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Original Article

The lncRNA *UCA1* Enhances Pancreatic Cancer EMT by Regulating miR-708-5p and miR-135b-5p: A Bioinformatics Approach

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Abstract

Background: Pancreatic cancer (PC) is an exceedingly malignant ailment that is not only characterized by its insidious onset and rapid progression but also by its poor therapeutic effects. Recently, emerging evidence has shed light on the significant role that non-coding RNAs (ncRNAs), particularly long ncRNAs (lncRNAs) and microRNAs (miRNAs), play in the pathogenesis of PC. This investigation aimed to construct a network of interactions between miRNAs, lncRNAs, and mRNAs, as well as to perform correlation analyses in the context of PC.

Methods: This study carried out in Kerman City, southeastern Iran in 2023. We utilized the GSE119794 dataset from the Gene Expression Omnibus (GEO) to analyze differentially expressed lncRNAs (DE-lncRNAs), miRNAs (DE-miRNAs), and mRNAs (DE-mRNAs). Following the identification of the DE-lncRNAs, DE-mRNAs, and DE-miRNAs, we proceeded to examine differentially expressed epithelial-mesenchymal transition (EMT) genes. Subsequently, we utilized the RNAInter database to predict interactions among lncRNAs, miRNAs, and mRNAs. Finally, we employed Cytoscape to visualize and analyze the constructed network.

Results: 14 DE-lncRNAs, 14 DE-miRNAs, 545 DE-mRNAs, and 65 DE-EMT from pancreatic cancer and its adjacent tissue RNA-Seq data were identified. 1184 EMT genes from dbEMT were obtained, among which 65 DE-EMT were assigned as EMT genes and correlated with tumor progression. One functional lncRNA (*UCA1*) was identified as a key functional lncRNA. The area under the ROC curve (AUC) of *UCA1* and miR-708-5p were 0.79 and 0.86, respectively. Thus, it is reasonable to believe that this prognostic risk model helps predict PC metastasis.

Conclusion: *UCA1* is a new lncRNA linked with EMT in PC and contributes to a better knowledge of the regulatory mechanisms related to lncRNAs in PC.

Keywords: Pancreatic cancer; Long noncoding RNA (lncRNA); Epithelial-mesenchymal transition (EMT); Biomarker

Introduction

Pancreatic cancer (PC) prevalence and mortality have increased rapidly in recent decades (1). Furthermore, PC will continue to be the leading cause of cancer-related deaths (2). Most deadly pancreatic malignancies are characterized as pancreatic ductal adenocarcinoma (PDAC) (3). Unfortunately, the survival rate after five years is less than 5% (4), and the prognosis for this cancer type remains poor (5). Unlike other gastrointestinal cancers, there is little evidence for PDAC risk factors, which can only be detected in 40% of cases (6).

A crucial factor influencing pancreatic tumor cell invasion and metastasis is the EMT process defined as the transformation of epithelial into mesenchymal cells (7). EMT allows the tumor-generating cells to develop the characteristics of highly malignant cells, including motility, invasiveness, and distant metastases, eventually leading to inadequate drug delivery (8, 9).

Two regulatory non-coding RNAs (ncRNAs), named microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), play an essential role in cell biological processes (10). MiRNAs, which have a length of 18 to 24 nucleotides, are involved in controlling the expression of many genes through their complementarity with the target mRNA sequences and accelerating their target degradation or preventing translation at the post-transcriptional level. MiRNAs affect a variety of physiological and neoplastic processes, such as cell proliferation, migration, invasion, metastasis, and EMT (11, 12). LncRNAs are defined as non-coding RNAs with more than 200 nucleotides in length. They exhibit a variety of biological roles in normal cell proliferation and differentiation through sponging miRNAs, thereby inhibiting the degradation of mRNAs targeted by miRNAs (13, 14). Similar to other malignancies, pancreatic cancer can develop because of ectopic expression of genes and regulatory RNAs (15). Remarkably, the deregulation of lncRNAs and miRNA expression has been implicated in helping the initiation and progression of PDAC (13, 16). According to previous studies, cross-regulation between lncRNAs and miRNAs plays an important role in cancer metastasis and EMT mechanisms (17, 18). Despite this, little research has been done on the miRNA-lncRNA-mRNA network and the development of PC. As a valuable method for identifying the transcriptomes of cancer tissues, RNA sequencing is being utilized to facilitate biomarker discovery and develop approaches for early detection and improved therapies (19).

In this study, we performed an analysis of the RNA expression profiles in PDAC from the Gene Expression Omnibus (GEO) database to screen the differentially expressed lncRNAs, miRNAs, and mRNAs that are related to PDAC and construct a miRNA-lncRNA-mRNA network by combining bioinformatics and correlation analyses.

Methods

Data collection and Gene expression analysis

This study conducted in 2023 in Kerman, southeastern Iran. We downloaded the human expression profiles of PC from NCBI-GEO (https://www.ncbi.nlm.nih.gov/gds), derived from research carried out by Jie Linet al. (20). The human expression profiles of PC have the accession number GSE119794. The expression data contained mRNA, lncRNA, and miRNA expression profiles, respectively. The samples were taken from the tumor tissue and the non-tumour tissue adjacent to it from 10 individuals with PC. The GPL11154 Illumina HiSeq 2000 (Homo sapiens) was the RNA-seq platform that was utilized to analyze this data.

Identification of differentially expressed Genes (DEGs)

All differentially expressed genes (DEGs) were discovered with the DESeq2 R package in Bio-

conductor. DESeq2 employs the mean expression intensity of every gene, considering all samples, as its filtering criterion, and it excludes all genes with mean normalized counts below a given filtering threshold from multiple testing adjustments. DESeq2 will automatically select a threshold that optimizes the count of genes identified at a user-specified objective. This package was used to mine statistically significant DEGs based on the difference in expression values between tumor and normal samples to investigate DEGs between adjacent normal tissue and tumor groups. In other words, DEGs were identified as significantly different between tumor and normal samples, including lncRNAs (DElncRNAs), mRNAs (DEmRNAs), and miRNAs (DEmiRNAs). A log fold change of |1| and an adjusted p-value threshold of 0.05 were used to define significant differential expression (21). The normalized matrix and clustering plots were placed in the supplementary file 1.

Identification of differentially expressed EMT genes (DE-EMT genes)

The dbEMT 2.0 database, accessed at http://www.dbemt.bioinfo-minzhao.org/, was used to retrieve the EMT genes. We obtained all of the human EMT genes associated with EMT using the dbEMT database (22). The DEGs (DElncRNAs and DEmRNAs) that were determined by expression analysis and the EMT genes that were gathered were compared with the help of the Venny 2.1 tool (found at https://bioinfogp.cnb.csic.es/tools/venny/). Finally, we determined DE-EMT genes as *list* α in this study.

Identification of DE-EMT genes for each DEmiRNA

RNAInter (RNA Interactome Database) is a web-based tool that promotes the formation of the interactome and expands our understanding of the biological activities and molecular processes of RNA, some interactions such as RNA–RNA, RNA–protein, and RNA–DNA/compound interactions in RNAInter (23). We chose categories related to miRNA, species belonging to Homo sapiens, interaction type relating to RNA-RNA interaction, detection method according to computational prediction, and confidence score interval ranging between 0.1 and 1, respectively. Based on the mentioned setting, we obtained all RNAs (list β) as interactors with DEmiRNAs in this study. Therefore, to deduce commonalities, the final list was obtained from *list* α across the *list* β by Venny 2.1. Therefore, the final list (*list* ω) contains DE-EMT genes targeted by DEmiRNAs. Some DEmiRNAs did not interact, so they were not considered in the study's continuation. Finally, lncRNA-miRNA-mRNA networks were constructed by Cytoscape 3.9.1 software.

Gene Set Enrichment Analysis (GSEA)

The Gene Set Enrichment Analysis (GSEA) provides insight into biological function, including pathways and gene ontologies (GO) in clouding: biological process (BP), cellular component (CC), and molecular function (MF). GO, and pathway enrichment analysis of *list* ω was conducted using the ToppGene database (https://toppgene.cchmc.org/), GOplot and clusterProfiler packages in R software with P.adjust <0.05.

Validation of genes: (Receiver operating characteristic) ROC analysis

To survey the diagnostic value of the expression of selected lncRNA and miRNA in PC individuals relative to healthy persons was utilized of the receiver operating characteristic (ROC) analysis. The area under the ROC curve (AUC) was computed to contrast the diagnostic value of the candidate genes. Roc analysis in the significance level of 0.05 (P<0.05) and the 95% confidence interval (95%CI) was statistically significant. This statistical analysis was conducted using the GraphPad Prism implement (version 9.1.0).

Validation of list ω and its related miRNAs and lncRNA

To validate the differentially expressed genes in this study, we analyzed an independent comprehensive PC dataset obtained from the microarray

method. This dataset encompassed the same experimental conditions as our study, investigating miRNA, mRNA, and lncRNA. The analysis was performed using the limma package in the R programming language. Genes with an absolute log2 transformed fold change (log2FC) value greater than or equal to 1 and an adjusted p-value less than or equal to 0.05 were considered significantly differentially expressed genes. Finally, we compared the *list ω*, miRNAs and *UCA1* that participated as upstream regulators in our study.

Results

Identification of DEmiRNAs, DEmRNAs and DElncRNAs

We summarized the analysis pipeline in Fig. 1. One RNA-Seq dataset (GSE119794) was used in this study. We identified 14 DEmiRNAs (11 up-regulated & 3 down-regulated), 14 DElncRNAs (11 up-regulated & 3 down-regulated) and 545 (412 up-regulated & 133 down-regulated) DEmRNAs by comparing the tumor groups (n=10) with the adjacent normal (n=10) tissue group using the DeSeq2 package (Supplementary File 1).

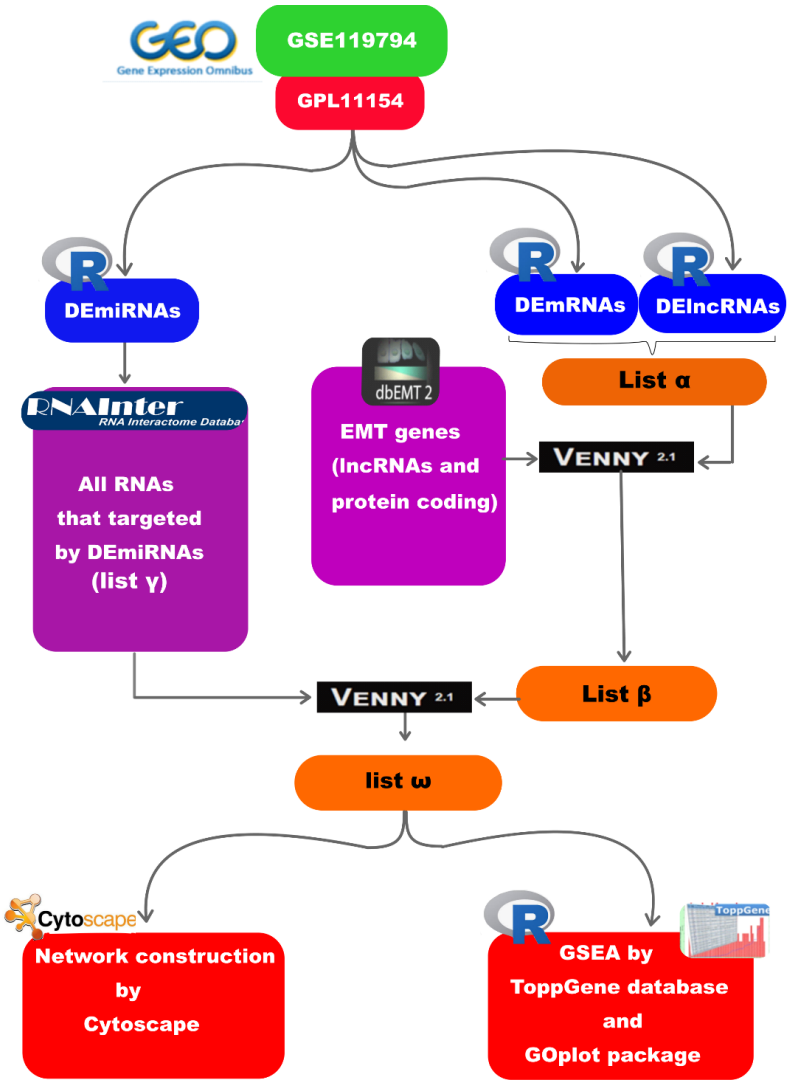

Fig. 1: The study's schematic flowchart for finding EMT biomarkers in pancreatic cancer

Identification of DE-EMT genes

We obtained 1184 EMT genes from dbEMT. To get DE-EMT contains DElncRNA and DEmRNA, we compared 1034 EMT and DElncRNAs and DEmRNAs. Finally, this study assigned 65 DE-EMT (listα) as EMT genes.

Finding DE-EMT for each DEmiRNAs

By RNAInter, we determined DE-EMT that interact with DEmiRNAs. Among 17 DEmiRNAs, only 11 DEmiRNAs (miR-509-3p, miR-708-5p, miR-205-5p, miR-135b-5p, miR-221-3p, miR-4443, miR-210-5p, miR-31-5p, miR-210-3p, miR-4521 and miR-139-5p) interact with DE-EMT based on computational prediction (Supplementary File 2). After conformation between obtained RNAs (*list* β) from RNAInter with DE-EMT (*list*α), we got the final list (*list* ω: including one lncRNA (*UCA1*) and 38 protein-coding genes) which are DE-EMT that interact with DEmiRNAs. Finally, miRNA-mRNA-lncRNA interactions were constructed by Cytoscape 2.9.2. (Fig. 2).

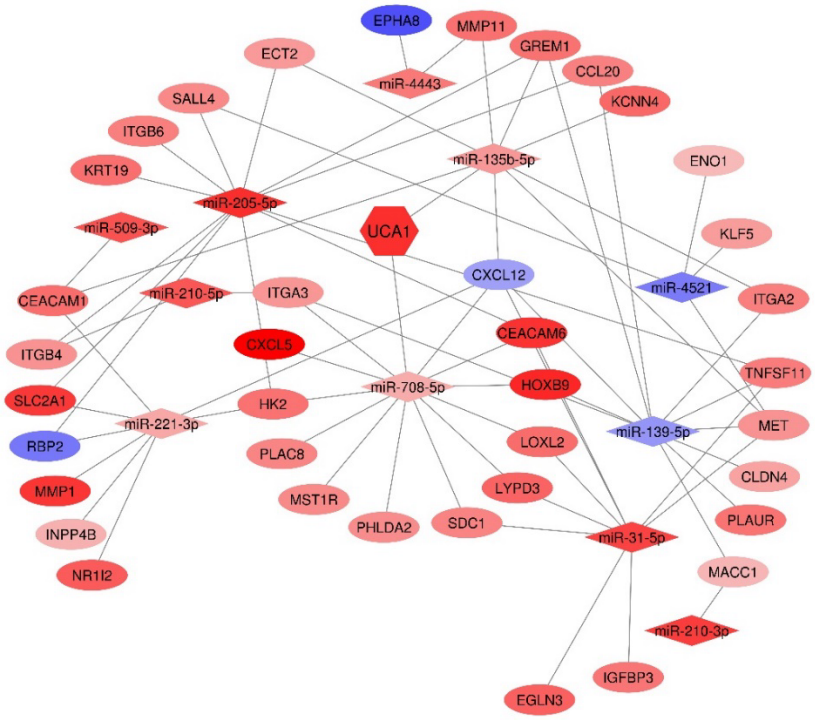

Fig. 2: Networks were constructed between DEmiRNAs and DE-EMT. The diamonds represent DEmiRNAs. The hexagon represents lncRNA. Red and blue color spectrum circles represent up & down-regulated mRNAs, respectively.

Gene Ontology (GO) and pathway enrichment analysis of list ω

The bar and chord plots were utilized to find the enriched GO and KEGG pathways with P adjust <0.05 respectively. In GO analysis, cell-matrix adhesion, cell adhesion mediated by integrin, response to hypoxia, response to decreased oxygen levels, and cell chemotaxisis were most prevalent in the BP category. In CC, protein complex involved in cell adhesion, integrin complex, basal part of cell, basal plasma membrane and apicolateral plasma membrane were more enriched. Moreover, integrin binding, laminin binding, cytokine activity, chemokine activity and extracellular matrix binding were of most noteworthy importance in the field of MF. (Fig. 3A). Besides,

KEGG pathway analysis indicated that ECM-receptor interaction, Rheumatoid arthritis, Arrhythmogenic right ventricular cardiomyopathy (ARVC), Hypertrophic cardiomyopathy (HCM), Pathways in cancer, Dilated cardiomyopathy, HIF-1 signaling pathway and Focal adhesion were more significant (Supplementary File 3) (Fig. 3B).

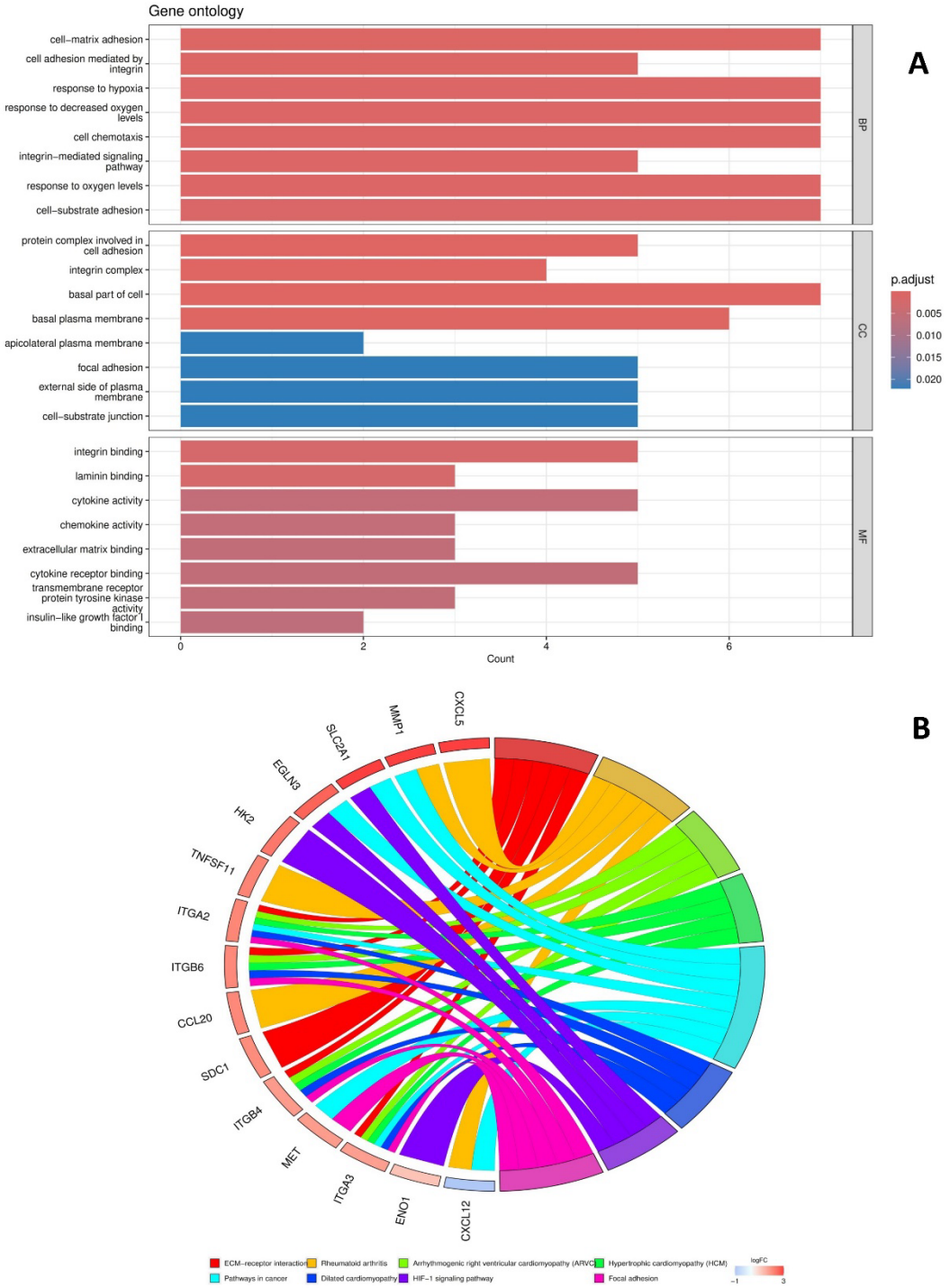

Fig. 3: GO (A) and KEGG pathway (B) enrichment analysis of list ω

ROC analysis

ROC analysis was employed to evaluate the accuracy of the selected genes forecast. ROC curves and AUC values were used to compare the diagnostic values of selected lncRNA and miRNA (Fig. 4 and Supplementary File 4). According to the ROC analysis, the diagnostic value of all genes was high; AUC is equal to 0.79, 0.86, and 0.88 for *UCA1*, miR-708-5p and miR-135b-5p, respectively, showing the expression levels of these genes have significant diagnostic values.

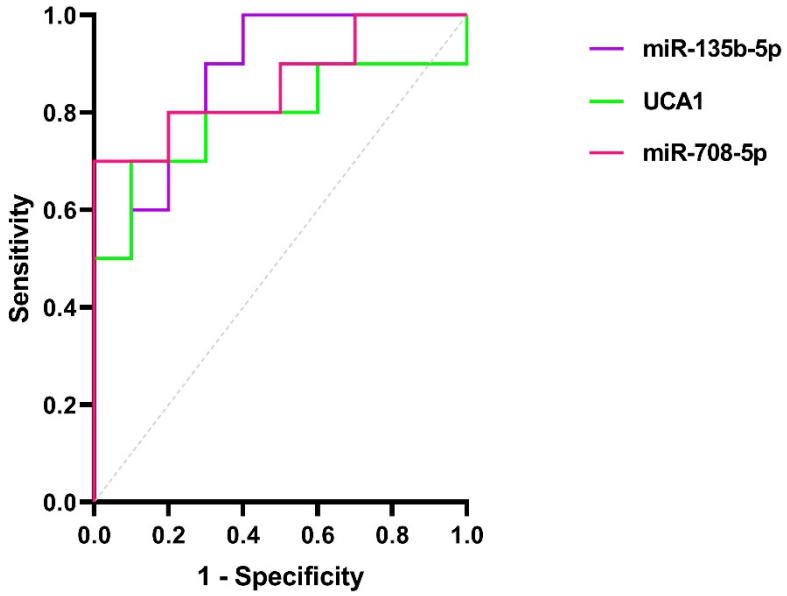

Fig. 4: ROC curves for *UCA1*, miR-708-5p and miR-135b-5p, the computed area under the curve AUC >0.7, $P<0.05$.

Validation of list ω and its related miRNAs and lncRNA

We selected the study GSE41372, which had two platforms. The first platform was GPL6244 (Affymetrix Human Gene 1.0 ST Array [transcript (gene)]), and the second platform was GPL16142 (NanoString nCounter Human miRNA assay [v1]). This study comprised 30 samples: 12 samples (6 normal and 6 tumor samples) for mRNA and lncRNA analysis, and 18 samples (9 normal and 9 tumor samples) for miRNA analysis. After analysis, we obtained the results and compared them with the final results of the current study. *UCA1* was also significantly up-regulated in both studies. Additionally, we compared the list of 38 genes involved in the EMT process from list ω with the significant genes of the study using the online tool Venn Diagram. Half of the list ω was confirmed (Fig. 5A). Furthermore, we examined the 11 miRNAs related to our final network and found that 4 miRNAs overlapped with this study (Fig. 5B). An interesting point was miR-135b-5p; which was the most significantly up-regulated miRNA in the study of interest.

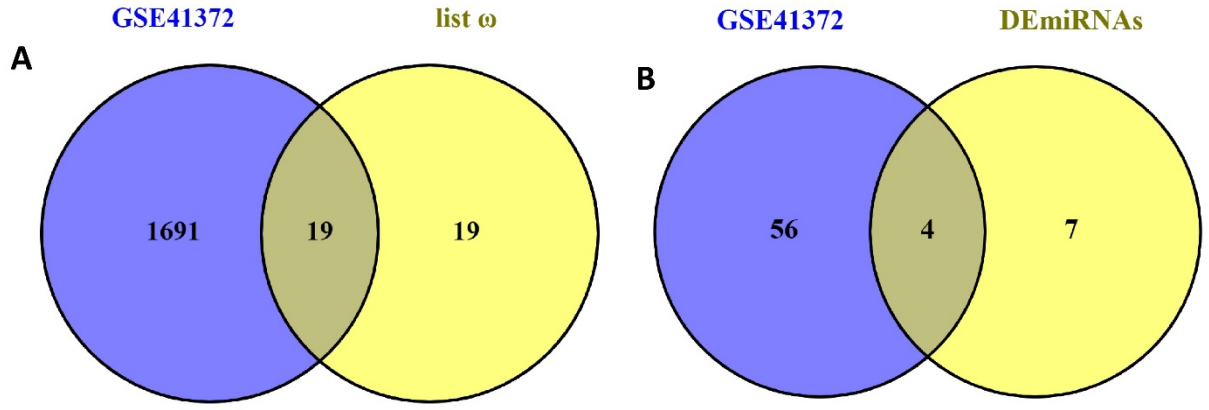

Fig. 5: We compared the list of 38 genes involved in the EMT process from the list ω **(A)** and miRNAs related to the final network **(B)** with the significant genes of the GSE41372 study

Discussion

The findings of this investigation unveiled the distinctive manifestation of long non-coding RNAs (lncRNAs), microRNAs (miRNAs), messenger RNAs (mRNAs), and EMT genes in pancreatic cancer (PC) and surrounding tissue. Additionally, a previously unidentified lncRNA (*UCA1*) was detected, which exhibits a correlation with the EMT process in PC. Consequently, comprehending the molecular mechanisms that underlie the EMT phenomenon in PC is of utmost importance in the advancement of effective methods for diagnosing and treating medical conditions.

To scrutinize the expression patterns of lncRNAs, miRNAs, mRNAs, and EMT genes in PC and surrounding tissue samples, RNA sequencing (RNA-seq) and bioinformatics techniques were employed in this study. The area under the receiver operating characteristic (ROC) curve (AUC) for *UCA1* and hsa-miR-708-5p was determined to be 0.79 and 0.86, respectively.

The outcomes of this study suggested that *UCA1* is an unprecedented lncRNA that plays a role in the regulation of the EMT process in PC. Moreover, *UCA1* could serve as a potential biomarker and target for therapeutic interventions in PC.

DCM, although not directly associated with pancreatic cancer, does share some common mutated genes, such as *LMNA* and *MT-TL1*, which are also found in the pancreas and potentially play a role in pancreatic development and function.

Furthermore, the HIF-1 signaling pathway encompasses the hypoxia-inducible factor 1 (*HIF-1*), a transcription factor is responsible for governing the expression of genes that are implicated in cellular adaptation to low oxygen levels, specifically in situations of hypoxia (24).

One of the methodologies employed for the examination of pancreatic cancer involves the analysis of the genes implicated in its manifestation and behaviour. By comprehending the genetic attributes of pancreatic cancer cells and their interactions within the surrounding milieu, researchers can acquire novel insights into the biology and pathology of this ailment.

These findings posit that *CXCL5*, *HOXB9*, *CEACAM6*, *MMP1*, *SLC2A1*, and *NR12* constitute a selection of the most pivotal genes engaged in the regulation of pancreatic cancer, thereby exhibiting the potential to serve as biomarkers and therapeutic targets for this disorder.

Antecedent investigations have demonstrated an appreciable upsurge in the expression of *CXCL5* within pancreatic cancer tissues when juxtaposed with normal pancreatic tissues, thereby correlating with unfavourable survival rates and prognoses among pancreatic cancer patients (25).

CXCL5 facilitates the growth and spread of pancreatic cancer by augmenting the proliferation, migration, and invasion of malignant cells. The process of EMT is involved in both the initiation and progression of pancreatic cancer, as well as its resistance to chemotherapy (26).

Furthermore, *CXCL5* has the potential to affect the tumor microenvironment by attracting and activating immune suppressive cells like neutrophils and macrophages. These cells possess the ability to release factors that foster tumor growth and impede the anti-tumor immune response (27).

There is a possibility that *CXCL5* establishes a positive feedback loop that amplifies the malignant characteristics of pancreatic cancer cells and shields them from immune-mediated assault. The expression of *HOXB9* is altered in both pancreatic cancer tissues and cell lines, and its presence is associated with the prognosis and survival of individuals with pancreatic cancer. *HOXB9* is down-regulated in pancreatic cancer tissues and cell lines and that overexpression of *HOXB9* inhibits the proliferation, migration, and invasion of pancreatic cancer cells by blocking the progression of the cell cycle during the G0/G1 phase (28).

On the other hand, *HOXB9* is up-regulated in pancreatic cancer tissues and cell lines, and that knockdown of *HOXB9* reduces pancreatic cancer cell proliferation, migration, and invasion by inducing apoptosis and inhibiting EMT (28, 29).

Previous studies have demonstrated a noteworthy augmentation in the expression of *CEACAM6* in pancreatic cancer tissues relative to normal pancreatic tissues. Additionally, this upregulation is linked with unfavourable survival outcomes and prognosis in patients afflicted with pancreatic cancer (30).

Furthermore, *CEACAM6* has the potential to exert an influence on the tumor microenvironment by recruiting and activating immune suppressive cells, including neutrophils and macrophages. These immune cells possess the capability to secrete factors that foster tumor development while simultaneously suppressing the anti-tumor immune response (31).

CEACAM6 might facilitate the advancement of pancreatic cancer and the dissemination of cancer cells by heightening their proliferation, migration, and invasive properties. Epithelial-mesenchymal transition (EMT) has been implicated in the initiation and progression of pancreatic cancer, as well as its resistance to chemotherapy.

CEACAM6 may promote pancreatic cancer growth and metastasis by enhancing the proliferation, migration, and invasion of cancer cells. EMT has been implicated in the initiation and progression of pancreatic cancer, as well as its resistance to chemotherapy

Moreover, *CEACAM6* may also affect the tumor microenvironment by recruiting and activating immune suppressive cells, such as neutrophils and macrophages, which can secrete factors that support tumor growth and inhibit anti-tumor immunity (32).

The expression of *SLC2A1* is notably elevated in pancreatic cancer tissues in comparison to normal pancreas tissues. Furthermore, this increased expression is linked to unfavourable survival rates and prognoses in individuals diagnosed with pancreatic cancer (33).

The promotion of pancreatic cancer growth and metastasis may be facilitated by *SLC2A1* through its ability to augment the proliferation, migration, and invasion of cancerous cells. Consequently, a positive feedback loop may be established by *SLC2A1*, which amplifies the malignant behaviour of pancreatic cancer cells and shields them from immune attacks (34).

However, more studies are needed to confirm the function of these genes in pancreatic cancer and to elucidate the molecular mechanisms and pathways by which it affects pancreatic cancer cells and the tumor microenvironment.

Conclusion

A comprehensive bioinformatics study was carried out to discover lncRNA, miRNA, and mRNA in PC that exhibited differential expression. It was discovered by integrated in silico analysis that a unique mRNA-miRNA-lncRNA

regulatory network could be established. Within this network, lncRNA (*UCA1*), miR-708-5p, miR-135b-5p, and genes exhibited significant predictive values for PC metastasis. In addition, the data provided some essential hints that may be used in the future for PC molecular and mechanistic research. Experiments in vivo and in vitro will be required to complete the necessary further validation.

Journalism Ethics considerations

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Not applicable for this study.

Data availability

Data and supplementary materials related to this article can be obtained from the corresponding author on reasonable request.

Conflict of interests

The authors declare that they have no competing interests.

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