Original Article

Long non-coding RNA BANCR promotes pancreatic ductal adenocarcinoma cell growth and metastasis via affecting epithelial-mesenchymal transition

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Abstract: Background: Long non-coding RNAs (lncRNA) have been shown to play critical roles in the development and progression of cancer. Recent studies reported that lncRNA BANCR was associated with proliferation and metastasis for melanoma and lung cancer. However, the role of BANCR in pancreatic ductal adenocarcinoma (PDAC) remains unclear. Methods: Quantitative real-time PCR (qRT-PCR) was used to detect the expression levels of BANCR in PDAC tissues. RNA interference (RNAi) was used to investigate the biological functions of BANCR. The effect of BANCR on proliferation and metastasis were evaluated by CCK-8 and Transwell assays. Expression of BANCR targets were determined by qRT-PCR and western blot. Results: Our results showed that BANCR was markedly upregulated in PDAC tissues relative to adjacent non-tumor tissues. Clinicopathologic analysis revealed that high BANCR expression was correlated with lymphnode metastasis, advanced tumor stage, and shorter overall survival of PDAC. Multivariate regression analysis suggested that BANCR overexpression could act as an independent unfavorable prognostic factor. Additionally, decreased expression of BANCR significantly suppressed the PDAC cell proliferation and metastasis in vitro via affecting epithelial-mesenchymal transition. Conclusions: These results demonstrated that high expression of lncRNA BANCR is involved in progression of PDAC and may represent a novel therapeutic target for the treatment of PDAC patients.

Keywords: Long non-coding RNA, BANCR, pancreatic ductal adenocarcinoma, proliferation, migration, invasion, epithelial-mesenchymal transition

Introduction

Pancreatic cancer is the fourth leading cause of cancer deaths among men and women and is responsible for 6% of all cancer-related deaths [1]. The most common type of pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC), an aggressive and highly invasive tumor type [2]. Despite advances in surgical and medical therapies, PDAC remains one of the most aggressive tumors with an overall cumulative 5-year survival rate <5% [3]. The high mortality rate is primarily due to the high frequency of metastatic disease; over 80% of patients diagnosed with PDAC present too late for curative treatment due to metastasis [4]. Therefore, it is of great importance to understand the underlying biological mechanism to achieve early detection and effective treatment of pancreatic cancer.

Genome-wide transcriptional studies found that only approximately 1% of the human genome serves as blueprints for proteins, whereas a much larger proportion of the genome is transcribed into non-coding RNAs [5]. Among these non-coding RNAs are long non-coding RNAs (lncRNAs) which are more than 200 nucleotides in length with little protein-coding potential [6]. In recent years, several lncRNAs have been shown to be involved in tumor progression. For example, Zhang et al showed that upregulation of lncRNA MALT1 correlated with tumor progression and poor prognosis in clear cell renal cell carcinoma [7]. Han et al indicated that low expression of lncRNA PANDAR could predicted a poor prognosis of non-small cell lung cancer and affected cell apoptosis by regulating Bcl-2 [8]. Wang et al found that lncRNA AOC4P suppressed hepatocellular carcinoma metastasis by
BANCR expression in PDAC

enhancing vimentin degradation and inhibiting epithelial-mesenchymal transition [9].

BRAF-activated non-coding RNA (BANCR), a 693-bp IncRNA, was firstly identified by Flockhart RJ et al through massively parallel complementary DNA (cDNA) sequencing screen for transcripts affected by the oncogene BRAFV600E expression [10]. Recent studies suggested that the dysregulation of IncRNA BANCR play critical roles in tumor progression. For example, Wang et al showed that IncRNA BANCR expression was upregulated in papillary thyroid carcinoma and promoted cell proliferation and activated autophagy [11]. Su et al found that IncRNA BANCR promoted cell growth and metastasis and associated with poor prognosis in retinoblastoma [12]. However, Jiang et al revealed that IncRNA BANCR levels were downregulated in lung cancer and decreased expression of BANCR promoted cell proliferation and migration via MAPK pathways [13]. Shi et al found that IncRNA BANCR expression was significantly downregulated in colorectal cancer and downregulated BANCR expression promoted cell proliferation via downregulated expression of p21 [14]. These observations suggested that BANCR may serve as important regulators in tumorigenesis. Thus, the aim of this study was to explore the function of IncRNA BANCR in PDAC progression.

Materials and methods

Tissue specimens

A total of 49 paired PDAC tissues and their adjacent non-tumor tissues were obtained from the patients who underwent surgery at the The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology between 2013 and 2015. None of the patients received radiotherapy or chemotherapy before surgery. The diagnosis of PDAC was histopathologically confirmed. The samples were collected and immediately snap frozen in liquid nitrogen and stored at -80°C until use. The study was approved by the Research Ethics Committee of Henan University of Science and Technology and written informed consent was obtained from all patients.

Cell culture and RNA interference

Human pancreatic cancer cell lines (PANC-1 and AsPC-1) were obtained from American Type Culture Collection (ATCC). All of the cell lines were grown and maintained in DMEM medium (Invitrogen) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 mg/ml streptomycin (Invitrogen) at 37°C in a 5% CO₂ atmosphere.

IncRNA BANCR small interfering RNA (si-BANCR) and non-targeting small interfering RNA (si-NC) were purchased from Sigma-Aldrich. Pancreatic cancer cells were transfected with siRNA by using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. The cells were harvested for further assays 48 h after transfection.

CCK-8 assay

Cell proliferation was quantified using the Cell Counting Kit-8 (CCK-8, TaKaRa). Briefly, 100 μl of cells were seeded into a 96-well plate at a concentration of 1000 cells per well and were incubated at 37°C. At daily intervals (24 h, 48 h, 72 h, and 96 h), the optical density was measured at 450 nm using a microtiter plate reader (Quant BioTek Instruments). The results represent the average of three replicates under the same conditions.

Transwell migration and invasion assay

Cell migration ability was assessed using 6.5-mm transwell chambers with a pore size of 8 μm. Cell invasion was assessed using the Chamber matrigel invasion 24-well DI kit (BD). The assays were performed according to the manufacturer’s instructions. Briefly, 2.5 × 10⁴ cells from each group were suspended in serum-free medium and were seeded into the upper chamber. The lower chamber was filled with medium containing 10% FBS. After incubation for 24 h, the migrated/invaded cells in the lower chamber (below the filter surface) were fixed in 4% paraformaldehyde, stained with 0.1 mg/ml crystal violet solution, and counted under a microscope. Five random visual fields were counted for each well, and the average was determined. The experiments were performed in triplicate.

RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated with TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. Total RNA was reverse transcribed
BANCR expression in PDAC

in a final volume of 10 μl using random primers under standard conditions for the PrimeScript RT reagent Kit (TaKaRa). We used the SYBR Premix Ex Taq (TaKaRa) to determine BANCR expression levels, following the manufacturer’s instructions. Results were normalized to the expression of GAPDH. The primers were as follows: BANCR forward primer 5'-ACAGGACTCCATGGCAAACG-3'; BANCR reverse primer 5'-ATGAAGAAAGCCTGGTGCAGT-3'. All experiments were performed using the $2^{-\Delta\Delta Ct}$ method. Each experiment was performed in triplicate.

**Western blot**

Cells were lysed in RIPA buffer with protease inhibitors and phosphatase inhibitors. The protein extracts were loaded onto a 10% sodium SDS-PAGE gel and transferred to a PVDF membrane. The blots were probed with primary antibodies (Abcam) followed by the HRP-conjugated secondary antibody. Following three Tris-buffered saline containing 0.1% Tween-20 (TBST) washes, the membranes were developed using ECL Plus (Millipore) and exposed to X-ray film. GAPDH served as the loading control.

**Statistics**

All the statistical analyses were performed using SPSS 18.0 statistical software package. Results were presented as mean ± SD. Differences between groups were analyzed using Student’s t test or one-way ANOVA analysis. The correlations between BANCR and
BANCR expression in PDAC

Results

**IncRNA BANCR was upregulated in PDAC tissues and associated with poor prognosis**

The expression of IncRNA BANCR in 49 paired PDAC tissues and adjacent non-tumor tissues from patients were detected by qRT-PCR. Compared with the levels of the adjacent non-tumor tissues, a significant upregulation of BANCR was observed in PDAC tissues ($P<0.05$, Figure 1A).

To further explored the relationship between the clinical parameters and expression levels of IncRNA BANCR, the 49 PDAC patients were divided into two groups based on the median value of relative BANCR expression. As shown in Table 1, BANCR expression was correlated with lymphnode metastasis and tumor stage ($P<0.05$). However, there were no significant correlations between BANCR expression and other clinicopathologic factors including gender, age, tumor size and tumor differentiation ($P>0.05$). We further investigated the correlation of BANCR expression with overall survival (OS) of PDAC patients, Kaplan-Meier analyses showed that the 5-year OS rate of high BANCR expression group was significantly poorer than that of low BANCR expression group ($P<0.05$; Figure 1B).

Univariate survival analysis showed that lymphnode metastasis, tumor stage and IncRNA BANCR expression were statistically significant risk factors affecting the PDAC patients' OS ($P<0.05$; Table 2). Multivariate analysis using the Cox proportional hazard model for all variables that were significant in the univariate analysis confirmed that the status of lymphnode metastasis, tumor stage and the level of BANCR expression were independent prognostic factors for PDAC patients ($P<0.05$; Table 2). Taken together, these observations indicated that increased expression of BANCR is associated with the progression of PDAC.

**Suppressing IncRNA BANCR expression inhibited PDAC cell proliferation, migration and invasion**

To identify the potential role of IncRNA BANCR in tumor progression, we investigated the impact of BANCR on PDAC cell proliferation. BANCR was downregulated in the PDAC cell lines via expression of si-BANCR transfection ($P<0.05$, Figure 2A). CCK-8 assay was used to determine the role of BANCR on the proliferation of PDAC cells. We found that decreased expression of BANCR significantly inhibited cell proliferation of PANC-1 and AsPC-1 cells compared with the si-NC group ($P<0.05$; Figure 2B).

<table>
<thead>
<tr>
<th>Variable</th>
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<tr>
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<td>$P$</td>
<td>Risk ratio</td>
<td>95% CI</td>
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<tr>
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<td>$\geq$60 vs $&lt;60$</td>
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<td>Tumor size</td>
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<td>Differentiation grade</td>
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<td>Lymph node</td>
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Furthermore, transwell migration and invasion assays were performed to explore the role of BANCR on the metastasis of PDAC cells. Transwell migration assay showed that downregulated expression of BANCR dramatically suppressed cell migration ability in PANC-1 and AsPC-1 cells (P<0.05; Figure 2C). Similarly, transwell invasion assay demonstrated that knockdown BANCR expression significantly inhibited the invasion capacity of PANC-1 and AsPC-1 cells (P<0.05; Figure 2D). These findings suggested that decreased lncRNA BANCR could inhibit the growth and metastasis of PDAC cells in vitro.

**LncRNA BANCR influenced PDAC cell epithelial-mesenchymal transition**

As epithelial-mesenchymal transition (EMT) process playing a key role in cancer cells invasion and metastasis, and previous study showed that IncRNAs were involved in tumor invasion via regulating EMT [15]. In the present study, we determined the expression of the EMT-induced markers in IncRNA BANCR downregulated PDAC cells. qRT-PCR results showed that decreased expression of BANCR could increase E-cadherin expression and decrease Vimentin expression in PDAC cells (P<0.05; Figure 3A). In addition, western blot assay suggested that downregulated expression of BANCR stimulated E-cadherin expression and reduced Vimentin expression in PDAC cells compared to si-NC group (P<0.05; Figure 3B). These findings suggested that IncRNA BANCR contributed to PDAC cell growth and metastasis may partly via affecting EMT process, and further experiments are needed to elucidate the potential mechanism.

**Discussion**

Identifying novel molecules that take part in PDAC formation and progression may be helpful for improving the diagnosis, prevention and treatment of this disease. The relationship between IncRNAs and tumors has currently become one of the focuses of cancer studies [16]. Abnormal expression of IncRNAs has been reported in PDAC. For example, Ye et al found...
that high expression of AFAP1-AS1 was associated with poor survival and short-term recurrence in pancreatic ductal adenocarcinoma [17]. Li et al indicated that IncRNA HOT-TIP promoted progression and gemcitabine resistance by regulating HOXA13 in pancreatic cancer [18]. Zheng et al suggested that IncRNA LOC389641 promoted progression of pancreatic ductal adenocarcinoma and increased cell invasion by regulating E-cadherin in a TNFRSF10A-related manner [19]. Thus, the identification of these dysregulated IncRNAs will undoubtedly enhance our knowledge of how IncRNAs function in the progression and metastasis of PDAC and could be used as a new diagnostic or therapeutic target.

In conclusion, our study demonstrated that IncRNA BANCR was increased expression in PDAC and BANCR was likely to be a useful biomarker for this disease. Additionally, our data indicated that BANCR could promote PDAC cell proliferation, migration and invasion partly via regulating EMT process. These findings suggested that IncRNA BANCR could act as an oncogene in PDAC progression and would not only be a novel prognostic biomarker but also a potential therapeutic target for PDAC treatment.
Disclosure of conflict of interest

None.

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References


