Anatomical Features and Cell-Cell Interactions in the Human Limbal Epithelial Stem Cell Niche

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ABSTRACT **Epithelial stem cells of the ocular surface are essential for the maintenance of corneal transparency and therefore for vision. Human corneal/limbal epithelial stem cells (LESCs) are believed to reside in the limbus, the interface between the peripheral cornea and neighboring conjunctiva. A specific anatomical microenvironment called the *niche* regulates the proliferative and differentiation potential of LESCs and their daughter cells. This review covers multiple structural and functional aspects of the human limbal epithelial stem cell niche, including: anatomical features of the niche, composition of the local extracellular matrix, soluble factors and signaling pathways, interactions with surrounding stromal niche cells and melanocytes.**

KEY WORDS **cell interactions, cornea, epithelial stem cells, human, limbus, stem cell niche**

I. INTRODUCTION

The cornea is a highly specialized tissue that transmits and refracts light onto the retina. It also forms a protective barrier between the inner eye and the environment. Its outermost layer is composed of a multilayered squamous and stratified epithelium. The human corneal epithelium is continuously maintained by a population of epithelial stem cells located at the corneal periphery, in a region called the *limbus*. Anatomically, the limbus corresponds to the transition area located at the interface between the transparent central cornea and the conjunctiva. The limbus is a 1 mm-wide ring of tissue demarcated on the corneal side by the termination of the Bowman's layer. The limbal epithelium, the thickest of the ocular surface, is composed of 7-10 layers of nonkeratinized and stratified epithelial cells. Unlike the central cornea, Langherans cells (the antigen-presenting cells of the ocular surface) and melanocytes are also observed within the limbal epithelium.

Epithelial cells populating the superficial layer of the limbal epithelium highly express microvilli on their apical surface and tight junctions on the lateral sides. Basal cells of the limbal epithelium appear smaller and less columnar than basal cells of the corneal epithelium. It is widely accepted that, in human, a subpopulation of these basal cells corresponds to limbal epithelial stem cells (**LESC**s) that continuously regenerate the central corneal epithelium. Limbal epithelial progenitors can be discriminated from their differentiated progeny by their small size,[1] basal location, and the expression of a panel of putative stem cell markers. These include transporters such as ABCG2 and ABCB5,[2-4] transcription factors such as C/EBPδ,[5] Bmi-1,[6] p63α and its ΔNp63α isoform,[7-9] Pax6, cell adhesion molecules and receptors including N-cadherin, integrin α9 and β1,[10] Frizzled (**Fz**)7,[11,12] Notch-1,[12] or cytokeratins such as CK15,[13] CK14 and CK19.[14] In vitro, epithelial progenitors are small and circular, characterized by a high nucleus cytoplasm ratio and by the generation of colonies containing tightly packed epithelial cells.[15] These cells are maintained in a quiescent state during normal corneal homeostasis, but present the greatest proliferative potential and the ability to generate holoclones cultured on a feeder layer consisting of growth-arrested irradiated mouse fibroblasts (3T3).[16-18]

LESCs are compartmentalized within the limbus in a specific and highly regulated microenvironment called the *niche,* which maintains the epithelial lineage and the progenitors in a quiescent state.[19-23] Like in other epithelial

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OUTLINE

niches such as the hair follicle,[24] the intestinal crypt[25] or the terminal bronchioles of the epithelial airway,[26] the limbal stem cell niche consists of various aspects, such as distinct anatomical features, a variety of secreted soluble factors, a specific composition of the local extracellular matrix (**ECM**), biomechanical properties and interactions with surrounding "niche cells."[27,28] These molecular and structural aspects of the human limbal stem cell niche will be covered in the present review.

II. ANATOMICAL FEATURES OF THE HUMAN LIMBAL EPITHELIAL STEM CELL NICHE

A. Palisades of Vogt and Limbal Crypts

"Palisades" is a term assigned by Vogt to describe radial striae observed at the human limbus. Despite considerable variations from one individual to the other, the limbal palisades of Vogt measure 0.31 mm in length and 0.04 mm in width and are most frequently observed at the upper and lower limbal arcs.[29,30] The palisades of Vogt are easily identified in moderately or darkly pigmented individuals because of a concentration of melanin-containing cells and limbal melanocytes lining the interpalisade ridges (Figure 1).[30-32] However, as reported by Goldberg and Bron, in some lightly pigmented individuals, limbal palisades could not be observed at all. It has also been shown that distribution of the palisades from one eye to the other is symmetrical in the same individual. Townsend et al reported that the limbal palisades were, among 200 individuals analyzed, mainly located at the superior and inferior quadrants, with a thinner and longer ultrastructure for the superior quadrants.[33] These structures were mostly attenuated or undetectable in the horizontal quadrants, appeared more discrete with age, and their morphology was affected by limbal stem cell deficiency.[34,35]

The shape of the palisades is also varied; they have been described as long and narrow rectangles that sometimes appear as tiny circles and ovals. Histologically, the interpalisades

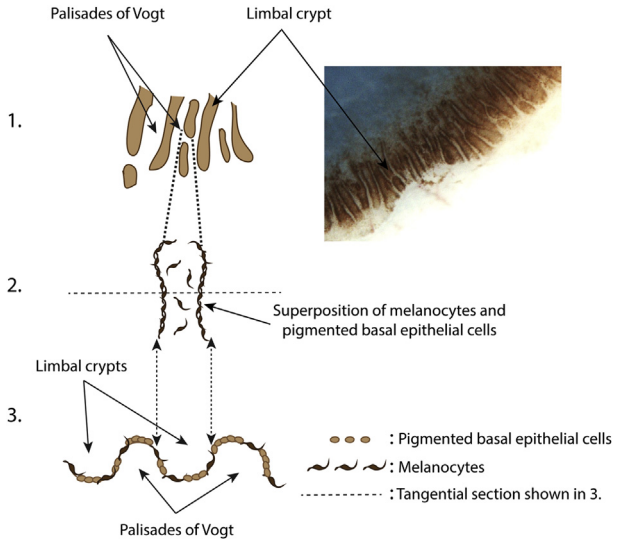

Figure 1. Identification of limbal crypts in pigmented individuals Limbal crypts are easily identified in pigmented donors and are delimited by two highly pigmented lines. (2) Magnified area shown in (1). (3) Tangential section shown in (2) highlighting the superposition of limbal melanocytes and pigmented basal epithelial cells at the edge of the crypts observed macroscopically in (1).

appear as thick ridges filled by epithelial cells and correspond to the limbal crypts described by Shortt et al (Figure 2A).[36]

The palisades of Vogt are highly populated by a radially oriented vascular complex. It has been proposed that the palisadal vessels supply metabolic needs to the large number of epithelial cells populating the interpalisade ridges.[37] Transmission electron micrographs of the basement membrane zone of the human limbal epithelium located between the limbal palisades revealed some discontinuities and focal interruptions, suggesting a possible route for direct stromal/epithelial interactions.[31,37]

In 2007, Shortt et al characterized the interpalisadal grooves observed by Goldberg and Bron and named them "limbal crypts."[36] Limbal crypts (**LC**s) were described by the authors as "distinct invaginations of epithelial cells tended from the peripheral cornea into the corneal limbal stroma." These structures are similar to the rete pegs of the epidermis and correspond to downward projections of the limbal epithelium into the limbal stroma between the palisades of Vogt (Figure 2A). High-resolution microscopy, including scanning electron microscopy (**SEM**) analysis on decellularized corneal limbal biopsies, revealed the manner in which the limbal stroma encloses the LCs laterally. Immunohistochemistry highlighted the presence of a complex vascular plexus that is intimately associated with the LCs. The limbal stroma that surrounds the LCs is also highly vascularized and contains a high population of stromal cells.

As previously observed for the limbal palisades of Vogt, there is a regional variation in distribution of LCs. LCs seem to be predominantly located in the superior and inferior limbal quadrants and could not be observed in the horizontal meridian of all individuals studied.[36] Immunohistochemical analysis of the newly identified stem cell markers Fz7