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Long noncoding RNA PCAT-1 accelerates the metastasis of pancreatic cancer by repressing RBM5

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Abstract. – OBJECTIVE: The role of long noncoding RNAs (lncRNAs) is vital in tumor progression. Our study aims to identify the role of PCAT-1 in the metastasis of pancreatic cancer.

PATIENTS AND METHODS: Real time-quantitative polymerase chain reaction (RT-qPCR) was used to measure PCAT-1 expression in 50 pancreatic cancer patients' tissues. Furthermore, to identify the function of PCAT-1 in pancreatic cancer *in vitro* wound healing assay and transwell assay were conducted. Besides, RT-qPCR and Western blot assay were performed to explore the underlying mechanism.

RESULTS: The expression level of PCAT[illegible] significantly upregulated in pancreatic cancer[illegible]ples compared with adjacent tissues. Moreo[illegible] cell migration and cell invasion were inhibited [illegible] knockdown of PCAT-1 in pancreati[illegible]ncer cells. Moreover, the mRNA and prote[illegible]ssion of RBM5 was upregulated via kno[illegible]wn o[illegible]AT-1 in pancreatic cancer cells. Furth[illegible]ore, the[illegible]M5 expression level was negative[illegible]ted t[illegible] expression level in pancr[illegible]tic [illegible]ssues.

CONCLUSIONS: Thi[illegible]study [illegible]ests that PCAT-1 acts as an o[illegible]ene in pa[illegible]tic cancer and promotes [illegible]tastasis via [illegible]biting RBM5, which mig[illegible]e a[illegible] therapeutic strategy in pancreatic [illegible]ancer.

Key Words:
Long no[illegible]ding R[illegible], PCAT-1, Pancreatic cancer, RBM5.

[illegible]ction

Pancr[illegible]ancer is the most aggressive and le[illegible] m[illegible]ldwide. Approximately 46,420 p[illegible] were newly diagnosed with pancreatic cancer, a[illegible]590 cases died of pancreatic cancer in America[illegible]014, which may be the second most common cause of cancer-related deaths by 2030[1,2]. Despite significant advances have been made in understandi[illegible]arcinogene[illegible]ncreatic cancer and new[illegible]tic treatmen[illegible]pancreatic cancer remain[illegible]uge[illegible] for the society. In 2005-2011, the 5-year survival[illegible]remained only 8%, which wa[illegible]4-5% higher t[illegible]hat in 1975-1989[3]. Most [illegible]reatic cancer patients died eventually because [illegible]cancer meta[illegible]sis. Therefore, it's urgent to understand the u[illegible]lying mechanism of pancreatic [illegible]r metasta[illegible]and to find out potential therapeu[illegible]

Defi[illegible]as endogenous cellular RNAs, long non-coding RNAs (lncRNAs) have been reported [illegible]an important role in tumorigenesis and [illegible]mor progression. For example, by regulating vasculogenic angiogenesis, lncRNA MALAT1 is reported to promote tumorigenicity and cell metastasis in gastric cancer[4]. Overexpression of lncRNA GAS5 inhibits the progression of non-small cell lung cancer through inhibiting miR-23a[5]. LncRNA LINC00092 acts as an important driver of metastatic progression in the progression of ovarian cancer[6]. Repression of lncRNA NEAT1 promotes the development of prostate cancer by disturbing the cell cycle and inhibiting the proliferation of prostate cancer cells[7]. Moreover, lncRNA NR_036575.1 acts as an oncogene in thyroid cancer by promoting cell proliferation and cell migration and could be applied as a potential biomarker and therapeutic target[8]. However, the clinical role and the biological mechanisms of PCAT-1 in the development of pancreatic cancer remain unexplored.

In our study, we found out that PCAT-1 expression level was remarkably higher in pancreatic cancer tissues. Moreover, downregulation of PCAT-1 inhibited cell migration and invasion in a pancreatic cancer cell *in vitro*. Moreover, our further experiment revealed that PCAT-1 functioned in pancreatic cancer development by regulating RBM5.

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Patients and Methods

Tissue Specimens and Cell Lines

50 pancreatic cancer patients received surgery at The First Hospital of Jilin University, and their tissue samples were enrolled for this research. Tissues got from the surgery were stored immediately at –80°C. Written informed consent was achieved from patients. This study was approved by the Ethics Committee of The First Hospital of Jilin University. Human pancreatic cancer cell lines BXPC3, CFPAC-1, Panc-1 and Capan-2 were got from the Institute of Biochemistry and Cell Biology, Chinese Academy of Science (Shanghai, China), and an immortalized pancreatic ductal epithelial cell line H6C7 was offered by Prof. Ming-sound Tsao (Ontario Cancer Institute, Toronto University, Toronto, Canada). Culture medium was Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) with 10% fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD, USA) and penicillin. Besides, the incubator for cell culture contained 5% CO_2 at 37°C.

Cell Transfection

Then shRNA (lentiviral small hairpin targeting PCAT-1 was cloned into the pcD 1 (GenePharma, Shanghai, China) and the e vector was taken as control. They were packag in 293T cells for following transfe f pancre atic cancer cells with Lipofecta (Invitrogen, Carlsbad, CA, USA). time antitative polymerase chain rea (RT- used to detect the transfe tio y.

RNA Extraction a RT-qPCR

The total RNA ated by usi TRIzol reagent (Invitroge arlsb A, USA). And then, the total RNA s reverse-t bed to cDNAs through reve Transcription K TaKaRa Biotechnology Ltd. lian, China). Following are the prim using RT-qPCR: PCAT-1 forward 5′-TGAG ATCT TGGAACC-3′, reverse 5′-GG TCTC CTGCTTTA-3′; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers forward GGGAGCCAAAAGGGTCAT-3′ and reverse 5′-GAGTCCTTCCACGATACCAA-3′. The cycle was as follows: 30 sec at 95°C, 35 sec for 40 cycles at 95°C, 35 sec at 60°C.

Western Blot Analysis

Reagent radioimmunoprecipitation assay (RIPA) (Beyotime, Shanghai, China) was utilized to extract protein from cells. Protein concentrations were quantified by bicinchoninic acid (BCA) protein assay kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was d to extract the target proteins which wer red to polyvinylidene difluoride (P F) mem s (Millipore, Billerica, MA, Cell Signa Technology provided us rabbit APDH, ra anti-RBM5, and goat a rabbit dary body (CST, Danvers, USA). Ima ware (NIH, Bethesda, M USA) s appli or the assessment of the in ex sion.

Transwell A y

5×10^4 t d cells w ormed to top chamber um pore s insert (Corning, Cornin NY,) added with 200 μL serum-free DMEM. inserts were previously co th 50 μg gel (BD Biosciences, Kin Lakes, NJ, USA). The bottom chamber s added wi DMEM containing FBS. These ls were cul d for 48 h. Then, the top sur- of chamb was wiped by cotton swab and im ecooling methanol for 20 min. Crysta t was used for staining of the inserts.

ical Analysis

Statistical Product and Service Solutions (SPSS) 18.0 (PASW Statistics for Windows, Chicago, IL, USA) was performed for the statistical analysis. In this study, the Student *t*-test was used. The statistical significance was defined as $p<0.05$.

Results

PCAT-1 Expression Level in Pancreatic Cancer Tissues

RT-qPCR was conducted for detecting PCAT-1 expression in 50 patients' tissues. Results showed that PCAT-1 was significantly upregulated in tumor tissue samples when compared with adjacent tissues (Figure 1).

PCAT-1 Expression Level in Pancreatic Cancer Cells

Then PCAT-1 expression was detected in pancreatic cancer cells by RT-qPCR. The results showed that PCAT-1 expression level of pancreatic cancer cells was higher than that of H6C7 (Figure 2A). According to the expression level in the cells, we chose CFPAC-1 and Panc-1 cell lines for shRNA transfection. The transfection efficiency was detected by RT-qPCR (Figure 2B).

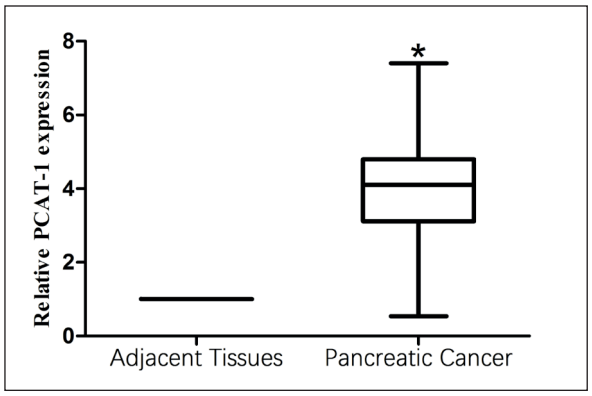

Figure 1. Expression levels of PCAT-1 in pancreatic cancer tissues. PCAT-1 expression was significantly increased in the pancreatic cancer tissues compared with adjacent tissues. Data are presented as the mean ± standard error of the mean. *p<0.05.

Knockdown of PCAT-1 Inhibited Migrated Ability in Pancreatic Cancer Cells

The results of wound healing assay revealed that knockdown of PCAT-1 reduced the migrated distance of CFPAC-1 pancreatic cancer cells (Figure 3A). Besides, the knockdown of PCAT-1 reduced the migrated distance of Panc-1 pancreatic cancer cells (Figure 3B).

Knockdown of PCAT-1 Inhibited Invaded Ability of Pancreatic Cancer Cells

The outcome of the transwell assay also revealed that PCAT-1 was knocked down in CFPAC-1 pancreatic cancer cells, the number of invaded cells was remarkably decreased in CFPAC-1 pancreatic cancer cells (Figure 4A). Moreover, the number of invaded cells was remarkably decreased via knockdown of PCAT-1 in Panc-1 pancreatic cancer cells (Figure 4B).

The Association Between RBM5 and PCAT-1 in PC3 Pancreatic Cancer Tissues and Cells

Our further experiments revealed that RBM5 was remarkably lowexpressed in pancreatic cancer tissues compared with adjacent tissues (Figure 5A). The correlation analysis revealed that the negative association was found between RBM5 and PCAT-1 expression in pancreatic cancer tissues (Figure 5B). Besides, the RT-qPCR results showed that RBM5 was upregulated in PCAT-1 shRNA group compared with the empty vector group *in vitro* (Figure 5C). Western blot assay showed that the protein level of RBM5 could be upregulated by knocking down PCAT-1 (Figure 5D).

Discussion

Numerous researches suggest that lncRNAs are critical regulators in the progression of pancreatic cancer. For instance, downregulation of lncRNA HOST2 represses cell proliferation and promotes cell apoptosis in pancreatic cancer, which may offer a potential therapeutic target for pancreatic cancer[9]. Regulated by ALKBH5, lncRNA KCNK15-AS1 inhibits cell migration and cell motility in pancreatic cancer[10]. Through

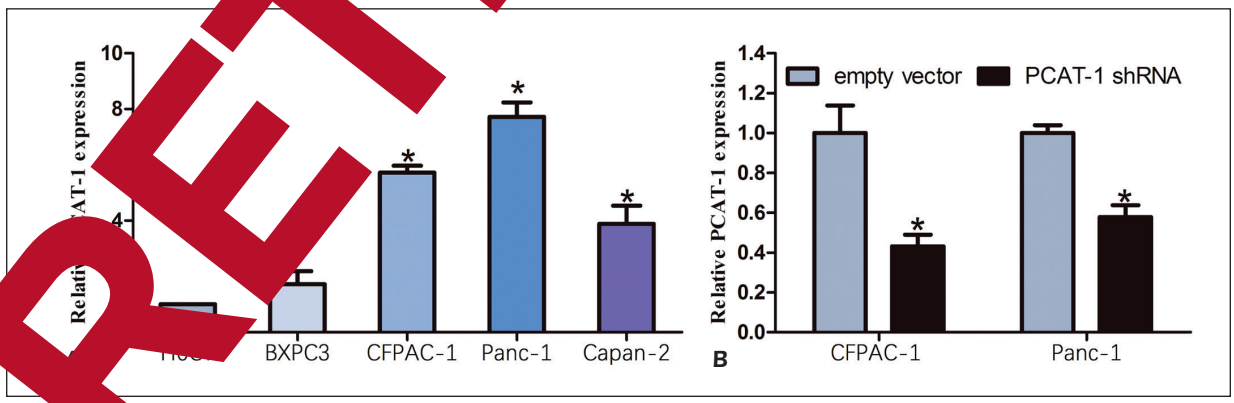

Figure ression levels of PCAT-1 in pancreatic cancer cell lines. ***A,*** RT-qPCR results showed that expression levels of PCAT-1 were higher in the human pancreatic cancer cell lines compared with H6C7 (immortalized pancreatic ductal epithelial cell line). ***B,*** The PCAT-1 expression of CFPAC-1 and Panc-1 pancreatic cancer cells was lower in PCAT-1 shRNA group than that in empty vector group. GAPDH was used as an internal control. Data are presented as the mean ± standard error of the mean. *p<0.05.

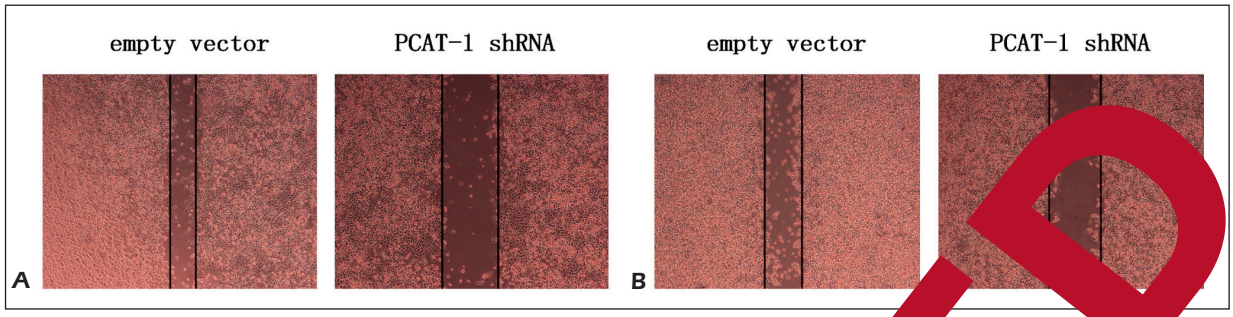

Figure 3. Knockdown of PCAT-1 inhibited pancreatic cancer cell migration. ***A,*** Wound h[illegible]g assay showed that k[illegible]ckdown of PCAT-1 significantly decreased cell migrated distance of CFPAC-1 pancreatic cancer[illegible] ***B,*** W[illegible] healing assay showed that knockdown of PCAT-1 significantly decreased cell migrated distance of Panc-1 pan[illegible] cells.

targeting miR-221/SOCS3, lncRNA GAS5 suppresses cell proliferation, cell metastasis and gemcitabine resistance in pancreatic cancer[11]. LncRNA H19 promotes cell proliferation and cell migration in pancreatic cancer which is modulated by miR-194[12]. In addition, lncRNA MEG8 enhances the epigenetic induction of the epithelial-mesenchymal transition in pancreatic cancer cells[13].

Some evidence has proved that prostate [illegible]ancer-associated ncRNA transcripts 1 (PC[illegible] firstly discovered in prostate cancer, p[illegible]ipates in the progression of several canc[illegible] For instance, PCAT-1 plays an important r[illegible] in tumorigenesis of hepatocellular[illegible]inoma b[illegible] modulating TP53-miR-215-PCA[illegible] axis[15]. Upregulation of PCAT-1 pro[illegible]s me[illegible]asis of osteosarcoma by interacti[illegible]th EZ[illegible] pressing p21 expression[16]. PC[illegible]nces cell proliferation and invad[illegible]he ab[illegible]f cervical cancer which is ass[illegible]ed with po[illegible]gnosis of patients[17]. In add[illegible]regulation o[illegible]CAT-1 promotes the dev[illegible]pmen[illegible]ladder cancer and may be a pot[illegible]tial bioman[illegible]d therapeutic target[18]. Ou[illegible]udy revealed th[illegible]PCAT-1 was upregulat[illegible]oth pancrea[illegible] cancer samples and ce[illegible] Bes[illegible]after PCAT-1 was knocked down, pancreatic c[illegible] cell migration and invasio[illegible]cities were [illegible]d to be inhibited. The [illegible]ts above indicated that PCAT-1 promoted [illegible]tastasis of p[illegible]creatic cancer and might act as [illegible]oncogene.

[illegible]cated on[illegible] cancer inhibitor region 3p21.3, R[illegible]notif 5 (RBM5) participates in the pro[illegible]ion of several carcinomas[19]. For example, the expression level of RBM5 is signifi[illegible]lownregulated in lung adenocarcinoma [illegible]d [illegible] be used as a diagnostic marker for these patients[20,21]. RBM5 functions as a tumor suppressor gene in the progression of gastric cancer by enhancing the activity of p53 transcription[22]. RBM5 is significantly down-expressed in pancreatic ductal adenocarcinoma which is related to the clinicopathological characteristic of the patients[23]. In addition, RBM5 depresses tumorigenesis of gliomas by inhibiting Wnt/beta-catenin signaling and inducing cell apoptosis[24]. Our further experiments identified that RBM5 expression could be upregulated by knockdown of PCAT-1. Moreover, RBM5 expression in pancreatic cancer tissues

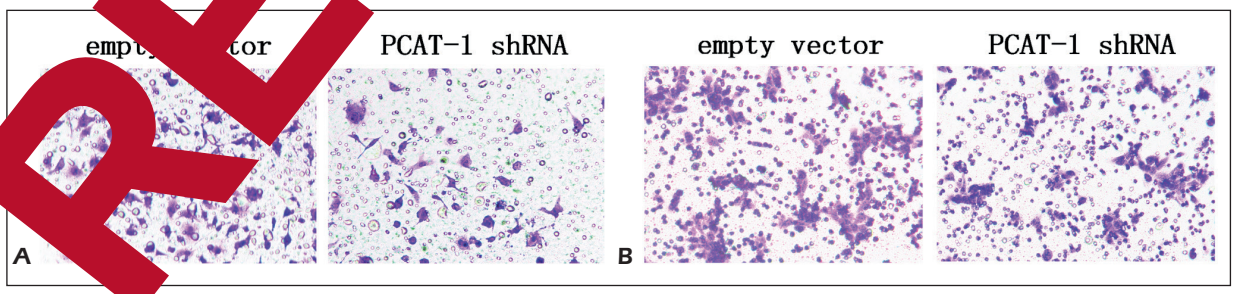

Figure 4. Knockdown of PCAT-1 inhibited pancreatic cancer cell invasion. ***A,*** Transwell assay showed that number of invaded cells was significantly decreased via knockdown of PCAT-1 in CFPAC-1 pancreatic cancer cells (40×). ***B,*** Transwell assay showed that number of invaded cells was significantly decreased via knockdown of PCAT-1 in Panc-1 pancreatic cancer cells (40×).

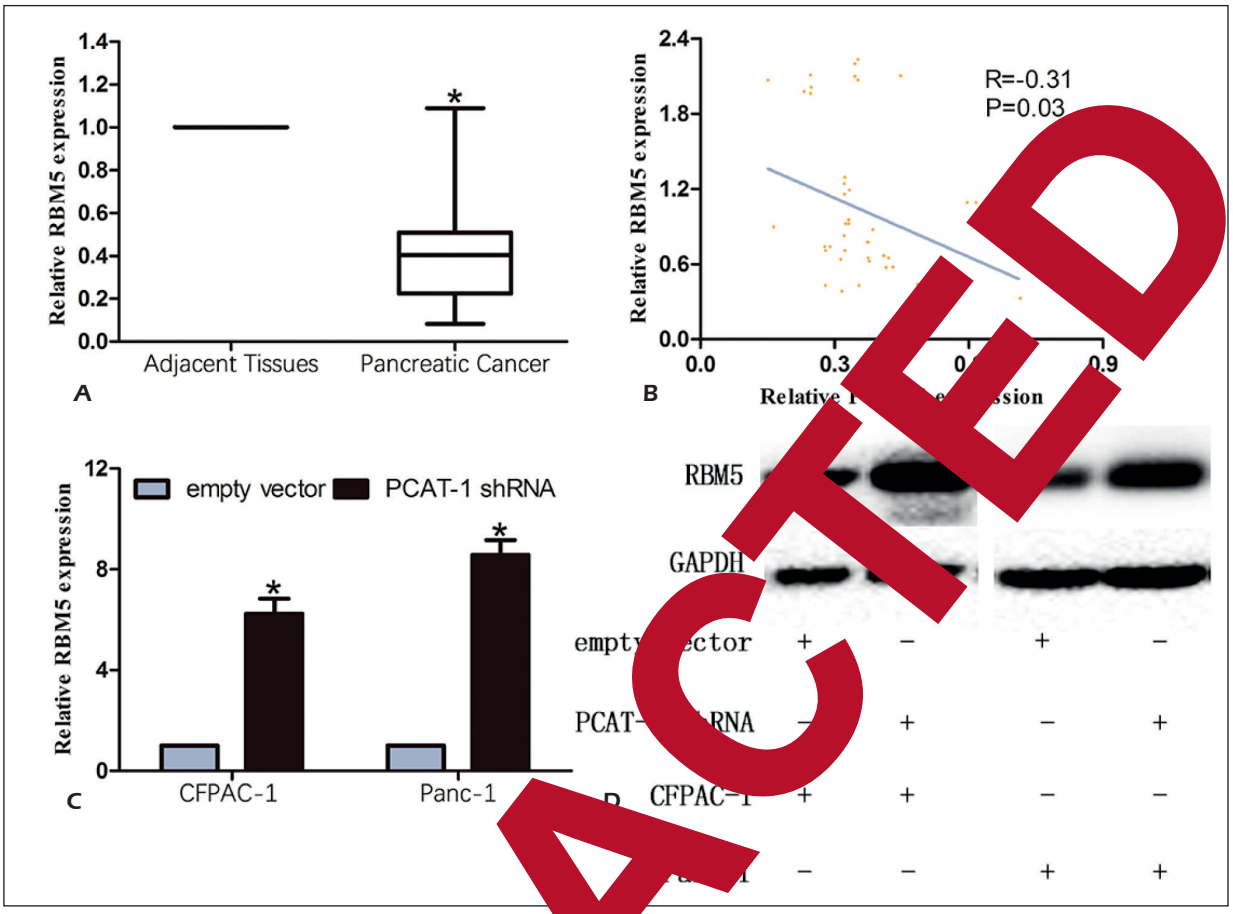

Figure 5. The association between PCAT-1 and RBM5 in pancreatic cancer tissues and cells. ***A,*** RBM5 was significantly downregulated in pancreatic cancer tissues compared with adjacent tissues. ***B,*** The negative correlation between the expression level of RBM5 and PCAT-1 in pancreatic cancer tissues. ***C,*** qPCR results showed that RBM5 expression was higher in PCAT-1 shRNA compared with the empty vector in pancreatic cancer cells. ***D,*** Western blot assay revealed that RBM5 protein expression of pancreatic cancer cells was increased by PCAT-1 shRNA compared with empty vector. The results represent the average of three independent experiments. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

was negatively related with PCAT-1 expression. All the results above suggested that PCAT-1 might promote tumorigenesis of pancreatic cancer via suppressing RBM5.

Conclusions

In PC, PCAT-1 is remarkably upregulated and PCAT-1 could enhance cell invasion and migration in pancreatic cancer by repressing RBM5 expression. Suggesting that PCAT-1 may serve as a candidate target that contributes to therapy for pancreatic cancer.

Conflict of Interests

The Authors declared that they have no conflict of interests.

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