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Effects of Keratinocyte Growth Factor 2(KGF-2) on Keratinocyte Growth, Migration and on Excisional Wound Healing

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Abstract Keratinocyte growth factor 2 (KGF-2) is a member of the FGF family that is mainly synthesized by mesenchymal cells and acts predominantly on epithelial cells in a paracrine manner. It is known to play an important role in fetal limb and lung development; skin wound healing and prostatic epithelial cell growth. The KGF-2 coding sequence were isolated from human kidney cDNA library, revealing that the Kgf-2 gene is also expressed in the kidney apparatus. Purified from prokaryotic *E. coli* cells, the effects of the recombinant KGF-2 protein in cultured keratinocyte were analyzed by using MTT assay and *in situ* TUNEL assay. Interestingly, results revealed that KGF-2 promoted keratinocyte cell growth by stimulating cell proliferation and attenuating cell apoptosis. These findings supported a few evidences that KGF-2 could contribute to alveolar epithelial cells against apoptosis. Cell migration assays for the first time revealed that KGF-2 could stimulate keratinocyte cell migration *in vitro*. In addition, in the pilot animal test, recombinant KGF-2 incorporated within the hydrogel dressing exhibited significantly stimulatory effect on cutaneous wound healing. These combined effects implicate a potential therapeutic application of human recombinant KGF-2 in the future.

Key words KGF-2, keratinocyte cell proliferation, cell apoptosis, cell migration, wound healing, therapeutic application
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Keratinocyte growth factor 2(KGF-2), also named fibroblast growth factor 10(FGF-10), is a member of fibroblast growth factor(FGF) super family, a growing group of structurally related polypeptide mitogens, which currently include more than 20 different members. Among the FGF family members, KGF-2 exhibits close structural and functional similarities to KGF-1(FGF-7)[1, 2]. It was first isolated as an epithelial mitogen from rat embryos by homology-based polymerase chain reaction in 1996[3]. Afterwards, Emoto group[4] and Jimenez group[5] respectively isolated the cDNA sequence encoding human KGF-2. This cDNA encodes a protein of 208 amino acids including a classical signal peptide and has high sequence identity with the rat Kgf-2 (95.6%). By *in situ* radioactive hybridization, this human gene was mapped to the chromosome 5p12-p13[4]. FGFs appear to play important roles as peptide growth factors in both developing and adult tissues for a variety of cells of mesoderm, ectoderm and endoderm origin. They have multiple biological activities involved in angiogenesis, mitogenesis, cellular differentiation and repair of tissue injury[6]. In contrast to most of the other FGFs, which have wide cell targets, KGF-2 and KGF-1 were highly specific mitogens for epithelial cells.

KGF-2 is widely expressed in developing and adult tissues. With an antisense ^{35}S-labeled Kgf-2 cDNA probe, *in situ* hybridization assay determined that discrete labeling was observed in several regions of rat embryo, including the posterior pituitary, the first cervical vertebra, sacral and coccygeal segments of the spinal cord, the duodenum, and the lung [3]. KGF-2 is preferentially expressed in the preadipocytes but not the mature adipocyte fraction and has mitogenic activity for primary preadipocytes [6]. Decreased cell numbers and proliferative activity were observed in the white adipose tissue of Kgf-2$^{-/-}$ mouse embryos [7]. The addition of exogenous recombinant KGF-2 could induce proliferation in isolated human

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hair-follicle cells [8]. Forced Kgf-2 expression was found to stimulate cell proliferation of both the luminal and glandular epithelia and increase the number of BrdU-labeled cells *in ovo*. Moreover, cell proliferation was downregulated by blocking KGF-2 signaling in the proventriculus [9]. Histological examination of Kgf-2$^{-/-}$ newborn mouse skin revealed abnormalities in epidermal morphogenesis: The number of proliferating cells in the basal layer of epidermis decreased; the granular layer was hypoplastic and lacked distinctive keratohyaline granules and tonofibrils[10].

These studies reveal that KGF-2 protein functions as a mitogen for epithelial cells, significantly stimulating epithelial cell proliferation *in vivo* or in primary cultured cells. These data evoked us to think whether it could also enhance cells growth by attenuating cell apoptosis. In this study, we isolated the cDNA sequence encoding KGF-2 from human kidney cDNA library. The recombinant KGF-2 was highly expressed in *E. coli* cells and efficiently purified. Moreover, *in vitro* proliferation assay suggested that it was highly active. Furthermore, *in situ* TUNEL assay suggested that KGF-2 could attenuate H_2O_2-induced cell apoptosis in the keratinocyte cell line. Cell migration assay with tranwell plate for the first time demonstrated that it could also stimulate keratinocyte cell migration *in vitro*. In addition, in the pilot animal test, recombinant KGF-2 incorporated within the hydrogel dressing exhibited significantly stimulatory effect on cutaneous wound healing.

1 Materials and methods

1.1 Construction, expression, and purification of recombinant KGF-2

The PCR reaction was carried out for 31 cycles at 95℃ for 1 min, 55℃ for 1 min, and 72℃ for 1.5 min. Primers are designed based on the cDNA sequence published on web NCBI(AB002097, gi: 2440220) and the signal peptide sequence was omitted. The sense primer is as following: 5′ TCGAATTCCAAGCC - CTTGGTCAGGAC 3′ adding an *Eco*R Ⅰ site at the 5′ end. And the antisense primer is: 5′ GTAAGCTT- CTATGAGTGTACCACCATTGGAA 3′ containing a *Hin*d Ⅲ site. The PCR product was digested with *Eco*R Ⅰ and *Hin*d Ⅲ (TakaRa) and then fractionated on a 1% agarose gel, recovered and cloned in frame into the vector named pT$_7$450 at *Eco*R Ⅰ and *Hin*d Ⅲ sites, which was constructed in our laboratory based on pET-32a (Novagen). The cloned Kgf-2 gene was verified by sequencing and the constructed plasmid was transformed into Origami (DE3) cell (Novagen). After induction with IPTG, the culture temperature was adjusted to 25℃ for 6～8 h incubation and then the cell pellets were harvested.

The cell lysate of *E. coli* expressing the recombinant KGF-2 was applied directly to a heparin column (Heparin Sepharose™ CL-6B, Amersham Pharmacia Biotech) pre-equilibrated with PBS containing 0.2 mol/L NaCl (pH 7.4), and proteins bound to the column were eluted with a linear gradient of 0.2～2.0 mol/L NaCl in PBS(pH 7.4). The fraction containing the recombinant KGF-2 was desalted and concentrated with a centrifugal filter device(Centricon Plus-20, PL-10, 10000NMWL, Millipore).

1.2 Cell culture

HaCaT cells (simultaneous human immortal keratinocyte cell line[11]) were grown in DMEM: F12 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 mg/L streptomycin (Amresco). H460 cells (human lung cancer cell line) were grown in RPMI1640 medium supplemented with 10% newborn bovine serum and the same antibiotics. HeLa(Henrietta Lacks strain of cancer cells) and CV-1 (monkey kidney fibroblast cell line) cells were grown in DMEM medium supplemented with 10% newborn bovine serum and the same antibiotics. All the cell lines were cultivated in a humidified atmosphere containing 5% CO_2 at 37℃

1.3 MTT assay

MTT assays were conducted according to the method of Tim[12] and the instructions of ATCC(MTT Cell Proliferation Assay Instructions). Cells were trypsinized and adjusted density to 5×10^4 cells/ml by the respective incubation media. The suspended cells were seeded at 5×10^3 cells(100 μl) per well in 96-well cell culture plate(Costar) and incubated for 24 h. Then the cells were gently washed with DMEM: F12 or RPMI 1640 or DMEM twice to remove the serum. Purified KGF-2 was serial diluted in the same serum free medium and added into the plate. After 3～5 days stimulation, 50 μl of 1 g/L MTT(Amresco) was added to each well followed by incubation for 4 h at 37℃. The products, formazan crystals, were solubilized with 100 μl DMSO, and the A was determined using a Bio-Rad microtiter reader at A_{570}. For cell apoptosis analysis, MTT assay was done as above using H_2O_2 as an apoptotic agent for 1 h after appropriate time of KGF-2 treatment.

1.4 TUNEL assay

TUNEL was made out according to the protocol of DeadEnd™ fluorometric TUNEL system (Promega, USA). Briefly, HaCaT cells were trypsinized and adjusted density to 5×10^4 cells/ml by the appropriate medium with 10% FBS. 1 ml of the suspended cells was then seeded on the slides pretreated with gelatin and incubated for 24～48 h. After being rinsed with serum-free medium twice, the cultivation medium was changed to serum free medium with appropriate concentrations of KGF-2 and cultivated for 2～24 h followed by H_2O_2 (50 μmol/L) treatment for 1 h to induce cell apoptosis. TUNEL assay was performed afterwards according to the protocol. Apoptotic and total cells were counted at 5 different areas and the percentages of apoptotic cells were calculated as [(positive cells/total cells) ×100%].

1.5 Flow cytometry analysis

HaCaT cells were trypsinized and adjusted density to 5×10^4 cells/ml by the appropriate medium with 10% FBS. 10 ml of the suspended cells was then seeded in 10 cm dishes. Being serum-starved for 24 h, cells were cultured with or without 0.5 g/L KGF-2 for 24 h, and then collected by trypsinization or treated with H_2O_2 for 1 h before collection. According to the manufacturer's instructions, cell cycle and apoptosis analysis was performed with an FACS (fluorescence-activated cell sorting) system. The proliferation index (PI) was determined from the flow cytometry analysis data according to the following formula: PI=(G2/M+S) ÷(G0/G1+S+G2/M)×100%[13]

1.6 Cell migration

Cell migration assays were performed with 24-well transwell plate(8.0 μm pore size, Costar) according to a modified protocol described by Leavesley *et al*[14]. Briefly, 200 μl HaCaT cells were seeded in the upper chamber (5×10^4 cells/ml) while the lower chamber was supplemented with serum free DMEM : F12 medium containing indicated concentrations of KGF-2 or serum free DMEM : F12 medium supplemented with 0.1% BSA for control. Cells were allowed to migrate from upper chamber to the lower chamber for 20 h. Nonmigratory cells were removed from the upper surface with cotton swabs. Cells that had migrated through the membrane were then fixed with 4% paraformaldehyde for 30 min and stained by 0.5% crystal violet for 30 min. After extensive washing, the stained cells were solubilized with 500 μl of 1% SDS and A_{570} value was determined.

1.7 Wound healing studies

Wound healing studies were carried out in the mid-dorsal region of Sprague Dawley rats according to the experiments of Staiano-Coico *et al*[15] with some modifications. Briefly, 20 Sprague Dawley rats about 2 months old were tested. Animals received food and water *ad libitum*. All manipulations were performed using aseptic techniques. On the day of wounding, animals were anesthetized with an intraperitoneal injection of 2% sodium pentobarbital at a ratio of 40 mg/kg body weight. The dorsal regions were shaved and the surgical area was disinfected with 70% alcohol. 10mm-diameter full thickness excisional wounds were made on the 20 rats' backs, with 6 wounds on the two sides of each. Then the rats were randomly divided into five groups (n =4) as follows: blank control, vehicle control, 12.5 mg/L KGF-2 treatment group, 2.5 mg/L KGF-2 treatment group, and 0.5 mg/L KGF-2 treatment group. Medicals were topically deployed daily on the open wounds.

Wound diameters were measured everyday. At 1, 4, 8 and 12 d post-wounding, rats were euthanized by over doses of sodium pentobarbital. Skin samples containing the wounds in the center were harvested and fixed in neutral formaldehyde. After fixation the skin biopsy was embedded in paraffin.

1.8 Histological analysis

Paraffin sections were dewaxed by melting for 30～60 min at 60℃, cleared in xylene three times for 5 min, and re-hydrated in water solutions containing decreasing percentages of ethanol and stained with hematoxylin-eosin following a standard procedure.

1.9 Statistical analysis

Results were statistically evaluated by Student's t-test as indicated. The values were expressed as $\bar{x}\pm s$. $P<0.05$ were interpreted as indicating significant differences.

2 Results

2.1 Construction, expression, and purification of human recombinant KGF-2

The cDNA coding for mature KGF-2 was isolated at about 530 bp from the human kidney cDNA library (Figure 1a, lane *4*). The correspondent PCR product was ligated into the vector plasmid pT_7 450 and the correct plasmid construction $pT_7$450-kgf-2 was verified by restriction enzyme digestion(Figure 1b, lane *6*) and sequencing (data not shown). Like other FGF family members, KGF-2 is a heparin-binding growth factor.

So we applied the Heparin Sepharose™ CL-6B affinity column to purify the recombinant KGF-2 protein. The interest protein was eluted at approximately 1 mol/L NaCl and the purification efficiency was remarkable (Figure 1c, lane *8*, *9* and *10*).

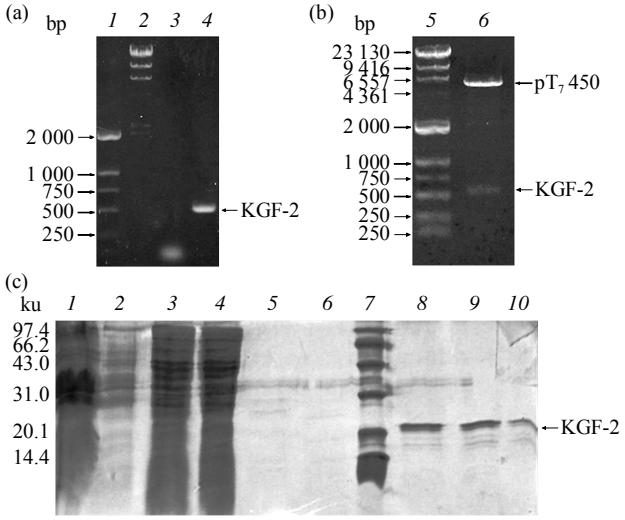

Fig. 1 Cloning and expression of Kgf-2 gene

(a), (b) Kgf-2 gene was isolated from the human kidney cDNA library and cloned into the vector pT_7450. *1*: DNA marker DL2000; *2*: λ/*Hin*d Ⅲ digestion; *3*: PCR product from other library; *4*: PCR product from the kidney cDNA library; *5*: Mixture of DL2000 and λ/*Hin*d Ⅲ digestion; *6*: Plasmid fractions digested with *Eco*R Ⅰ and *Hin*d Ⅲ. (c) Analysis of KGF-2 expression and purification. *1*: Cell pellet of whole; *2*: Cell pellet of sonication; *3*: Supernatant of sonication; *4*: Fraction of flow-through; *5*, *6*: Elution peels at less than 1 mol/L NaCl which represent nucleic acid; *7*: Standard protein marker; Lane *8*～*10*: Eluates of KGF-2.

2.2 KGF-2 stimulates keratinocyte cell proliferation

KGF-2 was thought to be an epithelial mitogen. The results depicted in Figure 2 revealed that KGF-2 could significantly stimulate the cell line HaCaT proliferation. The stimulatory effects of KGF-2 to the other three cell lines were almost indiscernible, suggesting that KGF-2 was a strong specific mitogen to the keratinocyte epithelia. It has the maximal stimulatory effect for cell proliferation at about 0.5 mg/L (Figure 2a) on day 3 (Figure 2b). Therefore we applied this concentration of KGF-2 in the following proliferation experiments unless exceptionally requested. Researches discovered that binding of FGFs to heparin or heparan sulfate proteoglycans (HSPGs) can facilitate FGF signal transduction by oligomerizing and presenting the ligands to high-affinity FGFRs [16], and stabilizing KGF-2 proteins in the cells. Consistent with findings by others, adding heparin (1 mg/L) could enhance its proliferation stimulatory effect (Figure 2c). This proliferative effect shown by MTT assay was verified by flow cytometry analysis, which showed that KGF-2 treatment increased cells in S phase (Figure 2d), and the PI value was increased significantly likewise (57.82% *vs*. 45.87% representing KGF-2 treated *vs*. untreated control respectively).

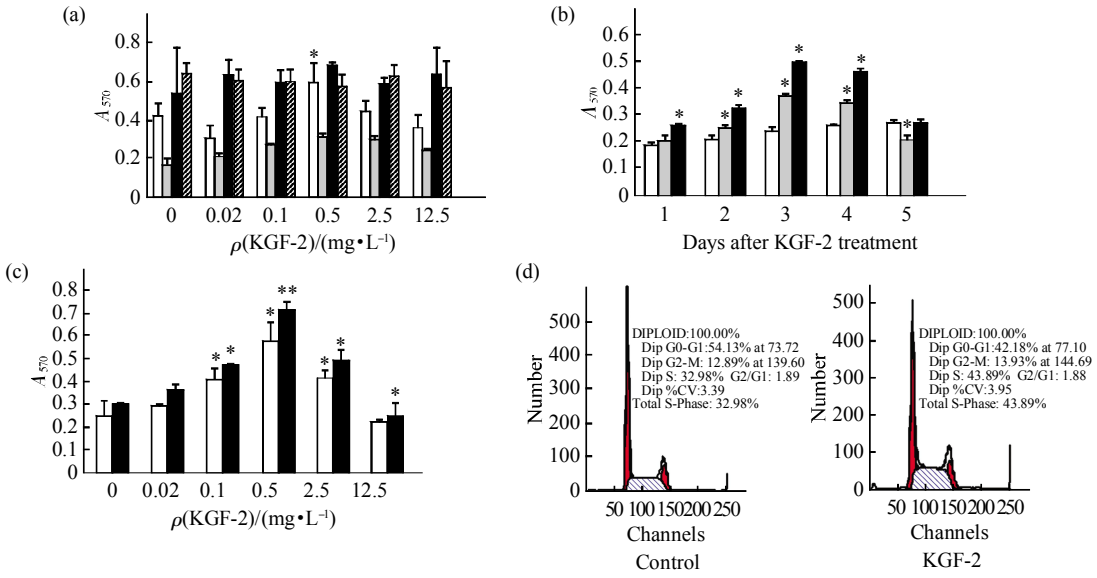

Fig. 2 KGF-2 could stimulate cell proliferation in HaCaT cell line

(a) KGF-2 could significantly stimulate HaCaT cell proliferation. It got the highest proliferation stimulatory effect at the concentration of 0.5 mg/L. □: HaCaT; ▨: HeLa; ■: H460; ▨: CV-1. (b) Time course of stimulatory effect of KGF-2 on HaCaT cell proliferation indicated that it got the highest proliferation stimulatory effect on day 3. □: Control; ▨: H–; ■: H+. (c) Heparin(1 mg/L) could enhance the stimulatory effect of KGF-2 on HaCaT cell proliferation. □: H–; ■: H+. (d) FACS analysis revealed that KGF-2 treatment increased the percentage of cells in S phase significantly. H–: Cell proliferation effect of KGF-2 without heparin; H+: Cell proliferation effect of KGF-2 with heparin. (The results represented means of triplicates. $^{**}P < 0.001$, $^{*}P < 0.05$.)

2.3 KGF-2 attenuates apoptosis of keratinocyte cells

Cell proliferation and apoptosis usually take place at the same time in cell growth. To address the effect of KGF-2 on cell apoptosis, we again employed MTT assay in which H_2O_2 was applied as an apoptotic agent [17]. MTT assay results indicated that the proliferating cells reduced after H_2O_2 induction, whereas KGF-2 recruited proliferating cells (Figure 3a). Similarly, analyzing with FACS(fluorescence activated cell sorter) disclosed that H_2O_2 decreased cells in S phase, droved cells to the gap period, while treatment with KGF-2 before H_2O_2 management markedly restored cells in the S phase (Figure 3c). In addition, *in situ* fluorometric TUNEL assay showed that KGF-2 could significantly protect cells from confronting apoptosis (Figure 3b and Figure 4). These data revealed that KGF-2 could attenuate apoptosis of keratinocyte cells.

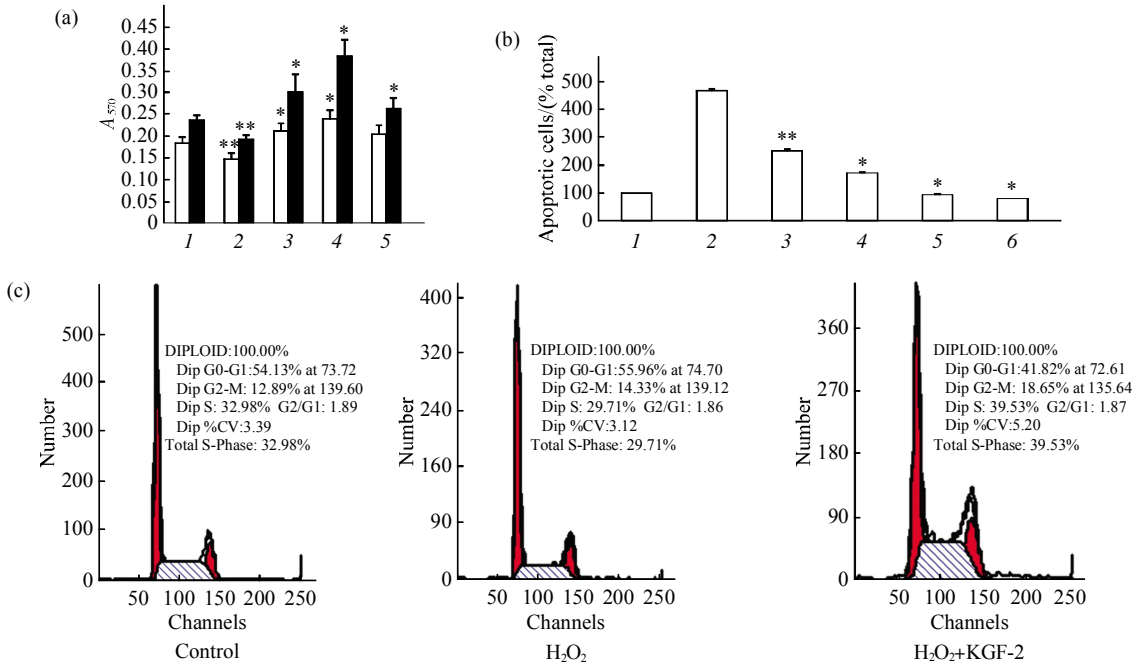

Fig. 3 MTT and TUNEL assay results of KGF-2 on H_2O_2-induced apoptosis

(a) MTT assay indicated that the proliferating cell number reduced after H_2O_2 treatment and KGF-2 management could recruit the proliferating cells, where 24 h treatment at the concentration of 0.5 mg/L had the most pronounced effect(*P < 0.05 concerning KGF-2 pretreatment *vs*. H_2O_2 treatment directly; **P <0.05 concerning H_2O_2 treatment *vs*. control). □: 12 h; ■: 24 h. *1*: Control; *2*: H_2O_2; *3*: H_2O_2+KGF-2(2.5 mg/L); *4*: H_2O_2+KGF-2(0.5 mg/L); *5*: H_2O_2+KGF-2(0.1 mg/L). (b) Total and apoptotic cells were counted. Data were normalized to control, which was assigned a value of 100% and all others were expressed as a percent of that value. Long as 17 h treatment of KGF-2 before H_2O_2 inducing could attenuate cell's apoptosis. (Results presented $\bar{x} \pm s$ of 5 different areas. **P < 0.001, *P < 0.05). *1*: Control; *2*: H_2O_2; *3*: KGF-2+H_2O_2(0 h); *4*: KGF-2+H_2O_2(2 h); *5*: KGF-2+H_2O_2(17 h); *6*: KGF-2+H_2O_2(24 h). (c) FACS analysis revealed that KGF-2 could revive the apoptotic cells induced by H_2O_2.

2.4 KGF-2 promotes migration of keratinocyte cells

Cell migration is an important part in wound healing and other pathogenesis. KGF-2 is supposed to be a wound healing candidate regent, but little is known on the effect of KGF-2 on cell migration. To

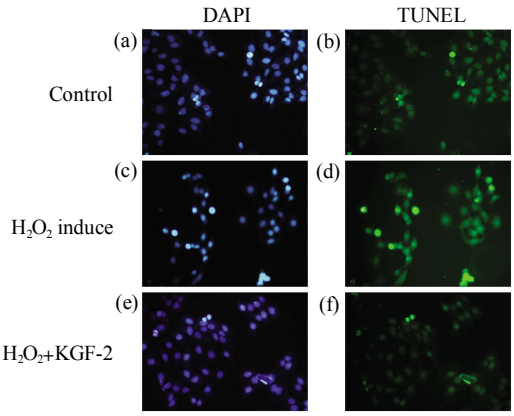

Fig. 4 Representative apoptosis labeling results of KGF-2 on HaCaT detected by DeadEnd™ Fluorometric TUNEL System

0.5 mg/L KGF-2 could significantly protect cells from apoptosis (e, f). (a, c and e) DAPI labeling of control and KGF-2 treated cells respectively. (b, d and f) DeadEnd™ Fluorometric TUNEL System labeling of untreated controls and KGF-2 treated cells respectively. a, b represent control cells that were treated with neither H_2O_2 nor KGF-2. c, d represent apoptotic cells induced by H_2O_2 without KGF-2 protection, e, f represent cells that were treated with KGF-2 (2.5 mg/L) for 24 h followed by H_2O_2 inducing. The brilliant green fluorescence means the cells confronting apoptosis.

test whether KGF-2 could promote cultured keratinocytes cell migration, we applied the migration assays using transwell chambers. Cells were allowed to migrate from the upper chamber to the lower chamber for 20 h. This time point was chosen based on our previous studies(data not shown). As shown in Figure 5, the HaCaT cells migrated faster through the filter within 20 h in wells treated with KGF-2 than in control wells. Numbers of migrated cells, measured by crystal violet staining, were related with the concentration.

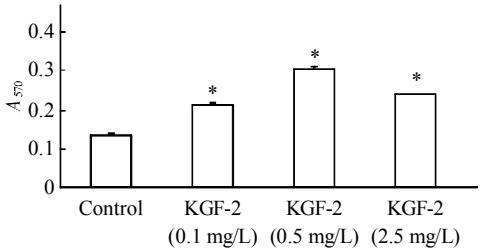

Fig. 5 KGF-2 promotes migration of keratinocytes

Indicated concentrations of KGF-2 were added into the lower chamber of the 24-well transwell plates and the cells(1×10^4 cells) were added in the upper chamber to carry out the migration assays. Results showed that as little as 0.1 mg/L KGF-2 could promote HaCaT cell migration and it had the strongest migration stimulatory effect at the concentration of 0.5 mg/L. (All the values represent $\bar{x} \pm s$ from triplicate samples. *$P < 0.001$).

2.5 Effect of exogenous KGF-2 on wound healing

0.5 mg/L KGF-2 treatment accelerated wound closure in Rat full thickness excisional surgery. As shown in Figure 6e, wounds treated with 0.5 mg/L KGF-2 mainly got 100 percent healed at 12 d post-wounding while wounds in other groups healed less than 90% mostly. HE staining of the 4 d post-wounding skin specimen revealed that the density of basal cells was significantly higher in the KGF-2 treated wounds and the granulation tissue collagens were much more conferted too versus control (Figure 6a, b, c and d).

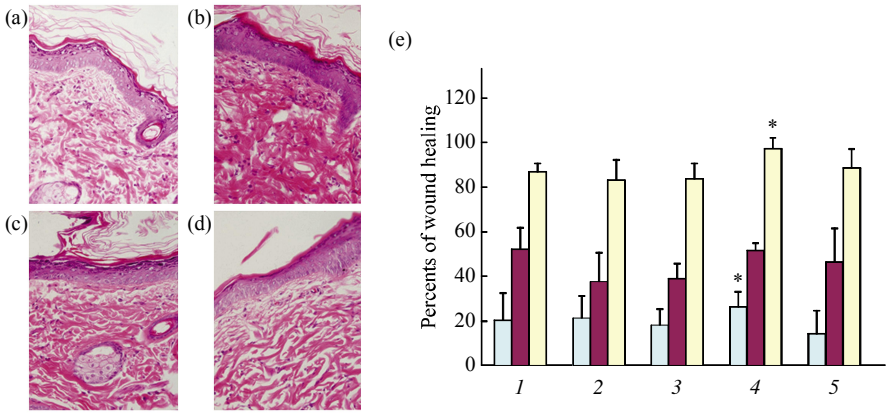

Fig. 6 KGF-2 improved wound healing in Rat full thickness excisional surgery

Exogenous recombinant KGF-2 was applied on healing wounds. Wound diameters were measured everyday. Skin samples containing the wounds in the center were harvested at 1, 4, 8 and 12 day post-wounding. 4 d post-wounding specimens HE staining revealed that the density of basal cells was significantly higher in the KGF-2 treated wounds and the granulation tissue collagens beneath the basal cells were much more conferted. (a) Control specimen. (b) Specimen of 0.5 mg/L KGF-2 treated wound. (c) Specimen of 12.5 mg/L KGF-2 treated wound. (d) Specimen of 2.5 mg/L KGF-2 treated wound. (e) 0.5 mg/L KGF-2 treatment almost got 100 percent healed at 12 d post-wounding while wounds in other groups healed less than 90% mostly. Graphs display the percentage of the healed wound. Bars indicate $\bar{x} \pm s$ of pooled data (*$P < 0.05$ concerning KGF-2 treatment versus control. The hydrogel vehicle treatment had no significant effect on wound healing versus control). *1*: Hgelbase; *2*: Hgel+KGF-2 (12.5 mg/L); *3*: Hgel+KGF-2 (2.5 mg/L); *4*: Hgel+KGF-2 (0.5 mg/L); *5*: Control. □: day4; ■: day8; □: day12.

3 Discussion

KGF-2 is a recently identified fibroblast growth factor. It is mainly synthesized by mesenchymal cells and acts predominantly on epithelial cells in a paracrine manner [1]. Its actions are dependent on its binding to the iiib isoform of the cell-surface FGF receptor 2(FGFR2iiib)[2], which is mainly expressed on epithelial origin. KGF-2 is known to be expressed in the normal lung, heart, skin, brain, and prostate [1, 2, 4, 18]. In our study, we isolated the cDNA sequence encoding KGF-2 from the human kidney cDNA library, revealing that the Kgf-2 gene is also expressed in the kidney.

Mature Kgf-2 gene has two cystines within the coding sequence, which might form a disulfide bond. So we finally chose the cell line Origami(DE3) as host, which greatly enhanced disulfide bond formation in

the cytoplasm and promoted the expression efficiency significantly. MTT assay and FACS flow cytometry assay were carried out on HaCaT to view the purified protein's activity. And we also surveyed its proliferative effects on other three cell lines. Results indicated that KGF-2 could specifically stimulate the epithelial keratincyte cell proliferation.

There are quite many studies about the proliferation stimulatory effect of KGF-2. But there are few studies addressed its function on cell apoptosis. What is the function of KGF-2 on cultured keratinocyte cell apoptosis? As shown in Figure 3, KGF-2 treatment significantly protected cells from apoptosis. And a fluorometric TUNEL system that detects degradation of genomic DNA for the apoptotic cells by the DNA end labeling[19] got the same results. We also saw many cells treated with H_2O_2 presented morphological changes: cell size became smaller, cell morphology grew rounder and even the nucleus were more rippled. KGF-2 consumption restituted cell morphology conspicuously(data not shown).

Our study supported the accumulating evidence showing that KGF-2 contributes to cell against apoptosis. Tao and colleagues found that in Kgf-2-null mice, the mutant epithelium showed a dramatic decrease in proliferation using a BrdU incorporation assay, but no prominent changes in the apoptotic cell numbers were detected in Kgf-2-null eyelids compared with their wild-type littermates using an *in situ* TUNEL assay[20]. Nevertheless, Upadhyay and colleagues found that KGF-2 could attenuate H_2O_2-induced DNA damage in alveolar epithelial cell (AEC)[17]. They got similar results about KGF-2 in inhibiting asbestos[21] and mechanical stretch-induced[22] alveolar epithelial cell apoptosis and concluded that KGF-2 could protect cells from apoptosis by mechanisms that involve activation of Ras/RAF-1/ERK1/2 pathway.

Comparable to the above results, our study discovered that KGF-2 exposure for as little as 2 h could protect HaCaT cells from H_2O_2-induced apoptosis (Figure 3). It suggested that KGF-2 could improve HaCaT cell growth by stimulating cell proliferation and meanwhile attenuating cell apoptosis.

However, the exact pathways involved in inhibition of apoptosis by KGF-2 still remained to be illuminated because of the complicated mechanisms for apoptosis. Activation of the Ras/RAF-1/ERK1/2 pathway has also been implicated by other factors in cell apoptosis[23]. Some other pathways such as the PI3K-Akt pathway were also suggested to be important for the antiapoptotic effect. In the chondrogenic cell line ATDC5, both pathways were implicated in inhibition of apoptosis by IGF-I[24]. Nevertheless, it should be noted that there are also indications for the involvement of Akt-independent pathways *via* PI3K in inhibition of apoptosis[25]. In addition, KGF-1, the homologue of KGF-2, could carry out protective effect on H_2O_2-induced apoptosis by significantly decreasing the permeability of the uninjured monolayers and completely preventing the increase in permeability caused by H_2O_2[26]. Thus, future studies are necessary to address the role of KGF-2 on cell apoptosis and to determine the exact mechanisms it employed.

Cell migration is an important part in wound healing and other pathogenesis. Studies indicated that KGF-2 was required for both proliferation and coordinated migration of epithelial leading edge cells *in vivo* by studying the Kgf-2$^{-/-}$ knockout mice. Researchers observed that addition of KGF-2 protein could up-regulate the expression of activin βB, which is probably associated with epithelial movement as it can regulate actin fiber formation[20]. Our study did support the migration stimulatory effect of exogenous KGF-2 in cultured keratinocyte (Figure 5).

The healing of wounds proceeds in three overlapping phases: inflammation, granulation tissue formation, and matrix formation and remodeling[27]. Growth factors are important tissue repair-signaling peptides up-regulated during the earliest phases of acute wound healing. Growth factor deficiencies lead to impaired wound healing, as reduced levels of numerous growth factors have been observed in chronic wounds when compared with normal acute wounds. However, 5～7 days are required before peak levels of growth factors are reached in wounds. The exogenous addition of recombinant growth factors could provide the necessary signals for fibroblast migration and proliferation thereby shorten the wound healing process[28～30]. Steed *et al.*[31] first reported a successful clinical trial using recombinant platelet-derived growth factor-BB (PDGF-BB) to accelerate closure of diabetic foot ulcers. Tsuboi's experiments indicated that recombinant bFGF was capable of significantly improving the degree of dermal healing in db/db mice by promoting the growth of granulation tissues and increasing infiltrated macrophages and fibroblasts, capillary number and tensile strength[32]. As the expression level of KGF-2

was significantly up-regulated at various times during wound healing [33, 34] and it was supposed to be a candidate which could compensate for the wound healing defects in KGF-1 knockout mice [35], it is proposed be a potential wound healing agent.

Growth factors applied to an open wound in a liquid formulation makes containment within the wound difficult and equal distribution throughout the wound nearly impossible [36]. Therefore we applied a hydrogel dressing which could improve the handling and delivery of growth factors for open wound models and the released KGF-2 was able to diffuse toward the wound site.

Our observations showed that topical application of KGF-2 daily displayed increases in epidermal proliferation and collagen deposition, both of which playing important roles in skin wound healing, and resulted in accelerated wound closure (Figure 6), suggesting that KGF-2 promoted wound healing mainly by enhanced reepithelialization and granulation tissue deposition in the early phase of wound healing. This is different from that of bFGF, which had little or no effect on the degree of reepithelialization [32]. The situation that KGF-2 could stimulate reepithelialization is generally recognized due to its direct effect on keratinocytes. The enhanced granulation tissue deposition induced by KGF-2 is speculated to be an indirect action including release of growth factors (PDGF, TGF, FGF) from the newly formed keratinocytes at the wound site [37] in respect that epithelial cells are the exclusive target cell type for KGF-2.

In summary, the recombinant KGF-2 prepared from *E. coli* cell is able to stimulate cell proliferation and attenuate cell apoptosis. It has modest migration stimulatory effect and is capable of accelerating excisional wound healing. It will be also interesting in the future to determine whether in pathological situations leading to wound healing defects, such as diabetes, the requirement for KGF-2 is more pronounced than in normal animals.

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角质细胞生长因子 2 对细胞生长、移行及创伤愈合的作用

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摘要 角质细胞生长因子 2(KGF-2)是成纤维细胞生长因子超家族的一员，由间质细胞合成并分泌，能特异促进上皮细胞增殖、分化与迁移，对脊椎动物多种组织和器官的发育起重要调控作用．通过 PCR 从人的肾组织 cDNA 文库中克隆分离获得了 KGF-2 的 cDNA，表明该因子在成人肾中有表达．采用大肠杆菌表达并纯化重组蛋白用于生物学功能研究的结果显示：KGF-2 在体外不仅能够促进角质细胞的生长和增殖，而且对其凋亡具有抑制作用，还对细胞的移行具有影响．在动物实验中，KGF-2 能促进皮肤切除产生的伤口愈合，提示该蛋白质可以作为创伤治疗或辅助用药的候选分子．

关键词 角质细胞生长因子 2，细胞生长，细胞凋亡，细胞移行，伤口愈合，治疗应用
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