Original Article

Identification and validation of the microRNAs and hub genes for pancreatic ductal adenocarcinoma by an integrated bioinformatic analysis

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Background: In the progression of pancreatic ductal adenocarcinoma (PDAC), aberrant micro RNAs (miRNAs) expression plays a crucial role. This study sought to identify and validate the key miRNAs and potential target genes involved in PDAC. A bioinformatic analysis was conducted to determine their potential use as biomarkers and therapeutic targets.

Methods: Gene profiling data sets (GSE41372 and GSE32688) were retrieved from the Gene Expression Omnibus database. Differentially expressed miRNAs (DEMs) with a P value <0.05, and |fold change| >2 was identified. The prognostic value of the DEMs was accessed using the online server Kaplan-Meier plotter. Further, gene ontology terms and Kyoto Encyclopedia of Genes and Genomes pathway analyses were performed using DAVID 6.7. The protein-protein interaction analyses were conducted with STRING, and miRNA-hub gene networks were constructed using Cytoscape software. The PDAC cells were transfected with miRNA inhibitors or mimics. Cell Counting Kit-8 assays and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining were used to examine cell proliferation and apoptosis, respectively. Wound-healing assays were performed to evaluate cell migration.

Results: Three DEMs (hsa-miR-21-5p, hsa-miR-135b-5p, and hsa-miR-222-3p) were identified. High expression levels of hsa-miR-21-5p, hsa-miR-135b-5p, or hsa-miR-222-3p predicted poor overall survival in PDAC patients. The pathway analysis revealed that the predicted target genes of the DEMs were closely related to several signaling pathways (including 'pathways in cancer', 'miRNAs in cancer', 'platinum drug resistance', 'lipid and atherosclerosis', and 'MAPK signaling pathway'). The MYC proto-oncogene (*MYC*), phosphate and tensin homolog gene (*PTEN*), poly(ADP-ribose) polymerase 1 (*PARP1*), von Hippel-Lindau (*VHL*), and fork head box p3 (*FOXP3*) were identified as potential target genes. The inhibition of hsa-miR-21-5p, hsa-miR-135b-5p, or hsa-miR-222-3p expression decreased cell proliferation. The overexpression of hsa-miR-21-5p, hsa-miR-135b-5p, or hsa-miR-222-3p facilitated PDAC cell migration.

Conclusions: This study constructed the miRNA-hub gene network, which provides novel insights into the PDAC progression. Although further research is required, our results offer clues for new potential prognostic markers and therapeutic targets of PDAC.

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Keywords: Pancreatic ductal adenocarcinoma (PDAC); micro RNA (miRNA); hub genes; bioinformatic analysis

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Introduction

With an overall 5-year survival rate of only 3–9%, pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies (1,2). It is estimated that PDAC will become the second leading cause of cancer-associated mortality by 2030 (3). Surgical resection continues to be the only potentially curative option for PDAD. Due to the insidious onset and early metastasis of PDAC, >80% of patients suffer from locally advanced invasion or metastasis clinically; thus, <20% of PDAC patients are eligible for surgical resection (4-6). Adjuvant and neoadjuvant therapies can improve the clinical outcomes of PDAC patients, but due to the lack of effective biomarkers for early diagnosis, recurrence, and prognosis, sometimes timely medical treatment was not available, which leads to a poor prognosis (7,8). Exploring molecular bases could extend understandings of the pathogenesis, diagnosis, and therapeutic possibilities of PDAC. The diagnostic and/or therapeutic value of cell free DNA (9), circulating tumor DNA (10), circulating tumor cells (9), circulating monocytes (11), exosomes (12), metabolic changes (13,14), microbiome (15), and tumor-specific micro RNAs (miRNAs) (16) has been suggested in latest discoveries and studies. Tumor-specific miRNAs are gaining widespread attention as critical regulators in PDAC.

Highlight box

Key findings

- High expression of hsa-miR-21, hsa-miR-135b, and hsa-miR-222 resulted in the poor overall survival of PDAC patients.

What is known and what is new?

- Dysregulated miRNAs play a fundamental role in the progression or suppression of various types of cancer, including PDAC.
- The miRNA-hub gene network may provide useful information to understand the miRNAs regulation mechanism, which suggests the potential role of DEMs and hub genes to be utilized as prognostic biomarkers in PDAC.

What is the implication, and what should change now?

- The regulatory mechanism of DEMs and their hub genes should be further studied.

MiRNAs are a class of small endogenous single-stranded, non-coding, short RNA molecules, approximately 21–25 nucleotides in length (17). By binding to the 3'-untranslated region of messenger RNAs (mRNAs), miRNAs play important regulatory roles in gene expression, leading to direct mRNA degradation or protein translation repression (18). Researchers found that miRNAs regulate at least 30% of protein-coding genes, and that approximately 50% of miRNA genes exist in cancer-related genomes (19). MiRNAs are key mediators of various critical biological processes (BPs), including cell differentiation, survival, apoptosis, migration, invasion, and DNA repair (20). Numerous studies have shown that some dysregulated miRNAs play a fundamental role in the progression or suppression of various types of cancer (21), including PDAC (22). Thus, we focused on tumor-specific miRNAs on PDAC progression in the current study. Several previous studies on bioinformatics analysis have investigated the differentially expressed miRNAs (DEMs) in PDAC. Zhou and coworkers (23) elucidated the potential biomarker and prognostic targets in PDAC via a bioinformatics analysis and included 4 datasets from the Gene Expression Omnibus (GEO) database. However, only GSE41369 was used to screen differentially-expressed miRNAs, the other 3 datasets were used for screening differentially expressed genes, so the differentially-expressed miRNAs have not been cross-verified by other datasets. In another bioinformatics analysis (24), in which one data set containing 9 PDAC samples and 9 normal samples were employed for miRNA-seq analysis to identify the DEMs, a total of 32 DEMs were identified. However, only one microarray data set with small sample size is included, and the function of DEMs has not been verified. A comprehensive bioinformatics analysis with multiple data sets, more samples, functional miRNA-mRNA interactive networks, and function verification of identified DEMs in PDAC based on paired human tissues need to be conducted.

The present study analyzed the DEMs between PDAC tumor tissues and normal pancreatic tissue samples using bioinformatic methods. In brief, 2 miRNA expression profile data sets were selected from the GEO database, and the DEMs were screened using the GEO2R analytic

tool. Subsequently, we performed target gene prediction, functional, and pathway enrichment analyses, and constructed protein-protein interaction (PPI) and miRNA-target gene networks. A survival analysis of the DEMs and their target genes was performed to identify their prognostic value. Finally, the effects of the 3 identified miRNAs (hsa-miR-21-5p, hsa-miR-135b-5p, and hsa-miR-222-3p) on the cell viability, apoptosis, and migration of the PDAC cells were evaluated. The current study sought to explore and identify the potential role and molecular mechanisms of dysregulated miRNAs in PDAC progression. Our findings may provide useful information for cancer diagnosis and therapy. We present the following article in accordance with the MDAR reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-192/rc).

Methods

MiRNA microarray human data sets

In the discovery step, data sets were acquired from the GEO database (www.ncbi.nlm.nih.gov/geo) to compare miRNA expression between human PDAC tissues and human pancreatic tissues. The titles and abstracts of the collected data sets were screened, and the full information of the selected data sets was further assessed. After a systematic review, 2 miRNA expression profile data sets (GSE41372 and GSE32688) were selected for further research. The GSE41372 data set, which uses the GPL16142 platform (NanoString nCounter Human miRNA assay), contained 9 PDAC tumor samples and 9 normal pancreatic tissue samples. The GSE32688 data set, which uses the GPL7723 platform (multispecies Affymetrix GeneChip miRNA Array), contained 25 PDAC tumor samples and 7 normal pancreatic tissue samples. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Screening for DE-miRNAs

The online GEO2R (LIMMA package of R software) was used to compare the differences between the PDAC tumor samples and normal pancreatic samples from each data set. An adjusted P value <0.05 and |fold change| >2 was set as the thresholds to identify the statistically significant results. The overlapping DEMs in the 2 data sets were considered the candidate DEMs.

Survival analysis of the DEMs

The prognostic value of the DEMs in the PDAC patients was analyzed using Kaplan-Meier plotter online software (www.kmplot.com) (25). Several databases are included in the database, including GEO, the European Genome-phenome Archive (EGA), and The Cancer Genome Atlas (TCGA). In brief, using the auto-selected best cut-off values, patient samples were divided into two groups (high expression versus low expression), the DEMs or their target genes were entered, and Kaplan-Meier survival curves were generated. Additionally, hazard ratios with 95% confidence intervals and log-rank P values were calculated. A P value <0.05 was considered statistically significant.

Prediction of target genes for DEMs

The potential target genes of the selected DEMs were predicted using miRNet (www.mirnet.ca/), a miRNA-centric network visual analytics platform that provides miRNA-target interaction networks from 11 different miRNA databases. A manual network amplifying and pruning function can be implemented. The program uses hypergeometric tests to perform a gene set enrichment analysis with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Gene Ontology (GO) and pathway enrichment analysis

DAVID 6.8 (https://david-d.ncifcrf.gov/) was used to perform the functional annotation and KEGG pathway analyses for the predicted targets of the candidate DEMs (26,27). The top 25 enriched KEGG pathways were kept for each comparison. A P value <0.05 was considered statistically significant.

PPI network and miRNA-target network construction

The STRING database (https://string-db.org/) was used to visualize the PPI network for the potential target genes (28). Only the validated interactions with a combined score of >0.4 were considered significant, and the PPI network was visualized. Subsequently, Cytoscape software 3.7.2 was used to identify the hub genes based on the degree of connectivity (29). The top 30 hub genes were screened to construct the PPI network of the target genes using Cytoscape, after which the miRNA-hub gene network was also constructed.

Analysis of target gene expression using the TNMplot database

The TNMplot (https://www.tnmplot.com) is an open-access bioinformatics tool used to compare differential gene expression in tumor, normal, and metastatic tissues (accessed on 18 February 2023) (30). This analysis was applied to mRNA expression levels for paired pancreatic tumor tissues and adjacent normal tissues. The Mann-Whitney U-test was used to determine statistical significance.

Cell culture

The PANC-1 PDAC (TCHu 98) cell line was obtained from the Shanghai Institute of Biochemistry and the Cell Biology. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum at 37 ℃ in a 5% carbon dioxide incubator.

Cell transfection

The candidate miRNA mimics, inhibitors, and negative controls were synthesized and purified by Guangzhou RiboBio Co., Ltd. (Guangzhou, China). The sequences of the mimics and inhibitors are shown in Table S1. The PANC-1 cells were seeded and incubated for 12 h to allow for adhesion. The cells were transfected with miRNA mimics or control mimics, miRNA inhibitors or control inhibitors of hsa-miR-21-5p, hsa-miR-135b-5p, and hsa-miR-222-3p using Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA) at a final concentration of 50 nM.

CCK-8 assays

Cell viability was monitored using a Cell Counting Kit-8 (CCK-8) kit (Beyotime, Shanghai, China) in accordance with the manufacturer's instructions. After transfection with the miRNA or control inhibitors for 24, 48, or 72 h, 10 μL of CCK-8 reagent was added to the culture medium of the PANC-1 cells for an additional 2 h before the analysis. The absorbance (optical density) was measured at 450 nm.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays

Apoptosis was determined using TUNEL assays and 4',6-diamidino-2-phenylindole (DAPI; Beyotime). In brief, the PANC-1 cells were transfected with the miRNA or control inhibitor for 48 h. The cells were then incubated with TUNEL solution (Beyotime) for an additional 1 h. The nuclei were counterstained with DAPI.

Wound-healing assays

Wound-healing assays were performed to analyze the migration of the PANC-1 cells. The cells (2×10^5/per well) were seeded in 6-well culture plates for adhesion overnight. A 200-μL pipette tip was used to create the cell scratches. Next, the cells were cultured in serum-free DMEM for 24 h. Pre-migration and migration images were captured with a light microscope.

Statistical analysis

Numerical data processing was performed with GraphPad Prism Software version 8.0 and Excel. All the results were expressed as the mean ± standard deviation. All the experiments were repeated at least 3 times. Differences between 2 groups were evaluated using parametric two-tailed unpaired Student's *t*-test. The log rank test was employed to compare survival curves. The non-parametric Mann-Whitney U test was utilized for gene expression between paired tumor and adjacent normal tissues. A P value <0.05 was considered statistically significant.

Results

Identification of DEMs and their target genes

To identify the DEMs in human PDAC tissues compared to normal pancreatic tissues, GSE41372 and GSE32688 were downloaded from the GEO database. A total of 64 and 29 DEMs were identified in the GSE41372 and GSE32688 data sets, respectively (Tables S2,S3). These DEMs, which included 61 upregulated miRNAs and 29 downregulated miRNAs (*Figure 1A*), are graphically represented in volcano plots (*Figure 1B*). Ultimately, 3 upregulated DEMs (hsa-miR-21, hsa-miR-135b, and hsa-miR-222) were found to be overlapping in both the data sets and identified as candidate DEMs (*Table 1*).

Survival analysis of DEMs

To validate the clinical relevance of the 3 candidate DEMs in PDAC, the survival data were analyzed using the Kaplan-

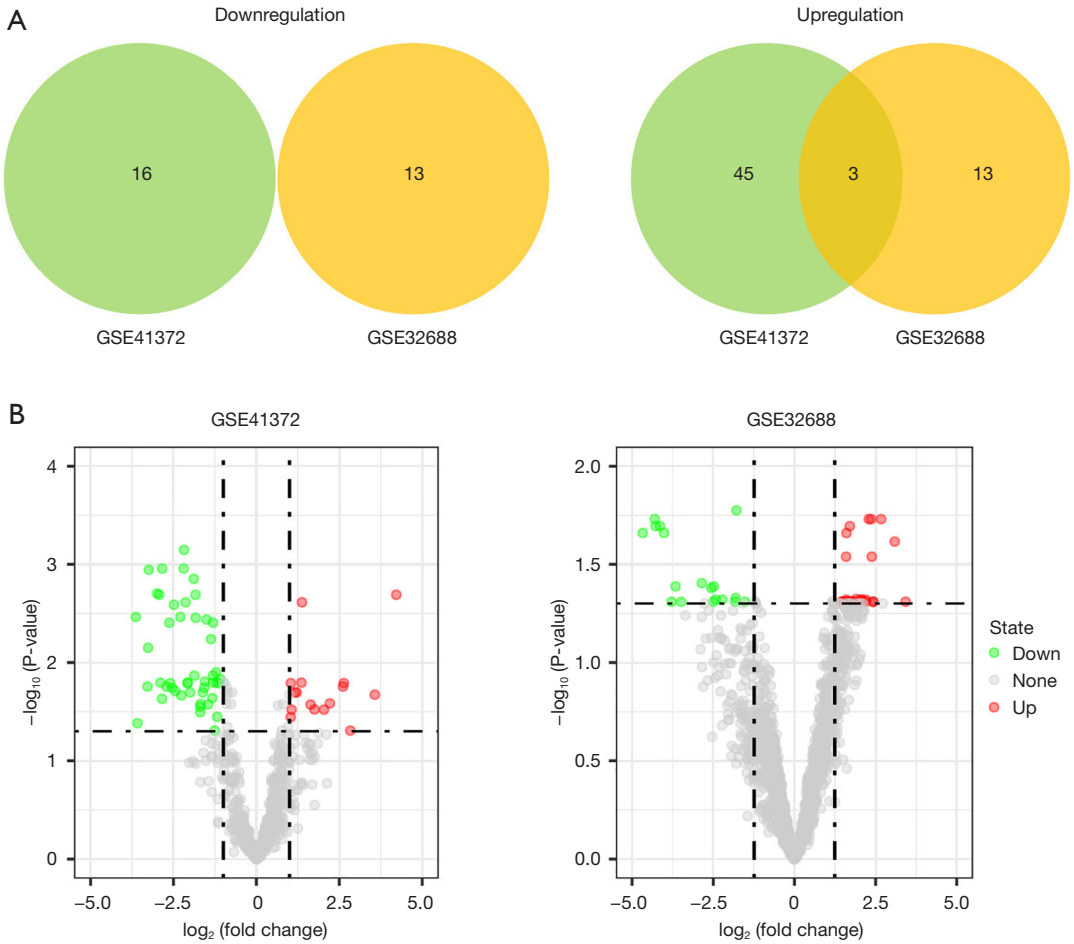

Figure 1 Identification of potential DEMs in the GSE41372 and GSE32688 data sets. (A) Venn diagrams of the overlapping downregulated (left) or upregulated (right) DEMs. (B) Volcano plots of the $\log_2$ (fold change) and $-\log_{10}$ (adjusted P value) of DEMs. DEM, differentially expressed miRNA.

Table 1 Overlapping DEMs in the GSE41372 and GSE32688 data sets

Common DEM	Log_2FC	t	B	P value	Adj.P.Val
GSE41372					
hsa-miR-21	2.99254	−5.53617	2.9787	1.90E−05	0.001978
hsa-miR-135b	2.81999	−8.41107	8.6657	4.63E−08	3.37E−05
hsa-miR-222	1.57231	−3.80002	−0.8929	1.09E−03	0.018069
GSE32688					
hsa-miR-21	3.41842	−4.43458	1.4843	8.13E−05	0.0202
hsa-miR-135b	3.44254	−4.87457	2.6836	2.14E−05	0.0186
hsa-miR-222	2.00441	−3.53755	−0.8569	0.00112	0.049

DEM, differentially expressed miRNA; FC, fold change; Adj.P.Val, adjust P value.

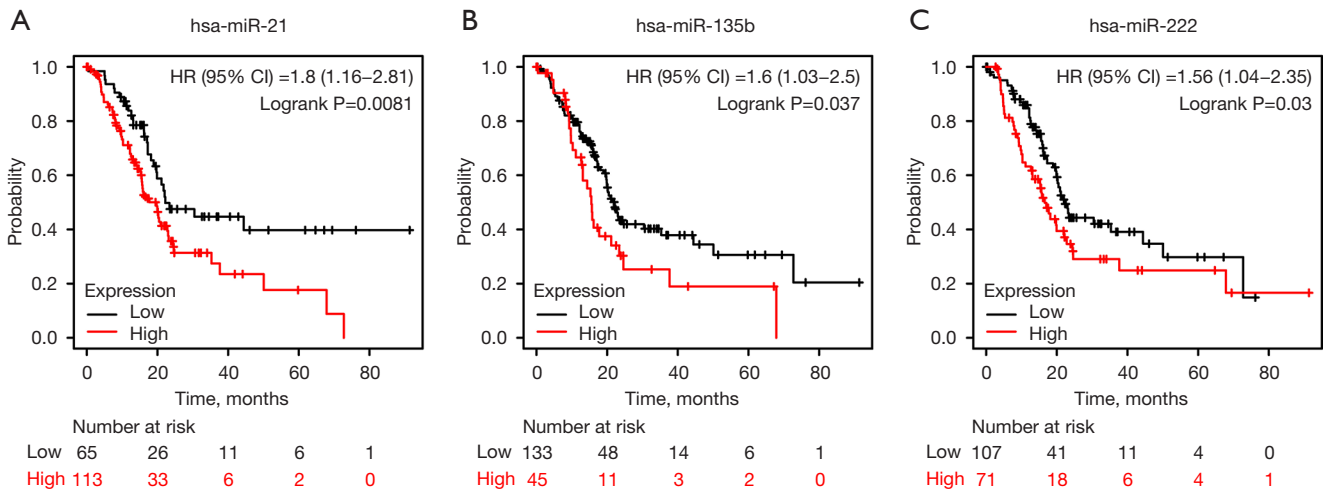

Figure 2 Kaplan-Meier survival analysis for PDAC patients based on different expression of DEMs. Kaplan-Meier survival curves of hsa-miR-21 (A), hsa-miR-135b (B), and hsa-miR-222 (C) in PDAC from the Kaplan-Meier plotter database. PDAC, pancreatic ductal adenocarcinoma; DEM, differentially expressed miRNA.

Meier plotter database, which contained 178 PDAC patients from TCGA database. As *Figure 2* shows, the survival analysis demonstrated that the PDAC patients with high expressions of hsa-miR-21, hsa-miR-135b, and hsa-miR-222 had significantly worse overall survival than those with low expressions (log-rank P=0.0081, 0.037, and 0.03, respectively). Thus, all 3 miRNAs were selected as final candidate DEMs for further study.

The target genes of candidate DEMs

The potential target genes of the candidate DEMs were subsequently predicted using miRNet. Ultimately, 1089 potential target genes were yielded for the 3 candidate DEMs. There were 612, 83, and 394 unique genes for hsa-miR-21-5p, hsa-miR-135b-5p, and hsa-miR-222-3p, respectively. The visualized miRNA-mRNA regulatory networks are shown in *Figure 3*, and all predicted target genes are summarized in Table S4.

Functional annotation and pathway enrichment analysis

The GO terms contain 3 non-overlapping categories: BP, cellular component (CC), and molecular function (MF). In the BP category, the most enriched functions for the target genes of the 3 candidate miRNAs were the regulation of apoptotic signaling pathway, myeloid cell differentiation, and reproductive structure development (*Figure 4A*). In the CC category, the most enriched GO functions were nuclear chromatin, transcription regulator complex, and cell leading edge (*Figure 4A*). In the MF category, the most significant enriched GO functions were ubiquitin-like protein transferase activity, DNA-binding transcription activator activity (RNA polymerase II-specific), and DNA-binding transcription activator activity (*Figure 4A*).

A KEGG analysis was carried out to analyze the enriched pathways of these target genes. The top 20 highly enriched KEGG pathways for the target genes are presented in *Figure 4B*, the top 5 enriched pathways were 'pathways in cancer', 'miRNAs in cancer', 'platinum drug resistance', 'lipid and atherosclerosis', and 'MAPK signaling pathway'.

Construction of PPI network and hub gene identification

A PPI network of the target genes for the 3 candidate DEMs was constructed using the STRING online tool. We identified the top 30 hub genes with the highest degrees of connectivity. after analyzing the data from STRING using the cytoHubba APP in the Cytoscape software (*Table 2*). The networks of the hub genes with the top 30 hub genes with highest degrees of connectivity were visualized using Cytoscape (*Figure 5A*). The top 10 ranked hub genes were MYC proto-oncogene (*MYC*), epidermal growth factor receptor (*EGFR*), phosphate and tensin homolog gene (*PTEN*), vascular endothelial growth factor A (*VEGFA*), signal transduction and transcription activator-3

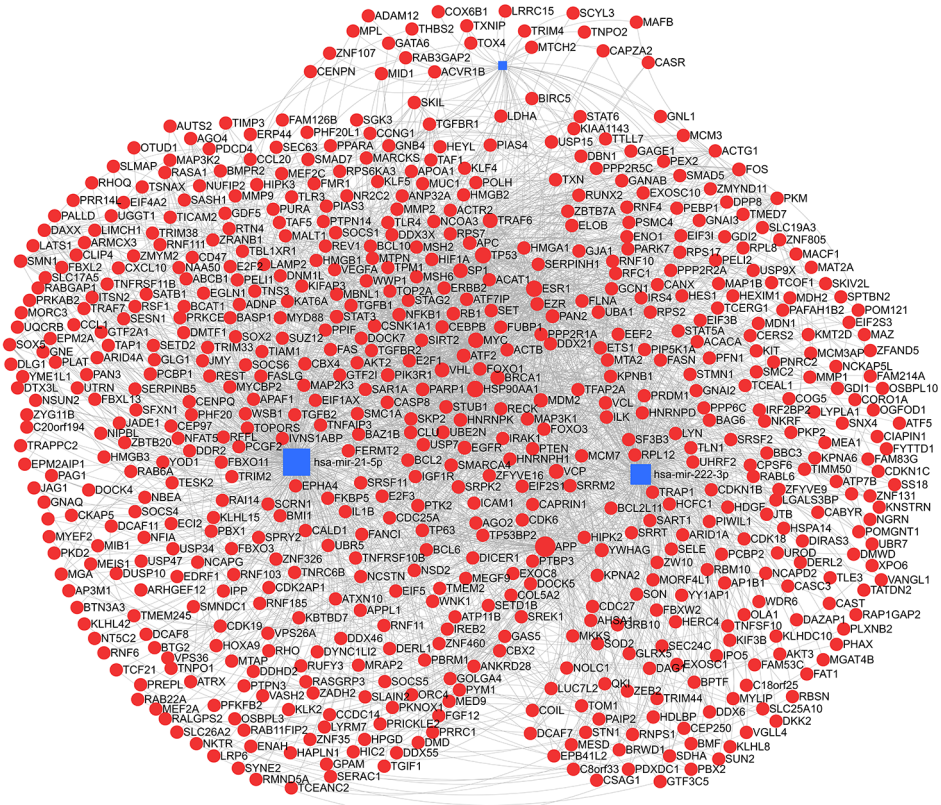

Figure 3 Potential target genes of hsa-miR-21-5p, hsa-miR-135b-5p, and hsa-miR-222-3p predicted by miRNet.

(*STAT3*), breast-cancer susceptibility gene 1 (*BRCA1*), epidermal growth factor receptor 2 (*ERBB2*), SWI/SNF-related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (*SMARCA4*), matrix metallopeptidase 9 (*MMP9*), and ubiquitin-conjugating enzyme E2 N (*UBE2N*). Among these hub genes, MYC had the highest node degree of connection (degree = 220).

Subsequently, a miRNA-hub gene network was constructed. As *Figure 5B* shows, hsa-miR-129-5p interacted with all 30 hub genes, hsa-miR-135b-5p interacted with MYC and forkhead box transcription factor O1 (*FOXO1*), and hsa-miR-222-3p interacted with *MYC*, *FOXO1*, *PTEN*, *SMARCA4*, *UBE2N*, *FOXO3*, and dicer 1, ribonuclease III (*DICER*).

Expression of the hub genes for DEMs

The TNMplot platform was used to explore the differential expression of the hub genes between the PDAC and adjacent normal tissues. As *Figure 6* shows, among the top 30 hub genes, only the expression of *MYC*, *PTEN*, poly (ADP-ribose) polymerase 1 (*PARP1*), von Hippel-Lindau (*VHL*), and fork head box p3 (*FOXP3*) was significantly decreased in the human PDAC tissues compared to that in the adjacent paired normal tissues (*Figure 6*). The expressions levels of interleukin 1 beta (*IL1B*), phosphoinositide-3-kinase regulatory subunit 1 (*PIK3R1*), *FOXO3*, nuclear factor kappa B subunit 1 (*NFκB1*), insulin like growth factor 1 receptor (*IGF1R*), *DICER1*, transforming growth factor beta 1 (*TGFβ1*), *FOXO1*, and STIP1 homology and U-box containing protein 1 (*STUB1*) did not differ significantly between the groups. The expression levels of *EGFR*, *VEGFA*, *STAT3*, *BRCA1*, *ERBB2*, *SMARCA4*, *MMP9*, *UBE2N*, S-phase kinase associated protein 2 (*SKP2*), toll like receptor 4 (*TLR4*), specificity protein 1 (*SP1*), caspase-8 (*CASP8*), suppressors of cytokine signaling 1 (*SOCS1*), hypoxia-inducible factor-1α (*HIF1A*), *MMP2*, and retinoblastoma 1 (*RB1*) were significantly more increased in the tumor tissues than the adjacent normal tissues. Thus, 5 hub genes (*MYC*, *PTEN*, *PARP1*, *VHL*, and *FOXP3*) were identified as the potential regulatory targets of the candidate DEMs.

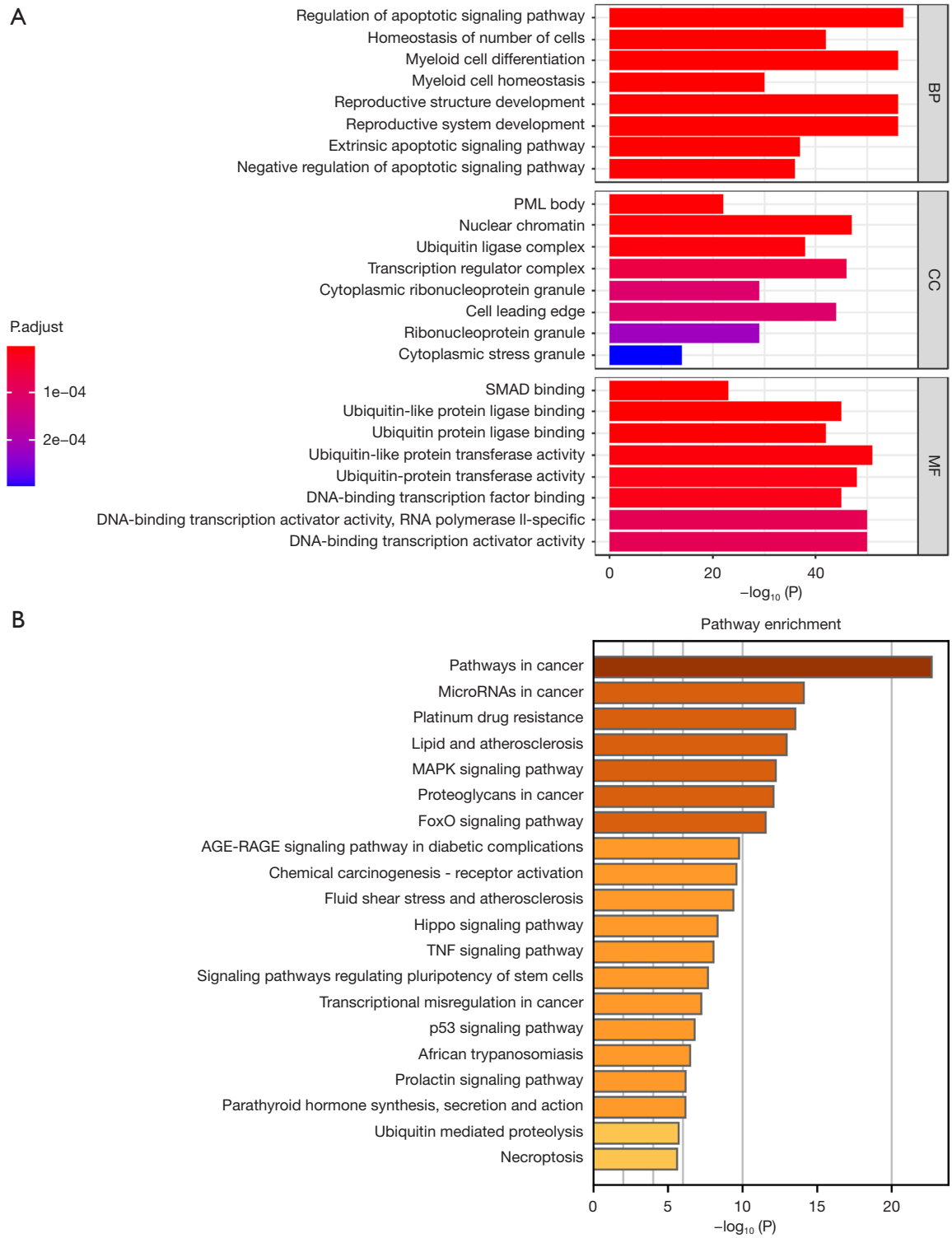

Figure 4 GO and KEGG analysis of the potential target genes for the DEMs. (A) Enriched BPs, CCs, and MFs of the potential target genes. (B) KEGG pathway enrichment analysis of the potential target genes. DEMs, differentially expressed miRNAs. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cellular component; MF, molecular function.

Table 2 The top 30 hub genes with the highest degrees of connectivity in the PPI network of the DEMs

Gene symbol	Degree	Gene symbol	Degree	Gene symbol	Degree
MYC	220	*IL1B*	100	*HIF1A*	78
EGFR	210	*TLR4*	94	*MMP2*	78
PTEN	186	*SKP2*	92	*VHL*	74
VEGFA	158	*SP1*	90	*FOXP3*	72
STAT3	156	*FOXO3*	88	*IGF1R*	72
BRCA1	124	*PIK3R1*	86	*DICER1*	72
ERBB2	118	*CASP8*	84	*RB1*	72
SMARCA4	110	*SOCS1*	82	*TGFB1*	68
MMP9	106	*PARP1*	80	*FOXO1*	68
UBE2N	104	*NFKB1*	78	*STUB1*	66

PPI, protein-protein interaction; DEM, differentially expressed miRNA.

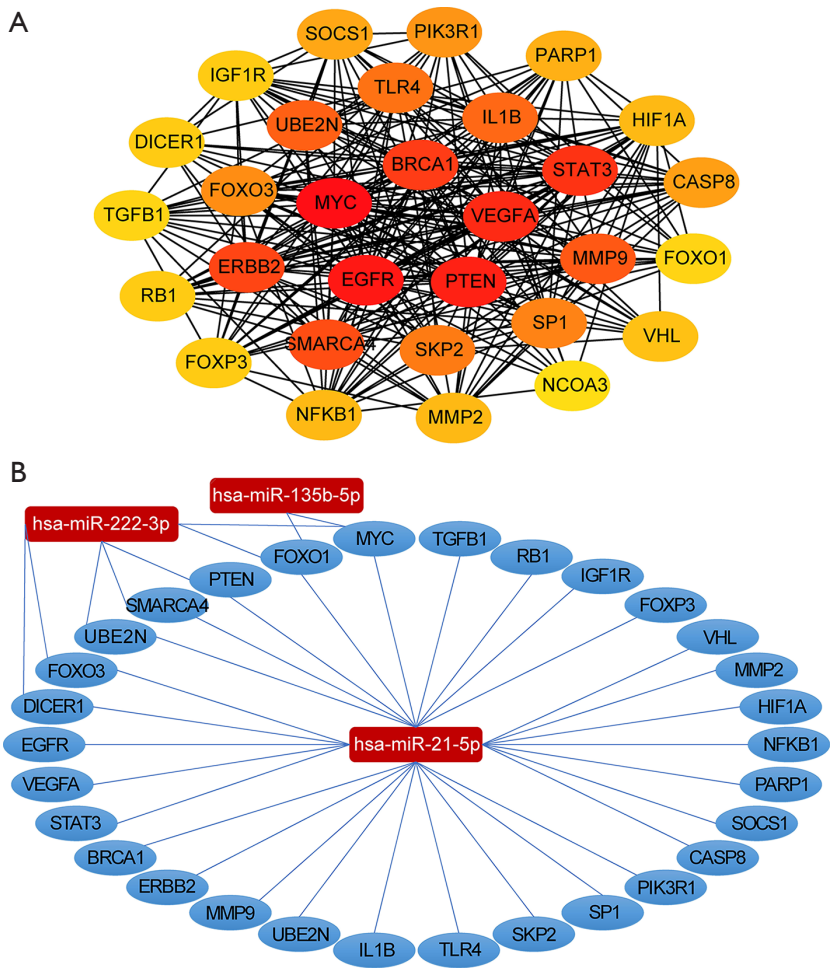

Figure 5 The hub gene PPI networks and the miRNA-hub gene networks. (A) The PPI networks of the top 30 hub genes. (B) The miRNA and hub gene networks. PPI, protein-protein interaction.

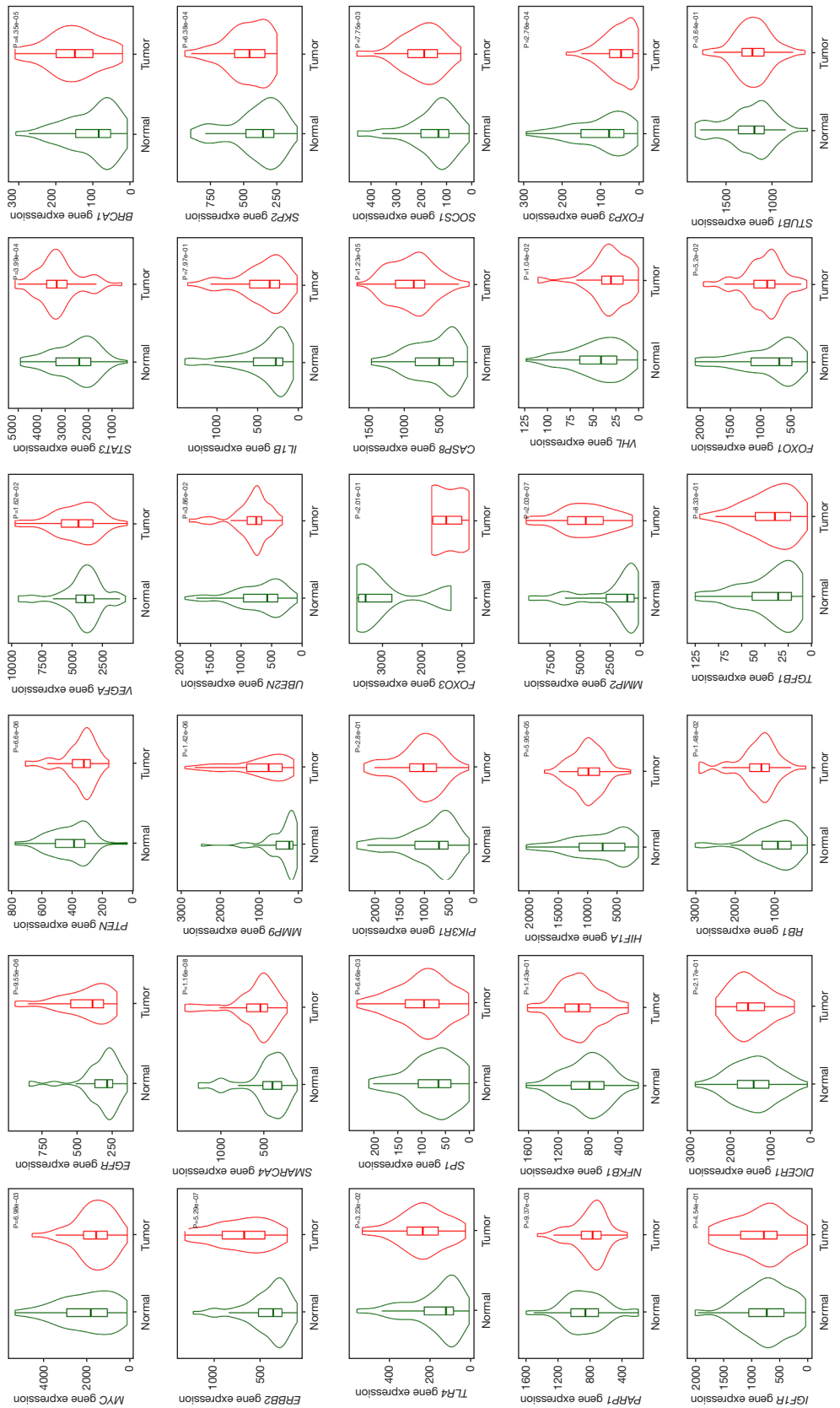

Figure 6 Expression of potential target genes with the top 30 hub genes with highest degrees of connectivity from the TNMplot database.

Effects of DEMs on PDAC cell proliferation, apoptosis, and migration

To explore the effect of hsa-miR-21-5p, hsa-miR-135b-5p, and hsa-miR-222-3p on the proliferation, apoptosis, and migration of PDAC cells, CCK-8 assays, TUNEL staining, and wound-healing assays were performed. As *Figure 7A* shows, the expression of miRNAs was successfully inhibited by specific miRNA inhibitors. The results revealed that treatment with inhibitors of all 3 miRNAs repressed the proliferation of the PANC-1 cells at 24, 48, and 72 h (*Figure 7B*). However, the cells did not undergo apoptosis when treated with the 3 miRNA inhibitors (*Figure 7C*). As shown in *Figure 7D*, the transfection efficiency of miRNA mimics was determined to be satisfactory. Further studies revealed that transfection with hsa-miR-21-5p, hsa-miR-135b-5p, or hsa-miR-222-3p mimics facilitated the migration of the PANC-1 cells (*Figure 7E,7F*).

Discussion

PDAC is a lethal and aggressive malignancy with minimal therapeutic options. Due to the lack of definite molecular mechanisms in cancer progression and effective targetable carcinogenic drivers for treatment, the prognosis of PDAC patients remains poor. Thus, the potential molecular signatures involved in the process of carcinogenesis and tumor progression of PDAC need to be explored urgently.

In the present study, an integrated microarray analysis revealed that 3 upregulated DEMs (hsa-miR-21, hsa-miR-135b, and hsa-miR-222) changed consistently in both the GSE41372 and GSE32688 data sets. The Kaplan-Meier survival analysis demonstrated that the high expression of hsa-miR-21, hsa-miR-135b, and hsa-miR-222 resulted in the poor overall survival of PDAC patients. The bioinformatics analysis indicated that the top 30 hub genes with the highest degrees of connectivity were enriched in the following pathways, including 'pathways in cancer', 'miRNAs in cancer', 'platinum drug resistance', 'lipid and atherosclerosis', and 'MAPK signaling pathway'. These target genes were selected to construct the PPI and miRNA-hub gene regulatory network. Among these top 30 hub genes, only the expressions levels of *MYC*, *PTEN*, *PARP1*, *VHL*, and *FOXP3* were significantly decreased in the PDAC tissues compared to the adjacent normal tissues. Thus, these 5 genes were identified as potential target genes for the candidate DEMs. Ultimately, we found that hsa-miR-21-5p, hsa-miR-135b-5p, and hsa-miR-222-3p regulated the proliferation and migration of the PDAC cells.

MiRNAs have been reported to be closely associated with tumor initiation and development, including proliferation, invasion, metastasis, and other malignant phenotypes. The role of mir-21-5p in pancreatic cancer has only been reported in a few studies. Upregulated MiR-21-5p in PDAC could deregulate the checkpoints of the cell cycle, promote the growth of cancer cells, and down-regulate the tumor suppressor gene expression (16). It acts as an oncogenic miRNA and a specific miRNA for the identification of pancreatic tumors (31). It has been reported that miR-21-5p is significantly more upregulated in the blood plasma of PDAC patients than in healthy individuals, and an increased expression level of miR-21-5p predicts poor prognosis in patients with PDAC (32). M2 macrophage-derived exosomes elevate miR-21-5p expression, which stimulates the differentiation and activity of pancreatic cancer stem cells by targeting Krüppel-like factor 3 (*KLF3*) (33). In our study, PDAC patients with high miR-21-5p expression in tumor tissues had worse overall survival rates than those with low miR-21-5p expression, and miR-21-5p expression promoted the cell migration of PDAC cells. *MYC*, *PTEN*, *PARP1*, *VHL*, and *FOXP3* were predicted to be the potential target genes of miR-21-5p, indicating that miR-21-5p might regulate the proliferation and migration of PDAC cells by targeting these 5 genes. Previously, 2 studies have reported that miR-135b-5p is upregulated and functions as oncogene in pancreatic cancer, promotes the migration, invasion, and epithelial-mesenchymal transition (EMT) of pancreatic cancer cells by targeting *KLF4* and/or nuclear receptor subfamily 3 group C member 2 (*NR3C2*), and the high expression of miR-135b-5p predicts the poor prognosis of patients (34,35). Our research showed that the high expression of miR-135b-5p was associated with the poor prognosis of PDAC patients and may regulate the proliferation and migration of PDAC cells by targeting *MYC*. Hsa-miR-222-3p has been reported to be significantly upregulated in M2 macrophage-derived extracellular vesicles in the chemoresistance of pancreatic cancer and enhances chemoresistance through TSC1 inhibition and PI3K/AKT/mTOR activation (36). Our study suggests that the high expression of hsa200222-3p indicates poor survival and slightly facilitates the migration of PDAC cells. *MYC* and *PTEN* may be targets of hsa-miR-222-3p.

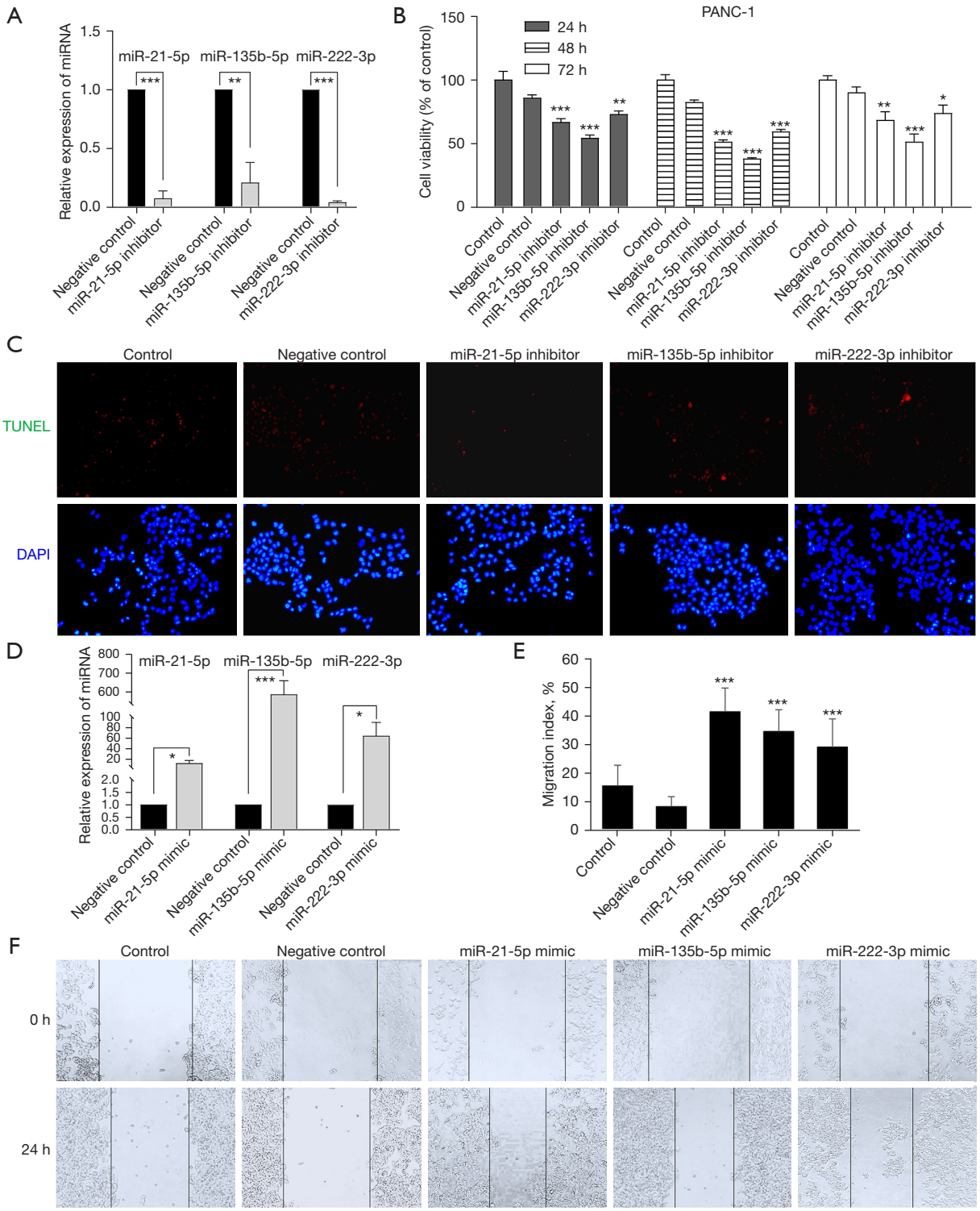

Figure 7 Effects of mimics and inhibitors of hsa-miR-21-5p, hsa-miR-135b-5p, and hsa-miR-222-3p on cell viability, apoptosis, and migration of PANC-1 cells. (A) Transfection efficiency of the miRNA inhibitors. (B) Effect of specific miRNA inhibitors on cell viability detected by CCK-8 assays. (C) Effect of specific miRNA inhibitors on apoptosis detected by TUNEL/DAPI double staining assays (200×). (D) Transfection efficiency of the miRNA mimics. (E) The statistics of wound healing assay. (F) Representative images of cell migration detected by wound-healing assays (100×). *, P<0.05; **, P<0.01; ***, P<0.001 compared with negative control.

Conclusions

In the present study, a bioinformatic analysis and experimental validation were conducted, and 3 metastasis-associated DEMs (hsa-miR-21-5p, hsa-miR-135b-5p, and hsa-miR-222-3p) were successfully identified as potential biomarkers for PDAC progression and prognosis. Further, we constructed a miRNA-hub gene networks that may extend understandings of the miRNA regulation mechanisms, which suggests the potential use of hub genes as prognostic biomarkers in PDAC.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-192/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-192/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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