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In vitro transdifferentiation of human skin keratinocytes to corneal epithelial cells

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Abstract

Background aims. Skin keratinocytes (SKs) share the same surface ectodermal origin as that of corneal epithelium. In this study, the plasticity of epidermal keratinocytes was exploited to generate corneal epithelial–like cells, which might serve as an alternative source of autologous tissue for the treatment of bilateral limbal stem cell deficiency. *Methods.* Skin samples were subjected to collagenase digestion to isolate SKs and transdifferentiated to corneal epithelial–like cells using limbal fibroblast conditioned medium (LFCM). SKs and transdifferentiated corneal epithelial cells (TDCECs) were characterized using immunofluorescence and fluorescence-activated cell sorting. The propensity for expression of angiogenic genes in TDCECs was compared with cultured oral mucosal epithelial cells (COMEC) *in vitro*. RT^2 quantitative polymerase chain reaction profiler array was performed to study the signaling pathways involved in the transdifferentiation process. *Results.* The TDCECs obtained from SKs showed corneal epithelial–like morphology and expressed corneal epithelial markers, CK3 and CK12. Hematoxylin-eosin and immunohistochemistry showed stratified layers of TDCECs expressing CK 3/12, confirming the corneal epithelial phenotype. We found that the expression of several angiogenic and epithelial mesenchymal transition factors were down-regulated in TDCECs compared with COMEC, suggesting a lower capacity to induce angiogenesis in TDCECs. There was considerable difference in the signaling mechanisms between TDCECs and SKs on testing by RT^2 profiler array, signifying differences at the global gene profile. The comparison of TDCECs and limbal derived corneal epithelial cells showed similar gene expression. *Discussion.* Our study shows that SKs have the potential to transdifferentiate into corneal epithelial–like cells using LFCM.

Key Words: *bilateral limbal stem cell deficiency, corneal epithelial cells, limbal fibroblast conditioned medium, skin keratinocytes, transdifferentiation*

Introduction

The corneal epithelium is one of the outermost layers of eye. It is exposed to the external environment and is subjected to many stress factors. This includes drying due to excessive exposure as in cases of facial palsy, scanty secretion in Sjogren syndrome and chemical or thermal injuries. Normally there exists a reserve of stem cell population in the palisades of Vogt of limbus, which rejuvenates the corneal epithelium and helps to maintain the transparency and avascularity essential for good vision. This is threatened in limbal stem cell deficiency (LSCD), whether it is unilateral or bilateral.

Conventional methods of stem cell transplantation employ kerato-limbal allograft, conjunctival-limbal allograft and conjunctival-limbal autograft [1]. For unilateral LSCD, replacement of stem cell population to correct the corneal epithelial defects of the diseased eye, autologous limbal tissue transplanta-

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tion from contralateral eye is used [2]. Cultivated oral mucosal epithelial transplantation (COMET) and cultivated limbal epithelial transplantation on amniotic membrane are current surgical alternatives for stabilizing the ocular surface. Advantages of *ex vivo* expansion over conventional methods include the autologous nature of the tissue source and the small amount of limbal or mucosal biopsy needed.

In bilateral LSCD, however, because of extensive damage of both the eyes, transplantation of cultivated oral mucosal epithelial cells (COMEC) are frequently used for treatment. Oral explants are sources of limbal stem cell equivalents because corneal epithelial–like cells can be generated under the proper culture conditions [3]. The main advantage of COMET in treating bilateral LSCD is its autologous nature. The transplantation of allogenic tissue necessitates postoperative immunosuppression, leading to frequent occurrence of rejection and failure [4]. However, COMET is frequently associated with neovascularization of the transplanted graft, which remains a major drawback [5,6].

Because both the corneal epithelium and the skin are derivatives of the ectoderm and stem cells of both these tissues express stem cell markers p63α and ABCG2, skin is likely to be an improved option for transdifferentiation to develop corneal epithelial grafts [7]. Skin is the largest organ and spans an area of about 2 m^2. It is also an easily obtainable source. Skin keratinocytes (SKs) have self-renewal potential and can be maintained without further terminal differentiation under laboratory conditions [8,9]. Epidermal adult stem cells (EpiASC) are harbored in niches in a state of stemness and quiescence. Isolation of EpiASC from the skin by digesting tissue with proteases, trypsin and dispase has been reported earlier [10]. In the interfollicular epidermis, stem cells are dispersed throughout the basal layer expressing CK5 and CK14 similar to basal limbal epithelial cells [11,12]. An earlier study demonstrated the transdifferentiation of corneal epithelial cells into epidermis under the influence of embryonal dermis [13]. The possibility of transdifferentiation to corneal epithelium from various autologous sources has been studied in animal models. EpiASC has been transplanted in a goat model for reconstructing the damaged corneal surface [14]. Corneal epithelial–like cells were generated from Rhesus epidermal cells under the influence of corneal tissue [9]. Previous studies have shown the potential of skin-derived precursors to transdifferentiate into the corneal epithelial phenotype. However, these studies used explant cultures that contained both the keratinocytes and fibroblasts and showed a predominance of mesenchymal markers in the cultures, suggesting higher frequency of dermal fibroblasts than keratinocytes [15,16]. Keratinocytes have greater potential for epithelial differentiation [17], and thus we hypothesized that the SKs might have an enhanced differentiation potential for the corneal epithelial phenotype in serum-free culture. The findings of Blazejewska et al. [18] showed that adult murine HF keratinocyte stem cells are capable of differentiating into corneal epithelial–like cells *in vitro* when exposed to a limbus-specific microenvironment. Nieto-Miguel et al. [19] showed that human adipose tissue–derived mesenchymal stromal cells acquire corneal epithelial phenotype by subjecting them to an *in vitro* corneal microenvironment. In the present study, SKs were transdifferentiated into the corneal epithelial phenotype using human limbal fibroblast conditioned media (LFCM). The characterization of transdifferentiated corneal epithelial cells (TDCECs) revealed that appropriate cornea specific signals were activated *in vitro* to direct transdifferentiation from SKs into corneal epithelial cells.

Methods

Ethics statement

Human skin samples were collected from the Oculoplasty Department, Medical Research Foundation, Sankara Nethralaya (Chennai, India) from patients undergoing eyelid surgery. Informed consent from all the donors or the next of kin was obtained. Samples used for research were in accordance with the principles outlined in Declaration of Helsinki. Placental tissues were collected after obtaining parental written informed consent from patients undergoing cesarean delivery for use of these samples in research. Cadaveric eyes were collected after obtaining consent from all donors or the next of kin for use of these samples in research by the C U Shah Eye Bank, Sankara Nethralaya, according to established guidelines. Oral tissues were collected from patients who underwent oral mucosal graft for bilateral LSCD at the Sankara Nethralaya Eye Hospital (Chennai, India), and the informed consent of donor or donor family were obtained. The study was conducted after obtaining approval from the ethics sub-committee (institutional review board) of the Sankara Nethralaya Eye Hospital for all the steps in which human tissue was used (Ethical clearance no: Ethics No: 67-2004-P).

Collection of donor skin

Skin tissues of 10 mm^2 from donors ($n = 3$) aged between 20 and 50 years were collected in Dulbecco's Modified Eagle's Medium (DMEM; 11885084, Invitrogen; 1000 mg/L glucose, 2 mmol/L L-glutamine, pyridoxine, sodium bicarbonate) with 1% antibiotics (10 000 units penicillin, 10 mg streptomycin, 25 mg