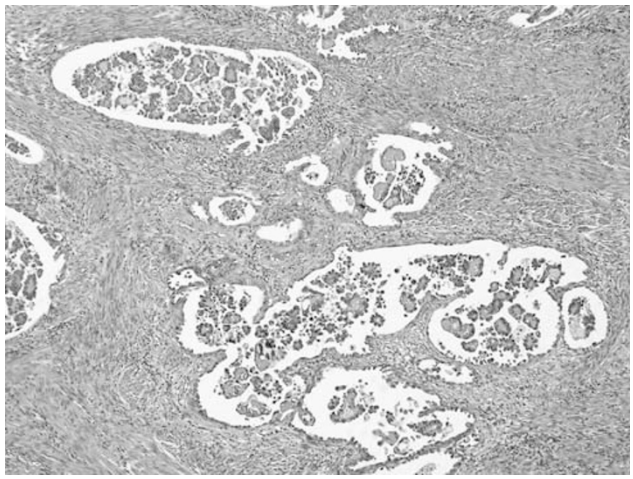

Figure 1 Clear cell carcinoma, morphologic features. A full color version of this figure is available at the *Modern Pathology* journal online.

30 min (BAF250a) and 20 min (p53). For BAF250a, slides were incubated with the primary antibody (BAF250a, PSG3; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at 1:750 dilution. This antibody is a mouse monoclonal antibody that was raised against a recombinant protein that corresponds to amino acids 600–1018 of the human BAF250a protein. For p53, slides were incubated with the prediluted primary antibody (clone DO-7; Leica), a mouse anti-human monoclonal antibody. The Bond Polymer Refine detection system was used for visualization. This ready-to-use system is a biotin-free, polymeric horseradish peroxidase (HRP)-linked antibody conjugate system, and contains a peroxide block (to limit endogenous peroxidase activity), post-primary IgG linker reagent (to link mouse antibodies), poly-HRP reagent (to localize rabbit antibodies), the substrate chromogen (3′,3-diaminobenzidene tetrahydrochloride) and hematoxylin counterstain. Slides were the dehydrated, cleared and coverslipped. Staining patterns were interpreted using different scoring methods (Figures 1b–f). Immunohistochemical staining for BAF250a was scored using a previously described system that incorporates both the extent and intensity of staining.[25] The *extent* was scored on a four-tiered semiquantitative scale based on the estimated percentage of tumor epithelial cells displaying *any* immunoreactivity: 0 (0–9%), 1 (10–25%), 2 (26–50%) and 3 (51–100%). Intensity of BAF250a immunoreactivity was similarly scored on a four-tiered scale (0–3). The final score for each case was obtained by multiplying the 'extent' score by the 'intensity' score, with potential final scores that thus ranged from 0 to 9. A final score of 0 was considered to be negative (Figure 1f), a score of 1–3 was considered weakly positive (Figure 1d) and a score of 4–9 was considered to be strongly positive (Figure 1e). BAF250a is a nuclear protein that is expressed to varying degrees in most human cells. As nuclear expression of BAF250a is an expected finding in lymphocytes, endothelial cells and stromal cells,[15,18] these cells served as internal positive controls for assay validity. P53 staining status was assessed based on previously described concepts on staining patterns that most likely correlate with an underlying *TP53* mutation and/or have prognostic significance.[36–38] Cases were classified either as displaying a 'p53-wild-type' pattern of staining (p53-wt: focal and/or weak and/or heterogeneous staining pattern; Figure 1c) or as 'p53 + ' (strong, 3 +, diffuse expression in at least half of tumoral nuclei (Figure 1b), or complete absence of staining in tumoral nuclei in the setting of wt staining of background non-epithelial cells: null phenotype).

Statistical Analysis

Kaplan–Meier survival curves were generated for overall survival and progression-free survival, defined by the period between primary treatment and death or relapse. Comparisons between survival curves were performed using log-rank tests. Cox regression analyses were used to assess relationships between clinicopathologic factors, including p53 and BAF250a expression, and outcome using multivariate and univariate models. Univariate analyses using Fisher's exact, Pearson's χ^2 and Student's *t*-tests were also used to compare between subgroups as appropriate. Spearman's correlation tests were used to assess the relationships between p53 and BAF250a expression. A *P*-value of <0.05 was considered to be statistically significant for all analyses.

Results

Clinical Description of Patients

The 50 patients ranged in age from 50 to 85 (mean 67.8) years. Their International Federation of Gynecology and Obstetrics stage distribution was as follows: stage I ($n=19$; IA, $n=18$, including one pT0; IB, $n=1$), stage II ($n=8$), stage III ($n=14$; IIIA, $n=6$; IIIB, $n=1$; IIIC, $n=7$)) and stage IV ($n=9$). In total, 48 patients underwent a total hysterectomy with bilateral salpingo-oophorectomy, and 2 did not undergo a primary surgical procedure other than the diagnostic biopsy. Regional lymphadenectomy was performed in 43 patients, with only pelvic nodes removed in 10 patients, and both pelvic and paraaortic nodes removed in 23 patients. In 10 patients, lymph nodes were positive for metastatic disease. Among the 19 patients with stage I disease, lymphadenectomy was performed in 13 (pelvic only in 3; pelvic and paraaortic in 10). For the eight stage II patients, lymphadenectomy was performed in seven (pelvic only in two; pelvic and paraaortic in three). Overall, directed peritoneal biopsies and/or omentectomy was performed in only 16 of the 27 stage I or II patients. Adjuvant treatments included chemotherapy and adjuvant radiotherapy ($n=9$), radiotherapy only ($n=12$) and chemotherapy only ($n=10$); one patient received chemotherapy only without surgical resection; one patient received neoadjuvant chemotherapy, surgery and adjuvant chemotherapy. Seven patients received no further treatment after surgery. In 10 patients, adjuvant management is unknown. Follow-up was available in 43 patients (median duration 31 months, range 1–104 months): 25 were without evidence of disease, 9 were dead of disease, 8 were alive with disease and 1 was dead of other causes. There were 11 relapses, occurring 1 to 27 months (mean 11.2 months) after primary surgical resection. Relapse sites were in the vagina ($n=2$), pleura ($n=1$), inguinal/groin region ($n=2$), supraclavicular lymph node ($n=1$), kidney ($n=1$), bone ($n=2$), abdominal soft tissue ($n=1$) and lungs ($n=1$). There were only three recurrences in patients with stage I or II

disease, including two patients with stage IA tumors and one patient with a stage II tumor. The clinicopathologic features of this data set are detailed elsewhere.[39]

P53

In all, 34% of cases (17/50) were p53+; the remaining cases had a p53-wt immunophenotype. Of the 17 p53+ cases, 15 showed diffuse, strong immunoreactivity in ≥90% of tumor cells (Figures 1–3). In two cases, 50–75% of tumor cells were strongly positive; there were no cases with a p53-null phenotype. On univariate analyses, p53+ and p53-wt cases showed no significant differences regarding average patient age, architectural grade, predominant architectural pattern, necrosis, mitotic index, average depth of myometrial invasion, and frequencies of lymphovascular invasion, tumor-positive lymph nodes, any extrauterine extension of tumor and distant metastatic disease (Table 1). The 'Architectural grade,' as was recently proposed in ovarian clear cell carcinomas,[40] is a three-tiered system that is based solely on tumor architecture. At least 90% of grade A tumors are composed of well-differentiated tubulocystic and/or papillary patterns; grade C tumors have at least 10% of the tumor composed of solid masses or individual infiltrating tumor cells; and grade B tumors do not fit either of the aforementioned descriptions. In ovarian clear cell carcinomas, grade A tumors were found to be the most prognostically favorable group, and grade C the least favorable.[40] Follow-up information was available in 16 of the 17 p53+ cases and in 27 of the 33 p53-wt cases. Of the 11 recurrences in the entire data set, 73% were in the p53+ group ($P=0.008$). Seven of the 27 patients in the low-stage (stages I and II) group had p53+ cancers. Of note, there were three relapses among these 27 patients, and the tumors in all three cases were p53+. A possible role for p53 expression in abdominopelvic dissemination of disease was also evaluated. Of the 23 cases with extrauterine extension (stages III and IV), 20 had intra-abdominal disease. Of these 20 cases, 8 were p53+, which was not significantly different from the 7 of 27 early-stage cases that were p53+ ($P=0.36$), or the 9 of 30 cases (low- and high-stage) without intra-abdominal disease ($P=0.55$). However, if 'intra-abdominal disease' is restricted to 'intraperitoneal disease,' that is, exclusive of high-stage cases that were upstaged solely owing to lymph node, supradiaphragmatic or retroperitoneal involvement, only 14 of the 23 high-stage cases qualified for this designation. Of these 14 cases, 7 were p53+ as compared with only 1 of the other 9 high-stage cases ($P=0.08$), and 7 of the 27 early-stage cases ($P=0.17$). On univariate analysis, the median overall survival for the p53-wt patients (83months) was longer than the p53+ patients (63 months), a difference that approached but which did not attain statistical significance ($P=0.07$). Also on univariate analysis, the median progression-free survival for the p53+ group (56.1 months) was significantly lower than that in the p53-wt group (88 months) ($P=0.01$). On multivariate analyses, p53 expression was not associated with reduced overall or progression-free survival (Table 2).

BAF250a

In all, 10 (20%) of the 50 cases were BAF250a−. Seven cases were classified as 'weakly positive' (immunoreactivity scores of 1–3), and the remaining 33 cases (66%) as strongly positive (scores of 4–9) (Figures 4–6). On univariate analyses, BAF250a− and BAF250a+ cases showed no significant differences

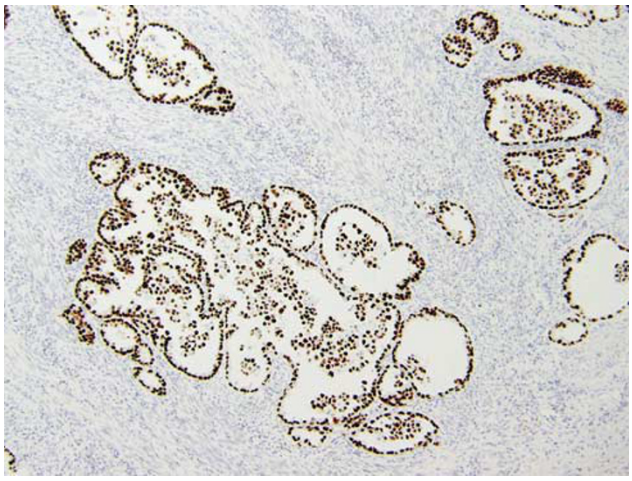

Figure 2 Clear cell carcinoma, classified as 'p53+' due to strong diffuse immunoreactivity.

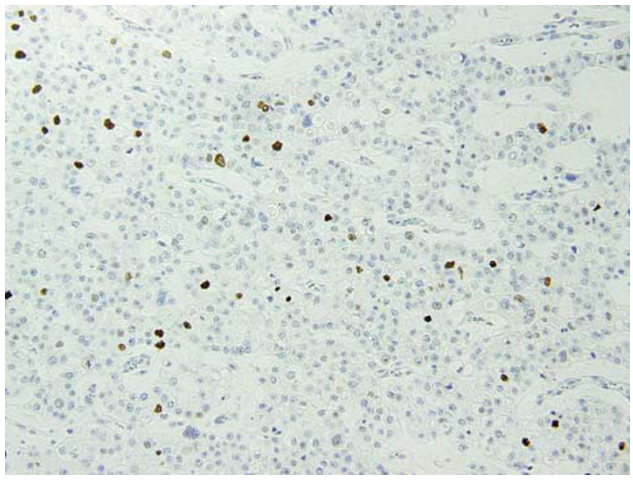

Figure 3 Clear cell carcinoma, classified as displaying a 'p53-wild type' immunophenotype due to heterogeneous, sporadic and non-confluent pattern of p53 immunoreactivity.

Table 1 Classification of cases based on their patterns of p53 and BAF250a immunoreactivity

Parameters	*P53+*	*P53-wt*	P-*value*	*BAF250a−*	*BAF250a+*	P-*value*
Number of cases	17	33	NA	10	40	NA
Mean age (years)	70	66.6	NS	68.9	67.5	NS
FIGO stage						
I and II (uterus-confined tumor)	7	20	NS	7	20	NS
III and IV (extrauterine disease)	10	13		3	20	
Architectural grade[a]						
A	9	13	NS	4	18	NS
B	1	8		3	6	
C	8	11		3	16	
Lymphovascular invasion						
Positive	10	12	NS	5	17	NS
Negative	7	20		5	22	
Distant metastases						
Positive	3	6	NS	0	9	NS
Negative	14	27		10	31	
Myometrial invasion						
>30%	8	19	NS[b]	4	19	NS[b]
≤30%	9	14		6	21	
Mean	6.5	21.5		17.4	12.2	
Relapses[c]						
Positive	8	3	0.008	1	10	NS
Negative	8	26		7	25	
Mean MI	6.5	3.2	NS	5.8	4	NS
Lymph nodes[d]						
Positive	5	5	NS	2	8	NS
Negative	11	19		8	22	

Abbreviations: NA: not applicable; NS: not statistically significant.
[a]Architectural grade as per the Yamamoto *et al*[40] criteria: at least 90% of grade A tumors are composed of well-differentiated tubulocystic and/or papillary patterns; grade C tumors have at least 10% of the tumor composed of solid masses or individual infiltrating tumor cells; and grade B tumors do not fit either of the aforementioned descriptions.
[b]Also not statistically significant at the 50% threshold.
[c]Only for cases with follow-up.
[d]Only cases with lymphadenectomy and for which results were available.

regarding average patient age, architectural grade, mitotic index, predominant architectural pattern, necrosis, average depth of myometrial invasion, stage, and frequencies of lymphovascular invasion, tumor-positive lymph nodes, any extrauterine extension of tumor, relapses/recurrences and distant metastatic disease. Follow-up information was available in 8 of the 10 BAF250a− cases and in 35 of the 40 BAF250a+ cases. On univariate and multivariate analyses, patients with BAF250a− and BAF250a+ tumors displayed no statistically significant differences in overall or progression-free survival.

There was no significant correlation between p53 and BAF250a expression ($r = -0.03$). The p53+/BAF250a−, p53+/BAF250a+, p53-wt/BAF250a+ and p53-wt/BAF250a− composite immunophenotypes were identified in 8%, 26%, 54% and 12% of cases, respectively. There was no composite profile that was associated with reduced overall or progression-free survival on univariate or multivariate analysis.

Discussion

Somatic mutations that may activate oncogenes and/or inactivate tumor suppressor genes are a well-known feature of malignant neoplastic processes. The most commonly altered gene in human neoplasms is the *TP53* gene, which encodes the p53 protein.[41] P53 is a potent tetrameric transcription factor that regulates net cell growth and genomic integrity by activating or repressing several target genes, thereby influencing a myriad of cellular pathways, including those that are normally suppressive of oncogenesis.[27,28,41] *TP53* mutations, as assessed from direct mutational analysis or inferred from p53 protein overexpression, are well recognized in endometrial serous carcinomas, up to 96% of which have been reported to display somatic *TP53* mutations.[42] P53 overexpression, as assessed by immunohistochemical techniques and as scored using contemporary criteria, have been reported in approximately 37.5% of high-grade endometrial endometrioid carcinomas[43] and lower proportions of low- and intermediate-grade endometrioid carcinomas.[38] P53 alteration is also recognized as a negative prognostic factor in endometrial carcinomas in general, although the degree to which this is independent of histotype is unclear.[38,44]

P53 overexpression in endometrial clear cell carcinomas has been studied previously only in small data sets and predominantly in analyses that were not focused on assessing its prognostic significance. For example, studies by Alkushi *et al*,[45] Lax *et al*[46] and Vang *et al*,[47] in which a total of 26 tumors were analyzed, reported extensive and moderate/intense levels of p53 immunoreactivity in an average of 27% of cases. Arai *et al*,[48] in contrast, reported that 76.9% of 13 tumors showed p53 immunoreactivity, with an average labeling index of 46.4%, but the proportion of cases that displayed extensive and intense levels of immunoreactivity was not stated. On the other end of the spectrum, An *et al*[6] found strong and diffuse p53 expression in only 1 (9%) of 11 endometrial clear cell carcinomas. The clinicopathologic significance of p53 overexpression in endometrial clear cell carcinomas has only recently been explored. Reports of an overrepresentation of high-stage cases in the BAF250a− subset of endometrial clear cell carcinoma,[20] and the finding of p53 overexpression in a subset of those BAF250a− cases,[5] raised the possibility that p53

Table 2 Relationship of clinicopathologic factors, including p53 and BAF250a expression, and survival

Parameter	*Number of patients*	*Survival (months), median ± s.e.*	*95% CI*	*Progression-free survival*		*Overall survival*	
				P-*value (uni)*	P-*value (multi)*	P-*value (uni)*	P-*value (multi)*
BAF250a							
Positive (weak + strong)	35	65.6 ± 8.1	49.5–81.7	NS	NS	NS	NS
Negative	8	88.2 ± 14.5	59.8–116.5				
Positive (strong)	28	67.6 ± 8.6	50.7–84.4	NS	NS	NS	NS
Negative (weak + zero)	15	80.8 ± 11.7	57.9–103.8				
P53							
Positive	17	56.1 ± 12.7	37.1–81.4	0.01	NS	0.0	NS
Wild-type	25	88.2 ± 5.3	77.8–98.6				
Age							
>65 years	25	70.6 ± 9.3	52.2–88.8	0.02	0.02	0.04	0.023
≤65 years	18	75.6 ± 10.7	54.7–96.5				
FIGO stage							
I and II	26	91.2 ± 7.1	77.3–104.8	0.002	NS	0.003	0.0022
III and IV	17	16.5 ± 2.0	12.5–20.5				
Architectural pattern in >50% of tumor							
Glandular	17	71.8 ± 10.5	51.2–92.5	NS	NS	0.7	NS
Papillary	12	64.4 ± 15.8	33.3–95.4				
Solid	10	37.4 ± 3.4	30.5–44.1				
Cystic	4	60.3 ± 18.5	24.1–96.6				
Lymph nodes							
Positive	8	13.7 ± 2.8	8.2–19.1	0.002	NS	NS	NS
Negative	29	84.1 ± 8.1	96.1–101.4				
Lymphovascular invasion							
Positive	20	70.1 ± 11.1	48.4–91.8	NS	NS	NS	NS
Negative	22	76.4 ± 8.9	58.9–93.9				
Myometrium invasion							
>30%	19	61.7 ± 11.1	40.1–83.3	NS	NS	0.01	NS
≤30%	24	83.9 ± 9.1	66.1–101.7				
Mitotic index							
>4	12	68.9 ± 13.4	42.7–95.2	NS	NS	0.014	NS
≤4	31	77.3 ± 7.6	61.3–91.2				
Architectural grade[a]							
A + B	24	77.1 ± 9.4	58.6–95.5	0.09	NS	0.02	NS
C	19	32.6 ± 3.6	25.6–39.6				

Abbreviations: NS: not statistically significant (all *P*-values between 0.05 and 0.09999 are listed for informational purposes); CI: confidence interval; s.e.: standard error; FIGO: International Federation of Gynecology and Obstetrics; uni: univariate analysis; multi: multivariate analysis.
[a]Architectural grade as per the Yamamoto *et al*[40] criteria: at least 90% of grade A tumors are composed of well-differentiated tubulocystic and/or papillary patterns; grade C tumors have at least 10% of the tumor composed of solid masses or individual infiltrating tumor cells; and grade B tumors do not fit either of the aforementioned descriptions.

overexpression has some prognostic significance, and prompted us to do this study. In a recent abstract, Delair *et al*[49] compared the clinicopathologic features of 16 endometrial clear cell carcinomas with ($n = 8$) and without ($n = 8$) p53 overexpression, and found that p53-overexpressing cases were associated with advanced stage and worsened patient outcomes. In this study, we assessed a relatively large group of cases, and found p53 overexpression to be a negative prognostic factor, but whose independence from other variables could not be demonstrated.

There are several possible explanations for p53 overexpression in clear cell carcinoma. The first possibility is that the cases were erroneously classified, and are actually endometrial serous carcinomas or mixed clear cell/serous carcinomas. Although this possibility cannot be entirely eliminated, we have subjected our cases to rigorous review for diagnostic accuracy, and consider it

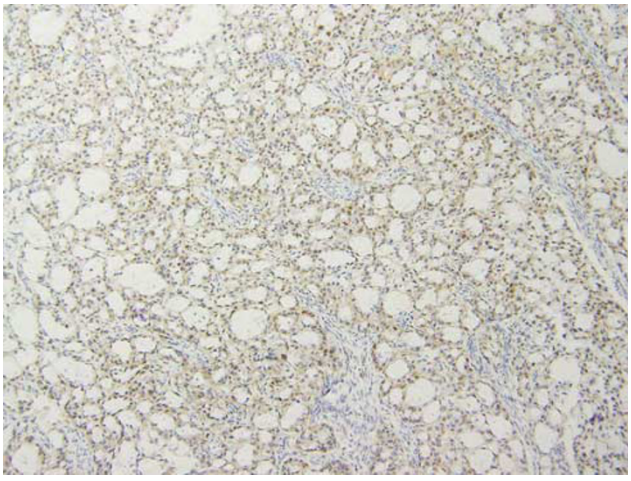

Figure 4 Clear cell carcinoma, BAF250a—weakly positive.

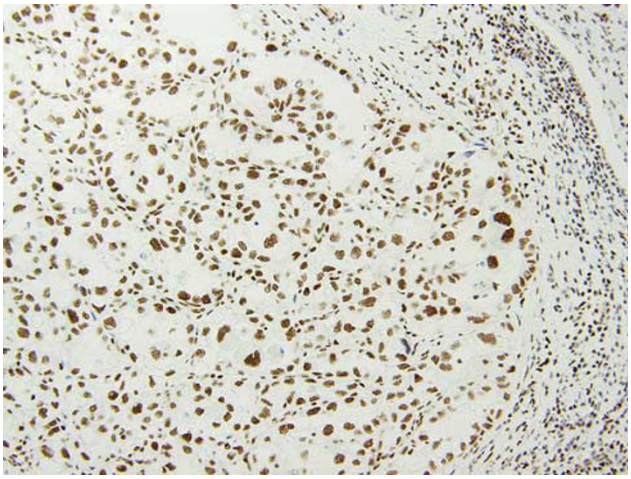

Figure 5 Clear cell carcinoma, BAF250a—strongly positive; background inflammatory and stromal cells are also positive.

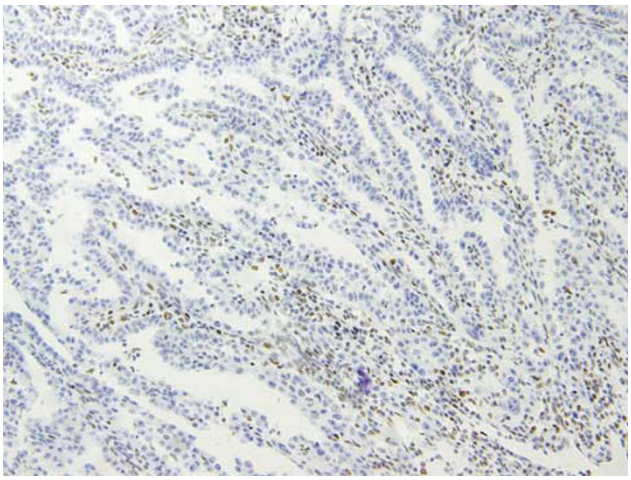

Figure 6 Clear cell carcinoma, BAF250a−; background inflammatory and stromal cells retain BAF250a expression.

unlikely. Furthermore, although the proportions vary, almost every study that has evaluated the issue has identified strong and diffuse p53 immunoreactivity in at least a small subset of clear cell carcinomas.[6,45–48] The second possibility is that *TP53* mutations are acquired in a subset of clear cell carcinomas as a form of neoplastic progression, akin to endometrioid carcinomas, in which the rate of p53 overexpression increases in a grade- and stage-dependent manner.[50,51] In this study, we did not find any association between p53 overexpression and stage, tumor mitotic index, necrosis and predominant architectural pattern. We also applied an architectural grading system that has been found to be of prognostic value in ovarian clear cell carcinoma,[40] and found no association between this grading system and p53 overexpression. All these findings suggest that the p53-overexpressing subset of clear cell carcinoma are not morphologically distinct, and as such are not recognizable on routine sections. A third possibility is that the p53-immunoreactive cases are clear cell carcinomas at the morphologic level, but actually evolved from other histotypes, including endometrial serous carcinomas, and accordingly retain the primary molecular events in the antecedent histotype. A study by An *et al*[6] provides support for this possibility. An *et al*[6] analyzed two cases of mixed serous/clear cell carcinomas, and found identical *TP53* mutations in the two morphologically distinct components.[6] In addition, in one case of a mixed clear cell/endometrioid carcinomas, identical mutations in *PTEN* and *TP53*, as well as microsatellite instability, were identified in both components.[6] They concluded that the tumors that are definable as clear cell carcinoma at the morphologic level are actually fairly heterogeneous, and may 'arise via different pathogenetic pathways.'[6] The notion that some endometrial carcinomas may have evolved from other histotypes is gaining increasing recognition, and may explain morphologically ambiguous, hybrid or mixed carcinomas.[52,53] Our findings provide some preliminary data in support of the notion that p53+ clear cell carcinomas may have a higher propensity for peritoneal dissemination than p53-wt cancers,[49] but this requires confirmation. The fact that p53+ cases may be associated with recurrences, especially in low-stage cases, suggests that this marker may be used to define the subset of patients in need of more aggressive management *a priori.*

We also assessed the significance of BAF250a expression in the largest data set of endometrial clear cell carcinoma reported to date. There are several preclinical lines of evidence that indicate that chromatin remodeling complexes and their subunits interact with p53 to facilitate the tumor suppressor functions of each.[16,29–35] One example of this phenomenon is the known interaction of BAF250a with HIC1 (hypermethylated in cancer 1), an epigenetically regulated transcriptional repressor whose interaction with p53 suppress cancer development.[29,35] *In vitro* models indicate that BAF250a directly binds p53, possibly recruiting p53 to the larger chromatin remodeling complex,

and leading to the transcriptional regulation of their downstream target genes.[16] Guan *et al*[16] reported a statistically significant inverse correlation between the mutational statuses of the *ARID1A* and *TP53* genes in tumor samples of ovarian clear cell carcinomas and endometrial endometrioid, carcinomas, and that these molecular events were mutually exclusive in the samples that they evaluated. We speculated that *ARID1A* and *TP53* act as collaborative, tumorigenesis-preventing gatekeepers, a mutation of only one of which is required for cancer development.[16]

We found no significant correlation between p53 overexpression and BAF250a loss. These immunophenotypes were not found to be mutually exclusive, and the BAF250a−/p53+ phenotype was identified in 8% of cases. A recent immunohistochemical analysis of 111 endometrial endometrioid carcinomas reported similar findings: no significant association between BAF250a expression and p53 overexpression was found.[19] However, it must be noted that for both genes, immunohistochemical analyses are strong but imperfect surrogate indicators for an underlying mutation. For example, in one of the seminal studies of *ARID1A* in ovarian clear cell carcinoma, only 73% of cases with an *ARID1A* mutation showed a loss of expression of the BAF250a protein as assessed by immunohistochemistry, and 11% of cases without an *ARID1A* mutation were BAF250a−.[15] Similarly, a subset of endometrial endometrioid carcinomas shows strong p53 overexpression without an underlying *TP53* mutation.[54] In these cases, p53 tumor suppressor function may be inactivated by epigenetic mechanisms that prevent it from binding with the chromatin remodeling machinery, but the protein may still accumulate owing to an increased half-life attributable to altered binding with its negative regulators, such as MDM2. Loss of BAF250a expression did not have prognostic significance in this study, a substantially larger data set than our previous report.[20] In their study of endometrial endometrioid carcinomas, Rahman *et al*[19] reported similar findings. We also assessed the significance of reduced BAF250a expression, and did not identify any significant correlations between 'weak' BAF250a expression and any clinicopathologic parameters. Such reduced expression without complete loss of function has been identified for ARID1A, BRG1 and SNF in some steroid-refractory leukemias.[55,56] As with the p53-overexpressing cases, the BAF250a− clear cell carcinomas were not morphologically distinct.

In summary, a subset of endometrial clear cell carcinomas express p53 and these cases are apparently not definable by their morphologic features. P53 expression may be a negative prognostic factor, but it is unclear if this is independent of other variables. Although ours is a relatively large cohort for the histotype (the second largest group of centrally reviewed pure endometrial clear cell carcinomas ever reported from the United States), it still represents a relatively small data set relative to the frequencies of the events being measured. As such, additional studies are required to clarify whether the molecular interactions between p53 and chromatin remodeling complexes or the expression patterns of either protein have clinical significance.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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