Biochemical and Biophysical Research Communications 456 (2015) 225–231

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Fibroblast growth factor 10 protects neuron against oxygen–glucose deprivation injury through inducing heme oxygenase-1

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ARTICLE INFO

Article history:
Received 12 November 2014
Available online 22 November 2014

Keywords:
FGF10
Cerebral ischemia
HO-1
Neuroprotection

ABSTRACT

Fibroblast growth factors (FGFs) are a family of structurally related heparin-binding proteins with diverse biological functions. FGFs participate in mitogenesis, angiogenesis, cell proliferation, development, differentiation and cell migration. Here, we investigated the potential effect of FGF10, a member of FGFs, on neuron survival in oxygen–glucose deprivation (OGD) model. In primary cultured mouse cortical neurons upon OGD, FGF10 treatment (100 and 1000 ng/ml) attenuated the decrease of cell viability and rescued the LDH release. Tuj-1 immunocytochemistry assay showed that FGF10 promoted neuronal survival. Apoptosis assay with Annexin V + PI by flow cytometry demonstrated that FGF10 treatment reduced apoptotic cell proportion. Moreover, immunoblotting showed that FGF10 alleviated the cleaved caspase-3 upregulation caused by OGD. FGF10 treatment also depressed the OGD-induced increase of caspase-3, -8 and -9 activities. At last, we found FGF10 triggered heme oxygenase-1 (HO-1) protein expression rather than hypoxia-inducible factor-1 (HIF-1), AMP-activated protein kinase (AMPK) signaling and extracellular signal-regulated kinases 1/2 (ERK1/2) signaling. Knockdown of HO-1 by siRNA partly abolished the neuroprotection of FGF10 in OGD model. In summary, our observations provide the first evidence for the neuroprotective function of FGF10 against ischemic neuronal injury and suggest that FGF10 may be a promising agent for treatment of ischemic stroke.

1. Introduction

Fibroblast growth factors (FGFs) are a family of structurally related heparin-binding polypeptides with potent mitogens and chemoattractants in various cells [1]. In human, FGF gene family consists of at least 23 different members (FGF1–23) [1]. FGFs are grouped into six subfamilies based on the similarities and differences among their structural and functional characteristics [1]. FGFs interact with FGF receptors, which are a family of tyrosine kinase membrane bound receptors with extracellular ligands, and trigger transducing extracellular cues to elicit diverse biological responses such as mitogenesis, angiogenesis, cell proliferation, development, differentiation and cell migration. According to the current literature, FGFs participate in physiopathological processes of numerous diseases, including cardiovascular diseases, diabetes, obesity, kidney diseases and cancer [1].

FGF10 is a member of FGFs. It was cloned from rat embryos in 1996 and is required for embryos development [2]. Moreover, FGF10 is indispensable for adipose, limb, lung and prostate development [3–5]. FGF10 is a mitogen for many types of cells. FGF-10 promotes epithelial cell motility, differentiation and migration [6], and induces keratinocytes proliferation and differentiation [7]. The activation of FGF receptor 2 and mitogen-activated protein kinase pathways might be one of major mechanisms of FGF10 action [8]. FGF10 also induces the prompt phosphorylation of ERK1/2 and consequently stimulate DNA synthesis [8]. It was reported that FGF10 does not activate Akt, protein kinase C, and signal transducer and activator of transcription-3 in human endometrial carcinoma cells [8]. FGF10 activates branch morphogenesis and epithelial stem cells [9,10], suggesting a key role of FGF10 in regenerative medicine [11].

Currently, it is well-accepted that FGFs play important roles in ischemic diseases. FGF2, also known as basic FGF (bFGF), improves vascularization in skin flap ischemia [12] and chronic myocardial ischemia [13]. Intracoronary administration of recombinant FGF-2 is effective, safe and well tolerated in human with ischemic heart disease [14]. Serum levels of FGF23 was associated with early mortality in cardiogenic shock [15]. FGF2 was upregulated and released from the liver and adipose tissue in myocardial injury, contributing to myocardial protection [16,17]. In cerebral ischemic rodent model, FGF2, FGF13 and FGF18 are neuroprotective by

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http://dx.doi.org/10.1016/j.bbrc.2014.11.063
0006-291X/

reducing infarct volumes and behavioral deficits [18–20]. However, whether FGF10 has biological functions in ischemia-induced physiopathological changes has not been studied previously. In this study, we tested the effect of FGF10 on primary neurons and PC12 neuron cell line with oxygen–glucose deprivation (OGD) stimuli, which is a common model for studying molecular mechanisms of ischemic stroke injury *in vitro*.

2. Materials and methods

2.1. Reagents

Recombinant human FGF10 (catalog: 345-FG-025) was purchased from R&D Systems (Minneapolis, MN). Antibodies against heme oxygenase-1 (HO-1), hypoxia-inducible factor-1 (HIF-1), AMP-activated protein kinase (AMPK), extracellular signal-regulated kinases 1/2 (ERK1/2) and tubulin were purchased from Abcam (Cambridge, MA). Antibody against caspase-3 was purchased from Cell Signaling Biotechnology (Danvers, MA). LDH cytotoxic kit was purchased from Promega (Medison, WI). Cell viability assay (Cell Counting Kit-8) was from Dojindo Molecular Technologies Inc. (Kumamoto, Japan). Caspase-3, -8 and -9 activities kits were purchased from Beyotime Institute of Biotechnology (Haimen, China). Dichloro-dihydro-fluorescein diacetate (DCFH) was purchased from Sigma (Carslbad, CA). Superoxide O_2^- and MnSOD kits were purchased from Cell Biolabs (San Diego, CA). Annexin V + PI kits were purchased from Life Technologies Inc. (Gaithersburg, MD).

2.2. Cell culture

Primary mouse cortical neurons and PC12 neuronal cells were used in this study. Male C57BL/6 mice were purchased from Sino-British SIPPR/BK Lab Animal Ltd. (Shanghai, China). All experiments were performed in adherence with the National Institutes of Health guidelines on the use of laboratory animals and were approved by the Scientific Investigation Board of the Second Military Medical University. Primary mouse neuronal cells were prepared from the cerebral cortex of E17 or E18 mouse embryos as described previously [21,22]. Briefly, the dissociated cortical cells were added to poly-L-lysine-coated culture plates and maintained in Neurobasal medium supplemented with 2% B27. Glial growth was suppressed by addition of 5-fluoro-2-deoxyuridine and uridine (10 μM), yielding cultured cells with >90% neurons, which was confirmed by NeuN staining. After 6 days *in vitro* (DIV), the neurons were used for experiments. PC12 neuronal cells were purchased from ATCC (American Type Culture Collection, Manassas, VA) and used in experiments with siRNA transfection. PC12 cells were maintained in DMEM media supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 U/ml) at 37 °C in a humidified atmosphere of 5% CO_2/95% air.

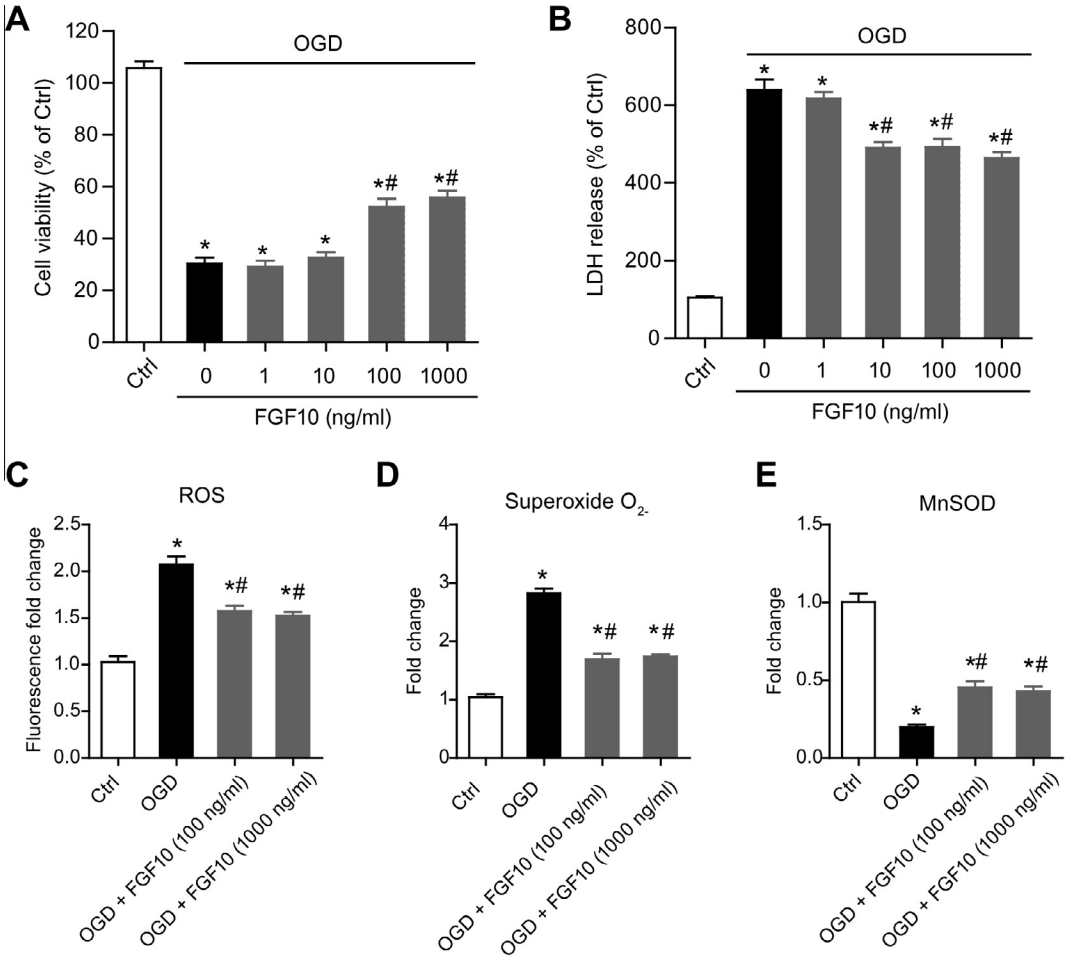

Fig. 1. FGF10 ameliorates OGD induced cell injury and oxidative stress in cortical neurons. (A and B) Effect of FGF10 (1–1000 ng/ml) on cell viability (A) and LDH release (B) in OGD-treated primary cultured neurons. $^*P < 0.05$ vs Ctrl (control), $^\#P < 0.05$ vs OGD, $n = 8$ per group. (C–E) Effect of FGF10 (100 and 1000 ng/ml) on intracellular levels of ROS (C), superoxide O_2^- (D) and MnSOD (E). $^*P < 0.05$ vs Ctrl (control), $^\#P < 0.05$ vs OGD, $n = 8$ per group.

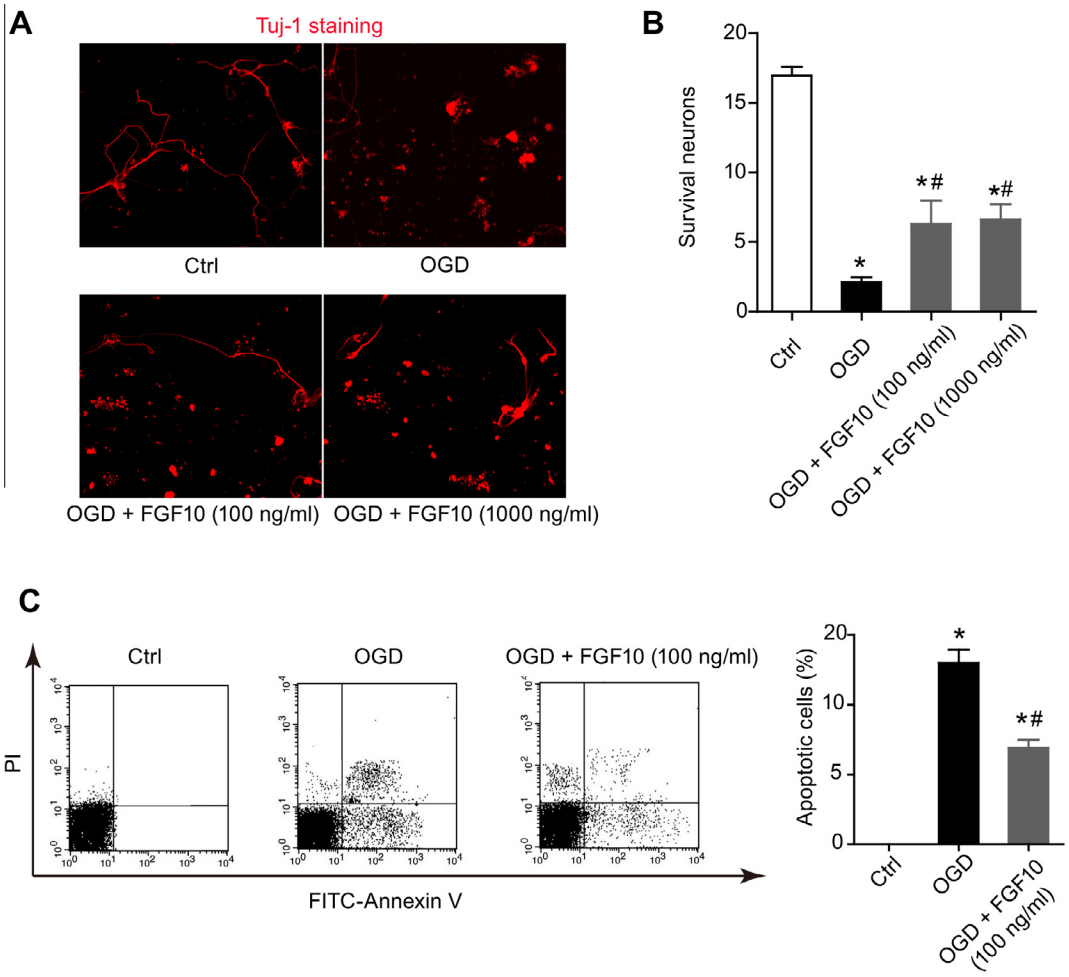

Fig. 2. FGF10 promotes survival of cortical neurons upon OGD condition. (A) Tuj-1 staining was performed to evaluate the effect of FGF10 (100 and 1000 ng/ml) on neuron survival upon OGD (4 h). (B) Quantitative analysis of Tuj-1 positive neurons after OGD challenge. $^{*}P < 0.05$ vs Ctrl (control), $^{\#}P < 0.05$ vs OGD, $n = 8$ per group. (C) Apoptosis was evaluated by flow cytometry with FITC-Annexin V + PI assay. Annexin^{+}/PI^{-} cells were considered as early stage apoptotic cells while Annexin^{+}/PI^{+} cells were considered as late stage apoptotic cells. $^{*}P < 0.05$ vs Ctrl (control), $^{\#}P < 0.05$ vs OGD, $n = 3$ per group.

2.3. Oxygen–glucose deprivation (OGD) model and FGF10 treatment

OGD model was prepared as described previously [23]. Briefly, the cultured neurons were washed three times and incubated with glucose-free Earle's balanced salt solution (EBSS) and placed within chamber with <1% oxygen for 2 or 4 h to establish OGD conditions. Control neurons were placed in EBSS containing glucose (25 mM) and incubated under normal culture conditions for the same period. For FGF10 treatment, FGF10 dissolved in saline was added into culture disks to reach final concentration (1, 10, 100 and 1000 ng/ml). Saline was used as vehicle control.

2.4. Cell viability assay

Cell viability was evaluated by a non-radioactive cell counting kit-8 (CCK-8) assay as described previously [24]. Cells were treated with OGD or OGD + FGF10 for 4 h. Then, cells were incubated with 10 μl of CCK-8 solution for 1 h at 37 °C. The optical density at 450 nm was analyzed in a microplate reader (Tecan, Durham, NC). Experiments were performed in duplicate.

2.5. LDH release assay

Cytotoxic activity was determined by a lactate dehydrogenase (LDH) release assay as described previously [25]. Briefly, the LDH in the culture medium (released LDH) was quantified according to the instructions of manufacturer. The relative amount of released LDH was used to reflect the cell injury.

2.6. Annexin V + PI assay

Annexin V + PI was performed as described previously [26]. Cells were subjected with OGD stimuli with or without FGF21 incubation for 4 h. Then, 5×10^5 cells were harvested and washed with Phosphate Buffered Saline (PBS). Cells were suspended in 500 μl binding buffer and then incubated for 5 min at room temperature in the dark with 10 μl Annexin-V-FITC and 10 μl PI additions. Flow cytometry analysis was performed in FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). The Annexin V^{+}/PI^{-} cells were considered as apoptotic cells.

2.7. Immunoblotting

Immunoblotting analyses of cell-extracts were performed as described previously [27]. Cells were lysed with RIPA buffer with protease inhibitors. Samples were separated in 12% SDS–PAGE, and transferred onto PVDF membranes at 100 V for 1–2 h. After blocking by 5% evaporated milk, membranes were incubated with primary antibodies (HO-1, 1:1000; tubulin, 1:3000) diluted in TBS containing 1% w/v bovine serum albumin and followed by

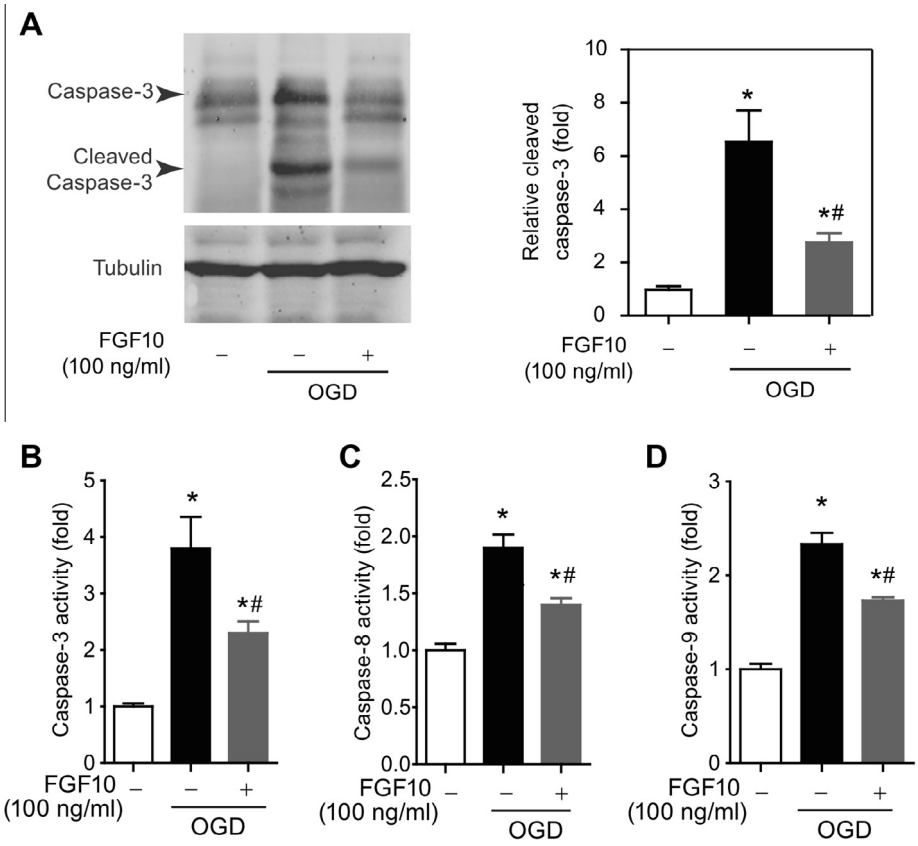

Fig. 3. FGF10 suppresses the activation of pro-apoptotic cascades in cortical neurons under OGD condition. (A) Representative images and quantitative analysis of immunoblotting assay on the cleaved caspase-3 in neurons upon OGD stimuli. Tubulin was used as a loading control. $^{*}P < 0.05$ vs Ctrl (control), $^{\#}P < 0.05$ vs OGD, $n = 3$ per group. (B–D) Effect of FGF10 (100 ng/ml) on activities of caspase-3, -8 and -9. $^{*}P < 0.05$ vs Ctrl (control), $^{\#}P < 0.05$ vs OGD, $n = 6$ per group.

HRP-labeled secondary antibody. The signal of immunoblotting was detected using the enhanced chemiluminescence system.

2.8. Assays for reactive oxygen species (ROS), superoxide O_2^- and MnSOD

Cells were challenged by OGD with or without FGF10 treatment for 4 h and then incubated with the DCFH probe for 1 h. After washing 3 times by PBS, the fluorescence intensity was measured by microplate reader at an excitation wavelength of 488 nm to reflect the intracellular ROS level [28]. Intracellular superoxide O_2^- and MnSOD were measured as described previously [29,30]. Cells were lysed and the MDA and MnSOD concentrations of each sample were detected according to the manufacturer's instruction.

2.9. siRNA-mediated knockdown

To knock down HO-1, PC12 cells were transfected with siRNA targeting to HO-1 (catalog: sc-35554, Santa Cruz Biotechnology) or siRNA-Ctrl (scramble) using Lipofectamine® LTX & Plus Reagent (Invitrogen) following the manufacturer's instructions. After 72 h, the efficiency of HO-1 knockdown was tested by immunoblotting. The siRNA-transfected cells were challenged by OGD stimuli with or without FGF10 for 4 h. Then, the following biological examinations were performed.

2.10. Statistical analysis

Data are expressed as mean ± SEM. Differences were evaluated by two-tailed Student's *t* test or ANOVA followed by Tukey's post-hoc test with GraphPad Prism 5.0 version. Statistical significance was set at $P < 0.05$.

3. Results

3.1. FGF10 treatment ameliorates OGD induced cell injury and oxidative stress in mouse cortical neurons

OGD treatment (4 h) induced potent cell injury in primary cortical neurons, evidenced by the decreased cell viability (Fig. 1A) and enhanced LDH release (Fig. 1B). FGF10 ranging from 1 to 1000 ng/ml was added into culture medium. FGF10 had no effect on cell viability at 1 and 10 ng/ml, but significantly attenuated the OGD-induced decrease of cell viability at 100 and 1000 ng/ml (Fig. 1A). FGF10 also did not rescued the LDH release at 1 ng/ml, but retarded LDH releases at 10, 100 and 1000 ng/ml (Fig. 1B). So we used FGF at 100 or 1000 ng/ml in the following experiments. As expected, OGD stimuli significantly increased intracellular ROS (Fig. 1C) and superoxide O_2^- levels (Fig. 1D). OGD also reduced MnSOD level (Fig. 1E), which is a nuclear-encoded mitochondrial antioxidant enzyme [31]. FGF10 treatment (100 and 1000 ng/ml) partly blocked these changes induced by OGD (Fig. 1C–E).

3.2. FGF10 treatment promotes neuronal survival in mouse cortical neurons upon OGD challenge

Next, Tuj-1 immunocytochemical staining was used to detect live neurons after OGD challenge (Fig. 2A). OGD treatment for 4 h caused a remarkable reduction of neuron survival, whereas FGF10 treatment (100 and 1000 ng/ml) significantly rescued

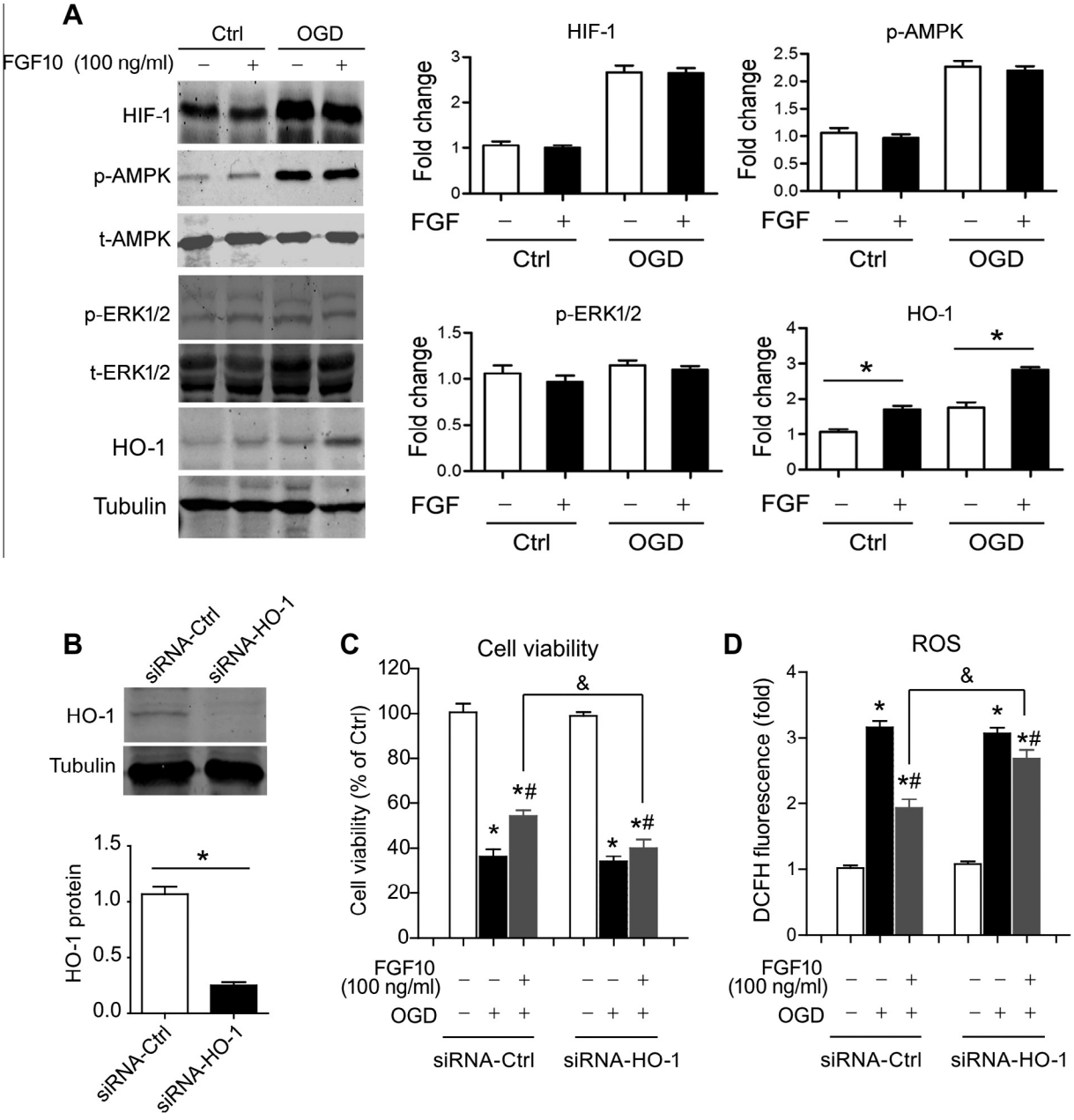

Fig. 4. HO-1 upregulation contributes to the neuroprotection of FGF10. (A) Effect of FGF10 on protein levels of HIF-1, p-AMPK, p-ERK1/2 and HO-1. $^{*}P < 0.05$ vs without FGF10, $n = 4$ per group. (B) siRNA mediated knockdown of HO-1 in PC12 neuronal cells. $^{*}P < 0.05$ vs siRNA-Ctrl, $n = 4$ per group. (C and D) The neuroprotection of FGF10 on cell viability (C) and ROS production (D) was impaired in siRNA-HO-1 transfected cells. $^{*}P < 0.05$ vs without OGD; $^{\#}P < 0.05$ vs without FGF10; $^{\&}P < 0.05$ vs siRNA-Ctrl, $n = 6$ per group.

neuronal survival (Fig. 2B). Annexin V/PI assay showed that OGD for 2 h induced obvious apoptosis (Annexin V^{+}/PI^{-} cells + Annexin V^{+}/PI^{-} cells, Fig. 2C). FGF10 treatment reduced the proportion of apoptotic cells (Fig. 2C).

3.3. FGF10 treatment suppresses the activation of pro-apoptotic signaling cascades in cortical neurons upon OGD challenge

Caspase cascades activation is a critical step in the initiation of apoptosis in diverse biological systems [32]. We investigated the cleavage of caspase-3, a well-characterized apoptotic molecular marker. OGD treatment induced caspase-3 cleavage in neurons, which was partly blocked by FGF10 treatment (Fig. 3A). Activities of caspase-3, -8 and -9 were also measured. As expected, OGD stimuli induced activities of caspase-3 (Fig. 3B), caspase-8 (Fig. 3C) and caspase-9 (Fig. 3D). FGF10 treatment attenuated the induction of activities of caspase-3 (Fig. 3B), caspase-8 (Fig. 3C) and caspase-9 (Fig. 3D) by OGD.

3.4. FGF10 induces HO-1 to protect neuron against OGD injury

We examined the influences of FGF-10 on several stress responsors, including HIF-1, AMPK, ERK1/2 and HO-1. OGD treatment did not alter p-ERK1/2 level, but enhanced HIF-1, p-AMPK and HO-1 levels (Fig. 4A). However, FGF-10 did not modulate HIF-1 and p-AMPK levels; it only increased HO-1 expression (Fig. 4A). Interestingly, we found that FGF10 treatment upregulated HO-1 even in control condition (Fig. 4A). HO-1, one of the major intracellular

anti-oxidant enzymes, is a stress response protein [33–35]. These changes raised the possibility that HO-1 upregulation might contribute to the neuroprotection of FGF10. We tested this hypothesis in PC12 neuronal cells, a widely used neuronal cell line for studying of molecular mechanisms because conventional transfection techniques have been of limited effectiveness in primary culture neurons. We successfully knocked down HO-1 in PC12 neuronal cells using siRNA (Fig. 4B). The neuroprotection of FGF10 on cell viability (Fig. 4C) and ROS level (Fig. 4D) in HO-1 knockdown PC12 cells was significantly lower than that in control cells (siRNA-Ctrl transfected cells). However, FGF10 still partly rescued cell viability (Fig. 4C) and reduced ROS level (Fig. 4D) in HO-1 knockdown PC12 cells, suggesting that the HO-1 upregulation by FGF10 is not the only molecular mechanism for its neuroprotection.

4. Discussion

According to our knowledge, this is the first report of the neuroprotective effect of FGF10. In the present study, we demonstrated that treatment of FGF10 was able to promote cell survival, decrease apoptosis and depress ROS increase upon OGD stress in neurons. Moreover, we revealed a molecular mechanism of neuroprotection of FGF10 that FGF10 protected neurons against OGD-induced ischemic injury via upregulating HO-1, a well-known antioxidant molecule.

The first important finding of our study is that FGF10 treatment protects neurons from ischemic stress induced injury. Previously, some other members of FGFs, such as FGF2, FGF13 and FGF18, have been demonstrated to be neuroprotective in rodent cerebral ischemic models [18–20]. FGF2 is one of the most studied members in FGFs. FGF2 was mainly located in astrocytes and increased after focal cerebral ischemia in rats [36]. Even post-ischemic administration of FGF2 was sufficient to improve sensorimotor function, reduce infarct size and enhance neurogenesis in middle cerebral artery model [37,38]. Moreover, transplantation of FGF-2 gene-transferred bone marrow stromal cell intracerebrally 24 h after transient MCAO improved neurological functional recovery compared with MSCs transplantation alone [39]. FGF13 and FGF18 also exhibited neuroprotection in rodent cerebral ischemic models [18–20]. However, the role of FGF10 in ischemia-related diseases is still unknown. In this study, we demonstrated that FGF10 treatment at 100 and 1000 ng/ml displayed a remarkable neuroprotection. These results suggest that FGF10 may be a promising therapy for cerebral ischemic damage. Nevertheless, our results were obtained from *in vitro* model. Whether FGF10 would be effective *in vivo* or even in human needs more studies in future.

The second important finding of our study is that FGF10 induces HO-1 expression in neurons. HO-1 is a heme-catabolizing enzyme that not only catabolizes the hemoglobin-heme into biliverdin, carbon monoxide and iron, but also acts as an anti-inflammatory protector against oxidative injurious stimuli [33,34,40]. The endogenous HO-1 upregulation upon cerebral ischemia was considered as an adaptive response against ischemia-induced oxidative injury [30,33]. Furthermore, it seems that HO-1 can activate intracellular pro-survival signaling pathways such as Akt [41]. Previously, FGF1 was found to increase HO-1 in spinal cord astrocytes and improve motor neuron survival [42]. We found FGF10 is able to induce HO-1 protein expression in primary cortical neurons under both normal and OGD conditions. Additionally, knockdown of HO-1 compromised the neuroprotective effect of FGF10. It should be noted that the neuroprotective effect of FGF10 was not totally blocked in HO-1-knowdown neuron cells. We consider that the induction of HO-1 may be not the only molecular mechanism contributes to the neuroprotective effect of FGF10. Some pro-survival mechanisms, such as Akt pathway, may participate in this process.

In conclusion, we demonstrate that FGF10 protects against cell injury, suppresses ROS level and inhibits apoptosis in OGD model. The upregulation of HO-1 induced by FGF10 is important for the neuroprotection of FGF10. These results indicate that FGF10 treatment may be a promising agent for cerebral ischemic injury.

References

[1] N. Itoh, D.M. Ornitz, Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease, J. Biochem. 149 (2011) 121–130.
[2] M. Yamasaki, A. Miyake, S. Tagashira, et al., Structure and expression of the rat mRNA encoding a novel member of the fibroblast growth factor family, J. Biol. Chem. 271 (1996) 15918–15921.
[3] H. Min, D.M. Danilenko, S.A. Scully, et al., Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless, Genes Dev. 12 (1998) 3156–3161.
[4] A.A. Thomson, G.R. Cunha, Prostatic growth and development are regulated by FGF10, Development 126 (1999) 3693–3701.
[5] H. Sakaue, M. Konishi, W. Ogawa, et al., Requirement of fibroblast growth factor 10 in development of white adipose tissue, Genes Dev. 16 (2002) 908–912.
[6] P.A. Jimenez, M.A. Rampy, Keratinocyte growth factor-2 accelerates wound healing in incisional wounds, J. Surg. Res. 81 (1999) 238–242.
[7] C. Marchese, A. Felici, V. Visco, et al., Fibroblast growth factor 10 induces proliferation and differentiation of human primary cultured keratinocytes, J. Invest. Dermatol. 116 (2001) 623–628.
[8] F. Taniguchi, T. Harada, Y. Sakamoto, et al., Activation of mitogen-activated protein kinase pathway by keratinocyte growth factor or fibroblast growth factor-10 promotes cell proliferation in human endometrial carcinoma cells, J. Clin. Endocrinol. Metab. 88 (2003) 773–780.
[9] H. Zhao, S. Li, D. Han, et al., Alk5-mediated transforming growth factor beta signaling acts upstream of fibroblast growth factor 10 to regulate the proliferation and maintenance of dental epithelial stem cells, Mol. Cell. Biol. 31 (2011) 2079–2089.
[10] F. Rochais, R. Sturny, C.M. Chao, et al., FGF10 promotes regional fetal cardiomyocyte proliferation and adult cardiomyocyte cell cycle reentry, Cardiovasc. Res. (2014), http://dx.doi.org/10.1093/cvr/cvu1232.
[11] N. Itoh, H. Ohta, Fgf10: a paracrine-signaling molecule in development, disease, and regenerative medicine, Curr. Mol. Med. 14 (2014) 504–509.
[12] C. Rinsch, P. Quinodoz, B. Pittet, et al., Delivery of FGF-2 but not VEGF by encapsulated genetically engineered myoblasts improves survival and vascularization in a model of acute skin flap ischemia, Gene Ther. 8 (2001) 523–533.
[13] K. Sato, R.J. Laham, J.D. Pearlman, et al., Efficacy of intracoronary versus intravenous FGF-2 in a pig model of chronic myocardial ischemia, Ann. Thorac. Surg. 70 (2000) 2113–2118.
[14] R.J. Laham, N.A. Chronos, M. Pike, et al., Intracoronary basic fibroblast growth factor (FGF-2) in patients with severe ischemic heart disease: results of a phase I open-label dose escalation study, J. Am. Coll. Cardiol. 36 (2000) 2132–2139.
[15] J. Poss, F. Mahfoud, S. Seiler, et al., FGF-23 is associated with increased disease severity and early mortality in cardiogenic shock, Eur. Heart J. Acute Cardiovasc. Care 2 (2013) 211–218.
[16] S.Q. Liu, D. Roberts, A. Kharitonenkov, et al., Endocrine protection of ischemic myocardium by FGF21 from the liver and adipose tissue, Sci. Rep. 3 (2013) 2767.
[17] T. Romacho, M. Elsen, D. Rohrborn, et al., Adipose tissue and its role in organ crosstalk, Acta Physiol. (Oxf.) 210 (2014) 733–753.
[18] I. Kiprianova, K. Schindowski, K. Schindowski, K. Schindowski, O. von Bohlen und Halbach, et al., Enlarged infarct volume and loss of BDNF mRNA induction following brain ischemia in mice lacking FGF-2, Exp. Neurol. 189 (2004) 252–260.
[19] J.L. Ellsworth, R. Garcia, J. Yu, et al., Fibroblast growth factor-18 reduced infarct volumes and behavioral deficits after transient occlusion of the middle cerebral artery in rats, Stroke 34 (2003) 1507–1512.
[20] D.L. Yao, K. Masonic, D. Petullo, et al., Pretreatment with intravenous FGF-13 reduces infarct volume and ameliorates neurological deficits following focal cerebral ischemia in rats, Brain Res. 818 (1999) 140–146.
[21] P. Wang, T.Y. Xu, K. Wei, et al., ARRB1/beta-arrestin-1 mediates neuroprotection through coordination of BECN1-dependent autophagy in cerebral ischemia, Autophagy 10 (2014) 1535–1548.
[22] P. Wang, T.Y. Xu, Y.F. Guan, et al., Nicotinamide phosphoribosyltransferase protects against ischemic stroke through SIRT1-dependent adenosine monophosphate-activated kinase pathway, Ann. Neurol. 69 (2011) 360–374.
[23] P. Wang, Y.F. Guan, H. Du, et al., Induction of autophagy contributes to the neuroprotection of nicotinamide phosphoribosyltransferase in cerebral ischemia, Autophagy 8 (2012) 77–87.
[24] P. Wang, T.Y. Xu, Y.F. Guan, et al., Vascular smooth muscle cell apoptosis is an early trigger for hypothyroid atherosclerosis, Cardiovasc. Res. 102 (2014) 448–459.

[25] O.A. Alrob, S. Sankaralingam, C. Ma, et al., Obesity-induced lysine acetylation increases cardiac fatty acid oxidation and impairs insulin signalling, Cardiovasc. Res. 103 (2014) 485–497.
[26] L. Lencesova, S. Hudecova, L. Csaderova, et al., Sulphide signalling potentiates apoptosis through the up-regulation of IP3 receptor types 1 and 2, Acta Physiol. (Oxf.) 208 (2013) 350–361.
[27] K.M. Gomes, J.C. Campos, L.R. Bechara, et al., Aldehyde dehydrogenase 2 activation in heart failure restores mitochondrial function and improves ventricular function and remodelling, Cardiovasc. Res. 103 (2014) 498–508.
[28] Y.G. Wang, Y. Li, C.Y. Wang, et al., L-3-*n*-Butylphthalide protects rats' cardiomyocytes from ischaemia/reperfusion-induced apoptosis by affecting the mitochondrial apoptosis pathway, Acta Physiol. (Oxf.) 210 (2014) 524–533.
[29] N.W. Rajapakse, F. Karim, N.E. Straznicky, et al., Augmented endothelial-specific L-arginine transport prevents obesity-induced hypertension, Acta Physiol. (Oxf.) 212 (2014) 39–48.
[30] A.A. Shokeir, A.M. Hussein, N. Barakat, et al., Activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and Nrf-2-dependent genes by ischaemic pre-conditioning and post-conditioning: new adaptive endogenous protective responses against renal ischaemia/reperfusion injury, Acta Physiol. (Oxf.) 210 (2014) 342–353.
[31] L.A. Macmillan-Crow, D.L. Cruthirds, Invited review: manganese superoxide dismutase in disease, Free Radic. Res. 34 (2001) 325–336.
[32] D.W. Nicholson, Caspase structure, proteolytic substrates, and function during apoptotic cell death, Cell Death Differ. 6 (1999) 1028–1042.
[33] R. Gozzelino, V. Jeney, M.P. Soares, Mechanisms of cell protection by heme oxygenase-1, Annu. Rev. Pharmacol. Toxicol. 50 (2010) 323–354.
[34] A. Corcoran, J.J. O'Connor, Hypoxia-inducible factor signalling mechanisms in the central nervous system, Acta Physiol. (Oxf.) 208 (2013) 298–310.
[35] F. Zhang, S. Wang, M. Zhang, et al., Pharmacological induction of heme oxygenase-1 by a triterpenoid protects neurons against ischemic injury, Stroke 43 (2012) 1390–1397.
[36] O.Y. Wei, Y.L. Huang, C.D. Da, et al., Alteration of basic fibroblast growth factor expression in rat during cerebral ischemia, Acta Pharmacol. Sin. 21 (2000) 296–300.
[37] Q. Li, D. Stephenson, Postischemic administration of basic fibroblast growth factor improves sensorimotor function and reduces infarct size following permanent focal cerebral ischemia in the rat, Exp. Neurol. 177 (2002) 531–537.
[38] Z.L. Wang, S.M. Cheng, M.M. Ma, et al., Intranasally delivered bFGF enhances neurogenesis in adult rats following cerebral ischemia, Neurosci. Lett. 446 (2008) 30–35.
[39] N. Ikeda, N. Nonoguchi, M.Z. Zhao, et al., Bone marrow stromal cells that enhanced fibroblast growth factor-2 secretion by herpes simplex virus vector improve neurological outcome after transient focal cerebral ischemia in rats, Stroke 36 (2005) 2725–2730.
[40] Q. Li, Y. Zhu, H. Jiang, et al., Up-regulation of heme oxygenase-1 by isoflurane preconditioning during tolerance against neuronal injury induced by oxygen glucose deprivation, Acta Biochim. Biophys. Sin. (Shanghai) 40 (2008) 803–810.
[41] D. Qi, C. Ouyang, Y. Wang, et al., HO-1 attenuates hippocampal neurons injury via the activation of BDNF-TrkB-PI3K/Akt signaling pathway in stroke, Brain Res. 1577 (2014) 69–76.
[42] M.R. Vargas, M. Pehar, P. Cassina, et al., Fibroblast growth factor-1 induces heme oxygenase-1 via nuclear factor erythroid 2-related factor 2 (Nrf2) in spinal cord astrocytes: consequences for motor neuron survival, J. Biol. Chem. 280 (2005) 25571–25579.