Original Research

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Effects of miR-192-5p on stem cell-like phenotype of pancreatic cancer cells via ATXN7

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Abstract

Pancreatic cancer is one of the deadliest cancers in the world. Cancer stem cells (CSC) play a critical role in the genesis, progression, metastasis, chemical resistance, and recurrence of tumors. Cumulative studies have shown that miR-192-5p regulates the stem cell-like phenotype in malignancies. However, the mechanism of the effect of miR-192-5p on the stem cell-like phenotype of pancreatic cancer remains to be studied. In this study, we predicted through starBase database that the expression of miR-192-5p was abnormally high in tissues. After the stem cell-like cells of SW1990 cells enriched in Serum-free Medium (SFM), RT-qPCR revealed significantly high expression of miR-192-5p in these cells. In addition, the effects of miR-192-5p on SW1990 cell proliferation and stem cell-like properties were examined by CCK-8, EdU staining, clone formation and sphere formation assay, respectively. The results showed that overexpression or knockdown of miR-192-5p significantly upregulated or decreased the proliferation activity, EdU positive cell proportion, number of clones and number of spheres in SW1990 cells. We further predicted the downstream target of miR-192-5p by PITA, RNA22 and miRmap, and verified it by dual-luciferase reporter gene assay and Western blotting. The results showed that ATXN7 was the downstream target of miR-192-5p, and miR-192-5p negatively regulated the expression of ATXN7. The database predicted that ATXN7 expression was significantly lower in pancreatic cancer tissues and negatively correlated with miR-192-5p expression. The results of functional experiments showed that the overexpressing ATXN7 decreased the proliferation activity, EdU positive cell percentage, number of clones and number of spheres of SW1990 cells. However, simultaneous overexpressing miR-192-5p and ATXN7 rescued these phenotypes of SW1990 cells. In conclusion, the present study found that miR-192-5p was abnormal and highly expressed in pancreatic cancer, and miR-192-5p promoted pancreatic cancer cell proliferation and stem cell-like phenotype by inhibiting the expression of ATXN7.

Keywords: pancreatic cancer; stem cell-like phenotype; proliferation; miR-192-5p; ATXN7

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Introduction

Pancreatic cancer is one of the most devastating and fatal malignant tumors in the world, with a poor prognosis and high mortality, showing a close parallel correlation between morbidity and mortality[1]. Currently, less than 10% of pancreatic cancer patients are diagnosed at an early stage. Therefore, most patients lack the opportunity to receive surgical treatment due to they are diagnosed at a later stage[2]. The high mortality rate of pancreatic cancer is mainly due to a variety of factors, such as family history, genetics, smoking and chronic pancreatitis[1]. The key reason for the high mortality rate is that most patients are diagnosed at an advanced stage[3]. Another challenge of pancreatic cancer therapy is that pancreatic cancer patients have poor response to radiotherapy or chemotherapy[4]. However, only 15% of tumors are suitable for resection due to late diagnosis[5]. The accumulation of cancer stem cells (CSC) is an important factor in pancreatic cancer chemoresistance, recurrence and metastasis[6-8]. Therefore, exploring the regulatory mechanism of CSC in pancreatic cancer is of great significance to the treatment of pancreatic cancer.

microRNA (miRNA) is a small non-protein coding RNA. More and more evidences show that miRNAs are important in carcinogenesis through post-transcriptional gene silencing. The dysregulation of miRNAs is related to many cancers, and the role of some miRNAs is to inhibit tumors or oncogenes[9,10]. miR-192-5p has an important regulatory effect on the malignant biological behavior of a variety of tumors, and has a dual role[11-13]. For example, Ji et al. showed that miR-192-5p was down-regulated in human bladder cancer cell lines and tissues, and that miR-192-5p inhibited the growth of bladder cancer cells by targeting YY1[14]. In contrast, Martyna et al. showed that miR-192-5p promotes chemical resistance maintenance, invasion, epithelial-mesenchymal transformation, and immune escape in squamous cell carcinoma[15]. In addition, some studies have shown that miR-192-5p can regulate stem cell-like characteristics of malignant tumors. Gu et al. showed that miR-192-5p Silencing by Genetic Aberrations Is a Key Event in Hepatocellular Carcinomas with Cancer Stem Cell Features[16]. Loss of miR-192-5p initiates a hyper glycolysis and stemness positive feedback in hepatocellular carcinoma[17]. However, the mechanism of miR-192-5p on stem cell-like characteristics of pancreatic cancer remains to be elucidated.

In this study, we used bioinformatics database to predict the expression level of miR-192-5p in pancreatic cancer tissues and analyze its correlation with the prognosis of pancreatic cancer patients. Moreover, we investigated the effects of miR-192-5p on the proliferation and stem cell-like properties of pancreatic cancer cells. It aims to provide basic experimental evidence and targets for the treatment of pancreatic cancer.

Materials and Methods

Cell culture

Normal pancreatic ductal epithelial cells lines HPDE and Human pancreatic cancer cell lines CFPA-1， SW1990， PANC-1 and PATU8988, were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). The cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich, Shanghai, China) supplemented with 10% fetal bovine serum (Life Technologies, Grand Island, NY, USA) at 37°C with 5% CO_2. The morphology, growth curve, and mycoplasma detection of pancreatic cancer cell lines were determined one month prior to the experiment, according to the cell line verification test recommendation from ATCC.

Cell transfection

For miR-192-5p overexpression or knockdown,

an miR-192-5p mimic or inhibitor and corresponding NC (miR-NC) were purchased from GenePharma (Shanghai, China). SW1990 cells were transfected with either the miR-192-5p mimic, miR-192-5p inhibitor or miR-NC at a final concentration of 50 nM using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. Cells were used for miR-192-5p expression analysis or other experiments after 48 h of transfection. For ATXN7 overexpression, recombinant lentiviral overexpressing ATXN7 (GenePharma) was used to increase ATXN7 expression, with empty carrier recombinant lentiviral serving as an NC.

Bioinformatics analysis

In short, we used the starBase database to predict the expression levels of miR-192-5p and ATXN7 in pancreatic cancer and normal pancreatic cancer tissues, the survival analysis of the prognosis of pancreatic cancer patients, and the correlation between the expression of miR-192-5p and ATXN7 in pancreatic cancer. PITA, RNA22, and miRmap databases were used to predicted downstream targets of miR-192-5p.

Flow cytometry analysis

Cells were harvested, disaggregated to a single cell suspension, and stained as described previously. The antibodies PE anti-human CXCR4 and ALDH1 (a dilution of 1:100, Miltenyi Biotech Inc., Bergisch Gladbach, Germany) were added to 1×10^6 cell/100 μL in PBS, and incubated for 20 min on ice in dark. After washing twice with PBS, samples were resuspended in 400 μL PBS and analyzed on a BD Aria II flow cytometer (BD Immunocytometry Systems, San Jose, CA, USA). Forward-scatter and Side-scatter profiles were used to eliminate cell doublets. Positive CXCR4 and ALDH1 sub-populations were identified by comparison of fully-stained samples to FMo (fluorescence-minus-one) controls.

RT-qPCR assay

Total RNAs were isolated from cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA). The PCR reaction was performed on the Bio-Rad Icycler Pcr Thermal Cycler (Hercules, CA, USA) using SYBR™ Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). U6 serves as an internal reference. The primer sequence is as follows: miR-192-5p Forward 5'-CACAGGGCTCTGACCTATGA-3'; Reverse 5'-GGCATTGAGGCGAACATACC-3', U6 Forward 5'-CCCTTCGGGGACATCCGATA-3'; Reverse 5'-TTTGTGCGTGTCATCCTTGC-3'.

Western Blot assay

Cells were washed three times with cold PBS and lysed on ice in RIPA buffer with protease inhibitors PMSF. The protein concentrations were determined using the BCA method (Beyotime Biotechnology, Haimen, China). A total of 30 μg of protein was separated by 10% SDS-PAGE and electro-blotted onto NC membranes using a semi-dry blotting apparatus. After blocking in 3% BSA, the membranes were incubated overnight at 4°C with the primary antibodies. The membranes were then incubated in the secondary antibodies for 1 h at room temperature on a shaker. The protein bands were visualized by using a commercially available enhanced chemiluminescence kit (Thermo Scientific, Hudson, NH, USA). GAPDH or β-Actin was used as a loading control. Antibodies used for western blot analyses were as follows: ATXN7, Nanog, Oct4 and Sox2 (Cell Signaling Technology, Beverly, MA, USA).

Dual-Luciferase reporter gene assay

To construct luciferase reporter vectors, 3' UTR fragments from ATXN7 cDNA containing the predicted miR-192-5p-binding sites were amplified by PCR and subcloned downstream of the luciferase gene in the PYr-MirTarget luciferase vector (Ambion, Austin, TX, USA). The 3' UTR of ATXN7 with or without the mutant

sequence were then amplified. For dual-Luciferase reporter gene assays, T293 cells were cultured in 24-well plates and co-transfected with 50 ng vector containing firefly luciferase sequence with 25 ng miR-192-5p or control DNA, using Lipofectamine 2000 reagent. At 48 h post-transfection, relative luciferase activity was calculated by normalizing firefly luminescence to Renilla luminescence using Dual-Luciferase Reporter Assays (Promega, Madison, WI, USA), according to the manufacturer's instructions.

CCK-8 assay

For CCK-8 assay, SW1990 cells (1×10^4) were seeded into 96-well plates and incubated overnight. Medium was removed and cells were washed three times with PBS. DMEM (90 μL; Gibco, Grand Island, NY, USA) and CCK-8 reagent (10 μL) were added to each well and incubated for 1.5 h at 37°C. A microplate reader was used to measure optical density at 450 nm.

EdU staining assay

Forty-eight h after transfection, the cells were labeled with EdU (100 μL/well) for 2 h. A cell fixative solution (100 μL/well) was added into the wells. After incubating the plate at room temperature for 30 min, the cells were washed and incubated with 2 mg/mL glycine for 5 min. The cells were incubated with 100 μL/well of penetrant (PBS containing 0.5% TritonX-100) for 10 min. Subsequently, the cells were washed with PBS and incubated with 1×Apollo staining solution in conditions devoid of light for 30 min, treated with the penetrant, and washed with methanol. Cells were added with DAPI reaction solution (100 μL/well) for decolorized incubation in oscillator at room temperature for 30 minutes in conditions devoid of light. An anti-fluorescence quench solution was added to the plate (100 μL/well). After staining, the cells were observed under a fluorescence microscope and photographed.

Colony formation assay

Well-dispersed single cells were seeded into 6-well plate (500 cells/well) and subjected to drug treatment for 48 h at 37℃. Fresh medium was then replaced, followed by consecutive culture for another 10 days. Colonies were fixed with 3% formaldehyde briefly and stained with 0.5% crystal violet for 15 min (Sigma, MO, USA).

Sphere formation assay

Sphere formation assay was performed as described elsewhere[18]. In brief, single cell suspensions were washed twice using serum-free PBS and plated in 24-well ultralow attachment plates (Corning, Steuben County, New York, NY, USA) at a density of 250 cells in culture media supplemented with 1% N_2 supplement (Gibco, Carlsbad, CA, USA), 2% B27 supplement (Gibco, Carlsbad, CA, USA), 20 ng/mL human platelet growth factor (Sigma-Aldrich, St. Louis, MO, USA), 100 ng/mL epidermal growth factor (Gibco, Carlsbad, CA, USA) at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

Statistical analysis

Statistical analysis was performed using the SPSS 17.0 software (SPSS, Chicago, USA). Data were expressed as mean ± standard deviation (SD). Three replicates were included in each independent experiment. Student's t-test and ANOVA were used for statistical analysis. Statistical significance was regarded as $P<0.05$.

Results

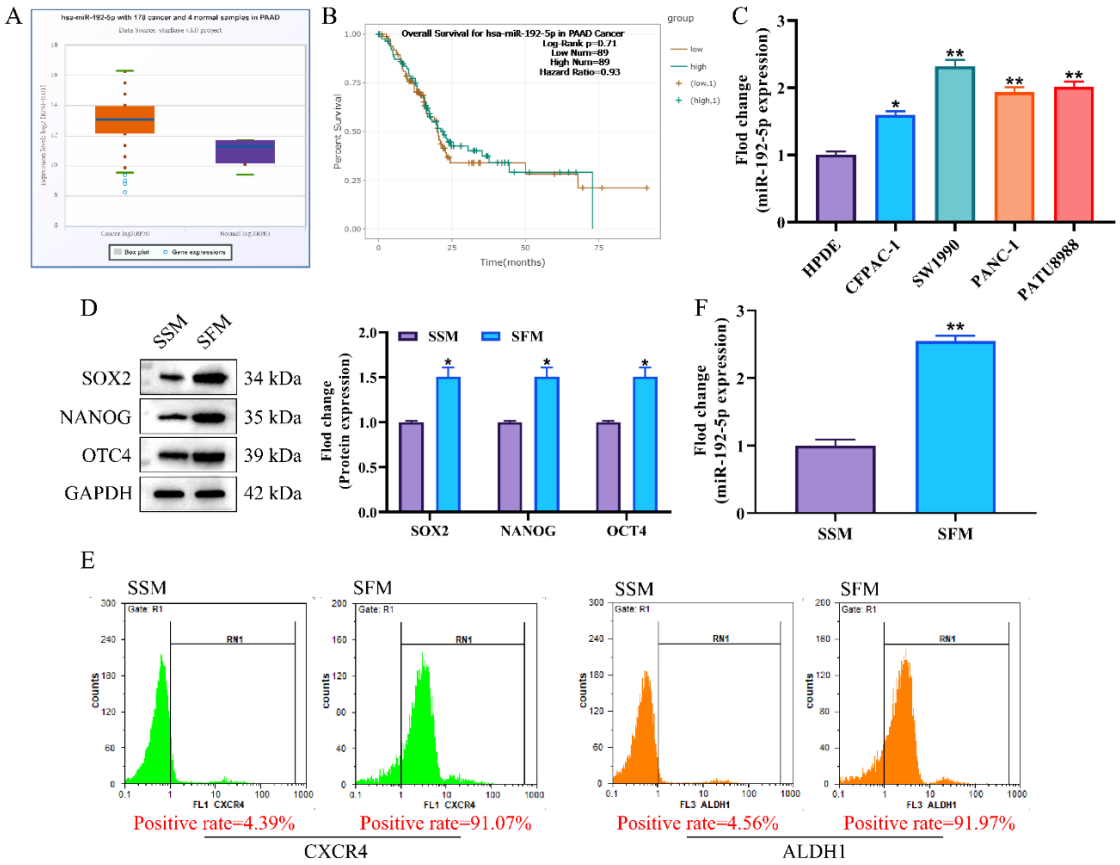

Figure 1 miR-330-5p downregulates in Erastin- and RSL3-induced ferroptosis in CRC cells. (A)-(D) MTT assay was used to analysis cell viability for HCT116 and HT29 cells after stimulating with increasing concentration of Erastin (0, 2.5, 5, 10, 20 μM) and RSL3 (0, 0.05, 0.1, 0.5, 1 μM). (E)-(F) QRT-PCR was used to detect miR-330-5p expression after stimulating with increasing concentration of Erastin (0, 2.5, 5, 10, 20 μM) and RSL3 (0, 0.05, 0.1, 0.5, 1 μM). **P*<0.05, ***P*<0.01, ****P*<0.001.

Differential expression of miR-192-5p in pancreatic cancer

First, we predicted the expression difference of miR-192-5p in pancreatic cancer and normal tissue, and its correlation with patient prognosis through starBase database. The results showed that miR-192-5p was highly expressed in pancreatic cancer tissues compared with normal pancreatic tissues (Fig. 1A). However, there was no significant correlation between the high and low expression of miR-192-5p and the survival of pancreatic cancer patients (Fig. 1B). We also verified the expression level of miR-192-5p at the cellular level. The results showed that the expression level of miR-192-5p in pancreatic cancer cells was significantly higher than that in normal pancreatic ductal epithelial cells, and the expression of miR-192-5p was the highest in

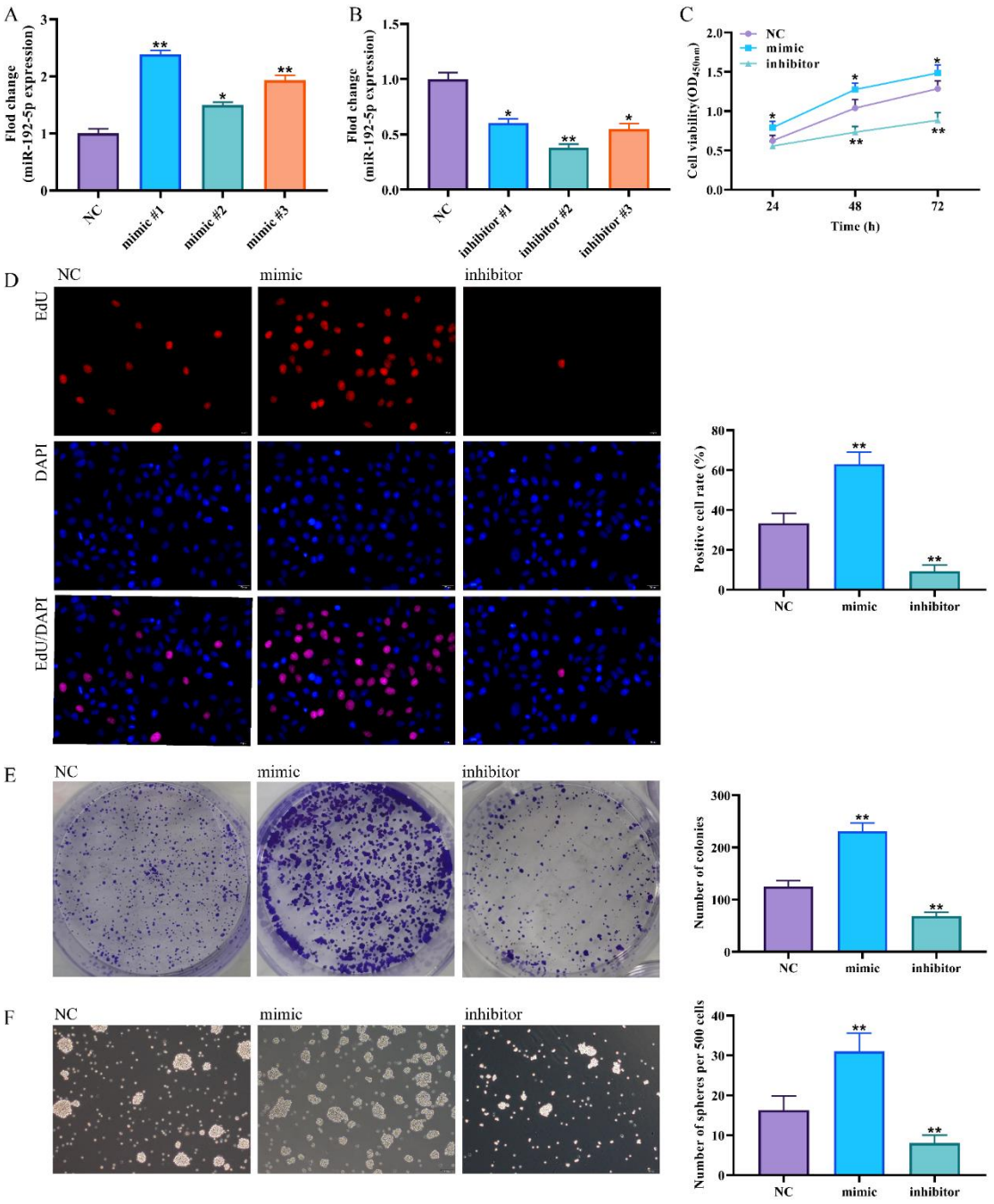

Figure 2 miR-192-5p promoted SW1990 cell proliferation and stem cell-like phenotype. The transfection efficiency of (A) miR-192-5p mimic and (B) miR-192-5p inhibitor in SW1990 cells was detected by RT-qPCR. (C) The proliferation activity of SW1990 cells in each group was analyzed by CCK-8 kit. (D) EdU staining was used to detect the proportion of EDU positive cells in SW1990 cells. (E) Clonal formation assay was used to observe the clonal formation ability of SW1990 cells. (F) The spheres-forming efficiency of SW1990 cells was detected by spheres forming assay. n=3, data were expressed as mean ± SD. Comparison with NC group, $^{*}P<0.05$, $^{**}P<0.01$.

SW1990 cells (Fig. 1C). We selected SW1990 cells for subsequent experiments We further enriched stem cells in SW1990 cells by SFM. The results showed that compared with the SSM group, the protein expressions of SOX2, Nanog and OTC4 were significantly increased in the

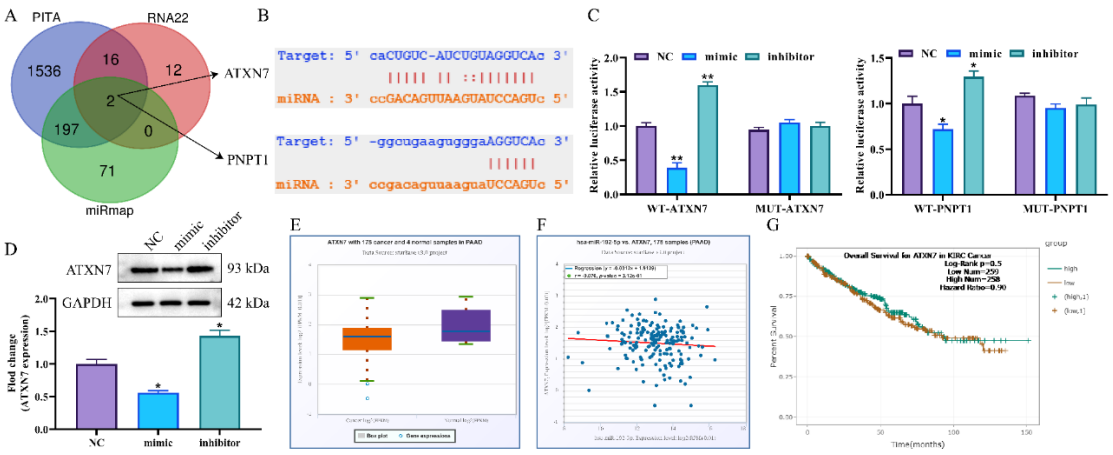

Figure 3 ATXN7 is a downstream target of miR-192-5p. (A) Venn plots of Pita, RNA22 and MIRMAP predicted results. (B) (B) miR-192-5p binding sequence with ATXN7 and PNPT7, respectively. (C) Dual-luciferase reporter gene assay was performed to verify the targeting relationship between miR-192-5p and ATXN7 and PNPT7. (D) The effect of miR-192-5p on the expression of ATXN7 was detected by Western blotting. The starBase database was used to predict (E) the difference in the expression of ATXN7 in pancreatic cancer and normal tissues, (F) the correlation between miR-192-5p and ATXN7 expression, and (G) the correlation between the expression level of ATXN7 and the prognosis of pancreatic cancer. n=3, data were expressed as mean ± SD. Comparison with NC group, $^{*}P<0.05$, $^{**}P<0.01$.

SFM group (Fig. 1D). Flow cytometry results also showed that the positive proportion of 1E). It is suggested that the stem cell-like stemness of SW1990 cells in SFM group was significantly higher than that of SSM, and we successfully enriched the stem cells of SW1990 cells. In addition, we also found that the expression level of miR-192-5p in the SFM group was significantly higher than that in the SSM group (Fig. 1F). These results suggest that miR-192-5p may be involved in the regulation of pancreatic cancer development and stem cell-like phenotype.

Effects of miR-192-5p on the biological behavior of SW1990 cells

We further investigated the effects of miR-192-5p on SW1990 cell proliferation and stem cell-like phenotype. First, we exogenously regulated the expression level of miR-192-5p in SW1990 cells. As shown in Fig. 2A-B, after transfection with miR-192-5p mimic or miR-192-5p inhibitor, the expression level of miR-192-5p in SW1990 cells

CXCR4 and ALDH1 in SFM group was significantly higher than that in SSM group (Fig. was significantly higher or lower than that in the negative control group. In addition, miR-192-5p mimic #1 and miR-192-5p inhibitor #2 had the best transfection effect. Therefore, miR-192-5p mimic #1 and miR-192-5p inhibitor #2 were selected for transfection in subsequent experiments. CCK-8 results showed that, compared with the NC group, the proliferation activity of SW1990 cells in the mimic group was significantly increased, and the proliferation activity of SW1990 cells in the inhibitor group was significantly decreased (Fig. 2C). In a consistent manner, the EdU results showed that the proportion of EDU positive cells in SW1990 cells of the mimic group was significantly higher than that of the NC group, and the proportion of EdU positive cells in SW1990 cells of the inhibitor group was significantly lower than that of the NC group (Fig.2D). The results of the clone formation assay also showed that,

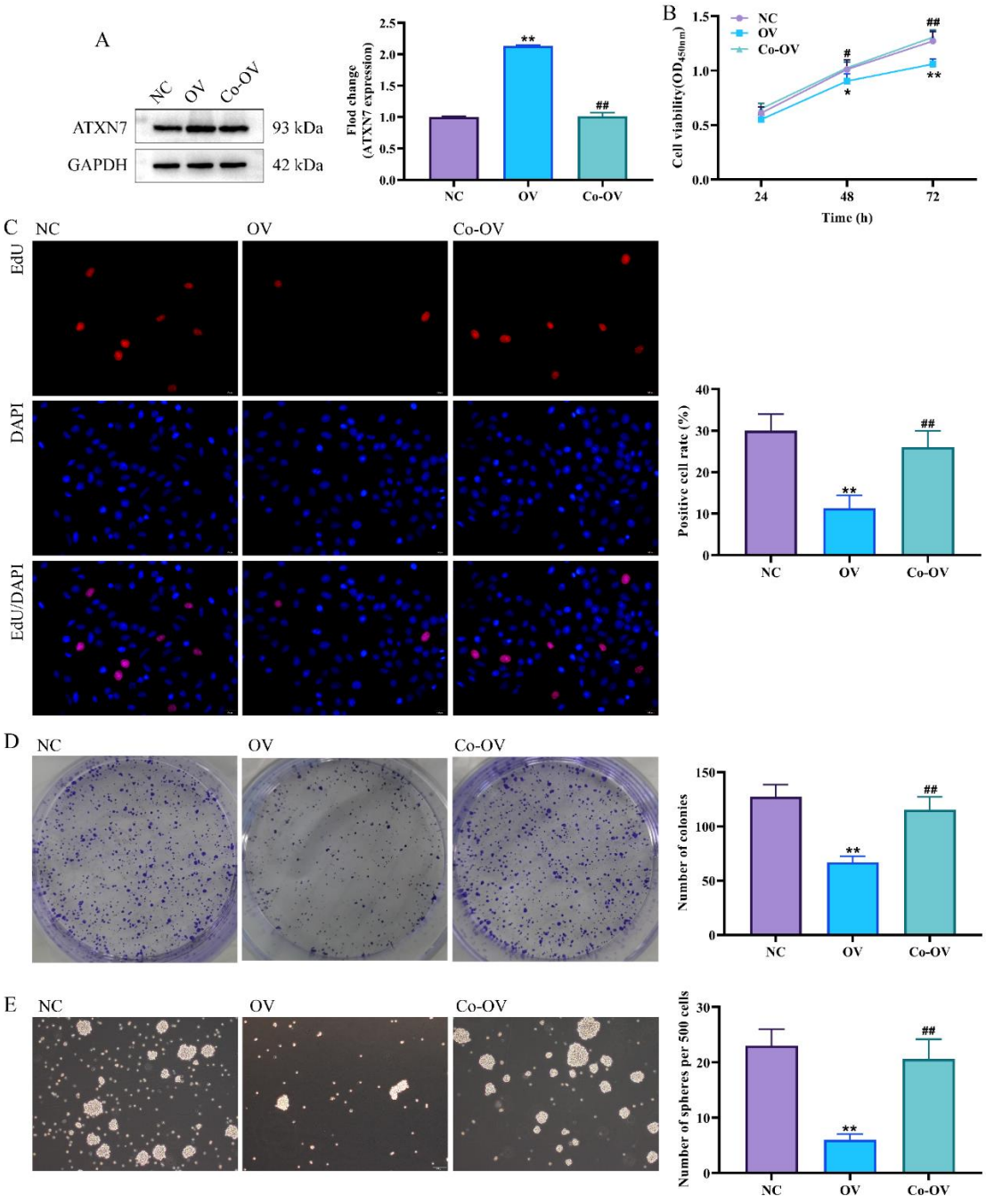

Figure 4 Effects of miR-192-5p on proliferation and stem cell-like phenotype of SW1990 cells via ATXN7. (A) ATXN7 expression in SW1990 cells was detected by Western blotting. (B) The proliferation of SW1990 cells in each group was detected by CCK-8 kit. (C) EDU staining showed the proportion of EDU positive cells in SW1990 cells in each group. (D) Cloning number of SW1990 cells in each group was detected by clone formation assay. (E) Spheres forming assay was used to evaluate the stem-like properties of SW1990 cells in each group. n=3, data were expressed as mean ± SD. Comparison with NC group, $^{*}P<0.05$, $^{**}P<0.01$; Comparison with OV group, $^{\#}P<0.05$, $^{\#\#}P<0.01$.

compared with the NC group, overexpression of miR-192-5p significantly increased the number

of clones, while knockdown of miR-192-5p was vice versa (Fig.2e). The results of spheres formation experiment showed that the number of spheres composed by mimic was significantly higher than that of NC and inhibitor group, and the number of spheres composed by NC group was higher than that of inhibitor group (Fig.2F). These results indicated that miR-192-5p promoted the proliferation and stem cell-like phenotype of SW1990 cells.

ATXN7 is a downstream target of miR-192-5p

We continued to explore the downstream target of miR-192-5p. As shown in Fig. 3A, the downstream targets of miR-192-5p were predicted by PITA, RNA22 and MIRMAP, and VENN was performed for the prediction results of the three databases. The results showed that there were two potential targets, ATXN7 and PNPT1, in the intersection of the three starBase. The potential binding sequences of miR-192-5p with ATXN7 and PNPT1 are shown in Fig. 3B. Subsequently, we mutated the binding sites of miR-192-5p with ATXN7 and PNPT1, and performed dual-luciferase reporter genes assay. The results showed that, compared with the NC group, overexpression of miR-192-5p significantly inhibited luciferase activity of ATXN7 and PNPT1 wild-type vectors, but had no significant effect on mutant vector, and knockdown of miR-192-5p achieved the opposite effect (Fig. 3C). Notably, the effect of miR-192-5p on ATXN7 was better than that of PNPT1. Therefore, ATXN7 was selected for subsequent verification. Western blotting results showed that the expression level of ATXN7 in the MIMIC group was significantly lower than that in the NC group, while the effect of the inhibitor group was opposite (Fig.3D). Further, we predicted the expression level and prognostic correlation of ATXN7 in pancreatic cancer tissues by starBase database. The results showed that the expression level of ATXN7 in pancreatic cancer tissues was significantly higher than that in normal pancreatic tissues (Fig. 3F), and there was a negative correlation between miR-192-5p and ATXN7 expression in pancreatic cancer tissues (Fig. 3G). In addition, as with miR-192-5p, there was no significant correlation between high and low expression of ATXN7 and survival in pancreatic cancer patients (Fig. 3H). These results suggest that ATXN7 is a downstream target of miR-192-5p in pancreatic cancer, and miR-192-5p can negatively regulate the expression level of ATXN7.

Effects of miR-192-5p on proliferation and stem cell-like phenotype of SW1990 cells via ATXN7

We further investigated the effect of miR-192-5p on the malignant biological behavior of SW1990 cells via ATXN7. Western blotting results showed that, compared with the NC group, overexpression of ATXN7 significantly up-regulated the expression level of ATXN7 in SW1990 cells, while overexpression of miR-192-5p and ATXN7 at the same time would rescue the expression of ATXN7 (Fig. 4A). CCK-8 results showed that the proliferation activity of SW1990 cells in the OV group was significantly lower than that in the NC group, and lower than that in the Co-OV group (Fig. 4B). EdU staining results showed that the proportion of EdU positive cells in SW1990 cells in the OV group was significantly higher than that in the NC and Co-OV groups, and there was no significant difference between the NC and Co-OV groups (Fig. 4C). Similarly, the results of the clone formation experiment showed that, compared with the NC group, the overexpression of ATXN7 significantly reduced the clone number of SW1990 cells, while the clone number in the Co-OV group was Rescue (Fig. 4D). Moreover, spheres forming assay results showed that the number of spheres-forming SW1990 cells was significantly reduced after the overexpression of ATXN7, while the number of spheres-forming SW1990 cells was rescued after the

overexpression of miR-192-5p and ATXN7 (Fig. 4E). These results suggested that miR-192-5p promoted the proliferation and stem cell-like phenotype of SW1990 cells by inhibiting the expression of ATXN7.

Discussion

Recent studies have shown that many of the genetic and epigenetic changes behind the aggressive and destructive behavior of cancer cells are composed of discrete cancer cells with stem-cell properties called CSCs[19,20]. However, the identification of effective CSC markers and the development of new therapies that can selectively target pancreatic cancer CSCs lag behind other cancers[21]. By weakening the protein levels of various factors required for stem cell function, the ability of miRNAs to regulate stem cell fate and differentiation opens a new field of miRNA regulation of CSC function[22]. Our research shows for the first time that miR-192-5p is highly expressed in pancreatic cancer tissues and cell lines. In addition, the expression level of ATXN7 and miR-192-5p is negatively correlated in pancreatic cancer tissues, and miR-192-5p negatively regulates the expression level of ATXN7 through targeting. Functional tests have shown that miR-192-5p promotes pancreatic cancer cell proliferation and stem cell-like properties by inhibiting the expression of ATXN7. In summary, these findings indicate that the altered expression of miR-192-5p can be used as a potential disease biomarker for pancreatic cancer diagnosis and prognosis.

Despite the use of multimodal treatment methods, pancreatic cancer is still a highly lethal malignant tumor. miRNA has been proven to negatively regulate target mRNA in a sequence-specific manner, and is a key regulator of various carcinogenic processes (such as cell proliferation, differentiation, invasion, and metastasis), and can play a role in suppressing tumors or oncogenes[23-25]. Therefore, elucidating the potential mechanism of miRNA in tumor development may provide valuable diagnostic and therapeutic strategies for malignant tumors. miR-192-5p has been explained to have an important regulatory role in a variety of malignant tumors. For example, miR-192-5p acts as a tumor suppressor in colon cancer and simvastatin activates miR-192 to inhibit cancer cell growth[26]. MiR-192-5p reverses cisplatin resistance by targeting ERCC3 and ERCC4 in SGC7901/DDP cells[27]. MiR-192 inhibits cell proliferation and induces apoptosis in human breast cancer by targeting caveolin 1[28]. We described for the first time that miR-192-5p acts as a carcinogen in pancreatic cancer and promotes the proliferation of pancreatic cancer cells. In addition, we also found that miR-192-5p can promote the stem cell-likeness of pancreatic cancer cells. The CSC model has been proposed to explain the high recurrence rate of cancer and subsequent resistance to current systemic treatments[29,30]. CSC has been identified in many solid malignancies, including pancreatic cancer, and has important clinical significance, because targeting the CSC population may be essential to prevent tumor recurrence and spread[31,32]. Previous literature has shown that miR-192-5p has a role in lung cancer stem cell-like properties[16,17]. However, its role in pancreatic cancer stem cell-like properties remains to be studied. We have also clarified the regulatory mechanism of miR-192-5p in pancreatic cancer stem cell-likeness for the first time. Moreover, we also found that ATXN7 is the downstream target of miR-192-5p.

ATXN7 is one of autosomal dominant cerebellar ataxia (ADCA) which is a heterogeneous group of neurodegenerative disorders characterized by progressive degeneration of the cerebellum, brain stem and spinal cord. ADCA is caused by the expansion of the CAG repeats, producing an elongated polyglutamine tract in the corresponding protein[33]. The expanded repeats are variable in size and unstable, usually increasing in size when transmitted to successive generations[34]. This locus has been mapped to chromosome 3, and it has been determined that

the diseased allele associated with spinocerebellar ataxia-7 contains 38–130 CAG repeats (near the N-terminus), compared to 7–17 in the normal allele[35]. The encoded protein is a component of the SPT3/TAF9/GCN5 acetyltransferase and TBP-free TAF-containing chromatin remodeling complexes, and it thus plays a role in transcriptional regulation[36]。 However, few studies have reported on the role of ATXN7 in cancer. We demonstrate for the first time that ATXN7 acts as a tumor suppressor in pancreatic cancer, inhibiting pancreatic cancer cell proliferation and stem cell-like properties. ATXN7 may be an important molecule in future tumor research and may be involved in a variety of malignant biological behaviors of cancer cells, especially epithelial mesenchymal transformation, because it is involved in the regulation of cytoskeleton.

In conclusion, we reveal for the first time the regulatory role of miR-192-5p/ATXN7 molecular axis in pancreatic cancer. Emphatically, ATXN7 is likely to be a key focus of our future oncology research, as it has been poorly studied in oncology. Moreover, miR-192-5p can be used as a therapeutic target for pancreatic cancer due to its carcinogenicity in pancreatic cancer.

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