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Abstract. A growing body of evidence confirms that long non-coding RNAs (lncRNAs) have an important role in biological processes by regulating gene expression at multiple levels. Dysregulated lncRNAs may be potential prognostic biomarkers or targets for the development of cancer treatments. However, the prognostic role of an lncRNA signature in pancreatic cancer has not been investigated. Pancreatic cancer lncRNA expression profiles from The Cancer Genome Atlas (TCGA) were analyzed in the current study. The prognostic value of differentially expressed lncRNAs (DElncRNAs) was evaluated via the Kaplan-Meier method. A risk score model was established based on the potential prognostic lncRNAs. The biological functions of lncRNAs were predicted by functional enrichment analysis. Then, an lncRNA-mRNA co-expression network was established and predicted the function of the lncRNAs. Seven DElncRNAs that were significantly associated with the prognosis of pancreatic cancer were identified. Patients were classified into high-risk and low-risk groups using a risk score based on a three-lncRNA signature. There was a significant difference in overall survival (OS) between the groups (median OS 1.33 vs. 3.65 years; log-rank test, P=0.0000). Cox regression analysis and ROC curves demonstrated that the three-lncRNA signature may be an effective independent prognostic biomarker in patients with pancreatic cancer. The functional enrichment analysis showed that lncRNA AL137789.1, one component of the three-lncRNA signature, may be associated with tumor immune responses. In the present study, a novel three-lncRNA signature that was established that may be useful in predicting survival among patients with pancreatic cancer. These lncRNAs may be involved in tumor immunity and thus affect the prognosis of patients.

Introduction
Pancreatic cancer (PC) is one of the most malignant tumor types, with a 5-year overall survival rate of <6% (1). Globally, pancreatic cancer is the fourth most common cause of cancer-associated mortality. By 2030, pancreatic cancer may surpass breast cancer as the second most deadly tumor in the US (2). Patients with pancreatic cancer with no obvious clinical symptoms are frequently not diagnosed until advanced tumor stages. In recent years, with the development of molecular diagnoses and targeted therapies, there has been a huge improvement in diagnosis and treatment. However, pancreatic cancer, as a highly heterogeneous disease, still lacks effective diagnostic and prognostic biomarkers (3). To improve the rate of early diagnosis and extend the survival time of patients with pancreatic cancer, it is essential to identify effective tumor biomarkers for early diagnosis and prognosis risk stratification. This will not only help clinicians choose effective and individualized treatment programs, it will also be conducive to future investigations into the molecular mechanisms of the occurrence and development of pancreatic cancer.

Due to the development of genomic and transcriptomic sequencing techniques, an increasing number of non-coding RNAs (ncRNAs), which differ from protein-coding RNAs, have been identified. ncRNAs are divided into two categories according to size: Short ncRNAs and long ncRNAs (lncRNAs). lncRNAs are defined as >200 nucleotides in length (4). The Human Genome Project has indicated that protein-coding genes account for <2% of the total sequence of the genome (5). Our understanding of lncRNAs is also very limited because the number of ncRNAs, including lncRNAs, is huge. It has been reported that the majority of lncRNAs do not have gene regulatory functions; however,

Novel three-lncRNA signature predicts survival in patients with pancreatic cancer

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there is growing evidence to support the idea that IncRNAs may regulate gene expression at multiple levels, including genomic imprinting and transcriptional and post-transcriptional regulation (6). Chen et al (7) demonstrated that IncRNA small nuclear RNA host gene 20 promotes the proliferation and migration of lung cancer cells by silencing p21 expression. Additionally, the IncRNA DPP10 antisense RNA 1 inhibits the metastasis of colorectal cancer by upregulating TIMP metallopeptidase inhibitor 3, which suggests that this IncRNA could be used as a new therapeutic target for colorectal cancer (8). In recent years, a novel mechanistic type of IncRNA, known as a competing endogenous RNA (ceRNA), has been reported (6). IncRNAs of this class harbor microRNA (miR) response elements and can compete with mRNAs to communicate with miRNAs and to regulate the expression of genes (9). The study demonstrated that the IncRNA colon cancer associated transcript 2 regulates miR-145 expression by inhibiting its maturation in colon cancer cells (10). Another study reported that long intergenic non-protein coding RNA 941 acted as a ceRNA for miR-34a, leading to Snail1 upregulation and epithelial-mesenchymal transition activation in hepatocarcinogenesis (11).

Regarding pancreatic cancer, it has been reported that several IncRNAs, including HOX transcript antisense RNA (12), HOXA distal transcript antisense RNA (13), metastasis associated lung adenocarcinoma transcript 1 (14,15) and long intergenic non-protein coding RNA, regulator of reprogramming (16), are differentially expressed between pancreatic cancer tissues and normal tissues. These findings indicate that IncRNAs may be potential diagnostic or prognostic biomarkers for pancreatic cancer. However, a single IncRNA biomarker is often limited in its diagnostic and prognostic predictive value. Recent studies have reported that several IncRNA signatures may improve prognosis predictions for certain malignant tumors, including lymphoma (17), colorectal (18), esophageal (19) and lung cancer (20). However, the prognostic role of IncRNAs in pancreatic cancer has not been investigated.

To determine an effective signature of IncRNAs for predicting pancreatic cancer survival, IncRNA expression profiles from a large number of patients with pancreatic cancer were obtained and analyzed by repurposing the publicly available Cancer Genome Atlas (TCGA) database in this study.

**Materials and methods**

**Data processing.** The IncRNA expression data and corresponding clinical data of pancreatic cancer were obtained from the TCGA database (portal.gdc.cancer.gov/) using the search terms ‘Project = TCGA-PAAD’. The data included IncRNA expression profiles of 178 cancer tissue samples and 4 normal tissue samples from 177 patients with pancreatic cancer. Analysis of differentially expressed IncRNAs (DEIncRNAs) between pancreatic tumor tissues and normal tissues was performed using the edgeR package (bioconductor.org/packages/release/bioc/html/edgeR.html) in R (software version 3.1.4; r-project.org) (21). IncRNAs satisfying the conditions log₂fold change (FC)>1.0 and P<0.05 were considered to be differentially expressed. Unsupervised hierarchical cluster analysis using the gplots package (cran.r-project.org/web/packages/gplots/index.html) in R (software version 3.1.4; r-project.org) revealed that normal tissues could be distinguished from pancreatic cancer tissues based on the DEIncRNA patterns. Unsupervised hierarchical cluster analysis evaluated the degree of variation of DEIncRNA patterns from pancreatic cancer tissues and normal tissues.

**Association of DEIncRNAs with patient prognosis.** Patients with no prognostic information were excluded and then combined the DEIncRNA expression profiles with the corresponding survival prognostic information. The prognostic value of each DEIncRNA was evaluated using a Kaplan-Meier curve and the log-rank method. Patients were divided into the high expression and low expression groups for each of the IncRNAs according to the median value of the expression. The DEIncRNAs that were significantly associated with overall survival (OS) were identified as potential prognostic IncRNA biomarkers.

**Cox regression analysis and the establishment of a prognostic risk score model.** The potential prognostic IncRNAs obtained by Kaplan-Meier survival analysis were included in a Cox regression analysis. In the Cox regression analysis, it was attempted to identify the best signature associated with patient survival by using the least number of IncRNAs. Then, a prognostic risk model consisting of multiple pancreatic cancer IncRNAs was established, and a risk score was computed as

\[
\text{Risk score} = \sum_{i=1}^{N} (E_i \times C_i)
\]

follows:

\[
\text{Risk score} = (N, \text{the number of prognostic IncRNAs}; E_i, \text{the expression value of IncRNA}_i; C_i, \text{the estimated repression coefficient of IncRNA}_i \text{in the multivariable Cox regression analysis}).
\]

The risk score for each patient was calculated, and all patients were divided into two groups (a high-risk group and a low-risk group) according to the median risk score. The difference in prognosis between the high-risk group and the low-risk group was evaluated by the Kaplan-Meier method. Then, to evaluate the prognostic risk score model for the 3- and 5-year survival rates of patients with pancreatic cancer, the receiver operating characteristic (ROC) curve was analyzed. The area under the ROC curve (AUC) was calculated from the ROC curves.

**Establishment of an IncRNA-mRNA co-expression network and IncRNA functional predictions by bioinformatics analysis.** An IncRNA-mRNA co-expression network using the WCGNA package in R. The co-expression relationships between the DEIncRNAs and DEprotein-coding genes were calculated using Pearson correlation coefficients (the cut-off of Pearson correlation coefficient was >0.6). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of the co-expressed protein-coding genes with DEIncRNAs were performed to predict the biological function of DEIncRNAs using the DAVID Bioinformatics Tool version 6.8 (david.ncifcrf.gov/). Enrichment analysis was performed using the functional annotation chart and was limited to GO terms in the ‘Biological Process direct’ and KEGG pathways categories. Functional annotations with a P<0.05 and an enrichment score of >2 were considered significant.
Statistical analysis. T-test for continuous variables and χ² test for categorical variables were performed to assess the relationship between the prognostic risk score and clinical features. In the χ² analysis, when the total number of samples was <40 or the expected value of the sample as <1, Fisher exact test was used. Univariate and multivariate Cox regression analyses were performed to verify if the predictive indicators of prognostic risk score calculated from multiple lncRNAs were independent of other clinical features. Statistical analyses were performed using IBM SPSS statistics software program version 22.0 (IBM Corp., Armonk, NY, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristic. In this study, expression profiles of 178 samples of pancreatic cancer tissue and 4 samples of normal tissue were used to identify DElncRNAs. These samples were from 177 patients with pancreatic cancer included the following clinical and pathological characteristics: Age, sex, tumor site, tumor size, grade, stage, TNM status, chronic pancreatitis, smoking, alcohol consumption and diabetes (Table I).

DElncRNAs between pancreatic tumor tissues and normal tissues. According to the screening criteria (P<0.05 and |log₂ FC|>2.0), a total of 92 DElncRNAs were identified between pancreatic tumor tissues and normal tissues, including 3 (3.3%) upregulated and 89 (96.7%) downregulated lncRNAs. In order to visually display DElncRNA between the two groups, the results were presented as a volcano plot (Fig. 1). Unsupervised hierarchical cluster analysis showed that normal tissues could be distinguished from pancreatic cancer tissues based on the DElncRNA patterns (Fig. 2).

Identification of potential lncRNA biomarkers significantly associated with OS from DElncRNAs. Kaplan-Meier curves and the log-rank method were used to identify potential lncRNA biomarkers significantly associated with OS from the DElncRNAs. The results showed that seven lncRNAs [AC008033.3, AC079015.1, MIR600 host gene (MIR600HG), AP003086.1, FAM53B antisense RNA 1 (FAM53B-AS1), DNAH17 antisense RNA 1 (DNAH17-AS1), AL137789.1; Table II] were significantly associated with pancreatic cancer prognosis. Of these, five were positively associated with OS (AC008033.3, AC079015.1, MIR600HG, AP003086.1 and FAM53B-AS1; Fig. 3A-E), and two were negatively associated with OS (DNAH17-AS1 and AL137789.1, Fig. 3F and G).

Establishment of a three-lncRNA signature associated with pancreatic cancer patient survival. Three lncRNAs (MIR600HG, AL137789.1, AC079015.1) were selected from seven potential prognostic lncRNAs for logistic regression analysis according to the algorithm by trying to incorporate fewer lncRNAs, while achieving the best fit for patient's survival. Then, a formula was established using these three-lncRNAs to assess prognostic risk as follows: Risk score = (0.3073 x expression value of AL137789.1) + (-0.2044 x expression value of AC079015.1) + (-0.7195 x expression value of MIR600HG). The risk score for each patient was calculated and all patients were divided into a high-risk group (88 cases) and

| Characteristic | Number of patients |
|---------------|--------------------|
| Age (years)   |                    |
| <60/≥60       | 54/123             |
| Sex           |                    |
| Male/female   | 97/80              |
| Tumor site    |                    |
| Head/other/NA | 138/28/11          |
| Tumor size    |                    |
| <4/≥4 cm/NA   | 96/68/13           |
| Grade         |                    |
| I/IIA/IIB/III/IV | 21/28/117/3/4    |
| T             |                    |
| T1/T2/T3/T4/Tx | 8/24/141/3/1      |
| N             |                    |
| N0/N1/Nx      | 49/123/5           |
| M             |                    |
| M0/M1/Mx      | 79/4/94            |
| Chronic pancreatitis |        |
| None/yes/NA   | 126/14/37         |
| Smoking (years) |                |
| <3/≥3/NA      | 84/58/35          |
| Drinking      |                    |
| None/yes/NA   | 64/101/12         |
| Diabetes      |                    |
| None/yes/NA   | 108/37/32         |

NA, not available; T, tumor; N, node; M, metastasis.

Figure 1. Volcano plot of differentially expressed lncRNAs. Red dots represent upregulated lncRNAs and green dots represent downregulated lncRNAs. lncRNA, long non-coding RNA; FC, fold change; FDR, false discovery rate.
a low-risk group (89 cases) according to the cut-off median risk score (Fig. 4A and B). The OS of the high-risk group was significantly shorter than that of the low-risk group (median OS 1.33 vs. 3.65 years; P<0.0001). The Kaplan-Meier survival curve is shown in Fig. 4C. As the risk score rose, the expression of AL137789.1 increased, and the expression of MIR600HG and AC079015.1 decreased (Fig. 4D). To evaluate the ability of the risk score to predict 3- and 5-year survival rates, a
A time-dependent ROC curve was used (Fig. 5). The results showed that the AUC for the lncRNA signature prognostic model was 0.742 for an OS of 3 years and 0.793 for an OS of 5 years. These results suggested an improvement in survival predictions based on the expression of these three pancreatic cancer lncRNAs.

**Association between the three-lncRNA signature risk score and clinicopathological characteristics.** Significant differences in tumor size, American Joint Committee on Cancer (AJCC) (22) stage, AJCC tumor status (T) and AJCC node status (N) were identified between patients in high and low risk scores; however, there was no difference in age, sex, tumor site, grade, AJCC metastasis status, chronic pancreatitis or smoking between the patients with high and low risk scores (Table III).

**Prognostic value of the three-lncRNA signature is independent of other clinicopathological factors.** Furthermore, univariate analyses were used to test the prognostic value of the three-lncRNA signature on OS. The results showed that risk score [hazard ratio (HR)=2.442; P<0.001], tumor site
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(HR=0.343; P=0.004), grade (HR=1.584; P=0.035), AJCC stage (HR=2.295; P=0.001), AJCC T (HR=1.774; P=0.051), and AJCC N (HR=1.968; P=0.008) were associated with OS in patients with pancreatic cancer (Table IV). These significant indicators from univariate analyses were subsequently included in multivariate Cox regression analyses (because AJCC stage is not independent of the AJCC T and AJCC N indicators, AJCC stage was excluded as an indicator). The results of multivariate Cox regression analyses indicated that risk score (HR=1.909; P=0.007) is an independent prognostic factor for pancreatic cancer and that a high risk score is associated with a poor prognosis (Table IV).

Establishment of an lncRNA-mRNA co-expression network and lncRNA functional predictions. To investigate the potential biological functions of the DElncRNAs, a co-expression network was established between DElncRNAs and DEprotein-coding genes (Fig. 6A). The expression of 153 protein-coding genes from 1,452 DEMRNAs was highly correlated with 18 DElncRNAs (Pearson correlation coefficient >0.60). IncRNA AL137789.1, which is part of the three-lncRNA signature, was also in this co-expression network. Subsequently, the co-expressed protein-encoding genes were subjected to GO and KEGG pathway enrichment analyses, with the background of the whole human genome. GO functional annotation suggested that 153 protein-coding genes were significantly enriched in 63 GO terms. The top 20 GO terms are presented in Fig. 6B. KEGG functional annotation suggested that protein-coding genes were significantly enriched in 40 KEGG pathways. The top 20 pathways are presented in Fig. 6C. The results from GO and KEGG analyses were associated with immune responses involving T, B and natural killer cells. Taken together, these results suggested that lncRNAs may be involved in the tumor immune response via interactions with protein-coding genes.

Discussion

The traditional view suggests that non-coding RNAs do not affect gene expression. However, these non-coding RNAs regulate the expression of genes at different levels, and it has been reported that they can even encode proteins involved in biological processes (23). Increasing numbers of abnormally expressed lncRNAs have been identified in a variety of tumors (6). These lncRNAs have complex biological functions, including tumor promotion and tumor suppression, which may be closely associated with tumorigenesis and tumor progression.

It has been reported that the lncRNAs HOXA11 antisense RNA (24), DiGeorge syndrome critical region gene 5 (25), maternally expressed 3 (26) and hepatocellular carcinoma upregulated long non-coding RNA (27) promote metastasis and tumor cell proliferation and are associated with poor prognosis in cancer patients. However, the lncRNAs CPS1 intronic transcript 1 (28), cancer susceptibility 2 (29), long intergenic non-protein-coding RNA 589 (30) and growth arrest specific 5 (31) can inhibit the proliferation and metastasis of
Table III. Association between the risk score of three-long non-coding RNAs signature and clinicopathological characteristics.

| Characteristic          | Number of patients (%) | Low risk | High risk | $\chi^2$ | P-value |
|------------------------|------------------------|----------|-----------|----------|---------|
| Total patients         |                        | 89 (100) | 88 (100)  |          |         |
| Age (years)            |                        |          |           | 0.077    | 0.782   |
| <60                    |                        | 28 (31.5)| 26 (29.5) |          |         |
| ≥60                    |                        | 61 (68.5)| 62 (70.5) |          |         |
| Sex                    |                        |          |           | 0.950    | 0.330   |
| Male                   |                        | 52 (58.4%)| 45 (51.1%)|          |         |
| Female                 |                        | 37 (41.6%)| 43 (48.9%)|          |         |
| Tumor site             |                        |          |           | 0.202    | 0.889   |
| Head                   |                        | 67 (75.3)| 71 (80.7) |          |         |
| Others                 |                        | 14 (15.7)| 14 (15.9) |          |         |
| NA                     |                        | 8 (9.0)  | 3 (3.4)   |          |         |
| Tumor size (cm)        |                        |          |           | 5.796    | 0.016   |
| <4                     |                        | 54 (60.7)| 42 (47.7) |          |         |
| ≥4                     |                        | 27 (30.3)| 41 (46.6) |          |         |
| NA                     |                        | 8 (9.0%) | 5 (5.7%)  |          |         |
| Grade                  |                        |          |           | 0.005    | 0.945   |
| $G_1+G_2$              |                        | 62 (69.7)| 61 (69.3) |          |         |
| $G_3+G_4$              |                        | 26 (29.2)| 25 (28.4) |          |         |
| $G_X$                  |                        | 1 (1.1)  | 2 (2.3)   |          |         |
| AJCC stage             |                        |          |           | 4.192    | 0.041   |
| I+IIA                  |                        | 31 (34.8)| 18 (20.5) |          |         |
| IIIB+III+IV            |                        | 56 (62.9)| 68 (77.3) |          |         |
| NA                     |                        | 2 (2.2)  | 2 (2.3)   |          |         |
| AJCC T                 |                        |          |           | 6.116    | 0.013   |
| $T_1+T_2$              |                        | 22 (24.7)| 10 (11.4) |          |         |
| $T_3+T_4$              |                        | 66 (74.1)| 78 (88.7) |          |         |
| $T_X$                  |                        | 1 (1.1)  | 0 (0.0)   |          |         |
| AJCC N                 |                        |          |           | 5.256    | 0.022   |
| $N_0$                  |                        | 31 (34.8)| 18 (20.5) |          |         |
| $N_1$                  |                        | 54 (60.7)| 69 (78.4) |          |         |
| $N_X$                  |                        | 4 (4.5)  | 1 (1.1)   |          |         |
| Chronic pancreatitis   |                        |          |           | 0.013    | 0.910   |
| None                   |                        | 65 (73.0)| 61 (69.3) |          |         |
| Yes                    |                        | 7 (7.9)  | 7 (8.0)   |          |         |
| NA                     |                        | 17 (19.1)| 20 (22.7) |          |         |
| Smoking (years)        |                        |          |           | 1.049    | 0.306   |
| <3                     |                        | 45 (50.6)| 39 (44.3) |          |         |
| ≥3                     |                        | 26 (29.2)| 32 (36.4) |          |         |
| NA                     |                        | 18 (20.3)| 17 (19.3) |          |         |
| Alcohol consumption    |                        |          |           | 0.004    | 0.951   |
| None                   |                        | 32 (36)  | 32 (36.4) |          |         |
| Yes                    |                        | 51 (57.3)| 50 (56.8) |          |         |
| NA                     |                        | 6 (6.7)  | 6 (6.8)   |          |         |

Table III. Continued.

| Characteristic          | Number of patients (%) | Low risk | High risk | $\chi^2$ | P-value |
|------------------------|------------------------|----------|-----------|----------|---------|
| Diabetes               |                        |          |           | 1.651    | 0.199   |
| None                   |                        | 51 (57.3)| 57 (64.8) |          |         |
| Yes                    |                        | 22 (24.7)| 15 (17.0) |          |         |
| NA                     |                        | 16 (18.0)| 16 (18.2) |          |         |

NA, not available; AJCC, American Joint Committee on Cancer; T, tumor; N, node; M, metastasis.

Figure 4. Three-lncRNA risk score analysis of the TCGA patients. The distribution of the three-lncRNA risk score, overall survival status and lncRNA expression signature were analyzed in the entire TCGA patient cohort (n=177). (A) Three-lncRNA risk score distribution; (B) patient overall survival status and survival length. The dotted line divides the patients into low-risk and high-risk groups. Patients were divided into two groups based on median risk score. (C) Kaplan-Meier curves for low- and high-risk patients with pancreatic cancer. (D) Risk heatmap of the three-lncRNA expression profiles. As the risk score rose, the expression of AL137799.1 increased, and the expression of MIR600HG and AC079015.1 decreased. Rows represent lncRNAs in the signature, and columns represent patients. TCGA, The Cancer Genome Atlas; MIR600HG, MIR600 host gene.
tumor cells, which indicates a favorable survival. The role of certain lncRNAs, such as taurine upregulated 1 (TUG1), in tumors is controversial. Studies have reported that TUG1 contributes to tumor progression (32-34) via its role in the promotion of cell proliferation, metastasis and inhibition of cell apoptosis. Thus, it is thought to act as an oncogene in various cancers, such as ovarian (35), thyroid (34) and colorectal cancer (36). However, other studies have reported that the lncRNA TUG1 is a tumor suppressor (37,38). A similar controversy occurred for the lncRNA prostate cancer associated 3 (PCA3) (39,40). However, ncRNA accounts for 80% of the whole genome, and the general understanding of the function of lncRNAs is still superficial. Many lncRNAs have not been functionally investigated.

In pancreatic cancer, several single lncRNA biomarkers have been reported, including lncRNA PVT1 oncogene (41), CCDC26 lncRNA (42) and long intergenic non-protein coding RNA, p53 induced transcript (43). The same controversy that occurred around PCA3 in prostate cancer may also occur in pancreatic cancer. The expression of lncRNAs is relatively low, thus it may be easy to introduce bias when using a single lncRNA as a biomarker. Utilizing a combination of multiple potential lncRNA biomarkers could improve accuracy. In this study, a three-lncRNA signature that is associated with OS was established by analyzing the RNA-sequencing data from 178 patients with pancreatic cancer from the TCGA database.

Table IV. Univariate and multivariate analyses of the three-long non-coding RNA signature in the prediction of pancreatic cancer overall survival.

| Univariate analysis | HR (95% CI) | P-value |
|---------------------|-------------|---------|
| Risk score (high vs. low) | 2.442 (1.576-3.784) | 0.000 |
| Age, years (<60 vs. <60) | 1.291 (0.820-2.035) | 0.270 |
| Sex (female vs. male) | 0.900 (0.597-1.357) | 0.616 |
| Tumor site (other vs. head) | 0.343 (0.165-0.712) | 0.004 |
| Tumor Size (≥4 cm vs. <4 cm) | 1.016 (0.666-1.549) | 0.943 |
| Grade (G1 + G2 vs. G3 + G4) | 1.584 (1.032-2.432) | 0.035 |
| AJCC stage (IIB + III + IV vs. I + IIA) | 2.295 (1.383-3.808) | 0.001 |
| AJCC T (T1 + T2 vs. T3 + T4) | 1.774 (0.997-3.159) | 0.051 |
| AJCC N (N1 vs. N0) | 1.968 (1.191-3.252) | 0.008 |
| AJCC M (M1 vs. M0) | 0.571 (0.078-4.192) | 0.581 |
| Chronic pancreatitis (yes vs. none) | 1.008 (0.460-2.208) | 0.983 |
| Smoking (3 years vs. <3 years) | 0.941 (0.595-1.490) | 0.797 |
| Alcohol consumption (yes vs. none) | 1.249 (0.805-3.159) | 0.322 |
| Diabetes (yes vs. none) | 0.902 (0.518-1.569) | 0.322 |

| Multivariate analysis | HR (95% CI) | P-value |
|----------------------|-------------|---------|
| Risk score (high vs. low) | 1.909 (1.195-3.050) | 0.007 |
| Age, years (<60 vs. <60) | 0.415 (0.194-0.885) | 0.023 |
| Sex (female vs. male) | 1.643 (1.052-2.567) | 0.029 |
| Tumor site (other vs. head) | 0.937 (0.496-1.769) | 0.841 |
| Tumor Size (≥4 cm vs. <4 cm) | 1.596 (0.877-2.904) | 0.126 |
| Grade (G1 + G2 vs. G3 + G4) | 1.643 (1.052-2.567) | 0.029 |
| AJCC stage (IIB + III + IV vs. I + IIA) | 1.909 (1.195-3.050) | 0.007 |
| AJCC T (T1 + T2 vs. T3 + T4) | 0.415 (0.194-0.885) | 0.023 |
| AJCC N (N1 vs. N0) | 1.596 (0.877-2.904) | 0.126 |
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| Alcohol consumption (yes vs. none) | 1.249 (0.805-3.159) | 0.322 |
| Diabetes (yes vs. none) | 0.902 (0.518-1.569) | 0.714 |

HR, hazard ratio; CI, confidence interval; AJCC, American Joint Committee on Cancer; T, tumor; N, node; M, metastasis.

Figure 5. ROC curves of the multivariate logistic regression model with or without risk score using the three-lncRNA signature to predict (A) 3-year and (B) 5-year survival. ROC, receiver operating characteristic; OS, overall survival; AUC, area under the curve.
Figure 6. Co-expression network and functional enrichment results. (A) Co-expression network between DElncRNAs and DEprotein-coding genes. lncRNAs, pink hexagons; mRNAs, green ovals. (B) Top 20 significantly enriched GO terms from the co-expressed protein-coding genes and DElncRNAs. (C) Top 20 significantly enriched Kyoto Encyclopedia to Genes and Genomes pathways from the co-expressed protein-coding genes and DElncRNAs. DE, differentially expressed; lncRNA, long non-coding RNA; GO, Gene Ontology.
knowledge, these three lncRNAs have not been previously reported in any cancer. The expression of all three lncRNAs was significantly lower in tumor tissues than in normal tissues. Further, the expression of these lncRNAs is closely associated with the OS of patients with pancreatic cancer. Lower expression of AC079015.1 and MIR600HG was associated with poorer OS, whereas lower expression of lncRNA AL137789.1 was associated with favorable survival. A calculation for prognosis risk score in patients with pancreatic cancer was established according to the expression of the three lncRNAs. The formula indicates that a higher risk score is associated with a poorer prognosis. Multivariate analyses demonstrated that the three-lncRNA signature was an independent prognostic predictor of survival for patients with pancreatic cancer patient. Further, ROC analyses showed that the AUC of the three-lncRNA signature was 0.742 for the prediction of 3-year OS and 0.793 for the prediction of 5-year OS. These results demonstrate that these three lncRNAs have a high accuracy in predicting the prognosis of patients with pancreatic cancer. In analyses of the association between risk score and clinicopathological characteristics, high risk scores were significantly associated with larger tumor size and higher TNM stage. This indicated that high risk is associated with tumor progression from another perspective.

Thus far, the function of these three lncRNAs is unknown. A lncRNA-mRNA co-expression network was used to predict the function of lncRNAs by functional analysis of protein-coding genes. The GO and KEGG enrichment results implied that the DElncRNAs may be involved in tumorigenesis through effects on the tumor immune response. Unfortunately, AL137789.1 was the only lncRNA from three-lncRNA signature that was involved in the co-expression network. AL137789.1 may increase the malignancy of pancreatic cancer by affecting the tumor immune microenvironment, despite the current lack of supporting evidence. A large number of studies have demonstrated that tumor biological behavior is regulated by the tumor immune microenvironment. In pancreatic cancer, the tumor immune microenvironment is more complicated due to the rich stromal environment of the pancreas. Tumor-infiltrating lymphocytes and cytokines secreted from the stroma constitute the main components of the tumor immune microenvironment, and have a role in the immune regulation of tumors. However, lncRNAs may be involved in certain tumor immunity processes and may aid in tumor cell immune evasion (44,45). Notably, studies have reported that lncRNAs are involved in the human immune system, including roles in dendritic cells, T cells and macrophages (44,45). Jiang et al (45) reported that the lncRNA Inc-epidermal growth factor receptor stimulates T-regulatory cell differentiation, thus promoting hepatocellular carcinoma immune evasion. Another study reported that the lncRNA IFNG antisense RNA 1, which is expressed by Th1 cells, specifically decreases the differentiation of T-cells toward the Th2 phenotype (46).

In summary, the current study established a three-lncRNA signature associated with pancreatic cancer by examining lncRNA expression profiles from the TCGA database. Further analysis demonstrated that the three-lncRNA signature may be an independent prognostic biomarker of survival of patients with pancreatic cancer. Functional predictions revealed that one of these three lncRNAs, AL137789.1, may be associated with the tumor immune response.

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Availability of data and materials
The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors’ contributions
BW and ZZ designed the experiments and analyzed the data; YB and XW analyzed the data; KW, FC and JG performed lncRNA functional predictions; JF and ZS performed survival analysis; all authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate
Ethical approval was obtained from the Second Hospital of Jiaxing (Jiaxing, China).

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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