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Effects of keratinocyte growth factor on skin epithelial differentiation of human amnion epithelial cells

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ARTICLE INFO

Article history:
Accepted 23 October 2012

Keywords:
Amnion cells
Differentiation
Epithelial
Keratin
Keratinocyte growth factor
Stemness
Skin

ABSTRACT

The aim of the present study was to determine the effects of KGF on the differentiation of cultured human amnion epithelial cells (HAECs) towards skin keratinocyte. HAECs at passage 1 were cultured in medium HAM's F12: Dulbecco's Modified Eagles Medium (1:1) supplemented with different concentrations of KGF (0, 5, 10, 20, 30 and 50 ng/ml KGF). Dose–response of KGF on HAECs was determined by morphological assessment; growth kinetic evaluation; immunocytochemical analysis; stemness and epithelial gene expression quantification with two step real time RT-PCR. KGF promotes the proliferation of HAECs with maximal effect observed at 10 ng/ml KGF. However, KGF decreased the stemness genes expression: Oct-3/4, Sox-2, Nanog3, Rex-1, FGF-4, FZD-9 and BST-1. KGF also down-regulates epithelial genes expression: CK3, CK18, CK19, Integrin-β1, p63 and involucrin in cultured HAECs. No significant difference on the gene expression was detected for each Nestin, ABCG-2, CK1 and CK14 in KGF-treated HAECs. Immunocytochemical analysis for both control and KGF-treated HAECs demonstrated positive staining against CK14 and CK18 but negative staining against involucrin. The results suggested that KGF stimulates an early differentiation of HAECs towards epidermal cells. Differentiation of KGF-treated HAECs to corneal lineage is unfavourable. Therefore, further studies are needed to elucidate the roles of KGF in the differentiation of HAECs towards skin keratinocytes.

1. Introduction

Keratinocyte growth factor (KGF) is a unique member of fibroblast growth factor family (FGF-7) and has been found to be secreted by the stromal cells of a variety of tissues, including cornea, skin, lung and mammary gland [1,2]. KGF binds with high affinity to KGF receptor (KGFR), an alternative splicing variant of the FGF receptor 2 (FGFR2), which is expressed exclusively on the epithelial cells [3]. It does not act on the stromal cells (fibroblasts) nor endothelial cells [1].

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0305-4179/$36.00
http://dx.doi.org/10.1016/j.burns.2012.10.019

KGF has been identified as a mediator for proliferation and a modulator for migration and differentiation processes in squamous epithelia [1]. It plays a role in early differentiation for skin keratinocytes and inhibits terminal differentiation of the cultured keratinocytes [4]. In vivo study demonstrated that transformed keratinocytes expressing KGF can cause hyperthickening and alter the differentiation pattern of epidermal tissue [5]. However, the effects of KGF on the differentiation processes are independent of keratin gene regulation [6]. It does not regulate keratin genes expression in cultured skin keratinocytes nor differentiating corneal cells [2,6]. Furthermore, KGF has been used to differentiate human bone marrow-derived mesenchymal stem cells to epithelial cells [7]. It stimulates the differentiation of the prostate epithelial stem cells by increasing the number of cells expressing their differentiation markers such as prostate acid phosphate and androgen receptor [8]. However, to the best of our knowledge, there are still limited studies done on the effects of exogenous KGF on cultured HAECs. Since KGF is a potent paracrine mitogen specific for epithelial cells such as epidermal keratinocytes, information on how KGF regulates the stemness genes and epithelial genes expression in HAECs as well as the differentiation potential of HAECs, are still unknown and remain to be resolved.

Previously, we have shown that human amnion epithelial cells (HAECs) have stem cell-like characteristics and have expressed the pluripotent and multipotent markers: Oct-4, Sox-2, FGF-4, Rex-1, Nanog3, ABCG2, Nestin, Vimentin and BST-1 [9–12]. Cultured HAECs also differentially expressed the epithelial genes [9]. They have low expression level of major histocompatibility complex antigens and have a less restricted differentiation potential [13]. They have been shown to differentiate into cells originating from 3 germ layers, namely, ectoderm-derived neural cells, mesoderm-derived cardiomyocytes and endoderm-derived pancreatic cells and hepatocytes [9,12].

Since skin and HAECs are ectoderm-derived cells, HAECs also have a potential to differentiate to skin. Regauer et al. [14] showed that during late gestation, human amnion generated stratified layer that expressed skin epidermal keratins. Later on, Fliniaux et al. [15] demonstrated that mouse amnion could transform to skin, hair follicles and sebaceous gland upon association with the embryonic hair-forming dermis. During the embryonic epidermal development, prior to commitment to stratification, the single layered surface ectoderm expresses cytokeratin 8/18 (CK8/CK18) which are markers for simple epithelium [16]. The expression of CK5/14 and p63 marked the onset of the stratification when the developing epidermis is still single layered [16]. Progressively, several layers of suprabasal differentiated cells appear with the expression of cytokeratin differentiated markers such as CK1/CK10 and cornified cell envelope such as involucrin, loricrin and filaggrin [17,18].

We have also recently shown that epidermal growth factor (EGF) played a role in regulating HAECs differentiation and proliferation [19]. In the present study, we determined the effects of KGF on the differentiation of HAECs towards skin keratinocytes. The degree of differentiation was determined via the quantitative gene expression of stemness and epithelial genes, morphological analysis, growth kinetic evaluation and immunocytochemistry. Epilife medium supplemented with Human Keratinocyte Growth Supplements (HKGS) were used to induce HAECs to skin epithelial cells and used as a positive control. Epilife medium has been shown to greatly extend the in vitro lifespan of human epidermal keratinocytes.

2. Materials and methods

This study was approved by the Universiti Kebangsaan Malaysia Research and Ethics Committee (Approval Project Code: FF-272-2007). Informed, written consent was obtained from donors prior to collection. Human term placentas were obtained from uncomplicated, elective caesarean section from healthy mothers of age ranging from 20 to 40 years.

2.1. Isolation and culture of cells

The amnion layer was mechanically peeled off from the chorion and washed several times with phosphate buffered saline (PBS, Invitrogen, USA) to remove the red blood cells. It was then cut into 2 cm × 2 cm and processed as the previous methods [3]. Briefly, ten ml of 0.05% trypsin containing 0.2 g/l of EDTA (Invitrogen, Gibco/BRL) was used to dissociate HAECs from 15 pieces of amnion (2 cm × 2 cm) and incubated at 37 °C with constant agitation. Trypsin was inactivated by adding 10 ml of F12: DMEM (1:1) supplemented with 10% heat-inactivated foetal bovine serum (FBS, Invitrogen; FD + 10%FBS). The suspension from the first 10 min was discarded to exclude cell debris. The cells from the second and third 30 min of digestion were collected and washed with PBS. HAECs were then plated on 6-well plates at 20,000 cells/cm^2 seeding density, in culture medium F12:DMEM (1:1) supplemented with 10% FBS, 10 ng/ml epidermal growth factor (EGF, Peprotech, USA), 1% antibiotic–antimycotic (Invitrogen), 1% Glutamax (Invitrogen) and 1% Vitamin C (Merck, Germany). The culture plates were incubated in a humidified incubator at 37 °C with 5% CO_2.

HAECs from the initial culture (P0) were subcultured to passage 1 (P1) with a split ratio of 1:3. Various concentrations of human recombinant keratinocyte growth factor, KGF (Peprotech, USA) (0 ng/ml KGF, 5 ng/ml KGF, 10 ng/ml KGF, 20 ng/ml KGF, 30 ng/ml KGF and 50 ng/ml KGF) were added to the medium F12:DMEM (1:1) supplemented with 2% FBS, 1% antibiotic–antimycotic, 1% Glutamax and 1% Vitamin C. HAECs were also cultured in Epilife medium (Cascade Biologics (Gibco), USA) supplemented with Human Keratinocyte Growth Supplement (HKGS, Cascade Biologics (Gibco), USA) which acts as an induction medium to induce HAECs to skin epithelial cells. Epilife is a medium that supports the growth of skin keratinocytes and used as a control. After 7 days, the cells were trypsinized using 0.125% trypsin-EDTA (Invitrogen). The cell viability and cell number of HAECs were determined by using haemocytometer and trypan blue dye exclusion (Invitrogen).

2.2. Cell cycle analysis using flow cytometry

To determine the effects of KGF on the cell cycle distribution, HAECs at passage 1 (P1) cultured in 0 ng/ml KGF, 5 ng/ml KGF, 10 ng/ml KGF, 20 ng/ml KGF, 30 ng/ml KGF and 50 ng/ml KGF were trypsinized after 7 days of culture. A total number of