Experimental Eye Research 127 (2014) 9–13

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Serial explant culture provides novel insights into the potential location and phenotype of corneal endothelial progenitor cells

Jennifer Walshe [a, *], Damien G. Harkin [a, b, c, 1]

[a] Queensland Eye Institute, 140 Melbourne St, South Brisbane, QLD 4101, Australia
[b] School of Biomedical Sciences, Queensland University of Technology, 2 George Street, Brisbane, Queensland 4001, Australia
[c] Institute of Health and Biomedical Innovation, 60 Musk Avenue, Kelvin Grove, Queensland 4059, Australia

ARTICLE INFO

Article history:
Received 15 April 2014
Accepted in revised form 4 July 2014
Available online 14 July 2014

Keywords:
cornea
endothelium
limbus
progenitor
explant
nestin
telomerase
glypican-4

ABSTRACT

The routine cultivation of human corneal endothelial cells, with the view to treating patients with endothelial dysfunction, remains a challenging task. While progress in this field has been buoyed by the proposed existence of progenitor cells for the corneal endothelium at the corneal limbus, strategies for exploiting this concept remain unclear. In the course of evaluating methods for growing corneal endothelial cells, we have noted a case where remarkable growth was achieved using a serial explant culture technique. Over the course of 7 months, a single explant of corneal endothelium, acquired from cadaveric human tissue, was sequentially seeded into 7 culture plates and on each occasion produced a confluent cell monolayer. Sample cultures were confirmed as endothelial in origin by positive staining for glypican-4. On each occasion, small cells, closest to the tissue explant, developed into a highly compact layer with an almost homogenous structure. This layer was resistant to removal with trypsin and produced continuous cell outgrowth during multiple culture periods. The small cells gave rise to larger cells with phase-bright cell boundaries and prominent immunostaining for both nestin and telomerase. Nestin and telomerase were also strongly expressed in small cells immediately adjacent to the wound site, following transfer of the explant to another culture plate. These findings are consistent with the theory that progenitor cells for the corneal endothelium reside within the limbus and provide new insights into expected expression patterns for nestin and telomerase within the differentiation pathway.

The corneal endothelium resides as a monolayer of cells on the posterior surface of the cornea and maintains corneal clarity by transporting fluid from the inner corneal layers to the aqueous humour. Endothelial dysfunction therefore results in a swollen and hazy cornea, with associated vision loss. Currently, the only treatment for this condition is a full or partial thickness corneal transplant using tissue obtained from deceased donors. To overcome some of the drawbacks associated with the transplantation of donor corneal tissue, we and other groups are developing methods for growing sheets of corneal endothelium in the laboratory that could be potentially implanted into patients with endothelial dysfunction (Ozcelik et al., 2013; Proulx and Brunette, 2012; Madden et al., 2011). Several groups have now demonstrated the effectiveness of transplanted laboratory-produced endothelial sheets for clearing corneas in animal models of endothelial dysfunction (Mimura et al., 2013), however, sufficient expansion of primary human corneal endothelial cells (HCECs) to produce these sheets is technically challenging. HCECs are considered to be non-replicative in the adult but can be induced to divide to a limited extent *in vitro* (Joyce, 2012). Unfortunately, primary cultured HCECs tend to become senescent or transition to a mesenchymal phenotype within days or after several passages depending on the donor age and culture conditions (Peh et al., 2011).

Three major culture methods have been described for isolating and expanding HCECs. Konomi et al. (2005) favour a method in which the cells are placed straight into adherent culture following enzymatic disaggregation. Yokoo et al. (2005) also subject the cells to enzymatic disaggregation but then place them into a medium that promotes the formation of floating spheres. Cell monolayers are then derived from these spheres under different culture conditions. This method is called a sphere-forming assay and is proposed to promote the isolation of immature endothelial cells. The third method involves placing pieces of intact endothelium (tissue explants) into medium that promotes migration and expansion of

* Corresponding author. Tel.: +61 7 3239 5031; fax: +61 7 3844 1914.
E-mail addresses: jennifer.young@qei.org.au, jenniferyoung100@gmail.com (J. Walshe), d.harkin@qut.edu.au (D.G. Harkin).
[1] Tel.: +61 7 3239 5023; fax: +61 7 3844 1914.

http://dx.doi.org/10.1016/j.exer.2014.07.002
0014-4835/

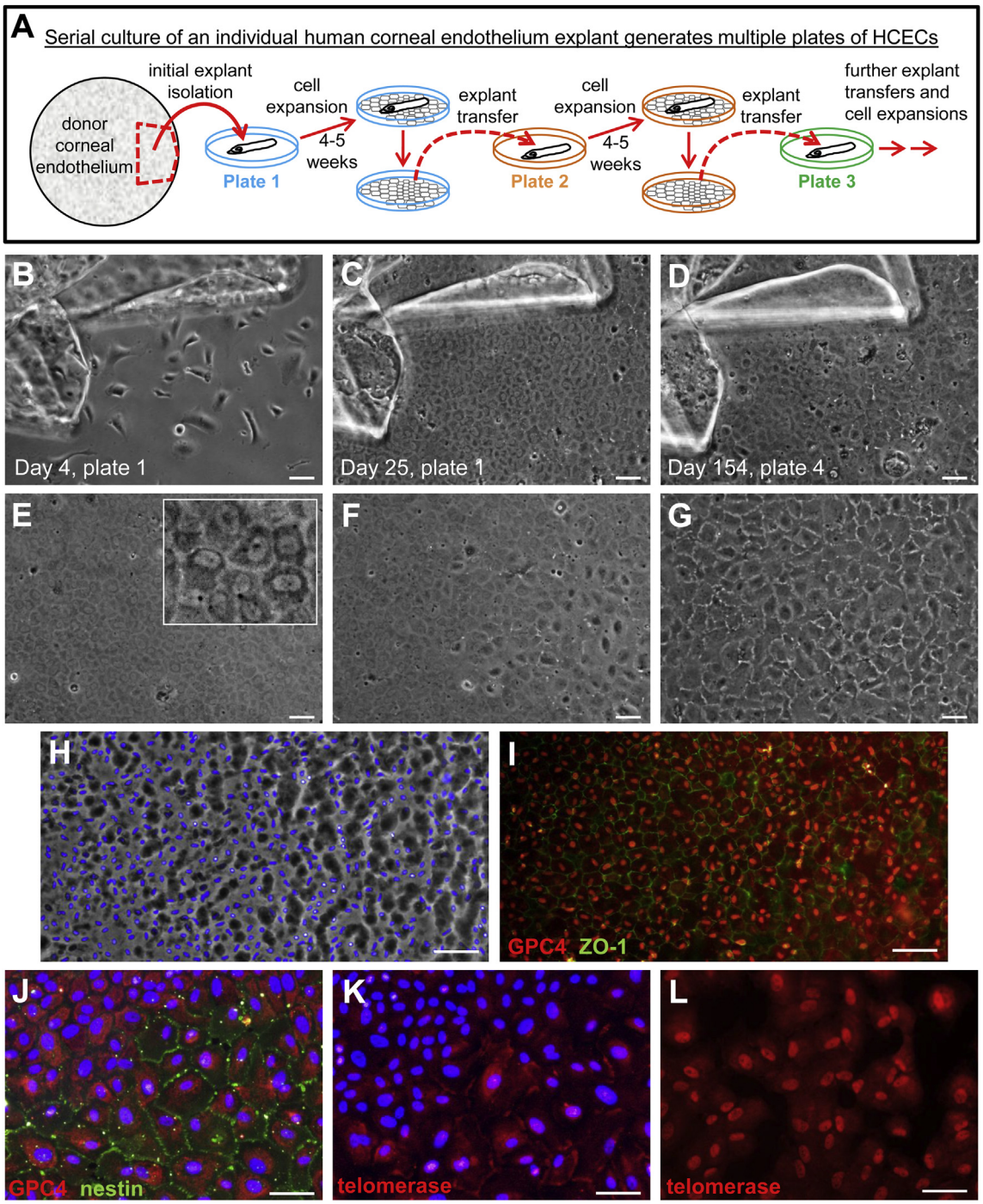

Fig. 1. Demonstration of serial cultures derived from an explant of corneal endothelium. (A) Schema illustrating the method used to generate multiple cultures of primary non-passaged human corneal endothelial cells from an individual endothelium explant taken from the cornea of a 29 year old donor. The explant was transferred to 7 new plates over a period of 6 months, generating sheets of small, primitive cells in each plate. (B) The endothelium explant in its first plate, after 4 days in culture. Cells are seen migrating out from one end of the explant. (C) A densely-packed sheet of small cells surrounds the explant at 25 days. (D) This figure shows the explant in its 4th plate, 154 days after isolation from the cornea. (E) A sheet of small, primitive cells generated by the explant in its 3rd plate. Under close examination the cells exhibit a high nucleus to cytoplasm ratio (see 3 × enlarged inset). (F) Primitive cells are arranged in a colony-type formation that surrounds the explant. A population of larger cells emerge from the margins of this colony. The transition between small (on the left) and larger cells (on the right) is evident in this figure. (G) Larger cells generated at the edges of the primitive cell population are polygonal and form a monolayer in a cobblestone pattern typical of corneal endothelial cells. (H) A sample of fixed human endothelium taken from a similar corneal location to that of the serially-cultured explant. The tissue has been photographed under phase contrast and Hoechst-stained nuclei appear blue. Cells towards the centre of the endothelium (to the left of photo) are densely packed while the cells towards the extreme periphery (to the right of photo) are less numerous and irregularly-spaced. (I). Corresponding fluorescence image to that in 1K. Cell border expression of ZO-1 is patchy at the extreme periphery of the corneal endothelium (to the right of photo). GPC4 immunoreactivity is detected in central and peripheral endothelial cells. (J) Immunodetection of GPC4 in cells generated by the explant indicates that they are of corneal endothelial origin. The stem/progenitor cell marker nestin is immunodetected in the larger cell population. (K) Telomerase is predominantly immunodetected in the cytoplasm of both the small and large cells immediately surrounding the explant. (L) Telomerase is immunodetected in the nuclei of migratory cells at the edges of the expanding cell population. Primary antibodies: GPC4, Aviva Systems Biology ARP64505; ZO-1, Invitrogen 339100; telomerase, Novus Biologicals NB110-89471SS; nestin, R&D Systems MAB1259. Scale bars = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)