BMC Cancer

RESEARCH

Open Access

# Plasma-derived exosomal long noncoding RNAs of pancreatic cancer patients as novel blood-based biomarkers of disease

Xiaomeng He[1,2†], Litian Chen[3†], Yang Di[4†], Wenyang Li[5†], Xin Zhang[1], Zhihui Bai[1,2], Zhefeng Wang[1], Shanshan Liu[2], Christopher Corpe[6] and Jin Wang[1,2*]

## Abstract

**Background** Pancreatic cancer (PaCa) is one of the most intractable and fatal malignancies and is associated with the dysregulation of long noncoding RNAs (lncRNAs), which are a large class of noncoding RNAs larger than 200 nt that act as competing endogenous RNAs or sponges for miRNAs to induce tumour biological behaviours. However, their clinical value in treating pancreatic cancer has been poorly explained, but they are essential for improving the prognosis of PaCa patients.

**Methods** We analysed the plasma-derived exosomal lncRNA profiles of PaCa patients by using whole-transcriptome sequencing analysis and identified significantly differentially expressed lncRNAs, including LINC01268, LINC02802, AC124854.1, and AL132657.1. In the current study, the expression levels of four plasma-derived exosomal lncRNAs in PaCa plasma were validated via quantitative real-time polymerase chain reaction (qRT–PCR). The relationship between the expression of the four lncRNAs and the clinicopathological features of patients with PaCa was also evaluated.

**Results** We demonstrated that exosomal LINC01268, LINC02802, AC124854.1 and AL132657.1 were highly expressed in PaCa plasma compared with those in normal controls; moreover, they were positively correlated with the serum expression of carbohydrate antigen 19–9 (CA19-9). The receiver operating characteristic curves (AUCs) of the four lncRNAs were 0.8421, 0.6544, 0.7190, and 0.6321, and the AUC value of the combination of the four exosomal lncRNAs increased to 0.8476, with a sensitivity of 0.72 and specificity of 0.89. These results suggested that the plasma-derived exosomal genes LINC01268, LINC02802, AC124854.1, and AL132657.1 may be novel diagnostic markers for PaCa.

**Conclusions** Our research demonstrated that the plasma-derived exosomal lncRNAs of PaCa patients are novel blood-based biomarkers of disease.

**Keywords** Biomarker, Diagnosis, Exosome, Long noncoding RNA, Pancreatic cancer

†Xiaomeng He, Litian Chen, Yang Di and Wenyang Li contributed equally to this work.

*Correspondence:
Jin Wang
wjincityu@yahoo.com
Full list of author information is available at the end of the article

## Introduction

Pancreatic cancer (PaCa) is one of the most intractable and fatal malignancies and has high morbidity and mortality, as well as a low 5-year survival rate of approximately 10% worldwide [1]. Fewer than 20% of patients can undergo curative resection because they are diagnosed at an early stage, and most PaCa patients have cancer spread by metastasis [2] because effective biomarkers have not yet been determined for the early detection of PaCa. The diagnosis of PaCa relies mainly on the level of carbohydrate antigen (CA 19–9 and CA125), as well as the results of pancreatic imaging and histopathological findings in surgical specimens [3, 4]. Ki-67 is used to detect PaCa tissue samples after puncture or surgery via immunohistochemistry and is an important indicator of whether a tumour is benign or malignant; moreover, a Ki-67 index < 10% indicates a benign tumour [5]. CA 19–9 and CA125 are tumour biomarkers in serum that are used for the diagnosis and postoperative monitoring of PaCa [6]. CA 19–9 is a cell surface glycoprotein complex, and the clinical normal reference value of CA19-9 is 0–37.0 U/mL, which is helpful for the diagnosis and prognosis of PaCa [7]. CA 125 is also a type of cell surface glycoprotein, and the clinical normal reference value range of CA125 is 0–35.0 U/mL. CA125 was investigated to be predictive of surgical resectability in two studies, with cut-off values ranging between 34 and 35 U/ml [8]. The levels of CA19-9 and CA125 in patients' venous blood serum can be determined by using the Beckman Dxi800 automatic chemiluminescence method; however, they have insufficient diagnostic specificity and sensitivity for PaCa. Therefore, novel biomarkers with high accuracy are needed to improve clinical decision-making related to PaCa.

Noncoding RNAs (ncRNAs) function as diagnostic and prognostic markers in PaCa [3, 4]. For example, microRNAs (miRNAs), which are ncRNAs with a length of 19–25 nucleotides, have the potential to serve as blood-based markers for PaCa diagnosis and prognosis [3] and to discriminate low-grade benign lesions from high-grade invasive lesions in pancreatic cystic fluid [4]. Long noncoding RNAs (lncRNAs) are ncRNAs with a length of more than 200 nucleotides (nt) that lack protein-coding ability [9, 10] and are involved in the regulation of gene expression at the transcriptional and posttranscriptional levels [10, 11]. Dysfunctional lncRNAs play important roles in determining cell fate and disease occurrence [12] in many cancers, including breast [13], lung [14], colorectal [15], liver [16], and pancreatic cancer [17, 18].

Previous studies have shown that lncRNAs can be key regulators of PaCa tumorigenesis [19] and that the regulation of the epithelial–mesenchymal transition pathway in PaCa cells is a promising biomarker for tumour metastasis [20]. Moreover, lncRNAs may serve as potential markers for the early detection, screening, and prognostic monitoring of PaCa. For example, exosomal LINC01111 in plasma was positively correlated with overall survival but negatively correlated with TNM stage in PaCa patients [21]. LINC01638 and ABHD11-AS1 are highly expressed in the plasma of PaCa patients and distinguish patients with pancreatic ductal adenocarcinoma (PDAC) from healthy controls [22, 23], and ABHD11-AS1 is more effective when combined with CA19-9 than when combined with ABHD11-AS1 alone [23], which shows the best diagnostic performance for PaCa.

Four lncRNAs, including LINC01268, AC245595.1 (LINC02802), AC124854.1, and AL132657.1 (according to whole-transcriptome sequencing of the plasma-derived exosomal lncRNAs in patients with PaCa) are upregulated in PaCa tissues compared to normal tissues [24]. To further explore the potential of plasma lncRNAs as tumour markers in PaCa, we verified the expression levels of LINC01268, LINC02802, AC124854.1, and AL132657.1 in the plasma of PaCa patients and healthy controls by using quantitative real-time polymerase chain reaction (qRT−PCR) analysis, which identified these lncRNAs as being novel candidate biomarkers for the diagnosis and prognosis of PaCa.

## Materials and methods

### Study population and blood samples

All of the peripheral blood from healthy controls and preoperative and postoperative patients with PaCa was collected at Shanghai Jiaotong University School of Medicine Xinhua Hospital (Shanghai, China) and Huashan Hospital of Fudan University (Shanghai, China) between 2020 and 2023. Patients with pancreatic cancer were eligible for this study if they had a pathological diagnosis of pancreatic ductal adenocarcinoma and had not undergone any treatment before surgery. The blood was stored in sodium heparin tubes (BD Vacutainer) and centrifuged at 2,000 × g for 10 min at 4 °C within 2 h. The separated plasma was subpackaged into new tubes and stored at -80 °C. All of the study subjects provided written informed consent for the collection of blood samples in accordance with the protocol approved by the Ethics Committees of Shanghai Public Health Clinical Center (No: 2020-S027-02).

### Identification of exosomes from plasma using transmission electron microscopy

Exosomes or other extracellular vesicles (EVs) were isolated from plasma by a novel spin column-based method as described previously [25]. Briefly, 1 mL of prefiltered (0.8 μm) plasma from patients was mixed with 2 × binding buffer (XBP) and added to an exoEasy membrane

affinity rotation column (QIAGEN, Cat. 77,044). After centrifugation, the flow-through was discarded, and the column was washed with 3.5 mL of wash buffer (XWP). After another centrifugation, the column was eluted with 100 μL of buffer in PBS, and the exosomes or EVs were collected with a 100 kDa pore size ultrafilter (Cat. VS0142, Sartorius). Finally, 10 μL of exosome suspension solution was dropped onto a copper grid for fixation with 3% glutaraldehyde solution for 30 min, followed by the addition of 4% acetic acid oxygen dye solution and 1% methylcellulose solution for observation of the ultrastructure of the exosomes under transmission electron microscopy (TEM) (Philips CM-120, Holland).

#### Western blot analysis

Total proteins were extracted from the exosomes or cells using RIPA lysis buffer supplemented with proteinase and phosphatase inhibitors (Sangon, Shanghai, China). Twenty micrograms of protein were loaded and separated by SDS–PAGE. After the separated proteins were transferred to nitrocellulose filter membranes (PALL, USA) and blocked with 5% nonfat milk, the membranes were incubated with primary antibodies, including rabbit anti-CD63 (Cat. D264579, Sangon Biotech), rabbit anti-CD9 (Cat. D290251, Sangon Biotech), and rabbit anti-Calnexin (Cat. D262986, Sangon Biotech), overnight at 4 °C. Subsequently, the membranes were washed with PBST and then incubated with secondary antibodies (Sangon, Shanghai, China) for 1.5 h. Finally, enhanced chemiluminescence (ECL) reagent (Beyotime, Shanghai, China) was used to visualize the protein bands.

#### Plasma-derived exosomal RNA purification and RNA sequencing

Plasma-derived exosomal RNA was extracted with TRIzol reagent (Life Technologies) and an exoRNAseasy Serum/Plasma Midi Kit (QIAGEN, Cat. 77,044) according to the manufacturer's instructions [26]. The concentration of each RNA sample was quantified by using a NanoDrop-2000 (Thermo Scientific). RNA sequencing of five PDAC cancer plasma samples and five control samples and follow-up bioinformatics analysis were performed by Cloud-Seq Biotech (Shanghai, China). The short reads were aligned to the reference genome (hg38 from Ensembl 84) by using the HISAT program, and the differentially expressed lncRNAs between PaCa and normal control plasma were identified via FC (fold change) $\geq 2.0$ and $p < 0.05$.

#### Quantitative real-time PCR analysis of plasma-derived exosomal lncRNAs

Reverse transcription of each sample was conducted by using the PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa, Cat. RR047A), and qRT–PCR analysis was performed by using TB Green® Premix Ex Taq™ II (TaKaRa, Cat. RR820A) in a Roche LightCycler® 480 II System according to the manufacturer's instructions with several modifications. The plasma RNA was incubated with the RT mixture at 37 °C for 2 h and 85 °C for 5 s to synthesize cDNA after being treated with gDNA eraser at 42 °C for 8 min. Afterwards, 40 μL of water was added to 20 μL of reverse transcription (RT) reaction, and 4 μL of the diluted RT product was mixed with 5 μL of SYBR Green, 0.5 μL of forwards primer (10 μM), and 0.5 μL of reverse primer (10 μM) in a final volume of 10 μL. The reaction mixture was kept at 95 °C for 1 min, 95 °C for 5 s, and 60 °C for 30 s for a total of 45 cycles. The relative expression of the lncRNAs was calculated by using the $2^{-\Delta\Delta CT}$ method with normalization to 18S rRNA. The values are presented as the means ± SDs from three independent reactions, and the primers used for qRT–PCR are shown in Table S1.

#### Statistical analysis

All of the statistical analyses were performed with SPSS 26.0 (IBM, Endicott, NY, USA) and GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). Student's t test was performed to test for significant differences between individual groups for quantitative data. One-way ANOVA was used to compare the differential expression of the four lncRNAs between healthy controls and between preoperative and postoperative PaCa patients. Spearman correlation analysis was used to evaluate the correlation coefficient (r) between four lncRNAs and clinical characteristics and the correlation coefficient of four lncRNAs between plasma and tumour tissues of PaCa patients. We constructed a receiver operating characteristic (ROC) curve and calculated the area under the curve (AUC) to evaluate the predictive values of each individual lncRNA and the combination of the four lncRNAs for the patients and controls. A $P$ value $< 0.05$ was considered to indicate statistical significance.

## Results

#### Patient characteristics

Among the 148 patients enrolled in the study, 70 were normal controls, and 78 were diagnosed with PaCa. The clinical features of these patients at diagnosis, including age, sex, and disease stage, were retrospectively collected and are summarized in Table S2. Among the 78 patients with PaCa, univariate analysis via SPSS showed that the tumour stage did not differ according to age, sex, tumour diameter, or Ki-67-positivity. Serum CA19-9 (tumour stage I-II: 156.9 U/mL; tumour stage III-IV: 698.6 U/mL) and CA125 (tumour stage I-II: 16.0 U/mL; tumour stage III-IV: 21.9 U/mL) were significantly differentially

expressed among the tumour stages of 78 patients with PaCa ($p < 0.05$) (Table 1).

### Identification of exosomes from plasma and whole-transcriptome sequencing analysis of differentially expressed exosomal lncRNAs in plasma from PaCa patients

Exosomes from the plasma of patients with PaCa were identified by transmission electron microscopy and immunoblotting. The typical disc-shaped structure of exosomes from plasma was first observed via transmission electron microscopy (Fig. S1A). At the same time, CD63 and CD9, which are specific proteins on the surface of exosomes, were analysed by immunoblotting. Calnexin was also selected as a negative control for exosomes and was detected on BxPC-3 cell membranes. Finally, we found that the CD63 and CD9 proteins were significantly enriched in plasma-derived exosomes from the patients (Fig. S1B).

Next, to identify potential plasma biomarkers of PaCa, whole-transcriptome sequencing was further used to detect a large number of transcripts in plasma exosome samples from five PDAC patients and five normal controls (NCs). Our results demonstrated that thirty exosomal lncRNAs were upregulated (Table S3), and 436 exosomal lncRNAs were downregulated, in plasma from PaCa patients (fold change $\geq \pm 2.0$, and $p < 0.05$) compared with those from the controls [24].

### Quantitative real-time polymerase chain reaction analysis of candidate lncRNAs in the tumour tissue and plasma of PaCa patients

qRT–PCR was used to analyse the differentially expressed lncRNAs in PaCa tumour tissues, and four lncRNAs (LINC01268, LINC02802, AC124854.1, and AL132657.1) were upregulated in the tumour tissues of 78 patients with PaCa compared to the paracancerous tissues (Fig. S2A–D). To identify lncRNAs that can be used as potential biomarkers in PaCa plasma, we detected the expression of lncRNAs in the plasma of 78 PaCa patients and 70 healthy volunteers via quantitative real-time reverse transcription polymerase chain reaction (qRT–PCR) and analysed the expression levels of the standardized lncRNAs through the Student's t test. Compared with those in the healthy controls, the plasma levels of LINC01268 ($p < 0.0001$) (Fig. 1A), LINC02802 ($p = 0.0021$) (Fig. 1B), AC124854.1 ($p = 0.0017$) (Fig. 1C), and AL132657.1 ($p = 0.0016$) (Fig. 1D) in patients with PaCa were significantly increased by 73.63, 60.27, 11.25, and 34.63 times, respectively. These four differentially expressed plasma lncRNAs may be used as biomarkers for the noninvasive diagnosis of PaCa. We further analysed the correlation between the expression levels of the four lncRNAs in the plasma and tumour tissues of PaCa patients (Fig. S2E-H). We found positive correlations between the expression levels of LINC01268 ($r = 0.3068$, $p = 0.0063$), LINC02802 ($r = 0.2258$, $p = 0.0468$), AC124854.1 ($r = 0.2367$, $p = 0.0369$), and AL132657.1 ($r = 0.2239$, $p = 0.0488$) in the plasma and tumour tissues of PaCa patients.

**Table 1** Clinical characteristics of 78 PaCa patients stratified by tumour stage

| Clinical feature | Tumour stage of PaCa patients ($n = 78$) | | |
|---|---|---|---|
| | (I-II, $n = 59$) | (III-IV, $n = 19$) | $p$ value |
| Gender (male/female) | 34/25 | 14/5 | 0.211 |
| Age (years) | 65 (61–73) | 60 (56–69) | 0.136 |
| Diameter of tumour (cm) | 3.5 (2.8–5.0) | 3 (2.5–4.0) | 0.911 |
| Ki-67 positive rate (%) | 25 (10–50) | 15 (10–20) | 0.082 |
| CA19-9 (U/mL) | 156.9 (17.3–370.5) | 698.6 (90.3–1115) | **0.049** |
| CA125 (U/mL) | 16 (10.7–23.6) | 21.9 (12.69–40.2) | **0.004** |

*PaCa* pancreatic cancer, *CA19-9* carbohydrate antigen 19–9, *CA125* carbohydrate antigen 125

### Plasma-derived exosomal lncRNAs as biomarkers in PaCa

We performed receiver operating characteristic (ROC) curve analysis to evaluate the diagnostic value of these four plasma lncRNAs in PaCa to further assess their biological functions. An area under the ROC curve (AUC) closer to 1 reflects a more substantial difference between PaCa patients and healthy controls. The results showed that the AUCs of LINC01268, LINC02802, AC124854.1, and AL132657.1 were 0.8421, 0.6544, 0.7190, and 0.6231, respectively, with sensitivities of 0.69, 0.63, 0.59, and 0.51, respectively, and specificities of 0.89, 0.64, 0.86, and 0.74, respectively. These lncRNAs can be used as good diagnostic and prognostic indicators of PaCa, especially LINC01268, which has excellent diagnostic value in PaCa (Fig. 2A-D and Table 2). In addition, we further analysed the combined diagnostic value of these four plasma lncRNAs, and the results showed that, compared with those of the single diagnostic value of LINC001268, the AUC of the combined diagnostic value of the four plasma lncRNAs increased to 0.8476, and the sensitivity increased to 0.72 (Fig. 2E and Table 2), thus indicating that the combined diagnosis of LINC01268, LINC02802, AC124854.1, and AL132657.1 was more effective. Moreover, we also analysed the expression levels of the four lncRNAs in the plasma between preoperative and postoperative patients. Compared with those in preoperative plasma, the expression of LINC01268 ($p = 0.0244$) and AC124854.1 ($p = 0.0498$) was differentially repressed in the postoperative plasma of PaCa patients (Fig. 3).

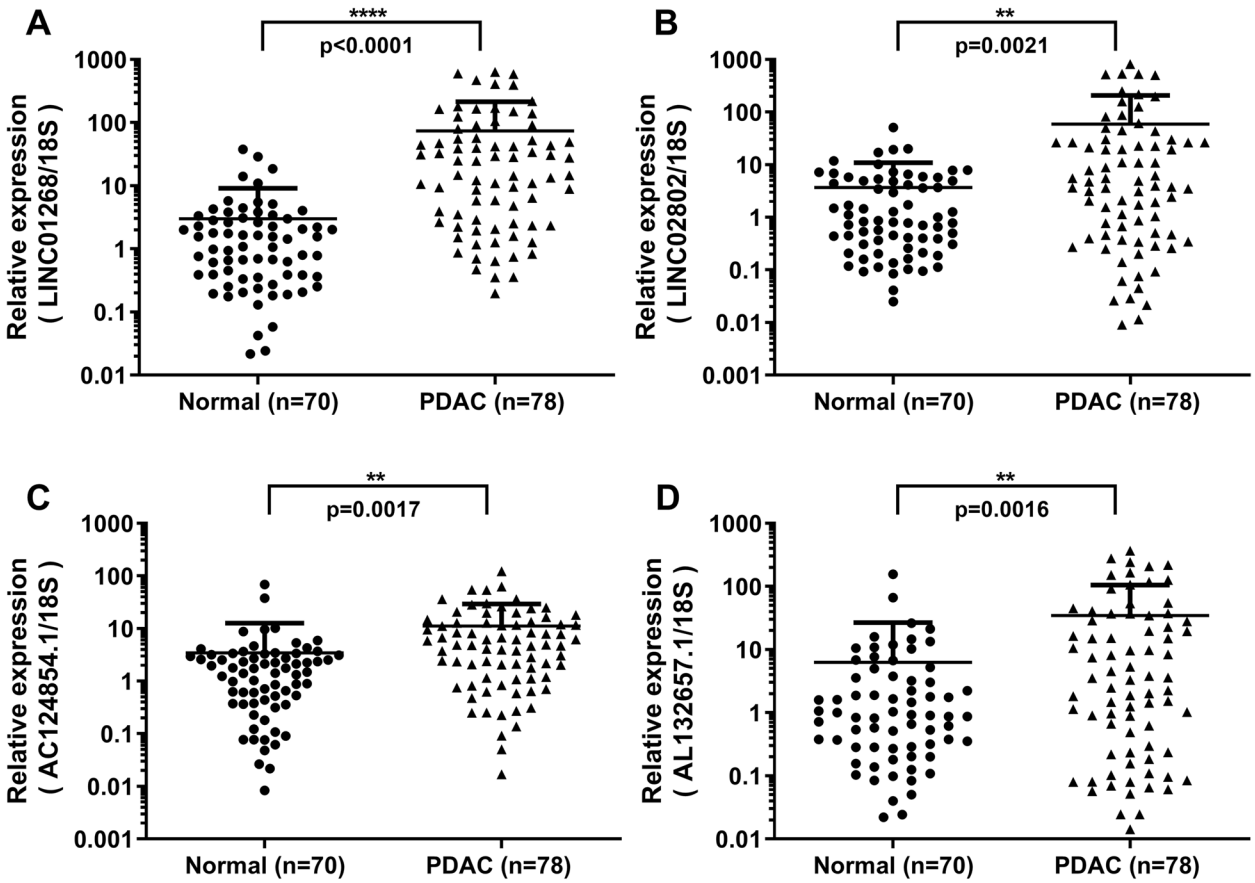


**Fig. 1** The relative expression of four lncRNAs in the plasma of PaCa patients and healthy controls. LINC01268 (**A**), LINC02802 (**B**), AC124854.1 (**C**), and AL132657.1 (**D**). PaCa, pancreatic cancer; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$

### Plasma-derived exosomal lncRNAs were correlated with the serum expression of CA19-9

The 78 patients with PaCa were divided into low- and high-expression groups based on the median expression levels of LINC01268, LINC02802, AC124854.1, and AL132657.1. Spearman's correlation analysis was subsequently used to evaluate the associations between the expression of the four plasma lncRNAs and the clinicopathological features of PaCa (Table 3). The results showed that LINC01268 ($r = 0.4593$, $p < 0.0001$), LINC02802 ($r = 0.3593$, $p = 0.0012$), AC124854.1 ($r = 0.3757$, $p = 0.0007$), and AL132657.1 ($r = 0.2674$, $p = 0.0179$) were positively correlated with the serum expression of CA19-9 (Fig. 4 and Table 3). In particular, LINC01268 ($r = -0.1023$, $p = 0.3726$), LINC02802 ($r = -0.1203$, $p = 0.2942$), AC124854.1 ($r = -0.1132$, $p = 0.3239$), and AL132657.1 ($r = -0.1285$ $p = 0.2623$) were negatively associated with tumour stage; however, these associations were not statistically significant due to the small sample size. These results provide evidence that four plasma lncRNAs may be related to the early stage of PaCa and can be used as biomarkers combined with CA19-9 for the diagnosis and prognosis of PaCa.

## Discussion

PaCa is an aggressive tumour with a very poor prognosis. Early screening and prognosis analysis are the key factors for improving the survival rate of patients with PaCa [27]. LncRNAs are abnormally expressed in various types of cancers, and differentially expressed lncRNAs in the plasma or serum have become an emerging focus area in noninvasive diagnostic applications [23], including those for intrahepatic cholangiocarcinoma [28], gallbladder carcinoma [29], glioblastoma [30], and prostate carcinoma [31]. Although the functions and molecular mechanisms of most lncRNAs are still unknown, increasing evidence has shown that lncRNAs may play important roles in the occurrence, progression, and metastasis of tumours [32, 33]. Increasing number of lncRNAs have been found to be related to the prognosis of PaCa. Pan et al. has reported that LINC01111, which is a tumour suppressor, was negatively correlated with TNM stage

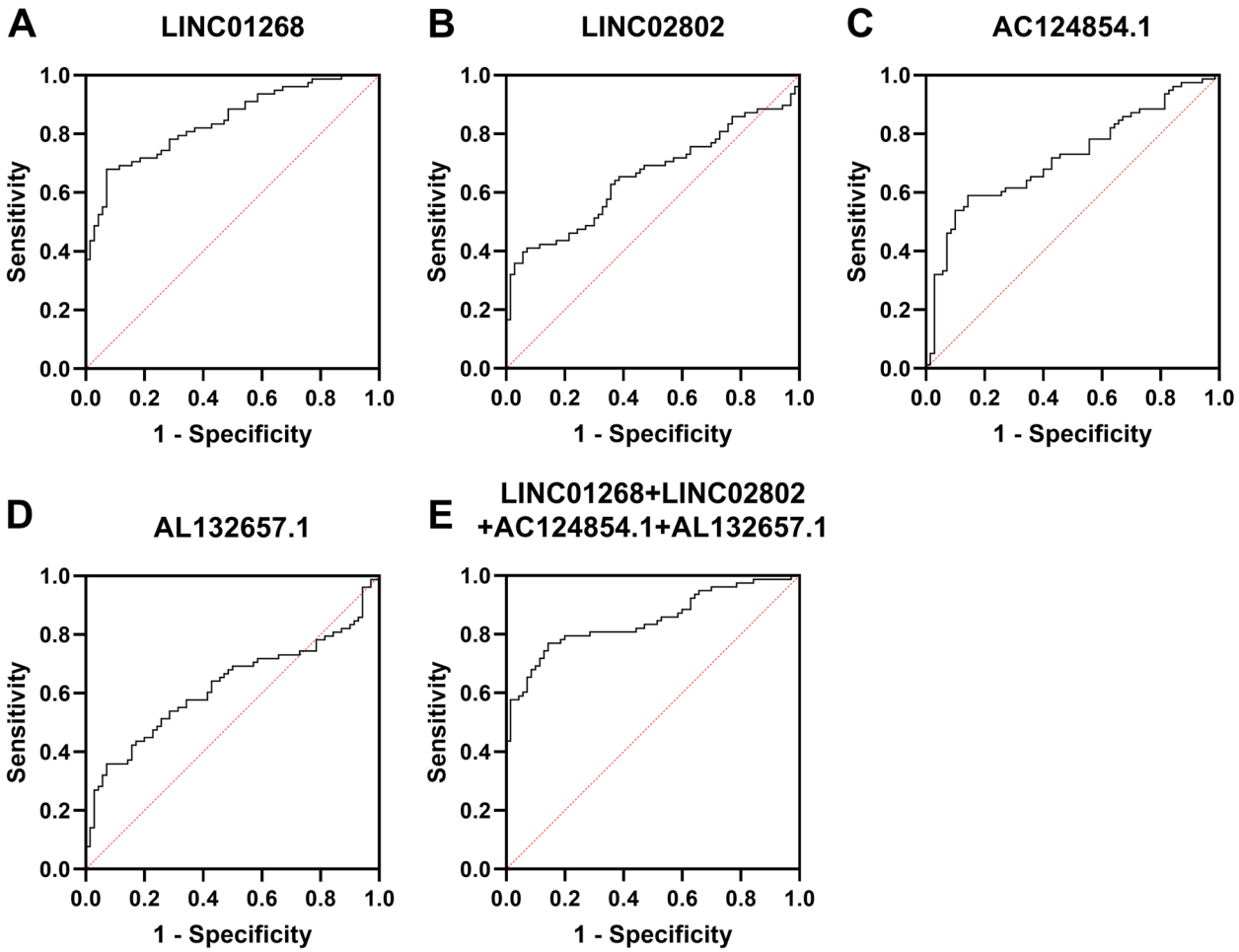


**Fig. 2** The ROC curve shows the diagnostic potential of the expression levels of lncRNAs in the plasma of patients with PaCa. LINC01268 (**A**), LINC02802 (**B**), AC124854.1 (**C**), AL132657.1 (**D**), and the four lncRNA combinations (**E**). ROC, receiver operating characteristic

**Table 2** The diagnostic efficiency of plasma lncRNAs (LINC01268, LINC02802, AC124854.1, and AL132657.1) in PaCa patients

| Biomarker | Sensitivity | Specificity | AUC (95% CI) | *p* value |
|---|---|---|---|---|
| LINC01268 | 0.69 | 0.89 | 0.8421 (0.7801–0.9041) | < 0.0001 |
| LINC02802 | 0.63 | 0.64 | 0.6544 (0.5656–0.7432) | 0.0013 |
| AC124854.1 | 0.59 | 0.86 | 0.7190 (0.6363–0.8018) | < 0.0001 |
| AL132657.1 | 0.51 | 0.74 | 0.6231 (0.5317–0.7145) | 0.0099 |
| LINC01268 + LINC02802 + AC124854.1 + AL132657.1 | 0.72 | 0.89 | 0.8476 (0.7846–0.9106) | < 0.0001 |

*PaCa* pancreatic cancer, *AUC* area under the ROC curve, *CI* confidence interval

but positively correlated with survival rate and may be a negative prognostic factor for PaCa [21]. Decreased expression levels of LINC00671, LINC00261, and SNHG9 in PaCa patients were associated with clinical stage, whereas increased expression levels of LINC00346, LINC00578, and LINC00673 were associated with poor prognosis [34]. We also found that immune-related lncRNAs, such as ZEB2-AS1 and LINC02325, have good predictive effects on five-year survival and are positively associated with CA19-9 and tumour size [18].

In this study, we first analysed the expression levels of the top ten differentially expressed lncRNAs (LUCAT1, LINC02802, LINC00887, AC124854.1, AL132657.1, LINC01268, AL365361.1, AL592071.1, AC079015.1, and

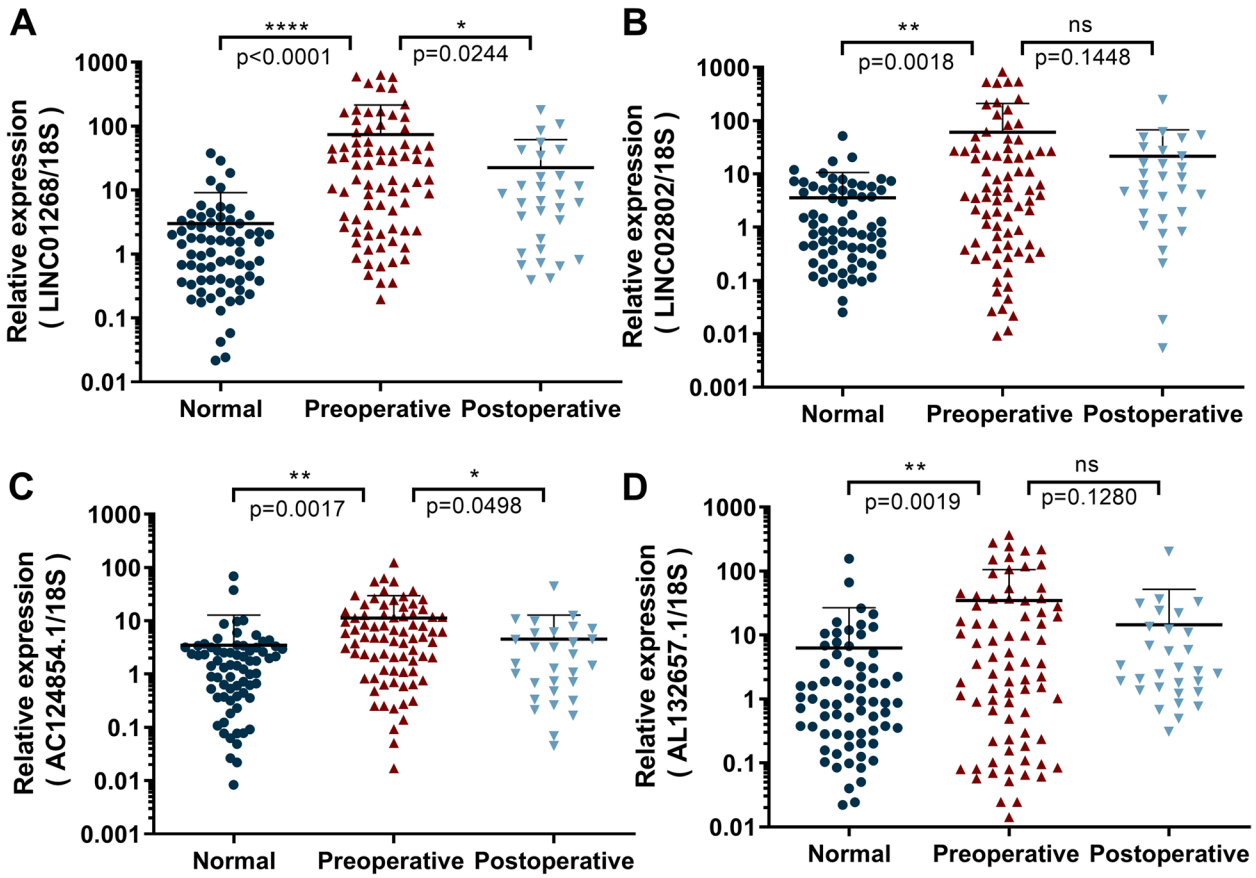


**Fig. 3** The expression levels of four lncRNAs in the plasma of healthy controls and in the preoperative and postoperative plasma of PaCa patients. Comparison of the differential expression levels of LINC01268 (**A**), LINC02802 (**B**), AC124854.1 (**C**), and AL132657.1 (**D**) in the plasma of healthy controls and in the preoperative and postoperative tissues of PaCa patients determined by qRT–PCR (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$)

**Table 3** Correlations between the expression levels of four plasma lncRNAs and clinical characteristics in 78 PaCa patients

| LncRNA | | Tumour stage | | r | *P* value | CA19-9 (U/mL) | | r | *P* value | CA125 (U/mL) | | r | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I/II | III/IV | | | ≤ 37 | > 37 | | | ≤ 35 | > 35 | | |
| **LINC01268** | High | 34 | 5 | -0.1023 | 0.3726 | 3 | 36 | **0.4593** | **< 0.0001** | 33 | 6 | 0.0329 | 0.7750 |
| | Low | 25 | 14 | | | 19 | 20 | | | 32 | 7 | | |
| **LINC02802** | High | 31 | 8 | -0.1203 | 0.2942 | 5 | 34 | **0.3593** | **0.0012** | 29 | 10 | 0.1337 | 0.2432 |
| | Low | 28 | 11 | | | 17 | 22 | | | 36 | 3 | | |
| **AC124854.1** | High | 31 | 8 | -0.1132 | 0.3239 | 8 | 31 | **0.3757** | **0.0007** | 31 | 8 | 0.1161 | 0.3116 |
| | Low | 28 | 11 | | | 14 | 25 | | | 34 | 5 | | |
| **AL132657.1** | High | 32 | 7 | -0.1285 | 0.2623 | 7 | 32 | **0.2674** | **0.0179** | 30 | 9 | 0.0890 | 0.4383 |
| | Low | 27 | 12 | | | 15 | 24 | | | 35 | 4 | | |

*PaCa* pancreatic cancer, *CA19-9* carbohydrate antigen 19-9, *CA125* carbohydrate antigen 125, *High* high-expression groups, *Low* low-expression groups

SNHG15) (Table S3) in ten pairs of PaCa tumour tissues and paracancerous tissue samples by qRT–PCR and found that LINC01268, LINC02802, AC124854.1 and AL132657.1 were significantly differentially expressed in the ten PaCa tumour tissue samples compared to their paracancerous tissue samples ($p < 0.05$) (Fig. S3). Thus,

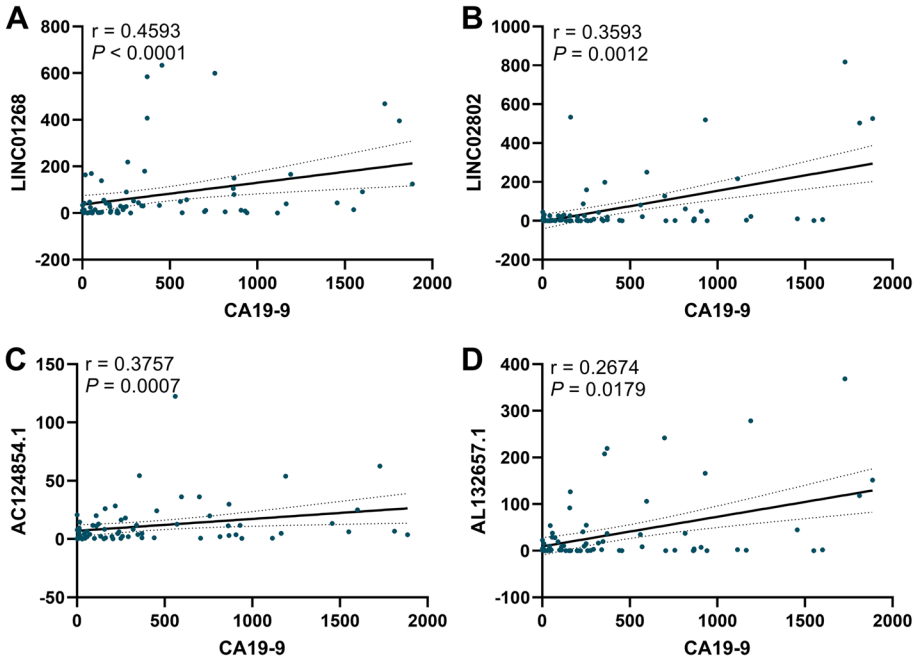


**Fig. 4** Correlation between the expression of lncRNAs and CA19-9 in PaCa patients. LINC01268 (**A**), LINC02802 (**B**), AC124854.1 (**C**), and AL132657.1 (**D**)

we selected these four lncRNAs as candidate lncRNA biomarkers of PaCa for further analysis. Next, we found that the expression levels of four different lncRNAs (LINC01268, LINC02802, AC124854.1, and AL132657.1) were significantly changed in the plasma of PaCa patients. To evaluate whether these lncRNAs could be used as prognostic factors in PaCa patients, we performed ROC analysis, and the results showed that four different lncRNAs could serve as good diagnostic and prognostic indicators of PaCa, and the combined diagnostic capability of four different lncRNAs was greater, which indicated that LINC01268, LINC02802, AC124854.1, and AL132657.1 may represent novel diagnostic and prognostic markers of PaCa. Spearman's correlation coefficient analysis demonstrated that LINC01268, LINC02802, AC124854.1, and AL132657.1 were positively correlated with the expression level of CA19-9, which is a poor prognostic indicator of PaCa [6]. LINC02802 plays a key role in preventing the antiangiogenic effects of miR-486-5p, and this inhibition is attributed to decreased expression of MAML3 [35]. Bioinformatics analysis also demonstrated that LINC02802, which is an immune-related lncRNA, is associated with the progression of cervical cancer and poor prognosis [36]. LINC01268 is an independent prognostic immune-related marker that can reduce the proliferation and metastasis of cancer cells in neuroblastoma [37]. Increased plasma levels of LINC01268 are associated with poor prognosis in patients with myelofibrosis [38], and LINC01268 positively regulates SOS1 expression by sponging miR-217 to promote acute myeloid leukaemia cell viability and cell cycle progression (but inhibit apoptosis) [39]. We also found that LINC01268, which is an oncogene, can promote epithelial–mesenchymal transition (EMT) in PaCa through the miR-217-KIF2A-PI3K/AKT axis [24].

In summary, we demonstrated that the expression levels of LINC01268, LINC02802, AC124854.1, and AL132657.1 were significantly altered in the plasma of PaCa patients, and their expression levels were positively correlated with CA19-9 levels. ROC analysis demonstrated that these lncRNAs have high diagnostic value for PaCa. We also found that the expression levels of four lncRNAs in plasma and tissues were positively correlated in patients with PaCa ($p < 0.05$). Therefore, the four plasma-derived exosomal lncRNAs may function as potential biomarkers in patients with PaCa. Although our findings show the feasibility of this approach in designing blood-based lncRNA biomarker panels, we also recognize that a small cohort of patients was tested in the present study, which has limited clinical implications for developing PaCa screening at this time. In the future, the potential mechanisms of these four exosomal lncRNAs (LINC01268, LINC02802, AC124854.1, and AL132657.1) also require further in-depth exploration for use in identifying tumorigenesis as potential diagnostic and therapeutic targets for PaCa.

## Conclusion

This study highlights the potential value of four exosomal lncRNAs (LINC01268, LINC02802, AC124854.1, and AL132657.1) in diagnosing pancreatic cancer.

### Abbreviations

| | |
|---|---|
| ABHD11-AS1 | Abhydrolase domain containing 11-antisense RNA 1 |
| AKT | AKT serine/threonine kinase |
| AUC | Area under the ROC curve |
| CA | Carbohydrate antigen |
| EMT | Epithelial–mesenchymal transition |
| KIF2A | Kinesin family member 2A |
| lncRNAs | Long noncoding RNAs |
| MAML3 | Master-like transcriptional coactivator 3 |
| miRNAs | MicroRNAs |
| PaCa | Pancreatic cancer |
| PI3K | Phosphoinositide-3-kinase |
| ROC | Receiver operating characteristic curve |
| SNHG9 | Small nucleolar RNA host gene 9 |
| SOS1 | SOS Ras/Rac guanine nucleotide exchange factor 1 |
| TNM | Tumour node metastasis |
| ZEB2-AS1 | Zinc finger E-box binding homeobox 2-antisense RNA 1 |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-12755-z.

Supplementary Material 1: Supplementary Table S1. The sequence of primers used for qRT–PCR of the lncRNAs in PaCa. F, forwards primer; R, reverse primer. Supplementary Table S2. Clinical characteristics of 78 PaCa patients. Supplementary Table S3. Partial overexpression of lncRNAs in the plasma of PaCa patients. Supplementary Table S4. Raw data of clinical features of 78 PaCa patients. Supplemental Figure S1. Exosomes from plasma were identified by TEM and immunoblots. (A) Transmission electron microscopy image of the exosome. Scale bar = 100 nm. (B) The expression levels of CD63, CD9 and Calnexin on the exosomes or BxPC-3 cells were analysed by immunoblots. Supplementary Figure S2. The expression levels of four lncRNAs in the tumour and paracancerous tissues of PaCa patients and the correlation of the expression between plasma and tumour tissues. The expression levels of LINC01268 (A), LINC02802 (B), AC124854.1 (C), and AL132657.1 (D) in the tumour and adjacent tissues of PaCa patients (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Spearman correlation analysis of LINC01268 (E), LINC02802 (F), AC124854.1 (G), and AL132657.1 (H) in plasma and tumour tissues from PaCa patients. Supplemental Figure S3. The expression levels of the top ten differently expressed lncRNAs (Class: lincRNA: LUCAT1, LINC02802, LINC00887, AC124854.1, AL132657.1, LINC01268, AL365361.1, AL592071.1, AC079015.1, and SNHG15) in the ten pairs of PaCa tumour tissue and paracancerous tissue samples by qRT-PCR analysis.

### Acknowledgements

We are grateful to all the authors for their useful discussion of the manuscript.

### No potential conflicts of interest were disclosed

No potential conflicts of interest were disclosed.

### Authors' contributions

JW designed the study. XH and SL carried out the experiments. XH, LC and YD collected the clinic samples. XH, LC, YD, WL, XZ, ZB, and ZW analyzed the data. XH was mainly responsible for statistical analysis and drafted the manuscript. JW, WL, CC, and YS revised the manuscript. All the authors have read and approved the final manuscript.

### Funding

This research was supported by the National Natural Science Foundation of China (Grant No. 81672383), the Science and Technology Commission of Shanghai (Grant No. 20Y11900700), the Special Research Fund of Youan Medical Alliance for the Liver and Infectious Diseases (Grant No. LM202020), and the Shanghai Public Health Clinical Center (Grant No. KY-GW-09).

### Availability of data and materials

The datasets that were used and/or analysed during the current study are available from the corresponding author upon reasonable request.

## Declarations

### Ethics approval and consent to participate

Blood sample collection was approved by the Ethics Committee of Shanghai Public Health Clinical Center, and all of the research subjects provided written informed consent.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

### Author details

[1]Central Laboratory, Zhongshan Hospital (Xiamen), Fudan University, Xiamen, Fujian 361015, China. [2]Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508, China. [3]Present Address: Department of Hepatobiliary Surgery, Shanghai Jiaotong University School of Medicine Xinhua Hospital, Shanghai 200092, China. [4]Present Address: Department of Pancreatic Surgery, Huashan Hospital, Fudan University, Shanghai, China. [5]Present Address: Department of Physiology and Pathophysiology, Hexi University School of Medicine, Zhangye, Gansu 734000, China. [6]King's College London, Nutritional Science Department, Waterloo, London SE19NH, UK.

Received: 18 October 2023 Accepted: 2 August 2024
Published online: 06 August 2024

### References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7–33.
2. Taherian M, Wang H. Critical issues in pathologic evaluation of pancreatic ductal adenocarcinoma resected after neoadjuvant treatment: a narrative review. Chin Clin Oncol. 2022;11(3):21.
3. Wang J, Chen J, Chang P, LeBlanc A, Li D, Abbruzzesse JL, Frazier ML, Killary AM, Sen S. MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. Cancer Prev Res (Phila). 2009;2(9):807–13.
4. Wang J, Paris PL, Chen J, Ngo V, Yao H, Frazier ML, Killary AM, Liu CG, Liang H, Mathy C, et al. Next generation sequencing of pancreatic cyst fluid microRNAs from low grade-benign and high grade-invasive lesions. Cancer Lett. 2015;356(2 Pt B):404–9.
5. Lee L, Igarashi H, Fujimori N, Hijioka M, Kawabe K, Oda Y, Jensen RT, Ito T. Long-term outcomes and prognostic factors in 78 Japanese patients with advanced pancreatic neuroendocrine neoplasms: a single-center retrospective study. Jpn J Clin Oncol. 2015;45(12):1131–8.
6. Yang Y, Ding Y, Gong Y, Zhao S, Li M, Li X, Song G, Zhai B, Liu J, Shao Y, et al. The genetic landscape of pancreatic head ductal adenocarcinoma in China and prognosis stratification. BMC Cancer. 2022;22(1):186.
7. Chen J, Wang H, Zhou L, Liu Z, Tan X. A combination of circulating tumor cells and CA199 improves the diagnosis of pancreatic cancer. J Clin Lab Anal. 2022;36(5):e24341.
8. Diab HMH, Smith HG, Jensen KK, Jorgensen LN. The current role of blood-based biomarkers in surgical decision-making in patients with localised pancreatic cancer: a systematic review. Eur J Cancer. 2021;154:73–81.
9. Wang Y, Li Z, Zheng S, Zhou Y, Zhao L, Ye H, Zhao X, Gao W, Fu Z, Zhou Q, et al. Expression profile of long non-coding RNAs in pancreatic cancer and their clinical significance as biomarkers. Oncotarget. 2015;6(34):35684–98.
10. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell. 2009;136(4):629–41.

11. Shi X, Sun M, Liu H, Yao Y, Song Y. Long non-coding RNAs: a new frontier in the study of human diseases. Cancer Lett. 2013;339(2):159–66.
12. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009;10(3):155–9.
13. Kong X, Duan Y, Sang Y, Li Y, Zhang H, Liang Y, Liu Y, Zhang N, Yang Q. LncRNA-CDC6 promotes breast cancer progression and function as ceRNA to target CDC6 by sponging microRNA-215. J Cell Physiol. 2019;234(6):9105–17.
14. Jiang N, Meng X, Mi H, Chi Y, Li S, Jin Z, Tian H, He J, Shen W, Tian H, et al. Circulating lncRNA XLOC_009167 serves as a diagnostic biomarker to predict lung cancer. Clin Chim Acta. 2018;486:26–33.
15. Ren J, Ding L, Zhang D, Shi G, Xu Q, Shen S, Wang Y, Wang T, Hou Y. Carcinoma-associated fibroblasts promote the stemness and chemoresistance of colorectal cancer by transferring exosomal lncRNA H19. Theranostics. 2018;8(14):3932–48.
16. Feng J, Yang G, Liu Y, Gao Y, Zhao M, Bu Y, Yuan H, Yuan Y, Yun H, Sun M, et al. LncRNA PCNAP1 modulates hepatitis B virus replication and enhances tumor growth of liver cancer. Theranostics. 2019;9(18):5227–45.
17. Hao L, Wu W, Xu Y, Chen Y, Meng C, Yun J, Wang X. LncRNA-MALAT1: a key participant in the occurrence and development of cancer. Molecules. 2023;28(5):2126.
18. Ma Y, He X, Di Y, Liu S, Zhan Q, Bai Z, Qiu T, Corpe C, Wang J. Identification of prognostic immune-related lncRNAs in pancreatic cancer. Front Immunol. 2022;13:1005695.
19. Huang X, Zhi X, Gao Y, Ta N, Jiang H, Zheng J. LncRNAs in pancreatic cancer. Oncotarget. 2016;7(35):57379–90.
20. Ma Y, Di Y, Li Q, Zhan Q, He X, Liu S, Zou H, Corpe C, Chen L, Wang J. LncRNAs as epigenetic regulators of epithelial to mesenchymal transition in pancreatic cancer. Discov Oncol. 2022;13(1):61.
21. Pan S, Shen M, Zhou M, Shi X, He R, Yin T, Wang M, Guo X, Qin R. Long noncoding RNA LINC01111 suppresses pancreatic cancer aggressiveness by regulating DUSP1 expression via microRNA-3924. Cell Death Dis. 2019;10(12):883.
22. Lu H, Ye J, Zhang L, Li M, Lu S, Yang D, Hu W. Downregulation of LINC01638 lncRNA inhibits migration and invasion of pancreatic ductal adenocarcinoma cells by reducing TGF-beta signaling. Mol Med Rep. 2019;20(5):4533–9.
23. Liu Y, Feng W, Liu W, Kong X, Li L, He J, Wang D, Zhang M, Zhou G, Xu W, et al. Circulating lncRNA ABHD11-AS1 serves as a biomarker for early pancreatic cancer diagnosis. J Cancer. 2019;10(16):3746–56.
24. Liu S, Di Y, Li Q, Chen L, Ma Y, He X, Corpe C, Zhang X, Xu J, Wang J. Exosomal lncRNA LINC01268 promotes pancreatic cancer progression via the miR-217-KIF2A-PI3K/AKT axis. Genes Dis. 2023;10(5):1799–801.
25. Enderle D, Spiel A, Coticchia CM, Berghoff E, Mueller R, Schlumpberger M, Sprenger-Haussels M, Shaffer JM, Lader E, Skog J, et al. Characterization of RNA from exosomes and other extracellular vesicles isolated by a novel spin column-based method. PLoS ONE. 2015;10(8):e0136133.
26. Li Z, Chen Z, Hu G, Zhang Y, Feng Y, Jiang Y, Wang J. Profiling and integrated analysis of differentially expressed circRNAs as novel biomarkers for breast cancer. J Cell Physiol. 2020;235(11):7945–59.
27. Goral V. Pancreatic cancer: pathogenesis and diagnosis. Asian Pac J Cancer Prev. 2015;16(14):5619–24.
28. Wu L, Yang J, Ke RS, Liu Y, Guo P, Feng L, Li Z. Impact of lncRNA SOX9-AS1 overexpression on the prognosis and progression of intrahepatic cholangiocarcinoma. Clin Res Hepatol Gastroenterol. 2022;46(9):101999.
29. Fu C, Nie L, Yin T, Xu X, Lu W. LncRNA EPIC1 promotes proliferation and inhibits apoptosis of gallbladder cancer cells by interacting with LET. Ann Hepatol. 2021;26:100563.
30. Wu X, Jiang T, Huang R, Xiao X. LncRNA GAS8-AS1 downregulates lncRNA NEAT1 to inhibit glioblastoma cell proliferation. Brain Behav. 2021;11(6):e02128.
31. Ren S, Wang F, Shen J, Sun Y, Xu W, Lu J, Wei M, Xu C, Wu C, Zhang Z, et al. Long non-coding RNA metastasis associated in lung adenocarcinoma transcript 1 derived miniRNA as a novel plasma-based biomarker for diagnosing prostate cancer. Eur J Cancer. 2013;49(13):2949–59.
32. Ye D, Liu H, Zhao G, Chen A, Jiang Y, Hu Y, Liu D, Xie N, Liang W, Chen X, et al. LncGMDS-AS1 promotes the tumorigenesis of colorectal cancer through HuR-STAT3/Wnt axis. Cell Death Dis. 2023;14(2):165.
33. Wu T, Li X, Yan G, Tan Z, Zhao D, Liu S, Wang H, Xiang Y, Chen W, Lu H, et al. LncRNA BCAR4 promotes migration, invasion, and chemo-resistance by inhibiting miR-644a in breast cancer. J Exp Clin Cancer Res. 2023;42(1):14.
34. Zhang B, Li C, Sun Z. Long non-coding RNA LINC00346, LINC00578, LINC00673, LINC00671, LINC00261, and SNHG9 are novel prognostic markers for pancreatic cancer. Am J Transl Res. 2018;10(8):2648–58.
35. Rosano S, Parab S, Noghero A, Cora D, Bussolino F. Long non-coding RNA LINC02802 Regulates in vitro sprouting angiogenesis by sponging microRNA-486-5p. Int J Mol Sci. 2022;23(3):1653.
36. Ye J, Chen X, Lu W. Identification and experimental validation of immune-associate lncRNAs for Predicting prognosis in cervical cancer. Onco Targets Ther. 2021;14:4721–34.
37. Liu DH, Yang X, Guo JF, Meng H, Shen SH. Immune-related lncRNAs, LINC01268 and CTB-31O20.2, as favorable prognostic markers for glioma inhibition. Transl Cancer Res. 2022;11(4):823–34.
38. Fantini S, Rontauroli S, Sartini S, Mirabile M, Bianchi E, Badii F, et al. Increased plasma levels of lncRNAs LINC01268, GAS5 and MALAT1 correlate with negative prognostic factors in myelofibrosis. Cancers (Basel). 2021;13(19):4744.
39. Chen B, Li Y, Nie Y, Tang A, Zhou Q. Long non-coding RNA LINC01268 promotes cell growth and inhibits cell apoptosis by modulating miR-217/SOS1 axis in acute myeloid leukemia. Braz J Med Biol Res. 2020;53(8):e9299.


## Publisher's Note