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Original Paper

A candidate precursor to serous carcinoma that originates in the distal fallopian tube

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Abstract

The tubal fimbria is a common site of origin for early (tubal intraepithelial carcinoma or TIC) serous carcinomas in women with familial *BRCA1* or *2* mutations (BRCA+). Somatic *p53* tumour suppressor gene mutations in these tumours suggest a pathogenesis involving DNA damage, *p53* mutation, and progressive loss of cell cycle control. We recently identified foci of strong p53 immunostaining — termed 'p53 signatures' — in benign tubal mucosa from BRCA+ women. To examine the relationship between p53 signatures and TIC, we compared location (fimbria vs ampulla), cell type (ciliated vs secretory), evidence of DNA damage, and *p53* mutation status between the two entities. p53 signatures were equally common in non-neoplastic tubes from BRCA+ women and controls, but more frequently present (53%) and multifocal (67%) in fallopian tubes also containing TIC. Like prior studies of TIC, p53 signatures predominated in the fimbriae (80–100%) and targeted secretory cells (HMFG2+/p73−), with evidence of DNA damage by co-localization of γ-H2AX. Laser-capture microdissected and polymerase chain reaction-amplified DNA revealed reproducible *p53* mutations in eight of 14 fully-analysed p53 signatures and all of the 12 TICs; TICs and their associated ovarian carcinomas shared identical mutations. In one case, a contiguous p53 signature and TIC shared the same mutation. Morphological intermediates between the two, with *p53* mutations and moderate proliferative activity, were also seen. This is the first report of an early and distinct alteration in non-neoplastic upper genital tract mucosa that fulfils many requirements for a precursor to pelvic serous cancer. The p53 signature and its malignant counterpart (TIC) underline the significance of the fimbria, both as a candidate site for serous carcinogenesis and as a target for future research on the early detection and prevention of this disease.

Keywords: BRCA; p53; fallopian tube neoplasms; serous carcinoma; ovarian neoplasms; fimbria

Introduction

Ovarian cancer is diagnosed in approximately 22 200 women yearly in the United States and causes approximately 16 210 deaths, with a worldwide incidence and mortality of 190 000 and 114 000 respectively [1,2]. The pathogenesis of ovarian cancers is diverse. One pathway entails multiple genetic events paralleling a histological progression from benign to malignant, including borderline and low-grade malignancies and endometrioid carcinomas. Defects in mismatch repair (microsatellite instability) and mutations in *KRAS/BRAF, beta catenin*, and *pTEN* characterize this pathway. The second involves mutations in the *p53* tumour suppressor gene, a rapid progression to malignancy, and characterizes serous carcinomas [3,4]. Because they involve serosal surfaces with rapid peritoneal spread, serous carcinomas are the most lethal form of epithelial ovarian cancer [5–7].

Experimental data support an origin in the ovarian surface epithelium, and some prior reports identified focal accumulations of p53 protein in cortical ovarian inclusion cysts and tubal mucosa of women with, or at risk for, ovarian carcinoma. However, a precursor to high-grade serous ovarian cancer has not been demonstrated and universally accepted [8–13]. Three recent studies localized both BRCA+ and sporadic (non-familial) tubal carcinomas to the fimbria

[14–16]. Protocols using systematic analysis of the fimbriated end have associated tubal intraepithelial carcinoma (TIC) with many peritoneal and ovarian serous carcinomas [17].

DNA damage results in activation of specific response pathways, and the coordination of cell cycle arrest requires a functional p53 protein. The relationship between DNA damage and subsequent *p53* mutations is unknown, but one study has proposed that the former exerts a selective pressure on the latter, ultimately leading to loss of cell cycle control [18,19]. Mutations in *p53* commonly occur in cancer but may be seen earlier in both preinvasive neoplasia and clonal epithelial expansion [20,21]. In a recent analysis of fallopian tubes from BRCA+ women, we discovered small segments of strongly p53-positive, benign-appearing epithelium. This study sought to determine if these alterations in p53 staining, which we termed 'p53 signatures' shared other attributes with early tubal cancer.

Methods

Overall study design and rationale

The institutional review board at Brigham and Women's Hospital approved the study. The first goal was to determine the prevalence of p53 signatures in three different populations. The second was to determine if p53 signatures shared certain qualities (in addition to p53 staining) with TIC, including tubal location, tubal cell type, evidence of DNA damage, and *p53* mutations.

Patient selection

Subjects consisted of consecutively treated women in three groups, including: (1) 41 women with *BRCA1* or *2* mutations (BRCA+) undergoing prophylactic salpingo-oophorectomy; (2) 58 consecutive women undergoing procedures for other pelvic conditions, including uterine leiomyomata, cervical and uterine malignancies, and benign ovarian tumours; and (3) 17 women with TIC, with or without ovarian or pelvic involvement [17].

Histological analysis of fallopian tubes

Patients in all groups were eligible for inclusion if both fallopian tubes were entirely removed and submitted for histological analysis. The fimbriae were extensively sectioned by a protocol that extensively examined the fimbriated end (SEE-FIM protocol) [14,15]. All sections were stained with haematoxylin and eosin and examined histologically.

Immunohistochemistry

All sections of each fallopian tube were stained and the following procedures carried out:

(1) A monoclonal antibody to p53, targeting an epitope in amino acids 21–25 of the protein, was used to localize p53 protein (OP43; Oncogene Research Products, San Diego, CA, USA). A positive score required strong nuclear staining (obscuring nuclear detail) for at least 12 consecutive nuclei, which excluded common and presumably physiological staining that is usually limited to no more than two to three consecutive nuclei in the tubal epithelium. Virtually all p53 signatures exceeded this threshold by a few to hundreds of cells.
(2) A marker of proliferation (MIB1 corresponding to Ki-67; M7240; DAKO, Carpinteria, CA, USA) was used to determine the proliferative activity of p53 signatures and to distinguish them from TICs, which are highly proliferative [22]. A positive score for MIB1 required intense nuclear staining in over 80% of the nuclei in at least a portion of a given p53 signature [15].
(3) Selected cases were analysed further for antibodies to localize the following proteins:
    - Antibodies to biomarkers distinguishing ciliated (p73, LhS28) from secretory (HMFG2) cell phenotypes (McKeon F, unpublished data) were used to determine if p53 signatures shared the same cell phenotype as TIC [23,24].
    - Staining for γ-H2AX, the phosphorylated form of the core histone H2AX that localizes to the vicinity of, and is recognized as a marker for, double-stranded DNA breakage (Upstate Cell Signaling Solution, Charlottesville, VA, USA; monoclonal JBW301, 1 : 200) was compared between p53 signatures and TICs. H2AX is phosphorylated by the ATM kinase at a unique serine residue (S139) in its *C*-terminus at sites of DNA double-strand breaks [25–27]. Punctate intranuclear staining was interpreted as a positive signal.
    - Cyclin E, the over-expression of which can promote unscheduled S phase entry and enhance genomic instability, was also compared [28]. Cyclin E staining was scored as weak (<25% of cell nuclei), moderate (25–50%) or strong (>50%).

Antigen–antibody complexes were localized with the Envision technique using peroxidase and diaminobenzidine as the reporter, as previously described [14]. Two observers screened all of the slides after p53 staining. Discrepancies in interpretation of p53 signatures were resolved by group review.

Analysis for *p53* mutations

Some p53 signature-positive foci and intraepithelial carcinomas were analysed for *p53* mutations. In all cases, normal mucosa from the same tissue served as a control. When present, a remote tumour site, either ovarian or peritoneal, was also selected for analysis (Table 2). Laser-capture microdissection isolated p53 signatures and control somatic DNA

from selected cases using the PALM microbeam instrument. Genomic DNA was amplified by polymerase chain reaction (PCR), using tailed primers designed to amplify exons 2, 3, 5–9, and 11 of *p53*. A secondary amplification was performed using T3 and T7 primers specific to the tail sequence used in the primary amplification. PCR products were then sequenced from both strands using T3 and T7 primers. Data were analysed using the Mutation Surveyor programme (Soft Genetics, State College, PA, USA). Candidate mutations found by the software were compared to a reference database for cancer-associated *p53* mutations (Universal Mutation Database, http://www.umd.be:2072/IFAMTP53A.shtml [accessed on 25 October 2006]).

Because formalin fixation can introduce spurious mutations into somatic DNA, all *p53* mutation-positive exons were re-sequenced from a replicate PCR-amplified product [29]. Samples were scored as *p53* mutation-positive only if an identical mutation was identified in products of both amplifications.

Analysis of data

The following attributes of the p53 signatures were analysed and, where appropriate, compared with normal epithelium and TIC:

- Frequency in the three patient groups
- Number found in each tube pair
- Side (right or left) involved
- Location (fimbria vs proximal tube)
- Epithelial phenotype (ie secretory vs ciliated)
- *p53* mutation status, in a subset
- Localization patterns/frequency of γ-H2AX and cyclin E.

Results

p53 signatures are common in non-neoplastic fallopian tubes, irrespective of BRCA status: p53 signatures were identified in 24% and 33% of women with BRCA mutations and other disorders, respectively (Table 1). The latter included 19 women with the following postoperative diagnoses: cervical neoplasia (three), endometrial polyps (two), endometrial hyperplasia or adenocarcinoma (four), leiomyomata (three), leiomyosarcoma (one), ovarian adhesions (one), ovarian fibroma (one), and benign ovarian epithelial tumours (four). Individual p53 signatures were typically limited to a single plical surface or sulcus (Figures 1 and 2), similar in distribution to TICs. The intensity of p53 immunostaining was similar in both p53 signatures and TICs (Figures 1 and 2). However, p53 signatures lacked the epithelial stratification and atypia of TICs and had a lower frequency (<25%) of nuclear MIB1 immunostaining (Figure 1).

p53 signatures predominate in the fimbriated end of the fallopian tube

In patients from all groups in which the side was specified ($n = 33$), p53 signatures were found in the right, left, and both tubes in 54%, 30%, and 16%, respectively. The fimbria was involved in 80%, 89%, and 100% of women with *BRCA* mutations, other disorders and TICs, respectively (Table 1). p53 signatures were associated with a higher mean age in tubes from BRCA+ cases and other disorders (Table 1), but the differences in mean age were not significant (Student's *t* test).

p53 signatures are more frequently present in association with TICs

Fifty-three percent of TICs contained at least one p53 signature; multiple p53 signatures were over twice as common in association with TICs relative to non-neoplastic tubes (67% vs 20–32%). In one case (Table 2, case 7), a p53 signature merged with a TIC, both sharing the same *p53* mutation (Figure 2). In two cases (Table 2, cases 7 and 8 and Figure 2) p53-positive foci were identified with moderate atypia and MIB1 staining (25–50%) and were classified as intermediate between a p53 signature and TIC.

p53 signatures target the non-ciliated (secretory) cell phenotype

Sections were stained for HMFG2, LhS28, and p73. p53 signatures immunostained strongly for HMFG2, a marker that, in the fallopian tube, is specific for secretory tubal epithelial cells and spares ciliated epithelium

Table 1. Frequency and distribution of p53 signatures

				Location			Mean age (variance)	
Subjects	**No.**	**p53 signature, No (%)**	**Multifocal**	**Fim, No (%)**	**Fim + Prox, No (%)**	**Prox, No (%)**	**p53+**	**p53 (−)**
BRCA+[*]	41	10 (24)	2 (20)	8 (80)	0	2 (20)	51 (39)	47 (75)
Others[†]	58	19 (33)	6 (32)	15 (78)	2 (11)	2 (11)	60 (60)	58 (118)
TIC[‡]	17	9 (53)	6 (67)	6 (100)	0	0	60 (125)	60 (128)

[*] History of heterozygous mutation in *BRCA1* or *BRCA2* gene.
[†] Consecutive bilateral salpingectomies for diseases other than ovarian cancer.
[‡] Bilateral salpingectomies for pelvic cancer with co-existing tubal intraepithelial carcinoma (TIC).
Fim = fimbria only; Fim + Prox = fimbria and proximal tube; Prox = proximal tube only.
Differences in proportion of cases involving fimbria vs proximal tube were significant at $p < 0.001$ (χ^2 analysis).

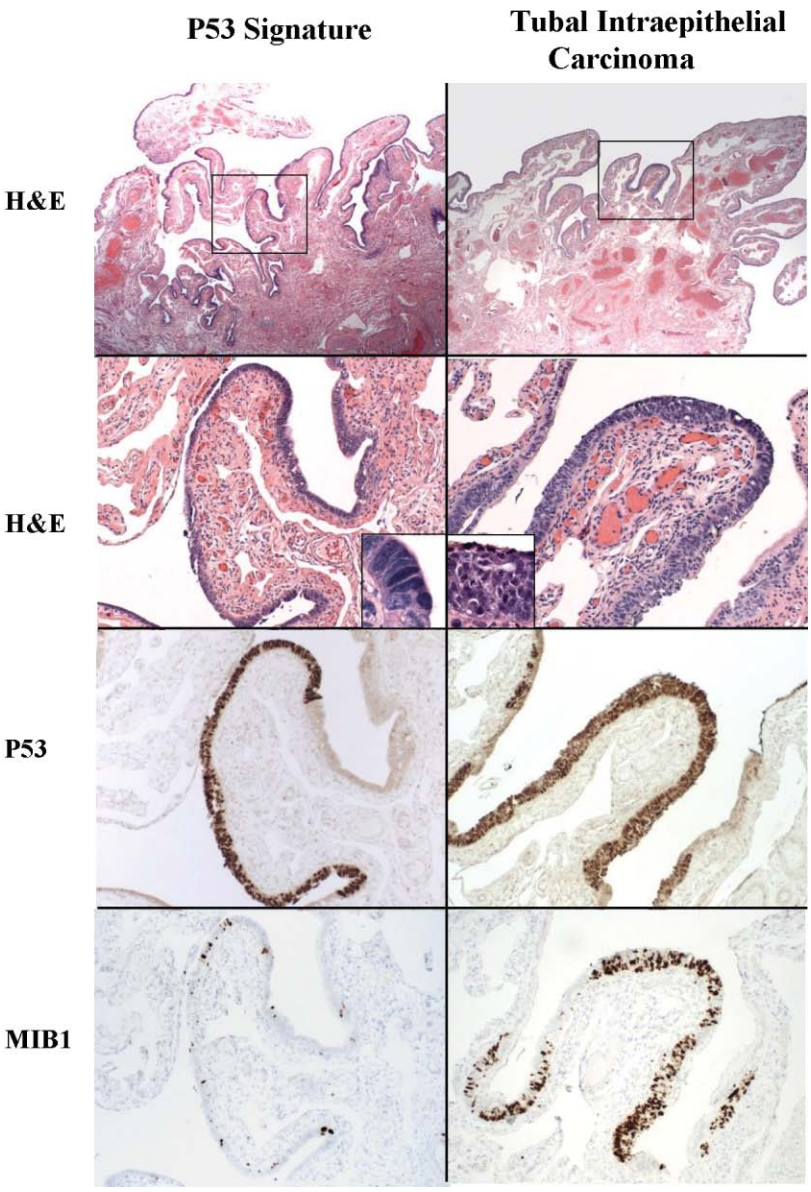

Figure 1. Comparison of a p53 signature from a fallopian tube removed incidentally at hysterectomy for a benign ovarian cystadenoma (left) and a tubal intraepithelial carcinoma (TIC) from a BRCA+ woman (right). Both are intensely p53 immuno-positive. Both localize to a single plica in the fimbria (boxes) and exhibit nuclear enlargement (insets), but are distinguished by epithelial stratification and increased proliferative activity (MIB1) in the intraepithelial carcinoma

(Figure 3). This pattern of antigen localization was identical to that seen in intraepithelial carcinomas of the fallopian tube (Figure 3) and serous carcinomas (not shown) and was consistent with prior associations between the secretory cell phenotype and serous carcinoma [30].

p53 signatures and TICs share histochemical evidence of double-strand DNA breakage relative to adjacent uninvolved salpingeal mucosa

A subset of normal fimbrial mucosa, p53 signatures, TICs, and associated tubal and ovarian carcinomas was immunostained for γ-H2AX. Figure 4 shows examples of the staining. In p53 signatures, discrete punctuate intranuclear immunostaining was seen, typically in two or more foci per nucleus of the p53-positive cells (Figure 4), a staining distribution previously described for this biomarker [25,26]. TICs and invasive serous cancers demonstrated a similar pattern of staining (Figure 4).

p53 signatures, and TIC, with or without ovarian carcinoma are associated with mutations in the *p53* tumour suppressor gene

Data were analysed from exons 2, 3, 5–9, and 11 on laser-capture microdissected DNA from 23 p53 signatures from ten cases, 13 TICs from 13 cases and, in the latter, one tubal and eight corresponding ovarian cancers. In all, a paired normal control epithelium was analysed. Reproducible *p53* sequence data were obtained from 14/23 p53 signatures and all of the TICs analysed. The higher rate of mutation confirmation in the TICs and corresponding carcinomas reflects the higher amount of input DNA

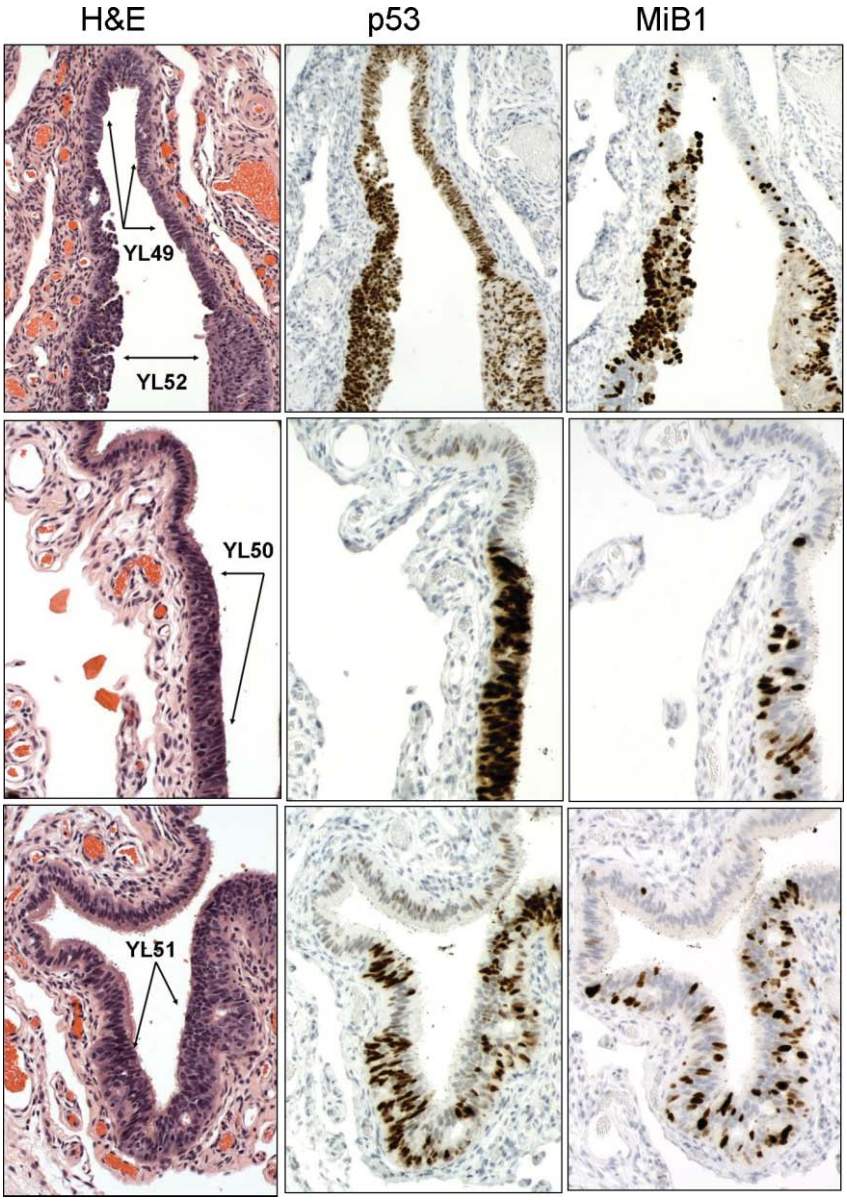

Figure 2. Multiple foci of p53 accumulation with *p53* mutations in a BRCA+ fallopian tube with TIC (YL52). Both signature (YL49) and carcinoma (YL52) are p53 immuno-positive; however, the signature is distinguished by both a lack of atypia in the haematoxylin and eosin (H&E) stained section and a low proliferative activity by MIB1 immunohistochemistry. Two other p53 positive foci (YL50, YL51) also contain *p53* mutations and exhibit MIB1 staining activity intermediate between the p53 signature and intraepithelial carcinoma (Table 2, case 7)

and is consistent with prior reports of formalin-fixed, paraffin-embedded material [29]. Overall, eight of the 14 p53 signatures (57%) scored *p53* mutation-positive (Table 2, cases 1–7). *p53* mutations were identified in all of the TICs, and all TIC/ovarian or tubal carcinoma pairs shared an identical *p53* mutation (Table 2, cases 7–19).

Of the eight *p53* mutations detected in p53 signatures, six were missense, one was a splice, and one was a frameshift mutation. Three of the six transitions resulting in missense mutations occurred at CpG dinucleotides within the p53 signature group. Of 13 mutations detected in TIC (and corresponding ovarian cancers when present) six were missense (of which two occurred at CpG dinucleotides), five were frameshift, and one each were nonsense and splice mutations.

Intraepithelial carcinomas are distinguished from p53 signatures by up-regulation of cyclin E and MIB1

The percentage of MIB1 positive nuclei in normal mucosa varied from 5 to 20%. Nuclear cyclin E staining varied from none to over 50% of cell nuclei and stained from mild to moderate in intensity. In p53 signatures, frequency of staining for both antigens was equal or less than that seen in normal mucosa, consistent with normal or suppressed DNA proliferation (Figures 1, 2, and 4). In contrast, intraepithelial and invasive serous carcinomas of the tube exhibited increased frequencies of both cyclin E and MIB1 staining (Figure 4) [14].

Table 2. *p53* Mutations in p53 signatures, TICs, and ovarian carcinomas

Case	Pathology	Code	Epithelium	Base change	Designation	Codon	Effect	OVCA/total citations*
1	OV CA	YL14	Normal	None	None			
		YL15	P53SIG	542G > A	R175H	175	Missense	60/979
2	Cystadenoma	YL22	Normal	None	None			
		YL23	P53SIG	817C > T	R273C	273	Missense	29/555
3	EMCA	YL25	Normal	None	None			
		YL26	P53SIG	743G > A	R248Q	248	Missense	30/727
4	HNPCC	YL29	Normal	None	None			
		YL30	P53SIG	770T > C	L257P	257	Missense	0/10
5	OV CA	YL35	Normal	None	None	—	—	
		YL36	P53SIG	IVS5-1G > T	IVS5-1G > T	NA	Splice	
		YL43	P53SIG	461G > T	G154V	154	Missense	1/52
6	BRCA + EMOID	YL45	Normal	None	None			
	Ca (tube)	YL46	P53SIG	583A > T	I195F	195	Missense	0/24
7	BRCA +	YL07	Normal	None	None	—	—	—
	TIC	YL49	P53SIG	1004delG	1004delG	335	Frameshift	—
		YL52	TIC	1004delG	1004delG	335	Frameshift	—
		YL50	INTER	991delC	991delC	330	Frameshift	—
		YL51	INTER	743G > A	R248Q	248	Missense	30/727
8	TIC	YL18	Normal	None	None			
	TUB CA	YL19	INTER	482del3	482del3	160	Frameshift	
	OV CA	YL20	TIC	482del3	482del3	160	Frameshift	
		YL21	TUB CA	482del3	482del3	160	Frameshift	
9	BRCA +	YL09	Normal	None	None			
	TIC	YL10	TIC	542G > A	R175H	175	Missense	60/979
10	BRCA +	YL12	Normal	None	None			
	TIC	YL13	TIC	451C > T	P151S	151	Missense	2/78
11	OV CA	MP0	Normal	None	None	—	—	—
		MP1	TIC	162delC	162delC	54	Frameshift	
		MP2	Ov CA	162delC	162delC	54	Frameshift	
12	OV CA	MP3	Normal	None	None	—	—	
		MP4	TIC	493C > T	Q165X	165	Nonsense	2/40
		MP5	TIC	493C > T	Q165X	165	Nonsense	
13	OV CA	MP6	Normal	None	None	—	—	
		MP7	TIC	747G > T	R249S	249	Missense	4/314
		MP8	OV CA	747G > T	R249S	249	Missense	
14	OV CA	MP9	Normal	None	None	—	—	—
		MP10	TIC	IVS9 + 1G > A	IVS9 + 1G > A	NA	Splice	
		MP11	OV CA	IVS9 + 1G > A	IVS9 + 1G > A	NA	Splice	
15	OV CA	MP12	Normal	None	None	—	—	—
		MP13	TIC	996delC	996delC	332	Frameshift	
		MP14	OV CA	996delC	996delC	332	Frameshift	
16	OV CA	MP15	Normal	None	None	—	—	—
		MP16	TIC	749del9	749del9	249	Frameshift	
		MP17	OV CA	749del9	749del9	249	Frameshift	
17	OV CA	MP18	Normal	None	None	—	—	
		MP19	TIC	838A > G	R280G	280	Missense	3/32
		MP20	OV CA	838A > G	R280G	280	Missense	
18	OV CA	MP21	Normal	None	None	—	—	
		MP22	TIC	830G > T	C277F	277	Missense	4/39
		MP23	OV CA	830G > T	C277F	277	Missense	
19	OV CA	MP22	Normal	None	None	—	—	
		MP23	TIC	542G > A	R175H	175	Missense	60/979
		MP24	OV CA	542G > A	R175H	175	Missense	

P53SIG = p53 signature; INTER = proliferative activity and atypia intermediate between p53 signature and TIC (see Figure 2); OV CA = ovarian carcinoma; TUB CA = tubal carcinoma; TIC = tubal intraepithelial carcinoma; EMCA = coexisting endometrial carcinoma; HNPCC = prophylactic adnexectomy for hereditary non-polyposis coli syndrome; EMOID = endometrioid carcinoma.

* Determined by the Universal p53 Mutation Database (http://www.umd.be:2072/IFAMTP53A.shtml); the right hand column depicts the number of ovarian cancer citations for a given mutation/total citations in the database.

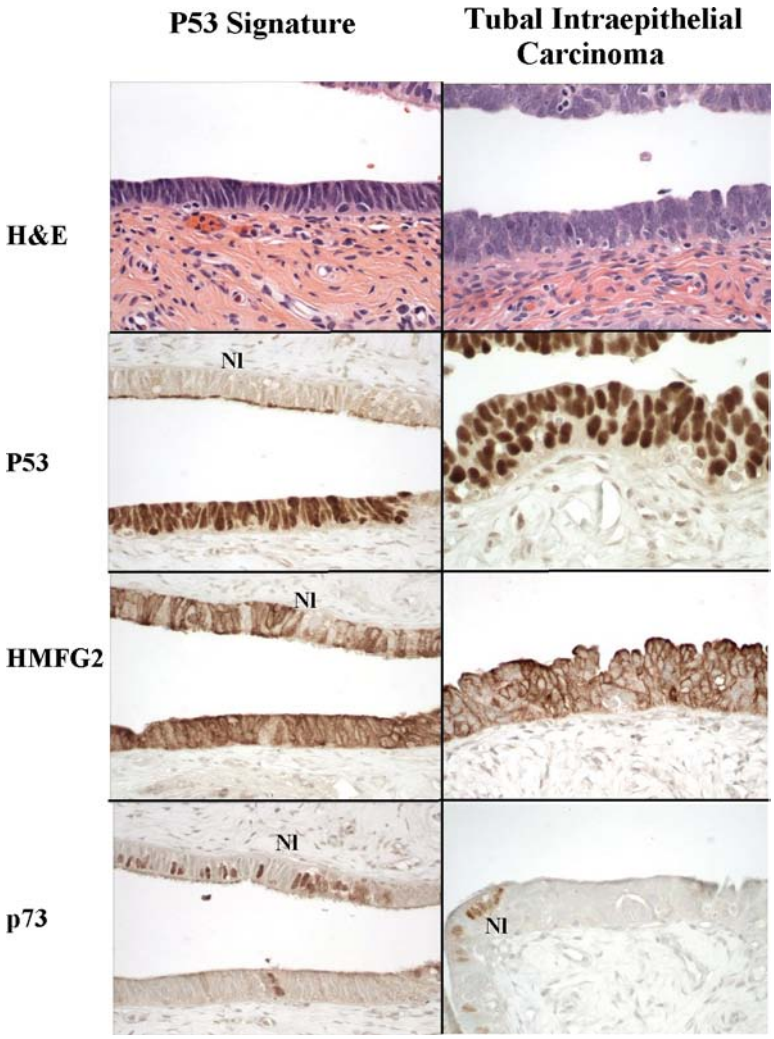

Figure 3. Secretory cell-specific immunostaining of a p53 signature from an incidental salpingectomy (left panels) compared to an intraepithelial carcinoma of the fallopian tube from a BRCA+ woman (right panels). Both the p53 signature and the intraepithelial carcinoma are intensely p53 positive. Both stain strongly positive for HMFG2, characteristic of a secretory cell phenotype. In the left panels, nuclear staining for p73 and staining for HMFG2 are mutually exclusive in both signature and normal epithelium. A small focus of ciliated cells adjacent to intraepithelial carcinoma is illustrated in the lower right panel for reference. Nl = adjacent normal mucosa

A direct transition between p53 signature and TIC was uncommon. However one frameshift mutation was shared by a contiguous p53 signature and TIC (Table 2, case 7 and Figure 2). In addition, three p53 positive foci that were morphologically intermediate between the two were seen (Table 2, cases 7 and 8 and Figure 2).

Discussion

This study was prompted by the incidental discovery of p53-positive normal tubal mucosa during histological surveillance of prophylactic salpingo-oophorectomy specimens from BRCA+ women [15]. Thus, we designed a protocol to systematically section fallopian tubes and to determine the frequency of this entity, termed the 'p53 signature', and its relationship to TIC [15]. Recent studies have identified the distal fallopian tube as a common site of involvement by serous carcinoma in BRCA+ women [15–17]. We have recently shown that, irrespective of BRCA status, many tumours classified as ovarian or peritoneal serous carcinoma co-exist with a TIC in the fimbria and share identical *p53* mutations [17]. Because a precursor to ovarian and peritoneal carcinomas has never been described, we viewed the p53 signature, and its early neoplastic counterpart in the tube (TIC), as part of a candidate pathway for not only tubal but also ovarian and peritoneal serous cancers [8].

Like TIC, the p53 signature localizes predominantly to the fimbriated end, a preferred site for BRCA+ and sporadic tubal carcinomas [14–17]. The p53 signature is also more commonly associated with TIC. However, it is equally common in non-neoplastic tubes from women with and without *BRCA* mutations, suggesting that the p53 signature occurs independently of BRCA status. The p53 signature also targets secretory cells, which are the presumed cells of origin for serous carcinomas of the fallopian tube (Figure 3) [30]. Why

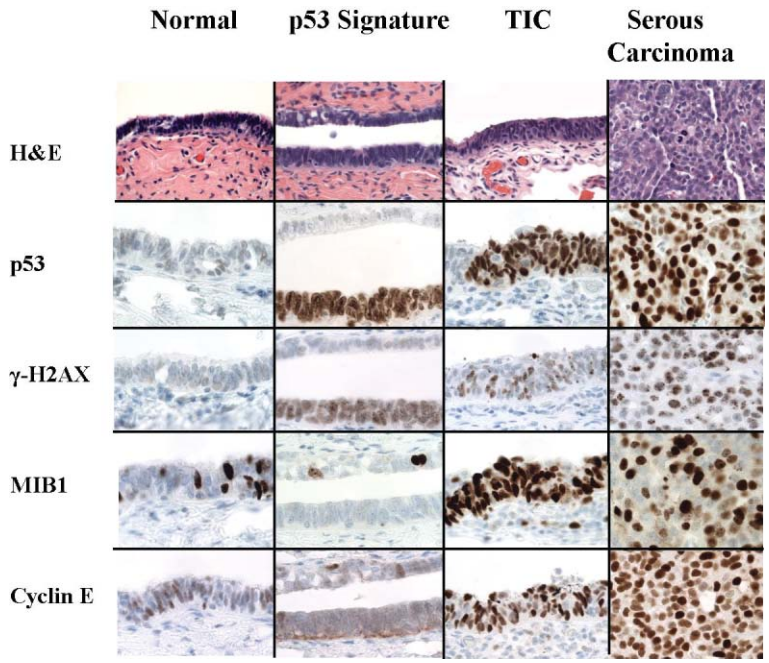

Figure 4. Co-localization of p53, γ-H2AX, MIB1, and cyclin E in normal mucosa, p53 signature, tubal intraepithelial carcinoma, and serous carcinoma. In contrast to normal mucosa, p53 signatures and neoplastic epithelia display discrete nuclear punctate staining for γ-H2AX. Intraepithelial and invasive serous carcinomas exhibit, in addition, increased proliferative activity (MIB1) and increased cyclin E staining

the tubal secretory cell would be more susceptible to the development of p53 signatures and serous carcinomas is unclear, but similar tumours of the chicken oviduct also arise in the secretory cell population [31]. Interestingly, the tubal secretory cell phenotype does not characterize lower-grade serous tumours (borderline malignancies), the latter exhibiting a mixture of both ciliated and secretory differentiation that typifies the ovarian cortical inclusion cyst (Parast M, unpublished data).

The third similarity between the p53 signature and TIC is the presence of *p53* mutations, many of which have been reported previously in serous carcinomas. The most common mutations in both p53 signatures and TICs/ovarian cancers were missense, followed by frameshift, splice, and nonsense mutations. This frequency is similar to that described by Leitao *et al*, who reported missense, splice, and nonsense mutations in 50%, 23%, and 19% of 26 mutation-positive early serous cancers [32]. Moreover, the fraction of mutations that were transitions at CpG dinucleotides in this study (5/21 [24%]) correlates well with the frequency described in the Universal Mutation Database for ovarian cancer (304/1892 [16%]). p53 immunostaining has previously been principally associated with missense mutations but, in recent studies of tumours with frameshift mutation in *p53*, the majority have been immunopositive for p53 [33–35]. This demonstrates that truncated forms of p53 are expressed in these tissues and can be detected by the antibody that is reactive against the extreme *N*-terminus (amino acids 21–25).

This finding, coupled with the localization of γ-H2AX, suggests that p53 signatures are initiated by DNA damage (Figure 4) [24,26]. DNA strand breakage can arise during DNA replication, ionizing radiation, and after exposure to a wide range of DNA-altering agents [36]. Unrepaired double-stranded DNA breaks are associated with genomic instability and neoplasia; triggering the accumulation of proteins involved in DNA repair, including cell cycle checkpoint activation. Of particular note, particularly in the context of emerging models for DNA damage and repair in carcinogenesis, is the co-localization of the γ-H2AX and p53 immunostaining. Recent publications indicate that, in early tumourigenesis (before genomic instability and malignant conversion occur), human cells activate an ATM/ATR-regulated DNA damage response network in reply to DNA damage, which can be incited by a multiplicity of stimuli. This sequence of responses typically results in growth arrest. The p53 signature, which demonstrates a low level of proliferative activity, is consistent with this phase of the process. However, mutations compromising this cell cycle checkpoint, including defects in the ATM-Chk2-p53-BRCA1 pathway, would permit increased cell survival, followed in some cases by genomic instability and tumour progression [18]. This subsequent genomic instability would be integrated with up-regulation of cyclin E, which typifies intraepithelial and invasive carcinomas, shown in Figure 4. Based on the small number of signatures analysed, it would be premature to equate all p53 signatures with *p53* mutations. However, we propose that the p53 signatures described in this report signify unrepaired double-stranded DNA breaks and that *p53* mutations in this setting can coexist before loss of the cell cycle control that characterizes intraepithelial carcinoma (Figure 4). The role

of BRCA in the early stage of this process remains unclear, because p53 signatures are equally prevalent in benign tubes of BRCA+ and control women. Further studies will be required to determine now BRCA status influences risk of progression from p53 signature to TIC.

Excluding a *BRCA* mutation, the most compelling risk factor for ovarian cancer is ovulation, including uninterrupted ovulation seen with nulliparity and increased number of lifetime ovulatory cycles. Conversely, risk is reduced with increasing number of pregnancies and oral contraceptive use [8]. Proposed mechanisms by which ovulation influences risk include the development of ovarian inclusion cysts, stimulation of the ovarian cortex, and ovarian surface epithelium (OSE) by oestrogens, repair of OSE after follicle rupture, and promotion of clonal growth by growth factors produced during wound healing [8]. In one animal model (ewes), oxidants are released during follicle rupture, which exert their greatest effect on the OSE in the immediate vicinity of the follicle with diminishing impact at greater distances from the site of release. OSE cells closest to the site of rupture undergo DNA fragmentation and apoptosis, whereas those more remote have milder forms of DNA damage, fail to undergo DNA repair, and accumulate p53 [37,38]. Because p53 signatures are most prevalent in the tubal mucosa in the vicinity of the ovarian surface (fimbria), they represent another possible sequel to ovulation-related oxidative stress. Given the increasing support for the fimbria as an initiation site for pelvic serous carcinoma, studies elucidating the mechanism by which the p53 signature develops could prove particularly relevant to the pathogenesis, and possibly prevention, of this disease.

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