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Stathmin 1 and p16^{INK4A} are sensitive adjunct biomarkers for serous tubal intraepithelial carcinoma

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HIGHLIGHTS

- A significant fraction of STIC lesions can be negative for p53 immunostaining.
- STMN1 and p16 are sensitive and specific biomarkers for STIC.
- The addition of STMN1 and p16 to Ki-67 and p53 stains improves diagnostic accuracy of STIC.

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ABSTRACT

Objective. To credential Stathmin 1 (STMN1) and p16^{INK4A} (p16) as adjunct markers for the diagnosis of serous tubal intraepithelial carcinoma (STIC), and to compare STMN1 and p16 expression in p53-positive and p53-negative STIC and invasive high-grade serous carcinoma (HGSC).

Methods. Immunohistochemistry (IHC) was used to examine STMN1 and p16 expression in fallopian tube specimens ($n = 31$) containing p53-positive and p53-negative STICs, invasive HGSCs, and morphologically normal FTE (fallopian tube epithelium). STMN1 and p16 expression was scored semiquantitatively by four individuals. The semiquantitative scores were dichotomized, and reported as positive or negative. Pooled siRNA was used to knockdown p53 in a panel of cell lines derived from immortalized FTE and HGSC.

Results. STMN1 and p16 were expressed in the majority of p53-positive and p53-negative STICs and concomitant invasive HGSCs, but only scattered positive cells were present in morphologically normal FTE. Both proteins were expressed consistently across multiple STICs from the same patient and in concomitant invasive HGSC. Knockdown of p53 in immortalized FTE cells and in four HGSC-derived cell lines expressing different missense p53 mutations did not affect STMN1 protein levels.

Conclusions. This study demonstrates that STMN1 and p16 are sensitive and specific adjunct biomarkers that, when used with p53 and Ki-67, improve the diagnostic accuracy of STIC. The addition of STMN1 and p16 helps to compensate for practical limitations of p53 and Ki-67 that complicate the diagnosis in up to one third of STICs.

1. Introduction

High-grade serous carcinoma (HGSC) is the most common form of epithelial ovarian cancer and typically presents at an advanced stage when current therapies are rarely curative [1]. A growing body of literature now supports the fallopian tube fimbria as the site of origin for a majority of HGSCs [2–8]. The chief argument for a tubal origin of HGSC is the presence of occult non-invasive carcinomas in the distal end of the fallopian tube (i.e., fimbria), designated serous tubal intraepithelial carcinoma (STIC). Morphologic and genetic evaluation of STICs have shown a high degree of similarity to concomitant ovarian or peritoneal carcinomas [1]. In particular, similar to HGSC, virtually all STICs harbor *TP53* mutations, which are identical to *TP53* mutations in the affiliated ovarian carcinomas, supporting their clonal relationship [9]. A similar precursor lesion in the ovary containing a *TP53* mutation has not been shown. The most common *TP53* mutations are missense (61%), while non-sense mutations are present in the remainder of cases [9,10]. Missense *TP53* mutations are correlated with strong diffuse staining of

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p53 in STICs and HGSCs, while complete absence of p53 immunoreactivity correlates with non-sense mutations, which produce a truncated protein that is not detected by the p53 antibody ("null mutation") [9].

In addition to invasive HGSC cases, STICs are also found in 5–10% of fallopian tubes removed prophylactically from women who are at high risk for developing ovarian cancer, including those women with BRCA mutations and/or those with a strong family history of ovarian cancer [11,12]. Detection of STICs in the high risk population has been greatly enhanced by comprehensive pathologic assessment of fallopian tubes through the use of the SEE-FIM (Sectioning and Extensively Examining the FIMbriated end) protocol, and risk-reducing bilateral salpingo-oophorectomy (RRSO) [7]. An accurate and sensitive diagnosis of STICs in the high-risk population may impact their subsequent clinical management, and can prompt early clinical action including increased surveillance, additional surgical staging or adjuvant chemotherapy [13,14].

Currently, STICs are diagnosed in many cases using an algorithm that combines morphologic evaluation and immunohistochemistry for p53 and Ki-67 [13]. However, the histologic diagnosis can be challenging when the morphologic changes are subtle, and lack reproducibility [13,15]. Accordingly, strong and diffuse p53 immunoreactivity may be the most contributory component of the diagnostic algorithm. However, in the presence of a null mutation, p53 immunoreactivity is completely absent, and this can occur in 20%–50% of STICs [11]. This diagnostic pitfall necessitates the development of additional biomarkers to aid in diagnosis of p53-negative STICs.

Previous studies have shown that overexpression of other oncogenic proteins can also be associated with STIC and HGSC. Karst et al. reported that Stathmin 1 (STMN1), a cytoplasmic phosphoprotein that regulates microtubule dynamics, is strongly and diffusely immunoreactive in STICs and a large proportion of HGSCs, but not in non-neoplastic FT epithelium [16]. Similarly, $p16^{INK4A}$ (p16), a cyclin-dependent kinase IV inhibitor, has been shown to be overexpressed in STIC [17] and HGSC [18,19]. However, neither of these studies specifically addressed the expression of these proteins in p53-negative STICs. The primary objective of this study was to compare STMN1 and p16 expression in p53-positive and p53-negative STICs and HGSCs, and to credential these proteins as adjunct biomarkers for the diagnosis of STIC.

2. Materials and methods

This study was approved by the Institutional Review Boards at the Cedars-Sinai Medical Center (CSMC), Brigham and Women's Hospital (BWH), Dana-Farber Cancer Institute (DFCI), and Yale University.

2.1. Case selection

The cases for this study were obtained from the Departments of Pathology at CSMC, BWH, and Yale University. Tubal sections were cut from paraffin blocks from 31 patients whose original pathology reports indicated the presence of STIC. These H&E slides were reviewed concurrently by two pathologists (MSH, RD) to confirm the presence of STICs and possibly invasive carcinoma in the deeper tissue sections cut for this study. STICs were diagnosed based on established morphologic features, including loss of ciliated cells, loss of cell polarity, epithelial stratification and tufting, nuclear enlargement and pleomorphism, nuclear hyperchromasia, prominent nucleoli, and increased mitotic figures. Lesions fulfilling the morphologic criteria were then examined for p53 and Ki-67 reactivity in subsequent serial sections. Lesions with >10% Ki-67-positive nuclei were considered proliferative. p53 expression was evaluated for strong diffuse immunoreactivity (positive for mutation, "p53 positive STIC") or a complete absence of staining (positive for a null mutation, "p53-negative STIC") in the area of atypia; scattered cells immunoreactive for p53 were considered a negative result (p53 wild type). For p53-null lesions, the presence of scattered immunoreactive stromal cells and/or non-neoplastic fallopian tube cells was noted to confirm that the antibody and immunostaining technique were adequate (i.e., positive internal control).

The tissue sections in this study were obtained from patients whose age at the time of surgery ranged between 44–75 years (mean = 63, median = 65). For detailed information about the patients' age, FIGO stage, and BRCA1/2 status please refer to Supplemental Table 1. Of the 31 cases, 20 cases contained p53-positive STICs, and 11 contained p53-negative (null) STICs. Six of the 31 cases contained only one STIC (four p53-positive, two p53-negative), while the remaining 25 cases contained at least two STICs (16 p53-positive, nine p53-negative) of which two per case were used for analysis; therefore, 56 STICs in total were examined in this study. Multiple STICs in one tissue section presented an opportunity to examine whether STMN1 and p16 immunoreactivity is concordant across multiple lesions in the same patient. In the 25 cases where two arbitrary STICs were evaluated, the in situ lesions were marked "A" and "B" by one author (MN) to ensure that the same lesions were evaluated by multiple subsequent reviewers. For statistical analyses, only one STIC per case was used (unless comparing group A to group B), and the STICs used from either group A or B were selected randomly using the "RANDBETWEEN" function in Microsoft Excel. Twenty-four of the 31 total cases with STIC also contained an invasive tumor component (17 p53-positive, seven p53-negative). Of note, there were three cases, which contained STICs with no evidence of invasive HGSC in the tubes or in the peritoneum (marked "in situ" in Supplemental Table 1). Morphologically normal tubal epithelium consisting of secretory and ciliated cells was represented in every case.

2.2. Immunohistochemistry

Immunohistochemical staining was performed using Envision Plus Horseradish Peroxidase system (DAKO, Carpinteria, CA, USA) as previously described [16]. Sections were incubated with primary antibody using the conditions specified in Supplemental Table 2. Secondary antibody was applied for 30 min, followed by DAB. Studies were interpreted in conjunction with appropriate positive (Supplemental Table 2) and negative (incubation without a primary antibody) controls. Additionally, scattered cell immunoreactivity by all biomarkers in non-neoplastic epithelium was used an internal positive control.

2.3. Analysis of p16 and STMN1 immunostaining

The p16 and STMN1 immunostains were scored independently by four individuals (MN, AMK, MSH, RD), to evaluate the extent of immunoreactivity (percent of positive cells). STICs (single or multiple) and invasive carcinomas were marked accordingly on the H&E slides as described above. The scoring criteria for p16 and STMN1 were adopted from Phillips et al. [19] and Karst et al. [16], respectively (summarized in Supplemental Table 3). In brief, the distribution of immunoreactivity for both p16 and STMN1 was scored semiquantitatively as follows: 0 (negative or occasional positive cells), 1+ (<10% cells positive), 2+

Table 1
STMN1 and p16 immunostaining in p53-positive and p53-negative STIC and invasive serous carcinoma.

Morphologic feature	p53 status	n	STMN1 positive	p^*	p16 positive	p^*
STIC (n = 31)[a]	Positive	20	17 (85%)	1.000	17 (85%)	0.210
	Negative	11	9 (82%)		7 (63%)	
Invasive carcinoma (n = 24)[b]	Positive	17	16 (94%)	1.000	16 (94%)	0.507
	Negative	7	7 (100%)		6 (86%)	
Morphologically benign FTE (n = 31)[c]	Positive	20	0 (0%)	1.000	0 (0%)	1.000
	Negative	11	0 (0%)		0 (0%)	

[a] vs. [b]; ($p = 0.158$); Fisher's exact test.
[a], [b] vs. [c]; ($p < 0.001$), Chi-square.
p^* (p53 pos vs. p53 neg); Fisher's exact test.