

## A candidate precursor to pelvic serous cancer (p53 signature) and its prevalence in ovaries and fallopian tubes from women with BRCA mutations

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### Abstract

**Background.** Early serous carcinomas predominate in the fimbria of women with BRCA mutations (BRCA+). An entity in non-neoplastic mucosa sharing several properties of early serous carcinomas – the “p53 signature” – has been described in the distal fallopian tube and proposed as a precursor to serous carcinomas. This study compared the prevalence of p53 signatures in ovarian cortical inclusion cysts (CICs) and fallopian tubes from BRCA+ women and explored their relationship.

**Design.** All tissues from 75 completely excised ovaries and tubes obtained during prophylactic surgery were studied by conventional microscopy, immunostaining for p53, and in selected cases,  $\gamma$ -H2AX (DNA damage). P53 signatures were defined as 12 or more consecutive p53-positive secretory cell nuclei. Their prevalence in fallopian tubes and CICs was recorded, compared to an existing database of consecutive women without a suspicion of BRCA+ or ovarian cancer, and correlated with the number of CICs.

**Results.** Tubal p53 signatures were detected in 29 of 75 cases (38%); 20 of 30 (66%) signatures examined were  $\gamma$ -H2AX-positive. One ovary contained a small  $\gamma$ -H2AX negative p53 signature on the ovarian surface; no p53 signatures were identified in CICs. The prevalence of BRCA+ p53 tubal signatures was similar to that of women with unknown BRCA status (38 v 33%). Presence of p53 signatures did not correlate with number of CICs.

**Conclusions.** p53 signatures were common in the fallopian tubes of BRCA+ women, were not identified in CICs, and did not correlate with the latter. The tubal p53 signature merits serious consideration as an important early event in serous carcinogenesis in BRCA+ women.

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**Keywords:** Ovarian neoplasms; BRCA; Serous carcinoma; Carcinogenesis; Fallopian tube neoplasms; Precancer; Precursor; p53

### Introduction

Approximately 25,000 women in the United States develop ovarian cancer each year, and over one-half die of their disease

[1]. The majority of these tumors are epithelial and have been traditionally presumed to arise from the ovarian surface epithelium (OSE). OSE has been defined as a modified mesothelium with Müllerian characteristics. One theory has held that a mesothelial to Müllerian transformation or “metaplasia” occurs in these cells, leading to Müllerian inclusions in the ovarian cortex. Another proposes that salpingeal epithelial cells are transported to the ovarian surface and give rise to inclusion

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cysts. In either model, inclusions resulting from these origins occasionally undergo neoplastic transformation, leading to a wide range of epithelial neoplasms in the ovary [2,3]. This model is most strongly supported by tumors that are indisputably ovarian in origin, including mucinous, endometrioid, and low-grade serous neoplasms. Many of these tumors are confined to the ovary when discovered and do not involve the ovarian surface. In contrast to the above tumors, serous carcinomas have a more widespread distribution when detected, including the involvement of peritoneal surfaces [4]. Although some are associated with endometriotic cysts, the usual presentation for these tumors is bilateral ovarian involvement, including the ovarian surface, hence their designation as ovarian surface epithelial tumors [4].

Although an origin in the OSE has been proposed for these tumors, little direct evidence has emerged to link high-grade serous carcinomas to this epithelium. This has been due in part to the extensive nature of most of these tumors, precluding assignment of a site of origin. Studies searching for early neoplasia in the ovarian surface epithelium have yielded conflicting results. Earlier studies reported focal p53 immuno-positive inclusion cysts in the ovaries of women with serous carcinomas, postulating an origin from these sites [5]. Similar changes were not found in the OSE of women with BRCA mutations [6]. Others have described dysplastic changes in the ovarian surface epithelium [2,7]. Other candidate sites of origin, including the peritoneum, have been proposed for cases with minimal ovarian involvement. The fallopian tube has been proposed as a primary site for a minority of serous carcinomas, with an estimated ovarian to fallopian tube serous cancer prevalence of 50:1.

Despite the low reported incidence of tubal serous carcinoma in the literature, the potential role of the fallopian tube in the pathogenesis of ovarian serous carcinomas has not gone unnoticed [8]. Recent studies of women with hereditary mutations in the BRCA1 and BRCA2 genes have reported detecting early carcinomas in the fallopian tube in a significant percentage of cases. Recently, Finch et al described a plausible origin in the fallopian tube for five of their seven cases, in the form of a non-invasive (intra-epithelial) carcinoma [9]. Medeiros et al reported that of five consecutive early carcinomas detected in BRCA+ women, all were in the fallopian tube, and were either in the fimbria or ampullary region, similar to other reports [10,11]. A follow-up study by Kindelberger et al reported that approximately one-half of carcinomas classified as ovarian serous neoplasms were associated with intra-epithelial carcinomas of the fimbria [12].

Because p53 mutations occur in over 80% of high-grade pelvic serous carcinomas and because they are associated with accumulation of p53 protein, efforts to identify the early steps in pelvic carcinogenesis have concentrated on identifying p53 immuno-positive ovarian cortical inclusions. One report described p53 positive inclusions in a small number of normal-appearing ovaries adjacent to advanced-stage ovarian cancers [5]. Another study reported a statistically significant increase in p53 staining of cortical inclusion cysts in prophylactically removed ovaries for increased carcinoma risk versus normal ovaries [13]. In contrast, another study systematically examined

the ovaries from BRCA1 positive women and failed to identify p53-positive inclusions [6].

Recently, we described an entity in the distal fallopian tube that shares many attributes with serous carcinomas, including strong p53 positivity, fimbrial location, secretory cell phenotype, evidence of DNA damage by localization of  $\gamma$ -H2AX, p53 mutations, and occasionally, direct continuity with intra-epithelial carcinoma (Table 1). This entity, termed the “p53 signature” has been proposed as an early (or latent) precursor to tubal and some pelvic serous cancers [14].

This study was designed to complement the above studies by expanding the number of fallopian tubes analyzed for p53 signatures in BRCA+ women, determine if p53 signatures were present in the ovaries of the same population, and determine if a relationship existed between the presence of p53 signatures and another possible risk factor for pelvic serous cancer, the cortical (Müllerian) inclusion cyst of the ovary (CIC).

## Materials and methods

### Case material

This study was approved by the institutional review board at Brigham and Women’s Hospital. Consecutive cases of prophylactic salpingo-oophorectomies for a hereditary mutation in the BRCA1 or BRCA2 gene accessioned between February 2005 and December 2006 were examined. Controls consisted of a consecutively analyzed series of women with unknown BRCA status who did not have ovarian cancer, as previously reported [14].

### Tissue processing and pathologic review

All fallopian tubes and ovaries were entirely submitted for histologic exam. Fallopian tubes were analyzed using a procedure for sectioning and extensively examining the fimbriated end (SEE-FIM protocol), as previously described [10]. The fimbria was amputated, sectioned lengthwise and submitted with cross-sections of the proximal tube at 2–3 mm intervals. Ovaries were sectioned at 2–3 mm intervals. All ovarian sections were reviewed and scored for the presence of cortical (Müllerian) inclusion cysts (CICs). The number of CICs was totaled for each ovary and a mean number for the entire group was computed.

### Immunohistochemistry for the p53 and $\gamma$ -H2AX antigens

A monoclonal antibody to p53 was used to localize p53 protein (OP43, Oncogene Science, Cambridge, MA) in all sections from the fallopian tubes and

Table 1

Evidence supporting the distal fallopian tube as a source of pelvic epithelial malignancy in women with heterozygous BRCA mutations

1. The distal fallopian tube is the dominant site for tubal carcinogenesis in all women irrespective of known genetic risk factors.[11]
2. Up to 100% of early carcinomas detected in prophylactic salpingo-oophorectomies from BRCA+ women have involved the distal tube. [9,10,19]
3. Identical p53 mutations have been confirmed in both early tubal carcinomas and remote tumors of the ovary in women with pelvic serous carcinoma. [12]
4. p53 positive tubal “dysplasias” have been described in BRCA+ women. [28]
5. A candidate precursor (p53 signature) sharing location (distal tube), p53 mutations, secretory cell type, and DNA damage with pelvic serous cancer commonly occurs in BRCA+ women (and controls).[14]
6. p53 signatures frequently co-exist with early tubal serous cancer in BRCA+ women and have been demonstrated in continuity with malignant epithelium. [14]
7. p53 signatures or other candidate precursors are rare in ovarian cortical inclusion cysts of BRCA+ women (current study).[6]

ovaries. A positive score required strong immunostaining obscuring nuclear detail. A requirement of at least 12 consecutive p53-positive secretory cell nuclei was arbitrarily imposed to exclude sporadic staining, which is common, presumably physiologic, and usually limited to no more than 2–3 consecutive nuclei in the tubal epithelium. Although a threshold of 12 nuclei was established as a minimum, when detected, a minority of p53 signatures were at this threshold (Fig. 1).

$\gamma$ -H2AX, the phosphorylated form of the core histone H2AX, localizes to the vicinity of and is recognized as a marker for double-stranded DNA breakage (Upstate Cell Signaling Solution, Charlottesville, VA; monoclonal JBW301, 1:200).  $\gamma$ -H2AX is phosphorylated by the ATM/ATR kinases at a unique serine residue (S139) in its C terminus at sites of DNA double strand breaks. [15,16] The antibody staining was performed on cases with p53 signatures to determine the presence of co-localizing staining patterns [17,18]. Punctate intranuclear staining was interpreted as a positive signal.

#### Statistical analysis

The following correlations were made: 1) frequency of p53 signatures in fallopian tubes and ovaries; 2) location in ovary versus tube, 3) location within ovary (CICs vs ovarian surface epithelium) or tube (fimbria versus tubal cross-sections); 4) comparison with women who did not have ovarian cancer or a history thereof; 5) evidence of DNA damage as determined by immunostaining for  $\gamma$ -H2AX; and 6) relationship to cortical inclusion cysts. We compared the mean number of CICs in women with versus without the p53 signature using the Student's T-test.

## Results

#### Material reviewed

Ovaries and fallopian tubes from 75 women were included in the study. The mean age of the population was 48 years with a range from 35 to 76. All ovaries and fallopian tubes were examined completely. Six cases harbored a malignancy, including two invasive and four intra-epithelial carcinomas. A

total of 486 and 605 tissue blocks from fallopian tubes and ovaries respectively from BRCA+ women were evaluated by immunohistochemical staining for p53. An average of 6.4 (range 2–13) and 8.2 (range 2–21) tubal and ovarian sections per case respectively were examined.

#### Frequency and location of p53 signatures in tubes and ovaries

Twenty-nine of the 75 BRCA cases (38%) harbored at least one p53 signature in the fallopian tube (Fig. 1). The prevalence of p53 signatures in the BRCA+ women (38%) is similar to that reported in women whose BRCA status is negative or unknown (33%) (Fig. 2) [14]. A total of 40 signatures were identified in the fallopian tubes; nine cases contained more than one p53 signature. Examples of p53 signatures are shown in Figs. 1 and 3. Of these 40 signatures, 30 occurred in the fimbriated end (75%) and 10 occurred in the proximal tube (25%). The average age of the women in the cases without p53 signatures (49 years) was similar to those with p53 signatures (48 years). Immunostaining of cortical inclusion cysts did not disclose any p53 signatures. One focus of moderate p53 positivity was identified on the ovarian surface in a cuboidal non-ciliated surface epithelium (Figs. 2 and 3).

#### Correlation with evidence of DNA damage ( $\gamma$ -H2AX)

Of the 30 signatures from BRCA+ women stained with  $\gamma$ -H2AX, 20 were positive with variable punctate nuclear staining pattern (66%). Examples of positive immunohistochemical results for  $\gamma$ -H2AX localization with p53 are shown in Fig. 3. The one p53-positive focus in the OSE was negative for  $\gamma$ -H2AX (Fig. 3).

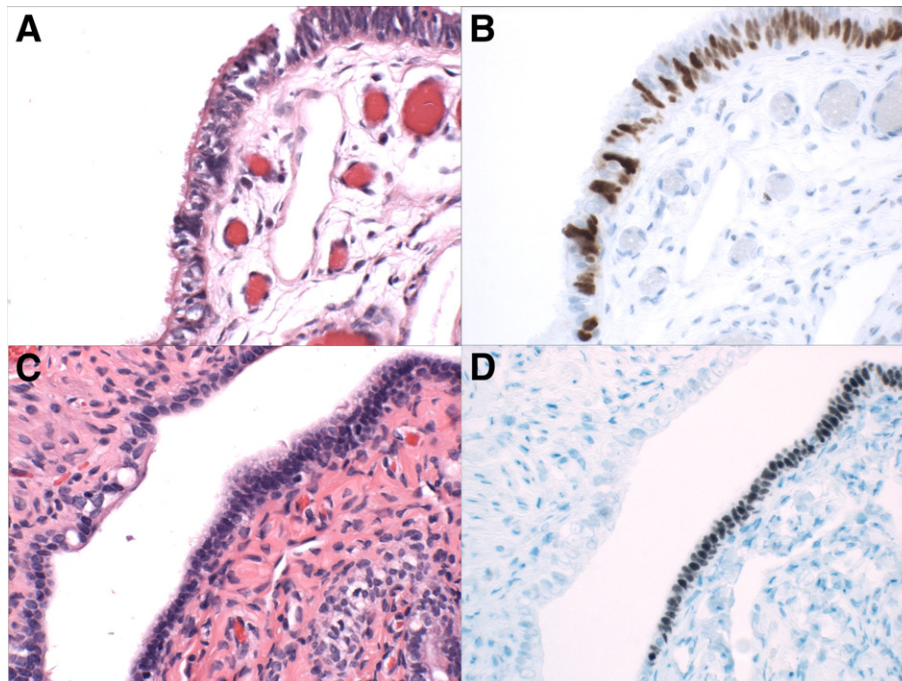


Fig. 1. Benign-appearing salpingeal epithelium (A and C) with strong nuclear staining for p53 (p53 signatures, B and D). Interrupted staining is due to the co-existing non-staining ciliated cells (B) in contrast to continuous staining (D) in a homogeneous population of secretory cells.

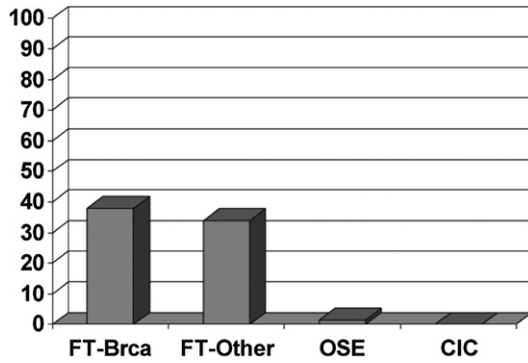


Fig. 2. Graphic depiction (from left to right) of the prevalence (percentage) of p53 signatures in tubes from BRCA+ women (FT-Brca), consecutive women untested for BRCA or negative for ovarian cancer from a prior study (FT-Other), [14], BRCA+ OSE (OSE), and BRCA+ ovarian CICs (CIC).

#### Correlation between p53 signatures and CICs

Overall, the average number of CICs identified per ovary was 6.6 (range 0–60). The differences in means between women

with and without signatures was calculated. Among women with the p53 signature, the mean number of CICs was 6.38, in contrast to 6.86 for women without p53 signatures, which was not statistically significant ( $p=0.87$ ).

#### Discussion

There is an emerging shift in the relative frequencies of ovarian and fallopian tube cancer in BRCA+ women that has been generated by studies of women undergoing prophylactic salpingo-oophorectomy [9–11,14,19]. In contrast to symptomatic BRCA+ women with ovarian cancer whose tumors have been classified as ovarian in over 90%, early cancers detected in the asymptomatic women have been attributed to the fallopian tubes in up to 100% of studies [19,20]. Supporting this origin has been a recently described entity in the benign tubal mucosa – the p53 signature – that shares several attributes with early serous carcinomas (tubal intra-epithelial carcinomas), including strong staining for p53, predominately distal (fimbria) tubal location, involvement of secretory cells, and evidence of DNA damage with frequent p53 mutations [14]. However, controversy exists

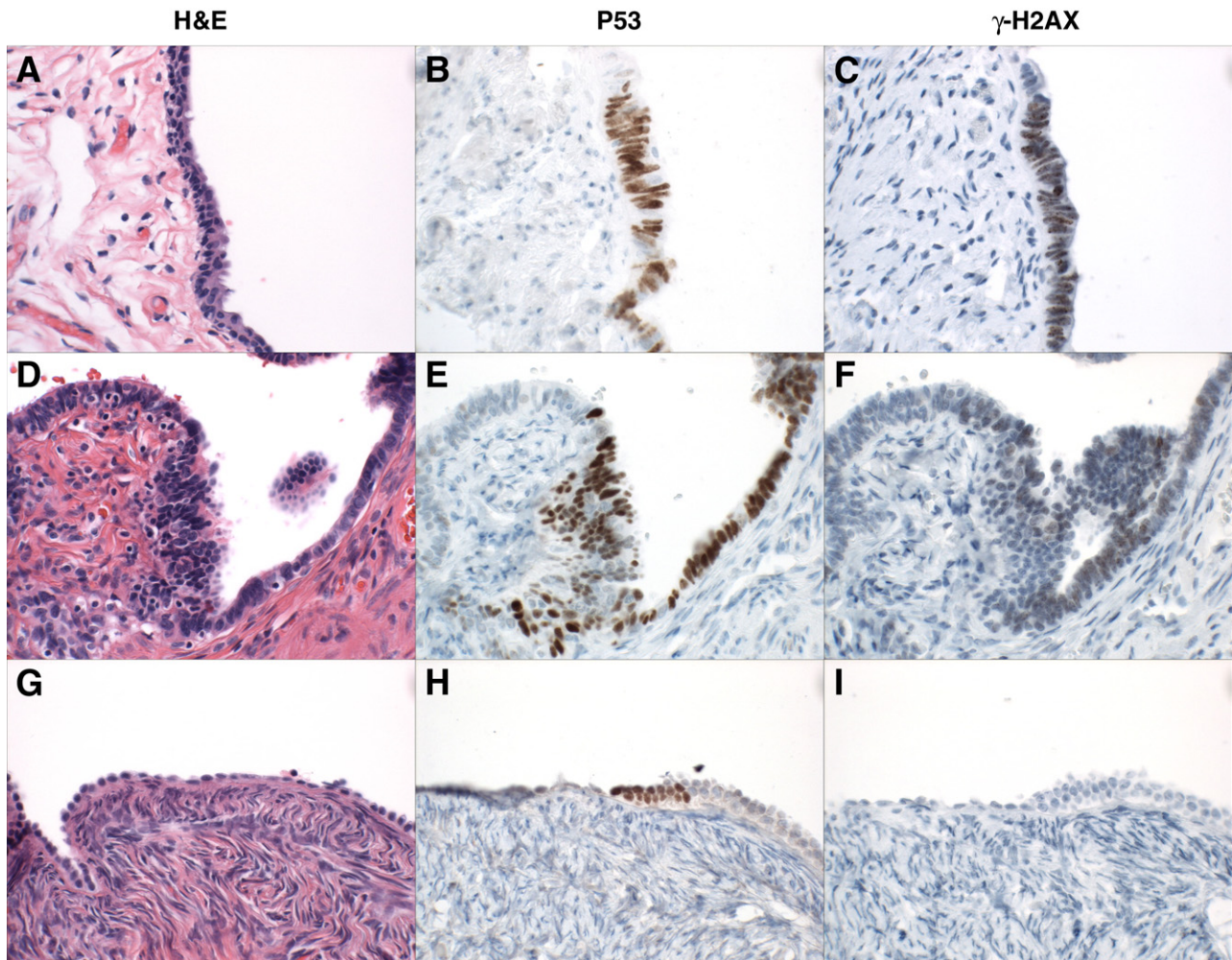


Fig. 3. Correlation of p53 signatures and  $\gamma$ -H2AX staining, a marker for DNA damage. Hematoxylin and eosin stained tube (A, D) and ovary (G), and respective p53 immunostaining (B, E, and H) and localization of  $\gamma$ -H2AX (C, F, and I). Note the lack of  $\gamma$ -H2AX localization in the ovary (I).

over the magnitude of cases of serous carcinoma deserving assignment to the tube versus ovary. This is the first study to systematically compare the prevalence of this candidate precursor lesion in Müllerian epithelium of the fallopian tube and ovary from the same patient population and to correlate the presence of CICs with p53 signatures in either site.

The frequency of the p53 signature in this series of BRCA+ fallopian tubes was very similar to that of consecutively accessioned fallopian tubes from women with neither a strong family history of ovarian cancer nor concurrent ovarian cancer described in an earlier report (Fig. 2) [14]. The similarity in frequency of p53 signatures in the two groups suggests that the initiation of DNA damage and p53 accumulation is independent of BRCA mutation status and may serve as a generic early event in the pathogenesis of serous carcinomas arising in the distal fallopian tube.

The degree to which CICs play a role in serous carcinogenesis in BRCA+ women has been controversial. There is no question that the Müllerian epithelium in OSE, CICs or endometriosis is directly or indirectly responsible for a range of mucinous, endometrioid and low-grade serous tumors. The degree to which BRCA status plays a role in the pathogenesis of these tumors is unclear, inasmuch as cancer risk in these patients is attributed primarily to the high-grade serous carcinomas [21]. However, in a recent study, we found that 2 of 7 presumed primary tubal carcinomas in women with BRCA mutations were of the endometrioid type, suggesting that endometrioid carcinogenesis is enhanced, if to a lesser degree [19]. Investigators have focused principally on p53 mutations in CICs as an early event. A prior report has described p53 positive CICs adjacent to ovarian carcinomas [5]. A recent study has also implicated involvement of ovarian inclusions in this pathway by the discovery of p53 positive benign epithelia, often in concert with ovarian cancers [22]. However, p53 positive CICs were not documented in another study of a similar population [6]. The findings in this study are consistent with the last study; only one p53 positive focus was detected on the ovarian surface and it was not associated with immunohistochemical evidence of DNA damage. No CICs contained p53 signatures.

Although we and others have not demonstrated a compelling scenario of p53 mutations in CICs, the role of the OSE in serous carcinogenesis remains an important, if unproven, concept. Animal models of ovarian epithelial carcinogenesis have focused on genotoxic damage imposed by ovulation, in which OSE cells in the proximity of the ovulatory event undergo DNA damage [23]. A similar scenario would explain the localization of p53 signatures and serous carcinomas to the distal fallopian tube, where the potential genotoxic impact of ovulation would be highest [14]. In this study, we found only one focus of p53-positive epithelium in the ovarian surface, but the number could be underestimated if OSE were lost during tissue processing, as is often the case. Another report described focally intense p53 staining in the OSE of 14% of prophylactic specimens from BRCA+ women in contrast to none of controls, and linked it to expression of BTAK, which is over-expressed in immortalized human OSE cells and a high percentage of ovarian cancers [24,25]. Whether this staining pattern has biologic significance is

unclear, inasmuch as we did not see evidence of DNA damage in the one p53-positive focus identified in the OSE in this study, and the p53 mutational status of these foci is unknown. Nevertheless, the experimental evidence supports a role of the OSE in the development of ovarian cancer. While a distal tubal origin can be implicated in nearly one-half of “ovarian” serous carcinomas, the origin of nearly one-fourth of these tumors – surface carcinomas without either an obvious intra-parenchymal ovarian source or endosalpingeal involvement – remains unresolved and could be explained by an origin in the OSE [12].

The recent studies implicating the fallopian tube in serous carcinogenesis and the presence of a candidate precursor in this site provide a compelling endorsement for further research to resolve the risk factors involved in this model of serous carcinogenesis. The high frequency of p53 signatures is in keeping with most precursor models, in which the early events are far more common than cancer outcomes [26]. The overall risk of ovarian cancer has been reported as high as 60% in BRCA+ women, and the odds of detecting an early malignancy in prophylactic specimens approximately 6 percent [27]. The high frequency of p53 signatures in fallopian tubes from all women, combined with the much higher risk of cancer in BRCA+ women, suggests that a germline BRCA mutation may serve as a promoter, enhancing the risk of transition from a p53 signature to malignancy. However, further studies are needed to determine if p53 signatures represent more than one biologic entity. In a prior study, we found that 8 of 14 p53 signatures contained p53 mutations, an approach that is complicated by the small amount of target tissue and low recovery of DNA. In this study, immunostaining for  $\gamma$ -H2AX was employed as a surrogate marker for DNA damage, a feature characteristic of serous carcinomas, with 66% of cases scoring positive. We are currently investigating the possible role of immunostaining with this marker to predict the presence of a p53 mutation.

The strength of association between the distal fallopian tube and serous carcinogenesis is a compelling endorsement for an epidemiologic analysis of the p53 signature, in women both with and without genetic risk factors for pelvic serous cancer (Table 1). Studies that systematically catalogue the frequency of p53 signatures and divine their epidemiologic correlates in these populations will clarify the role of this entity as a surrogate marker for ovarian cancer risk and shed light on its causation.

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