

Original Article

Overexpression of EIF5A2 correlates with tumor progression and predicts poor prognosis in cervical cancer

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Abstract: Background: Eukaryotic translation initiation factor 5A2 (EIF5A2) has been demonstrated to play important roles in tumor progression. However, the role of EIF5A2 in cervical cancer remains unknown. This study aimed to investigate the expression and clinicopathological significance of EIF5A2 in cervical cancer. Methods: The mRNA expression level of EIF5A2 was analyzed in 10 pairs of cervical cancer and adjacent non-cancerous tissues by semi-quantitative real time-PCR assay. Immunohistochemistry was performed to assess EIF5A2 protein expression in 124 paraffin-embedded, archived cervical cancer samples. Survival analysis and Cox regression analysis were performed to investigate the clinicopathological significance of EIF5A2 expression. Results: Our data revealed that EIF5A2 was frequently overexpressed in cervical cancer tissues compared to normal adjacent noncancerous cervical tissues. Moreover, high expression of EIF5A2 is strongly associated with advanced FIGO stage ($P=0.005$) and parametrial invasion ($P=0.041$). Cervical cancer patients with higher Parametrial invasion expression had substantially shorter overall ($P=0.038$) and recurrence-free ($P<0.001$) survival times than patients with lower parametrial invasion expression. Multivariate analysis revealed that parametrial invasion might be an independent prognostic factor for breast cancer patients ($P=0.017$). Conclusions: Our study showed that EIF5A2 expression was positively associated with progression and aggressiveness of cervical cancer, and that EIF5A2 could serve as an independent prognostic marker in cervical cancer patients.

Keywords: EIF5A2, cervical cancer, progression, prognosis

Introduction

Human cervical cancer is one of the most common malignancies in female reproductive system, and the third leading cause of cancer related mortality in women, with approximately 500,000 new cancer cases among women and 274,000 cancer related mortality, representing a serious health threat to women worldwide [1]. Although various treatments for cervical cancer, such as chemotherapy, radiation and radical surgery, have been used and have been improved in the past decades, the clinical outcome of patients with advanced-stage diseases remains unsatisfactory [2]. This is largely because of a lack of effective and specific biomarkers that predict the progression of cervical cancer [3]. Thus, it is important to identify new genes and molecules that can effectively dis-

tinguish patients with favorable prognosis from those with poor prognosis, and to develop new therapy options for cervical cancer patients.

Eukaryotic translation initiation factor 5A (EIF5A) is a small molecular-sized protein classified in the eIF family. The EIF5A gene is conserved in all organisms from bacteria to humans, except in eubacteria [4]. EIF5A functions mainly as an elongation factor in mRNA translation by facilitating the formation of the first peptide bond during the translation initiation step. It can serve as a shuttle protein regulating the nucleus-cytoplasmic transport of mRNAs in cells as well [5]. It is the only known protein to contain the unusual amino acid hypusine, which is synthesized on eIF5A at a specific lysine residue from the polyamine spermidine by two catalytic steps [6]. The EIF5A

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Table 1. Correlation between the clinicopathologic features and expression of EIF5A2

Characteristics	Group	EIF5A2 expression		p	
		Total			
		Low	High		
Age(y)	≤55	75	49	26	0.478
	>55	49	35	14	
FIGO stage	IB1	72	56	16	0.005
	IB2-IIIB	52	28	24	
Differentiation	≤4 cm	67	45	22	0.726
	>4 cm	57	39	18	
Tumor diameter	1/2	74	53	21	0.261
	3	50	31	19	
Parametrial invasion	No	103	75	38	0.041
	Yes	21	9	12	
LN Metastasis	No	85	76	33	0.203
	Yes	15	8	7	
Total		124	84	40	

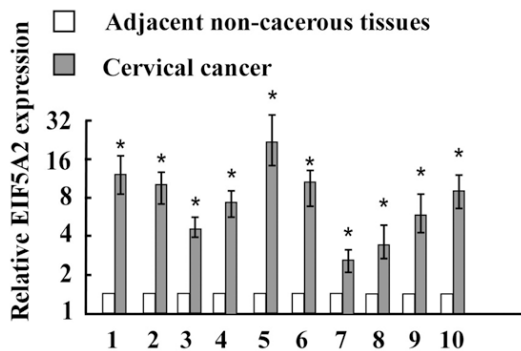


Figure 1. Expression of EIF5A2 mRNA is increased in cervical cancer tissues compared with non-cancerous tissues by qRT-PCR.

gene has two isoforms, EIF5A1 and EIF5A2. The EIF5A2 is located on chromosome 3q26, a region where amplification is observed in different human malignancies [7]. It is suggested that EIF5A2 expression is elevated in multiple cancers, and that up-regulation of EIF5A2 expression contributes to proliferation of cancer cells [8]. Additionally, EIF5A2 overexpression promotes tumorigenesis both in vitro and in vivo and is also positively correlated to an advanced stage of the disease [9]. Recently, EIF5A2 was shown to contribute to tumor metastasis and angiogenesis via hypoxia or gene amplification [10]. However, the expression and clinical relevance of EIF5A2 in cervical cancer have not been determined.

In the present study, we aimed to investigate the expression of EIF5A2 in cervical cancer and its relationship with clinical parameters and prognosis in cervical cancer patients. The results showed that EIF5A2 is significantly upregulated in cervical cancer, and overexpression of EIF5A2 is closely associated with the FIGO stage and parametrial invasion in cervical cancer. Multivariate Regression analysis revealed that EIF5A2 might be considered as an independent biomarker for cervical cancer prognosis. Collectively, our findings strongly suggested that EIF5A2 plays an important role in the development and progression of human cervical cancer, and might be a useful predictive marker of prognosis in cervical cancer patients.

Materials and methods

Tissue specimens and patient information

A total of 124 cervical cancer paraffin-embedded specimens from female patients, which had been histopathologically and clinically diagnosed as cervical cancer at the Panyu Central Hospital from 2005-2009, were used in the present study. Tumor stage was defined according to the pathological classification system of the International Federation of Gynecology and Obstetrics (FIGO). For the use of these clinical materials for research purposes, prior patients' consents and approval from Panyu Central Hospital Institutional Review Board were obtained. Clinical information on the samples is summarized in **Table 1**. The median age at the time of surgery was 49 years (range 21-77 years). The percentage of tumor purity in sections adjacent to the regions used for RNA extraction was estimated during routine histopathological analysis.

RNA extraction, reverse transcription and real-time PCR

Total RNA from fresh surgically obtained tumor tissues and their adjacent noncancerous tissues was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's recommendations. The RNA was treated with DNase, and 2 µg of total RNA was used for cDNA synthesis using random hexamers. Real-time reverse transcription polymerase chain reaction (qRT-PCR) was carried out using SYBER green kit in a Light Cycler system (Roche

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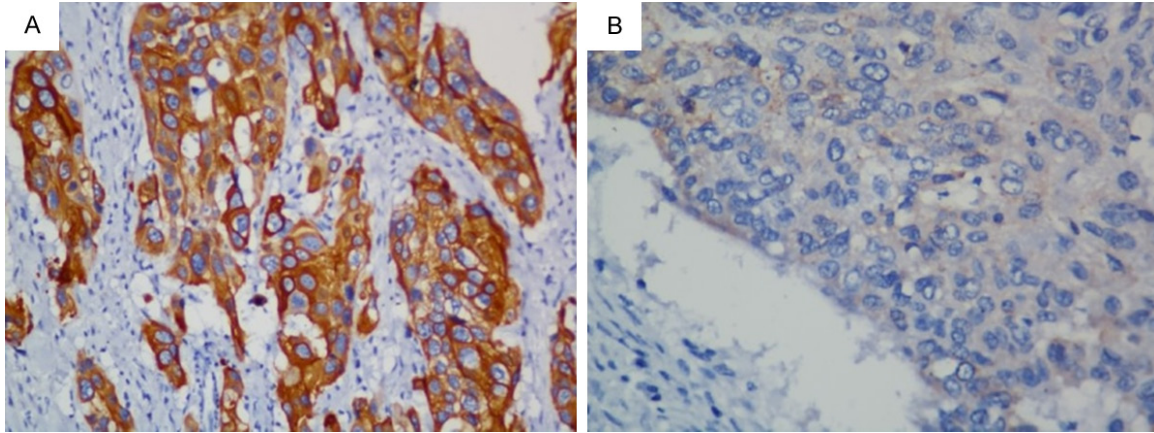


Figure 2. Expression of EIF5A2 protein in tissue by immunohistochemistry. A. Positive expression of EIF5A2 in cervical cancer tissues. B. Weak staining of EIF5A2 in cervical cancer tissues.

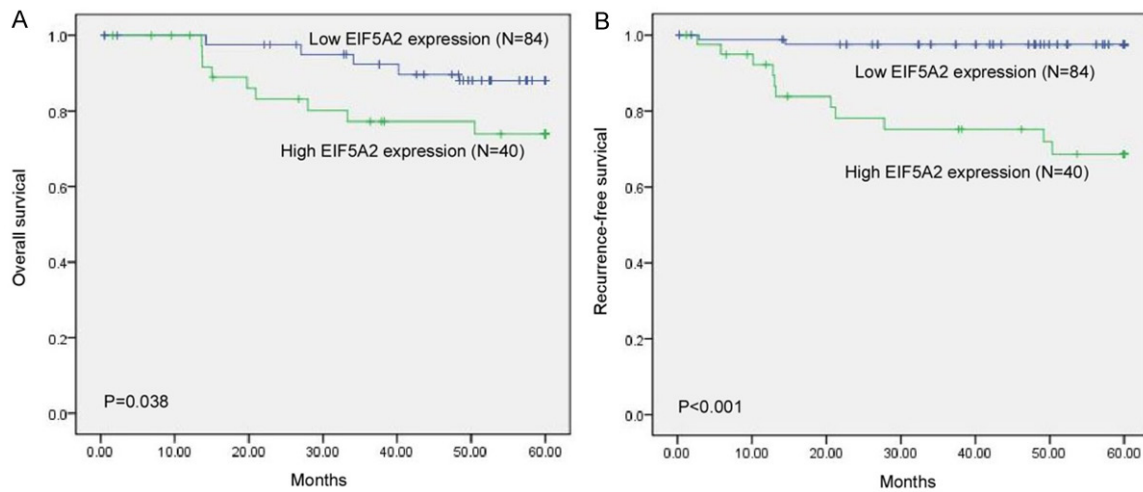


Figure 3. Kaplan-Meier curves stratified according to EIF5A2 expression in cervical cancer patients. A. EIF5A2 expression and patients' overall survival. B. EIF5A2 expression and patients' recurrence-free survival.

Applied Science). For the evaluation of the relationship between EIF5A2 and b-actin (internal control), the primer selected were as follows: EIF5A2, forward, 5'-CCCTGCTGAC AGAACT-GGT-3' and reverse, 5'-TTGCACACATGACAGACACC-3'; b-actin, forward, 5'-GCTCTTTCCAGCCTTCCTT-3', and reverse, 5'-CGGATGTCAACTTCACACTT-3'.

Immunohistochemistry

Immunohistochemical analysis was done to measure EIF5A2 protein expression in 124 human cervical cancer tissues. Briefly, paraffin embedded specimens were cut into 4 μ m sections and baked at 60°C for 2 hours, followed by deparaffinized with xylenes and rehydrated. Antigenic retrieval was done by submerging

the Sections into EDTA antigenic retrieval buffer and microwaving. The sections were then treated with 3% hydrogen peroxide in methanol to quench the endogenous peroxidase activity, followed by incubation with 1% bovine serum albumin to block the nonspecific binding. Sections were then incubated with anti-EIF5A2 rabbit polyclonal antibody (1:100, Abcam) overnight at 4°C. For negative controls, the primary antibody was replaced by normal goat serum. After washing, the tissue sections were treated with biotinylated anti-rabbit secondary antibody (Abcam), followed by a further incubation with streptavidin-horseradish peroxidase complex (Abcam). The tissue sections were immersed in 3-amino-9-ethylcarbazole and counterstained with 10% Mayer's hematoxylin, dehydrated and mounted in Crystal Mount.

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Table 2. Multivariate analysis of different prognostic parameters in patients with cervical cancer

	Overall survival		Recurrence-free survival	
	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Age (>55 y vs ≤55 y)				
FIGO Stage (IB2-IIA vs IB1)	4.429 (0.214-12.577)	0.004	4.507 (1.115-10.097)	0.015
Tumor diameter (>4 cm vs ≤4 cm)				
Tumor grade (Grade 3 vs 1/2)				
Parametrial invasion (yes vs no)				
LN Metastasis (yes vs no)	2.297 (0.625-7.097)	0.029		
EIF5A2 level (high vs low)	2.034 (0.179-7.820)	0.017		

The degree of immunostaining of formalin-fixed, paraffin-embedded sections was reviewed and scored by two independent observers. The proportion of the stained cells and the extent of the staining were used as criteria of evaluation. One score was given according to the percent of positive cells as: ≤5% of the cells, 1 point; 6-35% of the cells, 2 point; 36-70% of the cells, 3 point; ≥71% of the cells, 4 point. Another score was given according to the intensity of staining as: 0 point, negative; 1 point, weak staining (light yellow); 2 point, positive staining (yellowish brown); and 3 point, strong staining (brown). A final score was then calculated by multiple the above two scores. If the final score was equal or bigger than four, the tumor was considered high expression; otherwise, the tumor was considered low expression.

Statistical analysis

All statistical analyses were carried out using the SPSS 13.0 statistical software package. The chi-square test and Fisher's exact test were used to analyze the correlation between EIF5A2 expression and the clinicopathologic characteristics. Survival curves were plotted by the Kaplan Meier method and compared by the log-rank test. The significance of various variables for survival was analyzed by the Cox proportional hazards model in the multivariate analysis. $P < 0.05$ in all cases was considered statistically significant.

Results

EIF5A2 is upregulated in cervical cancer tissues

To explore the potential role of EIF5A2 in the tumorigenesis of cervical cancer, the expres-

sion of the EIF5A2 mRNA in 10 pairs of cervical cancer and adjacent non-cancerous tissues was determined by real time-PCR. These results indicated that EIF5A2 is upregulated in cervical cancer tissues (**Figure 1**).

EIF5A2 expression is associated with cervical cancer progression

To further explore whether EIF5A2 upregulation is associated with clinicopathological characteristics of cervical cancer, we examined the EIF5A2 expression status in 124 paraffin-embedded, archived cervical cancer tissues by immunohistochemistry (IHC) (**Figure 2**). IHC revealed that increasing EIF5A2 staining was positively correlated with advancing FIGO stage ($P = 0.005$) and parametrial invasion ($P = 0.041$). Taken together, these results suggested that upregulation of EIF5A2 is associated with cervical cancer progression.

EIF5A2 expression level is associated with the patient survival

Assessment of patient survival by the Kaplan-Meier analysis indicated an adverse correlation between EIF5A2 expression and overall survival ($P = 0.038$) and recurrent-free survival time ($P < 0.001$) of patients with cervical cancer (**Figure 3**). Multivariate Cox proportional hazards model analysis revealed that EIF5A2 expression was an independent prognostic factor for overall survival ($P = 0.017$) (**Table 2**).

Discussion

In the present study, we provided the first evidence that overexpression of EIF5A2 protein is associated with poor prognosis in cervical cancer patients. Our data showed that EIF5A2 is upregulated in cervical cancer specimens in

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compared with that in adjacent non-cancerous tissues. Moreover, analysis of 124 archived cervical cancer samples revealed that EIF5A2 expression is significantly correlated with FIGO stage, parametrial invasion, and clinical prognosis in these patients. Furthermore, Cox regression analysis showed that higher EIF5A2 expression was an independent prognostic indicator of shorter survival in cervical cancer patients. These findings strongly suggested that lower expression of EIF5A2 would provide a selective advantage in prognosis for cervical cancer patients.

EIF5A is an abundant, small, highly conserved, RNA-binding protein that associates with ribosomes and with the cytoskeleton, and shuttles between the nucleus and cytoplasm. It contains the amino acid hypusine, which appears to be essential to most of EIF5A's functions [11]. EIF5A2 is an isoform of EIF5A, and the expression of EIF5A2 is rare in most normal tissues but conspicuous in many malignancies. The association and functions of EIF5A2 have been studied in several tumors. EIF5A2 was first identified as an elevated expressed gene by analysis of an amplified chromosomal region from ovarian cancer [7]. Aberrant EIF5A2 expression is associated with tumor progression, increased likelihood of recurrence after surgery or radiotherapy [12]. EIF5A2 was reported to be upregulated by Hh signaling pathway via cis-acting elements in prostate cancer cells [13]. EIF5A2 promotes tumorigenesis, tumor cell proliferation and apoptosis of various cancers, such as esophageal squamous cell carcinoma, hepatocellular carcinoma, and bladder cancer [4], indicating its oncogenic role in tumor development. Thus, we assumed that EIF5A2 might promote cervical cancer progression through a similar mechanism. The present study showed that EIF5A2 is overexpressed in cervical cancer in compared with that in adjacent non-cancerous tissues, and that high EIF5A2 expression is correlated with advanced tumor stage, indicating that EIF5A2 is involved in the progression of cervical cancer.

Lymph node invasion and remote metastasis are EIF5A2-related pathological features that have attracted attention from a mechanistic point of view. EIF5A2 overexpression is detected in hepatocellular carcinoma margins [14]. Interestingly, EIF5A2 is one of only three genes

found to be predictive of lymph node metastasis in gastric cancer, while downregulation of EIF5A2 by miR-30b targeting suppresses tumor migration and invasion in gastric cancer cells [15]. Association of EIF5A2 with metastasis is also seen in hepatocellular carcinoma. Tang et al. found that ectopic expression of EIF5A2 could enhance cancer cell migration and invasion of hepatocellular carcinoma cells, while inhibition of EIF5A2 by small interfering RNA or deoxyhypusine synthase inhibitor GC7 decrease cell motility in vitro [14]. Therefore, we speculated that EIF5A2 might be correlated with tumor invasion in cervical cancer. Although we revealed that EIF5A2 expression was not correlated with lymph nodes metastasis, which may be due to the small sample size, high expression of EIF5A2 was associated with parametrial invasion. Thus, EIF5A2 might be a positive regulator of cervical cancer development and progression; however, the exact mechanism needs further investigation.

Remarkably, our study indicated that EIF5A2 expression was significantly correlated with clinical prognosis in cervical cancer patients. Our results were supported by previous studies. Wei et al. demonstrated that EIF5A2 overexpression predicts tumor metastatic potential in patients with localized invasive bladder cancer [16]. In addition, Bao et al. suggested that EIF5A2 promoted the chemoresistance to doxorubicin via regulation of EMT in colon cancer cells [17]. Li et al. showed that overexpression of EIF5A2 is associated with up-regulation of HIF1a, metastasis, and shorter survival times of patients with esophageal squamous cell carcinoma [10]. These studies together suggested that EIF5A2 overexpression predicts poor clinical outcomes in cancer patients.

In conclusion, we found that EIF5A2 overexpression is correlated with cervical cancer progression and progressive phenotype. Moreover, our data indicated that EIF5A2 might be an independent prognostic marker in cervical cancer patients. Thus, EIF5A2 protein expression might be a useful marker for stratifying cervical cancer patients' prognosis as well as an effective novel criterion for selection of therapeutic options.

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Disclosure of conflict of interest

None.

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