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Original Research

Construction of a paclitaxel-related competitive endogenous RNA network and identification of a potential regulatory axis in pancreatic cancer

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ABSTRACT

Background: Increasing numbers of studies have elucidated the role of competitive endogenous RNA (ceRNA) networks in carcinogenesis. However, the potential role of the paclitaxel-related ceRNA network in the innate mechanism and prognosis of pancreatic cancer has not been identified.

Methods: Comprehensive bioinformatics analyses were performed to identify drug-related miRNAs (DRmiRNAs), drug-related mRNAs (DRmRNAs) and drug-related lncRNAs (DRlncRNAs) and construct a ceRNA network. The ssGSEA and CIBERSORT algorithms were utilized for immune cell infiltration analysis. Additionally, we validated our paclitaxel-related ceRNA regulatory axis at the gene expression level; functional experiments were conducted to explore the biological functions of the key genes.

Results: A total of 182 mRNAs, 13 miRNAs, and 53 lncRNAs were confirmed in the paclitaxel-related ceRNA network. In total, 6 mRNAs, 4 miRNAs, and 6 lncRNAs were identified to establish a risk signature and exhibited optimal prognostic effects. The mRNA signature can predict the abundance of immune cell infiltration and the sensitivity of different chemotherapeutic drugs and may also have a guiding effect in immune checkpoint therapy. A potential PART1/hsa-mir-21/SCRN1 axis was confirmed according to the ceRNA theory and was verified by qPCR. The results indicated that PART1 knockdown markedly increased hsa-mir-21 expression but inhibited SCRN1 expression, weakening the proliferation and migration abilities.

Conclusions: We hypothesized that the paclitaxel-related ceRNA network strongly influences the innate mechanism, prognosis, and immune infiltration of pancreatic cancer. Our risk signatures can accurately predict survival outcomes and provide a clinical basis.

Introduction

Pancreatic cancer is a malignant tumor with increasing incidence and high mortality, and the worldwide cumulative number of new cases and deaths in 2020 exceeded 100,000 [1]. Many patients have already lost the opportunity for surgery when they are diagnosed because of the untimely detection of tumors. Additionally, the drug resistance of chemotherapy is a challenge to many physicians and patients, making the treatment effect unsatisfactory [2]. Therefore, research on drug resistance mechanisms and the exploration of ideal prognostic markers are critical.

Increasing evidence has elucidated the critical role of various types of RNAs in the innate mechanism of cancer occurrence and progression [3]. LncRNAs are noncoding RNAs that are longer than 200 nucleotides, and miRNAs are noncoding single-stranded RNA molecules with a length of approximately 22 nucleotides encoded by endogenous genes

Abbreviations: TCGA, the cancer genome atlas; GEO, gene expression omnibus; GC, Genome Consortium; ssGSEA, single-sample gene set enrichment analysis; AUC, area under the curve.

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[4]. Emerging medical literature has highlighted that lncRNAs are involved in the regulation of cancer biology, including proliferation, migration, cell cycle, and immune infiltration [5]. Interestingly, recent studies indicate crosstalk between lncRNAs and mRNAs through the sponging of miRNAs [6,7]. The mediators of this interaction are called miRNA response elements (MREs), and the mutual regulation of lncRNAs-miRNAs-mRNAs is defined as a competing endogenous RNA (ceRNA) network [8]. Recently, an increasing number of studies have elucidated the potential role of ceRNA networks in carcinogenesis [9]. Yang et al found that the lncRNA LINC01133 regulated APC expression by competitively sponging miR-106a-3p and thus promoted gastric cancer progression and metastasis [10]. The lncRNA GSTM3TV2 was also found to function as a ceRNA and competitively sponge let-7, facilitating gemcitabine resistance in pancreatic cancer [11]. In the past, most studies of ceRNAs in pancreatic cancer focused on cell and animal experiments. The development of sequencing technology in recent years has made it possible to analyze high-throughput data and construct high-throughput ceRNA networks, which may provide new insights into pancreatic cancer pathology and therapeutic strategies.

In our current study, comprehensive bioinformatics analysis was performed to conduct drug sensitivity-related investigations and identify paclitaxel-related ceRNA networks. In addition, we comprehensively explored the potential role of the mRNA signature in prognosis, immune landscape and therapeutic sensitivity, with a focus on chemotherapy effects. The concrete analysis process is shown in Fig. 1.

Materials and methods

Data collection

The compound activity and RNA-seq data of 60 cancer cells listed by the National Cancer Institution were downloaded from the CellMiner database. The sensitivity data of paclitaxel were selected for correlation analysis to identify the correlated lncRNAs, miRNAs, and mRNAs. In addition, to further construct our ceRNA network and perform clinical correlation analysis, we downloaded the transcriptome and clinical data of pancreatic cancer patients from The Cancer Genome Atlas (TCGA) database. All clinical samples included were eventually diagnosed as pancreatic cancer and must have completed follow-up data and clinicopathological data (sex, age, race, T stage, N stage, M stage, total stage, and alcohol). After excluding patients with missing clinical information and expression information, 154 patients with complete information were included in our follow-up analysis (Detailed clinical data is

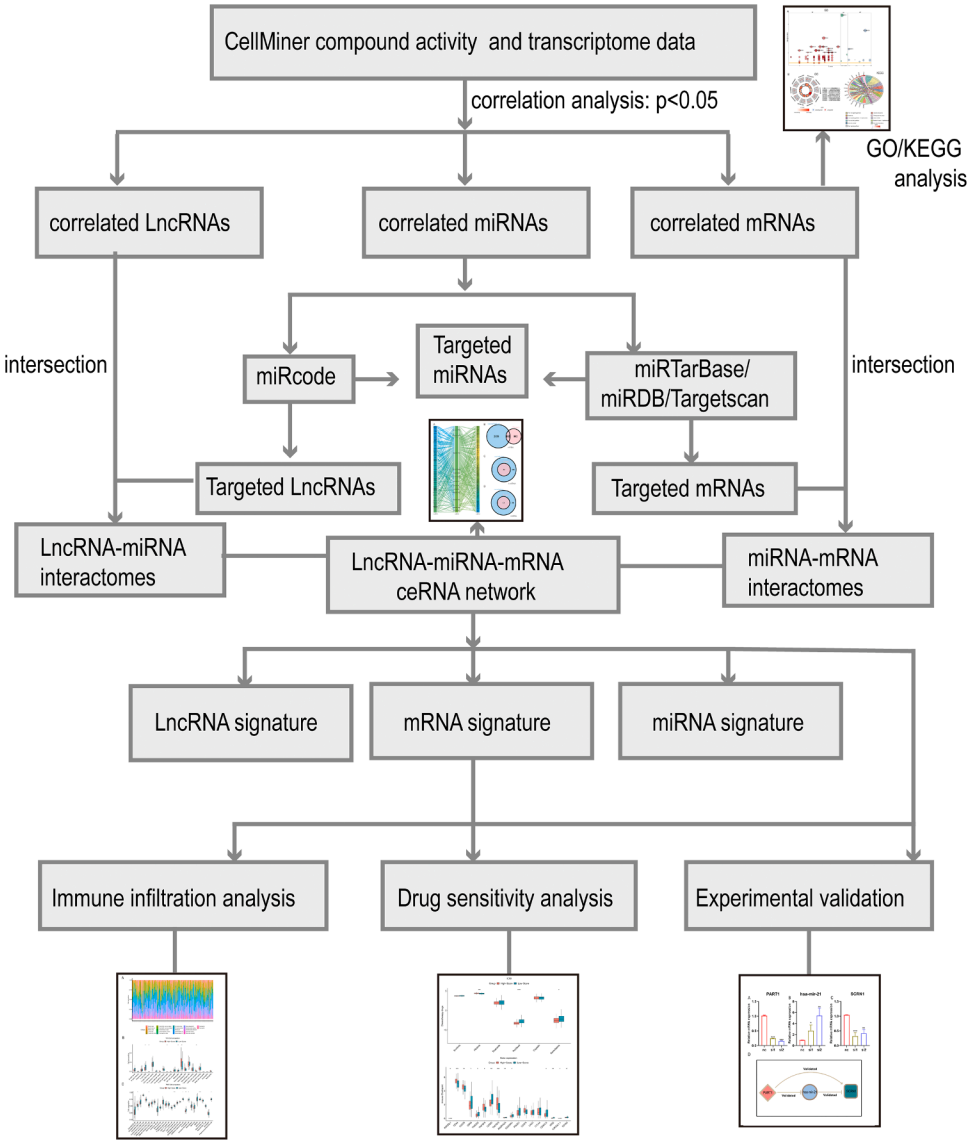

Fig. 1. Research design and analysis process.

summarized in Table S1 and Table S2). Transcriptome data from the Gene Expression Omnibus (GEO) database were used as an external cohort for verification.

Drug sensitivity analysis

Transcriptome data and drug sensitivity data of paclitaxel were used for correlation analysis. LncRNAs, miRNAs or mRNAs with $|r| > 0.2$ and $p < 0.05$ were confirmed to be drug-related.

Construction of the ceRNA network

To identify an accurate and stable ceRNA network, we used multiple databases to make predictions for targeted mRNAs and lncRNAs based on drug-related miRNAs (DRmiRNAs) and took the intersection with our drug-related mRNAs (DRmRNAs) and drug-related lncRNAs (DRlncRNAs). The miRNA-targeted mRNAs were predicted by miRDB, TargetScan, and miRTarBase. Only the overlapping mRNAs of the three databases were intersected with the drug-related mRNAs to identify the terminal targeted mRNAs. The interactions between DRmiRNAs and DRlncRNAs were confirmed by miRcode, which contains complete and integrated information about miRNA-targeted lncRNAs. The R package "ggalluvial" was used to identify the ceRNA network of paclitaxel.

Functional annotation analysis

We performed functional enrichment analysis to explore the potential biological functions and pathways in which DRmRNAs may be involved. The functional enrichment analysis mainly included Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, and the R package "clusterProfiler" was used to conduct the enrichment analyses.

Establishment of a significant risk signature

To further explore the prognostic value of key molecules of the ceRNA network in pancreatic cancer, we established a risk stratification method for lncRNAs, miRNAs, and mRNAs. Univariate Cox regression was used to initially screen for valuable prognostic molecules, and multivariate Cox regression was used to establish a risk signature. The R package "Survival" was used to perform the univariate and multivariate Cox regression analyses. In addition, survival curves were used to verify the ability of the risk stratification method to distinguish different risks, while receiver operating characteristic (ROC) curves were used to identify the stability and accuracy of the model.

Immune infiltration and drug sensitivity analyses

Single-sample gene set enrichment analysis (ssGSEA) and CIBERSORT algorithms were used to estimate the immune infiltrating cell abundance of 154 pancreatic cancer patients from the TCGA database. Twenty-eight and 22 tumor-infiltrating immune cells were estimated by the ssGSEA and CIBERSORT algorithms, respectively. Furthermore, we compared the immune cell infiltration abundance between the high- and low-risk groups according to the mRNA risk signature. We then constructed a risk stratification method based on significantly different immune cells.

Cell culture and quantitative RT–PCR

One human pancreatic cancer cell line (PANC-1) was chosen to knock down the expression of PART1. The cDNA of PANC-1 cells was extracted to detect the expression of hsa-mir-21 and SCRN1. PANC-1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 1% antifungal. The primer sequences of the lncRNAs and mRNAs were as follows: PART1 forward: 5′-CCCTTTCACTATGAAGGACC-3′, PART1 reverse 5′- ATTTACCCGTCCAGTTCTG-3′; SCRN1 forward: 5′- CAGAGATGAAGTGCAAGAGG -3′, SCRN1 reverse: 5′- GAAATGTAAGTGCACTCAACC -3′; and GAPDH forward: 5′-CAGGAGGCATTGCTGATGAT-3′, and GAPDH reverse: 5′-GAAGGCTGGGGCTCATTT-3′. The miRNA reverse transcription primers and qPCR primers were all designed by RIBOBIO company (Guangzhou, China). We normalized our expression data by using GAPDH and U6 as controls and calculated the relative mRNA expression level by the $2^{-\Delta\Delta Ct}$ method.

Transwell migration assay

We cultured PANC-1 cells in a Transwell chamber for 24 h. The upper transwell chamber was filled with 200 μl of serum-free medium and PANC-1 cells (5×10^4 cells), and the lower chamber was filled with 500 μl of medium with 20% FBS. The transwell chambers were fixed, stained and washed with paraformaldehyde, 1% crystal violet and PBS. We then captured and counted the migrating cells in 5 random areas per chamber.

EdU assay

PANC-1 cells (3×10^4 cells) were cultured in 96-well plates for 12 h and incubated with EdU working solution for two hours (BeyoClick^{TM} EdU Cell Proliferation Kit with Alexa Fluor 594, Shang Hai, China). After fixation, membrane rupture and nuclear staining, we used a fluorescence microscope (OLYMPUS, Tokyo, Japan) to capture fluorescence images.

Results

Identifying DRlncRNAs, DRmiRNAs, and DRmRNAs of paclitaxel

With the cutoffs of $|r| > 0.2$ and $p < 0.05$ for the correlation analysis of drug sensitivity, the DRlncRNAs, DRmiRNAs, and DRmRNAs of paclitaxel were identified. A total of 2502 DRmRNAs, 13 DRmiRNAs, and 194 DRlncRNAs were confirmed.

Construction of a paclitaxel sensitivity-related ceRNA network

Based on the prediction of the miRNA–mRNA pairs from miRDB, TargetScan, and miRTarBase, as well as the miRNA-lncRNA pairs from miRcode, the main components of the ceRNA network of paclitaxel were identified by intersection with DRmiRNAs and DRlncRNAs. A total of 182 mRNAs, 13 miRNAs, and 53 lncRNAs were confirmed in the ceRNA network of paclitaxel. The Sankey diagram shows the detailed information of paclitaxel's ceRNA network (Fig. 2A), while the Venn diagram is used to show the construction process of the ceRNA network (Fig. 2B–D).

The risk stratification signature exhibited optimal prognostic competence and stability

A total of 182 mRNAs, 13 miRNAs, and 53 lncRNAs were included in the univariate and multivariate Cox regression analyses to construct a risk stratification signature. Six mRNAs, 4 miRNAs, and 6 lncRNAs were reserved to establish a risk signature (TCGA cohort) (Fig. 3). Surprisingly, the three prognostic models all showed strong stability and prediction accuracy. The risk score curves show the distribution and expression of patient risk ratios under the three models (Fig. 3A–C), and the survival curves show the prognostic prediction ability of the model (Fig. 3D–F). The low-risk group in the mRNA model showed a longer survival time than the high-risk group, and the same conclusion was reached in the miRNA model and the lncRNA model. We drew ROC curves to evaluate the accuracy of the model. The 1-, 2-, and 3-year areas under the curve (AUCs) of the mRNA model were 0.74, 0.82, and 0.83,

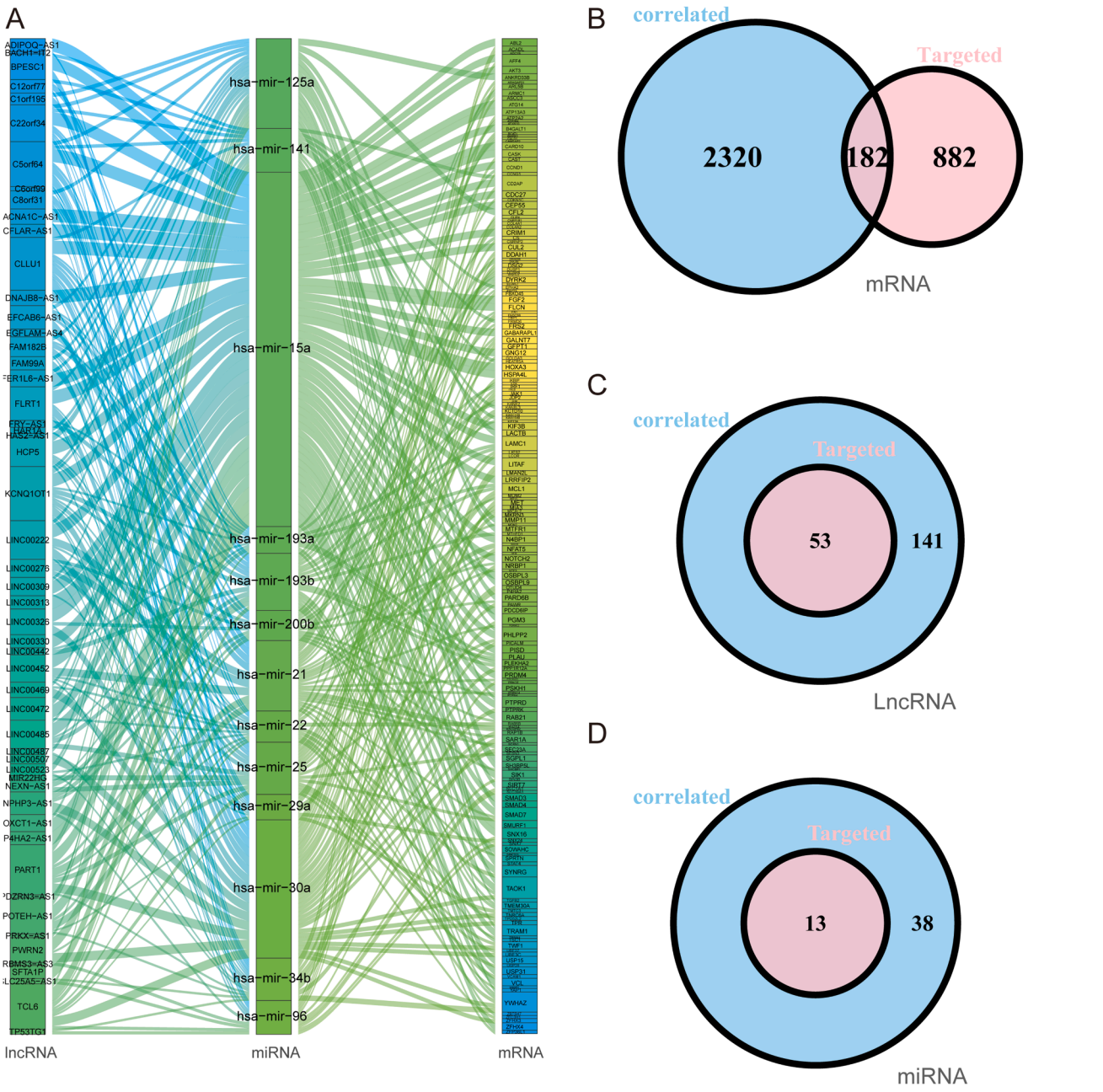

Fig. 2. Drug-related ceRNA network of paclitaxel. (A) The Sankey diagram shows the detailed information of paclitaxel's ceRNA network. (B) The Venn diagram shows the intersection of DRmRNAs and targeted mRNAs. (C) The Venn diagram shows the intersection of DRlncRNAs and targeted LncRNAs. (D) The Venn diagram shows the intersection of DRmiRNAs and targeted miRNAs.

respectively (Fig. 3G). Similarly, the miRNA model and the lncRNA model also exhibited high AUCs at 1, 2, and 3 years (Fig. 3H, I), further indicating the robustness and accuracy of our model. In view of the universal applicability of coding genes in detection and application, subsequent analysis of immune infiltration and other analyses were based on the mRNA model. In addition, we validated our mRNA model in two GEO cohorts, and the result was consistent with that in the TCGA cohort (Fig. S1).

Functional annotation

To explore the potential functions of DRmRNAs in pancreatic cancer, we conducted enrichment analysis to explore the biological functions and related pathways of these DRmRNAs. GO analysis indicated that the DRmRNAs were mainly enriched in functions correlated with cell proliferation, migration and adhesion, including "epithelial cell proliferation", "cell–cell junction organization", "focal adhesion assembly" and "cell junction assembly" (Fig. S2A, S2B). Additionally, KEGG analysis indicated that the DRmRNAs are primarily involved in the "PI3K–Akt signaling pathway", "Hippo signaling pathway", "Proteoglycans in cancer", "Ras signaling pathway" and "Adherens junction", which are associated with carcinogenesis and progression of pancreatic cancer (Fig. S2C).

The risk stratification signature exhibited a critical influence on the immune landscape and drug sensitivity

To further evaluate immune cell infiltration in pancreatic cancer, two immune infiltration assessment methods, CIBERSORT and ssGSEA, were used to calculate the immune cell abundance. A convolutional

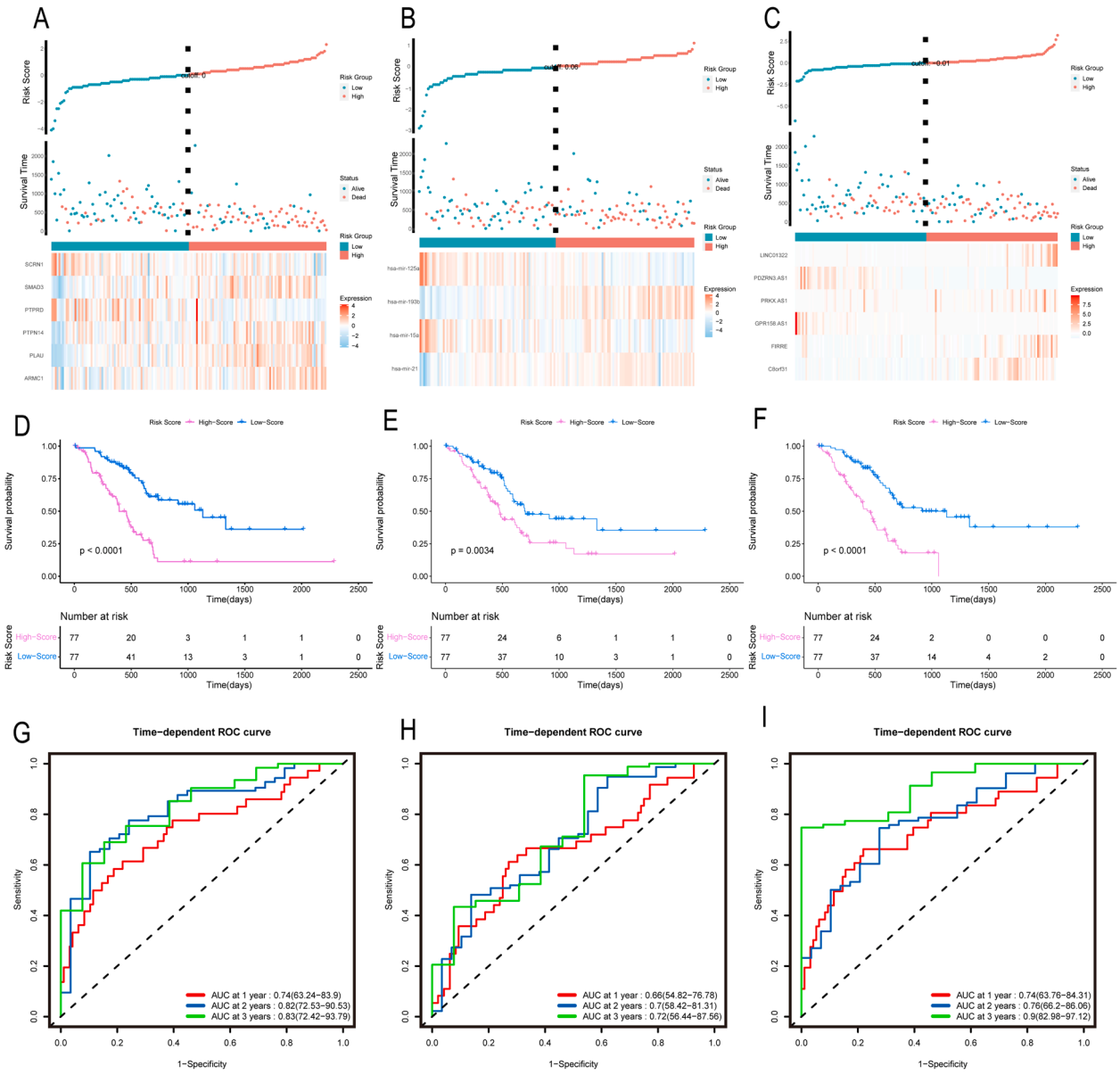

Fig. 3. The risk stratification signature exhibited optimal prognostic competence and stability. (A–C) Risk score curves of 3 risk signatures showing the distribution of each patient's risk score and survival time. (D–F) The survival curves of the 3 risk signatures reflect the prognosis of patients with different risk scores. (G–I) The ROC curves of the 3 risk signatures verified the accuracy of our risk signatures.

histogram was used to show the proportion of different immune cells (Fig. 4A), and the box plot exhibited the difference in the immune cell proportion between the high- and low-risk groups of the mRNA model (Fig. 4B, C). Overall, the low-risk group exhibited a higher proportion of immune cells than the high-risk group. In CIBERSORT's assessment results, 5/6 differential immune cells ($p < 0.05$) exhibited higher abundance in the low-risk group (Fig. 4B). Similarly, in the ssGSEA results, 5/8 differential immune cells ($p < 0.05$) exhibited higher abundance in the low-risk group (Fig. 4C), which was consistent with the prognostic results. To further explore the potential role of these differentially distributed immune cells in pancreatic cancer, we conducted univariate Cox regression on immune cells and found that only three immune cells in the ssGSEA results had prognostic capabilities (Fig. S3A). Multivariate Cox regression identified 2 immune cells in the immune cell risk model (Fig. S3B), and the results suggested that the low-risk group had a better survival time (Fig. S3C). The model exhibited good predictive ability, and its AUC was lower than that of our mRNA model, which further illustrates the superiority of our mRNA model (Fig. S3C).

We further explored the immune checkpoint expression and IC50 difference of several commonly used chemotherapeutic drugs between the high- and low-risk groups (mRNA signature). The results suggested that three drugs, nilotinib, paclitaxel, and gemcitabine, showed differences in sensitivity between different risk groups (Fig. 5A), and most immune checkpoints exhibited differences between different risk groups (Fig. 5B), suggesting that our risk model may have a certain indicative effect on the use of chemotherapy drugs and immune checkpoint treatment drugs.

Exploration of the potential regulatory axis and experimental verification in pancreatic cancer

According to the ceRNA hypothesis, miRNA expression is negatively correlated with lncRNA or mRNA expression. In addition, lncRNAs can also positively regulate mRNA expression indirectly. To select the most meaningful ceRNA regulatory axis, we only chose mRNAs, miRNAs or lncRNAs that are significantly related to the prognosis of pancreatic

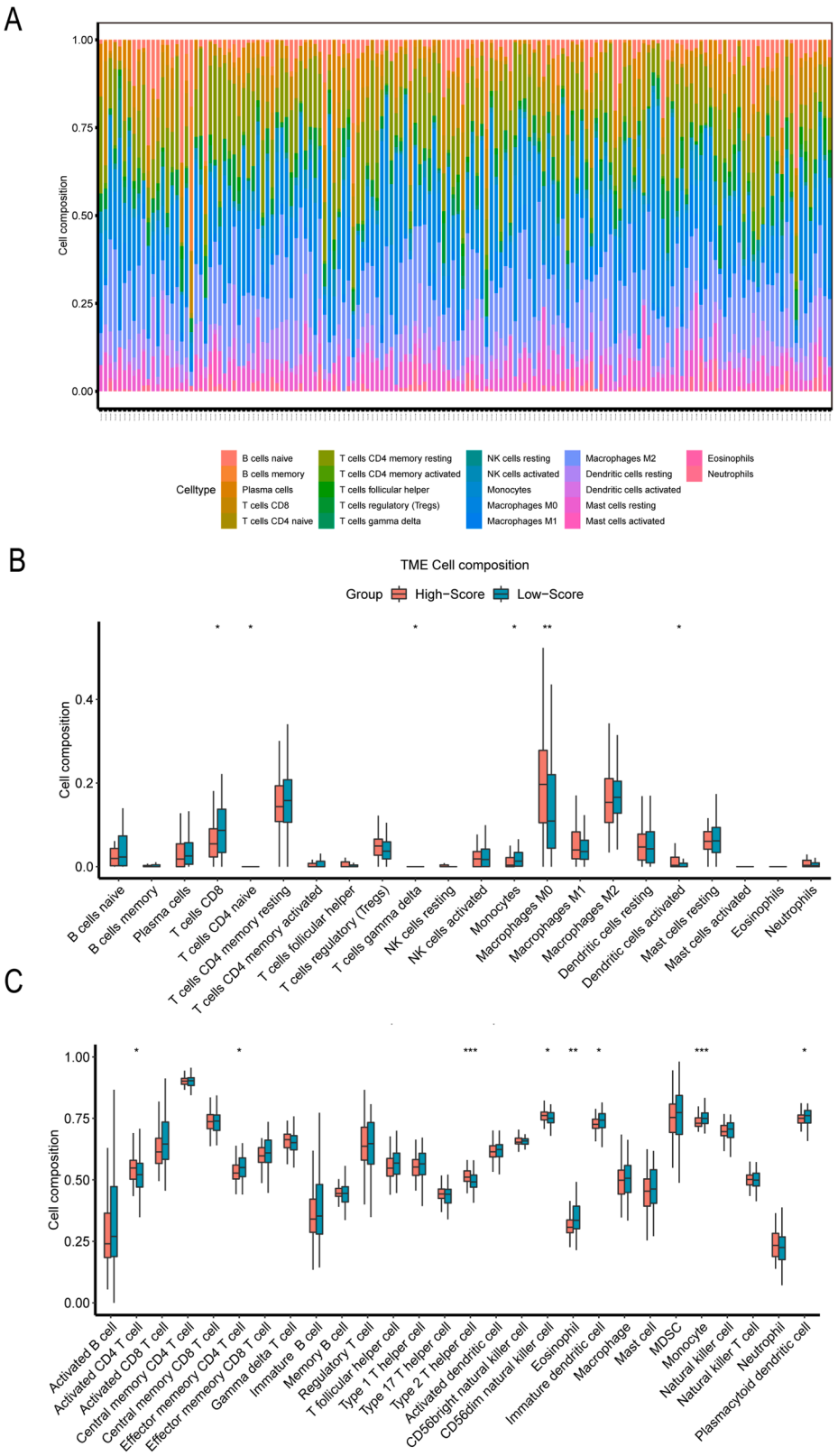

Fig. 4. The risk stratification signature exhibited critical influence on the immune landscape. (A) The convolution histogram shows the proportion of immune cells in different samples. (B) Immune cell abundance analysis between the high- and low-risk groups (CICBERSOFT). (C) Immune cell abundance analysis between the high- and low-risk groups (ssGSEA).

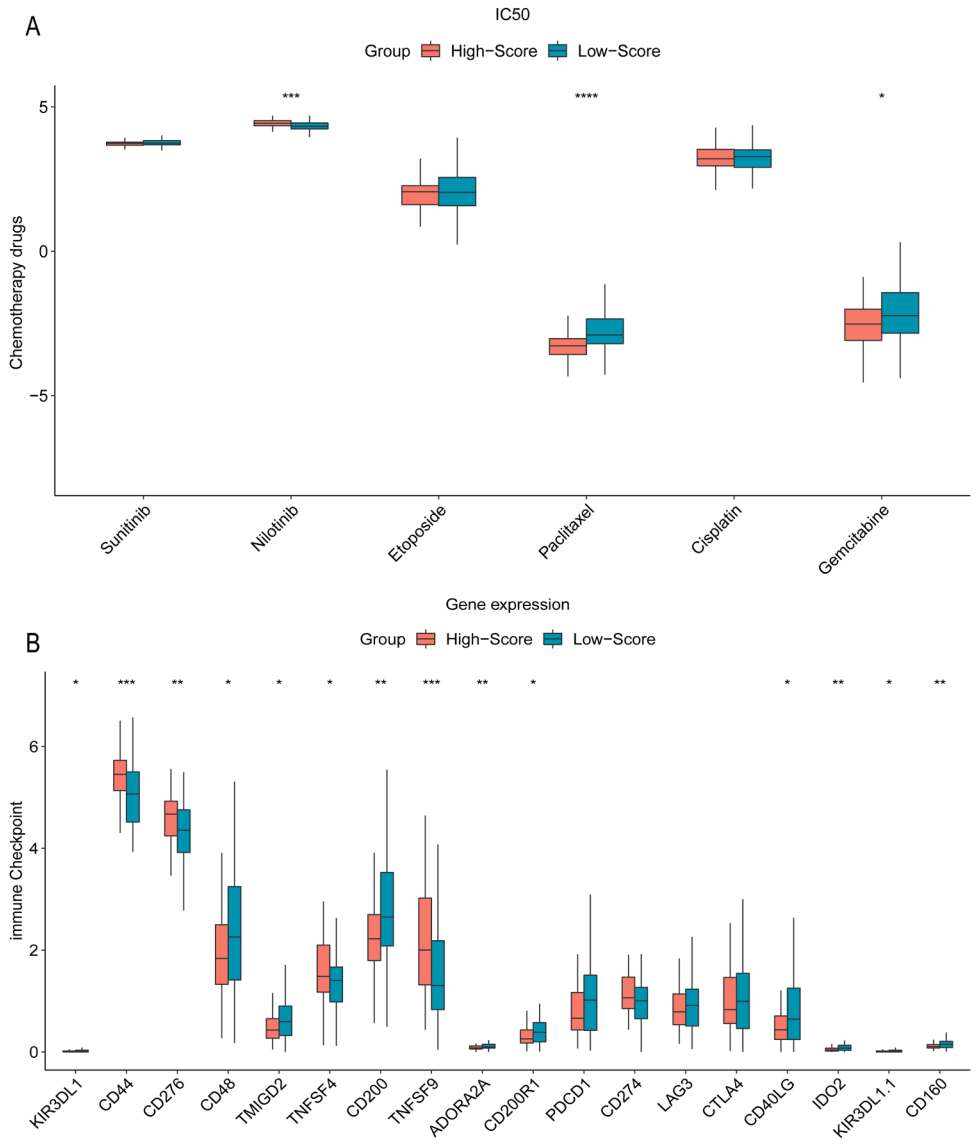

Fig. 5. The potential predictive competence of the risk signature for chemotherapy and immune therapy. (A) Competency of our risk signature to predict the IC50 values of 6 chemotherapy drugs. (B) Correlation analysis between the risk score and crucial immune checkpoint expression levels.

cancer. Cox analysis indicated that only hsa-mir-21, hsa-mir-125a, hsa-mir-15a, and hsa-mir-193b among the miRNA categories met the inclusion criteria. Furthermore, we established possible regulatory axes based on these four miRNAs. In the regulatory axis centered by hsa-mir-125a, we identified 7 lncRNAs and 3 mRNAs with prognostic value from the previously established ceRNA (Fig. S4). Further correlation analysis showed that HCP5 (lncRNA), LACTB (mRNA) and MTFR1 (mRNA) expression was negatively correlated with hsa-mir-125a, while HCP5 expression was positively correlated with LACTB and MTFR1 (Fig. 6A, B). We identified HCP5/hsa-mir-125a/(LACTB or MTFR1) as possible regulatory axes. Similarly, in the regulatory axis centered on hsa-mir-193b, FRY-AS1 and TSC1 were negatively correlated with hsa-mir-193b, while FRY-AS1 and TSC1 were positively correlated (Figs. S4, 6C, 6D). Therefore, we identified FRY-AS1/hsa-mir-193b/TSC1 as a possible regulatory axis. We used the same method to identify PART1/hsa-mir-21/SCRN1 as a potential regulatory axis (Figs. S5, 6E, 6F). However, no mRNA negatively correlated with hsa-mir-15a was found in the regulatory axis. To conclude, 3 regulatory axes were identified as potential regulatory mechanisms (Fig. 6G–I). Furthermore, we identified PART1/hsa-mir-21/SCRN1 as the possible adjustment axis for verification. We knocked down the expression of PART1 in PANC-1 cells (Fig. 7A) and detected the expression changes of downstream hsa-mir-21 and SCRN1 (Fig. 7B, C). The results indicated that PART1 knockdown markedly increased hsa-mir-21 expression but inhibited SCRN1 expression, which suggested the existence of a PART1/hsa-mir-21/SCRN1 regulatory axis (Fig. 7D).

Knockdown of PART1 notably inhibited the proliferation and migration of cancer cells in pancreatic cancer

After knocking down the expression of PART1 in PANC-1 cells, we tested the changes in its biological behavior. We found that the proliferation ability of the PART1 knockdown group was significantly lower than that of the control group (Fig. 8A), and its migration ability was also significantly inhibited (Fig. 8B), which suggested that PART1 may be a potential oncogene in pancreatic cancer.

Discussion

Generally, pancreatic cancer remains a malignancy with a high fatality rate and poor prognosis [1]. Immunotherapy has not had much of an impact on pancreatic cancer patients, and relatively few new

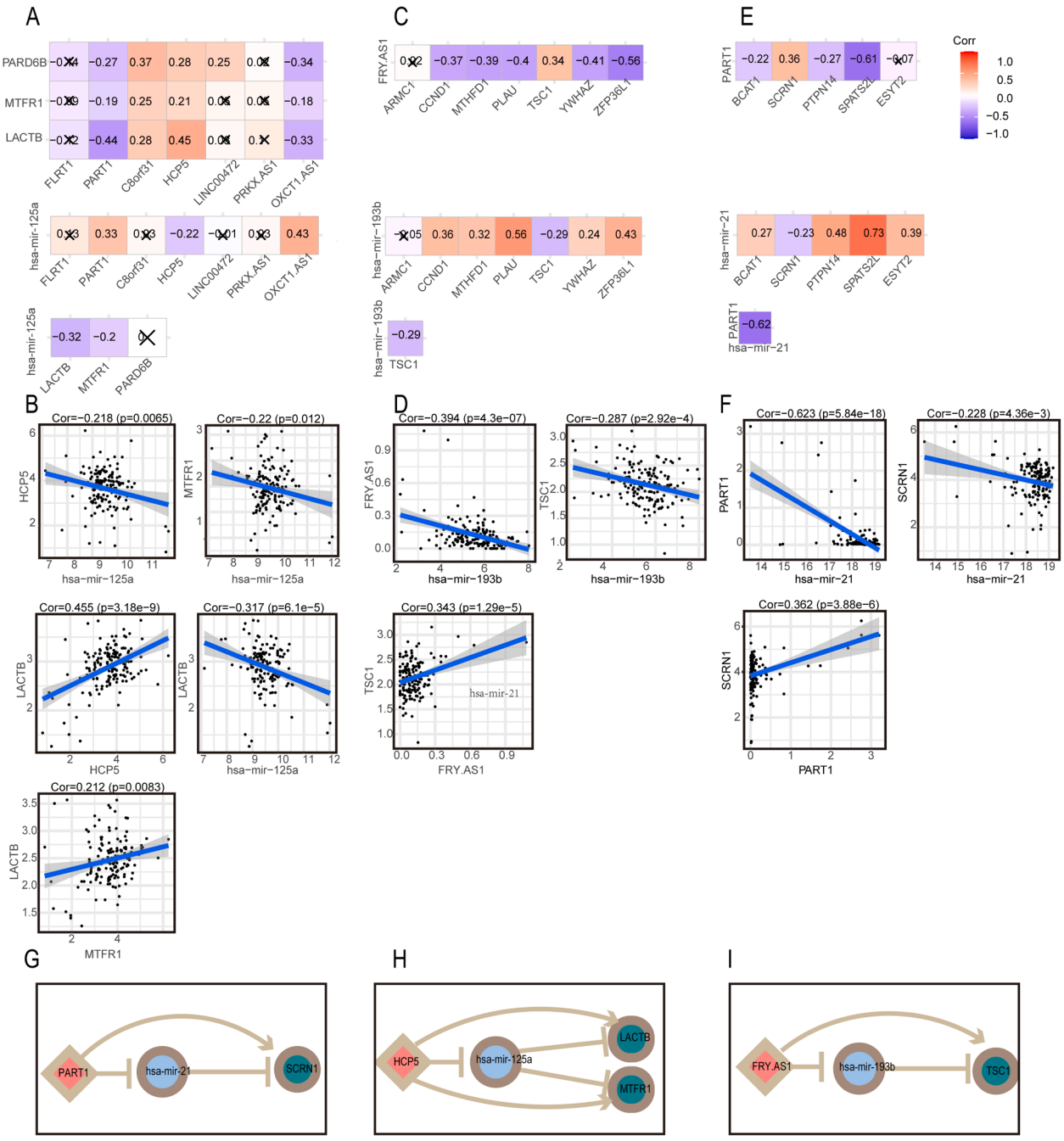

Fig. 6. Exploration of the potential regulatory axis and experimental verification in pancreatic cancer. (A, B) Correlation analysis based on the hsa-miR-125a-related ceRNA network. (C, D) Correlation analysis based on the hsa-mir-193b-related ceRNA network. (E, F) Correlation analysis based on the hsa-miR-21-related ceRNA network. (G) Potential regulatory axis based on hsa-mir-125a. (H) Potential regulatory axis based on hsa-mir-193b. (I) Potential regulatory axis based on hsa-mir-21.

treatments have been developed in recent decades [2]. Further exploration of more effective and accurate prognostic biomarkers and underlying mechanisms is needed to promote rapid progress in the diagnosis and treatment of pancreatic cancer. Noncoding RNAs acting as oncogenes or tumor suppressors in cancer biology have been studied in recent years [12], and the mutual regulation between lncRNAs and mRNAs binding to the same miRNA through the MRE has been defined as a ceRNA network [8]. The dysregulation of the ceRNA regulatory network and ceRNA molecules could promote the progression of various diseases, including malignancies. Although our understanding of ceRNA regulatory networks is still relatively simple, further research on this topic will help deepen our understanding of diseases and develop new therapies [8].

Conjugated paclitaxel is a typical and commonly used drug for pancreatic cancer. Drug sensitivity analysis and ceRNA network construction based on paclitaxel may play a guiding role in pancreatic cancer drug development and prognosis judgment. In the current study, expression data and paclitaxel sensitivity data from the CellMiner database were utilized to identify DRlncRNAs, DRmiRNAs, and DRmRNAs and to construct a ceRNA network. Risk signatures of mRNAs, miRNAs, and lncRNAs were established, and we elucidated the special role of the mRNA risk signature in immune regulation and drug sensitivity. Additionally, based on the results of the RNA mutual regulation network and correlation analysis verification, we constructed four potential ceRNA regulation axes, which may provide new insights for the understanding of pathogenesis and drug development in pancreatic

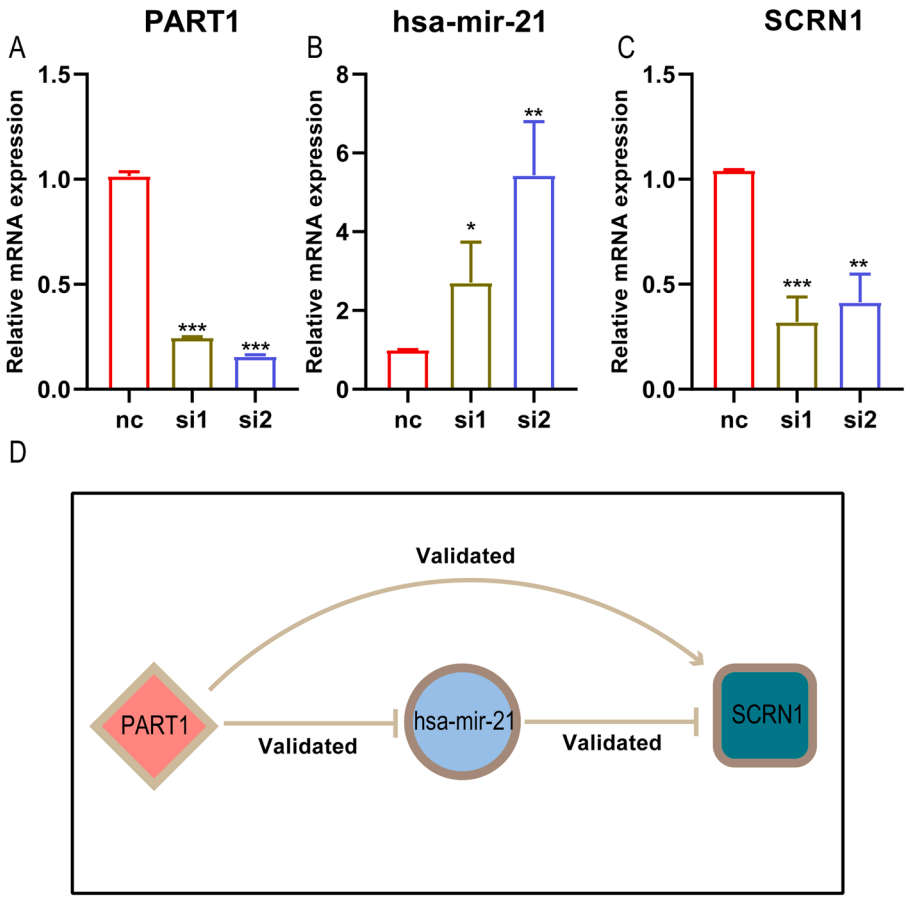

Fig. 7. Experimental verification of the potential regulatory axis in pancreatic cancer.
(A) PART1 expression levels were knocked down. (B) PART1 knockdown increased hsa-mir-21 expression. (C) PART1 knockdown inhibited the expression of SCRN1. (D) The validated PART1/hsa-miR-21/SCRN1 regulatory axis.

cancer. mRNAs can regulate the biological expression of oncogenes or tumor suppressors by serving as ceRNAs, which are beneficial or harmful to tumor progression [13]. In our study, 6 mRNAs (SMAD3, PTPRD, PTPN14, PLAU, ARMC1, and SCRN1) were found to be associated with the prognosis of pancreatic cancer and were used to construct a risk signature. SMAD3 is a typical oncogene that has been reported by many scientists. Tang et al reported that SMAD3 inhibited E4BP4-mediated NK-cell proliferation and promoted cancer progression [14]. PTPRD and PTPN14 act as tumor suppressors and are correlated with a better prognosis of cancer [15,16]. Interestingly, PLAU was found to induce fibroblast conversion and to activate the uPAR/Akt/NF-κB/IL8 pathway in esophageal squamous cell carcinoma [17]. ARMC1 is a dual-localization protein related to mitochondrial localization, and it has not been reported to be related to tumor progression [18]. SCRN1 is a novel biomarker that has been reported to be correlated with colorectal cancer [19], gastric cancer [20], and prostate cancer [21]. In our ceRNA network, SCRN1 could interact with hsa-mir-21 and was a critical component of the PART1/has-mir-21/SCRN1 regulatory axis, which was validated in our cytology experiment. To conclude, these mRNAs may play an irreplaceable role in the physiology, pathology and treatment of pancreatic cancer, and further cytological and histological experiments are needed to verify their applicable effect.

LncRNAs are long noncoding RNAs that can have a significant impact on human health and disease [22]. Recent research has focused on transcriptome changes in various tumors, and some researchers have found that lncRNAs can promote tumor occurrence by functioning as ceRNAs [23]. We identified 6 lncRNAs that have a significant influence on survival from the ceRNA network and used them to construct a lncRNA-related risk signature. Our risk signature exhibited extremely high prognostic prediction capabilities, with a three-year AUC higher than 0.9, revealing the nonnegligible role of lncRNAs in pancreatic cancer. Additionally, we identified 3 lncRNAs from the ceRNA regulatory axis that may be critical to the drug sensitivity and pathophysiological process of pancreatic cancer and verified the potential role of PART1. We concluded that PART1 was able to facilitate the proliferation and migration of pancreatic cancer cells and was a potential oncogene. Subsequent investigation is needed to confirm its clinical value and practical application ability. miRNAs lie in a pivotal position of the ceRNA network, as they regulate the expression level and biological status of different ceRNAs through the shared MRE [8]. Previous research has focused on the influence of miRNAs on the internal mechanism of tumorigenesis [24]. Based on paclitaxel sensitivity analysis, our research built a ceRNA network and identified ceRNA regulatory axes with miRNAs as the pivot, filling the gap in pancreatic cancer drug sensitivity research to a certain extent. In addition, we revealed that hsa-mir-21 may be involved in the occurrence and development of pancreatic cancer, and its regulation of the biological behavior of pancreatic cancer and its internal mechanism are worthy of further experimental exploration.

Research on the immune microenvironment has become a research hotspot in recent years, and increasing evidence shows that the immune microenvironment plays a crucial role in immune regulation and immunotherapy [25]. Our research has discovered the important role of the mRNA model in immune cell regulation and immune infiltration abundance prediction. In addition, we further explored the potential applicable capacity of the mRNA model in immune checkpoint expression and the sensitivity of commonly used chemotherapeutics, further highlighting the reliability and practicality of the paclitaxel-based

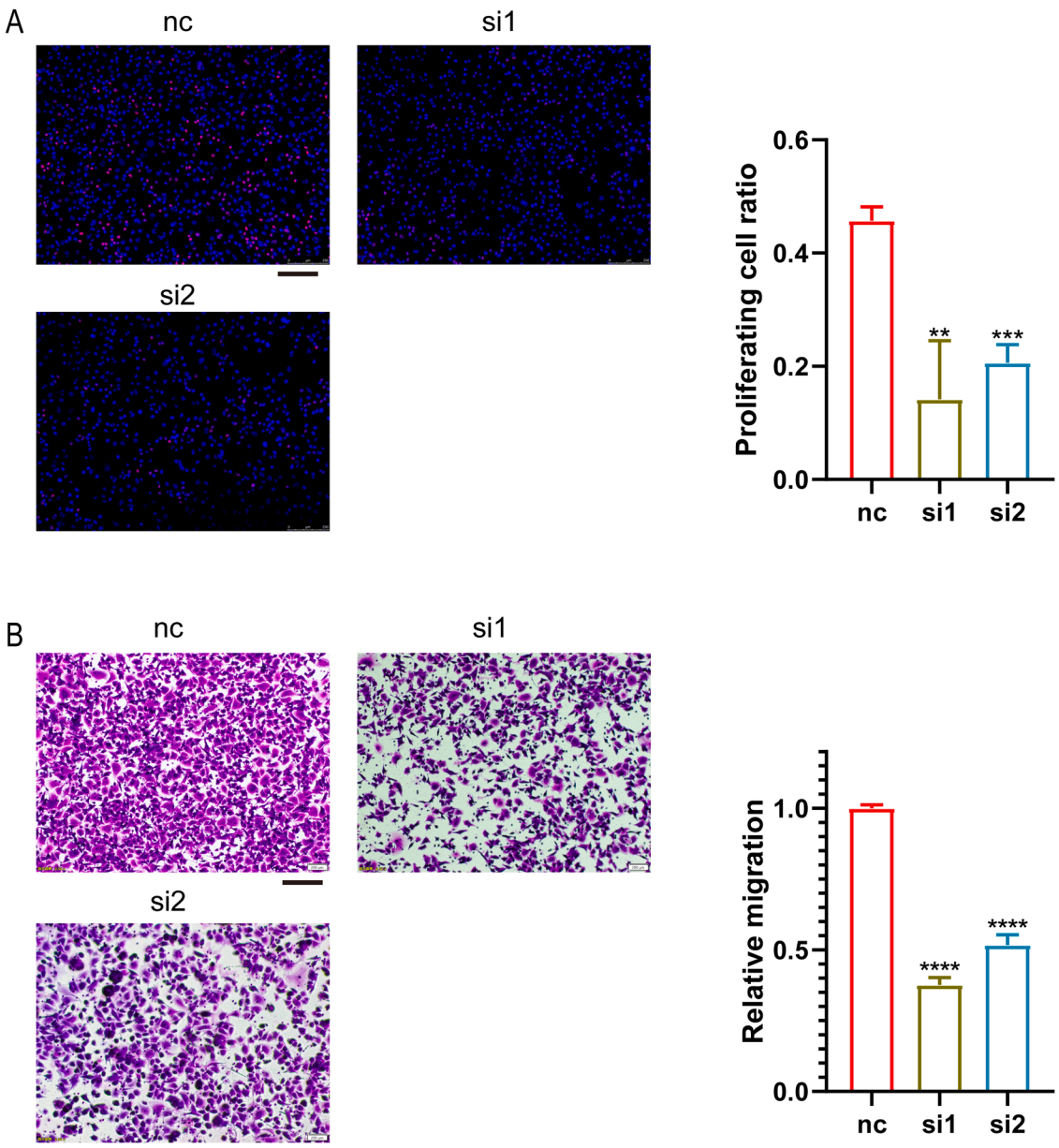

Fig. 8. Knockdown of PART1 inhibited the proliferation and migration of cancer cells in pancreatic cancer. (A) The proliferation ability of the PART1 knockdown group was inhibited. (B) The migration ability of the PART1 knockdown group was downregulated.

signature.

Our research has some benefits. On the one hand, this is the first study to identify key molecules related to paclitaxel sensitivity and construct a ceRNA network based on drug sensitivity data, which may provide new ideas and insights for future research. Additionally, we yielded several new regulatory networks for paclitaxel and verified their reliability in experiments, which provided new targets for intrinsic drought mechanism research. Furthermore, we proposed different types of prognostic features related to paclitaxel, all of which have good prognostic competence, and our mRNA signature also exhibits exemplary performance in chemotherapy sensitivity and immune infiltration judgment.

Our research also has some limitations. First, our validation set and training set came from different platforms, and differences in data processing methods may cause instability of the results. Second, our patient data volume is relatively small, which warrants further verification in a larger external data set. Our merit is that our ceRNA regulatory axis was supported by cell experiments, and the evidence is more sufficient.

Conclusion

In conclusion, we comprehensively analyzed the drug sensitivity data and constructed a paclitaxel-related ceRNA network as well as a ceRNA regulatory axis. The paclitaxel-related mRNA signature can predict the abundance of infiltrating immune cells and the sensitivity to different chemotherapeutic drugs. Future research is needed to further explore the underlying mechanisms of these regulatory axes and develop new targets for clinical treatment.

Fig. S1 Validation of the mRNA risk signature in two GEO cohorts. (A) Validation of the mRNA risk signature in GSE57495. (B) Validation of the mRNA risk signature in GSE62452.

Fig. S2 Functional annotation of DRmRNAs in pancreatic cancer. (A, B) GO analysis of the DRmRNAs. (C) KEGG analysis of the DRmRNAs.

Fig. S3 Exploration of the immune cell signature in pancreatic cancer. (A, B) Univariate and multivariate regression analyses of immune

cells. (C) The survival curves of the immune cell signature reflect the prognosis of patients with different risk scores. (D) The ROC curves of immune cell signatures verified the accuracy.

Fig. S4 Screening the prognostic mRNAs and LncRNAs in the potential regulatory axis. (A, B) Screening the prognostic mRNAs and lncRNAs in the hsa-miR-125a-related regulatory axis. (C, D) Screening the prognostic mRNAs and lncRNAs in the hsa-mir-193b-related potential regulatory axis.

Fig. S5 Screening the prognostic mRNAs and LncRNAs in the potential regulatory axis. (A, B) Screening the prognostic mRNAs and lncRNAs in the hsa-miR-21-related regulatory axis. (C, D) Screening the prognostic mRNAs and lncRNAs in the hsa-mir-15a-related potential regulatory axis.

CRediT authorship contribution statement

Si Yuan Lu: Data curation, Writing – original draft, Writing – review & editing. **Jie Hua:** Data curation, Writing – original draft, Writing – review & editing. **Jiang Liu:** Data curation, Writing – original draft, Writing – review & editing. **Miao Yan Wei:** Data curation, Writing – original draft, Writing – review & editing. **Chen Liang:** Data curation, Writing – original draft, Writing – review & editing. **Qing Cai Meng:** Data curation, Writing – original draft, Writing – review & editing. **Bo Zhang:** . **Xian Jun Yu:** Conceptualization, Writing – review & editing. **Wei Wang:** Writing – review & editing, Conceptualization, Writing – review & editing. **Jin Xu:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

The Institutional Research Ethics Committee of Fudan University Shanghai Cancer Center (FUSCC) approved this study, and written informed consent was obtained from all patients prior to the investigation. The ethics approval number was 050432-4-1212B. We confirmed that the experiment on human tissue samples was performed in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Availability of data and materials

Access to all databases used in the present study is publicly available. GEO: https://www.ncbi.nlm.nih.gov/geo/; TCGA: https://portal.gdc.cancer.gov/); ICGC: https://icgcportal.genomics.cn/.

Acknowledgment

Not applicable.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2022.101419.

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