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Stathmin 1, a marker of PI3K pathway activation and regulator of microtubule dynamics, is expressed in early pelvic serous carcinomas

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ABSTRACT

Background. Most high-grade pelvic serous carcinomas (HGPSCs) arise from fallopian tube epithelium (FTE). To date, few markers have been shown to characterize FTE transformation. Stathmin 1 (STMN1) is a candidate oncogene whose activity is influenced by p53, p27Kip1 (p27), and PI3K/Akt pathway activation. As a microtubule destabilizing protein, STMN1 regulates cytoskeletal dynamics, cell cycle progression, mitosis, and cell migration. This study examines the expression of STMN1 and its negative regulator p27 along the morphologic continuum from normal FTE to invasive carcinoma.

Methods. STMN1 and p27 expression were examined by immunohistochemistry (IHC) in benign ($n=12$) and malignant ($n=13$) fallopian tubes containing normal epithelium, morphologically benign putative precursor lesions ("p53 signatures"), potential transitional precursor lesions ("proliferative p53 signatures"), tubal intraepithelial carcinoma (TIC), and/or invasive serous carcinoma. STMN1 expression was further assessed in 131 late-stage HGPSCs diagnosed as primary ovarian and in 6 ovarian cancer cell lines by IHC and Western blot, respectively.

Results. STMN1 expression was absent in benign FTE and infrequently detected in p53 signatures. However, it was weakly expressed in proliferative p53 signatures and robustly induced upon progression to TIC and invasive carcinoma, typically accompanied by decreased p27 levels. STMN1 was expressed in >80% of high-grade serous ovarian carcinomas and cell lines.

Conclusions. STMN1 is a novel marker of early serous carcinoma that may play a role in FTE tumor initiation. Our data are consistent with a model by which STMN1 overexpression, resulting from loss of p27-mediated regulation, may potentiate aberrant cell proliferation, migration, and/or loss of polarity during early tumorigenesis.

Introduction

High-grade pelvic serous carcinoma (HGPSC) is a lethal malignancy for which there is no curative therapy. The vast majority of patients are diagnosed with metastatic disease, at which point cytoreductive surgery and chemotherapy remain the only treatment options [1]. Despite initial high response rates, most patients recur and ultimately succumb to chemoresistant disease [2]. Our ability to detect and effectively treat HGPSC is hampered by a poor understanding of the molecular events underlying its pathogenesis. Recent studies suggest that most HGPSCs, including a significant proportion diagnosed as "primary ovarian", arise from fallopian tube epithelium and a model for HGPSC development has been described based on pathological studies of women genetically predisposed to ovarian cancer due to germline BRCA mutations [3]. These studies identified a benign appearing lesion in the tubal mucosa that appears to precede development of tubal intraepithelial carcinoma (TIC). The putative precursor, called a "p53 signature", occurs in the fimbrial region of the fallopian tube and is characterized by secretory cell composition, somatic TP53 mutation, and accumulation of DNA damage [4,5]. The hypothesis that p53 signatures and TICs are precursors of HGPSC is supported by the finding that these lesions often share identical p53 mutations with co-existing HGPSC, indicative of clonality, and that TICs have shorter telomeres than co-existing HGPSC, suggesting that they represent an earlier disease stage rather than mucosal spread or metastasis from an advanced tumor [6,7]. However, the genetic alterations driving tumor initiation in this setting remain uncertain. Answers may soon be forthcoming with the emergence of large scale

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genomic profiling data of serous ovarian carcinomas, such as The Cancer Genome Atlas [8]. With insight from such genomic analyses, we may identify relevant genetic alterations and query their roles at each stage of the HGPSC progression model in order to identify drivers of tumor initiation and progression.

The current model of fallopian tube carcinogenesis has been well described histologically [9], but few molecular markers other than p53 and Ki-67 have been shown to characterize the transformation of fallopian tube secretory epithelial cells (FTSECs). Norquist et al. recently reported that p53 mutation, clonal proliferation, and loss of p27 expression occur in preneoplastic lesions of the fallopian tube epithelium [10]. However, the consequences of p27 loss in this setting have not been explored. To determine how p27 loss may contribute to tumorigenesis, we sought to examine the expression of its direct downstream targets. Among several candidates, Stathmin1 (STMN1) was of particular interest because it has been shown to regulate cell division, motility, and migration; all of which are critical processes in tumorigenesis. In this report we identify STMN1 as a novel marker of FTSEC transformation and serous tumor initiation. STMN1 is a ubiquitous cytoplasmic phosphoprotein that regulates microtubule dynamics. Microtubules are protein polymers, comprised of α/β tubulin heterodimers, that constitute a major portion of the cytoskeleton. They exist in a constant state of polymerization and depolymerization referred to as "dynamic instability". STMN1 depolymerizes microtubules and is required for all cellular processes involving microtubule rearrangement, most notably mitosis. At the onset of mitosis, STMN1 rapidly destabilizes interphase microtubules, allowing them to be reorganized into a mitotic spindle [11]. During spindle assembly, STMN1 activity is repressed through phosphorylation, thus enabling repolymerization [12]. Following chromosomal segregation, STMN1 is once again activated in order to disassemble the spindle and begin cytoskeleton reconstruction. STMN1 activity is therefore critically important for both successful M-phase entry and timely M-phase exit.

Independent from its role in cell division, STMN1 has also been shown to regulate cell motility, enhance cell migration, and promote metastasis [13,14]. The goal of this study was to determine whether STMN1, and its negative regulator p27, are relevant markers of early neoplasia in the fallopian tube.

Methods

This study was approved by the Institutional Review Boards at the Brigham and Women's Hospital (BWH), Dana-Farber Cancer Institute (DFCI), and Beth Israel Deaconess Medical Center (BIDMC).

Case selection

The following cases were retrieved from the 2004–2010 Department of Pathology archives of BWH and BIDMC: (1) 13 cases of HGPSC clinically diagnosed as stage III–IV fallopian tube carcinoma ($n=2$, 1 unilateral, 1 bilateral) or ovarian papillary serous adenocarcinoma ($n=11$, all bilateral), each with involvement of both the ovary and fallopian tube; and (2) 76 histologically benign appearing fallopian tubes collected from prophylactic bilateral salpingo-oophorectomies or total abdominal or vaginal hysterectomies performed mainly on patients with germline BRCA1/2 mutations or a personal or family history of breast cancer. Patient ages ranged from 50 to 73 years for the HGSPC group and 29 to 69 years for the histologically benign group. Patients were unselected for BRCA mutational status.

Immunohistochemistry (IHC)

Immunohistochemical staining was performed using Envision Plus/Horseradish Peroxidase system (DAKO, Carpinteria, CA, USA). Formalin-fixed paraffin-embedded tissue sections were de-waxed, rehydrated, and incubated in hydrogen peroxide solution for 30 min to block endogenous peroxidase activity. Antigen retrieval was carried out by pressure cooker treatment in citrate buffer (pH 6.0) for 40 min. Sections were incubated with primary antibody using the conditions specified in Supplemental Table 1. Secondary antibody was applied for 30 min, followed by DAB for 5 min.

Evaluation of p53 Signatures, proliferative p53 Signatures, and TICs

Two adjacent serial sections from each case were immunostained for p53 and Ki-67 (Supplemental Table 1) and evaluated for the presence of p53 signatures, proliferative p53 signatures, and TIC. A p53 signature was defined as ≥12 consecutive FTSECs having strong nuclear p53 staining, normal morphology, and a low Ki-67 proliferation index (<10% positive nuclei) [15]. "Proliferative p53 signatures" are putative transitional precursor lesions bearing features intermediate between a p53 signature and TIC [9]. A proliferative p53 signature was defined as ≥12 consecutive FTSECs having strong nuclear p53 staining, mild cytological atypia, and a moderately elevated proliferation index (10–50% positive nuclei). TIC was defined as a region of FTSECs exhibiting significant nuclear atypia, loss of cell polarity, and a high Ki-67 index (>50% positive nuclei). All cases were reviewed by two pathologists (MSH and RD).

STMN1 and p27 immunostaining

For all cases containing a p53 signature, proliferative p53 signature, or TIC, two adjacent serial sections were immunostained for STMN1 and p27 (Supplemental Table 1). In each of these cases, the normal epithelium was also evaluated for STMN1 and p27 immunoreactivity. STMN1 staining was scored as 0 (all cells negative), 1+ (scattered rare cells = <10% positive cells), 2+ (focal or multifocal staining = 10–75% positive cells), or 3+ (diffuse staining = >75% positive cells). In subsequent data analyses, 0 and 1+ were considered to be "STMN1-negative", while 2+ and 3+ were categorized as "STMN1-positive". Appropriate positive and negative (incubation with secondary antibody only) controls were stained in parallel with each round of immunohistochemistry (Supplemental Table 1).

Tissue microarray (TMA)

A TMA was constructed from 131 cases of high-grade late-stage (FIGO III–IV) serous ovarian adenocarcinoma from patients who underwent cytoreductive surgery at BWH during 1999–2005, as previously described [16,17]. Each case was represented by quadruplicate cores, 0.8 mm in diameter. The TMA was immunostained for STMN1 and each core was scored as described in the previous section.

Cell lines

Six ovarian cancer cell lines (OVCAR-5, OVCAR-8, OV-90, HEYA8, IGROV1, and SKOV3) were used in this study and maintained as previously described [16]. Immortal FTSEC lines were generated from freshly resected fallopian tubes obtained from the BWH Department of Pathology, collected from patients with benign gynecological conditions as recently described [18,19]. Briefly, FTSECs were dissociated by incubating fimbria in Eagle's Minimum Essential Medium (Cellgro, Manassas, VA, USA), supplemented with 1.4 mg/ml Pronase (Roche Diagnostics, Indianapolis, IN, USA) and 0.1 mg/ml DNAse (Sigma-Aldrich, St Louis, MO, USA), for 48–72 h at 4 °C. Cells were seeded onto plates coated with human placental collagen (Sigma-Aldrich) and cultured in Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F-12 1:1 (Cellgro) supplemented with 2% Ultroser G serum substitute (Pall Life Sciences, Ann Arbor, MI, USA) and 1% penicillin/streptomycin. FTSECs were immortalized via transduction with retroviral vectors expressing human Telomerase Reverse Transcriptase (hTERT) [20],

mutant Cyclin-Dependent Kinase 4 (CDK4R24C) [21], and either dominant negative TP53 [21] or shRNA targeting TP53 [22,23] (Addgene plasmids #1774, 11254, 9058, and 10671; Addgene, Cambridge, MA, USA).

Western blot

Whole cell lysates were prepared using NETN-150 lysis buffer (20 mM Tris–HCl [pH 8.0], 1 mM EDTA, 0.5% NP-40, 150 mM NaCl). Proteins were separated by SDS-PAGE on 4–12% Tris–Glycine gels (Invitrogen, Carlsbad, CA, USA), electroblotted onto nitrocellulose membranes (Invitrogen), and blocked with 5% BSA-Tween-20 (Boston Bioproducts, Worcester, MA, USA) for 1 h. Membranes were incubated in STMN1 primary antibody (1:1000, Cell Signaling Technology, Danvers, MA, USA) overnight at 4 °C, followed by HRP-conjugated secondary antibody for 1 h. Bound antibody was detected by HyGLO Chemiluminescent HRP Antibody Detection Reagent (Denville Scientific, South Plainfield, NJ, USA). Membranes were re-probed for GAPDH as a loading control.

Results

Fallopian tube morphology

The morphologic continuum from histologically benign ("normal") fallopian tube epithelium through TIC to invasive serous carcinoma is characterized by several features: increased nuclear/cytoplasmic ratio, enlarged nuclei with prominent nucleoli, lack of ciliated cells, epithelial stratification, and loss of polarity (Fig. 1A–D) [15]. Morphologically benign putative precursors referred to as "p53 signatures" (Fig. 1B) are thought to precede transformation to intraepithelial carcinoma. These lesions are histologically unremarkable but can be distinguished from normal epithelium by intense nuclear p53 immunostaining, which persists throughout the carcinogenic sequence (Fig. 1E–H). A marked

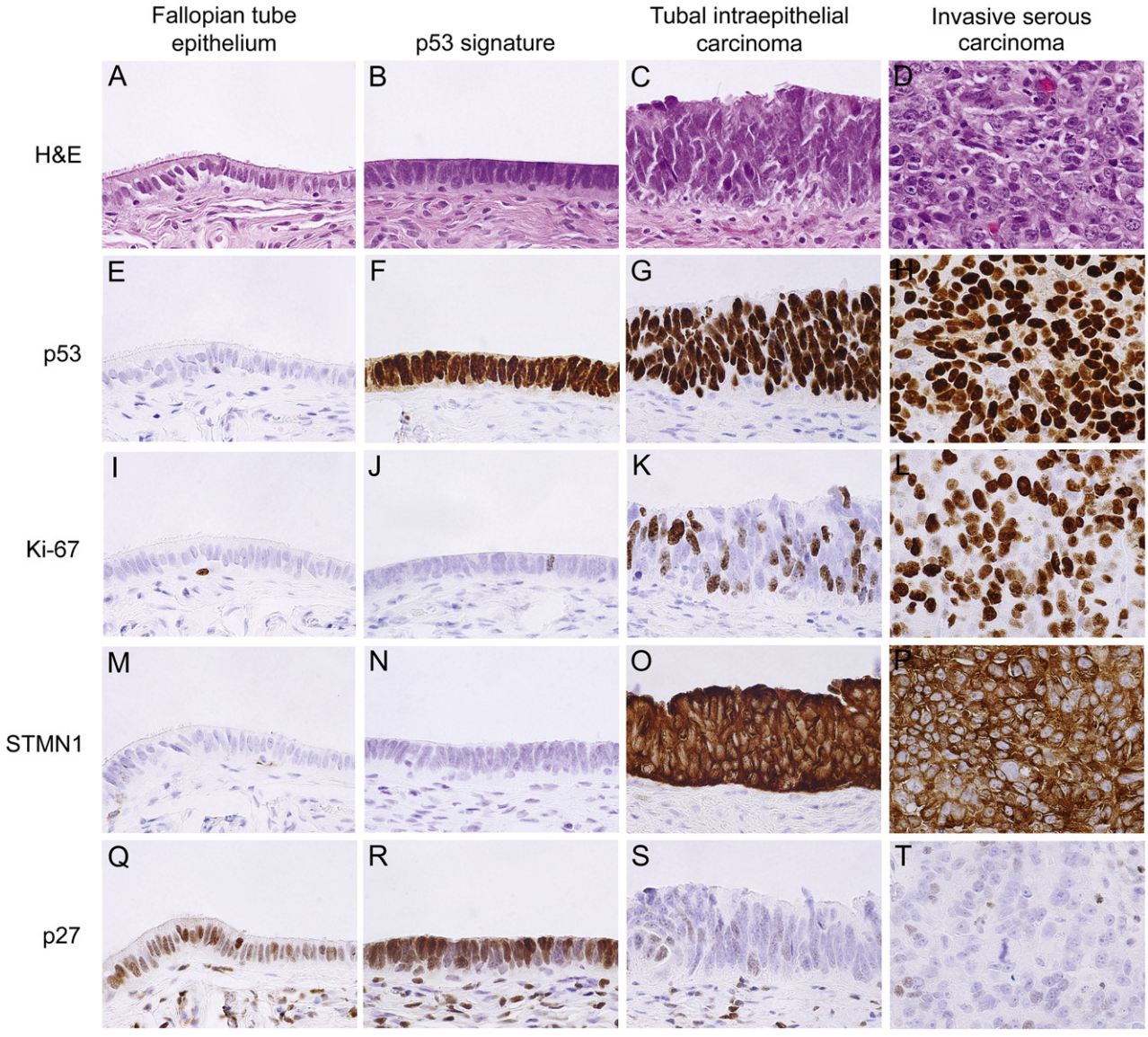

Fig. 1. STMN1 expression across the morphologic continuum from benign fallopian tube epithelium to invasive serous carcinoma. (A–D) Histological transition from normal epithelium to benign p53 signature, TIC, and invasive HGPSC. (E–H) Intense nuclear p53 staining characterizes the p53 signature, TIC, and invasive tumor; (I–L) Ki-67 staining identifies proliferating cells in the TIC and invasive tumor; (M–P) STMN1 expression is absent from benign epithelium but is strongly induced upon progression to TIC; (Q–T) p27 expression is frequently lost in STMN1-positive lesions. All images are from one representative case.

increase in proliferative activity, identified by Ki-67 staining, marks the transition from benign p53 signature to TIC and remains present in invasive serous carcinoma (Fig. 1I–L).

Frequency of p53 signatures, proliferative p53 signatures, and TICs

In order to study protein expression patterns across the morphologic continuum from normal to malignant fallopian tube epithelium, we first examined 13 cases of HGPSC. All 13 cases were found to contain regions of both normal fallopian tube epithelium and TIC in addition to the invasive tumor. Immunostaining for p53 and Ki-67 revealed that 7 (54%) of the HGPSC cases also contained a p53 signature and 6 cases (46%) contained a proliferative p53 signature (Table 1). In 5 cases, both p53 signatures and proliferative p53 signatures were observed in the same patient.

We next looked for p53 signatures by evaluating 76 fallopian tubes diagnosed as benign with absence of ovarian or peritoneal malignancy. By immunostaining for p53 and Ki-67, we identified 11 cases (15%) containing a p53 signature and 1 case (1%) containing a proliferative p53 signature (Table 1). The number of p53 signatures observed in malignant cases (54%) was consistent with previous reports [6,10,24,25]. The number observed in benign fallopian tubes (15%) was lower than that reported by other groups [6,10,24] but was consistent with the number reported by Shaw et al. [25]. This may be attributed to the fact that, like Shaw et al., we did not stain multiple sections when evaluating samples for p53 signatures.

STMN1 is expressed in intraepithelial lesions and HGPSC

In an effort to further characterize the protein expression changes associated with serous carcinoma pathogenesis, we immunostained benign and malignant fallopian tubes for STMN1 and p27, two proteins intimately involved in cell cycle progression. STMN1 is a modulator of microtubules dynamics that is critically important for mitosis and whose activation is strongly linked to growth factor receptor signaling [26]. p27 is a cyclin-dependent kinase inhibitor, governing G_0- to S-phase transition, that negatively regulates STMN1 [14].

STMN1 immunostaining of morphologically normal tubal mucosa was largely negative (Fig. 1M) with rare immunoreactive cells (not shown). Staining, where present, was diffusely cytoplasmic and restricted to the secretory cell compartment. STMN1 expression remained negative in most (13 of 18) p53 signatures but was strongly immunoreactive in all TICs and invasive serous carcinomas (Fig. 1N–P, Table 1). STMN1 staining in TICs and tumors varied from focal (10–75% positive cells) to diffuse (>75% positive cells), but was consistently present in all cases (Fig. 2). Interestingly, varying levels of STMN1 expression were also observed in proliferative p53 signatures (Figs. 2 and 3, Table 1).

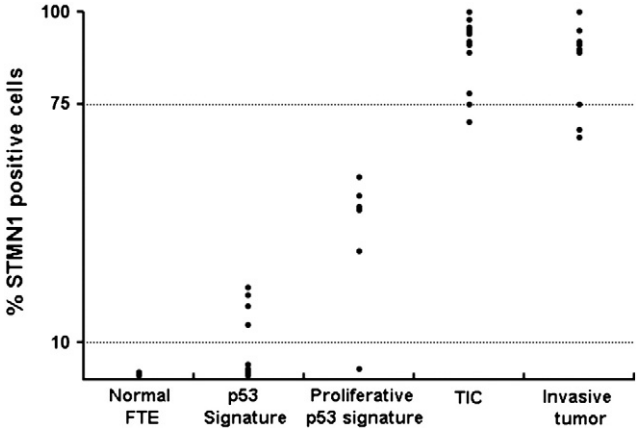

Fig. 2. Distribution of STMN1-positive cells in benign and malignant fallopian tube epithelium. STMN1 staining was negative (<10% positive cells) in both normal FTE and most p53 signatures, but positive (10–100%) in proliferative p53 signatures, TIC, and HGPSC.

In contrast, normal fallopian tube epithelium and p53 signatures were largely positive for p27 with >50% of cells exhibiting nuclear staining in nearly all cases (Fig. 1Q–R, Table 1). However, TICs and invasive carcinomas showed reduced p27 levels when compared to morphologically benign epithelium ($P=0.000$, Student's T-test) (Fig. 1S–T, Table 1). p27 was also reduced in some, but not all, proliferative p53 signatures ($P<0.05$, Student's T-test) (Fig. 3, Table 1). Of note, stromal cell nuclei stained strongly for p27, providing an internal positive control. In several cases the transition from benign to malignant epithelium was clearly delineated by a dramatic induction of STMN1 and a marked decrease in p27 expression, as illustrated in Fig. 4. Additionally, robust STMN1 staining was observed in several stretches of epithelium that were morphologically consistent with TIC but exhibited a lower Ki-67 index than expected, suggesting that these lesions were indeed proliferative (Fig. 4). To determine whether the apparent inverse relationship between STMN1 and p27 expression was statistically significant, we conducted contingency table analyses. When all putative precursors and malignant lesions (p53 signatures, proliferative p53 signatures, TICs and invasive tumors) were considered, STMN1 expression clearly correlated with low p27 levels (Fisher's exact test, $P=0.000$). However, when only putative precursor lesions (p53

Table 1
STMN1 and p27 immunostaining in benign and malignant fallopian tubes.

	STMN1		p27	
	Negative (%)*	Positive (%)*	Low (<50% positive nuclei) (%)	High (>50% positive nuclei) (%)
Findings associated with malignancy (N=13)				
Normal FTE (n=13)	13/13 (100)	0/13 (0)	0/13 (0)	13/13 (100)
p53 signature (n=7)	6/7 (86)	1/7 (14)	0/7 (0)	7/7 (100)
Proliferative p53 signature (n=6)	1/6 (17)	5/6 (83)	2/6 (33)	4/6 (66)
TIC (n=13)	0/13 (0)	13/13 (100)	11/13 (85)	2/13 (15)
Invasive tumor (n=13)	0/13 (0)	13/13 (100)	10/13 (77)	3/13 (23)
Benign/incidental findings (N=12)				
Normal FTE (n=12)	12/12 (100)	0/12 (0)	0/11 (0)	11/11 (100)
p53 signature (n=11)	7/11 (64)	4/11 (36)	1/10 (10)	9/10 (90)
Proliferative p53 signature (n=1)	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)

*STMN1 scoring system.
Negative=score 0 (all cells negative) or 1+ (<10% positive cells).
Positive=score 2+ (10–75% positive cells) or 3+ (>75% positive cells).
FTE, fallopian tube epithelium; TIC, tubal intraepithelial carcinoma.

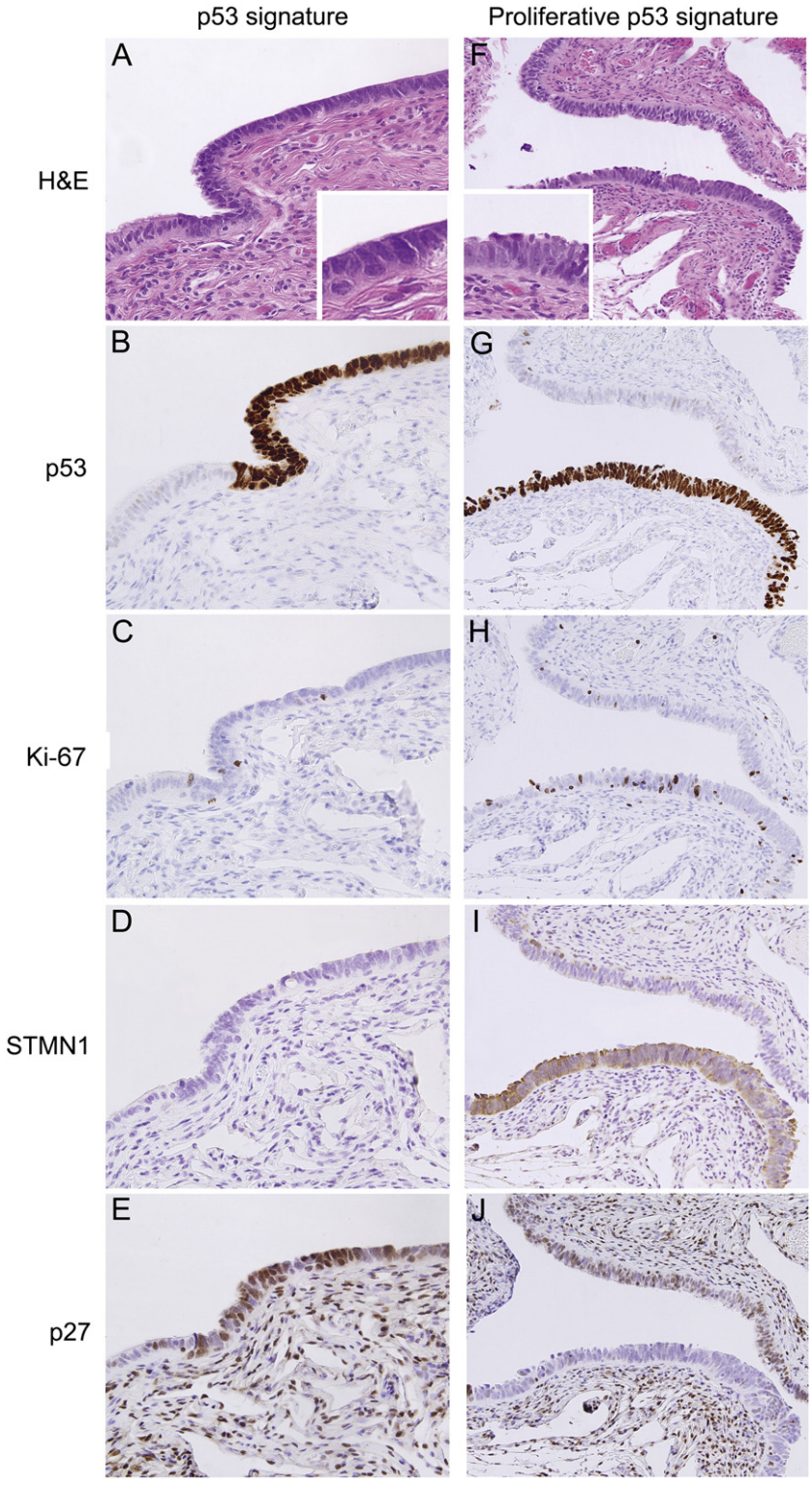

Fig. 3. STMN1 is expressed in putative precursors to HGPSC. A p53 signature (A–E) and proliferative p53 signature (F–J) from the same patient. The p53 signature is non-proliferative, STMN1-negative, and p27 positive. Proliferative p53 signatures are transitional lesions exhibiting features intermediate between a p53 signature and TIC. Here, mildly increased proliferative activity is accompanied by expression of STMN1 and reduced p27.

signatures and proliferative p53 signatures) were considered, the trend was less significant (Fisher's exact test, $P = 0.059$), possibly due to small sample size. Among the 18 p53 signatures examined, only 4 were STMN1-positive and, of these, one exhibited low p27 expression. Among the 7 proliferative p53 signatures examined, 6 were STMN1-positive and, of these, 2 had reduced p27 expression. Notably, low p27 levels were not observed in any STMN1-negative putative precursors or malignant lesions.

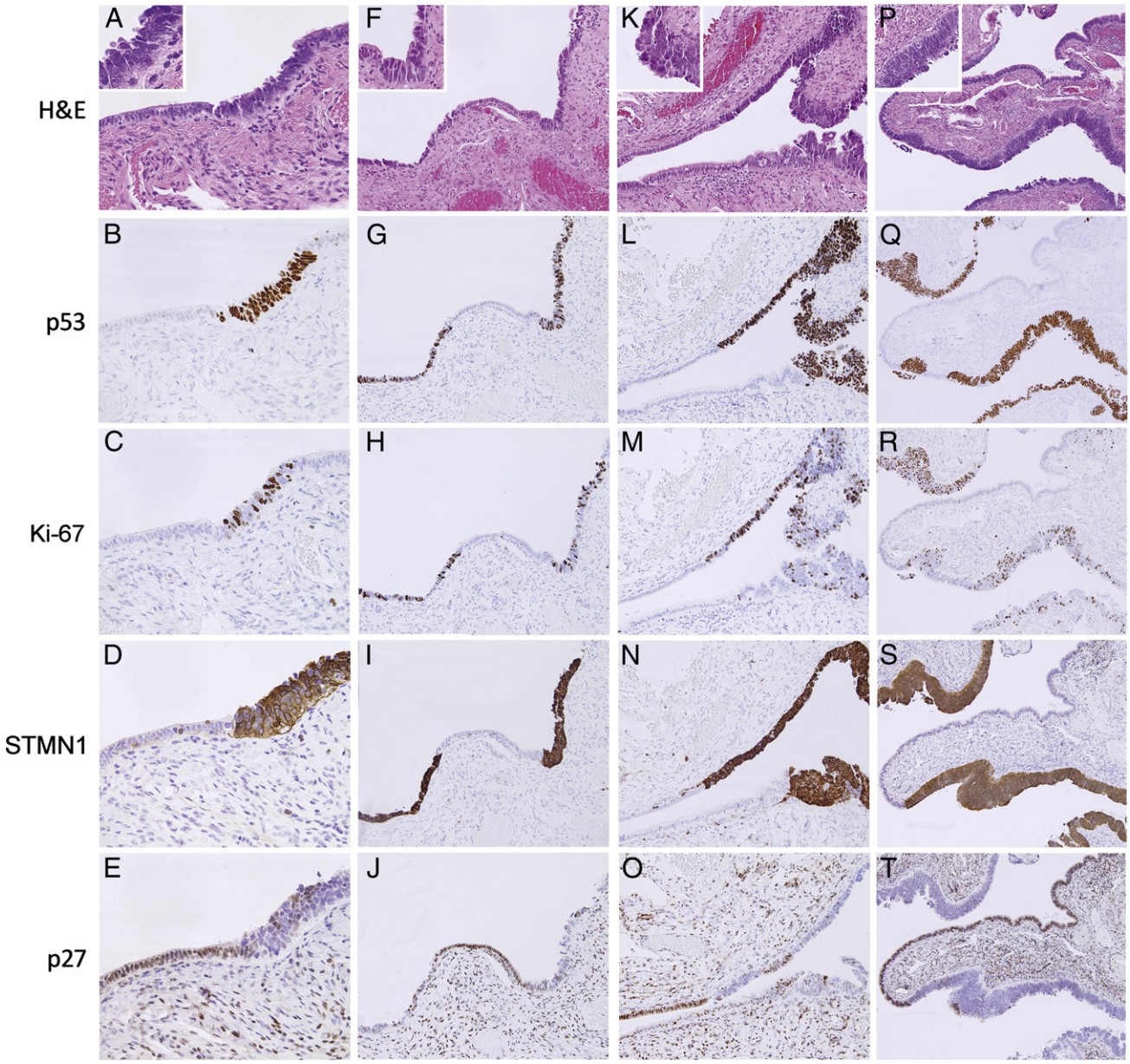

Fig. 4. Examples of reciprocal STMN1 and p27 expression in TICs. (A–T) Coordinated changes in STMN1 and p27 levels occur at the transitions from benign to malignant epithelium. Note the continuity of STMN1 staining throughout each lesion, even in regions with variable Ki-67 immunoreactivity.

STMN1 is strongly expressed in invasive high-grade serous ovarian carcinoma

TMA analysis was used to assess STMN1 expression in a large panel of primary and metastatic high-grade late-stage serous ovarian carcinomas. Of 131 primary tumors, 16% (21/131) was STMN1-negative (score 0 or 1+) and 84% (110/131) was positive (score 2+ or 3+) (Fig. 5A). The percentage of STMN1 positive cells did not correlate with overall survival or response to chemotherapy. Nor did it differ significantly between the primary and corresponding metastatic disease site (data not shown).

STMN1 protein levels were also examined in 6 ovarian carcinoma cell lines and 2 immortalized, non-transformed FTSEC lines by Western blot (Fig. 5B). The Müllerian origin of these lines was confirmed by immunoblotting for PAX8, a lineage marker expressed by FTSECs and serous ovarian carcinomas [18,19,27–29]. Five out of 6 ovarian cancer cell lines expressed STMN1 whereas the protein was undetectable in immortal FTSEC lines, consistent with their low proliferation rates.

Discussion

This study identifies STMN1 as a novel marker of fallopian tube epithelial transformation, characterizing the transition from benign to malignant mucosa. Generally speaking, terminally differentiated or quiescent (G_0) cells express low levels of STMN1 [26]. Accordingly, we did not observe STMN1 staining in normal fallopian tube epithelium, nor in most p53 signatures, which are both defined by a low Ki-67 proliferation index (Figs. 1 and 2). However, STMN1 was expressed in "proliferating p53 signatures" — potential precursor lesions exhibiting increased Ki-67 positivity compared to p53 signatures, albeit lower than that typically seen in TICs (Figs. 2 and 3). STMN1 induction in these lesions possibly marks the

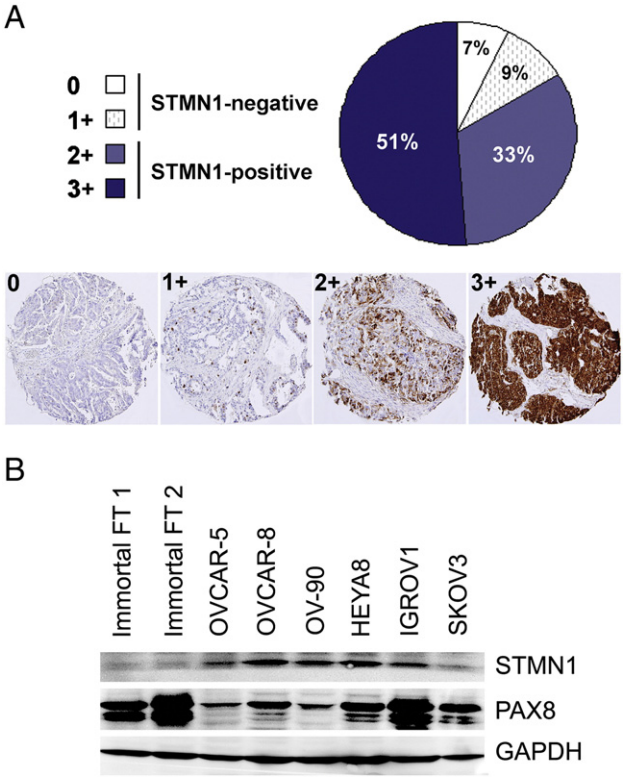

Fig. 5. STMN1 is strongly expressed in serous ovarian carcinomas. (A) In a tissue microarray analysis of 131 high-grade serous ovarian carcinomas, 16% of samples was STMN1-negative [score 0 (all cells negative) or 1+ (<10% positive cells)] while 84% was STMN1-positive [score 2+ (10–75% positive cells) or 3+ (>75% positive cells)]. (B) 5/6 ovarian cancer cell lines express high levels of STMN1 whereas 2 immortalized FTSEC lines are negative, determined by Western blotting. The Müllerian origin of the cell lines was confirmed by PAX8 immunoblotting. GAPDH is a loading control.

transition from benign precursor to proliferative lesion. Alternatively, STMN1 expression in this setting could reflect a loss of TP53 tumor suppressor function. TP53 mutations are present within an overwhelming majority of HGPSCs [30] and about half have already occurred by the p53 signature stage [6]. Wild-type p53 transcriptionally represses STMN1, and mutant p53 can impair this negative regulation, leading to increased STMN1 levels [31–33]. Silencing STMN1 expression reportedly inhibits proliferation, viability, and clonogenicity of mutant TP53 breast cancer cells *in vitro*, recapitulating a wild-type TP53 phenotype [34].

We observed strong STMN1 expression in TICs and invasive serous carcinomas, which is not unexpected given their high mitotic index. However, we were surprised by how dramatically STMN1 expression increased at the juncture between benign and malignant epithelium (Fig. 4). STMN1 expression clearly correlated with increased Ki-67, a proliferation marker associated with late S-phase [35]. However, there were usually more STMN1-positive than Ki-67-positive cells (Figs. 1 and 4), implying that STMN1 is not merely a mitotic cell marker but, rather, identifies cells with proliferative potential, including cycling interphase cells that have exited G_0 but may not be actively dividing. This is consistent with STMN1's role in maintaining microtubule fluidity during interphase. It is important to note that STMN1 expression in early lesions (proliferative p53 signatures and TICs) was not uniquely observed in cases of HGPSC, but also occurred in incidental lesions in the absence of overt malignancy (data not shown). This suggests that STMN1 induction does not merely reflect a cellular response to the tumor microenvironment, but is truly associated with tumor initiation. Moreover, given the possibility that some TICs may represent mucosal spread rather than precursor lesions in cases of advanced disease, it is significant that a similar pattern of STMN1 expression was observed in incidental TICs (data not shown). Accordingly, STMN1 may be a useful supplemental marker for determining the cell cycle status of seemingly benign yet atypical lesions in the fallopian tube.

Whether STMN1 expression directly contributes to fallopian tube epithelial transformation or is merely an indicator of cellular transformation events remains to be determined. STMN1 expression has been closely linked to phosphatidylinositol 3-kinase (PI3K)-mediated signal transduction. Therefore, STMN1 induction in tubal lesions may indicate the activation of this pathway. At least two studies have identified STMN1 as a robust biomarker of PI3K activation. The first, conducted by Saal et al., generated an IHC-based gene expression signature of PI3K activation using >100 breast cancer biopsies [36]. Of 246 candidate genes, STMN1 was the most reliable surrogate marker of PI3K pathway activation and was readily detectable by IHC, thereby compensating for a lack of suitable antibodies against more obvious markers such as phosphorylated AKT. A second study, published by Andersen et al., employed phosphoprofiling to identify drug-specific biomarkers of responsiveness to PI3K small molecule inhibitors [37]. Among the most prominently inhibited phosphoproteins following PI3K inhibitor treatment were the cytoskeletal machinery proteins, including STMN1. These studies suggest that STMN1 expression or phosphorylation status may be clinically useful for predicting susceptibility to anti-PI3K pathway therapy.

In several cases we examined, STMN1 induction at the transition from benign to malignant fallopian tube epithelium coordinated with reduced p27 expression (Fig. 4), suggesting that p27 down-regulation may be required for the initiation of FTSEC proliferative activity. This observation is consistent with a recent study by Norquist et al., which reported decreased p27 expression in the "p53 foci" of BRCA1/2 mutation carriers but not in those of normal controls. p53 foci are essentially variants of the p53 signature, defined by Norquist et al. as focal p53 staining (>75% positive cells) without specific reference to Ki-67 index [10,25]. It is difficult to compare our findings to theirs because they separated p53 foci according to BRCA mutational status rather than Ki-67 expression level. However, both of our studies identify p27 down-regulation as an early event in serous tumor pathogenesis. Reduced p27 expression has also been described in pre-malignant lesions of the oral mucosa (oral dysplasia) and prostate (prostatic hypertrophy), and in non-invasive tumors such as ductal breast carcinoma *in situ* [38–40]. As a cyclin-dependent kinase inhibitor, p27 inhibits G0- to S-phase transition by binding to CyclinE–CDK2 complexes [41]. It is normally degraded during G1-phase, enabling CyclinE–CDK2 to activate the transcription of G1- to S-phase genes [42]. Regulation of p27 activity is complex and involves multiple upstream signaling pathways, including the PI3K, SRC, and MAPK pathways. Aberrant p27 depletion in tumor cells is typically attributed to reduced transcription, increased degradation, or altered subcellular localization [35].

Coordinated changes in STMN1 and p27 expression may be significant for at least two reasons. Firstly, STMN1 expression in FTSECs could be interpreted as direct evidence of cell cycle entry, and this event likely follows a release of p27-mediated restrictions on cell proliferation. Secondly, p27 has been shown to directly bind to and inhibit STMN1 in the context of cell migration [13]. Microtubule dynamics are important not only for mitosis but also for cell motility. p27 appears to interfere with STMN1's depolymerizing ability, thus impairing the cytoskeletal remodeling required for cell movement [14]. STMN1 expression in "proliferative p53 signatures" and TICs could reflect an acquisition of migratory potential by FTSECs. The ability to migrate is important during early tumorigenesis because it supports anchorage independence and enables neoplastic cells to move away from their site of origin. This is particularly relevant in the setting of pelvic serous carcinoma where rapid spread of malignant cells over peritoneal membranes often occurs at very early stages of the disease. Baldassarre et al. demonstrated that p27 overexpression inhibits sarcoma cell motility and this effect can be reversed by co-expression of STMN1 [14]. Furthermore, they found that a low

p27/STMN1 ratio in sarcoma tumors correlated with increased metastasis. However, we cannot eliminate the possibility that other upstream regulators, aside from p27, are driving STMN1 expression in the setting of fallopian tube epithelial transformation.

In conclusion, our study has identified STMN1 as a novel marker of serous tumorigenesis in the fallopian tube. The dynamics of STMN1 expression observed in early tubal lesions suggest that STMN1 plays a critical role in FTSEC cell cycle progression. Its induction in pre-neoplastic cells may signal cell cycle entry and identify pre-mitotic cells with proliferative potential. In addition, STMN1 expression might contribute to HGPSC pathogenesis by relaying oncogenic growth signals to the cytoskeleton or by potentiating early-stage cell migration and loss of polarity.

Supplementary materials related to this article can be found online at doi:10.1016/j.ygyno.2011.05.021.

Conflict of interest statement
The authors declare no conflicts of interest.

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