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Moving epithelia: Tracking the fate of mammalian limbal epithelial stem cells

Nick Di Girolamo*, [1]

School of Medical Sciences, University of New South Wales, Sydney, 2052 NSW, Australia

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ABSTRACT

Lineage tracing allows the destiny of a stem cell (SC) and its progeny to be followed through time. In order to track their long-term fate, SC must be permanently marked to discern their distribution, division, displacement and differentiation. This information is essential for unravelling the mysteries that govern their replenishing activity while they remain anchored within their niche microenvironment. Modern-day lineage tracing uses inducible genetic recombination to illuminate cells within embryonic, newborn and adult tissues, and the advent of powerful high-resolution microscopy has enabled the behaviour of labelled cells to be monitored in real-time in a living organism. The simple structural organization of the mammalian cornea, including its accessibility and transparency, renders it the ideal tissue to study SC fate using lineage tracing assisted by non-invasive intravital microscopy. Despite more than a century of research devoted to understanding how this tissue is maintained and repaired, many limitations and controversies continue to plague the field, including uncertainties about the specificity of current SC markers, the number of SC within the cornea, their mode of division, their location, and importantly the signals that dictate cell migration. This communication will highlight historical discoveries as well as recent developments in the corneal SC field; more specifically how the progeny of these cells are mobilised to replenish this dynamic tissue during steady-state, disease and transplantation. Also discussed is how insights gleaned from animal studies can be used to advance our knowledge of the fundamental mechanisms that govern modelling and remodelling of the human cornea in health and disease.

Contents

Abbreviations: AP, Alkaline phosphatase; BM, Basement membrane; β-Gal, β-galactosidase; CFP, Cerulean fluorescent protein; ECM, Extracellular matrix; FP, Fluorescent proteins; GFP, Green fluorescent protein; HRP, Horseradish peroxidase; K, Keratins; LRC, Label-retaining cells; LESC, Limbal epithelial stem cells; LSCD, Limbal stem cell deficiency; RFP, Red fluorescent protein; SC, Stem cells; TAM, Tamoxifen; TDC, Terminally differentiated cells; TAC, Transient amplifying cells; YFP, Yellow fluorescent protein.

* Tel.: +61 2 93852538; fax: +61 2 93851389.
E-mail address: n.digirolamo@unsw.edu.au.

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1. Introduction

Blindness from corneal disease affects 10-million individuals globally (Whitcher et al., 2001). The possibility of restoring vision with a trephined donor cornea was proposed by Erasmus Darwin (Charles Darwin's grandfather) nearly 250 years ago and successfully performed in humans for over a century (Zirm, 1906), and prior to that in animals (Bigger, 1837). For the more difficult to treat patients, including those with a disease called limbal stem cell deficiency (LSCD), a donor graft is not a viable option as their SC are depleted or their 'niche' residence destroyed by chemical, mechanical, or surgical trauma, or as a consequence of genetic and inflammatory diseases. The challenge for clinicians is to replenish the SC pool; previously achieved by transplanting large sectors of limbal tissue (Kenyon and Tseng, 1989). Today, *ex vivo* expansion and delivery of SC on biodegradable or synthetic scaffolds is standard practice. However, grafts still fail and success beyond 5–10 years is not well documented (Shortt et al., 2007a; Di Girolamo et al., 2009; Rama et al., 2010; Baylis et al., 2011; Sangwan et al., 2011; Bobba et al., 2015). Patients with LSCD suffer physical and psychological distress including falls, fractures, depression and premature mortality, and the economy is burdened from the loss of income and high health care costs (Geerling et al., 2002), particularly as the most common cause (chemical injury), typically occurs in patients of working age. Restoring corneal health and vision in patients with LSCD is a global initiative with researchers focused on improving current and developing new long-lasting therapies. Notably, a standardised therapeutic intervention has not been devised for this debilitating disease; this is not surprising as the disease varies in aetiology and severity. Therefore, in the future one might anticipate that a suite of procedures will become available to tailor treatment on a case-by-case basis. However, before we can adequately address this medical dilemma, a more comprehensive understanding of the basic biology of LESC is warranted.

Of the external organs, the cornea is perhaps the perfect tissue to study the long-term fate of its epithelial cells; firstly because it's SC are spatially segregated from their differentiated progeny, and secondly because they are thought to contribute to life-long tissue maintenance. Other advantages include its translucency and accessibility, rendering cell dynamics through this clear 'window' readily observable with non-invasive high-resolution intravital microscopy.

Over a century of research has been devoted to understanding the structural and functional relationship of the mammalian cornea and its resident cells, and despite the constant flow of literature, the field continues to be plagued with limitations and controversies especially in regards to how and from where the cornea receives its signals that direct structural organisation during embryogenesis, how it self-perpetuates during adult live, and how it's SC orchestrate the many activities that govern corneal health for exquisite vision. This article will address these voids in our knowledge base and showcase new scientific discoveries and technological advances within the field. However, the overarching goal is to highlight seminal studies that have informed modern-day fate mapping of cells within this highly specialised structure and to discuss how this data has been instrumental in detailing where SC reside, how they divide, how their daughters differentiate and migrate, how long they survive, and how they behave during steady-state, wound-healing and transplantation. While this information may seem trivial, it is anticipated clues will be divulged as to (i) whether the corneal epithelium contains one or multiple SC reservoirs, (ii) identification of novel SC markers and the signals that trigger cell egress from the niche, and (iii) identification of proteins that regulate 'stemness'.

1.1. Structure and function of the cornea

The adult mammalian cornea is composed of three distinct and functionally diverse cellular layers; the externalised anterior region consists of a multilayered non-keratinised squamous epithelium with proliferating basal cells, which in man rest on a thick (8–15 μm) basement membrane (BM)-like structure called Bowman's layer. Overlying these cells are suprabasal 'wing' epithelia, and above these cells are flat post-mitotic superficial epithelia. The posterior cornea contains a monolayer of specialised endothelial cells attached to Descemets' membrane which play a key role in fluid exchange across this tissue. Wedged between the epithelium and endothelium is an avascular connective tissue stroma that makes up 90% (~500 μm) of the cornea and is comprised of an orderly array of collagen lamellae interspersed with resident keratocytes and sympathetic nerves with axons that pierce the BM and terminate between the epithelia. Circumscribing the cornea is a 1 mm wide transition zone otherwise known as the limbus; this region partitions the cornea from the