

Original Article

Signet-ring cell in papilloma virus type 6 and 16-related non-neoplastic squamous cell lesion

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Abstract: Signet-ring cell has been used largely to refer to adenocarcinoma. Primary squamous cell lesions with signet-ring cell morphology are extremely uncommon. We reported the first case of condyloma acuminata with signet-ring cell of perianal skin in a 34-year-old Chinese woman. Typical signet-ring cells and gray-blue material were observed in parabasal and intermediate stratum. The large cytoplasmic vacuoles of signet-ring cell were negative for periodic-acid Schiff (PAS), Alcian blue (AB), mucicarmin, AE1/AE3, EMA, MUC2, MUC5ac, HMB45, p16^{INK4a} and CEA. EMA and AE1/AE3 immunopositivity draw the outline of signet-ring cells. The gray-blue material and septum of small vacuoles also expressed AE1/AE3, indicating that they might contain intermediate filaments. The blocking staining pattern of p16^{INK4a} encouraged the diagnosis of high-grade squamous intraepithelial lesion which carries a significant risk of invasive cancer development if not treated. HPV type 6 and 16 infections were confirmed by Hybrid Capture HPV test. Due to the similarities in molecular profiles it is conceived that signet-ring cell in HPV-related squamous cell lesion may represent a rare variant of koilocyte.

Keywords: Signet-ring cell, HPV, squamous cell lesion, immunohistochemistry, condyloma acuminata

Introduction

Signet-ring cell is used to describe cells with an eccentric nucleus that is compressed to the cellular border by a large cytoplasmic vacuole [1]. The presence of signet-ring cell has been traditionally associated with mucin-producing adenocarcinomas, most commonly of gastric, colonic or mammary origin [2-4]. Primary squamous cell lesion with signet-ring cell morphology is very rare. The importance of recognizing it lies mainly in differentiating it from several other cutaneous and metastatic neoplasms which have the capacity to show signet-ring cell changes, including but not limited to, basal cell carcinoma (BCC), malignant melanoma, metastatic gastric adenocarcinoma and breast carcinoma [5]. There is now convincing evidence that signet-ring cell can be observed in primary squamous cell carcinoma (SCC) [6, 7]. Although the exact etiology of primary SCC with signet-ring cell is still unknown, human papilloma virus

(HPV) appears to be one of the most potent inducers [8]. Here, we report the first case of HPV type 6 and 16-related non-neoplasia squamous cell lesion with signet-ring cell morphology.

Case presentation

A 34-year-old healthy Chinese woman (gravida 1, para 1) presented to the gynecology clinic with a 3-month history of nodal lesion in perianal skin, which had been increased in size in the past 3 weeks. Physical examination revealed a 7 mm diameter polypoid lesion. The nodule was resected under local anesthesia and then processed together with other routine biopsy materials from cervix to avoid the artifactual nature of the vacuolization. The formalin-fixed and paraffin-embedded (FFPE) tissue sections were studied by using hematoxylin and eosin staining, histochemistry, immunohistochemistry and HPV type-specific PCR and

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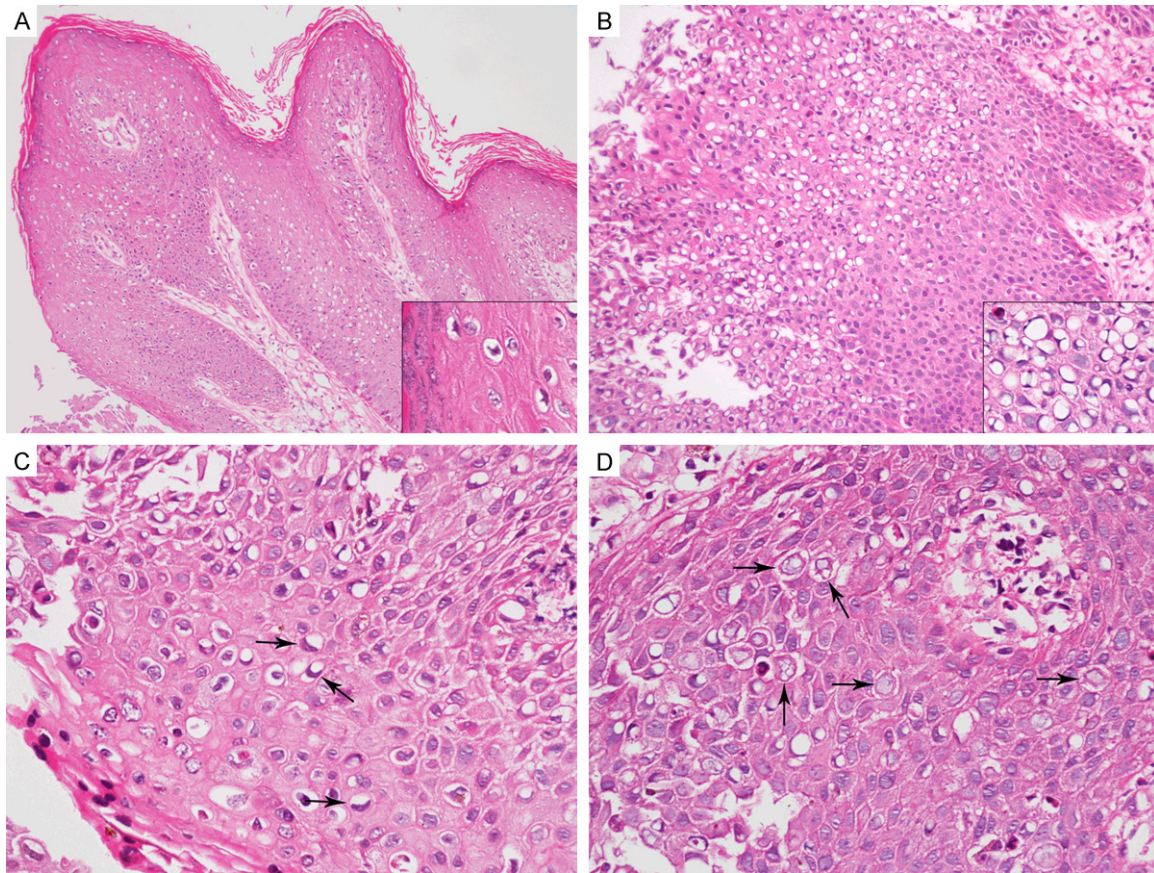


Figure 1. Histopathologic examination showed koilocytes in the superficial layer of squamous cell (A. original magnification $\times 100$; inserts $\times 400$). Typical signet-ring cells were observed (B. original magnification $\times 100$; inserts $\times 400$), and some of them (black arrows) contained hyperchromatic nuclei (C. original magnification $\times 400$). In the parabasal stratum of squamous cell, gray-blue material (black arrows) was detected in the cytoplasm (D. original magnification $\times 400$).

genotyping. Evaluation of all results was performed by two independent pathologists who were blinded regarding patient details.

Histopathology

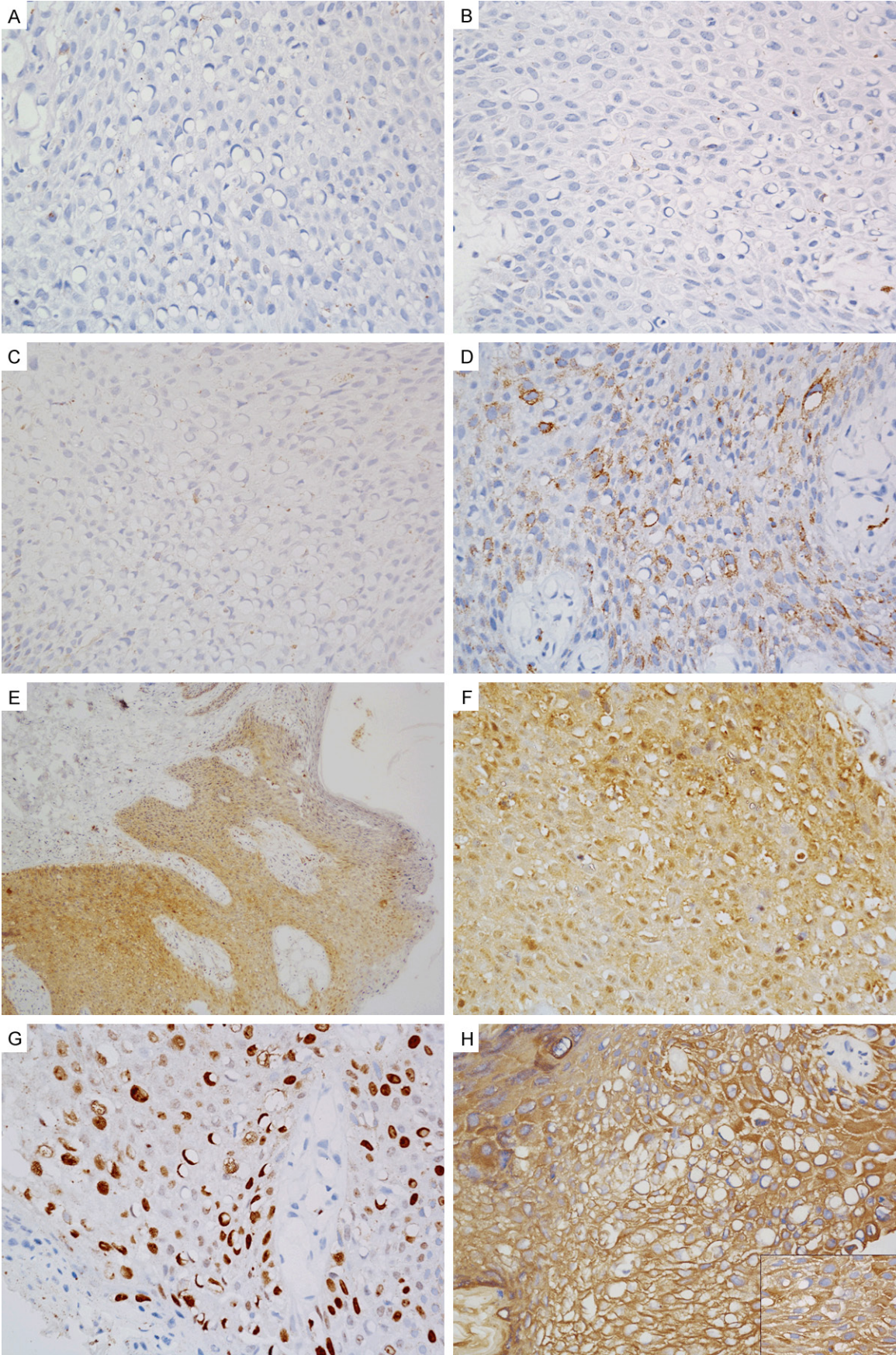
Epithelial cell hyperchromasia and crowding were seen. Some superficial and intermediate squamous cells exhibited enlarged nucleus with raisin-like nuclear membrane and prominent perinuclear vacuolation, characteristics of koilocyte (**Figure 1A**). Noteworthy, diffuse signet-ring cells were observed in the intermediate or parabasal stratum (**Figure 1B**). These signet-ring cells contained prominent vacuoles, which were sharply demarcated and appeared empty. However, most of signet-ring cells in this case are different from the malignant counterparts in their relative normal nuclei, the others scattered in the superficial stratum contained

enlarged and hyperchromatic nuclei (**Figure 1C**). Another intriguing finding is that cytoplasm of some parabasal cells contained basophilic material (**Figure 1D**).

Histochemistry and immunohistochemistry

The paraffin-embedded sections were stained with periodic-acid Schiff (PAS), Alcian blue, mucicarmin to explore the nature of signet-ring cell and gray-blue material. However, both of them were negative for the three histochemical staining, suggesting that neither mucin and mucopolysaccharides nor glycogen existed (**Figure 2A-C**). Signet-ring cells and koilocytes were positive for EMA (**Figure 2D**) but negative for MUC2, MUC5ac, HMB45 and CEA (data not shown). The scoring of p16^{INK4a} generally includes both nuclear and cytoplasmic staining, and is classified as negative, discontinu-

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Figure 2. The signet-ring cells were negative for PAS, Alcian Blue and mucicarmine (A-C. original magnification $\times 400$). They expressed EMA rather than MUC2, MUC5ac, HMB45 and CEA (D. original magnification $\times 400$). The block staining pattern of p16^{INK4a} was similar to that in high-grade squamous intraepithelial lesion of cervix (E, F. original magnification $\times 400$). Ki-67 immunopositivity was detected in most of koilocytes and signet-ring cells (G. original magnification $\times 400$). A strongly positive staining for cytokeratin AE1/AE3 was obtained in septum of small vacuoles and gray-blue material (H. original magnification $\times 400$; inserts $\times 400$).

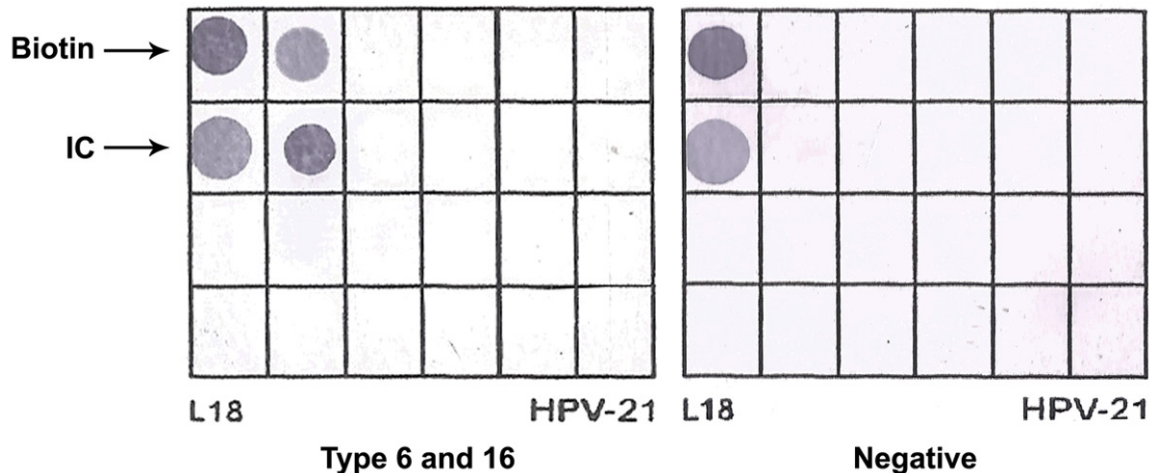


Figure 3. HPV type-specific PCR and genotyping result showed HPV type 6 and 16 infections.

ous, or diffuse/continuous [9-11]. At the periphery of the lesion, p16^{INK4a} was negative (**Figure 2E**). Strong and diffuse block pattern of p16^{INK4a} was observed in the area composed of atypical epithelial cells (**Figure 2E, 2F**). Nuclear staining observed in epithelial cells above the parabasal layer was considered Ki-67 positivity. More than 50% of signet-ring cells and koilocytes in the middle and upper one-third of the epithelium expressed Ki-67 (**Figure 2G**). Cytokeratin AE1/AE3 drew the outlines of signet-ring cells and septum of small vacuoles (**Figure 2H**). The gray-blue cytoplasmic material also showed positivity for AE1/AE3 (**Figure 2H**). CK7 and CK20 were negative in this specimen (data not shown).

HPV type-specific PCR and genotyping

DNA was extracted and purified from FFPE tissue sections by using TIANamp FFPE DNA Kit (TIANGEN, Beijing, China). The operation was performed according to the manufacturer's protocol. Subsequently, HPV DNA was amplified with the L1 consensus HPV PGMY09/PGMY11 primer set as described previously [12]. PCR was performed with a 25 μ l reaction system, which contained 1 μ l DNA template and 0.75 μ l DNA Taq polymerase. Amplification was carried

out for 40 cycles in the CFX96 Touch™ Real-Time PCR Detection System (BIO-RAD, USA). HPV genotyping was performed by using HPV GenoArray test kit (HybriBio, Chaozhou, China), which makes use of flow-through hybridization and gene chips to identify 15 high-risk HPV types (HPV type 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68) and 6 low-risk HPV types (HPV type 6, 11, 42, 43, 44 and 81). The result of genotypes indicated that this patient was co-infected with HPV type 6 and 16 (**Figure 3**).

Discussion

To the best of our knowledge, this is the first report of HPV-related non-neoplasia squamous cell lesion with signet-ring cell morphology. Increasing evidence has shown that signet-ring cell is no longer restricted to adenocarcinoma. A set of malignant or benign lesions of squamous cell origin has been demonstrated to exhibit signet-ring cell morphology (**Table 1**) [1, 5, 7, 8, 13-18].

The exact etiology of SRC-containing lesion is still unknown. However, accumulating evidence indicate the particularly strong association between HPV and squamous cell neoplasia.

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Table 1. Summary of primary squamous cell lesions with signet-ring cell morphology

Case	Age/ Sex	Pathologic Diagnosis	Location	HC		IHC		Etiology	EM	Reference
				Positive	Negative	Positive	Negative			
1	69/M	SRSCC	Forehead	N/A	Kreyberg Stain	keratin	N/A	N/A	N/A	[13]
2	N/A	SRSCC	Pyriiform Sinus	N/A	N/A	N/A	N/A	N/A	N/A	[14]
3	74/F	SCC	Cervix	No	AB, Mucicarmine, PAS	Cytokeratin	Vimentin, LCA, UEA I	No <i>Chlamydia trachomatis</i> infection	Empty spaces	[7]
4	84/F	SCC	Cervix	No	AB, Mucicarmine, PAS	Cytokeratin	Vimentin, LCA, UEA I	No <i>Chlamydia trachomatis</i> infection	Empty spaces	
5	35/F	CIN3	Cervix	No	AB, Mucicarmine, PAS	Cytokeratin	Vimentin, LCA, UEA I	No <i>Chlamydia trachomatis</i> infection	Moderately electron-dense flocculent material/empty spaces	
6	37/F	CIN3	Cervix	No	AB, Mucicarmine, PAS, Giemsa	Cytokeratin	Vimentin, LCA, UEA I	No <i>Chlamydia trachomatis</i> infection	Not done	
7	50/M	SRSCC	Neck	PAS (septum between vacuoles)	Mucicarmine, PAS (vacuoles)	AE1/AE3, MAK 6 Ker, Ker 903, CAM 5.2, CEA (weak), EMA (weak), Ki-67	Leu M1, S-100, HMB-45, Actin, Vimentin, SMA	Not done	Rough ER cisternal dilatation	[15]
8	79/F	SCC	Right cheek	No	Colloidal iron, PAS	cytokeratin	No	Not done	no stained material	[5]
9	82/M	SCC	Left temple	No	Colloidal iron, PAS	cytokeratin	No	Not done	no stained material	
10	83/M	SCC	Right ear	No	Colloidal iron, PAS	cytokeratin	No	Not done	no stained material	
11	80/M	SCC	Forehead	No	Colloidal iron, PAS	cytokeratin	No	Not done	no stained material	
12	87/M	SCC	Frontal scalp	No	Colloidal iron, PAS	cytokeratin	No	Not done	no stained material	
13	N/A	SCC	N/A	No	Colloidal iron, PAS	cytokeratin	No	Not done	no stained material	
14	76/M	SCC	Forehead	No	Colloidal iron, PAS	cytokeratin	No	Not done	no stained material	
15	84/F	SRSCC	Upper lip	No	Mucicarmine, PAS	keratin, P63, EMA	No	Not done	Not done	[16]
16	66/F	SCC	Sole	AB, Colloidal iron	PAS	AE1/AE3, EMA, CK5/6	CK7, CK20, CEA, BerEP4, S100, Her2-neu, ER, Ki-67	HPV 18	Not done	[8]
17	67/M	SCC	Left lateral canthus	No	AB, Mucicarmine, PAS	CK5/6, p63, EMA	CK7, Ck20, CEA	Not done	Not done	[1]
18	83/M	SRSCC	Left hand	Not done	Not done	No	CK20, CEA, vimentin, HMB45, Melan A, desmin	Not done	Not done	[17]
19	64/M	SRSCC	Left lung	No	AB, PAS	CK5/6, p16, p40, p63, EMA	TTF-1, Napsin A, calretinin, WT-1, D2-40, CD56, SYN	No HPV infection	Not done	[18]
20	34/F	Condyloma acuminata	Perianal skin	No	AB, Mucicarmine, PAS	AE1/AE3, p16 ^{INK4a} , Ki-67, EMA	CEA, MUC2, MUC5ac, CK7, CK20, HMB45	HPV type 6 and 16	Not done	present study

SRSCC: signet-ring squamous cell carcinoma; SCC: squamous cell carcinoma; CIN: cervical intraepithelial neoplasia; HC: histochemistry; IHC: immunohistochemistry; AB: Alcian Blue; PAS: periodic-acid Schiff; ER: endoplasmic reticulum.

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HPV type-6, 11, 16, 18, 31, 35 and 39 are most commonly observed in squamous cell lesions [19]. Including the present study, three cases have been submitted to HPV genotyping, and HPV type 6/16 and type 18 infections are confirmed in two of them respectively [8, 18]. The HPV proteins E6 and E7 are thought to play an important role in this process by their interaction with the P53 protein and the RB-susceptibility gene product [125, 159, 169]. Consistent with previous studies, our case also exhibited overexpression of p16^{INK4a} (block staining pattern) and a high Ki-67 index, which indicate a high-grade squamous intraepithelial lesion [9, 10].

Although there are no significant differences in histological appearance, signet-ring cell in squamous cell lesion clearly distinct from their counterparts in adenocarcinoma because of negativity for PAS, AB, and mucicarmine or colloidal iron (**Table 1**). The results of electron microscopy reveal that most of vacuoles contain moderate electron-dense flocculent material, others are empty [7]. Study further demonstrates that dilated endoplasmic reticulum serves as one of the most important components of vacuoles [15]. The septum of vacuoles includes intermediate filaments, which is in agreement with positivity for AE1/AE3 [15]. In contradiction to above-mentioned findings, an elaborate study suggests that signet-ring cell in SCC with mucinous metaplasia is positive for AB and colloidal iron rather than PAS [8]. However, these signet-ring cells also show reactivity for AE1/AE3, EMA and CK5/6 and negativity for CEA, which denote a squamous cell origin phenotype [8]. Mature squamous cells do not contain mucinous. However, human epidermal stem cells are capable of differentiation into both keratinocytes and mucin producing cells in vitro [20]. We hypothesize that persistent HPV infection can trigger the epidermal stem cell to differentiate toward mucinous producing cells. During this process, the genomic changes may increase susceptibility of these transitioning cells to the harmful microenvironment, which inhibit the normal cell cycle and then create a pool of self-renewing cells that no longer enter a postmitotic differentiated state. Further accumulation of other alterations eventually leads to malignant transformation of signet-ring cells. Several studies of signet-ring cell generally encourage this view. There is now

convincing evidence that cervical squamous cell can serve as a cellular source of signet-ring cell [7]. In an adenosquamous carcinoma of cervix, it is tempting to speculate that signet-ring cell may be derived from multipotential immature cells because neoplastic cells express both squamous and adenocarcinoma hallmarks, including low molecular weight cytokeratin, high molecular weight cytokeratin, MUC5AC and p63 [21]. Furthermore, HPV DNA is detected in several cases of primary cervical adenocarcinoma with signet-ring cell morphology [6, 22, 23]. Our study provides the first analysis of signet-ring cell in HPV-related benign squamous cell lesion. Koilocyte and signet-ring cell express the same panel of immunohistochemical markers, raising the possibility that the later may represent a rare variant of koilocyte. However, further experimentation will be required to determine the details of the relationship between signet-ring cell and koilocyte.

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Disclosure of conflict of interest

None.

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