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Upregulation of MUC4 in Cervical Squamous Cell Carcinoma: Pathologic Significance

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Summary

MUC4 is a transmembrane glycoprotein more highly expressed in cervical dysplasia than benign cervical epithelium. We sought to determine whether MUC4 expression differs between benign and malignant cervical tissue. Fifty-eight patients with benign, dysplastic, or malignant cervical pathology were identified retrospectively, and representative sections were stained with a mouse monoclonal anti-MUC4 antibody. Semiquantitative analysis was performed on benign, dysplastic, and malignant regions by scoring staining intensity (0: negative, 1: weak, 2: moderate, and 3: strong) and distribution (focal <10%, multifocal = 10%–60%, diffuse ≥60%). In samples with benign glycogenated squamous epithelium, only the parabasal cells had MUC4 staining, and 48.5% had an intensity of 2 or 3. All samples with immature squamous metaplasia were positive through the entire epithelial thickness. Cervical intraepithelial neoplasia (CIN) 1 samples had variable staining with an intensity similar to glycogenated squamous epithelium but distribution similar to squamous metaplasia. All CIN 3 (n = 21) and invasive squamous cell carcinomas (n = 17) had increased MUC4 staining intensity ($P < 0.001$ and $P < 0.001$) and increased diffuse staining ($P < 0.001$ and $P < 0.001$) compared with the limited staining in glycogenated squamous epithelium. In contrast, no differences in staining were observed between benign endocervical glands, adenocarcinoma *in situ*, and invasive adenocarcinoma. These expression patterns suggest that MUC4 is a lineage marker in benign cervical tissue that may have aberrant expression in squamous dysplasia and carcinoma. Further studies may elucidate the role of MUC4 in the development of squamous cell cervical cancer.

Keywords

MUC4; Cervical cancer; Squamous cell carcinoma; Adenocarcinoma

The American Cancer Society estimates that 11,070 women in the United States would be diagnosed with cervical cancer in 2008, and 3,870 women will die of their disease (1). Though

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progress has been made in prevention and treatment of early-stage disease, fewer treatment options are available for women with recurrent or advanced disease. Elucidating the molecular pathways involved in cervical cancer development may lead to novel therapies.

Mucins are membrane-bound or membrane-secreted glycoproteins found in epithelial cells including the lining of the endocervical canal (2–5). Twenty different mucins have been found in humans, which serve to protect normal epithelial tissue throughout the body (2–4). Many mucins have been shown to play a role in cell proliferation, differentiation, and survival, suggesting that abnormal mucin expression may have a role in the pathogenesis of cancer (3, 4).

Recently, it has been noted that MUC4, a membrane-bound mucin, is upregulated in several different malignancies including pancreatic, ovarian, and breast cancer (6–10). Our recent studies have shown that ErbB2 (also known as HER2/neu) signaling is regulated by MUC4 in pancreatic tumor cells (11). In addition, rat Muc4/sialomucin complex serves as a ligand for rat p185/neu, a homolog of ErbB2 (12). Activation of this receptor can lead to an increase in signaling molecules that stimulate cell proliferation and the dissociation of cell-cell adhesions, promoting metastatic potential (13,14). Variable ErbB2 expression in some types of cervical cancer has been examined in attempts to correlate expression with prognosis (15–20).

Prior studies have provided evidence that MUC4 is upregulated in squamous cervical dysplasia (21). In addition, a study of only glandular lesions of the cervix found MUC4 expression in benign endocervical glands and 38% of adenocarcinoma *in situ* (AIS) and 75% of adenocarcinoma (AC) (22). No studies have evaluated the expression of MUC4 in squamous cell carcinoma (SCC) of the cervix. Thus, we sought to evaluate MUC4 expression in benign and malignant cervical epithelia. Differential expression could suggest a role for MUC4 in the signaling pathways involved in cervical cancer and lead to further studies investigating new potential treatments.

METHODS

Tissue Samples

Institutional Review Board approval was obtained from the respective institutions. Tissue samples from 58 patients with cervical pathologic diagnoses of benign cervical tissue (n = 10), CIN 3 (n = 15), invasive SCC (n = 17), AIS (n = 8), and AC (n = 8) were identified.

Immunohistochemistry

A mouse monoclonal antibody, 8G7, which recognizes a tandem repeat sequence of human MUC4 was used according to a previously described protocol (23,24). Briefly, 5- μ m sections of formalin-fixed paraffin-embedded tissues were cut and deparaffinized in xylene and rehydrated in graded alcohol. Antigen retrieval was performed by heating the slides for 15 minutes in citrate buffer (pH 6.0). After 3 washes in phosphate-buffered saline (PBS), the endogenous peroxidase activity was quenched with 0.3% H₂O₂ and the sections were blocked for nonspecific protein binding by incubating with normal serum. The sections were incubated overnight at 4°C with 1:1000 dilution of anti-MUC4 monoclonal antibody 8G7 and washed 3 times with PBS containing 0.05% Tween 20. Slides were then incubated with peroxidase-labeled anti-mouse IgG from the Impress kit (Vector Laboratories) and developed using 3,3'-diamino benzidine (Sigma). The slides were counterstained with hematoxylin, dehydrated in graded alcohol, and mounted with Vectamount permanent mounting media (Vector Laboratories).

Staining Analysis

The staining was evaluated by pathologists at Massachusetts General Hospital, University of Nebraska Medical Center, and Creighton University, and subsets of immunostains were reanalyzed by the same pathologists to confirm accuracy. Each tissue type (benign, dysplastic, and malignant) present on a slide was graded on intensity of staining on a scale of 0 to 3 (0 = no staining, 1 = light staining, 2 = moderate staining, and 3 = strong staining). The distribution of staining was categorized as none, focal (<10%), multifocal (10%–60%), and diffuse (≥60%). The Pearson χ^2 test was used to compare proportions with an $\alpha \leq 0.05$ defining significance. These tests were performed on STATA, version 10 software.

RESULTS

The staining profiles of benign, dysplastic, and neoplastic squamous epithelium are summarized in Table 1. Benign glycogenated squamous epithelium was present in 33 samples, and 26 of these (78.8%) had MUC4 staining, though it was predominantly confined to the parabasal cells (Fig. 1A). The parabasal cells exhibited variable intensity (0 in 21.2%; 1 in 30.3%; 2 in 33.3%; and 3 in 15.2%) and distribution of staining (7/33 [21.2%] were negative, 12/33 [36.4%] had focal staining, 10/33 [30.3%] had multifocal staining, and 4/33 [12.1%] had diffuse staining).

All samples ($n = 11$) with immature squamous metaplasia stained positively. The staining pattern was different from that observed in glycogenated squamous epithelium in that the entire thickness of the epithelium stained diffusely in all cases ($P < 0.001$; Fig. 1B) with the intensity of staining being strong in 6 of 11 cases and moderate in 5 of 11 ($P = 0.002$).

Seven samples had areas of CIN 1, and 6 of these (85.7%) showed diffuse positivity but variable staining intensity (0 = 14.3%; 1 = 28.6%; and 2 = 28.6%; 3 = 28.6%; Fig. 1C). The intensity of CIN 1 samples was similar to glycogenated squamous epithelium ($P = 0.677$) but less intense than immature squamous metaplasia ($P = 0.017$). However, the distribution of staining for CIN 1 was more diffuse than that of glycogenated squamous epithelium ($P < 0.001$) and similar to that of squamous metaplasia ($P = 0.197$). All CIN 3 samples ($n = 21$) showed strong MUC4 staining, and the distribution of staining in the tissue with CIN 3 was diffuse in 18 of 21 (85.7%) of the samples, both higher than in glycogenated squamous epithelium ($P < 0.001$ and $P < 0.001$; Fig. 1D). All invasive SCCs ($n = 17$) were strongly positive, 76.5% displaying strong staining (intensity of 3) and the remaining showing moderate staining, more than in glycogenated squamous epithelium ($P < 0.001$; Fig. 1E). The majority of invasive SCCs also stained diffusely (10/17 = 58.8%; $P < 0.001$), whereas 5 and 2 of 17 (29.4% and 11.8%) showed multifocal or focal staining, respectively ($P < 0.001$).

The staining profiles of benign endocervical glands, AIS, and invasive AC are summarized in Table 2. Benign endocervical glands, present in 38 samples, always stained positively. In most cases, the staining was moderate (17/38 = 44.7%) or strong (12/38 = 31.6%) and diffuse (26/38 = 68.4%; Fig. 2A) and primarily noted at the apical border of the cells.

The majority of AIS samples showed strong or moderate (intensity of 2 or 3: 11/12 = 91.6%) and diffuse (10/12 = 83.3%) MUC4 staining (Fig. 2B). This was not significantly different from the profile on benign endocervical glands where the majority also stained diffusely and with an intensity of 2 or 3 ($P = 0.247$ and $P = 0.316$, respectively). Only 1 AIS sample was negative (8.3%). The pattern of staining in invasive AC was also not different from normal endocervical glands. Six of the 7 (85.7%) ACs stained positively; 5 displaying strong and 1 moderate positivity ($P = 0.583$), with the staining pattern being either multifocal (2/7) or diffuse (4/7; $P = 0.561$).

DISCUSSION

The present report provides the first evidence for MUC4 expression in SCC of the cervix. Furthermore, our data fit with a progression of minimal staining in benign glycogenated epithelium to variable staining in CIN 1, and extensive staining in CIN 3 and SCC. Though the difference in the distribution and intensity of MUC4 staining between glycogenated squamous epithelium and SCC is clear, there is no marked difference between immature squamous metaplasia and SCC. Cervical glandular tissue, including benign endocervical glands, AIS, and AC, stained strongly and diffusely, more similar to immature squamous metaplasia than glycogenated squamous epithelium. This suggests that MUC4 may be a lineage marker of the glandular origin of metaplastic cells and is conserved when HPV is introduced and cells undergo dysplastic change. These changes may trigger perpetual expression of MUC4, contributing to the diffusely positive staining we see in invasive SCC.

MUC4 staining was diffuse and intense in SCC whereas positive staining was virtually absent in normal glycogenated epithelium with the exception of basal cells. Our results localize MUC4 expression to the parabasal cells of benign ectocervical and endocervical tissue. These findings are consistent with results of other studies that used immunohistochemistry and *in situ* hybridization to demonstrate MUC4 expression in benign endocervical glandular epithelium in at least half of the samples analyzed (5,21,22,25). Despite a small sample size, 6 of the 8 ectocervical samples studied by Gipson et al. (5) were positive for MUC4 expression, a finding independent of the menstrual cycle. In contrast, results from Audie et al. (25) suggest that there is increased MUC4 expression in the endocervix in the luteal phase of the menstrual cycle (5). Given the dynamic properties of the cervical epithelium it is also possible that the shift in MUC4 intensity or pattern may reflect morphologic changes associated with metaplasia.

In contrast to the diffuse staining found in immature benign squamous metaplasia in our study (>60%), a previous report identified a variable pattern of staining with only 10% to 50% of cells stained (21). These differences could be attributed to the antibodies used, polyclonal versus the more specific monoclonal antibody 8G7 (24). The Lopez-Ferrer study is the only report before ours to investigate and show any evidence of MUC4 staining in dysplastic cervical squamous epithelium (21). The authors suggested that the increased MUC4 expression in benign endocervical tissue and squamous dysplasia may support the hypothesis that endocervical glands serve as a site for progenitor cells that may develop or differentiate into squamous dysplasia (21). Together these findings provide more evidence to suggest MUC4 expression may be perpetuated or turned on during dysplastic conversion.

Only one other group has looked at MUC4 staining in cervical ACs (22). Their study found that 38% of AIS and 75% of AC were positive for MUC4, leading to the proposal that a lower level of MUC4 in AIS may help to discern the diagnosis. However, we observed MUC4-positive staining in 91.7% of AIS and 85.7% of AC and found no marked difference in staining pattern between benign endocervical glands and AIS or AC.

Given the diffuse MUC4 staining in immature squamous metaplasia, MUC4 staining in SCC cannot be used to make a diagnosis. Nonetheless, the pattern of MUC4 expression may still provide important information about the development of cervical malignancies. Of note, endocervical glands stained more intensely than glycogenated squamous epithelium. Therefore, it may simply be that the cells undergoing squamous metaplasia have not yet lost the MUC4 expressing phenotype present in the reserve cell layer as they develop into mature squamous cells. MUC4 is lost in mature squamous epithelium, but perhaps with the development of malignancy, MUC4 expression is reactivated or never lost.

One hypothesis for a potential role for MUC4 in the setting of malignancy may be mediated through the ErbB2 receptor. MUC4 has 3 endothelial growth factor-like domains that can

potentially serve as ligands for the ErbB2 receptor (11,26,27). Previous studies have noted variable ErbB2 expression patterns or amplification of the gene in cancers of the uterine cervix, yet the presence of the ErbB2 receptor in certain groups of patients with cervical cancer offers a potential mechanism through which aberrant MUC4 expression could lead to the pathogenesis of malignancy. In one study, 100% of 19 ACs were positive but only 45.5% of 70 SCCs and 16.7% of 37 adenosquamous carcinomas were positive for ErbB2 (17). These observations were corroborated in a smaller study of only 5 samples in which ErbB2 overexpression correlated significantly with ACs (20). In contrast, a 1994 study looking at only SCC of the cervix found that ErbB2 was amplified in only 14% whereas a more recent study reported ErbB2 expression in 26% of squamous cell cervical carcinomas (15,16). Of the total number of ACs, 41% expressed ErbB2 (16). Whether or not increased MUC4 and ErbB2 activation promotes cervical carcinogenesis is yet to be determined.

The relationship between ErbB2 expression and prognosis in cervical cancer is unclear as the results from different studies have been contradictory (17–20). ErbB2 expression in cervical cancer was shown to be associated with poor survival in a series of 126 patients (17). A more recent study of 21 patients found that patients with tumors expressing ErbB2 also showed a trend toward worse survival (18). However, a very recent study by Califano and colleagues (19) contradicted these results; in a series of 25 patients they found that those diagnosed at earlier stages were more likely to exhibit ErbB2 expression although no difference in survival was observed between ErbB2-positive or negative tumors. Another group found that patients with tumors exhibiting increased ErbB2 and decreased epidermal growth factor receptor expression had significantly improved survival (20). Though the above studies show that some groups of patients with cervical cancer express ErbB2 receptors and MUC4 has been proposed to be a ligand for this receptor, it is unknown whether MUC4 binds this receptor specifically in the setting of cervical cancer (12,15–20). Further characterization of MUC4 and ErbB2 expression in cervical cancer could help delineate avenues of targeted treatment.

Despite advances in the prevention and treatment of cervical cancer, there are still limited options for those with advanced or recurrent disease. Biologic therapies that target signaling pathways important in the pathogenesis of cervical cancer allow more individualized and therefore effective treatments for patients. These data provide evidence that MUC4 expression is consistently upregulated in malignant SCC of the cervix, similar to immature squamous metaplasia, and markedly more so than benign glycogenated squamous epithelium. In contrast, the expression in ACs is not markedly different from expression in normal endocervical glands. Further work on the role of MUC4 may lead to the development of targeted therapies that benefit those with recurrent or advanced cervical cancer.

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References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics. *CA Cancer J Clin* 2008;58:71–96. [PubMed: 18287387]
2. Gendler SJ, Spicer AP. Epithelial mucin genes. *Annu Rev Physiol* 1995;57:607–34. [PubMed: 7778880]
3. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer* 2004;4:45–60. [PubMed: 14681689]
4. Moniaux N, Escande F, Porchet N, et al. Structural organization and classification of the human mucin genes. *Front Biosci* 2001;6:D1192–206. [PubMed: 11578969]

5. Gipson IK, Ho SB, Spurr-Michaud SJ, et al. Mucin genes expressed by human female reproductive tract epithelia. *Biol Reprod* 1997;56:999–1011. [PubMed: 9096884]
6. Rakha EA, Boyce RW, Abd El-Rehim D, et al. Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. *Mod Pathol* 2005;18:1295–304. [PubMed: 15976813]
7. Ponnusamy MP, Venkatraman G, Singh AP, et al. Expression of TAG-72 in ovarian cancer and its correlation with tumor stage and patient prognosis. *Cancer Lett* 2007;251:247–57. [PubMed: 17210225]
8. Swartz MJ, Batra SK, Varshney GC, et al. MUC4 expression increases progressively in pancreatic intraepithelial neoplasia. *Am J Clin Pathol* 2002;117:791–6. [PubMed: 12090430]
9. Andrianifahanana M, Moniaux N, Schmied BM, et al. Mucin (MUC) gene expression in human pancreatic adenocarcinoma and chronic pancreatitis: a potential role of MUC4 as a tumor marker of diagnostic significance. *Clin Cancer Res* 2001;7:4033–40. [PubMed: 11751498]
10. Arul GS, Moorghen M, Myerscough N, et al. Mucin gene expression in Barrett's oesophagus: an in situ hybridisation and immunohistochemical study. *Gut* 2000;47:753–61. [PubMed: 11076872]
11. Singh AP, Moniaux N, Chauhan SC, et al. Inhibition of MUC4 expression suppresses pancreatic tumor cell growth and metastasis. *Cancer Res* 2004;64:622–30. [PubMed: 14744777]
12. Carraway KL, Ramsauer VP, Haq B, et al. Cell signaling through membrane mucins. *Bioessays* 2003;25:66–71. [PubMed: 12508284]
13. Singh AP, Chaturvedi P, Batra SK. Emerging roles of MUC4 in cancer: a novel target for diagnosis and therapy. *Cancer Res* 2007;67:433–6. [PubMed: 17234748]
14. Jepson S, Komatsu M, Haq B, et al. Muc4/sialomucin complex, the intramembrane ErbB2 ligand, induces specific phosphorylation of ErbB2 and enhances expression of p27(kip), but does not activate mitogen-activated kinase or protein kinaseB/Akt pathways. *Oncogene* 2002;21:7524–32. [PubMed: 12386815]
15. Mitra AB, Murty VV, Pratap M, et al. ERBB2 (HER2/neu) oncogene is frequently amplified in squamous cell carcinoma of the uterine cervix. *Cancer Res* 1994;54:637–9. [PubMed: 7905784]
16. Rosty C, Couturier J, Vincent-Salomon A, et al. Overexpression/amplification of HER-2/neu is uncommon in invasive carcinoma of the uterine cervix. *Int J Gynecol Pathol* 2004;23:13–7. [PubMed: 14668544]
17. Nevin J, Laing D, Kaye P, et al. The significance of Erb-b2 immunostaining in cervical cancer. *Gynecol Oncol* 1999;73:354–8. [PubMed: 10366459]
18. Niibe Y, Nakano T, Ohno T, et al. Prognostic significance of c-erbB-2/HER2 expression in advanced uterine cervical carcinoma with para-aortic lymph node metastasis treated with radiation therapy. *Int J Gynecol Cancer* 2003;13:849–55. [PubMed: 14675323]
19. Califano D, Losito S, Pisano C, et al. Significance of erb-B2 immunoreactivity in cervical cancer. *Front Biosci* 2006;11:2071–6. [PubMed: 16720293]
20. Lee CM, Lee RJ, Hammond E, et al. Expression of HER2neu (c-erbB-2) and epidermal growth factor receptor in cervical cancer: prognostic correlation with clinical characteristics, and comparison of manual and automated imaging analysis. *Gynecol Oncol* 2004;93:209–14. [PubMed: 15047238]
21. Lopez-Ferrer A, Alameda F, Barranco C, et al. MUC4 expression is increased in dysplastic cervical disorders. *Hum Pathol* 2001;32:1197–202. [PubMed: 11727258]
22. Baker AC, Eltoum I, Curry RO, et al. Mucinous expression in benign and neoplastic glandular lesions of the uterine cervix. *Arch Pathol Lab Med* 2006;130:1510–5. [PubMed: 17090193]
23. Moniaux N, Varshney GC, Chauhan SC, et al. Generation and characterization of anti-MUC4 monoclonal antibodies reactive with normal and cancer cells in humans. *J Histochem Cytochem* 2004;52:253–61. [PubMed: 14729877]
24. Chauhan SC, Singh AP, Ruiz F, et al. Aberrant expression of MUC4 in ovarian carcinoma: diagnostic significance alone and in combination with MUC1 and MUC16 (CA125). *Mod Pathol* 2006;19:1386–94. [PubMed: 16880776]
25. Audie JP, Tetaert D, Pigny P, et al. Mucin gene expression in the human endocervix. *Hum Reprod* 1995;10:98–102. [PubMed: 7745080]
26. Moniaux N, Nollet S, Porchet N, et al. Complete sequence of the human mucin MUC4: a putative cell membrane-associated mucin. *Biochem J* 1999;338(Pt 2):325–33. [PubMed: 10024507]

27. Carraway KL III, Rossi EA, Komatsu M, et al. An intramembrane modulator of the ErbB2 receptor tyrosine kinase that potentiates neuregulin signaling. *J Biol Chem* 1999;274:5263–6. [PubMed: 10026131]

FIG. 1. MUC4 staining in representative sections of cervical tissue in the squamous lineage: (A) benign glycogenated squamous epithelium, (B) immature squamous metaplasia, (C) CIN 1, (D) CIN 3, and (E) invasive squamous cell carcinoma (left to right).

FIG. 2. MUC4 staining patterns in representative sections of cervical tissue of glandular lineage: (A) benign endocervical glands, (B) adenocarcinoma *in situ*, and (C) invasive adenocarcinoma.

TABLE 1

MUC4 staining in cervical tissues of squamous lineage

Cell type	n	Intensity, n (%)	P	Distribution, n (%)	P
Glycogenated squamous epithelium	33	0 = 7/33 (21.2%)		Negative = 7/33 (21.2%)	
		1 = 10/33 (30.3%)		Focal = 12/33 (36.4%)	
		2 = 11/33 (33.3%)		Multifocal = 10/33 (30.3%)	
		3 = 5/33 (15.2%)		Diffuse = 4/33 (12.1%)	
Immature squamous metaplasia	11	0 = 0		Negative = 0	
		1 = 0		Focal = 0	
		2 = 5/11 (45.5%)		Multifocal = 0	
		3 = 6/11 (54.5%)	0.002*	Diffuse = 11/11 (100%)	<0.001 [†]
CIN 1	7	0 = 1/7 (14.3%)		Negative = 1/7 (14.3%)	
		1 = 2/7 (28.6%)		Focal = 0	
		2 = 2/7 (28.6%)	0.678*	Multifocal = 0	<0.001 [†]
		3 = 2/7 (28.6%)	0.017 [‡]	Diffuse = 6/7 (85.7%)	0.197 [‡]
CIN 3	21**	0 = 0		Negative = 0	
		1 = 0		Focal = 2/21 (9.5%)	
		2 = 0	<0.001*	Multifocal = 1/21 (4.8%)	<0.001 [†]
		3 = 21/21 (100%)	N/A [‡]	Diffuse = 18/21 (85.7%)	0.188 [‡]
Invasive squamous cell carcinoma	17	0 = 0		Negative = 0	
		1 = 0		Focal = 2/17 (11.8%)	
		2 = 4/17 (23.5%)	<0.001*	Multifocal = 5/17 (29.4%)	<0.001 [†]
		3 = 13/17 (76.5%)	N/A [‡]	Diffuse = 10/17 (58.8%)	0.014 [‡]

* P value reflecting proportion of samples with intensity 2–3 compared with glycogenated epithelium.

** Although initially only 15 patients with CIN 3 were identified, but some of those with invasive SCC had areas of CIN 3, so the total number of samples with CIN 3 was 21.

[†] P value reflecting proportion with diffuse staining compared with glycogenated epithelium.

[‡] P value reflecting proportion of samples with intensity 2–3 compared with squamous metaplasia; in the case of the comparison for intensity, not calculable because proportions were both 100%.

TABLE 2

MUC4 staining patterns in cervical tissue of glandular lineage

Cell type	n	Intensity, n (%)	P	Distribution, n (%)	P
Normal endocervical glands	38	0 = 0	0.247*	Negative = 0	0.316†
		1 = 9/38 (23.7%)		Focal = 4/38 (10.5%)	
		2 = 17/38 (44.7%)		Multifocal = 8/38 (21.1%)	
Adenocarcinoma <i>in situ</i>	12	3 = 12/38 (31.6%)	0.583*	Diffuse = 26/38 (68.4%)	0.561†
		0 = 1/12 (8.3%)		Negative = 1/12 (8.3%)	
		1 = 0		Focal = 0	
Invasive adenocarcinoma	7	2 = 1/12 (8.3%)	0.583*	Multifocal = 1/12 (8.3%)	0.561†
		3 = 10/12 (83.3%)		Diffuse = 10/12 (83.3%)	
		0 = 1/7 (14.3%)		Negative = 1/7 (14.3%)	
		1 = 0		Focal = 0	
		2 = 1/7 (14.3%)		Multifocal = 2/7 (28.6%)	
		3 = 5/7 (71.4%)		Diffuse = 4/7 (57.1%)	

* P value reflecting proportion of samples with intensity 2–3 compared with normal endocervical glands.
† P value reflecting proportion of samples with diffuse staining compared with normal endocervical glands.