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Detection of high-risk human papillomavirus DNA and immunohistochemical expressions of p16, vimentin, ER, and PR in primary endocervical and endometrial adenocarcinomas

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Summary

Objective: The aim of this study was to explore a panel of useful markers in differential diagnosis of primary endocervical adenocarcinoma (ECA) and endometrial adenocarcinoma (EMA). *Materials and Methods:* Thirty-three ECAs and 31 EMAs were collected and examined for high-risk human papillomavirus (hr-HPV) (16/18) DNA using in situ hybridization, and for p16, vimentin, ER, PR expression using immunohistochemistry (IHC). *Results:* Detection rate of hr-HPV (16/18) DNA in ECA (72.7%, 24/33) was significantly higher than that in EMA (12.9%, 4/31) ($p < 0.01$). Twenty-four of 33 (72.7%) cases of ECA, but only five of 31 (16.1%) cases of EMA showed high expression of p16. Twenty-three of 24 (95.8%) hr-HPV DNA-positive ECA and all four (100.0%) hr-HPV DNA-positive EMA showed high levels of p16 expression. High expression rates of vimentin (90.3%, 28/31), ER (58.1%, 18/31), and PR (71.0%, 22/31) in EMA were significantly higher than those in ECA, respectively ($p < 0.01$). *Conclusion:* Detection of hr-HPV DNA combined with immunohistochemical expressions of p16, vimentin, ER, and PR have important value in differential diagnosis between ECA and EMA.

Key words: hr-HPV DNA; p16; Vimentin; ER/PR; In situ hybridization; Immunohistochemistry; Endocervical adenocarcinoma; Endometrial adenocarcinoma.

Introduction

Endocervical adenocarcinoma (ECA) accounts for 15%-25% of all cervical cancers and the incidence appears to be increasing. Most ECA exhibit a hybrid of endometrioid and mucinous features, or even a dominant or pure endometrioid or mucinous differentiation, therefore ECA and endometrial adenocarcinoma (EMA), share many common morphological characteristics. Only when adenocarcinoma is alone confined to the cervix or the corpus (usually lower uterine segment), the primary ECA or EMA are readily diagnosed. When both the corpus and cervix are involved and precursor lesions are lacking, determining the primary site of a uterine adenocarcinoma can be problematic in hysterectomy specimens [1]. Many recent publications indicated that most ECA are high-risk human papillomavirus (hr-HPV)-related tumors, and diffuse/strong p16 expression can be regarded as a surrogate marker of the presence of hr-HPV [1, 2]. In contrast, most EMA are considered etiologically unrelated to HPV infection, and usually show high levels of vimentin, ER/PR expressions [3-7]. Therefore it is entirely possible to complete the morphological differential diagnosis between ECA and EMA. In the present study, the authors investigated the detection of hr-HPV (16/18) DNA by in situ hybridization (ISH) and expressions of p16, vimentin, ER, and PR by immunohistochemistry (IHC). Their aim was to explore a panel of useful markers in differential diagnosis of primary ECA and EMA.

Materials and Methods

Tissue samples

Thirty-three ECA (22 usual endocervical type, three endometrioid type, three villoglandular type, two intestinal type, two serous type, one adenosquamous carcinoma) and 31 EMA (27 endometrioid type, four serous type) were collected for this study from archives (2010-2012) of the Department of Pathology, Zhongnan Hospital of Wuhan University. The average age of the patients with ECA was 43.8 years (from 27 to 64), and 53.6 years for patients with EMA (from 28 to 80).

Detection of HPV DNA

ISH for hr-HPV (16/18) DNA was performed in all the cases. The hr-HPV (type 16/18) ISH detection kit was utilized. The procedures included slice processing, dewaxing and hydration, microwave heating, hybridization, signal amplification, and chromogenic development [7, 8]. Controls included positive tissue sections of cervical squamous cell carcinoma and negative tissue sections of EMA for hr-HPV (16/18) DNA detection. Cases with brown precipitation or a discrete punctate reaction product (when the copies of viruses were low) in the nucleus were interpreted as positive.

IHC

The expressions of p16, vimentin, ER, and PR were determined according to the manufacturer's instruction. Brown staining in cyto-

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plasm/ nucleus (p16), cytoplasm (vimentin) and nucleus (ER, PR) was accepted as presence of immunoreactivity. The percentage of positive cells was assessed under ×400 microscopy. The expression was classified as following grades: (1) -, if none were stained positive or positive staining cell < 5%; (2) 1+, positive cell occupied 6%-25%; (3) 2+, 26%-50%; (4) 3+,>50%. - and 1+ were categorized as low expression, and 2+ and 3+ were scored as high (over) expression [9]. The cervical squamous cell carcinoma, fibrosarcoma and breast carcinoma tissue section was used as positive control for p16, vimentin, and ER/PR, respectively. The PBS solution was used to replace the primary antibody as negative control.

Statistical analysis

The SPSS 11.0 statistics program was used for data analysis. The chi-square test was used and $p < 0.05$ was accepted as statistically significant.

Results

The detection of HPV (16/18) DNA in ECA and EMA

A total of 24 of the 33 (72.7%) ECA were found to be hr-HPV (16/18) DNA-positive by ISH, but the difference of detection rate for hr-HPV (16/18) DNA was not significant in histological subtypes ($p > 0.05$). Only four of 31(12.9%) EMA were hr-HPV (16/18) DNA-positive, but detection rate of hr-HPV (16/18) DNA in serous type (75.0%, 3/4) was significantly higher than that of endometrioid type (1/27, 3.7%) ($p < 0.01$). Statistically, detection rate of hr-HPV (16/18) DNA in ECA was significantly higher than that in EMA ($p < 0.01$) (Table 1) (Figures 1A, 2A).

Table 1. — *Detection of hr-HPV (16/18) DNA and expression of p16 in ECA and EMA (n, %).*

Group	n	hr-HPV DNA (+)	p16 expression* Low	High
Cervical adenocarcinoma	33	24 (72.7%)	9 (27.3)	24 (72.7)
endocervical type	22	17 (77.3%)	6 (27.3)	16 (72.7)
villoglandular type	3	2 (66.7%)	1 (33.3)	2 (66.7)
endometrioid type	3	2 (66.7%)	0 (0.0)	3 (100.0)
intestinal type	2	2 (100.0%)	0 (0.0)	2 (100.0)
serous type	2	1 (50.0%)	1 (50.0)	1 (50.0)
andenosquamous carcinoma	1	0 (0.0%)	1 (100.0)	0 (0.0)
Endometrial adenocarcinoma	31	4 (12.9%)	26 (83.9)	5 (16.1)
endometrioid type	27	1 (3.7%)	25 (92.6)	2 (7.4)
serous type	4	3 (75.0%)	1 (25.0)	3 (75.0)
Total	64	28 (43.8%)	35 (54.7)	29 (45.3)

* - and 1+ were categorized as low expression;
2+ and 3+ were scored as high expression.

The expression of p16 and correlation between hr-HPV DNA detection and expression of p16 in ECA and EMA

Twenty-four of 33 (72.7%) cases showed high expression of p16 in ECA, but only five of 31 (16.1%) cases in EMA exhibited high expression of p16. The difference was significant between the former and the latter ($p < 0.01$).

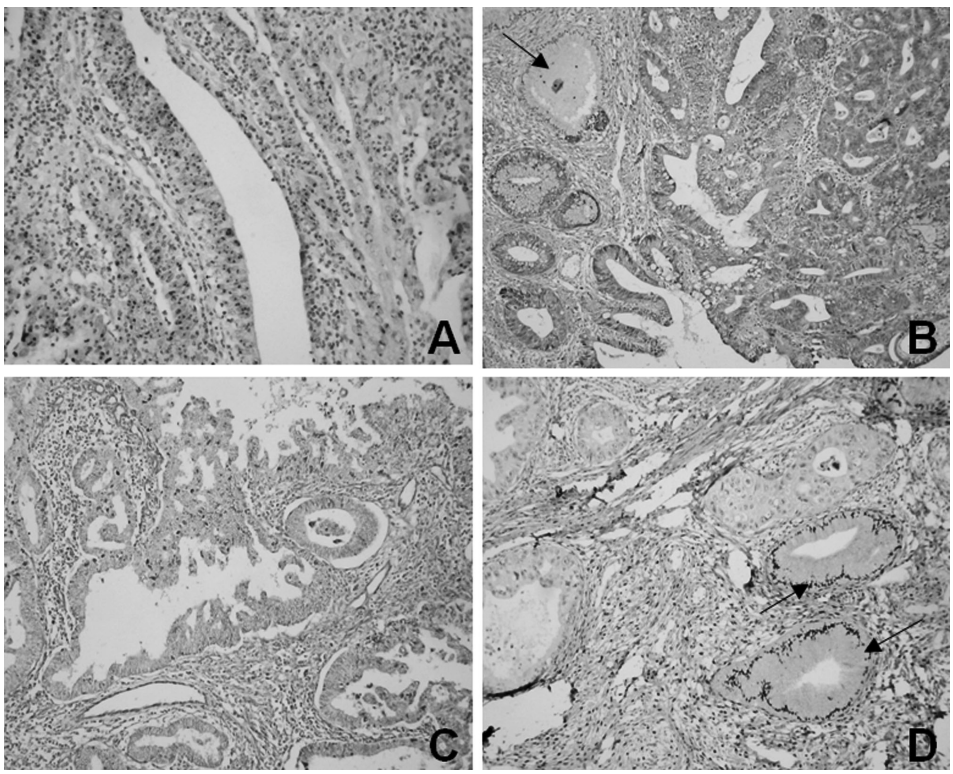

Figure 1. — Detection and expression of hr-HPV (16/18) DNA, p16, vimentin, and ER in ECA. (A) The hr-HPV (16/18) DNA detection demonstrate brown punctate positive reaction product within tumor cell nuclei of ECA. In situ hybridization ×20. (B) The p16 staining shows diffuse cytoplasmic and nuclear expression in case of hr-HPV DNA (+) ECA. Normal endocervical glands (black arrow) exhibit negative staining (×10). (C) The vimentin staining shows negative expression in ECA. The endometrial stroma exhibits positive staining (×10). (D) The ER staining shows negative expression in ECA. The endometrial stroma and residual endocervical glands (black arrows) are visualized as positive control (×10).

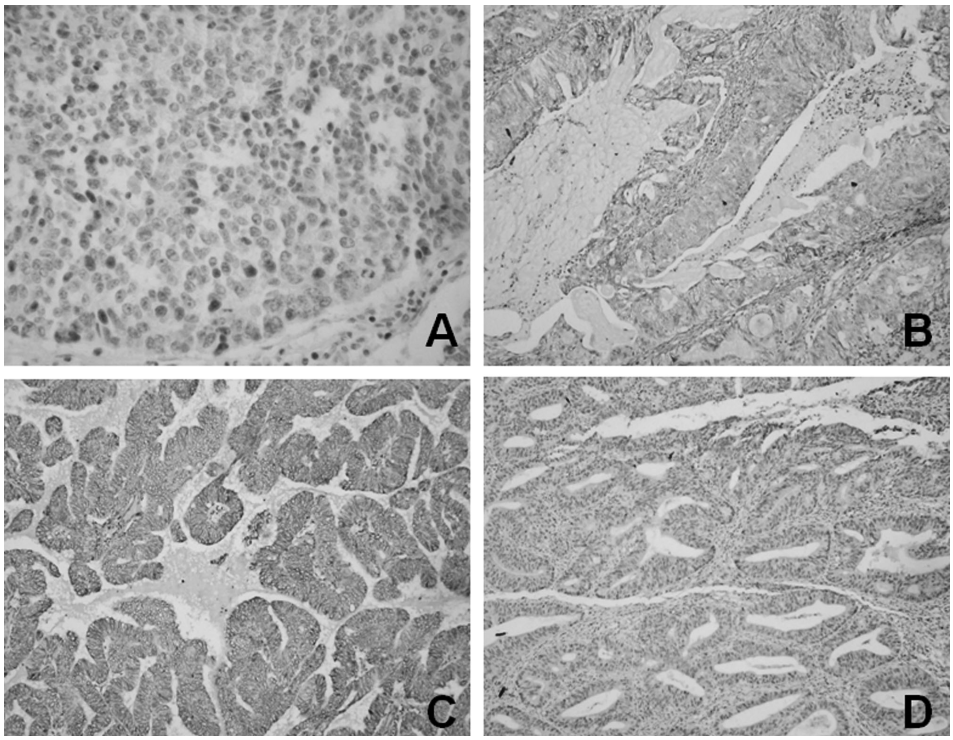

Figure 2. — Detection and expression of hr-HPV (16/18) DNA, p16, vimentin, and PR in EMA. (A) The hr-HPV (16/18) DNA detection demonstrates brown positive precipitation within tumor cell nuclei of some EMA. In situ hybridization ×40. (B) The p16 protein staining shows focal positive cytoplasmic and nuclear expression in case for hr-HPV DNA(-) EMA (×10). (C) The vimentin staining shows diffuse cytoplasmic expression in EMA (×10). (D) The PR staining shows diffuse positive expression within tumor cell nuclei of endometrial adenocarcinoma (×10).

Table 2. — *Correlation between hr-HPV(16/18) infection and p16 high expression in ECA and EMA.*

Group	hr-HPV (16/18) DNA detection (n)	p16 expression high (n)	X^2	*p*
ECA	+, 24	23	5.13	< 0.05
	-, 9	1		
EMA	+, 4	4	11.2	< 0.01
	-, 27	1		

High levels of p16 expression did not correlate with various histological subtypes of ECA ($p > 0.05$), but the rate of p16 high expression in serous type (75.0%, 3/4) was higher than that in endometrioid type (7.4%, 2/27) of EMA ($p < 0.01$) (Table 1) (Figures 1B, 2B).

Twenty-three of 24 (95.8%) hr-HPV DNA-positive ECA cases showed high expression of p16, but only 1 of 9 (11.1%) hr-HPV DNA-negative ECA cases showed p16 high expression. All 4 (100.0%) hr-HPV DNA-positive EMA cases presented p16 high expression, but 1 of 27 (3.7%) of hr-HPV DNA-negative EMA cases exhibited p16 high expression. Regarding the incidence of p16 high-level expression, there was significant difference between hr-HPV DNA-positive and -negative cases of ECA ($p< 0.05$) and EMA ($p< 0.01$), respectively (Table 2) (Figure 1B, 2B).

The expression of vimentin, ER, and PR in ECA and EMA

All 33 cases of ECA showed low expression of vimentin (no detection in 32 cases, weak positive expression in one case). Twenty-eight of 31 (90.3%) EMA cases presented high levels of vimentin expression, but difference for high levels of vimentin expression was significant between endometrioid (96.3%, 26/27) and serous type (50.0%, 2/4) of EMA ($p < 0.01$). Statistically, the rate of high expression of vimentin in EMA was higher than that in ECA, and the difference was dramatic ($p < 0.01$) (Table 3) (Figure 1C, 2C).

All 33 cases of ECA showed low expression of ER (no detection in 32 cases and minimal positive expression in one case). ER High expression rate was 58.1% (18/31) in EMA, but high expression rate in endometrioid type (18/27, 66.7%) was higher than that of serous type (0/4, 0.0%) ($p < 0.05$). The high expression rate of ER in EMA was evidently higher than that in ECA ($p < 0.01$) (Table 3) (Figure 1D).

All 33 cases of ECA exhibited low expression (negative staining) of PR. High expression rate of PR was 71.0% (22/31) in EMA, but difference of high expression rate between endometrioid type (81.5%) and serous type (0.0%) was significance ($p < 0.01$). Statistically, the high expression rate of PR in EMA was significantly higher than that in ECA ($p < 0.01$) (Table 3) (Figure 2D).

Discussion

Infection of hr-HPV is the main causative event in the development of cervical cancer and hr-HPVs have been detected

Table 3. — *Expressions of vimentin, ER, and PR in endocervical and endometrial adenocarcinoma (n, %).*

Group	n	Vimentin expression*		ER expression*		PR expression*	
		Low	High	Low	High	Low	High
Cervical adenocarcinoma	33	33 (100.0)	0 (0.0)	33 (100.0)	0 (0.0)	33 (100.0)	0 (0.0)
endocervical type	22	22 (100.0)	0 (0.0)	22 (100.0)	0 (0.0)	22 (100.0)	0 (0.0)
villoglandular type	3	3 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)
endometrioid type	3	3 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)
intestinal type	2	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)
serous type	2	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)
andenosquamous carcinoma	1	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)
Endometrial adenocarcinoma	31	3 (9.7)	28 (90.3)	13 (41.9)	18 (58.1)	9 (29.0)	22 (71.0)
endometrioid type	27	1 (3.7)	26 (96.3)	9 (33.3)	18 (66.7)	5 (18.5)	22 (81.5)
serous type	4	2 (50.0)	2 (50.0)	4 (100.0)	0 (0.0)	4 (100.0)	0 (0.0)
Total	64	36 (56.3)	28 (43.7)	46 (71.9)	18 (28.1)	42 (65.6)	22 (34.4)

* –and 1+ were categorized as low expression; 2+ and 3+ were scored as high expression.

in up to 99.7% of squamous cell carcinoma [10]. At present, although infection frequency and viral type of hr-HPV are distinct in many studies, most ECAs (~90.0%) have been regarded as hr-HPV-related tumors. In contrast, infection of hr-HPV is rarely seen in EMA. Plunkeff *et al.* found that 78.0% (39 of 50) ECAs contained hr-HPV DNA, compared with 2.0% (1 of 50) EMAs that was positive for hr-HPV DNA [6]. Hadzisejdć *et al.* reported that 81 out of 89 (91.0%) tested positive for hr-HPV DNA in ECA, in which, statistically significant predominance of single hr-HPV (type 18) infections in adenocarcinoma in situ (AIS) and endocervical adenocarcinoma (AC) whereas multiple hr-HPV (16/18 type) infections were more abundant in AC compared with AIS [8]. In cervical cancer associated with hr-HPV infection, functional inactivation of Rb by HPV E7 protein results in an accumulation of p16 protein because normally Rb inhibits transcription of p16 [10,11]. As a consequence, most ECA exhibit a diffuse positivity of p16, whereas EMA (especially endometrioid type) is usually negative or there is focal positivity [3, 5, 12, 13]. Expression of p16 protein is associated with HPV oncogenic potential in cervical and genital lesions [14]. Thus, p16 protein is also a useful marker to help determine the tumor origin such as ECA and EMA (endometrioid type). In the present study, detection rate of hr-HPV (16/18) DNA was 72.7% (24/33) in ECA, compared with 12.9% (4/31) in EMA. High p16 expression rate of ECA (72.7%, 24/33) was significantly higher than that of EMA (16.1%, 5/31). The authors also found that the rate of p16 high expression was 95.8% (23/24) in hr-HPV-positive cases of ECA, and all four (100.0%) hr-HPV DNA-positive EMA presented p16 high expression. The present results and other studies suggest that hr-HPV DNA detection and p16 expression level could be a useful adjunct to distinguish between ECA and EMA. There have been some controversial reports regarding the role of the p16 protein in the differential diagnosis of ECA and EMA. Recently, Saad *et al.* proposed that the expression of p16 in undifferentiated carcinoma of the uterus does not exclude its endometrial origin. They found that diffuse/strong positive staining for p16 was seen in 40/50 (80%) cases of ECA and 14/28 (50%) cases of undifferentiated endometrial carcinoma. At present, distinguishing between undifferentiated endometrial carcinoma and endocervical adenocarcinoma, both of which share diffuse p16 expression, should rely on simultaneous detection of human papilloma virus in the latter [15]. Because most ECAs are hr-HPV-related tumors and the diffuse p16 positivity can be regarded as a surrogate marker of the presence of hr-HPV, p16 marker is currently the most important focus of attention and has been most widely applied in the field of gynecologic pathology [16].

Vimentin is an intermediate filament protein normally expressed in mesenchymal cells, but the aberrant expression of vimentin can be found in epithelial cancer cells [17]. For a long time, vimentin is a useful and reliable marker to identify the primary ECA and EMA (especially endometrioid type). Azumi *et al.* have indicated that as a diagnostic reagent, antibodies to vimentin are of the greatest application in the diagnosis of carcinoma of uncertain primary site, whereas strong co-expression of vimentin and keratin may be a clue to renal, endometrial, and thyroid carcinomas [18]. Many studies showed that the positive expression rates for vimentin were significantly different between primary ECA and EMA [19]. The positive expression rate of vimentin in the former was approximately 7%-14% compared with 62%-93% in the latter [20-22]. In the present study, 32 cases did not have any expression of vimentin and only one case was weak positive expression in 33 cases of ECA. In contrast, the high expression rate for vimentin was 90.3% (28/31) in EMA, especially in EMA of endometrioid type with high expression rate of 96.3% (26/27). The present research results strongly suggest that vimentin is indeed a valuable marker in the differential diagnosis of ECA and EMA.

The endometrium is one principal target tissue of the pituitary-gonadal axis, but has also been recognized as an endocrine organ. Human endometrium expresses ER and PR, which are related to autocrine and paracrine processes that respond to estrogen and progesterone. The ER and PR expres-

sion and distribution pattern may play an important role in endometrial function and pathogenesis [23]. Therefore, EMA (especially endometrioid type) is typically hormone-dependent. Many studies reported that the EMA (endometrioid type) significantly expresses ER and PR. In contrast, ECA usually have absent or limited expression of ER and PR [1, 7, 22-24]. According to literature, positive expression rates of ER and PR in EMA were 67% - 97% and 89% - 96%, compared with 4% - 20%, and 4% - 21% in ECA, respectively [20-22]. The present results show that the high expression rates of ER and PR were 58.1% and 71.0%, respectively, in the EMA, and 66.7% and 81.5%, especially in the endometrioid type of EMA. However, ER and PR expressions were low in ECA (the vast majority did not express), with no high-expression cases. The high expression rates of ER and PR in EMA was evidently higher than that in ECA ($p < 0.01$). The present results were consistent with what have been reported in the literature.

The present study demonstrated once again that the detection of hr-HPV DNA combined with immunohistochemical expressions of p16, vimentin, ER, and PR are useful for differential diagnosis between ECA and EMA and clarifying the origin of uterine adenocarcinomas.

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