ORIGINAL ARTICLE

ARID1A Mutations in Endometriosis-Associated Ovarian Carcinomas

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ABSTRACT

BACKGROUND

Ovarian clear-cell and endometrioid carcinomas may arise from endometriosis, but the molecular events involved in this transformation have not been described.

METHODS

We sequenced the whole transcriptomes of 18 ovarian clear-cell carcinomas and 1 ovarian clear-cell carcinoma cell line and found somatic mutations in *ARID1A* (the AT-rich interactive domain 1A [SWI-like] gene) in 6 of the samples. *ARID1A* encodes BAF250a, a key component of the SWI–SNF chromatin remodeling complex. We sequenced *ARID1A* in an additional 210 ovarian carcinomas and a second ovarian clear-cell carcinoma cell line and measured BAF250a expression by means of immunohistochemical analysis in an additional 455 ovarian carcinomas.

RESULTS

ARID1A mutations were seen in 55 of 119 ovarian clear-cell carcinomas (46%), 10 of 33 endometrioid carcinomas (30%), and none of the 76 high-grade serous ovarian carcinomas. Seventeen carcinomas had two somatic mutations each. Loss of the BAF250a protein correlated strongly with the ovarian clear-cell carcinoma and endometrioid carcinoma subtypes and the presence of *ARID1A* mutations. In two patients, *ARID1A* mutations and loss of BAF250a expression were evident in the tumor and contiguous atypical endometriosis but not in distant endometriotic lesions.

CONCLUSIONS

These data implicate *ARID1A* as a tumor-suppressor gene frequently disrupted in ovarian clear-cell and endometrioid carcinomas. Since *ARID1A* mutation and loss of BAF250a can be seen in the preneoplastic lesions, we speculate that this is an early event in the transformation of endometriosis into cancer. (Funded by the British Columbia Cancer Foundation and the Vancouver General Hospital–University of British Columbia Hospital Foundation.)

In the United States, ovarian cancer ranks as the fifth deadliest cancer among women.[1] Of the several subtypes of epithelial ovarian cancer, high-grade serous carcinomas are the most common, accounting for approximately 70% of all cases of epithelial ovarian cancer in North America.[2] Although ovarian clear-cell carcinoma is the second most common subtype in North America (accounting for 12% of cases and an even higher percentage in Japan[3]) and is the second leading cause of death from ovarian cancer,[2] it is relatively understudied. Ovarian clear-cell carcinoma is defined on the basis of histopathological findings, including a predominance of clear cells and "hobnail" cells.[4] Ovarian clear-cell carcinomas have a low mitotic rate,[5,6] are genetically stable, and do not exhibit the complex karyotypes or chromosomal instability of high-grade serous carcinomas[5,7-9] that may contribute to their lack of sensitivity to platinum-based chemotherapy.[10-12] Although ovarian clear-cell carcinoma does not respond well to conventional platinum–taxane chemotherapy for ovarian carcinoma, this remains the adjuvant treatment of choice, because effective alternatives have not been identified. Both ovarian clear-cell and endometrioid carcinomas are associated with endometriosis.[13,14] The genetic events associated with the transformation of endometriosis into ovarian clear-cell carcinoma and endometrioid carcinoma are unknown.

The SWI–SNF chromatin remodeling complex, present in all eukaryotes, is involved in the regulation of many cellular processes, including development, differentiation, proliferation, DNA repair, and tumor suppression.[15] The complex uses ATP to mobilize nucleosomes, thereby modulating the accessibility of promoters to transcriptional activation or repression. BAF250a, the protein encoded by *ARID1A* (the AT-rich interactive domain 1A [SWI-like] gene),[16,17] is one of the accessory subunits of the SWI–SNF complex believed to confer specificity in regulation of gene expression. Mutations or other aberrations in *ARID1A* have not been described in ovarian carcinomas; however, an *ARID1A* rearrangement has been found in a breast-cancer cell line, an *ARID1A* deletion has been identified in a lung-cancer cell line, and it has been suggested that *ARID1A* is a tumor-suppressor gene.[18]

We used data derived from the whole-transcriptome sequencing (RNA sequencing) of 18 ovarian clear-cell carcinomas and an ovarian clear-cell carcinoma cell line to identify variants in *ARID1A*, as previously described.[19,20] We then studied this gene in a larger cohort of patients with ovarian carcinoma and associated endometriosis. The results suggest that *ARID1A* is a tumor suppressor in ovarian clear-cell and endometrioid carcinomas.

Methods

Patients and Samples

Ovarian clear-cell carcinomas from 18 patients, obtained from the OvCaRe (Ovarian Cancer Research) frozen-tumor bank, and 1 ovarian clear-cell carcinoma–derived cell line (TOV21G)[21] were selected as the discovery cohort for RNA sequencing. Approval from the hospital's institutional review board was obtained to permit the use of these samples for RNA-sequencing experiments.

To determine the frequency of *ARID1A* mutations in ovarian clear-cell carcinoma and other subtypes of ovarian cancer, we performed targeted exon resequencing in the discovery cohort, as well as in a mutation-validation cohort, consisting of 210 samples: samples of ovarian clear-cell carcinoma from 101 patients (independent of the 19 samples used in RNA sequencing for the discovery cohort), samples of endometrioid carcinoma from 33 patients, samples of high-grade serous carcinoma from 76 patients, and the ovarian clear-cell carcinoma–derived cell line ES2.[22] Ten specimens of ovarian clear-cell carcinoma came from Johns Hopkins University, 29 from the Centre Hospitalier de l'Université de Montréal–Hôpital Notre-Dame, and 42 from the Australian Ovarian Cancer Study (AOCS); all others were obtained from the OvCaRe frozen-tumor bank. All patients from both the discovery and mutation-validation cohorts provided written informed consent to have their tumors and germ-line DNA used for research, including genomic studies. Details regarding the consents and other approvals by the institutional review boards are supplied in the Supplementary Appendix (available with the full text of this article at NEJM.org).

Pathological Review

All tumor samples were reviewed independently by a gynecologic pathologist before mutational analysis was performed. In cases in which the review diagnosis differed from the diagnosis at the source institution, the samples were further reviewed by another gynecologic pathologist, who

acted as an arbiter. Both review pathologists were unaware of the results of the genomic studies.

PAIRED-END RNA SEQUENCING AND ANALYSIS

RNA sequencing and analysis were performed as previously described.[19,20] For details, see the Methods section in the Supplementary Appendix.

TARGETED EXON RESEQUENCING OF *ARID1A* AND MUTATION VALIDATION

Genomic DNA from samples in both the discovery and mutation-validation cohorts were subjected to targeted exon resequencing. Selected *ARID1A* variants (those with truncating changes or radical missense mutations[23] with an allele frequency of >10%) detected by means of exon resequencing were validated in tumor DNA by means of Sanger sequencing. In most cases, germ-line DNA (from formalin-fixed paraffin-embedded sections, blood samples, or cultured fibroblasts) was also analyzed by means of Sanger sequencing (see Table 3 in the Supplementary Appendix). Full details are provided in the Supplementary Appendix.

IMMUNOHISTOCHEMICAL ANALYSIS OF BAF250A

Immunohistochemical staining for BAF250a was performed in all samples except 42 ovarian clear-cell carcinoma specimens from the AOCS and 4 from Johns Hopkins University. A total of 455 additional ovarian-carcinoma samples — including 132 ovarian clear-cell carcinomas, 125 endometrioid carcinomas, and 198 high-grade serous carcinomas — from a previously described tissue microarray[6] were used for an immunohistochemical validation cohort and were analyzed for BAF250a expression. All normal gynecologic tissues showed moderate or intense nuclear immunoreactivity for BAF250a. Tumors were scored positive for BAF250a if tumor cells showed definite nuclear staining and negative if tumor nuclei had no immunoreactivity but endothelial and other nontumor cells from the same samples showed immunoreactivity. Cases in which neither normal cells in the stroma nor tumor cells were immunoreactive were considered to be the result of technical failure. Details of the staining protocol are provided in the Supplementary Appendix. Additional immunohistochemical staining for hepatocyte nuclear factor 1β (HNF-1β) and estrogen receptor was performed on whole sections for two tumors with contiguous atypical endometriosis, as previously described.[24]

LASER-CAPTURE MICRODISSECTION, DNA ISOLATION, AND CLONING

In two tumors with identified *ARID1A* mutations, sections of atypical (contiguous) and distant endometriosis were identified by a gynecologic pathologist. Laser-capture microdissection was used to isolate endometriotic epithelium. DNA extracted from these cells was analyzed by means of sequencing for the mutations seen in the tumor (see the Supplementary Appendix).

RESULTS

ARID1A MUTATIONS

The RNA-sequencing data, including the number of mapped sequencing reads and potential nonsynonymous sequence variants, are summarized in Table 1 in the Supplementary Appendix. RNA sequencing of the 19 samples in the discovery cohort resulted in the detection of the following nucleotide mutations (and corresponding amino acid mutations) (also shown in Table 1 and Fig. 1): three somatic nonsense mutations — C4201T (Q1401*), C5164T (R1722*), and C1680A (Y560*) (stars denote a stop codon); two somatic indels (insertion–deletion) — 6018-6020delGCT and 5541insG; one somatic missense mutation — T5953C (S1989P) (found in the same sample as the 5541insG mutation); and one gene rearrangement involving *ARID1A* and the neighboring gene *ZDHHC18* (encoding the zinc-finger DHHC domain-containing protein 18). The fusion ends of this rearrangement map to a homozygous deletion involving most of the *ARID1A* gene (Fig. 1 in the Supplementary Appendix).

All predicted variants of *ARID1A* were validated with the use of Sanger sequencing of DNA from the source tumors, except for the deletion–rearrangement, which was validated with the use of microarray data (Affymetrix SNP 6.0) (Table 3 in the Supplementary Appendix). The finding of multiple types of mutations in a single gene, *ARID1A*, in ovarian clear-cell carcinoma led us to further explore *ARID1A* in this cancer type. Since mutations in *PIK3CA* (the phosphoinositide-3-kinase, catalytic, alpha polypeptide gene), *CTNNB1* (the catenin beta-1 gene), *KRAS* (the v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue gene), and *TP53* (the tumor protein p53 gene) are recurrent in ovarian clear-cell carcinoma,[25] we analyzed the RNA-sequencing data and performed a polymerase-chain-reaction assay for the presence of

Table 1. Results of RNA Sequencing and Exon Resequencing of the Discovery Cohort of 19 Specimens of Ovarian Clear-Cell Carcinoma (Including a Cell Line).*

Sample No.	*ARID1A* Mutation	Exon Resequencing	RNA Sequencing	Immunohistochemical Test for BAF250a Expression	Predicted *ARID1A* Status	Other Somatic Mutations in Oncogenes or Tumor Suppressors
		no. of reads containing mutation/ total no. of reads at mutation position (%)				
CCC01	6018-6020delGCT	223/1529 (15)	10/40 (25)	Positive	Heterozygous†	
CCC02	404delC‡	Not applicable§	No coverage	Negative	Mutation with loss of heterozygosity¶	*CTNNB1* C110G (S37C)
CCC03	5518delG‡	395/1725 (23)	1/2 (50)	Positive	Heterozygous†	
CCC04	Deletion and rearrangement	Not applicable	6/6 (100)	Negative	Deletion and rearrangement (homozygous)	
CCC06	C4201T (Q1401*)	100/914 (11)	8/26 (31)	Positive	Heterozygous†	
CCC09	C5164T (R1722*)	1132/1513 (75)	30/30 (100)	Negative	Mutation with loss of heterozygosity	
CCC10	3948delG‡	166/758 (22)	No coverage	Negative	Heterozygous†	
CCC13	5541insG	395/1518 (26)	23/97 (24)	Negative	Heterozygous‖	*CTNNB1* C110G (S37C)
CCC13	T5953C (S1985P)	339/1093 (31)	25/60 (42)	Negative	Heterozygous‖	*CTNNB1* C110G (S37C)
CCC14	C1680A (Y560*)	1411/2651 (53)	3/6 (50)	Negative	Heterozygous	
CCC05	None			Positive		*KRAS* G38A (G13D)
CCC67	None			Positive		
CCC68	None			Positive		
CCC66	None			Positive		
CCC69	None			Positive		
CCC70	None			Negative		
CCC71	None			Positive		
CCC72	None			Positive		
CCC73	None			Positive		
TOV21G cell line	1645insC‡	484/1821 (27)	5/34 (15)	Negative	Heterozygous†	*PIK3CA* C3139T (H1047Y), *KRAS* G37T (G13C)

* The mutations listed are nucleotide mutations, followed by amino acid mutations (if known) in parentheses, with a star indicating a stop codon. *CTNNB1* denotes the catenin beta-1 gene, *KRAS* the v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue gene, and *PIK3CA* the phosphoinositide-3-kinase, catalytic, alpha polypeptide gene (Entrez Gene record numbers NM_001904.3, NM_004985.3, and NM_006218.2, respectively).

† The percentage of reads containing a mutation in samples CCC01, CCC03, CCC06, and CCC10 and the TOV21G cell line suggests some form of allelic imbalance, with extra copies of the wild-type allele.

‡ The *ARID1A* mutations in samples CCC02, CCC03, CCC10, and the TOV21G cell line were not initially identified or discovered through RNA sequencing.

§ For sample CCC02, no reads were available from the exon resequencing of exon 1, but Sanger sequencing showed a dominant peak from the mutation.

¶ For sample CCC02, the predicted *ARID1A* status is based on microarray data (Affymetrix SNP 6.0).

‖ For sample CCC13, the two somatic mutations in *ARID1A* can be found both in the trans configuration (on independent alleles) and in the cis configuration (on the same alleles). For details, see Figure 4 in the Supplementary Appendix.

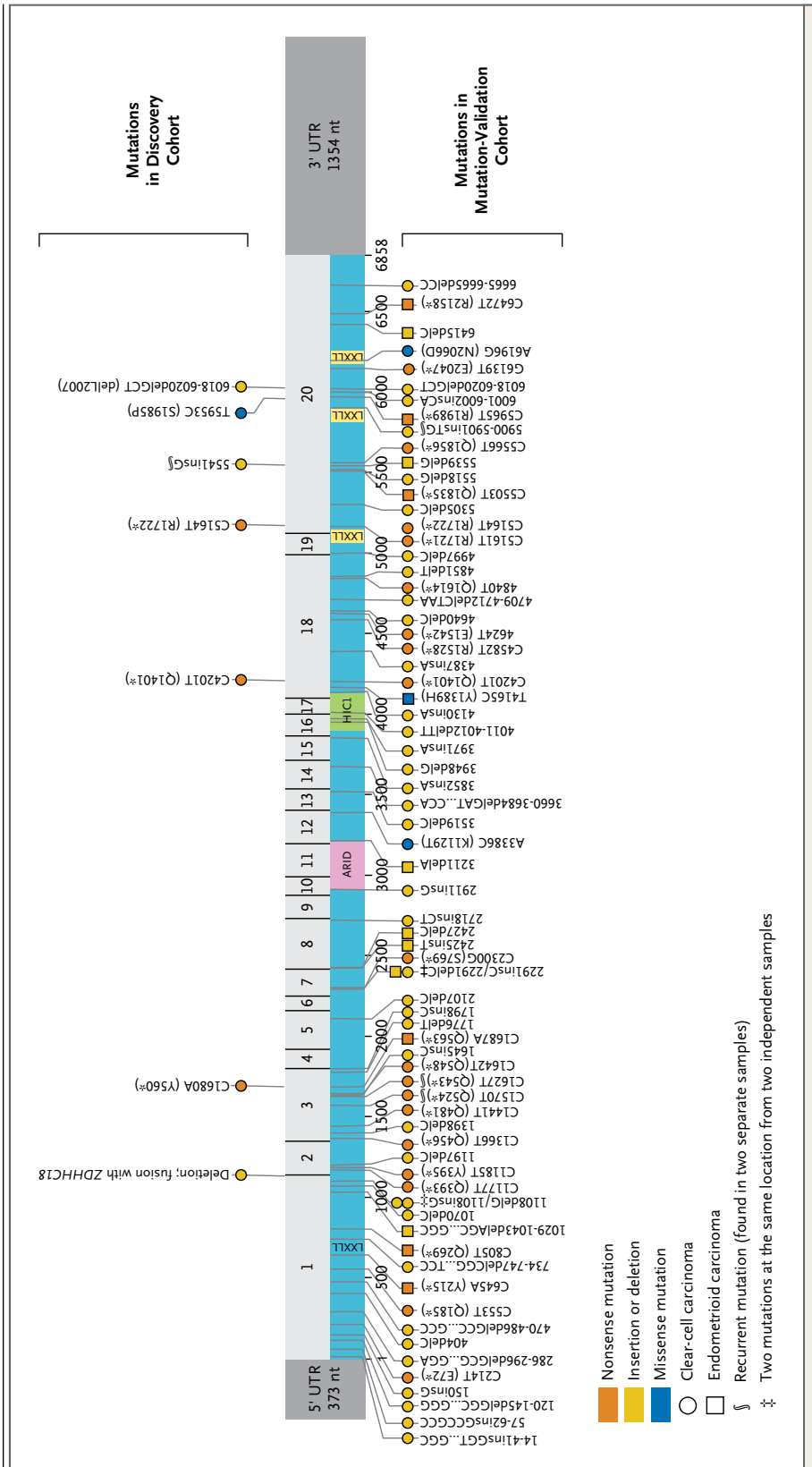

Figure 1. Mutations Found in *ARID1A* and the BAF250a Protein It Encodes.

The 20 exons of *ARID1A* are represented (as numbered gray boxes) above a schematic of the BAF250a protein (the blue segment, with the ARID [AT-rich interactive domain] DNA-binding domain in pink, the HIC1 [hypermethylated in cancer 1] binding domain in green, and the three C-terminal leucine-rich LXXLL motifs that facilitate interaction with glucocorticoid receptor in yellow). The nucleotide mutations (with corresponding amino acid mutations in parentheses) listed above the schematic are those identified by means of transcriptome sequencing (RNA sequencing) of the 18 samples of ovarian clear-cell carcinoma and the TOV21G cell line in the discovery cohort, and those listed below the schematic were identified in subsequent validation efforts with the use of targeted exon resequencing and Sanger sequencing of genomic DNA from the 210 ovarian-cancer samples in the mutation-validation cohort. All unique somatic mutations detected in samples of ovarian clear-cell carcinoma, endometrioid carcinoma, and high-grade serous carcinoma are shown. Numbers 1 through 6858 below the schematic indicate the nucleotide (nt) position, starting with the A in the ATG start codon for *ARID1A* in position 1 (based on the sequence given in record number NM_006015.4 in Entrez Gene; also see Table 1 in the Supplementary Appendix). UTR denotes untranslated region.

variants in these genes (Table 1). Whole-transcriptome sequence data for the 19 samples of the discovery cohort have been deposited at the European Genome–Phenome Archive (accession number, EGAS00000000075).

ARID1A mutation frequency in ovarian clear-cell carcinomas and other ovarian-cancer subtypes was established through targeted exon resequencing of the mutation-validation cohort of 210 samples of various subtypes of ovarian carcinomas and 1 ovarian clear-cell carcinoma cell line, along with the original discovery cohort of 18 samples of ovarian clear-cell carcinoma and 1 ovarian clear-cell carcinoma cell line. *ARID1A* mutations were identified in 55 of 119 (46%) ovarian clear-cell carcinomas, 10 of the 33 (30%) endometrioid carcinomas, and none of the 76 high-grade serous carcinomas (Table 2, and Table 3 in the Supplementary Appendix). A total of 17 samples (12 of ovarian clear-cell carcinoma and 5 of endometrioid carcinoma) each had two validated *ARID1A* mutations. In addition, the ovarian clear-cell carcinoma cell line TOV21G had a truncating mutation in *ARID1A* (1645insC).

We analyzed germ-line DNA from 55 samples (47 ovarian clear-cell carcinomas and 8 endometrioid carcinomas) in the discovery and mutation-validation cohorts for the presence of 65 truncating mutations (53 found in ovarian clear-cell carcinomas and 12 found in endometrioid carcinomas). In all 55, the mutations were found to be somatic. On this basis, we made the assumption that 12 subsequent truncating mutations (10 in ovarian clear-cell carcinoma and 2 in endometrioid carcinoma) would be somatic (i.e., predicted to be somatic without germ-line DNA testing) (Table 3 in the Supplementary Appendix). The presence of *ARID1A* mutations showed a strong association ($P<0.001$ by Fisher's exact test) with the two ovarian-cancer subtypes associated with endometriosis (ovarian clear-cell carcinoma and endometrioid carcinoma).

BAF250a PROTEIN EXPRESSION

The correlation between *ARID1A* mutations and BAF250a expression was evaluated by means of immunohistochemical staining for BAF250a in 182 tumors for which formalin-fixed, paraffin-embedded sections were available in the discovery cohort and the mutation-validation cohort: 73 ovarian clear-cell carcinomas, 33 endometrioid carcinomas, and 76 high-grade serous carcinomas. The

Table 2. Mutational Status in the Discovery and Mutation-Validation Cohorts (Excluding the Two Cell Lines), According to Carcinoma Type.

Mutational Status	Ovarian Clear-Cell Carcinoma				Endometrioid Carcinoma				High-Grade Serous Carcinoma			
	Total	Immunohistochemical Test for BAF250a Expression			Total	Immunohistochemical Test for BAF250a Expression			Total	Immunohistochemical Test for BAF250a Expression		
ARID1A mutation		*negative*	*positive*	*not available*		*negative*	*positive*	*not available*		*negative*	*positive*	*not available*
One somatic nonsense or indel mutation	41	19	9	13	5	1	4	0	0	0	0	0
Two somatic nonsense or indel mutations	10	5	0	5	4	4	0	0	0	0	0	0
One somatic nonsense or indel mutation, one missense mutation	2	2	0	0	1	0	1	0	0	0	0	0
One missense mutation	1	0	1	0	0	0	0	0	0	0	0	0
Other mutation (deletion and rearrangement)	1	1	0	0	0	0	0	0	0	0	0	0
Total	55	27	10	18	10	5	5	0	0	0	0	0
Normal *ARID1A*	64	4	32	28	23	2	21	0	76	1	75	0
Overall total	119	31	42	46	33	7	26	0	76	1	75	0

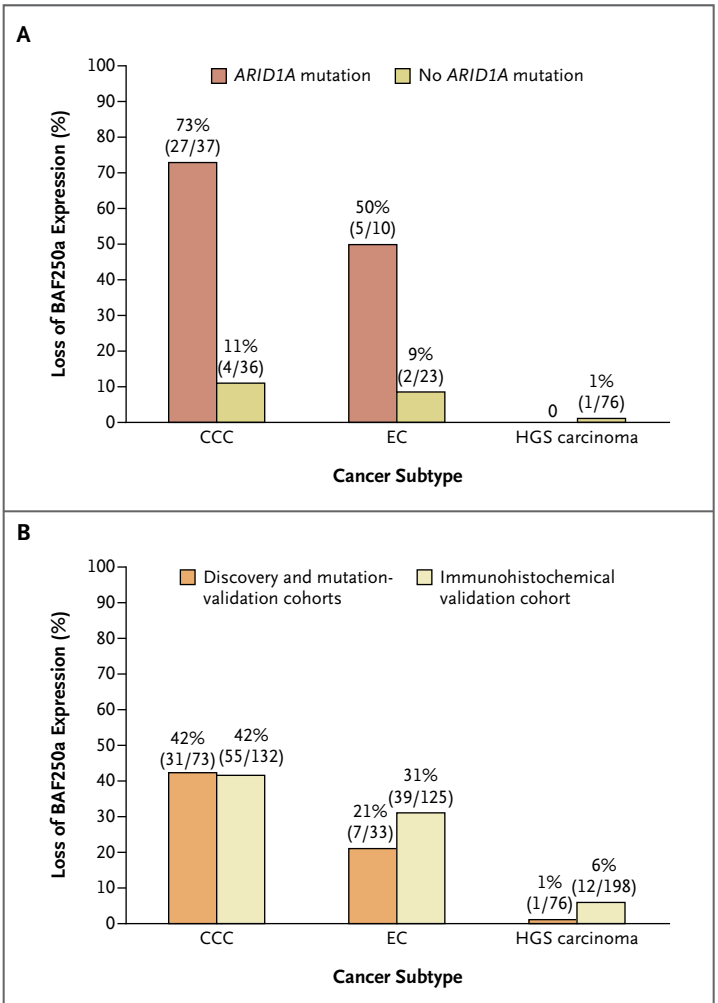

Figure 2. Results of Immunohistochemical Analyses of BAF250a Expression.

The percentages of tumors (with number and total number in parentheses) from three subtypes of ovarian cancer — clear-cell carcinoma (CCC), endometrioid carcinoma (EC), and high-grade serous (HGS) carcinoma — from the discovery and mutation-validation cohorts that showed loss of BAF250a expression are shown in Panel A for samples with and samples without *ARID1A* mutations and in Panel B for samples in the discovery and mutation-validation cohorts and samples in the immunohistochemical validation cohort. The rate of BAF250a loss was higher among CCC specimens with an *ARID1A* mutation than among those without an *ARID1A* mutation ($P<0.001$); the same was true for EC specimens ($P=0.02$). The loss of expression was also consistently more common in CCC and EC (the two endometriosis-associated carcinomas) than in HGS carcinoma when assessed in the discovery and mutation-validation cohorts and again in the immunohistochemical validation cohort (Panel B), with $P<0.001$ for all comparisons. All P values were calculated with the use of Fisher's exact test.

presence of mutations was significantly associated with BAF250a loss in endometriosis-associated cancers ($P<0.001$ by Fisher's exact test). A total of 27 of 37 samples (73%) and 5 of 10 samples (50%) of ovarian clear-cell carcinoma and endometrioid carcinoma, respectively, with an *ARID1A* mutation showed a loss of BAF250a expression, as compared with 4 of 36 samples (11%) and 2 of 23 samples (9%), respectively, without an *ARID1A* mutation (Fig. 2A and Table 2). Loss of BAF250a expression was strongly associated with the endometriosis-related ovarian cancers — with 31 of 73 samples (42%) of ovarian clear-cell carcinoma and 7 of 33 samples (21%) of endometrioid carcinoma showing a loss of expression — as compared with high-grade serous carcinomas, for which 1 of the 76 samples (1%) had loss of expression ($P<0.001$ by Fisher's exact test) (Fig. 2B). *ARID1A* mutations were not significantly associated with the presence of endometriosis in 86 ovarian clear-cell carcinomas and 33 endometrioid carcinomas (Table 5 in the Supplementary Appendix).

The immunohistochemical validation cohort was also assessed for BAF250a expression (Fig. 2B). This analysis revealed that 55 of the 132 samples (42%) of ovarian clear-cell carcinoma, 39 of the 125 samples (31%) of endometrioid carcinoma, and 12 of the 198 samples (6%) of high-grade serous carcinoma lacked BAF250a expression. These findings are in agreement with the proportions observed in the discovery and mutation-validation cohorts. No significant associations with absence of BAF250a expression were noted on the basis of age of presentation, stage of disease (low or high), or disease-specific survival within any of the cancer subtypes, as assessed by means of Welch's analysis of variance, Fisher's exact test, and the log-rank statistic, respectively ($P>0.05$ for all analyses).

ANALYSIS OF *ARID1A* IN ENDOMETRIOSIS ASSOCIATED WITH OVARIAN CANCER

Two patients with ovarian clear-cell carcinomas (samples CCC13 and CCC23) carrying *ARID1A* mutations had contiguous atypical endometriosis (Fig. 3, and Fig. 3 in the Supplementary Appendix). For one of the two patients, the specimen was heterozygous for an *ARID1A* truncating mutation (G6139T [E2047*]) in exon 20. This mutation was also found in 17 of 42 clones derived from atypical endometriosis but in none of 52 clones from a distant endometriotic lesion ($P<0.001$ by Fisher's exact test) (Fig. 3C). Epithelial samples of both the ovarian clear-cell carcinoma and atypical endometriosis had loss of BAF250a expression, whereas expression was maintained in the distant endometriotic lesion (Fig. 3B). HNF-1β was expressed in the ovarian clear-cell carcinoma but not in the

contiguous atypical or distant endometriosis, and estrogen receptor was expressed in both the contiguous and distant endometriosis but not in the ovarian clear-cell carcinoma, as was expected.[24] Thus, atypical endometrium could be distinguished from the distant endometrium only on the basis of loss of BAF250a expression, which correlated with the presence of an *ARID1A* mutation.

For the other patient, the sample of ovarian clear-cell carcinoma had two somatic mutations in *ARID1A* (and loss of BAF250a expression): both these mutations, along with a *CTNNB1* missense mutation, were present in the tumor and the adjacent atypical endometriosis but not in a distant endometriotic lesion (Fig. 3B in the Supplementary Appendix).

DISCUSSION

Overall, 46% of patients with ovarian clear-cell carcinoma and 30% of those with endometrioid carcinoma had somatic truncating or missense mutations in *ARID1A;* no *ARID1A* mutations were found in any of the 76 specimens of high-grade serous carcinoma analyzed. Loss of *ARID1A* expression was also specific to the subtype of ovarian cancer, with loss of nuclear BAF250a expression seen in 36% of ovarian clear-cell carcinomas and endometrioid carcinomas, but only 1% of high-grade serous carcinomas. Our initial mutation-screening assays involving RNA sequencing in the discovery cohort identified seven somatic mutations in *ARID1A* in the 19 samples; four additional mutations were subsequently identified when these samples were analyzed by means of amplicon-exon resequencing. Most likely, the additional mutations had not been seen in the RNA-sequencing data owing to their transcripts being rapidly targeted for nonsense-mediated decay[26] or the inherently decreased sensitivity of the assay to mutations at the 5′ end of transcripts. Thus, although RNA sequencing is a useful tool for discovery, targeted exon resequencing may be more appropriate for the determination of true mutation frequency.

ARID1A is located at 1p36.11.[27] This chromosomal region is commonly deleted in tumors, and it has been suggested that deletion regions encompassing 1p36 could contain tumor-suppressor genes.[28,29] Rearrangements and deletions in *ARID1A* have been identified in a primary breast-cancer cell line and a lung-cancer cell line, respectively,[18] and the loss of BAF250a has also been observed in cervical- and breast-carcinoma cell lines.[30] In a study by Wang and colleagues,[31] the screening of 241 tumors revealed that *ARID1A* transcript levels are decreased in approximately 6% of cancers in general and in 30% of renal carcinomas and 10% of breast carcinomas, specifically; however, none of the 14 ovarian cancers showed loss of expression, probably because they were predominantly the high-grade serous subtype.

The *ARID1A* mutations identified in our study were mostly truncating mutations, which were evenly distributed across the gene. The presence of mutations is strongly correlated with the loss of BAF250a protein (Table 2 and Fig. 2A). Loss of BAF250a expression was seen in 73% and 50% of samples of ovarian clear-cell carcinoma and endometrioid carcinoma with an *ARID1A* mutation, respectively, and in only 11% and 9% of samples without an *ARID1A* mutation, respectively. Seventeen of the mutation-positive samples had two *ARID1A* mutations; in all but one of the specimens with two mutations for which immunohistochemical data were available, BAF250a expression was not detected. That single exception (an endometrioid carcinoma) had both a C-terminal truncating mutation and a missense mutation; either of these changes could produce a detectable protein. A single sample of ovarian clear-cell carcinoma had *ARID1A* loss and rearrangement resulting in the homozygous deletion of the gene. Three other cases of ovarian clear-cell carcinoma also appear to be characterized by loss of heterozygosity, on the basis of the frequency of mutant alleles and wild-type alleles (Table 3 in the Supplementary Appendix) and subsequent loss of BAF250a expression. However, the majority of cancers with somatic *ARID1A* mutations and loss of BAF250a expression appear to have a wild-type allele present. Data from exon resequencing and RNA sequencing show excellent agreement between the fraction of mutant and wild-type alleles at both the DNA and RNA levels (Table 1), suggesting that epigenetic silencing is not a significant factor. Post-transcriptional or post-translational regulation or dominant negative effects of the mutations are possible, albeit untested, explanations for the lack of protein expression in these heterozygous cases.

The presence of BAF250a immunoreactivity in 15 samples positive for an *ARID1A* mutation (all but 1 of which had truncating mutations) may in-

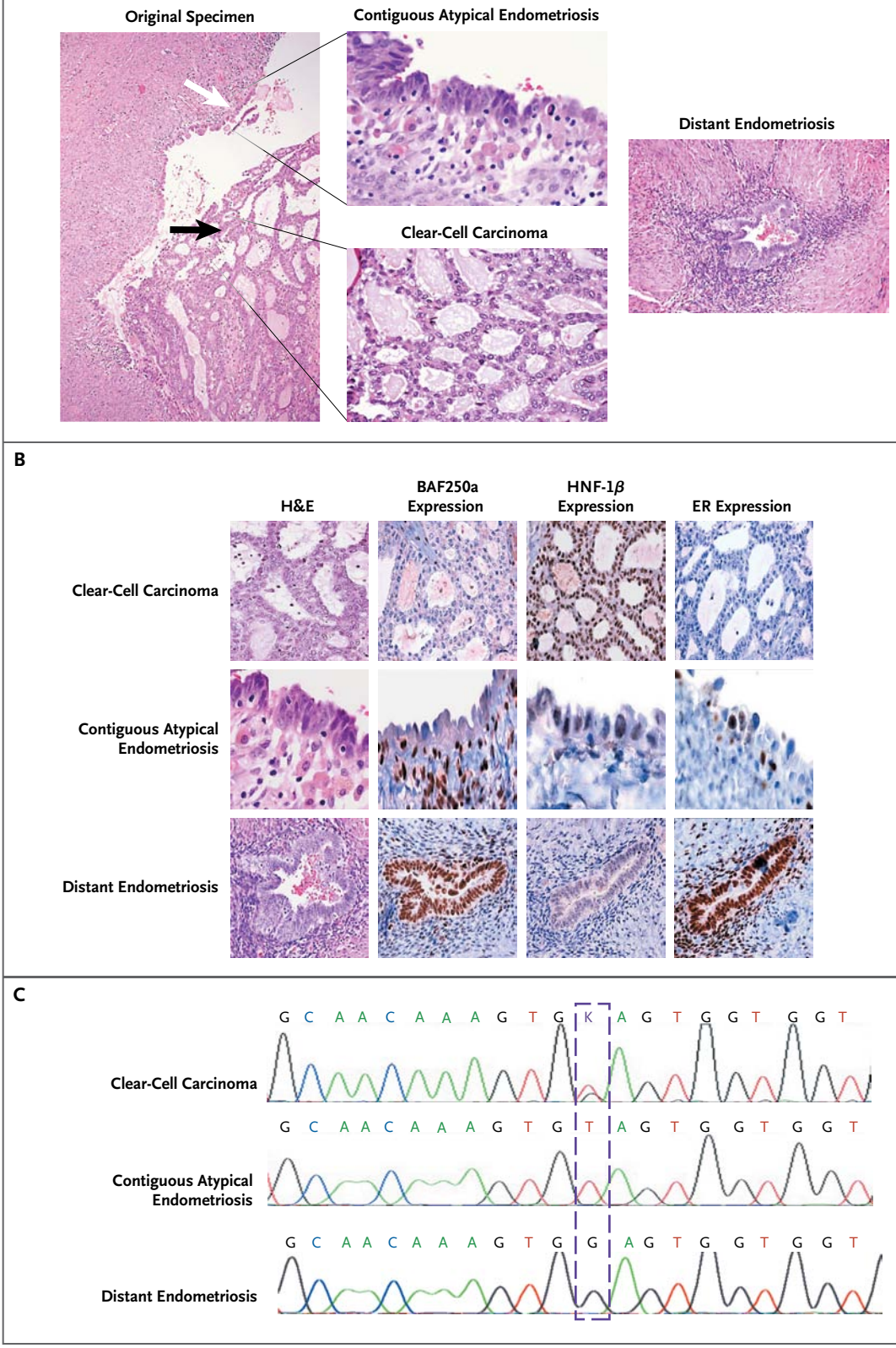

Figure 3 (facing page). Analysis of Ovarian Clear-Cell Carcinoma and Associated Endometriosis in a Study Patient.

Panel A shows a section (hematoxylin and eosin [H&E]) on which a clear-cell carcinoma (black arrow) has arisen in an endometriotic cyst (white arrow). The same section, viewed at a higher magnification, shows regions of the clear-cell carcinoma and contiguous atypical endometriosis. A region of distant endometriosis from the same patient is also shown. Panel B shows the results of immunohistochemical staining of the epithelial portions of tissue specimens shown in Panel A for expression of BAF250a, hepatocyte nuclear factor 1β (HNF-1β), and estrogen receptor (ER). BAF250a immunoreactivity is lost in both the clear-cell carcinoma and the contiguous atypical endometriosis but is maintained in the distant endometriosis. Both regions of endometriosis differ from the carcinoma in their lack of HNF-1β expression (with weak expression in the contiguous atypical endometriosis) and maintenance of estrogen-receptor expression. Panel C shows sequencing chromatograms for the clear-cell carcinoma and polymerase-chain-reaction (PCR) clones of microdissected material from the contiguous atypical endometriosis and distant endometriosis, from which DNA was extracted. The carcinoma and contiguous atypical endometriosis show nucleotide variation corresponding to G6139T (as indicated with the dashed box); the tumor shows a heterozygous peak at that location, whereas the atypical endometriosis is homozygous for the substitution (in 17 of 42 clones). In contrast, the distant endometriosis shows wild-type sequence (in all 52 clones analyzed). None of the PCR clones from the distant endometriosis showed variation from the wild-type sequence.

dicate that haploinsufficiency is pathogenic, as has been reported in mice.[32] Alternatively, immunohistochemical detection of a truncated but nonfunctional BAF250a protein may account for the immunostaining results. The antibody used in the assay targets a region of 111 amino acids (amino acids 1216 through 1326) in the middle of the protein, and 7 of the 15 specimens that were positive for loss of BAF250a expression had mutations that would result in truncation distal to the epitope.

The mutations are common in ovarian carcinomas that are associated with endometriosis (ovarian clear-cell carcinoma and endometrioid carcinoma) but not in the unrelated high-grade serous carcinoma. This suggests that the mutations may be pathogenic, rather than random, events. Mutations in the *PTEN* gene (encoding the phosphatase and tensin homologue) have been described in 20% of endometriotic cysts,[33] and conditional expression of either oncogenic *Kras* or deletion of the *Pten* tumor suppressor in the ovarian surface epithelium in mice was found to induce endometriosis.[34] Expression of oncogenic *Kras* accompanied by simultaneous loss of *Pten* resulted in widely metastatic ovarian carcinoma; however, *KRAS* mutations are not seen in human cases of endometriosis and are uncommon in endometriosis-associated ovarian cancers in humans. By comparing ovarian clear-cell carcinomas to their contiguous atypical endometriotic lesions in two patients, we show that the same mutations may be present in the putative precursor lesions and in the tumors. In contrast, the distant endometriotic lesions do not have *ARID1A* mutations. In the case of ovarian clear-cell carcinoma described in Figure 3, the mutation (G6139T [E2047*]) was present before the atypical endometriosis resulted in the development of the immunophenotype associated with the cancer (estrogen-receptor–negative, HNF-1β–positive[24]), suggesting that the mutation is an early event in neoplastic transformation. Taken together, these data suggest that *ARID1A* is a classic tumor-suppressor gene. Unlike *BRCA* or *TP53* mutations, which can be found in the germ-line DNA, all truncating *ARID1A* mutations were somatic. Deletion of *ARID1A* on one allele results in embryonic lethality in mice.[32]

Mutations in *ARID1A* and loss of BAF250a expression were seen preferentially in ovarian clear-cell carcinomas and endometrioid carcinomas, cancers that do not feature the chromosomal instability, nearly ubiquitous *TP53* mutations, and frequent abnormalities in *BRCA* (associated with early breast cancer) seen in high-grade serous carcinomas.[5,35] It is possible that defects in genes that alter the accessibility of transcription factors to chromatin, such as *ARID1A*, in addition to mutations in the WNT and PI3 kinase pathways,[25] will help to define ovarian clear-cell carcinomas and endometrioid carcinomas. If such a model is correct, other abnormalities affecting the *ARIDIA* locus or dysregulation of other chromatin-remodeling genes may be found in ovarian clear-cell and endometrioid carcinomas that are negative for an *ARID1A* mutation. This idea is supported by the clinical similarities between ovarian clear-cell carcinomas positive for and those negative for an *ARID1A* mutation.

The mechanism by which somatic mutations in *ARID1A* enable the progression of benign endometriosis to carcinoma is unclear; however, our findings are consistent with a critical role for

ARID1A mutations in the genesis of a substantial fraction of ovarian clear-cell and endometrioid carcinomas.

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