Original Article
FGF2 shows therapeutic effects in Alzheimer's disease animal model via suppressing PI3K/Akt mediated ER stress

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Abstract: Background: Alzheimer's disease (AD) is one of the leading causes of dementia in elderly people. The hippocampal amyloid-β (Aβ) accumulation was recognized as the pathological basis for AD. FGF2 showed therapeutic effects in neurodegenerative diseases. However, FGF2's effects on AD and specific mechanism are still unclear. Methods: APP/PS1 transgenic mice were selected as the AD animal model. Mice received intranasal administration of FGF2 and oral administration of PI3K inhibitor GDC-0941. Morris water maze test was utilized to assess the spatial learning ability; ELISA was used to detect the concentration of FGF2 in CSF; immunofluorescence was applied to evaluate the neurogenesis; the hippocampal CA1 region synaptic transmission and plasticity was evaluated by long-term potentiation (LTP); Western blotting was used to exam the expressions of Aβ species, activation of ER stress, PI3K/Akt pathway and PERK/BACE1 pathway. Results: FGF2 administration dramatically improved the spatial learning ability as well as hippocampal LTP of APP/PS1 transgenic mice. Pathologically, FGF2 treatment attenuated Aβ species accumulation in hippocampus. Mechanically, PI3K/Akt pathway was activated while ER stress was suppressed. As a result, PERK signaling of ER stress was inhibited, leading to down-regulation of BACE1. Moreover, PI3K was recognized as the target for FGF2 which was testified by GDC-0941 treatment. The PI3K inhibitor significantly impaired FGF2's effects on spatial learning ability, hippocampal LTP and Aβ species accumulation. Furthermore, after treatment of the PI3K inhibitor, ER stress was less affected and PERK/BACE1 pathway was then activated. Conclusions: FGF2 treatment could be used as a potential alternative therapy for AD, PI3K/Akt pathway regulated ER stress is involved in the mechanism.

Keywords: Alzheimer's disease, fibroblast growth factor2, PI3K/Akt, endoplasmic reticulum stress

Introduction

In worldwide, Alzheimer's disease (AD) is one of the most frequent neurodegenerative diseases and takes responsibility for over 80% dementia in elderly population [1]. Loss of mental and behavioral function and decline of learning ability are the featured clinical manifestations of AD. It is now generally accepted that beta-amyloid plaques accumulation, neurofibrillary tangles and neuron loss are the characterized pathological features of AD [2]. It was found that amyloid-β peptides aggregation is one of the key initiators inducing the onset of AD [3]. After APP (amyloid-β precursor protein) are cleaved by BACE1 (β-secretase)

and γ-secretase complexes, Aβ species including Aβ40, Aβ42 and smaller peptides such as Tau are formed [4]. These peptides are neurotoxic and induce pathological changes of AD.

As one of the important organelles, endoplasmic reticulum (ER) executes fundamental biological progresses such as calcium homeostasis, protein folding and post-translational modification. Under certain pathological conditions, oxidative stress for instance, the normal function of ER could not be maintained and ER stress is induced [5]. It was reported that ER stress was triggered in the brains of AD which was evidenced by up-regulation of ER stress markers [6]. These results suggested that ER

stress is probably involved in the onset of AD. In several recent studies, it was showed that one of the ER stress transducers, PERK (RNA-activated protein kinase-like ER kinase), could elevate the expression of BACE1 [7] which subsequently enhance the levels of Aβ species.

Fibroblast growth factor2 (FGF2) belongs to a large protein family which bind to heparin and heparan sulfate [8]. It is believed that FGF2 could modulate various biological functions in a wide range of cell types. FGF2 is one of the typical neurogenic factors that regulate the adult neurogenesis by affecting proliferation and cell fate in central nerve system [9]. It was reported by several studies that exogenous FGF2 exerted neuroprotective effects against neurodamage and neurogeneration in vivo and in vitro [10, 11]. Though several mechanisms were indicated, the specific mechanisms were still unclear. Moreover, the exact therapeutic effect of FGF2 in AD remains unknown. In a recent study, notably, it was suggested that FGF inhibited ER stress by activating PI3K/Akt signaling pathway. This result provided us clues to perform the further investigation.

Thus, it is reasonable for us to speculate that FGF2 exerts its neuroprotective in AD by inhibiting PERK-induced Aβ species formation in central nervous system. This inhibiting effect was possibly mediated by PI3K/Akt pathway. This study was designed to testify the above hypothesis. In this study, APP/PS1 transgenic was used. Exogenous FGF2 was used to treat the animals. The spatial learning, neurogenesis, Aβ species clearance and hippocampal long-term potentiation (LTP) were examined. Furthermore, for mechanism, both of PI3K/Akt and PERK/BACE1 pathways were also evaluated. We believe that results from this study would not only rich our knowledge of mechanism of AD, but also provide novel basis for potential clinical application of FGF2 in treatment of AD.

Material and methods

Animal and treatment

The double APP/PS1 transgenic male mice were originated from Jackson Laboratory (Bar Harbor, USA) and the wild type counterpart were obtained from Animal Experimental Center of Zhejiang University. The expressions of APP and PSEN1 genes were confirmed by PCR

prior to implement of the experiments. The average age of the mice was (6.2±0.4) months. The mice were raised in an artificial environment with controlled temperature and 12-hour dark-light cycle. The mice got a free access to standard food and tap water. In order to investigate the effects of FGF2 on AD, dosages of 0.5 μg/g FGF2 (Sigma-Aldrich, USA) were administrated intraintranasally to the animals according to the descriptions of several previous studies [12]. Before FGF2 administration, part of mice were treated with the PI3K inhibitor GDC-0941 (Sigma-Aldrich, USA) at 75 mg/Kg orally for 21 days. All animal experimental procedures were carried out according to the National Institutes of Health (NIH) guideline for care and use of laboratory animals.

Morris water maze test

The water maze test was carried out in accordance with previous descriptions [13]. The water maze test apparatus was a pool with 120 cm diameter and white interior walls, containing water at 24°C. A 10 cm-diameter platform was submerged 1 cm under water surface in the apparatus. The mice were trained to swim and climb onto the platform before the tests. 120 seconds were allowed for the mice to search the platform. The mice were gently guided to the platform if they failed to find the platform within 120 seconds. 20 seconds were allowed for the mice to stay on the platform. Spending time for platform searching was recorded.

Hippocampal electrophysiology

Hippocampal slice was prepared in accordance with previous studies [14]. The artificial cerebrospinal fluid (ACSF) solution was prepared. The ACSF was composed of 206 mmol/L sucrose, 1.25 mmol/L NaH_2PO_4, 1mmol/L $CaCl_2$, 2 mmol/L KCl, 2 mmol/L $MgSO_4$, 26 mmol/L $NaHCO_3$, 10 mmol/L D-glucose, 1 mmol/L $MgCl_2$ with pH at 7.4 and mOsm at 315 mmol/L. The brains from mice were removed after sacrifice and submerged in ice-cold ACSF. With a vibroslicer, the hippocampal slices (350 μm) were made. Single slice was submerged in continuously perfusing ACSF in the recording chamber. Conditioning stimuli were delivered by a bipolar stimulating electrode (FHC Inc, USA) placed in the Schaffer collaterals. For recording, borosilicate glass recording elec-
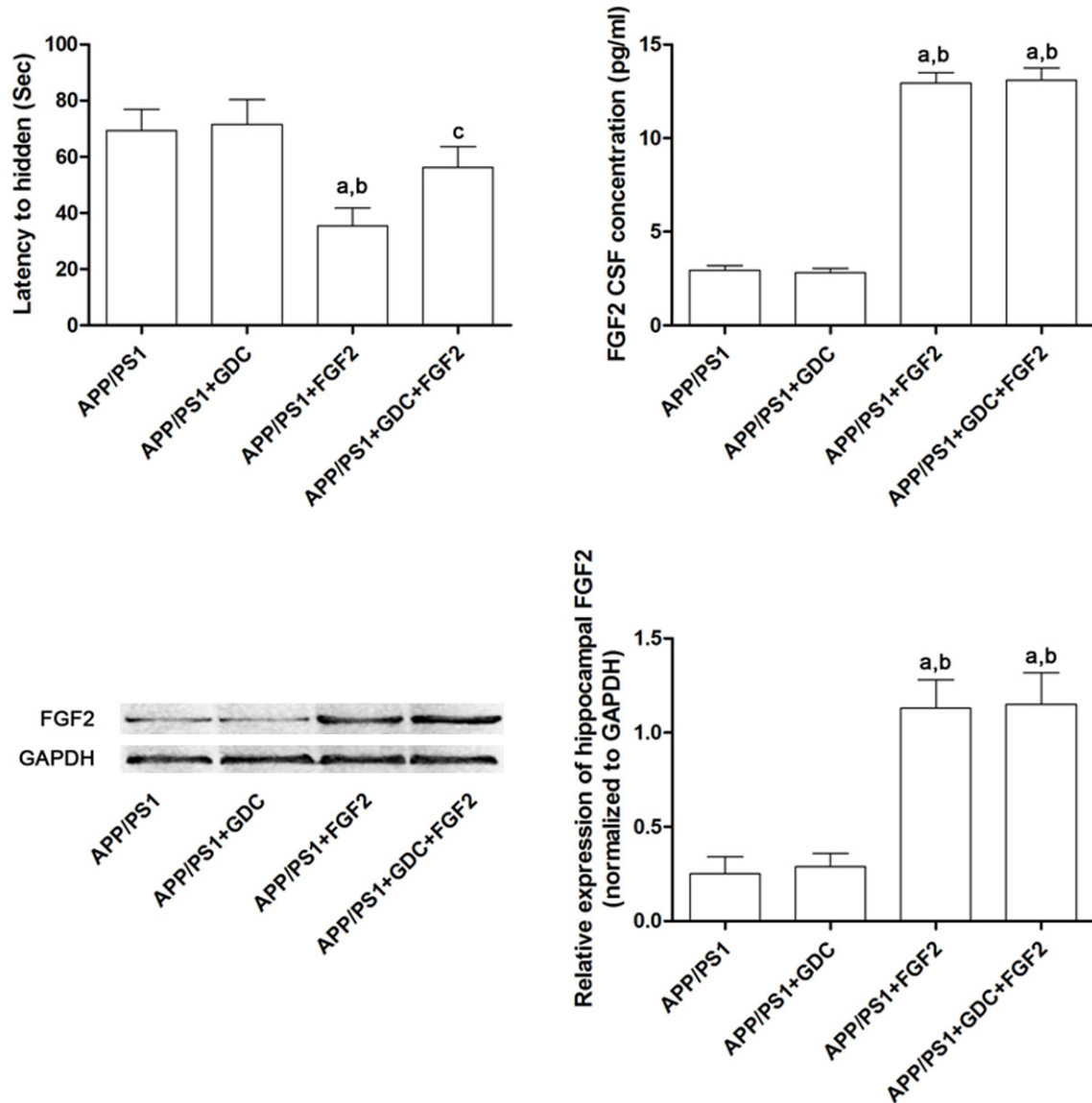
Figure 1. Left part of the upper panel of this figure demonstrated the results from Morris water maze tests. The time consumed by mice to locate the hidden platform was recorded. The longer time spend in locating hidden platform, the more spatial learning ability was impaired. Columns on the right part of the upper panel indicate the ELISA assay detected FGF2 concentrations in CSF collected from mice. The lower panel shows the FGF2 levels in hippocampus harvested from mice brain. On the left part, the immunoblots of FGF2 and the internal reference GAPDH were shown. Columns on the right part indicated the quantification of the immunoblots, representing the relative expression levels of FGF2 in hippocampus. [[a]Compared with APP/PS1; [b]Compared with APP/PS1+GDC, differences were statistically significant ($P<0.05$)].

trodes were placed in the stratum radiatum of hippocampal CA1 region. The field excitatory postsynaptic potentials (fEPSP) in the hippocampal CA1 region was recorded after high frequency stimuli (HFS) at 100 Hz. The initial slope of the FESPS was expressed in a percentage manner (fEPSP/basal level) in bar graphs which quantified the magnitudes of LTP.

ELISA assay

The concentration of FGF2 in cerebrospinal fluid (CSF) was determined by ELISA assay. The mice FGF2 ELSIA kit (R&D, USA) was used to detect the FGF2 concentration in collected mice CSF according to the instructions provided by the manufacturer.
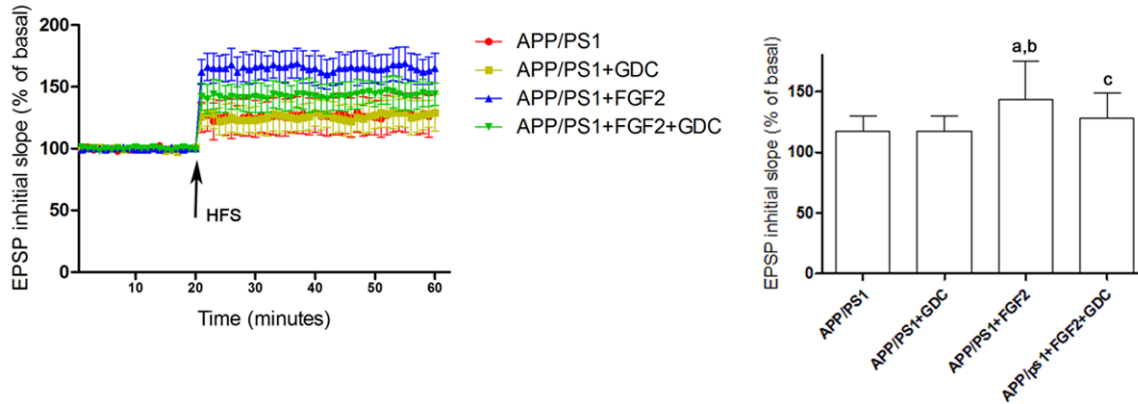
Figure 2. Left of this figure demonstrated the line chart presenting the time course and magnitude of LTP recorded in the CA1 stratum radium of hippocampal slice prepared from APP/PS1 mice received different treatments in different colors. The black arrow is pointing at the time when HFS at 100 Hz was applied. Columns of the bar graph on the right part of this figure demonstrated the average magnitude of recorded LTP in a mean ± SD manner. [[a]Compared with APP/PS1; [b]Compared with APP/PS1+GDC; [c]Compared with APP/PS1+FGF2, differences were statistically significant (P<0.05)].

Immunohistochemistry

1 hour prior to sacrifice, BudU (Sigma-Aldrich, 100 mg/Kg) was administrated intraperitoneally to mice. The neurogenesis in hippocampus slice was evaluated by immunohistochemistry using specific antibody against BrdU (Abcam) and NeuN (Abcam). Corresponding Alexa Fluor 488 and Alexa Fluor 594 conjugated secondary antibodies were used to incubate the slices of hippocampus. The images were captured under a fluorescent microscope. Mean fluorescent intensity (MFI) was used to quantify the expression levels.

Western blotting

The harvested hippocampus tissue was homogenized and the total protein was extracted by ProteoPrep® Total Extraction Sample Kit (Sigma-Aldrich, USA) according to manufacturer's instructions. The concentration of protein samples was detected by BCA method using a BCA kit (Thermo, USA). After loaded to the SDS (sodium dodecyl sulfate) gel, the protein was separated by vertical electrophoresis and then transferred to PVDF (polyvinylidene difluoride) membranes. Antibodies against Akt (Abcam, USA), phosphorylated Akt (p-Akt, Abcam, USA), GRP78 (CST, USA), PERK (CST, USA), phosphorylated PERK (p-PERK, CST, USA), BACE1 (Abcam, USA), Aβ40 (Santa Cruz, USA), Aβ42 (Santa Cruz, USA) and GAPDH (Santa Cruz, USA) at 4°C overnight. Corresponding peroxidase- conjugated secondary antibodies were used to de-

tect the specific bands which were observed by using an enhanced chemiluminescence (ECL) Western blotting analysis system (Bio-Rad, USA).

Statistical considerations

The statistical analysis on the data acquired from this study was processed by the statistics software SPSS (ver.16.0). The differences between groups were analyzed by using Student's t test and ANOVA. When P<0.05, the differences were considered statistically significant.

Results

FGF2 treatment significantly improved the spatial learning ability of APP/PS1 transgenic mice but impaired by GDC-0941 administration

As shown in **Figure 1**, intranasal treatment significantly enhanced the FGF2 concentration in CSF and hippocampus. Also shown in **Figure 1**, The Morris water maze test for spatial learning was performed 1 week after administration. Time consumed by mice to locate the hidden platform (latency to hidden) was recorded and analyzed. The result showed that compared with untreated APP/PS1 mice, mice received FGF2 treatment showed significantly shorter latency to hidden. However, after administrated with the PI3K inhibitor GDC-0941, the latency to hidden was dramatically prolonged compared with FGF2 treatment. The intranasal
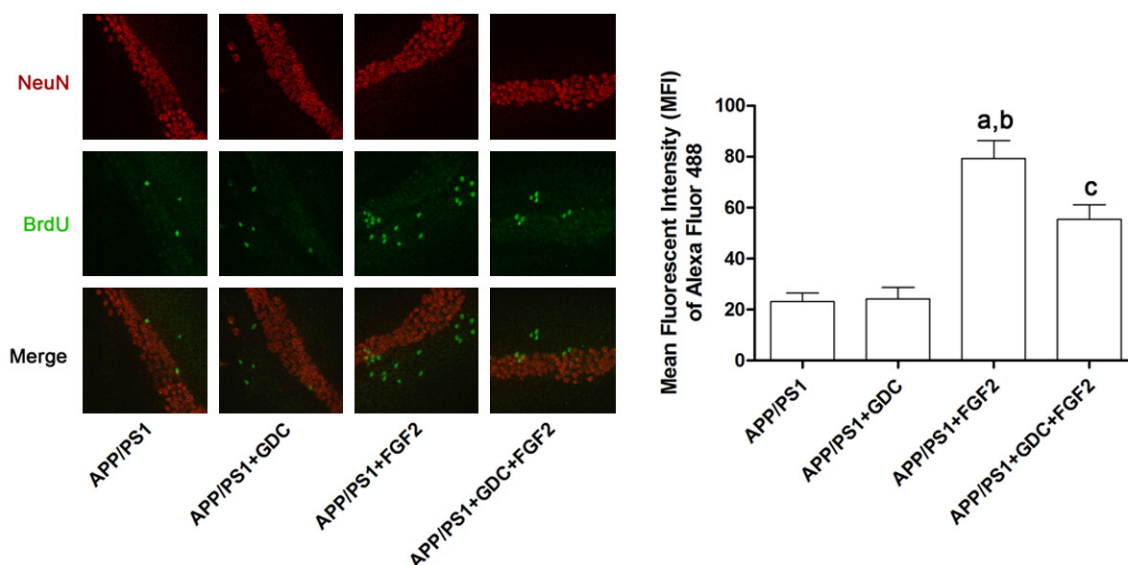
Figure 3. After the brains were harvested from APP/PS1 mice received different treatments, the hippocampus were SLICED and subjected to immunofluorescence. The left part of this figure showed the captured fluorescent images of NeuN and BrdU which were tagged by Alexa Fluor 594 and Alexa Fluor 488 respectively. The merged images were also demonstrated. Columns on the right part of this figure indicated the mean fluorescent intensity of Alexa Fluor 488 calculated by software ImagePro. [aCompared with APP/PS1; bCompared with APP/PS1+GDC; ccompared with APP/PS1+FGF2, differences were statistically significant (P<0.05)].

administration elevated FGF2 concentration in CSF and hippocampus tissue, which are also demonstrated in **Figure 1**.

Hippocampal LTP was improved by FGF2 treatment which was reversed by PI3K inhibitor

It was believed that the LTP is one of the markers of experience- dependent modification of behaviors. We examined the LTP in the Schaffer-collateral pathway to hippocampal CA1 region to evaluate the synaptic transmission and plasticity which are involved in learning and memory abilities. According to several previous studies, the LTP was impaired in APP/PS1 transgenic mice. In this study, as demonstrated in **Figure 2**, FGF2 treatment showed significant improving effects on LTP in hippocampal slice from APP/PS1 transgenic mice. However, the GDC-0941 oral treatment dramatically impaired this improving effect.

PI3K inhibitor administration inhibited FGF2's effects of neurogenesis and Aβ species clearance in APP/PS1 transgenic mice

The suppressed neurogenesis and increased Aβ species accumulation in hippocampus are believed as the pathological basis of AD. In this

study, we assessed the neurogenesis by BrdU assay and the Aβ species accumulation by immunoblots. As demonstrated in **Figure 3**, FGF2 intranasal treatment elevated neurogenesis and inhibited the expression of Aβ species in hippocampal CA1 region. Nevertheless, the following PI3K inhibitor GDC-0941 administration impaired FGF2's neurogenesis improving and Aβ species clearing effects in hippocampus from APP/PS1 transgenic mice.

PI3K/Akt pathway activation and subsequent PERK/BACE1 pathway inhibition were the supposed mechanism of FGF2's therapeutic effects on AD

According to previous studies, activation of PI3K/Akt could suppress the ER stress. As one of the three signaling transducers of ER stress, PERK signaling was supposed to be inhibited. Indeed, as shown in **Figure 4**, after PI3K/Akt pathway was activated by FGF2, the expression of ER stress marker, GRP78, was reduced. Furthermore, the phosphorylation of PERK was also suppressed. Correspondingly, as the downstream effecter of PERK, expression level of BACE1 in hippocampal tissue was reduced. To testify the signaling transduction, we introduced the PI3K inhibitor GDC-0941 by oral
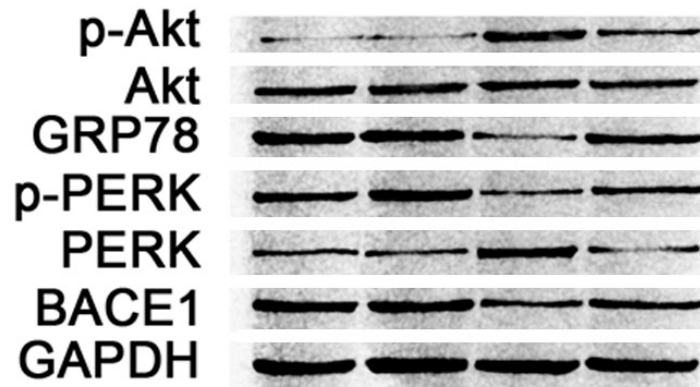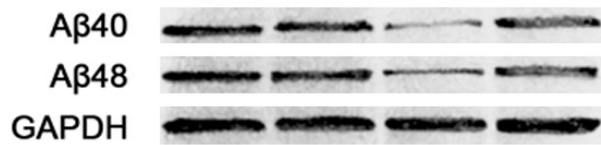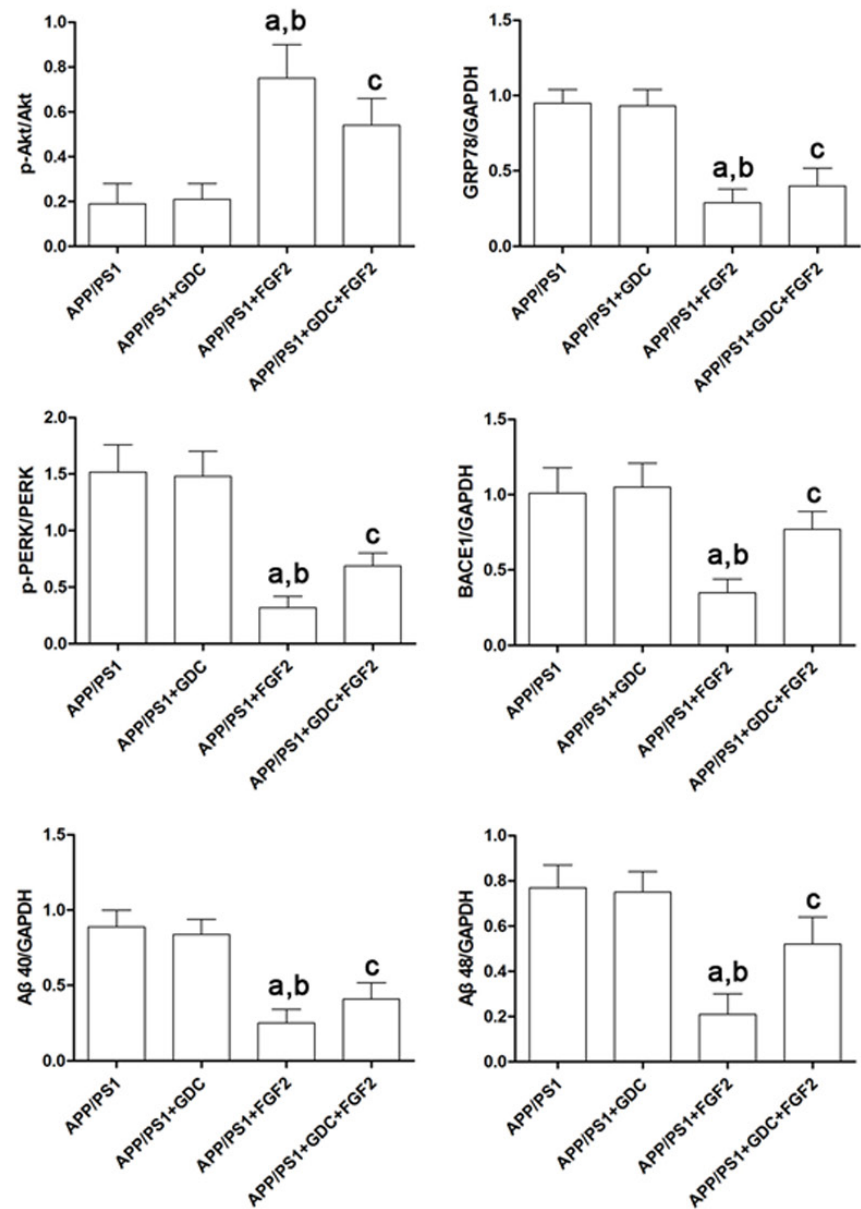
Figure 4. The expression levels of proteins extracted from hippocampal tissue were measured by Western blotting. The left part of the upper panel of this figure demonstrated the immunoblots of p-Akt, Akt, GRP78, p-PERK, PERK, BACE1 and GFPDH respectively. Columns on the right part of upper panel of this figure indicated the quantification of Akt phosphorylation, PERK phosphorylation, relative expression of GRP78 and BACE1 respectively. The left part of this figure showed the immunoblots of the Aβ proteins, namely Aβ40 and Aβ42 respectively. Columns on the right part of the lower panel of this figure indicated the relative expression levels of Aβ40 and Aβ42 respectively. [aCompared with APP/PS1; bCompared with APP/PS1+GDC; ccompared with APP/PS1+FGF2, differences were statistically significant (P<0.05)].

administration. The results turned out that the inhibitor dramatically blocked the activation of PI3K/Akt pathway and thus the activation of PERK/BACE1 pathway was not restrained in the ER stress.

Discussion

As one of the most common neurodegenerative diseases, AD is causing dementia in mainly elderly people. The progressive loss of mental, learning, memory, behavioral and other basic functions impaired the life quality of AD patients and causing heavy social burdens [15]. Continuous neurodegeneration and amyloid plaques formation are generally considered as the causes of AD [16]. It is believed that the Aβ species deposition in the hippocampus is not only the characterized both pathological change and basis of AD. The APP/PS1 transgenic mice are well documented as an ideal AD animal model, which is characterized by Aβ species deposition in hippocampal area [17]. The spatial learning is disabled in APP/PS1 mice. In this study, APP/PS1 mice were used to investigate the therapeutic effect on FGF2. The results turned out that FGF2 intranasal administration significantly improved the spatial learning ability as well as hippocampal LTP of APP/PS1 transgenic mice. Pathologically, Aβ species expressions were reduced and neurogenesis was increased in hippocampal CA1 region. Further investigation indicated that PI3K/Akt activation- induced PERK/BACE1 deactivation was the one of the molecular mechanisms involved.

As one of the members of FGF family, FGF2 was documented to exert neuronal protective and repairing effects in metabolic, ischemic and traumatic brain injury [18]. In animal models of stroke and excitotoxic neuronal damage, exogenous FGF2 supplementation has been proved protective [19]. In a recent study, the FGF2 gene was transferred to APP/PS1 transgenic mice, as a result, the gene transfer attenuated

pathological status and improved neural function. These results indicated that FGF2 could be a potential alternative therapy for AD and possibly other neurogenerative diseases [20]. In the present study, we administrated FGF2 to APP/PS1 transgenic mice intranasally which elevated local FGF2 concentration in both CSF and hippocampus. The results showed that the spatial learning ability as well as hippocampal LTP of AD animal model improved significantly. Furthermore, the Aβ species accumulation and neurogeneration were also dramatically attenuated after FGF2 treatment. Though several mechanisms such as NMDA receptor regulation, calcium homeostasis attenuation and detoxifying enzymes activity adjustment were indicated, the specific molecular mechanisms of FGF2's neuroprotective effects are still unknown.

During ER stress, multiple normal biological processes, including protein folding, protein post-translational modification and massive gene translation are suspended. The stressful signals are then transduced through mainly three ER transducers, namely ATF6, IRE1 and PERK, to initiate various biological effects [21]. Accumulating evidences have indicated that the neuronal degeneration and dysfunction are associated with ER stress. It was reported that ER stress was activated in neurons from AD brains [22]. Furthermore, neurons under ER stress were found with abundant Aβ species [23]. These results indicated that the APP was cleaved into Aβ species in ER stress [24]. In this study, we found that ER stress was activated, and correspondingly, Aβ proteins were accumulated in hippocampus of APP/PS1 transgenic mice.

PI3K/Akt pathway is considered to participate in regulating cell survival under stressful conditions [25]. FGF2 is believed as one of the initiator to activate PI3K/Akt pathway [26]. Activated Akt is capable of activating a number of downstream biological responses including inhibit-

ing ER stress. A previous study found that neurons display elevated expression of p-PERK and its down-stream effectors along with increased Tau protein [27]. Moreover, a recent study pointed out that PERK signaling activation would lead to expression elevation of BACE1 which takes responsibility in cleaving APP into Aβ proteins [28]. We speculate that FGF2 treatment activates PI3K/Akt pathway which further inhibits PERK signaling activation, resulting in reduction of BACE1 and Aβ species accumulation.

In this study, we testified our above speculation with the introduction of PI3K inhibitor GDC-0941. The inhibitor was administrated to APP/PS1 transgenic mice orally after they received FGF2 treatment. The inhibitor impaired FGF2's therapeutic effects of improving spatial learning ability, increasing hippocampal neurogeneration and reducing Aβ accumulation. Moreover, PI3K inhibitor also deactivated Akt and subsequently made FGF2 loss its inhibitory effects on ER stress signaling transduction, leading to elevated expression of BACE1 which cleaves APP.

In conclusion, FGF2's therapeutic effects in AD animal model were observed and confirmed. The spatial learning ability was improved while hippocampal Aβ species accumulation was attenuated by intranasal treatment of FGF2. Mechanically, FGF2 was found to activate PI3K/Akt pathway which further inhibited PERK/BACE1 pathway, leading to impaired Aβ species formation in hippocampus.

Disclosure of conflict of interest

None.

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References

[1] Laakkonen ML, Raivio MM, Eloniemi-Sulkava U, Saarenheimo M, Pietila M, Tilvis RS and Pitkala KH. How do elderly spouse care givers of people with Alzheimer disease experience the disclosure of dementia diagnosis and subsequent care? J Med Ethics 2008; 34: 427-430.

[2] Gouras GK and Beal MF. Metal chelator decreases Alzheimer beta-amyloid plaques. Neuron 2001; 30: 641-642.

[3] Baglietto-Vargas D, Moreno-Gonzalez I, Sanchez-Varo R, Jimenez S, Trujillo-Estrada L, Sanchez-Mejias E, Torres M, Romero-Acebal M, Ruano D, Vizuete M, Vitorica J and Gutierrez A. Calretinin interneurons are early targets of extracellular amyloid-beta pathology in PS1/AbetaPP Alzheimer mice hippocampus. J Alzheimers Dis 2010; 21: 119-132.

[4] Li Q and Sudhof TC. Cleavage of amyloid-beta precursor protein and amyloid-beta precursor-like protein by BACE 1. J Biol Chem 2004; 279: 10542-10550.

[5] Scheper W, Nijholt DA and Hoozemans JJ. The unfolded protein response and proteostasis in Alzheimer disease: preferential activation of autophagy by endoplasmic reticulum stress. Autophagy 2011; 7: 910-911.

[6] Unterberger U, Hoftberger R, Gelpi E, Flicker H, Budka H and Voigtlander T. Endoplasmic reticulum stress features are prominent in Alzheimer disease but not in prion diseases in vivo. J Neuropathol Exp Neurol 2006; 65: 348-357.

[7] Devi L and Ohno M. PERK mediates eIF2alpha phosphorylation responsible for BACE1 elevation, CREB dysfunction and neurodegeneration in a mouse model of Alzheimer's disease. Neurobiol Aging 2014; 35: 2272-2281.

[8] Nandy SB, Mohanty S, Singh M, Behari M and Airan B. Fibroblast Growth Factor-2 alone as an efficient inducer for differentiation of human bone marrow mesenchymal stem cells into dopaminergic neurons. J Biomed Sci 2014; 21: 014-0083.

[9] Anderson G and Maes M. Reconceptualizing adult neurogenesis: role for sphingosine-1-phosphate and fibroblast growth factor-1 in coordinating astrocyte-neuronal precursor interactions. CNS Neurol Disord Drug Targets 2014; 13: 126-136.

[10] Hashimoto M, Sagara Y, Langford D, Everall IP, Mallory M, Everson A, Digicaylioglu M and Masliah E. Fibroblast growth factor 1 regulates signaling via the glycogen synthase kinase-3beta pathway. Implications for neuroprotection. J Biol Chem 2002; 277: 32985-32991.

[11] Zhu SP, Wang ZG, Zhao YZ, Wu J, Shi HX, Ye LB, Wu FZ, Cheng Y, Zhang HY, He S, Wei X, Fu XB, Li XK, Xu HZ and Xiao J. Gelatin Nanostructured Lipid Carriers Incorporating Nerve Growth Factor Inhibit Endoplasmic Reticulum Stress-Induced Apoptosis and Improve Recovery in Spinal Cord Injury. Mol Neurobiol 2015; 2: 2.

[12] Wang Z, Zhang H, Xu X, Shi H, Yu X, Wang X, Yan Y, Fu X, Hu H, Li X and Xiao J. bFGF inhibits ER stress induced by ischemic oxidative injury

via activation of the PI3K/Akt and ERK1/2 pathways. Toxicol Lett 2012; 212: 137-146.

[13] Bromley-Brits K, Deng Y and Song W. Morris water maze test for learning and memory deficits in Alzheimer's disease model mice. J Vis Exp 2011.

[14] Li S, Jin M, Zhang D, Yang T, Koeglsperger T, Fu H and Selkoe DJ. Environmental novelty activates beta2-adrenergic signaling to prevent the impairment of hippocampal LTP by Abeta oligomers. Neuron 2013; 77: 929-941.

[15] Sindi S, Mangialasche F and Kivipelto M. Advances in the prevention of Alzheimer's Disease. F1000Prime Rep 1000; 7: 7-50.

[16] Retraction. Winton et al., Intraneuronal APP, not free Abeta peptides in 3xTg-AD mice: implications for Tau versus Abeta-mediated Alzheimer neurodegeneration. J Neurosci 2015; 35: 3724.

[17] van Groen T, Kiliaan AJ and Kadish I. Deposition of mouse amyloid beta in human APP/PS1 double and single AD model transgenic mice. Neurobiol Dis 2006; 23: 653-662.

[18] Yoshimura S, Teramoto T, Whalen MJ, Irizarry MC, Takagi Y, Qiu J, Harada J, Waeber C, Breakefield XO and Moskowitz MA. FGF-2 regulates neurogenesis and degeneration in the dentate gyrus after traumatic brain injury in mice. J Clin Invest 2003; 112: 1202-1210.

[19] Noshita T, Murayama N, Oka T, Ogino R, Nakamura S and Inoue T. Effect of bFGF on neuronal damage induced by sequential treatment of amyloid beta and excitatory amino acid in vitro and in vivo. Eur J Pharmacol 2012; 695: 76-82.

[20] Kiyota T, Ingraham KL, Jacobsen MT, Xiong H and Ikezu T. FGF2 gene transfer restores hippocampal functions in mouse models of Alzheimer's disease and has therapeutic implications for neurocognitive disorders. Proc Natl Acad Sci U S A 2011; 108: 31.

[21] Liu Z, Lv Y, Zhao N, Guan G and Wang J. Protein kinase R-like ER kinase and its role in endoplasmic reticulum stress-decided cell fate. Cell Death Dis 2015; 30: 183.

[22] Volgyi K, Juhasz G, Kovacs Z and Penke B. Dysfunction of Endoplasmic Reticulum (ER) and Mitochondria (MT) in Alzheimer's Disease: The Role of the ER-MT Cross-Talk. Curr Alzheimer Res 2015; 12: 655-672.

[23] Liu B, Zhu Y, Zhou J, Wei Y, Long C, Chen M, Ling Y, Ge J and Zhuo Y. Endoplasmic reticulum stress promotes amyloid-beta peptides production in RGC-5 cells. Cell Stress Chaperones 2014; 19: 827-835.

[24] Liu X, Xu K, Yan M, Wang Y and Zheng X. Protective effects of galantamine against Abeta-induced PC12 cell apoptosis by preventing mitochondrial dysfunction and endoplasmic reticulum stress. Neurochem Int 2010; 57: 588-599.

[25] Padiya R, Chowdhury D, Borkar R, Srinivas R, Pal Bhadra M and Banerjee SK. Garlic attenuates cardiac oxidative stress via activation of PI3K/AKT/Nrf2-Keap1 pathway in fructose-fed diabetic rat. PLoS One 2014; 9:

[26] Ojeda L, Gao J, Hooten KG, Wang E, Thonhoff JR, Dunn TJ, Gao T and Wu P. Critical role of PI3K/Akt/GSK3beta in motoneuron specification from human neural stem cells in response to FGF2 and EGF. PLoS One 2011; 6: 24.

[27] van der Harg JM, Nolle A, Zwart R, Boerema AS, van Haastert ES, Strijkstra AM, Hoozemans JJ and Scheper W. The unfolded protein response mediates reversible tau phosphorylation induced by metabolic stress. Cell Death Dis 2014; 28: 354.

[28] Rozpedek W, Markiewicz L, Diehl JA, Pytel D and Majsterek I. Unfolded Protein Response and PERK kinase as a new therapeutic target in the pathogenesis of Alzheimer's disease. Curr Med Chem 2015; 17: 17.