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*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 MALAT1 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

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

BRAF-activated non-coding RNA (BANCR), a 693-bp lncRNA, was firstly identified by Flockhart RJ et al through massively parallel complementary DNA (cDNA) sequencing screen for transcripts affected by the oncogene $BRAF^{V600E}$ expression [10]. Recent studies suggested that the dysregulation of lncRNA BANCR play critical roles in tumor progression. For example, Wang et al showed that lncRNA BANCR expression was upregulated in papillary thyroid carcinoma and promoted cell proliferation and activated autophagy [11]. Su et al found that lncRNA BANCR promoted cell growth and metastasis and associated with poor prognosis in retinoblastoma [12]. However, Jiang et al revealed that lncRNA 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 lncRNA BANCR expression was significantly downregulated in colorectal cancer and downregulated BANCR expression promoted cell proliferation via downregualted 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 lncRNA 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% $CO_2$ atmosphere.

lncRNA 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, $2.5 \times 10^4$ 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

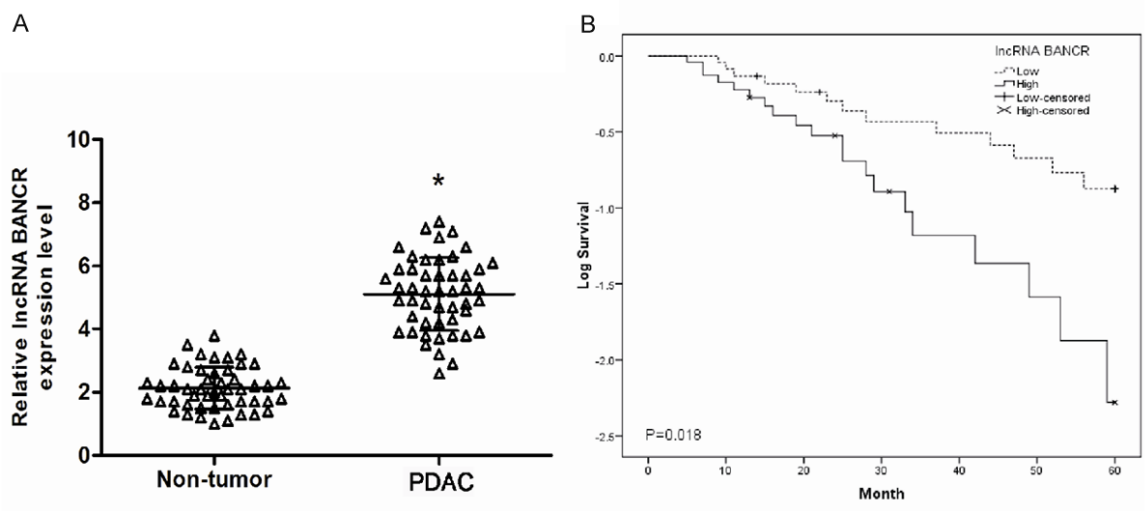


**Figure 1.** Relative expression of lncRNA BANCR in PDAC and its clinical significance. A. The expression of lncRNA BANCR was examined in 49 paired PDAC tissues and adjacent non-tumor tissues by qRT-PCR. GAPDH was used as an internal control. B. Kaplan-Meier curve presenting the overall survival of PDAC patients exhibiting high or low lncRNA BANCR expression. High lncRNA BANCR expression levels in PDAC tissues were associated with a poor prognostic outcome (log-rank test). *P<0.05.

**Table 1.** Correlation between lncRNA BANCR expression and clinicopathological features of PDAC patients (n=49)

| Parameters | Group | Total | lncRNA BANCR expression | | *P* value |
|---|---|---|---|---|---|
| | | | High | Low | |
| Gender | Male | 31 | 17 | 14 | 0.483 |
| | Female | 18 | 8 | 10 | |
| Age (years) | <60 | 27 | 15 | 12 | 0.482 |
| | ≥60 | 22 | 10 | 12 | |
| Tumor size (cm) | <2 | 9 | 4 | 5 | 0.662 |
| | ≥2 | 40 | 21 | 19 | |
| Tumor differentiation | Wel/moderate | 31 | 13 | 18 | 0.095 |
| | Poor | 18 | 12 | 6 | |
| Lymph nodes metastasis | Absence | 30 | 10 | 20 | 0.002 |
| | Presence | 19 | 15 | 4 | |
| Tumor stage | I+II | 23 | 6 | 17 | 0.001 |
| | III+IV | 26 | 19 | 7 | |

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

**Table 2.** Univariate and multivariate analysis of overall survival in PDAC patients

| Variable | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | Risk ratio | 95% CI | *P* | Risk ratio | 95% CI | *P* |
| Gender | 1.183 | 0.619-2.475 | 0.409 | | | |
| Male vs Female | | | | | | |
| Age (years) | 1.635 | 0.472-3.063 | 0.315 | | | |
| ≥60 vs <60 | | | | | | |
| Tumor size | 1.472 | 0.694-2.733 | 0.518 | | | |
| ≥2 vs <2 | | | | | | |
| Differentiation grade | 2.217 | 0.867-6.175 | 0.093 | | | |
| Poor vs Wel/moderate | | | | | | |
| Lymph node | 3.064 | 1.683-7.935 | 0.014 | 2.738 | 1.586-6.817 | 0.009 |
| Presence vs Absence | | | | | | |
| Tumor stage | 3.627 | 2.014-8.573 | 0.007 | 3.218 | 1.893-7.794 | 0.009 |
| III+IV vs I+II | | | | | | |
| lncRNA BANCR | 2.635 | 1.422-6.843 | 0.005 | 2.375 | 1.317-6.108 | <0.001 |
| High vs Low | | | | | | |

clinicopathological features were analyzed using chi-square test. Survival curves were plotted using the Kaplan-Meier method and the log-rank test. P<0.05 was considered statistically significant.

## Results

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

The expression of lncRNA 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 lncRNA 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 lncRNA 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 lncRNA BANCR expression inhibited PDAC cell proliferation, migration and invasion*

To identify the potential role of lncRNA 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**).

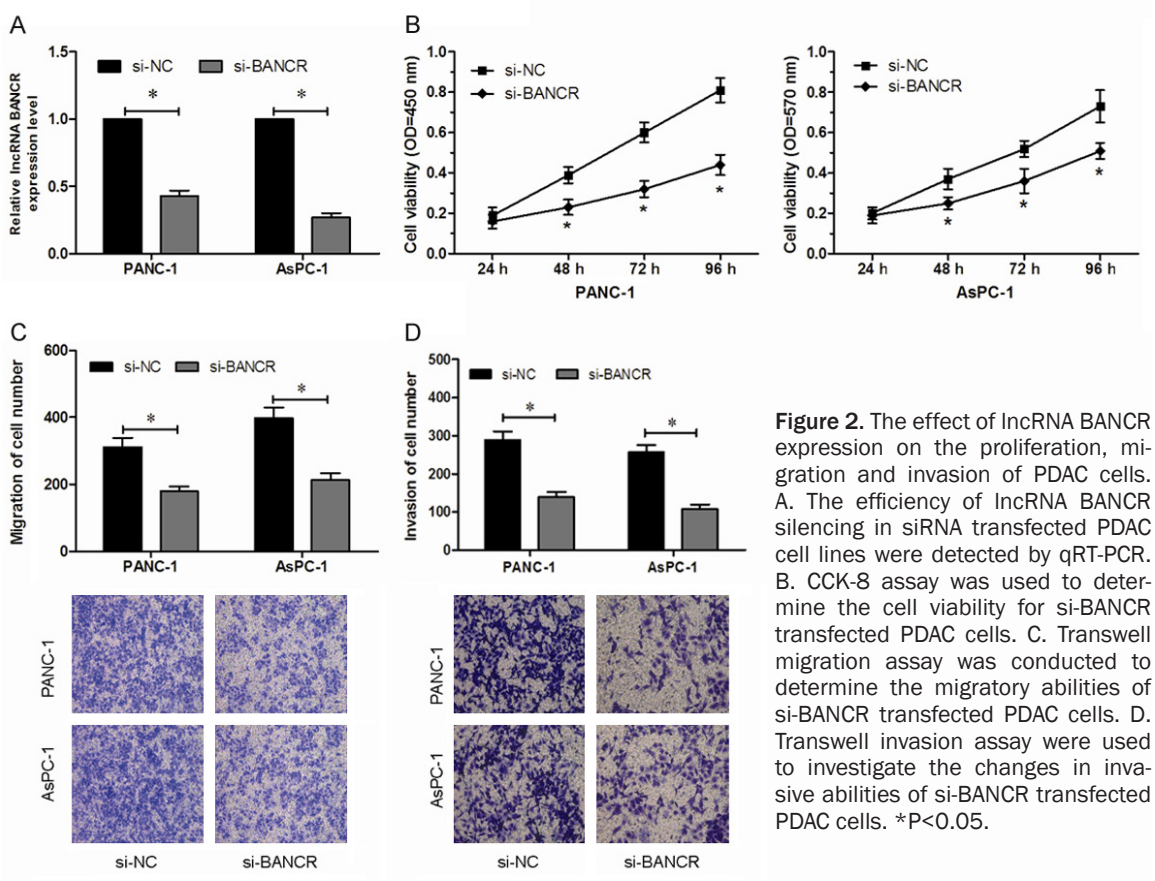


**Figure 2.** The effect of lncRNA BANCR expression on the proliferation, migration and invasion of PDAC cells. A. The efficiency of lncRNA BANCR silencing in siRNA transfected PDAC cell lines were detected by qRT-PCR. B. CCK-8 assay was used to determine the cell viability for si-BANCR transfected PDAC cells. C. Transwell migration assay was conducted to determine the migratory abilities of si-BANCR transfected PDAC cells. D. Transwell invasion assay were used to investigate the changes in invasive abilities of si-BANCR transfected PDAC cells. *P<0.05.

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 lncRNAs were involved in tumor invasion via regulating EMT [15]. In the present study, we determined the expression of the EMT-induced markers in lncRNA 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 lncRNA 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 lncRNAs and tumors has currently become one of the focuses of cancer studies [16]. Abnormal expression of lncRNAs has been reported in PDAC. For example, Ye et al found

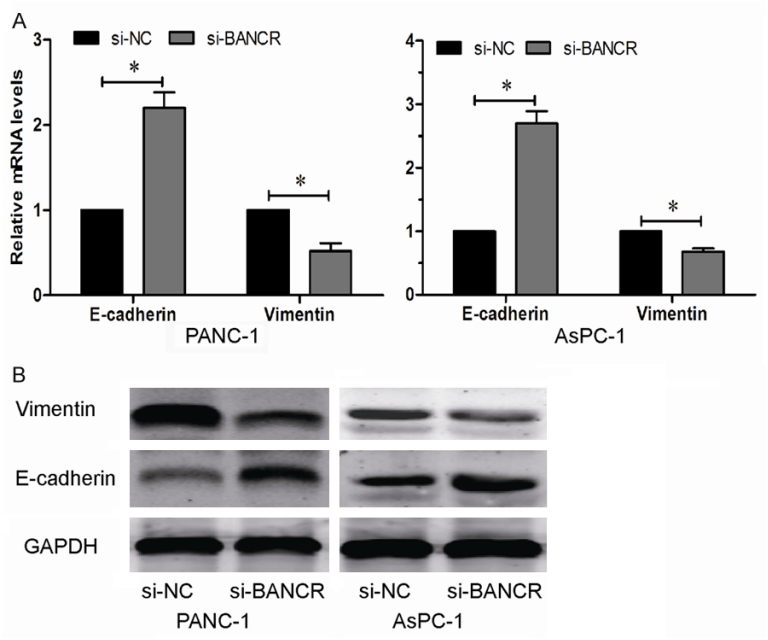


**Figure 3.** lncRNA BANCR regulates PDAC cell growth and metastasis by affecting EMT. A. qRT-PCR assay was used to detect E-cadherin and Vimentin expression in si-BANCR transfected PDAC cells. B. Western blot assay was used to explore E-cadherin and Vimentin expression in si-BANCR transfected PDAC cells. GAPDH was used as an internal control. *P<0.05.

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 lncRNA HOTTIP promoted progression and gemcitabine resistance by regulating HOXA13 in pancreatic cancer [18]. Zheng et al suggested that lncRNA 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 lncRNAs will undoubtedly enhance our knowledge of how lncRNAs function in the progression and metastasis of PDAC and could be used as a new diagnostic or therapeutic target.

In the present study, we found that the expression levels of lncRNA BANCR was significantly upregulated in PDAC tissues. A higher expression of BANCR was detected in PDAC patients with lymphnode metastasis and advanced tumor stage. The increased expression of lncRNA BANCR was associated with poor overall survival. Multivariate Cox hazard regression analysis identified high BANCR expression as an independent indicator of unfavorable prognosis. In addition, we demonstrated that downregulated expression of BANCR led to the significant inhibition of cell proliferation, migration and invasion of PD-AC. Taken together, these findings suggested that BANCR could function as a tumor oncogene and may be useful as a novel prognostic or progression biomarker for PDAC.

The growth and metastasis of cancer cells are landmark events that involve many changes in cellular behavior, and lead to different steps of the metastatic cascade [20]. Although lncRNA BANCR could inhibit proliferation, migration and invasion capacity of PDAC cells, the underlying mechanism was still unclear. Sun et al found that lncRNA BANCR was decreased in NSCLC and could promote NSCLC cell metastasis by affecting epithelial-mesenchymal transition [21]. EMT is a key step toward cancer metastasis, a biological process where epithelial cells lose their polarity and undergo transition into a mesenchymal phenotype. Loss of E-cadherin expression is a hallmark of EMT process and is likely required for enhanced tumor cell motility [22]. In this study, our results revealed that the expression of E-cadherin was increased and Vimentin was decreased following BANCR knockdown. Those results suggested that inhibitory effects of BANCR on PDAC cell growth and metastasis were partly via affecting EMT process, and further experiments were needed to elucidate the potential mechanism.

In conclusion, our study demonstrated that lncRNA 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 lncRNA BANCR could act as an oncogene in PDAC progression and would be not only a novel prognostic biomarker but also a potential therapeutic target for PDAC treatment.

## Disclosure of conflict of interest

None.

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