

# Keratinocyte Growth Factor Improves Epithelial Structure and Function in a Mouse Model of Intestinal Ischemia/Reperfusion

Yujiao Cai<sup>1</sup>, Wensheng Wang<sup>1</sup>, Hongying Liang<sup>1</sup>, Lihua Sun<sup>1</sup>, Daniel H. Teitelbaum<sup>2</sup>, Hua Yang<sup>1,2\*</sup>

<sup>1</sup> Department of General Surgery, Xinqiao Hospital, Third Military Medical University, Chongqing, China, <sup>2</sup> Department of Surgery, the University of Michigan Medical School, Ann Arbor, Michigan, United States of America

## Abstract

**Background:** Intestinal ischemia/reperfusion (I/R) induces the desquamation of the intestinal epithelium, increases the intestinal permeability, and in patients often causes fatal conditions including sepsis and multiple organ failure. Keratinocyte growth factor (KGF) increases intestinal growth, although little is known about KGF activity on intestinal function after intestinal I/R. We hypothesized that KGF administration would improve the intestinal function in a mouse model of intestinal I/R.

**Methods:** Adult C57BL/6J mice were randomized to three groups: Sham, I/R group and I/R+KGF group. Mice were killed on day 5, and the small bowel was harvested for histology, wet weight, RNA and protein content analysis. Epithelial cell (EC) proliferation was detected by immunohistochemistry for PCNA, and apoptosis was determined by TUNEL staining. The expressions of Claudin-1 and ZO-1 were detected by immunohistochemistry. Epithelial barrier function was assessed with transepithelial resistance (TER).

**Results:** KGF significantly increased the intestinal wet weight, contents of intestinal protein and RNA, villus height, crypt depth and crypt cell proliferation, while KGF resulted in the decrease of epithelial apoptosis. KGF also stimulated the recovery of mucosal structures and attenuated the disrupted distribution of TJ proteins. Moreover, KGF attenuated the intestinal I/R-induced decrease in TER and maintained the intestinal barrier function.

**Conclusion:** KGF administration improves the epithelial structure and barrier function in a mouse model of intestinal I/R. This suggests that KGF may have clinical applicability.

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\* E-mail: hyang@umich.edu

## Introduction

Intestinal ischemia/reperfusion (I/R) injury occurs in a variety of important clinical conditions [1–2]. The pathophysiology of intestinal I/R injury includes direct cellular damage due to ischemia and delayed dysfunction and damage as a result of the activation of inflammatory pathways [1]. The intestinal damage that ensues includes injury to the mucosal structure and barrier function.

Keratinocyte Growth Factor (KGF), also known as FGF7, is a known mitogenic growth factor, and expressed by T-cell receptor  $\gamma\delta$  intraepithelial lymphocytes (IEL) in the mucosal layer [3]. KGF receptors (KGFR) have been detected in high numbers in the gastrointestinal tract, which indicates that the gut can both synthesize and respond to KGF [4–6]. It is believed that KGF plays a critical role in the intestinal epithelial growth and maintenance. Studies have shown that KGF has a number of beneficial effects. A study from our group indicated that recombinant human KGF (rHuKGF) significantly improves the

epithelial barrier function in a mouse model of total parenteral nutrition (TPN) [7]. In a mouse model of short bowel syndrome (SBS), 55% of the mid-small intestine was removed, and our results revealed IEL-derived KGF was significantly increased [8]. Studies have demonstrated that exogenous KGF could ameliorate the mucosal injury in several colitis animal models [9–11], while KGF null mice were more susceptible to dextran sulfate-induced colonic injury than their wild-type counterparts, and in the absence of KGF, healing was delayed [12]. Moreover, a study showed rHuKGF could increase the gas exchange and improve the mechanics of the lung in the oleic acid (OA) induced lung injury [13]. All these results suggest that KGF plays a critical role in the intestinal mucosal protection and repair in chronic intestinal injury. However, the effects of KGF on the intestinal mucosa in the ischemia/reperfusion (I/R) induced acute intestinal injury remain unclear. In the present study, we hypothesized that KGF could attenuate the damage to the epithelial structure and function in a mouse model of intestinal I/R.

## Materials and Methods

### Animals

Studies reported here conformed to 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 the whole protocol was approved by this committee. Male, 6–8-week-old, specific pathogen-free, C57Bl/6 mice were purchased from the Laboratory Animal Center (Third Military Medical University, Chongqing, P.R. China), maintained in temperature, humidity and light-controlled conditions, and then randomized into sham group, I/R group and I/R+KGF group. Recombinant human KGF rHuKGF was intraperitoneally given in the I/R+KGF group at 5 mg/kg/day once daily for 5 days before the operation.

### Intestinal ischemia-reperfusion injury

After intraperitoneal anesthesia with 40 mg/kg pentobarbital, the abdomen was opened at the midline, and the superior mesenteric artery (SMA) was occluded for 20 min using non-traumatic vascular clamps, followed by reperfusion for 0 h, 6 h, 24 h and 72 h. Animals in the sham group underwent identical procedures without SMA occlusion (Sham). At the end of surgery, mice were given *ad libitum* access to water and food. There was no significant difference in the survival between the I/R+KGF group and the I/R group. In all experiments, at least 6 animals were included in each group and experiment was repeated at least three times.

### Histological examination

Segments of jejunum were harvested, fixed in 4% paraformaldehyde and used for histological examination. Tissues were dehydrated in the ethanol and embedded in the paraffin. Sections were obtained for hematoxylin-eosin (H&E) staining. The intestinal mucosal injury was evaluated under a light microscope according to the criteria described by Chiu et al [14] and graded from 0 to 5. Histological findings were assessed and scored by a pathologist blind to the experiment. The villus height and the depth of crypt were measured using a calibrated micrometer. A mean of 7 different fields were selected for the measurement of villus height and crypt depth.

### Measurements of mucosal wet weight and content of RNA and protein

At the time of tissue harvest, 10 cm of jejunum was obtained, weighed and used for the measurement of intestinal RNA and protein content. Intestinal mucosal RNA was determined by spectrophotometry using a modified Schmidt-Tannhauser method as described by Munro and Fleck [15]. Protein determination was performed by using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA). RNA is expressed in  $\mu\text{g}/\text{cm}$  segment of intestine and proteins are expressed in  $\text{mg}/\text{cm}$  segment of intestine.

### TUNEL staining

TUNEL staining was performed according to the manufacturer's instructions (*In Situ* Cell Death Detection Kit; Roche, Germany). In brief, the paraffin-embedded sections were deparaffinized with xylene followed by absolute ethanol, 95% ethanol, 85% ethanol, 75% ethanol and 70% ethanol. The sections were washed with phosphate-buffered saline (PBS) for 5 min and treated with protease K (20  $\mu\text{g}/\text{mL}$ ) for 20 min at room temperature. After washing in PBS twice, these sections were

incubated with 50  $\mu\text{l}$  of TUNEL reaction solution in a humidified environment at 37°C for 60 min. Following washing in PBS thrice, these sections were treated with 50  $\mu\text{l}$  of converter-POD in a humidified environment at 37°C for 30 min. Following washing in PBS thrice, sections were incubated with 100  $\mu\text{l}$  of substrate at room temperature for 10 min. Mounting was performed after washing in PBS thrice. The number of TUNEL positive cells was counted under a microscope (400 $\times$ ), and the apoptotic cells in each section were counted in 7 independent fields followed by averaging.

### Western blot assay

Proteins were extracted from the intestinal mucosa followed by homogenization by sonication and centrifugation at 14,000 rpm for 15 min at 4°C. Twenty-five micrograms of protein were separated on the 10% SDS polyacrylamide gel and transferred to 0.2- $\mu\text{m}$  nitrocellulose membrane which was then blocked in TBST (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween 20) containing 5% milk for 1 h. Blots were incubated with anti-KGF antibody (catalogue no.bs-0734R; Beijing Biosynthesis Biotechnology Co., Ltd.) overnight at 4°C. After washing, the membrane was incubated with HRP-conjugated secondary antibodies (Cell Signaling) and then visualized with an enhanced chemiluminescence (Cell Signaling).  $\beta$ -tubulin (Sigma, Dorset, UK) was used as an internal control. Optical density (OD) was determined, and OD of target protein was normalized by that of  $\beta$ -tubulin and presented as percentage of control.

### Detection of Epithelial Proliferation

Tissues were fixed in 4% paraformaldehyde and cut into 5- $\mu\text{m}$  sections which were treated with 0.5% hydrogen peroxide in methanol, blocked for 45 min, and then incubated with anti-PCNA antibody (catalogue no.10205-2-AP; Proteintech) or purified rabbit IgG (10 mg/ml; negative control) overnight at 4°C. These sections were incubated with biotinylated goat anti-rabbit IgG for 60 min, and then with streptavidin-enzyme conjugate (Vector Laboratories Inc). The peroxidase activities were developed with diaminobenzidine (DAB). After counterstaining with hematoxylin, histological examination was done under a light microscope (400 $\times$ ).

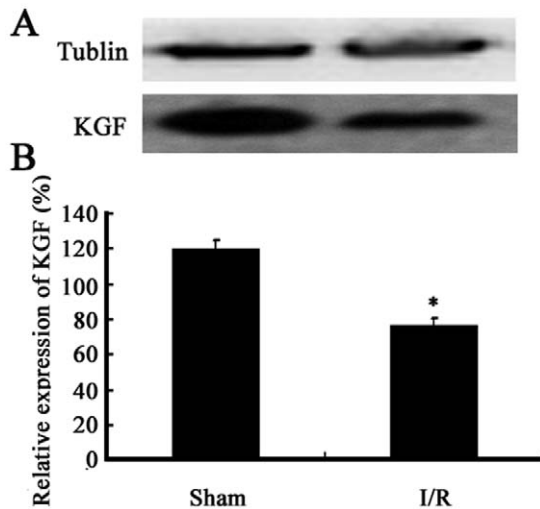
The crypt cell proliferation rate was calculated as the ratio of the number of crypt cells positive for PCNA to the total number of crypt cells. The total number of proliferating cells per crypt was defined as a mean of proliferating cells in 10 crypts.

### Immunohistochemistry

Anti-Claudin-1 antibody (catalogue no.ab-15098; Abcam Inc.) and anti-ZO-1 antibody (catalogue no.21773-1-AP; Proteintech) were used for immunohistochemistry staining as described above. After counterstaining with hematoxylin, the localization of Claudin-1 and ZO-1 was examined under light microscope(630 $\times$ ).

### Detection of intestinal permeability

The transmembrane resistance (TER,  $\Omega\cdot\text{cm}^2$ ), an indicator of intestinal epithelial barrier function and tissue viability [16], was determined using the Ohm's law. The permeability of the small intestine was evaluated by TER. Tissues were bathed on the serosal and mucosal sides with Ringer solution. Bathing solutions were oxygenated (95%  $\text{O}_2$ -5%  $\text{CO}_2$ ) and circulated in water-jacketed reservoirs at 37°C. After a 20-min equilibration period in Ussing chambers, detection was done for up to 1.5 h. TER was calculated from the spontaneous potential difference (PD) and short-circuit current.



**Figure 1. Change in KGF expression in a mouse model of acute intestinal I/R.** (A) Decreased expression of KGF was confirmed by western blot assay in mice early after acute intestinal I/R, when compared with the sham group. Tubulin was used as an internal control. (B) Spearman rank correlation of KGF protein level with acute intestinal I/R in mice. \* $P < 0.05$  vs sham group,  $n = 6$  per group. doi:10.1371/journal.pone.0044772.g001

#### Statistical analysis

Data are expressed as mean  $\pm$  standard deviation (SD). Statistics were performed using SPSS version 13.0 for windows. Results were analyzed using analysis of variance (ANOVA). A value of  $P < 0.05$  was considered statistically significant.

#### Results

##### Changes in KGF expression in a mouse model of acute intestinal I/R

Western blot assay was performed to detect the KGF expression. Results showed that the KGF expression significantly decreased by  $35.9 \pm 4.5\%$  early after intestinal I/R when compared with the sham group ( $P < 0.05$ ) (Figure 1). This suggests that KGF involves in the intestinal mucosal injury after intestinal I/R.

##### Morphological examination of intestine

Animals were sacrificed at 0 h, 6 h, 24 h and 72 h after surgery. The results in histological examination and classification of the

lesions are summarized in Table 1. Results showed that there were no lesions in the intestinal mucosa in the sham group, whereas the intestine of I/R group (6 h) presented with grade 5 and 4 injuries of the intestinal mucosa, which were more severe than those in the I/R (6 h) +KGF group, characterized by the loss of villi or epithelial shedding from the villi. Grade 2 and 3 injuries were observed in the I/R (6 h) +KGF group, with partly epithelial lifting down the sides of the villi (Figure 2).

##### Intestinal wet weight and content of RNA and protein

As KGF markedly improved the intestinal morphology at 6 h after I/R, this time point was chosen for the following experiments. The mucosal wet weight of the jejunum was significantly increased in the I/R (6 h) +KGF group ( $561.3 \pm 10.2$  mg/10 cm) when compared with the I/R group ( $405.7 \pm 8.9$  mg/10 cm) at 6 h after intestinal I/R ( $P < 0.05$ ) (Table 2). As shown in Table 2, there was significant difference in the RNA content between the I/R (6 h)+KGF group ( $37.5 \pm 5.4$   $\mu$ g/cm) and the I/R group ( $21.2 \pm 4.3$   $\mu$ g/cm) at 6 h after intestinal I/R ( $P < 0.05$ ). The changes in the protein content of jejunum mucosa were similar to those in RNA content, and significant difference was also noted between the I/R (6 h) +KGF group ( $2.57 \pm 0.14$  mg/cm) and the I/R (6 h) group ( $1.84 \pm 0.29$  mg/cm) ( $P < 0.05$ ) (Table 2).

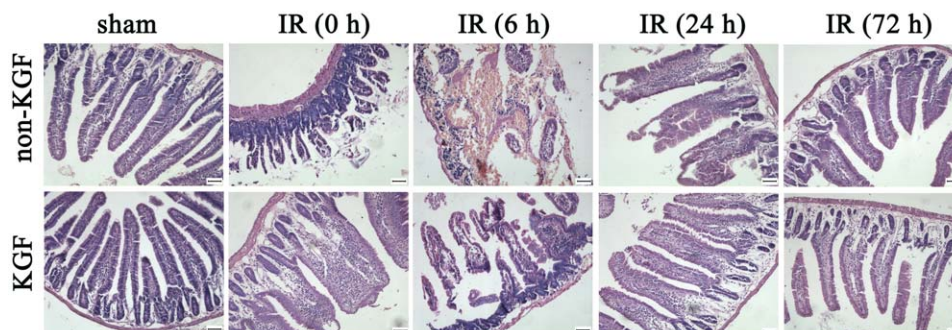
##### Intestinal epithelial proliferation

There was an increase in both villus height and crypt depth after KGF treatment. KGF treatment led to a significant increase in the jejunal villus height ( $382 \pm 62$   $\mu$ m) when compared with the I/R (6 h) group ( $247 \pm 41$   $\mu$ m) ( $P < 0.05$ ). The crypt depth was also significantly greater in the I/R (6 h) +KGF group ( $103 \pm 18$   $\mu$ m) than that in the I/R group ( $56 \pm 12$   $\mu$ m) at 6 h after intestinal I/R ( $P < 0.05$ ) (Figure 2, 3).

PCNA-positive cells were all distributed in the crypt of Lieberkuhn of the small intestine. In addition, KGF significantly increased the number of PCNA positive cells in the I/R (6 h)+KGF group ( $47.9 \pm 5.6\%$ ) when compared with the I/R group ( $24.6 \pm 3.1\%$ ) at 6 h after I/R ( $P < 0.05$ ) (Figure 4). There was no difference in the location of PCNA positive cells between groups, and all positive cells were found in the crypts.

##### Intestinal epithelial apoptosis

To determine whether KGF could attenuate the increase of intestinal epithelial apoptosis due to I/R, TUNEL staining was performed. Results showed that the highest amount of TUNEL positive cells was noted at 6 h after intestinal I/R ( $51 \pm 8.3\%$ ) when compared with the sham group ( $7 \pm 1.6\%$ ) ( $P < 0.05$ ) (Figure 5), but



**Figure 2. Histological changes in intestinal I/R mice with KGF treatment by H&E staining.** More severe mucosal injury was observed in I/R group than in I/R+KGF group, while no mucosal injury was noted in the sham group; Original magnification:  $\times 200$ ,  $n = 6$  per group. Scale bar = 50  $\mu$ m. doi:10.1371/journal.pone.0044772.g002

**Table 1.** Classification of mucosa lesions in the experimental animals\*.

| Animals | Mucosal lesion severity |           |                |
|---------|-------------------------|-----------|----------------|
|         | Sham group              | I/R group | I/R+ KGF group |
| 1       | 0                       | 4         | 2              |
| 2       | 0                       | 4         | 3              |
| 3       | 0                       | 5         | 2              |
| 4       | 0                       | 4         | 3              |
| 5       | 0                       | 5         | 2              |
| 6       | 0                       | 4         | 2              |

\*Tissues were harvested at 6 h after intestinal I/R.  
doi:10.1371/journal.pone.0044772.t001

KGF significantly attenuated the increase in TUNEL-positive cells ( $22 \pm 5.7\%$ ) when compared with the I/R (6 h) group ( $51 \pm 8.3\%$ ) ( $P < 0.05$ ) (Figure 5). These findings suggest that KGF could attenuate the increase in I/R-induced intestinal epithelial apoptosis.

### Changes in expression of tight junction proteins

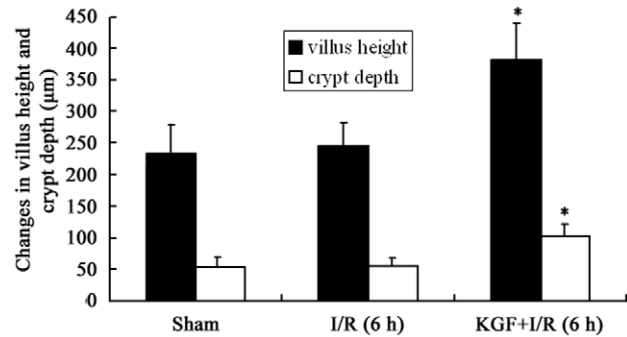
It has been found that tight junction (TJ) proteins are critical structural proteins in the maintenance of mucosal barrier function [17–18]. The impairment of mucosal barrier function is directly characterized by the aberrant expression of TJ proteins [17–19]. Immunohistochemistry was used to detect the expression of TJ proteins including Claudin-1 and ZO-1. The expression of Claudin-1 at 6 h and 24 h after intestinal I/R showed a significantly disrupted, diffuse staining pattern, while the normal chicken wire pattern was seen in the cross sections of the sham group (Figure 6). However, KGF improved the abnormal distribution of Claudin-1 following I/R, the reticular structures were more apparent and favorably maintained, especially in the I/R+KGF at 6 h or 24 h after intestinal I/R when compared with the I/R group at the corresponding time points (Figure 6). The ZO-1 exclusively localizes at the TJ of villous enterocytes, and an intense apical staining was found in the sham group (Figure 7). The apical staining of ZO-1 was obviously interrupted and partly disappeared in the I/R group, as compared to the sham group at 6 h after intestinal I/R, while the subtotal structure of ZO-1 was remained in the I/R (6 h)+KGF group, which was similar to the changes in the Claudin-1 expression (Figure 7). These results suggest that KGF can exert protective effect on the TJ proteins after acute intestinal I/R injury.

**Table 2.** Intestinal wet weight and contents of jejunal protein and RNA#.

|                                  | Sham            | I/R group       | I/R+ KGF group     |
|----------------------------------|-----------------|-----------------|--------------------|
| Protein (mg/cm)                  | $1.72 \pm 0.21$ | $1.84 \pm 0.29$ | $2.57 \pm 0.14^*$  |
| RNA ( $\mu\text{g}/\text{cm}$ )  | $14.6 \pm 2.3$  | $21.2 \pm 4.3$  | $37.5 \pm 5.4^*$   |
| Intestinal wet weight (mg/10 cm) | $392.5 \pm 7.3$ | $405.7 \pm 8.9$ | $561.3 \pm 10.2^*$ |

#Tissues were harvested at 6 h after intestinal I/R,  
\* $P < 0.05$  vs I/R group.

doi:10.1371/journal.pone.0044772.t002



**Figure 3.** Alterations in villus height and crypt depth in mice after KGF treatment. \*  $P < 0.05$  vs. I/R (6 h) group,  $n = 6$  per group.  
doi:10.1371/journal.pone.0044772.g003

### Transepithelial resistance

To further confirm the role of KGF in the maintenance of intestinal barrier function, transepithelial resistance (TER) was detected with Ussing chambers [16]. Because our study revealed that more severe destruction was found in the intestinal morphology, and the location of TJ proteins was disrupted at 6 h after acute intestinal injury, the intestinal barrier function was determined at 6 h after surgery. The baseline TER (0 min after mounting) was  $76.6 \pm 5.2 \Omega \cdot \text{cm}^2$  and  $31.7 \pm 2.8 \Omega \cdot \text{cm}^2$  in the sham group and I/R (6 h) group, respectively. I/R resulted in a significant decrease in the TER (58.6%) when compared with the sham group. KGF partially attenuated the decrease in TER following intestinal I/R (26.1%), although the TER remained lower than that in the sham group (Figure 8). All these findings suggest that KGF can significantly attenuate the I/R-induced epithelial barrier dysfunction.

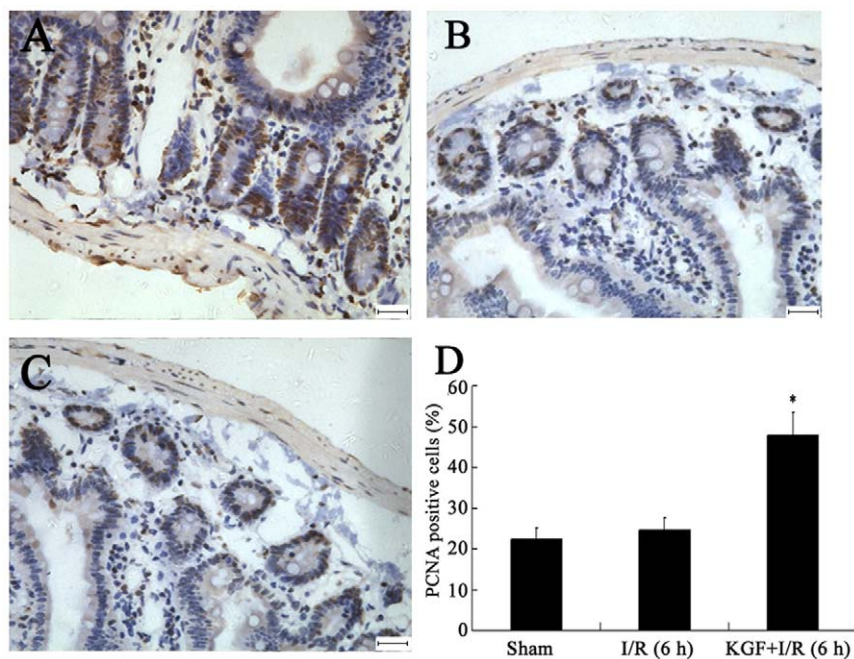
### Discussion

In this study, the effects of rHuKGF on the intestinal epithelial structure, function and growth were investigated in a mouse model of acute intestinal I/R. Our results demonstrated that KGF could exert protective effects in preventing the loss of epithelial structure and barrier function after acute intestinal I/R. Results also revealed KGF significantly improved the content of intestinal RNA, protein, the mucosal wet weight, increased the number of proliferating cells in the crypt, while decreased the intestinal epithelial apoptosis.

Intestinal I/R injury is a significant clinical problem arising from diseases or as a consequence of abdominal surgery [1]. A number of changes may occur in the intestinal mucosa after acute intestinal I/R, including a decline in the mucosal epithelial growth and function. Damage to the mucosa includes the epithelial shedding, bacterial translocation, disordered mucosal permeability, and alteration of the absorptive function [20–21]. In the present study, the histological features occurring in the I/R group were characterized by the shortening of the villi, loss of villous epithelium, multiple erosions, inflammatory cell infiltration, necrosis and hemorrhage into the intestinal wall. Our findings were consistent with those in previous studies on intestinal I/R in animals [22–23]. Our results indicated that the mucosal lesions (grade 4, 5) were more severe at 6 h after intestinal I/R and KGF significantly decreased the mucosal lesions (grade 2, 3), which suggested that KGF could improve the mucosal morphology in a mouse model of acute intestinal I/R.

The regulation of gastrointestinal cell growth and differentiation is complex and usually influenced by many factors [24]. KGF has

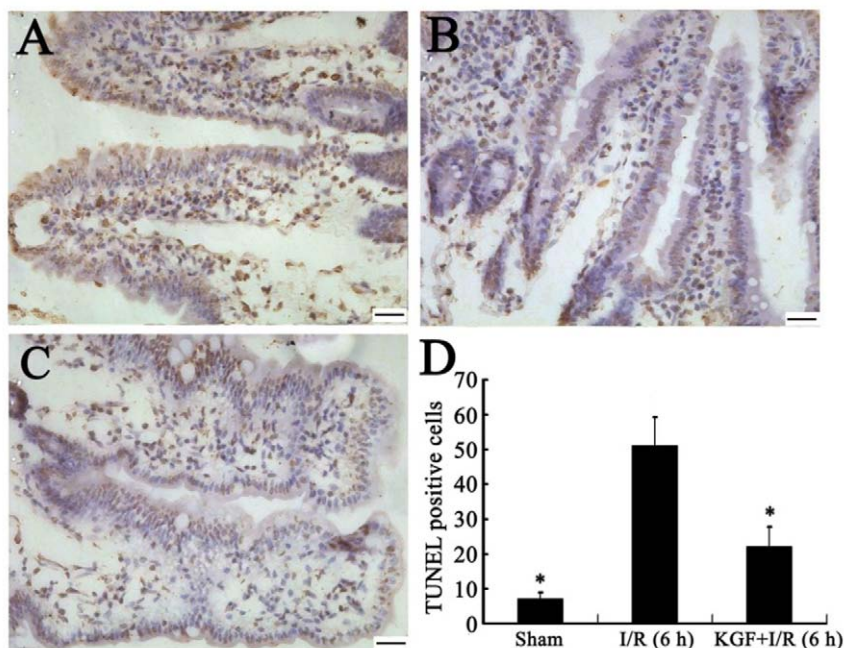




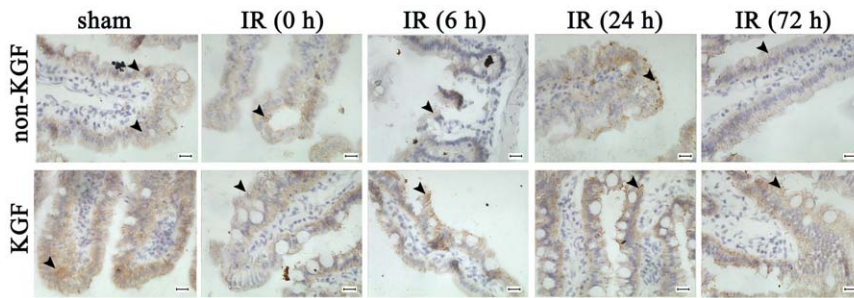
**Figure 4. Alterations in PCNA expression in small intestine of KGF treated mice by immunohistochemistry.** PCNA expression was significantly increased in I/R+KGF (6 h) group (A), as compared to the I/R (6 h) group (B) and sham group (C). Original magnification:  $\times 400$ ; (D) PCNA expression is expressed as means  $\pm$  SD,  $*P < 0.05$  vs I/R group,  $n = 6$  per group. Scale bar = 25  $\mu\text{m}$ . doi:10.1371/journal.pone.0044772.g004

been shown to regulate the proliferative response of the intestinal tract [25], which supports that KGF plays an important role in the intestinal epithelial growth and its maintenance. Studies have shown that KGF (rHuKGF) can improve the survival, body weight

loss, hematochezia, diarrhea, and the histopathology in a dextran sodium sulfate-induced inflammatory bowel disease (IBD) mouse model [9,26]. Exogenous KGF can prevent the mucositis caused by chemotherapy and radiation to the intestine [27]. KGF has also



**Figure 5. TUNEL staining of small intestine of mice after KGF treatment.** The number of TUNEL positive cells was counted at a high magnification ( $\times 400$ ). The number of TUNEL positive cells was significantly increased in the I/R group after 6 h intestinal I/R (A), as compared to the sham group (B); KGF significantly prevented the increase in TUNEL positive cells in the I/R(6 h)+KGF group (C) when compared with the I/R (6 h) group; (D) The number TUNEL positive cells is presented as mean  $\pm$  SD.  $*P < 0.05$  vs I/R group,  $n = 6$  per group. Scale bar = 25  $\mu\text{m}$ . doi:10.1371/journal.pone.0044772.g005



**Figure 6. Changes in Claudin-1 expression in acute intestinal I/R mice after KGF treatment by immunohistochemistry.** Claudin-1 expression was detected at 0 h, 6 h, 24 h and 72 h after I/R with or without KGF treatment, compared with the sham group. The reticular structures were significantly improved and maintained after KGF treatment at all stages of I/R injury when compared with the sham group. Arrow, Claudin-1 expression. Original magnification:  $\times 630$ ,  $n=6$  per group. Scale bar = 25  $\mu\text{m}$ . doi:10.1371/journal.pone.0044772.g006

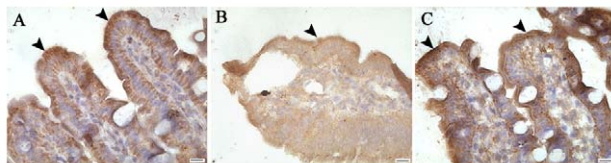
been shown to ameliorate the mucosal injury in an experimental model of intestinal inflammation in rats [9,11]. All these results suggest that KGF plays a critical role in the intestinal mucosal protection and repair in the chronic intestinal injury, which was also confirmed in a mouse model of acute intestinal I/R in our study.

In the present study, results showed that KGF expression significantly decreased in mice early after acute intestinal I/R, which suggested that KGF was involved in the acute intestinal I/R in mice. However, exogenous rHuKGF increased the PCNA-positive epithelial cells, villus height and crypt depth and attenuated the changes in the mucosal morphology. The mucosal wet weight, the content of intestinal RNA and protein were also found significantly increased by exogenous KGF in our study. A study from Johnson et al [28] showed that KGF significantly increased the villus height in a rat SBS model. The mucosal wet weight, the content DNA and protein in rats were also significantly increased by KGF at 3 days after an 85% small bowel resection in a rat model [28]. BrdU-positive epithelial cells further increased in the SBS group after KGF treatment when compared with the non-treated SBS group [8]. Our previous study also revealed that KGF significantly increased the intestinal wet weight, the content of intestinal DNA and protein in the TPN mice, and led to an increase in the villus height and crypt cell proliferation [7]. Additionally, our results revealed that KGF significantly reduced the intestinal epithelial apoptosis of the small intestine, although I/R actually induced the epithelial apoptosis and mucosal injury in the mouse intestine, which was consistent with findings in the study of Hung et al [29]. Taken together, our results showed that KGF could affect either the proliferation or apoptosis of the

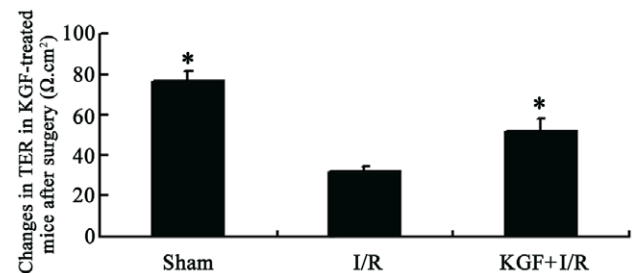
intestinal epithelial cells in the mice with intestinal I/R, and KGF may play an important role in this pathological condition.

TJs are the major determinants of paracellular permeability. Intestinal injury alters the distribution of TJ proteins *in vivo*, which is associated with the functional TJ (barrier function) deficiency. Although the changes in the epithelial TJ protein expression have been studied extensively in monolayers stimulated by cytokines, bacteria, or aspirin [30–35], much remains to be understood about the changes in the barrier function following acute intestinal I/R *in vivo*. In the present study, I/R resulted in the disruption of TJ proteins (Claudin-1 and ZO-1), which was consistent with previous findings [19,36–37]. The gut permits the absorption of nutrients while prevents the systemic contamination by luminal toxins and microbial products [38]. TER has been used to detect the tissue TJ integrity. Our results showed that the intestinal I/R caused an increase in the intestinal permeability. This result was supported by the findings of Higuchi et al [39], in which I/R in a rat model led to an increased intestinal conductance.

In the present study, TJ proteins including Claudin-1 and ZO-1 were damaged by intestinal I/R, KGF could attenuate this damages to TJ proteins. KGF also partly attenuated the intestinal barrier dysfunction after intestinal I/R. All these findings further confirmed that KGF played an important role in the maintenance of intestinal barrier function in acute intestinal I/R. This protective effect of KGF was also observed in our previous study in a mouse TPN model, in which KGF significantly attenuated the barrier dysfunction due to TPN [7]. However, the mechanism underlying the protective effect of KGF on the intestinal integrity is still poorly understood. Our findings provide evidence that KGF



**Figure 7. Changes in ZO-1 expression in acute intestinal I/R mice after KGF treatment by immunohistochemistry.** ZO-1 expression was detected in the sham group (A), at 6 h after I/R (B) and after KGF treatment (C). KGF maintained the intense apical staining for ZO-1 at 6 h after I/R when compared with the I/R (6 h) group. Arrow, ZO-1 expression. Original magnification:  $\times 630$ ,  $n=6$  per group. Scale bar = 25  $\mu\text{m}$ . doi:10.1371/journal.pone.0044772.g007



**Figure 8. Changes in TER of I/R mice after KGF treatment.** The permeability of the small intestine was evaluated by TER. Tissues were harvested at 6 h after intestinal I/R,  $*P<0.05$  vs. I/R group,  $n=6$  per group. doi:10.1371/journal.pone.0044772.g008

attenuates the disrupted distribution of TJ proteins, which may partially improve the functional TJ deficiency.

In conclusion, this study demonstrated that intestinal I/R caused profound changes in the small bowel epithelial morphology and physiology in a mouse model. These changes included the mucosal injury and the disruption in the TJ protein expression and the alteration in the epithelial barrier function. Moreover, KGF significantly attenuated these changes. These results demonstrate

that KGF can exert protective effects to prevent the loss of epithelial structure and barrier function after acute intestinal I/R.

## Author Contributions

Conceived and designed the experiments: CYJ DHT YH. Performed the experiments: CYJ WWS LHY. Analyzed the data: CYJ WWS LHY YH LS. Contributed reagents/materials/analysis tools: CYJ WWS LHY YH. Wrote the paper: DHT YH.

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