FGF21 Attenuates Neurodegeneration though Reducing Neuroinflammation and Oxidant-stress

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Abstract

Background It is reported that FGF21 can repair the nerve injury, but the specific mechanism is less studied. The present study was designed to investigate the effects and possible mechanisms of FGF21 on neurodegeneration of the aging in 20 month old C57BL/6 mice and the diabetes in C57BL/ksj -db/db mice which were susceptible to Alzheimer's disease (AD).

Methods The db/db mice and aging mice were used to verify the effects and possible mechanisms of FGF21 on neurodegeneration. These mice was processed with PBS, FGF21 or metformin once daily for several months. The related indicators of neurodegeneration were assessed by Quantitative Real Time-PCR, western blot and others.

Results There were loss of nerve cells and intracellular edema around hippocampus in db/db mice and aging mice which were inhibited by FGF21. In vivo investigation revealed that the procession of FGF21 led to suppress the aggregation of Tau and β-Amyloid1-42 which led to apoptosis in nerve cells. Meanwhile, FGF21 significantly decreased the expression of NF-κB, IL6 and IL8 ($p<0.05$) and increased anti-oxidant enzymes ($p<0.05$) in db/db mice. In addition, the phosphorylation of AKT and AMPKα were increased by FGF21 in db/db mice, which were considered as anti-inflammation and anti-oxidant stress pathway. The related indicators of neurodegeneration were also exhibited in aging mice which showed similar trends. The vitro experiment showed that the aggregation of Tau and β-Amyloid1-42 were increased by LPS, and hyperaggregation of Tau and β-Amyloid1-42 were inhibited by FGF21 in SHSY-5Y cells.

Conclusion It was concluded that FGF21 attenuated neurodegeneration by reducing neuroinflammation and oxidant stress though regulating the NF-κB pathway and AMPK pathway which enhanced the protective effect of mitochondria on nerve cells.

Background

Diabetes mellitus is a metabolic disease with multiple etiologies, characterized by chronic hyper-glycemia resulted from disturbance of insulin secretion or action, and it is associated with aging particularly. Recently, it is reported that the number of being living with diabetes maybe reach 600 million in 2035 over the world, and among these people, the risk of developing 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 has been illustrated that the neurodegeneration associated with aging and diabetes are pathological basis for AD, a progressive degenerative disease characterized by aggregation of Amyloid and hyperphosphorylation of Tau protein [3]. Diabetes and AD shared corporate abnormalities, including impaired glucose metabolism, increased oxidant stress, chronic inflammation, insulin resistance, hyperphosphorylation of Tau protein and aggregation of Amyloid [4]. The diabetes and aging mice model have provided a relevant animal model for diabetes and nerve damage, and developed many AD-like neuropathological features, including AKT inhibited activation, hyperphosphorylation of

Tau protein and glycogen synthase kinase–3β (GSK–3β) over-activation and so on [5,6]. The db/db mice were used usually [7]. Multiple studies have reported that the pathogenesis of AD has been described from inflammatory responses, lipid accumulation, cell apoptosis / necrosis and protein metabolic disturbance [8,9]. Therefore, these might be possible effective mechanism for the neurodegeneration about this disease.

Fibroblast growth factor 21 (FGF21) is an endogenic hormone which is expressed in liver, adipose tissue and pancreas and induced by starving stress or some pathological status. It is reported that FGF21 has many physiological functions, such as hypoglycemic, anti-inflammation, and anti-oxidant stress [10,11]. Metformin also has many physiological functions, which was used widely in diabetes and considered as potential anti-aging drugs, hence we used it as contrast drug. Evidences suggest that the β-klotho/FGFR receptor complex is responsible for the major regulative metabolic functions of FGF21 and expressed in the hypothalamus [12,13]. So FGF21 can perform function in the brain [14]. We hypothesized that FGF21 could not only reduce the burden of high blood glucose in blood circulation, but also reduce the injury caused by harmful substances and strengthen the protection for brain.

Increasing evidences suggest that nerve damage is mainly mediated by inflammatory response, oxidant-stress and apoptosis in both vitro and vivo [15]. Inflammation associated with oxidant stress results from the conflict in body's accept and resistance to injury, and it contributes many nerve diseases [16]. In addition, previous research has reported that inflammation induced by LPS, a strong inflammatory irritant, causes hippocampus damage by regulating protein expression involved in IL–6 and IL–8, which are important pro-inflammatory factors [17]. Studies have shown that the imbalance between the pro-inflammation and anti-inflammation results in mitochondria damage, cell apoptosis, and tissue necrosis [18]. Moreover, the increase in mitochondria damage causes the dysfunction of oxidant and antioxidant system, such as heme oxygenase–1 (HO–1), catalase (CAT), superoxide dismutase (SOD) and glutathion peroxidase–1 (Gpx–1), then Radical oxygen species (ROS) is accumulated largely. Conclusively, in the brain damage of diabetes and aging, inflammation and oxidant stress play a central role, which are activated by necrocytosis and metabolic dysregulation.

Adenosine Monophosphate Activated Protein Kinase α (AMPKα) is emerging as an important protein kinase that regulated metabolism and energy demand mainly, including mitochondia injury inducing peroxidation [19]. Sirtuin1 (SIRT1) associated with AMPKα, belonging to the mammalian sirtuin family, plays a pivotal role in many pathophysiological process, such as apoptosis, aging, inflammation and oxidation-stress though multiple factor, including PPARγ, NF-κB and others [20,21]. These proteins are involved in mediating the expression of nerve related 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 transfer process, not only related with the secretion of inflammatory factors and peroxide, but also participated in inhibiting neuroprotective process.

Therefore, the aim of the current study was to specifically evaluate the therapeutic effects on neurodegeneration and illuminate the mechanism about neurodegeneration which was treated by FGF21.

Methods

Preparation of PEGylated FGF21 (pFGF−21)

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) was grown in LB media containing ampicillin (100ug/ml). When the OD600 reach 0.4 to 0.6, IPTG（final concentration:0.25mmol/L）was added into the medium and then FGF21 protein was expressed at 25℃ for 6 hours. The FGF21 was purified by AKTA Purifier (GE Healthcare) with Ni Sepharose 6 Fast Flow chromatography. The FGF21 was PEGylated by methoxy polyethylene glycol (mPEG) (Sigma, USA) [22]. The purity of pFGF21 was analyzed by SDS-PAGE. The activity assay of pFGF−21 in vitro was performed by HepG2 cells which were seeded 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 pFGF−21 for another 24h. Glucose uptake was measured using a glucose assay kit (Beijing Kingkawk pharmaceutical CO, LTD).

Assessment of SH-SY5Y cells

Cell viability was measured by a modified CCK8 assay (BestBio, Shanghai). SH-SY5Y cells were seed on 96-well plates and incubated with LPS (100μg/ml, sigma, USA) and in the presence of pFGF21 (0, 0.1, 1, 10μmol/L) for 48h, then 100μl each well was add with CCK8 in another well and cells were incubated at 37℃ for 4h. Absorbance at 450nm were measured using ELISA. The cells experiment was done in 6-well plates again for testing the levels of protein by western blot.

Animals and treatment regimens

All procedures involving mice were accord with the Animal Care and Use Committee of Institute of Materia Medica, PR.CHINA. Male C57BL/ksj-db/db mice (7–8 weeks, Shanghai Silaike Experimental Animals LTD) and the C57BL/6 mice (7–8weeks) were bred and maintained under constant SPF conditions and temperature 23±2℃, humidity 50±5%, with 12h light and 12h dark cycles. In the mouse experiment, db/db mice were divided into 5 groups: (1) wild type group for db/db mice (wild type, n = 10), (2) db/db mice treated with PBS (model type, n = 10), (3) db/db mice treated with pFGF−21 at a dose of 0.5mg/kg (0.5mg/kg, n = 10), (4) db/db mice treated with pFGF−21 at a dose of 1.0mg/kg (1.0mg/kg, n = 10), (5) db/db mice treated with pFGF−21 at a dose of 2.0mg/kg (2.0mg/kg, n = 10), PBS or pFGF−21 were given into the mice once daily for four months. Blood samples were taken from each mouse of five groups for serum T-AOC, CAT and T- SOD measurements. The T-AOC, CAT and SOD were measured by T-AOC (A015−1−2), CAT (A007−1−1) and SOD (A001−3−2) Assay Kit which 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 seed before the experiment ending (n = 10), (2) 14 month mice were treated by PBS (n = 10), (3) 14 month

mice were treated by pFGF21 (1.0mg/kg, n = 10), (4) 14 month mice were treated by metformin (20mg/kg, Sigma, USA, n = 10). PBS, pFGF–21 or metformin were given into the mice once daily for six months. The related indicators 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 the experimental animals were removed one lobe in 4% formaldehyde solution and embedded in paraffin. 5um sections were cut and stained with hematoxylin and eosin. The others were flash frozen in liquid nitrogen and stored at –80°C for RNA and western blotting.

Immunohistochemisty 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 in methanol 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°C, 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.

Reverse Transcription and Real-time PCR

Total RNA was isolated from tissues (100mg) using TRIZOL (Invitrogen, Carlsbad, CA) to perform Reverse transcription and quantitative PCR using M-MLV and RT-qPCR kit (Invitrogen, Carlsbad, CA). Real-time PCR was carried out using the Eppendorf Realplex 4 instrument (Eppendorf, Hamburg, Germany). The β-actin was detected at the same time and used 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). The secondary antibody was used by Rabbit (1:7500, HAF008, R&D, USA) or Mouse (1:7500, HAF007, R&D, USA) IgG HRP-conjugated antibody. Blots were developed using an ECL kit (Amersham Biosciences, Piscataway, NJ) and exposed with Blue BasicAutorad Film (ISC BioExpress).

Statistical analysis

Statistical analysis was performed by SPSS 19.0, and date were expressed as mean ± SD. All data were 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 pFGF21 remits the brains injury in db/db mice

The activity of Pfgf21 was assayed by glucose uptake in HepG2 cells. Results showed the activity of pFGF21 was exhibited in Fig.1A. After injecting pFGF21 or PBS four months, the weight of the db/db mice with PBS was much heavier than the db/db mice with pFGF21. The wild mice were much lighter than the above groups (Fig.1B). But it was no difference in aging mice. Sections of mouse brains was stained with a hematoxylin and eosin (H&E) procedure. The brain processed with H&E revealed that the brain injury in the db/db mice was in contrast to the pFGF21 treated groups. Compared with the model type group, the loss of nerve cells and intracellular edema around hippocampus were inhibited (Fig.2A) and the aggregation of Tau and β-Amyloid$_{1-42}$ which were typical protein in chronic nerve damage were reduced obviously in pFGF21 treated group ($p<0.05$) (Fig.2B). There was clearly alleviating symptoms which were hippocampal neuron cells decreasing in number, cells apoptosis action increasing in the brains in pFGF21 treated groups. FGF21 regulated the expression of Bcl_2 and Bax which controlled the apoptosis pathway ($p<0.05$) (Fig.2C). The results indicated that nerve damage from the mice treated by FGF21 was far less than from the model group in db/db mice.

FGF21 attenuates the expression of NF-κB, IL6 and IL8 in db/db mice

According to the previous reports, db/db mice over-expressed inflammatory cytokines which might be potential risk factors about brain damage [23]. The Immunohistochemisty Analysis exhibited that the expression of IL6 was enhanced in db/db mice, and pFGF21 inhibited it (Fig.3A). Our result showed that the mRNA and protein expression level of the pro-inflammatory cytokines, NF-κB, IL6 and IL8, in the brain of the db/db mice which were treated by pFGF21 were lower than the model type group ($p<0.05$)

(Fig.3B,C). In the obvious reports, NF-κB translocated and accumulated which was inducing the expression of inflammatory cytokines in inflammation. In neurodegeneration of diabetes, FGF21 inhibited inflammatory action.

FGF–21 promotes the activities of anti-oxidant enzymes system in different treated doses

The antioxidant enzymes system, such as HO–1, SOD, T-AOC and CAT, were used as a biomarker of anti-oxidant stress to judge the status of the body. The result showed that compared with the model type group, the activities of SOD, T-AOC and CAT in the pFGF21 treatment increased which were similar to the wild type group in serum of the mice. The pFGF21 (1.0, 2.0mg/kg) significantly prevented the db/db mice inducing diabetic obesity model though the decrease of SOD and CAT activities ($p<0.01$). The activity of T-AOC was also promoted in db/db mice treated by pFGF21 (2.0mg/kg) ($p<0.05$), shown in Table.2. Similar results were occurred in brains. The expressions of mRNA about HO–1, SOD, CAT, and GPx1 were regulated by pFGF21 in brains (Fig.4A). From the research, the antioxidant enzymes system were regulated by pFGF21 which was proved be connected with anti-inflammation function and the survival of cells.

FGF21 activates AKT/NF-κB pathway and AMPKα/SIRT1 pathway to inhibit nerve injury in db/db mice

Chronic inflammation reaction was one of the major features in brain damage. To determine whether pFGF21 could inhibit inflammatory responses during brain damage, the assessed proteins were not only about inflammation, but also the proteins connected with FGF21 and NF-κB. It was observed that there were decreases of the levels of the phosphorylation of AKT in db/db mice compared with the wild type group. However, these were decreased in the pFGF21 groups ($p<0.01$) (Fig.4B). What' more, the phosphorylation of GSK–3β were up-regulated in brain tissues of the pFGF21 groups which were closed to the wild type group. The phosphorylation of AKT and GSK–3β could prevent NF-κB nuclear translocation producing inflammatory action.

As shown in Fig.4C, AMPKα/SIRT1/PPARγ signaling pathway not only participated in regulating the aggregation of tau and β-Amyloid, but also accelerated expression of BDNF helping repair nerve damage. It was connected with the repair of mitochondria function. The result was shown that the SIRT1, AMPKα, PPARγ and BDNF were up-regulated in the brain tissues of db/db mice treated by pFGF21 ($p<0.05$). The activation of AMPKα and SIRT1 inhibited inflammatory responses and oxidant stress. Compared with model type group, the inflammatory responses, oxidant stress injury and the apoptosis of cells were inhibited by the treatment of pFGF21.

FGF21 attentudes neurodegeneretion though anti-inflammation in aging mice.

To explore whether FGF21 could reverse aging cognitive deficits, we used the Morris water maze.

It was found that aging mice displayed prolonged escape latency to find uncover platform from the fourth day (Fig. 5A). After removing the platform on the seventh day, significant differences were found between the four groups in escape latency and crossing number (Fig.5B, C). We observed longer escape

latency and fewer crossing number in aging mice than the mice treated by FGF21 and metformin, indicating that FGF21 and metformin effectively restored the cognitive deficits. The injury levels was exhibited by H&E and the IL−6 and IL−8 levels were exhibited by immunohistochemistry. Compared with the model type group, the chronic inflammation, the loss of nerve cells and intracellular edema around hippocampus were inhibited by FGF21 and metformin (Fig.5D)

FGF21 activates AKT/NF-κB pathway and AMPKα/SIRT1 pathway to inhibit nerve injury in aging mice

The aggregation of Tau and β-Amyloid$_{1-42}$ were increased in aging mice, and the inflammation factors exhibited similar trends. But FGF21 and metformin inhibited these proteins (Fig.6A). What's more, it was observed that the levels of the phosphorylation of AKT, AMPKα in aging mice were lower compared with the wild type group. However, these were decreased in the FGF21 and metformin groups ($p<0.01$) (Fig.6B). The result was shown that the SIRT1 and BDNF were up-regulated in the brain tissues of aging mice treated by pFGF21 ($p<0.001$). But the effect of metformin was not obvious. Compared with model type group, the inflammatory responses, oxidant stress injury and the apoptosis of cells were inhibited by the treatment of FGF21 and metformin.

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

The effectiveness on nerve cells of LPS was assessed. As shown in Fig.7A, a vitro CCK8 assay was carried out to identify the effect of LPS which inhibited SH-SY5Y cells proliferation and survival. Our results showed that LPS had significant cytotoxic effects on nerve cells ($p<0.01$), which was prevented by pFGF21. What's more, the expression levels of proteins involved the Tau and β-Amyloid were assessed. As a result, we found that pFGF21 could significantly inhibit the aggregation of Tau and β-Amyloid$_{1-42}$ which was increased by LPS-induced inflammation though NF-κB and AMPKα pathway in SH-SY5Y cells (Fig.7B).

Discussion

In the previous finding, lowering glucose and lipid were thought as basic functions of FGF21, which were considered as the reason of anti-aging by FGF21 [24]. The activity of FGF21 we purified was verified by glucose uptaking which was used widely, and PEG-FGF21 has a longer half-life [25]. Our findings were suggested that the activity of the FGF21 prepared by us was enough to enhance the glucose uptake. And the weight of the db/db mice decreased after 4 months treatment by pFGF21 compared with treatment by PBS, but the weight of aging mice treated by FGF21 and metformin had no significant difference [26].

Type 2 diabetes was thought as a common chronic disease by insulin resistance or secretion reduction causing by islet β cell damage which was regulated by FGF21 [27]. Infusion of FGF21 in mice resulted 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 triggered maladaptive responses in mitochondria including reduced expression

of genes and proteins related to oxidative phosphorylation and inflammation [29]. Previous advances showed that the neurodegeneration were caused by chronic inflammation and oxidant stress, the important dangerous factors about many age-related disease [30]. However, the hypoglycemic drug insulin could not solve the problem of diabetes-induced neurodegeneration, so it was very important to find a long-term drug treating diabetes and improving its nerve injury, specifically hippocampus 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 were 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 were found a lot of diabetes complications such as nerve damage, hepatitis and myocarditis like aging [31]. Our experiment was shown that the symptom about nerve injury could be significantly improved by FGF21 in db/db and aging mice, and 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 brain function. These results were in agreement with the hippocampus histological changes that were detected with H&E staining, immunohistochemisty analysis and western blot of the brains [34]. In our present study, we found that the symptoms of brains which inflammation and the loss of nerve cells were abnormal in the hippocampus of db/db and aging mice, but FGF21 improved it [35]. This was confirmed the effect of pFGF21 on the nerve injury. Hence, in order to clarify how pFGF21 protect the brain, we explored the possible mechanism about inflammation, oxidant stress and some critical regulatory factors in the present study.

It was demonstrated that the aggregation of tau and β-Amyloid$_{1-42}$ were higher than normal mice, so it could be confirmed 20 month old mice and db/db mice as the model of neurodegeneration. And the FGF21 reduced the injury. In the present research, it has been suggested that NF-κB and the anti-oxidant enzymes system are necessary mediators of inflammation and oxidant stress injury [36,37]. In our study, we found that the levels of IL−6 and NF-κB in the brain tissues of the model mice were higher than in the normal mice. In contract, 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 were 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 were reported to be connected with nerve injury. It could promote the apoptosis of nerve cells, then induce the aggregation of the β-Amyloid. The result was a breakdown of neurotransmitter transmission [40,41]. AKT was an important metabolic regulator that was suppressed in metabolic block such as db/db mice,

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

AMPKα as AMP kinase to regulate energy metablism, is important to maintain mitochondria stabilize function and adaptability [43]. FGF21 interaction with the AMPK pathway is multifaceted [44]. The protection effects about the rate of AMP/ADP, anti-oxidant stress ability were characterized by the phosphorylation of AMPK. 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 promoted activated by FGF21, thus directly or indirectly promoted the expression of downstream proteins such as PPARY, which were connected with NF-κB [46]. The expression of BDNF preventing apoptosis of nerve cells and inhibiting protein tau polymerization was promoted by these proteins.

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 were 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 and treatment mechanism of FGF21 on neurodegeneration, then we used SH-SY5Y cells treated by LPS to test that FGF21 inhibited neurodegeneration though anti-inflammation and anti-oxidant stress, but we could not exclude the hypoglycemic effect of FGF21. The experiment should be used more nerve injury models and FGF21-associated gene mice, but our experiment proved the potential application value of FGF21 on neurodegeneration.

Conclusions

In this study, we researched the mechanisms about neurodegeneration in db/db and aging mice and the protection from nerve injury caused by neuroinflammation by FGF21. The research provided a new strategy about treating chronic nerve damage. In a conclusion, FGF21 inhibits the polymerization of tau andβ-Amyloid, promotes the expression of BDNF though anti-inflammation and anti-oxidant stress regulated by NF-κB and AMPK signaling pathway.

Abbreviations

AD: Alzheimer’s disease

GSK−3β: glycogen synthase kinase−3β

FGF21: Fibroblast growth factor 21

pFGF21: PEGylated 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

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 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

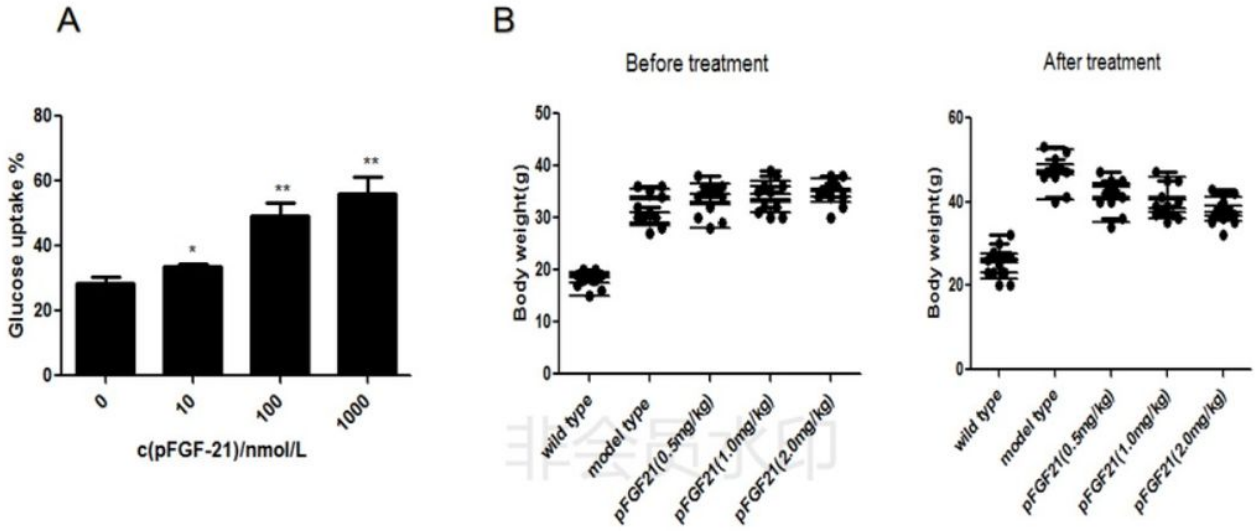

Fig .1. PEG-FGF21 decreases the weight of db/db mice. (A)Glucose uptake of HepG2 cells after treatment with different concentrations of pFGF21; (B) The weight of C57BL/6 mice and db/db mice treated by pFGF21 with different concentrations. The date were performed using one-way analysis of variance (ANOVA), followed by the student two-tailed t test. Significant as compared to pFGF21 (0nmol/L). *p＜0.05 ,**p＜0.01.

Figure 1

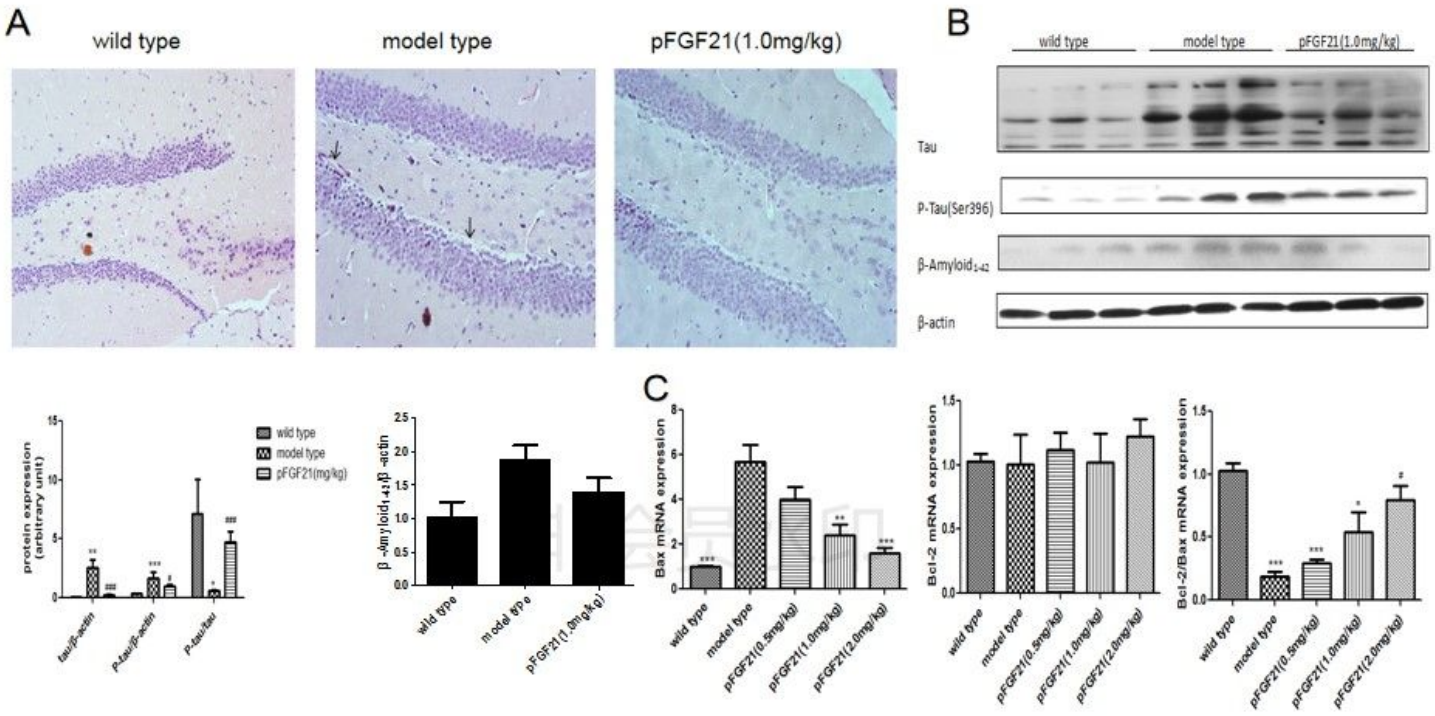

Fig.2. pFGF21 remits nerve cells damage and apoptosis in brains of db/db mice. (A)The injury levels of H&E in brains. (B)The levels of Tau and β-Amyloid1-42 in brains were measured by western bloting. (C)The mRNA expression of Bax and Bcl-2 in brains of each group. The date were performed using one-way analysis of variance (ANOVA), followed by the student two-tailed t test. Significant as compared to wild type. *p＜0.05 ,**p＜0.01,***p < 0.001. Significant as compared to model type. #p＜0.05 ,##p＜0.01.

Figure 2

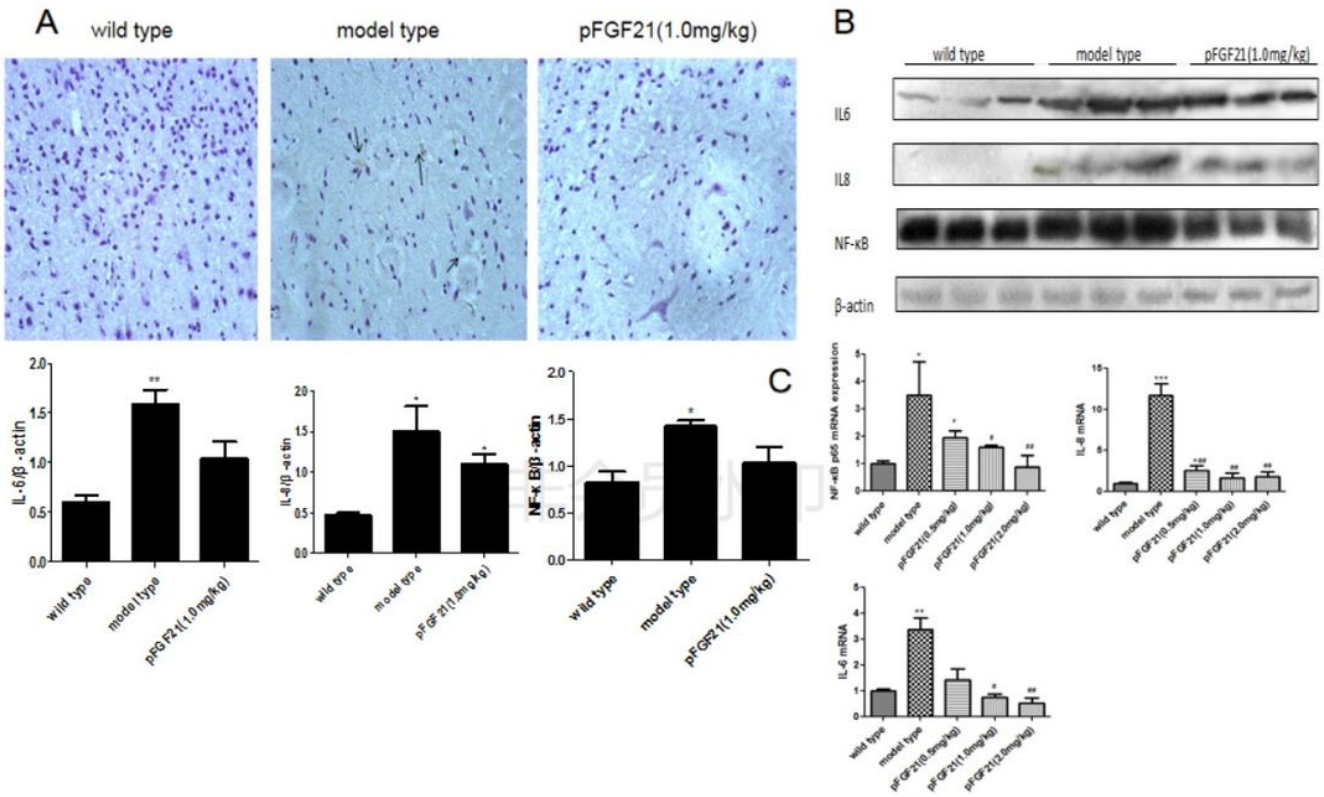
A
wild type
model type
pFGF21(1.0mg/kg)
B
wild type
model type
pFGF21(1.0mg/kg)
IL6
IL8
NF-κB
β-actin
C
IL-6/β -actin
IL-8/β -actin
NF-κ B/β -actin
wild type
model type
pFGF21(1.0mg/kg)
NF-κB p65 mRNA expression
IL-8 mRNA
IL-6 mRNA

Figure 3

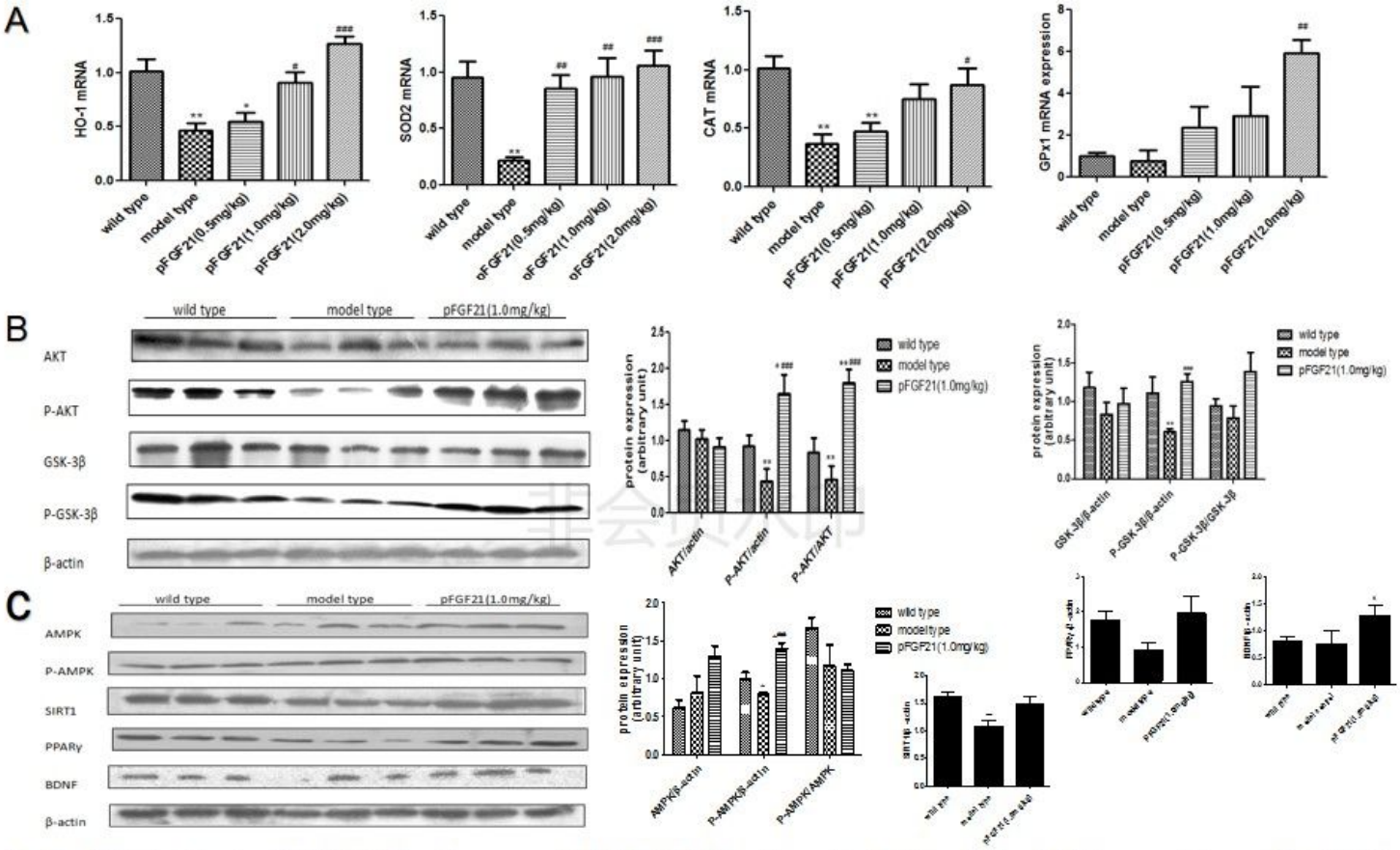

Fig.4. pFGF21 activates AKT/GSK-3β pathway and AMPKα/SIRT1 pathway in db/db mice to inhibit nerve injury. (A) pFGF21 increases the mRNA expression of the anti-oxidant enzyme system, including HO-1, CAT, SOD2 and GPx1, in brains. (B)The levels of AKT, GSK-3β and antophosphorylations in brains were measured by western blotting. (C)The levels of AMPKα, P-AMPKα, SIRT1, PPARγ, and BDNF in 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 wild type. *p＜0.05 ,**p＜0.01, ***p < 0.001. Significant as compared to model type. #p＜0.05 ,##p＜0.01, ###p＜0.001.

Figure 4

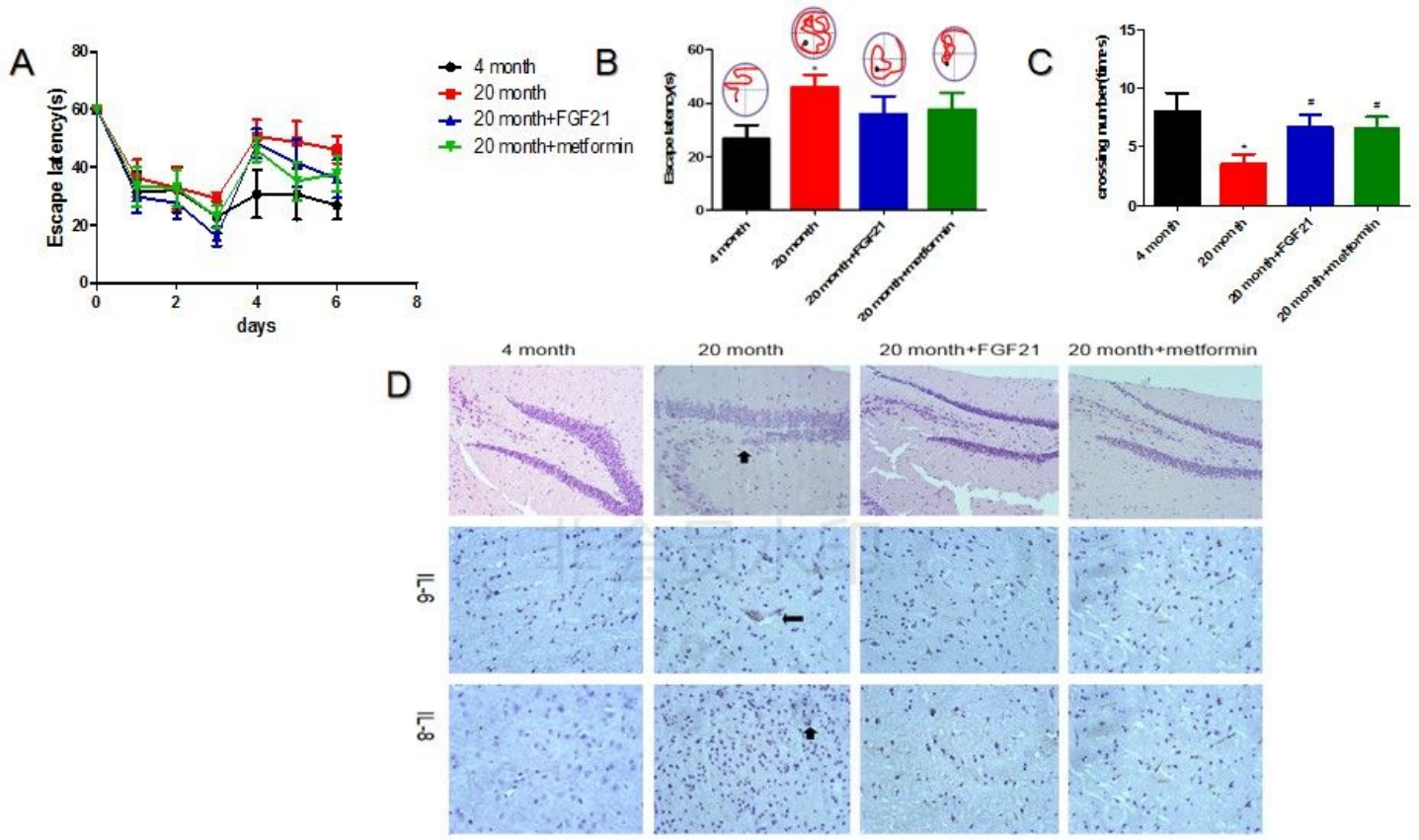

Fig.5. pFGF21 attentudes neurodegeneretion though anti-inflammation and anti-oxidant stress in 20 month old mice. (A)The escape latency travelled to the hidden platform for six consecutive days. (B)The escape latency travelled to the hidden platform on the seventh day. (C) The number of crossing the target quadrant on the seventh day. (D) The concentration of AGEs was assessed by elisa Kit in serum. (E) The injury levels was exhibited by H&E and the IL-6 and IL-8 levels were exhibited by immunohistochemistry. The date were performed using one-way analysis of variance (ANOVA), followed by the student two-tailed t test. Significant as compared to wild type. *p＜0.05 ,**p＜0.01, ***p < 0.001. Significant as compared to model type. #p＜0.05 ,##p＜0.01, ###p＜0.001.

Figure 5

Fig.6. pFGF21 attentudes inflammation oxidant stress though AKT and AMPK pathway in 20 month old mice. (A)The levels of Tau, P-Tau, β-Amyloid1-42, NF-κB, IL-6, and IL-8 were measured by western blotting. (B)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 wild type. *p＜0.05 ,**p＜0.01, ***p < 0.001. Significant as compared to model type. #p＜0.05 ,##p＜0.01, ###p＜0.001.

Figure 6

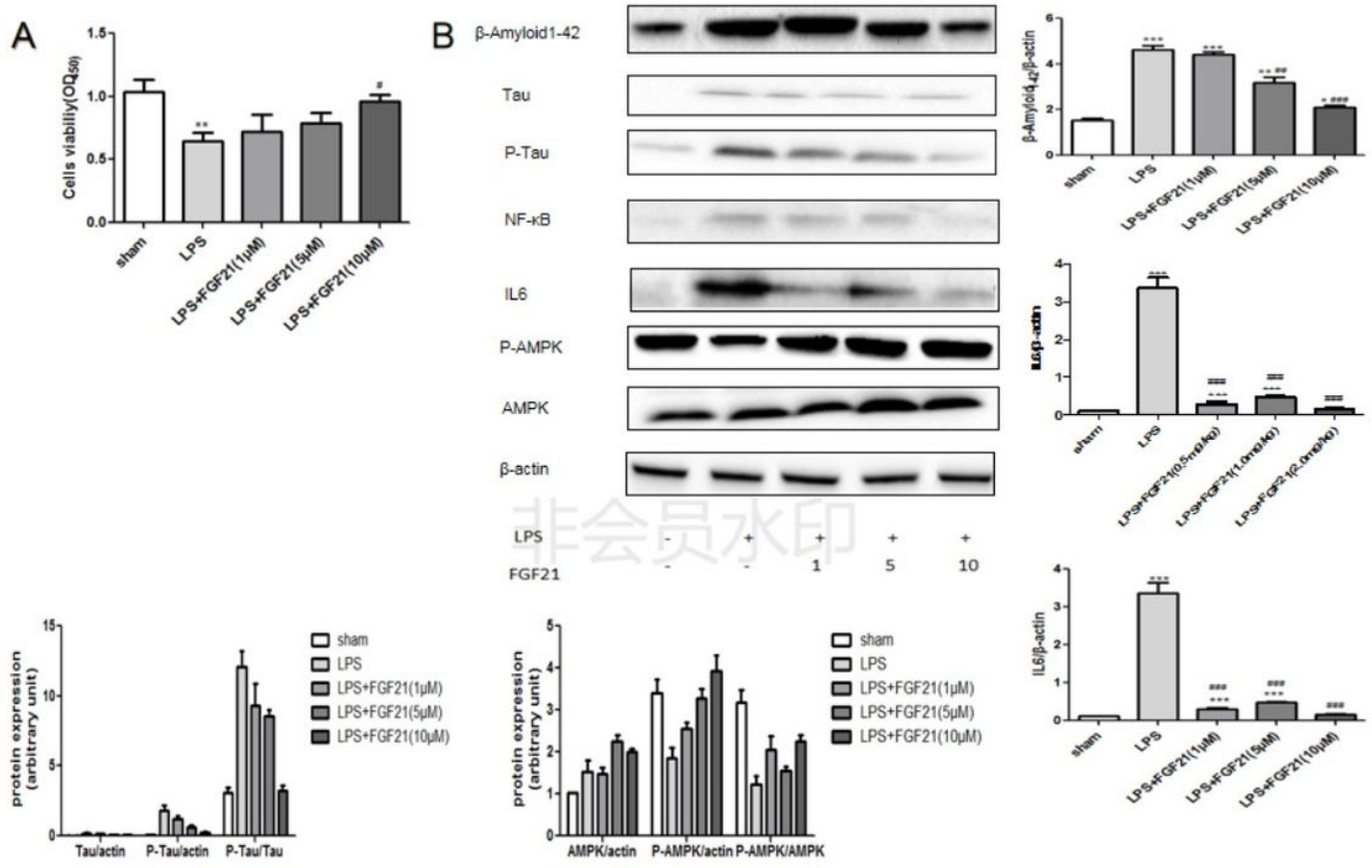

Figure 7

The effectiveness on nerve cells of LPS was assessed. As shown in Fig.7A, a vitro CCK8 assay was carried out to identify the effect of LPS which inhibited SH-SY5Y cells proliferation and survival.

Supplementary Files

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- renameda13c3.png