ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
December 2024; 23(6):727-737.
DOI: 10.18502/ijaai.v23i6.17381

MicroRNA-486-3p Targets Chymotrypsin C to Regulate Pancreatic Cancer Progression and Immunosuppressive Factor Expression

Yong Tao[1], Chengniu Chu[1], Dongjun Sun[2], Junfeng Xiang[1], Bo Wu[1], Cong Li[1], and Wei Cui[1]

[1] *Department of General Surgery, XuanCheng People's Hospital, Affiliated Xuancheng Hospital of Wannan Medical College, AnHui, China*
[2] *Department of Information, XuanCheng People's Hospital, Affiliated Xuancheng Hospital of Wannan Medical College, AnHui, China*

Received: 5 February 2024; Received in revised form: 17 June 2024; Accepted: 20 June 2024

ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is a common digestive system tumor with high mortality rates and a poor prognosis. Reports suggest that microRNA (miR)-486-3p in PDAC can be used as a diagnostic biomarker. This research aimed to elucidate the mechanisms by which miR-486-3p regulates PDAC progression.

miR-486-3p and chymotrypsin C (*CTRC*) expression in PDAC were measured using quantitative real-time polymerase chain reaction. Changes in the biological properties of PDAC cells were assessed by Transwell assay, scratch-wound assay, cell counting kit (CCK)-8 assay, and plate cloning assay. The protein expression of immunosuppressive factors (vascular endothelial growth factor, interleukin-6, and transforming growth factor-β) in PDAC cells was detected by western blot. Additionally, a subcutaneous graft tumor model was constructed to explore the influence of silencing miR-486-3p on PDAC in vivo.

PDAC showed a pronounced increase in miR-486-3p expression. Upregulation of miR-486-3p stimulated PDAC cell proliferation, migration, invasion, and immunosuppressive factor protein expression, whereas silencing miR-486-3p hindered PDAC malignant development. miR-486-3p targets and negatively regulates *CTRC* expression. Silencing *CTRC* partially rescued the restraining impact of silencing miR-486-3p on PDAC malignant progression. In vivo experiments also indicated that silencing miR-486-3p inhibited PDAC malignant progression and immunosuppressive factor expression in vivo.

In summary, miR-486-3p promotes immunosuppressive factor protein expression by targeting and negatively regulating *CTRC* expression, which in turn promotes PDAC malignant progression.

Keywords: Cell proliferation; Chymotrypsin C; Immunosuppressive factor; MicroRNA-486-3p; Pancreatic cancer

Corresponding Author: Wei Cui, PhD
Department of General Surgery, XuanCheng People's Hospital, Affiliated Xuancheng Hospital of Wannan Medical College, AnHui, China. Tel: (+86 1860) 5693 628, Fax: (+86 0563) 3033 568), E-mail: zhuang7241@163.com

* The first and second authors contributed equally to this study.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive type of digestive system tumor that has a very poor prognosis. Morbidity and mortality rates are

increasing year by year, posing a serious hazard to human health.[1,2] Statistics show that in 2020, more than 490 000 cases of PDAC were detected globally, accounting for 2.6% of all cancers and ranking 14th among them; however, deaths from PDAC accounted for 4.7% of all cancer deaths, ranking sixth.[3,4] Currently, the primary treatment for pancreatic cancer remains surgery.[5,6] Radical surgical resection combined with postoperative adjuvant therapy prolongs survival to some extent in patients with PDAC.[7,8] Unfortunately, PDAC has an insidious onset, and a large proportion of patients are diagnosed when the cancer is in an advanced stage, depriving them of the opportunity for surgery.[9,10] Therefore, clarifying the underlying molecular mechanisms of PDAC development and establishing methods for early diagnosis is paramount for enhancing the early detection rate of PDAC and diminishing recurrence and mortality rates.

MicroRNAs (miRs) are a type of short, noncoding RNA containing about 18 to 24 nucleotides.[11,12] It has been reported that miRs are aberrantly expressed in several tumors and can participate in the physiological and metabolic processes of tumor cells, making them potentially valuable in the early detection and treatment of tumors.[13,14] Numerous investigations have demonstrated that miR-486-3p is aberrantly expressed and affects the malignant progression of non–small cell lung cancer, oral cancer, and cervical cancer, among others.[15-17] Additionally, miR-486-3p has been found to suppress the expression of flotillin-2, thereby enhancing the proliferation and metastasis of skin squamous cell carcinoma.[18] Recent research has indicated that miR-486-3p was aberrantly expressed in PDAC and could be used as a diagnostic marker.[19] Nevertheless, the regulatory mechanism by which miR-486-3p affects PDAC progression is uncertain.

Chymotrypsin C (CTRC) is a serine protease produced by pancreatic cells.[20,21] It has been shown that upregulation of *CTRC* expression reduces chemotherapy resistance in gastric and colon cancer cells.[22] Currently, *CTRC* is not well studied in PDAC. This study began with an examination of miR-486-3p in PDAC. We then explored changes in the biological properties of PDAC cells after silencing or overexpressing miR-486-3p. We explored the impacts of silencing miR-486-3p and silencing *CTRC* on malignant development and immunosuppressive factor expression in PDAC. Finally, a subcutaneous graft tumor model was created to assess the influence of silencing miR-486-3p on tumor development in mice. This study will provide a novel reference for the study of miR-486-3p and *CTRC* in PDAC.

MATERIALS AND METHODS

Clinical Tissue Samples

Cancer and paracancer tissue samples (n = 52) were obtained from patients with PDAC admitted to Xuancheng People's Hospital. The tissues were collected randomly, cleaned, and stored in liquid nitrogen for backup. Patient inclusion criteria were as follows: (1) pathologically confirmed PDAC; (2) age≥18 years and gender neutral; (3) complete information; and (4) provision of written informed consent. The exclusion criteria were as follows: (1) any treatment, such as radiotherapy, chemotherapy, targeted therapy, or immunotherapy; (2) pathological diagnosis of benign tumors; (3) history of other malignant tumors; and (4) presence of extrapancreatic metastases. The Ethics Committee of Xuancheng People's Hospital approved the study, and all patients provided informed consent for sample acquisition.

Cell Culture and Transfection

The pancreatic cell line HPDE6-C7 and the PDAC cell lines (BxPC-3, PANC-1, and Capan-1) were sourced from the American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, NY, USA) with 10% fetal bovine serum (FBS; Gibco, NY, USA) and 1% penicillin and streptomycin (Gibco, NY, USA) at 37°C with 5% CO_2. The miR-486-3p mimics (mimics), miR-486-3p inhibitor (inhibitor), and short interfering *CTRC* RNA (si-*CTRC*) were supplied by RiboBio Co, Ltd (Guangzhou, China). BxPC-3 and PANC-1 cells were cotransfected using Lipofectamine 2000 (Invitrogen, CA, USA).

Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

RNA was extracted using TRIzol reagent (Invitrogen, CA, USA). After that, cDNA was obtained through reverse transcription with the inclusion of AMV reverse transcriptase (Invitrogen, CA, USA). SYBR Green qPCR Mix kit (TAKARA, Tokyo, Japan) was used for PCR amplification. The primer sequences were as follows:

miR-486-3p: F: 5′-GCCGAGCGGGGCAGCTCAGTA-3′, R: 5′-CGCAGGGTCCGAGGTATTC-3′

U6: F: 5′-CCCTTCGGGGACATCCGATA-3′, R: 5′-TTTGTGCGTGTCATCCTTGC-3′
CTRC: F: 5′-ATTGACTGGTGGGGCTTCAG-3′, R: 5′-CTTCCCCAGGAGTGGTTGTC-3′
β-actin: F: 5′-GCACCACACCTTCTACAATGAG-3′, R: 5′-GATAGCACAGCCTGGATAGCA-3′

Transwell Assay

Matrigel (Sigma-Aldrich, MO, USA) was diluted to a 1:6 mixture with serum-free medium, and 100 μL of the mixture was added to each Transwell chamber (Corning, MA, USA) and placed in the incubator overnight. The following day, the remaining liquid in the chambers was aspirated, and serum-free DMEM was used to hydrate the basement membrane. A total of 200 μL of cell suspension was added to the upper chamber, and an appropriate amount of DMEM was added to the lower chamber. Cells were incubated for approximately 12 hours. The Matrigel and cells in the upper chamber were then wiped away, and the membrane was exposed to 4% paraformaldehyde (Solarbio, Beijing, China) for 30 minutes. After that, the membrane was stained with 0.1% crystal violet (Beyotime, Shanghai, China) and rinsed twice with deionized water. A randomly selected field of view was photographed at high magnification, and the number of invading cells was counted.

Scratch-wound Assay

PDAC cells were inoculated into 6-well plates with predrawn horizontal lines after transfection treatment. When the cell density reached 70% to 90%, a 200-μL sterile lance tip was used to gently etch a vertical line perpendicular to the horizontal line at the base of the plate. DMEM was then added, and the cells were incubated. The healing of the scratch was observed at 0 and 24 hours after scratching.

Cell Counting Kit (CCK)-8 Assay

PDAC cells (2.0×10^4 cells/well) were seeded in 96-well cell culture plates after routine digestion and transfection. After cell attachment, 100 μL of complete medium with 10% CCK-8 solution (Solarbio, Beijing, China) was added to each well. Plates were incubated (37°C, 2 hours), and the OD_{450} was measured using a microplate reader to calculate the cell proliferation rate.

Plate Cloning Assay

After digestion and resuspension, 1000 cells were added to each well of a 6-well plate and incubated (37°C, 5% CO_2) for 2 weeks. When clonal cell clusters could be seen, the culture medium was removed, and the wells were rinsed twice with PBS. Cells were fixed with 4% paraformaldehyde (Solarbio, Beijing, China) for 20 minutes. The fixative was discarded, and cells were stained with 0.1% crystal violet (Sigma-Aldrich, MO, USA) for 15 minutes and then washed 3 times with deionized water. Images were acquired, and colonies were counted.

Western Blot

RIPA lysate (Solarbio, Beijing, China) was added to cells and tissues, and the lysates were collected. Protein concentrations were assessed using a BCA kit (Beyotime, Shanghai, China). After gel electrophoresis, the samples were transferred to polyvinylidene fluoride (PVDF) membranes (Invitrogen, CA, USA) and blocked. The membranes were rinsed and incubated overnight with primary antibody against vascular endothelial growth factor (VEGF), (Invitrogen, CA, USA; dilution 1:1000; MA5-32038), interleukin (IL)-6 (Invitrogen, CA, USA; dilution 1:1000; M620), or transforming growth factor-beta (TGF-β) (Invitrogen, CA, USA; dilution 1:1000; MA5-15065) at 4°C. Membranes were then incubated with sheep anti-rabbit secondary Immunoglobulin G (Invitrogen, CA, USA; dilution 1:10 000; 31460) for 1.5 hours. β-Actin (Invitrogen, CA, USA; MA1-140) was used as an internal reference. The membrane was washed 3 times and then developed.

In Vivo Experiment

Two groups of 5 mice each were randomly assigned. Each mouse was injected with 200 μL of BxPC-3 cell suspension (1.5×10^6 cells/mouse). The survival of mice was recorded every day, and the size of subcutaneous tumors was measured using vernier calipers on days 7, 14, 21, 28, and 35. Mice were euthanized after anesthesia on day 35, and tumors were excised and weighed.

Statistical Analysis

For each experiment, the results were determined by taking the mean ± standard deviation from at least 3 replicates. SPSS, version 26.0, software (IBM Corp) was used to perform ANOVA and Student *t* tests. $p<0.05$ indicated significant differences. Prism software, version 9.0 (GraphPad Software), was used for plotting.

RESULTS

Expression and Prognosis of miR-486-3p in PDAC

qRT-PCR results indicated significant upregulation of miR-486-3p in PDAC (Figure 1A). miR-486-3p expression was substantially lower in HPDE6-C7 cells than in PDAC cells (BxPC-3, PANC-1, and Capan-1) (Figure 1B). Notably, miR-486-3p levels differed in the PDAC cells; the highest levels were seen in BxPC-3 cells, followed by PANC-1 cells. Therefore, we chose BxPC-3 and PANC-1 cells for subsequent studies. Furthermore, the survival rate of patients with high miR-486-3p expression was markedly decreased compared with those with low expression (Figure 1C). miR-486-3p expression in patients with stages I and II PDAC was significantly lower than in those with stage III or IV PDAC (Figure 1D).

Overexpression of miR-486-3p Promotes Progression of PDAC

qRT-PCR confirmed that miR-486-3p was overexpressed, and this finding was used for subsequent functional experiments (Figure 2A). Transwell assay results indicated that miR-486-3p overexpression enhanced the invasion ability of PDAC cells (Figure 2B–2C). The cell scratch assay showed that miR-486-3p overexpression increased migration capability (Figure 2D-2E). miR-486-3p overexpression stimulated the proliferation of PDAC cells (Figure 2F). The plate cloning assay indicated a marked increase in the clone formation capacity of cells after miR-486-3p overexpression (Figure 2G–2H). Immunosuppressive factors, such as VEGF, IL-6, and TGF-β, were examined using western blot, and the expression of these proteins was significantly elevated when miR-486-3p was overexpressed (Figure 2I–2L).

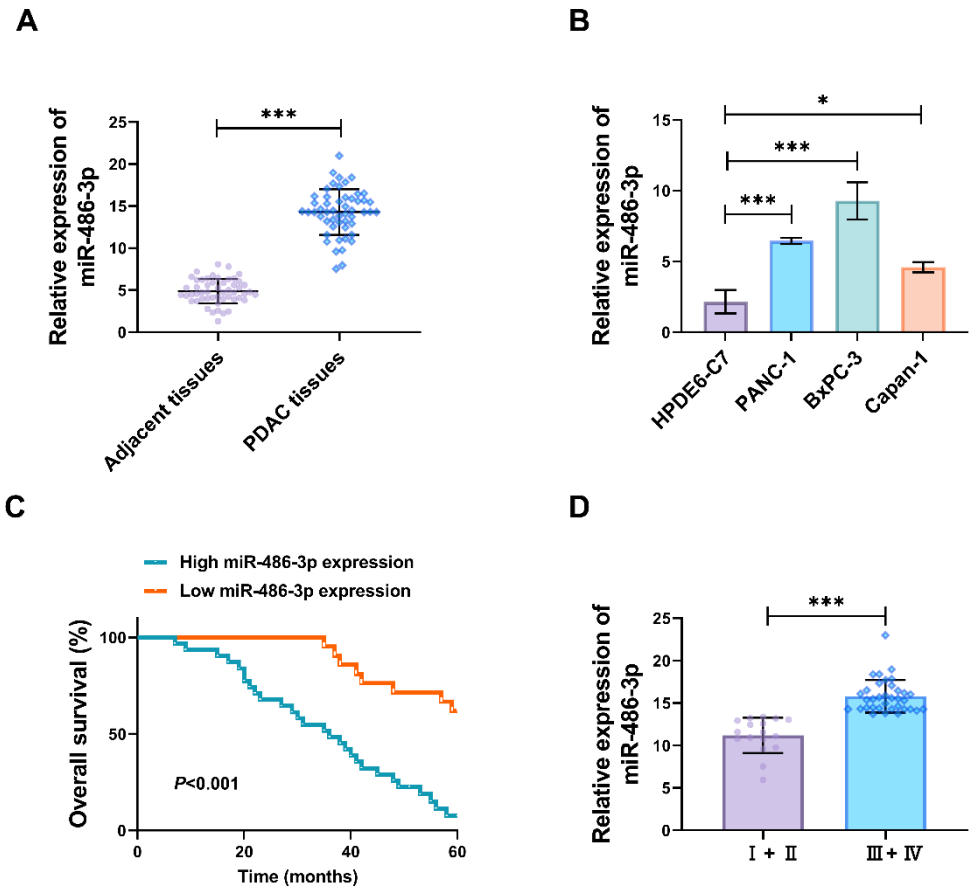

Figure 1. Expression and prognosis of microRNA (miR)-486-3p in pancreatic cancer (PDAC). (A) The miR-486-3p expression level in adjacent tissues and PDAC tissues was quantified utilizing quantitative real-time polymerase chain reaction (qRT-PCR). (B) qRT-PCR was implemented to measure miR-486-3p levels in different cells. (C) The connection between miR-486-3p and the prognosis of PDAC. (D) Clinical stages in correlation with miR-486-3p expression. *p<0.05, ***p<0.001.

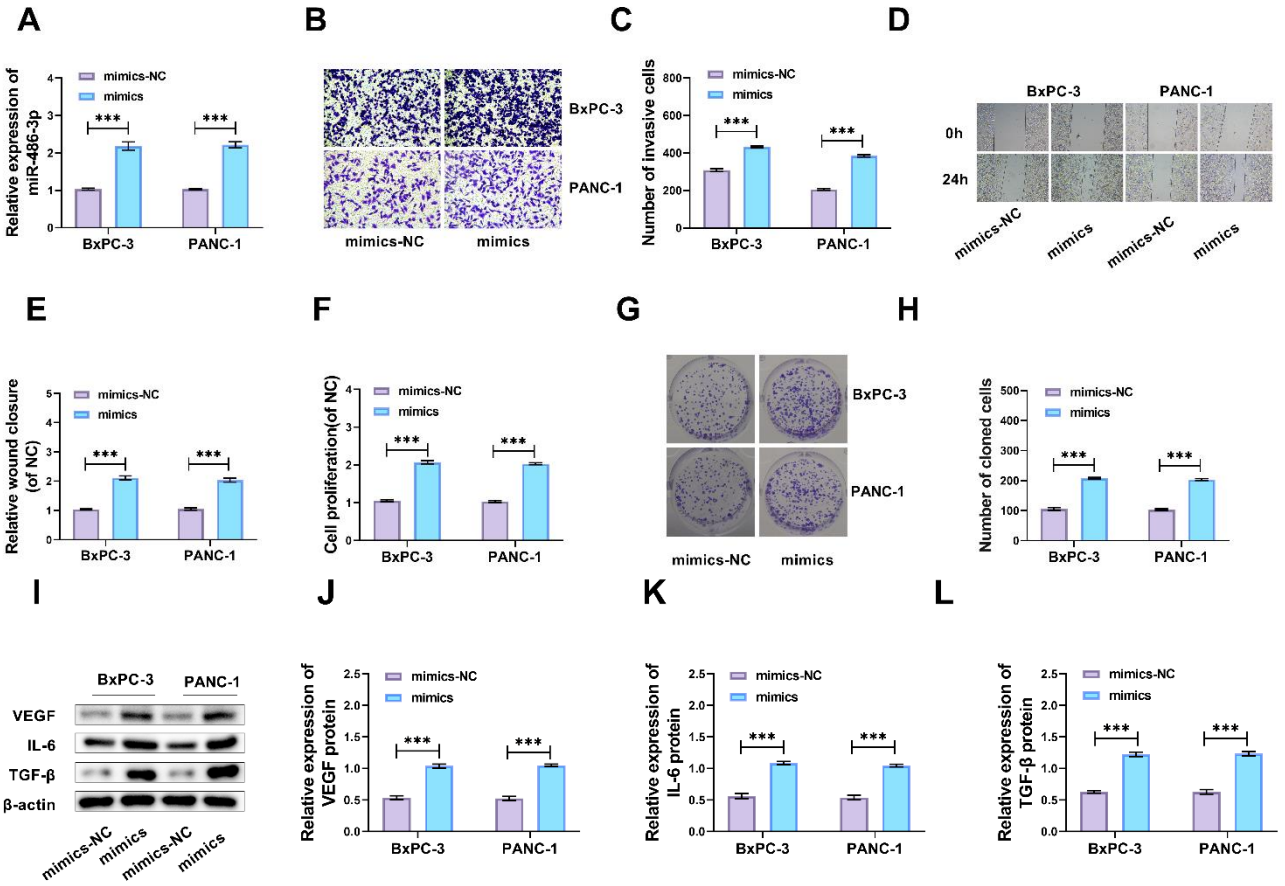

Figure 2. MicroRNA-486-3p overexpression on pancreatic cancer progression.

(A) The overexpression efficiency was measured utilizing quantitative real-time polymerase chain reaction (qRT-PCR). (B–C) The invasion capacities of BxPC-3 and PANC-1 cells were evaluated by Transwell assay. (D–E) Scratch-wound assay showed cell migration. (F) Utilizing a Cell Counting Kit (CCK)-8 assay monitored the cell proliferation. (G–H) The plate cloning experiment was applied to quantify the cell cloning formation of BxPC-3 and PANC-1 cells. (I–L) The contents of vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), and transforming growth factor (TGF)-β proteins were assessed by western blotting. ***p<0.001.

Silencing miR-486-3p Hinders the Progression of PDAC

Transfection of inhibitors into PDAC cells and verification of their silencing efficiency by qRT-PCR confirmed a significant decrease in miR-486-3p expression (Figure 3A). Silencing miR-486-3p resulted in a reduced invasion ability of PDAC cells (Figure 3B-3C). The cell scratch assay indicated that silencing miR-486-3p hindered cell migration ability (Figure 3D-3E). Cell proliferation capacity was impeded after silencing miR-486-3p (Figure 3F). The clone formation ability of PDAC cells was significantly decreased after silencing miR-486-3p (Figure 3G–3H). Western blot results revealed that silencing miR-486-3p led to a marked decline in VEGF, IL-6, and TGF-β expression compared with the control (Figure 3I–3L).

miR-486-3p Targets Negative Regulation of *CTRC*

TargetScanHuman, version 8.0 (https://www.targetscan.org/vert_80/), GEPIA (http://gepia.cancer-pku.cn/), and Home-miRWalk (http://mirwalk.umm.uni-heidelberg.de/) databases were used to predict downstream target genes of miR-486-3p, and 2 genes—*CTRC* and *syncollin* (*SYCN*)-were identified (Figure 4A). After silencing miR-486-3p, both *CTRC* and SYCN expression markedly increased, with the expression of *CTRC* being higher than that of SYCN; therefore, we selected *CTRC* for subsequent experiments (Figure 4B–4C). Figure 4D shows the *CTRC* 3′UTR-WT and *CTRC* 3′UTR-MUT nucleotide sequences. The expression of *CTRC* in PDAC tissues and cells was markedly lower than in normal tissues and cells (Figure

4E-4J). On silencing miR-486-3p, PDAC cells displayed a significant increase in *CTRC* protein expression (Figure 4K). Figure 4L shows the linear regression relationship between *CTRC* and miR-486-3p expression, with miR-486-3p negatively regulating *CTRC*.

Silencing of *CTRC* Expression Partially Attenuated the Inhibitory Effect of Silencing miR-486-3p on PDAC

Transfection of si-*CTRC* into PDAC cells and detection of the silencing efficiency of *CTRC* showed a marked decrease in *CTRC* protein expression (Figure 5A-B). Transwell assay results showed that silencing *CTRC* partially rescued the restraining impact of silencing miR-486-3p on invasion capacity (Figure 5C-5D). Silencing *CTRC* partially attenuated the restraining influence of silencing miR-486-3p on migration capacity (Figure 5E-5F). The CCK-8 assay showed that silencing *CTRC* was able to partially rescue cell proliferation ability (Figure 5G). After silencing miR-486-3p, silencing *CTRC* partially rescued the clone formation ability of cells (Figure 5H-5I). In addition, silencing *CTRC* partially rescued VEGF, IL-6, and TGF-β expression in PDAC cells (Figure 5J-5M).

In Vivo Experiment

Silencing miR-486-3p markedly inhibited tumor growth in terms of volume and weight (Figure 6A-6C). Western blotting was used to identify changes in the expression of immune factors, and miR-486-3p silencing significantly restrained VEGF, IL-6, and TGF-β protein expression (Figure 6D-6E).

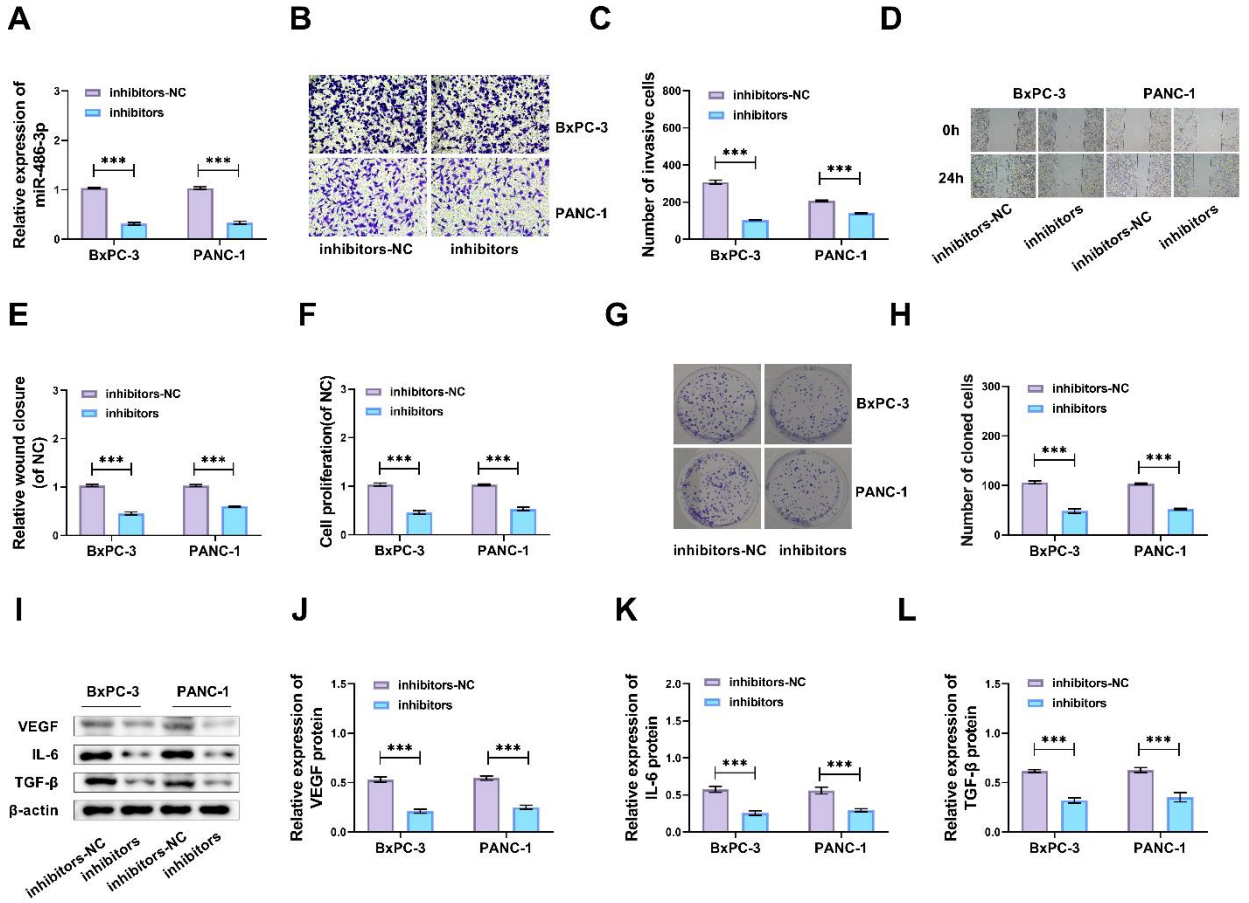

Figure 3. Silencing microRNA-486-3p on pancreatic cancer progression.

(A) The silencing efficiency was measured utilizing quantitative real-time polymerase chain reaction (qRT-PCR). (B–C) The invasion capacities of BxPC-3 and PANC-1 cells were evaluated by Transwell assay. (D–E) Scratch-wound assay showed cell migration. (F) Utilizing a Cell Counting Kit (CCK)-8 assay monitored the cell proliferation. (G–H) The clone formation abilities of BxPC-3 and PANC-1 cells were detected by plate cloning assay. (I–L) The contents of vascular endothelial growth factor (VEGF), interleukin (IL-6), and transforming growth factor-β (TGF-β) proteins were assessed by western blotting. ***p<0.001.

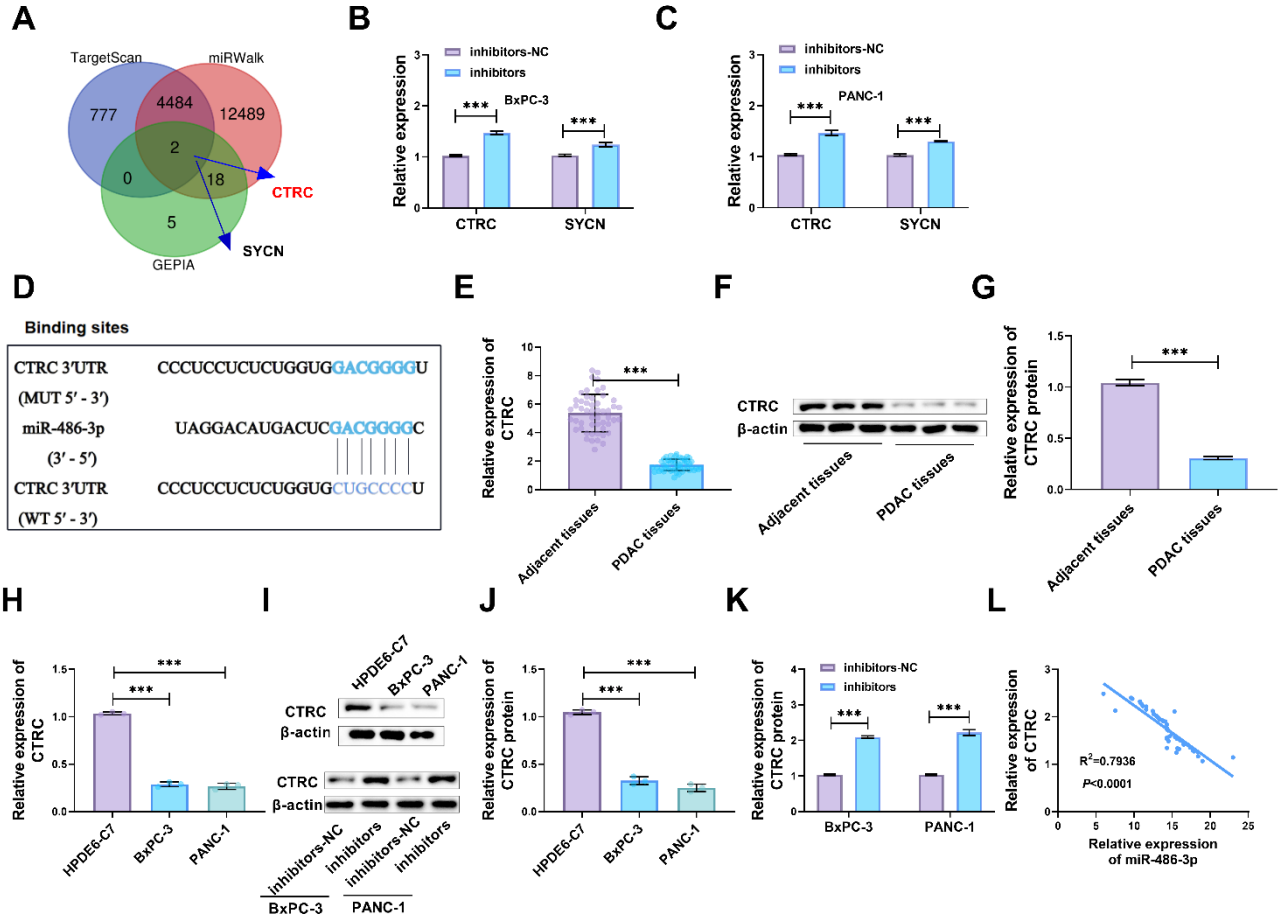

Figure 4. Screening and validation of microRNA (miR)-486-3p target genes.

(A) Obtain target genes through TargetScan, miRWalk, and GEPIA databases. (B-C) Chymotrypsin C (*CTRC*) and syncollin (*SYCN*) expression in BxPC-3 and PANC-1 cells were quantified utilizing quantitative real-time polymerase chain reaction (qRT-PCR). (D) The binding sites of miR-486-3p and *CTRC*. (E-G) *CTRC* expression in different tissues was quantified utilizing qRT-PCR and western blotting. (H-J) *CTRC* mRNA expression in different cells was quantified utilizing qRT-PCR and western blotting. (K) *CTRC* protein expression in BxPC-3 and PANC-1 cells was ascertained utilizing western blotting. (L) The connection between miR-486-3p and *CTRC*. ***$p<0.001$.

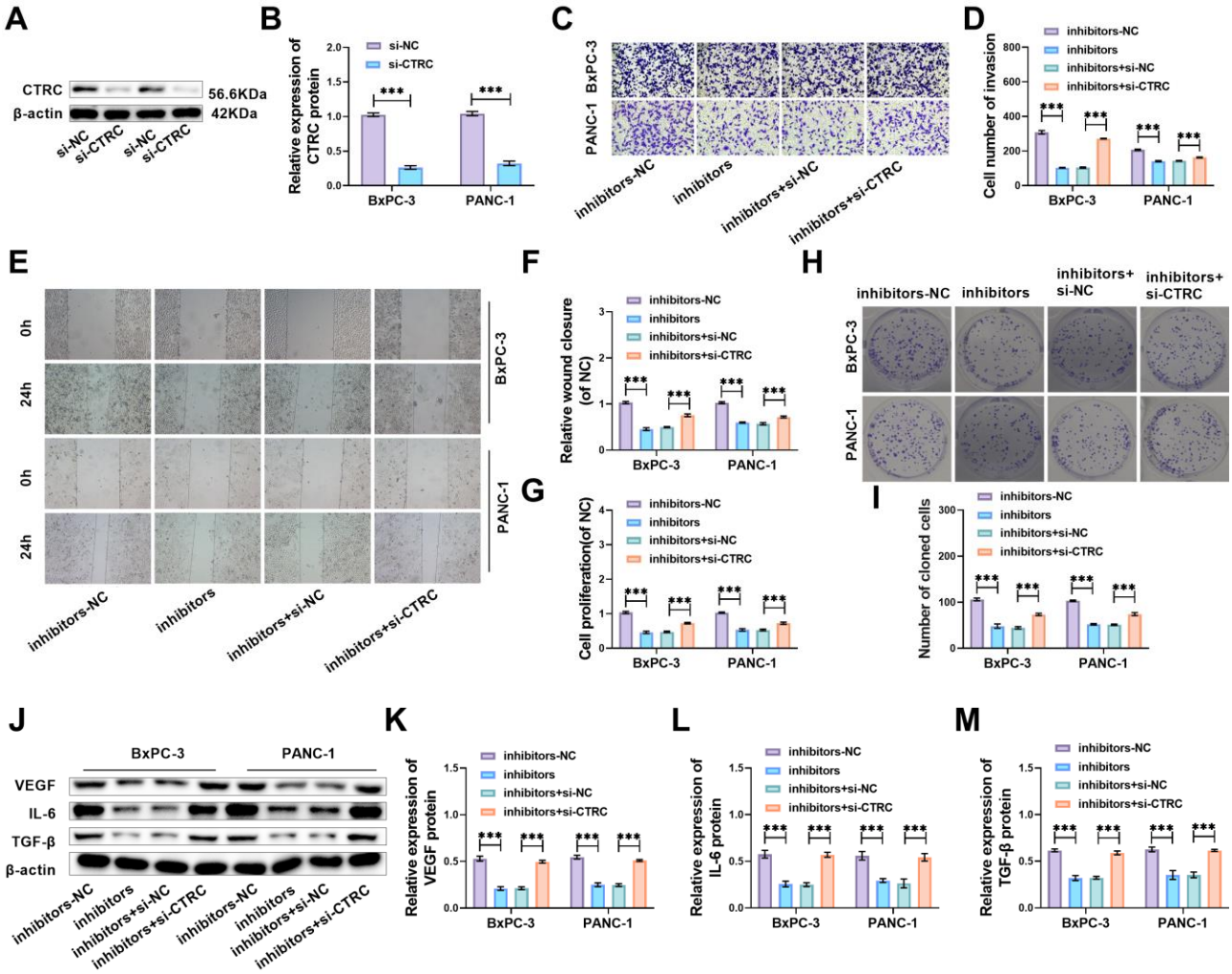

Figure 5. Silencing of Chymotrypsin C (*CTRC*) expression partially attenuated the inhibition of silencing microRNA (miR)-486-3p on pancreatic cancer (PDAC).

(A-B) *CTRC* protein expression in BxPC-3 and PANC-1 cells were ascertained utilizing western blotting. (C-D) The invasion capacities of BxPC-3 and PANC-1 cells were evaluated by Transwell assay. (E-F) The scratch-wound experiment revealed the migration capacities of BxPC-3 and PANC-1 cells. (G) Utilizing Cell Counting Kit (CCK)-8 assay monitored the cell proliferation. (H-I) The plate cloning experiment was applied to quantify the cell cloning formation. (J-M) The contents of vascular endothelial growth factor (VEGF), interleukin-6 (IL-6**), and transforming growth factor-β (TGF-β) proteins were scrutinized by western blot. ***p<0.001.**

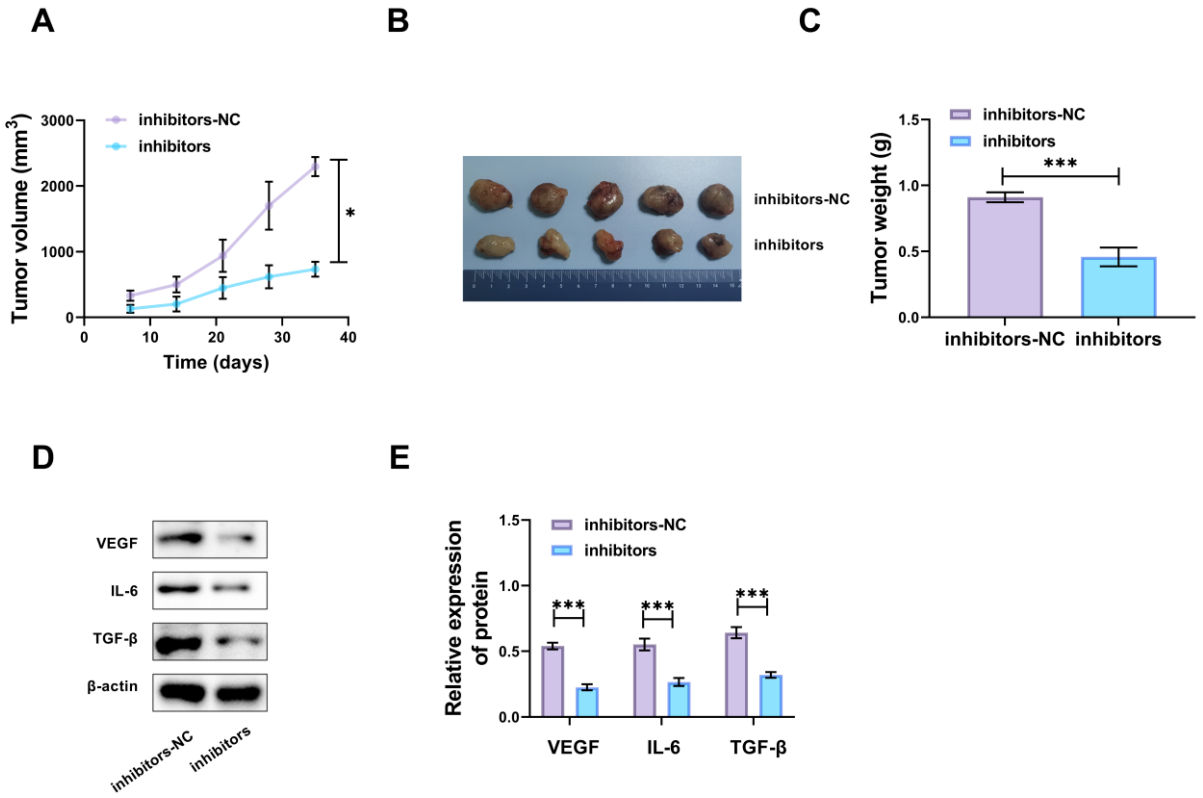

Figure 6. Silencing microRNA (miR)-486-3p on pancreatic cancer progression in vivo.
(A–C) Silencing miR-486-3p inhibited tumor volume (A) and weight growth (C). (D–E) The contents of vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), and transforming growth factor-β (TGF-β) proteins were scrutinized by western blot. *p<0.05, ***p<0.001.

DISCUSSION

Over the past few years, several investigations have attested to the fact that miRNAs are strongly associated with the progression of malignant tumors, specifically the promotion or inhibition of various biological behaviors of tumors by miRNAs.[23,24] Chen et al revealed that miR-486-3p overexpression drastically reduced the migration and infiltration of thyroid cancer cells, whereas silencing miR-486-3p promoted thyroid cancer progression.[25] In addition, Li et al reported that miR-486-3p was significantly upregulated in cutaneous squamous cell carcinoma and that its overexpression increased the proliferation of cancer cells.[18] Our study revealed that miR-486-3p was highly expressed in PDAC tissues and cells and that the overall survival rate was lower for patients with high expression. PDAC cells showed increased proliferation, migration, and invasion after miR-486-3p was overexpressed, whereas reduced miR-486-3p expression significantly inhibited the malignant progression of PDAC in vivo.

Our study used the GEPIA, TargetScanHuman, and Home-miRWalk databases to identify *CTRC* as a downstream gene of miR-486-3p. Wang et al found that upregulation of *CTRC* resulted in diminished migration of PDAC cells and that downregulation of *CTRC* expression enhanced the migration of PDAC cells.[26] Our findings revealed that silencing miR-486-3p resulted in upregulation of *CTRC* expression. Transfection of si-*CTRC* partially rescued the inhibitory effect of silencing miR-486-3p on PDAC progression, implying that miR-486-3p negatively regulates *CTRC*, which in turn is involved in the biological processes of PDAC.

Cytokines are a class of small-molecule proteins secreted by immune cells and many other types of cells, including lymphocyte factors and interferons, that monitor the host's immune response to infection.[27-29] Many cytokines are involved in immunosuppression in the tumor microenvironment, and it has been shown that VEGF is not only involved in the growth of tumor vasculature but also inhibits T-cell function, thus acting as an immunosuppressant.[30,31] IL-6 is part of the interleukin

cytokine family, and overproduction of IL-6 promotes cancer progression.[32,33] TGF-β modulates the activity of immune cells and controls inflammatory processes.[34,35] Our investigation revealed that miR-486-3p overexpression led to a marked increase in VEGF, IL-6, and TGF-β expression in PDAC cells, whereas inhibition of miR-486-3p expression led to a marked decrease in these factorsIL-6 in mice. This finding indicates that inhibition of miR-486-3p expression could reduce the secretion of immunosuppressive factors (VEGF, IL-6, and TGF-β), which augments the body's antitumor capabilities.

In summary, our study confirmed that miR-486-3p targets and negatively regulates *CTRC* and that miR-486-3p upregulation enhances immunosuppressive factor expression in PDAC cells and promotes the malignant progression of PDAC. We found that miR-486-3p and *CTRC* can be used as new biomarkers, which offers a new theoretical foundation for the early diagnosis and targeted therapy of PDAC.

STATEMENT OF ETHICS

This study was approved by the Ethics Committee of Xuancheng People's Hospital (2024-1w002-01).

FUNDING

This study was supported by the Anhui Province Clinical Medical Research Transformation Project (202204295107020059) and the Wannan Medical College University-Level Research Project Teaching Hospital Research Project (JXYY202222).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

The authors of this article would like to thank Tehran University of Medical Sciences and the National Institute for Medical Research Development (NIMAD) for their financial contributions, via grants 97-02-27 38998 and 977636, respectively.

REFERENCES

1. Tempero MA, Malafa MP, Al-Hawary M, Behrman SW, Benson AB, Cardin DB, et al. Pancreatic Adenocarcinoma, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021;19(4):439-57.
2. Gordon-Dseagu VL, Devesa SS, Goggins M, Stolzenberg-Solomon R. Pancreatic cancer incidence trends: evidence from the Surveillance, Epidemiology and End Results (SEER) population-based data. Int J Epidemiol. 2018;47(2):427-39.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-49.
4. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. Lancet. 2011;378(9791):607-20.
5. Khalaf N, El-Serag HB, Abrams HR, Thrift AP. Burden of Pancreatic Cancer: From Epidemiology to Practice. Clin Gastroenterol Hepatol. 2021;19(5):876-84.
6. McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG, McCain RS. Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. World J Gastroenterol. 2018;24(43):4846-61.
7. Chen X, Zeh HJ, Kang R, Kroemer G, Tang D. Cell death in pancreatic cancer: from pathogenesis to therapy. Nat Rev Gastroenterol Hepatol. 2021;18(11):804-23.
8. Mokdad AA, Minter RM, Zhu H, Augustine MM, Porembka MR, Wang SC, et al. Neoadjuvant Therapy Followed by Resection Versus Upfront Resection for Resectable Pancreatic Cancer: A Propensity Score Matched Analysis. J Clin Oncol. 2017;35(5):515-22.
9. Singhi AD, Koay EJ, Chari ST, Maitra A. Early Detection of Pancreatic Cancer: Opportunities and Challenges. Gastroenterology. 2019;156(7):2024-40.
10. Wood LD, Canto MI, Jaffee EM, Simeone DM. Pancreatic Cancer: Pathogenesis, Screening, Diagnosis, and Treatment. Gastroenterology. 2022;163(2):386-402.e1.
11. Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol. 2018;141(4):1202-1207.
12. Ye J, Xu M, Tian X, Cai S, Zeng S. Research advances in the detection of miRNA. Journal of pharmaceutical analysis. 2019;9(4):217-26.
13. Hill M, Tran N. miRNA interplay: mechanisms and consequences in cancer. Dis Model Mech. 2021;14(4):047662.
14. Chen L, Heikkinen L, Wang C, Yang Y, Sun H, Wong G. Trends in the development of miRNA bioinformatics tools. Brief Bioinform. 2019;20(5):1836-52.
15. Xie D, Zhang S, Jiang X, Huang W, He Y, Li Y, et al. Circ_CSPP1 Regulates the Development of Non-small

Cell Lung Cancer via the miR-486-3p/BRD9 Axis. Biochem Genet. 2023;61(1):1-20.
16. Chou ST, Peng HY, Mo KC, Hsu YM, Wu GH, Hsiao JR, et al. MicroRNA-486-3p functions as a tumor suppressor in oral cancer by targeting DDR1. J Exp Clin Cancer Res. 2019;38(1):281.
17. Ye H, Yu X, Xia J, Tang X, Tang L, Chen F. MiR-486-3p targeting ECM1 represses cell proliferation and metastasis in cervical cancer. Biomed Pharmacother. 2016;80:109-14.
18. Li X, Yuan Y, Wang Y, Xie K, Lu S, Chen F, et al. MicroRNA-486-3p promotes the proliferation and metastasis of cutaneous squamous cell carcinoma by suppressing flotillin-2. J Dermatol Sci. 2022;105(1):18-26.
19. Yu J, Li A, Hong SM, Hruban RH, Goggins M. MicroRNA alterations of pancreatic intraepithelial neoplasias. Clin Cancer Res. 2012;18(4):981-92.
20. Bence M, Sahin-Tóth M. Asparagine-linked glycosylation of human chymotrypsin C is required for folding and secretion but not for enzyme activity. FEBS J. 2011;278(22):4338-50.
21. Goettig P, Brandstetter H, Magdolen V. Surface loops of trypsin-like serine proteases as determinants of function. Biochimie. 2019;166:52-76.
22. Matsunaga T, Tsuchimura S, Azuma N, Endo S, Ichihara K, Ikari A. Caffeic acid phenethyl ester potentiates gastric cancer cell sensitivity to doxorubicin and cisplatin by decreasing proteasome function. Anticancer Drugs. 2019;30(3):251-9.
23. Hussen BM, Hidayat HJ, Salihi A, Sabir DK, Taheri M, Ghafouri-Fard S. MicroRNA: A signature for cancer progression. Biomed Pharmacother. 2021;138:111528.
24. Ali Syeda Z, Langden SSS, Munkhzul C, Lee M, Song SJ. Regulatory Mechanism of MicroRNA Expression in Cancer. Int J Mol Sci. 2020;21(5):1723.
25. Chen L, Xiong L, Hong S, Li J, Huo Z, Li Y, et al. Circulating Myeloid-derived Suppressor Cells Facilitate Invasion of Thyroid Cancer Cells by Repressing miR-486-3p. J Clin Endocrinol Metab. 2020;105(8):2704-18.
26. Wang H, Sha W, Liu Z, Chi CW. Effect of chymotrypsin C and related proteins on pancreatic cancer cell migration. Acta Biochim Biophys Sin (Shanghai). 2011;43(5):362-71.
27. Bayik D, Lathia JD. Cancer stem cell-immune cell crosstalk in tumour progression. Nat Rev Cancer. 2021;21(8):526-36.
28. Hirano T. IL-6 in inflammation, autoimmunity and cancer. Int Immunol. 2021;33(3):127-48.
29. Tie Y, Tang F, Wei YQ, Wei XW. Immunosuppressive cells in cancer: mechanisms and potential therapeutic targets. J Hematol Oncol. 2022;15(1):61.
30. Yang J, Yan J, Liu B. Targeting VEGF/VEGFR to Modulate Antitumor Immunity. 2018;9:978.
31. Patel SA, Nilsson MB, Le X, Cascone T, Jain RK, Heymach JV. Molecular Mechanisms and Future Implications of VEGF/VEGFR in Cancer Therapy. Clin Cancer Res. 2023;29(1):30-9.
32. Weber R, Groth C, Lasser S, Arkhypov I, Petrova V, Altevogt P, et al. IL-6 as a major regulator of MDSC activity and possible target for cancer immunotherapy. Cell Immunol. 2021;359:104254.
33. Uciechowski P, Dempke WCM. Interleukin-6: A Masterplayer in the Cytokine Network. Oncology. 2020;98(3):131-7.
34. Angioni R, Sánchez-Rodríguez R, Viola A, Molon B. TGF-β in Cancer: Metabolic Driver of the Tolerogenic Crosstalk in the Tumor Microenvironment. Cancers (Basel). 2021;13(3):401.
35. Kim IY, Kim MM, Kim SJ. Transforming growth factor-beta: Biology and clinical relevance. J Biochem Mol Biol. 2005;38(1):1-8.