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Original Article

The expression and significance of insulin like growth factor-II mRNA binding protein 3 (IMP3) and P16^{INK4a} in adenocarcinoma of the uterine cervix

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Abstract: Objective: To investigate the expression and significance of Insulin like growth factor-II mRNA binding protein 3 (IMP3) and P16^{INK4a} in adenocarcinoma of the uterine cervix. Methods: Immunohistochemical method was used to detect the expression of IMP3(SP method) and P16^{INK4a} (EnVision) in 25 cases of benign endocervical glands (BEG), 15 cases of endocervical adenocarcinoma in situ (AIS) and 56 cases of endocervical adenocarcinoma (ECA). To further assess the level of IMP3 protein expression, in situ hybridization detection of IMP3 mRNA was performed in 25 cases of ECA with IMP3 varying expression. Results: IMP3 expression was detected in 16.00% (4/25) of BEG, 80% (12/15) of AIS and 80.36% (45/56) of ECA, with significant difference among them (P<0.001). While P16^{INK4a} expression was detected in 0.00% (0/25) of BEG, 100% (15/15) of AIS and 83.93% (47/56) of ECA with significant difference among them (P<0.001). IMP3 and P16^{INK4a} positivity was most common in serous carcinoma (100%, 3/3, and 100%, 3/3) of all kinds of ECA, subsequently in the endometrioid carcinoma (85.71%, 6/7 and 71.43%, 5/7) and cervical endocarcinoma (73.91%, 34/46 and 84.78%, 39/46). There was significant difference among IMP3 expression of different differentiated ECA (P<0.05). No association was observed among IMP3 or P16^{INK4a} expression and age, tumor types and tumor FIGO stages (P>0.05). The IMP3+/P16^{INK4a}+ phenotype accounts for 64.29% (36/56) of ECA, while IMP3-/P16^{INK4a}-accounts for 3.57% (2/56). χ^2 analysis showed inconsistency between IMP3 and P16^{INK4a} expression in ECA (Kappa value 0.1, P=0.47). Conclusion: IMP3 and P16^{INK4a} would be helpful in identification of benign and malignant endocervical glandular lesions. IMP3 Expression is correlated with differentiation of ECA. Combination of IMP3 and P16^{INK4a} may be useful in diagnosis of ECA.

Keywords: The uterine cervix, adenocarcinoma, adenocarcinoma in situ, IMP3, P16^{INK4a}, immunohistochemistry

Introduction

Cervical carcinoma is a common malignant tumor among women. Recently, detection rate of cervical adenocarcinoma has increased with the application of the cervical carcinoma screening [1]. Distinction of benign endocervical gland (BEG) from endocervical adenocarcinoma in situ (AIS) and endocervical adenocarcinoma (ECA) may be challenging sometimes, especially for biopsy. Insulin-like growth factor II mRNA binding protein 3 (IMP3) is an embryo-protein, expressed in many malignant tumor, expressed differently according to the aggressive behavior of tumors, weakly or not expressed in borderline benign tumors or normal tissues [2-8]. P16^{INK4a} is an important tumor suppressor gene, which was related to high-risk HPV infection [9]. Many studies have found that cervical adenocarcinoma and cervical gland epithelial tumor expressed P16^{INK4a} protein and their genesis were related with HPV infection [6, 10-13]. Our aim was to evaluate immunohistochemical expression of IMP3 and P16^{INK4a} in benign and malignant endocervical glandular lesions as well as their relationship with clinicopathological characteristics of ECA.

Material and methods

Case selection

56 patients with ECA were identified from the files of the Department of Pathology of Guizhou provincial people's Hospital from June 2009 to June 2014. The specimens included cervical biopsies and hysterectomies. Their slices were

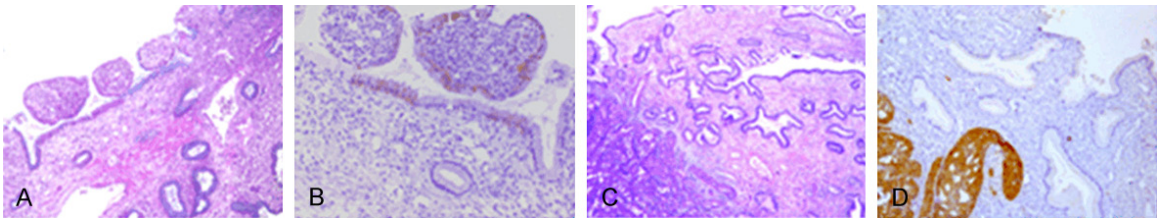

Figure 1. IMP3 and P16^{INK4a} expression in BEG. A: Chronic cervicitis with papillary erosion, HE, ×200. B: Focal and weak staning for IMP3 in endocervical glands of papillary erosion (SP, ×200). C: Normal epithelial adjacent to ECA, HE, ×100. D: Negative staning for P16^{INK4a} in normal epithelial adjacent to ECA. The lower-left coner showed positive P16^{INK4a} in ECA (Envision, ×200).

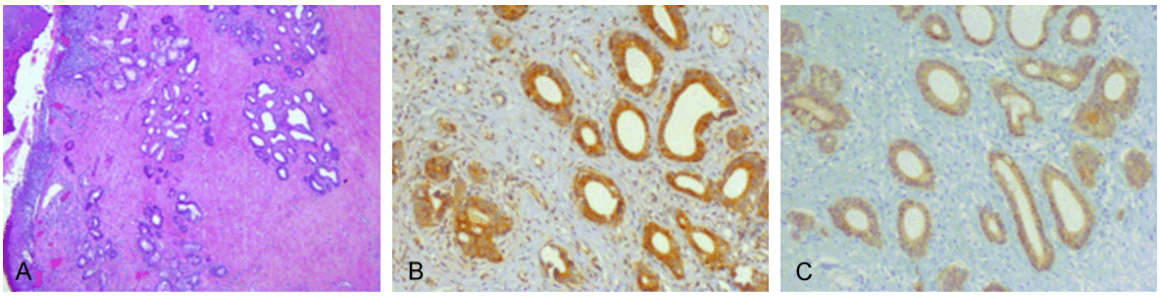

Figure 2. Positive staining of IMP3 and P16^{INK4a} in AIS. A: AIS (HE, ×200); B: Strong IMP3 expression in AIS (SP, ×200); C: Strong P16^{INK4a} expression in AIS (Envision, ×200).

read again by at least 2 senior pathologists. The patient age ranged from 30 to 70 years, with 34 from 30 to 47, 22 from 47 to 70 age group. The mean age was 47 years. In terms of histological classification, 46 out of 56 were cervical adenocarcinoma (including 2 minimal deviation adenocarcinoma), 7 endometrioid carcinoma and 3 serous adenocarcinoma. In terms of histological differentiation, 16 out of 56 patients were well differentiated, 25 intermediate/moderately differentiated and 15 poorly differentiated. 39 out of 56 patients have clear stage by FIGO (19 stage I, 11 stage II, 6 stage III, 3 stage IV). 15 patients diagnosed with AIS and 25 patients with BEG (5 precancerous lesions, 12 chronic cervicitis with papillary erosion, 5 micro-glandular hyperplasia and 3 tubal metaplasia) during the same term were included for control group.

Methods

Immunohistochemical study: All samples were fixed in 10% neutral formalin, embedded in paraffin, sectioned with 4 μm thickness. Then the slides were baked at 50°C for 2 hours. The expression of IMP3 were detected by SP immunohistochemical method (KIT form Beijing Zhongshan) using polyclonal antibodies bs-1521R (concentration were 1:1000, Beijing Aobosen Company). The expression of P16^{INK4a} was detected by EnVision immunohistochemical method (KIT from Dako Company with KIT number K5334) using antibodies (concentration were 1:25, Dako Company). Cervical squamous cell carcinoma tissues were used as a positive control. For negative control samples, the primary antibody was replaced by PBS.

In situ hybridization: Digoxigenin and biotin labeled IMP3 mRNA were composed by Tianjin Haoyang company. The sequence of probes was 5'TGGCA CCTTC CTATG ATGGC TCC, 5'AGCCT TGAAC TGAGC CTCTG GTGGT CC and 5'CTGGG CAACC TGGCA AGCAT AGAAG TG. Experiments were performed according to the instruction.

Microscopic evaluation

The percentage and intensity of positive cells was scored according to the following scheme: 0 (less than 5% of tumor cells); 1 (5-25% of tumor cells); 2 (26-50% of tumor cells); 3 (greater than 50% of tumor cells), and 0 (non-staining), 1 (yellow), 2 (yellowish brown), 3 (brown), respectively. And then multiply these

Table 1. Distribution of IMP3 and P16^{INK4a} staining with percentages of positive cells in BEG, AIS and ECA

Tissue	n	Expression of IMP3		*P* value	Expression of P16^{INK4}		*P* value
		Positive (%)	Negative (%)		Positive (%)	Negative (%)	
BEG	25	4 (16.00)	21	0.000*	0 (0.00)	25	0.000*
AIS	15	12 (80.00)	3	0.975△	15 (100.00)	0	0.189△
ECA	56	45 (80.36)	11	0.000▽	47 (83.93)	9	0.000▽

*: AIS vs BEG; △: ECA vs AIS; ▽: ECA vs BEG; IMP3 and P16^{INK4a} expression was negative or rare in BEG, with significant difference when comparing with that in AIS or ECA (P=0.000).

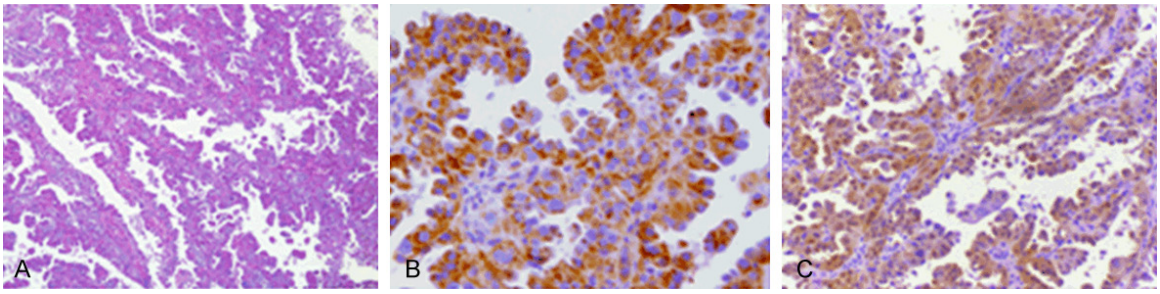

Figure 3. P IMP3 and P16^{INK4a} expression in ECA. A: Serous carcinoma (HE, ×200); B: Strong IMP3 expression in serous carcinoma (SP, ×200); C: Strong P16^{INK4a} expression in serous carcinoma (Envision, ×200).

two scores to get total score. When the total score were 0~1, 2~3, 4~6, >6, it would be identified as negative, weak positive, moderate positive and strong positive, respectively.

Statistical analysis

Fisher's Exact Test and χ^2 were performed using the SPSS software (version19.0). *P* values <0.05 were considered statistically significant.

Results

The expression of IMP3 and P16^{INK4a} in BEG, AIS and ECA

Positive immunostaining for IMP3 was identified in the cytoplasm, while positive immunostaining for P16 was identified in the cytoplasm, nucleus, or both. Both were presented with yellowish brown granule. IMP3 expression was detected in 4 out of 25 BEG (16.00%), 3 out of 12 papillary erosions (focal and weak positivity, **Figure 1**), 1 of 5 microglandular hyperplasia (focal and moderate positivity), none of precancerous lesions and tubal metaplasia. It was found in 80% (12/15) of AIS (**Figure 2**) and 80.36% (45/56) of ECA mainly with moderate or strong positivity, there are no significant difference between AIS and ECA (P>0.05). But the difference were significant comparing with BEG (P<0.001) (**Table 1**). P16^{INK4a} reactivity was found in 100% (15/15) of AIS (**Figure 2**) and 83.93% (47/56) of ECA mainly with strong positivity, but negative in BEG (**Figure 1**). Though the positive rate of P16^{INK4a} in AIS were higher than ECA, the difference was not significant (P>0.05). There are significant difference when comparing with BEG (P<0.001) (**Table 1**).

The expression of IMP3 and P16^{INK4a} in ECA and their relationship with clinicopathological characteristics of the tumors

IMP3 positivity was most common in serous carcinoma (100%, 3/3, **Figure 3**) of all kinds of subtypes, subsequently in the endometrioid carcinoma (85.71%, 6/7) and cervical endocarcinoma (73.91%, 34/46). IMP3 was expressed in 9 of 16 (47.37%) well-differentiated adenocarcinoma, 20 of 25 (80.00%) moderately differentiated adenocarcinoma, 14 of 15 (93.33%) of poorly differentiated adenocarcinoma, with significant difference among them (P<0.05). Tumors in FIGO IV showed most positive staining for IMP3 among different stages. P16^{INK4a} positivity was most common in serous carcinoma (100%, 3/3, **Figure 3**) of all kinds of ECA,

Table 2. Expression of IMP3 and $P16^{INK4a}$ in ECA, and their relationship with clinicopathological characteristics of ECA

Clinicopathological characteristics	n	IMP3		*P* value	$P16^{INK4a}$		*P* value
		Positive (%)	Negative (%)		Positive (%)	Negative (%)	
Age							
≤47	34	29 (85.29)	5	0.061	29 (85.29)	5	0.729
>47	22	14 (63.64)	8		18 (78.95)	4	
Tumor type							
Cervical adenocarcinoma	46	34 (73.91)	12	0.488	39 (84.78)	7	0.494
Endometrial adenocarcinoma	7	6 (85.71)	1		5 (71.43)	2	
Serous adenocarcinoma	3	3 (100.00)	0		3 (100.00)	0	
Tumor differentiation							
Well differentiated	16	9 (47.37)	7	0.015	11 (68.75)	5	0.134
Intermediate	25	20 (80.00)	5		23 (92.00)	2	
Poorly differentiated	15	14 (93.33)	1		13 (86.67)	2	
FIGO stage[a]							
I	19	16 (84.21)	3	0.259	16 (84.21)	3	0.553
II	11	8 (72.73)	3		10 (90.90)	1	
III	6	3 (50.00)	3		4 (66.67)	2	
IV	3	3 (100.00)	0		2 (66.67)	1	

a: 39 out of 56 patients have clear stage by FIGO.

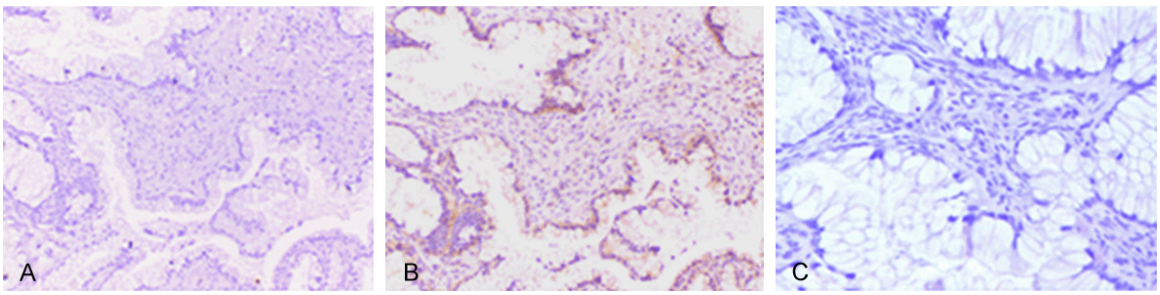

Figure 4. P IMP3 and $P16^{INK4a}$ expression in minimal deviation adenocarcinoma. A: Minimal deviation adenocarcinoma (HE, ×200); B: Focal IMP3 expression in minimal deviation adenocarcinoma (SP, ×200); C: Negative $P16^{INK4a}$ expression in minimal deviation adenocarcinoma (Envision, ×200).

subsequently in the cervical endocarcinoma (84.48%, 39/46) and endometrioid carcinoma (71.43%, 5/7). $P16^{INK4a}$ expression in moderately or poorly differentiated adenocarcinoma were higher in well-differentiated adenocarcinoma, but the difference were not significant (P>0.05). No association was observed among IMP3 or $P16^{INK4a}$ expression and age, tumor types and tumor FIGO stages (P>0.05) (**Table 2**).

Association between IMP3 expression and $P16^{INK4a}$ expression

The IMP3+/$P16^{INK4a}$+ phenotype accounts for 64.29% (36/56) of ECA, while IMP3-/$P16^{INK4a}$- accounts for 3.57% (2/56). Among 13 cases IMP3-ECA, $P16^{INK4a}$ was positive in 11 cases (82%). Among 9 cases $P16^{INK4a}$-ECA (5 endometrioid carcinoma, 2 cervical endocarcinoma, **Figure 4**), IMP3 was positive in 7 cases (78%). χ^2 association analysis showed inconsistency between IMP3 and $P16^{INK4a}$ expression in ECA (P=0.47) (**Table 2**).

Discussion

IMP3, a protein could bind to insulin-like growth factor, belongs to the insulin-like growth factor -II (IGF.II) messenger RNA-binding protein family, is expressed in epithelium, muscle and placental tissue during embryogenesis but rarely

found in adult benign tissues. Several studies have shown that IMP3 is expressed in a number of cancers and is associated with progression and prognosis of the tumor [2-7]. The P16 gene which is localized in chromosome 9p21, encodes for a protein with molecular weight of 16 KD, is a tumor suppressor gene and negative regulator of cell cycle. Mutation, expression deficiency or reduction of P16 gene was detected in many tumors [14, 15]. But P16 expression was high in ECA and was associated with high risk HPV infection [6, 9, 13]. Nowadays $P16^{INK4a}$ has been used to identify HPV infection and distinguish cervical intraepithelial neoplasia or cancer from normal cervical epithelium in clinical practice. Recently some studies found that IMP3 also expressed in cervical intraepithelial neoplasia, can serve as a new biomarker to predict progression of cervical intraepithelial neoplasia into invasive cancer [8].

However, the research about IMP3 and $P16^{INK4a}$ expression in cervical glandular epithelial lesions were very few. Li [6] et al. have found that IMP3 and $P16^{INK4a}$ were highly expressed in adenocarcinoma in situ of uterine cervix (93% and 100%), but no IMP3 and rare $P16^{INK4a}$ expression was detected in benign endocervical glands. They suggested that $P16^{INK4a}$ and IMP3 were a useful biomarker for identification of AIS from benign lesions. $P16^{INK4a}$ was positive in most AIS and ECA, negative in a few ECA, which indicates that most ECA were related to high risk HPV infection [9, 16]. Danialan [7] et al. have found that IMP3 expression were detected in the AIS and ECA but was focal positive in the benign endocervical glandular epithelium. In this study, IMP3 and $P16^{INK4a}$ were highly expressed in AIS and ECA, while $P16^{INK4a}$ was negative in BEG, IMP3 was focal positive in BEG. These results were coincident with the published literature [5-7] and suggest that IMP3 and $P16^{INK4a}$ would be helpful in identification of benign and malignant endocervical glandular lesions, may serve as antibodies for differential diagnosis in clinical practice. IMP3 may involve in oncogenesis of cervical intraepithelial neoplasia and cancer, but mechanism requires more research to confirm.

Both Zheng [4] et al. and Li [17] et al. have found that IMP3 expressed more common in endometrial serous carcinomas than other types of endometrial cancers. Yemelyanova et al. have showed that P16 expression was higher in uterine serous carcinomas than endometrial adenocarcinoma. In our study, IMP3 and $P16^{INK4a}$ expression were higher in serous carcomas than other types of ECA. The high expression of IMP3 and $P16^{INK4a}$ may associate to aggressive characteristics of serous carcinoma, which is a high degree and aggressive malignant tumor of female reproductive system. IMP3 positivity was more common in less differentiated tumors than in well differentiated tumors, and the difference was significant. These findings suggest the association between IMP3 expression and differentiation, which were coincident with the published literature [2]. Muller [9] et al. have found less p16 staining in poorly differentiated tumors than in more highly differentiated tumors as well as highly significant correlation between HPV infection and higher levels of $P16^{INK4a}$ staining. But Liushang [19] et al. have not found this pattern and significant difference, the same results as ours. The association between IMP3 expression and tumor grade was reported differently [17, 18]. Muller [9] et al. and Liushang [19] et al. have found that $P16^{INK4a}$ was expressed in all FIGO stage of ECA with lower positivity in higher grade. No significant difference was observed among IMP3 expression, $P16^{INK4a}$ expression in different FIGO stage when we analysis 33 cases with clear FIGO stage in our study.

In this study, we found ECA highly express IMP3 or/and $P16^{INK4a}$. Though no association was observed between IMP3 and $P16^{INK4a}$ positivity ($P>0.05$), IMP3 positivity is high in $P16^{INK4a}$ negative cases while $P16^{INK4a}$ positivity is also high in IMP3 negative cases. It suggests that combination of IMP3 and $P16^{INK4a}$ may be useful in diagnosis of ECA.

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Disclosure of conflict of interest

None.

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