Long Non-Coding RNA BANCR Is Overexpressed in Patients with Diabetic Retinopathy and Promotes Apoptosis of Retinal Pigment Epithelial Cells

BCD  Li Yin  
ABE  Zhaohui Sun  
BCEF  Qian Ren  
BDF  Xian Su  
ADE  Delong Zhang

Corresponding Author: Delong Zhang, e-mail: skcygo6@163.com
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Background: lncRNA BANCR participates in the pathogenesis of various types of human diseases; however, its involvement in diabetic retinopathy is unknown.

Material/Methods: In this study, the expression of lncRNA BANCR in plasma of patients with diabetic retinopathy, diabetic patients without complications, and healthy controls was analyzed by qRT-PCR. The accuracy plasma BANCR in diagnosing diabetic retinopathy was analyzed by ROC curve analysis. lncRNA BANCR expression vector and siRNA were transfected into the human retinal pigment epithelial cell line ARPE-19, and cell apoptosis was analyzed by cell apoptosis assay.

Results: We found that lncRNA BANCR was significantly upregulated in patients with diabetic retinopathy compared to diabetic patients without complications and healthy controls. Upregulation of lncRNA BANCR effectively distinguished patients with diabetic retinopathy from diabetic patients without complications and healthy controls. High-glucose treatment led to upregulated expression of BANCR in the human retinal pigment epithelial cell line ARPE-19. Overexpression of BANCR promoted and siRNA silencing inhibited the apoptosis of cells in the human retinal pigment epithelial cell line ARPE-19 in a high-glucose environment.

Conclusions: lncRNA BANCR is overexpressed in patients with diabetic retinopathy and can promote apoptosis of retinal pigment epithelial cells.

MeSH Keywords: Diabetic Retinopathy • Retinal Pigment Epithelium • RNA, Long Noncoding

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Background

Diabetic retinopathy is an ocular complication of diabetes mellitus that seriously affects the lives of diabetic patients [1]. Diabetic retinopathy affects 56.0% of type 1 diabetic patients and 30.3% of diabetic type 2 diabetic patients [2]. Among these patients, 11.2% cases of type 1 diabetic retinopathy and 2.9% cases of type 2 diabetic retinopathy are sight-threatening [2,3]. With the increasing prevalence of diabetes, the incidence rate of diabetic retinopathy is predicted to continuously and significantly increase in the near future [4,5]. Although progress has been made in the diagnosis of diabetic retinopathy [6,7], early diagnosis of this disease is still challenging, leading to poor treatment outcomes.

Long non-coding RNAs, or IncRNAs, are a subgroup of non-coding RNAs that contains more than 200 nucleotides [8]. A growing literature has revealed that IncRNAs not only participate in normal physiological processes, but also play pivotal roles in the development of various human diseases [9,10], including diabetic complications [11,12]. BANCR is a well-characterized IncRNA with key functions in cancer biology [13,14]. Our preliminary microarray data showed that IncRNA BANCR is also upregulated in blood specimens of patients with diabetic retinopathy, indicating its involvement in this disease. In the present study we observed that IncRNA BANCR was upregulated in patients with diabetic retinopathy, and found that overexpression of IncRNA BANCR can promote the apoptosis of retinal pigment epithelial cells.

Material and Methods

Cell line and human specimens

The ARPE-19 (ATCC® CRL-2302™) human retinal pigment epithelial cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA). Cells were cultured with ATCC-formulated DMEM: F12 Medium (Catalog No. 30-2006) containing 10% fetal bovine serum (FBS) at 37°C in a 5% CO₂ incubator. For the detection of IncRNA BANCR in a high-glucose environment, ARPE-19 cells were treated in culture medium containing 5, 10, 30, and 50 mM D-glucose for 6, 12, and 18 h before use.

Plasma samples were donated by 64 patients with diabetic retinopathy (nonproliferative stage), 62 diabetic patients without obvious complications, and 48 healthy volunteers. The 64 diabetic retinopathy and 62 diabetic patients were admitted to the First Hospital of Shijiazhuang from March 2016 to May 2018. Inclusion criteria were: 1) patients with diabetic retinopathy or diabetes only diagnosed in the First Hospital of Shijiazhuang; and 2) patients fully understood the experimental protocol and were willing to participate. Exclusion criteria were: 1) patients who were treated within 90 days before admission; and 2) patients who also had other diseases. The 48 healthy volunteers were enrolled from the physical examination center of the First Hospital of Shijiazhuang. No differences in basic information, except body mass index (BMI), were found among the 3 groups of participants (Table 1). The Ethics Committee of the First Hospital of Shijiazhuang approved this study. Participants read and understood the whole experimental protocol and signed informed consent.

Real-time quantitative PCR (RT-qPCR)

Following total RNA extraction from plasma using a Trizol reagent kit (Invitrogen, USA), cDNA was prepared using SuperScript III Reverse Transcriptase kit (Thermo Fisher Scientific). SuperScript III Platinum One-Step qRT-PCR Kit (Thermo Fisher Scientific) was used to prepare all PCR reaction systems. Thermal reaction cycles of PCR reactions were: 52 s at 95°C, then 28 s at 95°C, and 36 s at 56.5°C for 40 cycles. Sequences of primers used in PCR reactions were: 5’-ACAGGACTCCATGGCAAACG-3’ (forward) and 5’-ATGAAGAAAGCCTGGTGCAGT-3’ (reverse) for IncRNA BANCR; 5’-GACCTCTATGCCAACACAGT-3’ (forward) and 5’-AGTACTTGCGCTCAGGAGGA-3’ (reverse) for β-actin endogenous control using 2⁻ΔΔCT method.

Cell transfection

IncRNA BANCR expression pEGFPC3 vectors, shRNA (5’-CGGAAATAGACTGCAGCAAA-3’), and siRNA control were used in PCR reactions were: 5’-ACAGGACTCCATGGCAAACG-3’ (forward) and 5’-ATGAAGAAAGCCTGGTGCAGT-3’ (reverse) for IncRNA BANCR; 5’-GACCTCTATGCCAACACAGT-3’ (forward) and 5’-AGTACTTGCGCTCAGGAGGA-3’ (reverse) for β-actin. Ct values of IncRNA BANCR were normalized to β-actin endogenous control using 2⁻ΔΔCT method.

Table 1. Basic information of the 3 groups of participants.

|                        | Diabetic retinopathy | Diabetes | Control |
|------------------------|----------------------|----------|---------|
| Cases                  | 64                   | 62       | 48      |
| Gender                 |                      |          |         |
| Male                   | 35                   | 34       | 23      |
| Age range (year)       | 32–66                | 33–65    | 32–68   |
| Mean age (years)       | 48.3±6.5             | 47.9±6.9 | 48.8±6.1|
| Gender                 |                      |          |         |
| Female                 | 29                   | 28       | 25      |
| Mean BMI               | 26.3±2.2*            | 25.9±2.4*| 21.3±1.9|
| Smoking                | 30 (46.9%)           | 29 (46.8%)| 23 (47.9%)|
| Drinking               | 32 (50.0%)           | 28 (45.2%)| 22 (45.8%)|

* Compared with control group.
prepared by GenePharma (Guangzhou, China). For transfection, lipofectamine 2000 reagent (11668-019, Invitrogen, Carlsbad, USA) was mixed with the vectors and kept at room temperature for 30 min to form vector-reagent complexes. Then, ARPE-19 cells were added into the vector-reagent complexes and were incubated at 37°C for 6 h. After that, cells were washed by fresh culture medium to avoid cytotoxicity. Overexpression rate that higher than 190% (190–230%) and siRNA silencing rate below 50% (40% to 50%) was reached at 12 h after transfection.

Cell apoptosis assay

Cell apoptosis was detected after ARPE-19 cells were treated in culture medium containing 30 mM D-glucose for 6, 12, and 18 h. Briefly, serum-free cell suspensions (6×10^4 cells/ml) were prepared and were cultured in 6-well plates (10 ml in each well) for 48 h. After that, cells were digested by 0.25% trypsin. Cells were then harvested and dissolved in fresh cell culture medium. After staining with Annexin V-FITC (Dojindo, Japan) and propidium iodide (PI), apoptotic cells were detected by flow cytometry.

Statistical analysis

GraphPad Prism 6 was used. Data were recorded as mean ± standard deviation and compared by one-way analysis of variance and Tukey test, respectively. p<0.05 was used as the cut-off score to indicate statistical significance.

Results

**IncRNA BANCR was significantly upregulated in diabetic retinopathy patients**

We first detected the expression of IncRNA BANCR in plasma of 3 groups of participants. Compared with the diabetes and control groups, significantly upregulated expression levels of IncRNA BANCR were observed in plasma of patients with diabetic retinopathy (Figure 1, p<0.05). Compared with the control group, plasma levels of IncRNA BANCR were slightly increased in the diabetes group, but no significant difference was observed.

**Overexpression of IncRNA BANCR has diagnostic potentials for diabetic retinopathy**

With diabetic patients or healthy controls as true-negative subjects and diabetic retinopathy patients as true-positive subjects, ROC curve analysis was performed to investigate the diagnostic value of IncRNA BANCR for diabetic retinopathy. With healthy controls as true-negative subjects, the area under the curve (AUC) was 0.8580 (Figure 2A) (standard error: 0.03515; 95% confidence interval: 0.7891–0.9269; p<0.0001). With diabetic patients as true-negative subjects, the AUC was 0.6404 (Figure 2C) (standard error: 0.04424; 95% confidence interval: 0.5323–0.7485; p=0.01266).

**High-glucose environment upregulated the expression of IncRNA BANCR**

To investigate the effects of high-glucose environment on IncRNA BANCR expression in ARPE-19 cells, ARPE-19 cells were treated in culture medium containing 5 (control), 10, 30, and 50 mM D-glucose for 6, 12, and 18 h, and IncRNA BANCR expression was detected by RT-qPCR. Compared with 5 mM D-glucose, 30 and 50 mM D-glucose, but not 10 mM D-glucose, significantly upregulated IncRNA BANCR expression in a dose-dependent manner (Figure 3, p<0.05). In addition, longer treatment time of D-glucose showed stronger enhancing effects on IncRNA BANCR expression in ARPE-19 cells.

**IncRNA BANCR overexpression promoted, but BANCR siRNA silencing inhibited, the apoptosis of ARPE-19 cells**

Cell apoptosis was detected after ARPE-19 cells were treated in culture medium containing 30 mM D-glucose for 6, 12, and 18 h. Compared with control cells (C, cells without transfection) and negative control cells (NC, cells transfected with empty vectors or siRNA control), IncRNA BANCR overexpression significantly promoted (Figure 4A, p<0.05), but BANCR siRNA silencing significantly inhibited (Figure 4B, p<0.05), the apoptosis of ARPE-19 cells.

![Graph 1](image1.png)

Figure 1. IncRNA BANCR was significantly upregulated in diabetic retinopathy patients. Compared with the diabetes and control groups, significantly upregulated expression levels of IncRNA BANCR were observed in plasma of patients with diabetic retinopathy (*p<0.05).
Figure 2. Overexpression of lncRNA BANCR has diagnostic potentials for diabetic retinopathy. This figure shows the ROC curve analysis of the diagnostic value of lncRNA BANCR for diabetic retinopathy with diabetic patients (A) or healthy controls (B) as true-negative subjects, and the diagnostic value of lncRNA BANCR for diabetes with healthy controls as true-negative subjects (C).

Figure 3. High-glucose environment upregulated the expression of lncRNA BANCR. A high-glucose environment led to upregulated expression of lncRNA BANCR in a dose- and time-dependent manner (* p<0.05).
Figure 4. IncRNA BANCR overexpression promoted (A), while BANCR siRNA silencing inhibited (B), the apoptosis of ARPE-19 cells (* p<0.05).
Discussion

BANCR is a well-studied oncogenic IncRNA in cancer biology, but its involvement in other diseases is unknown. The major finding of our study is that IncRNA BANCR is likely to be involved in the apoptosis of retinal pigment epithelial cells, which is a major pathological change in the eyes of patients with diabetic retinopathy.

The high-glucose environment in the body of diabetic patients globally affects the expression of genes, including IncRNAs [15]. The altered expression of certain IncRNAs serves as mediators between pathological pathways to participate in the development of diabetic complications [16]. Diabetic retinopathy is a major type of diabetic complications that is also characterized by the dysregulated expression of IncRNAs [17]. It has been reported that IncRNA MEG3 is downregulated in diabetic retinopathy, and overexpression of IncRNA MEG3 may inhibit disease development by targeting TGF-β1 and VEGF [18]. In another study, IncRNA MALAT1 and HOTAIR were proved to be key players in the epigenetic regulation of gene expression in diabetic retinopathy [19]. Our study observed significantly upregulated expression of IncRNA BANCR in diabetic retinopathy patients but not in diabetic patients without obvious complications, indicating the specific involvement of IncRNA BANCR in the development of retinopathy in diabetic patients. The normal level of plasma glucose is around 5 mM. Interestingly, in our study, 10 mM failed to significantly affect the expression of IncRNA BANCR in ARPE-19 cells. However, D-glucose at 30 mM and 50 mM significantly promoted IncRNA BANCR expression. Therefore, a high-glucose environment can indeed regulate IncRNA BANCR expression. The unchanged plasma levels of IncRNA BANCR in diabetic patients in our study may be explained by the early stages of disease and relatively low blood glucose level.

Early diagnosis is critical for the recovery of diabetic retinopathy patients [20]. To evaluate the diagnostic value of IncRNA BANCR for diabetic retinopathy, our study only included patients at nonproliferative stage, which is the early stage of diabetic retinopathy. ROC curve analysis revealed that overexpression of IncRNA BANCR effectively distinguished diabetic retinopathy patients from diabetic patients and healthy controls. Therefore, IncRNA BANCR may serve as a biomarker for the early diagnosis of diabetic retinopathy. However, altered expression of IncRNA BANCR has been observed in many types of human cancers. Therefore, the existence of tumors should be excluded before the diagnosis of diabetic retinopathy, using IncRNA BANCR as a marker.

Apoposis of retinal pigment epithelial cells is a major pathological change in the eyes of patients with diabetic retinopathy [21]. Our study observed that overexpression of IncRNA BANCR promoted, but siRNA silencing inhibited, the apoptosis of cells of human retinal pigment epithelial cell line ARPE-19 in a high-glucose environment. Therefore, inhibition of IncRNA BANCR expression may serve as a potential therapeutic marker for the treatment of diabetic retinopathy. However, the molecular mechanism of the actions of IncRNA BANCR in the apoptosis of ARPE-19 cells is still unknown. Our study showed no significant response from several classic apoptotic pathways, such as Bcl2/Bax, after IncRNA BANCR overexpression or knockdown (data not shown). Our future studies will keep working on the molecular mechanism of its function.

Apoposis of different retinal cell types, such as ganglion cells, endothelial cells, pericytes, and pigment epithelial cells, are involved in the development and progression of retinopathy. Therefore, the upregulation of IncRNA BANCR may also be involved in the apoptosis of other cell types. Our future studies will investigate the involvement of IncRNA BANCR in the apoptosis of these cells.

Conclusions

In conclusion, IncRNA BANCR is overexpressed in the plasma of diabetic retinopathy patients. Inhibition of IncRNA BANCR inhibited the apoptosis of human retinal pigment epithelial cells. Therefore, IncRNA BANCR may serve as a potential therapeutic target for diabetic retinopathy.

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