



Original Article

Biological functions of PCAT-1/ SOX4 in cell proliferation and invasion of breast cancer



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Article Info

Abstract



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Breast cancer (BC) is one of the most common fatal cancers. Recent studies have identified the vital role of long noncoding RNA (lncRNAs) in the development and progression of BC. In this research, lncRNA PCAT-1 was studied to identify how it functioned in the metastasis of BC. PCAT-1 expression of tissues was detected by real-time quantitative polymerase chain reaction (RT-qPCR) in 50 BC patients. Cell proliferation, wound healing assay and transwell assay were used to observe the biological behavior changes of BC cells through knockdown or overexpression of PCAT-1. In addition, RT-qPCR and Western blot assay were performed to discover the potential target protein of PCAT-1 in BC. PCAT-1 expression level in BC samples was higher than that of adjacent ones. Besides, cell proliferation, migrated ability and cell invaded ability of BC cells were inhibited after PCAT-1 was silenced. Cell proliferation, migration and invasion of BC cells were promoted after PCAT-1 was overexpressed. In addition, SOX4 was downregulated after silence of PCAT-1 in BC cells, while SOX4 was upregulated after overexpression of PCAT-1 in BC cells. Furthermore, SOX4 was upregulated in BC tissues and was positively associated with PCAT-1. Our study uncovers a new oncogene in BC and suggests that PCAT-1 could enhance BC cell proliferation, migration and invasion via targeting SOX4, which provided a novel therapeutic target for BC patients.

Keywords: Long noncoding RNA, PCAT-1, BC, SOX4.

1. Introduction

Despite the tremendous progress in diagnosis and therapy of breast cancer (BC), BC remains a major health problem in the world. BC is the most common cancer affecting women worldwide, and the morbidity and mortality of BC are expected to increase significantly in the next few years [1]. Metastasis of BC affects the prognosis of BC which is the main cause of cancer-related death in BC patients. 30% of BC patients were diagnosed at early stage would inevitably relapse and metastasize after adjuvant treatment [2]. The mechanism of local invasion and metastasis of malignant tumors has not been fully understood, but it is known that it is a complex process consisting of a series of steps. MicroRNA (microRNA) and long non-coding RNA (lncRNA) are potential therapeutic targets.

More and more evidences have revealed the abnormal expression and function of lncRNA, which can influence the development of various malignant tumors, and directly participate in the proliferation, apoptosis, metastasis, immune regulation and drug resistance in tumors. lncRNA is involved in the regulation of various cellular functions, and its disorders are usually associated with human diseases, including cancer, such as lncRNA NEAT1, ROR, LINC00672, HOTAIR and so on [3-6].

Numerous reports have indicated that lncRNAs are closely related to the generation and development of BC. It

has been reported that regulated by p53 and EGFR signal pathway, lncRNA LINP1 promotes repair of DNA double-strand breaks and raises the sensitivity of cancer cells to radiotherapy in BC [7]. Shi et al. [8] reported that the proliferation and metastasis of BC cells could be inhibited by upregulation of lncRNA PTENP1 in BC Through suppression of miR-19b expression. Li et al. [9] found out that lncRNA LOXL1-AS1 inhibits cell proliferation, migration and invasion as well as induced apoptosis in BC via regulating miR-143-3p.

PCAT-1 is a newly identified cancer-related lncRNA located on chromosome 8. Many studies have shown that PCAT-1 plays an important role in the occurrence and development of many tumors. The purpose of this study was to investigate the expression of lncRNA PCAT-1 in BC and its effect on malignant biological behavior of cancer cells.

However, the clinical role and underlying mechanisms of PCAT-1 in the development of BC remain unexplored. In the present study, we performed function and mechanism assays to explore whether PCAT-1 functioned in the proliferation and metastasis of BC.

2. Materials and methods

2.1. Patients and clinical samples

A total of 50 BC patients were enrolled for human tis-

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sues who received surgery at our center. Before operation, written informed consent was achieved. No radiotherapy or chemotherapy for any patients before the operation. Tissues from the surgery were stored immediately at -80°C .

2.2. Cell culture

Human BC cells (MCF-7, LCC9, T-47D, SKBR3) and normal human breast cell line (MCF-10A) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The culture medium consisted of 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), Dulbecco's Modified Eagle Medium (DMEM) as well as 100 U/mL penicillin/streptomycin (Sigma, St. Louis, MO, USA). Besides, cells were cultured in an incubator containing 5% CO_2 at 37°C .

2.3. Cell transfection

Specific short-hairpin RNA (shRNA; BiosettiaInc., San Diego, CA, USA) against PCAT-1 was synthesized. Negative control shRNA was also synthesized. PCAT-1 shRNA (sh-PCAT-1) and negative control (control) were then used for transfection in LCC9 BC cells. 48 h later, real-time quantitative polymerase chain reaction (RT-qPCR) was used to detect transfection efficiency in these cells. Besides, lentivirus (BiosettiaInc., San Diego, CA, USA) against PCAT-1 (PCAT-1) was synthesized and then used for transfection in SKBR3 BC cells. Empty vector was used as control. 48 h later, RT-qPCR was used to detect transfection efficiency in these cells.

2.4. RNA extraction and RT-qPCR

The total RNA was separated by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), which was reverse-transcribed to cDNAs through reverse Transcription Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). Thermocycling conditions were as follows: pre-denaturation at 95°C for 5 min, denaturation at 95°C for 10 s, and annealing at 60°C for 30 s, a total of 35 cycles. Following are the primers used for RT-qPCR: PCAT1 primers forward 5'-TGAGAAGAGAAATCTATTGGAACC-3', reverse 5'-GGTTTGTC-TCCGCTGCTTTA-3'; β -actin, forward 5'-CCAACCGCGAGAAGATGA-3' and reverse 5'-CCA-GAGGCGTACAGGGATAG-3'.

2.5. Cell proliferation assay

Before transfection, cells (1000 cells/well) were seeded into 96-well plate for 12 h. After they were cultured at different times (0, 24, 48, and 72 h), 15 μL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was added to each well and incubated for 4 h. To stop the reaction, they were added with 100 μL dimethyl sulfoxide. Absorbance at 490 nm was assessed using an enzyme-linked immunosorbent assay (ELISA) reader system (Multiskan Ascent, LabSystems, Helsinki, Finland).

2.6. Wound healing assay

1.0×10^4 cells were seeded into a 6-well plate with cells in each well. Three parallel lines were made on the back of each well. After growing to about confluent of 90%, cells were scratched with a pipette tip and cultured in a medium. Cells were photographed under a light microscope after 0 and 48 h. Each assay was independently repeated in triplicate.

2.7. Transwell assay

24 h after transfection, 2×10^5 cells in 100 μL serum-free DMEM were transformed to top chamber of an 8- μm culture insert (Corning, Corning, NY, USA) coated with 50 μg Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). 20% FBS-DMEM was added to the lower chamber of the culture inserts. 24 h later, these inserts were treated by methanol for 30 min and stained with hematoxylin for 20 min. An inverted microscope ($\times 40$) was utilized for counting invaded cells in three random fields.

2.8. Western blot analysis

Reagent radioimmunoprecipitation assay (RIPA) (Beyotime, Shanghai, China) was utilized to extract protein from cells. Bicinchoninic acid (BCA) protein assay kit (TaKaRa Biotechnology Co., Ltd., Dalian, China) was chosen for quantifying protein concentrations. The target proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Then they were incubated with antibodies after being replaced with the polyvinylidene fluoride (PVDF) membrane (Roche, Basel, Switzerland). Cell Signaling Technology (CST, Danvers, MA, USA) provided us with rabbit anti- β -actin and rabbit anti-SOX4, as well as goat anti-rabbit secondary antibodies. Image J software (NIH, Bethesda, MD, USA) was applied for assessment of protein expression.

2.9. Statistical analysis

All statistical analyses were performed by GraphPad Prism 5.0 (La Jolla, CA, USA). The differences between two groups were compared by independent-sample t-test. The statistical significance was defined as $P < 0.05$.

3. Results

3.1. PCAT-1 expression level in BC tissues and cells

To determine the biological function of PCAT-1 in the tumorigenesis of triple-negative breast cancer, we detected PCAT-1 expression levels in 50 paired BC specimens by RT-qPCR. PCAT-1 was significantly upregulated in BC tissue samples compared with adjacent tissues (Figure 1A). PCAT-1 expression was also detected via RT-qPCR in four BC cell lines. PCAT-1 expression level in BC cells was higher than that of MCF-10A (Figure 1B).

3.2. Knockdown of PCAT-1 suppressed cell proliferation, migration and invasion in BC cells

To further investigate whether PCAT-1 is connected to the proliferation and metastasis of BC, we researched the

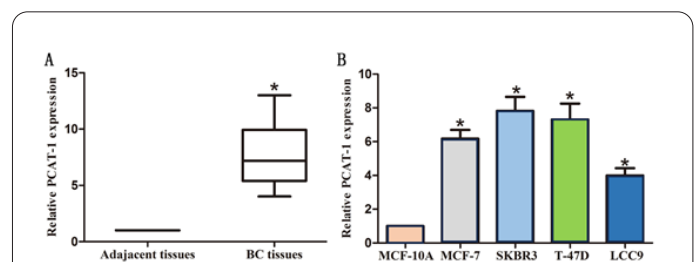


Fig. 1. Expression level of PCAT-1 was increased in BC tissues and cell lines. (A) PCAT-1 expression was significantly increased in the BC tissues compared with adjacent tissues. (B) Expression levels of PCAT-1 relative to β -actin were determined in the human BC cell lines and MCF-10A (normal human breast cell line) by RT-qPCR. Data are presented as the mean \pm standard error of the mean. * $P < 0.05$.

function of PCAT-1 in vitro. In this study, we chose SKBR3 BC cell line for the silence of PCAT-1. Then PCAT-1 expression was detected by RT-qPCR (Figure 2A). In this research, cell proliferation was examined via MTT assay after the knockdown of PCAT-1 in the SKBR3 cells. The results revealed that the cell growth ability was considerably reduced after the knockdown of PCAT-1 (Figure 2B). Moreover, the ability of cell migration was examined via wound healing assay after the knockdown of PCAT-1 in the SKBR3 cells. The results revealed that the rate of SKBR3 cell migration was considerably reduced after the knockdown of PCAT-1 (Figure 2C). The outcome of transwell assay also revealed that the number of invaded cells was remarkably decreased after PCAT-1 was silenced in BC cells (Figure 2D).

3.3. Overexpression of PCAT-1 promoted cell proliferation, migration and invasion in BC cells

LCC9 BC cell line was chosen for overexpression of PCAT-1. Then PCAT-1 expression was detected by RT-qPCR (Figure 3A). The results of MTT assay showed that overexpression of PCAT-1 significantly promoted cell growth ability of BC cells (Figure 3B). Moreover, results of wound healing assay showed that overexpression of PCAT-1 significantly promoted the ability of cell migration in BC cells (Figure 3C). The outcome of transwell assay also revealed that the number of invaded cells was remarkably increased after PCAT-1 was overexpressed in BC cells (Figure 3D).

3.4. The interaction between SOX4 and PCAT-1 in BC

Starbase v2.0 (<http://starbase.sysu.edu.cn/starbase2/rb-plncRNA.php>) was used to find target proteins of PCAT-1. RT-qPCR results showed that SOX4 was downregulated in sh-PCAT-1 group compared with control group (Figure 4A). Meanwhile, SOX4 was upregulated in PCAT-1 group

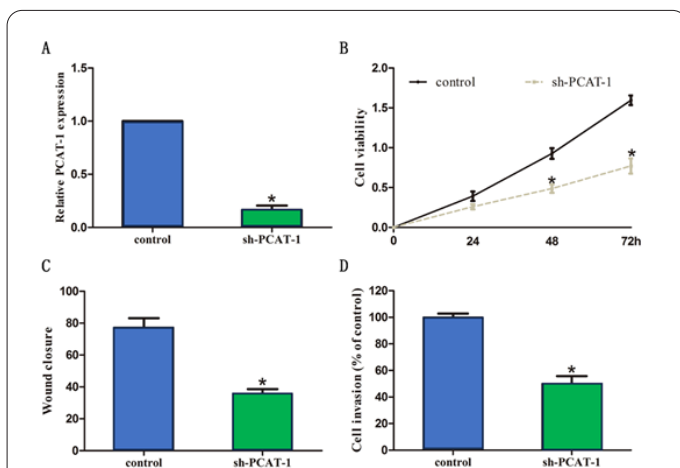


Fig. 2. Knockdown of PCAT-1 inhibited BC cell proliferation, migration and invasion. (A) PCAT-1 expression in BC cells transduced with PCAT-1 shRNA (sh-PCAT-1) and the negative control (control) was detected by RT-qPCR. β -actin was used as an internal control. (B) MTT assay showed that knockdown of PCAT-1 significantly repressed cell proliferation in BC cells. (C) Wound healing assay showed that silence of PCAT-1 significantly repressed cell migrated ability of BC cells (magnification: 40 \times). (D) Transwell assay showed that number of invaded cells was significantly decreased via silence of PCAT-1 in BC cells (magnification: 40 \times). The results represent the average of three independent experiments (mean \pm standard error of the mean). * $P < 0.05$.

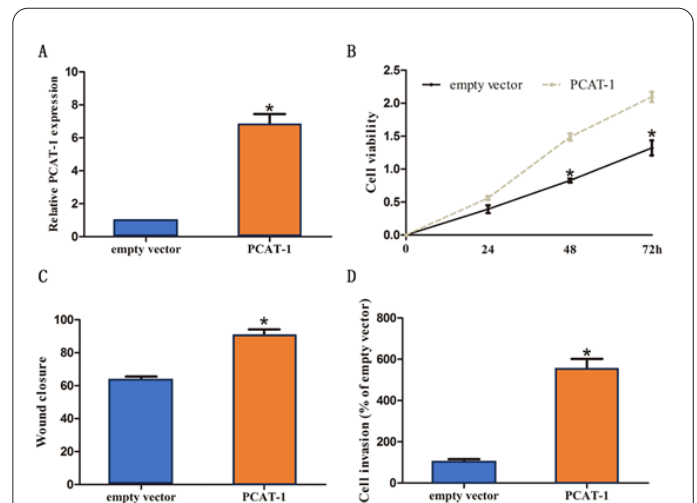


Fig. 3. Overexpression of PCAT-1 promoted BC cell proliferation, migration and invasion. (A) PCAT-1 expression in BC cells transduced with PCAT-1 lentivirus (PCAT-1) and the empty vector was detected by RT-qPCR. β -actin was used as an internal control. (B) MTT assay showed that knockdown of PCAT-1 significantly repressed cell proliferation in BC cells. (C) Wound healing assay showed that overexpression of PCAT-1 significantly promoted cell migration ability of BC cells (magnification: 40 \times). (D) Transwell assay showed that number of invaded cells was significantly increased via overexpression of PCAT-1 in BC cells (magnification: 40 \times). The results represent the average of three independent experiments (mean \pm standard error of the mean). * $P < 0.05$.

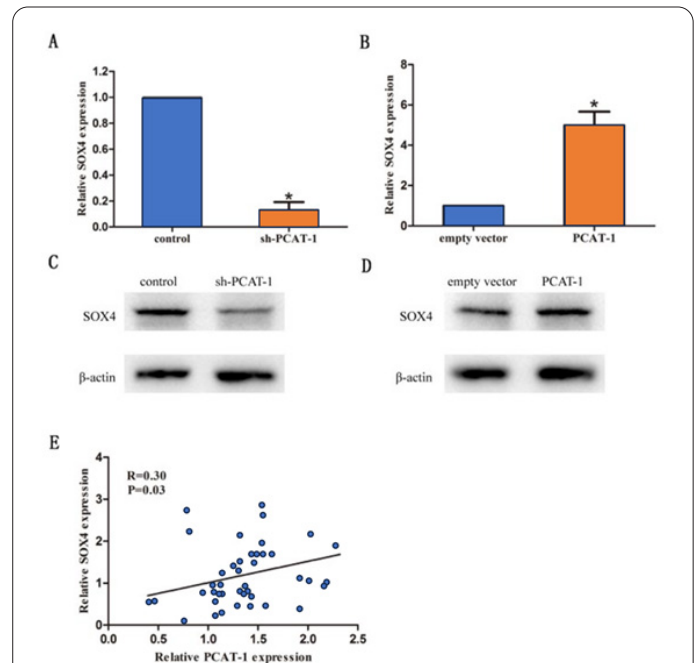


Fig. 4. Association between PCAT-1 and SOX4 in BC cells and tissues. (A) RT-qPCR results showed that SOX4 expression was lower in PCAT-1 shRNA (sh-PCAT-1) group compared with the negative control (control) group. (B) RT-qPCR results showed that SOX4 expression was higher in PCAT-1 lentivirus (PCAT-1) and the empty vector group. (C) Western blot assay revealed that SOX4 protein expression was decreased in PCAT-1 shRNA (sh-PCAT-1) group compared with the negative control (control) group. (D) Western blot assay revealed that SOX4 protein expression was increased in PCAT-1 lentivirus (PCAT-1) and the empty vector group. (E) The linear correlation between the expression level of SOX4 and PCAT-1 in BC tissues. The results represent the average of three independent experiments. Data are presented as the mean \pm standard error of the mean. * $P < 0.05$.

compared with empty vector group (Figure 4B). Western blot assay results showed that SOX4 was downregulated in sh-PCAT-1 group compared with control group (Figure 4C). Meanwhile, SOX4 was upregulated in PCAT-1 group compared with empty vector group (Figure 4D). In addition, correlation analysis demonstrated that SOX4 expression level positively correlated to PCAT-1 expression in BC tissues (Figure 4E).

4. Discussion

The progression of BC is a complex process consisting of a series of steps. The mechanisms of cell proliferation and cell invasion in BC have not been fully understood yet. However, it is known that lncRNA is a potential therapeutic target.

lncRNA is a kind of RNA whose transcript length exceeds 200 nt and does not encode proteins. Initially, this kind of RNA was considered as the "noise" of genomic transcription. With the discovery of HOTAIR function in 2017, the function of lncRNA has gradually become clear. lncRNA is often associated with invasion, metastasis and cell growth. Abnormal expression and functional changes of lncRNA can significantly affect the occurrence and development of various malignant tumors [10,11].

lncRNA can regulate the transcription of target genes, interfere with the splicing of RNA, inhibit the activity of RNA polymerase, and mediate the remodeling of chromatin. lncRNA can participate in the biological processes of embryonic development, cell cycle control and carcinogenesis. In tumors, the expression of lncRNA is closely related to the development of tumors. Upregulation of oncogenic lncRNA promotes the proliferation and metastasis of cancer cells [12].

PCAT-1 has been found in prostate cancer for the first time. It has been confirmed that the expression level of PCAT-1 is closely related to the occurrence of prostate cancer [13]. As a "molecular sponge" of microRNA-145-5p, PCAT-1 regulates the inhibitory effect of microRNA-145-5p on FACN1 by "absorbing" microRNA-145-5p, thereby promoting the proliferation, migration and invasion of prostate cancer and inhibiting the apoptosis of prostate cancer cells [14]. In hepatocellular carcinoma, the expression of PCAT-1 was significantly correlated with TNM grade, metastasis and histological grade of hepatocellular carcinoma. PCAT-1 can relieve the inhibitory effect of microRNA-129-5p on HMGB1 by inhibiting the expression of microRNA-129-5p, thus promoting the invasion and metastasis of hepatocellular carcinoma cells [15]. In our study, we first confirmed that PCAT-1 is abnormally expressed in BC specimens. Besides, knockdown of PCAT-1 inhibited cell proliferation, migration and invasion in BC cells, while overexpression of PCAT-1 promoted cell proliferation, migration and invasion in BC cells. The above results indicated that PCAT-1 promoted proliferation and metastasis of BC and might act as an oncogene.

To further identify the underlying mechanism of how PCAT-1 affects BC cell proliferation and invasion, we predicted and picked SOX4 as the potential target protein of PCAT-1 by using bioinformatic analysis and experimental verification. SOX4 is a member of the C subgroup of SOX transcription factor family. It is a key regulator of epithelial-mesenchymal transition (EMT) related to metastasis and invasion of tumors by binding specific protein molecules and 5'-(A/T) CAA (A/T) G-3' sequence in

DNA to participate in cell regulation, embryonic development. Studies have shown that SOX4 is highly expressed in endometrial cancer, gallbladder cancer, prostate cancer and other tumors. Silencing SOX4 can inhibit the metastasis and growth of melanoma and other tumors. Previous researches have indicated that SOX4 plays a promotive role in tumorigenesis [16-18]. In addition, Zhang et al. [19] found that SOX4 was highly expressed in BC tissues, and the expression level is correlated with lymph node metastasis. In the present study, SOX4 expression could be downregulated after knockdown of PCAT-1. Meanwhile, SOX4 expression could be upregulated after overexpression of PCAT-1. Moreover, SOX4 expression level positively correlated to PCAT-1 expression in BC tissues. All the results above suggest that PCAT-1 might promote proliferation and metastasis of BC *via* upregulating SOX4.

5. Conclusions

The above data identified that PCAT-1 was remarkably upregulated in BC patients. Besides, PCAT-1 could promote cell proliferation, migration and invasion in BC through upregulating SOX4. These findings suggest that PCAT-1 may contribute to therapy for BC as a candidate target.

Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

This study was approved by the ethics committee of The Second Hospital of Nanjing.

Informed consent

Signed written informed consents were obtained from the patients and/or guardians.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request

Authors' contributions

Tao Huang and Jizong Zhang designed the study and performed the experiments, Tao Huang collected the data, Jizong Zhang analyzed the data, Tao Huang and Jizong Zhang prepared the manuscript. All authors read and approved the final manuscript

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Non

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