Mol. Cells *36*, 316-321, October 31, 2013
DOI/10.1007/s10059-013-0093-y
eISSN: 0219-1032

Molecules and Cells
http://molcells.org
Established in 1990

The Effects of Adenoviral Transfection of the Keratinocyte Growth Factor Gene on Epidermal Stem Cells: an *In Vitro* Study

Xinping Li[1], Ling Liang[2], Pin Zhao[3], Kenzo Uchida[4], Hisatoshi Baba[4], Hong Huang[5], Wenfang Bai[1], Liming Bai[1], and Mingsheng Zhang[1,2,*]

Epidermal stem cells (ESCs) are characterized as slow-cycling, multi-potent, and self-renewing cells that not only maintain somatic homeostasis but also participate in tissue regeneration and repair. To examine the feasibility of adenoviral vector-mediated keratinocyte growth factor (KGF) gene transfer into *in vitro*-expanded ESCs, ESCs were isolated from samples of human skin, cultured *in vitro*, and then transfected with recombinant adenovirus (Ad) carrying the human KGF gene (AdKGF) or green fluorescent protein gene (AdGFP). The effects of KGF gene transfer on cell proliferation, cell cycle arrest, cell surface antigen phenotype, and β-catenin expression were investigated. Compared to ESCs transfected with AdGFP, AdKGF-transfected ESCs grew well, maintained a high proliferative capacity in keratinocyte serum-free medium, and expressed high levels of β-catenin. AdKGF infection increased the number of ESCs in the G0/G1 phase and promoted ESCs entry into the G2/M phase, but had no effect on cell surface antigen phenotype ($CD49f^+/CD71^-$). The results suggest that KGF gene transfer can stimulate ESCs to grow and undergo cell division, which can be applied to enhance cutaneous wound healing.

INTRODUCTION

Tissue-specific stem cells are present in most adult tissues and are thought to maintain tissue mass homeostasis and enable repair of tissue damage. Experimental evidences have shown that the epidermis, which forms the outer covering of mammalian skin, is maintained throughout life by proliferation of epidermal stem cells (ESCs), which are regarded as the progenitor cells of keratinocytes and are characterized by $CD49f^+/CD71^-$ expression (Barthel and Aberdam, 2005) and differentiation of progeny cells (Blanpain and Fuchs, 2009; Ghazizadeh and Taichman, 2001; Ito et al., 2005). A deficiency of ESCs has been reported to deregulate the process of re-epithelialization during cutaneous wound healing (Charruyer and Ghadially, 2009; Matsumoto et al., 2005).

Over the past 20 years, genetically engineered *ex vivo*-expanded adult stem cells have been investigated as an attractive tool for gene therapy, because these cells avoid some of the risks and disadvantages associated with direct *in vivo* delivery of viral vectors, non-viral vectors, and genetically modified *in vitro*-expanded differentiated cells. These genetically modified adult stem cells not only express the target protein but also enhance tissue regeneration and organ repair (Charruyer and Ghadially, 2009; Gnecchi, et al., 2005; Matsumoto, et al., 2005). ESCs have multipotent differentiation capability (Janes et al., 2002) and could possibly play an important role in the process of wound healing. They have already been shown to be beneficial in the formation of hair follicles and sweat glands (Charruyer and Ghadially, 2009). Therefore, these cells can be used for gene delivery in an adult stem cell-based gene therapy strategy.

Keratinocyte growth factor (KGF), a monomeric peptide that belongs to the fibroblast growth factor (FGF) family (Branski et al., 2007), is produced by cells of mesenchymal origin and mediates epithelial cell proliferation and differentiation in a variety of tissues such as lung and skin (Auf dem Keller et al., 2004; Yu et al., 2010). This paracrine action of KGF on epithelial cells is mediated through the KGF receptor, a splice variant of the FGF-2 receptor encoded by the FGF receptor gene. Indeed, KGF is a well-established mitogen for keratinocytes (Andreadis et al., 2001), and it has also been shown to promote early differentiation and inhibit terminal differentiation of cultured keratinocytes (Bao et al., 2005; Deters et al., 2005). However, the effects of KGF on ESCs are poorly understood. Specifically, there is little information on the proliferation and differentiation of ESCs after KGF infection.

The present study was designed to determine the *in vitro* proliferation capability of ESCs after KGF infection. For this purpose, we isolated human ESCs (hESCs) from human epider-

[1]Department of Physical Medicine and Rehabilitation, Guangdong Geriatric Institute, Guangdong Academy of Medical Sciences and Guangdong General Hospital, Guangzhou, Guangdong Province 510080, China, [2]The First Clinical College of Jinan University, Guangzhou 510632, China, [3]Changsha Medical University, Changsha 410219, China, [4]Department of Orthopaedics and Rehabilitation Medicine, Fukui University Faculty of Medical Sciences, Eiheiji Matsuoka, Fukui 910-1193, Japan, [5]School of Information, University of South Florida, USA
*Correspondence: mszrch@163.com

Received March 22, 2013; revised July 22, 2013; accepted August 1, 2013; published online October 22, 2013

Keywords: adenoviral vector, epidermal stem cells, keratinocyte growth factor

Springer

mis samples, cultured them *in vitro* before transfecting them with a recombinant adenovirus (Ad) carrying the human KGF gene, and examined the effects of KGF infection on hESCs. We also investigated the expression of β-catenin in this process.

MATERIALS AND METHODS

Adenoviral vector

AdKGF, a replication-deficient recombinant Ad carrying the human KGF gene under the control of the cytomegalovirus (CMV) promoter, and Ad green fluorescent protein (GFP), a replication-deficient recombinant Ad carrying GFP under the control of the CMV promoter, were prepared using the pAD-easy I system (Luo et al., 2007). The AdKGF virus titer was 1.8×10^{10} plaque-forming units (pfu) per milliliter. The AdGFP virus titer was 1.6×10^{10} pfu/ml.

Cell culture

Biopsy samples from foreskin were harvested for diagnostic purposes from young men (N = 4) aged 10-20 years and processed for the preparation of hESCs. Informed consent was obtained from all subjects or their parents. The institutional Ethics Review Council for Stem Cell Research (China) approved the study protocol, and the study strictly followed the institutional review board (IRB) guidelines of Guangdong General Hospital, Guangzhou, China.

All samples were processed using a previously described method (Dong et al., 2009; Tao et al., 2007). Briefly, after removal of fat and attached membranes, the skin was sliced into 10-mm wide strips, immersed in Dispase II solution (1 U/ml; Gibco, USA), and incubated overnight at 4°C. Subsequently, the epidermis was separated from the dermis by gently pulling apart the tissues using a pair of sterile forceps, triturated with a pipette, digested with a solution of 0.25% trypsin plus 0.02% ethylene diaminetetraacetic acid (Gibco) for 5 min at 37.0°C, and passed through a 200-μm nylon mesh. Then the cells were centrifuged at 1,200 rpm for 5 min, resuspended in a keratinocyte serum-free medium (K-SFM) (supplemented with 5 ng/ml epidermal growth factor and 50 mg/ml bovine pituitary extract; Gibco), and plated into 25-cm^2 culture flasks preprocessed using type IV collagen (100 μg/ml; Sigma, USA). Cells that did not attach after 10 min were discarded to separate the rapidly attaching stem cells from the slower adhering keratinocytes. The stem cells were passaged at 90% confluence, and cells between passages 3 and 4 were used for experiments.

Transduction with adenoviral vectors

The hESCs were plated at a density of 1×10^5 cells/cm^2 in six-well plates (BD Biosciences, USA) that had been preprocessed using type IV collagen and cultured overnight at 37.0°C in a humidified 5% CO_2 environment. When the stem cell density reached 70-80% confluent, hESCs were infected with vectors at multiplicity of infection (MOI) values of 50, 100, 150, or 200 for 48 h. After 48 h, GFP expression was observed under a fluorescence microscope (Olympus, Japan). The data were estimated by a single-blind method. Separate experiments were carried out in triplicate.

Western blot analysis

Western blot analysis for KGF transgene expression in cultured hESCs was carried out using whole cell lysates. Briefly, cells in six-well plates were rinsed with cold phosphate-buffered saline (PBS), drained, lysed with a buffer containing 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 0.1 mg/ml leupeptin, and 574 μM phenylmethylsulfonyl fluoride, and scraped into a 1.5-ml centrifuge tube. The samples were incubated for 15 min on ice and centrifuged at 8,000 × *g* for 15 min at 4°C, and the supernatant was collected. The protein content of whole cell lysate samples was quantified colorimetrically using the bicinchoninic acid protein assay (Pierce, USA). Whole cell lysate (5 μg) was loaded onto a 10% Tris-glycine gel (Beyotime, China). After electrophoresis, the protein was transferred onto a nitrocellulose membrane by electroelution. Immunodetection was performed with rabbit anti-KGF monoclonal antibody (Boster, China; dilution 1: 200) and mouse anti β-actin polyclonal antibody (Santa Cruz Biotechnology, USA dilution 1:2,000). The secondary antibodies were horseradish peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, dilution 1:5,000) or anti-mouse IgG (Santa Cruz Biotechnology, dilution 1:8,000). The nitrocellulose membrane was processed using enhanced chemiluminescence Western blotting detection reagents (Amersham Pharmacia Biotech, USA). Exposure was conducted using X-ray films (Eastman Kodak, USA).

Table 1. Primers used in real-time RT-PCT analysis

Gene	Primers	Annealing temperature
β-catenin	5′-GGGCGGCACCTTCCTACTCC-3′ 5′-AGCTCCCTCGCGGTTCAT-3′	60°C
β-actin	5′-GCTATCCAGGCTGTGCTAA-3′ 5′-GATGGAGTTGAAGGTAGTTT-3′	60°C

3(4,5 dimethylthiazol-2-yl)-2,5, diphenyltetrazolium bromide assay

The hESCs were seeded in 96-well plates (1×10^4 cells per well) and cultured in K-SFM containing adenoviral vectors at an MOI of 100. AdKGF-transfected and AdGFP-transfected cells were cultured at 37°C with 5% CO_2 for 24 h, and then the medium was changed with fresh K-SFM for continued culture at 37°C with 5% CO_2 for an additional 48 h. Cell proliferation was determined using the MTT [3(4,5 dimethylthiazol-2-yl)-2,5, diphenyltetrazolium bromide] assay. In brief, the cells were incubated with 20 μl of 1 mg/ml MTT (Sigma, USA) for 4 h, and the medium was subsequently removed. Dimethyl sulfoxide (150 μl) was added to each well, and plates were vortexed at low speed for 10 min to fully dissolve the blue crystals. The absorbance of each well was measured at 490 nm (OD_{490}) and directly correlated to cell number.

Flow cytometry

For cell cycle analysis, 5×10^3 hESCs were seeded in 6-well plates. When cell confluence reached 50-60%, hESCs were infected with vectors at an MOI of 100 for 24 h and further cultured in fresh K-SFM for 48 h. hESCs were harvested, resuspended in 250 μl cold PBS, then washed three times, and fixed with 0.5 ml ice-cold 70% ethanol for 12 h at -20°C. After washing with ethanol, the fixed cells were treated with 50 μg/ml RNAse A (MultiSciences, China), incubated with 50 μg/ml propidium iodide (PI; MultiSciences) at room temperature for 30 min, and then analyzed using a flow cytometer (Beckman Coulter, USA). The resulting data were analyzed in a blinded fashion using multicycle software (Beckman Coulter).

For analysis of cell phenotypes by cell surface antigen ex-

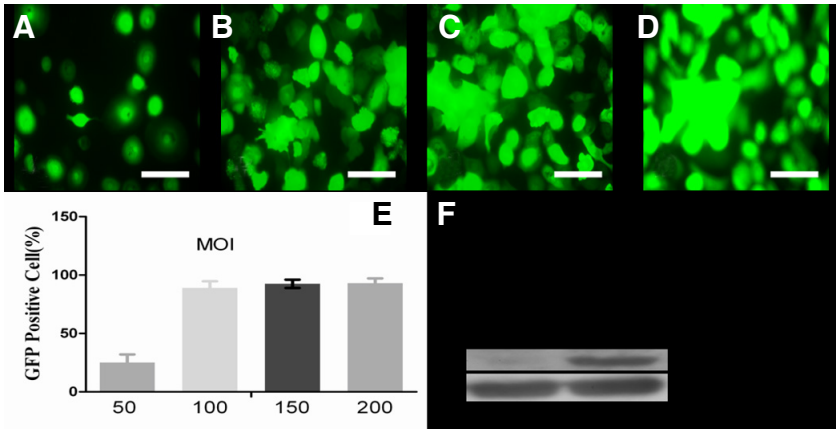

Fig. 1. Efficiency of transfer of adenoviral vector containing the green fluorescent protein gene (AdGFP) into human epidermal stem cells (hESCs). Values are mean ± SD (n = 2). (A-D) Photomicrographs of GFP expression in AdGFP-transduced hESCs. hESCs were transduced at different multiplicity of infection (MOI) values at 48 h. Scale bars: (A-D) = 100 μm. (A), MOI = 50; (B), MOI = 100; (C), MOI = 150; (D), MOI = 200; (E) GFP^+ cells were counted in three fields by microscopic observation to estimate the transduction efficiency. Results are represented as the percentage of GFP^+ cells at each MOI. (F) hESCs were infected with AdKGF, and KGF transgene expression in culture was determined by Western blot analysis using whole cell lysates.

pression, hESCs were seeded in 6-well plates (1×10^5 cells per well) and cultured in K-SFM containing AdKGF or AdGFP at an MOI of 100 for 24 h. The medium was then changed with a virus-free medium, and culture was continued for 48 h before cell harvesting. Cells were blocked in Fc block (Miltenyi Biotec, Australia) for 3 min at room temperature. The primary antibodies, rabbit anti-human CD49f and mouse anti-human CD71 (both from eBioscience, USA), were added, and cells were incubated on ice for 20 min. Negative control cells were exposed to IgG2b-PE or IgG1-APC (both from Santa Cruz Biotechnology). Excess antibodies were rinsed away, and antibodies were conjugated with secondary antibodies. Samples were analyzed with the flow cytometer (Beckman Coulter).

Real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis

hESCs were seeded in 6-well plates (1×10^6 cells per well) and cultured in K-SFM containing AdKGF or AdGFP at an MOI of 100 for 24 h. The medium was then replaced with virus-free medium, and culture continued for 48 h. Total RNA was extracted using the TRIZol reagent (Invitrogen, USA) from hESCs transduced with AdGFP or AdKGF and cultured for 48 h on collagen IV. Approximately 1 μg total mRNA was reverse transcribed to cDNA using the One Step RT-PCR Kit (Takana Biomedicals, Japan) according to the instructions of the manufacturer and amplified in a Perkin Elmer Thermal Cycler 480 (USA). PCR conditions were set as follows: 2 min at 95 °C followed by 39 cycles of 20 s at 95°C, 20 s at 60°C, and 30 s at 72°C, and a final extension at 72°C (5 min). The primers used in the RT-PCR are listed in Table 1. For each PCR product, a single narrow peak was obtained by melting curve analysis at the specific melting curve temperature, indicating specific am-plification. Samples were tested in triplicate, and the average values were used for quantification. For each sample, the ratio between the relative expression values of β-catenin and β-actin was calculated to compensate for variations in quantity or quality of starting mRNA, as well as for differences in the efficiency of the reverse transcription process. The relative amount of each gene was determined using the $2^{-\Delta\Delta}$Ct method (Livak and Schmittgen, 2001).

Statistical analysis

All data are presented as mean ± standard deviation (SD). We used a Student *t*-test for the comparisons of the results of the MTT assay. All other results were evaluated using two-way analysis of variance (ANOVA) followed by post hoc Tukey-Kramer test. A P value less than 0.05 was considered statistically significant.

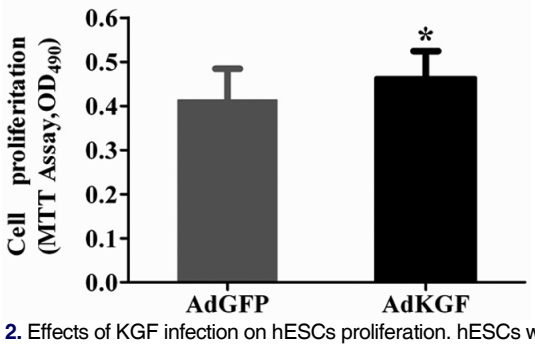

Fig. 2. Effects of KGF infection on hESCs proliferation. hESCs were cultured in a keratinocyte serum-free medium and transfected with AdKGF or AdGFP. After further culture for 12 h, the medium was changed, and after an additional 48 h in culture, cell growth was assessed by the MTT assay. Data are the mean values of triplicate determinations ± SD (n = 4); *P < 0.05, compared with the AdGFP group.

RESULTS

Adenoviral transduction of *in vitro*-expanded hESCs

The efficiency of adenoviral-mediated gene transfer into *in vitro*-expanded hESCs was examined using hESCs transfected with AdGFP at MOIs of 50, 100, 150, and 200. After 48 h, GFP expression was assessed under a fluorescence microscope. As shown in Fig. 1E, the transduction efficiency was > 50% at an MOI of 50 and > 90% at MOIs of 100, 150, and 200. KGF expression was assessed in AdKGF-transfected hESCs at an MOI of 100 using Western blot analysis and was confirmed in AdKGF-transfected hESCs (Fig. 1F).

KGF increases hESC proliferation

Next, we determined the effects of KGF on the cell growth rate

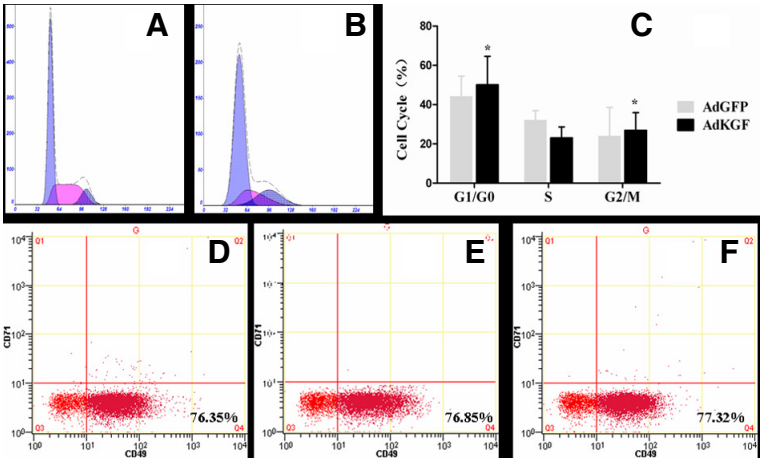

Fig. 3. Effects of KGF infection on the cell cycle distribution and cell surface antigen expression. hESCs were transfected with AdKGF or AdGFP and subjected to flow cytometric analysis to examine the effect of KGF on cell cycle distribution and cell surface antigen expression (n = 3). (A, B) Representative blots for the AdGFP group (A) and the AdKGF group (B). (C) The percentages of cells in the different cell cycle phases are shown, and significant differences between the AdGFP and AdKGF groups were as follows: the percentage of cells in the G0/G1 phase was lower in the AdGFP group than in the AdKGF group, but the percentage of cells in the G2/M phase was higher in the AdGFP group than in the AdKGF group. *P < 0.05. (D-F) Flow cytometric analysis showed no differences in CD49f or CD71 expression among cells of the untransfected group (D), the AdKGF group (E), and the AdGFP group (F).

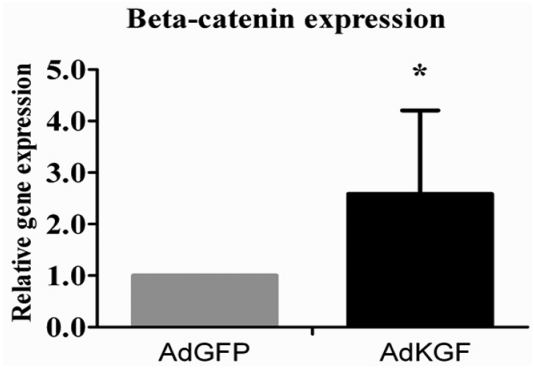

Fig. 4. Effects of KGF infection on β-catenin expression. hESCs were transfected with AdKGF or AdGFP. Total RNA was extracted after 48 h, and the β-catenin mRNA transcript level was determined by real-time RT-PCR and expressed as a fold-change from the level in the AdGFP group. The experiment was performed in triplicate. Data are mean ± SD.

of hESCs. The results in Fig. 2 showed that AdKGF infection increased the proliferation rate of hESCs to levels significantly higher than those of AdGFP-infected cells even after only 48 h.

Effect of KGF on cell cycle distribution and cell surface antigen expression

To confirm the MTT assay results, we determined the effect of KGF on cell cycle distribution and cell surface antigens. Cells were transfected with AdGFP or AdKGF. After 48 h, cell cycle analysis demonstrated that KGF arrested ESCs at the G0/G1 phase, and the fractions of G0/G1 phase in the AdKGF and AdGFP groups were 50.1 ± 9.57% and 44.1 ± 6.23%, respectively ($P < 0.05$). Furthermore, the percentages of hESCs at the G2/M phase in the AdKGF and AdGFP groups were 26.9 ± 2.53% and 22.8 ± 3.17%, respectively ($P < 0.05$; Figs. 3A-3C). The $CD49F^{+}/CD71^{-}$ cells are relatively quiescent in *vitro*, and populations of these cells have very high long-term proliferative capacity. Therefore, high expression of CD49f together with low expression of CD71 are considered markers of ESCs (Barthel and Aberdam, 2005). We also found that AdKGF infection had no significant effect on CD49f and CD71 expression relative to levels in control cells (Figs. 3D-3F), meaning that the percentages of $CD49f^{+}/CD71^{-}$ cells among AdKGF-infected hESCs remained unchanged.

Effects of KGF on β-catenin expression

The Wnt/β-catenin pathway is important in regulating ESC renewal and lineage selection. To investigate the involvement of β-catenin in the proliferation and differentiation of KGF-induced ESCs, hESCs were infected with AdGFP or AdKGF, and total RNA was extracted after 48 h and assayed for β-catenin expression by real-time RT-PCR. The level of β-catenin mRNA expression was increased > 2.5-fold in AdKGF-transfected cells compared to that in control cells (Fig. 4).

DISCUSSION

The present study demonstrated that AdKGF gene transduction into ESCs was feasible, and that the proliferation of ESCs was significantly increased by AdKGF infection. In addition, the results of cell cycle analysis supported this conclusion. Furthermore, AdKGF infection may influence hESC differentiation. These findings may be useful in the design of a new gene therapy strategy for cutaneous wound healing.

Ex vivo and *in vivo* strategies have been used to deliver therapeutic genes into cutaneous wounds (Keswani et al., 2004; Liechty et al., 1999; Wang et al., 2006). However, the disadvantages of these strategies include the local inflammatory response and random expression of the transgene in almost all cell types. Furthermore, previous studies reported the low efficiency of vector-mediated gene transfer *in vivo* (Lu and Ghazizadeh, 2007) and only transient *in vivo* gene expression after injection of vectors containing the exogenetic gene, probably due to inefficient transduction of ESCs (Kolodka et al., 1998). Therefore, genetically modified, *in vitro*-expanded adult stem cells are attractive alternatives for use in gene therapy. In this regard, one previous study demonstrated the formation of clonal units of epidermal structures after infusion of mouse ESCs transduced with retroviruses (Charruyer and Ghadially, 2009).

The cell cycle mainly proceeds in three phases, the G0/G1 phase, S phase, and G2/M phase. In general, the proportion of

S-phase cells is considered to represent the proliferative potential of a cell population. In this study, intriguingly, our results showed the percentage of AdKGF-transfected cells in the G2/M phase was higher than that in AdGFP-transfected cells, which conflicted with the results of our MTT assay. The MTT results showed that AdKGF infection significantly increased hESCs pro-liferation, whereas the proportion of AdKGF-transfected cells in the G0 and G2/M phases was higher than that of ADGFP-transfected cells. The reason for this discrepancy may be that AdKGF infection could shorten the S and G2/M phases to enhance cell proliferation.

Adult stem cells have been engineered for *ex vivo* gene therapy of various diseases (Ferrari et al., 2005), and the technique is relatively safe (Gregory et al., 2005). Transdifferentiated ESCs obtained from the skin (Watt et al., 2006) can both self-renew and produce differentiated progeny, which is beneficial in the formation of hair follicles and sweat glands (Qiao et al., 2008). Furthermore, KGF is known to regulate the migration, proliferation, differentiation, and survival of keratinocytes (Auf dem Keller et al., 2004). Several studies also suggested that KGF can increase the resistance of epithelial cells by altering the expression of mediators or antagonists of apoptosis (Buckley et al., 1998) or by altering the ability of cells to scavenge free radicals (Beeret et al., 2000). It is possible that injection of KGF gene-modified ESCs can promote wound healing. In the present study, genetically modified ESCs efficiently expressed KGF, and gene transfer of KGF into ESCs did enhance cell proliferation and maintenance of the stem cell phenotype. In addition, another study showed that KGF increased the number of progenitor/stem cells, which were considered the progenitor cells of epithelial cells, after irradiation *in vivo* (Braun et al., 2006), meaning that KGF may provide protection for ESCs against apoptosis triggered by unfavorable factors, such as inflammation. Thus, these results suggest that hESCs transduced with AdKGF may be useful in the treatment of chronic wounds.

In this study, AdKGF-transfected hESCs showed cell cycle arrest at the G0/G1 phase. In addition, AdKGF transfection did not alter the surface phenotype of the ESCs. The G1 phase represents the pre-differentiation arrest state at which cells can integrate the control of both proliferation and differentiation (Yun et al., 1983), and cells at the G1 phase can either reinitiate proliferation or differentiate. Furthermore, stem cells cannot self-differentiate. Thus, we suggest that KGF not only regulates cell proliferation, but also regulates maintenance of the stem cell phenotype by enhancing their resistance to stress, making ESCs less susceptible to their microenvironment. The results may explain in part why KGF increases the number of stem/progenitor cells after radiation (Lombaert et al., 2008). These findings also show that KGF infection of ESCs could be a strategy for selecting cells for the treatment of chronic wounds, because KGF-modified ESCs offer a source of stem cells and also release KGF to protect and expand ESCs, all while activating the cross-talk between epithelial and mesenchymal cells.

ESCs are tissue-specific stem cells mainly located in the basal layer and the bulge area of hair follicles (Ghazizadeh and Taichman, 2001; Ito et al., 2005). Although ESCs compose very little of the basal layer, they are quite important for epidermal maintenance and wound healing (Roh and Lyle, 2006). At least two signal transduction pathways have been implicated in stem cell fate determination, including the Notch signaling pathway and the Wnt signaling pathway (Gambardella and Barrandon, 2003). β-catenin is a downstream effector of the Wnt signaling pathway; a strong β-catenin signal pushes cells towards hair follicle differentiation, whereas a weak β-catenin signal in adult stem and progenitor cells directs differentiating cells to adopt an interfollicular epidermal fate (Huelsken et al., 2001; Zhu and Watt, 1999). To test the effects of KGF infection on ESCs differentiation, we analyzed β-catenin expression in total RNA from AdKGF-infected ESCs and observed upregulation of β-catenin RNA. This finding suggests that KGF infection affects the lineage selection process, although further experiments are needed to better understand these changes.

In summary, the present study reports adenoviral gene transfer of KGF into *in vitro*-expanded hESCs. AdKGF transduction enhanced ESCs proliferation and increased β-catenin expression. These findings suggest that our adult stem cell-based gene therapy strategy may be beneficial in the treatment of disorders characterized by low KGF activity.

ACKNOWLEDGMENTS

This work was supported by a Basic Research Grant S2012 010009308 and S2012010009498 from the Natural Science Foundation of Guangdong Province.

REFERENCES

Andreadis, S.T., Hamoen, K.E., Yarmush, M.L., and Morgan, J.R. (2001). Keratinocyte growth factor induces hyperproliferation and delays differentiation in a skin equivalent model system. FASEB J. *15*, 898-906.

Auf dem Keller, U., Krampert, M., Kümin, A, Braun, S., and Werner S. (2004). Keratinocyte growth factor: effects on keratinocytes and mechanisms of action. Eur. J. Cell Biol. *83*, 607-612.

Bao, S., Wang, Y., Sweeney, P., Chaudhuri, A., Doseff, A.I., Marsh, C.B., and Knoell, D.L. (2005). Keratinocyte growth factor induces Akt kinase activity and inhibits Fas-mediated apoptosis in A549 lung epithelial cells. Am. J. Physiol. Lung Cell Mol. Physiol. *288*, 36-42.

Barthel, R., and Aberdam, D. (2005). Epidermal stem cells. J. Eur. Acad. Dermatol. Venereol. *19*, 405-413.

Beer, H.D., Gassmann, M.G., Munz, B., Steiling, H., Engelhardt, F., Bleuel, K., and Werner, S. (2000). Expression and function of keratinocyte growth factor and activin in skin morphogenesis and cutaneous wound repair. J. Invest. Dermatol. Symp. Proc. *5*, 34-39.

Blanpain, C., and Fuchs, E. (2009). Epidermal homeostasis: a balancing act of stem cells in the skin. Nat. Rev. Mol. Cell Biol. *10*, 207-217.

Branski, L.K., Pereira, C.T., Herndon, D.N., and Jeschke, M.G. (2007). Gene therapy in wound healing: present status and future directions. Gene Ther. *14*, 1-10.

Braun, S., Krampert, M., Bodó, E,, Kümin, A., Born-Berclaz, C., Paus, R., and Werner, S. (2006). Keratinocyte growth factor protects epidermis and hair follicles from cell death induced by UV irradiation, chemotherapeutic or cytotoxic agents. J. Cell Sci. *119*, 4841-4849.

Buckley, S., Barsky, L., Driscoll, B., Weinberg, K., Anderson, K.D., and Warburton, D. (1998). Apoptosis and DNA damage in type 2 alveolar epithelial cells cultured from hyperoxic rats. Am. J. Physiol. *274*, 714-720.

Charruyer, A., and Ghadially, R. (2009). Stem cells and tissue-engineered skin. Skin Pharmacol. Physiol. *22*, 55-62.

Deters, A.M., Schröder, K.R., Smiatek, T., and Hensel, A. (2005). Ispaghula (Plantago ovata) seed husk polysaccharides promote proliferation of human epithelial cells (skin keratinocytes and fibroblasts) *via* enhanced growth factor receptors and energy production. Planta Med. *71*, 33-39.

Dong, R., Liu, X., Liu, Y., Deng, Z., Nie, X., Wang, X., and Jin, Y. (2007). Enrichment of epidermal stem cells by rapid adherence and analysis of the reciprocal interaction of epidermal stem cells with neighboring cells using an organotypic system. Cell Biol. Int. *31*, 733-740.

Ferrari, S., Pellegrini, G., Mavilio, F., and De Luca, M. (2005). Gene therapy approaches for epidermolysis bullosa. Clin. Dermatol. *23*, 430-436.

Gambardella, L., and Barrandon Y. (2003). The multifaceted adult

epidermal stem cell. Curr. Opin. Cell Biol. *15*, 771-777.
Ghazizadeh, S., and Taichman, L.B. (2001). Multiple classes of stem cells in cutaneous epithelium: a lineage analysis of adult mouse skin. EMBO J. *20*, 1215-1222.
Gnecchi, M., He, H., Liang, O.D., Melo, L.G, Morello, F., Mu, H., Noiseux, N., Zhang, L., Pratt, R.E., Ingwall, J.S., et al. (2005). Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat. Med. *11*, 367-368.
Gregory, C.A., Prockop, D.J., and Spees, J.L. (2005). Non-hematopoietic bone marrow stem cells: molecular control of expansion and differentiation. Exp. Cell Res. *306*, 330-335.
Huelsken, J., Vogel, R., Erdmann, B., Cotsarelis, G., and Birchmeier, W. (2001). Beta-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. Cell *105*, 533-545.
Ito, M., Liu, Y., Yang, Z., Nguyen, J., Liang, F., Morris, R.J., and Cotsarelis, G. (2005). Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. Nat. Med. *11*, 1351-1354.
Janes, S.M., Lowell, S., and Hutter, C. (2002). Epidermal stem cells. J. Pathol. *197*, 479-491.
Keswani, S.G, Katz, A.B., Lim, F.Y., Zoltick, P., Radu, A., Alaee, D., Herlyn, M., and Crombleholme, T.M. (2004). Adenoviral mediated gene transfer of PDGF-B enhances wound healing in type I and type II diabetic wounds. Wound Repair Regen. *12*, 497-504.
Kolodka, T.M., Garlick, J.A., and Taichman, L.B. (1998). Evidence for keratinocyte stem cells *in vitro*: long term engraftment and persistence of transgene expression from retrovirus-transduced keratinocytes. Proc. Natl. Acad. Sci. USA *95*, 4356-4361.
Liechty, K.W., Nesbit, M., Herlyn, M., Radu, A., Adzick, N.S., and Crombleholme, T.M. (1999). Adenoviral-mediated overexpression of platelet-derived growth factor-B corrects ischemic impaired wound healing. J. Invest. Dermatol. *11*, 3375-3383.
Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods *25*, 402-408.
Lombaert, I.M., Brunsting, J.F., Wierenga, P.K., Kampinga, H.H., de Haan, G., and Coppes, R.P. (2008). Keratinocyte growth factor prevents radiation damage to salivary glands by expansion of the stem/progenitor pool. Stem Cells *26*, 2595-2601.
Lu, Z., and Ghazizadeh, S. (2007). Loss of transgene following *ex vivo* gene transfer is associated with a dominant Th2 response: implications for cutaneous gene therapy. Mol. Ther. *15*, 954-961.
Luo, J., Deng, Z.L., Luo, X., Tang, N., Song, W.X., Chen, J., Sharff, K.A., Luu, H.H., Haydon, R.C., Kinzler, K.W., et al. (2007). A protocol for rapid generation of recombinant adenoviruses using the AdEasy system. Nat. Protoc. *2*, 1236-1247.
Matsumoto, R., Omura, T., Yoshiyama, M., Hayashi, T., Inamoto, S., Koh, K.R., Ohta, K., Izumi, Y., Nakamura, Y., Akioka, K., et al. (2005). Vascular endothelial growth factor-expressing mesenchymal stem cell transplantation for the treatment of acute myocardial infarction. Arterioscler. Thromb. Vasc. Biol. *25*, 1168-1173.
Qiao, R., Yan, W., Clavijo, C., Mehrian-Shai, R., Zhong, Q., Kim, K. J., Ann, D., Crandall, E.D., and Borok, Z. (2008). Effects of KGF on alveolar epithelial cell transdifferentiation are mediated by JNK signaling. Am. J. Respir. Cell Mol. Biol. *38*, 239-246.
Roh, C., and Lyle, S. (2006). Cutaneous stem cells and wound healing. Pediatr. Res. *59*, 100-103.
Tao, Q., Qiao, B., Lv, B., Zheng, C., Chen, Z., and Huang, H. (2009). p63 and its isoforms as markers of rat oral mucosa epidermal stem cells *in vitro*. Cell Biochem. Funct. *27*, 535-541.
Wang, X.T., Liu, P.Y., and Tang, J.B. (2006). PDGF gene therapy enhances expression of VEGF and bFGF genes and activates the NF-kappaB gene in signal pathways in ischemic flaps. Plast Reconstr. Surg. *117*, 129-139.
Watt, F.M., Lo Celso, C., and Silva-Vargas, V. (2006). Epidermal stem cells: an update. Curr. Opin. Genet. Dev. *16*, 518-524.
Yu, F.S., Yin, J., Xu, K., and Huang, J. (2010). Growth factors and corneal epithelial wound healing. Brain Res. Bull. *81*, 229-235.
Yun, K., Hoerl, B.J., and Scott, R.E. (1983). Efficient differentiation of proadipocyte stem cells on nonadherent surfaces: evidence for differentiation without DNA synthesis. J. Cell Physiol. *117*, 249-256.
Zhu, A.J., and Watt, F.M. (1999). Beta-catenin signalling modulates proliferative potential of human epidermal keratinocytes independently of intercellular adhesion. Development *126*, 2285-2298.