PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2004;10(24):3590-3596
World Journal of Gastroenterology
Copyright © 2004 by The WJG Press ISSN 1007-9327

• BASIC RESEARCH •

Enhanced anti-apoptosis and gut epithelium protection function of acidic fibroblast growth factor after cancelling of its mitogenic activity

Xiao-Bing Fu, Xiao-Kun Li, Tong Wang, Biao Cheng, Zhi-Yong Sheng

Xiao-Bing Fu, Biao Cheng, Zhi-Yong Sheng, Wound Healing and Cell Biology Laboratory, Burns Institute, 304 Medical Department, General Hospital of PLA, Trauma Centre of Postgraduate Medical College, Beijing 100037, China
Xiao-Kun Li, Tong Wang, Biopharmaceutical R&D Centre, Jinan University, Guangzhou 510632, Guangdong Province, China
Supported by the National Natural Science Foundation of China, No. 30170966, 30230370; National Basic Science and Development Program (973 Program), No.G1999054204; National High-Tech Development Program (863 Program), No.2001AA215131
Correspondence to: Professor Xiao-Bing Fu, M.D., Wound Healing and Cell Biology Laboratory, 304 Medical Department, General Hospital of PLA, Trauma Centre of Postgraduate Medical College, 51 Fucheng Road, Beijing 100037, China. fuxb@cgw.net.cn
Telephone: +86-10-66867396 **Fax:** +86-10-68480755
Received: 2004-04-04 **Accepted:** 2004-04-12

Abstract

AIM: Mitogenic and non-mitogenic activities of fibroblast growth factor (FGF) are coupled to a range of biological functions, from cell proliferation and differentiation to the onset of many diseases. Recent reports have shown that acidic fibroblast growth factor (aFGF) has a powerful anti-apoptosis function, which may have potentially therapeutical effect on gut ischemia and reperfusion injuries. However, whether this function depends on its mitogenic or non-mitogenic activity remains unclear. In this study, we identified the source of its anti-apoptosis function with a mutant, aFGF28-154 and observed its effect on reducing gut ischemia and reperfusion injury.

METHODS: aFGF28-154 was generated by amplification of appropriate DNA fragments followed by subcloning the products into pET-3c vectors, then they were expressed in BL21 (DE3) cells and purified on an M2 agarose affinity column. This mutant aFGF28-154 maintained its non-mitogenic activity and lost its mitogenic activity. With a dexamethasone (DEX)-induced mouse thymocyte apoptosis model *in vitro* and *in vivo,* we studied the anti-apoptotic function of aFGF28-154. Also, *in vivo* study was performed to further confirm whether aFGF28-154 could significantly reduce apoptosis in gut epithelium after gut ischemia-reperfusion injury in rats. Based on these studies, the possible signal transduction pathways involved were studied.

RESULTS: With a dexamethasone (DEX)-induced mouse thymocyte apoptosis model *in vitro* and *in vivo*, we found that the anti-apoptotic function of aFGF28-154 was significantly enhanced when compared with the wild type aFGF. *In vivo* study further confirmed that aFGF28-154 significantly reduced apoptosis in gut epithelium after gut ischemia-reperfusion injury in rats. The mechanisms of anti-apoptosis function of aFGF28-154 did not depend on its mitogenic activity and were mainly associated with its non-mitogenic activities, including the intracellular calcium ion balance protection, ERK1/2 activation sustaining and cell cycle balance.

CONCLUSION: These findings emphasize the importance of non-mitogenic effects of aFGF, and have implications for its therapeutic use in preventing apoptosis and other injuries in tissues and internal organs triggered by ischemia-reperfusion injury.

Fu XB, Li XK, Wang T, Cheng B, Sheng ZY. Enhanced anti-apoptosis and gut epithelium protection function of acidic fibroblast growth factor after cancelling of its mitogenic activity. *World J Gastroenterol* 2004; 10(24): 3590-3596
http://www.wjgnet.com/1007-9327/10/3590.asp

INTRODUCTION

Fibroblast growth factors (FGFs) are members of a family of polypeptides that are potent regulators of cell proliferation, differentiation, and function. They play very important roles in normal development, maintenance of tissues, wound healing and repair. They have also been implicated in a wide range of pathological conditions, including tumorigenesis and metastasis[1-4]. Acidic and basic fibroblast growth factors (aFGF and bFGF), the important members of this family, were named for their different isoelectric points. They have similar molecular weights and spectra of biological activities, and show approximately 55% amino acid similarity. aFGF and bFGF stimulate the proliferation of all cells of mesodermal organ, and many cells of neuroectodermal, ectodermal, and endodermal origin. Also, both FGFs are chemotactic and mitogenic for endothelial cells *in vitro*, inducing production of many factors involved in regulation of many functions[2-5]. Recent reports have shown that aFGF has a powerful anti-apoptotic function, which may have potentially therapeutical use in some diseases[5-13]. However, whether this function depends on its mitogenic or non-mitogenic activity remains unclear, and clarification of this function may help to find its new mechanisms and active domains. In the present study, we modified the wild type of aFGF and acquired a mutant, aFGF28-154, which kept its non-mitogenic activity and lost its mitogenic activity. By using the dexamethasone (DEX)-induced apoptotic model, we found that the anti-apoptosis function of the modified aFGF28-154 was significantly enhanced when compared with the wild type of aFGF. The results indicated that the powerful anti-apoptosis function of aFGF28-154 was associated with the intracellular calcium ion balance protection and ERK1/2 activation sustaining, which did not depend on its mitogenic activity. Further studies showed that aFGF might have a surveillance function at the checkpoint of cell cycle, for determination of stagnation, division or apoptosis. Finally, *in vivo* study confirmed that aFGF28-154 could significantly reduce apoptosis in gut epithelium after gut ischemia- reperfusion injury in rats.

MATERIALS AND METHODS

Protein preparation

Plasmids encoding aFGF28-154 were generated by amplification of appropriate DNA fragments, followed by subcloning the

products into pET-3c vectors. aFGF28-154 protein was expressed in BL21 (DE3) cells and purified on an M2 agarose affinity column (Sigma). We carried out SDS-PAGE and Western blot analysis with aFGF monoclonal antibody (Sigma) to determine the accuracy of protein expression.

Mitogenic activity assay

MTT method was conducted to test the mitogenic activity of both aFGFs. NIH 3T3 fibroblasts and PC12 cell lines from Wuhan University were cultured in DMEM (Hyclone) with 150 mL/L fetal bovine serum (Gibico) plus 50 mL/L equine serum (Hyclone) for PC12 culture. These cells were stimulated with aFGF and aFGF28-154 at a final concentration of 0, 1, 2, 3, 4, 5, 6, 8, 10 ng/mL for 24 h before *A* value detection.

Apoptosis models in thymocytes and treatment methods in vitro

Twenty homogeneous Balb/c mice aged four weeks were randomised into 4 groups: control group (group 1); DEX group (group 2), DEX+aFGF (Biopharmaceutical R&D Centre) group (group 3) and DEX+aFGF28-154 (group 4). DEX, if applied, was taken at a final concentration of 1 μmoL. aFGF and aFGF28-154, if applied, were taken at a final concentration of 100 ng/mL. In order to explore the relationship between dosage and effects, different dosages of aFGF28-154 from 50 to 200 ng/mL were used in another experiment. After collected under sterile conditions, thymocytes were cultured in RMPI1640 medium (Hyclone) supplemented with 100 mL/L fetal bovine serum (Gibico) for 5 h.

In *in vivo* study**,** Group 1 was injected with saline, group 2 with DEX 10 mg/kg, group 3 with DEX 10 mg/kg and aFGF10 μg/kg, and group 4 with DEX 10 mg/kg and aFGF28-154 10 μg/kg. Animals were fed for 5 h before collection of thymocytes.

Flow cytometry

Flow cytometry was performed using an FACScaliber (Becton Dickinson). Phosphatidylserine (PS), a sign of early apoptosis, was stained with Annexin V-FITC (Baosai), while DNA fragments of the necrotic cells were evaluated with PI apoptosis.

Morphological observation and gel electrophoresis analysis of apoptosis

Transmission electron microscope was used to morphologically visualize apoptosis and agarose gel electrophoresis was performed to find the damage of nuclear chromatin DNA.

Analysis of intracellular Ca^{2+} concentration

Confocal microscope was used to analyse the changes of intracellular Ca^{2+} concentration.

Apoptosis model in gut epithelium and treatment methods in vivo

Rat intestinal ischemia-reperfusion (I/R) injury was produced by clamping the superior mesenteric artery (SMA) for 45 min followed by reperfusion for 24 h. One hundred and fourteen Wistar rats were divided randomly into four groups, namely intestinal I/R plus saline treatment group (I/R+Saline), intestinal I/R plus wild type aFGF treatment group (I/R plus aFGF), intestinal I/R plus aFGF 28-154 group (I/R plus aFGF28-154) and normal control group (normal control). In groups 1, 2 and 3, SMA was separated and occluded for 45 min, then released for reperfusion for 24 h. At the onset of reperfusion, saline (0.5 mL) or aFGF (4 μg/rat), or aFGF28-154 (4 μg/rat) was injected through the jugular vein. In control group, SMA was separated without occlusion. After the animals were sacrificed, tissue biopsies were taken from the intestine at 30 min, 1, 2, 6, 12, and 24 h, respectively. Apoptosis in epithelium entericum was assayed with TUNEL technique and analyzed using light microscope. All animal experiments were carried out according to the Guidelines for Care and Use of Experimental Animals and approved by the internal ethical committees of the Trauma Centre of Postgraduate Medical College (Beijing).

Analysis of signal transduction in cells stimulated by DEX and both aFGFs in vitro

Cultured thymocytes were sampled to carry out Western blot analysis. Antibodies used were ERK1/2, p-ERK1/2, JNK1/2, p38 MAPK, c-Myc (Sigma), and Caspase-3 (Boster).

RESULTS

Protein preparation and mitogenic activation assay

Plasmids encoding aFGF28-154 were generated by amplification of appropriate DNA fragments, followed by subcloning the products into pET-3c vectors. aFGF28-154 protein was expressed in BL21 (DE3) cells and purified on an M2 agarose affinity column (Sigma). We carried out SDS-PAGE and Western blot analysis with aFGF monoclonal antibody (Sigma) to determine the accuracy of protein expression (Figure 1A , B). We tested both aFGF and aFGF28-154 for their effects on proliferation of NIH 3T3 fibroblasts and rat pheochromocytoma (PC12) cells. The results indicated that the latter significantly lost its mitogenic activity evidenced by MTT results (Figure 1C). Thus, we could perform a comparative research to clarify whether aFGF function depended on mitogenic activity.

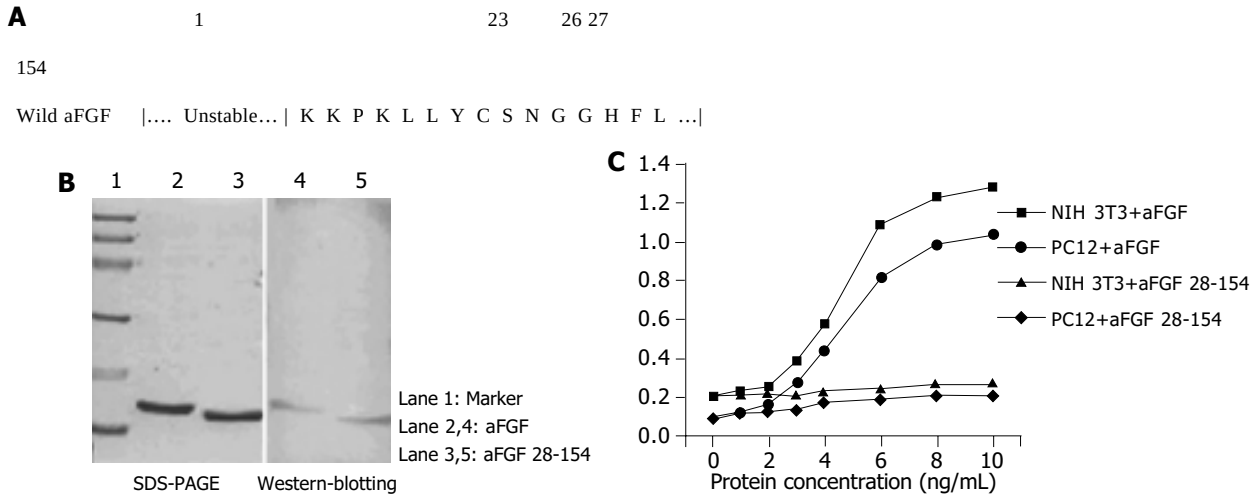

Figure 1 Loss of mitogenic activity in aFGF after modification. A: Sequence comparison between modified aFGF (aFGF28-154) and the wild type. B: Western blot analysis of purified aFGF and aFGF28-154. C: MTT assay of the mitogenic activity of both aFGFs.

Assay of anti-apoptotic activation of both aFGFs

We examined the anti-apoptotic effects of both aFGFs by a DEX-induced thymocyte apoptosis model in mouse, which was considered as one of the best characterized apoptosis research systems[14-16]. First, a number of typical apoptotic thymocytes with apoptotic bodies from the DEX-induced group were visualized by transmission electron microscope (Figure 2A, B). Second, agarose gel electrophoresis results of this group also indicated characteristic ladder bands of oligonucleosomal fragments (180-200 bp) produced by extracted chromatin DNA, while few were found in blank group and the DNA damage in DEX -induced group was much more serious than that in both aFGFs treated groups (Figure 2C). Third, Annexin V-FITC/PI staining was used to distinguish apoptotic and necrotic cells from normal ones by flow cytometry (Figure 2D). Our test indicated that under the stimulation of DEX, thymocytes showed apoptosis both *in vivo* and *in vitro* (44.40% and 20.07%, respectively) after 5-h culture. With the protection of aFGF, when exposed to DEX, the apoptosis rate was lower both *in vivo* and *in vitro* (33.71% and 18.31%, respectively). Under the same condition, however, the apoptosis rate was significantly lower in aFGF28-154 group than in DEX and aFGF groups both *in vivo* and *in vitro* (23.95% and 13.80%, respectively) (Figure 2D). The quantitative analysis of anti-apoptosis results and the dose-effect relationship of aFGF28-154 are summarized in Table 1. Fourth, we studied the changes of concentration of intracellular calcium ion (Ca^{2+}) by confocal microscope to further confirm the anti-apoptotic effects of both aFGFs on mouse thymocytes. High concentration of intracellular Ca^{2+} caused by influx increase was involved in triggering protease and endonuclease

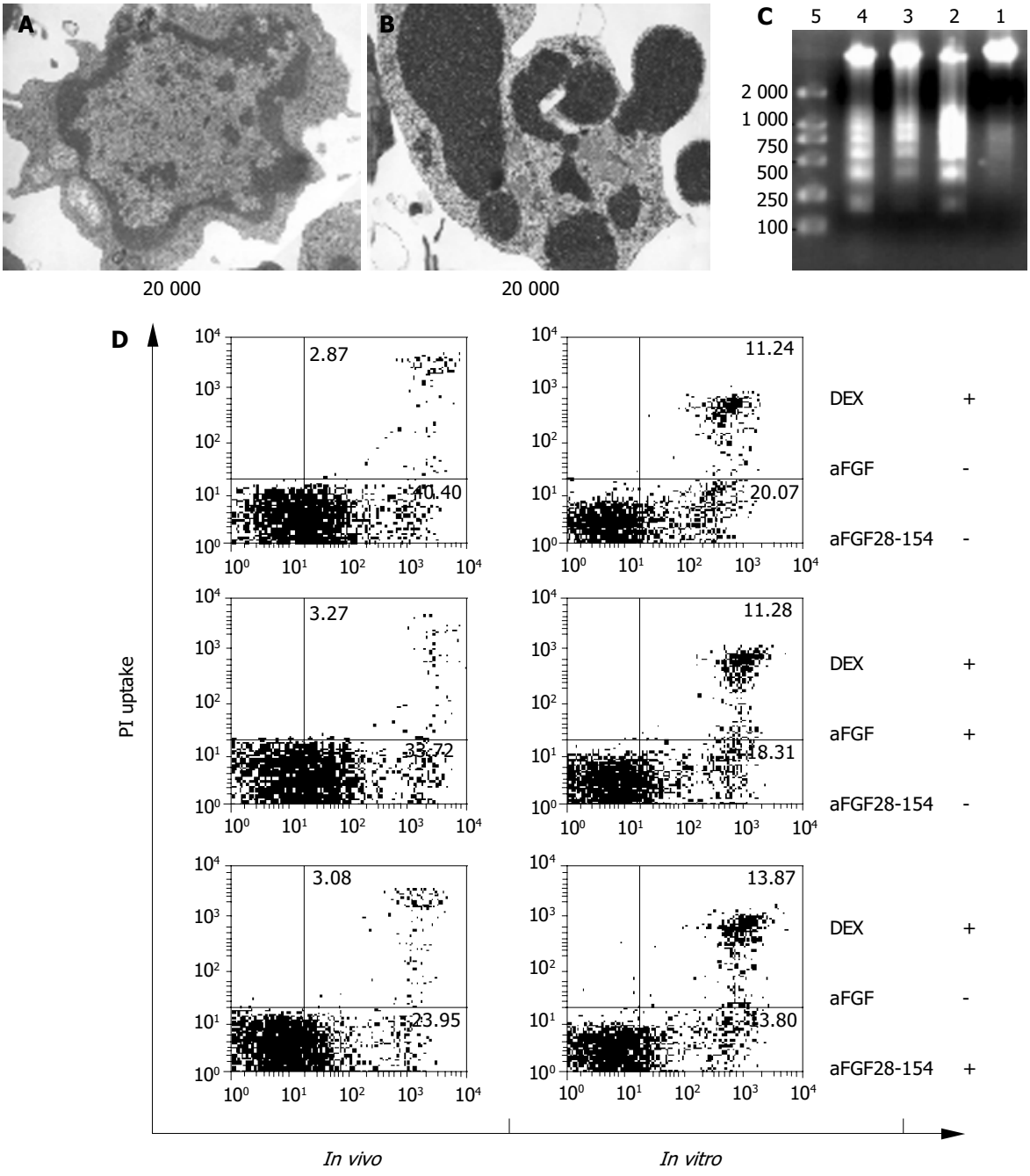

Figure 2 Comparative evaluation of the anti-apoptotic function of aFGF28-154 both *in vitro* and *in vivo*. A: TEM observation (×20 000) of DEX-induced apoptosis in mouse thymocytes. B: Typical apoptotic bodies in DEX-induced thymocytes. C: Damage of DNA analysis by agarose gel electrophoresis according to DNA ladder kit (Apoptosis DNA Laddering Kit-Ethidium Bromide, R&D Systems). Lane 1: DNA extracted from normal control group; lane 2: DNA from DEX only group; lane 3: DNA from DEX plus aFGF28-154 group; lane 4: DNA from DEX plus aFGF group; lane 5: DNA size markers (bp). D: Flow cytometry results.

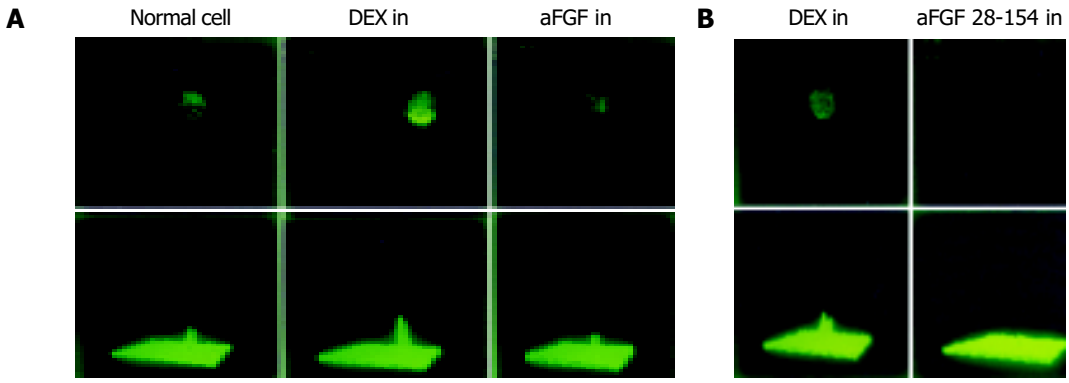

Figure 3 Balance of cytosolic calcium ion concentration protected by both aFGFs when stimulated by DEX. A: Mouse thymoytes from Ca^{2+} influx protected by wild type aFGF. B: Decrease of intracellular Ca^{2+} concentration caused by aFGF28-154.

activation and apoptosis. Hence, Ca^{2+} influx was considered as a sign of apoptosis occurrence[17-19]. In this study, the intracellular Ca^{2+} concentration in thymocytes increased quickly when exposed to DEX. When administrated with aFGF, however, Ca^{2+} concentration decreased to a lower level (Figure 3A). However, the test for aFGF28-154 showed a marked reduction of Ca^{2+} influx (Figure 3B).

Table 1 *In vitro* quantitative analysis of anti-apoptotic effects and dose-effect relationship of aFGF28-154 on thymocytes induced by DEX (mean±SD)

Groups	Conc. (ng/mL)	Apoptosis (%)
Normal control		1.37±0.39
DEX only		18.99±0.52[a]
DEX plus aFGF28-154	50	16.14±0.44[c]
	100	12.35±0.36[b]
	150	9.75±0.20[b]
	200	11.23±0.71[b]

[a]P<0.05 *vs* normal control; [c]P<0.05, [b]P<0.01 *vs* DEX only.

Gut epithelium apoptosis and treatment effects with both aFGFs in vivo

We investigated the therapeutic use of both aFGFs in gut ischemia-reperfusion injury in rats and found that the apoptosis rate of epithelium entericum was 5.83±1.47 in normal control rats, 46.16±4.06 in rats treated with saline, 39.66±3.56 in rats treated with wild type of aFGF and 40.66±2.73 in rats treated with aFGF28-154 2 h after ischemia-reperfusion injury (Table 2). The quantitative analysis of apoptosis in epithelium entericum in different groups of different time points is summarized in Table 2. The morphological examination with light microscope and immunohistochemical detection and quantification of apoptosis based on the labelling of DNA strand breaks confirmed that tissue edema, local necrosis (Figure 4A, C, E, G) and apoptosis in epithelium entericum were markedly reduced in both aFGFs treated rats (Figure 4B, D, F, H).

Table 2 *In vivo* quantitative analysis of apoptosis in epithelium entericum in different groups (mean±SD)

Time	Normal control (n = 6)	I/R+Saline (n = 36)	I/R+aFGF (n = 36)	I/R+aFGF 28-154 (n = 36)
Before ischemia	5.83±1.47	5.83±1.47	5.83±1.47	5.83±1.47
I/R 30 min		29.83±3.43[b]	23.83±2.78[c]	25.50±1.87[c]
I/R 60 min		39.33±4.32[b]	30.33±3.07[c]	33.44±5.60[c]
I/R 2 h		46.16±4.60[b]	39.66±3.56[c]	40.66±2.73[c]
I/R 6 h		31.33±3.72[b]	25.5±2.16[c]	26.00±2.61[c]
I/R 12 h		62.5±3.08[b]	48.50±2.74[c]	50.16±3.71[c]
I/R24 h		51.66±3.87[b]	41.33±2.94[c]	42.16±3.31[c]

[b]P<0.01 *vs* normal control; [c]P<0.05 *vs* I/R+Saline group. The percentage of positive apoptotic epithelium entericum was estimated after two hundred cells were counted in five different fields randomly chosen from preparation (n = 5). We did all analysis with SPSS. All data were presented as mean±SD. We used one-way analysis of variance and the t test where appropriate. Significance was defined as P<0.05.

Analysis of possible signal transduction pathway

In *in vitro* study, we analysed the changes of ERK1/2, p- ERK1/2, JNK1/2, p38 MAPK, c-Myc and Caspase-3. The results showed that only ERK1/2 pathway was activated in all of the three pathways during aFGF anti-apoptosis course (Figure 5A, B, C, D) and no phosphorylated JNK1/2 and p38 MAPK could be detected. In normal control thymocytes, steady ERK1/2 activation was observed, and such an activation was almost completely blocked when cells exposed to DEX. But both aFGFs attenuated this DEX-induced blockage, maintaining ERK1/2 activation, which is required for normal thymocyte cycle at a certain level (Figure 5A). P38 expression in control group was steady with time lapse (Figure 5B) and decreased gradually when cells exposed to DEX (Figure 5C). However, the total p38 MAPK expression decreased at 30 min and then a peak expression appeared at 60 min when the DEX-induced cells were protected with aFGF or aFGF28-154 (Figure 5D, E). The expression of c-Myc and Caspase-3 in thymocytes in the control group was kept at a stable level following the time course (Figure 5B). When stimulated merely by DEX, c-Myc was highly expressed in the first 30 min and the activated Caspase-3 increased following c-Myc (Figure 5C). These results indicated that DEX- induced thymocyte cell cycle turned out to be c-Myc-induced apoptosis. In the aFGF group, however, c-Myc was expressed only at 0 min, and then showed no significant change at other time points (Figure 5D). In the aFGF28-154 group, c-Myc showed a peak expression at 30 min, and then held at a relatively steady level (Figure 5E).

DISCUSSION

Currently, the anti-apoptotic effects of aFGFs have been established from the results of DNA ladder electrophoresis, Annexin V-FITC/PI staining, examination of Ca^{2+} concentration with confocal microscope, and *in vivo* study on gut ischemia-reperfusion injury in rats. But the results were far beyond our expectation. aFGF, especially the modified non-mitogenic aFGF28-154 with a powerful ability to reduce the apoptosis rate, made us to explore whether there were some special mechanisms involved in such an action.

To further investigate the mechanisms of the anti-apoptotic function of aFGF, we studied three protein kinases, including

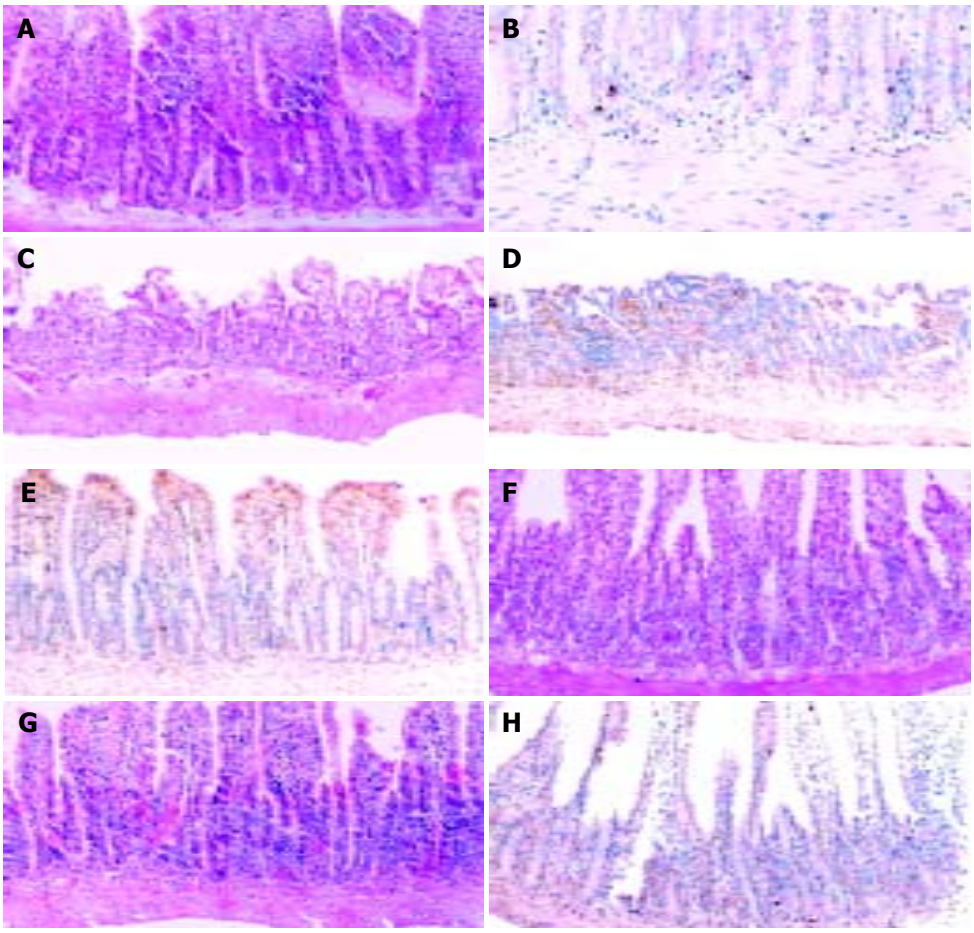

Figure 4 Morphological examination and immunohistochemical detection and quantification of apoptosis based on the labelling of DNA strand breaks (Roche Applied Science, Germany). A: Marked epithelial separation from the basement, subepithelial edema, haemorrhage, erosion. C: Necrosis in I/R plus saline control group. E and G: Tissue damage reduction in both aFGF and aFGF28-154 treated groups. B: Immunohistochemical detection and quantification of apoptosis in normal epithelium entericum. D: Increased number of apoptotic cells in epithelium entericum in rats treated with saline. F and H: Significant reduction of apoptotic cells in epithelium entericum in rats treated with both aFGF and aFGF28-154.

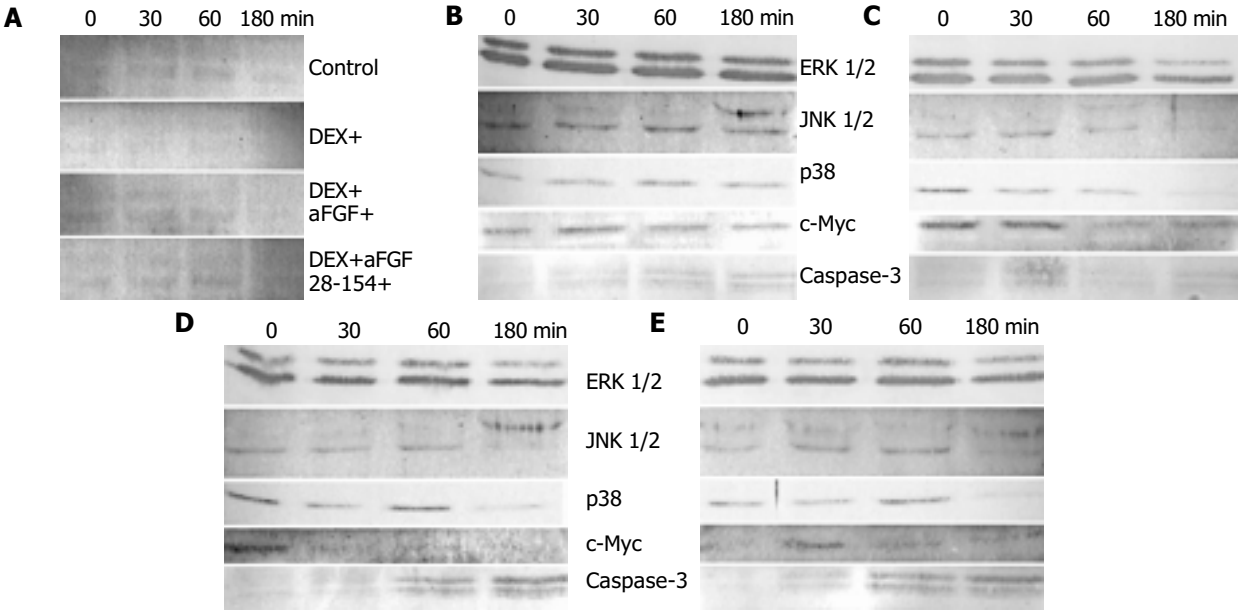

Figure 5 Function of aFGFs as a checker of cell cycle. A: Western blot analysis of the phosphorylation of ERK1/2. B: Western blot analysis of ERK1/2 MAPK, JNK1/2 and p38 MAPK, as well as c-Myc and Caspase-3 in control group. C: Analysis of thymocytes stimulated by DEX. D: Analysis of thymocytes under aFGF protection. E: Analysis of thymocytes under aFGF28-154 protection.

extracellular-signal-regulated kinase (ERK) 1/2, c-Jun-NH2-terminal kinase (JNK) 1/2, and p38 mitogen associated protein kinase (MAPK). According to the Western blot analysis on phosphorylation, we found that only ERK1/2 pathway was activated in all of the three pathways during aFGF anti-apoptosis course (Figure 5A) and no phosphorylated JNK1/2 and p38

MAPK could be detected. In normal control thymocytes, the steady ERK1/2 activation was observed, and such an activation was almost completely blocked when cells exposed to DEX. But both aFGFs attenuated this DEX-induced blockage, maintaining ERK1/2 activation, which is required for normal thymocyte cycle at a certain level (Figure 5A). ERK1/2 activation was crucial for aFGF to exert protective effects on many cell types[20,21]. DEX could induce mitochondrial membrane potential collapse and promote Ca^{2+} release from intracellular pool and the subsequent stimulation of a Ca^{2+} influx from extracellular environment, which contributes to apoptosis[16]. However, an ERK1/2 activation could protect mitochondrial membrane potential by downstreaming Bcl-X activation[22]. The fact that only ERK1/2 pathway was activated in all of the three pathways and Ca^{2+} influx reduction in both aFGF groups strongly suggested that the anti-apoptotic activity of aFGF involved in maintaining ERK1/2 activation and regulation of intracellular Ca^{2+} concentration.

A very interesting finding from the results of total p38 MAPK expression attracted our great attention. p38 expression in control group was steady with time lapse (Figure 5B) and decreased gradually when cells exposed to DEX (Figure 5C). However, the total p38 MAPK expression decreased at 30 min and then a peak expression appeared at 60 min when the DEX-induced cells were protected with aFGF or aFGF28-154 (Figures 5D, E). Normally, the activation of p38 MAPK often follows stress induction[23]. Although p38 MAPK was not markedly activated in all groups in our study, we noticed that according to a previous study by Lasa *et al*, dexamethasone could cause sustained expression of MAPK phosphatase 1 and phosphatase-mediated inhibition of p38 MAPK[24]. We also found p38 MAPK was constantly decomposed by DEX induction (Figure 5C). Because the cells in control group were not stressed and the other 3 groups were all exposed to DEX, we speculated that the peak expression of p38 MAPK at 60 min in both aFGF groups might reflect an unveiled stress, which was lagged in both aFGFs groups compared with the DEX group.

To investigate the anti-apoptotic function of aFGFs at protein level, we further examined the changes of c-Myc and Caspase-3. Thymocytes in control group were kept at a stable c-Myc level following the time course (Figure 5B). When stimulated merely by DEX, c-Myc was highly expressed in the first 30 min and the activated Caspase-3 increased following c-Myc (Figure 5C). These results indicated that DEX- induced thymocyte cell cycle turned out to be c-Myc-induced apoptosis. In aFGF group, however, c-Myc was expressed only at 0 min, and then showed no significant change at other time points (Figure 5D). In aFGF28-154 group, c-Myc showed a peak expression at 30 min, and then held at a relatively steady level (Figure 5E). c-Myc, in association with its partner Max, could function as a transcription factor to drive apoptosis when low quantities of survival factors such as IGF-1 and aFGF were present[25,26]. These results indicated that cell cycle was stagnated in aFGF group, and balanced in aFGF28-154 group. In both aFGF groups, Caspase-3 activation remained nearly null during first 30-60 min and was suddenly enhanced at 60 min and still kept at a high level until 180 min (Figure 5D, E). This activation is cohort with the p38 reaction during stress stimulation and especially with c-Myc expression in normal and both aFGF groups.

The above signal transduction analysis suggested that aFGF might serve as a "checker" for cell cycle while exerting anti-apoptotic function. These functions, including stagnation determination, cell division or apoptosis induction may be similar to those of p53, a typical checker of G1, S and G2/M phases of cell cycle, which contribute both to the cell cycle arrest and apoptotic functions by preventing replication of cells from DNA damage[27-30]. Our results illustrated that when exposed to apoptosis inducers, aFGF would also induce cell cycle arrest. This action was reflected by the stagnation of c-Myc expression in our study (Figures 5D, E). At the time interval of null c-Myc expression, thorough check up and repair might be conducted, thereby, new c-Myc would be expressed by aFGF induction. We regarded that this procedure as a "sentence procedure", reflected by p38 implied second stress, might be carried out by the combined effects of c-Myc and Caspase-3. C-Myc is a typical two-face protein, which is necessary not only for DNA-cleavage in G2 phase of cell cycle, but also for cell proliferation, when cells are protected by trophy factors[31]. If cells can pass through the examination and undergo a thorough "overhaul", they would be kept in normal cell cycle; whereas other non-recoverable cells would be "sentenced to death" with high activation of intracellular procaspase-3 by newly expressed c-Myc. Caspase-3 may finally cleave the damaged DNA and execute apoptosis.

Here we conclude that the anti-apoptotic function of aFGFs was enhanced after cancelling of its mitogenic activity and the anti-apoptotic function did not depend on its mitogenic activity. The action is associated with the protection of intracellular Ca^{2+} concentration balance, ERK1/2 pathway sustaining, and cell cycle balance. During the process of anti-apoptosis, aFGF may play a role as a "checker" for cell cycle. Furthermore, we speculate that there are two possible reasons for the anti-apoptotic function enhancement after aFGF modification. First, aFGF28-154 loses the ability of nuclear translocation, a typical aFGF characteristic[8], causing a longer interaction period with FGF receptors and higher efficiency for aFGF28-154 than the wild one. Second, the structural change of aFGF28-154 may be better exposed with its active domain, which contributes to some anti-apoptosis related activities.

FGF has been widely used in the fields of wound repair and regeneration and some other fields[32,33]. Because of its extensive distribution and broad mitogenic functions, the side-effects or potential tumour induction is of great concern for doctors. In this study, we demonstrate a genetically engineered method to abolish the mitogenic activity and maintain the non-mitogenic activity of aFGF. This may be helpful to reduce people's worry about its tumour induction and to take as many preventive measures as possible clinically. In short, the powerful anti-apoptotic function of aFGF28-154 may play an important role in prophylaxis and treatment of some immune system diseases or as a protector in preventing apoptosis in tissues and organs triggered by ischemia and reperfusion injury.

ACKNOWLEDGEMENTS

We thank Professor Yao-Ying Zeng, Drs. Xiao-Ping Wu, Jing-Xian Zhao, Tong-Zhu Sun, Ping Zhang, Dan Sun and Dan Chen for their critical comment and great technical assistance.

REFERENCES

1 **Galzie Z,** Kinsella AR, Smith JA. Fibroblast growth factors and their receptors. *Biochem Cell Biol* 1997; **75**: 669-685

2 **Nabel EG,** Yang ZY, Plautz G, Forough R, Zhan X, Haudenschild CC, Maciag T, Nabel GJ. Recombinant fibroblast growth factor-1 promotes intimal hyperplasia and angiogenesis in arteries *in vivo*. *Nature* 1993; **362**: 844-846

3 **Nurcombe V,** Ford MD, Wildschut JA, Bartlett PF. Developmental regulation of neural response to FGF-1 and FGF-2 by heparan sulfate proteoglycan. *Science* 1993; **260**: 103-106

4 **Fu X,** Sheng Z, Wang Y, Ye Y, Xu M, Sun T, Zhou B. Basic fibroblast growth factor reduces the gut and liver morphologic and functional injuries after ischemia and reperfusion. *J Trauma* 1997; **42**: 1080-1085

5 **Cuevas P,** Reimers D, Carceller F, Martinez-Coso V, Redondo-Horcajo M, Saenz de Tejada I, Gimenez-Gallego G. Fibroblast growth factor-1 prevents myocardial apoptosis triggered by

ischemia reperfusion injury. *Eur J Med Res* 1997; **2**: 465-468
6 **Cuevas P,** Carceller F, Reimers D, Gimenez-Gallego G. Fibroblast growth factor-1 inhibits medial smooth muscle cells apoptosis after balloon injury. *Neurol Res* 2000; **22**: 185-188
7 **Wan X,** Nass P, Duncan MD, Harmon JW. Acidic fibroblast growth factor overexpression partially protects 3T3 fibroblasts from apoptosis induced by synthetic retinoid CD437. *J Mol Med* 2001; **79**: 143-148
8 **Lozano RM,** Pineda-Lucena A, Gonzalez C, Angeles Jimenez M, Cuevas P, Redondo-Horcajo M, Sanz JM, Rico M, Gimenez-Gallego G. 1H NMR structural characterization of a nonmitogenic, vasodilatory, ischemia-protector and neuromodulatory acidic fibroblast growth factor. *Biochemistry* 2000; **39**: 4982-4993
9 **Yang YH,** Fu XB, Sun TZ, Jiang LX, Gu XM. bFGF and TGFβ expression in rat kidneys after ischemic/reperfusional gut injury and its relationship with tissue repair. *World J Gastroenterol* 2000; **6**: 147-149
10 **Fu XB,** Yang YH, Sun XQ, Sun TZ, Gu XM, Sheng ZY. Protective effects of endogenous basic fibroblast growth factor activated by 2, 3 butanedion moncime on functional changes of ischemic intestine, liver and kidney in rats. *Zhongguo Weizhongbing Jijiu Yixue* 2000; **12**: 69-72
11 **Yang YH,** Fu XB, Sun TZ, Jiang LX, Gu XM. The effect of exogenous basic fibroblast growth factor on hepatic endogenous basic fibroblast growth factor and fibroblast growth factor receptor expression after intestinal ischemia-reperfusion injury. *Zhongguo Weizhongbing Jijiu Yixue* 1999; **11**: 734-736
12 **Fu XB**, Yang YH, Sun TZ, Sun XQ, Gu XM, Chang GY, Sheng ZY. Effects of inhibition or anti-endogenous basic fibroblast growth factor on functional changes in intestine, liver and kidneys in rats after gut ischemia-reperfusion injury. *Zhongguo Weizhongbing Jijiu Yixue* 2000; **12**: 465-468
13 **Sun XQ,** Fu XB, Zhang R, Lü Y, Deng Q, Jiang XG, Sheng ZY. Relationship between plasma D (-)-lactate and intestinal damage after severe injuries in rats. *World J Gastroenterol* 2001; **7**: 555-558
14 **Wyllie AH.** Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 1980; **284**: 555-556
15 Voris **BP**, Young DA. Glucocorticoid-induced proteins in rat thymus cells. *J Biol Chem* 1981; **256**: 11319-11329
16 **McConkey DJ,** Nicotera P, Hartzell P, Bellomo G, Wyllie AH, Orrenius S. Glucocorticoids activate a suicide process in thymocytes through an elevation of cytosolic Ca2+ concentration. *Arch Biochem Biophys* 1989; **269**: 365-370
17 **McConkey DJ,** Zhivotovsky B, Orrenius S. Apoptosis-molecular mechanisms and biomedical implications. *Mol Aspects Med* 1996; **17**: 1-110
18 **Fernandez A,** Kiefer J, Fosdick L McConkey DJ. Oxygen radical production and thiol depletion are required for Ca (2+)-mediated endogenous endonuclease activation in apoptotic thymocytes. *J Immunol* 1995; **155**: 5133-5139
19 **Helmberg A,** Auphan N, Caelles C, Karin M. Glucocorticoid-induced apoptosis of human leukemic cells is caused by the repressive function of the glucocorticoid receptor. *EMBO J* 1995; **14**: 452-460
20 **Udayakumar TS**, Stratton MS, Nagle RB, Bowden GT. Fibroblast growth factor-1 induced promatrilysin expression through the activation of extracellular-regulated kinases and STAT3. *Neoplasia* 2002; **4**: 60-67
21 **Buehler A,** Martire A, Strohm C, Wolfram S, Fernandez B, Palmen M, Wehrens XH, Doevendans PA, Franz WM, Schaper W, Zimmermann R. Angiogenesis-independent cardioprotection in FGF-1 transgenic mice. *Cardiovasc Res* 2002; **55**: 768-777
22 **Boucher MJ,** Morisset J, Vachon PH, Reed JC, Laine J, Rivard N. MEK/ERK signaling pathway regulates the expression of Bcl-2, Bcl-X (L), and Mcl-1 and promotes survival of human pancreatic cancer cells. *J Cell Biochem* 2000; **79**: 355-369
23 **Min do S,** Shin EY, Kim EG. The p38 mitogen-activated protein kinase is involved in stress-induced phospholipase D activation in vascular smooth muscle cells. *Exp Mol Med* 2002; **34**: 38-46
24 **Lasa M,** Abraham SM, Boucheron C, Saklatvala J, Clark AR. Dexamethasone causes sustained expression of mitogen-activated protein kinase (MAPK) phosphatase 1 and phosphatase-mediated inhibition of MAPK p38. *Mol Cell Biol* 2002; **22**: 7802-7811
25 **Harrington EA,** Bennett MR, Fanidi A, Evan GI. c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. *EMBO J* 1994; **13**: 3286-3295
26 **Amati B,** Littlewood TD, Evan GI, Land H. The c-Myc protein induces cell cycle progression and apoptosis through dimerization with Max. *EMBO J* 1993; **12**: 5083-5087
27 **Green DR.** A Myc-induced apoptosis pathway surfaces. *Science* 1997; **278**: 1246-1247
28 **Winters ZE.** P53 pathways involving G2 checkpoint regulators and the role of their subcellular localisation. *J R Coll Surg Edinb* 2002; **47**: 591-598
29 **Adachi S,** Obaya AJ, Han Z, Ramos-Desimone N, Wyche JH, Sedivy JM. c-Myc is necessary for DNA damage-induced apoptosis in the G (2) phase of the cell cycle. *Mol Cell Biol* 2001; **21**: 4929-4937
30 **Young M,** Oger J, Blanchard MH, Asdourian H, Amos H, Arnason BG. Secretion of a nerve growth factor by primary chick fibroblast cultures. *Science* 1975; **187**: 361-362
31 **Kumagai A,** Dunphy WG. Repeated phosphopeptide motifs in Claspin mediate the regulated binding of Chk1. *Nat Cell Biol* 2003; **5**: 161-165
32 **Fu X,** Sun X, Li X, Sheng Z. Dedifferentiation of epidermal cells to stem cells *in vivo*. *Lancet* 2001; **358**: 1067-1068
33 **Fu X,** Shen Z, Cheng Y, Xe J, Guo Z, Zhang M, Sheng Z. Randomised placebo-controlled trial of use of topical recombinant bovine basic fibroblast growth factor for second-degree burns. *Lancet* 1998; **352**: 1661-1664

Edited by Wang XL **Proofread by** Zhu LH and Xu FM