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Expression levels and roles of EMC-6, Beclin1, and Rab5a in the cervical cancer

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Abstract. – OBJECTIVE: This study is to investigate the role of EMC-6 in the pathogenesis of cervical cancer, especially concerning its relationship with autophagy.

PATIENTS AND METHODS: Totally 100 invasive cervical cancer, 80 cervical intraepithelial neoplasia (CIN), and 80 normal cervical tissue samples were obtained. Expression levels of EMC-6, Beclin1, and Rab5a in the tissues were detected by immunohistochemistry.

RESULTS: Our results showed that positive staining of EMC-6 was mainly located in the nucleus. Compared with the normal cervical tissue, the positive rates of EMC-6 were significantly increased in the CIN and cervical cancer tissues. Moreover, the EMC-6 positive rate in the CIN tissue was higher than the cervical cancer tissue. No significant association was observed between the expression levels of EMC-6 and the clinicopathological features of cervical cancer, including age, FIGO staging, tumor size, tumor type, histological type, cell differentiation, and lymph node metastasis. Compared with the normal cervical tissue, the positive rate of Beclin1 in the CIN tissue was significantly declined, which was further significantly down-regulated in the cervical cancer tissue. However, the positive rate of Rab5a in the CIN tissue was significantly higher than the normal cervical tissue. Moreover, compared with the normal cervical and CIN tissues, the positive rate of Rab5a in the cervical cancer tissue was further significantly increased. EMC-6 was not associated with Beclin1 and Rab5a.

CONCLUSIONS: The expression level of EMC-6 is significantly elevated in cervical cancer, without significant correlation with Beclin1 and Rab5a. These findings might contribute to the understanding of the pathogenesis of cervical cancer and the involved role of EMC-6.

Key Words:
Cervical cancer; Autophagy; EMC-6; Beclin1; Rab5a.

Introduction

Cervical cancer is one of the most common cancers in women worldwide. In Xinjiang, China, the incidence of cervical cancer is as high as 490/100000, seriously affecting the patients' health and life[1,2]. Epidemiological studies show that the human papillomavirus (HPV) infection is closely related to more than 90% of the cervical cancer cases. However, the virus infection alone is not sufficient to cause cervical cancer[3]. Cellular and molecular mechanisms for the disease pathogenesis are still needed to be established.

Autophagy is a cellular homeostasis mechanism, and the autophagy dysfunction is associated with the development of malignant tumors[4-8] and the sensitivity to chemoradiation[9,10]. Endoplasmic reticulum (ER) membrane protein complex subunit 6 (EMC-6), an essential autophagic protein, is widely expressed in normal human tissues[4]. It has been shown that EMC-6 over-expression could induce the punctate distribution of GFP-LC3, and elevate the expression level of GFP-LC3II, initiating autophagy. On the other hand, EMC-6 knockout could decrease the expression level of endogenous LC3, and lead to the accumulation of autophagic precursors, thus blocking the autophagic process. Confocal microscopy has confirmed that EMC-6 might bind to the autophagic protein Beclin1 and the tumor metastasis-related protein Rab5a[11]. Beclin1 is one the most important autophagic proteins, which could enhance the autophagy to inhibit the tumor growth[12]. As a candidate tumor suppressor gene, the absence and/or down-regulated expression of Beclin1 have been reported in various human tumors. Rab5a is a transmembrane signal regulator, which plays important roles in the cell and plasma membrane transportation and in the function of the GDP/GTP cycle[13]. Disturbed Rab5a expression might lead to unlimited cell proliferation, which might end up with carcinogenesis.

In this study, the role of EMC-6 in the pathogenesis of cervical cancer, especially concerning its relationship with autophagy, was investigated. The expression levels of EMC-6, Beclin1, and Rab5a in

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the normal cervical, cervical intraepithelial neoplasia (CIN), and cervical cancer tissues were detected.

Patients and Methods

Tissue Samples

In this study, 100 invasive cervical cancer and 80 cervical intraepithelial neoplasia (CIN) samples were obtained from the patients who underwent surgical resection in the Affiliated Tumor Hospital of Xinjiang Medical University, from Jan 2013 to Jan 2014. These subjects received radical hysterectomy and pelvic lymphadenectomy, with no preoperative radiotherapy. The cytologic test in the peritoneal washes was performed during surgery. Pathological diagnosis was performed and reviewed by experienced pathologists, according to the FIGO2000 cervical cancer staging criteria. Another 80 normal cervical samples obtained from the hysterectomy due to hysteromyoma were used as control.

Immunohistochemistry

The expression levels of EMC-6, Beclin1, and Rab5a in the normal cervical, CIN, and cervical cancer tissues were detected by immunohistochemistry. The paraffin-embedded tissues were subjected to dehydration, clearance, and impregnation. The tissues were cut into 4-μm sections, which were incubated with anti-EMC-6 monoclonal antibody (1:50 dilution; provided by the Peking University Center for Human Disease Genomics, Beijing, China), anti-Beclin1 polyclonal antibody (1:100 dilution; Santa Cruz), and rabbit anti-human anti-Rab5a polyclonal antibody (1:50 dilution; Santa Cruz, Santa Cruz, CA, USA), respectively, at 4°C overnight. Then these sections were incubated with the peroxidase-labeled streptavidin kit (Zhongshan Biotechnology Co., Ltd., Beijing, China). The color development was performed with the DAB kit (Boster Biological Engineering Co., Ltd., Wuhan, Hubei, China). The known cervical cancer slides were used as positive control, and the negative control was treated with PBS instead of the primary antibodies.

Uniform brownish yellow granules in the cell membrane and cytoplasm were regarded as positive staining. Five fields at high magnification (×400) were randomly selected. The total cell number, as well as the cell numbers positive for EMC-6, Beclin1, and Rab5a, respectively, was counted. The staining results were obtained according to the percentage of positive cells: negative (-), 0-24%; weak positive (+), 25-74%; and strong positive (++), ≥ 75%.

Statistical Analysis

SPSS 21.0 software was used for the statistical analysis. Rank sum and χ^2 tests were used for group comparison. $p < 0.05$ was considered statistically significant.

Results

Expression Levels of EMC-6 in Normal Cervical, CIN, and Cervical Cancer Tissues

To investigate the expression levels of EMC-6 in the normal cervical, CIN, and cervical cancer tissues, immunohistochemical staining was performed. Our results showed that positive staining of EMC-6 was mainly located in the nucleus (Figure 1). Compared with the normal cervical tissue, the positive rates of EMC-6 were significantly increased in the CIN and cervical cancer tissues (both $p < 0.05$). Moreover, the positive rate of EMC-6 [the sum of the weak (+) and strong (++) positive rates] in the CIN tissue was 93.8% (75/80), which was higher than that in the cervical cancer tissue (64%, 64/100) (Table I).

On the other hand, the relationship between the EMC-6 expression levels and clinicopathological features of cervical cancer was also investigated. Our results showed that there was no significant association between the expression levels of EMC-6 and concerned clinicopathological features in the cervical cancer, including age ($p = 0.94$), FIGO staging ($p = 0.20$), tumor size ($p = 0.71$), tumor type ($p = 0.09$), histological type ($p = 0.23$), cell differentiation ($p = 0.23$), and lymph node metastasis ($p = 0.57$) (Table II). Taken together, these results suggest that the expression levels of EMC-6 are increased in the CIN and cervical cancer tissues, with a relatively higher positive rate for the CIN tissue. However, the EMC-6 expression level in the cervical cancer tissue is not associated with the clinicopathological features.

Expression Levels of Beclin1 in Normal Cervical, CIN, and Cervical Cancer Tissues

The expression levels of Beclin1 in the normal cervical, CIN, and cervical cancer tissues were next investigated with immunohistochemical staining. Our results showed that Beclin1 was mainly expressed in the cell membrane and cytoplasm, particularly exhibiting an uneven focal distribution

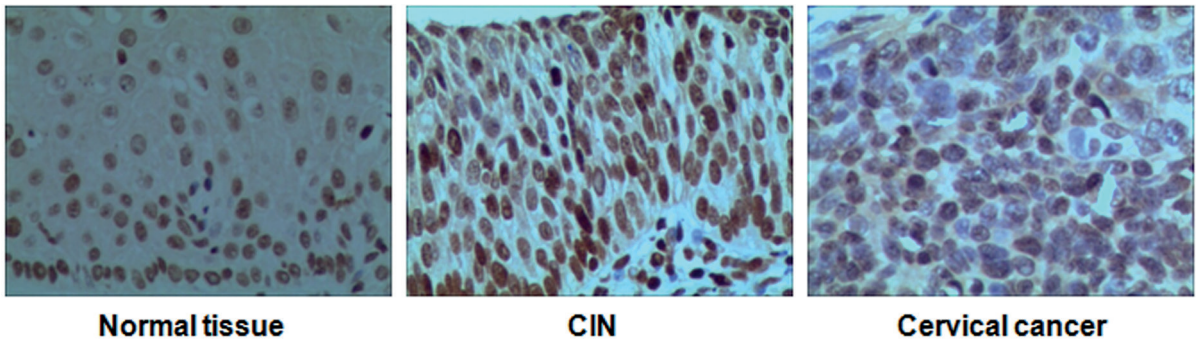

Figure 1 EMC-6 expression levels in the normal cervical, CIN, and cervical cancer tissues. The expression levels of EMC-6 in the normal cervical, CIN, and cervical cancer tissues were detected by immunohistochemistry (×400).

Table I. EMC-6 expression levels in the normal cervical, CIN, and cervical cancer tissues.

Group	N	EMC-6, N (%)			*p*
		-	+	++	
Normal tissue	80	15 (18.8%)	20 (25.0%)	45 (56.2%)	
CIN	80	5 (6.3%)	5 (6.3%)	70 (87.4%)	Compared with normal tissue, $p < 0.05$.
Cervical cancer	100	36 (36.0%)	18 (18.%)	46 (46.0%)	Compared with CIN, $p < 0.05$.

in the cervical cancer tissue (Figure 2). Compared with the normal cervical tissue, the positive rate of Beclin1 in the CIN tissue was significantly declined ($p < 0.05$), which was further significantly down-regulated in the cervical cancer tissue ($p < 0.05$) (Table III). Pairwise comparisons using rank sum test indicated significant differences between these three groups ($p < 0.01$). In particular, the positive rate of EMC-6 in the cervical cancer tissue was significantly lower than the normal cervical and CIN tissues (both $p < 0.05$) (down-regulated Beclin1 expression was often seen in the cervical cancer tissue, and two independent samples were missing in this group). However, no significant difference was observed in the Beclin1 positive rate between the CIN and normal cervical tissues ($p > 0.05$). Also, correlation analysis showed that no significant correlation was observed between the Beclin1 expression and the clinicopathological features of cervical cancer, including age, FIGO staging, tumor size, tumor type, histological type, cell differentiation, or lymph node metastasis ($p > 0.05$) (Table IV). These results suggest that the expression levels of Beclin1 are significantly declined in the CIN and cervical cancer tissues. However, the Beclin1 expression level in the cervical cancer tissue is not associated with the clinicopathological features.

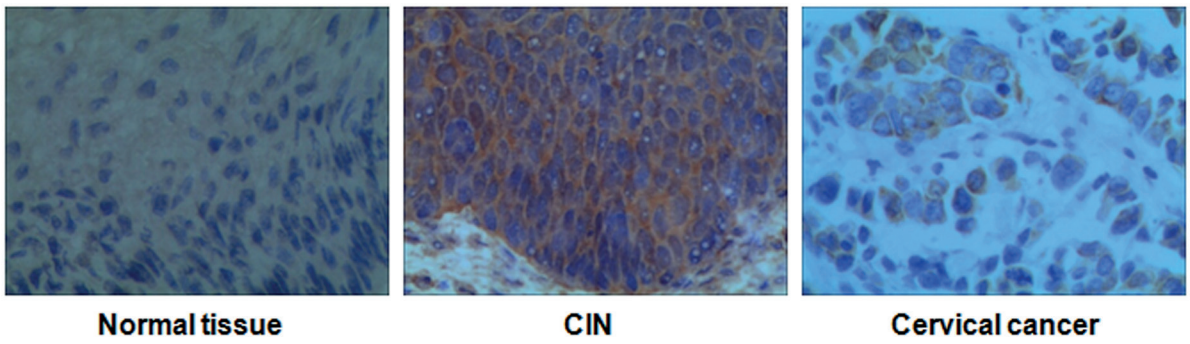

Figure 2. Beclin1 expression levels in the normal cervical, CIN, and cervical cancer tissues. The expression levels of Beclin1 in cervical cancer, NIC, and normal cervical tissues were detected by immunohistochemistry (×400).

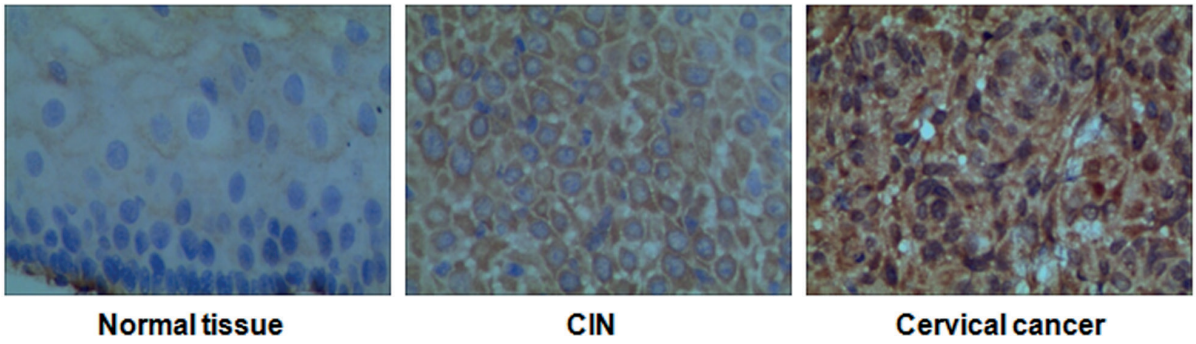

Figure 3. Rab5a expression levels in the normal cervical, CIN, and cervical cancer tissues. The expression levels of Rab5a in cervical cancer, NIC, and normal cervical tissues were detected by immunohistochemistry (×400).

Table II. Correlation between the EMC-6 expression levels and pathological features in cervical cancer.

	EMC-6, N (%)				
	N	-	+	++	*p*
Age					
≤ 45 years	53	19 (35.8%)	10 (18.9%)	24 (45.3%)	0.94
> 45 years	47	17 (36.2%)	8 (17.0%)	22 (46.8%)	
FIGO staging					
I	45	15 (33.3%)	5 (11.1%)	25 (55.6%)	0.20
IIa	55	21 (38.2%)	13 (23.6%)	21 (38.2%)	
Tumor size					
≤ 4 cm	47	18 (38.3%)	8 (17.0%)	21 (44.7%)	0.71
> 4 cm	53	18 (34.0%)	10 (18.9%)	25 (47.1%)	
Tumor type					
Exogenous tumor	56	20 (35.7%)	12 (21.4%)	24 (42.9%)	0.09
Endogenous tumor	33	8 (24.2%)	4 (12.1%)	21 (63.7%)	
Histological type					
Squamous cell carcinoma	82	29 (35.4%)	14 (17.1%)	39 (47.6%)	0.23
Adenocarcinoma	18	7 (38.9%)	4 (22.2%)	7 (38.9%)	
Celldifferentiation					
Moderate/high differentiation	61	22 (36.1%)	6 (9.8%)	33 (54.1%)	0.23
Poor differentiation	39	14 (35.9%)	12 (30.8%)	13 (33.3%)	
Lymph node metastasis					
Yes	66	23 (34.8%)	11 (16.7%)	32 (48.5%)	0.57
No	34	13 (38.2%)	7 (20.6%)	14 (41.2%)	

Expression Levels of Rab5a in Normal Cervical, CIN, and cervical Cancer Tissues

The expression levels of Rab5a in the normal cervical, CIN, and cervical cancer tissues were also investigated with immunohistochemical staining. Our results showed that Rab5a was mainly located in the cell membrane and cytoplasm, exhibiting an uneven focal distribution in the cervical cancer tissue (Figure 3). Significant differences were observed in the Rab5a expression levels between these three groups ($p < 0.05$) (Table V). The positive rates of Rab5a in cervical cancer and CIN tissues were significantly higher than the normal cervical tissue

Table III. Beclin1 expression levels in the normal cervical, CIN, and cervical cancer tissues.

Group	N	Beclin1, N (%)			p
		-	+	++	
Normal tissue	80	15 (18.8%)	25 (31.2%)	40 (50%)	
CIN	80	5 (6.3%)	50 (62.5%)	25 (31.2%)	Compared with normal tissue, $p < 0.05$.
Cervical cancer	100	38 (38.0%)	50 (50.0%)	12 (12.0%)	Compared with CIN, $p < 0.05$.

(both $p < 0.01$). However, no significant difference was observed in the Rab5a positive rate between the CIN and cervical cancer tissues ($p > 0.05$). Rank sum test for ranked data indicated no significant correlation between the Rab5a expression and the age of cervical cancer patients, FIGO staging, tumor size, or histological type ($p > 0.05$). However, the positive rate of Rab5a in the poorly-differentiated cervical cancer group was significantly higher than the moderately- and highly-differentiated cervical cancer group ($p < 0.05$). Moreover, the positive rate of Rab5a in cervical cancer patients with lymph node metastasis was significantly higher than the patients without lymph node metastasis ($p < 0.05$) (Table VI). These results suggest that the expression levels of Rab5a are significantly elevated in the CIN and cervical cancer tissues. Moreover, the Rab5a expression level is associated with the differentiation and metastasis status of cervical cancer.

Relationship Between Expression of EMC-6, Beclin1, and Rab5a in Normal Cervical, CIN, and Cervical Cancer Tissues

Relationships between the expression of EMC-6 and Beclin1, as well as EMC-6 and Rab5a, in the normal cervical, CIN, and cervical cancer

Table IV. Correlation between the Beclin1 expression levels and pathological features in cervical cancer.

Group	N	Beclin1, N (%)			p
		-	+	++	
Age					
≤ 45 years	53	22 (41.5%)	27 (51.0%)	4 (7.5%)	0.24
> 45 years	47	16 (34.1%)	23 (48.9%)	8 (17.0%)	
FIGO staging					
I	46	17 (37.0%)	22 (47.8%)	7 (15.2%)	0.61
IIa	54	21 (38.9%)	28 (51.9%)	5 (9.2%)	
Tumor size					
≤ 4 cm	47	18 (38.3%)	24 (51.1%)	5 (10.6%)	0.84
> 4 cm	53	20 (37.7%)	26 (49.1%)	7 (13.2%)	
Tumor type					
Exogenous tumor	56	19 (33.9%)	30 (53.6%)	7 (12.5%)	0.80
Endogenous tumor	33	19 (30.3%)	19 (57.6%)	4 (12.1%)	
Histological type					
Squamous cell carcinoma	82	30 (36.6%)	43 (52.4%)	9 (11.0%)	0.23
Adenocarcinoma	18	8 (44.4%)	7 (38.9%)	3 (16.7%)	
Cell differentiation					
Moderate/high differentiation	61	20 (32.8%)	34 (55.7%)	7 (11.5%)	0.31
Poor differentiation	39	18 (46.2%)	16 (41%)	5 (12.8%)	
Lymph node metastasis					
Yes	34	12 (35.3%)	16 (47.1%)	6 (17.6%)	0.42
No	66	26 (39.4%)	34 (51.5%)	6 (9.1%)	

Table V. Rab5a expression levels in the normal cervical, CIN, and cervical cancer tissues.

Group	N	Rab5a, N (%)			p
		-	+	++	
Normal tissue	80	60 (75%)	14 (17.5%)	6 (7.5%)	
CIN	80	25 (31.3%)	20 (25.0%)	35 (43.7%)	Compared with normal tissue, $p < 0.05$.
Cervical cancer	100	25 (25.0%)	37 (37.0%)	38 (38.0%)	Compared with CIN, $p < 0.05$.

Table VI. Correlation between the Rab5a expression levels and pathological features in cervical cancer.

Group	N	Rab5a, N (%)			p
		-	+	++	
Age					0.21
≤ 45 years	53	15 (28.3%)	21 (39.6%)	17 (32.1%)	
> 45 years	47	10 (21.3%)	16 (34.0%)	21 (44.7%)	
FIGO staging					0.80
I	45	10 (22.2%)	18 (40.0%)	17 (37.8%)	
IIa	55	15 (27.3%)	19 (34.5%)	21 (38.2%)	
Tumor size					0.32
≤ 4 cm	47	10 (21.3%)	17 (36.2%)	20 (42.5%)	
> 4 cm	53	15 (28.3%)	20 (37.7%)	18 (34.0%)	
Tumor type					0.81
Exogenous tumor	56	16 (28.6%)	23 (41.1%)	17 (30.3%)	
Endogenous tumor	33	9 (27.3%)	14 (42.4%)	11 (33.3%)	
Histological type					0.00
Squamous cell carcinoma	61	19 (31.1%)	26 (42.6%)	16 (26.3%)	
Adenocarcinoma	39	6 (15.4%)	11 (28.2%)	22 (56.4%)	
Cell differentiation					0.37
Moderate/high differentiation	82	18 (22.0%)	29 (35.3%)	35 (42.7%)	
Poor differentiation	18	7 (38.9%)	8 (44.4%)	3 (16.7%)	
lymph node metastasis					0.00
Yes	66	21 (31.8%)	28 (42.4%)	17 (25.8%)	
No	34	4 (11.7%)	9 (26.5%)	21 (61.8%)	

Table VII. Relationship between expression of EMC-6 and Beclin1 in normal cervical tissue.

	EMC-6, N (%)			R	p
	-	+	++		
Beclin1 (-)	5	6	4		
Beclin1 (+)	4	5	16	0.21	0.07
Beclin1 (++)	6	9	25		

Table VIII. Relationship between expression of EMC-6 and Rab5a in normal cervical tissue.

	EMC-6, N (%)			R	p
	-	+	++		
Rab5a (-)	13	14	33		
Rab5a (+)	1	4	9	0.07	0.56
Rab5a (++)	1	2	3		

tissues were analyzed with the nonparametric Spearman's rank correlation analysis. Our results indicated that, in the normal cervical tissue, the correlation coefficient between EMC-6 and Beclin1 was 0.21 ($p = 0.07$) (Table VII), indicating independent correlation. Independent correlation was also observed between EMC-6 and Rab5a in the cervical cancer tissue, with the correlation coefficient of 0.07 ($p = 0.56$) (Table VIII). Moreover, in the CIN tissue, no significant correlation was observed between EMC-6 and Beclin1 (Table IX), or between EMC-6 and Rab5a (Table X), with the correlation coefficients of 0.16 ($p = 0.19$) and 0.10 ($p = 0.36$), respectively. Furthermore, in the cervical cancer tissue, the expression of EMC-6 was correlated with neither Beclin1 (correlation

Table IX. Relationship between expression of EMC-6 and Beclin1 in CIN tissue.

	EMC-6, N (%)			R	p
	-	+	++		
Beclin1 (-)	2	1	2		
Beclin1 (+)	2	2	41	0.16	0.19
Beclin1 (++)	1	2	22		

Table XI. Relationship between expression of EMC-6 and Beclin1 in cervical cancer tissue.

	EMC-6, N (%)			R	p
	-	+	++		
Beclin1 (-)	18	6	14		
Beclin1 (+)	13	13	26	0.14	0.13
Beclin1 (++)	5	1	6		

Table X. Relationship between expression of EMC-6 and Rab5a in CIN tissue.

	EMC-6, N (%)			R	p
	-	+	++		
Rab5a (-)	2	2	21		
Rab5a (+)	2	1	17	0.10	0.36
Rab5a (++)	1	2	32		

Table XII. Relationship between expression of EMC-6 and Rab5a in cervical cancer tissue.

	EMC-6, N (%)			R	p
	-	+	++		
Rab5a (-)	7	10	8		
Rab5a (+)	14	5	18	0.05	0.63
Rab5a (++)	15	3	20		

coefficient of 0.05, $p = 0.14$) (Table XI) nor Rab5a (correlation coefficient of 0.05, $p = 0.63$) (Table XII). Taken together, these results suggest that the expression level of EMC-6 is not associated with Beclin1 and Rab5a in the normal cervical, CIN, and cervical cancer tissues.

Discussion

Autophagy starts with the formation of the semi-circular double- or multi-layer membrane in the cytoplasm, which subsequently surrounds a large number of cytoplasm and organelles (such as mitochondria and ER). The autophagosomes could fuse with the lysosomes, further degrading the cargoes with proteases. Phosphoinositide-3-kinase (PI3K) is an important autophagic pathway, which plays a role in promoting cell growth. In human tumors, the mutations of the proteins in the PI3K pathways have been reported. PI3K III and its product phosphatidylinositol have also been found to be associated with the autophagic signaling[7,8,11,14,15]. In yeast, PI3K III complex includes Apg6, Apg14, Vps15, and Vps34, which participate in the formation of autophagic vacuoles. Apg14 interacts with Apg6-Vps34, and Vps15 kinase modulates the activity of Vps34. Apg6-Apg14 unit contributes to the specificity of the PI3K complex during autophagy. On the other hand, Apg6 has been recognized as the homologous gene for Beclin1 in mammals. Rab5a is a small GTPase located on the plasma membrane, clathrin-coated vesicles, and early endosomes, which is involved in the ptdin3p complex to promote the formation of autophagic bodies. However, the exact role of Rab5a in the ptdin3p complex has not yet been clearly clarified.

EMC-6 is a gene newly discovered by Peking University Center for Human Disease Genomics. EMC-6 contains two conserved transmembrane domains, and interacts with Beclin1 and Rab5a, regulating the formation of autophagic bodies and promoting autophagic process[6]. EMC-6 recruits Rab5a onto the ER membrane, producing early endosomes and activating the PIK3C3 complex. The absence of EMC-6 would lead to the accumulation of autophagic precursors and attenuated autophagy. The expression of EMC-6 in the cancer tissues has not yet been fully investigated. In this study, the expression levels of EMC-6 in the normal cervical, CIN, and invasive cervical cancer tissues were detected by immunohistochemistry. Our results showed that the positive staining of EMC-6 was observed in all these tissues. Compared with the normal cervical tissue, the positive rates of EMC-6 were significantly elevated in the CIN and cervical cancer tissues. However, the positive rate of EMC-6 in the CIN tissue was higher than that the cervical cancer tissue. Based on these results, we speculate that EMC-6 might be involved in the continuous transformation of normal cervix-CIN-cervical cancer. In the normal cervical tissue, EMC-6 participates in the formation and maturation of autophagosome. The down-regulated EMC-6 expression could inhibit autophagy and lead to pre-autophagosome accumulation, and EMC-6 over-expression would si-

gnificantly enhance the autophagic process. Lack of EMC-6 expression would result in attenuated autophagic activity, which contributes to the pathogenic process from CIN to cervical cancer. Alternatively, down-regulated expression of EMC-6 might also be the consequence of cervical cancer. Similarly, over-expressed EMC-6 might enhance the autophagic process, which promotes the transformation from normal cervix to CIN. On the other hand, the elevated expression of EMC-6 might also be the consequence of CIN. This phenomenon might also be one of the reasons why CIN patients could be reversed to normal. Of course, further in-depth studies are still needed to elucidate this issue. The expression and role of EMC-6 in the pathogenesis of cervical cancer have been first reported herein. In cells, autophagic process degrades cytosolic proteins and intracellular organelles via the lysosomal pathway. Homeostasis of intracellular components would be maintained based on the recycling system, which keeps the cells at an optimal status.

Previous studies[16-20] have shown that, during the pathogenesis of cervical cancer, the maintenance of protein balance would provide cloning advantage. Cellular autophagy is negatively associated with the proliferation activity. Autophagy is one of the main pathways for long-cycle protein degradation, and the increased degradation rate could decrease the intracellular protein content. Similar results have been found by Rez et al[21] during tumorigenesis of pancreatic cancer in rat models. In azaserine-induced rat models of pancreatic cancer, the expanding and contracting rates of the newborn vesicles in the atypical tumor cells are 6-20 times higher than the normal tissue. In precancerous malignant cells, enhanced autophagic process could lead to a negative protein balance and inhibit cell growth, which represents one of the self-protection mechanisms. Further investigation shows that, at the 20th month, the autophagic capacity of pancreatic cells are greatly reduced, even to a lower level compared with the control group. During malignant transformation, the autophagic capacity would be significantly reduced, which, together with the declined sensitivity to the outside signals and drug treatments, greatly contribute to the growth of tumor cells. On the advanced stage, the inactivated autophagy would result in declined tolerance of tumor cells to ischemia and hypoxia, and the disturbed removal of damaged macromolecules and organelles might ultimately lead to cellular apoptosis or necrosis.

In this study, the positive rate of Beclin1 in the cervical cancer tissue was significantly lower than those in the CIN and normal cervical tissues. In line with this, Wang et al[22] have shown that the expression level of Beclin1 is declined in cervical cancer, indicating that the autophagic process is altered in the tumors, which might contribute to the disease deterioration. EMC-6 can bind to Beclin1 on the autophagic membrane, activating the autophagic process. However, no significant association was observed between the positive rates of EMC-6 and Beclin1 in the normal cervical, CIN, or cervical cancer tissue. The regulating mechanisms for the autophagic process are complicated, involving various signaling pathways, and further studies are still needed to investigate the interaction between these regulating factors.

Our results showed that, in cervical cancer, the expression levels of EMC-6 were not correlated with age, clinical staging, tumor size, tumor type, histological type, cell differentiation, or lymph node metastasis, suggesting that the EMC-6 decline might occur in the early stage of cervical cancer. Compare with the normal cervical tissue, the expression level of Rab5a was significantly elevated in the CIN tissue, which was further significantly increased in the cervical cancer tissue. These results suggest that Rab5a might be involved in the development from CIN to cervical cancer. EMC-6 is a potential member of the Rab5aIP superfamily, and EMC-6 knockout could lead to the absence of Rab5a. However, no significant association had been observed between the expression levels of EMC-6 and Rab5a in this study. Further studies are still needed to find out the exact relationship between these two factors.

Conclusions

Our results showed that, compared with the normal cervical tissue, the positive rates of EMC-6 were significantly increased in the CIN and cervical cancer tissues. Moreover, the EMC-6 positive rate in the CIN tissue was higher than the cervical cancer tissue. No significant association was observed between the expression levels of EMC-6 and the concerned clinicopathological features of cervical cancer. Also, the positive rates of Beclin1 were significantly declined in the CIN tissue, which was further significantly down-regulated in the cervical cancer tissue. Furthermore, the positive rate of Rab5a in the CIN tissue was significantly higher than the normal cervical tissue, which was further significantly increased in the cervical cancer tissue. Moreover, the

expression level of EMC-6 is not associated with Beclin1 and Rab5a in the normal cervical, CIN, and cervical cancer tissues. These findings might contribute to the understanding of the pathogenesis of cervical cancer, and provide the potential target for the genetic therapy of the disease.

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Conflict of interest

The authors declare no conflicts of interest.

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