KLF11 enhances the effect of liraglutide in high glucose-induced Schwann cells by regulating endoplasmic reticulum stress and autophagy through inactivation of P38 MAPK

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Abstract

Purpose: To investigate the effect of Kruppel-Like Factor 11 (KLF11) on diabetic peripheral neuropathy (DPN).

Methods: Schwann cells, RSC96, were treated with high glucose to induce DPN. Cell viability and apoptosis were evaluated by flow cytometry. Expressions of KLF11 and proteins involved in endoplasmic reticulum (ER) stress and autophagy were evaluated by western blot.

Results: The KLF11 was downregulated in high glucose-induced RSC96. Cell viability of RSC96 was decreased and apoptosis increased by high glucose. Overexpression of KLF11 restored viability and decreased apoptosis of high glucose-induced RSC96. High glucose-induced an increase in proteins involved in ER stress in RSC96, but reversed by KLF11 overexpression. Overexpression of KLF11 also attenuated high glucose-induced decrease in LC3 and Beclin1 expression in RSC96 cells. Phosphorylated p38 (p-p38) in high glucose-induced RSC96 decreased upon overexpression of KLF11. Liraglutide treatment increased cell viability and autophagy, decreased cell apoptosis, and inhibited ER stress in high glucose-induced RSC96. Furthermore, KLF11 enhanced the protective activity of liraglutide against high glucose-induced cytotoxicity in Schwann cells.

Conclusion: Overexpression of KLF11 exerts a neuroprotective effect against DPN by promoting autophagy and inhibiting ER stress via inactivation of p38 signaling, suggesting that KLF11 might be a target for DPN.

Keywords: KLF11, High glucose, Schwann cells, Diabetic Peripheral neuropathy, autophagy, ER stress, p38

INTRODUCTION

Diabetic peripheral neuropathy (DPN) is a common and refractory complication of diabetes [1], in which patients experience foot ulceration and amputation, fall-related injury, gait instability, and sensory loss [1]. DPN is characterized by dysfunction of the peripheral nerves with unclear
pathogenesis and increasing morbidity and mortality [2]. Schwann cell dysfunction has been implicated in abnormal nerve regeneration and repair associated with diabetes [3]. DPN and hyperglycemia stimulate Schwann cell apoptosis through pathological processes [4], and the regulation of these pathological processes reduces Schwann cell apoptosis, thus protecting against DPN [4].

Kruppel-like factor 11 (KLF11), a member of the KLF family, functions as a zinc finger transcription factor which regulates cell growth, apoptosis, oxidative stress, glucose metabolism, and other physiological processes [5]. Knockout of KLF11 enhanced cerebrovascular permeability and edema, which aggravated blood–brain barrier damage, indicating that KLF11 exerts neuroprotective effects on ischemic injuries [5]. KLF11 was involved in pancreatic beta cell function, and is associated with diabetes [6]. KLF11 repressed gluconeogenesis and reduced cellular glucose output, thereby improving hyperglycemia and glucose intolerance in diabetic mice [7]. Moreover, KLF11 expression was downregulated in sciatic nerve tissues of streptozotocin-stimulated diabetic peripheral neuropathy rats [8]. Therefore, it has been hypothesized that KLF11 is involved in the pathogenesis of DPN.

This study aims to examine the effect of KLF11 on Schwann cell apoptosis-related ER stress and autophagy, and the underlying mechanism, in order to identify potential therapeutic targets for DPN.

EXPERIMENTAL

Cell culture and treatment

The RSC96 was cultured in DMEM containing 10% fetal bovine serum (GIBCO, Island, NY, USA) in a 37 °C incubator. The cells were treated with 25, 50, or 125 mM glucose for 24 h before functional analyses.

Quantitative reverse transcription-polymerase chain reaction(qRT-PCR)

After glucose exposure, RNAs were isolated from RSC96 cells, and reverse-transcribed into cDNAs. RT-PCR analysis was carried out with the primers listed in Table 1.

Cell transfection, viability, and apoptosis

The RSC96 was seeded into 96-well plates and incubated with 125 mM glucose for 24 h. The cells were then transfected with pcDNA-KLF11 or pcDNA vector (Genepharma, Suzhou, China). Transfected cells were then treated with 0.1 μM liraglutide (Sigma-Aldrich, St. Louis, MO, USA). After 48 h, the cells were incubated with 10μL MTT solution (Beyotime, Beijing, China; final 0.5 mg/mL) for 4 h, and absorbance was measured at 570 nM. Transfected RSC96 cells were harvested, and stained with annexin-V-fluorescein and PI (Roche, Penzberg, Germany). Cell apoptosis was assessed by MTT assay.

Western blot assay

The RSC96 cells were lysed with RIPA lysis buffer (Beyotime) and centrifuged, and protein concentrations of the lysates were measured, separated, and transferred onto nitrocellulose membranes. The membranes were blocked and then probed with specific antibodies: anti-KLF11 and anti-β-actin (1:2000, Abcam, Cambridge, UK), anti-GRP-78 and anti-XBP-1 (1:2500, Abcam), anti-ATF-6 and anti-ATF-4 (1:3000, Abcam), anti-CHOP and anti-LC3 (1:3500, Abcam), anti-Beclin1, anti-p38 and anti-p-p38 (1:4000, Abcam). Membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:4500, Abcam), and the protein bands were visualized.

Statistical analysis

Data from at least three independent experiments were presented as mean ± SEM, and analyzed using Student’s t-test or one-way analysis of variance using SPSS software. P < 0.05 was considered statistically significant.

RESULTS

KLF11 was downregulated in Schwann cells induced with high glucose

KLF11 mRNA expression was decreased in RSC96 after glucose treatment (Figure 1 A). Glucose treatment also downregulated KLF11 protein expression in RSC96 cells (Figure 1 B). The data suggest an association between KLF11 and Schwann cell dysfunction.

Table 1: Primers used in PCR

<table>
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<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
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<tr>
<td>KLF1</td>
<td>5′-CTCCTGCAGGGCC-3′</td>
<td>5′-GGGGAACAGGCC-3′</td>
</tr>
<tr>
<td>β-actin</td>
<td>5′-CCGCTTCCCCCTCC-3′</td>
<td>5′-GTCCCGATGGTG-3′</td>
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KLF11 was downregulated in high glucose-induced Schwann cells. (A) After treatment with glucose, KLF11 mRNA expression in RSC96 cells was decreased; (B) Following treatment with glucose, KLF11 protein expression in RSC96 cells was decreased. *P < 0.05, ***p < 0.001 vs 0 mM glucose

KLF11 induced apoptosis of Schwann cells in high glucose conditions

The RSC96 in high glucose was transfected with pcDNA-KLF11, and an increased KLF11 protein expression in the transfected cells was observed (Figure 2 A). Treatment with high glucose decreased RSC96 cell viability (Figure 2 B), but increased the cell viability of RSC96 transfected with pcDNA-KLF11 (Figure 2 B). Moreover, increase in RSC96 cell apoptosis after treatment with high glucose was reversed by overexpression of KLF11 (Figure 2 C and D), thus demonstrating the anti-apoptotic effect of KLF11 in Schwann under high glucose conditions.

KLF11 repressed ER stress in Schwann under high glucose

The expressions of ER stress-related proteins, including GRP-78, XBP-1, ATF-6, ATF-4, and CHOP, increased in RSC96 treated with high glucose (Figure 3). However, the overexpression of KLF11 reduced GRP-78, XBP-1, ATF-6, ATF-4, and CHOP in high glucose-induced RSC96 cells (Figure 3), leading to the repression of endoplasmic reticulum stress.

KLF11 enhanced autophagy of Schwann cells under high glucose

The expression of autophagy-related proteins, i.e, LC3 and Beclin1, decreased in RSC96 cells exposed to high glucose (Figure 4). However, the overexpression of KLF11 increased LC3 and Beclin1 in high glucose-induced RSC96 (Figure 4). Therefore, KLF11 has a pro-autophagic effect on Schwann cells under high glucose.
**KLF11 repressed p38 signaling in Schwann under high glucose**

Protein expression of p38 in RSC96 cells was not affected by exposure to high glucose (Figure 5). However, phosphorylated p38 was upregulated in RSC96 cells after exposure to high glucose (Figure 5). Furthermore, the overexpression of KLF11 decreased the phosphorylated p38 levels in RSC96 treated with high glucose (Figure 5).

**Figure 5:** KLF11 repressed the activation of p38 signaling in RSC96 treated with high glucose. The overexpression of KLF11 attenuated high glucose-induced increase in p-p38 in RSC96. ***P < 0.001 vs control; +++p < 0.001 vs high glucose-treated Schwann cells with pcDNA empty vector transfection

**KLF11 enhanced the protective effect of liraglutide against high glucose-induced cytotoxicity in Schwann cells**

Liraglutide increased the viability (Figure 6 A) and decreased the cell apoptosis (Figure 6 B and C) of RSC96 treated with high glucose. However, the overexpression of KLF11 enhanced the liraglutide-induced increase in cell viability (Figure 6 A) and decrease in apoptosis (Figure 6 B and C) of high glucose-induced RSC96 cells. KLF11 overexpression further reduced liraglutide-induced decreases in GRP-78, XBP-1, and ATF-6 expression in high glucose-induced RSC96 cells (Figure 6 D). Moreover, KLF11 enhanced the liraglutide-induced increases in LC3 and Beclin1 in RSC96 cells treated with high glucose (Figure 6 E).

**Figure 6:** KLF11 enhanced the protective activity of liraglutide against high glucose-induced cytotoxicity in Schwann cells. (A) Overexpression of KLF11 enhanced the liraglutide-induced increase in cell viability of RSC96 treated with high glucose; (B) Overexpression of KLF11 enhanced the liraglutide-induced decrease in cell apoptosis of RSC96 treated with high glucose; (C) Relative apoptosis rates of high glucose-induced RSC96 cells with or without pcDNA-KLF11 transfection and liraglutide treatment; (D) The overexpression of KLF11 enhanced the liraglutide-induced increase in LC3 and Beclin1 expression in RSC96 treated with high glucose. ***P < 0.001 vs control; +++p < 0.001, +++p < 0.01, +++p < 0.001 vs high glucose-treated Schwann cells with pcDNA empty vector transfection; #p < 0.05, ##p < 0.01, ###p < 0.001 vs HG+0.1 μM liraglutide

In this study, RSC96 cell viability decreased and cell apoptosis increased, following treatment under high glucose. A previous study showed that KLF11 was downregulated in a rat model of DPN [8]. The expression of KLF11 decreased in RSC96 treated with high glucose. Moreover, overexpression of KLF11 increased the viability and repressed the apoptosis of RSC96 cells treated with high glucose. These results suggest that KFL11 plays an anti-apoptotic role against diabetic peripheral neuropathy. Diabetic hyperglycemia stimulates ER stress in peripheral nerve cells, and contributes to the development of diabetic peripheral neuropathy [10]. Expressions of ER stress-related proteins increased in the Schwann cells exposure to high glucose conditions, and downregulation of ER stress-related proteins reduced the apoptosis of Schwann cells [11]. Thus, KLF11 protects against endoplasmic reticulum stress in chondrocytes [12]. Overexpression of KLF11 attenuated high glucose-induced increases in ER

**DISCUSSION**

Schwann is sensitive to insulin and glucose concentrations, and Schwann cell dysfunction is implicated in the development of DPN [4]. A previous study showed that KLF11, a diabetes-related transcription factor, was downregulated in rats with diabetic peripheral neuropathy [8]. The effect of KLF11 on Schwann cells was investigated, and it was discovered that high glucose treatment reduced Schwann cell viability and promoted cell apoptosis [9], and high glucose-treated Schwann has been used as model of DPN [9].
stress-related proteins in RSC96 cells, protecting against high glucose-induced ER stress in Schwann cells. High glucose reduced autophagosomes and the expression of autophagy markers in Schwann cells [13]. Defects in autophagy induced endoplasmic reticulum stress, and increased cell apoptosis [14]. High glucose-induced decreases in LC3 and Beclin1 expression were restored by the overexpression of KLF11, indicating that KLF11 has a pro-autophagic effect on diabetic peripheral neuropathy. Liraglutide reduced glucolipotoxicity-induced inflammation and dysfunction in RSC96 cells [15], while KLF11 enhanced the protective role of liraglutide against high glucose-induced cytotoxicity in Schwann cells by increasing cell viability and autophagy, and decreasing cell apoptosis and endoplasmic reticulum stress. However, high glucose-induced oxidative stress and inflammation also contributed to the apoptosis of Schwann cells in DPN [4]. The effect of KLF11 on oxidative stress and the inflammation of high glucose-treated RSC96 cells should be investigated in further research.

MAPK regulated autophagy through ERK signaling; inhibition of MAPK produced antinociceptive action in diabetic neuropathy [16]. Activation of MAPK contributes to Schwann cell apoptosis [17], as well as inactivation of p38 MAPK relieved oxidative stress, inflammation, and peripheral nerve dysfunction in diabetic peripheral neuropathy [18]. In addition, KLF11 inhibits p38 MAPK and prevents osteoarthritis [12]. In this study, phosphorylated p38 in RSC96 cells treated under high glucose conditions was downregulated by overexpression of KLF11, indicating the suppressive effect of KLF11 on p38 signaling in diabetic peripheral neuropathy.

CONCLUSION

The findings of this study show that KLF11 exerts anti-diabetic effect in RSC96 cells treated with high levels of glucose by promoting cell autophagy, as well as suppressing apoptosis and endoplasmic reticulum stress. Inactivation of p38 signaling is also implicated in KLF11-mediated Schwann cell dysfunction in high glucose conditions. However, the in vivo effect of KLF11 on streptozotocin-induced diabetic animals should be investigated in further research.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

This work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Yuanyuan Xiao, Zhifeng Jiang, Chaoyu Zhu, and Fusong Jiang designed the study; Yuanyuan Xiao, Zhifeng Jiang, Chaoyu Zhu, Fusong Jiang, Qingge Gao, Shouxia Li, and Wenjing Song supervised the data collection, and analyzed and interpreted the data. Yuanyuan Xiao and Li Wei prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript for publication.

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