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Review

The diagnosis of limbal stem cell deficiency

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ABSTRACT

Limbal stem cells (LSCs) maintain the normal homeostasis and wound healing of corneal epithelium. Limbal stem cell deficiency (LSCD) is a pathologic condition that results from the dysfunction and/or an insufficient quantity of LSCs. The diagnosis of LSCD has been made mainly based on medical history and clinical signs, which often are not specific to LSCD. Methods to stage the severity of LSCD have been lacking. With the application of newly developed ocular imaging modalities and molecular methods as diagnostic tools, standardized quantitative criteria for the staging of LSCD can be established. Because of these recent advancements, effective patient-specific therapy for different stages of LSCD may be feasible.

1. Introduction

Limbal stem cells (LSCs) are adult stem cells that further differentiate into corneal epithelium. Functional LSCs are essential for maintaining the integrity of the corneal surface and transparency of the cornea. The limbus is the transition area between the transparent cornea and the opaque sclera [1]. Normal limbus and LSCs act as a barrier against the invasion of conjunctival epithelial cells onto the cornea [2–4]. In the past four decades, most studies of LSCD have focused on the biology of LSCs and their function in maintaining the regeneration of the corneal epithelium under healthy and pathologic conditions.

LSCs reside in the basal layer of epithelium within the limbal area [5,6]. Studies have provided direct evidence of LSC survival and self-maintenance at the limbus by using transgenic mice that expressed ubiquitous green fluorescent protein [7,8]. The palisades of Vogt, a limbal structure that is shown to harbor a high density of LSCs, provide the niche microenvironment that is necessary for the survival and function of LSCs, and maintenance of their stemness. It might represent the collective influence of coexisting local stromal cells, the extracellular matrix, local vasculature and soluble growth factors [4,6,9–12]. LSCs are also found outside of the palisades of Vogt in adult and fetal human eyes, including limbal epithelial crypts and limbal epithelial pit [7,13,14].

Direct damage to LSCs and/or the destruction of their niche microenvironment leads to limbal stem cell deficiency (LSCD). As a result, the barrier function of the limbus is compromised, and the corneal epithelium is replaced with conjunctival epithelial cells, which is the hallmark of LSCD. Neovascularization could occur within the corneal epithelium and stroma, and the cornea becomes opaque eventually, leading to vision loss and blindness [3,15].

Accurate diagnosis of LSCD is crucial because appropriate treatments can prevent progression of the condition and further damage to the ocular surface. For example, penetrating keratoplasty cannot restore sight to an eye blinded by LSCD before functional LSCs are restored [16]. In the past several decades, diagnosis of LSCD was predominantly made based on the patient's medical history and clinical signs. However, inherent limitations are associated with the interpretation of clinical signs [17]. For instance, the presence of a fibrovascular pannus may be caused by previous infectious keratitis rather than LSCD. Moreover, there is no consensus on the methods to stage the severity of LSCD. With the application of newly developed ocular imaging techniques and progress in identifying molecular diagnostic markers and developing new tests based on these markers, the diagnosis of LSCD is coming into a new era.

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To review the recent advances in the diagnosis of LSCD, we performed a systematic search on PubMed for English language literature published before March 31, 2017. The following combined search terms were used: “limbal stem cell deficiency” AND “diagnosis,” “limbal stem cell deficiency” AND ”in vivo confocal microscopy,” “limbal stem cell deficiency” AND “optical coherence tomography,” “limbal stem cell deficiency” AND “impression cytology.” Publications that are not related to the diagnosis of LSCD, or are published in non-English language were excluded. We collected data on diagnostic methods (clinical findings, in vivo confocal microscopy, anterior segment optical coherence tomography [OCT], impression cytology, or detection for various epithelial molecular markers). This literature review summarizes the diagnostic methods currently used in the diagnosis of LSCD and reports recent advances in the diagnosis and classification of LSCD. Based on these findings, a diagnostic approach for LSCD is proposed in the near future to overcome the limitations using the current diagnostic methods. We hope that this review will further emphasize the need to include objective testing using in vivo imaging modalities.

2. Etiology

The two pathologic mechanisms of LSCD are the direct destructive loss of LSCs and the loss of the limbal microenvironment/niche needed for LSC survival. The delicate niche of LSCs plays an important role in maintaining the LSC pool. Pathologic processes leading to either the direct loss of the LSC pool or the dysfunction of the limbal niche can result in the same phenotype of LSCD.

The etiology of LSCD can be primary, resulting from genetic mutations that lead to LSC dysfunction or destruction. The known causes of primary LSCD include aniridia [18,19], congenital epidermal dysplasia [20,21], dyskeratosis congenital [22], keratitis associated with multiple endocrine deficiencies [3], Turner syndrome [23], lacrimo-auriculo-dento-digital (LADD) syndrome [24], and xeroderma pigmentosum [25].

LSCD can also be secondary, resulting from external factors that directly destroy LSCs, damage the stem cell niche, or both. Common causes include chemical or thermal injuries [5,15,26–28]; chronic inflammation and cicatricial process from mucous membrane pemphigoid [28,29], Stevens-Johnson syndrome [30,31], graft-versus-host disease [32] and chronic limbitis [33,34]; iatrogenic injury caused by ocular surgeries, radiation, cryotherapy, or systemic chemotherapy [28,35–38]; drug-induced toxicity such as that caused by mitomycin C, 5-fluorouracil, and sulfur mustard [39–41]; and contact lens wear [28,42,43]. LSCD secondary to other ocular surface disorders has also been reported; these disorders include extensive microbial infection [28], neurotrophic (neural and ischemic) keratopathy [44], bullous keratopathy [45], and extensive ocular surface tumors [46].

Animal models have been established to investigate the pathogenesis of LSCD. Transcription factor PAX6 (paired box 6) is critical in anterior segment and corneal development. Mutations in PAX6 lead to aniridia and LSCD both in humans and mice [47,48]. In the $Pax6^{+/-}$ mouse model with heterozygous $Pax6^{+/Sey\text{-}Neu}$ mutant allele on a congenic CBA/Ca genetic background, unstable corneal homeostasis and features of progressive corneal deterioration are observed. This mouse model has been used to investigate the effect of *Pax6* genotype and age on corneal epithelial cells, and to explore the pathogenesis of LSCD [49]. Another mouse model of LSCD was created by using topical administration of benzalkonium chloride at high concentrations [50]. Sulfur mustard exposure induces severe ocular injury and late-onset LSCD in humans. Both rabbit [51] and mouse [52] models of sulfur mustard gas injury have been created. Information obtained from studies of these animal models will shed light on different mechanisms by which LSCD develops and aid in the development of appropriate treatments for LSCD arising from different causes.

3. Clinical presentations

3.1. Symptoms

Patients suffering from LSCD may present with a wide variety of symptoms related to poor epithelial wound healing and recurrent erosions. Patients often experience chronic conjunctival redness, decreased vision, photophobia, foreign body sensation, tearing, blepharospasm, and recurrent episodes of pain from recurrent epithelial breakdown. The pain, photophobia, and discomfort are often debilitating. However, most of these symptoms are nonspecific and inadequate to make the diagnosis correctly.

3.2. Clinical findings under slit-lamp biomicroscopy

Slit-lamp biomicroscopy has been the most commonly used method to make the diagnosis of LSCD. Examination under white light without fluorescein staining provides very limited information to make a correct diagnosis of LSCD. Examination under cobalt blue light using fluorescein staining is essential to detect the subtle signs of LSCD, particularly in the mild or early stage of LSCD (Fig. 1F and G).

LSCD may be progressive or stationary, diffuse or sectoral (partial). Clinical manifestations of LSCD vary depending on the severity and extent of limbal involvement.

3.2.1. Mild stage

In the mild stage of LSCD, slit-lamp examination findings include dull/irregular corneal surface with loss of light reflex, corneal epithelial opacity and loss of limbal palisades of Vogt.

3.2.1.1. Epithelial opacity. Compared with transparent corneal epithelium seen in normal cornea (Fig. 1A and B), a dull and irregular reflex of the epithelium that varies in thickness and transparency [53] is usually seen on the affected corneal surface. These abnormal epithelial cells may be a mixture of metaplastic corneal epithelial cells and conjunctival epithelial cells, or only conjunctival epithelial cells without neovascularization [54,55]. The irregular opacified epithelium can be identified under white light with careful examination, but is better visualized using fluorescein staining under cobalt blue light.

3.2.1.2. Epithelial staining. Fluorescein allows visualization of the abnormal cells and their pattern of distribution under cobalt blue light. Fluorescein tends to pool on the affected area because the abnormal conjunctival/metaplastic epithelium layer tends to be thinner and lacks cell-cell tight junctions [56]. The late staining on the abnormal area remains after 10 min and can be visualized even after rinsing with balanced salt solution or eye wash. In the mild or early stage of LSCD, stippling fluorescein staining may be present [43,53]. As the disease becomes more severe, a clear line of demarcation may sometimes, but not always, be visible between the area covered by the corneal and conjunctival epithelial cells in sectoral LSCD (Fig. 1F and G).

3.2.1.3. Loss of palisades of Vogt. The palisades of Vogt are more commonly seen in the superior and inferior limbus. In the early or mild stage of LSCD, there may be flattening at the limbus in the region of palisades of Vogt or loss of palisades of Vogt. However, this presentation is not reliably found in all cases. Loss of palisades

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