Journal of Cancer Research and Clinical Oncology (2019) 145:1165–1178
https://doi.org/10.1007/s00432-019-02863-w

ORIGINAL ARTICLE – CANCER RESEARCH

Clinical impact of circulating LAPTM4B-35 in pancreatic ductal adenocarcinoma

Zixuan Yang[1] · Norbert Senninger[1] · Isabelle Flammang[1] · Qifa Ye[2] · Sameer A. Dhayat[1]

Received: 14 December 2018 / Accepted: 12 February 2019 / Published online: 18 February 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Purpose LAPTM4B is upregulated in a wide range of cancers associated with poor prognosis. However, the clinical impact of LAPTM4B as diagnostic and prognostic marker in pancreatic ductal adenocarcinoma (PDAC) remains unknown. Thus, the aim of the present study was to investigate the expression of LAPTM4B as circulating marker in PDAC.

Methods Expression analysis of LAPTM4B-35 in pancreatic tissue and preoperative blood serum samples of 169 patients with PDAC UICC Stages I–IV ($n=98$), chronic pancreatitis ($n=41$), and healthy controls ($n=30$) by immunohistochemistry, Western blot, and ELISA. Descriptive and explorative statistical analyses of LAPTM4B-35's potential as diagnostic and prognostic marker in PDAC.

Results Expression of LAPTM4B-35 was significantly increased in tumor tissue and corresponding blood serum samples of patients with PDAC (each $p<0.001$) and it could well discriminate PDAC from healthy controls and chronic pancreatitis ($p<0.001$; $p=0.0037$). LAPTM4B-35 in combination with CA.19-9 outperforms the diagnostic accuracy with an AUC of 0.903 ($p<0.001$), sensitivity of 82%, and specificity of 92%. Kaplan–Meier survival analysis revealed an improved overall survival in PDAC UICC I–IV with low expression of circulating LAPTM4B-35 (17 versus 10 months, $p=0.039$) as well as an improved relapse-free survival in curatively treated PDAC UICC I–III (16 versus 10 months; $p=0.037$). Multivariate overall and recurrence-free survival analyses identified LAPTM4B-35 as favorable prognostic factor in PDAC patients (HR 2.73, $p=0.021$; HR 3.29, $p=0.003$).

Conclusion LAPTM4B-35 is significantly deregulated in PDAC with high diagnostic and prognostic impact as circulating tumor marker.

Keywords Pancreatic ductal adenocarcinoma · Circulating LAPTM4B-35 · Prognostic marker

Abbreviations

AUC	Area under the curve
CA.19-9	Cancer antigen 19-9
HR	Hazard ratio
CI	Confidence interval
IHC	Immunohistochemistry
ELISA	Enzyme-linked immune-sorbent assay
LAPTM4B	Lysosome-associated protein transmembrane-4beta
PDAC	Pancreatic ductal adenocarcinoma
ROC	Receiver operating characteristic
SEM	Standard error of mean
UICC	Union internationale contre le cancer

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00432-019-02863-w) contains supplementary material, which is available to authorized users.

✉ Sameer A. Dhayat
sameer.dhayat@ukmuenster.de

Zixuan Yang
Zixuan.Yang@ukmuenster.de

Norbert Senninger
Senning@ukmuenster.de

Isabelle Flammang
Isabelle.Flammang@ukmuenster.de

Qifa Ye
yqf_china@163.com

[1] Department of General, Visceral and Transplantation Surgery, University Hospital Muenster, Albert-Schweitzer-Campus 1 (W1), 48149 Münster, Germany

[2] Institute of Hepatobiliary Diseases of Wuhan University, Zhongnan Hospital of Wuhan University, Wuhan, China

Background

Pancreatic ductal adenocarcinoma (PDAC), as one of the most lethal solid malignancies in developed countries, is the fourth leading cause of cancer deaths among both males and females in the USA in 2015 and is predicted to become the 2nd leading cause of cancer-related death by 2030 (Bray et al. 2018; Rahib et al. 2014). In 2018, estimated 55,440 new cases and 44,330 deaths of PDAC patients go along with a 5-year overall survival of about 8% for all Union for International Cancer Control (UICC) stages (Bray et al. 2018; Rahib et al. 2014; Siegel et al. 2018). This dismal prognosis of PDAC is due to late diagnosis at an advanced tumor stage, aggressive tumor dissemination, resistance to current therapy regimens, and lack of specific biomarkers and screening methods (Neuzillet et al. 2015). Radical surgical resection followed by adjuvant chemotherapy remains the gold standard of curative treatment. However, at the time of diagnosis, less than 20% of PDAC patients are amenable to curative-intended surgery with low adjuvant chemotherapy response rates of less than 50% (Hidalgo 2010; Kamisawa et al. 2016). Therefore, early detection of PDAC at surgically manageable stages is crucial and offers the best chance of increasing survival.

In 2000, Liu et al. published the identification of a new hepatocellular carcinoma (HCC)-related gene named as Lysosome-associated protein transmembrane-4beta (LAPTM4B) (Liu et al. 2000). LAPTM4B belongs to the mammalian 4-tetra-transmembrane spanning protein superfamily and is localized mainly to the late endosome and lysosome as well as in plasma membrane and internal organelles such as Golgi apparatus (Shao et al. 2003; Vergarajauregui et al. 2011). LAPTM4B gene is mapped to chromosome 8q22.1 with a messenger RNA (mRNA) consisting of seven exons. LAPTM4B encodes two protein isoforms with molecular weights of 35 and 24 kDa, respectively (Shao et al. 2003). LAPTM4B-35, but not LAPTM4B-24, is upregulated in a wide range of cancers associated with poor prognosis including hepatocellular cancer, extrahepatic cholangiocarcinoma, gallbladder cancer, breast cancer, ovarian carcinoma, endometrial cancer, prostate cancer, lung cancer, colon cancer, and gastric cancer (Cheng et al. 2015; Kang et al. 2012; Kasper et al. 2005; Meng et al. 2010; Qiao and Hu 2015; Xiao et al. 2013; Yang et al. 2010a; Yin et al. 2011; Zhang et al. 2014; Zhou et al. 2008, 2011). Interestingly, LAPTM4B polymorphism was associated with cancer risk in most of these cancer entities with the allele LAPTM4B*2 as risk factor (Xia et al. 2015). Moreover, LAPTM4B contributes to tumorigenesis by promoting cell proliferation, migration, and invasion. In addition, it could reduce apoptosis, assist drug resistance and is also involved in cell-cycle progression, differentiation, and DNA damage repair (Kasper et al. 2005; Li et al. 2010; Meng et al. 2016).

In PDAC, LAPTM4B was found to be expressed immunohistochemically at high levels in tissue samples of PDAC patients associated with poor survival (Zhang et al. 2012). Recently, Wang et al. could demonstrate that LAPTM4B polymorphism was associated with the susceptibility of PDAC in a large Chinese case–control study (Wang et al. 2017). However, the clinical impact of circulating LAPTM4B protein as diagnostic and prognostic marker in PDAC and its discriminating power in different tumor stages remains unknown. Furthermore, simple and cost-effective measurement of protein tumor markers in serum by enzyme-linked immune-sorbent assay (ELISA) represents the standard to monitor patient progress in most hospitals. Thus, the aim of the present study was to investigate the expression of LAPTM4B-35 protein as circulating tumor marker in PDAC.

Patients and methods

Patients and samples

A biobank of tissue and blood samples combined with a clinical follow-up database was maintained prospectively by the Department of General, Visceral and Transplantation Surgery of the University Hospital Muenster, Germany. Between 2014 and 2018, tissue specimens and blood serum samples of 169 patients with PDAC UICC Stage I (2× tissue, 5× serum); PDAC UICC Stage II (19× tissue, 11× serum); PDAC UICC Stage III (9× tissue, 24× serum); PDAC UICC Stage IV (27× serum); chronic pancreatitis (24× tissue, 17× serum); benign age-matched healthy controls (13× tissue, 17× serum) were collected. The study group of benign, non-inflammatory pancreatic specimens consisted of 13 tissue samples of patients with serous cystadenoma of the pancreas, that have been taken during surgery in fair distance to the cystadenoma as they function as the healthy control group in the tissue analysis. Patients that received immunosuppression, chemo- or radiotherapy before blood sampling and/or surgery were excluded to avoid potential influences on LAPTM4B-35 expression. Patients with a second tumor entity or pancreatic neuroendocrine tumor were excluded as well. Collection, processing and storage of venous blood samples and intraoperatively obtained tissue samples were performed under standardized conditions as described previously (Dhayat et al. 2015). All tissue specimens showed > 60% viable cells and < 20% necrosis with high percentage of cancer cells in guided macrodissection of PDAC samples proofed by an experienced pathologist. PDAC patients were staged according to the eighth edition of the UICC TNM classification of malignant tumors (Amin

et al. 2017). Ethical approval for tissue and/or blood serum collection was obtained by the Ethics committee of the University Muenster (1IXHai, 11.8.2011) and all patients provided informed written consent. All patients with suspicion of resectable PDAC underwent radical resection and were assigned to pancreatic head resection, total pancreatectomy, or pancreatic left resection followed by approved adjuvant therapy. Excisional tumor biopsy was performed in patients with unresectable metastasized PDAC followed by palliative therapy. Clinical data, histopathological information, and survival follow-up data were collected for all patients. The primary endpoints of this study were overall survival and recurrence-free survival.

Immunohistochemistry

Briefly, after deparaffinization and antigen retrieval using the cooker steaming method for 30 min, endogenous peroxidase activity was quenched by being exposed to 3% H2O2 for 12 min at room temperature. The slices were then blocked for 45 min at room temperature with 5% bovine serum albumin (BSA). Afterwards, slides were incubated with the primary antibody anti-LAPTM4B-35 (rabbit polyclonal, 1:80; abcam, Cambridge, UK) for 60 min in a humid chamber and then incubated with the horseradish peroxidase (HRP)-conjugated secondary detection antibody for 30 min at room temperature. The sections were then incubated with AEC Substrate Chromogen (Dako North America, Inc. CA, USA), counterstained with Haematoxylin and finally dehydrated, cleared, and mounted with neutral gum. Negative controls without primary antibodies and liver as positive control were included in all experiments using the same experimental conditions. Immunohistochemical staining was evaluated by a pathologist and was investigated in a blinded manner using light microscopy (Eclipse E1000M and NIS-Elements D3.1 imaging software, Nikon, Tokyo, Japan). Staining intensity was evaluated by Image-Pro Plus 6.0 software. (Media Cybernetics, Inc., MD, USA).

Western blotting

Cryopreserved tissue samples were dissociated in RIPA lysis buffer with Protease Inhibitor Cocktail (Cell Signaling Technology, Cambridge, UK) using a TissueLyserLT bead mill (Qiagen, Hilden, Germany). The lysates were cleared by centrifugation at 14,000*g* for 45 min at 4 °C. After quantification using PierceTM BCA Protein Assay Kit (ThermoScientificTM, Waltham, MA, USA), proteins were separated by sodium dodecyl sulfate (SDS) 10% polyacrylamide gel electrophoresis and blotted to a blocked PVDF membrane (Merck, Darmstadt, Germany). The primary antibody anti-LAPTM4B-35 (anti-rabbit, 1:1000, ab202301, abcam, Cambridge, UK) was diluted in blocking buffer and incubated overnight at 4 °C. Mouse anti-GAPDH (1D4, BioLegend Inc., San Diego, CA, USA) was used as a loading control at 1:5.000. The membrane was incubated with secondary antibody IgG-HRP (anti-rabbit, 1:14.000, A6154, Sigma-Aldrich) for 1 h at room temperature. After three washings, peroxidase was detected using ImmobilonTM ECL western blotting substrate (Millipore, Schwalbach, Germany).

Sandwich enzyme-linked immune-sorbent assay

Quantification of biomarker protein in blood serum samples was conducted with ELISA for LAPTM4B-35 (ready-to-use kit MBS760462, 1:2, MyBioSource Inc., San Diego, CA, USA) according to the manufacturer's instructions in duplicate. Briefly, 5 ml of venous blood was collected under standardized conditions and was separated into serum and cellular fractions by centrifugation at 1000*g* for 20 min. Anti-LAPTM4B-35 antibody was pre-coated onto 96-well plates. Set standard, test samples diluted with standard dilution at 1:2, and biotin conjugated detection antibody diluted with antibody dilution buffer at 1:100 were added to the wells subsequently, and washed with wash buffer. Horse radish peroxidase (HRP)-Streptavidin Conjugate (SABC) working solution diluted with SABC dilution buffer at 1:100 was added. Tetramethylbenzidine (TMB) substrates were used to visualize HRP enzymatic reaction. Finally, the absorbance of each well was recorded at a wavelength of 450 nm absorption, using a microplate reader (Biomed Dr Wieser GmbH, Salzburg, Austria) immediately after adding acidic stop solution. The professional software Curve Expert version 1.4 (Daniel G. Hyams, Hyams Development, Starkville, MS, USA) was used to calculate the LAPTM4B concentration based on a corresponding standard curve.

Statistical analysis

Explorative and descriptive statistical analysis were performed with Microsoft® Excel for Mac Version 16 (Microsoft Corp., Redmond, WA, USA), SPSS® Statistics Version 17 (IBM Corp. Armonk, NY, USA), and GraphPad Prism 7 (GraphPad Software, INC, La Jolla, CA, USA). The results are presented as mean with standard error of mean (SEM). Patients' dataset was performed by one-way analysis of variance (ANOVA), followed by least significant difference (LSD) multiple comparison tests when p was significant. To test the correlation between the clinicopathological data we used the Fisher's

two-tailed exact test and whenever appropriate the χ^2 test. Survival data were tested for significance using log-rank test for the comparison of relapse-free survival and overall survival rates between the groups with high and low expression of LAPTM4B-35 in PDAC samples and plotted for Kaplan–Meier curves. The primary end points were overall survival and relapse-free survival, as measured from the date of surgery to the time of cancer-related death or tumor relapse, or the last follow-up, respectively. Data of patients who were still alive and without evidence of tumor relapse at the end of the study were censored. The diagnostic potential of LAPTM4B-35 was analysed by the receiver operating characteristic (ROC) method. Area under the ROC curve (AUC) with its corresponding 95% confidence interval (CI) was used as an accuracy index for evaluating the diagnostic performance. A Cox proportional hazards regression model was used to estimate hazard ratios and 95% CIs. Variables with significant p values in the univariate analysis were included in the multivariate analysis. Values for $p \leq 0.05$ were considered to be statistically significant.

Results

Clinicopathologic characteristics

A total of 169 patients including PDAC UICC Stage I ($n = 7$), II ($n = 30$), III ($n = 33$) and IV ($n = 28$), chronic pancreatitis ($n = 41$), and healthy controls ($n = 30$) were analyzed (Table 1). There was no difference in age, gender, body mass index, smoking, alcohol consumption, and CEA between PDAC and its control groups of chronic pancreatitis, and healthy volunteers. However, they were distinguishable in pre-surgical diabetes mellitus ($p = 0.05$), pre-surgical chronic pancreatitis ($p < 0.001$), and pre-surgical CA.19-9 expression ($p < 0.001$). Subgroup analysis of all PDAC patients ($n = 98$) revealed prognostic impact of early UICC Stage with a median overall survival of 39 months versus 9.0 months for UICC Stage IV ($p < 0.001$), histological grading ($p = 0.016$), metastasis, nodal invasion, type of surgery, and adjuvant chemotherapy (each $p < 0.001$) (Table 2).

Clinicopathologic characteristics of 57 curatively treated PDAC patients were summarized in Table 3. Improved overall survival could be shown for no pancreatitis before surgery ($p = 0.025$), low grading ($p = 0.031$), no synchronous nodal invasion ($p = 0.017$), and adjuvant chemotherapy ($p < 0.001$), while histological grading ($p = 0.023$) and adjuvant chemotherapy ($p < 0.001$) correlated with recurrence-free survival.

Western blot and immunohistochemical analysis of LAPTM4B-35

Analysis of LAPTM4B-35 by Western blot showed a strong overexpression in cryopreserved PDAC tissue versus healthy and chronic pancreatitis tissues (each $p < 0.001$) (Fig. 1). Correspondingly, the immunohistochemical staining demonstrated significant higher levels of LAPTM4B expression in PDAC tissues (Fig. 2). LAPTM4B-35 was expressed predominantly in the epithelial pancreatic ductal cells, with significant overexpression in PDAC compared with Non-PDAC groups (each $p < 0.001$). LAPTM4B-35 expression in chronic pancreatitis was significantly higher than in healthy controls ($p = 0.009$). In total, exocrine acinar cells and stromal pancreatic cells showed only weak cytoplasmic positivity of LAPTM4B-35. The highest cytoplasmatic LAPTM4B-35 expression was detected in columnar epithelium of intralobular ducts in PDAC samples followed by interlobular ducts with cuboidal or columnar epithelium of larger ducts.

ELISA analysis of circulating LAPTM4B-35

Blood analysis of LAPTM4B-35 protein in PDAC of all UICC Stages revealed a significant overexpression of LAPTM4B-35 compared to healthy controls and chronic pancreatitis blood samples ($p < 0.001$) (Fig. 3). Subgroup analysis of circulating LAPTM4B-35 in the different PDAC UICC Stages demonstrated a significant overexpression of LAPTM4B-35 in UICC Stage I ($p = 0.016$), UICC Stage II, UICC Stage III, and UICC Stage IV (each $p < 0.001$) compared to healthy controls. Besides, it also revealed significant upregulation in PDAC UICC Stage II ($p = 0.001$), UICC Stage III ($p = 0.001$), and UICC Stage IV ($p = 0.005$) compared to chronic pancreatitis.

Diagnostic potential of circulating LAPTM4B-35

Circulating LAPTM4B-35 shows potential as a good discriminator between PDAC and healthy controls with excellent accuracy (AUC = 0.91 (CI 0.84–0.98), $p < 0.001$, sensitivity: 0.88, specificity: 0.82) and between PDAC and chronic pancreatitis with fair accuracy (AUC = 0.73 (CI 0.60–0.86), $p = 0.0037$, sensitivity: 0.59, specificity: 0.64) (Fig. 4). Subgroup analysis of each UICC Stage I–IV confirms excellent AUC values versus healthy controls as well as fair AUC values versus chronic pancreatitis (Supplemental Fig. 1). Circulating LAPTM4B-35 could differentiate PDAC from Non-PDAC controls including healthy controls and chronic pancreatitis with fair accuracy (AUC = 0.77 (CI

Table 1 Clinicopathologic characteristics of the study groups

Category	Total ($n=169$)	Healthy controls ($n=30$)	Chronic pancreatitis ($n=41$)	PDAC UICC Stage I ($n=7$)	PDAC UICC Stage II ($n=30$)	PDAC UICC Stage III ($n=33$)	PDAC UICC Stage IV ($n=28$)	p value
Age (years)								0.108
Median	65	64	60	72	65.5	68	64	
Range	26–87	42–87	36–80	66–75	42–82	43–84	26–82	
≤60	62	9	21	0	11	10	11	
>60	107	21	20	7	19	23	17	
Gender								0.306
Female	68	17	15	1	11	12	12	
Male	101	13	26	6	19	21	16	
Smoker								0.128
Yes	63	10	19	0	9	16	9	
No	106	20	22	7	21	17	19	
Alcohol abuse								0.087
Yes	20	1	8	0	2	7	2	
No	149	29	33	7	28	26	26	
Body mass index								0.499
Median	24.6	26.2	24	25.65	24.8	24	24.45	
Range	16.7–47.6	17.3–33.1	18.5–33.6	23.1–30.1	19–37	19–33.6	16.7–47.6	
≤25	98	13	27	4	19	20	15	
>25	71	17	14	3	11	13	13	
Pre-surgical diabetes mellitus								**0.050**
Yes	47	4	10	3	12	6	12	
No	122	26	31	4	18	27	16	
Pre-surgical pancreatitis								**<0.001**
Yes	59	0	41	0	7	10	1	
No	110	30	0	7	23	23	27	
Pre-surgical CA.19-9 (U/ml)								**<0.001**
Median	68.85	8.2	24.5	48.7	81.5	190.7	282.95	
Range	0.6–48715.2	1.6–42.9	0.6–48715.2	35.7–350	9.1–3136.1	2.6–18517.4	4–20640	
≤30	43	8	18	0	6	6	5	
>30	73	1	10	5	16	20	21	
Pre-surgical CEA (ng/ml)								0.198
Median	2.1	0.9	2.15	4.45	2.2	2.1	2	
Range	0.2–14.2	0.2–4.6	0.3–9.3	1.2–7.44	0.2–6.8	0.2–10.5	0.5–14.2	
≤5	72	9	16	3	13	17	14	
>5	16	0	2	3	2	4	5	

$p<0.05$ indicates significance are in bold

0.65–0.89), $p=0.0024$, sensitivity: 0.70, specificity: 0.62). Diagnostic accuracy of LAPTM4B-35 is similar to CA.**19-9** (AUC = 0.76 (CI 0.60–0.91), $p=0.0037$; sensitivity: 0.85, specificity: 0.69). The best diagnostic accuracy to discriminate PDAC from non-PDAC patients could be achieved by a combination of LAPTM4B-35 and CA.**19-9** with an

Table 2 Clinicopathologic characteristics of PDAC patients

Category	Number of PDAC patients	Median overall survival (months)	95% CI	*p* value
Total	98	17	13.7–20.3	
Age (years)				
≤ 60	32	14.0	9.80–18.2	0.477
> 60	66	17.0	13.9–20.1	
Gender				
Female	36	16.0	11.7–20.3	0.065
Male	62	20.0	13.4–26.6	
Smoker				
Yes	34	14.0	10.0–18.0	0.454
No	64	18.0	14.5–21.5	
Alcohol abuse				
Yes	11	18.0	4.5–31.5	0.718
No	87	17.0	14.1–20.0	
Body mass index				
≤ 25	57	16.0	12.3–19.7	0.379
> 25	41	18.0	12.6–23.4	
Pre-surgical diabetes mellitus				
Yes	33	12.0	3.9–20.1	0.704
No	65	17.0	14.0–20.0	
Pre-surgical pancreatitis				
Yes	18	13.0	9.2–16.8	0.305
No	80	17.0	13.4–20.6	
Pre-surgical CA.19-9 (U/ml)				
≤ 30	19	16.0	11.5–20.5	0.863
> 30	62	17.0	11.9–22.1	
Pre-surgical CEA (ng/ml)				
≤ 5	47	18.0	12.8–23.2	0.944
> 5	14	20.0		
UICC stage				
I	7	39		
II	30	21.0	14.2–27.8	**< 0.001**
III	33	17.0	13.5–20.5	
IV	28	9.0	2.5–15.5	
Grading				
G2	36	21.0	17.2–24.8	**0.016**
G3	33	16.0	10.2–21.9	
Metastasis				
M0	70	20.0	17.3–22.7	**< 0.001**
M1	28	9.0	2.5–15.5	
Nodal invasion				
N0	23	34.0	14.4–53.6	**< 0.001**
N1	24	14.0	7.9–20.1	
N2	47	13.0	8.2–17.8	
Lymphatic invasion				
L0	40	22.0	17.1–26.9	0.153
L1	21	16.0	8.7–23.3	
Perineural invasion				
Pn0	18	23.0	0.7–45.3	0.074
Pn1	41	20.0	15.8–24.2	
Vene invasion				

Table 2 (continued)

Category	Number of PDAC patients	Median overall survival (months)	95% CI	*p* value
V0	51	21.0	15.1–26.9	0.495
V1	11	20.0	13.0–27.0	
Resection margin				
R0	43	22.0	17.8–26.2	0.821
R1	16	16.0	10.9–21.1	
Tumor size (cm)				
≤ 3	30	20.0	11.4–28.6	0.452
> 3	21	21.0	2.7–39.3	
Type of surgery				
Pancreatic head resection	44	21.0	11.0–31.0	**< 0.001**
Pancreatic left resection	10	23.0	15.5–30.5	
Total pancreatectomy	3	4.0		
Excisional biopsy	41	9.0	3.6–14.4	
Post-op chemotherapy				
Adjuvant	51	21.0	16.2–25.8	**< 0.001**
Palliative	37	11.0	1.9–20.1	
No	10	3.0	0–6.7	
Post-op radiotherapy				
Yes	13	20.0	17.3–22.7	0.095
No	85	16.0	13.4–18.6	

$p < 0.05$ indicates significance are in bold

Indication of median overall survival in months and 95% confidence interval (CI)

excellent AUC of 0.903 (CI 0.84–0.97, p < 0.001, sensitivity: 0.82, and specificity: 0.92, likelihood ratio: 10.66).

Prognostic impact of circulating LAPTM4B-35

Patients were categorized into low LAPTM4B-35 and high LAPTM4B-35 categories at the median cut-off of 366.64 ng/ml. Univariate Kaplan–Meier survival analysis revealed a significantly improved overall survival in all PDAC patients UICC Stage I–IV with low expression of circulating LAPTM4B-35 ($p = 0.039$; median survival: 17 months versus 10 months). Overall survival of curatively treated PDAC patients with low expression of circulating LAPTM4B-35 was improved as well, but did not reach significance ($p = 0.064$). Improved relapse-free survival correlated significantly with low expression of circulating LAPTM4B-35 ($p = 0.037$; median survival: 16 months versus 10 months) (Fig. 5). Univariate Cox regression overall and recurrence-free survival analyses identified high histological grading ($p = 0.035$; $p = 0.026$), lack of postoperative adjuvant chemotherapy (each $p < 0.001$), and LAPTM4B-35 ($p = 0.005$, $p < 0.001$) as unfavorable prognostic factors in resected PDAC patients. Pre-surgical pancreatitis ($p = 0.031$), and nodal invasion ($p = 0.007$) correlated with poor overall survival. Lymphatic invasion ($p = 0.046$) correlated with poor recurrence-free survival analyses (Table 4). Multivariate Cox regression overall and recurrence-free survival analyses identified LAPTM4B-35 (HR 2.73, $p = 0.021$; HR 3.29, $p = 0.003$) and postoperative adjuvant chemotherapy (HR 0.03, $p < 0.001$; HR 0.10, $p = 0.006$) as favorable prognostic factors. Grading (HR 2.63, $p = 0.018$) could be used as prognostic factor in curatively treated relapse-free PDAC patients (Table 5).

Discussion

Since decades, PDAC still remains a highly lethal tumor entity for which no reliable molecular targets exist to support detection at early tumor stage or predict response to treatment modalities. Although several cell signaling pathway alterations in PDAC with promising targeted molecule inhibitors are reported, extensive efforts are needed to bring the research to clinical application (Zhang et al. 2018). However, several clinical factors have been identified in PDAC patients (Lim et al. 2003). In accordance with the literature, low-tumor grading and adjuvant gemcitabine chemotherapy correlated to improved survival in PDAC with potential as independent prognostic markers in our study. Furthermore, we could reveal that circulating LAPTM4B-35 might be an independent prognostic marker and oncogenic target protein in PDAC patients. LAPTM4B-35 was significantly

Table 3 Clinicopathologic characteristics of curatively treated PDAC patients

Category	Disease-specific overall survival			Recurrence-free survival		
	Number of patients [death/censored]	Predicted median survival (months) [95% CI]	*p* value	Number of patients [death/censored]	Predicted median survival (months) [95% CI]	*p* value
Total	57 [29/28]			57 [36/21]		
Age (years)						
≤ 60	16 [8/8]	17 [7.1–26.9]	0.702	16 [13/3]	13 [9.7–16.3]	0.67
> 60	41 [21/20]	22 [17.1–26.9]		41 [23/18]	12 [9.5–14.5]	
Gender						
Female	20 [12/8]	17 [12.3–21.7]	0.074	20 [12/8]	12 [9.5–14.5]	0.945
Male	37 [17/20]	23 [12.9–31.1]		37 [24/13]	13 [10.1–15.9]	
Smoker						
Yes	20 [10/10]	17 [14.0–20.0]	0.254	20 [14/6]	13 [12.3–13.7]	0.186
No	37 [19/18]	23 [14.3–31.7]		37 [22/15]	12 [8.3–15.7]	
Alcohol abuse						
Yes	3 [1/2]		0.473	3 [2/1]	0	0.837
No	54 [28/26]	20 [15.1–24.9]		54 [34/20]	12 [10.0–14.0]	
Body mass index						
≤ 25	36 [19/17]	17 [13.4–20.6]	0.078	36 [21/15]	13 [12.1–13.9]	0.742
> 25	21 [10/11]	34 [13.4–54.6]		21 [15/6]	12 [8.1–15.9]	
Pre-surgical diabetes mellitus						
Yes	18 [9/9]	34 [16.6–51.4]	0.371	18 [11/7]	16 [5.3–26.7]	0.321
No	39 [20/18]	20 [15.7–24.3]		39 [25/14]	12 [10.2–13.8]	
Pre-surgical pancreatitis						
Yes	15 [10/5]	17 [11.7–22.3]	**0.025**	15 [11/4]	10 [7.1–12.9]	0.173
No	42 [19/23]	28 [17.3–38.7]		42 [25/17]	13 [10.4–15.6]	
Pre-surgical CA.19-9 (U/ml)						
≤ 30	10 [4/6]	23	0.363	10 [5/5]	12 [10.3–13.7]	0.515
> 30	31 [12/19]	34 [12.3–55.7]		31 [16/15]	16 [10.1–21.9]	
Pre-surgical CEA (ng/ml)						
≤ 5	23 [10/13]		0.289	23 [12/11]	17 [10.0–24.0]	0.304
> 5	8 [0/8]			8 [2/6]	12	
UICC stage						
I	7 [1/6]	39	0.101	7 [2/5]		0.062
II	28 [15/13]	21 [7.0–35.0]		30 [19/11]	12 [8.6–15.4]	
III	20 [13/7]	17 [13.7–20.3]		20 [15/5]	12 [10.0–14.0]	
Grading						
G2	30 [14/16]	23 [19.2–26.8]	**0.031**	30 [18/12]	14 [9.8–18.3]	**0.023**
G3	27 [15/12]	17 [10.0–24.0]		27 [18/9]	10 [6.3–13.7]	
Nodal invasion						
N0	18 [7/11]	36 [17.4–54.6]	**0.017**	18 [10/8]	16 [11.5–20.5]	0.138
N1	20 [11/9]	14 [12.2–15.8]		20 [12/8]	10 [5.8–14.2]	
N2	19 [11/8]	17 [10.9–23.1]		19 [14/5]	12 [9.8–14.2]	
Lymphatic invasion						
L0	39 [18/21]	23 [12.4–33.6]	0.372	39 [23/16]	12[9.6–14.4]	0.949
L1	18 [11/7]	20 [12.2–27.8]		18 [13/5]	13[11.2–14.8]	
Perineural invasion						
Pn0	18 [9/9]	23 [0.7–45.3]	0.064	18 [13/5]	16 [8.8–23.2]	0.342
Pn1	39 [20/19]	17 [12.8–21.2]		39 [23/16]	12 [10.0–14.0]	
Vene invasion						
V0	51 [24/27]	21 [15.1–26.9]	0.734	51 [31/20]	13 [10.7–15.3]	0.723

Table 3 (continued)

Category	Disease-specific overall survival			Recurrence-free survival		
	Number of patients [death/censored]	Predicted median survival (months) [95% CI]	*p* value	Number of patients [death/censored]	Predicted median survival (months) [95% CI]	*p* value
V1	6 [5/1]	20 [8.2–31.8]		6 [5/1]	12 [7.2–16.8]	
Resection margin						
R0	43 [21/22]	22 [17.8–26.2]	0.698	43 [26/17]	13 [10.0–16.0]	0.592
R1	14 [8/6]	16 [7.7–24.2]		14 [10/4]	12 [9.7–14.3]	
Tumor size (cm) [range]						
≤ 3	29 [16/13]	20 [11.7–28.3]	0.509	29 [20/9]	12 [9.7–14.3]	0.260
> 3	20 [7/13]	21 [2.7–39.3]		20 [10/10]	17 [15.2–18.8]	
Type of surgery						
Pancreatic head resection	44 [22/22]	21 [15.3–26.7]	0.083	44 [28/16]	13 [10.5–15.5]	0.183
Pancreatic left resection	10 [6/4]	23 [15.5–30.5]		10 [7/3]	12 [5.6–18.4]	
Total pancreatectomy	3 [1/2]	4		3 [1/2]	3	
Post-op adjuvant chemotherapy						
Yes	51 [26/25]	21 [16.2–25.8]	**< 0.001**	51 [33/18]	13 [11.1–14.9]	**< 0.001**
No	6 [3/3]	4 [2.4–5.6]		6 [3/3]	3	
Post-op radiotherapy						
Yes	9 [5/4]	20 [8.0–26.5]	0.514	9 [8/1]	10 [6.2–13.9]	0.188
No	48 [24/24]	21 [15.5–26.5]		48 [28/20]	13 [11.0–15.0]	

$p < 0.05$ indicates significance are in bold

Indication of median disease-specific overall survival and recurrence-free survival in months and 95% confidence interval (CI)

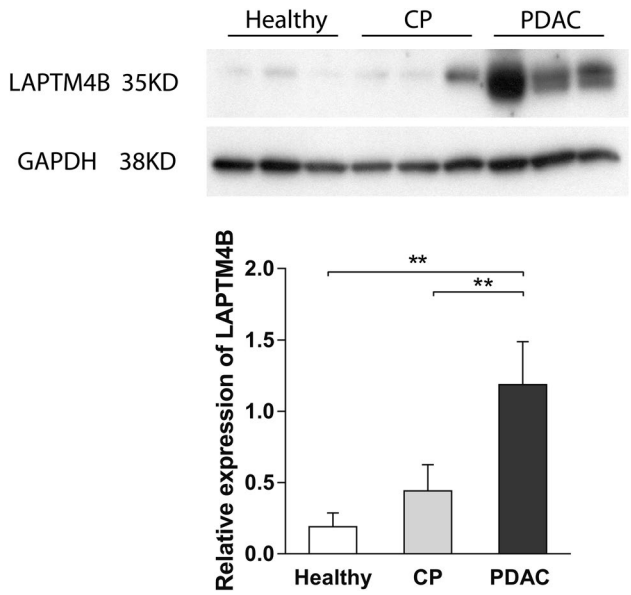

Fig. 1 Protein expression of LAPTM4B-35 in pancreatic tissue of healthy controls, chronic pancreatitis (CP), and PDAC by Western blotting. Asterisks (**) indicate a significant difference of $p \leq 0.01$ between PDAC patients and non-PDAC patients

upregulated in both, carcinoma tissue and blood circulation of early and advanced PDAC, suggesting that LAPTM4B-35 plays an important role in carcinogenesis and PDAC progression. The oncogenic role of LAPTM4B-35 in several solid carcinoma entities associated with high-tumor recurrence and poor postoperative survival is already well known (Cheng et al. 2015; Kang et al. 2012; Kasper et al. 2005; Meng et al. 2010; Qiao and Hu 2015; Xiao et al. 2013; Yang et al. 2010a; Yin et al. 2011; Zhang et al. 2014; Zhou et al. 2008, 2011). In accordance with the study of Zhang et al., immunohistochemical staining of LAPTM4B-35 was high in PDAC tissue samples with significant overexpression in epithelial pancreatic ductal cells. Validation of these semi-quantitative immunohistochemical staining results by Western blot analysis could confirm a significant overexpression of LAPTM4B-35 protein in macrodissected PDAC compared to non-PDAC controls in our study collective. So far, previous immunohistochemical and gene polymorphism analysis by polymerase chain reaction (PCR) indicates that LAPTM4B is highly expressed in PDAC tissue and its genetic polymorphism is positively correlated with poor prognosis in PDAC patients (Wang et al. 2017; Zhang et al. 2012). However, the value of circulating LAPTM4B-35 as circulating tumor marker for PDAC remained undefined.

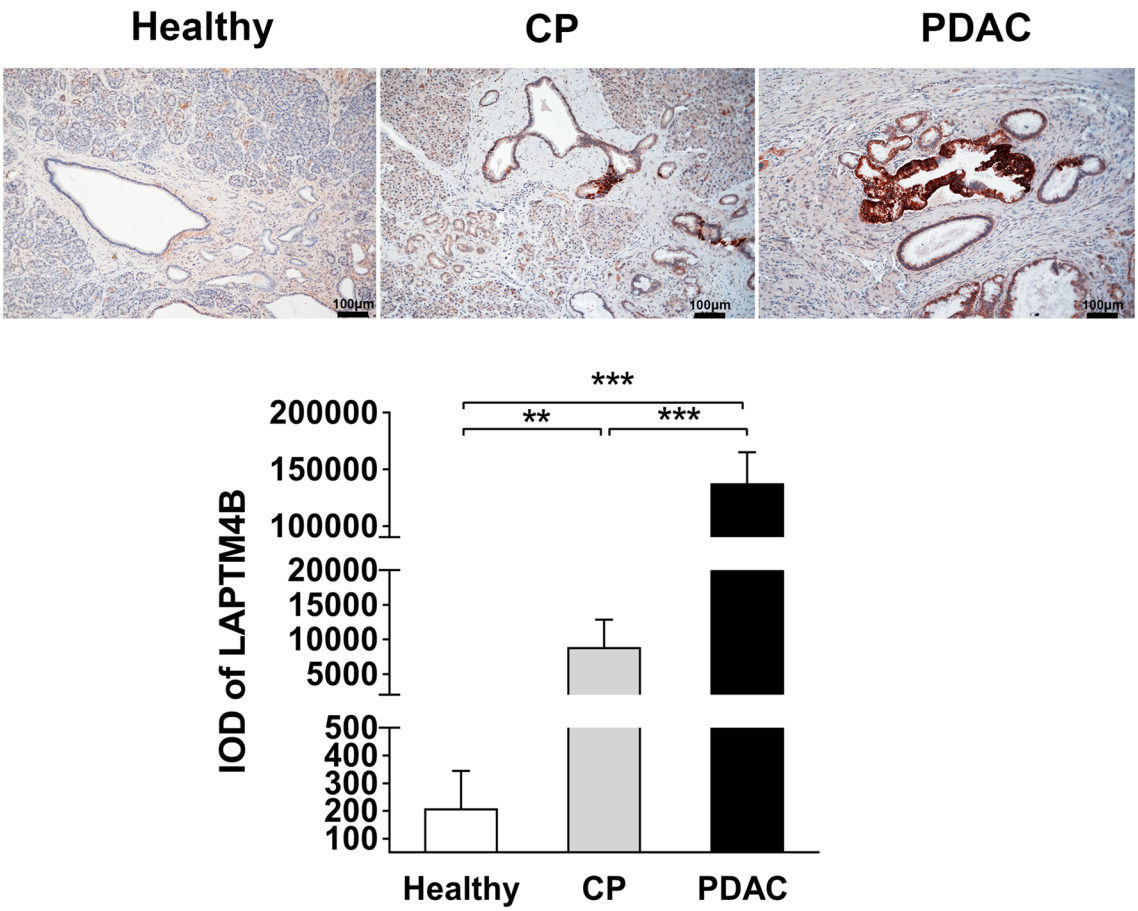

Fig. 2 Immunohistochemical staining of LAPTM4B-35 in healthy controls, chronic pancreatitis (CP), and PDAC. Asterisks (**) indicate a significant difference of $p = 0.009$. Asterisks (***) indicate a significant difference of $p < 0.001$. *IOD* integrated optical density

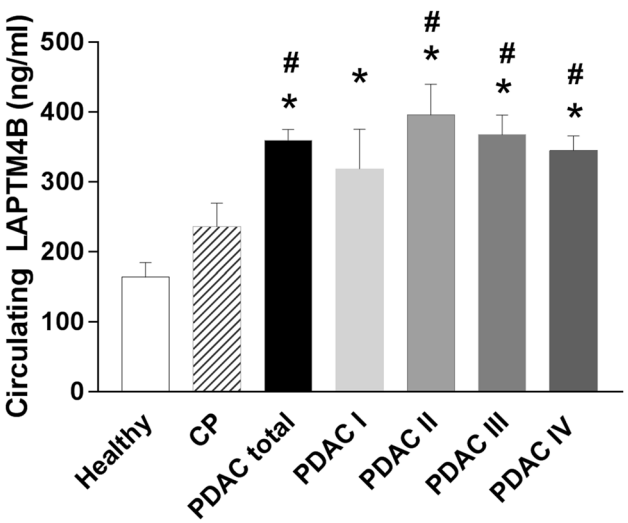

Fig. 3 Expression of circulating LAPTM4B-35 in healthy controls, chronic pancreatitis (CP), and PDAC Stages I–IV analyzed by enzyme-linked immune-sorbent assay (ELISA). Asterisk (*) indicates a significant difference of $p < 0.05$ between PDAC patients and healthy controls. Section sign (#) indicates a significant difference of $p < 0.05$ between PDAC patients and CP

Therefore, we performed targeted ELISA analyses on circulating LAPTM4B-35 of PDAC patients UICC Stages I–IV in comparison with age-matched healthy controls and patients with chronic pancreatitis. We could reveal that circulating LAPTM4B-35 was able to differentiate between PDAC UICC Stage I–IV and healthy pancreas with an excellent AUC suggesting its diagnostic value as non-invasive serum marker. Furthermore, the combination of circulating LAPTM4B-35 and cancer antigen 19-9 (CA.19-9) as the currently most common diagnostic biomarker for PDAC reached an excellent diagnostic accuracy with a sensitivity of 82%, and specificity of 92% in our patient collective. Besides its diagnostic impact, elevated LAPTM4B-35 expression correlated significantly with high tumor recurrence and poor overall survival in PDAC patients. In addition, circulating LAPTM4B-35 remained as an independent prognostic survival marker in multivariate analysis. Consequently LAPTM4B-35 has high diagnostic and prognostic impact as circulating tumor marker in PDAC. However, the

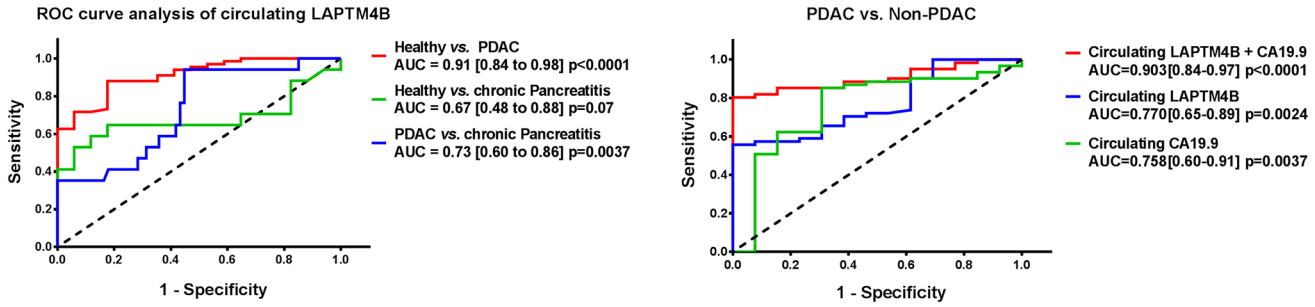

Fig. 4 Diagnostic potential of circulating LAPTM4B-35 and CA.**19-9** by receiver operating characteristic (ROC) analysis in PDAC and non-PDAC patients. $P < 0.05$ indicates significance. *AUC* area under the curve

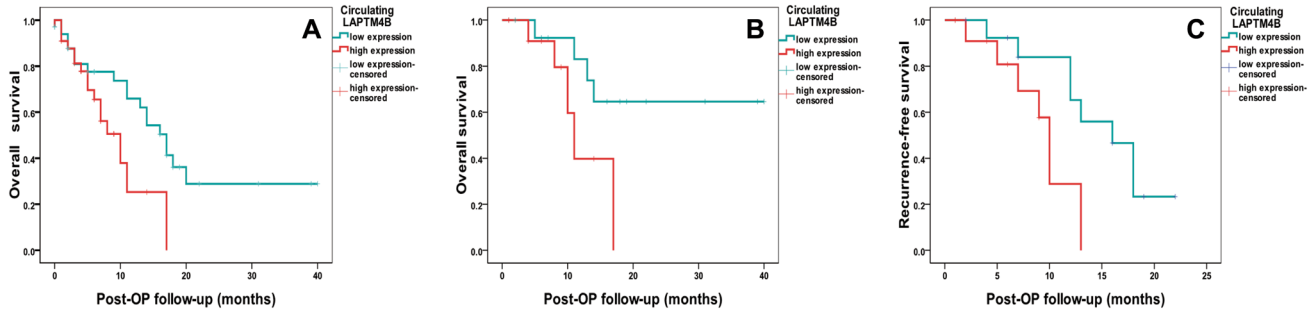

Fig. 5 Prognostic impact of circulating LAPTM4B-35 on overall survival ($p = 0.039$) in all PDAC patients UICC Stage I–IV (**a**) and on overall survival ($p = 0.064$) (**b**) and recurrence-free survival ($p = 0.037$) (**c**) in curatively treated PDAC patients UICC Stage I–III

ability of LAPTM4B-35 to discriminate PDAC in different UICC stages and its value as independent prognostic circulating biomarker for early PDAC stage remains undefined because of the limited statistical power. Taken into account that LAPTM4B-35 is upregulated in various malignancies, it seems that cancer specificity of LAPTM4B-35 is considerably high with commonly dysregulated genes and pathways, whereas its organ specificity and potential to differentiate between solid carcinoma entities are low. In vitro and in vivo studies revealed that overexpression of LAPTM4B promoted cell growth and proliferation by upregulation of cell-cycle regulators, such as cyclin D1 and E, and by activating proto-oncogenes, such as c-myc, the phosphatidylinositol 3-kinase/Akt signaling pathway, and the epidermal growth factor receptor (EGFR) signaling. Conversely, the use of antisense oligonucleotides against LAPTM4B suppressed growth and metastasis of human HCC xenograft in nude mice (Li et al. 2010; Liu et al. 2009; Tan et al. 2015; Yang et al. 2010b). Therefore, LAPTM4B seems to be a suitable membrane-associated intracellular target for new chemotherapeutic agents to be developed.

Because of the limited statistical power and the mono-centric study design with retrospective data analysis, further multi-center studies with a larger number of patients are required to reevaluate the impact of LAPTM4B-35 as diagnostic and survival biomarker in PDAC.

Conclusion

LAPTM4B-35 seems to be a promising tool in clinical management of PDAC patients. Further clinical research on LAPTM4B-35 is needed to define its potential as a circulating biomarker for earlier diagnosis of PDAC.

Table 4 Univariate Cox regression overall and recurrence-free survival analysis

Variable	Subset	Overall survival Hazard ratio (95% CI)	*p* value	Recurrence-free survival Hazard ratio (95% CI)	*p* value
Age	> 60/≤ 60	1.17 [0.52–2.65]	0.703	0.85 [0.43–1.69]	0.637
Gender	Male/female	0.50 [0.23–1.07]	0.075	1.03 [0.51–2.06]	0.937
Smoker	Yes/No	1.59 [0.63–0.73]	0.244	1.63 [0.82–3.24]	0.160
Alcohol abuse	Yes/No	0.48 [0.06–3.63]	0.481	1.14 [0.27–4.79]	0.857
Body mass index	> 25/≤25	0.50 [0.23–1.09]	0.082	1.11 [0.57–2.19]	0.753
Pre-surgical diabetes mellitus	Yes/No	0.69 [0.31–1.54]	0.368	0.69 [0.34–1.41]	0.311
Pre-surgical pancreatitis	Yes/No	2.47 [1.09–5.62]	**0.031**	1.70 [0.82–3.53]	0.154
UICC Stage	UICC3/UICC1–2	1.39 [0.65–2.98]	0.393	1.62 [0.82–3.19]	0.163
Grading	G3/G2	2.23 [1.06–4.70]	**0.035**	2.17 [1.10–4.29]	**0.026**
Nodal invasion	N1–2/N0	3.72 [1.44–9.60]	**0.007**	2.12 [0.99–4.56]	0.054
Lymphatic invasion	L1/L0	1.57 [0.75–3.26]	0.232	1.97 [1.01–3.85]	**0.046**
Perineural invasion	Pn1/Pn0	2.13 [0.94–4.82]	0.070	1.40 [0.69–2.83]	0.349
Vene invasion	V1/V0	1.19 [0.45–3.14]	0.723	1.19 [0.46–3.09]	0.736
Resection margin	R1/R0	1.18 [0.52–2.68]	0.699	1.26 [0.60–2.64]	0.543
Type of surgery	head/tail	0.94 [0.40–2.21]	0.892	1.24 [0.54–2.84]	0.613
Post-op adjuvant chemotherapy	Yes/No	0.03 [0.00–0.17]	**0.000**	0.06 [0.01–0.26]	**0.000**
Post-op radiotherapy	Yes/No	0.73 [0.27–1.95]	0.527	1.72 [0.78–3.81]	0.178
Pre-surgical CA.19-9 (U/ml)	> 30/≤30	0.59 [0.18–1.91]	0.376	0.72 [0.26–2.0]	0.524
Pre-surgical CEA (ng/ml)	> 5/≤5	0.04 [0.0–611.1]	0.507	2.24 [0.44–11.28]	0.329
LAPTM4B (ng/ml)	high/low	3.35 [1.35–8.33]	**0.005**	3.00 [1.41–6.37]	**0.000**

$p < 0.05$ indicates significance are in bold

Indication of hazard ratio and 95% confidence interval (CI)

Table 5 Multivariate Cox regression overall and recurrence-free survival analysis

Variable	Subset	Overall survival Hazard ratio (95% CI)	*p* value	Recurrence-free survival Hazard ratio (95% CI)	*p* value
Grading	G3/G2	2.13 [0.88–5.13]	0.093	2.63 [1.18–5.84]	**0.018**
Nodal invasion	N1–2/N0	2.72 [0.98–7.52]	0.054	1.03 [0.44–2.42]	0.951
Lymphatic invasion	L1/L0	1.23 [0.50–3.01]	0.654	2.01 [0.92–4.42]	0.081
Post-op adjuvant chemotherapy	Yes/No	0.03 [0.00–0.20]	**0.000**	0.10 [0.02–0.52]	**0.006**
LAPTM4B (ng/ml)	High/Low	2.73 [1.17–6.40]	**0.021**	3.29 [1.51–7.16]	**0.003**

$p < 0.05$ indicates significance are in bold

Indication of hazard ratio and 95% confidence interval (CI)

Acknowledgements We thank the patients and clinicians contributing to this study. Particularly, we thank Sabine von Rueden, Ludgera Weber-Koberg, Petra Ahrens, and Dr. med. Verena Raetzel (Department of General, Visceral and Transplantation Surgery, University Hospital Muenster) and Dr. med. Jan Rehkaemper (Gerhard Domagk Institute of Pathology, University Hospital Muenster) for their support and expert technical assistance. These results are part of the MD thesis of Zixuan Yang, who was supported by the China Scholarship Council (CSC).

Author contributions Conceptualization: SAD; methodology: ZY; SAD; validation: SAD; formal analysis: ZY, SAD; investigation: ZY, SAD, IF; resources: SAD, NS; writing-original draft preparation: SAD and ZY; writing-review and editing: SAD and ZY; visualization: ZY, SAD; supervision: SAD, NS, QJ; project administration: SAD; funding acquisition: NS, QJ.

Funding The authors declare that they have no funding to disclose.

Compliance with ethical standards

Conflict of interest The authors declare that they have has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Human and animal rights This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

Amin MB et al (2017) The Eighth Edition AJCC Cancer staging manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin 67:93–99. https://doi.org/10.3322/caac.21388

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394–424. https://doi.org/10.3322/caac.21492

Cheng XJ et al (2015) LAPTM4B-35, a cancer-related gene, is associated with poor prognosis in TNM Stages I–III gastric cancer patients. Plos One. https://doi.org/10.1371/journal.pone.0121559

Dhayat SA, Abdeen B, Kohler G, Senninger N, Haier J, Mardin WA (2015) MicroRNA-100 and microRNA-21 as markers of survival and chemotherapy response in pancreatic ductal adenocarcinoma UICC stage II. Clin Epigenet 7:132. https://doi.org/10.1186/s13148-015-0166-1

Hidalgo M (2010) Pancreatic cancer. N Engl J Med 362:1605–1617. https://doi.org/10.1056/NEJMra0901557

Kamisawa T, Wood LD, Itoi T, Takaori K (2016) Pancreatic cancer. Lancet 388:73–85. https://doi.org/10.1016/S0140-6736(16)00141-0

Kang Y, Yin MZ, Jiang W, Zhang HY, Xia BR, Xue YW, Huang YN (2012) Overexpression of LAPTM4B-35 is associated with poor prognosis in colorectal carcinoma. Am J Surg 204:677–683. https://doi.org/10.1016/j.amjsurg.2012.02.003

Kasper G et al (2005) The human LAPTM4b transcript is upregulated in various types of solid tumours and seems to play a dual functional role during tumour progression. Cancer Lett 224:93–103. https://doi.org/10.1016/j.canlet.2004.10.004

Li L et al (2010) LAPTM4B: a novel cancer-associated gene motivates multidrug resistance through efflux and activating PI3K/AKT signaling. Oncogene 29:5785–5795. https://doi.org/10.1038/onc.2010.303

Lim JE, Chien MW, Earle CC (2003) Prognostic factors following curative resection for pancreatic adenocarcinoma: a population-based, linked database analysis of 396 patients. Ann Surg 237:74–85. https://doi.org/10.1097/01.SLA.0000041266.10047.38

Liu J, Zhou R, Zhang N, Rui J, Jin C (2000) Biological function of a novel gene overexpressed in human hepatocellular carcinoma. Chin Med J (Engl) 113:881–885

Liu XR, Xiong FX, Wei XH, Yang H, Zhou RL (2009) LAPTM4B-35, a novel tetratransmembrane protein and its PPRP motif play critical roles in proliferation and metastatic potential of hepatocellular carcinoma cells. Cancer Sci 100:2335–2340. https://doi.org/10.1111/j.1349-7006.2009.01346.x

Meng FL, Yin MZ, Song HT, Yang H, Lou G, Zhou RL (2010) LAPTM4B-35 overexpression is an independent prognostic marker in endometrial carcinoma. Int J Gynecol Cancer 20:745–750. https://doi.org/10.1111/IGC.0b013e3181e02f90

Meng Y et al (2016) LAPTM4B: an oncogene in various solid tumors and its functions. Oncogene 35:6359–6365. https://doi.org/10.1038/onc.2016.189

Neuzillet C et al (2015) State of the art and future directions of pancreatic ductal adenocarcinoma therapy. Pharmacol Ther 155:80–104. https://doi.org/10.1016/j.pharmthera.2015.08.006

Qiao M, Hu GH (2015) Lysosome-associated protein transmembrane-4 beta-35 is a novel independent prognostic factor in small cell lung cancer. Tumor Biol 36:7493–7499. https://doi.org/10.1007/s13277-015-3467-2

Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM (2014) Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United. States Cancer Res 74:2913–2921. https://doi.org/10.1158/0008-5472.CAN-14-0155

Shao GZ et al (2003) Molecular cloning and characterization of LAPTM4B, a novel gene upregulated in hepatocellular carcinoma. Oncogene 22:5060–5069. https://doi.org/10.1038/sj.onc.1206832

Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. CA Cancer J Clin 68:7–30. https://doi.org/10.3322/caac.21442

Tan XJ, Sun Y, Thapa N, Liao YH, Hedman AC, Anderson RA (2015) LAPTM4B is a PtdIns(4,5)P-2 effector that regulates EGFR signaling, lysosomal sorting, and degradation. Embo J 34:475–490. https://doi.org/10.15252/embj.201489425

Vergarajauregui S, Martina JA, Puertollano R (2011) LAPTMs regulate lysosomal function and interact with mucolipin 1: new clues for understanding mucolipidosis type IV. J Cell Sci 124:459–468. https://doi.org/10.1242/jcs.076240

Wang B, Wang S, Liang G, Xu J, Zhou R, Zhang Q (2017) Association of lysosomal protein transmembrane 4 beta gene polymorphism with pancreatic carcinoma susceptibility in the Chinese population. Tumour Biol 39:1010428317705518. https://doi.org/10.1177/1010428317705518

Xia LZ et al (2015) The relationship between LAPTM4B polymorphisms and cancer risk in Chinese Han population: a meta-analysis. Springerplus. https://doi.org/10.1186/s40064-015-0941-7

Xiao M, Jia SS, Wang HB, Wang JS, Huang YX, Li ZG (2013) Overexpression of LAPTM4B: an independent prognostic marker in breast cancer. J Cancer Res Clin 139:661–667. https://doi.org/10.1007/s00432-012-1368-y

Yang H et al (2010a) LAPTM4B-35 is a novel prognostic factor of hepatocellular carcinoma. J Surg Oncol 101:363–369. https://doi.org/10.1002/jso.21489

Yang H, Xiong FX, Wei XH, Yang Y, McNutt MA, Zhou RL (2010b) Overexpression of LAPTM4B-35 promotes growth and metastasis of hepatocellular carcinoma in vitro and in vivo. Cancer Lett 294:236–244. https://doi.org/10.1016/j.canlet.2010.02.006

Yin MZ et al (2011) Over-expression of LAPTM4B is associated with poor prognosis and chemotherapy resistance in stages III and IV epithelial ovarian cancer. J Surg Oncol 104:29–36. https://doi.org/10.1002/jso.21912

Zhang G, Liang Y, Huang Y, Chen Y, Zhou R (2012) Elevated lysosome-associated protein transmembrane-4beta-35 is an independent prognostic marker in pancreatic carcinoma. J Int Med Res 40:1275–1283. https://doi.org/10.1177/147323001204000406

Zhang HT et al (2014) Overexpression of LAPTM4B-35: a novel marker of poor prognosis of prostate cancer. Plos One. https://doi.org/10.1371/journal.pone.0091069

Zhang X, Shi S, Zhang B, Ni Q, Yu X, Xu J (2018) Circulating biomarkers for early diagnosis of pancreatic cancer: facts and hopes. Am J Cancer Res 8:332–353

Zhou L et al (2008) Expression of LAPTM4B-35: A novel marker of progression, invasiveness and poor prognosis of extrahepatic cholanglocarcinoma. Cancer Lett 264:209–217. https://doi.org/10.1016/j.canlet.2008.01.025

Zhou L, He XD, Yu JC, Zhou RL, Shan Y, Rui JA (2011) Overexpression of LAPTM4B-35 attenuates epirubucin-induced apoptosis of gallbladder carcinoma GBC-SD cells. Surgery 150:25–31. https://doi.org/10.1016/j.surg.2010.12.010

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.