

Predictive significance of combined LAPT_M4B and VEGF expression in patients with cervical cancer

Fanling Meng¹ · Shu Tan¹ · Tianbo Liu¹ · Hongtao Song² · Ge Lou¹

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Abstract Lysosome-associated protein transmembrane 4β-35 (LAPT_M4B-35) is overexpressed in several solid malignancies. This study determines the expression level of LAPT_M4B-35 in the cervical cancer during tumor development and progression. The present study investigated the clinicopathological significance of the coexpression of LAPT_M4B-35 and VEGF in patients with cervical cancer. Immunohistochemistry was used to evaluate the expression of LAPT_M4B-35 and VEGF in 62 cervical intraepithelial neoplasia (CIN) and 226 cervical carcinoma in comparison with 45 normal cervical specimens. The correlation of combined LAPT_M4B-35 and VEGF with clinicopathologic characteristics was analyzed using a chi-squared test. Patient survival was determined using Kaplan–Meier method and log-rank test. A Cox regression analysis was performed to determine the prognostic significance of the factors. Combined LAPT_M4B-35 and VEGF expression was significantly associated with FIGO stage ($P=0.014$), tumor histologic grade ($P=0.033$), lymph node metastasis ($P=0.045$), and recurrence ($P=0.010$). Kaplan–Meier survival analysis showed that patients with cervical cancer expressing both LAPT_M4B-35 and VEGF exhibited both poor overall survival (OS) and disease-free survival (DFS) ($P=0.015$ and $P=0.016$, respectively). Cox analysis demonstrated that combined LAPT_M4B-35 and VEGF expression was an independent factor for both

OS and DFS ($P=0.015$ and $P=0.016$, respectively). Overexpression of LAPT_M4B-35 combined with positive VEGF expression may serve as a new biological marker to predict the prognosis of cervical carcinoma patients.

Keywords Lysosomal protein transmembrane 4 beta · Vascular endothelial growth factor · Immunohistochemistry · Cervical carcinoma · Prognosis

Introduction

Cervical cancer (CC) is the fourth most common cancer in women worldwide, with the highest prevalence in developing countries where CC is the leading cause of death [1, 2]. Persistent infection of high-risk human papillomavirus (HPV) is considered the main cause for developing CC. HPV vaccines are effectively used to prevent type 16 and 18 HPV infections. However, the natural history of the disease indicates that an effect on cervical cancer incidence will not occur for decades because approximately 30–60 % of women who are infected with genital HPV do not develop cancer [3, 4]. Therefore, identifying valuable prognostic factors is necessary to improve procedures for early detection and treatment of CC.

Recently, lysosomal protein transmembrane 4 beta (LAPT_M4B), a novel cancer-related gene, was successfully cloned [5]. The LAPT_M4B gene has been demonstrated to encode the LAPT_M4B-35 protein with molecular weight of 35 kDa. Studies have shown that LAPT_M4B-35 is upregulated in several human cancers [6–13]. Our previous studies also indicated that the overexpression of LAPT_M4B is associated with poor prognosis in a large portion of CC tissues [14] and contributes to cellular proliferation, transformation, tumorigenesis, metastatic progression, and angiogenesis in CC cell line HeLa [15].

Fanling Meng and Shu Tan contributed equally to this work.

✉ Ge Lou
doctorlouge@126.com

¹ Department of Gynecology, The Affiliated Tumor Hospital, Harbin Medical University, Harbin, China

² Department of Pathology, The Affiliated Tumor Hospital, Harbin Medical University, Harbin, China

Angiogenesis is critical for cancer growth, invasion, and metastasis [16]. Vascular endothelial growth factor (VEGF) is a potent vascular active molecule that is important in regulating physiological and pathological angiogenesis [17]. An increasing amount of evidence suggests that VEGF has been widely assessed in many types of tumors, including CC [18–20]. Our previous study revealed that the downregulation of LAPT4B-35 by RNA interference significantly reduced VEGF protein expression in the CC cell line HeLa [15]. Therefore, further investigation is needed to better define the combined predictive values of these two potential prognostic factors in CC.

The present study investigated the potential correlation of the coexpression of LAPT4B-35 and VEGF in CC tissues using clinical findings and patient outcome. Our results indicate that the combined LAPT4B-35 and VEGF expression may be used as an independent marker for prognosis of CC in patients.

Materials and methods

The specimens ($n=333$) were obtained from patients at the Department of Gynecology of Harbin Medical University's affiliated tumor hospital between September 2006 and March 2008. Of the 333 patients, 45 had normal cervical epithelia, 62 had cervical intraepithelial neoplasia (CIN), and 226 had cervical carcinoma. The patients with cervical carcinoma underwent radical hysterectomy and pelvic lymphadenectomy. The tumor stages were evaluated following the International Federation of Gynecology and Obstetrics (FIGO) staging system. The clinicopathologic features in these patients included gender, age, clinical hepatic status, viral infection status, and tumor size. All patients with CC were monitored for survival analysis until 31 March 2013 (median, 57 months; range, 14–78 months). Table 1 summarizes detailed information of the tumor characteristics.

The approval from the Medical Ethical Committee of Harbin Medical University's affiliated tumor hospital was obtained before the research began.

Immunohistochemistry

After the collection of paraffin-embedded cervical specimens, all tissue blocks were cut to 4- μ m-thick slices using a microtome and affixed onto slides. Tissue sections were dewaxed in xylene and rehydrated by graded alcohol concentrations according to standard procedures. The sections were immersed in 0.3 % hydrogen peroxide (H_2O_2) for 15 min to block the activity of endogenous peroxidase. Then, LAPT4B-35 antigen retrieval was performed by heating the sections in a microwave for 15 min in 0.01 mol/L citrate buffer (pH 6.0). The slide was

incubated at room temperature for 2 h with anti-LAPT4B-35 antibody (Abcam, CA, USA) or anti-VEGF antibody (Santa Cruz, CA, USA) at a dilution of 1:100. The sections were incubated with a second biotinylated antibody, followed by horseradish peroxidase-conjugated streptavidin for 30 min. Then, 3,3'-diaminobenzidine tetrahydrochloride (Dako, Germany) was used to visualize the reaction. The slides were counterstained with Meyer's hematoxylin, and negative control slides were stained with normal rabbit IgG instead of primary antibodies.

Staining evaluation

LAPT4B-35 and VEGF expression levels were classified semi-quantitatively based on the total combined scores of positive-staining tumor cell percentage and staining intensity. The percentage of positive cells was scored as "0" if <5 % of the tumor cells were stained positive, "1" if 5–50 % were stained positive, and "2" if >50 % of the cells were stained positive. The staining intensity was scored as follows: "0" in cases of no or only weak staining, "1" in cases of moderate staining, and "2" in cases of strong staining. The staining intensity score plus the percentage of positive staining was used to evaluate expression levels, where 0–2 indicated low expression and 3–4 indicated high expression.

Statistical analysis

The χ^2 test was used to show differences in categorical variables. Patient survival and follow-up data were analyzed using the Kaplan–Meier method and log-rank test, respectively. P values of less than 0.05 were considered statistically significant. All analyses were carried out using the statistical software package SPSS 13.0 software (SPSS, Chicago, IL, USA).

Results

Expression of LAPT4B-35 and VEGF in patients with CC

Statistical analysis showed that LAPT4B-35 and VEGF expression was significantly elevated in CINs and CC compared with the normal controls ($P<0.05$, data not shown). Representative images of LAPT4B-35 and VEGF expression are shown in Fig. 1. The expression levels of LAPT4B-35 and VEGF in CC patients are summarized in Table 1. Based on the scoring system used in the present study, 156 (69.0 %) cases were both LAPT4B-35(+) and VEGF (+), 56 (24.8 %) cases were both LAPT4B-35(-) and VEGF(-), 9 (4.0 %) cases

Table 1 Expression of LAPTM4B-35 and VEGF in relation to selected clinicopathological characteristics of in CC patients

Variables patients, <i>n</i>	LAPTM4B-35 and VEGF expression				<i>P</i> value ^a	
	L(-)V(-)	L(+)V(-)	L(-)V(+)	L(+)V(+)		
All cases						
Age (years)						
<40	114	30	6	3	75	<i>P</i> =0.637
≥40	112	26	3	2	81	
Histological type						
SCC	172	42	8	3	119	<i>P</i> =0.668
Adenocarcinoma	54	14	1	2	37	
FIGO stage						
I	118	40	5	3	70	<i>P</i> =0.008
II	108	16	4	2	86	
Histological grade						
G1	54	24	3	2	25	<i>P</i> =0.001
G2-3	172	32	6	3	131	
Lymph node metastasis						
No	150	50	6	4	90	<i>P</i> <0.001
Yes	76	6	3	1	66	
Recurrence						
No	146	53	6	3	84	<i>P</i> <0.001
Yes	80	3	3	2	72	

FIGO International Federation of Gynecology and Obstetrics, *G1* well differentiated, *G2-3* moderately and poorly differentiated, *LAPTM4B* lysosomal protein transmembrane 4 beta, *VEGF* vascular endothelial growth factor, *SCC* squamous cell carcinoma, *CC* cervical cancer

^aChi-squared test

were LAPTM4B-35(+) and VEGF(-), and 5 (2.2 %) cases were LAPTM4B-35(-) and VEGF(+). According to the chi-squared test, the LAPTM4B-35 expression was

significantly associated with VEGF expression (*P*=0.05, Tables 2 and 3).

Fig. 1 Representative immunohistochemical staining of LAPTM4B-35 and VEGF in cervical specimens: **a** positive expression of LAPTM4B-35 in a cervical carcinoma; **b** negative expression of LAPTM4B-35 in a cervical carcinoma; **c** positive expression of VEGF in a cervical carcinoma; **d** negative expression of VEGF in a cervical carcinoma

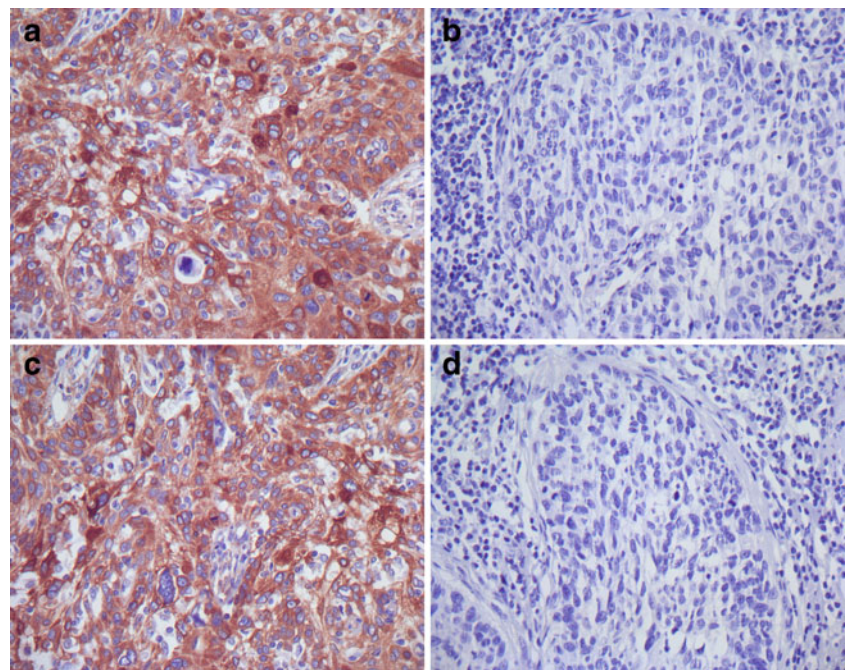


Table 2 Expression of LAPTM4B-35 and VEGF in CC patients

		LAPTM4B-35 expression		<i>P</i> value ^a
		Positive (n=165)	Negative (n=61)	
VEGF expression	Positive (n=161)	156	5	<i>P</i> <0.001
	Negative (n=65)	65	56	

LAPTM4B lysosomal protein transmembrane 4 beta, VEGF vascular endothelial growth factor, CC cervical cancer

^a Chi-squared test

Correlation of clinicopathologic parameters with LAPTM4B-35 and VEGF expression in CC

As shown in Table 1, the coexpression of LAPTM4B-35 and VEGF in CC was associated with FIGO stage, histological grade, lymph node metastasis, and tumor recurrence (*P*<0.05), but not with age or histological type (*P*>0.05).

Prognostic significance of LAPTM4B-35 and VEGF expression in CC

Univariate survival analysis revealed that CC with high expression of both LAPTM4B-35 and VEGF were correlated with shorter overall survival (OS) or disease-free survival (DFS) of patients (Fig. 2 and Table 3; both *P*<0.001).

Multivariate analysis showed that combined LAPTM4B-35 and VEGF expression was an independent prognostic marker for both OS and DFS of patients with CC (Table 4; *P*=0.001 and *P*<0.001, respectively).

Discussion

CC remains a serious health problem for women worldwide, and good prognostic factors are urgently needed [21]. In the present study, we analyzed the combined expression of LAPTM4B-35 and VEGF with the clinicopathologic characteristics in CC tissues. The results of this study indicate that the expression of both LAPTM4B-35 and VEGF were more

Table 3 Univariate survival analysis of OS and DFS in patients with CC

Variables	Number	OS		<i>P</i> value ^a	DFS		<i>P</i> value ^a
		Mean±SE (months)	95%CI		Mean±SE (months)	95%CI	
Age (years)							
<40	114	66±2	62–69	<i>P</i> =0.132	65±2	60–69	<i>P</i> =0.120
≥40	112	62±2	57–66		60±2	55–64	
Histological type							
SCC	172	65±2	62–69	<i>P</i> =0.004	64±2	61–67	<i>P</i> =0.007
Adenocarcinoma	54	57±3	51–63		55±3	49–62	
FIGO stage							
I	118	67±2	64–71	<i>P</i> =0.003	67±2	63–71	<i>P</i> <0.001
II	108	58±2	54–62		56±2	52–61	
Histological grade							
G1	54	72±2	68–76	<i>P</i> =0.001	71±2	67–76	<i>P</i> <0.001
G2-3	172	61±2	58–64		59±2	55–63	
Lymph node metastasis							
No	150	69±2	66–72	<i>P</i> <0.001	68±2	65–71	<i>P</i> <0.001
Yes	76	51±2	47–56		49±3	43–54	
LAPTM4B-35 and VEGF expression							
LAPTM4B-35(-), VEGF(-)	56	76±1	74–78	<i>P</i> <0.001	76±1	73–78	<i>P</i> <0.001
LAPTM4B-35(+), VEGF(-)	65	71±3	65–78		70±4	62–78	
LAPTM4B-35(-), VEGF(+)	5	67±2	63–70		65±3	59–71	
LAPTM4B-35(+), VEGF(+)	156	58±2	61–66		56±2	52–60	

FIGO International Federation of Gynecology and Obstetrics, G1 well differentiated, G2-3 moderately and poorly differentiated, LAPTM4B lysosomal protein transmembrane 4 beta, VEGF vascular endothelial growth factor, SCC squamous cell carcinoma, CC cervical cancer, OS overall survival, DFS disease-free survival

^a Log-rank test

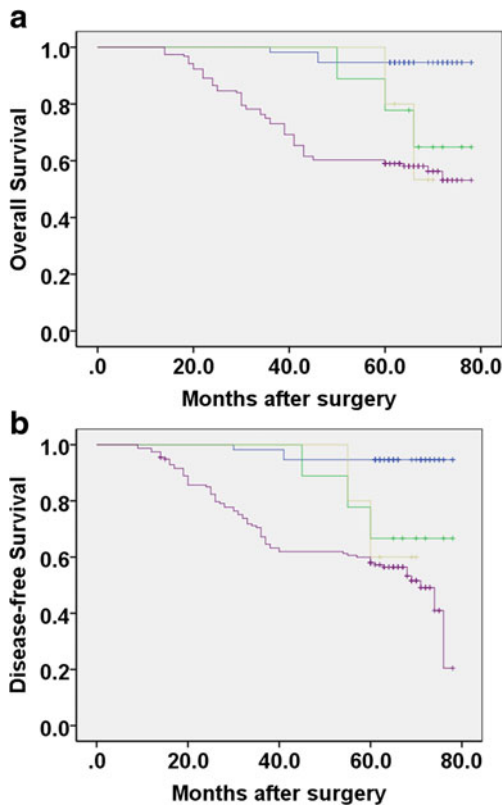


Fig. 2 Kaplan–Meier analysis of overall survival and disease-free survival related to the expression of LAPTM4B-35 and VEGF. **a** Overall survival curves of the cervical carcinoma patients; **b** disease-free survival curves of the cervical carcinoma patients; LAPTM4B-35(-) and VEGF(-), blue line; LAPTM4B-35(-) and VEGF(+), yellow line; LAPTM4B-35(+) and VEGF(-), green line; LAPTM4B-35(+) and VEGF(+), purple line

common in CC tissues than in CINs and normal tissues. Moreover, we observed that the coexpression of LAPTM4B-35 and VEGF is associated with portal vein invasion, tumor recurrence, and patient prognosis. Our results therefore suggest that the coexpression of VEGF and LAPTM4B-35 was widely activated in CC, making it a possible pivotal marker for progression and prognosis in CC patients.

Angiogenesis plays a key role in tumor invasion and metastasis [22]. Angiogenesis regulators have been found owing to the increase in angiogenesis studies since the early 1990s. Of these regulators, VEGF is recognized as an active member of one of the main signaling pathways in angiogenesis. This regulator is involved in tumor angiogenesis and in the invasion of malignant tumor cells [23, 24]. VEGF is significantly overexpressed in a large number of human tumor types, such as gastric cancer [25], colorectal cancer [26], lung cancer [27], breast carcinoma [28], and prostate carcinoma [29]. Several studies have demonstrated that high VEGF protein expression is a valuable predictor of cervical cancer prognosis [30–32]. Consistent with these researches, the VEGF has clinical outcomes both in terms of overall survival and disease-free survival (Fig. 2a, b and Table 3; both $P < 0.001$).

LAPTM4B-35 is significantly overexpressed in several malignant tumors. Current studies revealed that LAPTM4B-35 might be important for tumor development, progression, and metastasis through the activation of some proto-oncogenes, such as c-myc, c-jun, and c-fos [7]. Moreover, recent data indicate that LAPTM4B is involved in signal transduction pathways, thus enhancing tumor cell malignant transformation [33, 34]. These findings provide important evidence to explicate the mechanism by which LAPTM4B-35 contributes to oncogenesis and tumor development. Tang et al. evaluated the expression of LAPTM4B and CD34 proteins in CC tissues through immunohistochemistry, and the results revealed that LAPTM4B protein could be used in predicting patient prognosis and in angiogenesis [35]. Our previous study indicated that downregulated LAPTM4B also exhibited decreased migration and invasion activity, as well as significantly reduced VEGF expression [15]. LAPTM4B-35 expression, accompanied by the overexpression of VEGF, promotes growth and proliferation of CC cell HeLa. Therefore, LAPTM4B-35 and VEGF both exist in the CC. With this, we hypothesized that coexpressing LAPTM4B-35 and VEGF may lead to CC progression, angiogenesis, and poor prognosis. In accordance with this concept, our results demonstrated that patients with

Table 4 Multivariate survival analysis of OS and DFS in patients with CC

Variables	OS			DFS		
	Exp(B)	95 % CI	P^a	Exp(B)	95 % CI	P^a
Histological type	2.261	1.401–3.648	$P=0.001$	2.375	1.469–3.841	$P<0.001$
FIGO stage	2.517	1.891–3.350	$P<0.001$	1.652	1.005–2.716	$P=0.048$
Lymph node metastasis	2.513	1.573–4.014	$P<0.001$	2.107	1.314–3.378	$P=0.002$
LAPTM4B-35 and VEGF expression	1.727	1.263–2.361	$P=0.001$	1.845	1.344–2.532	$P<0.001$
Histological grade				1.889	0.894–3.991	$P=0.095$

FIGO International Federation of Gynecology and Obstetrics, LAPTM4B Lysosomal protein transmembrane 4 beta, VEGF vascular endothelial growth factor, CC cervical cancer, OS overall survival, DFS disease-free survival

^a Cox regression test

CC that coexpress LAPT4B-35 and VEGF have poor clinical outcomes for OS and DFS. In addition, combined LAPT4B-35 and VEGF expression may serve as an independent marker for the prognosis of patients with CC. This is the first study to investigate the association between LAPT4B-35 and VEGF coexpression and patient prognosis using CC tissues.

In conclusion, this study indicated that combined LAPT4B-35 and VEGF expression may provide important information for predicting CC prognosis and individualizing therapy for patients with CC.

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Compliance with ethical standards

Conflicts of interest None

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