Long Non-Coding RNA (LncRNA) CASC15 Is Upregulated in Diabetes-Induced Chronic Renal Failure and Regulates Podocyte Apoptosis

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Background: CASC15 has been recently characterized as an oncogenic lncRNA. This study aimed to investigate the role of CASC15 in diabetic patients complicated with chronic renal failure (DCRF).

Material/Methods: Levels of CASC15 in plasma derived from 3 groups of participants were measured by qPCR and compared by ANOVA and Tukey test. The interaction between CASC15 and miR-34c was analyzed by performing cell transfections. Cell apoptosis assay was performed to analyze the effects of transfections on the apoptosis of CIHP-1 cells (podocytes).

Results: We found that CASC15 in plasma was upregulated in DCRF compared with diabetic patients (no obvious complications) and healthy controls. Upregulation of CASC15 distinguished DCRF patients from healthy controls and diabetic patients. High D-glucose environment induced the upregulation of CASC15 in cells of the human podocyte cell line CIHP-1. Overexpression of CASC15 did not affect miR-34c in CIHP-1 cells, but bioinformatics analysis showed that CASC15 can sponge miR-34c. Overexpression of CASC15 led to an increased apoptotic rate of CIHP-1 cells, and miR-34c overexpression led to a decreased apoptotic rate of CIHP-1 cells. In addition, CASC15 overexpression attenuated the effects of miR-34c overexpression on cell apoptosis.

Conclusions: Therefore, CASC15 is upregulated in DCRF patients and promotes the apoptosis of podocytes by sponging miR-34c. Our study adds to our understanding of the pathogenesis of DCRF and suggests that CASC15 could serve as a potential therapeutic target of DCRF.

MeSH Keywords: Apoptosis • Kidney Failure, Chronic • MicroRNAs

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CLINICAL RESEARCH

Background

Diabetes is one of the most prevalent diseases in both developing and developed countries [1]. With changes in modern society, the number of people living with this disease has doubled during the past 20 years [1]. It is predicted that this number will continuously increase at least until 2030 [2]. Currently, diabetes is a major public health burden [3]. Compared to healthy adults without diabetes, a 50% higher risk of death was observed in adults with diabetes, largely due to the fact that almost all diabetic patients will develop complications during the progression of disease [4]. At present, prevention of diabetic complications is still critical for long-time survival of patients [5].

The long-term high-glucose environment in diabetic patients can seriously affect functions of the most important organs and cause development of multiple diabetic complications, such as diabetic chronic renal failure (DCRF) [6]. Under high-glucose conditions, wound-healing of kidney tissue is usually unsuccessful and chronic renal failure is common [6]. DCRF patients usually suffer from multiple functional disorders of the kidney, such as the apoptosis of renal cells [7]. Therefore, inhibition of renal apoptosis is a potential therapeutic approach for clinical treatment. It has been reported that DCRF requires the involvement of multiple genetic factors [8,9]. In a recent study, Liu et al. showed that miR-34c can target Notch signaling pathways to inhibit the apoptosis of podocytes induced by high glucose [10]. CASC15 is a well-studied oncogenic lncRNA; however, the role of CASC15 in renal injuries is unknown [11]. In our preliminary experiments, bioinformatics analysis revealed the potential targeting effect between CASC15 and miR-34c. This study aimed to investigate the exact interactions between CASC15 and miR-34c in DCRF.

Material and Methods

Research subjects

Research subjects of the present study included 50 DCRF patients (DCRF group, 26 males and 24 females; age 36–67 years, mean age 51.9±6.0 years), 50 diabetic patients without obvious complications (Diabetes group, 26 males and 24 females; age 35–68 years, mean age 52.3±6.5 years), and 50 healthy volunteers (Control group, 26 males and 24 females; age 36–68 years, mean age 52.0±6.3 years). All participants were selected at the Second Affiliated Hospital of Nanchang University between April 2016 and April 2018. All patients were diagnosed according to the criteria proposed by the Chinese Medical Association. This study was approved by the hospital Ethics Committee. Patient inclusion criteria were: 1) newly diagnosed cases, 2) no therapies were performed within 100 days before admission, and 3) CRF caused by diabetes without

complications. Exclusion criteria were: 1) complications were observed, and 2) other severe diseases were observed. The participants were informed about the details of the study and all of them signed written informed consent.

Plasma specimens

Before the initiation of therapies, 5 ml of blood was extracted under fasting conditions. To prepare plasma samples, blood was placed into EDTA tubes, which were centrifuged for 20 min at 1200 g to collect the supernatant.

Podocytes

The human podocyte cell line CIHP-1 (Ximbio) was used in this study. The reason we used podocytes is that glomerular function requires the involvement of podocytes, and chronic renal failure results in dysfunctional podocytes. CIHP-1 cells were cultivated in a mixture of 10% FBS and 90% RPMI-1640 under the conditions of 37°C, 95% humidity, and 5% CO_2. For high-glucose treatment, we added 5, 10, 20, and 30 mM D-glucose (Sigma-Aldrich) to the medium, and cells were harvested at 12, 24, and 48 h after the initiation of treatment.

Transient cell transfections

The CASC15 expression vector was constructed using pcDNA3.1 vector (Sangon, Shanghai, China). Negative control (NC) miRNA and miR-34c mimic were from Sangon. CIHP-1 cells were counted. The transient transfections were performed using lipofectamine 2000 reagent (Invitrogen, USA) to transfect 15 nM vectors (empty vector as NC group) or 35 nM miRNAs (NC miRNA as NC group) into 2×10^6 cells. The following experiments were performed using cells harvested at 24 h after transfection. For all transfections, control (C) cells were untransfected cells.

RNA extractions and qPCR

Total RNAs in 0.3 ml plasma and 2×10^5 cells were extracted using RNAzol (Sigma-Aldrich). To harvest miRNAs, 85% ethanol was used to precipitate all RNA samples. All RNA samples were digested with DNase I for 80 min at 37°C to completely remove genomic DNA. The digested DNA samples were reverse-transcribed into cDNA using Tetro Reverse Transcriptase (Bioline), and cDNA was used as a template to prepare qPCR mixtures using Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent Technologies). With 18S rRNA as an endogenous control, the expression levels of CASC15 were detected. To measure the expression levels of miR-34c, all steps were completed using the All-in-OneTM miRNA qRT-PCR Detection Kit (GeneCopoeia). U6 was used as the endogenous control. The $2^{-\Delta\Delta CT}$ method was used to process all data. All PCR reactions were performed in triplicate.
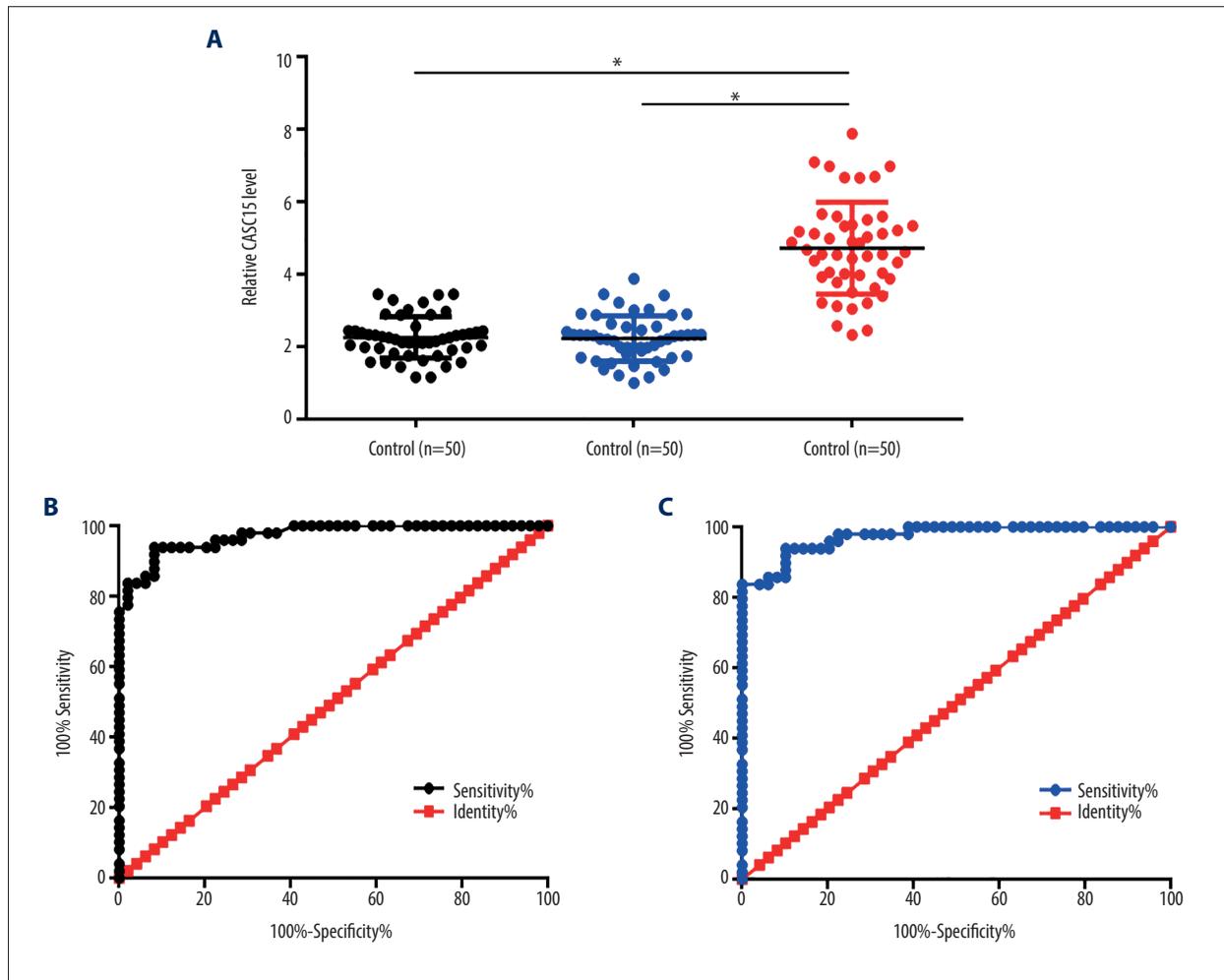
Figure 1. Plasma CASC15 was upregulated in DCRF and showed diagnostic values. Levels of CASC15 in plasma derived from 3 groups of participants (DCRF, Diabetes, and Control) were measured by qPCR and compared by ANOVA (one-way) and Tukey test (**A**). In ROC curve, true-positive cases were DCRF patients and true-negative cases were either diabetic patients (**B**) or healthy controls (**C**). Experiments were performed in 3 replicates and mean values are presented, * p<0.05.

Cell apoptosis analysis

CIHP-1 cells were harvested at 24 h after transfection and counted. To prepare a single-cell suspension, 4×10^3 cells were mixed with 1 ml of the cell culture medium. Cells were transferred to a 6-well plate with 2 ml cell suspension per well, followed by the addition of 30 mM D-glucose. Cells were cultivated for 48 h under the aforementioned conditions. After that, cells were washed with PBS, and 0.25% trypsin digestion was performed. Finally, cells were stained with Annexin V-FITC and propidium iodide (PI) for 20 min in the dark, and flow cytometry was then performed to separate apoptotic cells.

Statistical analysis

The mean values of 3 biological replicates were calculated and were used in all data analyses. Differences were explored among different patient and cell groups by performing ANOVA (one-way) and Tukey test. The diagnostic analysis was performed using ROC curves. In ROC curves, true-positive cases were DCRF patients and true-negative cases were either diabetic patients or healthy controls. p<0.05 was statistically significant.

Results

Plasma CASC15 was upregulated in DCRF and showed diagnostic values

Levels of CASC15 in plasma derived from 3 groups of participants (DCRF, Diabetes, and Control) were measured by qPCR and compared by one-way ANOVA and Tukey test. Comparing to the Control group, significantly higher plasma levels of CASC15 were observed in the DCRF group but not in the
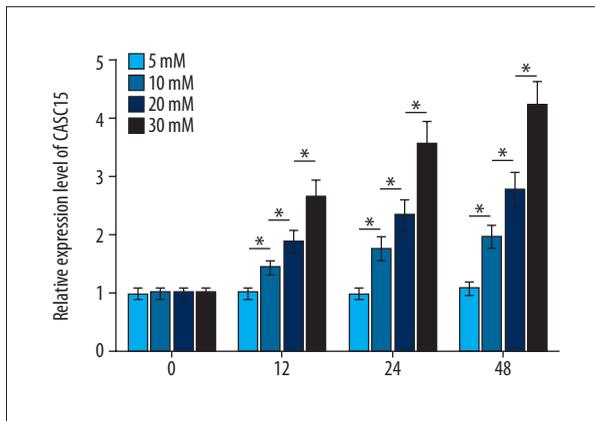
Figure 2. Upregulation of CASC15 was induced by high glucose in CIHP-1 cells. CIHP-1 cells were treated with 5, 10, 20, and 30 mM D-glucose for 12, 24, and 48 h, followed by measuring the expression levels of CASC15 by qPCR. Data were compared by ANOVA (one-way) and Tukey test. Experiments were performed in 3 replicates and mean values are presented, * p<0.05.
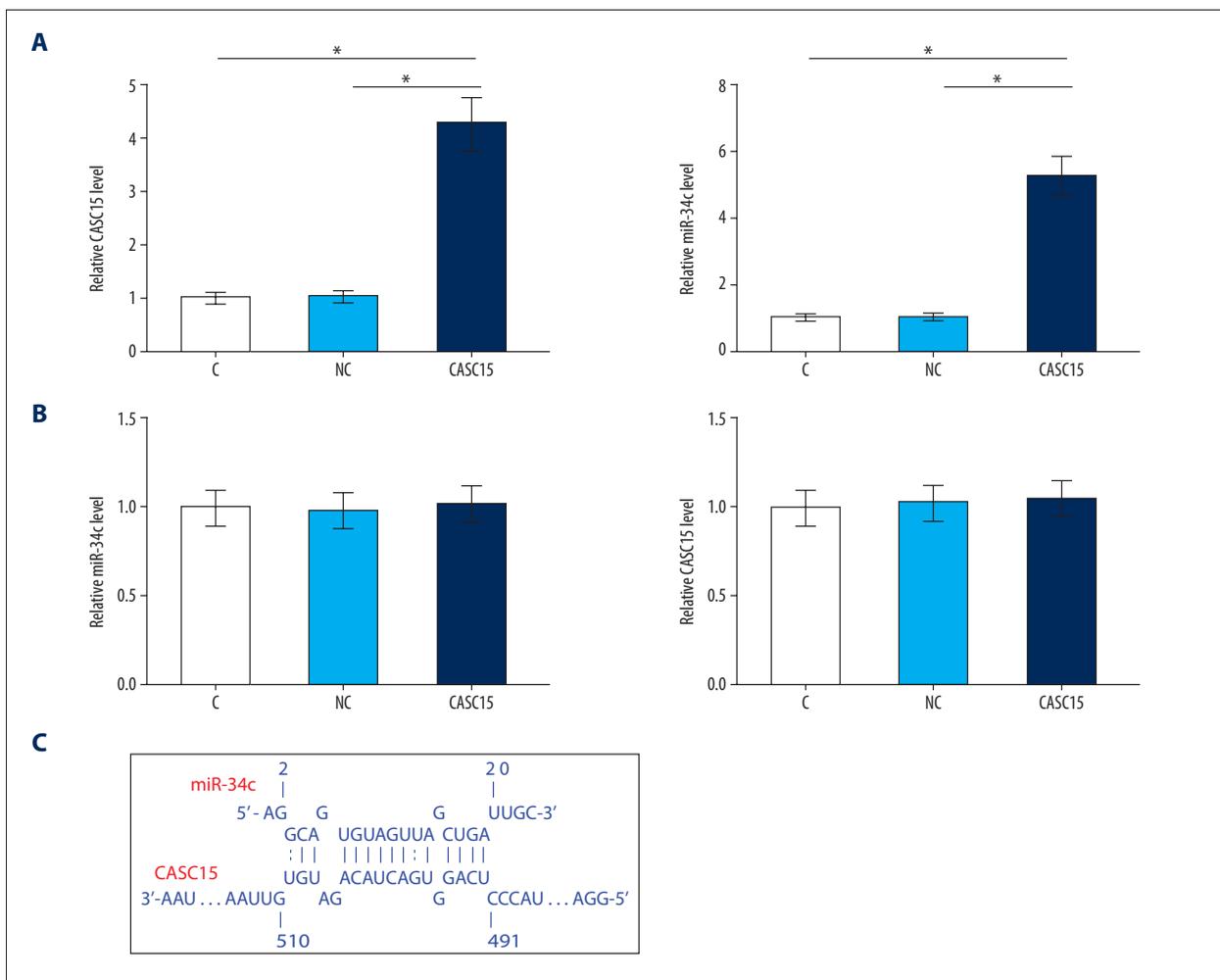

Figure 3. CASC15 can sponge miR-34c. CIHP-1 cells were transfected with the CASC15 expression vector and miR-34c mimic. Overexpression of CASC15 and miR-34c was confirmed at 24 h after transfection by qPCR (**A**). The interaction between CASC15 and miR-34c was analyzed by qPCR (**B**). IntaRNA was used to predict the interaction between miR-34c and CASC15 (**C**). Experiments were performed in 3 replicates and mean values are presented, * p<0.05.
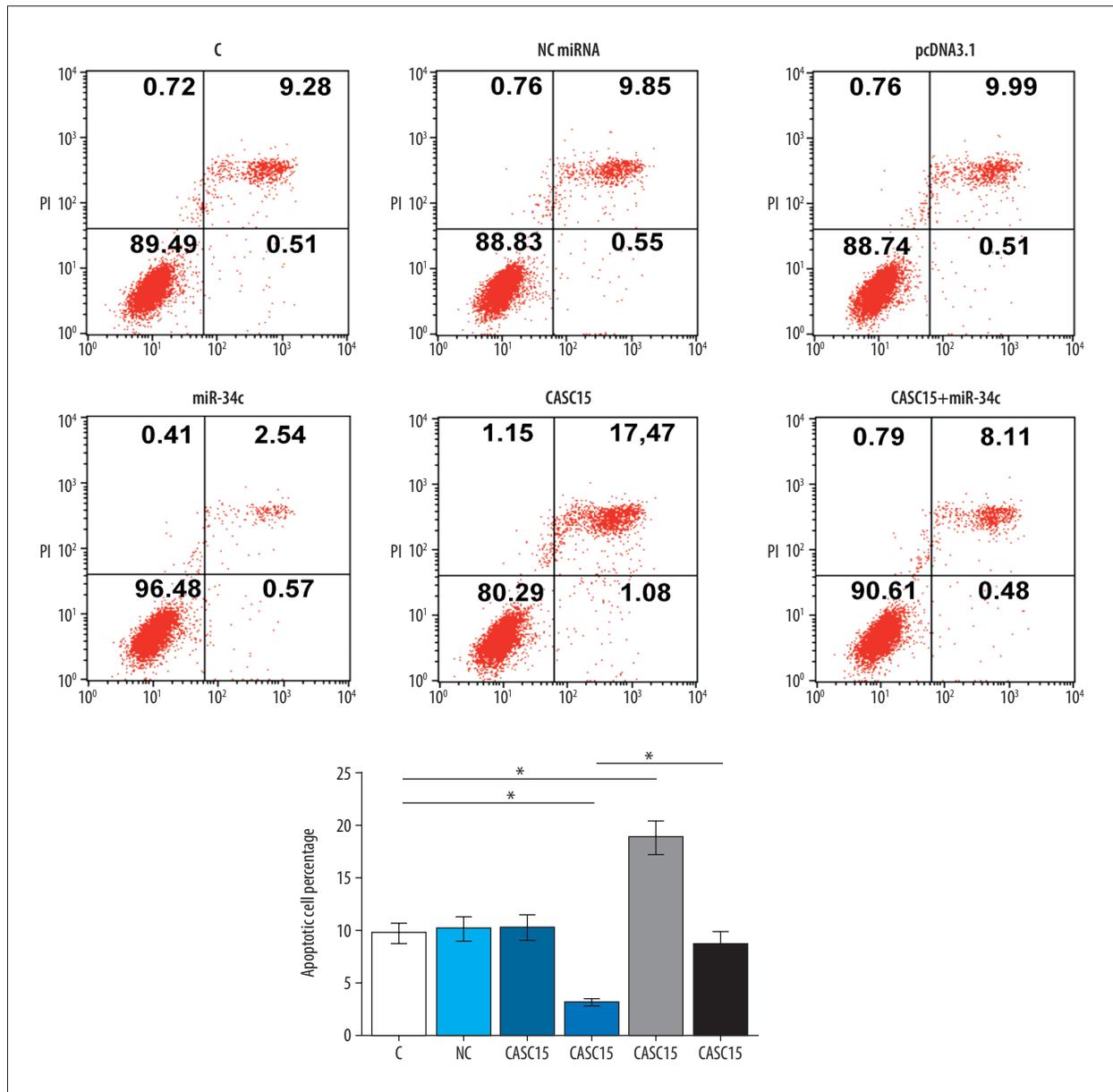
Figure 4. CASC15 attenuated the effects of miR-34c on cell apoptosis. Cell apoptosis assay was performed to analyze the effects of transfections on apoptosis of CIHP-1 cells. Experiments were performed in 3 replicates and mean values are presented, * p<0.05.

Diabetes group (Figure 1, p<0.05). The diagnostic value of plasma CASC15 for DCRF was analyzed by ROC curve. AUC>0.65 indicates potential diagnostic value. With diabetic patients as true-negative cases, the AUC was 0.97 (95% confidence interval: 0.95–0.99; standard error: 0.013). With healthy controls as true-negative cases, the AUC was 0.97 (95% confidence interval: 0.95–1.00; standard error: 0.013). AUC>0.65 indicated diagnostic values. Therefore, plasma CASC15 can serve as a diagnostic marker for DCRF.

Upregulation of CASC15 was induced by high glucose in CIHP-1 cells

CIHP-1 cells were treated with 5, 10, 20, and 30 mM D-glucose for 12, 24, and 48 h, followed by measuring the expression levels of CASC15 by qPCR. As shown in Figure 2, expression levels of CASC15 in CIHP-1 cells were upregulated by D-glucose in a dose- and time-dependent manner (Figure 2, p<0.05). However, 5 mM D-glucose failed to affect CASC15 expression, possibly because 5 mM is within the normal range.

CASC15 can sponge miR-34c

CIHP-1 cells were transfected with the CASC15 expression vector and miR-34c mimic. Expression levels of CASC15 and miR-34c were measured at 24 h after transfection. Compared to the C and NC groups, expression levels of CASC15 and miR-34c were significantly increased after transfections (Figure 3A, p<0.05). However, overexpression of CASC15 and miR-34c failed to affect the expression of each other (Figure 3B). Bioinformatics analysis using IntaRNA showed that miR-34c can bind CASC15 from position 492 to 509 (Figure 3C).

CASC15 attenuated the effects of miR-34c on cell apoptosis

Cell apoptosis assay was performed to analyze the effects of transfections on apoptosis of CIHP-1 cells. Compared to the C and NC groups, overexpression of CASC15 led to increased apoptotic rate of CIHP-1 cells and miR-34c overexpression led to a decreased rate. In addition, CASC15 overexpression attenuated the effects of miR-34c overexpression on cell apoptosis (Figure 4, p<0.05).

Discussion

In this study, we investigated the functions of CASC15 in DCRF and also analyzed the clinical value. We found that CASC15 was upregulated in DCRF and can sponge miR-34c to promote the apoptosis of podocytes.

Previous studies have revealed many lncRNAs involved in the development of renal disorders. For instance, lncRNA Erbb4-IR can target miR-29b to promote renal injury under a high-glucose environment [12]. lncRNA MALAT1 was reported to be upregulated in diabetic patients, and downregulation of MALAT1 can promote recovery of renal functions in a rat model [13]. In another study, Wang et al. showed that p53 can downregulate lncRNA ZEB1-AS1 to participate in renal fibrosis in patients with diabetic nephropathy [14]. CASC15 is a well-characterized oncogenic lncRNA [11,15]. CASC15 is upregulated in cancers, and the overexpression of CASC15 promotes cancer progression by regulating cancer cell behaviors, such as inhibition of cell apoptosis [15]. The present study is the first to report the upregulation of CASC15 and the increased cell apoptosis rate after CASC15 overexpression. Therefore, CASC15 can contribute to the development of DCRF. Our study also showed that CASC15 can have opposite roles in regulating cell apoptosis in different diseases.

Our study did not observe the upregulation of CASC15 in plasma derived from diabetic patients. However, a high-glucose environment led to significantly upregulated expression of CASC15 in podocytes. The possible reason is that the upregulated CASC15 in podocytes fails to affect the plasma levels of CASC15 in diabetic patients. With the development of DCRF, CASC15 is further upregulated and more CASC15 is released into blood, resulting in increased plasma levels of CASC15.

This study showed that the upregulation of plasma CASC15 could be used to assist in diagnosis of DCRF, but the accuracy remains to be further analyzed. We showed that CASC15 can interact with miR-34c, while overexpression experiments showed that CASC15 and miR-34c have no effects on the expression of each other. Therefore, it appears that miR-34c does not target CASC15. It is known that lncRNAs can sponge miRNAs to attenuate their functions [16,17]. The main role of a miRNA sponge is to inhibit the roles of miRNA. This study revealed that CASC15 attenuated the effects of miR-34c on cell apoptosis. Therefore, CASC15 can sponge miR-34c to promote the apoptosis of podocytes.

CASC15 is dysregulated in multiple clinical disorders, such as different types of cancers and cardiac hypertrophy [11,15,18–20]. Therefore, the use of CASC15 as a diagnostic marker for DCRF should exclude the possibility of these clinical disorders. In addition, this study was limited by its small sample size. Studies with larger sample sizes are needed to confirm our conclusions.

Conclusions

In conclusion, CASC15 was upregulated in DCRF and can sponge miR-34c to promote the apoptosis of podocytes. Our study provides novel insights into the molecular mechanism of DCRF and suggests potential therapeutic targets.

Availability of data and materials

The analyzed datasets generated during the study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University. The research was carried out in accordance with the World Medical Association Declaration of Helsinki. All patients and healthy volunteers provided written informed consent prior to their inclusion in the study.

Conflict of interests

None.

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