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Role of Long Non-Coding RNA (LncRNA) LINC-PINT Downregulation in Cardiomyopathy and Retinopathy Progression Among Patients with Type 2 Diabetes

Authors' Contribution:

Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCF **Tianjian Zha***
CDE **Fuzeng Su***
ABC **Xiaolong Liu**
DFG **Chunfeng Yang**
AEG **Lihua Liu**

Department of Burns, Wound Repair Surgery, People's Hospital of Xinjiang Uygur Autonomous Region, Urumqi, Xinjiang, P.R. China

* Tianjian Zha and Fuzeng Su contributed equally to this work and should be considered as equal first coauthors

Corresponding Author: Xiaolong Liu, e-mail: xiaolongliuu@163.com

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Background: Despite the acknowledgement that LncRNA LINC-PINT may inhibit tumor cell invasion in human cancers, it is not yet determined when it comes to diabetes and its related complications.





Material/Methods: There were 244 patients with T2D and 126 healthy volunteers were admitted to People's Hospital of Xinjiang Uygur Autonomous Region Hospital. Fasting blood (5 mL) was obtained from the patients and controls a day after admission. The diabetes patients' fasting blood was extracted once every 6 months during follow-up. The total RNA was extracted and then used for detecting the expression of LINC-PINT.

Results: A comparison was made in this study, where LINC-PINT did not experience significant downregulation level in the majority of those suffering diabetes complications when in contrast to healthy controls, while LINC-PINT expression was found in diabetics. The follow-up study showed that LINC-PINT was downregulated in patients who developed cardiomyopathy and retinopathy or both but not in patients who developed other complications. Treatment with high glucose limited the extent of LINC-PINT expression in the ARPE-19 and AC16 cells. While the overexpression of LINC-PINT increased the viability of ARPE-19 and AC16 cells, siRNA-mediated silencing of LINC-PINT elicited the opposite effect.

Conclusions: Hence, we concluded that the overexpression of LINC-PINT may exhibit inhibitory effects on the progression of cardiomyopathy and retinopathy among patients with type 2 diabetes.

MeSH Keywords: **Diabetes Complications • Diabetic Neuropathies • Tissue Survival**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/918358>

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Background

The occurrence of diabetes is caused by altered insulin secretion and/or insulin action and hyperglycemia is one of the major clinical manifestations [1]. In 2010, it was observed that diabetes affected 285 million adults, which accounts for about 6.4% of the global size of the population [2]. With changes in people's lifestyle, more than 439 million adults are predicted to be affected by diabetes by the year 2030, which accounts for 7.7% of the population [2]. Diabetes features high blood sugar concentration, which results in long-term damage or failure of most of the vitals [3] like the heart [4], eyes [5], and kidney [6]. At present, the prevention of diabetes-related complications is still an important challenge in the treatment of diabetes.

lncRNA is a functional RNA containing at least 200 nucleotides. Lung cancer cannot progress without the participation of a vital player, lncRNAs, which is well established for the past few years. To be specific, as a regulator of key tumor genes, lncRNAs regulate tumor behavior biologically, exerting both transcriptional regulation and post-transcriptional regulation. lncRNAs have been recently reported to function significantly in multiplication, metastasis, maintenance of stem cells, epithelial transformation to mesenchymal cells, and a wide range of other biological processes, and thus promise to treat patients living with non-small cell lung cancer. They are signal biological markers. The progression of diabetes globally affects the expression of lncRNAs [7], which are a series of non-protein-coding RNA transcripts with pivotal functions in human diseases [8]. Certain differentially expressed lncRNAs have been proven to have critical functions in the progression of diabetes-related complications [9]. LINC-PINT is a well-studied lncRNA that is linked with tumor suppression in human cancers [10,11]. There have been no reports on its involvement in diabetes and the accompanying complications. We carried out a 6-year follow-up study to show that the downregulation of LINC-PINT is correlated with the progression of cardiomyopathy and retinopathy among patients with type II diabetes (T2D).

Material and Methods

Study participants

Approximately 244 patients with T2D and 126 healthy volunteers were admitted to People's Hospital of Xinjiang Uygur Autonomous Region Hospital from May 2010 to May 2012. The inclusion criteria for the patients included the following: 1) patients with T2D but with no relevant complications; 2) patients diagnosed for the first time; 3) patients who have completed the treatment as well as the 6-year follow-up. The exclusion criteria for the patients was as follows: 1) patients who simultaneously suffer from T2D and other diseases; 2) patients

who have undergone treatment before admission; 3) patients who died during the follow-up. All the healthy volunteers received systemic physiological examination simultaneously and all physiological parameters were within the normal range.

The 244 T2D patients included 137 males and 107 females (aged 31 to 67 years), and their average age was 48.2 ± 5.6 years. The 126 healthy volunteers included 68 males and 58 females (aged 30 to 67 years), and their average age was 48.9 ± 5.3 years. The trials were started with a formal statement of ratification from the Ethics Committee of People's Hospital of Xinjiang Uygur Autonomous Region. All participants signed informed consent forms.

Follow-up study

A 6-year follow-up was conducted for all the patients involved in the study after admission in order to record the occurrence of diabetes-related complications in a timely manner. Patients were asked to visit our hospital every 6 months. According to the follow-up data, diabetic cardiomyopathy-only, retinopathy-only, nephropathy-only, diabetic lung and diabetic foot ulcer occurred in 26, 27, 33, 17, and 16 cases, respectively. On the other hand, 40 cases developed multiple complications including diabetic cardiomyopathy and/or diabetic retinopathy, 48 cases developed multiple complications without diabetic cardiomyopathy or diabetic retinopathy, and 37 developed had no complications.

Human specimens and cell lines

Fasting blood (5 mL) was obtained from the patients and controls a day after admission. The diabetes patients' fasting blood was extracted once every 6 months during follow-up. The plasma samples were prepared via conventional methods (2000 g centrifuge for 10 minutes and stored at -80°C). ARPE-19 and AC16 cell lines were used *in vitro* herein. ARPE-19 cells were purchased from ATCC (Manassas, VA, USA) and cultivated in ATCC-formulated DMEM: F12 Medium (Catalog No. 30-2006) supplemented with 10% fetal bovine serum (FBS) in an incubator (37°C , 5% CO_2). AC16 cells were purchased from EMD Millipore and cultivated in DMEM containing 1% penicillin and streptomycin as well as 12% FBS in an incubator (37°C , 5% CO_2).

Real-time quantitative polymerase chain reaction (RT-qPCR)

The total RNA was extracted and then used for detecting the expression of LINC-PINT. *In vitro* transcription was carried out via SuperScript III Reverse Transcriptase and the PCR solution was prepared via Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix. Expression of LINC-PINT was standardized to the endogenous GAPDH control via $2^{-\Delta\Delta\text{CT}}$ method.

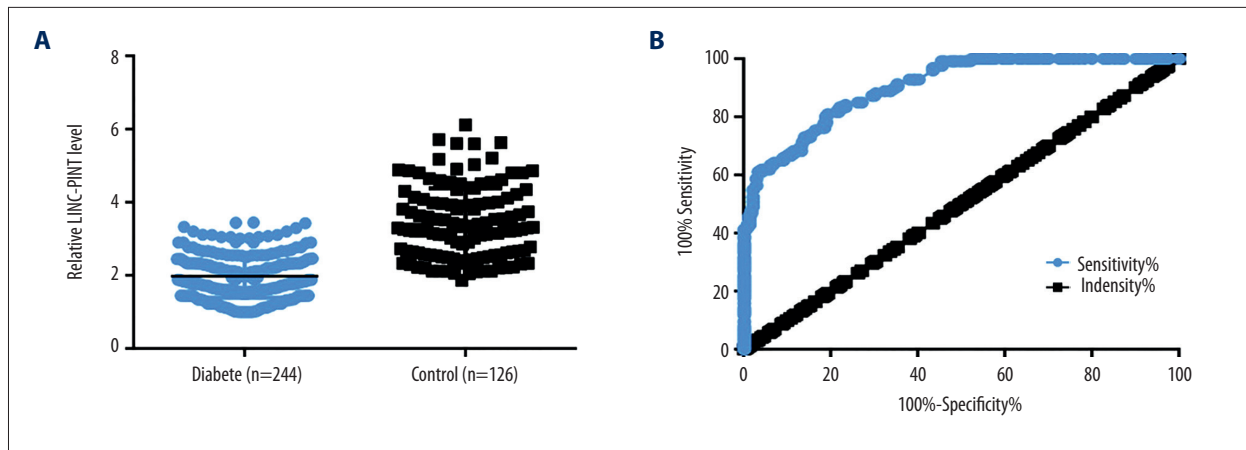


Figure 1. Downregulated expression of LINC-PINT distinguished type 2 diabetes patients from healthy controls. The results of RT-qPCR showed that (A) plasma LINC-PINT was downregulated in diabetes patients without obvious complications than in healthy controls ($* P<0.05$), (B) and ROC curve analysis showed that downregulated expression of LINC-PINT distinguished diabetes patients from healthy controls.

Cell transfection

LINC-PINT expressing vectors, empty vectors, LINC-PINT siRNA, siRNA, LINC-PINT primers and endogenous control GAPDH were all designed and combined to form syntheses by Sangon Biotech (Shanghai) Co., Ltd. To transfect cells with Lipofectamine 2000 and 10 nM of vectors or 50 nM of siRNAs followed. Treatment with Lipofectamine 2000 reagent as well as transfection with empty vector or siRNA was taken as control and negative control, respectively.

MTT assay

We obtained the expression level of LINC-PINT at 24 hours after transfection via RT-qPCR. The cells were collected when the over-expression rate and knockdown rate reached 200% and 50%, respectively, and these cells were used for MTT assay. Briefly, the cell suspensions were prepared with a cell density of 5×10^4 cells/mL and then transferred to a 96-well plate (0.1 mL per well), followed by addition of D-glucose until the final concentration reached 20 mM. Following that, the cells were cultured at 37°C and 5% CO₂ for 6 hours and MTT (10 μ L) was added into each well. Thereafter, the cells were cultured for another 4 hours and optical density (OD) values were measured at 570 nm.

Statistical analysis

All experiments were carried out thrice in parallel and the data obtained were presented as mean \pm standard deviation. GraphPad Prism 6 software was used to analyze the data in the study. The ROC curve analysis with the healthy controls as true negative cases and diabetes patients as true positive cases was used to evaluate the diagnostic values of plasma LINC-PINT for diabetes. Unpaired *t*-test was brought into

operation to investigate the difference between patients and controls. One-way ANOVA and Tukey test were conducted to analyze the difference among groups. A *P* value of <0.05 denoted statistical significance.

Results

Difference in the expression of LINC-PINT between patients and healthy controls

RT-qPCR was used to determine the plasma levels of LINC-PINT in all the subjects. LINC-PINT was at a markedly higher plasma level in healthy controls rather than in patients (Figure 1A). ROC curve analysis was accomplished to place diagnostic values on plasma LINC-PINT in diabetes patients. The space under the curve was measured in terms of area as illustrated in Figure 1B (standard error; 95% confidence interval).

Downregulation of LINC-PINT in patients who developed cardiomyopathy and retinopathy during follow-up

According to the follow-up data, there were 26 cases of diabetic cardiomyopathy-only (DC), 27 cases of diabetic retinopathy-only (DR), 33 cases of diabetic nephropathy-only (DN), 17 cases of diabetic lung-only (DL), and 16 cases of diabetic foot ulcer-only (DFU). In addition, there were 40 cases with multiple complications including diabetic cardiomyopathy and/or diabetic retinopathy (MCCR), 48 cases developed multiple complications without diabetic cardiomyopathy or diabetic retinopathy (MC), and 37 cases had no complications (NC). As shown in Figure 2, LINC-PINT was demonstrated in the follow-up to be further downregulated in DC, DR, and MCCR groups instead of the other groups.

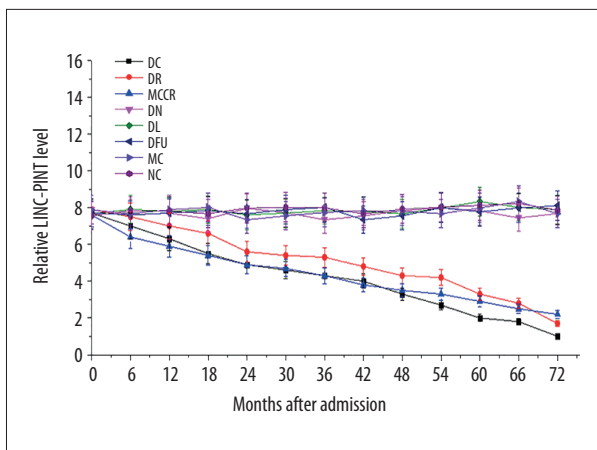


Figure 2. Further downregulation of LINC-PINT in patients who developed cardiomyopathy and retinopathy during follow-up. The results of the follow-up study showed that LINC-PINT was further downregulated in patients who developed diabetic cardiomyopathy (DC), diabetic retinopathy (DR), and multiple complications included diabetic cardiomyopathy and/or diabetic retinopathy (MCCR), but not in patients who developed other complications including diabetic nephropathy-only (DN), diabetic lung-only (DL), diabetic foot ulcer-only (DFU), complications by without diabetic cardiomyopathy or diabetic retinopathy (MC), and no complications (NC) (* $P < 0.05$).

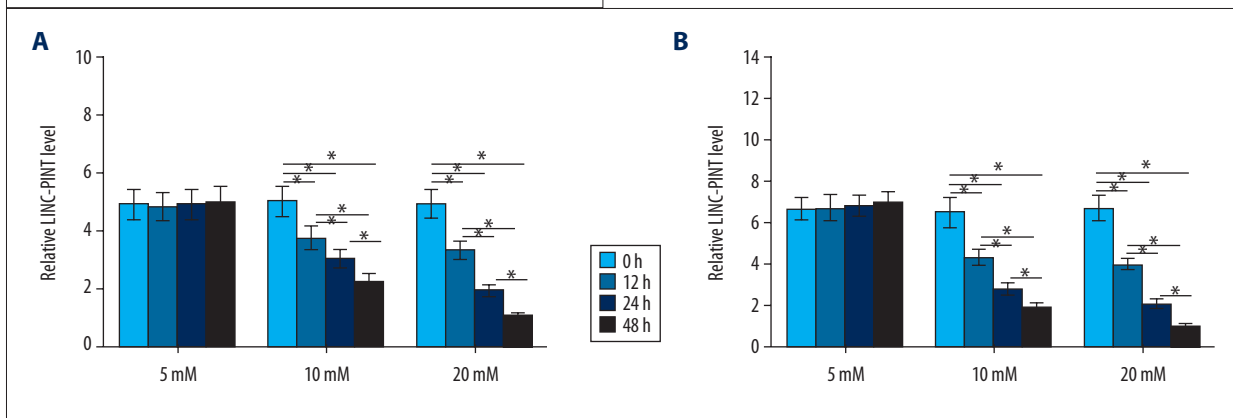


Figure 3. High glucose treatment led to inhibition of LINC-PINT expression in the ARPE-19 and AC16 cell lines. D-glucose treatment led to significantly inhibited expression of LINC-PINT in (A) the ARPE-19 cells and (B) AC16 cells in a dose and time dependent manner (* $P < 0.05$).

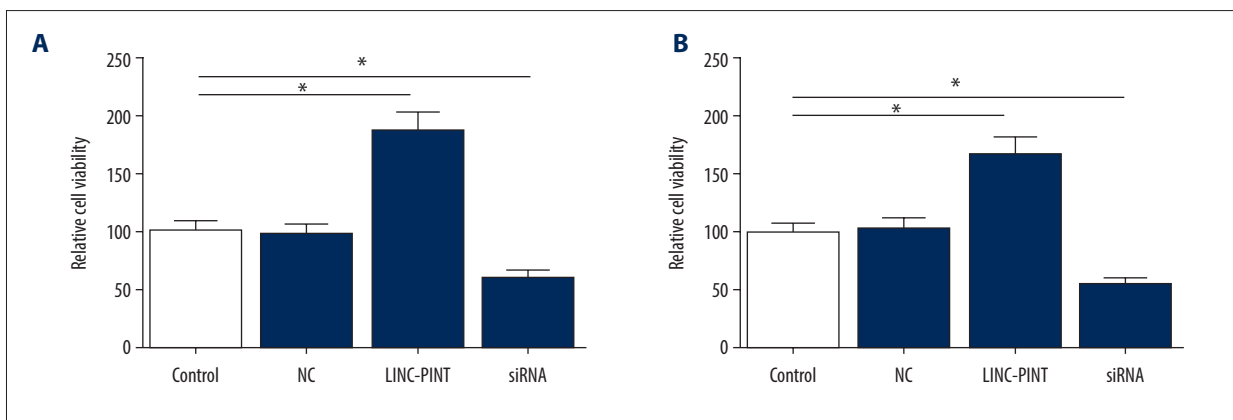


Figure 4. LINC-PINT increased the viability of ARPE-19 and AC16 cells treated with 20 mM D-glucose treatment. Overexpression of LINC-PINT led to increased cell viability while LINC-PINT siRNA silencing led to decreased cell viability of (A) ARPE-19 and (B) AC16 cells treated with 20 mM high-glucose (* $P < 0.05$).

Effect of high glucose treatment on the expression of LINC-PINT in ARPE-19 and AC16 cells

ARPE-19 and AC16 cells were treated with 5 (control), 10 and 20 mM of D-glucose for 12, 24 and 48 hours. The results of

RT-qPCR showed that the D-glucose treatment could significantly inhibit the expression of LINC-PINT in ARPE-19 (Figure 3A) and AC16 cells (Figure 3B).

Cell viability of ARPE-19 and AC16 cells by LINC-PINT after 20 mM D-glucose treatment

The results of the MTT assay showed that overexpression of LINC-PINT increases the viability of ARPE-19 (Figure 4A) and AC16 cells (Figure 4B) after treatment with 20 nM glucose ($P<0.05$) while silencing with LINC-PINT siRNA exerted the opposite effects.

Discussion

Due to unclear pathogenesis and lack of molecular targets, it is challenging to prevent and treat diabetes-related complications [12,13]. One crucial finding from the present study is that LINC-PINT can regulate the cell viability under high glucose conditions, which may contribute to the progression of cardiomyopathy and retinopathy among the patients with T2D.

LINC-PINT is an established tumor suppressor lncRNA which is downregulated in human cancers [10,11]. However, reports were seldom or never on its link to diabetes and the relevant complications. In the findings of the study, LINC-PINT levels were obviously downregulated in T2D patients by comparison with healthy controls. We also observed a difference in the pattern of altered LINC-PINT expression between these 2 groups. These results indicate that LINC-PINT is correlated with the progression of diabetes and plasma levels of LINC-PINT has several potential applications [14].

Although high level of blood sugar is the most frequent contribution to the occurrence of all diabetes-related complications [15], genome-wide studies have shown that various diabetes-related complications are accompanied by different DNA methylation and gene expression patterns [16–18], indicating that these complications have different molecular mechanisms. This study displayed the further downregulated expression of LINC-PINT in diabetes patients who developed cardiomyopathy and retinopathy. Our *in vitro* experiments provided more evidence that high glucose treatment resulted in reduced LINC-PINT expression in ARPE-19 and AC16 cells [19]. Therefore, there may be a correlation between the downregulation of LINC-PINT and a movement forward of diabetic cardiomyopathy

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and retinopathy, and thus, monitoring variations in the circulating levels of LINC-PINT in the blood plasma may provide predictive information about the occurrence of cardiomyopathy and retinopathy among patients with T2D [20].

As high glucose environment inhibits cell viability in most, if not all, important organs, improving cell viability under these conditions may alleviate the complications related to diabetes [21,22]. In this study, we indicated that LINC-PINT positively regulated the viability of ARPE-19 and AC16 cells under high glucose treatment. Hence, the overexpression of LINC-PINT can be considered as a novel strategy for the treatment and prevention of cardiomyopathy and retinopathy among patients with diabetes.

We focus this research on clinical practices of lncRNAs, particularly on their high possibilities in diagnosis, treatment, and medical prognosis in the progression of cardiomyopathy and retinopathy among patients with T2D. To sum up, lncRNAs are deemed to be crucial modulators of a variety of cellular processes, as well as active participants of manifold signaling pathways. Their roles in cellular processes are increasingly evidenced to vary from cell growth to metastasis, to maintenance of stem cells, and to cell suicide. It is believed that lncRNAs are likely to be of considerable value in clinical applications to T2D treatment with the development of biomedical research.

Conclusions

In brief, LINC-PINT is downregulated in diabetes patients and overexpression of LINC-PINT may inhibit the progression of cardiomyopathy and retinopathy among patients with T2D. Therefore, lncRNAs may reconstruct the specificity and sensitivity that cardiomyopathy and retinopathy cells have, and thereby are considered as new targets for the treatment of T2D diabetes complications. Furthermore, lncRNAs may even cure T2D diabetes.

Conflict of interests

None.

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