



# LAPTM4B-35 expression was correlated with favorable post-operation survival in pancreatic cancer patients

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**Background:** Pancreatic cancer (PC) is malignant and lethal with its invasiveness and metastasis. Till now, it's still very difficult to diagnose PC at its early stage due to lack of obvious symptoms. Therefore, there is an urgent need to find an efficient biomarker to help diagnose PC as early as possible. Lysosome-associated protein transmembrane 4 beta-35 (LAPTM4B-35) is a kind of transmembrane oncoprotein which has been proved to be overexpressed in a variety of solid tumors. Few studies have focused on the expression of LAPTM4B-35 and its function in PC patients. This study aimed to assess LAPTM4B-35 expression in PC and to investigate the relationship between LAPTM4B-35 expression with clinicopathological features and post-operation survival of PC patients.

**Methods:** We collected tumor tissues and paired non-cancerous tissues from 93 PC patients and evaluated LAPTM4B-35 expression through immunohistochemistry assay. And we also explored LAPTM4B-35 expression of mRNA and protein by means of reverse transcription-polymerase chain reaction (RT-PCR), real-time PCR and Western blotting in PC cell lines and frozen tumor tissues. We used chi-squared test and Fisher's exact test to evaluate the relationship between LAPTM4B-35 expression and clinicopathological factors. Kaplan-Meier survival analysis and Cox regression were used for survival analysis. A two-sided P value less than 0.05 was considered to be statistically significant.

**Results:** LAPTM4B-35 was found to be expressed in 60 of 93 (64.5%) tumor tissues and 7 of 93 (7.5%) paired non-cancerous tissues ( $P < 0.001$ ). LAPTM4B-35 expression was correlated with tumor differentiation ( $P = 0.039$ ), and LAPTM4B-35 expression was higher in tumor tissue of PC patients with moderate and well differentiation than those with poor differentiation. In addition, we also found a tendency that overexpression of LAPTM4B-35 was associated with more favorable clinicopathological features, including moderate and well differentiation, no lymph node metastasis, and early TNM stage and so on. In survival analysis, we found that patients with positive LAPTM4B-35 expression had a better post-operation survival than patients with negative LAPTM4B-35 expression ( $P = 0.039$ ). And in multivariate survival analysis, we also found that LAPTM4B-35 (positive *vs.* negative,  $P = 0.029$ , OR = 0.474, 95% CI: 0.242–0.927) and TNM stage (IV *vs.* I + II,  $P = 0.001$ , OR = 5.437, 95% CI: 1.940–15.237) were independent factors for post-operation survival of PC patients.

**Conclusions:** LAPTM4B-35 expression was correlated with favorable post-operation survival in PC patients. LAPTM4B-35 might act as a candidate biomarker for PC at its early stage.

**Keywords:** Immunohistochemistry; lysosome-associated protein transmembrane 4 beta-35 (LAPT<sub>M</sub>4B-35); pancreatic cancer (PC); post-operation survival

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## Introduction

Pancreatic cancer (PC) is notorious for its aggressive and malignant biological behaviors and is the fourth leading cause of cancer related death (1). Most PC patients cannot be diagnosed at early stage because their symptoms are not obvious, and a large amount of patients are not suitable for surgical resection due to its severe invasion and metastasis. For the patients who receive surgical resection, 5-year survival rate is less than 20% and 10-year survival rate even decreases to approximately 2% (2). So, it will be quite helpful if we can diagnose this disease as early as possible and elucidate the mechanisms underlying its invasive behaviors.

Lysosome-associated protein transmembrane 4 beta (LAPT<sub>M</sub>4B) was first cloned in hepatocellular carcinoma (HCC). *LAPT<sub>M</sub>4B* gene is located at chromosome 8q22.1 and it contains seven exons and six introns (3). *LAPT<sub>M</sub>4B* gene encodes a member of the mammalian 4-tetramembrane spanning protein superfamily. LAPT<sub>M</sub>4B-24 and LAPT<sub>M</sub>4B-35 are two proteins with different molecular weight encoded by *LAPT<sub>M</sub>4B* gene (4). LAPT<sub>M</sub>4B-35 has been found to be overexpressed in a variety of solid tumors compared with LAPT<sub>M</sub>4B-24, such as HCC (5-7), breast cancer (8), ovarian cancer (9), gastric cancer (10,11), cervical cancer (12), colorectal cancer (13) and so on, wherein LAPT<sub>M</sub>4B-35 was also found to be correlated with invasive behaviors of these cancers.

However, few studies have focused on the relationship between LAPT<sub>M</sub>4B and PC. Our study aimed to evaluate the expression of LAPT<sub>M</sub>4B-35 in tumor tissues and paired non-cancerous tissues in PC patients and to assess the correlation of LAPT<sub>M</sub>4B-35 expression with clinicopathological features or post-operation survival of PC patients, which may be helpful to elucidate the relationship between LAPT<sub>M</sub>4B-35 expression and PC.

## Methods

### *Patients and samples*

Tumor tissues and paired non-cancerous tissues were

obtained from ninety-three patients who were diagnosed as PC and received surgical resection during 2008–2014 at Peking University Cancer Hospital, Beijing, China. Tumor tissues and paired non-cancerous tissues were formalin-fixed immediately after resection and then embedded with paraffin. All patients had clinical records and none of them received chemotherapy or radiation therapy before surgery. These patients were followed up for at least 5 years after surgery. The detailed clinicopathological features of these 93 PC patients were described in *Table 1*. This study was approved by the Ethical Committee of Peking University Cancer Hospital (Ethical approval number: 2016KT41), and written informed consent was acquired from each patient prior to initiation.

### *Immunohistochemistry*

The 4 μm thick tissue sections were baked at 72 °C for 1 hour, and then dewaxed in xylene and rehydrated in graded alcohol concentrations. The 3% hydrogen peroxide was used to block the activity of endogenous peroxidase for 15 minutes. After PBS washing 3 times, the antigen retrieval was performed by heating the sections in a microwave for 10 min in 0.01 mol/L citrate buffer (pH =6.0). When cooling to room temperature, the sections were blocked by goat serum containing 5% BSA (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) for 1 hour at room temperature. Then the sections were incubated with LAPT<sub>M</sub>4B-35 antibody (gifted by Pro. RL Zhou, Department of Cell Biology, School of Basic Medical Sciences, Peking University, Beijing, China) at a dilution of 1:1,500 at 4 °C overnight. The following day, the ready-to-use EnVision™ reagent (EnVision™ detection system peroxidase/DAB, rabbit/mouse; Dako, Glostrup, Denmark) was used to bind the primary antibody. The 3,3'-diaminobenzidine tetrahydrochloride (Dako, Glostrup, Denmark) was used to visualize the reaction. Then the sections were counterstained with hematoxylin and dehydration was performed in the sequence of graded alcohol concentrations and xylene.

**Table 1** Clinicopathological features of the 93 pancreatic cancer patients<sup>†</sup>

Clinicopathological features	No. of patients	% of patients
Gender		
Male	55	59.1
Female	38	40.9
Age (years)		
<50	15	16.1
≥50	78	83.9
Tumor site		
Head and neck of pancreas	54	58.1
Body and tail of pancreas	39	41.9
Differentiation		
Moderate and well	46	56.8
Poor	35	43.2
Depth of tumor invasion		
T1 + T2	13	15.1
T3 + T4	73	84.9
Lymph node metastasis		
N0	38	41.8
N1-2	53	58.2
Distant metastasis		
M0	82	88.2
M1	11	11.8
Vascular invasion		
Absent	51	60.7
Present	33	39.3
TNM stage		
I + II	34	39.5
III + IV	52	60.5

<sup>†</sup>Unfortunately, there are some missing data of clinicopathological features of these 93 patients.

### Evaluation of staining

LAPTM4B-35 expression levels were classified semi-quantitatively based on the total combined scores of positive-staining tumor cell percentage and staining intensity by two independent pathologists who were blind

to all patients' clinical data. The percentage of positive cells (PP) was scored as 0 (negative), 1 (<25%), 2 (25–75%), and 3 (>75%) respectively while the staining intensity (SI) was scored as 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). The immunoreactivity score (IRS) was defined as the sum of PP and SI, and IRS <1 was regarded as “negative” while IRS ≥1 was “positive”.

### Cell lines and cell culture

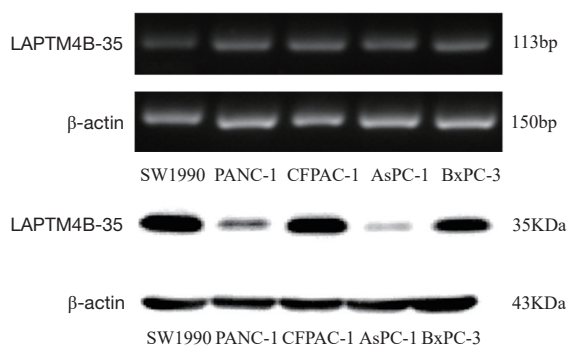
Five PC cell lines (SW1990, PANC-1, CFPAC-1, AsPC-1 and BxPC-3) were cultured in Dulbecco's modified Eagle's medium (DMEM, GibcoBRL, Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS), 100 µg/mL penicillin and 100 µg/mL streptomycin. And all cell lines were maintained at 37 °C in a humid condition with 5% CO<sub>2</sub>.

### Isolation of total RNA, reverse transcription-polymerase chain reaction (RT-PCR) and quantitative real-time PCR

We explored LAPTM4B-35 expression of mRNA level by means of RT-PCR and quantitative real-time PCR.

Total RNA of cell lines was isolated using Trizol reagent (Invitrogen, Life Technologies, Carlsbad, California, USA) while total RNA of 13 frozen tissues and paired non-cancerous tissues were isolated using RNeasy Mini Kit (Cot. 74104, QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. cDNA was synthesized from the 1 µg total RNA in a 20 µL reaction volume by RT using 5× All-In One RT MasterMix (Abm, Canada) with a procedure of 25 °C for 10 minutes, 42 °C for 50 minutes and 85 °C for 5 minutes.

RT-PCR amplification was performed using the following LAPTM4B-35 and beta-actin gene specific primers: LAPTM4B-35, 5'-CAAGAATTTCCCCACAACATC-3' (sense) and 5'-CGGCCTGGTGCTTTCTAATG-3' (antisense); and beta-actin, 5'-TTAGTTGCGTTACACCCTTTC-3' (sense) and 5'-ACCTTCACCGTTCCAGTTT-3' (antisense). Beta-actin was used to normalize LAPTM4B-35 gene expression. The PCR procedure was as follows: pre-denaturation at 94 °C for 5 minutes, 35 PCR cycles and a final elongation at 72 °C for 10 minutes. Each PCR cycle contained denaturation at 94 °C for 45 seconds, annealing at 60 °C for 45 seconds and extension at 72 °C for 45 seconds. PCR products were separated by gel electrophoresis on 2% agarose gel stained with GelRed (Biotium, USA). The band



**Figure 1** *LAPTM4B-35* mRNA and protein expression in pancreatic cell lines. (A) *LAPTM4B-35* gene expression was evaluated by RT-PCR in five pancreatic cancer cell lines. *LAPTM4B-35* gene was expressed at different levels in these cell lines, wherein *LAPTM4B-35* was found highly expressed in PANC-1, CFPAC-1 and BxPC-3 compared with SW1990 and AsPC-1; (B) differential expression of *LAPTM4B-35* protein in five pancreatic cancer cell lines was detected by western blot analysis. Expression level of *LAPTM4B-35* protein was different from RNA expression in these cell lines, that is, *LAPTM4B-35* protein was highly expressed in SW1990, CFPAC-1 and AsPC-1. *LAPTM4B-35*, lysosome-associated protein transmembrane 4 beta-35; RT-PCR, reverse transcription-polymerase chain reaction.

intensity was measured on an UVP EC3 Imaging System (Uvp Inc., Upland, CA, USA).

When performing real-time PCR, we used expressed Alu repeats (EAR) (14) as loading control instead of beta-actin with primer sequences of 5'-GAGGCTGAGGCAGGAGAATCG-3' (sense) and 5'-GTCGCCAGGCTGGAGTG-3' (antisense). And we performed EAR amplification using cDNA diluted 50 times. Real-time PCR was performed with pre-denaturation at 95 °C for 10 minutes, at 60 °C for 1 minutes and extension at 72 °C for 30 seconds. All reactions were performed in triplicate. The *LAPTM4B-35* expression was measured using  $2^{-\Delta\Delta CT}$  method.

#### Western blot analysis

Total protein was extracted from each cell line by 1× sodium dodecyl sulfate (SDS) lysis buffer. After denaturation at 95 °C for 5 minutes, equal amounts of protein (50 µg) were separated by 8% SDS-PAGE gel. After electrophoresis, the proteins were transferred onto polyvinylidene fluoride (PVDF) membrane. The

membranes were blocked with 5% skim milk for 2 hours at room temperature and then incubated by rabbit anti-human *LAPTM4B-35* antibody (1:800) and mouse anti-human beta-actin antibody (1:20,000, sigma, Cot. A4552) at 4 °C overnight. Beta-actin was used as a loading control. After being washed 3 times by PBST (phosphate-buffered saline with 0.1% Tween-20) buffer, the membranes were incubated with secondary horseradish peroxidase conjugated anti-rabbit and anti-mouse antibodies respectively at a dilution of 1:5,000 at room temperature for 2 hours. Finally, protein bands were evaluated with an enhanced chemiluminescence system.

#### Statistical analysis

The statistical analyses were performed using SPSS software, version 13.0 (SPSS, Chicago, IL, USA). Two-tailed chi-squared test ( $\chi^2$ ) or Fisher's exact test was used to compare *LAPTM4B-35* protein expression between PC tissues and paired non-cancerous tissues, and to evaluate the relationship between *LAPTM4B-35* expression and clinicopathological factors. The differential expression of *LAPTM4B-35* mRNA between tumor tissues and paired non-cancerous tissues was compared by using a nonparametric test. Kaplan-Meier survival analysis was used to evaluate patients' post-operation survival and P values were calculated by the log rank test. Multivariate survival analysis was performed with Cox proportional hazards regression model to identify the independent parameters affecting overall survival. In all cases, a two-sided P value less than 0.05 was considered statistically significant.

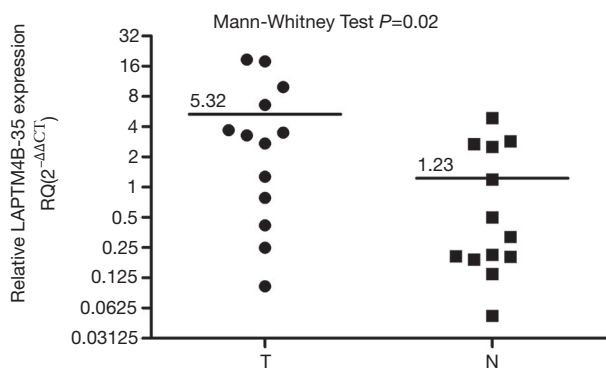
## Results

### *LAPTM4B-35* RNA and protein expression in pancreatic cell lines

We first examined RNA expression of *LAPTM4B-35* gene by RT-PCR in five pancreatic cell lines (SW1990, PANC-1, CFPAC-1, AsPC-1 and BxPC-3). The result showed that *LAPTM4B-35* gene was expressed at different levels in these cell lines, wherein *LAPTM4B-35* was found highly expressed in PANC-1, CFPAC-1 and BxPC-3 compared with SW1990 and AsPC-1 (shown in *Figure 1A*).

Furthermore, we evaluated the expression of *LAPTM4B-35* protein in 5 PC cell lines by western blotting assay and found that *LAPTM4B-35* protein was





**Figure 2** LAPT4B-35 mRNA expression in pancreatic cancer tumor tissues and paired non-cancerous tissues. LAPT4B mRNA expression in 13 pairs of pancreatic cancer tissues and paired non-cancerous tissues was tested by quantitative real-time PCR. The mRNA expression level of LAPT4B-35 was higher in cancer tissues than in pancreatic normal tissues. T means pancreatic cancer tissues, N means pancreatic normal tissues. LAPT4B-35, lysosome-associated protein transmembrane 4 beta-35; PCR, polymerase chain reaction.

expressed at different levels (shown in *Figure 1B*). We also noted that expression level of LAPT4B-35 protein was different from RNA expression in these cell lines, that is, LAPT4B-35 protein was highly expressed in SW1990, CFPAC-1 and AsPC-1. The difference between mRNA and protein expression might mainly be attributed to translational modification.

#### ***LAPT4B-35 RNA and protein expression in pancreatic tissues***

We assessed RNA expression of *LAPT4B-35* gene in 13 pairs of primary PC tissues and paired non-cancerous tissues by quantitative real-time PCR. As shown in *Figure 2*, we found that the mRNA expression level of LAPT4B-35 was higher in tumor tissues than in pancreatic normal tissues ( $P=0.02$ ).

We also examined expression of LAPT4B-35 protein by immunohistochemistry assay in tumor tissue and paired non-cancerous tissues from 93 PC patients. In these specimens, LAPT4B-35 was expressed in 60 of 93 (64.5%) tumor tissues and 7 of 93 (7.5%) paired non-cancerous tissues. And LAPT4B-35 protein was predominantly localized in the cytoplasm of PC cells. The expression of LAPT4B-35 in tumor tissues was markedly higher than in non-cancerous tissues ( $P<0.001$ ).

Representative images of LAPT4B-35 expression are shown in *Figure 3*.

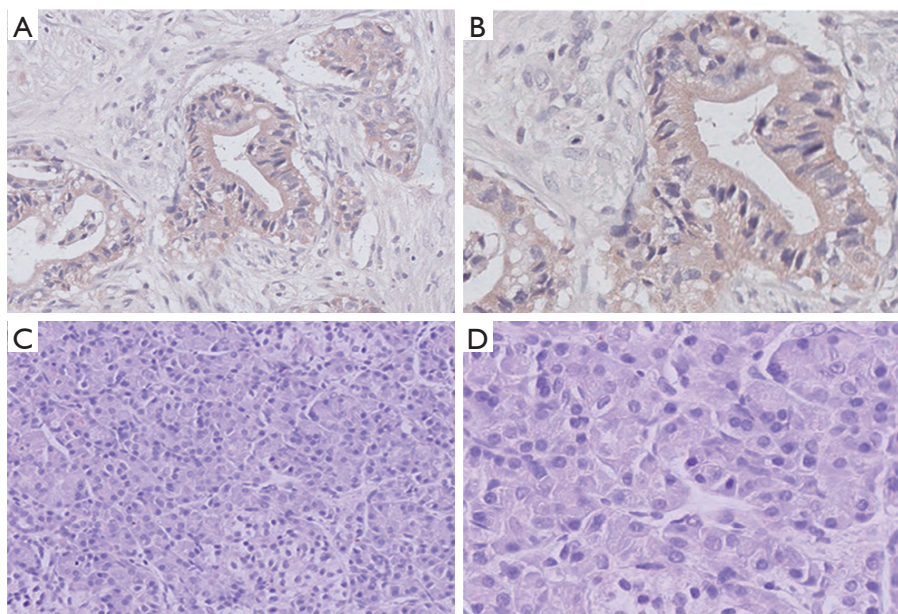
#### ***Correlation of LAPT4B-35 expression with clinicopathological parameters in PC patients***

Next, we assessed the relationship between LAPT4B-35 protein expression and clinicopathological features of PC. As shown in *Table 2*, we found that LAPT4B-35 expression was correlated with tumor differentiation ( $P=0.039$ ). Meanwhile, there was a tendency that LAPT4B-35 expression was a little higher in patients with more favorable clinicopathological features, including moderate and well differentiation, weaker tumor invasion, no lymph node metastasis, no distant metastasis, absence of vascular invasion and early TNM stage though without statistical difference (shown in *Figure 4*).

#### ***Correlation of LAPT4B-35 protein expression with post-operation survival of PC patients***

The follow-up time was measured from the operation date to the time of death or the last follow-up visit, which was from May 2008 to December 2015. During the follow-up period, 37 (39.8%) patients died of PC. Median survival time was 14.2 months (range from 2.5 to 62.7 months). The median survival time was  $17.64\pm 16.04$  months for LAPT4B-35 negative patients and  $19.2\pm 13.38$  months for LAPT4B-35 positive patients, respectively. We used Kaplan-Meier survival analysis and log rank test to evaluate the relationship between LAPT4B-35 expression or clinicopathological features and post-operation survival of PC patients. The survival curves showed that patients with positive LAPT4B-35 expression had a better post-operation survival than those with negative LAPT4B-35 expression ( $P=0.039$ ; *Figure 5*).

Various clinicopathological features that might affect overall survival were evaluated by univariate survival analysis, and we found that distant metastasis ( $P=0.006$ ), advanced TNM stage (IV *vs.* I + II,  $P=0.002$ ) and negative LAPT4B-35 expression ( $P=0.043$ ) were related to poor post-operation survival of PC patients. And multivariate Cox regression survival analysis further showed that LAPT4B-35 (positive *vs.* negative,  $P=0.029$ , OR =0.474, 95% CI: 0.242–0.927) and TNM stage (IV *vs.* I + II,  $P=0.001$ , OR =5.437, 95% CI: 1.940–15.237) were independent factors for post-operation survival of PC patients. For detailed information, please see *Table 3*.



**Figure 3** Representative immunohistochemical staining images for LAPT4B-35 protein expression in pancreatic cancer patients. LAPT4B-35 expression was shown in pancreatic cancer tissues (A,B) and paired non-cancerous pancreatic tissues (C,D). Magnification: A,C,  $\times 100$ ; B,D,  $\times 200$ . As shown, LAPT4B-35 protein expression was predominantly localized in the cytoplasm of pancreatic cancer cells. LAPT4B-35, lysosome-associated protein transmembrane 4 beta-35.

## Discussion

PC is a kind of aggressive carcinoma especially at its advanced stage and it is one of the most lethal human tumors. A study relating to familial PC has shown that PC might be quite heterogeneous with numerous gene deletions, amplifications and so on (15). Therefore, the development and progression of PC may involve various factors. It is important to find out markers to assist diagnosis, to monitor progression or to predict prognosis of PC patients.

Lysosome-associated protein transmembrane 4 beta (LAPT4B) is a kind of transmembrane oncoprotein that belongs to the mammalian LAPT4M family. Overexpression of LAPT4B has been proved in several kinds of solid tumors and has been related with malignant behaviors of these tumors. Furthermore, LAPT4B was found to be an independent prognosis factor in several kinds of tumors (5,7-13).

While, the mechanism of LAPT4B in tumor development and progression remains elusive. Overexpression of LAPT4B-35 can promote cell proliferation, cell migration and invasion in HCC through upregulating proliferation-promoting transcription

factors such as *c-Myc* (16). In NSCLC, LAPT4B increased NRF2 target genes such as heme oxygenase 1 (HMOX1) and the LAPT4B/NRF2 signaling axis modulated differentially in different cell lines with distinct mutational, genetic and epigenetic profiles and was being more activated in KRAS mutant lung cancer cells (17). And LAPT4B can play its role with other biomarkers. Overexpression of LAPT4B-35 in combination with positive argininosuccinate synthetase (ASS) expression was a negative prognostic marker for HCC patients (18), and combined overexpression of LAPT4B-35 and VEGF was an independent factor for both OS and DFS in cervical carcinoma patients (12).

LAPT4B is also related with multidrug resistance. Upregulation of LAPT4B-35 can motivate multidrug resistance by interaction with multidrug resistance 1 (MDR1) and activation of PI3K/AKT signaling pathway (19).

Recently, Klionsky *et al.* have found that LAPT4B can play a role in autophagy. In lung cancer cells, suppression of LAPT4B can cause an increase in LC3-II and p62 protein levels, which suggests decreased autophagosome turnover and fusion with lysosomes as well as abnormal autophagic flux. In addition, in serum deprived lung cancer cells, LAPT4B co-localized with LC3. LAPT4B was an

**Table 2** Correlation between LAPTM4B-35 expression and clinicopathological features of the 93 pancreatic cancer patients<sup>†</sup>

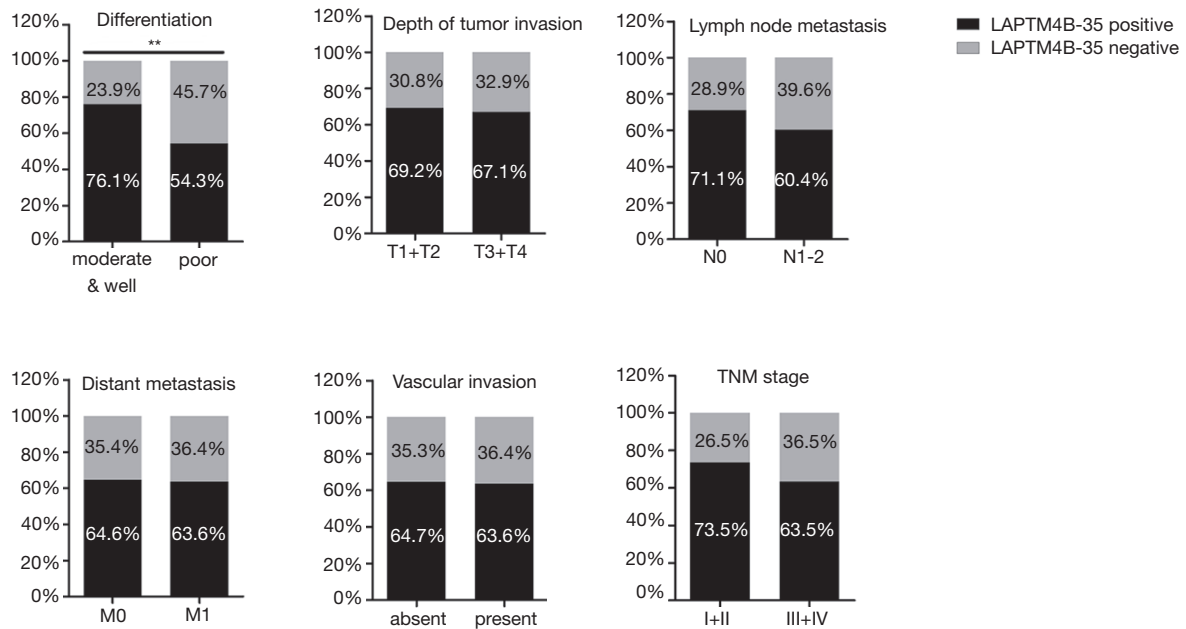
Clinicopathological features	No. of patients	Positive (%)	Negative (%)	P value
Gender				0.831
Male	55	35 (63.6)	20 (36.4)	
Female	38	25 (65.8)	13 (34.2)	
Age (years)				0.849
<50	15	10 (66.7)	5 (33.3)	
≥50	78	50 (64.1)	28 (35.9)	
Tumor site				0.713
Head and neck of pancreas	54	34 (63.0)	20 (37.0)	
Body and tail of pancreas	39	26 (66.7)	13 (33.3)	
Differentiation				0.039
Moderate and well	46	35 (76.1)	11 (23.9)	
Poor	35	19 (54.3)	16 (45.7)	
Depth of tumor invasion				0.881
T1 + T2	13	9 (69.2)	4 (30.8)	
T3 + T4	73	49 (67.1)	24 (32.9)	
Lymph node				0.293
N0	38	27 (71.1)	11 (28.9)	
N1-2	53	32 (60.4)	21 (39.6)	
Distant metastasis				0.948
M0	82	53 (64.6)	29 (35.4)	
M1	11	7 (63.6)	4 (36.4)	
Vascular invasion				0.920
Absent	51	33 (64.7)	18 (35.3)	
Present	33	21 (63.6)	12 (36.4)	
TNM stage				0.330
I + II	34	25 (73.5)	9 (26.5)	
III + IV	52	33 (63.5)	19 (36.5)	

<sup>†</sup>, unfortunately, there are some missing data of clinicopathological features of these 93 patients. LAPTM4B-35, lysosome-associated protein transmembrane 4 beta-35.

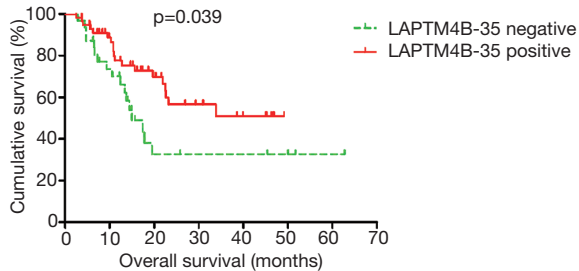
important mediator of autophagy for survival of lung cancer cells with serum starvation (17).

Few studies have focused on the expression of LAPTM4B and its function in PC, so the present study aimed to assess the relationship between LAPTM4B-35 expression and PC. In our study, we have detected high LAPTM4B-35 protein expression in one primary PDAC cell lines (BxPC-3) and

two metastatic PDAC cell lines (SW1990 and CFPAC-1). On the other hand, LAPTM4B-35 protein expression was significantly lower in PANC-1 (a primary PDAC cell line) and AsPC-1 (a metastatic PDAC cell line). From these findings, we cannot see a regular tendency, which may be attributed to multiple mutations during cellular immortalization. Even so, these cell lines can provide useful



**Figure 4** Correlation of LAPT<sub>M</sub>4B-35 expression and clinicopathological features of the 93 pancreatic cancer patients. LAPT<sub>M</sub>4B-35 expression was correlated with tumor differentiation ( $P=0.039$ ) and there was a tendency that overexpression of LAPT<sub>M</sub>4B-35 was associated with more favorable clinicopathological features. \*\*,  $P<0.05$ . LAPT<sub>M</sub>4B-35, lysosome-associated protein transmembrane 4 beta-35.



**Figure 5** Kaplan-Meier overall survival analysis of pancreatic cancer patients. Patients with positive LAPT<sub>M</sub>4B-35 expression had a better post-operation survival than those with negative LAPT<sub>M</sub>4B-35 expression ( $P=0.039$ ). LAPT<sub>M</sub>4B-35, lysosome-associated protein transmembrane 4 beta-35.

*in vitro* tools which can be manipulated for over-expression or knockdown so as to carry out subsequent functional or mechanism studies. We also noted that expression level of LAPT<sub>M</sub>4B-35 protein was different from RNA expression in these cell lines, and the difference might be attributed to translational modification and it may also be caused by regulation of upstream factors which might influence protein expression.

We also have found that expression of LAPT<sub>M</sub>4B-35

was higher in tumor tissues than paired non-cancerous tissues of PC patients. And there was a tendency that expression of LAPT<sub>M</sub>4B-35 was elevated in tumors with favorable clinicopathological features even though there was no statistical significance. LAPT<sub>M</sub>4B-35 was highly expressed in PC with moderate and well differentiation, relatively superficial depth of tumor invasion, no lymph node metastasis, no distant metastasis, absence of vascular invasion and early TNM stage. LAPT<sub>M</sub>4B-35 expression showed a gradually decreasing tendency with tumor progression, which has not been reported before.

Moreover, survival analysis showed that patients with positive LAPT<sub>M</sub>4B-35 expression had a better post-operation survival than those with negative LAPT<sub>M</sub>4B-35 expression, which was opposite to Dr. Zhou's research before (20). This difference might be due to the following reasons. Firstly, in Dr. Zhou's paper, LAPT<sub>M</sub>4B-35 protein was classified as low expression and high expression, while in our research we classified its expression as negative and positive. Different methods of identification may possibly cause different results. Secondly, it has been reported that LAPT<sub>M</sub>4B can play a dual function during tumor progression. The N-terminal part of LAPT<sub>M</sub>4B might be involved in signal transduction thus enhancing cell



**Table 3** Cox proportional hazard regression model analysis

Variables	Univariate analysis			Multivariate analysis		
	Relative risk	95% CI	P	Relative risk	95% CI	P
Gender (female vs. male)	0.776	0.394–1.527	0.463	0.685	0.341–1.379	0.289
Age ( $\geq 50$ vs. $< 50$ )	1.826	0.708–4.706	0.213	2.051	0.765–5.502	0.154
Differentiation (poor vs. moderate and well)	1.697	0.871–3.306	0.120			
Vascular invasion (present vs. absent)	1.674	0.840–3.334	0.143			
Distant metastasis (M1 vs. M0)	3.529	1.446–8.614	0.006			
TNM stage*						
III vs. I + II	1.785	0.847–3.763	0.128	1.576	0.743–3.344	0.236
IV vs. I + II	4.918	1.787–13.539	0.002	5.437	1.940–15.237	0.001
LAPTM4B-35 expression (positive vs. negative)	0.506	0.262–0.979	0.043	0.474	0.242–0.927	0.029

\*, as few patients were in stage I, we combined stage I and stage II together.

proliferation whereas the C-terminus might be responsible for the correct protein sorting to late endosomes and lysosomes (21). Thirdly, the different findings in the two studies might be caused by relatively small sample size and relatively short follow-up time. And in multivariate survival analysis, LAPTM4B-35 and TNM stage were independent factors for post-operation survival of PC patients. So, in clinical practice, we can evaluate the expression of LAPTM4B-35 in tumor specimen after surgery. If its expression is positive, then it might imply a favorable outcome, but we must combine routine postoperative re-examination to monitor the progression of PC patients. And in future we will expand the sample size and improve the post-operation follow-up to further validate our results.

Therefore, we inferred that LAPTM4B-35 might be up-regulated at the development stage of PC and doesn't participate much during the progression of this disease, which was not the same with other solid tumors (5,8,11). And this needs functional and mechanism validation. In Professor Zhou's lab, they have found that LAPTM4B-35 can be detected through enzyme-linked immunosorbent assay (ELISA) as exosome form in serum of HCC patients (unpublished data), so in future we will try to assess the LAPTM4B-35 level in PC patients, so as to assist diagnosis by combing other serum markers and imageological examination. Except CA19-9 and CEA, we can also combine LAPTM4B-35 with other serum biomarkers such as CD26, which has been explored extensively in PC patients by our team (22).

## Conclusions

In summary, we evaluated the expression of LAPTM4B-35 in PC tissues and paired non-cancerous tissues and assessed the relationship of LAPTM4B-35 expression with clinicopathological features and post-operation survival, respectively. We found that LAPTM4B-35 might be a potential biomarker for PC at its early stage, and LAPTM4B-35 expression was correlated with favorable outcome of PC patients. Furthermore, LAPTM4B polymorphism has been found to be associated with multiple kinds of tumors and increasing susceptibility to tumors (23-27). So, our team is also examining LAPTM4B polymorphism of PC patients. We hope to combine the results of this study with LAPTM4B polymorphism and to find a junction point so as to better understand the mechanisms underlying development and progression of PC.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

*Ethical Statement:* The study was approved by the Ethical Committee of Peking University Cancer Hospital (No. 2016KT41) and written informed consent was obtained from all patients.

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