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Original Article

Decreased expression of lncRNA TMED11P is correlated with progression and prognosis in pancreatic ductal adenocarcinoma

Zhenfei Zhu, Jian Zhou, Zixiang Zhang, Dechun Li

Department of General Surgery, The First Affiliated Hospital of Soochow University, Suzhou 215006, Jiangsu, PR China

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Abstract: Background: Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related mortalities in the USA and the sixth leading cause of mortality in China. Recent studies have shown that lncRNAs play important roles in carcinogenesis. The aim of this study was to explore the role of lncRNA TMED11P in PDAC. Methods: Quantitative real-time PCR was performed to investigate the expression of TMED11P in tumor tissues and corresponding normal tissues from 78 patients with PDAC. Results: The lower expression of TMED11P was significantly correlated with lymph node metastasis and more advanced TNM stage. Multivariate analyses revealed that TMED11P expression served as an independent predictor for overall survival. Conclusion: Our results suggest that TMED11P may serve as a candidate prognostic biomarker through growth regulation in human PDAC.

Keywords: Pancreatic ductal adenocarcinoma, lncRNA TMED11P, prognosis

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most leading causes of cancer related death. In USA, PDAC is the fourth malignancy of cancer deaths. The incidence of PDAC is growing recently [1]. It is estimated that there are about 53,070 new cases will be diagnosed and there are 41,780 cases will be died of PDAC in USA during 2016 [1]. While the incidence and mortality of PDAC is growing in China [2]. Due to be diagnosed in advanced stages and ineligible for potentially curative resection, only approximately 15% of the tumors can be surgically removed. The 5-year survival rate of PDAC remains 1% in recent 50 years [3]. Therefore, a thorough understanding of the molecular mechanisms underlying the progression of pancreatic cancer is urgently needed, which may support new opportunities for diagnosing at early stage and new therapeutic targets for improve this disappointed disease [3].

Long non-coding RNAs (lncRNAs) are RNA molecules that cannot be translated to protein and with more than 200 nucleotides [4, 5]. Recent evidence suggests that lncRNAs are not the genomic noise, but are involved in a variety of regulatory activities, including chromatin remodeling, transcriptional activation, decoy (transcriptional repressor), and RNA degradation [4-6]. Dysregulated expression of lncRNAs have been found in many cancers, such as breast cancer, colorectal cancer, liver cancer, PDAC and et al, which may serve as oncogenes or tumor suppressor genes, and promote carcinogenesis and cancer development [7-9]. For example, Tian et al showed that lncRNA MEG3 was decreased in osteosarcoma tissues and associated with clinical stage and distant metastasis. Furthermore, patients with lower expression of lncRNA MEG3 had a signicantly poor prognosis [10]. Fan et al found that lncRNA FGF14-AS2 was down-regulated in breast cancer, low expression of FGF14-AS2 negatively correlated with tumor size, lymph node metastasis and clinical stage, and decreased expression of FGF14-AS2 favored poor survival in breast cancer [11]. And the knowledge about PDAC is relatively fewer.

In the previous microarray study (GEO accession number GSE61166) [12], a new lncRNA transmembrane p24 trafficking protein 11, pseudogene (TMED11P) was found significantly downregulated in PDAC tissues comparing with noncancerous pancreatic tissues. Its gene is located at Chr4: 1115197-1123164, negative strand. The transcript is 545nt in length. Whether this lncRNA is related to carcinoma is unknown yet. Therefore, in this study, we investigated the expression level of TMED11P in PDAC tissues and explored the association between its expression and clinicopathologic characteristics of patients with PDAC.

Methods and materials

Patients and tissue samples

Seventy eight PDAC tissues and paired adjacent noncancerous pancreatic tissue specimens were obtained from patients who underwent surgery at the Department of General Surgery, the First Affiliated Hospital of Soochow University between April 2007 and December 2013. All tissue samples were snap frozen in liquid nitrogen immediately after surgical resection and were transferred to the freezer at -80°C before use. The diagnosis of PDAC was confirmed pathologically by the Department of Pathology of the First Affiliated Hospital of Soochow University. None of the patients received preoperative radiotherapy, chemotherapy, or any other cancer treatment. This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University. Written consent was obtained from the patients before surgery.

Cell lines and culture conditions

The human pancreatic cancer cell lines Bxpc-3, HPAC-1, SW1990, CFPAC-1, Aspc-1 and the mortal human pancreatic epithelial cell line hTERT-HPNE were obtained from the American Type Culture Collection (ATCC, Manassas, VA). These cells were cultured according to ATCC's instruction media, and supplemented with 10% fetal bovine serum (Invitrogen, Shanghai, China), 100 U/ml penicillin, and 100 mg/ml streptomycin (Invitrogen, Shanghai, China) in an incubator at 37°C with 5% CO_2.

RNA extraction and reverse transcription

The total RNA of the frozen tissue samples was extracted using RNAiso Plus (TaKaRa, Dalian, China) according to the manufacturer's instructions. The quantity of RNA was assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The ratio of the absorbances at 260 and 280 nm (A260/A280≥1.8) was used to assess the RNA purity. Qualified total RNA was then reverse transcribed using PrimeScript RT Master Mix (TaKaRa, Dalian, China) following the manufacturer's instructions.

qRT-PCR

The quantitative real-time polymerase chain reaction (qRT-PCR) was performed using SYBR Premix Ex Taq II (TaKaRa, Dalian, China) on th ABI 7500 Real-Time PCR System (Applied Biosystems). Briefly, the 20 μl reaction mixtures were incubated at 95°C for 30 s for the initial denaturation stage, followed by 40 cycles at 95°C for 5 s and 60°C for 34 s. Small nucleolar RNA U6 was used to normalize the target gene expression. The ΔCt method was used to calculate the relative expression of lncRNA TMED11P in BC tissues in comparison with paired normal tissues, respectively. Each sample was examined in triplicate. The primers used in this study were synthesized by Invitrogen with the sequences as follows: 5'-TCCTGGTTTGGGAATGTTTGTG-3' (forward) and 5'-ATAGAATGTCCCTTGCGGGC-3' (reverse) for TMED11P; 5'-GCGCGTCGTGAAGCGTTC-3' (Forward) and 5'-GTGCAGGGTCCGAGGT-3' (Reverse) for U6. All qRT-PCRs were performed in triplicate.

Statistical analysis

All statistical analyses were performed with SPSS 22.0 (IBM, Chicago, IL, USA) and GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA). The student t test was applied to test the difference of quantitative data. The chi-square test was used to exam the relationship between TMED11P expression level and clinicopathologic characteristics. The receiver operating characteristic curves (ROCs) were established to evaluate the value of TMED11P for PDAC diagnosis, and prediction of lymph node metastasis and distant metastasis, respectively. The survival curves were estimated using the Kaplan-Meier method. The log-rank test was used to estimate the significance of the differences between the survival curves. A Cox proportional hazards analysis was performed to calculate the hazard ratio (HR) and the 95%

Table 1. The relationship between clinicopathological characteristics of PDAC and TMED11P expression

Characteristics	TMED11P expression		χ^2	*p*
	High expression (21)	Low expression (57)		
Age				
≤58 years (30)	5	25	2.607	0.106
>58 years (48)	16	32		
Gender				
Male (52)	16	36	1.173	0.279
Female (26)	5	21		
Location				
Head (57)	16	41	0.142	0.707
Body and tail (21)	5	16		
Histological classificaiton				
I (14)	3	11	1.305	0.521
II (45)	11	34		
III (19)	7	12		
Tumor size				
I (7)	3	4	2.801	0.423
II (19)	7	12		
III (16)	3	13		
IV (36)	8	28		
Lymph nodes status				
Negative (41)	15	26	4.101	0.043
Positive (37)	6	31		
Metastasis				
No (62)	19	43	2.128	0.145
Yes (16)	2	14		
Stage				
I (26)	10	16	3.560	0.313
II (11)	3	8		
III (25)	6	19		
IV (16)	2	14		

confidence interval (CI) to evaluate the association between TMED11P expression and overall survival time (OS). A multivariate Cox regression was performed to adjust for other covariates. A $P<0.05$ was considered statistically significant.

Results

TMED11P was downregulated in pancreatic cancer

All of the 78 PDAC patients' medical records were reviewed. There were 26 females and 52 males, and aged from 32 to 78. And other clinicopathologic characteristics of the patients are summarized in **Table 1**.

The expression level of TMED11P was determined by qRT-PCR in 78 pairs of PDAC and adjacent noncancerous pancreatic tissues. The lncRNA TMED11P was found to be decreased in 57 PDAC tissues compared with noncancerous tissues, while only that was increased in 21 PDAC tissues (**Figure 1A**). Statistical analysis shown the lncRNA TMED11P was significantly downregulated in PDAC tissue (**Figure 1B**). Also, the expression level of TMED11P in PDAC cell lines and normal pancreatic ductal epithelial cell line was evaluated. As shown in **Figure 1C**, TMED11P was significantly downregulated in PDAC cell lines compared to HPDE.

Clincal relevance of downregulated TMED11P in pancreatic cancer

We next analyzed the relationship between the expression level of TMED11P and clinicopathologic parameters of patients with PDAC. As previous shown, the expression of TMED11P was decreased in 57 PDAC tissue specimens and increased in 21 PDAC tissue specimens. Accordingly, the 78 PDAC patients were divided into two groups: positive expression group (21 PDAC tissue specimens with increased TMED11P expression) and negative expression group (57 PDAC tissue specimens with decreased TMED11P expression). As **Table 1** indicates, downregulated TMED11P was significantly associated with lymph node metastasis (P=0.043). However, there were no significantly associations between TMED11P and other clinicopathologic characteristics, such as gender, age, tumor location, histological classification, primary tumor stage, distant metastasis, and TNM stage.

Kaplan-Meiers' survival analysis and log-rank tests were performed to further evaluate the correlation between TMED11P expression and and prognosis of PDAC. As shown in **Figure 2A**, TMED11P negative expression was associated

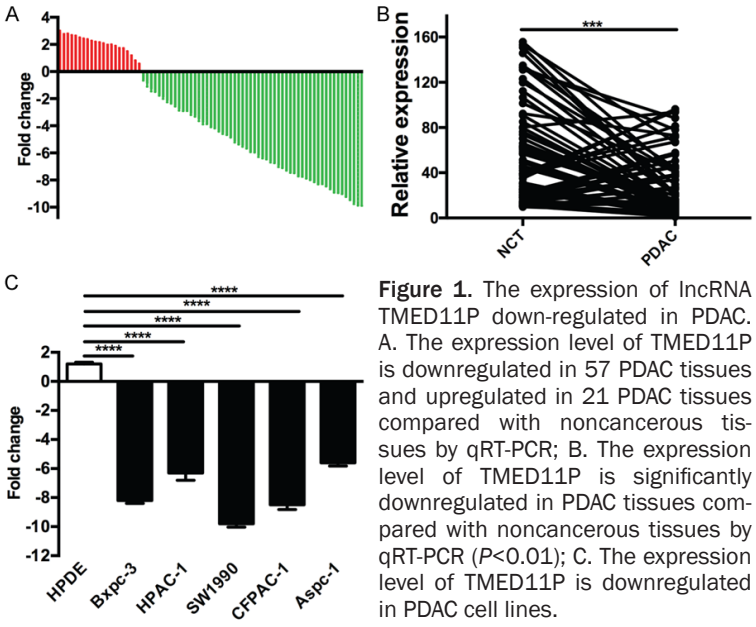

Figure 1. The expression of lncRNA TMED11P down-regulated in PDAC. A. The expression level of TMED11P is downregulated in 57 PDAC tissues and upregulated in 21 PDAC tissues compared with noncancerous tissues by qRT-PCR; B. The expression level of TMED11P is significantly downregulated in PDAC tissues compared with noncancerous tissues by qRT-PCR (P<0.01); C. The expression level of TMED11P is downregulated in PDAC cell lines.

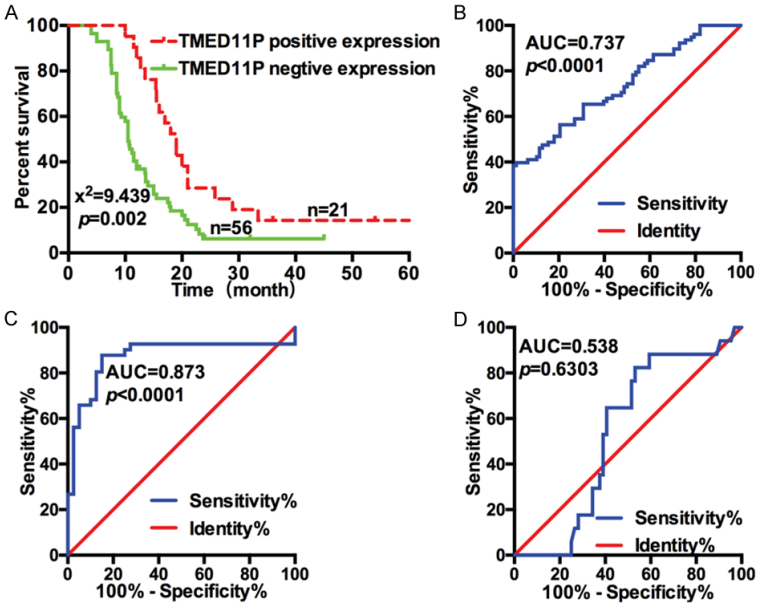

Figure 2. Clinical relevance of down-regulated TMED11P in PDAC. A. Kaplan-Meier's overall survival curve of PDAC according to the TMED11P relative expression, down-regulated TMED11P expression was associated with poor prognosis (log-rank, P=0.002). B-D: The receiver operating characteristic curves: B. Diagnostic value of TMED11P for differentiating between PDAC and noncancerous diseases; C. Predictive value of TMED11P for lymph node metastasis in PDAC patients; D. Predictive value of TMED11P for distant metastasis in PDAC patients.

with shorter overall survival (P=0.002). Univariate analysis identified five prognostic factors: tumor stage, lymph node metastasis, distant metastasis, TNM stage and TMED11P expression (**Table 2**). The other clinicopathological characteristics, such as age, gender, tumor location, and histological classification, were not statistically significant prognosis factors (**Table 2**). When it comes to multivariate analysis, TMED11P expression was a significant independent predictor of poor survival of PDAC patients (P=0.022), as well as tumor stage (P=0.039), lymph node metastasis (P=0.018) and distant metastasis (P=0.001) (**Table 2**).

Furthermore, we evaluated the diagnostic value and the prediction value for tumor stage, lymph node metastasis and distant metastasis of TMED11P expression. The area under the curve (AUC) analysis was used by TMED11P relative expression. As shown in **Figure 2B**, the AUC was up to 0.737 (95% CI=0.660-0.814, P<0.001), suggesting that TMED11P might serve as a diagnostic biomarker of PDAC. When using the cutoff value of 16.34, the sensitivity and specificity were 79.5 and 56.4%, respectively, and the Youden index was 0.359. In addition, we also found that TMED11P might be a promising biomarker for lymph node metastasis (**Figure 2C**). The AUC was 0.873 (95% CI=0.785-0.961, P<0.001). The Youden index was 0.728, and sensitivity and specificity were 87.8 and 85.0%, respectively, when taking 14.15 as the cutoff value. However, the relative expression of TMED11P was not a valuable predictive marker for distant metastasis (**Figure 2D**).

Table 2. Univariate and multivariate Cox regression analyses TMED11P for OS of PDAC patients (n=78)

Risk factor	Univariate		Multivariate	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Gender	1.240 (0.745, 2.062)	0.408	1.347 (0.766, 2.369)	0.301
Age	0.738 (0.456, 1.196)	0.217	0.915 (0.505, 1.658)	0.770
Location	1.271 (0.748, 2.158)	0.376	1.179 (0.612, 2.274)	0.622
T size	1.678 (1.309, 2.151)	0.000	2.315 (1.042, 5.144)	0.039
LN status	2.356 (1.456, 3.811)	0.000	2.819 (1.190, 6.678)	0.018
Metastasis	4.223 (2.298, 7.760)	0.000	19.254 (3.431, 108.041)	0.001
Stage	1,827 (1.437, 2.316)	0.000	0.385 (0.139, 1.061)	0.065
Histological classification	1.031 (0.736, 1.445)	0.858	1.072 (0.750, 1.533)	0.702
TMED11P	0.438 (0.253, 0.759)	0.003	0.501 (0.277, 0.905)	0.022

Discussion

PDAC is most malignant cancer with the lowest 5-year survival rate and the poorest prognosis [3]. Traditional clinical and pathologic parameters are valid, but not sufficient to make proper treatment decision and predict prognosis of PDAC [3, 13]. Therefore, novel parameters that is sufficient for choosing the optimal treatment and risk assessment of PDAC are of great importance in clinical practice [13-15]. Great effects have been made to explore the mechanism, new therapeutics and efficacious biomarker for treatment decision and prognosis prediction [14, 16].

Currently, lncRNAs are emerging as a diversal aspect of cancer biology [7, 8]. High throughput methods, such as microarray and RNA sequencing are used to discover lncRNAs that have essential roles in tumorigenesis and tumor progression [17-19]. Thousands of lncRNAs have been found dysregulated in PDAC [12, 20]. However, only few of these dysregulated lncRNAs' clinical relevance and function have been identified. For example, Qu et al showed downregulated lncRNA-ATB correlates with clinical progression and unfavorable prognosis in pancreatic cancer [21]. Li et al found HOTTIP was a crucial oncogene by promoting cell proliferation, invasion, and chemoresistance in PDAC. HOTTIP levels were upregulated in PDAC tissues and PDAC cell lines compared with pancreatic tissues. And HOTTIP silencing inhibites cell proliferation and invasion, and reinforces chemosensitivity of PDACs to gemcitabine. Furthermore, they found that HOTTIP promoted cell-cycle progression and epithelial-mesenchymal transition (EMT) by modulating HOXA3 *in vitro* [22]. Also, Lei et al identified PVT1 as a regulator of gemcitabine sensitivity in PDAC cells by using a genome-wide and *piggyBac* transposon-based genetic screening platform [23]. Since the functions of a large amount of PDAC associated lncRNAs are still unfamiliar, we attempted to find and explore their clinical relevance and functions in PDAC development.

In the present study, we confirmed that lncRNA TMED11P was decreased in PDAC tissues compared with adjacent normal pancreatic tissues. We also revealed that TMED11P was lowly expressed in PDAC cell lines compared with normal pancreatic epithelial cell lines. Additionally, the down-regulation of TMED11P expression correlated with lymph node metastasis. Furthermore, univariate analysis and multivariate analysis indicated that TMED11P could be an independent prognostic factor and decreased of TMED11P was correlated with unfavorable survival in PDAC. Additionally, the expression of TMED11P could be used as diagnostic biomarker and predict lymph node metastasis. More importantly, overexpression of TMED11P resulted in decreased proliferation of PDAC cells, which indicated that TMED11P might function as a tumor suppressor gene involved in PDAC tumorigenesis, and could be used as a therapeutic target for PDAC treatment. Furthermore, we also found overexpression TMED11P could suppress cell cycle progression and stall at the G1 phase. To the best of our knowledge, it was firstly reported that lncRNA TMED11P was involved in the progression and prognosis of cancer. However,

The mechanism of TMED11P transcripts in PDAC tumorigenesis and progression remains to be uncovered in the future.

In summary, lncRNA TMED11P is one of the downregulated lncRNAs in PDAC. Its abnormal expression can be used as a diagnostic biomarker, predict the progression of PDAC and it is an independent prognostic marker. Overexpression TMED11P inhibits PDAC proliferation. All of these show TMED11P is a potential new marker and a target for gene therapy in PDAC treatment.

Disclosure of conflict of interest

None.

Address correspondence to: Dechun Li, Department of General Surgery, The Second Affiliated Hospital of Soochow University, 188# Shizi Road, Suzhou 215006, Jiangsu Province, PR China. Tel: 86-512-6522-3637; Fax: 86-512-6778-0320; E-mail: dechunli@tom.com

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