SP/NK-1R Axis Promotes Perineural Invasion of Pancreatic Cancer and is Affected by lncRNA LOC389641

Tengfei Ji

Southern Medical University (People's Hospital of Huadu District)

Keqiang Ma

Southern Medical University (People's Hospital of Huadu District)

Hongsheng Wu

Southern Medical University (People's Hospital of Huadu District)

Tiansheng Cao# (✉ caotiansheng2088@sina.com)

Southern Medical University (People's Hospital of Huadu District)

Abstract

Background

Pancreatic cancer is a deadly disease with low overall survival during the past 30 years. Perineural invasion (PNI) was considered to be the main reason for poor prognosis. In the present study, we analyzed the role of substance P (SP)/neurokinin-1 receptor (NK-1R) and long non-coding RNA (lncRNA) LOC389641 on pancreatic cancer PNI.

Material and methods

Pancreatic cancer cell lines of BxPC-3 and MIAPaCa-2 were co-cultured with SHSY5Y cells, and then stimulated with SP to simulate the *in vivo* influence of pancreatic cancer by ganglion. The co-culture cells were transfected with overexpressed neurokinin-1 receptor (NK-1R), silenced NK-1R, overexpressed LOC389641, and silenced LOC389641. Enzyme-linked immunosorbent assay (ELISA) assay was performed to examine the concentration of SP in cells. The cell proliferation ability was assessed by cell counting kit-8 and 5-ethynyl-2'-deoxyuridine (EdU) assays. The wound-healing and Transwell assays were carried out to determine the cell migration and invasion analyses. Quantitative real-time PCR (qRT-PCR), western blot, immunofluorescence (IF) analyses were performed to evaluate the expression levels.

Results

Addition of SP in co-culture system positively regulates cell proliferation, migration, and invasion of pancreatic cancer PNI. SP significantly stimulated NK-1R/Akt/NF-κB signaling pathway. The concentration of 100 nmol/L for 24h was chosen to be optimal for SP treatment.NK-1R positively regulated the proliferation, migration, and invasion of pancreatic cancer PNI. The expression of lncRNA LOC389641 and mRNA tumor necrosis factor receptor SF10A (TNFRSF10A) was not affected by SP. Overexpressed and silenced LOC389641 can correspondingly change the effect of SP stimulation on pancreatic cancer PNI.

Conclusion

We found that SP/NK-1R and LOC389641 promote the cell progression of pancreatic cancer PNI. Moreover, we assumed that the pancreatic cancer PNI promoted by SP/NK-1R axis may be blocked by the TNFRSF10A/NF-κB pathway mediated by LOC389641.

1 Introduction

Pancreatic cancer is one of the deadliest malignant tumors, with an annual 432,242 related deaths in 2018 [1], which is continues to increase yearly[2]. Pancreatic cancer exhibits the hallmarks of the scarcity

of early symptoms, explosive outcomes, treatment resistance, poor prognosis, and high mortality rate [2–4]. Because of the chronic symptoms and severe abdominal pain, pancreatic cancer significantly affects the life quality of patients [5]. The treatment of cancers has improved substantially in the past decades, except pancreatic cancer.

Perineural invasion (PNI) is one prominent pathologic phenomenon of pancreatic cancer, almost occurring in all pancreatic cancer patients [6, 7].It is a process that cancer cells invade the surrounding nerves, which is observed in pancreatic cancer at an early stage and associated with pain, high tumor recurrence, and diminished overall survival[8]. PNI is defined as a characteristic of malignant tumor behavior and results in a poor prognosis of pancreatic cancer patients.

With the development of molecular treatment, PNI has been widely studied in terms of mRNA, non-coding RNAs (lncRNAs), and pathways. Studies have demonstrated that PNI can be reduced by targeting signaling pathways, revealing potential views on the treatment for pancreatic cancer [9, 10].

Neurokinin-1 receptor (NK-1R) is one tachykinin receptor distributed in peripheral tissues and nervous systems, involved in the biological functions of immune responses, neurogenic inflammation, pain, and depression[11]. By combining with substance P (SP), SP/NK-1R participated in the cancer pathophysiological actions [12–14], thus regulating the tumor cell proliferation, migration, metastasis, and angiogenesis[15]. The axis SP/NK-1R act by modulating signaling pathways, including ERK, Wnt, and Akt. Therefore, profound studies on SP/NK-1R regulating pathways in pancreatic cancer play essential roles.

Long non-coding RNA (lncRNA) has been widely studied in recent years. LOC389641 was reported to promote pancreatic ductal adenocarcinomaprogression and increase cell invasion by regulating E-cadherin with the possible involvement oftumor necrosis factor receptor SF10A (TNFRSF10A)[16]. However, if LOC389641 affects pancreatic cancer through SP/NK-1R axis is still uncovered. The present study studied the factors that influence pancreatic cancer cell progression in SP/NK-1R and the LOC389641 and its target gene of TNFRSF10A. This study might provide a promising therapeutic target for pancreatic cancer.

2 Materials And Methods

2.1 Cell culture

The pancreatic cancer cell lines of BxPC-3 and MIAPaCa-2 were bought from ATCC (Manassas, VA, USA) and cultured in RPMI 1640 supplemented with 10% fetal bovine serum at 37°C, 5% CO_2. The SH-SY5Y human neuroblastoma cell line was acquired from Kunming Institute of Zoology (Kunming, China). SPwas obtained from MCE (Shanghai, China). The *in vitro* analysis was performed in BxPC-3 (BxPC-3Ctrl) and MIAPaCa-2 (MIAPaCa-2Ctrl) cells and cultured with SH-SY5Y (SH-SY5Y-Vector),as well as SP and SH-SY5Y (SH-SY5Y-SP).

For the selection of optimal SP, the SP concentration of 0, 10, 25, 50, 100, and 150 nmol/L were cultured with BxPC-3 and MIAPaCa-2 cells for 24h. With the SP concentration of 20 nmol/L, the optimal culture times were selected from 0h, 12h, 24h, 36h, and 48h.

2.2 Transfections

The pancreatic cancer cell lines of BxPC-3 and MIAPaCa-2 were treated with 100nmol/L SP for 24h andthen transfected with NK-1R (NK-1R+SP), silenced NK-1R (siNK-1R+SP),LOC389641 (LOC389641+SP), and silenced LOC389641 (si-LOC389641+SP).

The vector of pGL3-Basic (Promega Corporation, Madison, USA) was used. The NK-1R DNA promoter sequences of NK-1R (forward primer, 5'-GGGGTACCTCTACCGTTTGAAATGGTCTTG-3' and reverse primer: 5'-CCCTCGAGGCAGTAGGTGGCTTGGAAGTA-3') and LOC389641 (forward primer, 5'-GGGGTACCTGGGAGGTGGGTAGTAGGG-3' and reverse primer: 5'-CCCTCGAGTGACACGGGATGAGACTTGG-3') were designed in Sangon Biotech(Shanghai, China). According to the manufacturer's instructions, the transfection was carried out using the Lipofectamine 2000 (Invitrogen, USA).

2.3 Enzyme-linked immunosorbent assay (ELISA) assay

Cell supernatants were collected after treatment with reagents. The concentration of SP was measured in Ctrl, SH-SY5Y-Vector, and SH-SY5Y-SP by standardized ELISA assay (Boster, Wuhan, China) according to the manufacturer's instructions.

2.4 Cell proliferation analysis

The cell proliferation ability of SP on BxPC-3 and MIAPaCa-2 was assessed by cell counting kit-8 (CCK-8; Glpbio; USA) and 5-ethynyl-2'-deoxyuridine (EdU; EdU Staining Proliferation Kit; Abcam, England) assays. The cells were cultured with CCK-8 reagent for 2 h at 37℃, and the absorbance OD450nm value was collected at 0 h, 12 h, 24 h, 36 h, and 48 h using the microplate reader (Biotech, China).

According to the manufacture's instruction, the EdU Proliferation Assay Kit (iFluor 647) (#ab222421; Abcam, Cambridge, MA, UK) was usedto detect the proliferation rates of BxPC-3 and MIAPaCa-2. In brief, 5×10^5 cells were cultured in 15mL tubes supplemented with culture medium containing 20 mMEdU reagent for 2 h. The cells were fixed with paraformaldehyde and stained with 4',6-diamidino-2-phenylindole (DAPI). Finally, the percentage of EdU-positive cells was analyzedand quantified by time-lapse fluorescence microscopy (Lonheart FX Automated Microscope, Winooski, VT, USA).

2.5 Wound-healing analysis

Approximately 3×10^5 BxPC-3 and MIAPaCa-2 cells and treatments were seeded in a 6-well plate and cultured until the cells reached about 80% confluence. Then, a straight line was scratched in each well using a 10-µl pipette tip. One day later, a microscopic camera captured the migration distance, and the migration rate was analyzed.

2.6 Transswell analysis

The migration and invasion analysis were performed using 24-well transwell chambers according to the manufacturer's instructions. Briefly, about 1×10^4 cells were plated into the top chamber of a transwell chamber (Corning, NY, USA) precoated with Matrigel matrix (BD, USA), and cultured at basal medium without FBS. Culture medium and 10% FBS were added to the down chamber. After culture, the invasion cells were fixed, stained, observed, andcounted under one Olympus microscope (40 ×).

2.7 Quantitative real-time PCR (qRT-PCR) analysis

The gene expression level of NK-1R was assessed in SH-SY5Y-SP with the SP concentrations of 0, 10, 25, 50, 100, and 150 nmol/L, and with the SP treatment times of 0h, 12h, 24h, 36h, and 48h. Moreover, the expression levels of NK-1R, LOC389641, and TNFRSF10A were assessed in pancreatic cancer cells and transfections.

The total RNA was extracted using TRIzol Reagent (Invitrogen, USA). The primers of NK-1R, LOC389641, and TNFRSF10A were designed by Primer5software and synthesized in Sangon Biotech (Shanghai, China) (**Table S1**). HiScript II One Step qRT-PCR SYBR Green Kit (#Q221-01; Vazyme Co. Ltd; Nanjing, China) evaluated the expression levels.

2.8 Western blot

The protein in cells was lysis by RIPA lysis buffer (#R0278, Sigma) and quantified by BCA Protein Assay Kit (Beyotime, Jiangsu, China). The protein expression level in collected cells and transfections were evaluated by the standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method. The membranes were blocked with 5% non-fat milk and incubated with the primary antibodies of NK-1R (#ab25445, 1:00, Abcam, Cambridge, UK), NF-κB p65 (#ab16502, 1:1000; Abcam), AKT (#ab28422, 1:1000; Abcam),phosphor-AKT (p-AKT) (#ab38449, 1:1000; Abcam), TNRSF10A (#ab157345; 1:1000; Abcam), GAPDH (#ab9485, 1:2500; Abcam),and HDAC1 (#ab41407; 1:1000; Abcam). Subsequently, the washed PVDF membranes were incubated with the corresponding HRP-conjugated secondary antibody (1:5000). The bands were observed under an ECL kit (Millipore, Germany).

2.9 Immunofluorescence (IF) analysis

The cells were fixed with 4% formaldehyde, blocked with 5% BSA, and incubated with anti-NK-1R, anti-TNFRSF10A fluorescent secondary antibody (Cell Signaling Technology) for 48 h. The cells were stained with DAPI and observed under a fluorescence microscope (Leica, Germany). Five randomly selected visuals in each group were observed, photographed, and calculated.

2.10 Statistical analysis

All data were analyzed and drawn using GraphPad Prism 8 (GraphPad Software, La Jolla, CA). One-way ANOVA or Student's t-test was used for comparisons between groups. Data were exhibited with mean±SD. $P<0.05$ was considered statistically significant.

3 Results

3.1 SP positively regulates cell proliferation, migration, and invasion of pancreatic cancer PNI

To evaluate the role of SP in pancreatic cancer cells, cell proliferation, migration, and invasion analyses were performed. Firstly, the SP concentration was evaluated in groups. As expected, the concentration of SP is higher in the SH-SY5Y-SP group than that in Ctrl and SH-SY5Y-Vector groups ($P<0.05$; Figure 1A). CCK-8 assessed the cell activity at OD450nm, and the result showed that SH-SY5Y-SP significantly increased the activity of BxPC-3 and MIAPaCa-2 compared with Ctrl and SH-SY5Y-Vector ($P<0.05$; Figure 1B). The EdU positive cells were significantly increased in the SH-SY5Y-SP group compared with Ctrl and SH-SY5Y-Vector groups ($P<0.05$; Figure 1C), revealing a promoted cell proliferation function by SP supplementation.The migration abilities were evaluated using Transwell and wound-healing assays. Compared with Ctrl and SH-SY5Y-Vector, the SP group's migration and invasion cell numbers were significantly promoted ($P<0.001$; Figure 1D, F). The wound-healing experiment revealed a significantly accelerated cell migration rate by SP ($P<0.05$; Figure 1E). As mentioned above, we concluded that SP positively regulated the proliferation, migration, and invasion of BxPC-3 and MIAPaCa-2 cells.

3.2 SP significantly stimulated NK-1R/Akt/NF-κB signaling pathway

As reported, the expression level of NK-1R was positively correlated with the concentration of SP. Therefore, we confirmedthe expression level of NK-1R using qRT-PCR, western blot, and IF in groups of Ctrl, SH-SY5Y-Vector, and SH-SY5Y-SP. The relative expression of NK-1R was significantly upregulated in SH-SY5Y-SP compared with Ctrl and SH-SY5Y-Vector ($P<0.001$; Figure 2A). As detected by western blot, the expression level of NK-1R was significantly increased in cells treated with SP (Figure 2B).As observed in Figure 2C, the fluorescence ratio of NK-1R was significantly promoted in SH-SH5Y-SP compared with Ctrl and SH-SY5Y-Vector ($P<0.05$; Figure 2C, D). These results demonstrated that the SP positively correlated with the expression of NK-1R.

NF-κB p65 is one of the transcription factors of NK-1R, which might also positively regulate with NK-1R. The western blot analyses were performed to determine the effect of the NK-1R/Akt/NF-κB signaling pathway in co-cultured cells.Therefore, we evaluated the expression levels of NF-κB p65 in Ctrl, SH-SY5Y-Vector, and SH-SY5Y-SP groups by western blot. We found that the expression of NF-κB p65 and p-AKT were upregulated in SH-SY5Y-SP compared with Ctrl and SH-SY5Y-Vector (Figure 2E, F).Taken together, we found that SP significantly stimulated the NK-1R/Akt/NF-κB signaling pathway.

3.3Selection of optimal SP concentration and treatment time

After confirming the role of SP on the regulation of NK-1R, NF-κB p65, and p-AKT, the optimal concentration and treatment time of SP were selected. Firstly, NK-1R, NF-κB p65, and AKT expression

level at the supplementary concentration of SP at 0, 10, 25, 50, 100, and 150 nmol/L were assessed. We found that the expression level of NK-1R was continuously enhanced when the addition of SP over 10 nmol/L. The expression level of NK-1R, NF-κB p65, and p-AKT were significantly increased when SP was added over 25nmol/L compared with that at 0nmol/L and 10nmol/L ($P<0.001$; Figure 3A-D). When the addition of SP over 100nmol/L, their expressions showed no significant difference. Therefore, we assumed 100nmol/L to be the optimal SP concentration.

Then, we evaluated the role of treatment time (0h, 12h, 24h, 36h, and 48h) of SP on the expression level of NK-1R, NF-κB p65, AKT, and p-AKT. We found that their expression was also continuously promoted with the increased treatment time (Figure 3E-H). Their expression levels were significantly increased at 12 h ($P<0.05$), 24 h($P<0.001$), 36 h($P<0.001$), and 48 h ($P<0.001$) compared with 0 h.We chose the addition of SP for 24h as the optimal treatment time.

3.4 NK-1R positively regulated the cell proliferation, migration, and invasion of pancreatic cancerPNI

After selecting optimal concentration and treatment time of SP, the role of NK-1R treated with SP on pancreatic cancer cell lines was further studied. As expected, the expression level of NK-1R was significantly increased in NK-1R+SP ($P<0.05$), and decreased in siNK-1R+SP ($P<0.001$) compared with NC-SP (Figure 4A, B). The protein expression of NF-κB p65 and p-AKT was positively correlated with the concentration of NK-1R (Figure 4C, D).

Then, we evaluated the role of NK-1R on cell progression. The cell viability at OD450nm and EdU positive cells were significantly increased in NK-1R+SP and inhibited in siNK-1R+SP ($P<0.05$; Figure 4E, F), revealing a promoted cell proliferation function by NK-1R supplementation. The migration and invasion abilities were significantly promoted in the NK-1R+SP group and inhibited by the siNK-1R+SP group ($P<0.05$; Figure 4G, H). Taken together, we concluded that SP treated with NK-1R positively regulated the proliferation, migration, and invasion of BxPC-3 and MIAPaCa-2 cells.

3.5 The expression of lncRNA LOC389641 and mRNA TNFRSF10A was not affected by SP

As reported, TNFRSF10A was the targeted gene of LOC389641, which was assumed to be one lncRNA regulating the metastasis of pancreatic cancer cells. Therefore, we evaluated the dysregulation of LOC389641 and TNFRSF10A in co-cultured cells of SH-SY5Y and SP. The expression level of lncRNA LOC389641 was evaluated by qRT-PCR; as well as the TNFRSF10A was assessed by qRT-PCR, western blot, and IF. The expression levels of LOC389641 and TNFRSF10A exhibited no significant differences among groups of Ctrl, SH-SY5Y-Vector, and SH-SY5Y-SP ($P>0.05$; Figure 5A). As observed in Figure 5B, C, the protein expression level of TNFRSF10A also exhibited no significant differences in groups.

3.6 Overexpressed and silenced LOC389641 can correspondingly change the effect of SP stimulation on

pancreatic cancerPNI

We assessed the role of LOC389641 and TNFRSF10A in pancreatic cancer cell lines by overexpressing and silencing them and culturing with SP.As exhibited in Figure 6A, the expression of LOC389641 was significantly upregulated in the LOC389641+SP and downregulated in the si-LOC389641+SP groups (P<0.001). As the targeted gene of LOC389641, TNFRSF10A exhibited an opposed expression trend with LOC389641 (Figure 6A-C).

Then, we assessed the functions of overexpressed and silenced LOC389641 on pancreatic cancer cell proliferation, migration, and invasion. The cell viability and proliferation ability were significantly promoted by overexpressed LOC389641 and inhibited by silenced LOC389641 (P<0.05; Figure 6D, E). As observed in Figure 6F, the migration cells significantly increased in the LOC389641+SP group and decreased in the si-LOC389641+SP group (P<0.001). The wound-healing assay was consistent with the Transwell assay on cell migration analysis (Figure 6G). The cell invasion was significantly promoted by LOC389641+SP and inhibited by si-LOC389641+SP (P<0.001). Taken together, we found that both overexpression and interference with LOC389641 can correspondingly change the effect of SP stimulation on pancreatic cancer PNI, which might through altering the expression of TNFRSF10A.

4 Discussion

As the prominent characteristic of pancreatic cancer, PNI provides an alternative route for metastatic spread and pain generation. It is associated with the poor prognosis of patients with pancreatic cancer[17]. PNI is also identified in some other cancers, including head and neck cancer [18], cervical cancer [19], rectal cancer [20], and prostate cancer [21]. For example, Huang *et al*. [9] and Ayala *et al*. [22] demonstrated that PNI mechanisms involve active and reciprocal interactions between carcinoma cells and adjacent nerve/ganglions in prostate cancer progression using the prostate cancer/dorsal root ganglia *in vitro* system. However, thedeep studies on PNI and pancreatic cancer progression was still inadequate.In the present study, we further studied the role of SP/NK-1R, lncRNA LOC389641 on pancreatic cancer PNI. Through the experiments, we can draw the following conclusions through the present study: (1) The SP/NK-1R was positively regulated the proliferation, migration, and invasion of pancreatic cancer cells. (2) Overexpressed and silenced LOC389641 can correspondingly change the effect of SP stimulation pancreatic cancer cell lines through regulating TNFRSF10A(Figure 7). Our present study revealed the role of SP/NK-1R and LOC389641/TNFRSF10A on PNI of pancreatic cancer cells for the first time, which might provide potential biomarkers for pancreatic cancer therapy.

As one of the most common members of the tachykinins neuropeptides family, SP functions through the G protein-coupled receptors of NK-1R, -2R, and -3R[23].As reported, SP have the roles of neurogenic inflammation, wound healing, pain transmission, and vasodilatation. Through activating NK-1R, it can induce tumor-initiating intracellular responses in various cancers, includinglung cancer [24], retinoblastoma [25], glioblastomas [26], breast cancer [27] and pancreatic cancer[28], and is correlated with cancer developmentand progression.The SP/NK-1R can substantially promote the activation of NF-

κB, which positively regulates the transcriptions factors of favor tumorigenesis. Considering this, researchers have put numerous studies on the role of SP/NK-1R on pancreatic cancer. For example, Muñoz *et al.* [13] demonstrated that theSP/NK-1R system is involved in developing pancreatic cancer and might be a promising therapeutic target.The present study found that the progression of pancreatic cancer cell lines cultured with human neuroblastoma cell lines was significantly regulated by SP and NK-1R.Not only in pancreatic cancer, but it also played essential roles in some other cancers. For example, SP/NK-1R promotes gallbladder cancer cell proliferation and migration [29]. Dong *et al.*[30] demonstrated that SP/NK-1R activation elevates the growth and migration of esophageal squamous cell carcinoma cells. SP/NK-1R promotes the tumor development and progression of oral cancer [31]. Although the role of SP/NK-1R has been widely studied, the underlying mechanisms is still not fully elucidated. Some studies correlated the SP/NK-1R with chronic inflammation [32, 33], but it still lacks confirmation.

Not only promotes the cell progression of pancreatic cancer,SP/NK-1R also upregulated the expression of NF-κB p65 and p-AKT. As reported, the inhibition of the NK-1R/Akt/NF-κB signaling pathway could cancel the proliferative effect of SP on gallbladder cancer [29], suggesting a driven role of NK-1R on tumor growth through Akt/NF-κB. This study was consistent with that in our present study, in which SP significantly promoted the NK-1R/Akt/NF-κB signaling pathway. The Akt/NF-κB associated signaling pathways was promoted by mRNAs, microRNA (miRNAs), and lncRNAsin pancreatic cancers. Akt/NF-κB signaling pathway was promoted by CCN1 [34], miRNA-23b-3p[35], and was involved in the tumorigenicity of pancreatic cancer.In the present study, we found that NK-1R/Akt/NF-κB signaling system affecting PNImight be a novel strategy for pancreatic cancer therapeutics.

With the broad studies of lncRNAs, mounting lncRNAs were demonstrated to play an essential role in cancer progression. LOC389641 was reported to promote pancreatic ductal adenocarcinomaprogression and increase cell invasion by regulating E-cadherin with the possible involvement of TNFRSF10A [16]. In the present study, we found that the co-culture of LOC389641 with SP has no impact on pancreatic cancer PNI. However, overexpressed or silenced LOC389641 can correspondingly change the effect of SP stimulation on pancreatic cancer PNI through regulating TNFRSF10A. TNFRSF10A is one of the genes involved in the NF-κBsignaling pathway. TNFRSF10A encodes TRAILR1, one TNF-related apoptosis-inducing ligand receptor that can induce NF-κB activation [36].Jeong*et al.* [37] suggested that activated AKT regulates NF-κB activation. Therefore, we assumed that the pancreatic cancer PNI promoted by SP/NK-1R axis may be blocked by the TNFRSF10A/NF-κB pathway mediated by LOC389641.

5 Conclusion

In conclusion, we provide evidence that the SP stimulated the NK-1R/Akt/NF-κB signaling pathway in pancreatic cancer cell lines co-cultured with SH-SY5Y. Moreover, NK-1R or LOC389641 promoted pancreatic cancer cell progression. We assumed that the pancreatic cancer PNI promoted by SP/NK-1R axis may be blocked by the TNFRSF10A/NF-κB pathway mediated by LOC389641.

Declarations

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Figures

Figure 1

SP significantly promoted cell proliferation, migration, and invasion. (A) The concentration of SP was measured using an ELISA kit. (B, C) Growth curves and cell proliferation rates were analyzed by CCK-8 and EdU staining assays in BxPC-3 and MIAPaCa-2 cells cultured with SP and SH-SY5Y. (D, F) Transwell assays tested the effects of SP on cell migration and invasion. (E) The wound-healing analysis was performed to evaluate the migration rate regulated by SP. $*P<0.05, ***P<0.001$, vs. SH-SY5Y-Vector group.

Figure 2

SP significantly increased the expression levels of NK-1R, NF-κB p65, and p-AKT. (A-D) The expression level of NK-1R was assessed using qRT-PCR, western blot, and IF. (E) The protein expression level of NF-κB p65 was evaluated using HDAC1 as the reference. (F) The protein expression level of AKT and p-AKT were evaluated using GAPDH as the reference. $*P<0.05, ***P<0.001$, vs. SH-SY5Y-Vector group.

Figure 3

Selection of optimal SP concentration and treatment time. (A-D) The optimal SP concentration was selected in 0, 10, 25, 50, 100, and 150 nmol/L. (A) exhibited the gene expression level of NK-1R under the graded concentration of SP. (B-D) exhibited the protein expression levels of NK-1R, NF-κB p65, AKT, and p-AKT under graded SP. (E-H) The optimal SP treatment time was selected in 0h, 12h, 24h, 36h, and 48h. (E) exhibited the gene expression level of NK-1R under graded treatment times of SP. (E-H) exhibited the protein expression levels of NK-1R, NF-κB p65, AKT, and p-AKT under graded treatment times of SP. $*P<0.05.$ $***P<0.001$, vs. 0 nmol/Lgroup or 0 h group.

Figure 4

NK-1R positively regulated the proliferation, migration, and invasion of pancreatic cancer cells. (A, B) The gene and protein expression of NK-1R in Ctrl, NC+SP, NK-1R+SP, and siNK-1R+SP groups. (C, D) The protein expression of NF-κB p65, AKT, and p-AKT was assessed in groups. (E, F) Growth curves and cell proliferation rates were analyzed by CCK-8 and EdU staining assays in Ctrl, NC+SP, NK-1R+SP, and siNK-1R+SP groups. (G, H) Transwell and wound-healing assays evaluated the cell migration ability. (I) The cell invasion was assessed by Transwell assay.*P<0.05,***P<0.001 vs Ctrl. #P<0.05,###P<0.001 vs. NC+SP group.

Figure 5

The expression of lncRNA LOC389641 and mRNA TNFRSF10A was not affected by SP. (A) The expression level of lncRNA LOC389641 and mRNA TNFRSF10A was evaluated by RT-PCR. (B, C) The protein expression level of TNFRSF10A was assessed by western blot and IF.

Figure 6

Overexpressed LOC389641 promoted the proliferation, migration, and invasion of pancreatic cancer cells. (A) The expression level of LOC389641 and TNFRSF10A was evaluated by qRT-PCR in NC+SP, LOC389641+SP, and si-LOC389641+SP groups. (B, C) The protein expression level was assessed using western blot and IF. (D, E) Growth curves and cell proliferation rates were analyzed by CCK-8 and EdU staining assays in NC+SP, LOC389641+SP, and si-LOC389641+SP groups. (F, G) Transwell and wound-healing assays evaluated the cell migration ability. (H) The cell invasion was assessed by Transwell assay.*P<0.05,***P<0.001, vs.NC+SP group.

Figure 7

The mechanism diagram of SP/NK-1R axis and lncRNA LOC389641 in pancreatic cancer.

Supplementary Files

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- TableS1.docx