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Original Article

Keratinocyte growth factor up-regulates Interleukin-7 expression following intestinal ischemia/reperfusion *in vitro* and *in vivo*

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Abstract: Background: Epithelial cell (EC)-derived Interleukin-7 (IL-7) plays a crucial role in control of neighboring intestinal intraepithelial lymphocytes (IEL) development and homeostasis, and IEL derived keratinocyte growth factor (KGF) promotes intestinal epithelial growth, which was regulated by EC-derived IL-7. On this basis, we hypothesize that there is a crosstalk between IELs and ECs, and KGF could regulate the EC-derived IL-7 expression, which should be associated with the protective effects by KGF on intestinal injury. Methods: Histological evaluation was performed in small intestine tissues of patients with intestinal obstruction and IL-7 expression was detected by immunofluorescence. Intestinal epithelial cells (LoVo) and adult C57BL/6J mice undergoing ischemia/reperfusion injury were treated with recombinant KGF. KGF, KGF receptor(KGFR) and IL-7 expressions were measured with western blot and immunofluorescence analysis. Results: IL-7 expression increased in the mild ischemia while decreased in severe ischemia small intestinal tissues of patients with intestinal obstruction. KGF expression significantly decreased while IL-7 expression increased early after acute intestinal I/R administration in a mouse model. KGF treatment significantly increased the IL-7 expression both *in vitro* and *in vivo*, while when the KGFR was blocked, the findings above were absent. In addition, our results showed changes of IL-7 expression at different stages after acute intestinal I/R administration, KGF treatment significantly attenuated the decreasing of IL-7 expression caused by acute intestinal I/R. Conclusion: KGF could up-regulate the IL-7 expression both *in vitro* and *in vivo* through KGFR pathway, which should have associated with the protective effects of KGF in intestinal injury.

Keywords: Keratinocyte growth factor, interleukin-7, ischemia/reperfusion, mouse, epithelial cells

Introduction

Intestinal ischemia/reperfusion (I/R) is encountered in a variety of clinical conditions, such as strangulation-obstruction of the intestine, sepsis, vascular surgery, hemorrhagic shock, small bowel transplantation, cardiopulmonary bypass, and abdominal aortic surgery. Severe intestinal ischemia induces the desquamation of the intestinal epithelium, increases the intestinal permeability, and in patients often causes fatal conditions including sepsis and multiple organ failure [1-5]. Thus, injury from intestinal ischemia/reperfusion (I/R) may cause a local and systemic inflammatory response [6, 7].

It is believed that KGF plays a critical role in intestinal epithelial growth and maintenance. Keratinocyte growth factor (KGF) which is expressed in γδ- TCR+-IEL (intraepithelial lymphocytes, IELs) has been shown to stimulate proliferation of a variety of epithelial cell (EC) lines [8, 9]. KGF receptors (KGFR) have been detected in high numbers in the gastrointestinal tract, indicating that gut can both synthesize and respond to KGF [10-12]. A marked increase in the proliferation of ECs from the foregut to the colon was found in rats with recombinant KGF treatment [12]. A study from our group found that recombinant KGF (rHuKGF) administration significantly improved the epithelial barrier function in a mouse model of

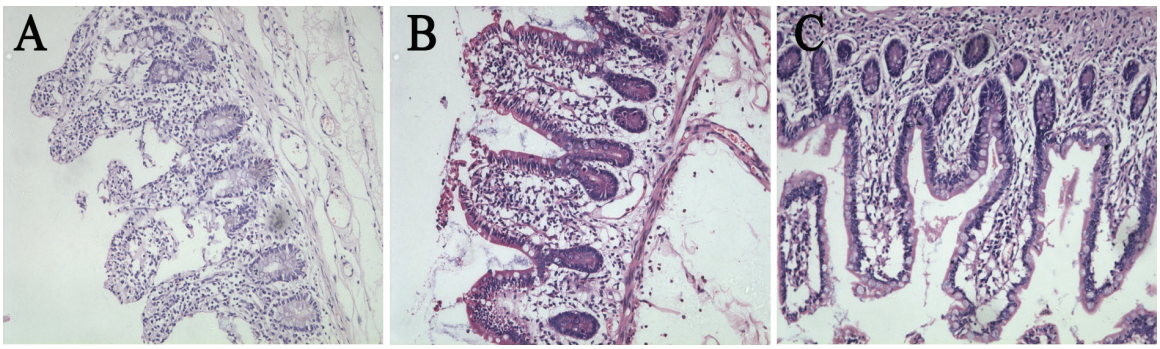

Figure 1. Histology changes in small intestine tissues of patients. More severe mucosal injury was observed in severe ischemia group (A) than mild ischemia group (B), while none in normal tissues (C); Original magnification × 200.

total parenteral nutrition (TPN) [13]. We also found that IEL-derived KGF was significantly increased in a model of villus hypertrophy by creating a short bowel syndrome (SBS) [14]. Resent studies found that administration of KGF (rHuKGF) could improve body weight loss, diarrhea, survival, histopathology and hematochezia in a dextran sodium sulfate-induced inflammatory bowel disease mouse model [15, 16]. KGF null mice are more susceptible to intestinal injury induced by dextran sulfate than their wild-type counterparts [17]. The intestinal damage is worsening and the healing is delayed in the absence of KGF [17]. All these results suggest that KGF plays a critical role in intestinal epithelial growth and maintenance. However, the mechanism involved in beneficial effects of KGF remains still unclear.

In the intestine, IL-7 is produced by intestinal EC, and in turn IL-7 receptors (IL-7R) have been detected on the IEL [18]. Recently, our findings and study of Watanabe M et al show that the administration of exogenous IL-7 significantly affected the IEL phenotype and led to alteration of IEL function, and also an increase in the IEL number [19, 20]. Furthermore, we found that EC-derived IL-7 over-expression was found a critical role in the phenotype and function of IEL and γδ-TCR+ IEL-derived KGF was noted to be significantly higher in an intestinal specific IL-7 transgenic mouse model [21]. Moreover, after bone marrow transplantation (BMT), KGF could lead to increased IL-7 production [22], and the protective effects of pre-BMT were improved by KGF administration on thymopoiesis [22].

These findings suggest that KGF and IL-7 are both important in intestinal epithelial growth and maintenance. Such studies, however, may not reveal the mechanism involved in the regulation of EC-derived IL-7. In this study, we hypothesized that there is a crosstalk between IEL and EC through the regulation between KGF and IL-7. KGF administration would influence the EC-derived IL-7 expression, which should be associated with the beneficial function of KGF on intestinal injury.

Materials and methods

Cell culture

Human intestinal epithelial LoVo Cells (ATCC CCL-229) were used in our experiments. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, 50 units/ml penicillin, and 50mg/ml streptomycin, refreshed every 48 h, and subcultured serially when 80% confluent. Cells were seeded at identical cell densities and were typically used 12–15 days after reaching confluence.

Cell treatments

Recombinant human KGF (rHuKGF) (Chongqing Zihe Pharm-Tec Co. Ltd) was used for treatment for cells and mice. Cells were grown on 6-well plates and incubated with KGF (0, 20, 40, 80, 100, 150 ng/ml respectively) for 48h. Then total protein extracts of cells were detected by Western blotting.

For the blockage of KGFR, cells were incubated with a specific FGFR antibody (R & D, Systems,

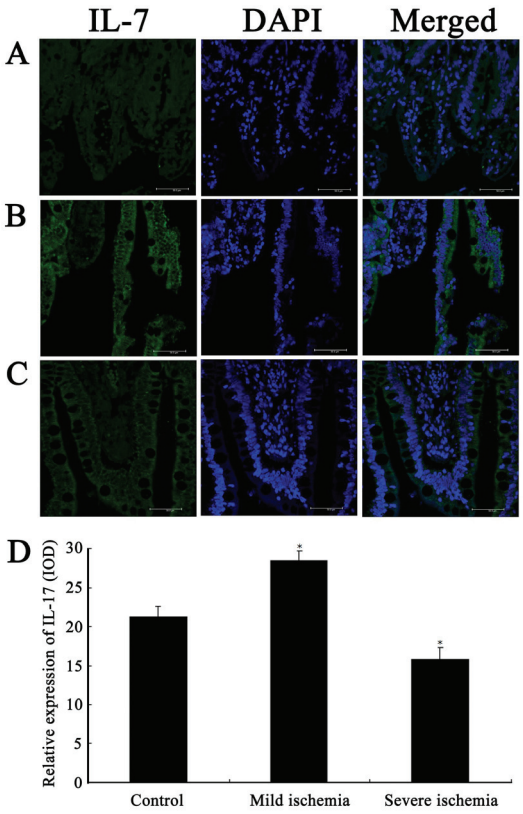

Figure 2. Alterations in IL-7 immunofluorenscence staining of small intestine tissues of patients. IL-7 expression was significantly decreased in severe ischemia small intestinal tissues (A), while increased in mild ischemia small intestinal tissues (B), as compared with normal tissues (C); (D) Arithmetic means ± SEM of IOD of IL-7 immunofluorenscence staining in small intestinal tissues of patients, * $P < 0.05$, between ischemia injury group and normal.

Inc.) for 2 h before incubation with KGF diluted in presence of the antibody. KGFR expression was silenced by using interfering RNA and the plasmids 335, 336 and 337 (Shanghai SunBio Medical Biotechnology Co., Ltd) were transfected into LoVo cells for silencing KGFR with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) following the manufacturer's instructions as previously.

Animals

The animal study was strictly based on the guidelines for the care and use of laboratory animals established by the University Committee on Use and Care of Animals at the Third Military Medical University and protocols were approved by the committee. 6-8 week-old, specific pathogen-free, male, C57Bl/6 mice weighing 20±3g were purchased from Laboratory Animal Center, Third Military Medical University, Chongqing, P.R. China, maintained in temperature, humidity, and light-controlled conditions, and then randomized into sham or I/R group. Recombinant human KGF (rHuKGF) administration to mice was given by daily intraperitoneal injection (5 mg/kg/day) for five days before the operation.

Intestinal ischemia-reperfusion model

After anesthesia (pentobarbital; 40 mg/kg; i.p.), laparotomy was performed at the midline, and superior mesenteric artery (SMA) was transiently occluded for 20 min using non-traumatic vascular clamps, with subsequent reperfusion for 0h, 6h, 24h, 72h as four subgroups. Animals underwent an identical procedure without SMA occlusion as the sham subgroup. Mice were allowed free access to water and liquid diet postoperatively. There was no significant difference in survival between treatment and control groups. Mice received above surgery were further assigned into two groups: KGF group and control group, in which rHuKGF and equal volume of normal saline were given by daily intraperitoneal injection (5 mg/kg/day) for five days before the operation, respectively. In all experiments, at least six animals were analyzed per subgroup.

Patients and histology

A total of 37 patients with intestinal obstruction undergoing small intestinal resection due to wall necrosis were enrolled. Our study was approved by the Clinical Ethical Committee of Xinqiao Hospital of Third Military Medical University. All subjects gave their written informed consent before biopsy sampling. Segments of small intestinal tissues of patients with intestinal obstruction were harvested fixed with 4% paraformaldehyde and used for histology studies. Tissues were then dehydrated with ethanol and embedded in paraffin. Sections were cut and stained with hematoxylin-eosin(H&E). Histological changes were assessed, and mucosal lesions were examined by two independent pathologists and classified according to the method developed by Chiu on the basis of hematoxylin-eosin staining.

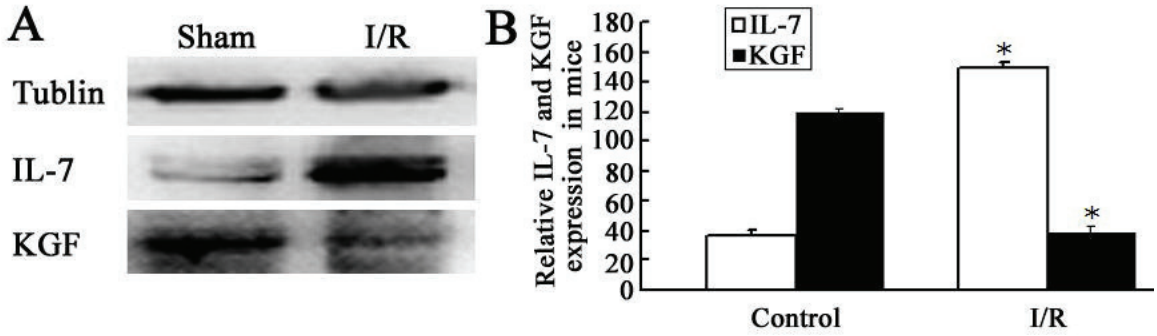

Figure 3. Changes of IL-7 and KGF expression in a mouse model of acute intestinal I/R.(A) Decreased expression of KGF and increased expression of IL-7 were confirmed by western blot in mice early after acute intestinal I/R, compared with sham. Tublin was used as an internal control. (B) Spearman rank correlation of KGF and IL-7 protein levels with acute intestinal I/R in mice. *indicates significant difference between I/R administration group and sham ($P < 0.05$), n = 6 per group.

Immunoblot

Proteins were extracted from treated LoVo cell lines or intestinal samples of mice and sonication with an ultrasonic probe, followed by centrifugation at 10,000 g for 10 minutes at 4°C. The supernatant was collected for Western blotting. Samples containing 30 μg of extracted protein, as determined by the Bradford protein assay (BioRad, Hemel Hempstead Hertfordshire, UK), were loaded on a 10% SDS polyacrylamide gel for protein fractionation by electrophoresis and then electro-transferred to a Polyvinylidene fluoride (PVDF, Milipore) membrane. Blots were blocked with 5% non-fat dry milk in TBS (pH8.0, containing 0.1% Tween-20), and probed with appropriate antibodies anti-KGF (catalogue no.bs-0734R; Beijing Biosynthesis Biotechnology Co.,Ltd.), anti-IL-7(catalogue no.ab-9628; Abcam Inc.), anti-KGFR (catalogue no.MAB-684, R & D; Systems, Inc.). After being washed, the membrane was incubated with HRP-conjugated secondary antibodies (Cell Signaling) and then visualized with enhanced chemiluminescence (Cell Signaling). β-tubulin (Sigma, Dorset, UK) was used as internal control. Densitometry analysis were preformed and normalized with β-tubulin and then presented as percentage of control.

Immunofluorescence

Sections of tissues were fixed for staining experiments. Sections were incubated with anti-IL-7 rabbit polyclonal antibody (catalogue no.bs-1811R; Beijing Biosynthesis Biotechnology Co., Ltd.) overnight at 4°C, then stained with FITC-conjugated goat anti-rabbit IgG. Nuclear staining for total cell counting was performed by 5 min addition of 1 mg/ml of DAPI (40, 60-diamidino-2-phenylindole) and the fluorescence signals were analyzed by recording and merging single-stained images, using confocal laser microscope (Leica TCS SP2). Images were processed by using Adobe Photoshop (Adobe Systems, San Jose, Calif., USA) and were analyzed by Leica's software system.

Statistical analysis

SPSS statistical package was used for data analysis. Data are expressed as mean ± SD. Results were analyzed by using ANOVA. Statistical significance was defined as $P < 0.05$.

Results

Histology changes

All of the 37 patients with intestinal obstruction underwent surgery of small intestinal resection due to wall necrosis, which are the conditions of intestinal I/R. Histological evaluation of patients with intestinal obstruction was performed according to the Chiu scoring method. Data related to scoring obtained by means of H&E staining as well as the microphotographs. In the group of small intestinal tissues with severe ischemia, denuded villi, disintegration of the lamina propria, and exposed capillaries were apparent (**Figure 1A**), while mild ischemia small intestinal tissues exhibited only capillary

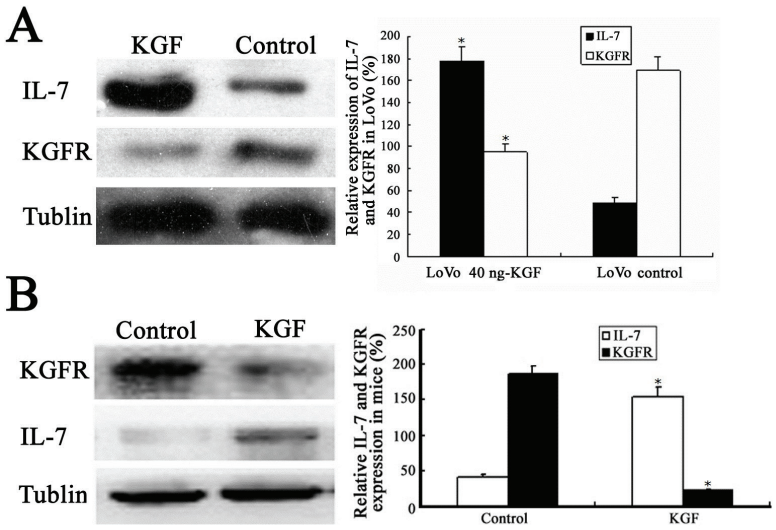

Figure 4. KGF administration regulates IL-7 and KGFR expression both in vivo and in vitro.(A)Increased expression of IL-7 and decreased expression of KGFR were confirmed by western blot in LoVo cells with KGF (40ng/ml) treatment. Tublin was used as internal control. * P < 0.05, between KGF treated group and control. (B) Increased expression of IL-7 and decreased expression of KGFR were confirmed by western blot in mice with KGF (5 mg/kg/ day×5d) treatment. Tublin was used as an internal control. *indicates significant difference between KGF treated group and control (P < 0.05).

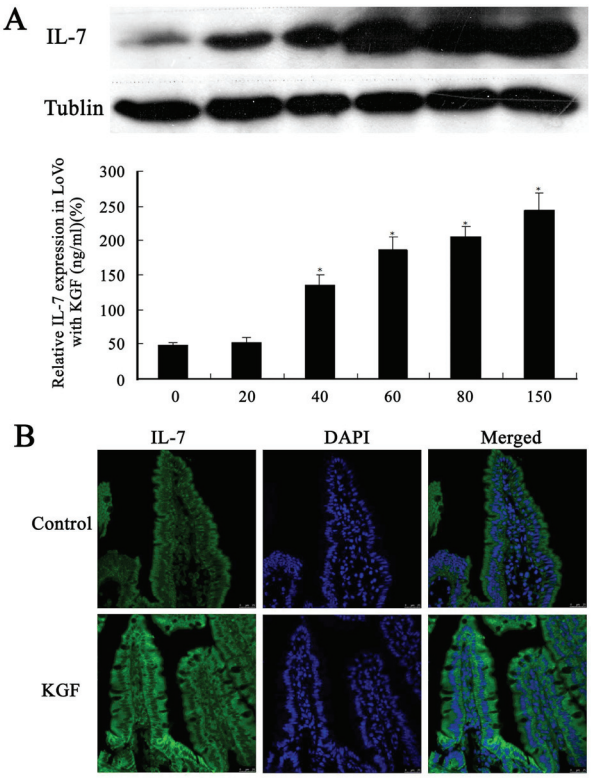

Figure 5. KGF administration results in an increased IL-7 expression both in vivo and in vitro.(A) Dose-dependent increased expression of IL-7 was confirmed by western blot in LoVo cells with KGF treatment. Tublin was used as an internal control. IL-7 expression increased nearly 4-folds when the concentration of KGF was at 150 ng/ml. *indicates significant difference between KGF treated group and control (P < 0.05). (B) Increased expression of IL-7 was confirmed by immunofluorenscence staining in mice with KGF treatment. * P < 0.05, between KGF treated group and control.

congestion and mild epithelial lifting from the lamina propria (**Figure 1B**). According to the Chiu scoring system, the injury in severe ischemia group and mild ischemia group were presented to grade 4 and grade 2 respectively. No mucosal injury was observed in the normal tissues, which exhibited normal mucosal architecture with intact villi and presented to grade 0(**Figure 1C**).

Changes of IL-7 expression in patients with intestinal obstruction

Immunofluorenscence staining was performed to detect the IL-7 expression in the small intestinal tissues with severe ischemia (**Figure 2A**), mild ischemia (**Figure 2B**) and normal tissues (**Figure 2C**) of patients with intestinal obstruction. Results showed that IL-7 expression was significantly increased (28.5±1.2 IOD) in mild ischemia intestinal tissues, decreased (15.9±1.4 IOD) in small intestinal tissues with severe ischemia, as compared with normal tissues (control) (21.2±1.4 IOD) ($p<0.05$) (**Figure 2D**).

Changes of IL-7 and KGF expression in mice

Western blotting was used to detect the KGF and IL-7 expression in a mouse model of intestinal I/R, and results showed that KGF expression decreased (**Figure 3A**) nearly two-thirds (118.5±3.4% in Control vs. 38.7±4.8%, $p<0.05$)

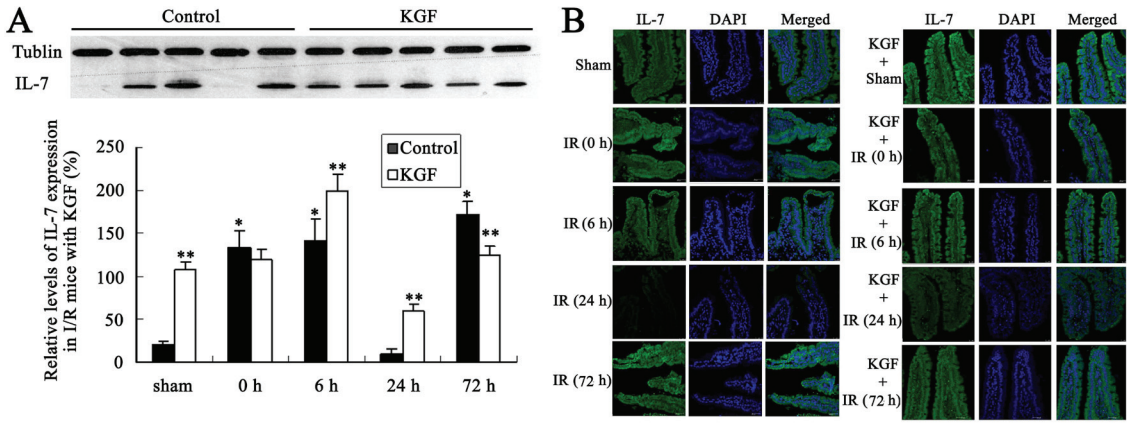

Figure 6. Changes of IL-7 expression in response to KGF treatment in a mouse model of ischemia-reperfusion injury. (A) Changes of IL-7 expression were confirmed by western blot in acute intestinal I/R mice with KGF (5 mg/kg/day×5d) treatment. Increased IL-7 expression was highest at 6h after I/R administration with KGF treatment, while decreased the lowest at 24h after I/R administration without treatment. * $P < 0.05$, between I/R administration group and sham, ** $P < 0.05$, between KGF treated group and untreated (control).(B)Confocal laser scanning microscopic analysis of IL-7 in mice intestinal paraffin sections stained with immunofluorenscence, showing specific FITC-related fluorescence in the cytoplasm. Visualization of the nuclei was evident by DAPI staining. At different time points (0, 6h, 24h and 48h after intestinal I/R administration), changes of IL-7 expression in mice were observed, with or without KGF treatment.

(**Figure 3B**) while IL-7 expression increased (**Figure 3A**) about 4-folds (36.5±3.9% in Control vs. 149.1±3.6%, $p<0.05$) (**Figure 3B**) early after I/R when compared with the sham group. These findings suggest that the changes of KGF and IL-7 expression are involved in intestinal mucosal injury after intestinal I/R.

KGF administration results in an increase expression of EC -derived IL-7 both in vivo and in vitro

Our previous study showed that the IEL derived KGF expression was increased in a mouse model with intestinal specific over-expression of IL-7, which suggests there is a crosstalk between EC and IEL, since the EC derived IL-7 affects the neighboring IEL derived KGF expression [21]. Hence, we hypothesized that recombinant KGF could alter the IL-7 expression in IECs. IL-7 expressions were studied in LoVo cells and intestinal mucosa in mice by Western blot. Our results showed that IL-7 expressions were found in LoVo cells and intestinal mucosa in mice (49.0±5.4%, 40.7±3.2%, respectively) (**Figure 4A** and **4B**).

1) KGF treatment results in an increase expression of EC-derived IL-7 in vitro: Results showed that KGF treatments of different concentrations (20, 40, 80, 100 and 150 ng/ml) for 48 h in the LoVo cells resulted in an increased IL-7 expression nearly 4-folds when the concentration of KGF was at 150 ng/ml (244.4±24.6%, compared with control 49.2±3.9%, $p<0.05$), showing a dose-dependent manner (**Figure 5A**).

2) KGF administration results in an increase expression of EC-derived IL-7 in a health mouse model: Western blot assay showed the IL-7 expression in the intestinal mucosa was dramatically increased nearly 4-folds (40.7±3.2% in Control vs. 153.8±14.7% KGF treated group $P<0.05$) following injection of rhKGF in normal mice (**Figure 4B**), which was also found in the results from immunofluorescence staining (**Figure 5B**).

3) Changes of IL-7 expression in response to KGF treatment in mice with ischemia-reperfusion injury: To further explore the changes of IL-7 expression in an intestinal ischemia/reperfusion mouse model at different time points, IL-7 expression was detected by using immunohistochemistry and Western blot. Results showed intestinal ischemia/reperfusion caused significant changes of IL-7 expression at different time points. Immediately and 6h after I/R administration, the IL-7 expression was elevated 133.2±20.2% immediately after I/R administration, and reached 141.5±24.9%

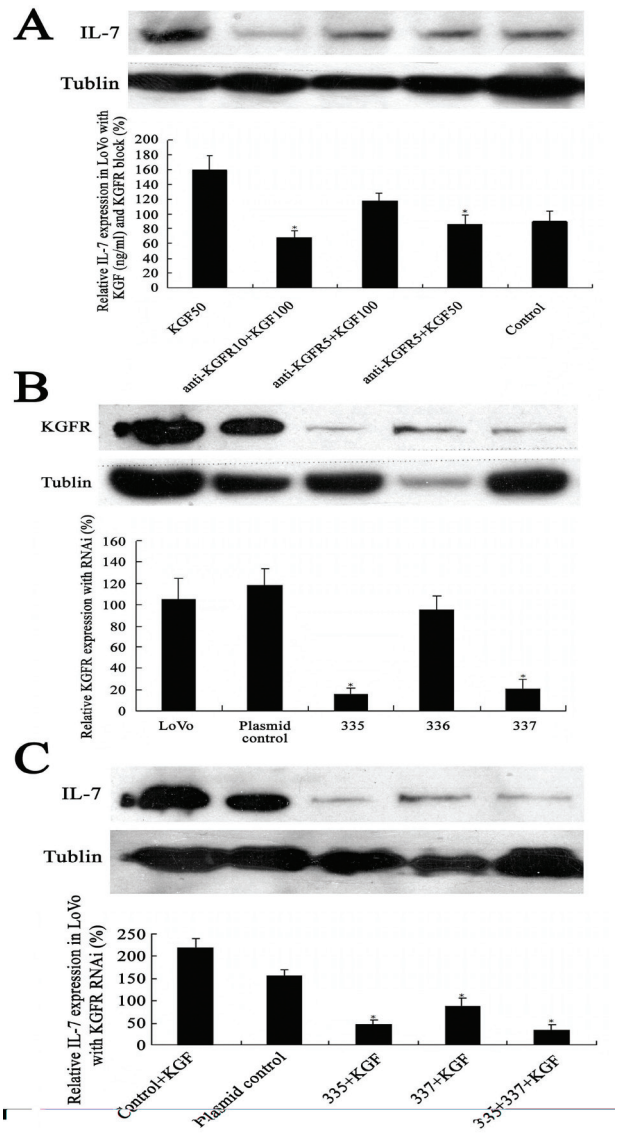

Figure 7. IL-7 is up-regulated by KGF through KGFR pathway. Tublin was used as an internal control. (A)Decreased expression of IL-7 was confirmed by western blot in LoVo cells after KGFR blockade with KGF treatment. Suppression of IL-7 expression was observed with dose dependent of KGFR antibody blockage (5μg/μl and10μg/μl) following KGF (50ng/ml and 100ng/ml) treatment. * P < 0.05, between KGFR blockade following KGF treated group and KGF (50ng/ml) treated group. (B) Reduced expression of KGFR was confirmed by western blot in LoVo cells following KGFR RNA interference. Plasmids 335, 336 and 337 were transfected into LoVo cells and KGFR expression was detected. Plasmid 335 and 337 can definitely inhibit the KGFR expression. Expressions of Tublin and KGFR in LoVo cells transfected with plasmid 336 were both very low, which suggested the plasmid 336 treated cells were unqualified for experiment. * P < 0.05, between KGFR RNA interference group and plasmid control. (C)Reduced expression of IL-7 was confirmed by western blot in LoVo cells following KGFR RNA interference. Decreased expressions of IL-7 were observed in LoVo cells following KGF treatment in response to RNA interference of KGFR by plasmid 335, plasmid 337 and plasmid 335+337. * P < 0.05, between plasmid+KGF group and control+KGF.

6 h after I/R administration, respectively, while significantly decreased to 8.8±6.3% at 24h and subsequent again, IL-7 expression increased to 171.9±15.6% at 72h, when compared with sham (19.5±5.1%), showed special changes of IL-7 expression at different time points after acute intestinal I/R administration. Moreover, KGF treatment further up-regulated IL-7 expression 106.7±9.3%, 199.8±21.3% and 59.8±7.9%, when compared with control groups in sham, at 6 and 24h after injury ($p<0.05$), while there was no significant IL-7 expression increasing immediately and 72h after injury (**Figure 6A**).

Immunofluorescence staining was employed to detect the location of IL-7 expression, and results also showed that the IL-7 expression changed at different time points in both controls and KGF treatment groups (**Figure 6B**), correlated well with the results of Western blot.

IL-7 is up-regulated by KGF through KGFR pathway

1) KGFR expression is found both in LoVo cells and intestinal mucosa in mice: Former study had shown that KGF treatment caused an increased production of intrathymic IL-7, signaling via KGFR pathway [22]. To confirm this result in intestine, KGFR expressions were studied in LoVo cells and intestinal mucosa in mice by Western blot. Our results showed KGFR expressions were found in LoVo cells 169.7±8.3% and intestinal mucosa in mice 186.2±11.2% respectively, while following KGF treatment, the KGFR expression was reduced in LoVo cells (94.4±5.2%) and intestinal mucosa (21.8±1.2%) in mice (**Figure 4A** and **4B**).

2) Changes of IL-7 expression after KGFR blockade : In this study, we found that recombinant KGF led to increased IL-7 expression, and KGFR expression was also found in both cell lines and

intestinal mucosa. We speculated the interaction between KGF and KGFR on the intestinal epithelial cells could initiate downstream signaling pathway resulting in the regulation of IL-7 expression. To confirm this hypothesis, the KGFR was neutralized with KGFR antibody and then exogenous KGF was used to stimulate LoVo cells. Results showed the suppression of IL-7 expression with dose dependent of KGFR antibody blockage (5 μg/μl and10 μg/μl) following KGF (50 ng/ml and 100ng/ml) treatment. The expression of IL-7 is 67.9±9.4% when KGFR antibody was given at 10 μg/μl, following KGF (100 ng/ml) treatment and IL-7 expression is 85.7±12.9% when KGFR antibody was given at 5 μg/μl, following KGF (50ng/ml) treatment respectively, which were both significantly different from that without KGFR blockage (159.2±20.3%, $p<0.05$), and following KGF (50ng/ml) treatment only (**Figure 7A**). This finding suggests that exogenous KGF can stimulate IL-7 expression in the LoVo cells which is mediated by the interaction between KGF and KGFR in IECs.

3) Changes of IL-7 expression after KGFR expression was silenced by using interfering RNA: To further confirm the pathway of KGF through KGFR to regulate IL-7 expression, KGFR expression was silenced by using interfering RNA, and then the effect of recombinant KGF on the IL-7 expression was investigated in the LoVo cells. The plasmids 335, 336 and 337 were transfected into LoVo cells and the KGFR expression was detected. Results showed the KGFR expression was dramatically reduced, (16.4±5.2% treated with 335 plasmid, 21.6±8.1% treated with 337 plasmid), compared to controls (118.4±15.6%, $p<0.05$) in the LoVo cells (**Figure 7B**). Following KGFR silencing by plasmids 335 and 337, LoVo cells were treated with 100 ng/ml KGF for 48 h, and significant reduction of IL-7 expression was noted (48.0±6.3% treated with 337 plasmid, 88.7±19.2% treated with 335 plasmid, 34.3±10.1% treated with 335 and 337 plasmid) compared to controls (218.8±20.8%, $p<0.05$) (**Figure 7C**). These results showed transfection of plasmid 335, plasmid 337 and plasmid 335+337 could result in obvious suppression of KGFR expression so that decreased IL-7 expression was observed in LoVo cells following KGF treatment. However, transfection of control plasmid had no influence on the expression of IL-7. These findings further confirm that KGF can regulate the IL-7 expression through interacting with KGFR in IECs.

Discussion

In this study, we found that IL-7 expression increased in the mild ischemia while decreased in severe ischemia small intestinal tissues of patients with intestinal obstruction. KGF expression significantly decreased while IL-7 expression increased in mice early after acute intestinal I/R. IL-7 and KGFR were both expressed in the intestinal epithelial cells, and KGF could up-regulate the IL-7 expression, which were confirmed in the intestinal mucosa among different time points after intestinal I/R and this up-regulation through interacting with KGFR.

IL-7 is produced by thymic epithelial cell and intestinal epithelial cell (EC) [18, 19]. Several studies have indicated that EC may play an important role in mucosal immune responses by helping to regulate intestinal intraepithelial lymphocytes (IEL) [23, 24]. The profound effects of IL-7 on developing and mature lymphocytes were implicated by recent study, showing the regulations by IL-7 not only in systemic but also in local immune [25]. IL-7 can promote growth and differentiation of many T cell phenotypes [26]. It is also essential for the differentiation of pre-T cells into mature thymocytes [27], which cannot be performed by any other known cytokine [27]. The absence of IL-7 results in greatly reduction of the lifespan of naive T cells and completely abolishing homeostatic proliferation of naive T-cells [28]. In vivo, administration of IL-7 has been demonstrated to enhance IEL functional capacity and population [29], and specific intestinal IL-7 overexpression significantly attenuated many IEL changes in phenotype and function after TPN administration [30]. Geiselhart et al. [18] have reported that IL-7 administration results in an increase in peripheral T cell numbers and altered function. A stimulation of lamina propria lymphocytes in exogenous IL-7 treated mice was observed [19]. All these data suggest that IL-7 may be essential for the ongoing maintenance of the IEL growth and function. However, the mechanisms of IL-7 production have remained unclear.

KGF is known as a mitogenic growth factor. It has been found to be expressed in the mucosal layer by T-cell receptor γδ+IEL [9]. KGFR is ubiquitously expressed in the mucosal epithelium of all gastrointestinal tract segments, and its baso-lateral position allows the receptor to lie in close proximity to IEL [31]. KGF plays an important role in the intestinal epithelial growth and maintenance. The mechanisms of KGF-induced gut growth are unknown. The intestinal epithelial cell proliferation is induced by KGF, which supports the hypothesis that KGFR signaling is crucial for the mitogenic stimulus correlated to gut adaptation. Studies show KGFR signaling is capable of activating several pathways that regulate cell proliferation and survival [32, 33]. KGF can expand thymic epithelium cells (TECs) [22, 34-36] and has been reported to increase IL-7 production in treated mice [22], and also potently augments thymopoiesis and protects from thymic damage [22, 37] by signaling via FGFR2IIIb [22, 39-40]. In this study, we found that IL-7 and KGFR were both expressed in the intestinal epithelial cells (IECs), and recombinant KGF could up regulate the IL-7 expression both *in vivo* and *in vitro*. When the KGFR was blocked, the above findings were absent. All these results suggest that KGF could up-regulate the IL-7 expression through interacting with KGFR pathway in IECs. Recent studies have demonstrated that the interactions between intestinal EC and mucosal lymphocytes are crucial in regulating maintenance intestinal function and immune response [19,41]. And these results were confirmed by our study.

In the present study, our results exhibited IL-7 expression changes response to the acute intestinal injury in whole 72h by I/R administration. Immediately and 6h after I/R administration, the IL-7 expression was elevated, while significantly decreased at 24h and subsequent again IL-7 expression increased at 72h, showed special changes of IL-7 expression at different stages after acute intestinal I/R administration. We also found that IL-7 expression was increased in the mild ischemia tissues, decreased in severe ischemia small intestinal tissues in human. No other studies about IL-7 expression in acute intestinal injury were available; Thiant et al found that IL-7 levels peaked at four- to fivefold over pre-conditioning values, on the occurrence of acute GVHD after reduced intensity conditioning (RIC) transplantation [42]. To evaluate changes in urinary chemokine / cytokine expression levels in dogs treated with cisplatin resulted in renal injury, increased IL-7 was observed on day 4 in a 28-day study [43]. Thus we hypothesis the pattern of IL-7 expression in the acute intestinal injury should be associated with injury of intestinal tissues and stage after acute intestinal I/R administration. Moreover, KGF treatment further up-regulated IL-7 expression in sham, at 6 and 24h after injury, while reduced by I/R administration. These results revealed that KGF could regulate IL-7 expression in vitro, in a health mouse model and an intestinal I/R administration mouse model. No difference in cellularity and thymic size was observed between KGF and PBS-treated IL-7$^{-/-}$-recipients of either congenic or allogeneic BMT [22]. And IL-7$^{-/-}$ recipients with KGF treatment have not increased thymopoiesis, showed a potential mechanism that increased intrathymic production of IL-7 by TECs was associated with the action of KGF in post-BMT immune reconstitution [22]. Our previous work also showed that TPN resulted in a loss of intestinal epithelial cell-derived IL-7, which is important in development of such IEL changes associated with TPN [21]. Fukatsu et al found that intestinal I/R changed in lymphocyte phenotypes, and also significantly reduced lymphocyte numbers in the lamina propria (LP), and the intraepithelial space [44]. In this study, we also observed decreased IL-7 expression in an intestinal I/R mouse model, which should be associated with IEL changes. These results may suggest that increased IL-7 expression regulated by KGF could improve the IEL functions may be associated with the beneficial effect of KGF on intestinal mucosa injury after acute intestinal I/R administration.

The regular balance between KGF and IL-7 in intestine has remained undetermined; however, our previous and present work may provide evidences that there is an interaction between KGF and IL-7 through crosstalk between IEL and IEC. Following KGF treatment, the KGFR expression significantly decreased, which suggests that there is the intrinsic feedback mechanism to regulate IL-7 and KGF expression.

In conclusion, this study demonstrates that KGF and IL-7 are both involved in the acute intestinal mucosal injury. KGF can up-regulate

the IL-7 expression both in health and acute I/R intestinal injury mouse model through KGFR pathway, which should be associated with the protective effects of KGF in intestinal injury. The findings further confirm the crosstalk between IELs and ECs.

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