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The utility of PAX8 and IMP3 immunohistochemical stains in the differential diagnosis of benign, premalignant, and malignant endocervical glandular lesions

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HIGHLIGHTS

- PAX8 stains strong and diffusely in benign endocervical glandular lesions, and is progressively lost in EC AIS and ECA.
- IMP3 antibody shows near absent staining in a cohort of benign endocervical glandular lesions
- IMP3 extent and intensity of staining gradually increase in carcinoma in situ to conventional invasive endocervical adenocarcinoma

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ABSTRACT

Glandular lesions of the endocervix can be diagnostically challenging and occasionally the differential diagnosis includes endocervical adenocarcinoma *in situ* (EC AIS) and well-differentiated endocervical adenocarcinoma (ECA). PAX8 and IMP3 are two markers which have not been well studied in the endocervix. Our aim was to evaluate their immunohistochemical (IHC) expression in benign and malignant endocervical glandular lesions as well as to compare them to the traditionally used panel (Ki-67, p16, CEA).

Design. We searched our surgical pathology files for a cohort of benign endocervical glandular lesions as well as premalignant and malignant groups including EC AIS and ECA. An IHC panel consisting of PAX8, IMP3, Ki-67, p16, and CEA was performed on all cases. Immunoreactivity was scored on a degree of positivity (S0 = no immunoreactivity, S1 = up to 10% cells, S2 = between 10 and 50% cells, S3 => 50% cells) and intensity (Int0 — absent, Int1 — mild/faint, Int2 — moderate, Int3 — strong).

Results. PAX8 showed diffuse positivity (S3) with at least a moderate intensity of staining (Int2) in the benign group. PAX8 was focal (S1) in ECA and faint (Int1), compared to EC AIS, which was moderate (S2) and faint (Int1). IMP3 expression was focal in the benign group (S1), moderate (S2) in EC AIS and moderate-to-diffuse (S2-3) in ECA. IMP3 intensity was faint (Int1) in benign lesions, moderate (Int2) in EC AIS, and strong (Int3) in ECA. Significant Ki-67, p16, and CEA expression was noted in the premalignant/malignant cohort.

Conclusion. PAX8 and IMP3 can be helpful in the differential diagnosis of benign vs. malignant endocervical glandular lesions. Our study, however, shows that there is some degree of overlap of staining in both the benign and malignant group. As such, PAX8 and IMP3 should always be interpreted with caution and in combination with the histomorphology.

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Introduction

There is a broad spectrum of benign glandular proliferations and changes of the endocervix including tunnel clusters (TC), endocervical laminar hyperplasia (ELH), microglandular hyperplasia (MGH), mesonephric hyperplasia (MNH) and tuboendometrioid metaplasia (TEM). While the separation from such commonly found benign proliferations from EC AIS and ECA is usually straightforward, in some cases this can be extremely challenging. A variety of IHC markers including CEA, Ki-67, p16 and p53 [1,2] have been reported in the literature to aid in this distinction. However, there are limitations to the utility of such an IHC panel. Benign lesions such as MGH have been reported to express CEA and p53 [1,3], while p16 expression has been reported in metaplasias of the endocervix [4].

PAX8 (paired box protein 8) is a recently described transcription factor on chromosome 2p13 that is involved in the development of the kidneys, eye, thyroid and reproductive system [5,6]. Recent literature has demonstrated PAX8 expression in benign endocervical and endometrial epithelium as well as in a majority of conventional endometrial carcinomas [6,7]. However there is limited literature evaluating PAX8 IHC expression in the endocervix [5,8–10].

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IMP3 (insulin-like growth factor-II mRNA-binding protein 3), also known as KOC (K-homologous domain containing protein overexpressed in cancer), is composed of a 580 amino acid protein encoded by 4350 nucleotides on an mRNA. The IMP3 gene is located on 7p11.5 and is expressed during embryogenesis but not in adult tissue [4]. Aberrant expression of IMP3 has been observed in a variety of carcinomas and dysplasias including the pancreas [11], cervix [12], colon [13], liver [14], head and neck [15], and the breast [16]. Recently, Cuizhen et al. [4] reported p16 and IMP3 expression in benign endocervical glands, TEM and EC AIS.

The aim of this study was to compare the IHC expression of PAX8 and IMP3 along with currently established markers (Ki-67, p16, and CEA) in benign endocervical glandular proliferations with that in EC AIS and ECA.

Materials and methods

Case selection

The surgical pathology files at Hartford Hospital were searched for cases of both benign and premalignant/malignant cervical lesions between 2005 and 2012. There were 23 cases in the benign cohort (15 hysterectomy, 8 biopsy specimens). The premalignant/malignant cohort consisted of 21 cases (12 hysterectomy, 9 biopsy specimens). All cases were processed routinely using 10% buffered formalin and cut at 4 μm. The benign cohort included: MGH (5 cases), ELH (5 cases), TEM (2 cases), cervical endometriosis (CEM) (5 cases), tunnel clusters (TC) (5 cases), and MNH (1 case). The premalignant and malignant cohorts included: EC AIS (8 cases) and ECA (11). One additional case of minimal deviation adenocarcinoma (MDA) metastatic to the small intestine and a poorly differentiated endocervical adenocarcinoma with signet ring features were also included for comparison.

Immunohistochemistry

Selected paraffin blocks from both cohorts were retrieved from our surgical pathology archives and unstained slides were cut at 4 μm in thickness. One unstained slide from each case was incubated with PAX8 polyclonal antibody. Another unstained slide was incubated with KOC polyclonal antibody. Benign kidney and a case of pancreatic adenocarcinoma were used as positive controls for the PAX8 and IMP3 antibodies, respectively. In addition, all cases were stained with currently utilized markers Ki-67, p16, and CEA antibodies for comparative study (Table 1). Each slide was examined for proper positive and negative internal controls. The extent (S) and intensity (Int) of immunoreactivity were scored independently by two pathologists (SM, RD) with excellent interobserver agreement (>80%) utilizing the following criteria: S0 = no immunoreactivity (absent), S1 = up to 10% of cells showing immunoreactivity (focal), S2 = between 10 and 50% of cells showing immunoreactivity (moderate), S3 = >50% cells showing immunoreactivity (diffuse), Int0 = no staining, Int1 = mild intensity, Int2 = moderate intensity, Int3 = strong intensity. PAX8 nuclear and IMP3 cytoplasmic immunoreactivity were considered positive, respectively. Only minimal background nonspecific staining was observed.

Table 1
PAX8 and IMP3 IHC antibody information.

Antibody	Source	Dilution	Antigen retrieval	Positive control
PAX8	ProteinTech, clone >1 PAX8 ab (polyclonal)	1:100	Leica Microsystems Bond Max	Benign kidney
IMP3	DAKO, clone 69.1 (polyclonal)	1:50	Leica Microsystems Bond Max	Pancreatic adenocarcinoma

Table 2
Cohort of benign endocervical lesions and their immunoreactivity for PAX8 and IMP3.

Endocervical lesion (no. of cases)	PAX8	IMP3
TC (5)	S3/Int2	S1/Int1
ELH (5)	S3/Int2	S1/Int1
MGH (5)	S2/Int2	S1/Int1
TEM (2)	S3/Int2	S1/Int1
MNH (1)	S3/Int3	S1/Int1
CEM (5)	S3/Int3	S1/Int1

TC, tunnel clusters; ELH, endocervical laminar hyperplasia; MGH, microglandular hyperplasia; TEM, tuboendometrioid metaplasia; CEM, cervical endometriosis; MNH, mesonephric hyperplasia.

Results

The results of the IHC patterns for both benign and malignant lesions are listed in Tables 2 and 3.

Benign endocervical glandular lesions

All benign endocervical glandular lesions (Images 1a, d, g, j, m, p) showed diffuse (S3) nuclear positivity for PAX8. The intensity of PAX8 expression was moderate to strong (Int2-3) in all cases (Images 1b, e, h, k, n, and q).

IMP3 showed positive cytoplasmic staining in all cases of benign endocervical glandular lesions, but the extent of positivity was focal (S1) and the intensity faint or mild (Int1) (Images 1c, f, i, l, o, r).

Ki-67 showed a range of staining. There was either absent (S0/Int0) or focal staining with up to moderate intensity (S1/Int2). CEA immunoreactivity was absent in all cases (S0/Int0). P16 was focal (S1) in all cases, but with variable intensity of staining (ranging from Int1 to Int3).

Endocervical adenocarcinoma in situ (EC AIS)

All cases of EC AIS (Image 2a) were positive for PAX8, but with 50% or less of tumor cells staining (S2) and additionally the intensity of immunoreactivity was mild (Int1) (Image 2b). IMP3 showed moderate immunoreactivity in both extent and intensity (S2/Int2) (Image 2c).

The extent of Ki-67 staining was moderate (S2), while the intensity was moderate to strong (Int2-3). P16 was diffuse (S3) with moderate intensity of staining (Int2) in EC AIS. CEA was moderate, both in extent and intensity of staining (S2/Int2).

Invasive endocervical adenocarcinoma (ECA)

Ten of twelve cases (83%) of ECA (Image 2d) showed focal (S1) and faint (Int1) intensity of staining for PAX8 (Image 2e). IMP3 immunoreactivity ranged from moderate to diffuse (S2-3) albeit with strong intensity (Int3) (Image 2f). The single case of metastatic, minimal deviation ECA to the small intestine (Image 2 g) showed focal extent of PAX8 staining (S1) (Image 2 h) with mild (Int1) intensity and moderate to diffuse (S2-3) extent of staining with strong (Int3) IMP3 intensity (image 2i). In contrast, the single case of poorly differentiated ECA with signet ring

Table 3
Cohort of malignant endocervical lesions and their immunoreactivity for PAX8 and IMP3.

Endocervical lesion (no. of cases)	PAX8	IMP3
EC AIS (8)	S2/Int1	S2/Int2
ECA (11)[a]	S1/Int1	S2-3/Int3
Metastatic MDA (1)	S1/Int1	S2-3/Int3
PD ECA with signet ring features	S2-3/Int2-3	S1/Int1

EC AIS, adenocarcinoma *in situ*; ECA, invasive adenocarcinoma, including minimal deviation adenocarcinoma (MDA); PD ECA, poorly differentiated endocervical adenocarcinoma.
[a] Two cases of ECA showing diffuse PAX8 immunoreactivity.