Expression, diagnostic value, and biological role of MicroRNA-4486 in pancreatic cancer

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Abstract: Objective: To investigate the expression characteristics and clinical diagnostic value of microRNA-4486 (miR-4486) in the serum of patients with pancreatic cancer, as well as its impact on the biological behavior of pancreatic cancer cells. Methods: A total of 40 patients diagnosed with pancreatic cancer from January 2018 to December 2024 were selected as the case group, and 40 healthy individuals during the same period were included as the control group. Real-time quantitative PCR (q RT-PCR) was used to detect the expression level of serum miR-4486. The human pancreatic cancer cell line PANC-1 was cultured in vitro and transfected with miR-4486 mimics and inhibitors, respectively. Cell proliferation, migration, and invasion capabilities were assessed using CCK-8 and Transwell assays. Results: The expression level of serum miR-4486 in patients with pancreatic cancer was significantly higher than that in the healthy control group ($P < 0.05$). High expression of serum miR-4486 was significantly associated with tumor size ≥ 3 cm, TNM stages III-IV, and lymph node metastasis (all $P < 0.05$). In vitro functional experiments showed that overexpression of miR-4486 promoted the proliferation, migration, and invasion of PANC-1 cells, whereas its inhibition significantly suppressed these phenotypes (all $P < 0.05$). Conclusion: miR-4486 is highly expressed in the serum of patients with pancreatic cancer and is closely related to tumor progression. It can regulate the proliferation, migration, and invasion of pancreatic cancer cells. These findings suggest that miR-4486 may serve as a potential diagnostic biomarker and therapeutic target for pancreatic cancer.

Keywords: Pancreatic cancer, MicroRNA-4486, Serum biomarker, Cell proliferation

1. Introduction

Pancreatic cancer is one of the digestive system malignancies with the poorest prognosis, with a 5-year survival rate remaining below 10%. This is primarily attributed to the lack of specific clinical manifestations in the early stages, difficulties in diagnosis, and its highly aggressive biological characteristics, including a high propensity for metastasis. The development and progression of pancreatic cancer involve multi-level and multi-step molecular regulatory processes, among which the dysregulation of the gene expression network is considered a crucial molecular basis. In recent years, the regulatory roles of non-coding RNAs, particularly microRNAs (miRNAs), in tumors have garnered extensive attention. MiRNAs are a class of endogenous small non-coding RNAs approximately 22 nucleotides in length. They can regulate gene expression at the post-transcriptional level by binding to the 3′-untranslated regions (3′-UTRs) of target mRNAs, thereby exerting dual biological functions as either tumor suppressor genes or oncogenes in processes such as tumor cell proliferation, apoptosis, invasion, and metastasis [1-2].

Previous studies have demonstrated that the aberrant expression of various miRNAs is closely associated with the occurrence and development of pancreatic cancer, participating in the regulation of biological behaviors including tumor cell proliferation, invasion, metastasis, and chemoresistance [3]. However, the expression profiles and underlying mechanisms of a considerable number of miRNAs in pancreatic cancer remain unclear, which limits their translational application in clinical diagnosis and treatment. MicroRNA-4486 (miR-4486), a miRNA reported in recent years, has been suggested to potentially participate in molecular regulatory processes related to tumor progression in cancers such as prostate cancer and colorectal cancer. Nevertheless, its expression characteristics, clinical significance, and biological functions in pancreatic cancer have not yet been systematically investigated [4]. Therefore, this study aims to explore the expression level and clinical diagnostic value of serum miR-4486 in pancreatic cancer, and to

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analyze its impact on the biological behaviors of pancreatic cancer cells through in vitro experiments, providing experimental evidence for its potential application as a molecular biomarker.

2. Materials and Methods

2.1. Study Subjects

A total of 40 patients diagnosed with pancreatic cancer at our hospital from January 2018 to December 2024 were selected as the case group. All patients were confirmed to have pancreatic ductal adenocarcinoma via postoperative pathology or needle biopsy. Concurrently, 40 healthy individuals undergoing physical examinations were recruited as the control group. In the control group, there were 23 males and 17 females, with an age range of 40–75 years and a mean age of (61.81 ± 9.23) years; 24 cases (60.00%) were aged ≥60 years. In the case group, there were 23 males and 17 females, with an age range of 42–78 years and a mean age of (62.42 ± 8.73) years; 24 cases (60.00%) were aged ≥60 years. There were no statistically significant differences in general characteristics such as gender and age between the two groups ($P > 0.05$), indicating comparability.

Inclusion criteria:

Case group: First-time diagnosis of pancreatic cancer without any prior anti-tumor treatment; age 18–80 years; complete clinical data.

Control group: No history of malignant tumors; age-matched with the case group; good general health status.

Exclusion criteria:

Combined with other malignancies;

Severe dysfunction of the heart, liver, or kidneys, or autoimmune diseases;

Pregnant or lactating women;

Combined with acute or chronic pancreatitis;

Incomplete clinical data.

2.2. Serum sample collection and processing

All subjects fasted for more than 8 hours prior to blood collection. In the morning, 5 mL of fasting venous blood was collected into vacuum tubes without anticoagulants. Samples were immediately placed on ice and processed within 2 hours to separate the serum. The clear upper serum layer was aspirated using sterile pipettes, aliquoted into 1.5 mL RNase-free centrifuge tubes (200 μL per tube) to avoid repeated freeze-thaw cycles, and subsequently stored in a −80°C ultra-low temperature freezer for subsequent RNA extraction.

2.3. RNA extraction and real-time quantitative PCR (qRT-PCR)

Total RNA in serum samples was isolated and extracted using TRI zol LS Reagent (Invitrogen, USA). The specific procedure was as follows: 200 μ L of serum was mixed thoroughly with 600 μ L of TRI zol LS and incubated at room temperature for 5 minutes. Subsequently, 160 μ L of chloroform was added, followed by vigorous shaking for 15 seconds and incubation on ice for 3 minutes. The mixture was then centrifuged at 12,000 r/min for 15 minutes at 4°C. The upper aqueous phase was collected and transferred to a new centrifuge tube, mixed with an equal volume of isopropanol, and precipitated overnight at -20°C. The next day, the sample was centrifuged under the same conditions for 15 minutes. After removing the supernatant, the precipitate was washed twice with 75% ethanol, air-dried, and resuspended in 20 μ L of RNase-free water. The reverse transcription of miRNA was performed using the mi RCURY LNA RT Kit (QIAGEN), with U6 snRNA serving as the endogenous control. Q RT-PCR was conducted using SYBR Green Master Mix (Roche, Switzerland) on a Quant Studio 5 Real-Time PCR System. For the reverse transcription and amplification of miR-4486, the forward primer sequence was 5′-GCCGAGGTGAGTGTGTTGGT-3′, and the reverse primer was provided with the kit. The forward primer for U6 was 5′-GCTTCGGCAGCACATATACTAAAAT-3′, and the reverse primer was 5′-CGCTTCAGAATTTGCGTGTCAT-3′. All samples were run in triplicate, and the expression level of miR-4486 was analyzed using the 2^-ΔΔ Ct method.

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2.4. Cell culture and transfection

The PANC-1 cell line was obtained from the Cell Resource Center of the Chinese Academy of Sciences, and its identity was verified by short tandem repeat (STR) analysis. The cells were maintained in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO2. Cells in the logarithmic growth phase were selected for experiments and seeded into 6-well plates at a density of 5 × 10^5 cells per well. Transfection was performed when cell confluence reached 60%–70%. The experiment included three groups: a blank control group, a miRNA mimic group, and a miR-4486 inhibitor group. Transfection was carried out using Lipofectamine 3000 reagent. The medium was replaced with complete medium after 6 hours, and transfection efficiency was assessed by q RT-PCR 48 hours post-transfection.

2.5. CCK-8 Assay

Forty-eight hours after transfection, cells from different treatment groups were seeded into 96-well plates at a density of 3,000 cells per well, with 5 replicate wells set for each group, along with blank control wells. At 24, 48, and 72 hours of incubation, 10 μ L of CCK-8 reagent (Dojindo, Japan) was added to each well and incubated for an additional 2 hours. The optical density (OD) of each well was measured at a wavelength of 450 nm using a microplate reader. This experiment was independently repeated three times.

2.6. Transwell Assay

Transwell chambers (Corning, 8 μ m pore size) were used to assess cell migration and invasion capabilities. For the migration assay, the upper chamber was not coated with Matrigel, whereas for the invasion assay, the upper chamber was pre-coated with Matrigel (diluted 1:8). Forty-eight hours after transfection, cells were starved for 12 hours, and the cell density was adjusted to 5 × 10^5 cells/m L. A 200 μ L cell suspension was added to the upper chamber, and 600 μ L of medium containing 20% FBS was added to the lower chamber. After 24 hours of incubation, the cells were fixed and stained. Cells were counted in 5 randomly selected fields under an inverted microscope.

3. Results

3.1. Detection of serum miR-4486 levels

Table 1 Comparison of relative expression levels of serum miR-4486 ($\bar{x} \pm s$)

Group	n	Relative expression of miR-4486	Group
Control group	40	1.00 ± 0.25	Control group
Case group	40	2.96 ± 1.32	Case group
t		9.231	t
P		< 0.001	P

Table 2. Association between serum miR-4486 expression levels and clinicopathological characteristics of pancreatic cancer (n, %)

Pathological characteristics		High expression group (n=20)	Low expression group (n=20)	x^2	P
Gender	Male	11(55.0%)	12(60.0%)	0.100	0.752
	Female	9(45.0%)	8(40.0%)		
Age	⩾60	13(65.0%)	11(55.0%)	0.400	0.527
	<60	7(35.0%)	9(45.0%)		
Tumor size	⩾3cm	15(75.0%)	7(35.0%)	4.800	0.028
	<3cm	5(25.0%)	13(65.0%)		

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Pathological characteristics		High expression group (n=20)	Low expression group (n=20)	x^2	P
TNM stage	Stage III-IV	16(80.0%)	8(40.0%)	6.667	0.010
	Stage I-II	4(20.0%)	12(60.0%)		
Lymph node metastasis	Present	16(80.0%)	9(45.0%)	5.000	0.025
	Absent	4(20.0%)	11(55.0%)		

Table 3. Effects of miR-4486 on the proliferation, migration, and invasion abilities of PANC-1 cells (±s)

Group	24h (O Dalue)	48h (O Dvalue)	72h (OD value)	Migration assay	Invasion assay
Sham	0.35±0.03	0.68±0.05	1.12±0.08	125.23±10.53	86.73±8.22
miR-4486inhibitor	0.28±0.02	0.45±0.04	0.69±0.06	65.81±7.22	42.12±5.65
miR-4486mimics	0.41±0.04	0.89±0.07	1.48±0.11	201.52±15.35	136.45±11.21
F	18.751	45.244	62.313	382.423	330.614
P	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001

The serum expression level of miR-4486 in the case group was significantly higher than that in the control group ($P<0.05$); detailed results are shown in Table 1.

3.2. Association between serum miR-4486 and clinicopathological characteristics

Based on the median expression level, pancreatic cancer patients were divided into a high miR-4486 expression group and a low miR-4486 expression group, with 20 cases in each. The results showed that miR-4486 expression levels were significantly correlated with tumor size, TNM stage, and lymph node metastasis ($P<0.05$), but showed no correlation with gender or age ($P>0.05$), as shown in Table 2.

3.3. Effects of miR-4486 on the proliferation, migration, and invasion abilities of PANC-1 cells

Compared with the control group, the OD values of PANC-1 cells in the miR-4486 inhibitor group decreased at different time points, and both the number of migrated and invaded cells were reduced. In contrast, the miR-4486 mimics group showed increased OD values as well as increased numbers of migrated and invaded cells. These differences were all statistically significant ($P<0.05$), as shown in Table 3.

4. Discussion

miR-4486 belongs to the microRNA family and plays a regulatory role by affecting vital cellular activities such as cell division and motility. The dysregulation of this molecule is significantly associated with the progression of malignant tumors. Studies have shown that miRNA expression profiles can serve as non-invasive tumor diagnostic biomarkers. These circulating miRNAs exist stably in serum and possess a certain degree of specificity and sensitivity, holding promise for the early detection of pancreatic cancer [5]. The results of this study indicate that, compared with healthy controls, the serum expression of miR-4486 in pancreatic cancer patients is significantly upregulated, suggesting that miR-4486 may serve as a potential serum biomarker for pancreatic cancer and provide new clues for early diagnosis.

miR-4486 may participate in tumor progression by regulating tumor-related signaling pathways. In this study, high serum miR-4486 expression was significantly associated with larger tumor size, advanced TNM stage, and a higher rate of lymph node metastasis, indicating that miR-4486 may be deeply involved in the local invasion and metastasis of pancreatic cancer. This is consistent with previously reported studies on the role of miRNAs in pancreatic cancer progression; for instance, miR-125a-5p and miR-361-3p are also considered to be involved in tumorigenesis and metastasis [6]. These cutting-edge studies provide a

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biological basis for the involvement of miRNAs in the formation of malignant phenotypes in pancreatic cancer, supporting the clinical correlation analysis results of the present study.

Functional experiments demonstrated that miR-4486 overexpression significantly enhanced the proliferation, migration, and invasion of pancreatic cancer cells, whereas inhibiting its expression significantly blocked these tumor-related phenotypes. This aligns with the functional performance of miR-4486 observed in other tumor types. For example, in gastric cancer models, miR-4486 affects cell survival and apoptosis via the JAK3/STAT3 signaling pathway [7], and in colorectal cancer cells, it regulates autophagy and chemoresistance by targeting ATG7 [8]. This suggests that miR-4486 may regulate cell behavior through various downstream molecular events. These previous mechanistic studies provide an important reference for the molecular basis of the in vitro data in this study.

Various miRNAs in pancreatic cancer have been confirmed to have oncogenic or tumor-suppressive roles and are being explored as potential biomarkers. For instance, studies have pointed out that miRNAs play an important role in tumor progression, metastasis, diagnosis, and prognosis in pancreatic cancer [9]. This study further supplements the biological and clinical evidence chain for the specific molecule miR-4486, emphasizing its oncogenic potential in the development and progression of pancreatic cancer, and extending the breadth and depth of existing miRNA research in pancreatic cancer.

Although this study provides a systematic chain of evidence in terms of mechanism and results, limitations still exist, such as the small sample size, single-center retrospective design, and the lack of further analysis on the correlation between miR-4486 and patient survival prognosis. In addition, the downstream target genes and signaling pathways of miR-4486 remain to be further verified. Future studies could verify its independent diagnostic value through multi-center large-sample cohorts, and combine in vivo experiments with high-throughput methods such as RNA-seq to elucidate the molecular mechanisms, thereby providing fundamental data for early diagnosis and treatment strategies.

5. Conclusion

This study reveals the oncogenic role of miR-4486 in pancreatic cancer. The high expression of serum miR-4486 in patients, which is significantly associated with tumor size, TNM stage, and lymph node metastasis, suggests its potential value in liquid biopsy. In vitro functional experiments demonstrate that miR-4486 promotes the proliferation, migration, and invasion of pancreatic cancer cells, providing causal evidence for its clinical relevance. These findings provide evidence for miR-4486 as a diagnostic biomarker and potential therapeutic target for pancreatic cancer, laying a foundation for future mechanistic and clinical application studies.

6. References

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