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LncRNA-NR_033515 promotes proliferation, fibrogenesis and epithelial-to-mesenchymal transition by targeting miR-743b-5p in diabetic nephropathy

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ABSTRACT

Diabetic nephropathy (DN) is a crucial microvascular complication of diabetes. Long non-coding RNAs (lncRNAs) participate in the occurrence and development of various diseases, but the function and regular mechanism of lncRNA-NR_033515 in DN is still unclear. In the present study, we demonstrated that the expression of NR_033515 was significantly increased in the serum of DN patients and was related to the different stages of DN. NR_033515 was also positively associated with diagnostic markers of DN (KIM-1 and NGAL). Overexpression of NR_033515 promoted proliferation, and inhibited apoptosis of MMC cells and increased the expression levels of proliferation-related genes. NR_033515 also accelerated the expression levels of fibrogenesis-related genes. TGF-β1 enhanced NR_033515-induced Epithelial-mesenchymal transition (EMT), while NR_033515 over-expression accelerated TGF-β1-induced EMT. Furthermore, we found that NR_033515 promoted cell proliferation and regulated P38, ASK1, Fibronectin, α-SMA, E-cadherin, and Vimentin expressions by miR-743b-5p. Therefore, our data indicated the potential role of NR_033515 in the proliferation, fibrogenesis and EMT in DN. NR_033515 could be a pivotal potential diagnostic and therapeutic target for the treatment of DN.

1. Introduction

Diabetes mellitus (DM) is a kind of metabolic disease resulting from defects in insulin secretion or insulin action. Due to chronic hyperglycemia, numerous complications occur in DM patients that produce long-term damage, dysfunction, and failure in many different organs, including the eyes, kidney, and the heart [1]. DN, identified as one of the most serious complications of DM, is characterized by the accumulation of extracellular matrix (ECM) proteins, thickening of basement membranes, renal fibrosis, and the activation of myofibroblasts [2,3]. Epithelial-to-mesenchymal transition (EMT), involved in the pathogenesis of renal interstitial fibrosis, podocyte dysfunction, and glomerulosclerosis, is an indispensable process during the development of DN [4]. During the EMT process, epithelial cells obtain mesenchymal cell features, resulting in the loss of epithelial markers, the acquisition of mesenchymal markers, and the increased deposition of ECM and renal interstitial fibrosis [5,6]. It has been reported that DN is the primary cause of end-stage renal disease (ESRD), accounting for millions of deaths in both developed and developing countries. Hyperglycemia is considered to be the driving force for the progression of DN, but actually, the pathogenesis of DN is extremely complex, involving many mechanisms and factors, such as inflammation and oxidative stress [7,8]. Current treatments for DN mainly include controlling blood glucose or interfering with the renin-angiotensin-aldosterone system by angiotensin-receptor blockers with very limited effects [9]. However, the exact cause of DN remains unclear.

Long non-coding RNAs (lncRNAs) play a key regulatory role in numerous biological processes and are a subset of RNAs of over 200 nucleotides that lack the ability to encode protein [10]. Previous studies have demonstrated that lncRNAs were involved in a variety of human diseases, including diverse cancers and metabolic disorders [11]. Recently, Weiping Lu et al. reported that lncRNAs (CYP4B1-PS1-001) were associated with the regulation of proliferation and fibrosis in DN [12]. However, whether the novel lncRNA-NR_033515 is involved in the development of DN remains largely unknown.

In this present study, the qRT-PCR results showed that compared to normal patients, lncRNA-NR_033515 was significantly up-regulated in the serum of DN patients, and the expression level was associated with the clinical stages of DN. We also found that the mRNA expression levels of KIM-1 and NGAL were up-regulated in the serum of DN patients, and their expression level exhibited a positive correlation with lncRNA-NR_033515, suggesting that KIM-1 and NGAL may act as two markers in the diagnosis of DN. Furthermore, we demonstrated that lncRNA-NR_033515 promoted cell proliferation and fibrogenesis, as well as

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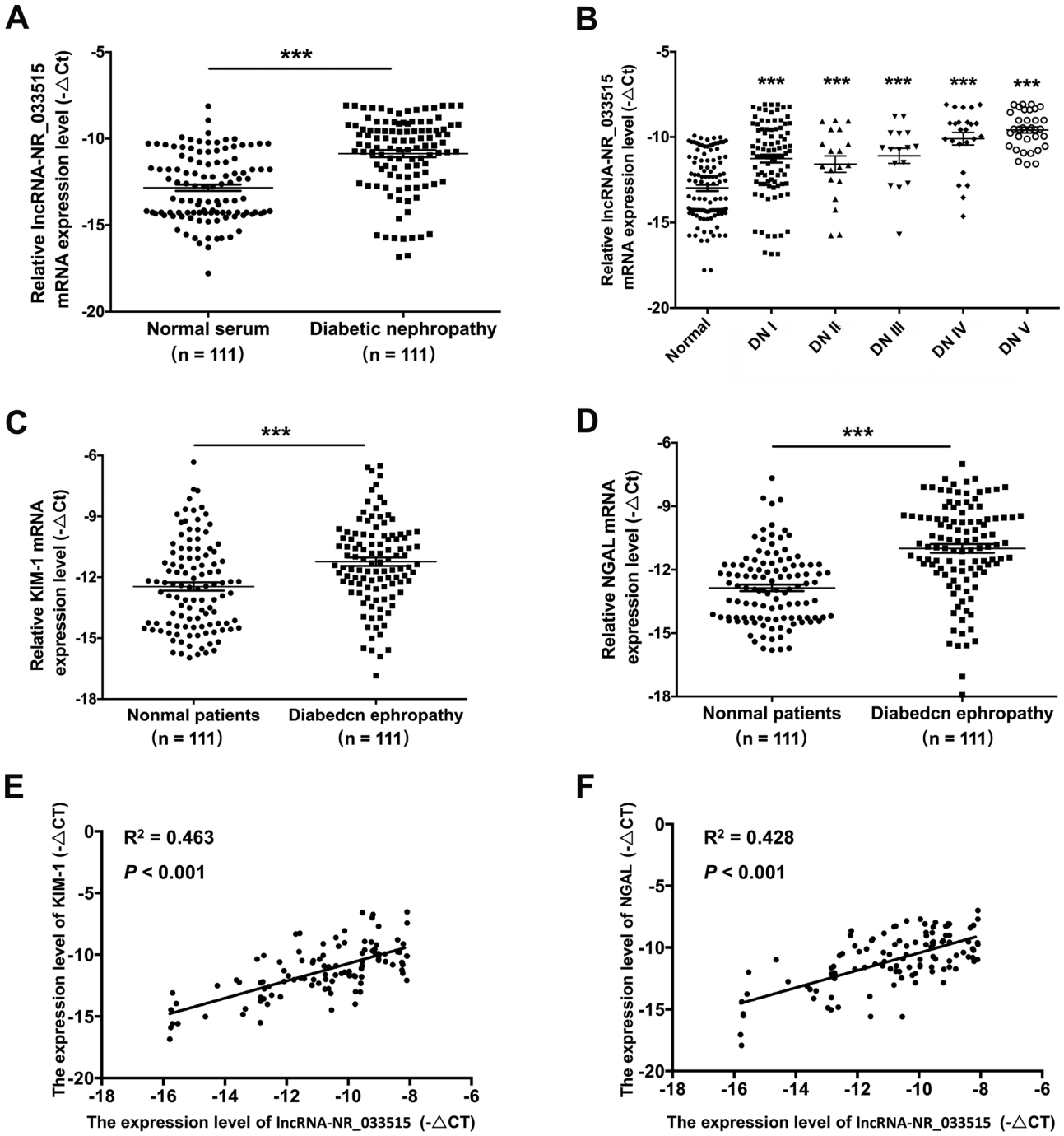

Fig. 1. LncRNA-NR_033515 was significantly up-regulated in the serum of DN patients. (A) The mRNA expression level of NR_033515 was detected by qRT-PCR in normal patient serum (n = 111) and DN patient serum (n = 111). The expression level was shown with the –ΔCt (CT_{GAPDH} - $CT_{objective\ gene}$) (***$P < 0.001$). (**B**) The graph shows the expression level of NR_033515 in different stages of DN (I stage, n = 18; II stage, n = 19; III stage, n = 16; IV stage, n = 24; V stage, n = 35) and the normal group (n = 37). Significant differences were observed between NR_033515 expression and clinical stages. The data were normalized to GAPDH, and the expression level was shown with the –ΔCt (CT_{GAPDH} - $CT_{objective\ gene}$) (***$P < 0.001$). **(C)** The mRNA expression level of KIM-1 was further measured by qRT-PCR in normal patient serum (n = 111) and DN patient serum (n = 111), (***$P < 0.001$). **(D)** NGAL expression was also detected by qRT-PCR (***$P < 0.001$). (**E**) The correlation analysis between NR_033515 and KIM-1 expression was analyzed by the Pearson's correlation algorithm (n = 111, $R^2 = 0.463$, $P < 0.001$). (**F**) The correlation analysis between NR_033515 and NGAL expression was analyzed by the Pearson's correlation algorithm (n = 111, $R^2 = 0.428$, $P < 0.001$).

increased production of PCNA, cyclin D1, p38 and ASK1 in high glucose conditions. We found that lncRNA-NR_033515 decreased the expression level of epithelial cell marker (E-cadherin) and increased the expression level of mesenchymal cell marker (Vimentin), indicating that it promoted the EMT process during DN. Additionally, NR_033515 promoted cell proliferation, fibrogenesis, and the EMT process by miR-743b-5p.

2. Results

2.1. LncRNA-NR_033515 was significantly up-regulated in serum of DN patients

According to previous results [13], microarray analysis of long noncoding RNA expression in diabetic nephropathy found that lncRNA-