Published in final edited form as:

Regen Med. 2015 January ; 10(1): 1–4. doi:10.2217/rme.14.66.

Restoring the cornea from limbal stem cells

Markus H Frank[†] and

Transplant Research Program, Boston Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, USA, markus.frank@childrens.harvard.edu

Natasha Y Frank[†]

Department of Medicine, VA Boston Healthcare System, Harvard Medical School, 1400 VFW Parkway, Boston, MA 02132, USA, nfrank@partners.org

Abstract

“...in light of the result that ABCB5 helps to amplify a PAX6-positive limbal stem cell population ... it will now be important to test whether ABCB5 selection could also enhance the conversion of skin epithelial stem cells to corneal epithelial stem cells.”

Keywords

ABCB5; adult stem cell; cornea; corneal epithelial stem cells; knockout mouse; KRT12; limbal stem cells; limbal stem cell deficiency; limbus; PAX6; skin; stem cell therapy; transplantation

Corneal disease due to limbal stem cell (LSC) deficiency (LSCD) is a major cause of blindness worldwide. Recently, the ATP-binding cassette transporter ABCB5 was shown to be required for normal LSC function in corneal development and repair. Moreover, ABCB5 was found to serve as a unique molecular marker allowing for prospective isolation of pure LSC populations from human tissue with the exclusive capacity to fully restore the cornea in preclinical models of LSC deficiency. These results and additional recent advances in this field suggest that therapeutic use of molecularly defined corneal epithelial stem cell populations derived from limbus or skin might optimize chances for successful clinical transplants.

Clarity and integrity of the cornea, the eye's outermost interface with the world, are essential for human vision, and corneal disease or injuries represent leading causes of blindness worldwide. Intact corneal epithelium serves several important functions, including preservation of tissue clarity and protection from pathogens. The cornea displays a high cellular turnover rate and differentiated cells are continuously replenished throughout an individual's lifespan by stem cell populations residing at the cornea's outer edge termed limbus, in other words, LSCs [1, 2]. Consequently, patients with LSCD due to injury or

[†]Authors contributed equally

Financial & competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

disease are unable to regenerate the corneal epithelium. Moreover, surrounding conjunctival epithelium typically migrates onto the corneal stroma in LSCD, resulting in corneal neovascularization, chronic inflammation and ultimately blindness due to irreversible corneal opacity.

Current therapeutic approaches to LSCD include transplantation of autologous or allogeneic limbal tissue and, more recently, transplantation of *in vitro* culture-expanded limbal cell populations containing variable numbers of LSCs. Autologous limbal cell grafts have yielded impressive results for treatment of LSCD [3] and have represented a pioneering breakthrough in the field of cell-based regenerative medicine. Nevertheless, in the setting of unavailability of suitable molecular markers for prospective LSC enrichment, therapeutic success has usually not been achieved in those cases where LSC numbers in heterogeneous cellular grafts were found to be low (<3%) [3], highlighting the need for identification of LSC surface markers suitable for enrichment to improve therapy.

LSCs comprise only a small subpopulation of cells among heterogeneous cell populations found in the limbus. They were first identified as slow-cycling label-retaining cells in mice based on 'pulse and chase' DNA-labeling approaches [4]. In humans, LSCs are characterized by expression of the nuclear transcription factor p63, which was found to be specifically expressed by quiescent basal limbal epithelial cells [5]. A subsequent quest for LSC markers led to the discovery of several additional molecules preferentially expressed in the basal limbal epithelial layer [6]; however, none has been proven useful for successful prospective isolation of LSC so far [7].

Based on our previous demonstration of expression of the ATP-binding cassette transporter and cell surface protein ABCB5 on quiescent tissue precursors in human skin [8], we recently investigated whether ABCB5 could also serve as a marker for LSCs [9]. In human limbal tissue, we detected ABCB5-expressing cells specifically localized to the Palisades of Vogt, a known LSC niche, with the majority of ABCB5-positive cells co-expressing the LSC-expressed transcription factor p63. Consistent with these findings, ABCB5 expression was significantly reduced in patients with LSCD compared with healthy controls. Similarly, in mice, *Abcb5* specifically marked the previously identified slow-cycling label-retaining LSC population. Importantly, *Abcb5* loss of function in newly generated *Abcb5*-knockout mice resulted in the loss of quiescent LSCs due to enhanced proliferation and apoptosis, identifying a critical role for ABCB5 in LSC maintenance. *Abcb5*-knockout mice also exhibited defective corneal differentiation manifested by increased corneal fragility, decreased corneal cellularity and diminished expression of the corneal differentiation markers PAX6 and KRT12, revealing a novel essential role of ABCB5 in normal corneal development. Confirming the therapeutic utility of ABCB5 as a novel molecular marker for mammalian LSC, ABCB5 monoclonal antibody-based purification of human or murine ABCB5-positive LSCs yielded purified LSC grafts that possessed the exclusive capacity to fully restore the cornea upon grafting to LSC-deficient mice, in xenogeneic or syngeneic transplantation models. This study demonstrated that the cell surface protein ABCB5 is essential for normal LSC function in corneal development and repair, through a critical antiapoptotic role required for stem cell maintenance and survival. Furthermore, our results showed that the capacity to fully restore the cornea is exclusively contained within the

ABCB5-positive LSC compartment, indicating that this pure LSC population has the potential to significantly improve therapy for corneal disease associated with LSCD.

To date, no molecular marker suitable for prospective LSC isolation for clinical use has been available, preventing the use of pure stem cell grafts in the clinic. Thus, our findings and the related ability to now isolate molecularly defined and pure adult LSC populations based on ABCB5 expression represent a critical first step toward clinical therapeutic trials of corneal epithelial stem cell transplantation.

Several opportunities and challenges arise from these novel findings that should guide future efforts directed at clinical translation. First, ABCB5 is unique as a molecular marker that allows for specific harvesting of LSCs from human limbal tissues and enables subsequent production of molecularly defined, pure and functionally active LSC preparations, an important prerequisite for further regulatory development towards use in human clinical trials as a somatic cell therapeutics for the treatment of LSCD. This novel approach, through which prospectively purified LSCs can be therapeutically administered at defined doses, promises to represent a critical advance toward improved cell-based LSCD therapy, because successful transplants for this disorder have been shown to require sufficient numbers of LSCs within grafts to induce regeneration and to result in positive long-term outcomes [3]. How can this approach be best translated to the clinic for patient benefit?

“...ABCB5-positive limbal stem cells might also be used as allografts in limbal stem cell deficiency patients with bilateral disease...”

In a most immediate instance of clinical translation of the existing preclinical results, one strategy could consist of harvesting autologous ABCB5-positive LSCs from the contralateral, unaffected eye of an LSCD patient with unilateral disease, allowing preparation of a pure LSC graft with optimal LSC numbers for therapeutic effect, in the absence of risks of immunological rejection. Because harvesting limbal tissue from the unaffected eye of a patient can carry risks to the healthy eye, including LSCD development following large-sized biopsies, the additional development of cell culture methods for *ex vivo* expansion of harvested ABCB5-positive LSCs prior to transplantation could serve to minimize such risks, as an additional route to clinical translation. Culture expansion and re-isolation of pure populations of ABCB5-positive LSCs might be based, for example, on further customization of already established techniques for expansion of adult ABCB5-positive stem cell populations derived from human skin [8]. *Ex vivo*-expanded, purified ABCB5-positive LSCs derived from healthy eye biopsies could then also be tested, like freshly patient-derived ABCB5-positive LSCs, as syngeneic grafts in clinical trials involving LSCD patients with unilateral disease. Furthermore, when alternatively derived from cadaveric limbal tissue, ABCB5-positive LSCs might also be used as allografts in LSCD patients with bilateral disease, a larger patient cohort for whom there exists currently no satisfactory long-term therapy.

In the case of LSC allotransplantation for bilateral LSCD, unlike for syngeneic transplantation for unilateral disease, LSC transplantation might normally be anticipated to require immunosuppression to prevent allograft rejection. Currently, human recipients of allogeneic limbal grafts (containing a mixture of LSC and more differentiated limbal cells)

are known to often develop transplant rejection, despite concurrent immunosuppressive therapy. However, based on the distinct antigenic composition and immunomodulatory function of adult stem cells, including ABCB5- positive cells, described in other tissues [10, 11], it is possible that purified allogeneic LSC grafts might prove to be less immunogenic, and display a higher capacity for resistance to and evasion of recipient immune rejection, compared with more differentiated limbal cell populations currently contained in clinically employed heterogeneous limbal cell grafts, with potentially lower requirements for immune suppression, and a lower incidence of allograft rejection for purified ABCB5-positive LSC grafts. Indeed, it is conceivable that contaminating non-LSC populations within currently employed heterogeneous grafts might represent the predominant drivers of allograft rejection, whereas purified stem cells might engraft and establish microchimerism across allogeneic barriers similar to findings involving other tissue precursors [12].

“Among other tissues under investigation, two recent studies have focused on skin as a potential source for cells, without or with genetic modification, with the capacity to serve, like limbal stem cells, as corneal epithelial stem cells in corneal regeneration.”

In the case of bilateral LSCD, where autologous LSCs are not available for transplantation and where current cadaveric limbal cell allografts are often unsuccessful, identification and use of alternative autologous tissue sources for corneal epithelial stem cells might provide for alternative treatment options. Among other tissues under investigation, two recent studies have focused on skin as a potential source for cells, without or with genetic modification, with the capacity to serve, like LSCs, as corneal epithelial stem cells in corneal regeneration. Winston Kao and colleagues demonstrated therapeutic potential of mouse vibrissae hair follicle bulge-derived stem cells as an autologous source for ocular surface reconstruction in murine recipients with LSCD, highlighting the niche as a key factor for determining stem cell fate and differentiation and indicating translational potential of the skin as tissue source to derive corneal epithelial stem cells [13]. Kang Zhang and colleagues identified PAX6 as a key molecular factor capable of reprogramming rabbit skin epithelial cells to give rise to corneal epithelial cells and re-establish a healthy cornea after transplantation into a rabbit model of LSCD [14]. Given these intriguing findings, in light of the result that ABCB5 helps to amplify a PAX6-positive LSC population, and given ABCB5 expression in human skin [8], it will now be important to test whether ABCB5 selection could also enhance the conversion of skin epithelial stem cells to corneal epithelial stem cells, even in the absence of forced PAX6 overexpression. If so, skin-derived human ABCB5-positive cells might then also provide for a novel promising source of autologous corneal epithelial stem cells for LSCD therapy, especially in LSCD patients with bilateral disease.

Acknowledgments

This work was supported by NIH/NCI grants R01CA113796, R01CA158467 and R01CA138231 to MH Frank and Department of Veterans Administration VA BLR&D 1101BX000516 and VA RR&D 1101RX000989 Merit Review Awards to NY Frank. Additionally, NY Frank is the recipient of a Harvard Stem Cell Institute (HSCI) seed grant. MH Frank is co-inventor of the ABCB5-related US patents 6,846,883 (Gene encoding a multidrug resistance human P-glycoprotein homologue on chromosome 7p15–21 and uses thereof) assigned to Brigham and Women's Hospital, Boston, Massachusetts, and licensed to Ticeba GmbH (Heidelberg, Germany) and Rheacell GmbH & Co.

KG (Heidelberg, Germany). MH Frank serves as scientific advisor to Ticeba GmbH and Rheacell GmbH & Co. KG.

Biographies

References

1. Davanger M, Evensen A. Role of the pericorneal papillary structure in renewal of corneal epithelium. *Nature*. 1971; 229(5286):560–561. [PubMed: 4925352]
2. Huang AJ, Tseng SC. Corneal epithelial wound healing in the absence of limbal epithelium. *Invest. Ophthalmol. Vis. Sci*. 1991; 32(1):96–105. [PubMed: 1702774]
3. Rama P, Matuska S, Paganoni G, Spinelli A, De Luca M, Pellegrini G. Limbal stem-cell therapy and long-term corneal regeneration. *N. Engl. J. Med*. 2010; 363(2):147–155. [PubMed: 20573916]
4. Cotsarelis G, Cheng SZ, Dong G, Sun TT, Lavker RM. Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. *Cell*. 1989; 57(2):201–209. [PubMed: 2702690]
5. Pellegrini G, Dellambra E, Golisano O, et al. p63 identifies keratinocyte stem cells. *Proc. Natl Acad. Sci. USA*. 2001; 98(6):3156–3161. [PubMed: 11248048]
6. Schlotzer-Schrehardt U, Kruse FE. Identification and characterization of limbal stem cells. *Exp. Eye Res*. 2005; 81(3):247–264. [PubMed: 16051216]
7. Notara M, Alatz A, Gilfillan J, et al. In sickness and in health: corneal epithelial stem cell biology, pathology and therapy. *Exp. Eye Res*. 2010; 90(2):188–195. [PubMed: 19840786]
8. Frank NY, Pendse SS, Lapchak PH, et al. Regulation of progenitor cell fusion by ABCB5 P-glycoprotein, a novel human ATP-binding cassette transporter. *J. Biol. Chem*. 2003; 278(47):47156–47165. [PubMed: 12960149]
9. Ksander BR, Kolovou PE, Wilson BJ, et al. ABCB5 is a limbal stem cell gene required for corneal development and repair. *Nature*. 2014; 511(7509):353–357. [PubMed: 25030174]
10. Frank MH, Sayegh MH. Immunomodulatory functions of mesenchymal stem cells. *Lancet*. 2004; 363(9419):1411–1412. [PubMed: 15121399]
11. Frank, MH. US455245. 2013.
12. Le Blanc K, Rasmusson I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet*. 2004; 363(9419):1439–1441. [PubMed: 15121408]
13. Meyer-Blazejewska EA, Call MK, Yamanaka O, et al. From hair to cornea: toward the therapeutic use of hair follicle-derived stem cells in the treatment of limbal stem cell deficiency. *Stem Cells*. 2011; 29(1):57–66. [PubMed: 20957740]
14. Ouyang H, Xue Y, Lin Y, et al. WNT7A and PAX6 define corneal epithelium homeostasis and pathogenesis. *Nature*. 2014; 511(7509):358–361. [PubMed: 25030175]