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The impact of tissue block sampling on the detection of p53 signatures in fallopian tubes from women with BRCA 1 or 2 mutations (BRCA+) and controls

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The tubal p53 signature is a putative precursor to pelvic serous carcinoma, but its frequencies in women with inherited mutations in the BRCA1 or BRCA2 genes (BRCA+) and controls has been controversial. An initial section and two levels (100–200 μm) from every block in BRCA+ (24) and control tubes (40) were stained for p53. The frequency of p53 signatures was computed between the populations and across the three levels from each block, and analyzed by Fisher exact test. A total of 17 (71%) BRCA+ and 20 (50%) control tubes were p53 signature positive ($P=0.12$); 21 and 16% of all tissue blocks sectioned harbored signatures ($P=0.29$), and 76 and 67% were found in the fimbria. In 49 and 32% of p53 signature positive cases in the two groups, the p53 signatures were not discovered until the second or third round of sectioning. In all, 38 and 40% of BRCA+ and control subjects harbored p53 signatures in more than one focus in a single block. In one case (BRCA+), a highly atypical proliferation was identified in one serial section. The p53 signatures are more common than previously reported and the frequency of detection increases as a function of sectioning through the tissue block, both in absolute frequency and in numbers of p53 signatures detected in a given block. There is a trend for a higher absolute frequency of p53 signatures (71 *vs* 50%; $P=0.12$) in BRCA+ subjects, but this is not reflected in a greater average number of p53 signatures or positive blocks per case. This study underscores the importance of systematic immunohistochemical examination of fallopian tubes when conducting epidemiological studies that compare the frequency of p53 signatures in different populations. Attention to this detail is critical when exploring risk factors germane to early serous carcinogenesis.
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In recent years, the pathogenesis of high-grade serous carcinomas of the ovary has come into sharper focus with the realization that a significant subset could arise in the distal fallopian tube.[1–4] Concurrent with this finding has been the description of a carcinogenic pathway in the fimbria that incorporates two entities, serous tubal intraepithelial carcinomas and a precursor termed the p53 signature.[5–7] Serous tubal intraepithelial carcinomas have been shown to be the earliest phase of serous carcinoma detected in ~80% of

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cancer-positive risk-reducing salpingo-oophorectomies in asymptomatic women with BRCA1 or BRCA2 mutations (BRCA+).[2,4] Moreover, intraepithelial carcinomas have been reported in association with from one-third to nearly one-half of high-grade serous carcinomas formerly classified as either ovarian or peritoneal in origin.[8,9] On the basis of the latter association, the possibility that a significant minority of high-grade pelvic carcinomas arise from the fallopian tube is now being addressed in the field of ovarian cancer research.[10,11]

The p53 signature is defined as a linear, strongly p53 immunopositive segment of tubal cells spanning at least 12 consecutive secretory cell nuclei.[5] It may coexist in continuity with carcinoma, and studies have shown that it shares with the latter an origin in the secretory cell, p53 mutations, fimbrial location, evidence of DNA damage, and in one study, an association with decreased parity.[12] Others have reported additional associations, including a negative relationship to oral contraceptive use.[13] The epidemiological connections are particularly intriguing because they imply an opportunity to explore the factors that may be operative in the earliest steps in serous carcinoma development.

In the original report by Lee *et al*,[5] and follow-up studies by Folkins *et al*[14] and Saleemuddin *et al*,[12] the frequency of p53 signatures in both BRCA+ subjects and controls was estimated to be between 35 and 40%. In a report by Shaw *et al*,[11] the rate was estimated to be between 23 and 25%. In both studies, the frequency of p53 signatures in BRCA+ women and controls was similar; however, although Saleemuddin *et al*[12] noted an association among p53 signatures, low parity and low basal metabolic index (BMI), Shaw *et al*[13] reported an inverse association with oral contraceptives and a direct association with BMI. Whether these differences reflect the populations studied, nature of the data collected or sampling is unclear. However, the accuracy of any study depends heavily on the uniformity and degree of tissue sampling.

The purpose of this study was to ascertain the effect of serial sectioning on the frequency of detection of p53 signatures. A series of BRCA+ cases and controls were selected and initial sections followed by two additional levels were obtained and immunostained. We report that the frequency of p53 signature detection is significantly increased with serial sections and that the number of positive cases is greatly influenced by this approach.

Materials and methods

This study was approved by the institutional review board (IRB) at the Brigham and Women's Hospital. Consecutive cases of risk-reducing salpingo-oophorectomy from asymptomatic BRCA+ women and bilateral salpingectomy from women undergoing hysterectomy for uterine leiomyomata were selected for analysis. In all cases, the fallopian tubes were submitted for histological examination in their entirety using the SEE-FIM protocol, as previously described.[15] In each case, one to four serial sections were taken from the tissue block. Then, three sections each were taken at 100–200 μm intervals, producing an initial section and two levels for analysis. Each level was immunostained for p53 using a monoclonal antibody to p53, targeting an epitope in amino acids 21–25 of the protein (OP43; Oncogene Research Products, San Diego, CA, USA) as previously described. Criteria for p53 signatures were the same as described previously, consisting of 12 or more consecutively strongly (that is, obscuring the nuclear chromatin detail) p53 staining secretory cell nuclei (Figure 1).

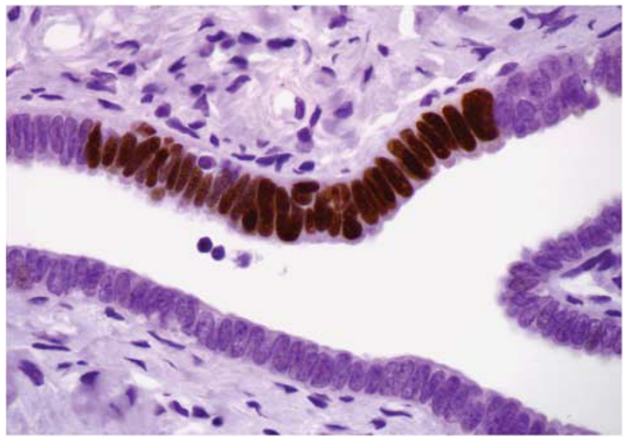

Figure 1 A p53 signature, seen here as linear staining of over 12 consecutive secretory cell nuclei.

Frequencies of p53-positive cells were computed for each set of sections in each group and differences in frequency were analyzed by the Fisher's exact test. The following parameters were analyzed and compared: (1) overall frequency of p53 signatures in each group; (2) relative number of tissue blocks scoring positive; (3) proportion positive in levels 1, 2 and 3; and (4) number of cases in which multiple sections scored positive. Evidence of any malignancy was also recorded if unexpected.

Results

A total of 24 BRCA+ and 40 control subjects were included in the study. Mean ages for each group were 49 (37–73) and 51 (42–76) years, respectively. Using age $\geqslant$50 years as a cutoff, the difference in frequency of p53 signatures under or over the age cutoff in either group or the groups combined was not significant (*P*-values 0.65–1.0; data not shown). An average of 5.8 and 3.9 tissue blocks were evaluated per patient from cases and controls, respectively. Table 1 summarizes the frequencies of each parameter in the case and control groups.

Table 1 Summary data for p53 signature frequencies in BRCA+ subjects and controls

Parameter	*Control*	*BRCA*	*Combined*
Total cases	40	24	64
Positive cases	20	17	37
Negative cases	20	7	27
P-value		0.12	
Total blocks	155	138	293
Positive blocks	25	29	54
Negative blocks	130	109	239
P-value		0.29	
p53S+ (level 1)	17	15	32
p53S+ (level 2)	8	6	14
P53S+ (level 3)	0	8	8
P-value		0.27	
p53S+ in multiple sections	10	11	21
p53S+ in two sections	8	8	16
p53S+ in three sections	2	3	5
P-value		0.62	
Fimbrial location*	67% (12/18)	76% (22/29)	72% (34/47)

Abbreviation: p53S+, p53 signature present.
P-values are calculated by the Fisher's exact test.
*Percent computed from the analysis of 18 control and 29 BRCA+ tubes.

A total of 17 (71%) and 20 (50%) of BRCA + and control tubes, respectively, harbored p53 signatures after the sectioning protocol outlined above ($P = 0.12$). Of all the tissue blocks analyzed in the cases and control groups, 21 and 16% harbored signatures, respectively. In the cases in which location could be determined, the majority, that is, 76 and 67%, were located in the fimbria (Table 1), a finding similar to a previous study from this group.[5]

Additional sections (second and third levels) had a dramatic effect on the frequency of blocks scoring positive for p53 signatures. In the cases, 15 blocks were initially positive, followed by an additional 6 (40% increase) and 8 (53% increase) in the second and third rounds of sectioning, respectively, effectively doubling the number of blocks that scored positive in the case group. In addition to the 17 blocks scoring positive in the first round in the control group, and additional 8 (49% increase) were detected in round two. Overall, 49 and 32% of positive blocks were detected in the two groups in the second or third round of sectioning, respectively. In all, 38 and 40% of BRCA + and control subjects harbored p53 signatures in more than one focus in a single block. Examples of the findings in different levels are illustrated in Figure 2. One BRCA + case harbored an epithelial atypia bordering on tubal intraepithelial carcinoma that was discovered before the study. No other cases were discovered during the study while the sections were being examined.

As Table 1 illustrates, although the percentage of cases scoring positive for at least one p53 signature was higher than that of the controls (70 *vs* 52%), a larger number of blocks per case was analyzed relative to controls. When the frequency of p53 signature detection per block was computed, the difference between the two groups was not significant.

Discussion

In most models of epithelial carcinogenesis, the precancerous lesion is a common phenomenon.[16,17] This reflects the fact that early steps in the pathway to malignancy are relatively frequent, but the combination of all the necessary steps rarely converges on a single cell. When it does, malignancy ensues. Human papillomavirus type 16 infection is common in the population, as is the precursors with which it is associated. However, corresponding malignancies are much less common, even in unscreened populations, reflecting the need for additional steps to bring about invasive cancer.[16] Similarly, PTEN mutations are frequent in the endometrial lining of asymptomatic women, affecting nearly one-half of them.[17] However, only a subset of these develop endometrial intraepithelial neoplasia, and overall, only 2–3% of the women develop endometrial cancer. These tumorigenic pathways require additional alterations in gene function for progression to take place, in keeping with established concepts of human carcinogenesis.[18]

The fallopian tube model for pelvic serous carcinogenesis involves the p53 signature, which like its counterpart in the endometrium, is a 'latent precursor'.[5,12] The p53 signatures are presumably early phenomena, as evidenced by their lack of proliferative activity and close resemblance to normal salpingeal epithelium. Nevertheless, they possess multiple features in common with tubal intraepithelial carcinomas and have been demonstrated on multiple occasions in continuity with, and sharing the same p53 mutations with, early tubal malignancies.[5,9] At this point, the p53 signature has no diagnostic significance to the practicing pathologist other than to be distinguished from tubal intraepithelial carcinomas; its value lying in its potential role as in early pelvic serous carcinogenesis.[12,13] In this context, the p53 signature is an attractive target for studying potential and unique risk factors that might influence the initial steps in serous carcinogenesis, as shown in recent preliminary studies.[12,13] The attraction of such studies lies in the novel association of pathobiology and epidemiology, wherein a specific set of early molecular events can be juxtaposed with epidemiological variables. The attraction lies in the opportunity to tease apart the variables associated with each step in the carcinogenic process. However, because studies

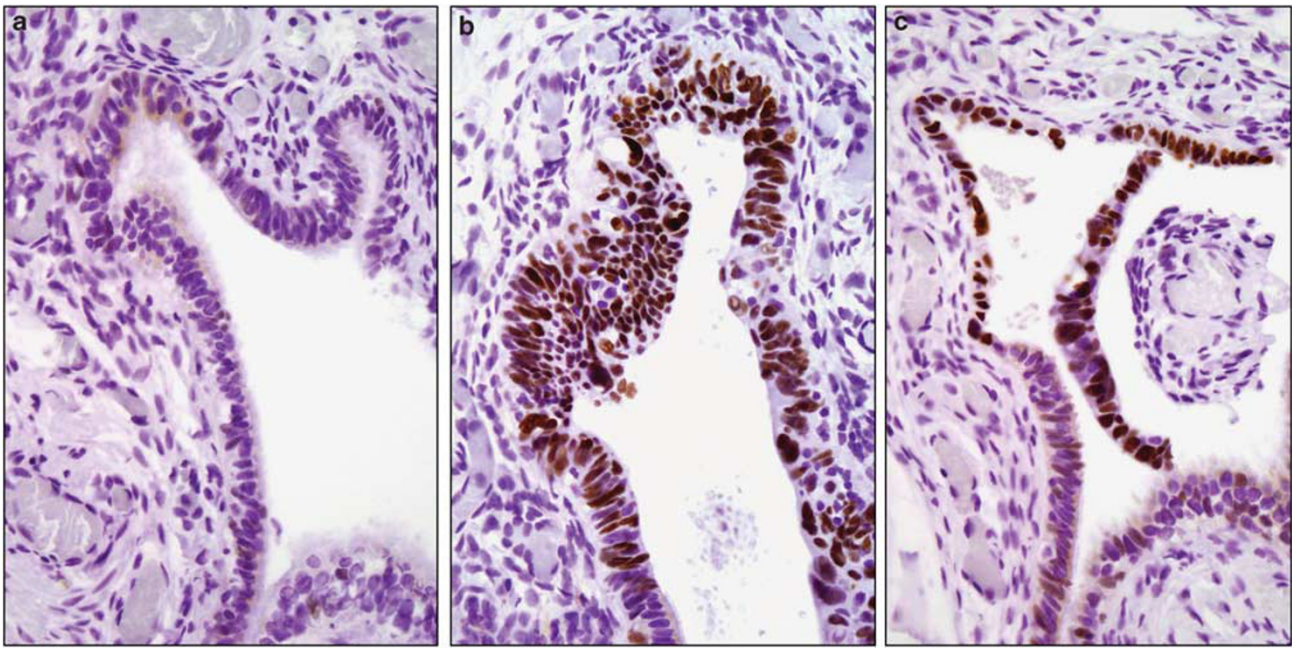

Figure 2 An initial (**a**) and serial levels (**b** and **c**) at 100–200 μm from a BRCA + fallopian tube immunostained for p53. The initial section is negative, followed by a p53 signature in the next level. The last level (**c**) retains some p53 staining.

of this type target very small precursor conditions, they are particularly prone to sampling error.

In this study, we attempted to be as equitable as possible in our handling of both cases and controls, using the SEE-FIM protocol for each. In this protocol, the entire tube was analyzed and the distal portion was sectioned longitudinally to maximize the amount of epithelium analyzed. We found that our previous estimation of p53 signatures in the population was an underestimation. In contrast to our previous finding of p53 signatures in 37 and 33% of BRCA + women and controls, the frequencies following an additional two levels of stained sections increased to 71 and 50%, respectively.[5,14] The frequency of detection increased as a function of sectioning through the tissue block, both in absolute frequency and in numbers of p53 signatures detected in a given block. The differences between the two populations were not significant, although there was a trend toward a higher frequency in the BRCA + women ($P = 0.12$). Importantly however, this difference was not reflected in a significantly greater number of p53 signatures or positive blocks per case. In other words, the mean number of p53 signatures found was not different and there was no evidence that BRCA + women were more susceptible to multiple signatures relative to the control population.

This study had several limitations. First, the areas of epithelium examined in each section in each case were not standardized, such that a ratio of p53 signatures detected per unit area analyzed was not computed. This is difficult to document, given the convoluted surface area of the tubal plica. Nevertheless, all fallopian tubes were sectioned under the same protocol and ample fimbrial tissue was available for review in the cases. Importantly, the findings were not markedly different in terms of comparative frequency in p53 signatures between the two populations, as shown in other studies. Another limitation was the relatively fewer blocks per case examined in the control group (3.9) *vs* the cases (5.8). However, the percentages of blocks positive in the two groups were 16 and 21, respectively, implying a comparable rate of positivity per unit of tissue examined.

The final limitation was that we were not able to calculate the risk factors for the p53 signatures under this new protocol, as an IRB (or funding) was not in place to conduct this study. As larger studies are conceived, attention to these limitations will be important, as well as ensuring that the fallopian tubes are examined as thoroughly as possible.

The deeper sections taken in this study did not uncover any additional neoplastic proliferations (tubal intraepithelial carcinomas) in either the BRCA + or control subjects. However, we have seen instances in which a third level of sections revealed a previously unappreciated malignancy and others have reported occasional discovery of new lesions, although uncommon.[15,19] Whether the optimal approach is multiple step sections through the tissue block or liberal fine sectioning of the fimbria and ampulla at the time of gross exam remains to be determined. However, the exceedingly small size of both serous cancer precursors and early serous cancers in the distal tube will mandate thorough examination in studies seeking to determine incidence and origin of pelvic serous cancer.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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