Stathmin-1 Expression as a Complement to p16 Helps Identify High-grade Cervical Intraepithelial Neoplasia With Increased Specificity

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Abstract: A fundamental controversy in using biomarkers to diagnose cervical cancer precursors [ie, squamous intraepithelial lesions (SIL)] is their lack of specificity for high-grade SIL [HSIL; cervical intraepithelial neoplasia grade 2/3 (CIN2/3)]. Stathmin-1 (STMN), a microtubule-destabilizing protein important in mitosis, is overexpressed in a variety of malignancies including those from the Müllerian tract and may be associated with poor outcome. However, the use of STMN as a diagnostic marker in cervical SILs has not been explored. A total of 193 cervical samples, including benign cervix and squamous and glandular lesions, were evaluated for STMN, p27 (a known cell cycle regulator inversely expressed with STMN in normal and many neoplastic tissues), p16, and Ki67 expression by immunohistochemical analysis. SILs were independently scored and classified as benign, low-grade SIL (LSIL/CIN1), and HSIL (separated into CIN2 or CIN3) on the basis of a majority diagnosis. Each diagnosis was correlated with biomarker expression independent of hematoxylin and eosin review. STMN was normally expressed in basal cells of the ectocervix and was absent in benign endocervix;"positive" STMN staining was defined as immunoreactivity in at least two thirds of the epithelial thickness. A majority hematoxylin and eosin diagnosis was obtained in 189/193 cases on initial independent review, with squamous epithelia ultimately classified as benign, CIN1, CIN2, and CIN3 in 25, 56, 11, and 16 cases, respectively. STMN was positive in 5/56 (9%) CIN1, 5/11 (45%) CIN2, 14/15 (93%) CIN3, all adenocarcinoma in situ (19/19), and all invasive squamous cell carcinoma (32/32) and adenocarcinoma (34/34) cases. In contrast, 40/56 (71%) CIN1, 11/11 (100%) CIN2, and 15/16 (94%) CIN3 lesions were p16 positive (defined as diffuse block staining in at least one third of the epithelial thickness). One CIN3 was negative for both p16 and STMN. Ki67 and p27 staining was variable in squamous lesions. These results demonstrate that STMN is overexpressed in virtually all cervical carcinomas and CIN3 lesions. In contrast to p16, which often stains LSIL and a small subset of reactive biopsies, STMN has greater specificity for CIN3 and has the potential to distinguish these latter lesions from the majority of low-grade precursors and negative/reactive cervical biopsies. STMN overexpression appears independent of proliferation, maturation, and human papilloma virus cytopathic effect and may be useful diagnostically in identifying HSIL. Further studies correlating STMN staining with lesion persistence or morphologic progression, in the context of CIN grade, are warranted.

Key Words: stathmin, p27, HPV, p16, cervix, CIN, SIL, squamous, adenocarcinoma

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A fundamental controversy in using biomarkers to diagnose cervical precursor lesions [ie, squamous intraepithelial lesions (SIL)] is their lack of specificity for high-grade SILs [HSILs; cervical intraepithelial neoplasia (CIN) 2/3]. Although diffuse p16 staining is traditionally used as a surrogate marker for high-risk human papilloma virus (HPV) infection, it has been shown to stain a significant number of low-grade SILs (LSILs), which often contain carcinogenic HPV.[1–4] Nevertheless, the distinction of HSIL (CIN2/3) from LSIL (CIN1) is clinically significant with treatment recommendations being linked specifically to risk for cancer or CIN3 outcome. LSIL/CIN1 will progress to invasive carcinoma in $<1\%$ of cases and is typically managed with Papanicolaou smear follow-up, whereas CIN2/CIN3 has a 5% to 20% risk of progression[5] and is usually treated with an excisional procedure (loop electrosurgical excision procedure or cone biopsy). Cervical adenocarcinoma in situ (AIS) portends a higher risk of concurrent or progression to invasive adenocarcinoma (ACA), with studies citing an incidence of up to 30%.[6,7] Therefore, adjuvant biomarker evaluation in SILs not only triages cases for immediate management but also helps to determine the required intensity of surveillance.

Recently, stathmin-1 (STMN) has gained significant attention as a prognostic biomarker for aggressive invasive

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and metastatic tumors from multiple organ sites including the cervix,[8] ovary,[9–11] endometrium,[12–14] breast,[15–18] stomach,[19] colon,[20,21] liver,[22–24] lung,[25,26] prostate,[27,28] and bladder,[29,30] among others.[31–37] STMN, also referred to as oncoprotein18, is a ubiquitous microtubule-destabilizing protein shown to be important during mitosis and has been implicated as a regulator of cell motility and migration.[29] Inhibition of STMN expression has been shown to decrease cellular proliferation and invasion, as cells accumulate in the G2/M phase of the cell cycle and undergo increased apoptosis.[19,29,38] $p27^{kip1}$ (hereafter p27) is a cell cycle inhibitor that has been implicated in cell motility and has been shown to bind and restrict STMN activity after p27 translocation to the cytoplasm.[39] In the absence of p27, STMN remains active, microtubules are less stable, and the cells have a higher propensity to be mobile and to invade. Such an association between STMN and p27 at the protein level has been shown in aggressive ovarian carcinomas, which demonstrate increased expression of STMN and a decreased expression of p27.[40]

Few studies have addressed STMN's role as a diagnostic biomarker or in the biological continuum encompassing normal, preinvasive, and invasive disease. Recently, Karst et al[40] demonstrated dramatic changes in STMN and p27 expression when comparing benign fallopian tube (FT) epithelium and intraepithelial and invasive carcinomas of the FT. In the normal FT and in nonproliferating precursor lesions (ie, p53 signatures), STMN expression was low to absent, and the expression of p27 was increased. In contrast, the converse was true in proliferating serous tubal intraepithelial lesions (STICs) and invasive carcinomas. These results not only have the potential to help understand the pathobiology behind serous pelvic carcinogenesis and new therapeutic targets but may also prove to be useful clinically for the practicing pathologist trying to diagnose a STIC.

The overexpression of STMN mRNA and protein has been demonstrated in invasive cervical carcinoma[8]; however, no one has evaluated STMN expression in intraepithelial lesions of the cervix. Therefore, the goal of this study was to investigate the expression of STMN in benign and precancerous lesions from the cervix with the intent to define its clinical utility in diagnosing high-risk lesions of the cervix, particularly in comparison with the more traditionally used biomarkers, p16 and Ki67, as well as p27, which has been shown to be upregulated in the presence of carcinogenic HPV.[41]

MATERIALS AND METHODS

Case Selection and Histologic Diagnoses

After approval from the Institutional Review Board at Brigham and Women's Hospital, cervical samples (n = 193) were retrieved from departmental archives and included non-neoplastic benign or reactive cervix (n = 25), noninvasive squamous (SIL/CIN; n = 83) and glandular (AIS; n = 19) lesions, invasive squamous cell carcinoma (SCC; n = 31), and invasive ACA (n = 34).

Hematoxylin and eosin (H&E)-stained biopsies were reviewed, and pathologic diagnosis was confirmed. All cervical biopsies were independently scored by 3 expert gynecologic pathologists (C.P.C., M.R.N., M.S.H.) and classified according to a majority diagnosis (≥ 2 of 3 agreeing) as benign/reactive changes, CIN1, CIN2, or CIN3; cases without majority agreement were reviewed together and majority opinion was obtained.

Immunohistochemistry

All cases were evaluated for STMN, p27, p16, and Ki67 expression by immunohistochemistry using standard protocols and as previously described.[40] Briefly, immunohistochemical analysis was performed using the Envision Plus/Horseradish Peroxidase system (Dako, Carpinteria, CA) and a polyclonal antibody to STMN (Cell Signaling Technology, Danvers, MA; 1:150 dilution), a monoclonal antibody to p27 (BD Transduction Laboratories, San Jose, CA; clone 57; 1:400 dilution), a prediluted monoclonal antibody to p16 [MTM Lab (9517); titer 1:2], and a monoclonal antibody to Ki67 [Dako; clone MIB1 (M7240); 1:200 dilution]. Paraffin-embedded sections were incubated in hydrogen peroxidase and absolute alcohol for 30 minutes to block endogenous peroxidase activity. Antigen retrieval was performed using pressure-cooker pretreatment in citrate buffer (pH = 6.0). Tissue sections were subsequently incubated with the primary antibody (STMN overnight; p27, p16 and Ki67 for 40 min) at 25°C. After rinsing with tris-buffered saline, the tissue was incubated using the Envision Plus secondary antibody for 30 minutes, followed by diaminobenzidine for 5 minutes. Appropriate positive (HSIL for p16, tonsil for Ki67, breast carcinoma for STMN, and colon carcinoma for p27) and negative (incubation with secondary antibody only) controls were stained in parallel for each round of immunohistochemistry.

Immunohistochemical stain interpretations were performed independent of the H&E diagnosis by 2 pathologists (B.E.H., M.S.H.). A summary of immunohistochemical scoring schema is provided in Table 1. Positive STMN staining in SIL biopsies was defined as cytoplasmic immunoreactivity in at least two thirds of the epithelial thickness for squamous epithelia (on the basis of the fact that the basal cell layer is uniformly positive in all cervical squamous epithelia; see also the Results section) and diffuse cytoplasmic staining in endocervical glands. For cervical carcinomas (SCC and ACA), STMN was evaluated for the amount of expression [scored as either negative (< 5%), patchy (5% to 50% of neoplastic cells), or diffuse (> 50% of neoplastic cells)] and for intensity [weak (1+), moderate (2+), or strong (3+)]. p16 was considered positive when continuous stretches (block staining) of nuclei with or without cytoplasmic reactivity were positive in at least one third of the epithelial thickness, as recently described by Darragh et al[42]; scattered individual cells and noncontinuous stretches of epithelium that were immunoreactive with p16 were scored as negative. p27 was evaluated for increased staining as follows: in the ectocervix, p27 staining in the basal layer

TABLE 1. Immunohistochemical Scoring Schema for All Sample Types

	STMN	p16	p27	Ki67
Ectocervix	Positive: ≥2/3 epithelial thickness Negative: <2/3 thickness	Positive: ≥1/3 epithelial thickness Negative: <1/3 thickness	Positive: staining in basal cell layer Negative: no staining of basal layer	Increased: ≥50% epithelial thickness Within normal limits: ≤50% epithelial thickness
Endocervix	Positive: diffuse staining Negative: absent or focal staining	Positive: diffuse staining Negative: absent or focal staining	Positive: >50% Negative: ≤50%	Increased: >50% Within normal limits: ≤50%
Carcinomas	Amount: Negative: <5% of tumor Patchy: 5%-50% of tumor Diffuse: >50% of tumor Intensity: Weak (1+) Moderate (2+) Strong (3+)	Amount: Negative: <5% of tumor Patchy: 5%-50% of tumor Diffuse: >50% of tumor Intensity: Weak (1+) Moderate (2+) Strong (3+)	Positive: >50% Negative: ≤50%	Increased: >50% Within normal limits: ≤50%

was considered increased, as no basal p27 positivity was seen in benign squamous epithelium; for invasive SCCs, AIS, and invasive ACA, increased staining was defined as staining in >50% of neoplastic nuclei. Ki67 was quantified in the carcinomas and AIS cases by estimating the percentage of neoplastic nuclei showing moderate to strong intensity of staining (< 50% = within normal limits; ≥ 50% = increased). In noninvasive squamous epithelia, the Ki67 index was scored as the extent of staining and reported as percentage of epithelial thickness (basal only or <50% = within normal limits; >50% = increased).

Statistical Analysis

The sensitivities and specificities of p16 and STMN were calculated for CIN2-3 (HSIL) as well as CIN3 only, using all cervical biopsies showing noninvasive SILs or a benign/reactive cervix. The positive predictive values (PPVs), negative predictive values (NPVs), and likelihood ratios were also calculated for HSIL/CIN2-3 for the same group of cervical biopsies (http://faculty.vassar.edu/lowry/clin1.html).

RESULTS

Morphologic Review

A majority (≥ 2) H&E diagnosis was obtained in 189/193 cases on the initial independent review by 3 pathologists (C.P.C., M.R.N., M.S.H.). The 4 cases without initial majority agreement were reviewed as a group to obtain the consensus diagnoses. Ultimately, biopsies of squamous epithelia were classified as benign (n = 25), CIN1 (n = 56), CIN2 (n = 11), or CIN3 (n = 16). Figure 1 illustrates the H&E diagnoses with complete (3/3; Figs. 1A–D) or majority (2/3; Figs. 1E–F) agreement, as well as examples of problematic cases without agreement on initial review (Fig. 1G–H).

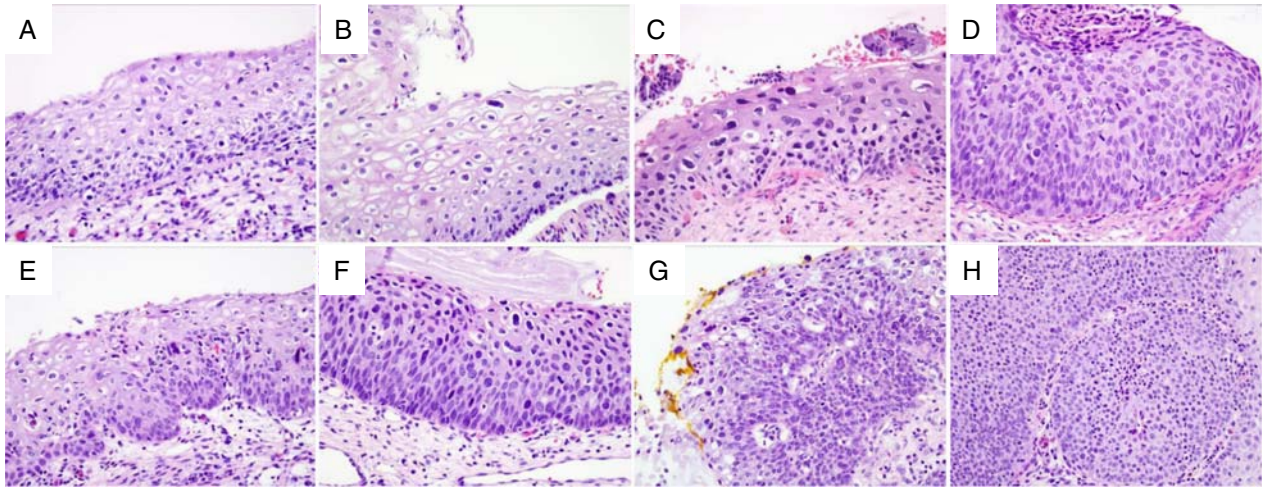

FIGURE 1. H&E consensus diagnoses. A–D, Initial 3/3 consensus for benign (A), CIN1 (B), CIN2 (C), and CIN3 (D). E and F, Initial 2/3 consensus for CIN1 (E) and CIN3 (F). G and H, No initial consensus, group review designated CIN2 (G) and CIN1 in squamous metaplasia (H).

TABLE 2. Summary of Immunohistochemistry Data for Cervical Samples

	STMN (%)	p16 (%)	Ki67 (%)	p27 (%)
Benign ecto	0/25 (0)	5/25 (20)	2/25 (8)	3/25 (12)
CIN1	5/56 (9)	40/56 (71)	26/55 (47)	9/56 (16)
CIN2	5/11 (45)	11/11 (100)	9/11 (82)	5/10 (50)
CIN3	14/15 (93)	15/16 (94)	12/16 (75)	13/15 (87)
SCC	32/32 (100)	31/32 (97)	27/32 (84)	21/31 (68)
Benign endo*	0/19 (0)	0/19 (0)	0/19 (0)	0/19 (0)
AIS	19/19 (100)	19/19 (100)	10/19 (53)	18/19 (95)
ACA	34/34 (100)	34/34 (100)	31/34 (91)	34/34 (100)

*Benign endocervix was evaluated in all of the AIS cases.
Ecto indicates ectocervix; endo, endocervix.

Nineteen samples showed AIS, 1 of which also had a coexisting HSIL/CIN3 lesion. Samples of SCC (N = 32) and invasive ACA (N = 34) were also included with complete consensus.

Immunohistochemistry

Squamous Cervical Epithelia

The staining results for STMN, p16, p27, and Ki67 in benign ectocervix, CIN1, CIN2, and CIN3 are summarized in Table 2.

STMN was normally expressed in basal cells of the ectocervix, whereas p27 was normally expressed in the mid to superficial layers of the ectocervix with a lack of basal-layer staining (Fig. 2). STMN was negative in all 25 benign/reactive cervical biopsies, and p27 showed aberrant basal-layer expression in 3/25 cases (12%). p16 and Ki67 were positive/increased in 5/25 (20%) and 2/25 (8%) biopsies classified as benign/reactive, respectively. One example of a reactive ectocervical biopsy with p16 positivity is shown in Figure 3, row 1.

STMN was positive in 24/82 (29%) SILs, with differential expression based on the grade of the lesion as

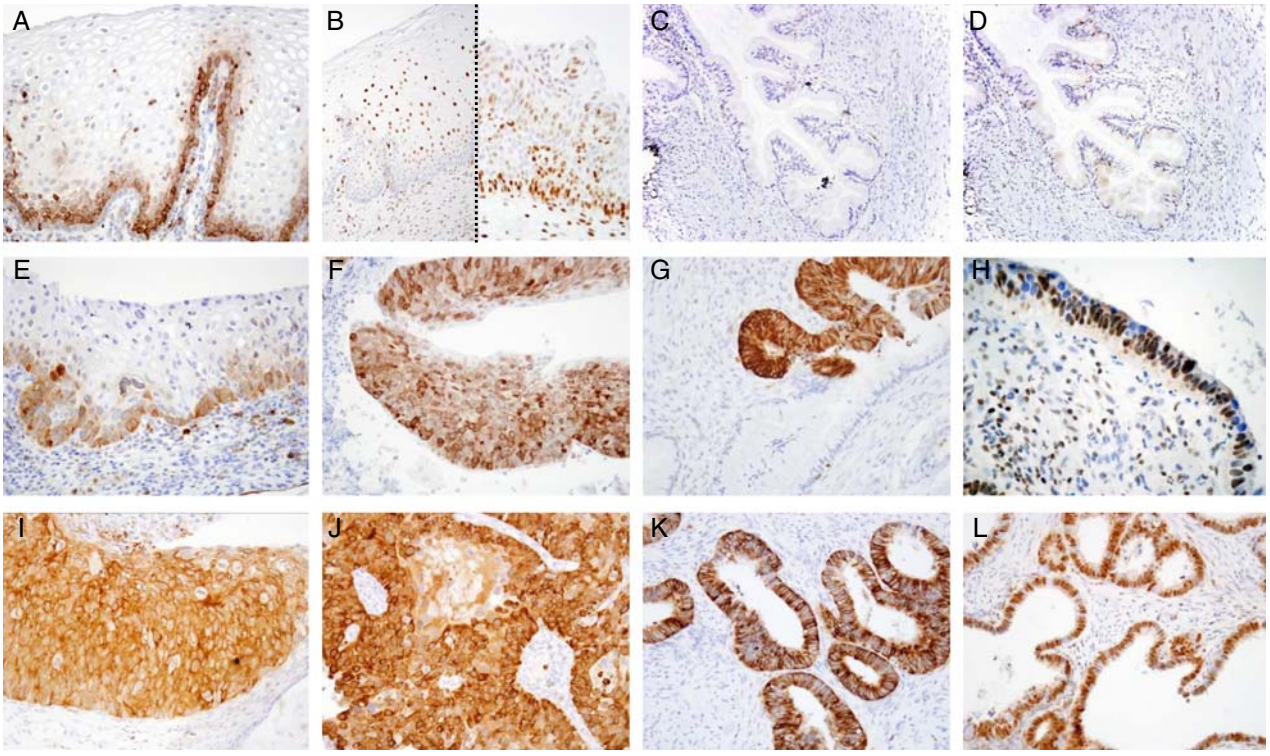

FIGURE 2. STMN and p27 immunohistochemistry. STMN normally stains the basal layer of the benign ectocervix (A). p27 stains the midlayer of mature squamous epithelium, but is absent in the basal layer (B, left), whereas in CIN3 p27 is positive in the basal layer (B, right). STMN (C) and p27 (D) are negative in the benign endocervix. STMN is negative in most CIN1 (E), is positive in some CIN2 (F), and almost all CIN3 (I) and SCC (J) cases. STMN (G and K) is positive in all AIS (G) and invasive ACA (K) cases. p27 (H and L) shows increased expression in glandular neoplasia as seen in this superficial AIS (H) and invasive ACA (L).

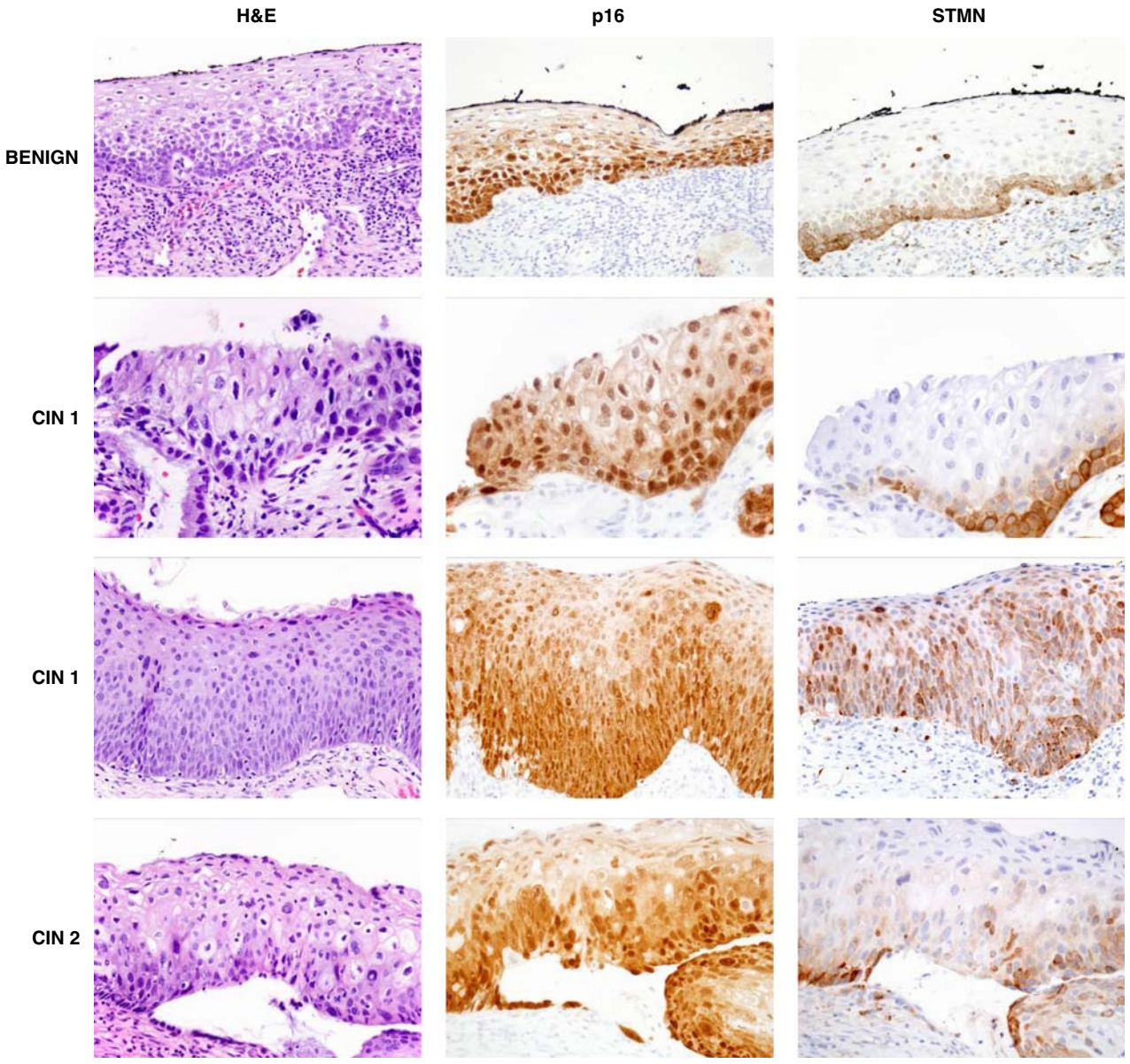

FIGURE 3. STMN and p16 in difficult cases. Four difficult cases (each row) showing H&E, p16, and STMN staining. Row 1 is a benign ectocervix with reactive epithelial changes, showing p16 positivity and STMN negativity. Row 2 is CIN1 showing p16 positivity and STMN negativity. Row 3 is another CIN1 showing both p16 and STMN positivity. Row 4 is CIN2 showing p16 positivity and STMN negativity.

follows: 5/56 (9%) CIN1, 5/11 (45%) CIN2, and 14/15 (93%) CIN3. p16 staining in these same cases was positive in 66/83 (80%) SILs, including 40/56 (71%) CIN1, 11/11 (100%) CIN2, and 15/16 (94%) CIN3 lesions. Of the 5 CIN1 cases that were STMN positive, 4 were also p16 positive. All CIN2/CIN3 cases that were STMN positive were also p16 positive. The one lesion classified as CIN3 that was p16 negative was also STMN negative. Overall, these results demonstrate that p16 immunoreactivity has greater sensitivity for SIL in general but that STMN may have greater specificity for high-grade lesions (HSIL).

Examples of difficult or problematic cases are illustrated in Figure 3, including benign or CIN1 that show immunoreactivity for p16 (Fig. 3, rows 1 to 3) and/or positivity for STMN (Fig. 3, row 3). An example of a CIN2 that was p16 positive and STMN negative is shown in Figure 3, row 4.

The results of statistical analysis to determine the sensitivity, specificity, PPV, and NPV for STMN for both CIN2-3 and CIN3 are presented in Table 3. For comparison, the same analyses were performed for p16. The specificity of STMN for both CIN2-3 (94%) and CIN3

TABLE 3. Sensitivity, Specificity, PPV, and NPV for STMN and p16 in CIN2-3 vs. CIN3

	STMN for CIN2-3	p16 for CIN2-3	STMN for CIN3	p16 for CIN3
Sensitivity (%)	73	96	93	94
Specificity (%)	94	44	89	39
PPV (%)	79	37	58	21
NPV (%)	92	97	99	97

(89%) is higher than that of p16 (44% and 39%, respectively), while retaining a similar sensitivity for CIN3 (93% for STMN vs. 94% for p16) but dropping off in sensitivity for CIN2-3 (73% for STMN vs. 96% for p16). The NPVs for both p16 and STMN are high and comparable for both CIN2-3 and CIN3 only groups; however, the PPV of STMN for CIN2-3 is significantly higher when compared with p16 (79% vs. 37%, respectively).

Glandular Cervical Epithelia

The immunohistochemical staining results for STMN and p27 in benign endocervix and AIS are included in Table 2.

STMN and p27 are both entirely absent in benign endocervical epithelium (Fig. 2). p16 was also typically negative in the benign endocervix, and Ki67 proliferative index was low (data not shown). All AIS cases were positive for STMN (n = 19), with the following distribution and intensity: patchy 2+ (n = 2), diffuse 2+ to 3+ (n = 17). All AIS cases were diffusely positive for p16 (n = 19; 2+ to 3+ intensity), and p27 was increased in 18/19 cases (95%). An elevated Ki67 index was seen in 10/19 (53%) AIS cases.

Invasive Cervical Carcinomas

All invasive SCCs were positive for STMN (n = 32) (Table 2), with the following distribution and intensity: patchy 1+ to 2+ staining (n = 4), 2+ diffuse staining (n = 9), and 3+ diffuse staining (n = 19). Similarly, 31/32 invasive SCCs were positive for p16, with the following distribution and intensity: patchy 2+ (n = 1), diffuse 2+ (n = 2), and diffuse 3+ (n = 28). p27 and Ki67 proliferative index were increased in 21/31 (68%) and 27/32 (84%) of SCCs, respectively.

All invasive ACA cases (n = 34) were positive for both STMN and p16 (Table 2), with the following distribution and intensity: STMN: patchy 2+ to 3+ (n = 2), diffuse 1+ (n = 1), diffuse 2+ to 3+ (n = 31); p16: patchy 2+ (n = 1), diffuse 2+ to 3+ (n = 33). All ACA cases had increased p27 expression. An elevated Ki67 index was seen in 31/34 (91%) ACAs.

DISCUSSION

Cervical biopsies performed to rule out cervical cancer and its precursor lesions are among the most common specimens encountered in gynecologic pathology. Although diagnoses may be made by evaluation of H&E-stained sections alone in most cases, a sufficient number of diagnostic dilemmas arise warranting the need for ancillary biomarkers to aid in diagnosis. SILs involving immature metaplasia, inflamed epithelium, and/or atrophic epithelium are some situations in which a pathologist may need to use immunohistochemical biomarkers.

Although many biomarkers have been studied in cervical biopsies,[42–47] the most widely and consistently used immunohistochemical stains in the cervix are p16 and Ki67, which are strongly and diffusely positive in most HSILs (CIN2/3). To date, p16 immunostaining remains the most reliable and sensitive (89% to 98%) adjuvant biomarker to help rule in or out SILs associated with carcinogenic HPV.[42,44,48] However, the specificity of p16 for CIN2/3 is limited by the high prevalence of carcinogenic HPVs across the entire spectrum of SIL. Another downside to p16 is that it has been known to stain a small subset of negative and reactive cervical biopsies.[2,44,49–51] High-risk HPV testing may also be of clinical value, but it is more expensive, time consuming, and may require sample send-out to an outside laboratory in smaller practices. Moreover, HPV typing is not specific for HSIL.[3,49]

In this study, 2 additional biomarkers, STMN and p27, were evaluated in benign and neoplastic cervical specimens, and STMN proved to be slightly less sensitive but more specific than p16 for HSILs (Table 3). Both STMN and p27 are cell cycle regulators that are known to play important opposing roles in cell cycle progression. STMN is a microtubule-destabilizing phosphoprotein that positively regulates cell cycle progression, and it is essential for cell division and proliferation.[52,53] p27 is a cyclin-dependent kinase inhibitor that negatively regulates cell cycle progression when localized to the nucleus and may also play a role in cell morphology and motility.[54] Some studies have shown that in the presence of HPV infection with E7 protein expression, p27 is actually overexpressed and sequestered to the cytoplasm.[41,55,56] This may suggest that p27, when localized to the cytoplasm, might actually promote migration and therefore have some oncogenic activity.

The results of this study demonstrate that STMN is overexpressed in virtually all cervical carcinomas and CIN3 lesions. In contrast to p16, STMN demonstrated greater specificity for CIN3, and it distinguished these lesions from the majority of low-grade precursor lesions and benign cervix, including those with reactive epithelial changes. Interestingly, STMN was positive in only a subset of CIN2 lesions, the reason for which is unclear. One explanation would be that STMN is more effective at identifying late (CIN3), rather than early (CIN2), phases of lesion progression. However, given the greater disparity in agreement regarding the biological potential of lesions classified as CIN2, it is also conceivable that STMN is actually more specific than histologic criteria in predicting behavior. Therefore, determining the real

reason for "inconsistent" staining patterns would require a follow-up study of STMN-positive versus STMN-negative lesions that were classified as CIN2 by morphology.

Karst et al[40] have also shown overexpression of STMN in precursor lesions of the FT (ie, STIC). In their study, they noted an inverse relationship between STMN and p27 staining; that is, in STICs with overexpression of STMN, p27 was negative. This is in contrast to the current findings in cervical tissues, in which increased expression of both STMN and p27 were present in the HPV-driven intraepithelial and invasive lesions. These findings are interesting from the biological perspective, as p27 has been shown to be overexpressed in the cytoplasm (in contrast to the nucleus) when the HPV E6/7 proteins are also expressed.[41,55,56] A similar concordant staining pattern for STMN and p27 has also been demonstrated in the oral cavity, another site affected by HPV infection.[55,56] Our observation of high p27 levels, most notably in AIS and invasive ACA, led us to believe that HPV oncogenic proteins lead to dysregulation of the cell cycle and loss of the inverse relationship with STMN expression.

As a diagnostic marker in SIL classification, the major potential advantage STMN appears to have is higher specificity relative to p16. In this study, p16 was positive in 80% (66/83) of the biopsies classified as SIL, including 71% (40/56) of CIN1 lesions, 100% (11/11) of CIN2 lesions, and 94% of (15/16) CIN3 lesions, whereas STMN was positive in only 29% (24/82) of the biopsies classified as SIL; however, this included a greater relative proportion of high-grade to low-grade lesions, as STMN was positive in only 9% (5/56) of CIN1 lesions, 45% (5/11) of CIN2 lesions, and 93% of (15/16) CIN3 lesions. In addition, p16 was immunoreactive in 5 cases classified by consensus (4 cases with agreement by 2 pathologists, and 1 case with agreement by all 3 pathologists) as benign/reactive. In hindsight, p16 expression may suggest that these 5 cases represent difficult to identify low-grade lesions; however, on the basis of the current College of American Pathologists' recommendations, a p16 would not have been performed because of the lack of significant cytologic atypia.[42] In this study, the lack of STMN immunoreactivity in these 5 p16-positive cases supports a benign diagnosis. Nevertheless, the findings still raise the question as to when the expression of a biomarker such as p16 or STMN confers clinical/prognostic and diagnostic significance.

Multiple studies have been conducted to determine whether the expression of p16 in SIL, especially low-grade lesions, can predict the potential for such lesions to "progress" to a higher-grade lesion.[1,2,43,50,51,57] In summary, these studies demonstrate that up to ~70% of CIN1 lesions are p16 positive, and of these lesions 42% to 60% will be followed by negative cytology or biopsy (regress), 9% to 40% of cases will persist as LSIL, and between 14% to 36% of cases will be followed by a high-grade SIL. Although nearly all cases with subsequent high-grade SIL were previously p16 immunoreactive and ultimately required treatment, there were also a significant subset of cases (greater than two thirds) in which the expression of p16 alone could potentially lead to overtreatment in a significant subset of women diagnosed with SIL. For this reason, the addition of the biomarkers, such as STMN, with increased specificity for HSIL could help more accurately diagnose and manage women with cervical SIL. One conceivable scenario would be a 2-tiered decision-making process whereby p16 is used to exclude both normal mucosa and lesions that do not contain carcinogenic HPVs. The addition of STMN would permit narrowing the proportion of p16-positive lesions that warrant consideration as HSIL. If used in concert with histologic evaluation there is the real possibility that a significant percentage of p16-positive reactive lesions and LSILs could be pulled out on the basis of lack of STMN positivity. Of course, evaluation of larger studies with follow-up data will be necessary to determine the different biological potentials of p16-postive/STMN-negative versus p16-postive/STMN-positive SILs. It is noteworthy that STMN did not provide any additional benefit over p16 staining when comparing invasive squamous lesions and all glandular lesions of the cervix; however, a previous study by Xi et al[8] has suggested that higher levels of STMN expression correlates with poor clinical outcome.

Caveats (ie, "pitfalls") worth noting when using STMN as a diagnostic biomarker in cervical squamous neoplasia is that it does stain the basal layer of normal benign ectocervix. Therefore, to correctly evaluate for STMN staining, one must have a well-oriented fragment of tissue. Although many poorly oriented biopsies are a problem on H&E staining for diagnosing dysplasia/SIL, STMN may not be helpful in this scenario. STMN may prove to be most reliable in concert with p16 in well-oriented cone biopsies and potentially in the evaluation of margins in cone/loop electrosurgical excision procedure specimens.

In summary, increased STMN expression is present in both high-grade cervical precursor lesions and frankly invasive carcinomas. STMN overexpression appears independent of proliferation, maturation, and HPV cytopathic effect and has potential to be useful diagnostically in identifying HSIL. p27 was less sensitive and specific for cervical neoplasia; however, in these HPV-associated samples we found that p27 may be concurrently increased with STMN, indicating aberrant regulation of cell cycle progression. Further studies correlating STMN and p16 staining with lesion persistence or morphologic progression, in the context of CIN grade, are warranted.

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