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Evidence for a latent precursor (p53 signature) that may precede serous endometrial intraepithelial carcinoma

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Both serous intraepithelial carcinoma and endometrial glandular dysplasia are associated with uterine serous carcinoma. Recently a candidate serous cancer precursor containing *p53* mutations (p53 signature) was described in the fallopian tube. We analyzed normal and neoplastic endometrium for a similar entity. In total 10 endometrial polyps involved by intraepithelial and/or invasive carcinoma and 137 benign polyps were studied. All were stained for p53 and MIB-1. A subset of p53 signatures and carcinomas were analyzed for γ-H2AX and *p53* mutations. p53 signatures were identified in 7 of 10 cases intraepithelial carcinoma and were multicentric in 2. In one case, the signature was in continuity with intraepithelial carcinoma. Of 137 benign polyps (4%), 6 contained p53 signatures. The MIB-1 fraction in most signatures was less than 5%, and ranged from 50 to 90% in carcinomas. DNA damage (γ-H2AX) was demonstrated in both p53 signatures and adjacent carcinomas but not in benign polyps. Shared identical *p53* mutations were found in paired signatures and carcinomas in two of three cases analyzed, including one case with multiple signatures. In one, a coexistent invasive serous cancer was not found to contain a *p53* mutation. In a third, a p53 signature and an invasive cancer harbored two different *p53* mutations. This is the first description of p53 signatures adjacent to carcinoma, suggesting a role for this entity in the genesis of serous malignancy. The significance of p53 signatures in benign conditions (polyps) remains to be determined. The role of the p53 signature in early serous neoplasia is discussed.
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There are approximately 40 000 new endometrial cancers annually in the United States.[1] Although endometrial serous carcinomas comprise a minority of these cancers, they are associated with significant mortality and result in a poor clinical outcome even in patients with early-stage disease.[2,3] Until recently, the earliest phase of uterine serous carcinoma was defined as serous intraepithelial carcinoma. However, despite the absence of stromal invasion, serous intraepithelial carcinoma was considered potentially malignant, with the capacity to spread beyond the uterus.[4] Recently, atypical glands have been described in the endometrium that are associated with serous intraepithelial carcinoma and contain mutations in the tumor suppressor gene *p53*.[5,6] This entity, termed endometrial glandular dysplasia, has been proposed as a precursor to serous intraepithelial carcinoma. Other mechanisms of serous cancer development, including clonal divergence from an existing endometriod carcinoma have also been described.[7,8]

A central molecular event in the pathogenesis of endometrial and pelvic serous carcinomas is a mutation in the tumor suppressor gene *p53*. Recent studies of pelvic serous cancer have linked a high percentage of early cancers in BRCA + women and many other ovarian and peritoneal carcinomas to an origin in the distal fallopian tube.[9–12] These early cancers, termed serous tubal intraepithelial carcinomas possess *p53* mutations that are identical to coexisting noncontiguous neoplasms, implying that one is a metastasis of the other. On the basis of these associations, the distal fallopian tube has become a candidate source of many pelvic serous cancers.[13,14]

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More compelling evidence of the distal fallopian tube as a source of pelvic serous cancer has been the identification of 'p53 signatures,' benign appearing mucosa that contains *p53* mutations.[15–17] Moreover, they predominate in the same location as early serous cancers (the fimbria), involve secretory cells, exhibit evidence of DNA damage (γ-H2AX+) and share epidemiologic characteristics with pelvic serous cancer.[18] This entity has emerged as a presumptive precursor to many serous cancers.[16]

Because of the strong association between the p53 signature in the fimbria and STIC, it is conceivable that endometrial serous carcinomas are preceded by a similar series of cellular events. The purpose of this study was to establish a relationship between p53 signatures in the endometrium and the earliest malignant endometrial serous lesion, endometrial intraepithelial carcinoma. We hypothesized that endometrial p53 signatures may represent a potential precursor to some cases of uterine serous carcinoma.

Materials and methods

Overall Study Design and Rationale

The institutional review board at Brigham and Women's Hospital approved the study. The goal of the study was to identify the earliest potential precursor of endometrial serous carcinoma and to determine its prevalence in both benign endometrial polyps and in association with serous intraepithelial carcinoma.

Case selection
Two different case groups were obtained from two different sources. The first consisted of nine uterine specimens (hysterectomies and endometrial biopsies) containing endometrial polyps harboring serous intraepithelial carcinoma, and in some cases, invasive serous carcinoma, that were retrieved by report review of cases at the Department of Surgical Pathology at CellNetix Pathology. An additional hysterectomy specimen harboring only invasive serous carcinoma in a polyp was collected from the Department of Pathology at Brigham and Women's Hospital. The second case group consisted of consecutively accessioned benign endometrial polyps obtained over a 2-year period in the Department of Pathology at Brigham and Women's Hospital.

Immunohistochemistry

Available blocks from all cases of serous intraepithelial and/or invasive serous carcinoma and benign polyps were sectioned and stained for p53 and MIB-1. A positive score for p53 required strong nuclear staining that obscured nuclear detail. In each case, a MIB-1 fraction, or percent of cells with intense nuclear staining, was reported. In all cases of serous intraepithelial carcinoma and in polyps in which discrete segments of p53 positivity were identified in morphologically benign glandular epithelium (p53 signatures), immunostaining for γ-H2AX was performed. γ-H2AX is the phosphorylated form of the core histone H2AX, and is a marker for double-stranded DNA breakage.[19] A positive score was based on punctate intranuclear staining.

Antigen retrieval was performed on 5-ím tissue sections in citrate buffer (20 mM, pH 6.0) at 120°C for 30 s. Sections were incubated with antibodies directed against p53 (DO-1, Immunotech, Westbrook, ME, USA) at a 1:1200 dilution, MIB-1 (corresponding to Ki-67; M7240, Dako, Carpintaria, CA, USA) at a 1:200 dilution, or γ-H2AX (JBW301, Upstate Cell Signaling Solution, Charlottesville, VA, USA) at a 1:300 dilution, at room temperature for 40 min as previously described.[15] Antigen–antibody complexes were localized using the EnVision system using horseradish peroxidase and 3,3′-diaminobenzidine (Dako).

Analysis of *p53* Mutations

A subset of matched serous intraepithelial carcinomas and/or invasive serous cancers and p53 signatures were analyzed for *p53* mutations. Benign appearing, endometrial glandular epithelium from the same tissue, not exhibiting immunoreactivity for p53, was used as a control. Laser-capture microdissected material was obtained using the PALM microbeam instrument. Genomic DNA was amplified by PCR using tailed primers designed to amplify *p53* exons 2, 3, 5–9 and 11. Secondary amplification was achieved using primers (T3 and T7) specific to the tail sequence used in the original amplification. Products from both strands were sequenced using T3 and T7 primers. The sequences were analyzed using the Mutation Surveyor program (Soft Genetics, State College, PA). Candidate mutations found were compared to a reference database for cancer-associated *p53* mutations (Universal mutation database).[15] To exclude spurious mutations introduced into somatic DNA as a consequence of formalin fixation, all *p53* mutation-positive exons were re-sequenced from a replicate-amplified product.[20]

Results

Case Characteristics and Identification of p53 Signatures

Nine serous intraepithelial carcinomas involving polyps were reviewed, including six that also harbored invasive serous cancer; an additional case that only contained invasive serous cancer in a polyp was also reviewed. p53 signatures were identified in seven of these cases, and were multiple in two (Table 1). Of 137 benign polyps reviewed, 6

Table 1 Characteristics of cases with serous cancer in an endometrial polyp

Case	Age	EIC present	Invasive cancer present	p53 signature	Extrauterine tumor
1	63	Yes	Yes	No	Cervix
2	74	Yes	Yes	1 signature	Omentum
3	77	Yes	Yes	3 signatures	
4	64	Yes	No	No	
5	67	Yes	Yes	1 signature	
6	82	Yes	No	2 signatures	Omentum
7	78	Yes	Yes	no	
8	57	Yes	Yes	1 signature	Ovary, peritoneum
9	65	Yes	No	No	
10	82	No	Yes	1 signature	Ovary, bowel serosa

EIC, endometrial intraepithelial carcinoma.
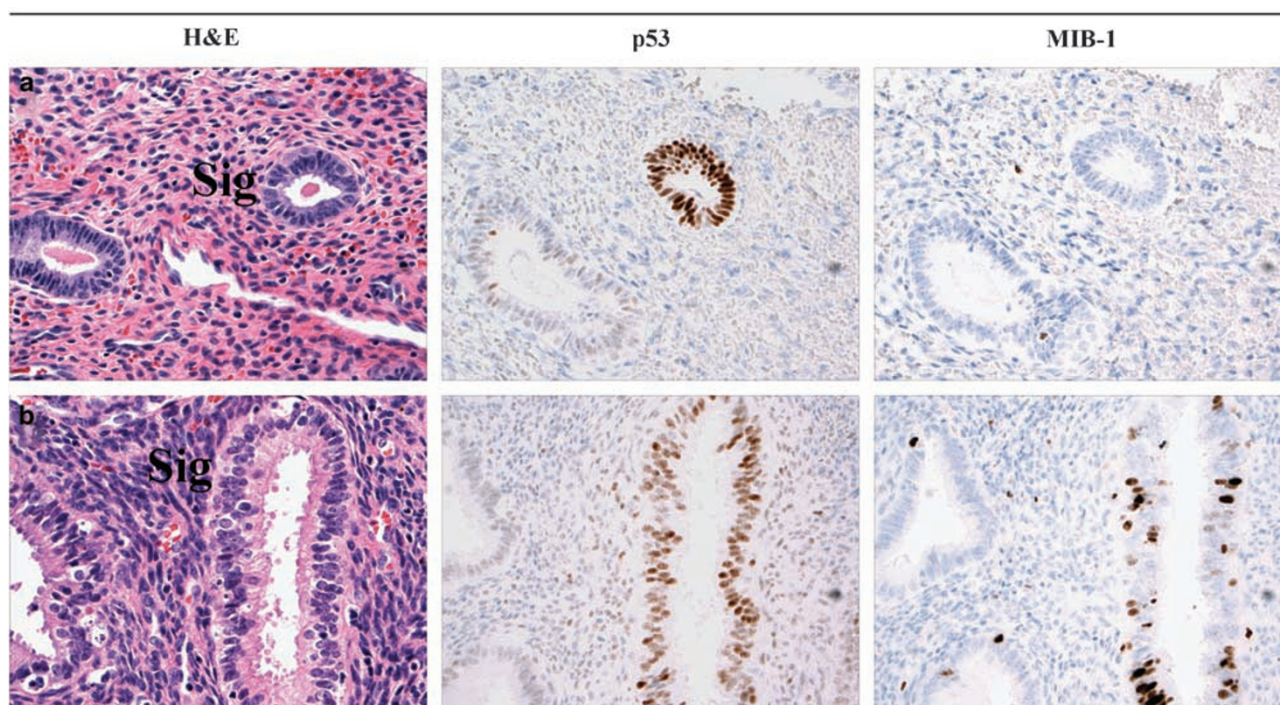
Figure 1 p53 signatures in benign polyps. Immunostaining for p53 highlights discrete glands that are morphologically indiscernible from the surrounding glands. The proliferative fraction of the p53 signature in panel (**a**) is 0%, similar to an adjacent p53-negative gland, whereas the signature in panel (**b**) exhibits a higher proliferative index.

were found to harbor p53 signatures. In most of these, the p53 staining was oriented circumferentially in a cross-section of a gland (Figure 1 , panels a and b; Figure 2, panel b). Occasionally, a signature could be seen as a discrete segment of linear p53-staining along the surface of a polyp (Figure 2, panel a). The MIB-1 fraction in p53 signatures in polyps with and without serous cancer ranged from 0 to 20%, but was less than 5% in most cases. In contrast, in serous intraepithelial carcinomas, the MIB-1 fraction ranged from 50 to 90%. Nuclear γ-H2AX dot-like immunostaining, indicative of DNA damage, was seen at least focally in all p53 signatures in cases with concurrent serous endometrial intraepithelial carcinoma. Serous intraepithelial and invasive carcinomas exhibited more intense

γ-H2AX staining (Figure 2). Of the six p53 signatures found in benign polyps, four showed no γ-H2AX staining, and two showed nonspecific staining that was indistinct from the surrounding p53-negative glands.

p53 Mutation Analysis

Four cases with serous intraepithelial carcinoma and p53 signatures were analyzed for *p53* mutations by the methods described above. Genomic DNA amplification was successful in three cases. The results are summarized in Table 2. In two cases, shared mutations were identified between p53 signatures and concurrent serous intraepithelial
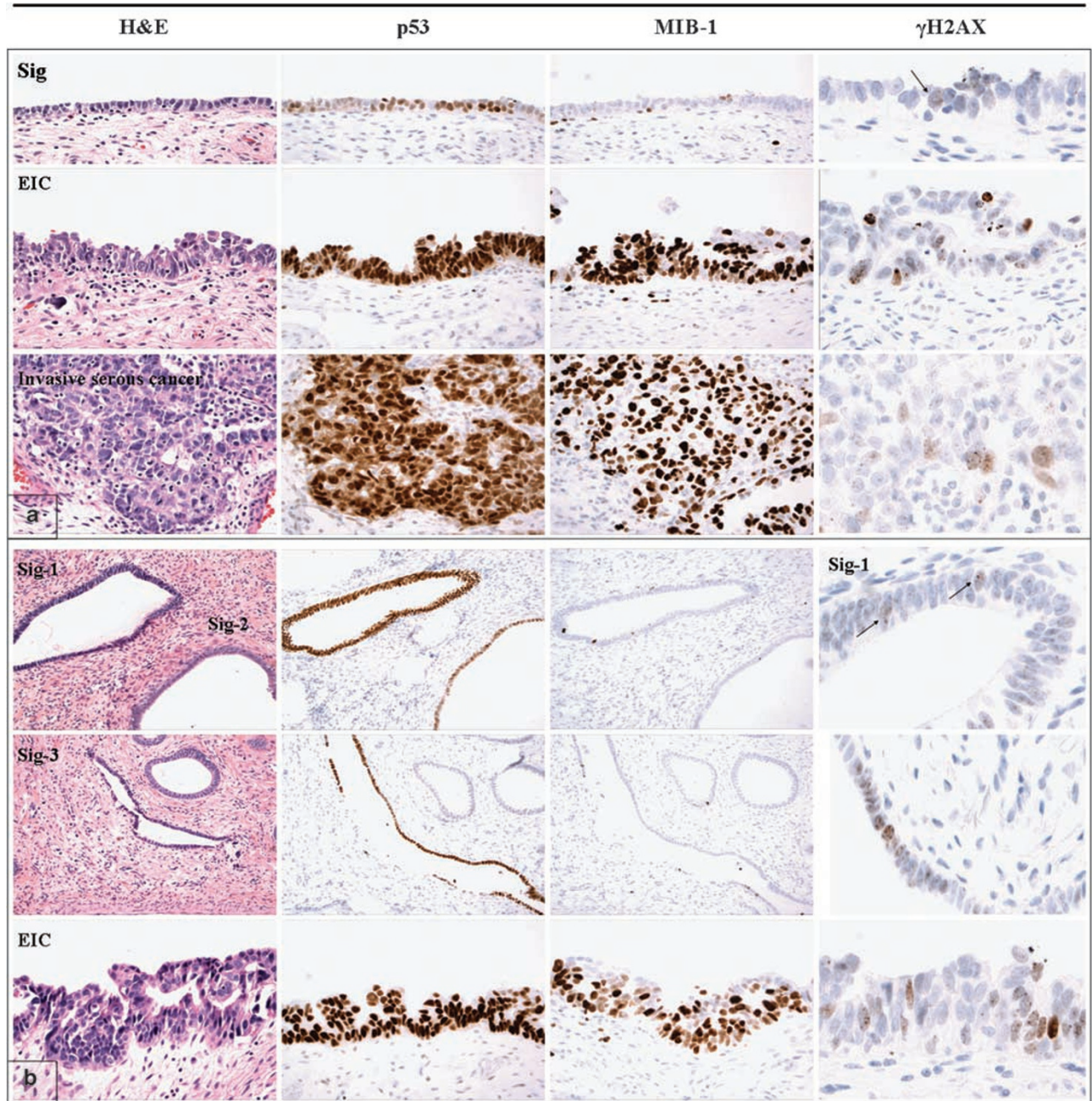
H&E	p53	MIB-1	γH2AX

Figure 2 Immunoprofile of matched endometrial p53 signatures and serous cancers in two cases (**a** and **b**). In both cases, the p53 signatures share intense nuclear p53 staining with the serous cancers. The proliferative indices of the p53 signatures (evidenced by MIB-1 staining) are consistently lower, however, than either serous endometrial intraepithelial carcinoma or the invasive cancer. Evidence of DNA damage (punctate nuclear γ-H2AX staining) is seen in the p53 signature as well as in the serous cancers.

carcinomas, including one case with three signatures. In one of these cases, an invasive serous cancer was present in which no *p53* mutation was found. The morphologic features and immunoprofiles of these two cases are illustrated in Figure 2. In a third case, a p53 signature was found to harbor a different mutation from the concurrent invasive serous cancer. There were no mutations found in the normal (control) epithelium in any of the three cases.

Discussion

The cause of uterine serous carcinoma is not clear but it has been associated with several pathologic entities, including a preexisting endometrioid carcinoma, serous intraepithelial carcinoma and endometrial glandular dysplasia.[4,7,8,21] One candidate precursor, termed endometrial glandular dysplasia, is linked to p53 mutations but does not stain diffusely for p53.[5,6,21] Thus its relationship to the
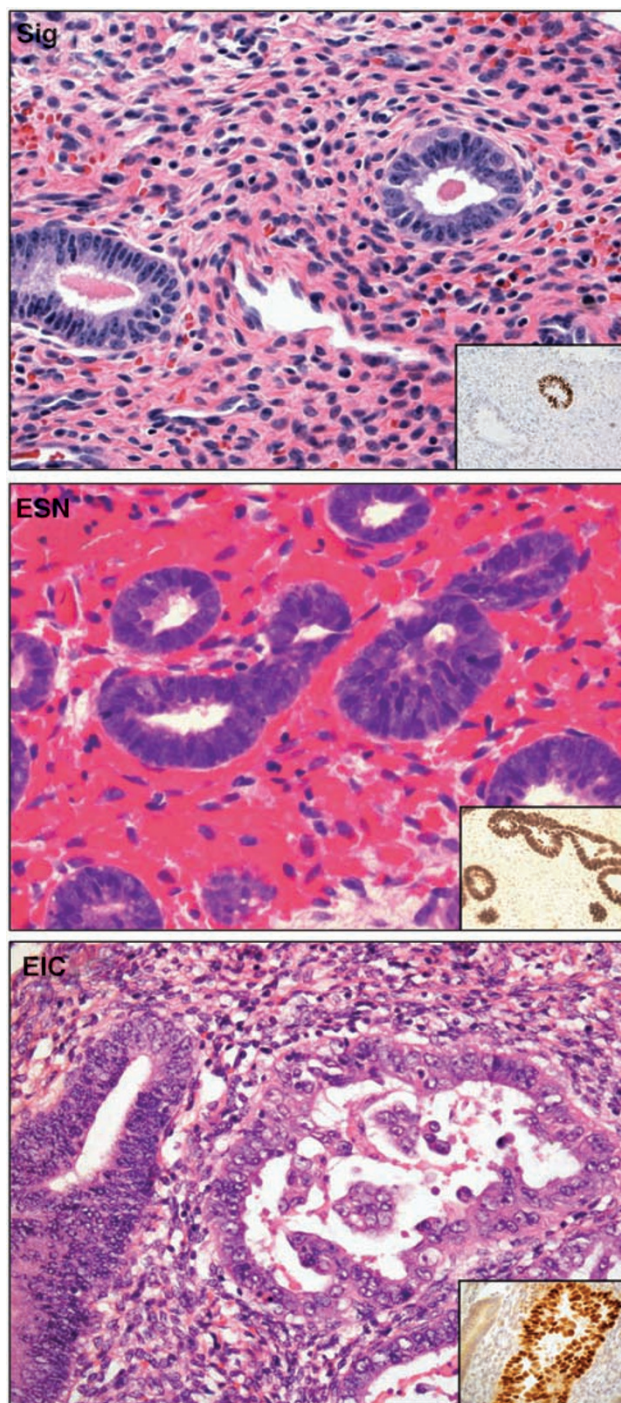
Figure 3 Three components of a possible serous carcinogenic sequence in the endometrium, including the p53 signature (Sig), a precancerous condition (early serous neoplasia or ESN) and serous endometrial intraepithelial carcinoma (EIC).

Table 2 *p53* mutations in endometrial p53 signatures and serous cancers

Case	Epithelium	p53 mutation	Mutation effect
3	Normal	None	
	EIC	818G>A	Arg to His
	p53 signature 1	818G>A	Arg to His
	p53 signature 2	818G>A	Arg to His
	p53 signature 3	818G>A	Arg to His
8	Normal	None	
	Invasive serous cancer	None	
	EIC	961delA	Frameshift
	p53 signature	961delA	Frameshift
10	Normal	None	
	Invasive serous cancer	715A>G	Asn to Asp
	p53 signature	646G>A	Val to Met

EIC, endometrial intraepithelial carcinoma.

Recent reports have linked the p53 signature to serous carcinogenesis in the fallopian tube, highlighting a series of properties that this process has in common with serous carcinoma of the fallopian tube, including location in the distal fallopian tube, involvement of the secretory cell phenotype, strong immunostaining for p53, *p53* mutations, evidence of DNA damage (punctate staining for γ-H2AX), physical association with serous intraepithelial carcinoma and shared risk factors with ovarian cancer.[15–18] Before this report, a similar entity had not been described in the endometrium.

This report describes foci of p53-positive benign epithelium in the endometrium raising the possibility that an entity similar to the p53 signature develops in the endometrial surface lining. The strongest case can be made for the p53-positive epithelia seen adjacent to serous intraepithelial carcinoma. First, these foci are typically immunopositive for γ-H2AX, defined as punctate immunostaining that presumably signifies a response to DNA damage. Second, γ-H2AX is invariably present in serous carcinoma. Third, based on the p53 sequence data, some p53 signatures are genetically related to their malignant counterparts. However, it is important to emphasize that not all p53 signatures shared the same mutation with the corresponding malignancy, consistent with the fact that the two entities are not always biologically related. We have previously shown that multiple p53 signatures, each with a different *p53* mutation, can be found adjacent to serous carcinomas, suggesting that multiple early events can occur, with only a subset progressing to malignancy.[15]

On the basis of findings in this report and the existence of other pathways to uterine serous cancer, the p53 signature associates with, and probably accounts for, only a subset of uterine serous malignancies. It should be emphasized that this study focused on classic examples of early serous carcinoma, primarily serous intraepithelial carcinoma. On the basis of this report, a subset of serous

p53 signature is unclear. A serous precancer that contained diffuse p53 staining and nuclear hyperchromasia, but had not achieved the level of nuclear atypia or proliferation seen in serous intraepithelial carcinoma, might link the p53 signature to malignancy (Figure 3). However, although such lesions are encountered, they have yet to be fully characterized.

malignancies appears to be related etiologically to a p53 signature. Not studied was the greater spectrum of these tumors, included those with mixed serous and endometrioid phenotypes including those with abrupt or gradual transitions from endometrioid to serous differentiation. These tumors appear much less likely to arise within p53 signatures. Nevertheless, all of these pathways appear to share a common feature, which is the emergence of a *p53* mutation. The mutation may develop before morphologic atypia (p53 signature), associate with minor epithelial atypia (endometrial glandular dysplasia) or emerge from a preexisting malignancy previously lacking a *p53* mutation, resulting in biphasic tumor patterns. When serous intraepithelial carcinoma is encountered, the presence of endometrial glandular dysplasia, the p53 signature, or both, appears more likely, as shown in this report.

Although the p53 signature appears to be a common entity in the fallopian tube, its frequency in the endometrium remains unclear, as does the significance of the p53 staining seen in the benign polyps. In contrast to the p53 signatures described in the fallopian tube, the p53-positive foci in the endometrial polyps did not stain for γ-H2AX. Thus, the significance of such changes, when found in otherwise benign glands, remains to be determined.

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Disclosure/conflict of interest

The authors state no conflict of interest.

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