Fibroblast Growth Factor 21 Ameliorates Hyperuricemic Nephropathy by Improving Oxidative Stress through Activating Akt/Nrf2 Signaling Pathway

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Abstract

Epidemiological investigations have shown an elevated expression of fibroblast growth factor 21 (FGF21) in the serum of patients with hyperuricemia. However, the effect of FGF21 on hyperuricemic nephropathy is still unknown. The purpose of this study, therefore, was to explore the effect and mechanism of action of FGF21 on hyperuricemic nephropathy. The level of FGF21 in PBMCs was determined in 10 patients with hyperuricemic nephropathy. Hyperuricemic mice models were induced in wild-type C57BL/6 and FGF21 knockout mice. Six mice in each group were treated with FGF21 at a dose of 1mg/kg and 5mg/kg for 30 days. For the *in vitro* studies, glomerular mesangial cells were exposed to lipopolysaccharide and monosodium uric acid to induce inflammation. This was followed by treatment with 100nM, 1000nM of FGF21 for 72 h to observe the therapeutic effect. The levels of FGF21 in patients with hyperuricemic nephropathy were elevated. Also, FGF21 knockout mice experienced more severe nephropathy compared to the WT mice. This was characterized by an increase in inflammatory factors and fibrosis in the kidney, which was reversed by exogenous FGF21 treatment. FGF21 recorded a significant therapeutic effect through the activation of Akt/Nrf2 signal pathway in both *in vivo* and *in vitro* studies. However, the effect increasing effect of FGF21 on Nrf2 was reduced by the addition of Akt inhibitor GSK690693. In conclusion, our study found for the first time that FGF21 can significantly improve hyperuricemic nephropathy through the promotion of the Akt/Nrf2 signalling pathway leading to improvement in oxidative stress.

Introduction

Hyperuricemic nephropathy is a kidney disease characterized by primary or secondary hyperuricemia accompanied by interstitial inflammation, fibrosis, renal calculi, and acute or chronic renal failure caused by uric acid (or urate) deposition in the kidney [1]. Uric acid is the final product of purine metabolism, and about 70% of it is excreted through the kidney [2]. When the concentration of uric acid exceeds the normal physiological range, it leads to various pathological reactions. Xanthine oxidoreductase (XOR) is an important enzyme involved in the degradation of purine nucleotides. It oxidizes hypoxanthine to xanthine, which in turn catalyzes the oxidation of xanthine to uric acid. In this process, reactive oxygen species (ROS) are produced as by-products with their accumulation leading to oxidative stress [3]. Studies have shown that oxidative stress can activate inflammation and fibrotic pathways leading to kidney damage [3–5]. Therefore, the inhibition of oxidative stress can significantly improve renal injury in hyperuricemia. Studies have also shown a yearly increase in the global incidence of hyperuricemia and it has become a metabolic disease second to diabetes and hyperlipidemia [6]. Selective XO inhibitors, febuxostat, and allopurinol are currently used in the clinical management of hyperuricemia and its related complications [7]. However, the long-term use of these drugs in some people leads to allergic reactions, progressive decline of red blood cells, diarrhoea, vomiting, and other adverse reactions accompanied by serious nephrotoxicity. These observations lead to apathy for these drugs in patients with hyperuricemic nephropathy [8, 9]. Therefore, it is necessary for the search for novel therapeutics for the management and treatment of hyperuricemic nephropathy.

FGF21 was first isolated from mouse embryos by Nishimura et al [10]. It has a signal protein with a conserved core domain (about 120 amino acids). These molecules have many functions, including embryo development, tissue regeneration, and maintenance of metabolic homeostasis [11]. FGF21 is a special member of the FGF family because it lacks the heparin domain but requires β -klotho to interact with FGFRs to exert its biological activity [12]. FGF21 is mainly expressed in liver tissues but can also be expressed in lymphatic tissues, fat tissues, skeletal muscle, and other tissues [10, 13]. FGF21 initially attracted the interest of the scientific community due to its ability to improve glucose and lipid metabolism. Studies have shown that exogenous FGF21 can significantly reduce blood sugar in diabetic rats, dogs, and monkeys [14–16]. In addition, the weight of obese model mice decreased significantly after FGF21 treatment [17]. In recent years, it has been found that FGF21 has significant anti-inflammatory effects leading to the inhibition of rheumatoid arthritis and inflammatory response in the pancreas induced by a high-fat diet and glucose [14, 17, 18]. Hence, FGF21 is an important factor in metabolic diseases. Hyperuricemia has been shown to be one of the main risk factors associated with these metabolic diseases [19]. However, the effect of FGF21 on hyperuricemia nephropathy has not been reported. Therefore, the purpose of this study is to determine the effect of FGF21 on the progression of nephropathy in hyperuricemia and to further explore the associated molecular mechanisms to provide new ideas for the treatment of hyperuricemic nephropathy.

Materials And Methods

Patients

All study participants signed written consent forms to be enrolled in the study with the confidentiality of their data assured. This study was approved by the Ethics Committee of the Endocrinology Department of Harbin First Hospital. Serum samples (20) were obtained from Harbin First Hospital, including 10 patients with hyperuricemic nephritis and 10 healthy individuals. The details of the patients with hyperuricemic nephropathy are as follows: patients 1 to 9, males, aged 28, 35, 35, 40, 45, 52, 55, 57, 59 with hyperuricemic interstitial glomerulonephritis, six of them were on allopurinol while three of them took febuxostat before the blood sample was taken. Patient 10, male, 65 years old, suffering from diabetes, hyperuricemia, and chronic renal failure who has been on febuxostat and insulin for a long time. The 10 healthy people were males, aged between 26 and 57 years old. Human peripheral blood mononuclear cells (PBMCs) were extracted with a PBMCs separation kit (Haoyang Biological products Technology Co., Ltd., Tianjin) followed by the Trizol method for the extraction of RNA or real-time quantitative PCR (qPCR) detection.

Animals

Male wild-type (WT) C57BL/6 mice, 8 weeks old, were purchased from Liaoning Changsheng Biological Co., Ltd. While FGF21 gene knockout C57BL/6 mice, 8 weeks old, were purchased from Beijing View Solid Biotechnology Co., Ltd. All mice were kept in cages fitted with air supply filters at 22 ~ 26°C, with a relative humidity of 40%~45% and a light-dark period of 12 hours, and were fed ad libitum. All animal

studies were conducted in accordance with the guidelines formulated by the Animal Protection and Utilization Committee of Northeast Agricultural University.

Acquisition of FGF21

Human FGF21 was selected for this study. Recombinant SUMO-FGF21 plasmid was added to the *E. coli* competent cell Rossetta (Bioengineering technology company, Shanghai) followed by bacteria fermentation. After the fermentation process, the cells were collected, the supernatant was extracted, and the SUMO-FGF21 protein was obtained by His Trap TM FF crude Colum (GE Health, USA) affinity chromatography. The SUMO-FGF21 complex was digested with SUMO protease for 8 hours followed by a second affinity chromatography to finally obtain the mature FGF21 protein. The molecular weight and purity of the FGF21 protein were analyzed by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and high-performance liquid chromatography (HPLC).

Grouping and establishment of hyperuricemic nephropathy model in mice

Mice models of hyperuricemic nephropathy were given adenine (160 mg/kg) and potassium oxysalt (2400 mg/kg) (Sigma-Aldrich, USA) [20] by gavage while the normal controls were given intragastric administration of saline at the same volume once a day for 60 days. The experimental animals were grouped into 5 as follows: normal control group, model control group, FGF21 gene knockout group, FGF21 low dose group, and FGF21 high dose group. The specific treatment regimens were as follows: low dose FGF21 (1mg/kg) group, (n = 6): FGF21 was injected intraperitoneally every day for 30 days; high dose FGF21 (5mg/kg) group, (n = 6): FGF21 was injected intraperitoneally every day for 30 days; normal control (n = 6), model control group (n = 6), FGF21 gene knockout group (n = 6): intraperitoneal injection of the same volume of normal saline every day for 30 days. At the end of the study, the mice were euthanized and blood and kidney samples were taken for further analysis.

Detection of renal function

Serum samples from the collected blood samples were isolated and sent to the Harbin Electric Power Hospital for the detection of renal function indices such as blood urea nitrogen (BUN), Serum creatinine (Scr), cystatin C (CysC), and β 2-microglobulin (β 2-MG).

Histopathological examination

Sample kidney tissues from the different experimental groups were fixed in 4% paraformaldehyde for 7 days, then dehydrated and embedded in paraffin. Tissue sections (7 μ m) were cut using a microtome (Lecia, Germany), and then fully stretched in a water bath at 42°C. The sections were then transferred to a glass slide for hematoxylin-eosin (HE) staining or Masson staining. Finally, the blue area stained by Masson staining was observed under an inverted optical microscope and was analyzed by Imagine J software.

Immunohistochemistry

The prepared paraffin sections were put into an oven at 60°C for one hour and then dewaxed in xylene and ethanol with different concentration gradients. After dewaxing, the antigen was repaired followed by incubation with anti F4/80 antibody (dilution: 1:200, Abcam, USA) at 4 °C for 12 hours. The slides were washed twice with PBS followed by incubation with goat anti-rabbit secondary antibody (R&D, USA) at 37 °C for 1 hour. Again, the slides were washed twice with PBS and the reaction visualized with diaminobenzidine (DAB) for 8 minutes. The reaction was terminated by rinsing with water followed by hematoxylin-eosin staining. Finally, the slide sections were observed under an inverted optical microscope and the results analyzed by Image J software.

Cell culture

Mouse Glomerular Mesangial cells (GMCs) (ATCC, USA) were cultured in Dulbecco's modified eagle's medium (DMEM) (70%) and Ham's F12 nutrient medium (25%) containing 5% fetal bovine serum (FBS) (Gibco, NY, USA). The concentration of penicillin and streptomycin in the culture medium was 100U/ml and cultured in a 37 °C incubator with 5% carbon dioxide. After fusion of the GMCs cells for about 80%, the culture medium was discarded, washed with PBS for 3 times, and a serum-free medium was added to the 24-well plate. The cells were given lipopolysaccharide (LPS) at a concentration of 500ng/ml followed by monosodium uric acid (MSU) (Sigma, USA) at a concentration of 0, 100, 200, 400, 800, and 1600ng/ml (2 wells per concentration). The cells were then stimulated for a period of 24 hours. Finally, protein expression of IL-1 β was estimated to determine the best concentration and period of action of MSU. After determining the optimal concentration and action time of MSU, 100nM and 1000nM of FGF21 were added to the cells to observe the therapeutic effect. For the study of inhibition of Protein kinase B (Akt), GMCs cells were grown in a medium containing 10 μ M GSK690693 (an Akt inhibitor) (Beyotime, Shanghai). At the end of the study, the cells were collected for qPCR, Elisa and Western blotting analysis.

Determination of β klotho in GMCs cells

GMCs cells in normal culture were collected, centrifuged at 1500r/min at 4°C for 5min, then washed with PBS for three times, and then resuspended with 500ul PBS. The cells were divided into control and experimental groups (two wells per each group), and anti-klotho antibody (Runmai Biotechnology, Shanghai, diluted at 1:100) was added and incubated at 37°C for 1h. After incubation, the cells were centrifuged and washed with PBS for three times. Donkey anti-sheep perCP antibody (Abcam, USA, diluted 1:1000) was added to the control group and experimental groups respectively and incubated for 1 h at room temperature in the dark. After incubation, the cells were washed with PBS for three times again, and the peak offsets of the different groups of cells were detected by flow cytometry.

qPCR

Total RNA was extracted from the kidney of the mice using Trizol reagent (Takara Company, Japan). cDNA was synthesized by reverse transcription and the expression of the target genes were analyzed by qPCR using Quanti Tet SYBR Green PCR kit (Takara Company, Japan). The relative content of the target genes was calculated relative to that of the normal group. The primers used in this study were as follows: GAPDH (human) forward: ACAACTTTGGTATCGTGGAAGG reverse: GCCATCACGCCACAGTTTC; β -actin

(mouse) forward: GGCTGTATTCCCCTCCATCG reverse: CCAGTTGGTAACAATGCCATGT; FGF21 (human) forward: TTTACCAGTCCGAAGCCCAC reverse: CCTGGTAGTGGCAGGAAGC; FGF21 (mouse) forward: CTGCTGGGGGTCTACCAAG reverse: CTGCGCCTACCACTGTTCC; NACHT, LRR and PYD domains-containing protein 3 (NLRP3) (mouse) forward: CTCCAACCATTCTCTGACCAG reverse: ACAGATTGAAGTAAGGCCGG; Collagen 1 (Col 1) (mouse) forward: GTGCTAAAGGTGCCAATGGT reverse: ACCAGGTTACCGCTGTTAC; α -smooth muscle actin (α -SMA) (mouse) forward: CGGGACATCAAGGAGAAACT reverse: CCCATCAGGCAACTCGTAA; interleukin (IL)-1 β (mouse) forward: TCGCCAGTGAAATGATGGCTTA reverse: GTCCATGGCCACAACAACCTGA; tumor necrosis factor (TNF)- α (mouse) forward: ATGAGCACTGAAAGCATGATC reverse: TCACAGGGCAATGCCAAAGTAGACCTGCCC; IL-6 (mouse) forward: ACTCACCTCTTCAGAACGAATTG reverse: CCATCTTTGGAAGGTTTCAGGTTG; IL-10 (mouse) forward: TGGACAACATACTGCTAACCGAC reverse: TGGACAACATACTGCTAACCGAC.

Elisa analysis

Radioimmunoprecipitation assay (RIPA) solution and protease inhibitor (Beyotime Biotechnology, Shanghai, China) were added to the preserved kidney tissues, ground, and crushed using medical tissue homogenizer to finally extracted the supernatant. The expressions of IL-1 β , TNF- α , IL-6, IL-10, reactive oxygen species (ROS), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GSH-PX), and hydroxyproline were detected by Elisa kit (Andy Gene Biotechnology Co., LTD, Beijing, China) after protein extraction from the kidney of the mice and GMCs. Nuclear proteins of tissues or cells are extracted by Nuclear Protein Extraction Kit (Beyotime Biotechnology, Shanghai, China).

Western blotting

The concentrations of the proteins extracted from the kidney tissues and GMCs were determined by BCA kit (Beyotime, Shanghai). Equal amounts of protein samples were separated by SDS-PAGE and then transferred to a nitrocellulose membrane. After sealing for 10 minutes at room temperature with rapid sealing solution, primary antibodies anti-NLRP3 (dilution: 1: 1000, Abcam, USA), anti-Col1 (dilution: 1: 1000, Abcam, USA), anti- α -SMA (dilution: 1: 1000, Abcam, USA), anti-nuclear factor E2-related factor 2 (Nrf2) (dilution: 1: 1000, Abcam, USA), anti-AKT (dilution: 1: 1000, Abcam, USA) and anti-p (Thr 308) AKT (dilution: 1: 1000, Abcam, USA) were added at 4°C for 12h. This was followed by incubation with a secondary antibody, horseradish peroxidase-coupled (HRP) (dilution: 1: 3000, R&D, USA) at 37°C for 1 h. Blots were developed with an Electro-Chemi-Luminescence (ECL) kit (Amersham Biosciences, Piscataway, NJ), and the results quantified using ImageJ software.

Statistical analysis

Data were analyzed with GraphPad Prism 7.0 software for windows. Mean $\pm$ SD values were calculated, and one-way ANOVA was used to test for significance at $P < 0.05$. Tukey's Post hoc analysis was further used for comparison between groups.

Results

Increased expression of FGF21 in both humans and mice with hyperuricemia

The expression of FGF21 mRNA was analyzed by qPCR. The results showed that the average mRNA content of FGF21 in patients with hyperuricemia was 0.83 times higher than that in healthy people, and the difference was significant ($P < 0.05$) (Fig. 1a). Again, the average mRNA content of FGF21 in PBMCs of hyperuricemic mice was 0.81 times higher than that of the normal group (Fig. 1b) ($P < 0.05$). The above results indicate that the levels of FGF21 in the serum increases in hyperuricemia. However, the effect of this observed upregulation is unknown warranting further exploration.

FGF21 ameliorates renal function damage in hyperuricemic mice

At the end of the animal experiment, serum samples of the mice were separated from whole blood samples for the detection of indices of renal function. The results showed that the renal function of mice in the model group was severely impaired characterized by a significant increase in BUN, Scr, CysC, and β 2-MG ($P < 0.01$), while the renal injury in the FGF21 gene knockout group was even more severe with significantly higher BUN, Scr and β 2-MG levels compared to the model group ($P < 0.05$). However, treatment with different doses of FGF21 was marked by a significant improvement in renal function indices in the hyperuricemic mice ($P < 0.001$) with the high dose recording a better therapeutic effect (Fig. 2a-d). H&E staining also revealed significant damage to the kidneys of both the model and FGF21 knockout mice. The damage was characterized by inflammatory cell infiltration between renal tissues, vacuolar degeneration, tubular atrophy, and glomerular hyperemia. These pathological injuries were, however, significantly improved after treatment with FGF21 (Fig. 2e).

FGF21 improves glomerulonephritis in hyperuricemic mice

Immune system activation and subsequent infiltration of macrophages play an important role in the occurrence and development of glomerulonephritis [21]. Therefore, we observed the expression of macrophages in the kidney of hyperuricemic mice by immunohistochemistry using F4/80 antibody. Increased macrophage infiltration in the kidney of the model and FGF21 knockout groups were recorded, an indication of severe inflammatory. This was however abated significantly after treatment with FGF21 ($P < 0.01$) (Fig. 3a, b). Again, FGF21 significantly reduced inflammatory factors such as IL-1 β , TNF- α , and IL-6 tied to increasing the levels of IL-10 compared with the model group ($P < 0.05$) (Fig. 3c, d). Interestingly, the levels of TNF- α (in kidney) and IL-6 (both in kidney and serum) in the FGF21 gene knockout group were significantly higher than those in the model group ($P < 0.01$) (Fig. 3c, d). Also, the levels of NLRP3, a marker of inflammation, were significantly increased at both gene and protein levels in both the hyperuricemic models and the FGF21 knockout groups compared to the normal group. Once again, a significant reduction of NLRP3 marked treatment with the low and high doses of FGF21 ($P < 0.05$) (Fig. 3e-g). These results intimate the anti-inflammatory effect of FGF21 in renal injury associated with hyperuricemia.

FGF21 improves renal fibrosis in hyperuricemic mice

In order to observe the degree of fibrosis in the kidney of the mice, Masson staining was used to determine the collagen content within the kidney. Compared with the normal group, the positive areas in the kidney of the model and the FGF21 knockout mice were significantly increased ($P < 0.01$) with the FGF21 knockout group recording the largest. This was, however, reduced significantly in the FGF21 group ($P < 0.01$) (Fig. 4a, b). Also, the hydroxyproline content in the kidney of the FGF21 treatment group was significantly lowered compared to the model group ($P < 0.01$) (Fig. 4c). In addition, markers of fibrosis such as Col1, α -SMA, and TGF- β were also detected. The mRNA and protein levels of Col1, α -SMA, and TGF- β in the model and FGF21 gene knockout groups were significantly higher compared with the normal group ($P < 0.01$). FGF21 treatment, however, was marked by a significant reduction in Col1, α -SMA, and TGF- β levels in the kidney both at the mRNA and protein levels ($P < 0.05$) (Fig. 4d-f). These results suggest that FGF21 can inhibit the development of renal fibrosis in hyperuricemic mice.

FGF21 ameliorates oxidative stress in hyperuricemic mice

After the observation that FGF21 could significantly improve hyperuricemic nephropathy, the possible contributing mechanisms were explored. Oxidative stress can promote inflammation and fibrosis [5], therefore, oxidative stress indices were detected. Compared with the normal group, the amount of ROS and MDA in the model and FGF21 gene knockout groups increased significantly ($P < 0.01$) with the FGF21 gene knockout group indices (MDA) being significantly higher ($P < 0.01$) (Fig. 5a, b). However, FGF21 treatment significantly inhibited the levels of ROS, MDA and increased the levels of antioxidant enzymes CAT, SOD, GR, and GSH-PX (Fig. 5c-f) ($P < 0.01$). The activation of the Nrf2 signal pathway plays an important role in the antioxidant process as it triggers the production of various antioxidant response elements (AREs) [22]. Western blotting results showed that compared with the model group, both the low and high doses of FGF21 significantly increased the nuclear accumulation of Nrf2 ($P < 0.05$). In addition, the level of pAKT/AKT was also significantly improved compared with the model group ($P < 0.01$) (Fig. 5g, h). However, whether FGF21 activates Nrf2 through AKT needs further proof.

FGF21 ameliorates oxidative stress through Akt/Nrf2 signal pathway in GMCs cells

The biological activity of FGF21 requires the participation of β klotho [12], therefore, the expression of β klotho in GMCs *in vitro* was detected by flow cytometry. The results showed a clear shift in peak in the experimental group compared with the blank control group, which proved that FGF21 can play a biological role in GMCs (Fig. 6a). Elisa results showed a gradual increase in the concentration of MSU and action time with the expression of IL-1 β with a peak concentration of MSU at 800ng/ml and an action time of 72h (Fig. 6b, c). These conditions were therefore used for the experimental model cells. We then detected the expression of inflammatory factors in the cells. Compared with the model group, the FL and FH groups significantly inhibited the expression of inflammatory factors such as IL-1 β , TNF- α , IL-6 ($P < 0.05$) and increase the expression level of anti-inflammatory factor IL-10 ($P < 0.05$) (Fig. 6d, e). Additionally, the relationship between FGF21 and oxidative stress in the GMCs was observed. Elisa results

showed that compared with the FH group, the FL group significantly inhibited the expression of ROS and MDA ($P < 0.01$), and increased the levels of expression of antioxidant enzymes CAT, SOD, GR, and GSH ($P < 0.05$) (Fig. 6f). The expressions of Nrf2, Akt, and pAkt in GMCs were also detected. The results showed that the Nrf2 and pAkt/Akt levels in the FL and FH groups were significantly higher compared to the model group ($P < 0.05$) (Fig. 6g-j). However, when GSK690693 was used to inhibit the expression of pAkt, there was no significant difference in the expression of Nrf2 between the FL, FH, and the model group. This is, however, indicative of the fact that FGF21 activates Nrf2 through Akt (Fig. 6i, j).

Discussion

In recent years, the incidence of hyperuricemic nephropathy is gradually increasing [6]. Traditional therapeutic drugs such as benzbromarone, allopurinol, and febuxostat are accompanied by severe hepatorenal toxicity, which is not suitable for all patients [8, 9]. Therefore, the development of a safe and effective therapeutic remedy for hyperuricemic nephropathy has aroused the interest of many researchers. In this study, we found that the mRNA content of FGF21 increased significantly in PBMCs of patients and mice with hyperuricemic nephropathy, which suggested that FGF21 might participate in the process of hyperuricemic nephropathy. Therefore to determine the effect of FGF21 in hyperuricemic nephropathy, low and high doses of FGF21 were used together with FGF21 gene knockout mice.

First of all, we found that FGF21 significantly improved renal dysfunction associated with hyperuricemia. This was manifested through the restoration of the levels of BUN, Scr, CysC, and β 2-MG, and an observed repairment of kidney injury evident from the H&E staining. It is worthy of note that these negative effects were more pronounced in the FGF21 gene knockout group than the model group. Studies have shown that abnormal uric acid metabolism promotes the development of chronic inflammation [23, 24]. Therefore markers of inflammation were determined in the kidney of the mice. An increasing number of macrophages is a characteristic of many inflammatory diseases due to the critical role of macrophages in the pathogenesis of chronic inflammatory [25]. The activation of macrophages leads to the secretion of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6, causing damage to the body [26]. Through the use of F4/80 immunohistochemistry in this study, we found that FGF21 significantly reduced the infiltration of macrophages in the kidney. Again the expressions of IL-1 β , TNF- α , and IL-6 were also significantly abated tied to an upsurge in the expression of anti-inflammatory factor IL-10 after FGF21 treatment. In addition, the deposition of local urate crystals can mediate inflammatory reaction through the activation of inflammatory corpuscle NLRP3 [27]. However, we also found that FGF21 intervention significantly inhibited the expression of NLRP3. These data are suggestive of the anti-inflammatory effect of FGF21 in kidney injury associated with hyperuricemia.

Fibrosis is also a frequent manifestation of hyperuricemia nephropathy due to the excessive deposition of extracellular matrix (ECM) in the kidney [28]. The ECM is mainly composed of collagen, fibronectin, and other proteins [29]. In this study, Masson staining revealed a significant increase in collagen fibres in the

model group. This was buttressed by western blotting results also recording a significant increase in α -SMA and Col1 in the kidney. After FGF21 treatment, however, these fibrotic markers were significantly decreased. TGF- β signalling pathway is the main pathway that leads to fibrosis in most chronic renal diseases [30]. In this study, FGF21 proved its anti-fibrotic effect by significantly inhibiting TGF- β .

Next, we explored the possible anti-inflammatory and anti-fibrotic mechanisms employed by FGF21. Studies suggest that oxidative stress plays an important role in the process of inflammation. ROS produced by oxidative stress can promote the aggregation and activation of NLRP3 inflammasome promoting the release of IL-1 β , IL-18, and other inflammatory factors which in turn induce the production of ROS [5, 31]. This results in a vicious cycle as ROS further activates TGF- β to aggravate the fibrotic process [32]. In this study, however, FGF21 significantly inhibited ROS levels in hyperuricemic nephropathy mice and MSU and LPS treated GMCs. FGF21, again, significantly increased antioxidant enzymes culminating in the observed improvement in oxidative stress. Nrf2 plays a key role in oxidative stress by binding to Keap1 to inhibit the expression of antioxidant enzymes. However, when stimulated by oxidative signals, Nrf2 uncouples with Keap1 and bind to AREs to up-regulate the expression of antioxidant enzymes [22]. Akt plays an important role in cell survival and apoptosis as it activates a variety of enzymes and transcription factors to regulate cell function [33]. Some studies have shown that Akt signalling pathway induced by cyclic stretching of mouse type 2 alveolar epithelial cells is inhibited in the presence of ROS inhibitor dibasic iodobenzene [34]. Other studies have also shown that FGF21 can promote the expression of Akt [35]. Therefore, it was speculated that FGF21 may regulate Nrf2 through Akt. In both *in vivo* and *in vitro* experiments, FGF21 significantly increased the phosphorylation level of Akt and the nuclear accumulation of Nrf2. However, when the Akt inhibitor GSK690693 was added to GMCs, the expression of Nrf2 was significantly decreased, which indicated that FGF21 increased the expression of Nrf2 through Akt leading to the inhibition of glomerulonephritis and renal fibrosis (Fig. 7). Finally, although FGF21 could not reduce the level of uric acid (data not shown), its significant improvement of renal injury is encouraging warranting further research focusing on the combination of FGF21 with other uric acid-lowering drugs.

In conclusion, this study has found for the first time that FGF21 can significantly improve renal injury in hyperuricemia. These beneficial effects are achieved by activating Akt/Nrf2 signal pathway to improve oxidative stress.

Declarations

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Conflict of interest statement

The authors have no relevant financial or non-financial interests to disclose.

Availability of data and material The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable

Author contributions All authors contributed to the study conception and design. Guiping Ren and Deshan Li designed the study. Xinghao Jiang, Shijie Liu and Dan Wang performed the research. Yeboah Kwaku Opoku wrote the paper. Yimeng Zou and Kai Kang analyzed the data. All authors read and approved the final manuscript.

Ethics approval

All study participants signed written consent forms to be enrolled in the study with the confidentiality of their data assured. This study was approved by the Ethics Committee of the Endocrinology Department of Harbin First Hospital. All animal studies were conducted in accordance with the guidelines formulated by the Animal Protection and Utilization Committee of Northeast Agricultural University.

Consent to participate Not applicable

Consent for publication Not applicable

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Figures

Figure 1

Enhanced levels of FGF21 in patients and mice with hyperuricemia. RNA was extracted from human and mouse PBMCs or mouse kidney for the detection of FGF21 by qPCR. HU: hyperuricemia, N: normal control. (a) qPCR analysis of FGF21 mRNA level in human PBMCs, n=10. (b) qPCR analysis of FGF21 mRNA level in mouse PBMCs, n=6. *P<0.05, compared with N.

Figure 2

FGF21 ameliorates renal function damage in hyperuricemic mice. N: normal control group; M: model control group; F^{-/-}: FGF21 gene knockout group; FL: FGF21 low dose group; FH: FGF21 high dose group. (a)-(d) Renal function indexes of hyperuricemic mice. The mean ± SD is plotted as indicated by the error bars, n=6. Significant as compared to N, ^{##}P<0.01; Significant as compared to M, *P<0.05, **P<0.01. (e) HE staining of kidney in each group of mice.

Figure 3

FGF21 improves glomerulonephritis in hyperuricemic mice. N: normal control group; M: model control group; F^{-/-}: FGF21 gene knockout group; FL: FGF21 low dose group; FH: FGF21 high dose group. (a) Immunohistochemical analysis of the expression of renal macrophages in hyperuricemic mice. (b) Quantitative analysis of immunohistochemical positive rate, n=3. (c) The relative content of IL-1β, TNF-α, IL-6 and IL-10 mRNA levels in renal, n=6. (d) Elisa analysis of IL-1β, TNF-α, IL-6 and IL-10 levels in serum,

n=6. (e) The relative content of NLRP3 mRNA levels, n=3 (f) Western blotting of NLRP3 and quantifications (g), n=3. The mean $\pm$ SD is plotted as indicated by the error bars. Significant as compared to N, #P<0.05, ##P<0.01; Significant as compared to M, *P<0.05, **P<0.01.

Figure 4

FGF21 improves renal fibrosis in hyperuricemic mice. N: normal control group; M: model control group; F^{-/-}: FGF21 gene knockout group; FL: FGF21 low dose group; FH: FGF21 high dose group. (a) Analysis of

Masson staining (b) Relative positive areas of the kidney in hyperuricemic mice, n=3 (c) Content of hydroxyproline in kidney of hyperuricemia mice, n=6. (d) The relative content of Col 1, α -SMA and TGF- β mRNA levels, n=3. (e) Western blotting of Col 1, α -SMA, TGF- β and quantifications (f), n=3. The mean $\pm$ SD is plotted as indicated by the error bars, n=3. Significant as compared to N, #P<0.05, ##P<0.01; Significant as compared to M, *P<0.05, **P<0.01.

Figure 5

FGF21 ameliorates oxidative stress in hyperuricemic mice. N: normal control group; M: model control group; F-/-: FGF21 gene knockout group; FL: FGF21 low dose group; FH: FGF21 high dose group. (a)-(f) Elisa analysis of ROS, MDA, CAT, SOD, GR and GSH-PX, n=6. (g) Western blotting analysis of Nrf2, Akt, pAkt and quantifications (h), n=3. The mean $\pm$ SD is plotted as indicated by the error bars, n=3. Significant as compared to N, ##P<0.01; Significant as compared to M, *P<0.05, **P<0.01.

Figure 6

FGF21 ameliorates oxidative stress through Akt/Nrf2 signal pathway in GMC cells. N: normal control group; M: model control group; F-/-: FGF21 gene knockout group; FL: FGF21 low dose group; FH: FGF21 high dose group. Cells were treated with LPS (500 ng/ml), MSU (800 ng/ml) and different concentrations of FGF21 (with or without GSK690693) for 72 hours. (a) Detection of β klotho expression in GMCs by flow cytometry. (b) Elisa analysis of IL-1 β content at different MSU concentrations, n=3. Significant as compared to 0, **P<0.01. (c) Elisa analysis of IL-1 β content with different action time, n=3. Significant as compared to 0, **P<0.01. (d) qPCR analysis of IL-1 β , TNF- α , IL-6 and IL-10, n=3. (e) Elisa analysis of IL-1 β , TNF- α , IL-6 and IL-10, n=3. (f) Elisa analysis of ROS, MDA, CAT, SOD, GR, GSH-PX, n=3. (g) Western blotting analysis of AKT, pAKT and quantifications (h), n=3. (i) Western blotting analysis of nuclear accumulation of Nrf2 and quantifications (j), n=3. The mean $\pm$ SD is plotted as indicated by the error bars, n=3. Significant as compared to N, #P<0.05, ##P<0.01; Significant as compared to M, *P<0.05, **P<0.01.

Figure 7

Schematic molecular mechanism underlying the improvement of hyperuricemic nephropathy by FGF21. FGF21 activates Akt to promote the uncoupling of Nrf2 and Keap1 making Nrf2 to combine with AREs to promote the release of antioxidant enzymes. Antioxidant enzymes inhibits the expression of ROS, NLRP3-mediated inflammation and the expression of TGF- β to improve fibrosis.

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