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Introduction of Stem Cells in Ophthalmology

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Abstract

Purpose: In this article, we review main clinical trials reported in the past decade.

Methods: Stem cells are relatively undifferentiated, with unlimited proliferative ability; self-renewal capability and also they can differentiate into specialized cells. Somatic stem cells in adult organisms are responsible for regenerating and self-renewing tissue. Many different stem cell types reside in the eye.

Results: One of the most important stem cells is limbal stem cell that retains in an undifferentiated state and exists in an optimal microenvironment or "niche". The importance of limbal stem cells in maintaining the corneal epithelium throughout life has long been recognized. Furthermore, stem cells in the retina have been suggested for treatment of the retinal degeneration, which generally results in constant visual disturbance or even blindness.

Conclusion: This review will briefly focus on the principal stem cells in the eye especially limbal stem cells.

Keywords: Limbal stem cell; Limbal stem cell deficiency; Transplantation; Retinal progenitor; Clinical trials

Introduction

Stem cells are undifferentiated cells with unlimited proliferative ability of self-renewal differentiating into differentiated cells. Limbal stem cells are nested in an optimal microenvironment or "niche". The importance of limbal stem cells in maintaining the corneal epithelium throughout life has long been recognized. Limbal epithelium consists of a non-keratinized stratified squamous epithelium and located between corneal and conjunctival epithelia [1-5]. It has been found to include a source of stem cells (SCs) known as corneal epithelial SCs or limbal stem cells (LSCs). Limbal Stem Cell Deficiency (LSCD) is a heterogeneous group of diseases in which the limbal epithelial stem cells (LESCs) are depleted by excessive damage or disease in the limbus to replenish the consistent corneal epithelial regeneration [6]. There are several procedures to address LSCD; some of the most global procedures are keratolimbal allograft (KLAL), conjunctival limbal autograft (CLAU) and simple limbal epithelial transplantation (SLET). In this review, we want to focus on stem cell field in the ophthalmology especially limbal stem cells.

Methods

We conducted a review of the literature using PubMed database to gather relevant English articles with the keywords "stem cell" and "eye" or "ophthalmology". Relevant articles during the past decade were selected.

Results

Limbal stem cells

Corneal epithelial stem cells: The ocular surface of the eye is covered with corneal epithelial surface, limbus, and conjunctiva [1]. Cornea has several roles such as: refraction, photo protection, transparency, and protection of internal ocular structures from the external environment [2,3]. Mature corneal epithelium is five or six layers of non-keratinizing stratified squamous epithelial cells with about 0.05 mm thickness and derived from the head surface ectoderm overlying the lens after invagination. The development of the cornea is a terminal inductive event in eye formation [4]. Conjunctival epithelium is the significant epithelium of the ocular

surface expanding from the posterior margin of the eyelids, posterior surface to the peripheral edge of the tarsal plate, then folds back to the sclera and then continues as the limbal epithelium [1-5]. Limbal epithelium consists of a non-keratinized stratified squamous epithelium and located between corneal and conjunctival epithelia [6]. It has been found to include a source of SCs known as corneal epithelial SCs or LSCs [7,8].

Concept of LSC: The repair and regeneration throughout the life of the adult cornea is responsible by LSC population [9]. In order to replenish the SC population, only one of the daughter cells is divided asymmetrically and can re-enter the niche and become a stem cell [6]. The characteristics of these stem cells are undifferentiated, slow-cycling, self-renewal, to have high proliferative potential, small size, and high nuclear to cytoplasm ratio [10-12]. The other cell is destined for differentiation to transient amplifying cell (TAC) which migrates to the corneal epithelium divides at an exponential rate and will finally differentiate into a post-mitotic cell (PMC) that can no longer multiply. The PMCs differentiate and mature into terminally differentiated cells (TDC) that represent the final phenotypic expression of the tissue type [6].

LSC niche: Stem cells in all renewable tissues are normally located in a unique and appropriate microenvironment called niche, which supports self-renewal and multipotential activity and nurtures the stem cells. SCs organized in a ridge-like structure around the circumference of the cornea, which have been suggested the ridge to be the rudimentary niche for LSC [13]. Some studies indicate that the adult LSC niche exists within the basal interpalisade epithelial papillae of the Palisades of Vogt which contains radially-oriented fibrovascular ridges. These are concentrated along the superior and inferior limbus and found at the corneoscleral limbus [14]. The function of normal SC depends more on their niche than their gene expression patterns. Interaction between stem cells and their niches are critical for regulating SC function such as quiescence, apoptosis, division, or differentiation of stem cells [15,16]. SC behavior is regulated by wide variety of cells, including neighboring cells, signaling molecules (integrins, Wnt/β-catenin,and Notch), local environmental factors such as extracellular matrix, and other intercellular contacts [17-19]. The precise molecular mechanism by which the stromal niche regulates limbal stem cells is just beginning to be understood [20].

Limbal epithelial stem cell markers: The ability to identify SCs in different organs has been prevented by nonspecific and unreliable markers. α-enolase is expressed in embryonic basal cells and localize to limbal basal epithelial cells without cytokeratin 3 (CK 3) and it is suggested as a potential stem cell marker [21,22]. The relation of LESC [23] can identify the existence of associated markers (e.g. ABCG2, vimentin, and cytokeratin 19) and the lack expression of differentiation markers (e.g. CK 3/12, connexin 43, and involucrin). Many of these markers are identified in early TACs. Consequently, small size of cell, high nucleus to cytoplasm ratio and other morphological, phenotypic and functional characteristics of stem cells are used with stem cell markers [24]. In Table 1, some important markers are described [25-34].

Markers	
ABCG2 [25-27]	ATP binding cassette transporter protein, in the limbal basal epithelium, 0.3-0.5% of cells in the limbal epithelium exhibit the side-population phenotype, to protect LSCs against oxidative stress induced by toxins
p63 [28-30]	a transcription factor, potential keratinocyte stem cell marker, DNp63α* is thought to be a highly expressed in the limbus during resting state, important for epithelial development
C/EBPδ [30,32]	CCAAT enhancer binding protein delta, a transcription factor, induces G0/G1 cell cycle arrest in mammary gland epithelial cells,
Bmi1 [33]	a repressor that to be expressed in the limbal epithelial side-population, a Polycomb group repressor involved in the self-renewal of various types of adult stem cell
Notch 1 [34]	a transmembrane receptor, maintaining cells in an undifferentiated state, localized to a small number of cells in the limbal epithelial basal layer, co-expressed with ABCG2

* DNp63α is one of 3 isoforms of p63 without an added transactivation domain (There are 6 isoforms of p63) has been shown to be more specific for LESCs than the other isoforms. [31] LSC: limbal stem cell. ATP: adenosine triphosphate

Table 1: Important stem cell markers

Limbal stem cell deficiency: etiology and classification

World Health Organization (WHO) estimates that 10 million of 45 million bilaterally blind people worldwide are the result of corneal involvement [35]. LSCD is a heterogeneous group of diseases in which the LESCs are depleted by excessive damage or disease in the limbus to replenish the consistent corneal epithelial regeneration. LSCD occurs in genetic or acquired disorders. There are hereditary or genetic causes, such as aniridia, keratitis associated with multiple endocrine deficiencies, epidermal dysplasia (ectrodactyly-ectodermal dysplasia-clefting syndrome, Keratitis-ichthyosis-deafness (KID) Syndrome) [36,37]. Aniridia(developmental dysgenesis of the anterior segment of the eye) caused by mutations in the *pax6* gene. *Pax6* is essential for eye development (oculogenesis) and maintenance of LSC function.

Most commonly, LESC deficiency is often caused by acquired factors. There are acquired causes, including ionizing and ultraviolet radiation, extensive microbial infection, contact lens (CL) wear, industrial accidents, after multiple resections of ocular surface

tumors and Johnson syndrome (SJS) and Ocular cicatricial pemphigoid (OCP) that appears through dysfunction in mucous membrane [38-40]. In other term, LSCD divided into primary or secondary. The primary, which does not present any identifiable external factors, is supported by an insufficient microenvironment. Here, there are some dysfunction/poor regulation of stromal microenvironment of limbal epithelial stem cells: congenital erythrokeratoderma, keratitis with multiple endocrine deficiency and poor nutritional or cytokine supply, neurotrophic keratopathy, peripheral inflammation and sclerocornea [41,42]. Secondary LSCD is acquired by external factors. Some causes of Secondary LSCD have also been described in acquired LSCD [40-42].

Clinical features of LSCD: Conjunctivalization of the cornea associated with goblet cells, superficial and deep vascularization, chronic inflammation, persistent epithelial defects and scarring are the essential clinical signs of LSCD [43-45]. The most important clinical trait of LSCD is conjunctivalization of the cornea that results in depletion of LESCs or loss of function by trauma or disease [46-48]. Tearing, reduced vision, chronic discomfort, chronic inflammation, stromal scarring, blepharospasm, photophobia, neovascularization, and persistent epithelial defects (PEDs) are clinical features of conjunctivalization [2,46]. It depends on the size of limbal damage, which has a pattern of partial ingrowths (partial LSCD) or will affect the whole cornea (total LSCD) [49]. Also, a variance in the thickness and transparency of the corneal epithelium on slit lamp examination may be seen [50]. The cause of the LSCD often dictates whether the disease is affecting one eye or both (unilateral or bilateral) [6].

Limbal stem cells transplantation

The aim of treatment for LSCD is to re-establish the physiologic and anatomic environment of the ocular surface by reconstruction of the corneal and conjunctival epithelium [36]. Some techniques to replace limbal stem cells have been reported. Currently, the main clinical procedures that are performed include CLAU, SLET, KLAL, and cultivated limbal stem cell transplantation (CLET) [51]. The source of stem cell in unilateral disorders is the healthy contralateral eye [4,52]. Subsequently, transplantation of allogeneic limbal SCs that are dissected from cadaveric donors or living tissue-matched eyes to treat bilateral LSCD [53,54]. Limbal deficiency in the donor eye is a potential serious risk to this procedure. Both of auto or allograft limbal tissues in transplantation have risks and benefits. Lid pathology, dry eye, uncontrolled systemic disorders and other different factors affect the achievement of LSC transplantation. Limbal transplantation is a definitive treatment for LSCD and may ameliorate the visual acuity of a patient with ocular surface disease [55]. The second strategy to treat LSCD is *ex vivo* expansion of limbal stem cells from a single small biopsy to transplant amniotic membrane (AMT), which forms the inner wall of the membranous sac surrounding the embryo during gestation. Anti-inflammatory and anti-angiogenic properties of amnion cause to use it in ocular surface reconstruction [56]. A number of clinical studies have shown transplantation of autologous, allogeneic limbal tissue or expanded cells in concomitance with AMT to promote the rapid re-epithelization required to restore corneas with LSCD [57-59]. In unilateral cases with total LSCD, transplantation of *ex vivo* cultured LSCs from the human eye (CLET) or a CLAU is utilized (conjunctival limbal auto-explant). Allo-explant limbal transplant performed in patients with bilateral total LSCD and it is extracted from a cadaveric or a living relative donor and then expanded in *ex vivo*. Recently, SLET has gained popularity in unilateral LSCD; in this technique, several pieces of one clock hour of contralateral healthy limbus are spread on a layer of AMT on the involved ocular surface [57-59].

Clinical trials of limbal stem cells transplantation: Variation of culture techniques, case selection between studies, performance of both autologous, allogeneic transplants in studies, not including the corneal surface, visual improvement in some studies and different follow-up periods limit the interpretation of clinical trial results from cultured LSC therapy [60-65]. Over the years, many different methods have been developed. The first stem cell autograft using conjunctival-limbal-corneal epithelium harvested from the healthy fellow eyes of patients with unilateral chemical burns was reported by Barraquer in 1965 [60]. In 1997, Pellegrini *et al.* [61] reported the first experience of the clinical use of *ex vivo* cultured limbal epithelial stem cells (LESCs) for treating corneal LESC deficiency. In 2003,Sangwan and colleagues published a study demonstrated reconstruction of the ocular surface in a case of severe bilateral partial LSCD with using autologous cultured conjunctival and limbal epithelium extracted from the healthy eye [62]. In addition, in 2006, they reported the clinical outcome of autologous cultivated limbal epithelial transplantation between March 2001 and May 2003.There were 88 eyes of 86 patients with limbal stem cell deficiency (LSCD). Sixty-four percent of patients were due to alkali burns and 69% eyes had total LSCD. Finally results shown 73.1% (57 eyes) were successful outcome with a stable ocular surface without conjunctivalization, 26.9% (21 eyes) had a considered failures and 10 patients were lost to follow-up [63]. The purpose of the study performed by Sangwan et al in 2011 was the efficacy of xeno-free autologous cell-based treatment with unilateral total limbal stem cell deficiency due to ocular surface burns treated between 2001 and 2010. A small limbal biopsy was taken from the healthy eye and the limbal epithelial cells were expanded *ex vivo* on human amniotic membrane using a xeno-free explant culture system. The resulting cultured epithelial monolayer and amniotic membrane substrate were transplanted on to the patient's affected eye. A completely epithelized, avascular and clinically stable corneal surface was seen in142 of 200 (71%) eyes in this retrospective study. An improvement in visual acuity, without further surgical intervention, was seen in 60.5% of eyes. All donor eyes remained healthy [64]. In 2005, Daya performed a study on 10 eyes of 10 patients with profound LSCD due to ectodermal dysplasia (3 eyes), Stevens-Johnson syndrome (3 eyes), chemical injury (2 eyes), thermal injury (1 eye), and rosacea blepharoconjunctivitis (1 eye) to investigate the outcome of *ex vivo* expanded stem cell allograft for LSCD. Seventy percent of eyes (7 of 10) had improved parameters, including vascularization, conjunctivalization, inflammation, epithelial defect, photophobia, and pain and 40% of eyes (4 cases of 7) had improved visual acuity [66]. A study by Nakamuraet al (2006) investigated 9 eyes from 9 patients with total limbal stem cell deficiency (2 eyes with Stevens-Johnson syndrome, 1 with chemical injury, 1 with ocular cicatricial pem-

phigoid, 1 with Salzmann corneal dystrophy, 1 with aniridia, 1 with graft-versus-host disease, and 2 with idiopathic ocular surface disease). The authors compared autologous serum (AS)-derived corneal epithelial equivalents with those derived from fetal bovine serum (FBS)-supplemented medium, so cultivated corneal epithelial transplantation can be used for the treatment of severe ocular surface disease. Allologous (7 cases) and autogenic (2 cases) AS-derived cultivated corneal epithelial equivalents were transplanted onto the ocular surface. During the follow-up period, the corneal surface of all patients remained stable and transparent, without significant complications and visual acuity improvement was seen in all eyes [67]. In 2006, Javadi *et al*. published a more detailed report on the early results of transplantation of autologous limbal stem cells cultivated on amniotic membrane (AM) in four eyes of 4 patients with total unilateral LSCD. After 5-13-month follow-up, visual acuity, corneal opacification, and vascularization improved in all cases [68]. In 2010, they performed keratolimbal allograft (KLAL) for treatment of 21 eyes of 20 patients with total LSCD and adequate tear production were included. Mean visual acuity improved. Graft survival rate was 61.9% at 1 year and 31% at 20 months [69]. Alex J. Shortt used a novel culture system without 3T3 feeder cells to determine the outcome of *ex vivo* cultured LESC transplantation. Allogeneic (7 eyes) and autologous (3 eyes) corneal LESCs were cultured on human amniotic membrane. Tissue was transplanted to the recipient eye after superficial keratectomy. The success rate was 60% with a successful outcome experienced [70]. In 2014, Vazirani with the cooperation of Sangwan performed a study on seventy eyes of 70 patients with unilateral, partial LSCD and reported the outcomes of autologous cultivated limbal epithelial transplantation using the healthy part of the affected eye or the fellow eye as a source of limbal stem cells in patients. In 36 eyes, the limbal biopsy was harvested from the healthy fellow eye (contralateral group) and in the remaining eyes from the healthy part of the limbus of the same eye (ipsilateral group). Clinical success was achieved in 70.59% of eyes in the ipsilateral group and 75% of eyes in the contralateral group. Outcomes are similar irrespective of whether the limbal biopsy is taken from the healthy part of the ipsilateral eye or the contralateral eye [65].

Stem cell therapy for retinal degenerative diseases

Introduction: Every year many people suffer from visual disturbance or even blindness caused by retinal detachment. The retina is a part of the central nervous system (CNS) consisting of neuronal cells (photoreceptors, horizontal cells, amacrine cells, bipolar cells and retinal ganglion cells) and glial cells (Müller glia is a specialized type of glial cell only present in the retina) [71]. Epidemiologists have found that damage to the retina can occur the entire age spectrum. For instance, the pediatric and young adult populations are affected by retinitis pigmentosa (RP) and middle-aged adults are affected by diabetic retinopathy (DR), and the elderly are affected by age related macular degeneration (AMD) [72,73]. Retinal neurodegenerative disorders divided into diseases affecting the inner retina, example glaucoma and can affect both bipolar cells and retinal ganglion cells (RGCs) [74]. Those affecting the outer retina often leads to the death of the photoreceptors. Retinal progenitor cells were identified as possible cell candidates for an accepted potential treatment strategy for retinal injury. These cells represent many of the properties associated with stem cells: 1) proliferation and expression of Nestin, a neuroectodermal stem cell marker, 2) multipotential property, and 3) self-renewal capacity [75]. Photoreceptors, intermediate neurons and Muller glia are differentiated in vitro to form sphere colonies of cell. Progenitor cells find themselves in an inhibitory environment and this is the result of the failure of retinal progenitor cells to renew retinal cells in the postnatal period [76]. Muller glia cells play a key role in the generation of multipotent precursor cells from embryonic retinal cells and these have the potential to become neurogenic retinal progenitor cells [77]. In retinal disease such as retinitis pigmentosa and age-related macular degeneration can use retinal progenitor cells for transplantation in the adult retina. Main sources of progenitor cells are: embryo, the bone marrow, neuronal genesis region, and eye (ciliary body epithelium, the iris, the ciliary marginal zone, and retina) [78].

Stem cells in retina: The ability of donor cells migrate into the desired location, to be alive after transplantation, and to differentiate into retinal cells are the three causes successful of stem cell therapy. Recent researches have shown embryonic SC (ESC), adult SC and induced pluripotent SC (iPSC) are three main types of stem cells being considered as the potential source for retinal repair and regeneration. These eye-derived PCs have the potential to differentiate into retina-specific cells in an allowable environment. IPSCs are somatic cells, which can be genetically reprogrammed to become ESC-like with the risk of tumor genesis. A sub-population of Muller glia with SC characteristics has been founded in the adult human retina. The aim of transplantation of functional retinal cells or stem cells is to restore vision by repopulating the degenerated retina via rescuing retinal neurons from further degeneration [79-81].

Target cell types for retinal degeneration treatment

Retinal pigmented epithelial cells (RPE): Human embryonic stem cells (HESCs) and human induced pluripotent stem cells (hiPSCs) can be differentiated into all retinal cell types [82] RPE plays a key role in maintenance of neural retinal function that can suggest retinal degeneration can be treated with sub-retinal injections of RPE cells. The improvement in stem cell differentiation techniques can make pluripotent stem cells differentiation into RPE cells. Extended monolayers of RPE can be isolated and transferred to a variety of substrates [83-85].

Photoreceptor: The retina includes highly specialized photoreceptors that capture the photons and transduce them into electrical signals. The ability to produce true multipotent neuroretinal progenitor cells (NRPCs) and being renewable are distinct advantages of human pluripotent stem cells (HPSCs) as sources of donor neuroretinal cell types [86]

Clinical studies: Recent studies on stem cell therapy in retinal diseases are summarized in Table 2.

Study	Retina disease	Outcomes
2003		
Van Meurs JC *et al.* [87]	AMD (clinical)	Postoperative vision with a 2-line increase in three patients
2004		
Otani A *et al.* [88]	Retinal degeneration	Treatment
2005		
Wang S *et al.* [89]	Photoreceptor loss	Survive and rescue photoreceptors using grafts
2006		
Lund RD *et al.* [90]	Retinal dystrophy	Improvement in visual performance was 100%
2007		
MacLaren RE *et al.* [91]	neovascular AMD (clinical)	Autologous RPE transplantation restores vision
2008		
Castanheira P *et al.* [92]	Retinal degeneration	Rod photoreceptor, bipolar and amacrine cell markers were expressed by grafted cells
2009		
Buchholz DE *et al.* [84]	Retinitis pigmentosa and AMD	iPSCs differentiate into functional RPEs
Lu B *et al.* [93]	Macular degeneration	The cells sustained visual function and photoreceptor integrity
2010		
Levkovitch-Verbin H *et al.* [94]	Glaucoma	BM-MSCs deliver neurotrophic factors and neuroprotection
West EL *et al.* [95]	Neural retina repair	Photoreceptors were present up to 12 months post-transplantation
2011		
Takeuchi K *et al.* [96]	AMD	Maintenance of visual acuity
Falkner-Radler *et al.* [97]	AMD	Autologous RPE sheet, Maintenance of visual acuity
2012		
Schwartz *et al.* [98]	AMD	Protection of retina in macular degeneration by replacement of the structural and trophic support provided by retinal pigment epithelium
Hu Y *et al.* [99]	RCS rat	Successful transplantation of hESC- RPE graft patch
2013		
K Homma *et al.* [100]	AMD and retiniti pigmentosa	Neural activity similar to native photoreceptors
Buchholz D *et al.* [101]	retinitis pigmentosa	HiPSCs less efficient in comparison with hESC
2014		
Hiroyuki Kamao *et al.* [102]	AMD	RPE cell sheets generated without any artificial cell sheets and performed subretinal injection into RCS
Adi Tzameret *et al.* [103]	retinal dystrophy	rescue of retinal function and significantly delayed photoreceptor degeneration
2015		
Britney O *et al.* [104]	AMD	hESCs and their differentiation maintaining into RPE using Xeno-Free derivation (a novel, synthetic substrate)
Chen ZG *et al.* [105]	AMD and Stargardt's macular dystrophy	increase in visual acuity

Table 2: Recent studies on stem cell therapy in retinal diseases. AMD: age related macular degeneration, RPE: retinal pigment epithelium, hESC: human embryonic stem cell, RCS: Royal College of Surgeons

Discussion

The focus of this review was principal stem cells in the eye especially limbal stem cells. One of the most important ocular stem cells is limbal stem cell that retains in an undifferentiated state and exists in an optimal microenvironment or "niche". The importance of limbal stem cells in maintaining the corneal epithelium throughout life has long been recognized. Moreover, stem cells in the retina as possible edge of the science, have been suggested for treatment of the retinal degeneration, which generally results in constant

visual disturbance or even blindness. However, the limbal stem cells have been studied deeply during previous days. When the patient is labeled as LSCD according to the clinical features, one of above-mentioned procedures could be done. In bilateral cases a KLAL or CLET procedure may be needed. In unilateral involvement, a CLAU or SLET could be savior. It seems that we need more studies in the field of ophthalmic stem cells

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