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Article

Clinical Significance of CA-199 and LINC01197 in Pancreatic Cancer

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Abstract: This study aimed to explore the expression and clinical significance of LINC01197 in the serum of patients with pancreatic cancer (PC). **Methods:** A total of 50 PC patients (patient group) treated in our hospital from March 2012 to April 2014 were collected, and another 50 healthy people (normal group) were collected for physical examination. The expression of LINC01197 in the serum of the two groups was detected by qRT-PCR method, and the expression of CA-199 in serum was detected by Roche automatic biochemistry. The expression and diagnostic values of CA-199 and LINC01197 in PC were analyzed, and the relationship between LINC01197 and the prognosis of PC patients was observed. **Results:** The expression of CA-199 in the patient group was significantly higher than in the normal group ($p < 0.001$). The area under the curve was 0.791 and 0.944, respectively. The incidence rate of Phases III + IV, lymphatic invasion, and distant metastasis in patients with low expression of LINC01197 is significantly higher than that in patients with high expression and has higher diagnostic value. With the progress of clinical staging, the expression of TNM gradually decreased and there were differences between groups ($p < 0.001$). Sperman test analysis found that the decreased TNM staging of LINC01197 gradually increased ($r = -0.816$, $p < 0.001$), and the area under the curve of LINC01197 distinguishing phase I and phase II + phase III + phase IV was 0.930. The 1-year survival rate and 5-year survival rate of patients in low expression group are lower than those in the high expression group (P1 year = 0.037, P5 year = 0.014). Distant metastasis is an independent prognostic factor for PC patients to survive for 1 to 5 years. Differentiation, TNM staging, and LINC01197 are independent prognostic factors for PC patients to survive for 5 years. **Conclusion:** The low expression of LINC01197 in PC patients indicates poor prognosis of patients and is expected to be a potential diagnostic and prognostic indicator of PC.

Keywords: LINC01197; pancreatic cancer; prognosis; diagnosis

1 Introduction

With the continuous development of modern society, people's living standard and diet structure have changed, and the morbidity of digestive tract diseases has increased year by year [1]. Pancreatic cancer (PC) is a malignant tumor with the lowest 5-year survival rate clinically. Studies of Allemani et al. [2] found that the 5-year net survival rate of PC decreased from 14.4% in 2000 to 9.9% in 2014 through monitoring the global cancer survival trend from 2000 to 2014, and the 5-year net survival rate of PC was the lowest among all cancers. How to improve the mortality of PC patients is one of the urgent problems for clinicians to solve at present [3]. Surgical treatment is the main treatment scheme for PC clinically, but very few PC patients can undergo surgical treatment clinically. Basically, more than 80% of PC patients admitted to hospital are developed to advanced stage of disease [4,5]. Therefore, early diagnosis of PC patients is very critical.

At present, the gold standard of PC is pathological biopsy clinically, magnetic resonance imaging/ magnetic resonance cholangiopancreatography (MRI/MRCP) is the most accurate except pathological

biopsy [6]. However, it is more expensive than pathological biopsy imaging analysis. Serological testing is a common and inexpensive testing method clinically. CA-199 is an important serological marker for PC diagnosis, but recent studies have found that the specificity of CA-199 is relatively low, and the expression of CA-199 in acute and chronic pancreatitis, bile duct obstruction, and hepatitis will change, and the expression is relatively low in poorly differentiated tumors [7–9]. Therefore, the diagnosis of PC is affected to some extent.

LncRNA (Long Non Coding RNAs) is a kind of long-chain non-coding RNA with a length of more than 200nts [10]. Most beginners think lncRNA has no ability to encode proteins. However, with the discovery of various functions of lncRNA in recent years, lncRNA has epigenetic changes, transcriptional regulation, and post-transcriptional modification, and it participates in the occurrence and development of various diseases through targeted regulation of microRNA and target proteins [11,12]. LINC01197 is a newly discovered lncRNA. Before this, LINC01197 was mentioned in the expression profile [13]. The expression of LINC01197 in PC is still unclear. In the research of Ling et al. [14], LINC01197 was found to be low expressed in PC, but whether it can be used as a potential prognosis and diagnostic indicator of PC is still unclear. Therefore, this study explores the clinical significance of LINC01197 in PC, providing potential diagnostic and prognostic indicators for clinical use.

2 Methods and Data

2.1 Patient Collection

A total of 50 PC patients (patient group) who were treated in our hospital from March 2012 to April 2014 were collected, and 50 normal people (normal group) who underwent physical examination in our hospital during the same period were also collected. The patient group consisted of 30 males and 20 females with an average age of 54.2 ± 5.3 years, the normal group consisted of 25 males and 25 females with an average age of 55.2 ± 4.1 years. There was no difference in gender and age between the two groups ($p > 0.05$). This study was approved by the Medical Ethics Committee of our hospital. Laboratory examination and imaging examination were normal in the normal group. The inclusion criteria of the patient group were as follows: The lesions of the patients could be detected and diagnosed as ductal pancreatic cancer by pathological biopsy and imaging. The patients met the 8th edition of TNM staging standard [15]. The clinical data of the patients were complete. The research purpose was to inform the patients and their family and sign an informed consent. The exclusion criteria for patients were as follows: the patients received corresponding anti-tumor treatment (radiotherapy, chemotherapy, and surgical treatment) before this study. The patients were pregnant women who expected to have a survival period of less than 1 month, but do not cooperate with follow-up.

2.2 Main Reagents and Instruments

TRIzol reagent (Invitrogen, Carlsbad, California, USA, 15596018), TransScript Green Two-Step qRT-PCR SuperMix (Beijing, China, TransGen Biotech, AQ201-01). CA-199 kit and automatic biochemical analyzer (Roche, Switzerland) and PCR instrument (Applied Biosystems, Foster City, California, 7500, 4366596, USA).

2.3 Collection and Detection of Samples

Altogether 5 mL of fasting peripheral venous blood was collected from the two groups, left standing for 30 min, centrifuged at 3000 rpm for 10 min, and the supernatant was collected and subpackaged for CA-199 and LINC01197 expression detection respectively. The detection scheme of LINC01197 was as follows: Total RNA was extracted from the collected serum by TRIzol reagent, and the purity, concentration, and integrity of total RNA were detected by ultraviolet spectrophotometer and agarose gel electrophoresis. TransScript® miRNA RT Enzyme Mix in TransScript Green Two-Step qRT-PCR SupeMix kit and 2 × TS miRNA Reaction Mix were used for reverse transcription. The experimental steps were tested according to the original kit. The amplification system was as follows: cDNA 1 μL, upstream and downstream primers 0.4 μL each, 2X

TransScript® Tip Green qPCR SuperMix 10 μL, and Passive Reference Dye (50X) were added, and Nuclease-free water was supplemented to 20 μL at last. The Amplification conditions were as follows: Pre-denaturation at 94°C for 30 s, denaturation at 94°C for 5 s, annealing extension at 60°C for 30 s, with a total of 40 cycles. Each sample was provided with 3 repeated holes. The experiment was conducted for 3 times. GAPDH was used as internal reference, and 2-△ct was used to analyze the data [16]. Experiments were carried out by ABI 7500 pcr instrument. CA-199 was analyzed by Roche automatic biochemical analyzer E170 module. The range of normal reference value is less than or equal to 37 U/mL.

2.4 Follow-Up of Patients

Patients were followed up for a period of 5 years. The follow-up methods were counted by telephones and clinic reexamination. In the first year of follow-up, follow-up was carried out at the 3rd, 6th, 9th, and 12th months respectively, and in the following 4 years, follow-up was carried out every 4 months.

2.5 Statistical Methods

In this study, SPSS20.0 software package was used to carry out statistical analysis on the collected data, GraphPad 7 software package was used to draw the required pictures, and K-S test was used to analyze the distribution of measurement data. The normal distribution data was expressed by mean ± standard deviation (Meas ± SD), the data that did not conform to the normal distribution were expressed by median, the quartile interval was used to describe P50 (P25–P75), and the nonparametric test was applied, which expressed by Z. Independent-samples *T* test was used for inter-group comparison, expressed by *T*, chi-square test was used for the counting data, expressed by 2, one-way analysis of variance was used for multi-group comparison, expressed by F, LSD-*t* test was used for pairwise comparison afterwards, ROC was used to plot the diagnostic value of LINC01197 and CA-199 in PC, sperman was used to analyze the relationship between LINC01197 and TNM staging of patients. The 5-year survival situation of patients was drawn by K-M survival curve, analyzed by Log-rank test, and independent risk factors affecting the prognosis of patients were analyzed by multivariate Cox regression. When $p < 0.05$, there was statistical difference.

3 Results

3.1 Expression and Diagnostic Value of LINC01197 and CA-199 in PC

The expressions of LINC01197 and CA-199 in PC between the patient group and the normal group were detected. It was found that the expression of CA-199 in the patient group was significantly higher than that in the normal group ($p < 0.001$), while the expression of LINC01197 in the patient group was significantly lower than that in the normal group ($p < 0.001$). The ROC curve analysis showed that both LINC01197 and CA-199 had higher value in diagnosis PC, and the area under the curve was 0.791 and 0.944 respectively. More details were shown in Tabs. 1 and 2, and Fig. 1.

Table 1: Expressions of LINC01197 and CA-199 in PC patients

Index	Normal group (n = 50)	Patient group (n = 50)	*T/Z* value	*p* value
LINC01197 expression	1.084 ± 0.083	0.805 ± 0.155	11.308	＜0.001
CA-199 (U/ml)	13.62 (8.49–18.90)	175.08 (20.11–405.98)	–5.016	＜0.001

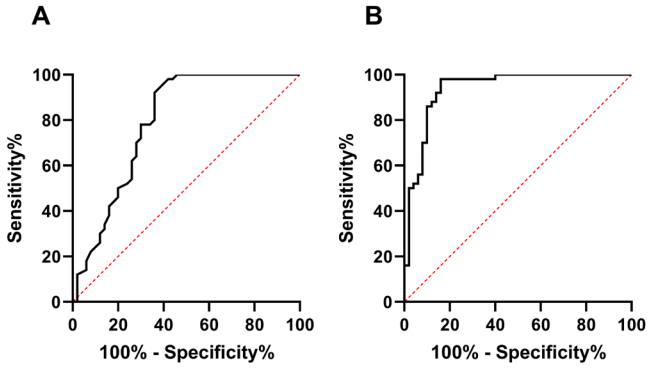

Figure 1: Area under the curve of LINC01197 and CA-199

The area under the curve of CA-199 was 0.833. B. The area under the curve of LINC01197 was 0.938.

Table 2: ROC parameters

Factor	AUC	Std	95% CI	*p* value	Specificity	Sensitivity	Youden index	Cut-off
LINC01197	0.944	0.027	0.886–0.997	<0.001	86.00%	100.00%	86.00%	>0.9335
CA-199	0.791	0.047	0.699–0.883	<0.001	64.00%	92.00%	–	–

3.2 Relationship between LINC01197 and Pathological Data of PC Patients

According to the median value of LINC01197 expression, it was divided into the high and low expression group. Analysis of the pathological data of patients in the two groups showed that there was no correlation between gender, age, lesion site, differentiation, vascular invasion, and high and low expression of LINC01197. However, patients with low expression of LINC01197 had significantly higher probability of Phases III + IV, lymphatic invasion, and distant metastasis than patients with high expression. Therefore, we analyzed the expression of LINC01197 in TNM staging, lymphatic invasion, and distant metastasis. In Phases III + IV, the expression of LINC01197 in patients with lymphatic invasion and distant metastasis was significantly lower than that in their corresponding groups, and ROC curve was drawn to find that LINC01197 has higher diagnostic value in TNM staging, lymphatic invasion, and distant metastasis. More details were shown in Tabs. 3 and 4, and Fig. 2.

Table 3: Relationship between expression of LINC01197 and pathological data

Parameter		LINC01197 expression		x2 value	*p* value
		Low expression	High expression		
Gender					
	Male (n = 30)	17 (68.00)	13 (52.00)	1.333	0.248
	Female (n = 20)	8 (32.00)	12 (48.00)		
Age					
	≥55 years old (n = 22)	13 (52.00)	9 (36.00)	1.299	0.255
	<55 years old (n = 28)	12 (48.00)	16 (64.00)		
Lesion site					
	Head of pancreas (n = 25)	14 (56.00)	11 (44.00)	0.720	0.396
	Rests (n = 25)	11 (44.00)	14 (56.00)		
Differentiation					
	Low differentiation (n = 25)	10 (40.00)	15 (60.00)	2.000	0.157
	Moderate + high differentiation (n = 25)	15 (60.00)	10 (40.00)		

TNM staging					
	Phase I + II (n = 23)	7 (28.00)	16 (64.00)	6.522	0.011
	Phase III + IV (n = 27)	18 (72.00)	9 (36.00)		
Lymphatic invasion					
	Yes (n = 19)	14 (56.00)	5 (20.00)	6.876	0.009
	No (n = 31)	11 (44.00)	20 (80.00)		
Vascular invasion					
	Yes (n = 20)	12 (48.00)	8 (32.00)	1.333	0.248
	No (n = 30)	13 (52.00)	17 (68.00)		
Distant metastasis					
	Yes (n = 18)	13 (52.00)	5 (20.00)	5.556	0.018
	No (n = 32)	12 (48.00)	20 (80.00)		

Table 4: ROC parameters

Factor	AUC	Std	95% CI	*p* value	Specificity	Sensitivity	Youden index	Cut-off
TNM staging	0.747	0.070	0.610–0.885	0.003	100.00%	51.85%	51.85%	<0.717
Lymphatic invasion	0.784	0.064	0.659–0.909	<0.001	84.21%	67.74%	51.95%	>0.797
Distant metastasis	0.825	0.057	0.713–0.936	<0.001	94.44%	59.38%	53.82%	>0.819

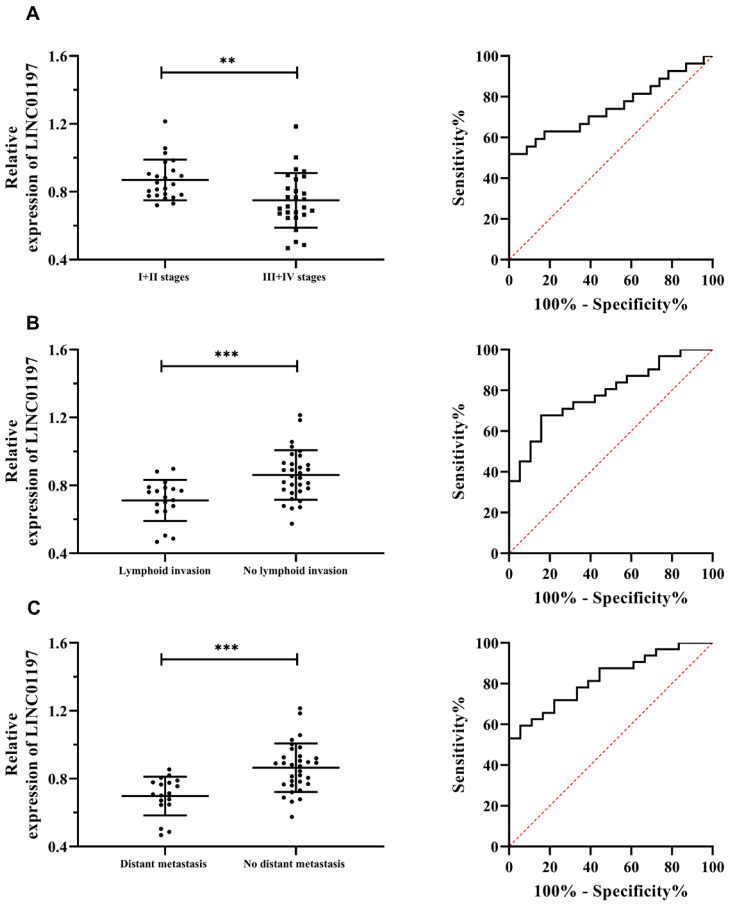

Figure 2: Expression of LINC01197 in patients with TNM staging, lymphatic invasion, and distant metastasis

A. The expression of LINC01197 in patients with Phases I + II is higher than that in patients with Phases III + IV, and the area under the curve is 0.747. B. The expression of LINC01197 in patients with lymphatic invasion is lower than that in patients without lymphatic invasion, and the area under the curve is 0.784. C. The expression of LINC01197 in patients with distant metastasis is lower than that in patients without metastasis, and the area under the curve is 0.825. ** indicates that $p < 0.01$ and *** indicates that $p < 0.001$.

3.3 Relationship between LINC01197 and Clinical Staging of Patients and Its Early Diagnostic Value

Previously, we analyzed the expression and diagnostic value in patients in phases I + II and III + IV. Here, we further analyzed the relationship between LINC01197 and clinical staging of patients. Firstly, we conducted sperman test analysis and found that TNM staging gradually increased with the decrease of LINC01197 ($r = –0.816$, $p < 0.001$). And through variance analysis, we found that the expression gradually decreased with the progress of clinical staging and there were statistical differences between the groups ($P < 0.001$). Finally, we drew ROC curve of LINC01197 in distinguishing early PC patients, and the result showed that the area under the curve of LINC01197 was 0.930. More details were shown in Fig. 3.

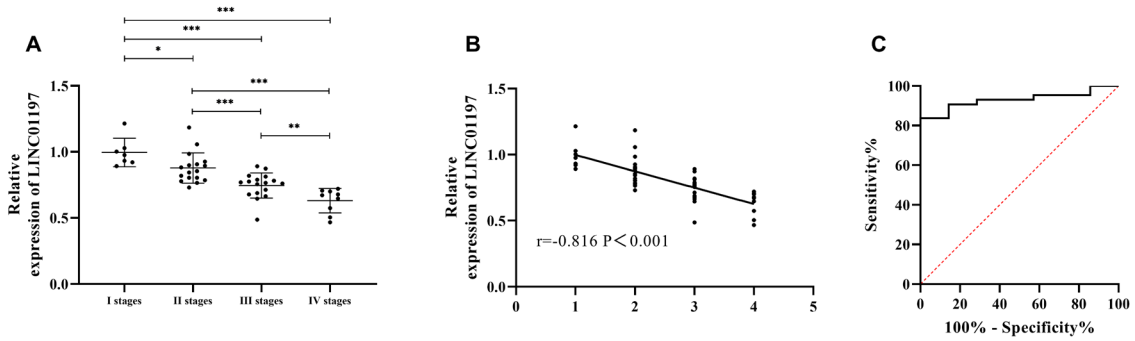

Figure 3: Diagnostic value of LINC01197 in clinical staging of PC patients

A. The expression of LINC01197 in PC of different staging. B. The correlation between LINC01197 and clinical staging. The diagnostic value of LINC01197 in distinguishing patients in phase I is 100.00% of the best specificity and 83.72% of the sensitivity when the cutoff value is 0.891. * indicates that $p < 0.05$, ** indicates that $p < 0.01$, and *** indicates that $p < 0.001$. 1 = Phase I, 2 = Phase II, 3 = Phase III, 4 = Phase IV.

3.4 Relationship between LINC01197 and Patient Survival

The patients were followed up for 5 years and all 50 patients were followed up, with a 5-year survival rate of 10%. Furthermore, we analyzed the 1-year survival rate and 5-year survival rate of patients in groups according to the median expression of LINC01197. It was found that the 1-year survival rate and 5-year survival rate of patients in low expression group were lower than those in high expression group (P1 year = 0.037, P5 year = 0.014). More details were shown in Fig. 4.

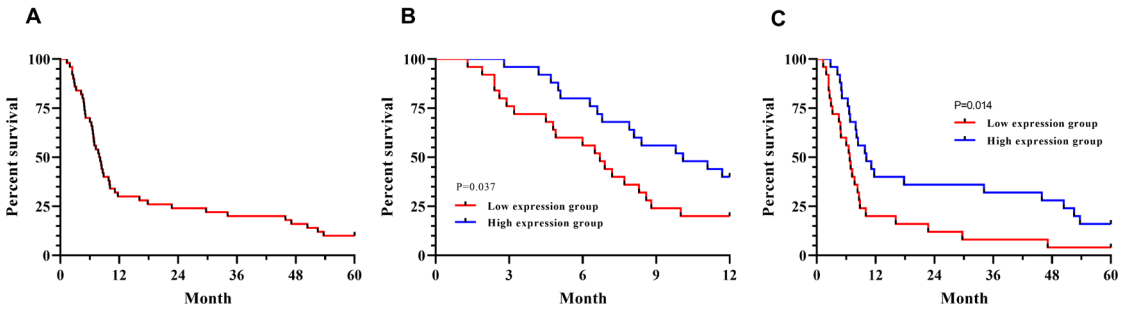

Figure 4: Survival analysis of LINC01197 and PC patients

The overall survival rate of PC patients.

The 1-year survival of patients in the high and low expression group of LINC01197.

C. The 5-year survival of patients with high and low expression of LINC01197.

3.5 Cox Regression Analysis of Prognosis

The pathological data of patients were collected to analyze independent factors affecting the prognosis of patients for 1 year and 5 years. Single factor Cox regression analysis showed that TNM staging, lymphatic invasion, distant metastasis, and LINC01197 were independent factors affecting the prognosis of patients for 1 year. Multiple factor analysis found that distant metastasis (HR: 0.374 (95CI%: 0.178–0.785)) was independent factor affecting the prognosis of patients for 1 year. Further analysis of the 5-year prognosis of patients revealed that differentiation, TNM staging, lymphatic invasion, distant metastasis, and LINC01197 were the factors affecting the 5-year prognosis of patients. Multivariate analysis found that differentiation (HR: 0.340 (95CI%: 0.169–0.681)), TNM staging (HR: 2.392 (95CI%: 1.156–4.947)), distant metastasis (HR: 0.406 (95CI%: 0.214–0.772)), and LINC01197 (HR: 0.432 (95CI%: 0.205–0.913)) were independent factors affecting the 5-year prognosis of patients. More details were shown in Tabs. 5 and 6.

Table 5: Assignment table

Factor	Assignment
Gender	Male = 1, female = 2
Age	≥55 = 1, <55 = 2
Lesion site	Head of pancreas = 1, rests = 2
Differentiation	Low differentiation = 1, moderate+high differentiation = 2
TNM staging	I + II = 1, III + IV = 2
Lymphatic invasion	Yes = 1, no = 2
Vascular invasion	Yes = 1, no = 2
Distant metastasis	Yes = 1, no = 2
LINC01197	<0.788 = 1, ≥0.788 = 2

Table 6: Analysis of multivariate Cox prognostic factors in 1 and 5 years

Factor	1-year single factor Cox		1-year multiple factor Cox		5-year single factor Cox		5-year multiple factor Cox	
	p value	HR (95CI%)	*p* value	HR (95CI%)	*p* value	HR (95CI%)	*p* value	HR (95CI%)
Gender	0.849	1.067 (0.548–2.079)			0.728	0.899 (0.493–1.638)		
Age	0.149	0.613 (0.315–1.191)			0.087	0.597 (0.331–1.078)		
Lesion site	0.631	0.850 (0.436–1.653)			0.271	0.718 (0.399–1.294)		
Differentiation	0.139	0.604 (0.310–1.177)			0.026	0.508 (0.280–0.921)	0.002	0.340 (0.169–0.681)
TNM staging	0.006	2.701 (1.335–5.462)	0.058	2.104 (0.974–4.545)	0.000	3.442 (1.751–6.765)	0.019	2.392 (1.156–4.947)
Lymphatic invasion	0.010	0.412 (0.211–0.806)	0.263	0.651 (0.308–1.379)	0.003	0.389 (0.207–0.732)	0.139	0.592 (0.295–1.185)
Vascular invasion	0.492	0.791 (0.405–1.546)			0.723	0.898 (0.494–1.631)		
Distant metastasis	0.001	0.318 (0.161–0.630)	0.009	0.374 (0.178–0.785)	0.001	0.352 (0.19–0.652)	0.006	0.406 (0.214–0.772)
LINC01197	0.041	0.496 (0.253–0.973)	0.683	0.849 (0.389–1.857)	0.016	0.477 (0.262–0.870)	0.028	0.432 (0.205–0.913)

4 Discussion

LncRNA is a hot research field in recent years. LncRNA is a kind of long-chain non-coding RNA that can participate in various biological functions of human body by regulating miR and related proteins [17,18]. More and more studies have found that lncRNA is closely related to the occurrence and development of various tumors. Studies of Nie et al. [19] have found that LncRNA-UCA1 can play a carcinogenic role by regulating miR-193a-3p in non-small cell lung cancer. Literature by Wei et al. [20] have reported that LncRNA XIST can promote PC cell proliferation by regulating miR-133a/EGFR. LINC01197 is a newly discovered lncRNA located on human 15q26.2 in recent years. It was found in previous studies by Ling et al. [14] that LINC01197 could inhibit PC cell proliferation by mediating Wnt/β-catenin signaling pathway. However, there has not been any relevant research on whether LINC01197 can become a clinical observation indicator of PC. Therefore, this study explores the expression of LINC01197 in PC patients to observe its potential clinical value and provide potential observation indicators for the treatment and diagnosis of clinicians.

In this study, we first compared the differences between LINC01197 and CA-199 in PC. CA-199 is a common tumor marker used in clinical screening of PC. Previous reports have shown that CA-199 has high sensitivity and specificity in PC, especially in predicting the prognosis and recurrence of PC patients undergoing surgery. However, there are also reports that [21] CA-199 expression has a low specificity, and its expression will also increase when some digestive system diseases occur, which is bound to reduce the specificity of CA-199. In addition, other studies have found that [22] CA-199 expression in serum of more than 30% PC patients will not increase. In this study, we detected the expression of CA-199 in serum of PC patients and found that the expression of CA-199 in the patient group was significantly higher than that in the normal group, while we found that the area under the curve was 0.791 by drawing ROC curve, which was basically consistent with other research results [23,24], and we further analyzed the expression of LINC01197 in PC. The results showed that the expression of LINC01197 was also differentially expressed in PC. The expression in PC patients was significantly lower than that in the normal group, and the area under the curve was 0.944, and the sensitivity was significantly higher than that of CA-199. This suggests

that LINC01197 has higher clinical value in diagnosing PC than CA-199, and may be a potential indicator for PC diagnosis. Therefore, we also analyzed the relationship between LINC01197 and PC pathological data. According to the expression of LINC01197, the patients in the low expression group of LINC01197 were divided into high and low expression groups. Through analysis, it was found that the patients in the low expression group of LINC 01197 had significantly higher probability of Phases III + IV, lymphatic invasion, and distant metastasis than the patients in the high expression group. Therefore, we further compared Phases I + II and III + IV, lymphatic invasion, and non-invasion, the expression of LINC01197 in serum of patients with distant metastasis and patients without metastasis was found to be different in each index, and it was also found that LINC01197 had a higher area under the curve of diagnostic value than 0.7 in distinguishing Phases I + II and III + IV, lymphatic invasion, and distal metastasis. Through the above studies, it can be shown that LINC01197 does exist low expression in PC and can be used as a potential indicator to distinguish PC from normal people, Phases I + II, Phases III + IV, lymphatic invasion, and distant metastasis, but whether it can be used as a diagnostic indicator to distinguish patients with phase I is still unclear.

At present, there are few patients with PC phase I clinically. The main reason we consider may be that the clinical characteristics of the early stage of PC onset are few and cannot be judged by clinical appearances. Most patients enter the advanced stage when they are admitted to hospital after the onset of the disease. Secondly, there is a lack of highly specific diagnostic indicators [25]. In this study, we further compared the relationship between LINC01197 and PCTNM staging. Through research, it is found that the expression of LINC01197 is different in different staging and there is a negative correlation between the expression of LINC01197 and PCTNM staging, namely, TNM staging are higher and more serious as the expression of LINC01197 gradually decreases. Analysis of ROC curve shows that LINC01197 has extremely high diagnostic value because its area under the curve of diagnosis I in PC is more than 0.9.

At the end of the study, we followed up the patients for 5 years, and the overall 5-year survival rate of the patients was 10%, which was basically consistent with the domestic and foreign reports [26,27]. Observing the 1-year and 5-year survival of patients in the LINC01197 high and low expression group, we found that the 1-year and 5-year survival of patients in the LINC01197 low expression group was lower than that of patients in the high expression group, which showed that LINC01197 could be used as a potential indicator of patient prognosis. Therefore, we collected patient pathological data to analyze the independent factors affecting the 1-year and 5-year survival of patients. Through analysis, we found that distant metastasis was an independent prognostic factor affecting the 1-year and 5-year survival of PC patients, while LINC01197 was not an independent prognostic factor in the short-term prognosis, but an independent prognostic factor for 5-year survival of patients. Previous reports [28] have shown that distant metastasis is an independent factor affecting the prognosis of PC patients, and our study found that LINC01197 can be used as an independent factor for the 5-year prognosis of PC patients. This result is the first time that LINC01197 has been found, suggesting that LINC 01197 is expected to become a potential observation indicator for the prognosis of PC patients.

However, we still have certain limitations in this study. Firstly, we have not collected serum samples from patients with benign pancreatic lesions. Whether LINC01197 can distinguish patients with benign pancreatic lesions from patients with PC still needs further study. Secondly, due to the small sample size, whether LINC01197 can be used as a prognostic indicator of PC still needs further study. Finally, the mechanism of LINC01197 affecting PC is still unclear. Besides influencing Wnt/β-catenin signaling pathway, whether it also affects other pathways leading to PC occurrence needs further study. Therefore, we hope to increase the type and number of samples in future research, further analyze the relevant mechanism of LINC01197 through bioinformatics, and carry out basic research to supplement and verify our research results.

To sum up, low expression of LINC01197 in PC patients indicates poor prognosis of patients and is expected to be a potential indicator for early diagnosis and prognosis observation of PC.

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