Medical Sciences

The role of corneal stroma: A potential nutritional source for the cornea

Lingling Zhang, Matthew C. Anderson, and Chia-Yang Liu*

Indiana University School of Optometry, Bloomington, IN 47405, USA

Corneal stroma plays a pivotal role in normal visual function. Anatomically, it is located between the outer epithelium and the inner endothelium and is the thickest layer of the cornea. Keratocytes in the stroma produce a variety of cellular products, including growth factors/cytokines, extracellular matrix (ECM) components, and kinases. These products support normal corneal development and homeostasis.

Corneal Stroma | Keratocytes | Cytokines | Extracellular Matrix | Kinases

Introduction

The cornea is a clear layer that covers the front portion of the eye, and plays numerous roles essential for the maintenance of clear vision and ocular health, including contributing most of the eye's focusing power, and helping to shield the rest of the eye from bacteria, dust, and other harmful particles. The structure of the human cornea consists of five layers, from anterior to posterior: corneal epithelium, Bowman's layer, corneal stroma, Descemet's membrane, and corneal endothelium. Each of these layers has its own role for maintaining normal visual function. In the stroma, specifically, there has been much work examining collagen fibril assembly and the maintenance of corneal transparency. Here, we summarize the function of corneal stroma from a new perspective: a potential nutritional source for the cornea.

Growth factors and cytokines

Communication between the epithelium and the stromal mesenchyme occurs during normal development, and this communication is sustained during adulthood to maintain homeostasis. Epithelial stratification is a complex and precise process that occurs during corneal development, and is highly dependent on this intercellular communication. Zhang et al. have shown that, in conditional knockout of *β-catenin* in corneal stroma via *Kera-rtTA* driver mice, growth factor bone morphogenetic protein 4 (Bmp4), released from the stroma, acts on corneal epithelium to trigger its stratification through activation of transcriptional factor p63. Before stratification, *Bmp4* is suppressed by Wnt/β-catenin signaling. At the onset of epithelial stratification, Wnt/β-catenin signaling is dampened, leading to the loss of *Bmp4* repression, and the subsequent initiation of epithelial stratification. In addition to *Bmp4*, expression levels of several other growth factors/cytokines in the stroma were also altered after knockout of *β-catenin*, but the functions of these growth factors/cytokines have not been yet elucidated. Therefore, this result suggests that the corneal stroma might be a potential reservoir of growth factors/cytokines for corneal development and homeostasis (1).

The production of growth factors/cytokines in the stroma also contributes to wound healing. After wounding, hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF) are markedly upregulated in stromal keratocytes, while expression of HGF receptor and KGF receptor are simultaneously upregulated in the corneal epithelium (2). Stromal keratocyte cell cultures and wounded corneal organ cultures *in vitro* showed HGF and KGF affected the stratification and differentiation of the epithelium, with HGF delaying and KGF accelerating epithelial coverage of the wound (3). Further studies have demonstrated that wounded corneal epithelial cells release IL-1 alpha and IL-1 beta, signaling stromal keratocytes to upregulate HGF and KGF to modulate healing of the wounded cells by regulating proliferation, motility, and differentiation (4).

Extracellular Matrix

ECM produced by stromal keratocytes also contributes to corneal development and homeostasis. Collagen is one of the main components of ECM. In a study by Sun et al, the function of collagen V was examined by ablating *Col5a1* in the stroma via the generation of conditional knockout mice, which were bred by the mating $Col5a1^{flox/flox}$ mice with *Kera*-Cre driver mice. The conditional knockout mice had abnormal corneas with decreased stromal thickness, decreased fibril concentration, increased fibril diameters, and disorganized lamellae, which resulted in corneal opacities. This study indicated that the central regulatory functions of collagen V play an important role in fibril and matrix assembly during tissue development (5). Moreover, collagen also contributes to corneal homeostasis. In alkali-burned or lacerated corneas, expression of collagen IV in the stroma of the injured corneas was increased, suggesting collagen IV may also contribute to the formation of basal lamina-like structures between the epithelium and the stroma (6).

Lumican is a proteoglycan, located in the ECM, that is essential for corneal transparency. Previous studies have demonstrated that lumican maintains corneal transparency by modulating the synthesis of collagen fibrils, promoting corneal epithelial wound healing, regulating expression of multiple collagen genes, and maintaining corneal homeostasis (7, 8). Due to these functions, lumican-null mice exhibit delayed wound healing. Additionally, anti-lumican antibodies can retard corneal epithelial wound healing in cultured mouse eyes (8). However, wound healing can be rescued by the addition of glycosylated lumican core proteins to the injured corneas (9). Further studies have revealed that the C-terminal domain of lumican binds the glycine-serine rich domain of transforming growth factor-β receptor 1 (ALK5) to promote epithelial wound healing by activation of pERK1/2 (10, 11).

Conflict of Interest: No conflicts declared.

* Corresponding Author: Chia-Yang Liu. Email: liuchia@iu.edu

Table 1. Various products produced by stromal keratocytes, as well as categorization and functions pertaining to ocular development and homeostasis.

Category	Product	Function
Growth factors and cytokines	Bmp4	Regulate corneal epithelial stratification (1)
	Hepatocyte Growth Factor	Modulate corneal epithelial wound healing (2-4)
	Keratocyte Growth Factor	Modulate corneal epithelial wound healing (2-4)
Extracellular matrix	Collagen V	Contribute to fibril and matrix assembly (5)
	Collagen IV	Contribute to basal lamina-like structure formation (6)
	Lumican	Regulate numerous collagen genes and modulate synthesis of collagen fibrils (7-8)
	Keratocan	Contribute to matrix assembly (12)
	Fibronectin	Modulate epithelial binding to denuded corneal surface during wound healing (13-14)
Kinases	IkB kinase β	Repress oxidative stress and attenuate fibrogenesis (16)

Keratocan is another protein located in the ECM that belongs to the small leucine-rich proteoglycan family. Corneal keratocan plays a pivotal role in matrix assembly, which is essential for corneal transparency. Keratocan-null mice have thinner corneal stromas with larger collagen fibril diameters and less organized fibril packing, compared to their wild-type counterparts. In addition, the corneas have an altered shape with narrower cornea-iris angles. This reveals that keratocan plays a unique role in maintaining normal corneal shape and ensuring normal vision (12).

Fibronectin is another prominent ECM component. Fibronectin mediates the binding of epithelial cells to denuded corneal surfaces via integrin receptors (13). Additionally, after anterior keratectomy in rabbit models, fibronectin expression in the stroma is increased, which may contribute to wound repair (13, 14).

Kinases

IkB kinase β (IKKβ) is a kinase active in inflammatory response signaling, as well as the regulation of epithelial migration during corneal wound healing (15). A recent study demonstrated that *IKKβ* knockout in stromal keratocytes caused failed recovery in over half of the wounded corneas. The mice developed recurrent haze with increased stromal thickness, severe inflammation, and apoptosis. This study suggests that IKKβ in keratocytes is required for corneal wound healing via the repression of oxidative stress and attenuating fibrogenesis (16).

Conclusion

The cellular products from stromal keratocytes are essential for corneal development and homeostasis (Table 1). The generation of corneal stroma-specific driver mice *Kera-Cre* and *Kera-rtTA* has made possible the examination of numerous stromal products and their roles, and will allow the functions of more stromal products to be elucidated in the near future. These will help us explore potential pharmaceutical mechanisms, which will advance the treatment of human eye disease in the future.

Acknowledgements

Source of financial support for the work: National Institutes of Health/National Eye Institute (NIH/NEI), R01-EY21501 & EY23086 to C.Y.L.

1. Zhang Y., Yeh L.K., Zhang S., Call M., Yuan Y., Yasunaga M., Kao W.W., Liu C.Y. 2015. Wnt/beta-catenin signaling modulates corneal epithelium stratification via inhibition of Bmp4 during mouse development. *Development* 142: 3383-3393.
2. Wilson S.E., Chen L., Mohan R.R., Liang Q., Liu J. 1999. Expression of HGF, KGF, EGF and receptor messenger RNAs following corneal epithelial wounding. *Exp Eye Res* 68: 377-397.
3. Carrington L.M., and Boulton M. 2004. Hepatocyte growth factor and keratinocyte growth factor regulation of epithelial and stromal corneal wound healing. *J Cataract Refract Surg* 31: 412–423.
4. Weng J., Mohan R., Li Q., Wilson S. 1997. IL-1 upregulates keratinocyte growth factor and hepatocyte growth factor mRNA and protein production by cultured stromal fibroblast cells: interleukin-1 beta expression in the cornea. *Cornea* 16: 465-471.
5. Sun M., Chen S., Adams S.M., Florer J.B., Liu H., Kao W.W., Wenstrup R.J., Birk D.E. 2011. Collagen V is a dominant regulator of collagen fibrillogenesis: dysfunctional regulation of structure and function in a corneal-stroma-specific Col5a1-null mouse model. *J Cell Sci* 124: 4096 -4105.
6. Ishizaki M., Shimoda M., Wakamatsu K., Ogro T., Yamanaka N., Kao C.W., Kao W.W. 2009. Stromal fibroblasts are associated with collagen IV in scar tissues of alkali-burned and lacerated corneas. *Current Eye Research* 16: 339-348.
7. Chakravarti S., Magnuson T., Lass J., Jepsen K., LaMantia C., Carroll H. 1998. Lumican regulates collagen fibril assembly: skin fragility and corneal opacity in the absence of lumican. *J Cell Biol* 141: 1277-1286.
8. Saika S., Shiraishi A., Saika S., Liu C.Y., Funderburgh J.L., Kao C.W., Converse R.L., Kao W.W. 2000. Role of Lumican in the Corneal Epithelium during Wound Healing. *Journal of Biological Chemistry* 275: 2607-2612.
9. Yeh L.K., Chen W.L., Li W., Espana E.M., Ouyang J., Kawakita T., Kao W.W., Tseng S.C., Liu C.Y. 2005. Soluble lumican glycoprotein

purified from human amniotic membrane promotes corneal epithelial wound healing. *Invest Ophthalmol Vis Sci* 46: 479-486.
10. Yamanaka O., Yuan Y., Coulson-Thomas V.J., Gesteira T.F., Call M.K., Zhang Y., Zhang J., Chang S.H., Xie C., Liu C.Y., et al. 2013. Lumican binds ALK5 to promote epithelium wound healing. *PLoS One* 8: e82730.
11. Gesteira T.F., Coulson-Thomas V.J., Yuan Y., Zhang J., Nader H.B., Kao W.W. 2017. Lumican Peptides: Rational Design Targeting ALK5/TGFBRI. *Sci Rep* 7: 42057.
12. Liu C.Y., Birk D.E., Hassell J.R., Kane B., Kao W.W. 2003. Keratocan-deficient mice display alterations in corneal structure. *J Biol Chem* 278: 21672-21677.
13. Stepp M.A., Spurr-Michaud S., Gipson I. 1993. Integrins in the wounded and unwounded stratified squamous epithelium of the cornea. *Invest Ophthalmol Vis Sci* 34: 1829-1844.
14. Tervo K., van Setten G.B., Beuerman R.W., Virtanen I., Tarkkanen A., Tervo T. 1991. Expression of tenascin and cellular fibronectin in the rabbit cornea after anterior keratectomy. *Invest Ophthalmol Vis Sci* 32: 2912-2918.
15. Chen L., Meng Q., Kao W., Xia Y. 2011. IKKβ Regulates Epithelium Migration during Corneal Wound Healing. *PLoS One* 6: e16132.
16. Chen L., Mongan M., Meng Q., Wang Q., Kao W., Xia Y. 2016. Corneal Wound Healing Requires IKB kinase beta Signaling in Keratocytes. *PLoS One* 11: e0151869.