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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, cell-to-cell interactions with surrounding stromal niche cells and melanocytes.

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

Outline

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 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 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 and N-cadherin[4,11,38] showed a strong positive signal for basal epithelial cells populating the LCs (Figure 3c and 3f), whereas ABCB5 is expressed at the surface of clusters of small and tightly packed epithelial cells (white arrows in figure 3i). High-magnification involving electron microscopy revealed the presence of a subpopulation of small and circular basal epithelial cells in the vicinity of stromal cell extensions in this specific limbal area. In vitro, cells isolated from LCs have the greatest proliferative potential and the ability to generate holoclones confirming these structures as a niche for the limbal epithelial progenitors.[31,36]

B. Limbal Epithelial Crypts

In 2005, Dua et al described the limbal epithelial crypt as radial extensions of the limbal palisades into the conjunctival stroma; the authors further proposed that the limbal epithelial crypt is a putative stem cell niche for the limbal epithelial progenitors. In this study, five human cadaveric corneas

aged between 17 and 75 years old were histologically serially sectioned. This unique anatomical structure was identified at the limbus of all specimens analyzed, with an estimation of six limbal epithelial crypts (**LEC**s) observed per specimen. LECs extended from the peripheral aspects of an interpalisade rete ridge and further extended into the conjunctival stroma as a solid chord of cells measuring up to 120 μm (Figure 2C and D). Immunohistochemical analysis revealed that all cells populating the LECs were highly positive (+ve) for the expression of the stem cell marker ABCG2.[39]

In 2007, Shanmuganathan et al further characterized the anatomy of the LEC in the human eye. Among 8 human corneoscleral rims analyzed, 74 LECs were identified with an occurrence that varied between donors ranging from 4 to 13.[40] The LECs varied in size and seemed to be uniformly distributed around the corneal circumference. For this reason, these observations do not correlate with the distribution of the palisades of Vogt confined within the superior and inferior segments of the limbus.[33] Immunohistochemistry revealed that cells populating the LECs were mainly CK3 negative (–ve), Ck19 +ve, CK14 +ve, CD34 –ve, Vimentin +ve, p63 +ve but positive for the expression of connexin 43 (Cx43).[40]

C. Focal Stromal Projections

Focal stromal projections (**FSP**s) have been described as finger-like projections of the limbal stroma into the limbal epithelium containing a central blood vessel (Figure 2B). Unlike the LCs describing interpalisadal grooves extending radially through the limbus, FSPs correspond to a focal protrusion of the limbal stroma into the limbal epithelium (Figure 2B). It has been shown that basal cells and epithelial cells directly adherent to FSPs were significantly smaller in diameter and had the highest nucleus/cytoplasm ratio when compared to suprabasal cells immediately adjacent to them.[36] These structures are located at the corneal side of the limbus but are distinct from the LCs.

Anatomical features of the human limbus have been further characterized and quantified by Molvaer et al.[41] They used serial sectioning as well as computer-assisted 3D reconstruction to describe and analyze the distribution of LCs, LECs and FSPs. In one eye analyzed, the authors observed that LECs (6

among 8), LCs (19 among 25), and FSPs (93 among 105) were localized in the superior limbal region, which does not accurately correlate with the superior/inferior distribution of the palisades of Vogt. Such difference could be explained by the important variability between individuals and the low number of donors (n=1) used to analyze the distribution of these structures around the human ocular surface.

III. Composition of the Limbal Extracellular Matrix

The extracellular matrix (**ECM**) of the limbal stroma, which provides mechanical strength, promotes intercellular communication and facilitates adhesion of basal epithelial and stromal cells, is an important element of the limbal stem cell niche. ECM components of the ocular surface have been characterised and reviewed by Mei et al.[42]

The ECM composing the limbal stroma that supports the epithelial progenitors presents some unique features and is believed to be essential in maintaining limbal stem/progenitors in their niche. The basement membrane is a unique component of the ECM that separates the epithelium from the stroma and supports important functions such as proliferation and cell differentiation.[43]

Ljubimov et al observed heterogeneity in the composition of the epithelial basement membrane of the limbus and the central cornea; the basement membrane of the central corneal epithelium was found to contain type IV collagen $\alpha3$ and $\alpha5$ chains, whereas the limbal epithelium contained $\alpha1$ and $\alpha2$ collagen IV and $\alpha2$, $\beta2$ laminin chains.[44] The importance of the limbal ECM was further demonstrated by the observation that limbal epithelial-like cells could be obtained from either embryonic stem cells or central corneal epithelial cells in the presence of specific limbal factors including collagen IV.[19] Moreover, it has been shown that murine-derived hair follicle stem cells have the ability to transdifferentiate into corneal epithelial cells when seeded on top of laminin 5-coated culture plates.[20]

Shlötzer-Schrehardt et al analyzed topographical variations of the basement membrane of the human ocular surface by immunohistochemistry. Interestingly, the basement membrane of the limbal epithelium presented a patchy immunoreactivity for laminin $\gamma3$ chain, BM40/SPARC and tenascin C,

which co-localized with ABCG2, p63, K19 positive and CK3, connexin 43, desmoglein, integrin α2 negative basal epithelial cell clusters.[45] Moreover, reactivity for tenascin C was the greatest at the epithelial stromal interface of the LEC.[46]

Vitronectin is a glycoprotein that is highly expressed within the limbal basement membrane but not in the central cornea or the conjunctiva. Echevarria et al reported that human limbal epithelial cells expanded on vitronectin-coated plates had the ability to generate large holoclone-like colonies and presented a higher colony-forming efficiency than cells expanded on non-coated plates.[47] These observations suggested a potential role of vitronectin in supporting LESCs in the native niche.

IV. Soluble Factors and Signaling Pathways

LESCs communicate with their microenvironment in order to maintain self-renewal and direct cell fate. Different studies have shown that the cross-talk between LESCs and the surrounding niche cells involving paracrine factors and their receptors is crucial for maintenance of the stem cell phenotype. These are described below.

A. Wnt Canonical Signaling Pathway

The wnt signal transduction pathway regulates crucial aspects of cell fate, such as migration, proliferation, differentiation, and polarity.[48] Wnt/β-catenin signaling plays a critical role in early stages of the embryonic development, but also has a role in the adult. Wnt are secreted glycoproteins that bind to the N-terminal extra cellular domain of the Fz receptor family. Upon activation, β-catenin is released from its inhibitory complex, accumulates and eventually translocates into the nucleus where it activates and suppresses specific target genes.

The Wnt/β-catenin signaling pathway has been demonstrated to be an important factor in various types of stem cell niches regulating stem cell proliferation and differentiation.[48-52] In the human ocular surface, Wnt2, Wnt6, Wnt11, Wnt16b are specific to the limbus where the LESC/progenitors are believed to reside. Moreover, nuclear localization of β-catenin has been observed

in only a very small subset of basal cells at the limbus. In vitro, activation of canonical wnt/β-catenin signaling increased the potential of LECs to generate secondary colonies that also maintained a stem cell phenotype, as shown by high expression of ABCG2 and ΔNp63α..[53]

Taken together, these findings suggest that Wnt signaling is present in the human ocular surface and plays a potential role in regulation of LESCs/progenitors. Recently, it has been observed that Fz7 was preferentially expressed by limbal basal epithelial cells to epithelial cells of the central cornea and was co-localized with N-cadherin and p63α positive clusters. In vitro, when Fz7 was knocked down in LECs, the expression of the stem cell markers ABCG2 and ΔNp63α was reduced significantly confirming the involvement of Fz7 in maintenance of the undifferentiated state of LESCs/progenitors.[11]

B. Stat3 and IL6 Signaling Pathway

In the human LCs, limbal stromal fibroblast-like cells are localized immediately beneath the limbal epithelium in close proximity to the LESC/progenitors. In order to identify potential paracrine factors of epithelial/stromal interaction, Notara et al developed an in vitro model of the limbal stem cell niche in which limbal fibroblasts were co-cultured with limbal epithelial cells in a serum-free culture system. In this model, limbal fibroblasts were able to support expansion of LECs that maintained stem cell characteristics with the ability to generate large holoclone like colonies, a high secondary colony forming efficiency and the expression of stem cell markers such as p63α and ABCG2. Interestingly, it has been shown that IL6 secretion by limbal fibroblasts was induced by LECs in the co-cultures but decreased with LECs differentiation.[54] In the native niche, immunohistochemistry revealed clusters of limbal epithelial cells and underlying stromal cells that were both positive for IL6, suggesting that IL6 could potentially be involved in stromal/epithelial cell interaction in vivo. Authors further observed that in the recipient LECs, IL6 induced Stat3 time-dependent phosphorylation and that inhibition of either IL6 or Stat3 led to significant reduction of generation of secondary colonies.[54]

V. Cell-Cell Interactions in the Human Limbal Stem Cell Niche

Interactions between stem cells and niche cells from mesenchymal lineages have been observed in numerous organs, such as the bone marrow, the intestinal crypt and the hair follicle, and are the subject of important investigations.[55-59]

A. Evidence of Limbal Epithelial Stromal Interactions

The limbal stroma is a complex environment that is highly vascularized, innervated but also populated by a mix of poorly characterized stromal cells exhibiting mesenchymal properties in culture. It is generally accepted that mesenchymal cells from the limbal stroma have a potential role in the maintenance and support of LESC and are therefore considered as an important element of the stem cell niche.[60-62]

Notara et al reported a spatial proximity between stromal cells underlying the LCs and tightly packed basal cells from the above epithelium. The authors suggest that such spatial arrangement at the stromal/epithelial interface could facilitate paracrine interactions. Later, Li et al observed that mesenchymal cells localized immediately beneath the limbal stroma, termed "limbal niche cells," had a greater potential to support expansion of LECs in vitro than cells localized deeper in the limbal stroma.[61] In another study, Chen et al demonstrated the efficiency of collagenase over dispase for the isolation of LESCs from cadaveric biopsies. They also observed that the collagenase isolation method, which preserves the basement membrane integrity, not only isolated epithelial pan-cytokeratin +ve cells but also their closely associated underlying vimentin +ve stromal cells.[62] Moreover, epithelial cells associated with Nanog, Sox2, Oct4, Rex1, CD34, Nestin-positive stromal cells highly expressed putative stem cell markers such as ABCG2, p63α and Bmi-1 and had the highest secondary clonogenic potential. The authors further reported that epithelial and stromal cells could reunite to generate spheres in matrigel and that such epithelial/stromal reunion was following the CXCR4/SDF-1 chemokine axis. Interestingly, when the stromal/epithelial reunion was disrupted using a CXCR4/SDF-1 inhibitor or a CXCR4 neutralizing antibody, the size of spheres generated, the secondary colony forming efficiency and the expression of stem

cell markers by epithelial cells decreased significantly.[63] Taken together, these observations suggest that direct epithelial/stromal contacts are crucial for maintenance of the LESCs properties in vitro and highlight the importance of vimentin +ve cells beneath the limbal epithelium as niche cells in the native tissue.

Direct stromal epithelial contacts have been further investigated in the human limbus using high-resolution imaging techniques.[31,64] Higa et al observed a subpopulation of vimentin +ve and AQP1 +ve stromal cells penetrating the epithelial basal membrane in the proximity of N-cad, K15 and p63 +ve epithelial clusters. In another study, volume electron microscopy and 3D reconstructions revealed a clear connection between small basal epithelial cells and underlying stromal extensions within the LCs (Figure 4). Such contacts were facilitated by focal basement membrane interruptions.[31,37]

B. N-cadherin-Mediated Cell-Cell Interactions

Hayashi et al observed by immunohistochemistry that a subpopulation of limbal basal epithelial cells and limbal melanocytes were positive for the expression of N-cadherin. They showed that N-cadherin +ve FACS-sorted epithelial cells had the greatest secondary colony-forming potential and exhibited significantly higher expression of stem cell-related markers such as ΔNp63α, CK15, Bmi-1 and ABCG2, whereas markers of cell differentiation such as CK12 and CK3 were upregulated in N-cadherin –ve epithelial cells. Therefore, they suggested N-cadherin as a +ve marker for LESCs and hypothesized that N-cadherin mediated homotypic cell-cell interaction with limbal melanocytes could maintain the epithelial progenitors in a quiescent state in the native niche.[65]

Later, Higa et al observed that N-cadherin +ve cells co-cultured with 3T3s were mainly located at the edges of colonies and were less differentiated than cells located at the center, as shown by a greater secondary colony-forming efficiency after separation of both cell populations by laser microdissection. Interestingly, they showed that N-cadherin was also expressed by 3T3s feeder cells, which had the ability to generate N-cadherin-mediated contacts with epithelial cells located at the periphery of the colony. When N-cadherin-mediated cell-cell interaction was disrupted in the feeders, secondary

proliferative potential of limbal epithelial cells decreased significantly, supporting the hypothesis that N-cadherin plays a pivotal role in the maintenance of the limbal epithelial progenitors in vitro.[38] Nevertheless, N-cadherin expression could not be detected in stromal cells located immediately beneath the limbal basement membrane and interacting with the epithelial progenitors in the niche.[64]

C. Melanocyte-Epithelial Cell-Cell Interaction

In the skin, normal melanocytic homeostasis is maintained and regulated by interactions with the surrounding epithelial cells, stromal fibroblasts, and the local extracellular matrix.[65] It has been shown in the human limbus that cells from the limbal stroma, in close vicinity to the above LESCs/progenitors, have the ability to support clonal growth of LECs that maintained expression of epithelial stem cell markers in vitro. These stromal cells called *limbal mesenchymal cells* or *limbal niche cells* have been proposed to support and maintain LESCs in their native microenvironment.

Pigmentation of the palisades of Vogt has been well described and has been attributed to a high population of melanocytes localized within the basal layer of the LCs, which also concentrate the LESCs/progenitors. It has been proposed that human limbal melanocytes (**hLMs**) could play a protective role against UVs by releasing melanin to the surrounding epithelial progenitors.[23,31,32] Volume electron microscopy revealed that small basal epithelial cells are closely associated with underlying stromal cells but also with the surrounding hLMs (Figure 4B). Localization of hLMs within clusters of compact basal epithelial cellsat the edge of the LC suggests that these cells could also act as a part of the niche, regulating the limbal epithelial progenitors.[66,67] Therefore, functional involvement of hLMs as niche cells has been investigated in vitro. After being successfully isolated and expanded in culture, hLMs were used as feeder cells for the expansion of LECs. Interestingly, it has been shown that hLMs had the ability to support clonal growth of LECs in vitro, and that LECs maintained expression of epithelial stem cell markers such as CK15, Bmi-1 and p63α.[67]

VI. Conclusions and Future Research Directions

Epithelial stem cells of the ocular surface are essential for maintenance of corneal integrity and vision. Understanding how various elements of the stem cell niche modulate the stem cell fate and basic knowledge of stem cell regulations are essential to improve effectiveness of corneal tissue engineering and limbal stem cell therapy. Elegant studies have provided evidence of slow cycling and label-retaining cells located at the limbus of the murine cornea.[68-72] In human, it has been shown that a fraction of limbal basal epithelial cells had the ability to generate holoclones in culture, confirming the limbus as a preferential niche site for LESCs.[16,18] Over the past decade, anatomical features of the human limbus have been described and proposed as putative niches for the epithelial progenitors. However, the generation of holoclones has so far been observed only when cells were isolated from the LCs after targeting the palisades of Vogt.[31] The epithelial stromal interface is the subject of considerable research, and there is still much to be discovered about the cell-cell interactions occurring in this specific area. In fact, direct contacts between stromal and epithelial cells have been observed both in vitro and in the human limbus. Similarly, direct mesenchymal-epithelial contacts have recently been identified in restricted areas of the rabbit limbus also showing a positive signal for chondroitin sulfate structures.[73] However, the exact function of such interaction in the native tissue remains unknown, and the stromal cell population capable of such contacts is poorly characterized.

In the skin, alterations in communications between melanocytes and cells of their microenvironment can lead to uncontrolled proliferation, transformation, and invasion.[65] In the cornea, limbal melanocytes that highly populate the basal layer of the LCs have been shown to successfully support the expansion of limbal epithelial cells in vitro by a mechanism not yet elucidated.[67] In vitro experiments suggested the importance of N-cadherin-mediated direct contacts between LECs and feeders. Moreover, immunohistochemical analysis highlighted the expression of N-cadherin in both limbal melanocytes and basal epithelial cells in the niche. Therefore, investigation of N-cadherin and its functional involvement in a LEC-melanocyte co-culture model would be the next

stage to elucidate the importance of such crosstalk and the modulation of the stem cell fate in the native tissue.

Wnt and BMP signaling have been found to control self-renewal and quiescence in numerous species and stem cell niches. Recent findings suggest a balance between Wnt and Bmp signaling between limbal epithelial progenitors and stromal cells in vitro.[74,75] Whereas Wnt signaling expression seems to promote epithelial expansion and proliferation, BMP and PCP signaling in stromal niche cells ensure maintenance of quiescence of the limbal epithelial progenitors. Therefore, investigation of signaling pathways involved in epithelial-melanocyte crosstalk in vitro would be essential to understand how limbal melanocytes promote proliferation of LECs in vitro and how these cells could be involved in the regulation of LESCs in the native human limbal stem cell niche.

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Figure legends

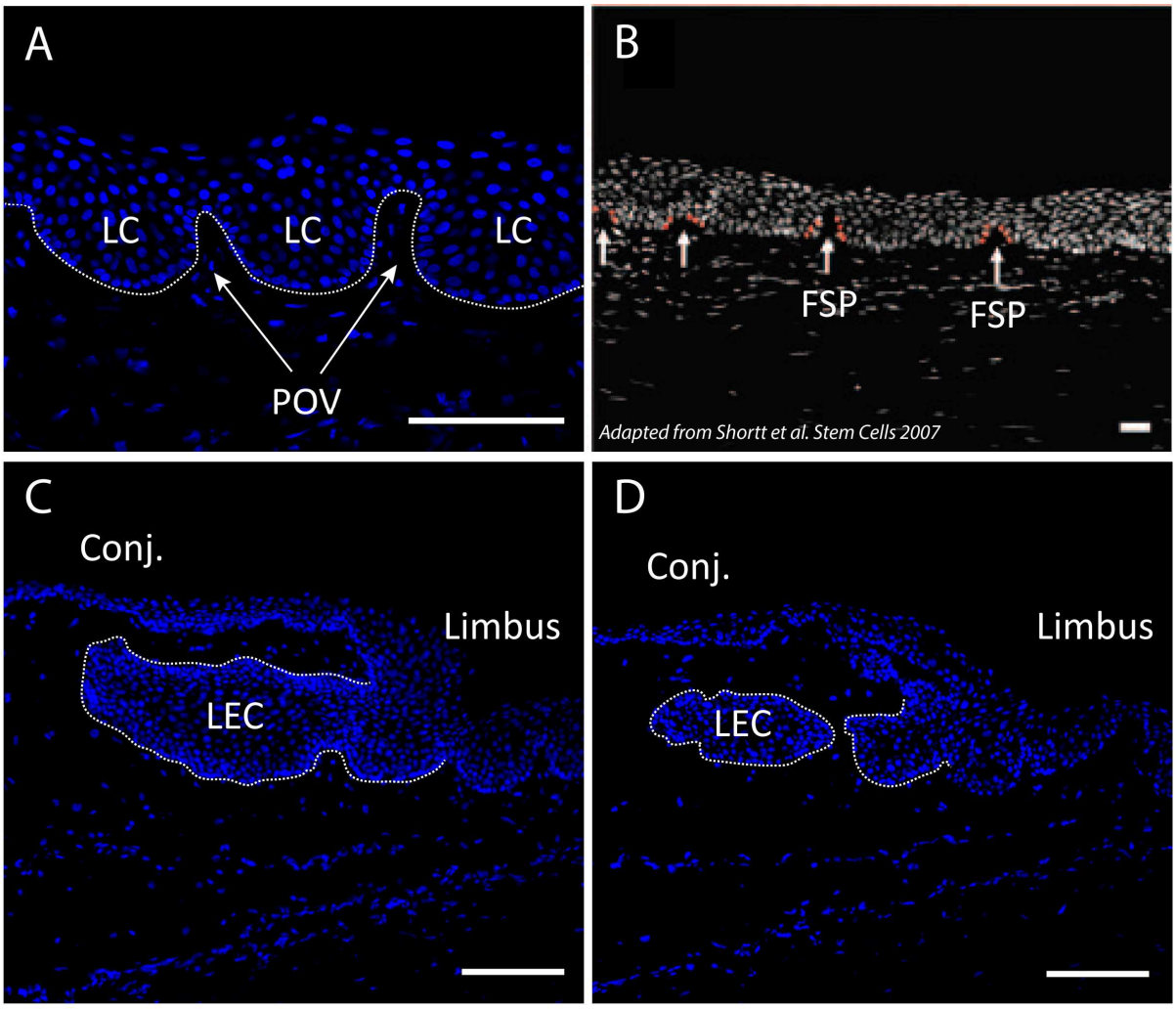
Figure 1. Identification of limbal crypts in pigmented individuals
Limbal crypts are easily identified in pigmented donors and are delimited by two highly pigmented lines. (2) Magnified area shown in (1). (3) Tangential section shown in (2) highlighting the superposition of limbal melanocytes and pigmented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

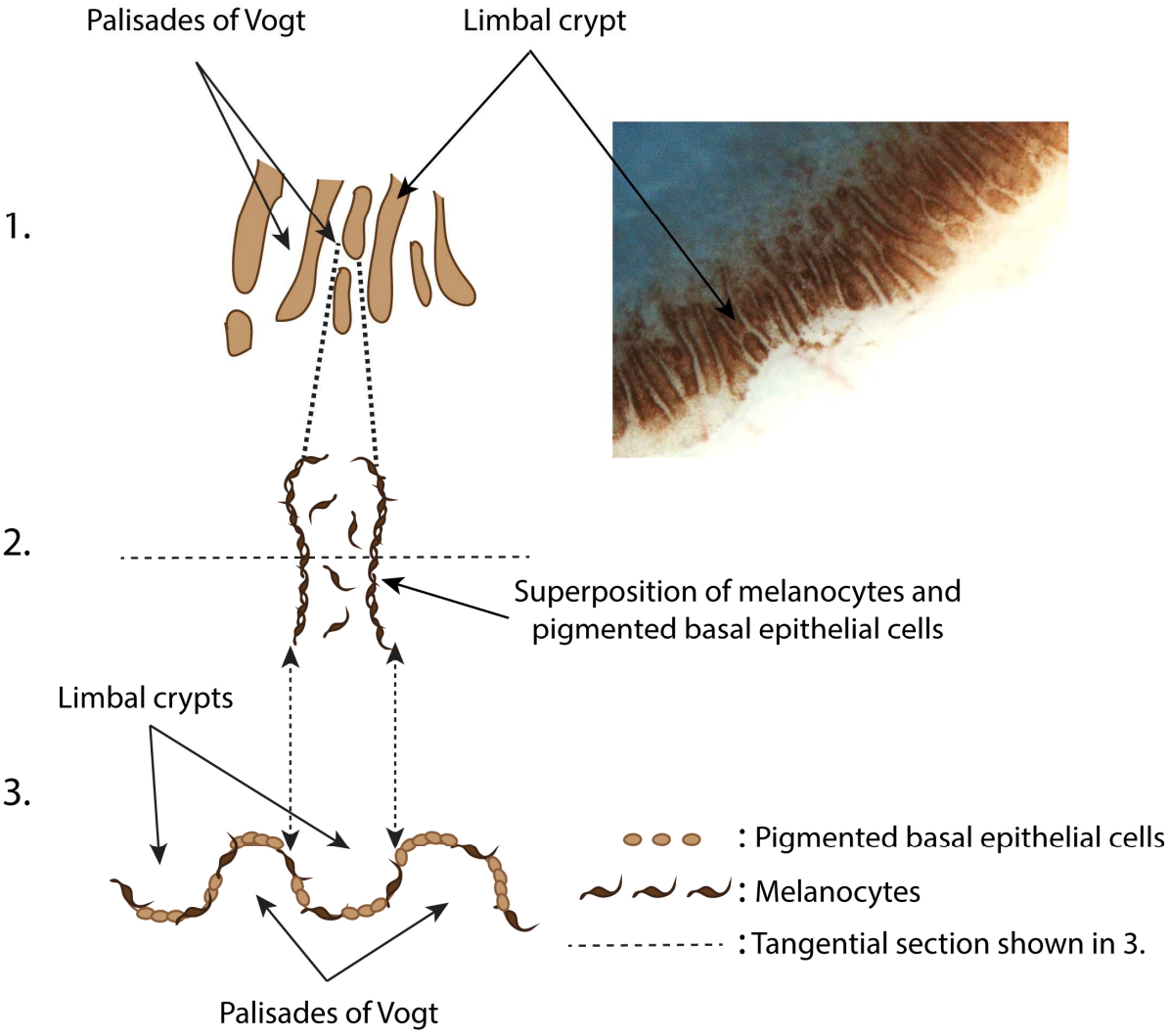
Figure 2. Anatomical features of the human limbal epithelium
Tangential histological sections of the limbal crypts (LC) (A) and focal stromal projections (FSP) (B) described by Shortt et al. (Reprinted from Shortt AJ et al[36] with permission of *Stem Cells*.) Limbal epithelial crypts (LEC) described by Dua et al appear as large extensions of the limbal epithelium into the limbal stroma (C). Serial sectioning (D) reveals detachment of the LEC from the limbus as a solid chord of cells. Scale bars: 100 μm A, C and D and 50 μm B.

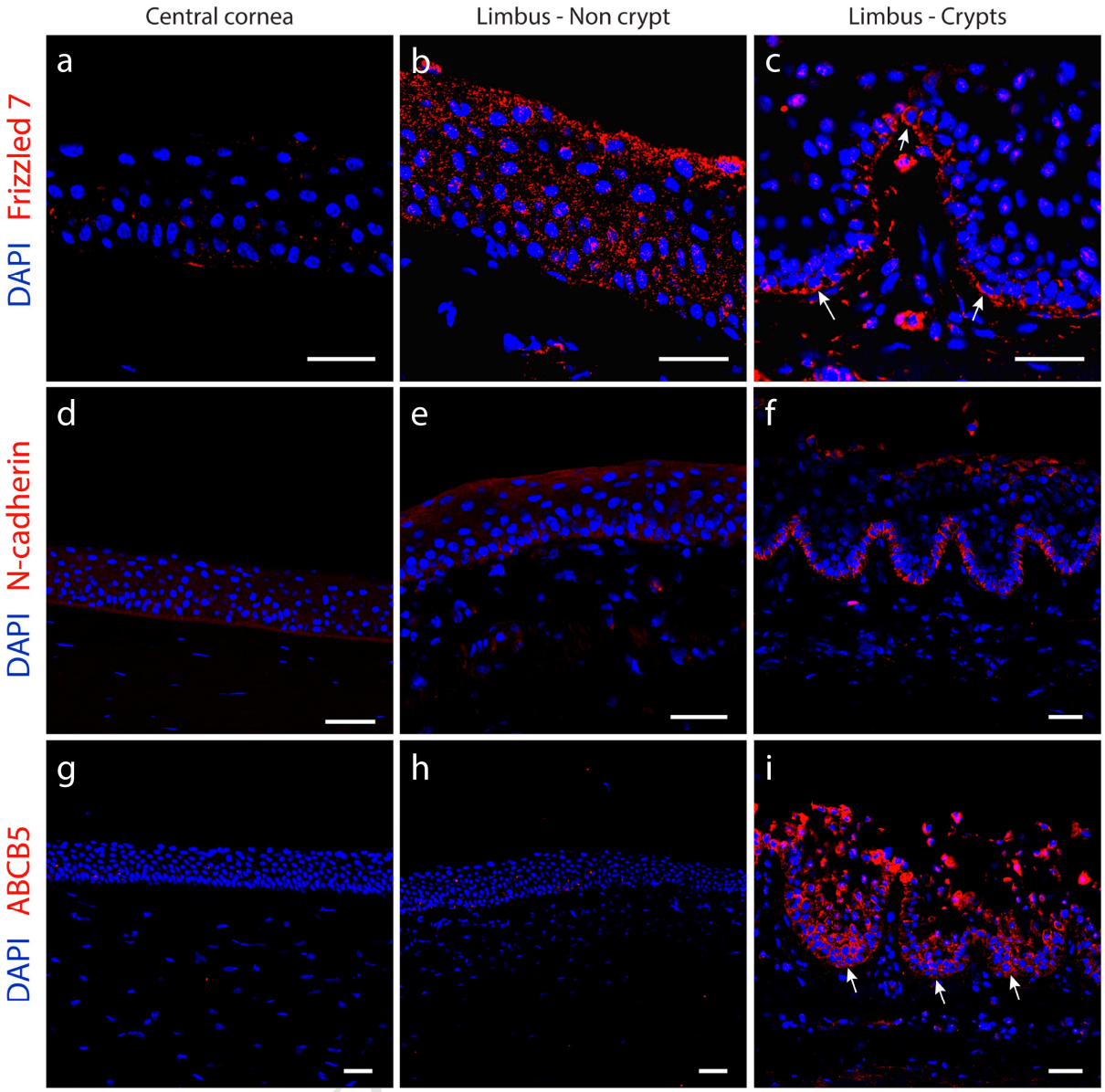
Figure 3. Immunofluorescence staining for the putative LESC markers Frizzled (Fz)7, N-cadherin and ABCB5 in the human ocular surface.
Immunohistochemistry shows positive staining for the newly identified LESC markers Fz7, N-cadherin and ABCB5 in cells populating the limbal crypts. Basal epithelial cells mainly express Fz7 and N-cadherin, whereas ABCB5 is observed in clusters of compact basal and suprabasal epithelial cells. Immunofluorescence signal appears weak for epithelial cells of the non-crypt rich limbus and absent for cells of the central cornea. Scale bars: 50 μm.

Figure 4. Simplified model of the LESC niche.

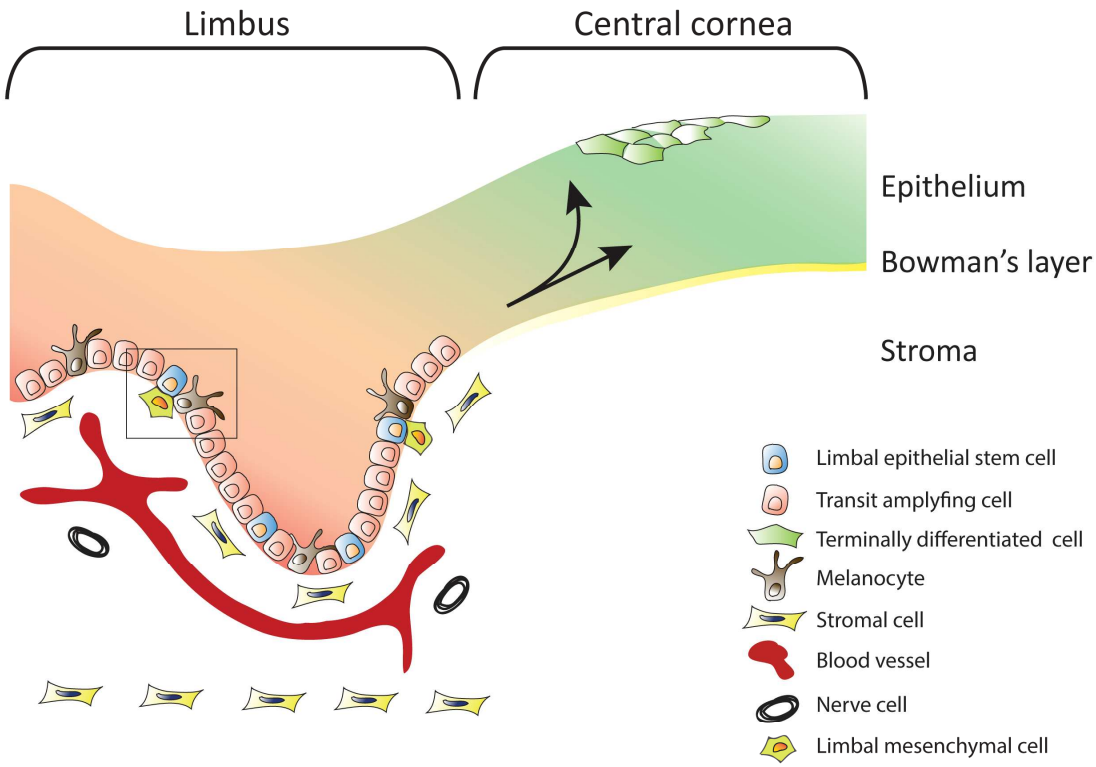
LESCs reside in the basal layer of the limbal epithelium. Daughter transit amplifying cells (TACs) divide and migrate centripetally towards the central cornea where they terminally differentiate and slough from the ocular surface. In the present model, LESCs are closely associated with both limbal mesenchymal cells and limbal melanocytes that also populate the limbal stem cell microenvironment. (B) 3D model at the cellular scale of the epithelial-mesenchymal-melanocyte cell interaction generated by volume electron microscopy and highlighted by the box in (A) (Reprinted from Dziasko MA et al[31] with permission of *Plos One*.)




Palisades of Vogt
Limbal crypt
1.
2.
Superposition of melanocytes and pigmented basal epithelial cells
Limbal crypts
3.
: Pigmented basal epithelial cells
: Melanocytes
: Tangential section shown in 3.
Palisades of Vogt



A



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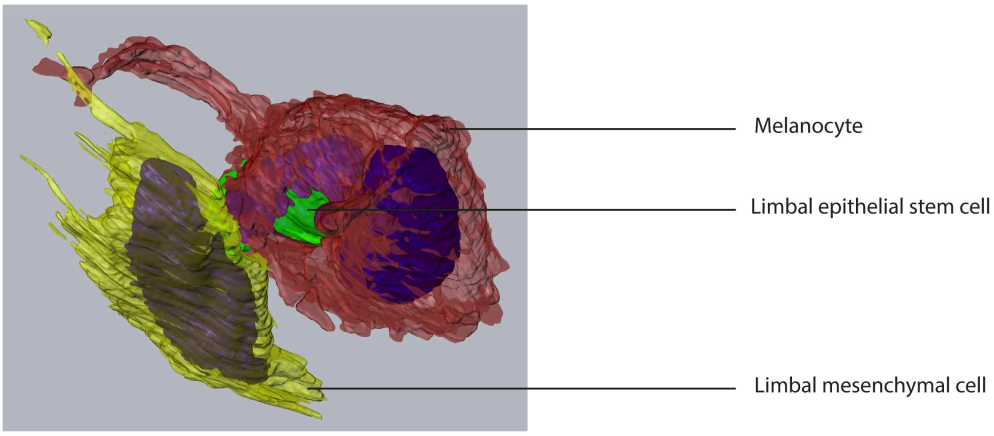

Adapted from Dziasko et al. PlosOne 2014