Biochemical and Biophysical Research Communications 390 (2009) 115–120

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

bFGF expression mediated by a hypoxia-regulated adenoviral vector protects PC12 cell death induced by serum deprivation

Hou-Wen Hu [a,e], Xiao-Kun Li [b,d], Rong-Yuan Zheng [c], Jian Xiao [b], Jin-Qi Zeng [c,*], Sheng T. Hou [b,c,e,*]

[a] School of Ophthalmology and Optometry, Wenzhou Medical College, Wenzhou City, Zhejiang Province, PR China
[b] Key Laboratory of Biotechnology & Pharmaceutical Engineering of Zhejiang Province, School of Pharmaceutical Science, Wenzhou Medical College, Wenzhou City, Zhejiang Province, PR China
[c] Department of Neurology, The First Affiliated Hospital and Research Institute of Experimental Neurobiology, Wenzhou Medical College, Wenzhou City, Zhejiang Province, PR China
[d] Engineering Research Center of Bioreactor and Pharmaceutical Development, Ministry of Education, Jilin Agricultural University, Changchun, PR China
[e] Institute for Biological Sciences, National Research Council of Canada, 1200 Montreal Road, Bldg M54, Ottawa, Ont., Canada K1A 0R6

ARTICLE INFO

Article history:
Received 17 September 2009
Available online 24 September 2009

Keywords:
Adenovirus
bFGF
Hypoxia
Hypoxia-responsive element
Gene expression
PC12 cells
Cerebral ischemia

ABSTRACT

Basic fibroblast growth factor (bFGF) is a known neuroprotectant against a number of brain injury conditions such as cerebral ischemia. However, bFGF also regulates a plethora of brain developmental processes and functions as a strong mitogen. Therefore, unregulated long-term expression of bFGF in brain may potentially be tumorigenic, limiting its utility in brain therapy. Here, we report the successful construction of an adenoviral vector (Ad-5HRE-bFGF) expressing bFGF under the regulation of five hypoxia-responsive elements (5HRE) and a minimal cytomegalovirus promoter (CMVmp). Following hypoxia treatment in a hypoxic chamber with less than 1% of oxygen, Ad-5HRE-bFGF induced a significant and time-dependent expression of bFGF protein and the fluorescent tag, humanized GFP (hrGFP) protein, in infected PC12 cells. In contrast, normoxia treatment evoked extremely low level of bFGF and hrGFP expression, demonstrating that the 5HRE-CMVmp cassette was effective in regulating the expression of bFGF gene in response to hypoxia. More importantly, bFGF expressed by the Ad-5HRE-bFGF viral vector under the regulation of hypoxia was significantly neuroprotective against PC12 cell death evoked by serum deprivation. Taken together, these studies demonstrated the feasibility to express bFGF in a hypoxia-regulated fashion to provide neuroprotection. The Ad-5HRE-bFGF can be further developed as an effective tool to provide neuroprotection against hypoxia-induced brain diseases, such as cerebral ischemia.

Introduction

bFGF as a survival-promoting neurotrophic factor for neurons has been extensively demonstrated in the literature [1–3]. During brain development, bFGF promotes brain patterning, neurite branching, morphogenesis and limb development [4–6]. In response to ischemic stroke in adult human brain, the expression of endogenous bFGF is up-regulated significantly in the penumbra region of the ischemic brain, suggesting bFGF plays a role in neuroprotection against ischemic damage to neurons [7]. Indeed, administering bFGF during ischemic stroke in rodent models of stroke reduced the cerebral infarct volume and enhanced brain function recovery, confirming bFGF's role in neuroprotection against ischemic insult [1,8,9]. Based on its neuroprotective properties, bFGF has been tested extensively in both pre-clinical and clinical settings to achieve brain protection against stroke [2,9,10].

However, bFGF also promotes mitogenic proliferation, tumor cell survival and contributes to angiogenesis during tumor development [5,11–13]. In fact, inhibition of bFGF has been shown to be beneficial in treating a number of cancers, such as prostate cancer and renal cancer [5]. Therefore, unregulated, long-term expression of bFGF as a brain therapy may produce many undesirable tumorigenic side effects, which can limit the clinical utility of bFGF as a safe neuroprotectant.

To overcome this, we hypothesized that using a patho-physiological specific inducer, such as hypoxia, to regulate bFGF expression in combination with a transient gene expression vector, such as the replication defective adenoviral vector, may improve bFGF's clinical benefit by reducing bFGF's tumorigenic side effects. Hypoxia-inducible factor 1α (HIF-1α) is a transcription factor that responds to hypoxia/ischemia to up-regulate the expression of several hypoxia responsive genes, such as VEGF, erythropoietin,

Abbreviations: bFGF, basic fibroblast growth factor; CMVmp, a minimal cytomegalovirus promoter; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HRE, hypoxia-responsive elements; hrGFP, humanized green florescent protein; MOI, multiplicity of infection; PI, propidium iodide.

* Corresponding authors. Fax: +1 613 941 4475 (S.T. Hou); +86 577 88069285 (J.-Q. Zeng).
E-mail addresses: doctorzjq@yahoo.com.cn (J.-Q. Zeng), sheng.hou@nrc-cnrc.gc.ca (S.T. Hou).

0006-291X/$ - see front matter Crown Copyright © 2009 Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.09.077

and enolase, through binding to the hypoxia-responsive element. Under normoxic conditions, hypoxia-inducible Factor 1α (HIF-1α) protein is rapidly degraded by the ubiquitin–proteasome pathway. Our previous studies have demonstrated the effectiveness of 5HRE enhancer element to modulate gene expression under hypoxia *in vitro* and *in vivo* using an adenoviral vector [14,15]. Others have also shown that hypoxia-regulated expression of growth factors such as VEGF can be beneficial against ischemic damage [16–18]. Replication-deficient recombinant adenovirus has low cytotoxicity, an ability to produce large quantities of viral particles, and to express genes in both dividing and non-dividing cells. More importantly, the adenoviral vector genome does not become incorporated into the host genome, and gene expression mediated by adenovirus is transient. This type of gene therapy vector is, therefore, an ideal tool to express bFGF under the regulation of hypoxia as therapeutics to the brain.

In the present study, we generated a replication defective adenoviral vector expressing bFGF under the regulation of a 5HRE-CMVmp cassette. Hypoxia-induced bFGF expression was confirmed and the protective effect of hypoxia-induced bFGF was demonstrated in PC12 cells which were protected against serum deprivation-induced death.

Materials and methods

Reagents and antibodies. All chemicals and reagents, unless stated otherwise, were purchased from Sigma (Burlington, ON). Rabbit polyclonal antibody against hrGFP was purchased from Stratagene (La Jolla, CA). The antibody to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was purchased from Chemicon (Temecula, CA). Mouse monoclonal antibody to bFGF was purchased from Millipore (Burlington, MT). Adeno-X™ rapid titer kit was purchased from Clon-

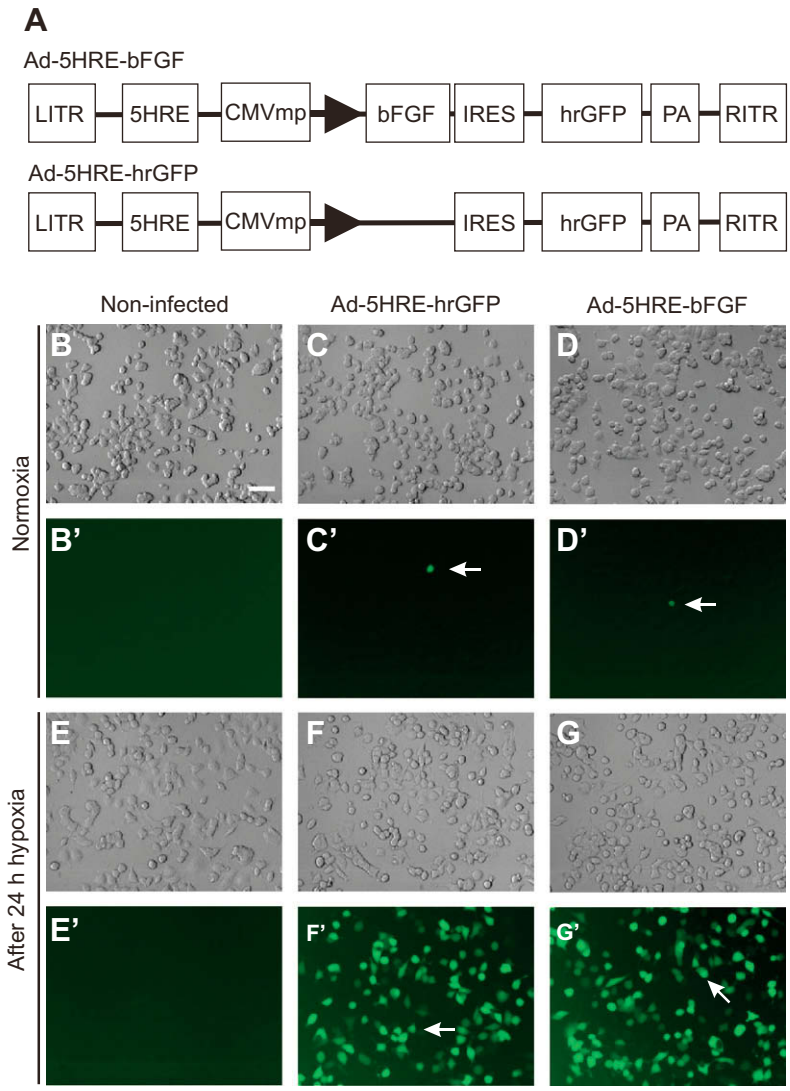

Fig. 1. Construction of hypoxia-inducible bFGF expression vector and determination of hrGFP expression under hypoxia. (A) A schematic diagram of constructed recombinant adenoviruses expressing bFGF (Ad-5HRE-bFGF) and hrGFP (Ad-5HRE-hrGFP). Five copies of HRE (5HRE), derived from VEGF, were inserted in front of the minimal human cytomegalovirus minimal promoter (CMVmp). LITR and RITR are left-end and right-end inverted terminal repeat, respectively, of serotype 5 adenoviral genome. IRES was from the *Encephalomyocarditis* virus internal ribosome entry site; hrGFP was derived from *R. reniformi*, while PA sequence was from early simian virus 40 poly(A) signal sequence. PC12 were infected with Ad-5HRE-hrGFP (C, C′, F, and F′) or Ad-5HRE-bFGF (D, D′, G, and G′) at 25 MOI. After incubation with the indicated adenoviral vector for 18 h, cells were either cultured under normoxic condition (21% oxygen) or exposed to hypoxic treatment in a hypoxic chamber (<1% oxygen) for 24 h. The expression of hrGFP was observed under a fluorescent microscope and high resolution digital images were taken as shown in panels B–G′. Panels B–D and B′–D′ show the phase contrast and hrGFP images, respectively, under the normoxia condition, when there were a few hrGFP positive cells (arrows). Panels E–G and E′–G′ show the effective expression of the hrGFP tag using these Ad vectors under hypoxia with the non-infected cells as a negative control (arrows indicate hrGFP positive cells). Scale bar = 50 μm.

tech (Mountain View, CA). Rat pheochromocytoma PC12 cells were obtained from American Type Cell Culture (ATCC).

Generation of recombinant adenoviral vectors. The recombinant adenoviral vector with five HRE enhancers and a CMV minimal promoter (CMVmp) controlling bFGF and humanized recombinant GFP (hrGFP) gene expression was constructed as follows. The firefly luciferase gene and SV40 poly(A) between the NcoI/SalI restriction sites of the pGL3/5HRE-CMVmp-Luc vector (a gift from Dr. Toru Shibata, Kyoto University, Japan) was replaced with the human bFGF full-length cDNA (Origene, Rockville, MD). The vector was then digested with KpnI and SalI and the resulting fragment containing the expression cassette of 5HRE-CMVmp-bFGF was sub-cloned into the KpnI/SalI restriction sites of pShuttle transfer vector from the AdEasy vector system (Qbiogene, Carlsbad, CA). The PCR-amplified fragment of IRES-hrGFP-poly(A) containing hrGFP cDNA from pShuttle-IRES-hrGFP-1 (Stratagene) was inserted into the SalI site of the constructed pShuttle/5HRE-CMVmp-bFGF vector. All plasmids were sequenced to confirm the authenticity of the obtained recombinant constructs.

Replication defective recombinant adenovirus Ad-5HRE-bFGF was generated from the constructed pShuttle vector using the AdEasy vector system (Qbiogene) according to the manufacturer's instructions. The recombinant adenovirus Ad-5HRE-hrGFP expressing only the reporter gene, hrGFP, under the regulation of 5HRE enhancer and CMVmp was used as a control and constructed by replacing bFGF-IRES-hrGFP sequence with IRES-hrGFP fragment. Both recombinant adenoviral particles were purified by two-step cesium chloride density gradient ultracentrifugation procedure. The preparations were dialyzed in dialysis buffer (10 mM Tris–Cl, pH 8.0, 2 mM $MgCl_2$ and 4% sucrose) and aliquots of the purified viruses were stored in 10% glycerol/dialysis buffer at −80 °C until use. The titer of each viral stock was determined on HEK293 cells by Adeno-X™ rapid titer kit (Clontech) according to the manufacturer's instructions. The plaque forming unit (pfu) for Ad-5HRE-bFGF was at 3.9×10^{10} pfu/ml and the Ad-5HRE-hrGFP had a concentration of 6×10^{10} pfu/ml.

Cell culture. PC12 cells were cultured using a previously described method [15]. Briefly, PC12 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated horse serum, 5% fetal bovine serum (Wisent) and 50 μg/ml gentamycin in a humidified atmosphere containing 5% CO_2 at 37 °C. The medium was changed twice weekly and cells were sub-cultured when they become confluent.

Adenoviral infection, hypoxia, and serum deprivation treatment. PC12 cells were seeded on poly-L-Lysine coated cell culture dishes/plates at a cell density of 1×10^5 cells/cm^2. After plating for 24 h, cells were infected with various concentrations of adenovirus at multiplicity of infection (MOI) ranging from 10 to 100. Optimal concentrations were empirically determined and a 25 MOI concentration was used for all the subsequent experiments which resulted in the infection of the majority of cells (about 90%, as assessed by hrGFP expression after 24 h of hypoxia treatment). Followed by 18 h of incubation with the adenoviral vector, cells were then transferred into an anaerobic chamber (Thermo Fisher Scientific, Ottawa, ON) for hypoxic treatment (with less than 1% O_2, and 5% CO_2 and balanced N_2). After hypoxia treatment for the indicated time, cells were taken out from hypoxic chamber for further analysis. To subject cells to serum deprivation treatment, culture medium containing full strength medium was removed and replaced with serum-free medium after carefully washing cells twice with PBS. Cells were collected after 48 h serum deprivation treatment and further analyzed for protein expression and cell death assay.

Western blotting. The procedures for Western blotting were as previously described [19]. Briefly, after treatment with hypoxia for the indicated time, cells were rinsed twice with cold PBS and collected using centrifugation. Cell pellets were lysed in 0.1 ml of RIPA buffer. Cell lysates containing 10 μg of the total protein was subjected to electrophoresis in a 15% SDS–PAGE gel, followed by transferring onto a nitrocellulose membrane in transfer buffer. The membrane was probed with a primary antibody at 4 °C overnight. After washing with 0.1% Tween 20 in TBS, horseradish peroxidase conjugated secondary antibody was applied to the membrane for 1 h at room temperature. Enhanced chemiluminescence detection of the target protein was performed using ECL Plus western blotting detection system (GE Healthcare, Baie d'Urfe Quebec) and exposed using a X-ray film.

Cell viability assays. The viability of PC12 cells was assayed using three methods: propidium iodide (PI) assay, nuclei counting, and Hoechst 33258 staining. (a) PI Assay: PI at a final concentration of 10 μg/ml was added to cells for 1 h at 37 °C. After the incubation, cells were examined under a florescent microscope. Digital images of a combination of phase contrast and PI were taken. The ratio of PI positive cells versus the total number of cells from at least five representative microscopic fields (200×) before serum deprivation (phase contrast) was calculated by counting the number of cells using an automated threshold method (Image J program located at http://rsbweb.nih.gov/ij/). (b) Cell nuclei counting: the culture medium (along with cellular debris) was removed by aspiration and replaced with 150 μl of cell counting buffer containing 10% zapoglobin, 0.5% Triton X-100, 2 mM $MgCl_2$, and 15 mM NaCl in 5 mM phosphate buffer, pH 7.4. This treatment dissolved cell membranes and cytoplasm and provided a uniform suspension of single intact nuclei. Nuclei were re-suspended by pipetting up-and-down 10 times using a P200 pipette set to 100 μl. Ten microliters of the nuclei mixture were transferred to a hemacytometer and only large and intact healthy nuclei were counted. Broken or damaged nuclei were not included in the counts, and (c) Hoechst 33258 staining: The cells were fixed directly on the plate with 4% fresh formaldehyde for 15 min. Cells were washed with PBS and stained with 200 μl of Hoechst 33258 dye which was dissolved in PBS to a final concentration of 5 μg/ml. After incubation for 10 min, cells were observed under a fluorescence microscopy (Zeiss, Toronto, ON). Digital images of hrGFP and Hoechst were taken. All these experiments were independently repeated at least three times. Statistical analysis was performed using ANOVA with Tukey's post hoc analysis for significant groups.

Results and discussion

Construction of Ad-5HRE-bFGF and Ad-5HRE-hrGFP vectors

Our previous studies have shown that the 5HRE combination is an optimal enhancer cassette to modulate CMVmp activity [15]. In the present study, two replication deficient adenoviral vectors were made containing the 5HRE-CMVmp cassette as shown in Fig. 1A, i.e. Ad-5HRE-bFGF and Ad-5HRE-hrGFP. The two constructs share all the components, except Ad-5HRE-hrGFP, which has no bFGF, serving as a control vector. The structures of the plasmid constructs are shown in Fig. 1A which included a 5HRE-CMVmp cassette as an enhancer/promoter and hrGFP controlled by an IRES element as a florescent tag. These plasmids were transfected into HEK293 cells containing the E1 gene product to generate replication defective adenovirus using the method as described in the Method section and previously by us [15]. Equal amount of each adenovirus at 25 MOI was used to infect the cultured PC12 cells.

Verification of hypoxia-regulated gene expression by Ad-5HRE-bFGF and Ad-5HRE-hrGFP vectors

Under normoxia condition, almost no hrGFP expression occurred as shown in Fig. 1B–D and B′–D′. After 6 h hypoxia (<1%

oxygen), a large number of GFP positive PC12 cells appeared in the culture indicating hypoxia-regulated expression of hrGFP and bFGF (Fig. 1E–G and E′–G′). To quantify the expression of bFGF and hrGFP induced by hypoxia, Western blotting was performed on the cell lysates derived from the infected PC12 cells. The intensities of Western blot bands were measured using Image J. The densities of bFGF and hrGFP were normalized against those of GAPDH which were used as internal loading controls. As shown in Fig. 2A, 6 h hypoxia treatment drastically induced the expression of hrGFP in PC12 cells infected with Ad-5HRE-hrGFP, while both hrGFP and bFGF were induced in PC12 cells infected with the Ad-5HRE-bFGF virus. In contrast, the expressions of bFGF and hrGFP under normoxia were at extremely low levels almost beyond detection shown using Western blotting (Fig. 2A). The time-dependent induction of bFGF and its reporter gene hrGFP using the Ad-5HRE-bFGF vector was determined as shown in Fig. 2B. Expression of both hrGFP and bFGF appeared after 3 h hypoxia treatment while the levels of hrGFP and bFGF under normoxia condition were extremely low and beyond detection using Western blotting (Fig. 2B). After 6 h hypoxia, the expression levels of bFGF and hrGFP were almost equally high and at a level close to 30-fold higher than the normoxia-treated PC12 cells, respectively. The expression of both hrGFP and bFGF after 24 h hypoxia treatment was maintained at more than 30-fold than those of the normoxia-treated PC12 cells (Fig. 2B–D). The expression levels of both hrGFP and bFGF were determined using Western blotting with GAPDH as an internal control as shown in Fig. 2C and D (**indicate statistical significance with $p < 0.01$, $n = 6$). Collectively, these two experiments demonstrated that the Ad-5HRE-bFGF and Ad-5HRE-hrGFP can mediate gene expression in response to hypoxia.

Expression of bFGF protects PC12 cells against serum-deprivation-induced death

To prove that hypoxia-induced bFGF was neuroprotective, PC12 cells were subjected to serum deprivation after infection with Ad-5HRE-bFGF or Ad-5HRE-hrGFP for 18 h followed by 6 h hypoxia (<1% oxygen). At this time, cells appeared healthy under the phase contrast microscope (Fig. 3A–C). Cells were then subjected to serum deprivation treatment by replacing the culture medium to DMEM containing 0% serum. After 48 h of serum deprivation, cells were stained with PI or Hoechst 33258 and examined under a phase contrast microscope. Serum deprivation caused a significant increase in cell death as shown in Fig. 3A′–C′. PC12 cells, either non-infected or infected with Ad-5HRE-hrGFP, were mostly detached from the culture dish and exhibited a shrunken morphology under the phase contrast microscope (Fig. 3A′ and B′). These cells were in fact dead and showed PI positive staining (Fig. 3A″ and B″). In contrast, most Ad-5HRE-bFGF infected cells remained healthy with normal cellular morphology (Fig. 3C′) and significantly fewer PI positive cells (Fig. 3C″, F and G; $p < 0.01$, $n = 6$ in both F and G). Co-localization of Hoechst 33258 with hrGFP expression, as shown in Fig. 3D–E, demonstrated that bFGF expressing cells were indeed having normal nuclei, confirming protection against serum deprivation, while Ad-5HRE-hrGFP infected cells showed condensed nuclei, indicating no protection.

Cellular viability following serum deprivation was quantified using both PI assay and a viable nuclei counting method as described in the Method section. Ad-5HRE-bFGF infected PC12 cells were significantly protected against serum deprivation-induced death compared with the non-treated and Ad-5HRE-hrGFP infected PC12 cells as determined using both methods (Fig. 3F and G, respectively; $p < 0.01$, $n = 6$).

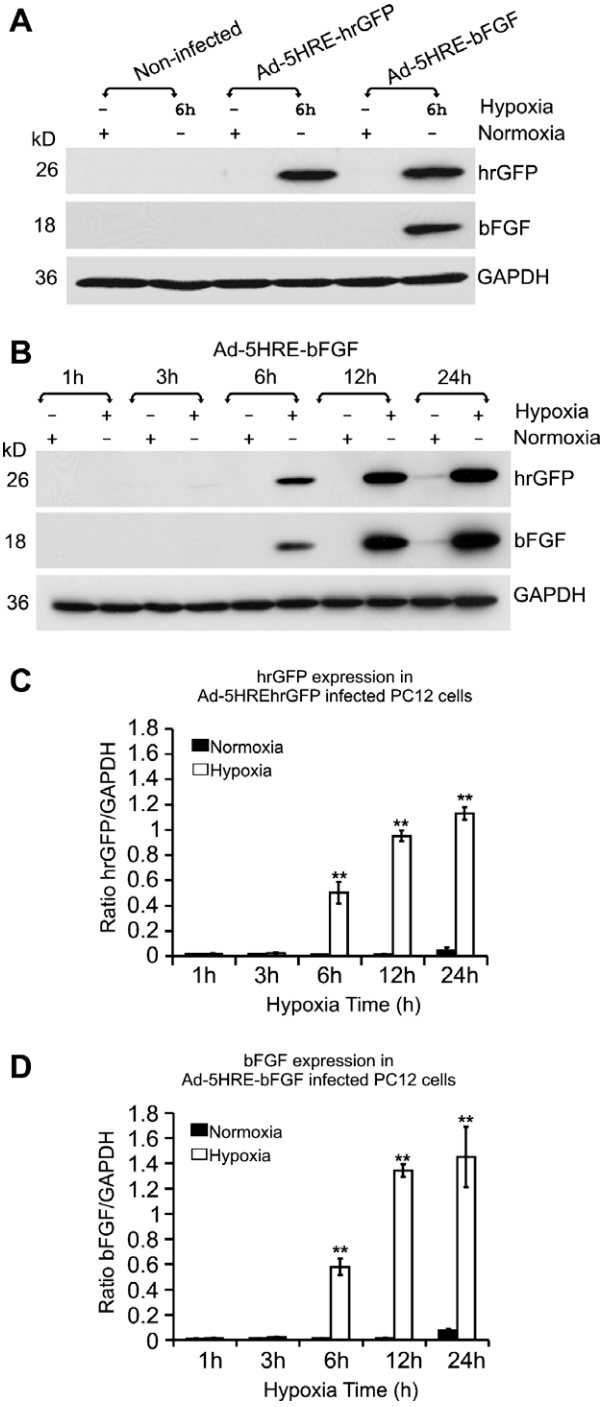

Fig. 2. Time-dependent, hypoxia-induced increase in bFGF expression by the Ad-5HRE-bFGF viral vector in PC12 cells. (A) Cultured PC12 cells were infected with either Ad-5HRE-bFGF or Ad-5HRE-hrGFP for 18 h before treatment with hypoxia (<1% oxygen) for 6 h. Proteins were immediately collected as described in the method section and 10 μg of the total proteins were subjected to Western blotting analysis to determine the expression levels of bFGF and hrGFP (panel A). GAPDH expression was determined using Western blotting to use as an internal loading control. Non-infected PC12 cells treated with normoxia and 6 h hypoxia were also used as controls (panel A). (B) After infection with 25 MOI of Ad-5HRE-bFGF for 18 h, PC12 cells were treated with hypoxia (<1% oxygen) for 1, 3, 6, 12, and 24 h as indicated in panel (B). After hypoxia, cells were collected for Western blotting analysis of the expression of bFGF and hrGFP. Hypoxia-induced bFGF and hrGFP expression mediated by the Ad-5HRE-bFGF virus was quantified using densitometry, aided by Image J software. The ratio of induction in bFGF and hrGFP was normalized against those of GAPDH. The data was plotted as shown in panels (C) and (D). At least three independent repeats were performed. Data in panels (C) and (D) represent means ± SEM. **Indicate statistical significance using one-way ANOVA followed by post hoc analysis using Tukey's test ($p < 0.01$).

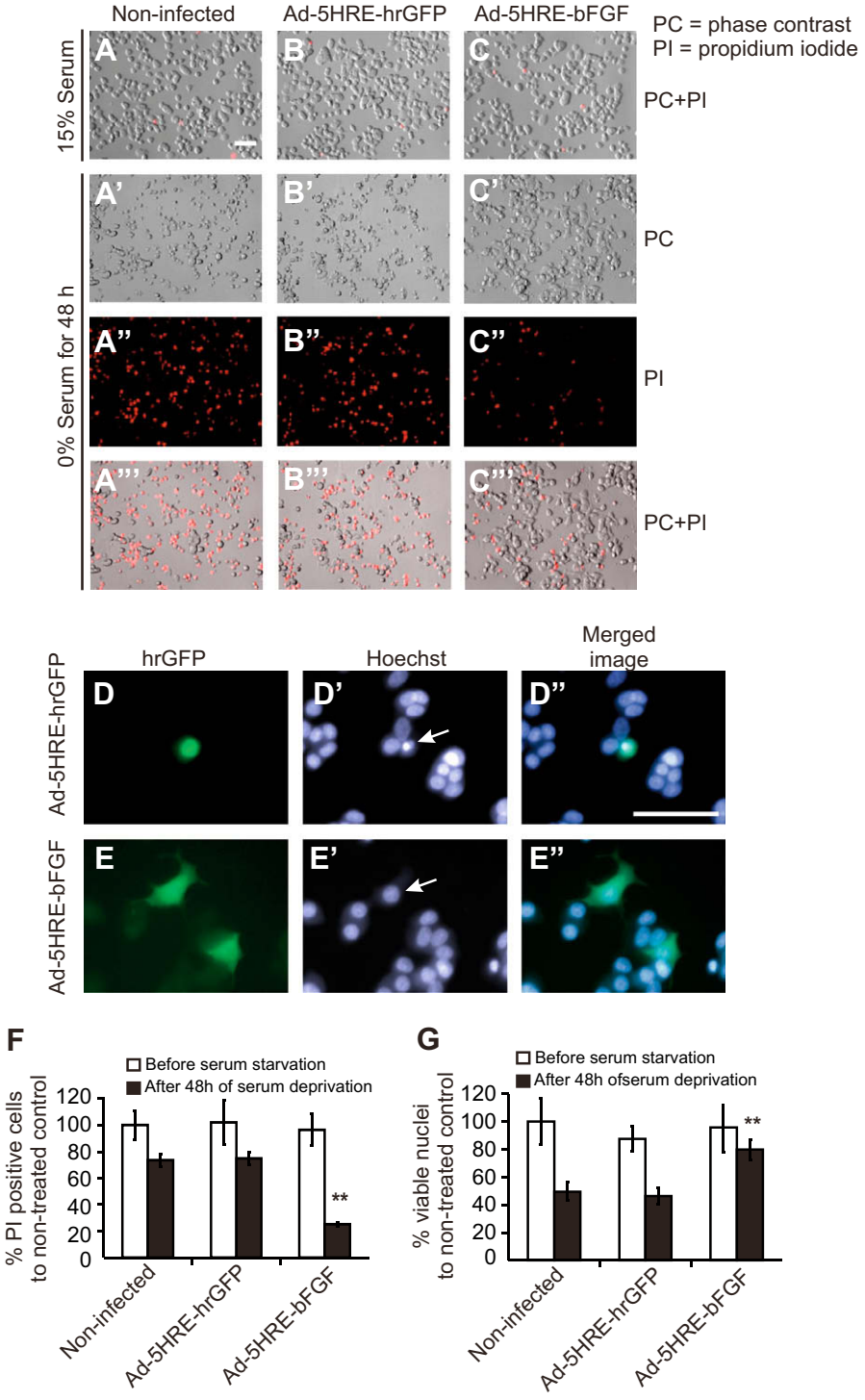

Fig. 3. Protection of PC12 cells against serum deprivation by Ad-5HRE-bFGF. PC12 cells were infected with 25 MOI of Ad-5HRE-hrGFP or Ad-5HRE-bFGF viral particles for 18 h followed by 6 h of hypoxia (<1% oxygen) (panels A–C). After the hypoxia treatment, the culture medium containing 15% serum was replaced with serum-free DMEM after washing the cells twice with PBS. Following 48 h of serum deprivation treatment, cells were examined under a phase contrast microscope (panels A′–C‴). Cells were also stained with PI (10 μg/ml) for 60 min and examined under a florescence microscope (red colored cells) (panels A″–C″). Images in panels A, B, C, A‴, B‴, and C‴ were the combined images of phase contrast and PI staining. Cell nuclei were stained with Hoechst 33258 and the co-localization of hrGFP fluorescence with Hoechst 33258 was determined under a florescence microscope (panels D–E″; arrows indicate nuclei). Cell viability was determined 48 h after serum deprivation using PI assay as shown in panels F and the nuclei counting method as shown in panel G. The percentage of cell death under serum starvation treatment was calculated by normalization against cells infected with the same viral vector before serum deprivation treatment. Data was analyzed using Prism 5.0 and plotted as shown. **Indicates significant difference using Student' *t*-test ($p < 0.01$). Scale bars = 50 μm.

Conclusions

Gene therapy is a promising approach for treatment of cerebral vascular diseases such as stroke [20]. Numerous pre-clinical studies have demonstrated that gene transfer of bFGF using adenoviral vector reduces infarct size and improves neurological deficit [9,10]. However, several challenges remain as to using bFGF-based gene therapy to treat stroke, for example, how to ameliorate the poten-

tial tumorigenic effect caused by the sustained expression of bFGF in the brain. These challenges require further investigation to increase target specificity and improve the safety and efficacy of the expression system and vector design is central to all of these. In the present study, we demonstrated the successful construction of an adenoviral vector which enabled bFGF expression regulated by hypoxia, a patho-physiological stimulus, and showed that hypoxia-induced bFGF expression was neuroprotective. Future studies will be designed to investigate the effect of using this vector in ischemic brains.

Acknowledgments

We thank Amy Aylsworth for technical assistance. Hou-Wen Hu was partially supported by a Doctoral Student Scholarship from the China Scholarship Council which allowed his research visit to the Institute for Biological Sciences, National Research Council of Canada. This project was supported by a grant from the National Natural Science Foundation of China (Grant No: 30670747) and funds from the Zhejiang Provincial Extremely Key Subject Building Project "Pharmacology and Biochemical Pharmaceutics 2008".

Reference

[1] J.M. Ren, S.P. Finklestein, Growth factor treatment of stroke, Curr. Drug Targets CNS Neurol. Disord. 4 (2005) 121–125.
[2] C.H. Chen, S.M. Poucher, J. Lu, P.D. Henry, Fibroblast growth factor 2: from laboratory evidence to clinical application, Curr. Vasc. Pharmacol. 2 (2004) 33–43.
[3] G.J. Chen, R. Forough, Fibroblast growth factors, fibroblast growth factor receptors, diseases, and drugs, Recent Pat. Cardiovasc. Drug Discov. 1 (2006) 211–224.
[4] K. Abe, H. Saito, Effects of basic fibroblast growth factor on central nervous system functions, Pharmacol. Res. 43 (2001) 307–312.
[5] A. Beenken, M. Mohammadi, The FGF family: biology, pathophysiology and therapy, Nat. Rev. Drug Discov. 8 (2009) 235–253.
[6] B. Reuss, H.O. von Bohlen und, Fibroblast growth factors and their receptors in the central nervous system, Cell Tissue Res. 313 (2003) 139–157.
[7] R. Issa, A. AlQteishat, N. Mitsios, M. Saka, J. Krupinski, E. Tarkowski, J. Gaffney, M. Slevin, S. Kumar, P. Kumar, Expression of basic fibroblast growth factor mRNA and protein in the human brain following ischaemic stroke, Angiogenesis 8 (2005) 53–62.
[8] H. Ay, I. Ay, W.J. Koroshetz, S.P. Finklestein, Potential usefulness of basic fibroblast growth factor as a treatment for stroke, Cerebrovasc. Dis. 9 (1999) 131–135.
[9] T. Watanabe, Y. Okuda, N. Nonoguchi, M.Z. Zhao, Y. Kajimoto, D. Furutama, H. Yukawa, M.A. Shibata, Y. Otsuki, T. Kuroiwa, S. Miyatake, Postischemic intraventricular administration of FGF-2 expressing adenoviral vectors improves neurologic outcome and reduces infarct volume after transient focal cerebral ischemia in rats, J. Cereb. Blood Flow Metab. 24 (2004) 1205–1213.
[10] Y. Liu, J.B. Lu, Z.R. Ye, Permeability of injured blood brain barrier for exogenous bFGF and protection mechanism of bFGF in rat brain ischemia, Neuropathology 26 (2006) 257–266.
[11] D. Ribatti, A. Vacca, M. Rusnati, M. Presta, The discovery of basic fibroblast growth factor/fibroblast growth factor-2 and its role in haematological malignancies, Cytok. Growth Factor Rev. 18 (2007) 327–334.
[12] R. Grose, C. Dickson, Fibroblast growth factor signaling in tumorigenesis, Cytok. Growth Factor Rev. 16 (2005) 179–186.
[13] A. Bikfalvi, Role of fibroblast growth factor-2 in tumor angiogenesis. Pathol. Biol. (Paris) 47 (1999) 364–367.
[14] S.T. Hou, S.X. Jiang, D. Huang, A. Desbois, A novel adenoviral vector-mediated neuronal selective gene expression in neonatal mouse brain in response to hypoxia, Neurosci. Lett. 419 (2007) 23–27.
[15] D. Huang, A. Desbois, S.T. Hou, A novel adenoviral vector which mediates hypoxia-inducible gene expression selectively in neurons, Gene Ther. 12 (2005) 1369–1376.
[16] K. Binley, O. Kan, J. White, S. Naylor, Exploiting the hypoxia response, Curr. Opin. Mol. Ther. 5 (2003) 650–656.
[17] H. Su, Y.W. Kan, Adeno-associated viral vector-delivered hypoxia-inducible gene expression in ischemic hearts, Methods Mol. Biol. 366 (2007) 331–342.
[18] F. Shen, Y. Fan, H. Su, Y. Zhu, Y. Chen, W. Liu, W.L. Young, G.Y. Yang, Adeno-associated viral vector-mediated hypoxia-regulated VEGF gene transfer promotes angiogenesis following focal cerebral ischemia in mice, Gene Ther. 15 (2008) 30–39.
[19] S.T. Hou, S.X. Jiang, A. Desbois, D. Huang, J. Kelly, L. Tessier, L. Karchewski, J. Kappler, Calpain-cleaved collapsin response mediator protein-3 induces neuronal death after glutamate toxicity and cerebral ischemia, J. Neurosci. 26 (2006) 2241–2249.
[20] Y. Chu, J.D. Miller, D.D. Heistad, Gene therapy for stroke: 2006 overview, Curr. Hypertens. Rep. 9 (2007) 19–24.