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# Identification of Common miRNAs Differentially Expressed in Periodontitis and Pancreatic Cancer

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## Abstract

*Background/Aim:* Periodontitis is a prevalent multifactorial, oral infectious disease and is considered a high-risk factor for pancreatic cancer. Nevertheless, there is limited understanding of the underlying epigenetic mechanisms governing this relationship. The aim of this study was to identify dysregulated miRNAs associated with periodontitis and pancreatic cancer, along with their related genes, signaling pathways, and compounds.

*Materials and Methods:* miRNA expression datasets for tissues affected by periodontitis and pancreatic cancer were obtained from the Gene Expression Omnibus database. miRNAs differentially expressed relative to normal tissues were detected, and those common to both datasets were determined. Further bioinformatics approaches were used to explore the association of common differentially expressed miRNAs with periodontitis and pancreatic cancer.

*Results:* Twenty shared, differentially expressed miRNAs were identified; 14 exhibited similar expression patterns in both diseases. Among these common differentially expressed miRNAs, 10 were found to be overexpressed. hsa-miR-155, hsa-miR-186, hsa-miR-765, hsa-miR-211 and hsa-miR-375 were the top miRNA nodes in the gene network, with hsa-mir-155 being the sole miRNA node in the transcription factor network. Top candidate miRNA-dysregulated genes included superoxide dismutase 2 (SOD2), nuclear FMR1 interacting protein 2 (NUFIP2), SFT2 domain-containing 2 (SFT2D2), thioredoxin-interacting protein (TXNIP), and cyclin D1 (CCND1), while top dysregulated transcription factors were Argonaute RISC catalytic component 2 (AGO2), AKT serine/threonine kinase 1 (AKT1), BCL6 transcription repressor (BCL6), breakpoint cluster region (BCR), and BRCA1 DNA repair associated (BRCA1). Relevant compounds for targeting these emerged, including 5-fluorouracil, gemcitabine, doxorubicin, ascorbate, diethylstilbestrol, and temozolomide.

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*Conclusion:* Our study suggests candidate molecular mechanisms linking periodontitis to pancreatic cancer, highlighting potential compounds that may target both diseases. These findings provide a foundation for guiding future fundamental and clinical research.

**Keywords:** Periodontitis, pancreatic cancer, network analysis, microRNA, integrated analysis.

## Introduction

Periodontitis, a multifactorial inflammatory disease caused by a complex infection, leads to the loss of connective tissue attachment and bone support for teeth, representing a significant cause of tooth loss in adults (1). Periodontal lesions are chronic wounds that may result in a considerable systemic inflammatory load. Furthermore, periodontal disorder can have adverse effects on overall health, and certain systemic conditions can increase the risk of periodontal disease (1, 2). In addition to pathogens in the biofilm, genetic and lifestyle/environmental factors such as age, race, male sex, body mass index, tobacco use, diabetes, nutrition, and limited access to dental care contribute to the onset and progression of periodontal diseases (1, 2).

In periodontitis, pathogens engage with host tissues and cells, leading to the release of a wide range of inflammatory cytokines, chemokines, proteolytic enzymes, reactive oxygen species, and other mediators, causing permanent deterioration of periodontal tissues. The systemic spread of infectious agents and inflammatory mediators from the oral cavity can result in a chronic and high-level systemic inflammatory condition. This condition can particularly contribute to the pathogenesis of distant inflammatory processes, such as cancer development. Various studies have shown a positive relationship between periodontal disease and the risk of carcinogenesis, supported by shared immune-inflammatory mechanisms, especially in different tissues, including the oral cavity, upper gastrointestinal system, lungs, and pancreas (1).

Over the past 25 years, the incidence of pancreatic cancer has more than doubled globally, becoming a leading cause of death. It constitutes over 2% of all malignancies and is commonly associated with modifiable risk factors such as smoking, obesity, diabetes, and alcohol consumption (2). Surgical intervention in this challenging-to-diagnose cancer is applied to only about 20% of patients, with 5-year survival rates varying between 2 and 9% (3). Early diagnosis is crucial as the chances of successful treatment increase when the disease shows no prominent symptoms in its initial stages. Chronic pancreatitis is frequently observed in individuals with pancreatic cancer, and a significant association has been identified between death related to the digestive system cancer and chronic periodontitis (4). Several prospective studies have demonstrated a high risk of pancreatic cancer in association with periodontal diseases or tooth loss (5).

MicroRNAs (miRNA) are small RNAs known to critically regulate gene expression. These miRNAs play a vital role in modulating cellular processes and mediating the pathogenesis of diseases. Identifying disease-associated miRNAs and their dysregulation patterns can provide a deeper understanding of molecular pathogenesis events (6). Moreover, such miRNAs can serve as potential biological markers or drug therapy targets (7). Integrated bioinformatics has successfully been applied to understand miRNA release in oral disorders, identifying these miRNAs as biological markers for periodontal diseases. At the same time, network analysis of miRNAs has identified differentially expressed miRNAs common in periodontitis, oral squamous cell carcinoma and prostate cancer (8, 9). Interestingly, dysregulated miRNAs in periodontitis are not limited to tissues alone; they can likely circulate through exosomal carriers, influencing gene expression in distant regions (10).

In this context, the present study aimed to conduct an integrated analysis of miRNA expression patterns in both

Table I. *Datasets used for analysis of periodontitis and pancreatic cancer in this study.*

| Disease | Accession no. | Platform | Cases, n | Controls, n | Total, n |
|---|---|---|---|---|---|
| Periodontitis | GSE54710 | GPL15159 | 159 | 41 | 200 |
| Pancreatic cancer | GSE43796 | GPL15159 | 26 | 5 | 31 |
| Pancreatic cancer | GSE60978 | GPL15159 | 51 | 6 | 57 |

periodontitis and pancreatic cancer. The goal was to identify candidate miRNA connections, associated genes, signaling pathways, and relevant compounds. The overarching objective was to enhance the comprehension of molecular mechanisms linking these disorders and provide a theoretical foundation for future basic and clinical study.

## Materials and Methods

*Microarray dataset.* The periodontitis and pancreatic cancer datasets were downloaded from the Gene Expression Omnibus (GEO) database (Table I) (https://www.ncbi.nlm.nih.gov/geo/). For analysis, the periodontitis miRNA expression dataset, GSE54710, and the pancreatic cancer miRNA expression datasets, GSE43796 and GSE60978 were identified.

*Differential expression of miRNA (Differentially expressed miRNA) analysis.* Microarray raw data for the specified samples were downloaded from the GEO database. GeneSpring Software (Agilent Technologies, Santa Clara, CA, USA) version 14.9_gx_pa was used to obtain differentially expressed miRNAs comparing periodontitis and normal gum tissues and comparing pancreatic cancer and normal pancreatic tissue. During the identification of differentially expressed miRNAs, statistical *t*-test analysis was performed between periodontitis and pancreatic cancer tissues and normal tissues. The criteria were established as follows: $p<0.05$, fold change >2.0, and the false-discovery rate was minimized using the Benjamini–Hochberg method for multiple testing correction.

*Analysis of shared differentially expressed miRNA and definition of common differentially expressed miRNA.* The differentially expressed miRNA lists for both diseases were analyzed using the R package VennDiagram to obtain shared differentially expressed miRNAs. These were regarded as mutual differentially expressed miRNAs and underwent additional analysis. miRNAs exhibiting a common expression tendency (both high and low expression) were defined as common differentially expressed miRNAs, while differentially expressed miRNAs with different expression tendencies (opposite expression) were excluded, as they do not contribute to disease-related studies and are not meaningful for scientific research.

*Construction of the common differentially expressed miRNA–target network and functional enrichment analyses.* Common differentially expressed miRNA target networks were created using miRNet 2.0 (https://www.mirnet.ca/). Target genes for the miRNA–gene network were obtained from three databases: miRTarBase v8.0, TarBase v8.0, and miRecords. To reduce network complexity and preserve key features showing network connections, a minimum network was chosen. All starting or query nodes were utilized in the calculation. To establish the minimum network, the shortest paths between pairs of starting nodes were identified, and nodes not included in these paths were eliminated. The same method was applied to create a common differentially expressed miRNA–transcription factor (TF) network utilizing TransmiR v2.0 (http://www.cuilab.cn/transmir) and a common differentially expressed miRNA–small molecule network based on data from SM2miR and PharmacomiR. The biological functions of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were evaluated using ShinyGO 0.80 software (http://bioinformatics.sdstate.edu/go/). The top 20 significantly enriched functions were

ranked with all the genes in the two minimum networks as the query set.

*Validation of common differentially expressed miRNAs.* The validation of miRNA regulation was performed using an independent dataset obtained from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/). TCGA Pancreatic Adenocarcinoma (TCGA-PAAD) and CPTAC-Brain, Head and Neck, Kidney, Lung, Pancreas, Uterus (CPTAC-3) datasets provide miRNA expression data in reads per million mapped for 325 patient samples and 42 control samples. Quantile normalization (11) was applied, followed by log2 transformation of the data. miRNA expression values were computed using the Bioconductor R package edgeR (12) to observe regulation directions, while the pROC R package (13) was used to determine the values of the area under the receiver operating characteristic (ROC) curve (AUC) for the selected miRNAs. The AUC ranges from 0.5 to 1, with higher values indicating greater diagnostic effectiveness.

## Results

*Identification of differentially expressed miRNAs and shared differentially expressed miRNAs.* After filtering, 52 miRNAs associated with periodontitis were identified in the analysis of the GSE54710 dataset. For the analysis of the GSE43796 and GSE60978 datasets, 743 miRNAs associated with pancreatic cancer were identified (Supplementary Table SI). When comparing periodontitis to controls, three differentially expressed miRNAs were overexpressed, and one was underexpressed. Similarly, in pancreatic cancer, 98 miRNAs were overexpressed, while 645 miRNAs were underexpressed. The intersection of the two differentially expressed miRNA lists was examined using a Venn diagram, which revealed a total of 20 shared differentially expressed miRNAs (Figure 1).

Related expression patterns in the two conditions are illustrated in Figure 2. When compared to control tissues in both conditions, similar expression patterns were evident for 14 shared differentially expressed miRNAs; 10 showed overexpression, and four exhibited low expression. Four shared differentially expressed miRNAs, namely *hsa-miR-1246*, *hsa-miR-24-1**, *hsa-miR-33a* and *hsa-miR-744*, demonstrated low expression in tissues affected by periodontitis compared to controls, while showing overexpression in pancreatic cancer tissues. In addition, two shared differentially expressed miRNAs, *hsa-miR-148*a and *hsa-miR-564*, exhibited opposite expression patterns, with overexpression in periodontitis and low expression in pancreatic cancer. The shared differentially expressed miRNAs are shown in Table II.

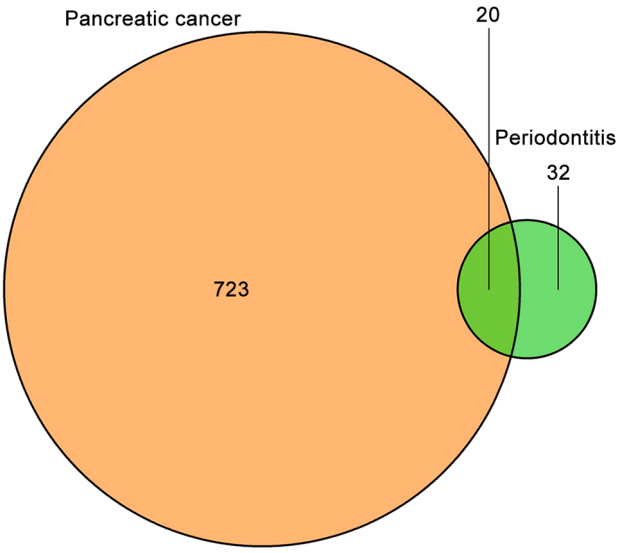


Figure 1. *Venn diagram showing shared differentially expressed miRNAs.*

*Common differentially expressed miRNA–gene network.* The common differentially expressed miRNA–gene minimum network, comprising 90 genes and 20 miRNAs, was a network with 369 edges (Figure 3, Supplementary Table SII). The differentially expressed miRNAs with the highest degree of connectivity in the network were *hsa-miR-155*-5p, *hsa-miR-186*-5p, *hsa-miR-765*, *hsa-miR-211-5p* and *hsa-miR-375*. A total of 90 gene nodes were found in the network. Among the top five gene nodes with the highest degree in the network were superoxide dismutase 2 (*SOD2*), nuclear FMR1-interacting protein 2 (*NUFIP2*),

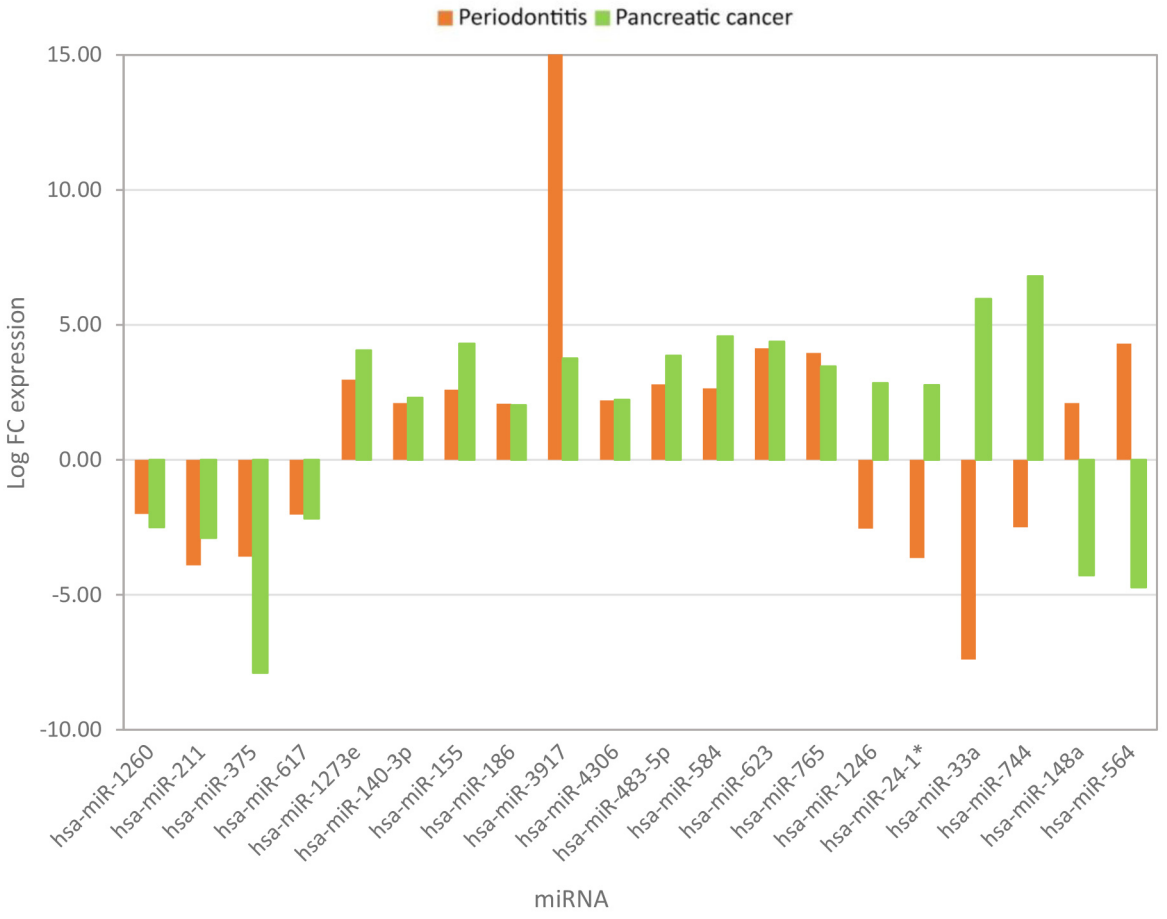


Figure 2. *Column chart showing log fold-change (FC) expression values for shared differentially expressed miRNAs in periodontitis and pancreatic cancer.*

Table II. *Shared differentially expressed miRNAs in periodontitis and pancreatic cancer.*

| Expression | Differentially expressed miRNA |
|---|---|
| Up-regulated in both | *hsa-miR-1273e, hsa-miR-140-3p, hsa-miR-155, hsa-miR-186, hsa-miR-3917, hsa-miR-4306, hsa-miR-483-5p, hsa-miR-584, hsa-miR-623, hsa-miR-765* |
| Down-regulated in both | *hsa-miR-1260, hsa-miR-211, hsa-miR-375, hsa-miR-617* |
| Opposite regulation | *hsa-miR-1246, hsa-miR-24-1*, hsa-miR-33a, hsa-miR-744, hsa-miR-148a, hsa-miR-564* |

SFT2 domain-containing 2 (*SFT2D2*), thioredoxin-interacting protein (*TXNIP*), and cyclin D1 (*CCND1*). The common differentially expressed miRNA-associated target networks and top nodes are summarized in Table III.

The top 20 enriched functions for each are listed in Figure 4. KEGG pathway analysis showed thyroid cancer, endometrial cancer, prostate cancer, colorectal cancer, pathways in cancer and other related pathways. The GO biological process analysis revealed the regulation of various processes, including cellular processes. The most enriched GO cellular components included the TF complex, lamellipodium, nucleoplasm and nuclear lumen.

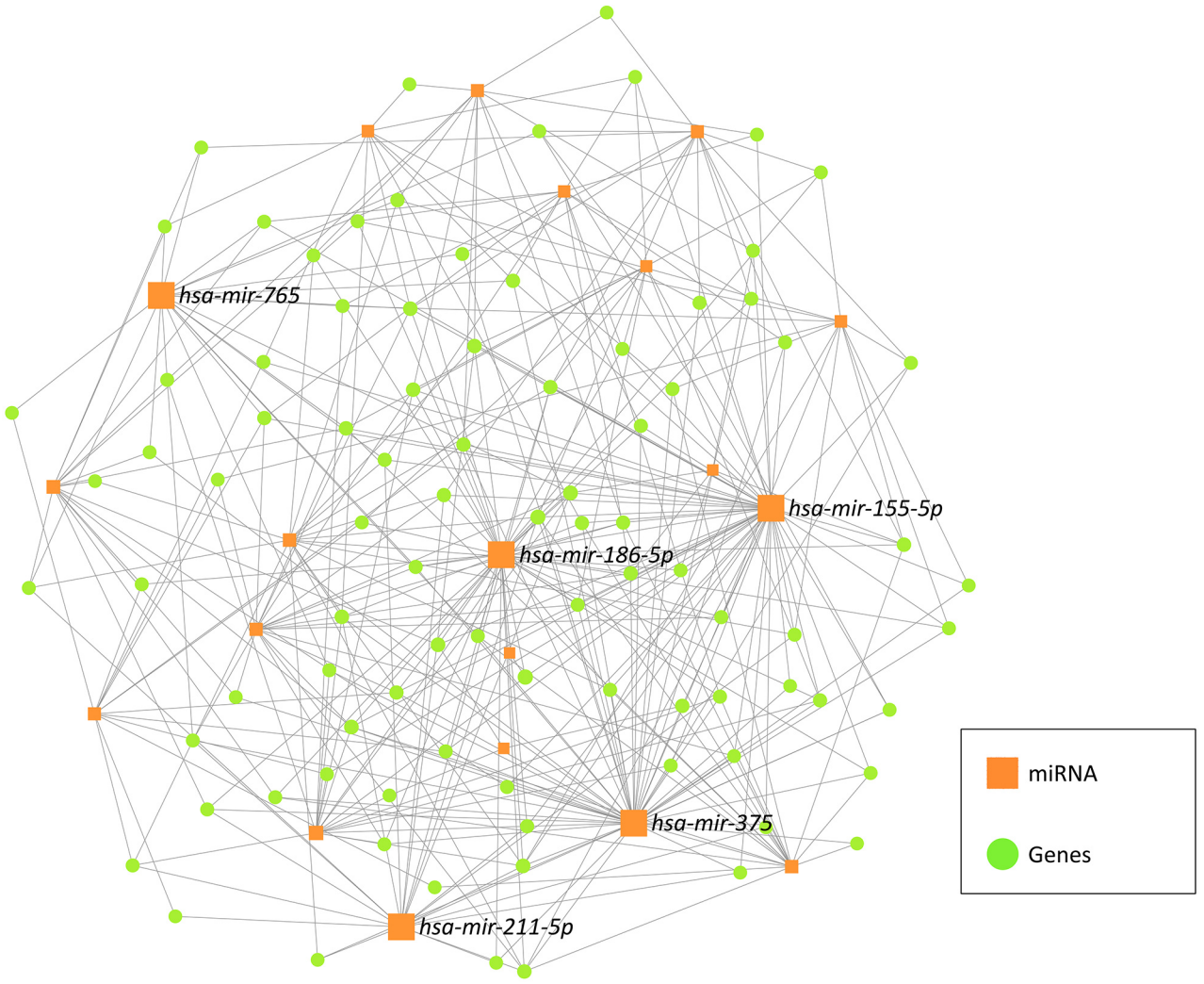


Figure 3. *miRNA–gene network for common differentially expressed miRNAs in periodontitis and pancreatic cancer.*

Among the most enriched GO molecular functions were functions related to binding, including enzyme, protein, TF, and chromatin binding.

*Common differentially expressed miRNA–TF network.* The common differentially expressed miRNA–TF minimum network represents a network consisting of 44 TFs and five miRNAs, with a total of 44 edges (Figure 5, Supplementary Table SIII). A total of 35 TFs were found in the network. Among the top TFs were Argonaute RISC catalytic component 2 (*AGO2*), AKT serine/threonine kinase 1 (AKT1), B-cell lymphoma 6 (BCL6), breakpoint cluster region (BCR), and BRCA1 DNA repair-associated (BRCA1), with a single miRNA node, *hsa-mir-155*, in the network (Figure 5, Supplementary Table SIII). The enriched KEGG and GO pathways are listed in Figure 6. KEGG pathway analysis showed pancreatic cancer, pathways in cancer, and other related pathways. GO biological process analysis encompassed processes involving both negative and positive regulation, such as RNA metabolic and transcriptional processes. The most enriched GO cellular components included the chromatin,

Table III. *The common differentially expressed miRNA-associated target networks and top nodes in periodontitis and pancreatic cancer.*

| | Top differentially expressed miRNA | | |
|---|---|---|---|
| Network | Up-regulated | Down-regulated | Top members |
| miRNA–gene | *hsa-miR-155*<br>*hsa-miR-186*<br>*hsa-miR-765* | *hsa-miR-211*<br>*hsa-miR-375* | *SOD2*<br>*NUFIP2*<br>*SFT2D2*<br>*TXNIP*<br>*CCND1* |
| miRNA–TF | *hsa-miR-155* | | *AGO2*<br>*AKT1*<br>*BCL6*<br>*BCR*<br>*BRCA1* |
| *miRNA–Compound* | *hsa-miR-155*<br>*hsa-miR-186*<br>*hsa-miR-765* | *hsa-miR-211*<br>*hsa-miR-375* | 5-Fluorouracil<br>Gemcitabine<br>Doxorubicin<br>Ascorbate diethylstilbestrol |

*AGO2:* Argonaute RISC catalytic component 2; *AKT1:* AKT serine/threonine kinase 1; *BCL6:* BCL6 transcription repressor; *BCR:* breakpoint cluster region; *BRCA1:* BRCA1 DNA repair-associated; *CCND1*: cyclin D1; *NUFIP2*: nuclear FMR1 interacting protein 2; *SFT2D2*: SFT2 domain-containing 2; *SOD2*: superoxide dismutase 2; *TXNIP*: thioredoxin-interacting protein.

chromosome, nucleoplasm and nuclear lumen. Among the most enriched GO molecular functions were functions related to binding, including enzyme, protein, TF, and chromatin binding.

*Common differentially expressed miRNA–small-molecule network.* The common differentially expressed miRNA–small-molecule minimum network represents a network consisting of six compounds and 11 miRNAs, with a total of 17 edges (Figure 7, Supplementary Table SIV). Included in this network were six compounds: 5-fluorouracil (5-FU), gemcitabine doxorubicin, ascorbate diethylstilbestrol, and temozolomide. The component with the highest degree and highest betweenness centrality in this network is 5-FU, followed by gemcitabine.

*Validation of common differentially expressed miRNAs.* The top common differentially expressed miRNAs identified were analyzed using pancreatic cancer data from TCGA as a validation set. The diagnostic efficacy and regulation patterns of these miRNAs in pancreatic cancer were evaluated. The regulatory patterns were observed to be co-up-regulated for *hsa-miR-765* and co-down-regulated for *hsa-miR-375* and *hsa-miR-211*, while the opposite was true for *hsa-miR-155* and *hsa-miR-186*. The ROC curves were generated for these common differentially expressed miRNAs, revealing that *hsa-miR-211* (AUC=0.675) and *hsa-miR-375* (AUC=0.594) had acceptable diagnostic accuracy for pancreatic cancer (Figure 8), whereas *hsa-miR-765* (AUC=0.462) exhibited low accuracy.

## Discussion

miRNAs are small RNA molecules with capable of regulating genetic material, controlling many biological processes by regulating gene expression (6). Some microorganisms can influence their host's gene expression, and these interactions are often complex and multifaceted. It has been shown that the periodontal pathogen *Porphyromonas gingivalis* can stimulate tumor formation and pancreatic tumor proliferation in humans (14). In addition, several scientific studies have suggested a potential relationship between periodontitis and pancreatic cancer (2-4). However, this relationship remains unclear, and a definitive cause-and-effect relationship has not been established.

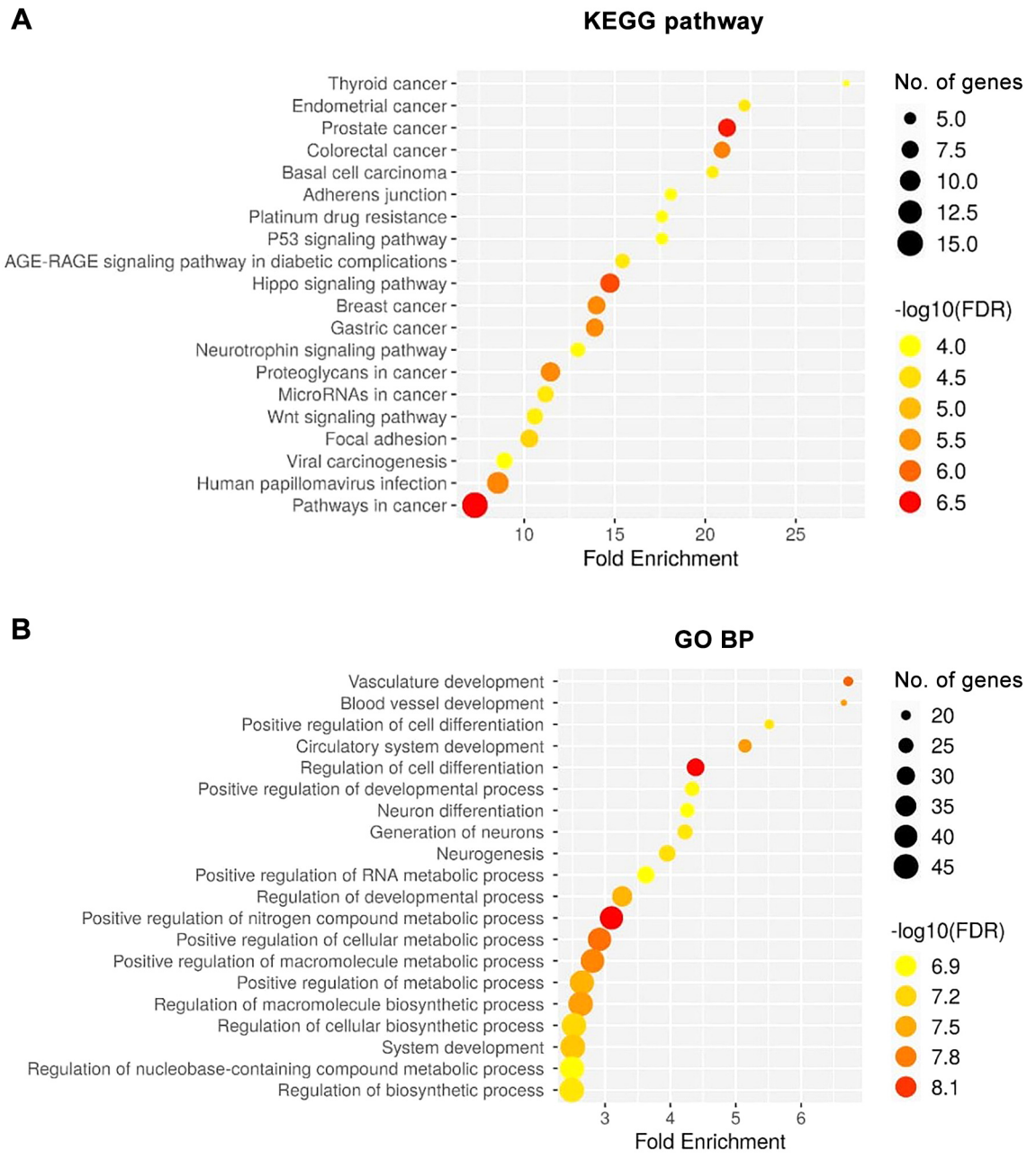


Figure 4. *Continued*

In this study, bioinformatic analyses were performed to explore the connection between periodontitis and pancreatic cancer. By identifying common dysregulated miRNAs in both conditions, we explored the regulatory mechanisms mediated by these miRNAs. Network analysis was employed to determine key candidate genes and that could be affected by the regulatory processes mediated by miRNAs. In addition, functional enrichment analysis on these genes determined the key pathways, molecular functions, and cellular components involved. Furthermore,

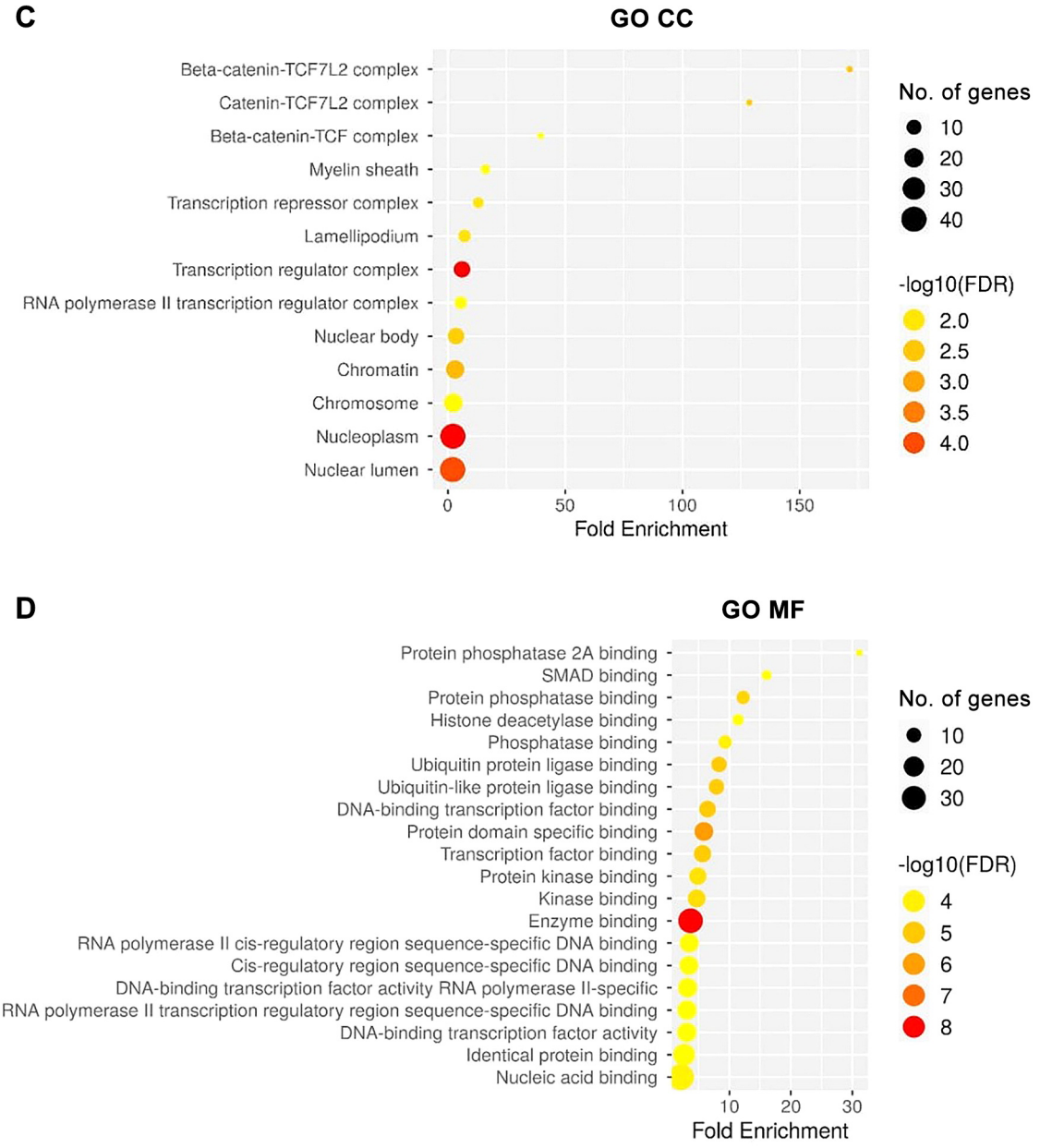


Figure 4. *Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analyses of genes associated with common differentially expressed miRNAs in periodontitis and pancreatic cancer. (A) KEGG pathway analysis. (B) Enriched biological processes (BP). (C) Enriched cellular components (CC). (D) Enriched molecular functions (MF). FDR: False-discovery rate.*

small molecule compounds linked with the common differentially expressed miRNAs were analyzed to investigate significant linkages between periodontitis and pancreatic cancer.

Oral infections can lead to inflammation in distant regions, and it is known that chronic inflammation triggers carcinogenesis (1). In our study, most differentially expressed miRNAs showed the same expression trend in

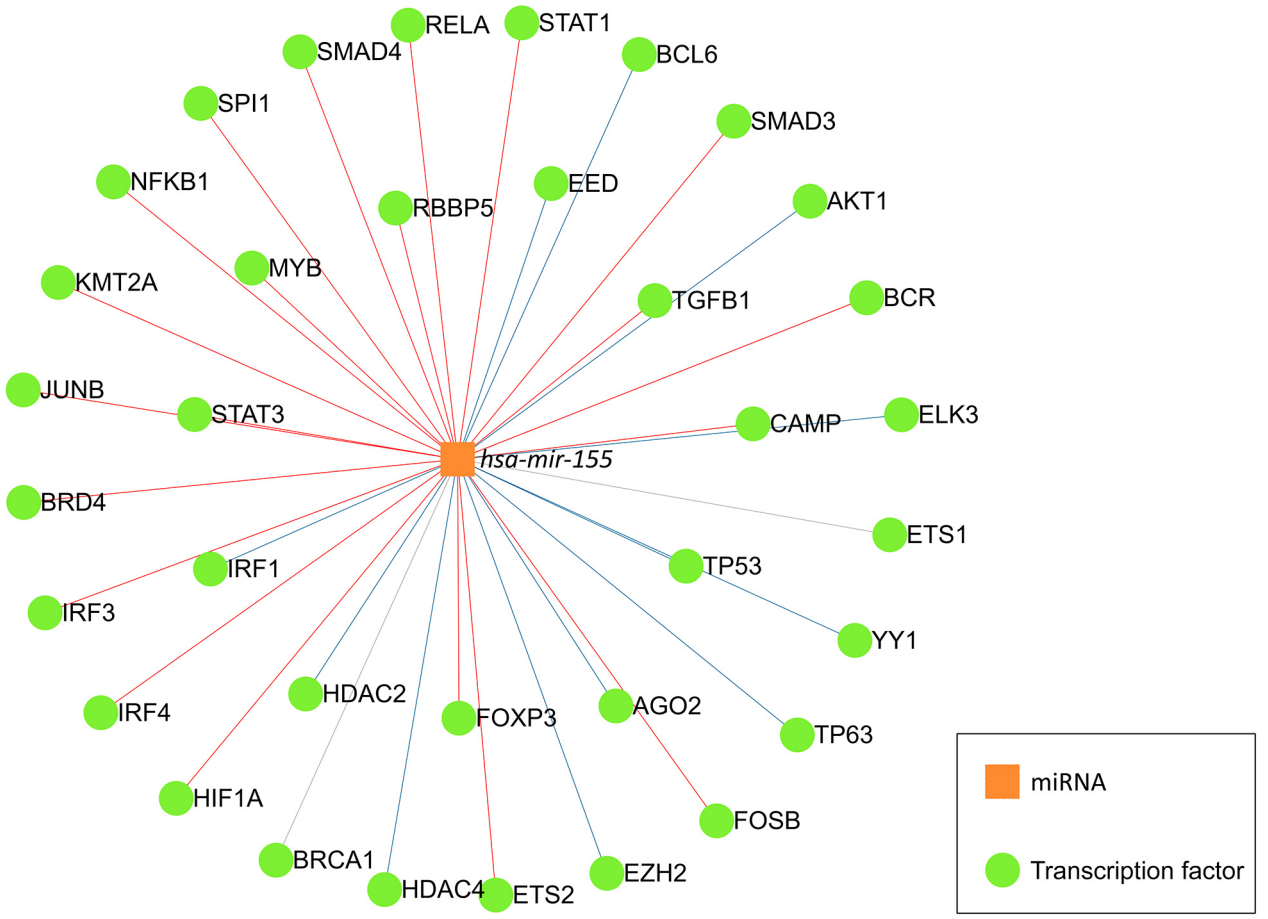


Figure 5. *miRNA–transcription factor network for common differentially expressed miRNAs in periodontitis and pancreatic cancer. AGO2: Argonaute RISC catalytic component 2; AKT1: AKT serine/threonine kinase 1; BCL6: BCL6 transcription repressor; BCR: BCR activator of RHO GEF and GTPase; BRCA1: breast cancer 1; BRD4: bromodomain-containing 4; CAMP: cathelicidin antimicrobial peptide; EED: embryonic ectoderm development; ELK3: ETS transcription factor ELK3; ETS1/2: ETS proto-oncogene 1/2; EZH2: enhancer of zeste 2 polycomb repressive complex 2 subunit; FOXP3: forkhead box P3; FOSB: FosB proto-oncogene; AP-1 transcription factor subunit; HDAC2/4: histone deacetylase 2/4; HIF1A: hypoxia-inducible factor 1 subunit alpha; IRF1/3/4: interferon regulatory factor 3/4; JUNB: JunB proto-oncogene; AP-1 transcription factor subunit; KMT2A: lysine methyltransferase 2A; MYB: MYB proto-oncogene, transcription factor; NFKB1: nuclear factor kappa B subunit 1; RELA: RELA proto-oncogene; RBBP5: RB binding protein 5; histone lysine methyltransferase complex subunit; SMAD3/4: SMAD family member 3/4; SPI1: Spi-1 proto-oncogene; STAT1/3: signal transducer and activator of transcription 1/3; TGFB1: transforming growth factor beta 1; TP53: tumor protein P53; TP63: tumor protein P63; YY1: YY1 transcription factor.*

both conditions, suggesting that the host's oral micro-environment may exhibit similar immune mechanisms against inflammation and cancer. This similarity in pro-inflammatory and pro-cancer responses implies a common pattern of miRNA dysregulation observed in these two diseases. Thus, these similar expression trends may indicate the presence of a shared miRNA regulatory pattern for the two diseases.

Our study identified several keys differentially expressed miRNAs in both periodontitis and pancreatic cancer, including *hsa-miR-155*, *hsa-miR-186*, and *hsa-miR-765* (up-regulated), as well as *hsa-miR-211* and *hsa-miR-375* (down-regulated). Among these, *hsa-miR-155*, a well-established regulator of inflammation and immune responses, was significantly up-regulated. This miRNA targets SRC homology 2 domain-containing inositol-5-


Figure 6. *Continued*

phosphatase 1 (*SHIP1*), thereby influencing inflammation, myeloid-derived suppressor cell activation, and tumor-associated macrophage polarization. These processes play pivotal roles in both periodontitis and cancer progression (15, 16). Similarly, *hsa-miR-186*, also up-regulated in our study, exhibits dual roles as either an oncogenic or tumor-

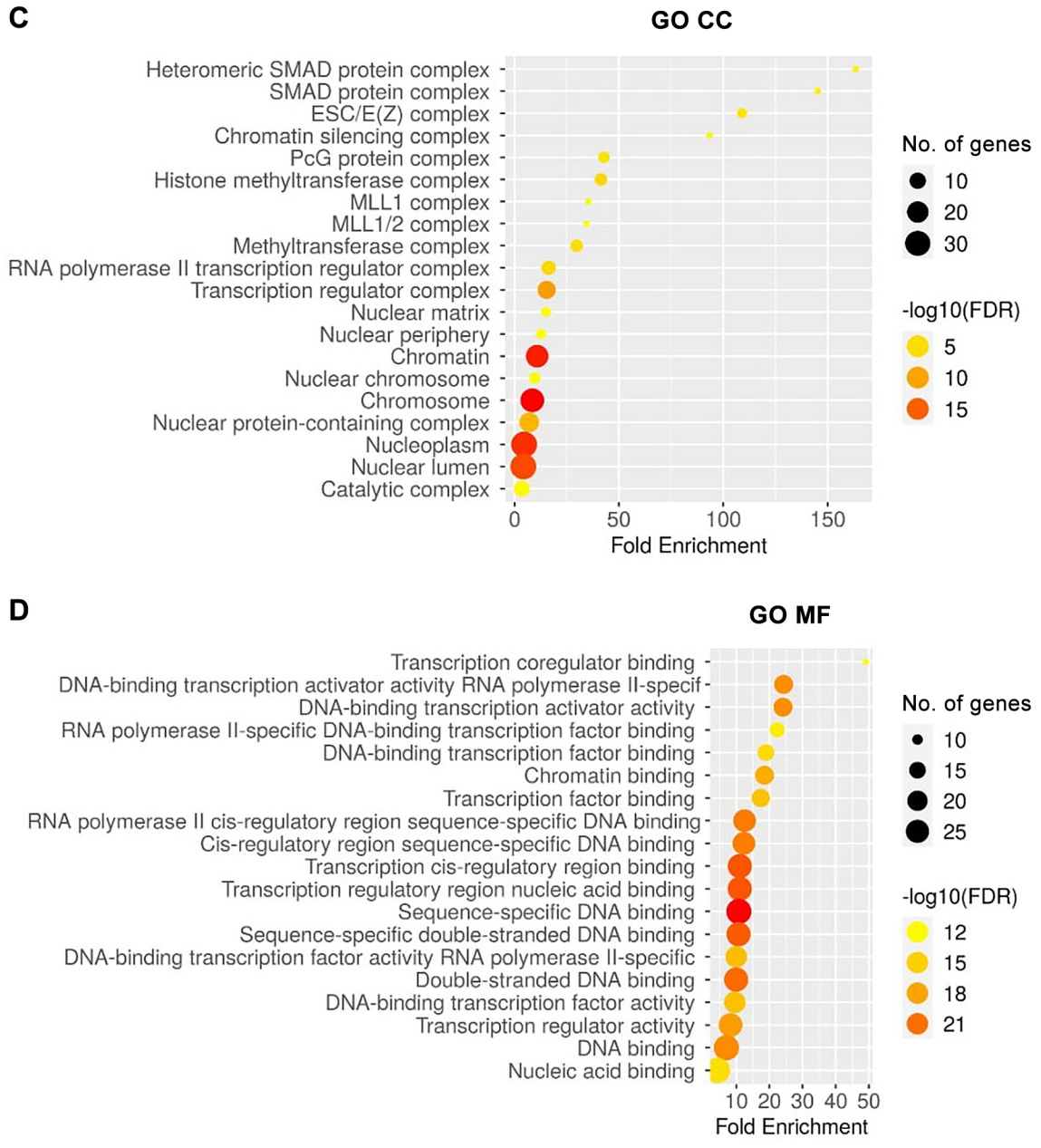


Figure 6. *Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analyses of genes associated with transcription factors associated with common differentially expressed miRNAs in periodontitis and pancreatic cancer. (A) KEGG pathway analysis. (B) Enriched biological processes (BP). (C) Enriched cellular components (CC). (D) Enriched molecular functions (MF). FDR: False-discovery rate.*

suppressive miRNA depending on the tissue context. Its dysregulation has been implicated in multiple cancer types, including pancreatic cancer, highlighting its potential as a therapeutic target (17). Another up-regulated miRNA, *hsa-miR-765*, was shown to promote malignancy in pancreatic cancer through interactions with long intergenic non-protein coding RNA 994 (LINC00994) and RUNT-related transcription factor 2 (RUNX2) (18). However, its

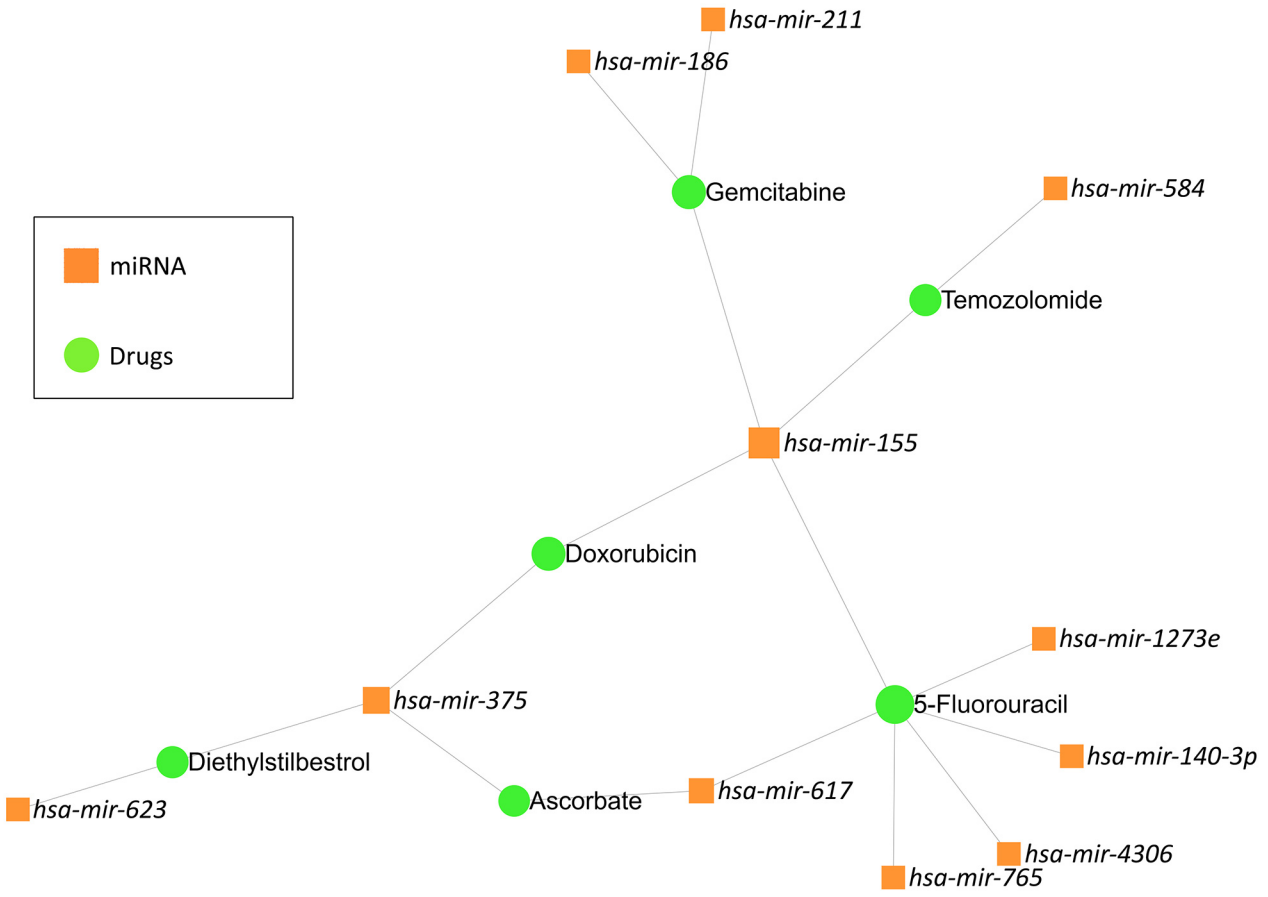


Figure 7. *miRNA–small-molecule network for common differentially expressed miRNAs in periodontitis and pancreatic cancer.*

role in periodontitis remains unexplored, warranting further investigation into shared molecular mechanisms.

Conversely, we found *hsa-miR-211* was down-regulated in both diseases. In periodontitis, it regulates inflammation-related pathways, while in pancreatic cancer, its down-regulation is associated with metastasis and resistance to gemcitabine treatment, suggesting its potential as a therapeutic target (19, 20). Similarly, *hsa-miR-375*, another miRNA we found to be down-regulated, is involved in immune regulation and is linked to pancreatic cancer proliferation and apoptosis. While its role in periodontitis remains poorly defined, its regulatory functions in inflammatory and oncological contexts highlight its value as a biomarker and therapeutic candidate (21, 22). Collectively, these findings underscore the importance of these miRNAs in shared pathological mechanisms, offering significant insights for both diagnostic and therapeutic strategies in periodontitis and pancreatic cancer.

Our research suggests that the underlying mechanism linking periodontitis to pancreatic cancer and other cancer types may involve disruptions in gene expression regulated by non-coding RNAs, particularly miRNAs. Through the common differentially expressed miRNA–gene network analysis, key central genes that may play a crucial role in the relationship between periodontitis and pancreatic cancer included *SOD2*, *NUFIP2*, *SFT2D2*, *TXNIP*, and *CCND1*. SOD2, a mitochondrial enzyme, converts superoxide anions into hydrogen peroxide, promoting epithelial–mesenchymal transition in cancer cells, and responding to microbial and

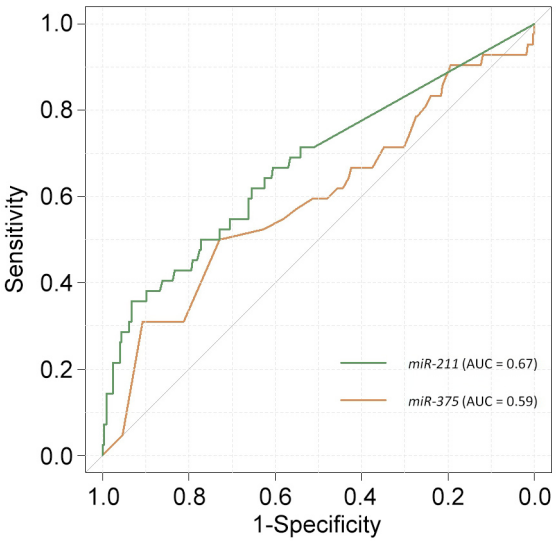


Figure 8. *The receiver operating characteristics curves for the top common differentially expressed miRNAs validated by The Cancer Genome Atlas dataset. AUC: Area under the receiver operating characteristics curve.*

mechanical signals in periodontal cells (23-25). *NUFIP2* is less studied but is associated with immune diseases and may be involved in immune response and immune cell function signaling pathways, with a potential link to pancreatic cancer (26). SFT2D2 is partially homologous to the yeast Sft2p protein and interacts genetically with yeast syntaxin 5 protein (Sed5p), exerting an influence on post-Golgi transport (27). The expression of the *SFT2D2* gene is altered in both periodontal diseases and pancreatic cancer, suggesting that it may play an important molecular role in both conditions (28, 29). *TXNIP* is a significant pathological regulator in diseases associated with glucose and lipid imbalances and inflammation. It has been recognized in various cancer types, including pancreatic cancer, where elevated glucose levels activate *p38*, mitogen-activated protein kinase and extracellular-regulated kinase pathways, supporting *TXNIP* expression and tumor development (30, 31). Moreover, the reactive oxygen species–*TXNIP*–NLR family pyrin domain-containing 3 inflammatory signaling pathway may play a critical role in the development of periodontal disease (32). *CCND1*, a key regulator of cell-cycle progression, facilitates the $G_1$/S transition, promoting cell proliferation and growth (33). Its overexpression is commonly found in tumors and is linked to poor prognosis in pancreatic cancer (34). It has been hypothesized that polymorphisms of *CCND1* may mediate oral cancer risk associated with periodontitis (35). These findings suggest that these genes may be crucial players in the molecular mechanisms of both periodontitis and pancreatic cancer and may serve as potential biomarkers or therapeutic targets.

The common differentially expressed miRNA–TF network identified AGO2, AKT1, BCL6, BCR, and BRCA1 as key TFs. *AGO2* may play a role in apoptotic processes in periodontitis and is considered a marker for distinguishing periodontitis (36). AGO2, a mediator of RNA silencing, promotes cell proliferation, migration, and cycle progression, and interacts with *KRAS* proto-oncogene, GTPase and epidermal growth factor receptor in pancreatic cancer, disrupting its function (37). AKT1, often hyperactive in cancer, enhances cell survival and growth, with a significant role in pancreatic cancer development and potential as a therapeutic target (38). It also regulates macrophage polarization and periodontal inflammation (39). BCL6, an oncogenic suppressor, is regulated by transcriptional and epigenetic factors, influencing miRNA expression and promoting apoptosis resistance, epithelial-mesenchymal transition, and migration in pancreatic carcinoma (40). BRCA1, critical for tumor suppression, exhibits loss of heterozygosity in *BRCA1* mutation carriers, contributing to pancreatic cancer progression (41, 42). These findings highlight shared pathways between pancreatic cancer and inflammatory diseases such as periodontitis.

In this analysis, small compounds targeting differentially expressed miRNAs were examined, with 5-FU, gemcitabine, doxorubicin, ascorbate, and diethylstilbestrol identified as having the highest correlation. Some miRNAs have been shown to increase resistance to 5-FU in human pancreatic cancer cells (43). Furthermore, 5-FU exacerbates periodontitis, impairing tissue repair and causing more periodontal damage due to its harmful effects on cell and

blood vessel regeneration (44). Gemcitabine, a widely used treatment for pancreatic ductal adenocarcinoma since 1997, remains a standard therapy for locally advanced and metastatic pancreatic ductal adenocarcinoma, despite its limited effect on overall survival (45). Over the past two decades, it has been commonly used either alone or in combination with other treatments such as fluoropyrimidines, platinum analogs, or the epidermal growth factor receptor inhibitor erlotinib (46). Further research is needed to explore the interactions of these compounds and their relationship to both pancreatic cancer and periodontitis.

The involvement of these miRNA-targeted genes and TFs in pancreatic cancer was predicted and validated using ShinyGO enrichment analysis. These analyses demonstrated that these genes are significantly implicated in multiple cancer-related KEGG pathways, influencing various biological processes and molecular functions. This aligns with earlier findings suggesting that periodontitis is a major risk factor for pancreatic cancer (2). They affect all cellular levels, ranging from the extracellular modulation of signaling molecules to the regulation of various TFs within the nucleus. Several GO molecular functions associated with TF binding were enriched, playing a vital role in the regulation of immune responses and inflammation (47, 48).

To validate the dataset obtained from the GEO database, we utilized the TCGA datasets. Among the top miRNAs, *hsa-miR-765* was observed to be up-regulated without significance, while *hsa-miR-375* and *hsa-miR-211* were validated as down-regulated, consistent with the GEO data. However, *hsa-miR-155* and *hsa-miR-186*, identified as up-regulated in the GEO dataset, were found to be down-regulated in the TCGA validation. A review of the literature reveals studies reporting that *hsa-miR-155* was up-regulated in pancreatic cancer (49, 50), whereas an analysis based on TCGA datasets demonstrated that *hsa-miR-155* was down-regulated in pancreatic cancer (51). Thus, the regulatory status of *hsa-miR-155* in pancreatic cancer remains controversial. Similarly, *hsa-miR-186* has been reported to exhibit dual roles as both a tumor suppressor and an oncogenic miRNA (52, 53), further complicating its regulatory status in pancreatic cancer. Future studies are warranted to elucidate the precise roles of these miRNAs in pancreatic cancer.

This research explored various factors, including differentially expressed miRNAs, common differentially expressed miRNA–gene interactions, TFs, and related compounds, to investigate the interconnected epigenetic mechanisms linking periodontitis and pancreatic cancer. A significant limitation of this study is the lack of investigation into other non-coding RNA types (long non-coding RNAs, circular RNAs, small non-coding RNAs), which may also play crucial roles in the mechanisms underlying these diseases. This highlights the need for further in-depth research in this area. Future studies should focus on elucidating the connections between common differentially expressed miRNA genes, TF pathways, and compounds through clinical studies, as well as *in vitro* and *in vivo* experiments. Such efforts might provide a more comprehensive understanding of the biological interactions between periodontitis and pancreatic cancer, ultimately aiding the development of more effective therapeutic strategies.

## Conclusion

The thorough analysis of common differentially expressed miRNAs in periodontitis and pancreatic cancer uncovered essential genetic and molecular mechanisms. These mechanisms involve miRNAs such as *hsa-mir-155*, *hsa-mir-186*, *hsa-miR-765*, *hsa-miR-211* and *hsa-mir-375*; genes such as *SOD2*, *NUFIP2*, *SFT2D2*, *TXNIP*, and *CCND1*; and TFs such as *AGO2*, *AKT1*, *BCL6*, *BCR*, and *BRCA1*. In addition, the study highlighted small-molecule compounds such as 5-FU, gemcitabine doxorubicin, ascorbate diethylstilbestrol, and temozolomide most associated with differentially expressed miRNAs. The identified core common differentially expressed miRNAs in this study might serve

as an effective guide for understanding the processes of transformation between inflammation and cancer and provide valuable insights for future research.

## Supplementary Material

Supplementary Tables available at: https://dx.doi.org/10.6084/m9.figshare.28525724

## Conflicts of Interest

The Authors declared that they have no conflict of interests including financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

## Authors' Contributions

Concept: DS-A, CU and NKS; design: DS-A, CU and NKS; data collection or processing: DS-A, CU, NKS and TO; analysis or interpretation: DS-A, CU, NKS and TO; literature search: DS-A, CU, NKS, TO, NC, SEK, AK and HS; Writing: DS-A, CU, NKS and TO.

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## Data Availability Statement

The datasets analyzed during the current study are available in the GEO (https://www.ncbi.nlm.nih.gov/geo/) and TCGA (https://portal.gdc.cancer.gov/) repositories. Data generated by the Authors are shown in this article or in the Supplementary Materials. Further data are available from the corresponding author upon reasonable request.

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