FGF21 Attenuates Neurodegeneration though Modulating Neuroinflammation and Oxidant-stress

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Abstract

Background It is reported that FGF21 can repair nerve injury, but the specific mechanism is less studied. The present study was designed to investigate the effects of FGF21 on neurodegeneration and possible mechanisms of the aging and diabetic mice, which were susceptible to Alzheimer's disease (AD). Methods The diabetic mice and aging mice were used to study the effects of FGF21 on neurodegeneration and possible mechanisms. These mice were administrated with PBS, FGF21 or metformin once daily for 4 or 6 months. Then the mechanism was verified in SH-SY5Y cells. The relative gene expressions for neurodegeneration were assessed by Quantitative Real Time-PCR, Western blot and others. Results FGF21 inhibited the loss of nerve cells and intracellular edema around hippocampus in diabetic mice and aging mice. In vivo results revealed that administration of FGF21 led to suppress the aggregation of Tau and β-Amyloid 1-42 , which resulted in apoptosis in nerve cells. Meanwhile, FGF21 significantly reduced the expression of NF-κB, IL6 and IL8 ($p<0.05$) and enhanced anti-oxidant enzymes ($p<0.05$) in diabetic mice. In addition, the phosphorylation of AKT and AMPKα was increased by FGF21 treated in diabetic mice, which were considered as anti-inflammation and anti-oxidant stress pathway. The relative gene expressions of neurodegeneration were also demonstrated in aging mice, which showed similar trends with diabetic mice. In vitro experiment showed that the aggregation of Tau and β-Amyloid 1-42 was increased by LPS in SH-SY5Y cells, and FGF21 inhibited the aggregation. Conclusion As shown above, FGF21 attenuated neurodegeneration by reducing neuroinflammation and oxidant stress though regulating the NF-κB pathway and AMPKα/AKT pathway, which enhanced the protective effect on mitochondria in nerve cells. Key words : FGF21, diabetes, neurodegeneration, inflammation, oxidant stress.

Background

Diabetes mellitus is a metabolic disease with multiple etiologies, characterized by chronic hyper-glycemia resulted from disturbance of insulin secretion or action, and associated with aging particularly. Recently, it is reported that the number of people living with diabetes maybe reach 600 million in 2035 over the world, and among these people, the risk of Alzheimer's disease (AD) is greatly increased [1]. The aging is also a risk factor for AD [2]. Aging and diabetes-associated brain damage have been paid much attention, which illustrated that the neurodegeneration associated with aging and diabetes is pathological basis for AD, a progressive degenerative disease characterized by aggregation of Amyloid and Tau protein [3]. Diabetes and AD shared corporate abnormalities, including impaired glucose metabolism, increased oxidant stress, chronic inflammation, insulin resistance, aggregation of Amyloid and Tau protein [4]. The diabetes and aging mice have provided a relevant animal model for nerve damage, and developed many AD-like neuropathological features, including inhibited AKT, aggregation of Tau protein and glycogen synthase kinase-3β (GSK-3β) over-activation [5,6]. The db/db mice were used usually [7]. Multiple studies revealed the pathogenesis of AD from inflammatory responses, lipid accumulation, cell apoptosis and protein metabolic disturbance [8,9]. Therefore, these might be possible effective mechanism for the AD associated neurodegeneration.

Increasing evidences suggest that nerve damage is mainly mediated by inflammatory response, oxidant-stress and apoptosis [10]. Inflammation associated with oxidant stress results from the conflict in body's accept and resistance to injury, which is attributed to many nerve diseases [11]. In addition, previous research has reported that inflammation induced by LPS, a strong inflammatory irritant, causes hippocampus damage by regulating protein expression of IL6 and IL8, two important pro-inflammatory factors [12]. Studies have shown that the imbalance between the pro-inflammation and anti-inflammation results in mitochondria damage, cell apoptosis, and tissue necrosis [13]. Moreover, the mitochondria damage causes the dysfunction of oxidant and antioxidant system, including hemeoxygenase-1 (HO-1), catalase (CAT), superoxide dismutase (SOD) and glutathion peroxidase-1 (Gpx-1), which results in accumulation of Radical oxygen species (ROS). Conclusively, inflammation and oxidant stress play central roles in the brain damage of diabetes and aging.

Adenosine Monophosphate Activated Protein Kinase α (AMPKα) is an important protein kinase, which mainly regulates metabolism and energy demand [14]. Sirtuin1 (SIRT1) associated with AMPKα, belong to the mammalian sirtuin family, plays a pivotal role in many pathophysiological process, such as apoptosis, aging, inflammation and oxidation-stress though multiple factor, PPARγ, NF-κB and others [15, 16]. These proteins mediate the expression of nerve relative proteins, such as Brain Derived Neurotrophic Factor (BDNF), Tau and β-Amyloid$_{1-42}$. Among the activating agents, NF-κB plays the critical role in signal transduction process, which not only relates with the secretion of inflammatory factors and peroxide, but also participates in inhibiting neuroprotective process.

Fibroblast growth factor 21 (FGF21) is an endogenic hormone expressed in the liver, adipose tissue and pancreas, which is induced by starving stress or some pathological status. FGF21 has many physiological functions, such as hypoglycemic, anti-inflammation, and anti-oxidant stress [17, 18]. Metformin also has many physiological functions, which is used widely in diabetes and considered as potential anti-aging drugs. Evidences suggest that the β-klotho/FGF receptor complex is responsible for the regulative metabolic functions of FGF21 and expressed in many organs including hypothalamus [19, 20]. Thus, FGF21 may perform function in the brain [21]. We hypothesize that FGF21 could not only reduce the burden of high blood glucose in blood circulation, but also alleviate the injury caused by harmful substances and strengthen the protection for brain.

It is reported that FGF21 can repair nerve injury, but the specific mechanism is less studied. Therefore, the aim of the current study was to specifically evaluate the therapeutic effects of FGF21 on neurodegeneration and illuminate the mechanism.

Methods

Preparation of FGF21

The recombinant P-SUMO vector containing FGF21 gene which was constructed in our laboratory was transformed into host bacterium, Rosetta (DE3). Single colonies of the Rosetta (containing the plasmid of

P-SUMO-FGF21) grew in LB media containing ampicillin (100ug/ml). When the OD600 reach 0.4 to 0.6, FGF21 protein expressed at 25℃ for 6 hours (IPTG final concentration: 0.25mmol/L). The FGF21 was purified by AKTA Purifier (GE Healthcare) [22]. The activity assay of FGF21 in vitro performed by HepG2 cells cultured as a materials of glucose uptake in a 96-well plate and incubated at 37℃ in a 5% CO_2 humidified atmosphere. When the number of the cells reached 70% to 80%, the cells were starved for 12h in a serum-free medium followed by stimulation without or with various concentrations of FGF21 for another 24h. We used a glucose assay kit (Beijing Kingkawk pharmaceutical CO, LTD) to measured glucose uptake.

Assessment of SH-SY5Y cells

Cell viability was measured by a modified CCK8 assay (BestBio, Shanghai). SH-SY5Y cells were cultured on 96-well plates and incubated with LPS (100μg/ml, sigma, USA) and in the presence of FGF21 (0, 1, 5, 10μmol/L) for 48h, then 10% CCK8 was added in wells and incubated at 37℃ for 4h. Absorbance at 450nm were measured using ELISA. The cells experiment repeated in 6-well plates for testing the levels of protein by Western blot.

Animals and treatment regimens

Male C57BL/ksj-db/db mice (7-8 weeks, blood glucose concentration≥20mmol/L, Shanghai Silaike Experimental Animals LTD) and the C57BL/6 mice (7-8weeks) fed and maintained under constant SPF conditions and temperature 23±2℃, humidity 50±5%, with 12h light and 12h dark cycles. In the mouse experiment, diabetic mice were divided into 5 groups : (1) wild type group for db/db mice (normal control, n=10), (2) diabetic mice treated with PBS (model control, n=10), (3) diabetic mice treated with FGF21 (0.5mg/kg, n=10), (4) diabetic mice treated with FGF21 (1.0mg/kg, n=10), (5) diabetic mice treated with FGF21 (2.0mg/kg, n=10). PBS or FGF21 injected into the mice once daily for four months. Blood samples were taken for serum T-AOC, CAT and T- SOD measurements. T-AOC (A015-1-2), CAT (A007-1-1) and SOD (A001-3-2) Assay Kits were purchased from Nanjing Jiancheng Bioengineering Institute, China.

In aging mice model, male C57BL/6 mice were divided into 4 groups: (1) 4 month C57BL/6 mice were fed before the experiment ending (n=10), (2) 14 month mice were treated by PBS (n=10), (3) 14 month mice were treated by FGF21 (1.0mg/kg, n=10), (4) 14 month mice were treated by metformin (20mg/kg, Sigma, USA, n=10). PBS, FGF21 or metformin were administrated in the mice once daily for 6 months. The relative situation of neurodegeneration were assessed by Water maze, Western blot and others.

Morris Water Maze

Before the experiment ending, the aging mice performed water maze test. Briefly, each mice was trained to find a hidden platform for six consecutive days, four trials per day with 120s rest period. The swimming pathway and escape latency were recorded in each trial. On the seven day, the swimming pathway, escape latency and number of platform quadrant crosses were examined in a 120s free swim period without platform.

H&E Staining

The brains of experimental animals were removed one lobe in 4% formaldehyde solution and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin. The others were flash frozen in liquid nitrogen and stored at -80℃ for Real Time-PCR and Western blot.

Immunohistochemisty (IHC) Analysis

After dehydration, sections were subjected to antigen retrieval in 0.01 mol/L citrate buffer (pH=6.0) by microwaving, and then placed in 3% hydrogen peroxide for 30min at room temperature. After blocking with 5% BSA, the sections were incubated with anti-IL6 antibody (1:100, DF6087, Affinity, USA) and anti-IL8 antibody (1:100, DF6998, Affinity, USA) overnight at 4℃, followed by the anti-Rabbit secondary antibody (1:200, R&D, USA). The reaction was visualized with DAB solution. After counterstaining with hematoxylin, the sections were enveloped and views under a light microscope.

Immunocytochemistry (ICC) Analysis

The cells were immobilized on glass slides, then the follow-up operation is basically the same as IHC. The primary antibodys were Tau (1:100, Abcom, ab32057, USA) and β-Amyloid$_{1-42}$ (1:100, bs-0107R, Bioss, China). The secondary antibody was anti-Rabbit antibody (1:200, R&D, USA)

Reverse Transcription and Real Time-PCR

Total RNA was isolated from tissues (100mg) using TRIZOL (Invitrogen, Carlsbad, CA) to perform Reverse transcription and Real Time-PCR. Real Time-PCR was carried out using the Eppendorf Realplex 4 instrument (Eppendorf, Hamburg, Germany). The β-actin was detected as an internal control. The primer sequences used were shown as Table 1.

Western Blotting

Tissues were lysed for total protein extraction in RIPA Lysis Buffer (Nanjing Jiancheng Bioengineering Institute, China) together with a protease inhibitor PMSF (Sigma-Aldrich Corporation, Saint Louis, MO, USA). The protein concentration was measured using the Pierce BCA protein Assay Kit (Thermo, USA). The membranes were block with the 5% skim milk in phosphate buffer saline (PBS) and probed with the following primary antibody overnight at 4C: AMPKα (1:1000, Cell Signaling Technology, D63G4, USA), P-AMPKα (Thr172) (1:1000, Cell Signaling Technology, 40H9, USA), AKT (pan) (1:1000, Cell Signaling Technology, C67E7, USA), P-AKT (Thr308) (1:1000, Cell Signaling Technology, D25E6, USA), Gsk-3β (1:1000, Cell Signaling Technology, D5C5Z, USA), P-GSK-3β (Ser9) (1:1000, Cell Signaling technology, D85E12, USA), Tau (1:1000, Abcom, ab32057, USA), P-Tau-s396 (1:1000, Abcom, ab151559, USA), BDNF (1:1000, Abcom, ab108319, USA), PPARγ (1:500, bs-0530R, Bioss, China), SIRT1 (1:500, bs-0921R, Bioss, China), β-Amyloid$_{1-42}$ (1:500, bs-0107R, Bioss, China), NF-κB (1:500, AF5006, Affinity, USA), IL6 (1:500, DF6087, Affinity, USA), IL8 (1:500, DF6998, Affinity, USA). We used Rabbit or Mouse (1:7500,

HAF008/HAF007, R&D, USA) IgG HRP-conjugated antibody as secondary antibody. Blots were developed using an ECL kit (Amersham Biosciences, Piscataway, NJ).

Enzyme-linked immunosorbent assays (ELISA)

The protein levels of Tau and β-Amyloid$_{1-42}$ in the supernatants of the brains tissue or cells homogenate were measured using ELISA, according to the double antibody sandwich method. Briefly, Tau antibody (Abcom, ab32057, USA) and β-Amyloid$_{1-42}$ (bsm-0107M, Bioss, China), was bonded to a solid state carrier, then incubated the supernatants of the brains tissue or cells homogenate, Tau antibody (bioss, bs-0157R, China) or β-Amyloid$_{1-42}$ (bs-0107R, Bioss, China), and anti-Rabbit secondary antibody (HAF008, R&D, USA) in turn. Color lipuid was added in wells and incubated at 37℃ for 0.5h. Finally stop buffer was added. Absorbance at 450nm were measured.

Statistical analysis

Statistical analysis performed by SPSS 19.0, and date showed as mean ± SD. All data performed using one-way analysis of variance (ANOVA), followed by Student two-tailed-t-text. Statistical significance was defined as P□0.05.

Results

Chronic administration of FGF21 remits the brains injury in diabetic mice

The activity of FGF21 was assayed by glucose uptake in HepG2 cells. Results showed that the glucose uptake rate was increased after adding FGF21 (Fig.1A). After injecting FGF21 or PBS for 4 months, the weight of the diabetic mice treated with PBS was much heavier than that of the diabetic mice treated with FGF21. The normal (C57BL/6) mice were much lighter than the above groups (Fig.1B). The brains processed with H&E revealed that the loss of nerve cells and intracellular edema around hippocampus in the model mice, which was contrast to that of normal group. FGF21 inhibited the loss of nerve cells and intracellular edema around hippocampus (Fig.2A). Furthermore, diabetes-induced nerve damage was verified by increase of Tau and β-Amyloid$_{1-42}$ (Fig.2B, C, and Extended Data Fig.1A). These elevations were prevented by FGF21 treatment. Next, FGF21 regulated the mRNA expressions of Bcl_2 and Bax, which control the apoptosis pathway ($p<0.05$) (Fig.2C). Hence, the results indicate that FGF21 prevents nerve damage in diabetic mice.

FGF21 reduces the expression of NF-κB, IL6 and IL8 in diabetic mice

According to the previous reports, diabetic mice over-expressed inflammatory cytokines, which might be potential risk factors for brain damage [23]. The IHC analysis exhibited that the expression of IL6 was enhanced in diabetic mice, and FGF21 inhibited the enhancement (Fig.3A). Our result showed that the mRNA and protein expression levels of the pro-inflammatory cytokines, NF-κB, IL6 and IL8 in the brains of diabetic mice treated by FGF21 were lower than that of untreated counterparts ($p<0.05$) (Fig.3B,C). In our

experiment, FGF21 alleviates inflammatory action though inhibiting the accumulation of NF-κB in neurodegeneration of diabetes.

FGF21 promotes the activities of anti-oxidant enzymes system

The antioxidant enzyme system is used as a biomarker of anti-oxidant stress to judge the status of the body oxidation/anti-oxidation. The result showed that activities of SOD, T-AOC and CAT in the serum of diabetic mice treated with FGF21 (1.0, 2.0mg/kg) were significantly enhanced compared with the untreated group ($p<0.05$), and the activity is similar to that of normal mice, which were shown in Table.2. Similar results occurred in brains. The mRNA expressions of HO-1, SOD, CAT, and GPx1 were down-regulated in the brains of diabetic mice, and FGF21 up-regulated them (Fig.4A). The results suggest that antioxidant enzymes system were regulated by FGF21, which is responsible for FGF21-induced anti-inflammation function and survival of cells.

FGF21 activates AMPKα/AKT pathway to inhibit nerve injury in diabetic mice

The relevant proteins were assessed to explore the mechanism of FGF21 inhibiting inflammatory responses during the brain damage. The levels of the phosphorylation of AKT and GSK-3β in diabetic mice were inhibited compared with those of normal control group. However, they were enhanced in the FGF21 groups ($p<0.01$) (Fig.4B). The results show that phosphorylation of AKT and GSK-3β was increased by FGF21 treatment in the nerve damage of diabetic mice. As shown in Fig.4C, the result showed that the expressions of AMPKα, SIRT1, PPARγ and BDNF were up-regulated in the brain tissues of diabetic mice treated by FGF21 compared with that of untreated diabetic mice ($p<0.05$). We observed significant differences in the phosphorylation levels of AMPKα among the diabetic groups treated with or without FGF21. The results indicates that FGF21 inhibits the inflammation and oxidant stress though AMPKα/AKT pathway, which regulate NF-κB signaling pathway.

FGF21 attentudes neurodegeneretion though anti-inflammation in aging mice.

We used the Morris water maze to explore whether FGF21 could reverse aging cognitive deficits. We found that untreated aging mice displayed prolonged escape latency to find uncover platform compared with aging mice treated by FGF21 and metformin from the fourth day (Fig. 5A). After removing the platform on the seventh day, we observed longer escape latency and fewer crossing number in aging mice than those in the mice treated by FGF21 and metformin, indicating that FGF21 and metformin effectively restored the cognitive deficits (Fig.5B, C). The nerve injury level was exhibited by H&E, and the IL6 and IL8 levels were shown by immunohistochemistry. Compared with the model group, FGF21 and metformin inhibited the loss of nerve cells and intracellular edema around hippocampus (Fig.5D). The protein levels of IL6 and IL8 were increased in untreated aging mice, FGF21 inhibited the expressions of them.

FGF21 activates AMPKα/AKT pathway to inhibit nerve injury in aging mice

Western blot analysis revealed that the aggregations of Tau and β-Amyloid$_{1-42}$ were increased in untreated aging mice, and FGF21 and metformin inhibited the aggregations of these proteins (Fig.6A, B, Extended data Fig.1B). Aging mice have a greater chance of developing neurodegeneration, thus we use aging mice to study the mechanism of FGF21 on nerve damage. To confirm the above findings, we text the relevant proteins about NF-κB and AMPKα *in vivo* in aging mice. As shown in Fig.6A, we found that administration of FGF21 significantly attenuated the upregulation of NF-κB, IL6 and IL8 protein expression in untreated aging mice by Western blot. These results verified the anti-inflammatory activity of FGF21 *in vivo*. Meanwhile, the levels of the phosphorylation of AKT and AMPKα in aging mice were reduced compared with those of young group. However, these were increased in the FGF21 and metformin groups ($p<0.01$) (Fig.6B). The results showed that the SIRT1 and BDNF were up-regulated in the brain tissues of aging mice treated by FGF21 compared with those of untreated aging group ($p<0.001$). The results indicate that the inflammatory responses, oxidant stress injury and mitochondria damage are inhibited by the treatment of FGF21 and metformin in the brains of aging mice.

FGF21 inhibits the aggregation of Tau and β-Amyloid$_{1-42}$ caused by LPS-induced inflammatory action in SH-SY5Y cells

To verify the critical role of inflammation in the neurodegeneration by FGF21, LPS was added in SH-SY5Y cells (Fig.7A). Interestingly, the addition of LPS increased the expressions of NF-κB and IL6 (Fig.7A), subsequently enhanced the aggregation of Tau and β-Amyloid$_{1-42}$ (Fig.7B and Extended data Fig.1C, D), which proved the critical role of NF-κB in inflammation-induced neurodegeneration in LPS-treated SH-SY5Y cells. Based on this finding, the anti-inflammatory, anti-oxidative, and anti-apoptotic activities of FGF21 continued to be investigated in the SH-SY5Y cells treated with LPS. As expected, the addition of FGF21 inhibited the expression of NF-κB, IL6, Tau and β-Amyloid$_{1-42}$ in the SH-SY5Y cells treated with LPS. The results showed that the activity of AMPKα was inhibited in SH-SY5Y cells treated by LPS, but FGF21 promoted the activity (Fig.7B). As a result, we find that FGF21 can significantly inhibit the aggregation of Tau and β-Amyloid$_{1-42,}$ which is increased by LPS-induced inflammation though NF-κB and AMPKα pathway in SH-SY5Y cells.

Discussion

In the previous finding, lower glucose and lipid are thought as basic functions of FGF21 [24]. The activity of FGF21 we purified was verified by glucose uptake which was used widely [25]. Our findings suggested that the activity of FGF21 was enough to enhance the glucose uptake. And the weight of the diabetic mice decreased after 4 months treatment by FGF21 compared with that of untreated counterparts, but the weight of aging mice treated by FGF21 and metformin had no significant difference [26].

Type 2 diabetes is thought as a common chronic disease by insulin resistance or secretion reduction causing by islet β cell damage which is regulated by FGF21 [27]. Infusion of FGF21 in mice results in higher ATP production, increased AMPK, AKT and PPARγ at protein levels, and elevated anti-inflammation and anti-oxidant enzymatic activities in heart [28]. Conversely, knockout of FGF21 or AMPK signaling in

mouse myocardial tissue triggers maladaptive responses in mitochondria including reduced expression of genes and proteins related to oxidative phosphorylation and inflammation [29]. Previous advances show that neurodegeneration is caused by chronic inflammation and oxidant stress, the important dangerous factors about many age-related disease [30]. However, the hypoglycemic drug insulin can't solve the problem of diabetes-induced neurodegeneration, so it is very important to find a long-term drug treating diabetes and improving its nerve injury. Metformin was considered as a hypoglycemic and potential anti-aging drug, which exhibited similar treatment pathway to FGF21 in aging mice. But the effect on inhibiting inflammation by metformin was stronger than FGF21 distinctly. It might be a risk of cancer.

Db/db mice are considered as a type of leptin deficiency diabetic mice, which were naturally associated with obesity, hyperglycemia and other diabetic characteristics. Due to long-term high glucose infiltration, db/db mice are found a lot of diabetes complications such as nerve damage, hepatitis and myocarditis like aging [31]. It was demonstrated that the aggregations of tau and β-Amyloid$_{1-42}$ in 20 month old mice and diabetic mice were higher than that of normal mice, so it could be confirmed 20 month old mice and diabetic mice as the model of neurodegeneration. And the FGF21 reduced the injury in our experiment. Our experiment was shown that the symptom about nerve injury could be significantly improved by FGF21 in db/db mice, which were assessed living with diabetes, and aging mice. The reason might be that FGF21 could not only decrease the blood glucose and fat, but also inhibit the inflammation and oxidant stress [32]. Which was proved in other studies, FGF21 had exhibited favorable long-acting hypoglycemic effect in animal experiments and the potential about treating diabetes [33]. In this study, we indicated that FGF21 could improve the brain functions. These results were in agreement with the hippocampus histological changes that were detected with H&E staining, IHC analysis and western blot of the brains [34]. In our present study, we found that the symptoms of the brains, inflammation and the loss of nerve cells, were abnormal around the hippocampus of diabetic and aging mice, but FGF21 improved it [35]. The effect of FGF21 on the nerve injury was confirmed. Hence, in order to clarify how FGF21 protect the brain, we explored the possible mechanism about inflammation, oxidant stress and some critical regulatory factors in the present study.

In the present research, it has suggested that NF-κB and anti-oxidant enzymes system are necessary mediators of inflammation and oxidant stress injury [36,37]. In our study, we found that FGF21 improved the brains damage though decreasing the pro-inflammatory factors and increasing anti-oxidant enzymes which may connected with macrophage [38,39], but the detailed mechanism remains to be further studied. All experiments performed that inflammation and oxidant stress injured the nerve cells which were prevented by FGF21. However, we cannot exclude the possibility that the inhibition of inflammation and oxidant stress was because of hypoglycemic effect of FGF21.

The aggregation of tau, one of the important biomarkers of the nerve injury, and antophosphorylation are reported to be connected with nerve injury. It can promote the apoptosis of nerve cells, then induce the aggregation of the β-Amyloid [40,41]. AKT is an important metabolic regulator that is suppressed in metabolic block such as diabetic mice, which can be activated by phosphorylating. GSK-3β, a factor that

regulates differentiation, proliferation and apoptosis of cells, can be inhibited activation by phosphorylating [42]. AKT and GSK-3β all are related with NF-κB signal pathway, and inducing the aggregation of tau and β-Amyloid which are different from that of normal group, the FGF21 treatment groups and metformin group.

AMPKα as AMP kinase to regulate energy metabolism, is important to maintain mitochondria stabilize function and adaptability [43]. FGF21 interacted with the AMPK pathway is multifaceted [44]. SIRT1 has a wide spectrum of metabolic and stress-tolerance properties which can protect cells against oxidant stress, regulate metabolism and slow down various age related disorders [45]. In our studies, AMPKα /SIRT1 signaling pathway was activated by FGF21, thus directly or indirectly promoted the expression of downstream proteins such as PPARY, which were associated with the expression of AKT and NF-κB [46].

It is well known that inflammation induced by LPS triggers the necrosis and apoptosis of cells [47]. In order to maintain living, self-protection mechanisms are provided in cells, including cellular fibrosis, autophagy, aggregation of some cells, and others [48]. These damages will cause many pathological phenomena. Enhancing anti-inflammatory function and reducing inflammatory action were methods of decreasing necrosis of cells. In this study, the cells viability was improved, the damages of cells were repaired, and the inflammatory action was inhibited [49]. However, we cannot exclude the possibility of negative feedback control.

In present research, diabetes and aging models were used to probe into the efficiency of FGF21 on neurodegeneration and the treatment mechanism, then we used SH-SY5Y cells treated by LPS to test that FGF21 inhibited neurodegeneration though anti-inflammation and anti-oxidant stress. Our experiment has proved the potential application value of FGF21 on neurodegeneration, and more nerve injury models and FGF21-associated gene mice could be used for the experiment.

Conclusions

In this study, we researched the mechanisms about neurodegeneration in diabetic and aging mice, and FGF21 protected the nerve injury from neuroinflammation. The research provided a new strategy about treating chronic nerve damage. In a conclusion, FGF21 inhibited the polymerization of tau and β-Amyloid, promoted expression of BDNF though anti-inflammation and anti-oxidant stress, which were regulated by NF-κB and AMPKα/AKT signaling pathway.

Abbreviations

AD: Alzheimer’s disease

GSK-3β: glycogen synthase kinase-3β

FGF21: Fibroblast growth factor 21

AMPKα: Adenosine Monophosphate Activated Protein Kinase α

SIRT1: Sirtuin1

SOD: superoxide dismutase

CAT: catalase

T-AOC: Total Antioxigenic Capacity

BDNF: Brain Derived Neurotrophic Factor

HO-1: heme oxygenase-1

Gpx-1: glutathion peroxidase-1

ROS: Radical oxygen species

APP: Amyloid beta precursor protein

Declarations

Ethics approval and consent to participate

All procedures involving mice were accord with the Animal Care and Use Committee of Institute of Materia Medica, PR.CHINA.

Consent for publication

Not applicable

Availability of data and materials

The datasets analyzed during the current study may be available upon reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions Contribution statement

Kai Kang performed the research, and analyzed the date. Pengfei Xu set up mouse model and provided useful experimental consideration. Mengxia Wang, Jian Chunyu and Xu Sun participated in date collection and analysis. Guiping Ren, Wei Xiao and Deshan Li contributed to the initial and consequent project discussion, manuscript discussion and revision. All the authors approved the final version of the manuscript.

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Tables

Due to technical limitations, tables are only available as a download in the supplemental files section

Figures

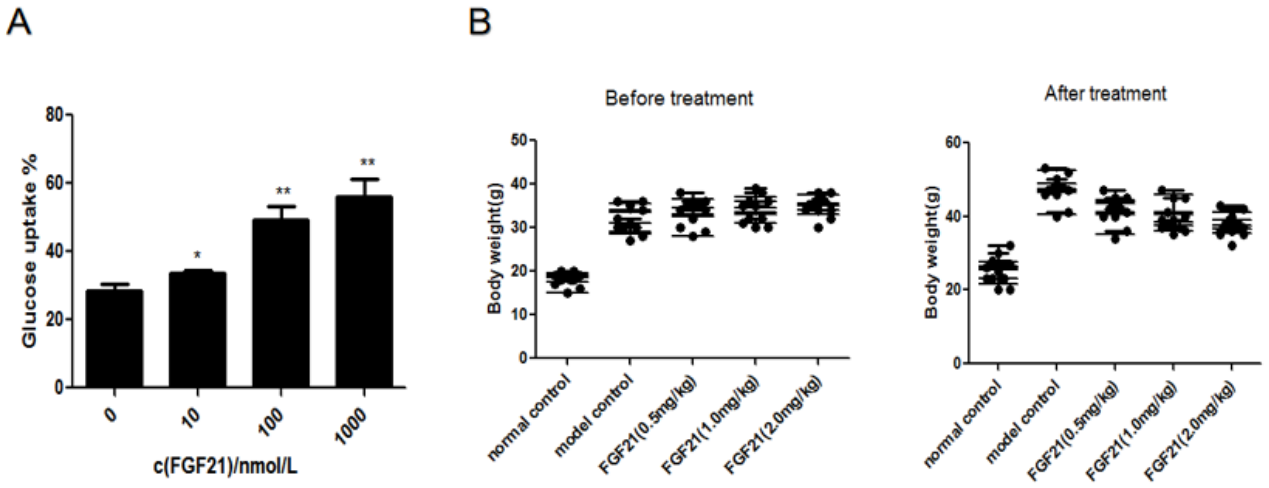

Fig .1. FGF21 decreases the weight of diabetic mice. (A)Glucose uptake of HepG2 cells after treatment with different concentrations of FGF21; (B) The weight of normal control mice and diabetic mice treated by FGF21 with different concentrations. The date were performed using one-way analysis of variance (ANOVA), followed by the student two-tailed t test. Significant as compared to FGF21 (0nmol/L). *$p<0.05$,**$p<0.01$.

Figure 1

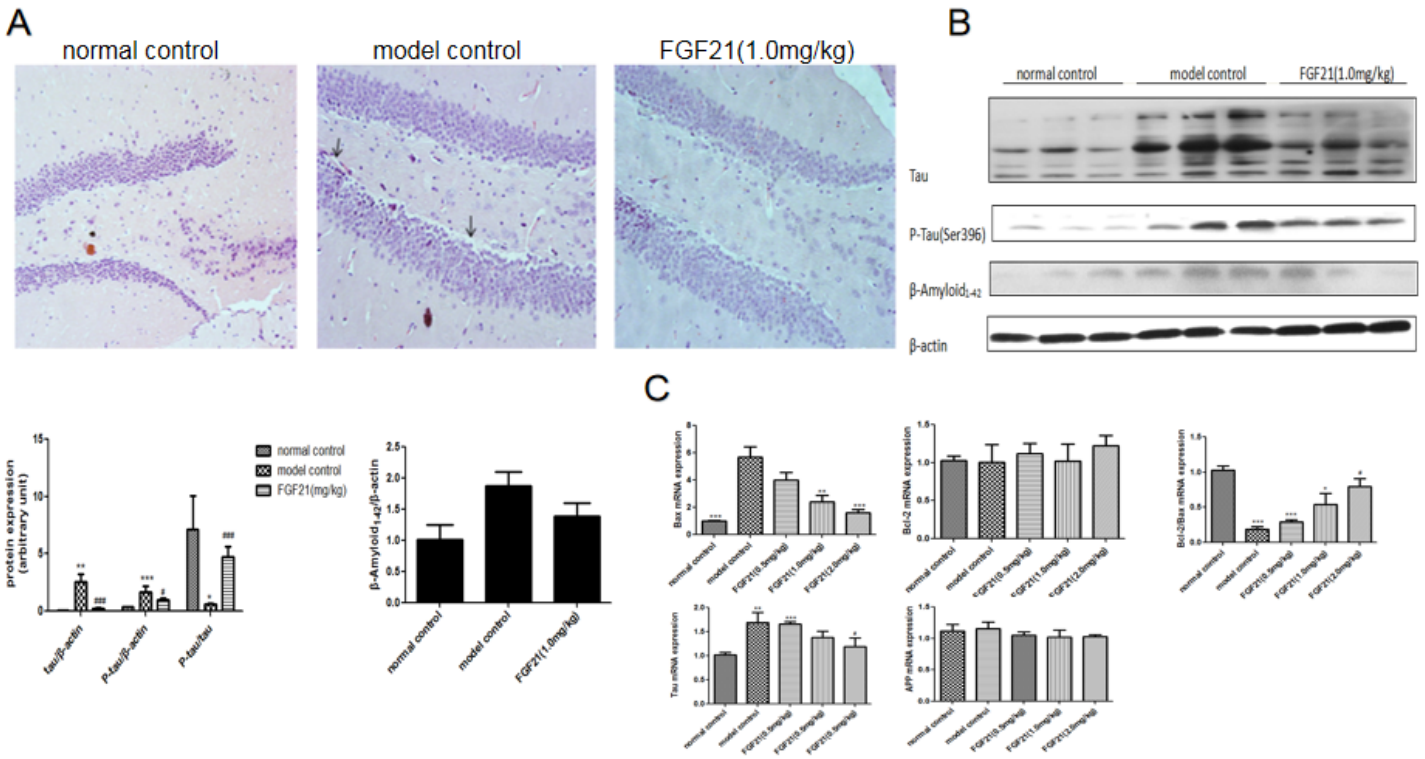

Fig.2. FGF21 remits nerve cells damage and apoptosis in the brains of diabetic mice(n=10/group). (A)The injury levels in the brains of H&E staining(20X magnification; bar = 50 mm). (B)The protein levels of Tau and β-Amyloid$_{1-42}$ in the brains were measured by western bloting. (C)The mRNA expression of Tau, APP, Bax and Bcl-2 in the brains of each group. The date were performed using one-way analysis of variance (ANOVA), followed by the student two-tailed t test. Significant as compared to normal control group. *p＜0.05 ,**p＜0.01,***p < 0.001. Significant as compared to model control group. #p＜0.05 ,##p＜0.01.

Figure 2

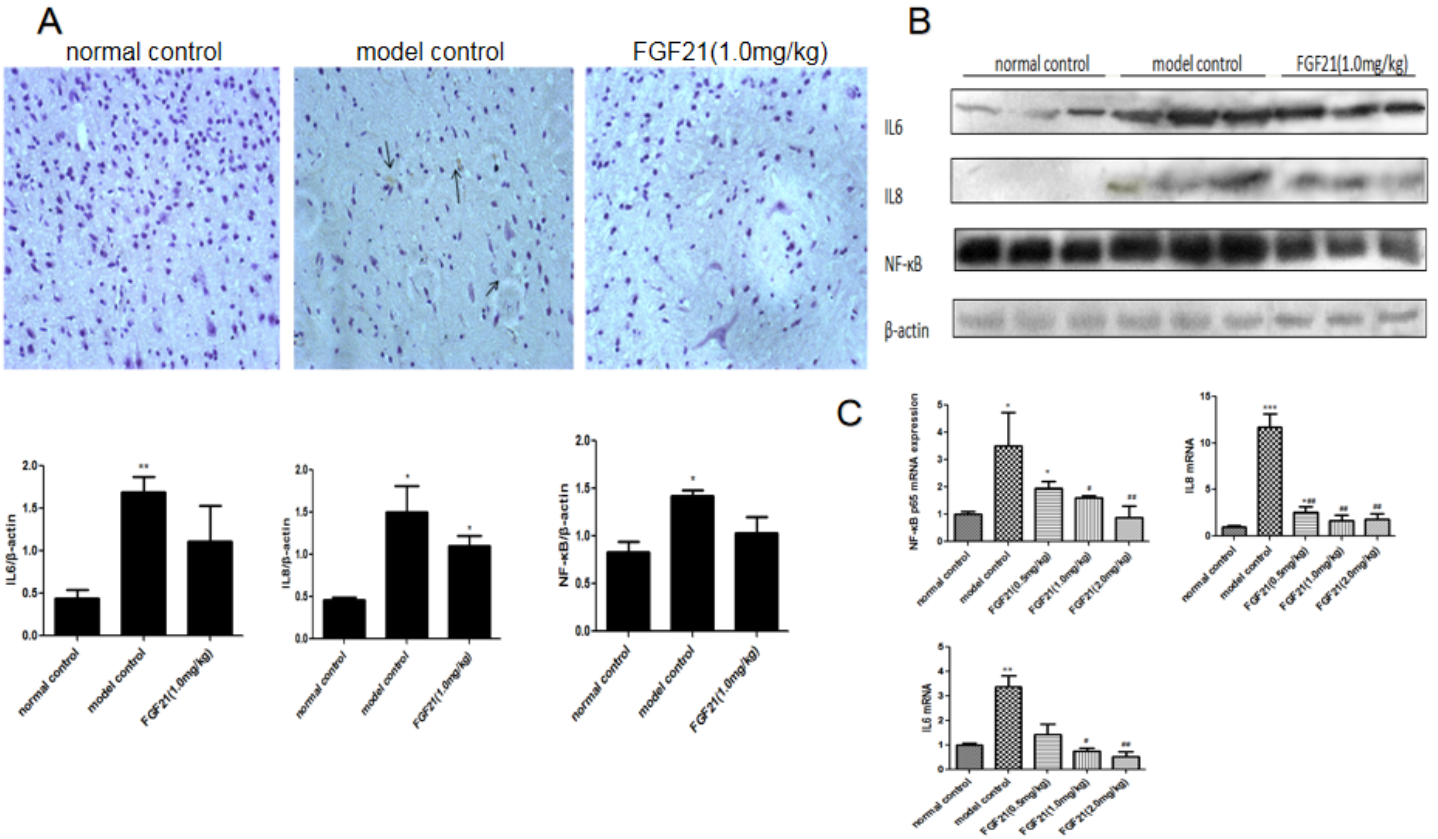

Fig.3. FGF21 attenuates the expression of NF-κB, IL6 and IL8 in the brains of diabetic mice (n=10/group). (A)The IL6 levels of each group mice were exhibited by immunohistochemistry(40X magnification; bar = 50 mm). (B) The levels of NF-κB, IL6 and IL8 in the brains were measured by western blotting. (C)The mRNA expressions of NF-κB, IL6 and IL8 in the brains of each group were assessed by realtime-PCR. The date were performed using one-way analysis of variance (ANOVA), followed by the student two-tailed t test. Significant as compared to normal control group. *p＜0.05 ,**p＜0.01,***p < 0.001. Significant as compared to model control group. #p＜0.05 ,##p＜0.01.

Figure 3

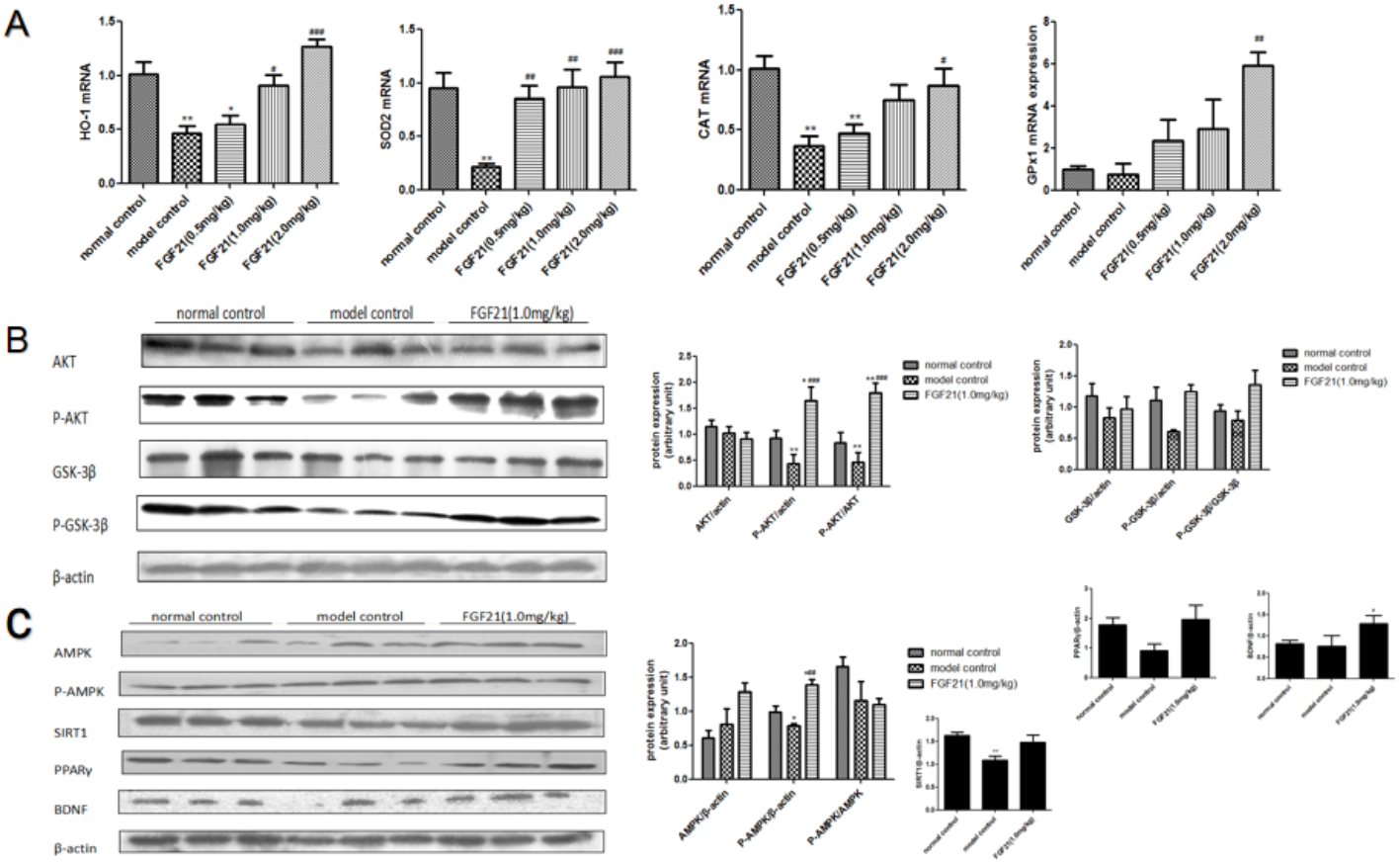

Fig.4. FGF21 activates AKT/GSK-3β pathway and AMPKα/SIRT1 pathway in diabetic mice (n=10/group). (A) FGF21 increased the mRNA expression of anti-oxidant enzyme system in the brains, including HO-1, CAT, SOD2 and GPx1. (B)The levels of AKT, GSK-3β and antophosphorylations in the brains were measured by western blotting. (C)The levels of AMPKα, P-AMPKα, SIRT1, PPARγ, and BDNF in the brains were measured by western blotting. The date were performed using one-way analysis of variance (ANOVA), followed by the student two-tailed t test. Significant as compared to normal control group. *p＜0.05 ,**p＜0.01. Significant as compared to model control group. #p＜0.05 ,##p＜0.01, ###p＜0.001.

Figure 4

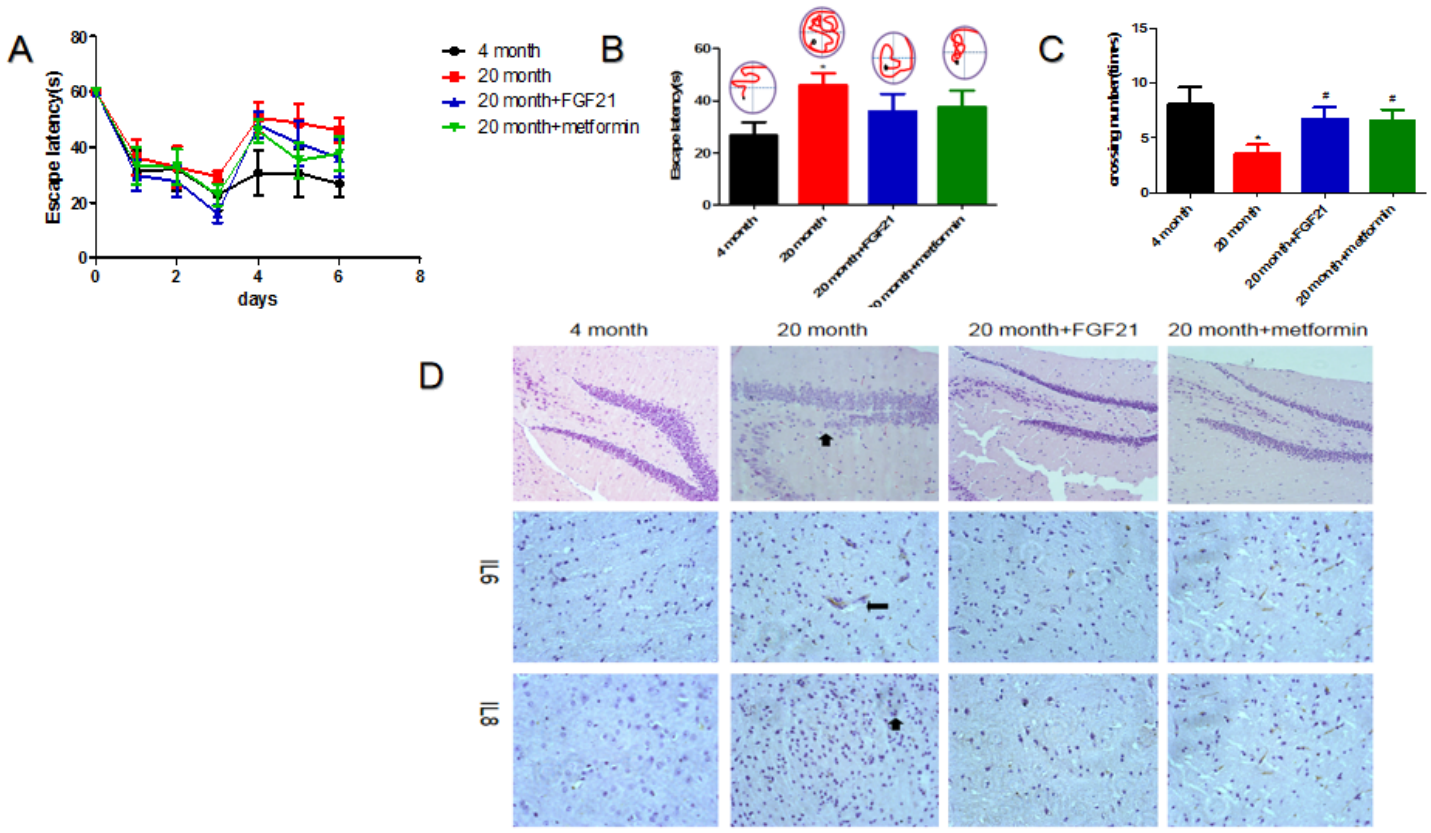

Fig.5. FGF21 attentudes neurodegeneretion though anti-inflammation in aging mice(n=7-8/group). (A)The escape latency travelled to the hidden platform for six consecutive days of each group mice. (B)The escape latency travelled to the hidden platform on the seventh day of each group mice. (C) The number of each group mice crossing the target quadrant on the seventh day. (D) The injury levels were exhibited by H&E staining (10X magnification; bar = 50 mm) and IL6, IL8 levels were exhibited by immunohistochemistry (40X magnification; bar = 50 mm) in the brains of each group mice. The FGF21(1.0mg/kg) and metformin(20mg/kg) were used. The date were performed using one-way analysis of variance (ANOVA), followed by the student two-tailed t test. Significant as compared to young mice. *p＜0.05 . Significant as compared to aging mice. #p＜0.05 .

Figure 5

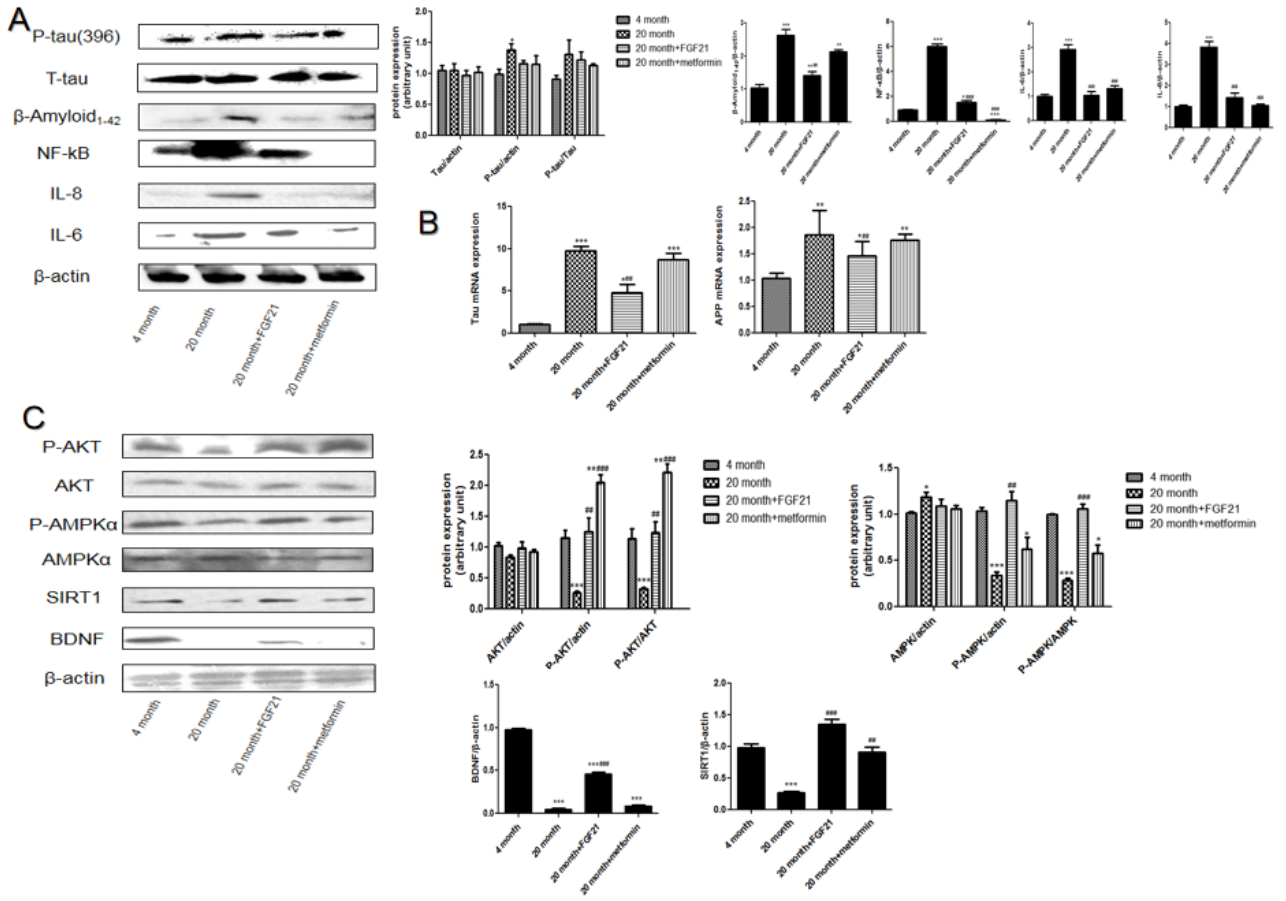

Fig.6. FGF21 attentudes nerve injury though AKT and AMPK pathway in aging mice (n=7-8/group). (A)The protein levels of Tau, P-Tau, β-Amyloid$_{1-42}$, NF-κB, IL6, and IL8 were measured by western blotting in the brains of each group mice. (B)FGF21 decreased the mRNA expression of Tau and APP in the brains. (C)The relative of AKT, P-AKT, AMPKα, P-AMPKα, SIRT1 and BDNF levels were compared with β-actin. The date were performed using one-way analysis of variance (ANOVA), followed by the student two-tailed t test. Significant as compared to young mice. *p＜0.05 ,**p＜0.01, ***p＜0.001. Significant as compared to aging mice. #p＜0.05 ,##p＜0.01, ###p＜0.001.

Figure 6

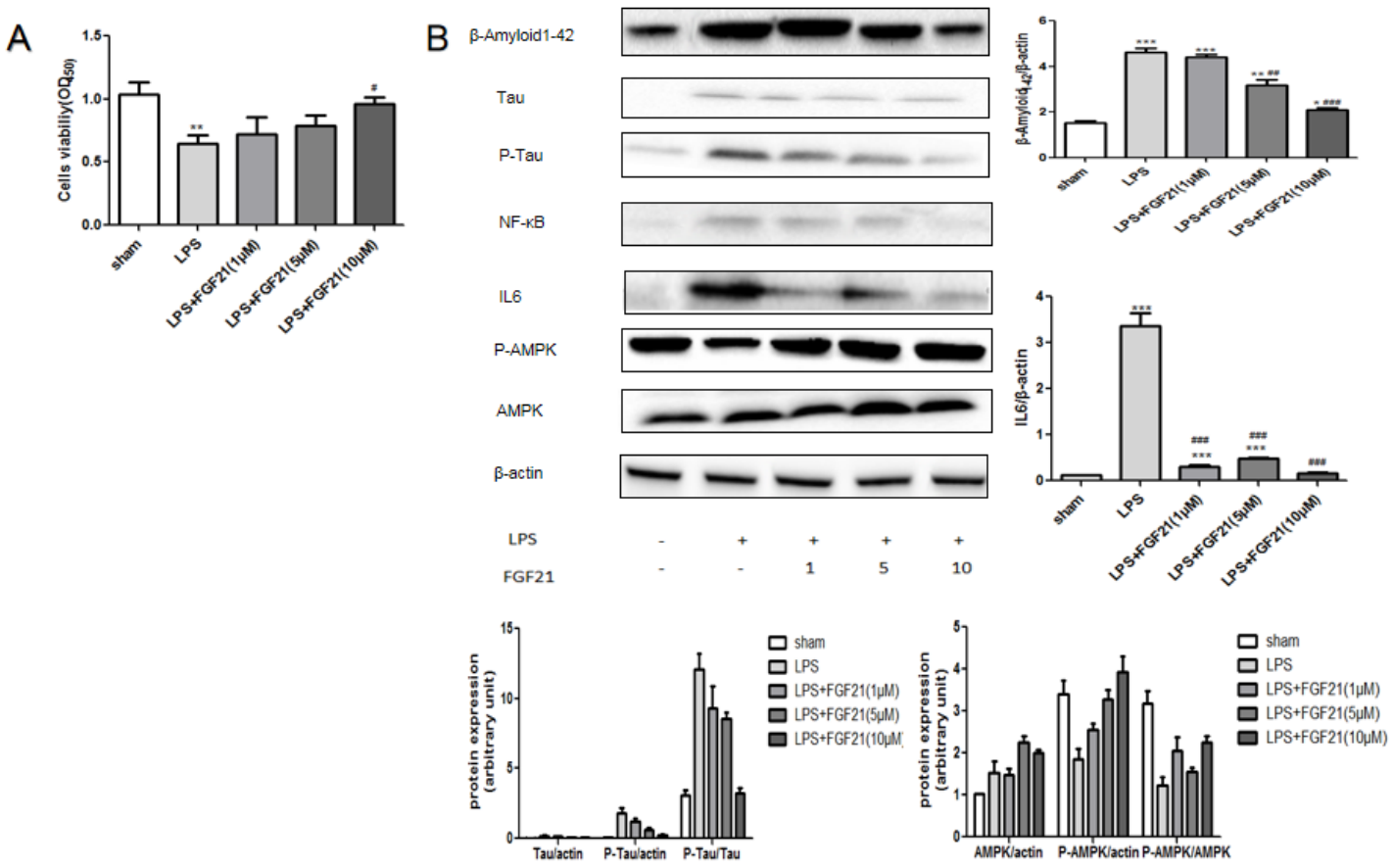

Fig.7. FGF21 attentudes neurodegeneretion though anti-inflammation and anti-oxidant stress in SH-SY5Y cells. (A) The cells viability treated by LPS and FGF21 was assessed by CCK8 Kit in SH-SY5Y cells. (B)The levels of β-Amyloid$_{1-42}$, Tau, P-Tau, NF-κB, IL6, AMPKα, P-AMPKα were measured by western blotting in SH-SY5Y cells.The date were performed using one-way analysis of variance(ANOVA), followed by the student two-tailed t test. Significant as compared to sham group. *p＜0.05 ,**p＜0.01, ***p < 0.001. Significant as compared to LPS group. #p＜0.05 ,##p＜0.01, ###p＜0.001.

Figure 7

Supplementary Files

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- Table1.jpg
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- Table2.jpg