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REVIEW

The integrin needle in the stromal haystack: emerging role in corneal physiology and pathology

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Abstract Several studies have established the role of activated corneal keratocytes in the fibrosis of the cornea. However, the role of keratocytes in maintaining the structural integrity of a normal cornea is less appreciated. We focus on the probable functions of integrins in the eye and of the importance of integrin-mediated keratocyte interactions with stromal matrix in the maintenance of corneal integrity. We point out that further understanding of how keratocytes interact with their matrix could establish a novel direction in preventing corneal pathology including loss of structural integrity as in keratoconus or as in fibrosis of the corneal stroma.

Keywords Cornea · Keratocytes · Corneal stroma · Extracellular matrix · Integrins · Keratoconus

The simple cellular organization of the cornea is belied by its fascinating specialization for transparency. The cornea has three main tissue layers, the outer epithelium, the stroma in the middle and the inner endothelial layer. The stromal connective tissue, the layer mainly responsible for the corneal configuration, constituting nearly 80 % of the thickness of the cornea, is extremely resilient, capable of resisting intraocular pressure and protecting the posterior structures of the eye. The stromal tissue consists of lamellae of orthogonally arranged collagen fibrils surrounded by proteoglycans; in between the lamellae of collagen are found the keratocytes. The near-orderly arrangement of collagen lamellae, the narrow diameter of the collagen fibrils, regular interfibrillar space, associated proteoglycans and the expression of crystalline proteins in the keratocytes (Birk 2001; Kao and Liu 2002; Jester et al. 2007; Hassell and Birk 2010) are some of the main factors responsible for corneal transparency. Consequently, the factors affecting corneal transparency such as fibrotic changes due to disease or injury are problems primarily associated with the stroma. Thus it is important to achieve further understanding of the mechanisms by which the stroma acquires and maintains its structural integrity and its transparency.

Loss of corneal transparency is the second leading cause of blindness affecting the general population worldwide (Whitcher et al. 2001). The lack of orderly arrangement of the newly deposited stromal extracellular matrix (ECM) after a penetrating wound of the cornea (McCally et al. 2007) or after inflammatory corneal diseases (herpes simplex virus, infectious ulcers, rosacea) is an impediment to the transmission of light through the cornea. Occurrence of edema in the stroma as in Fuchs' dystrophy or after surgical intervention is also known to hinder the transmission of light (Portellinha et al. 2001; Meek et al. 2003; Elhalis et al. 2010). The integrity of the stroma is important for many other clinically important topics as well; shape disorders of the cornea such as keratoconus, keratoglobus, pellucid marginal degeneration (Krachmer et al. 1984) often need surgical intervention, while correction of shape disorders of the cornea such as astigmatism is achieved by the multi-billion dollar spectacle and contact lens industry. The equally beneficial refractive surgery industry uses excimer laser to reshape the curvature of the stroma to achieve vision without glasses (McDonnell 1999).

Although several studies have expanded our understanding of the importance of structural components by which cornea remains transparent, very few investigations explore the role of keratocyte cells in a normal corneal stroma; they are generally considered quiescent having left the cell cycle (Francesconi et al. 2000; Jester et al. 2007). This is unexpected, considering that the keratocytes are the cells mainly

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responsible for the production of stromal extracellular matrix (ECM) (Cintron et al. 1983; Haustein 1983) and probably are mainly responsible for the unique arrangement and assembly of the matrix. A theme of importance that has not seen the limelight, mostly due to absence of in vivo studies, is the role of mechanical communication of keratocytes with their extracellular environment in maintaining corneal structural integrity. Cell-ECM interaction is critical for communicating the state of the mechanical environment to the cell as well as in mediating cellular response to a variety of stimuli (Pedersen and Swartz 2005). We discuss the role of keratocyte-integrins and ECM components in the maintenance of the stromal structure in a normal cornea and the restoration of stromal integrity after corneal disease or injury.

Constituents of corneal stromal ECM

Collagen is the primary constituent of the stromal matrix and include collagen types I, V, VI, VIII, XII, XIII and XIV (Quantock and Young 2008). The major collagen of the stroma is collagen type I and it forms heterotypic fibrils with collagen type V (Birk et al. 1988). Some collagens are detected in the stroma only during certain phases of corneal development and include type XIV collagen (may have a role in stromal fibrillogenesis (Young et al. 2002)), type XV collagen (may influence corneal avascularity (Saika et al. 2004)) and type XXIV collagen (may regulate type I collagen fibrillogenesis (Koch et al. 2003)).

In Mov13 mutant mouse, which does not synthesize collagen I, there was normal cellular organization during corneal morphogenesis, but the stroma lacked orthogonal organization of collagen lamellae and had markedly less collagen; also collagen fibrils present were of smaller fibril diameter than in normal cornea (Bard and Kratochwil 1987). Patients with Ehlers-Danlos syndrome due to COL5A1 haploinsufficiency or exon-skipping mutation in COL5A2 had thinner corneas. Collagen V regulates collagen fibril assembly and Col5a1-haploinsufficient mouse had opacity of cornea, thinner stroma, disorganized lamellae and fewer collagen fibrils with larger diameters (Segev et al. 2006; Sun et al. 2011). Lack of collagen type VIII leads to dysgenesis of the anterior segment in mice with protrusion of anterior chamber and thin corneal stroma (Hopfer et al. 2005). Further, there is increased expression of type XIII collagen in the myofibroblasts of stromal scar tissue in keratoconus corneas, indicating a role in wound healing (Maatta et al. 2006) (Table 1).

Table 1 Established functions of extracellular matrix components of the corneal stroma

Gene disruption / knockout / mutation	Corneal / stromal phenotype / disease	References
Collagen, type I, alpha 1	Less collagen, lack of orthogonal organization, smaller fibril diameter	(Bard and Kratochwil 1987)
Collagen, type V, alpha 1 / 2	Opacity of cornea, thinner stroma, disorganized lamellae, fewer collagen fibrils, larger fibril diameter; Ehlers-Danlos syndrome	(Segev et al. 2006; Sun et al. 2011)
Collagen, type VIII, alpha 1 and alpha 2	Dysgenesis of the anterior segment: keratoglobus-like protrusion, thin corneal stroma, thin Descemet's membrane, reduced number of endothelial cells; Fuch's endothelial dystrophy and posterior polymorphous corneal dystrophy	(Biswas et al. 2001; Hopfer et al. 2005)
Lumican / fibromodulin / PRELP / opticin	High myopia	(Wang et al. 2006; Majava et al. 2007)
Lumican	Corneal opacity, larger collagen fibril diameter, disorganized collagen arrangement	(Chakravarti et al. 1998)
Keratocan	Thinner stroma, larger collagen fibril diameter, less organized fibrils; cornea plana	(Pellegata et al. 2000; Liu et al. 2003)
N-acetylglucosamine-6-O-sulfotransferase	Thinner stroma, decreased interfibrillar spacing, disorganized collagen arrangement; macular corneal dystrophy	(Midura et al. 1990; Hayashida et al. 2006)
Decorin	Mild changes in collagen ultrastructure; congenital stromal dystrophy	(Bredrup et al. 2005; Zhang et al. 2009)
Biglycan	Mild changes in collagen ultrastructure	(Zhang et al. 2009)
Decorin and biglycan	Severe disruption of collagen fibril structure and organization	(Zhang et al. 2009)
Mimecan	No significant abnormalities	(Beecher et al. 2005)
Thrombospondin-1	Chronic edema and persistent opacity following penetrating corneal injury	(Blanco-Mezquita et al. 2013)
Osteopontin	Upon corneal injury: delayed wound healing, fewer myofibroblasts, reduced expression of TGF-β	(Miyazaki et al. 2008)
Hevin	Upon corneal injury: excessive apoptosis, aberrant wound healing, early corneal haze, severe chronic inflammation, stromal fibrosis	(Chaurasia et al. 2013)
Tenascin-C	Upon corneal injury: delayed stromal wound healing, impaired keratocyte migration, fewer myofibroblasts, less invasion of macrophages, reduced expression of TGF-β	(Matsuda et al. 1999; Sumioka et al. 2013)

Proteoglycans are found associated with collagen fibrils in the corneal stroma; they have a core protein with one or more glycosaminoglycan (GAG) side chains covalently attached. Corneal proteoglycans mainly belong to the small leucine-rich type proteoglycan (SLRP) gene family and include decorin, biglycan, lumican, keratocan, mimecan and fibromodulin. Decorin and biglycan have chondroitin / dermatan sulfate GAG chain attached to their core protein; while lumican, keratocan, mimecan and fibromodulin have keratan sulfate GAG chains. Decorin and keratocan are present in relatively high levels throughout the stroma in mature corneas (Chakravarti et al. 2006; Zhang et al. 2009). In contrast, post-natal keratocytes had low-immunoreactivity for mimecan (Chakravarti et al. 2006). Fibromodulin has been found in the mouse central cornea at post-natal day (P) 14 but becomes restricted to the limbus by P30 (Chen et al. 2010). Similarly, biglycan was expressed highly during corneal development but gradually decreased to low levels in mature cornea (Zhang et al. 2009) and lumican becomes restricted to posterior stroma in adult cornea (Chakravarti et al. 2006). These SLRPs have similar or homologous core protein and along with their GAG chains are also similar in size and thus considered to have a role in maintaining the regular distance between collagen fibrils in the corneal stroma (Hassell and Birk 2010). They are also involved in collagen fibril growth, fibril organization and ECM assembly (Chen et al. 2010; Chen and Birk 2013). SLRPs also regulate cell-matrix-interactions: for example, lumican modulated cell migration by binding to integrins (Lee et al. 2009; Zeltz et al. 2010); while decorin inhibited cell adhesion to fibronectin and thrombospondin (Winnemoller et al. 1991; Merle et al. 1997). Further, SLRPs have important role in modulation of inflammation (Moreth et al. 2012), and can regulate corneal wound healing (Mohan et al. 2011).

Loss of function mutations in genes encoding SLRPs lumican, fibromodulin, PRELP and opticin are associated with high myopia (Wang et al. 2006; Majava et al. 2007). Mice with null mutation in lumican develop corneal opacity with disorganized and abnormally thick collagen fibrils (Chakravarti et al. 1998); however, keratocan-deficient mice had normal corneal transparency despite thinner stroma, larger stromal fibril diameter and less organized collagen fibrils (Liu et al. 2003). People with mutations in the gene encoding keratocan suffer from cornea plana (Pellegata et al. 2000). Mutations in the gene that encodes a sulfotransferase that is vital for sulfation of keratan sulfate chains cause macular corneal dystrophy (Midura et al. 1990; Hayashida et al. 2006). Mutations in the decorin gene have been identified as the cause of corneal opacity as in congenital stromal dystrophy (Bredrup et al. 2005). Deficiency of either decorin or biglycan in mice caused only mild changes in stromal collagen ultrastructure; however deletion of both caused severe disruption of collagen fibril structure and organization (Zhang et al. 2009). Mimecan-deficient mice did not show significant abnormalities in corneal stromal architecture, indicating a lesser role for this proteoglycan (Beecher et al. 2005) (Table 1).

Matricellular proteins are non-structural ECM proteins that regulate cell function by interacting with and modulating the actions of cell-surface receptors, growth factors, cytokines, proteases, and matrix proteins (Bornstein and Sage 2002). Role of matricellular proteins such as hevin, connective tissue growth factor (CTGF / CCN2) and thrombospondin are less defined in a normal corneal stroma. However, matricellular proteins have an important role in wound healing (Kyriakides and Bornstein 2003) and their involvement in the corneal wound healing (Table 1) is discussed later in this review.

Identification of biological roles of various integrins

Cells interact with their extracellular matrix (ECM) mainly through integrin receptors. Integrins are glycoprotein heterodimers made of noncovalently associated α and β chains. The vertebrate family of integrins has 18 α subunits and 8 β subunits allowing 24 different heterodimers to exist, and are classified further into subgroups based either on their subunit composition or ligand-binding properties. They recognize and bind to aspartic acid or glutamic acid-based sequence motifs in various ligands, including collagen, fibronectin, laminin and growth factors (Hynes 2002; Barczyk et al. 2010). Mg^{2+}, Ca^{2+} and Mn^{2+} cations are also known to have a crucial role in binding of integrins to their ligands (Arnaout et al. 2002; Campbell and Humphries 2011).

Though integrins have been identified to have a role in several diseases such as cancer, infection, thrombosis and autoimmune disorders, identifying a specific integrin involved or assigning a precise role has been difficult because of the multifactorial nature of most diseases and due to the multiple integrins present on the cells and their redundant binding properties (Cox et al. 2010). However, whole-body deletion of individual integrin subunits is allowing the identification of unique function of each integrin subunit (Hynes 2002; Chen and Sheppard 2007; Takada et al. 2007); such deletions though are usually lethal and involve studying of embryonic or neonatal mice. Among the breakthroughs, for example, is the recognition of the critical role of the β1 integrin subunit in gastrulation; whole-body deletion of β1 integrin resulted in lethality shortly after embryo implantation (Fassler and Meyer 1995; Stephens et al. 1995). Whole-body deletion of the α5 integrin caused embryonic lethality around day 10–11 due to abnormality in the development of the posterior trunk with reduced mesoderm and defects in formation of the neural tube and vasculature (Yang et al. 1993). On the other hand, mice harboring a whole-body deletion of the β2 integrin were viable but developed chronic dermatitis and defective T cell function with impaired leukocyte adhesion, neutrophil

migration, leukocytosis and spontaneous infections (Scharffetter-Kochanek et al. 1998). So far, each of the 24 known integrin subunits have been inactivated resulting in various defects ranging from impaired bone healing, epidermal detachment, inflammation in skin and lungs, cerebral hemorrhage, kidney defect, heart defect and muscular dystrophy. In addition, the post-natal functions of individual integrins are also being deduced from a growing number of conditional cell-type-specific knockout animals that are becoming available, (see reviews (Hynes 2002; Chen and Sheppard 2007; Takada et al. 2007)). Though gene deletions allow us to understand the overall role of integrin subunits, the signaling mechanisms by which integrins regulate the function of various tissues have yet to make significant progress.

Established functions of integrins in the eye and their association to eye diseases

Though integrins are important for normal development and tissue homeostasis, their role in pathology / wound healing, largely perhaps, drives the interest in understanding their functions in the eye. This is because, unlike many other tissues, even minor disorganization of tissues of eye due to a pathology or injury can drastically affect normal vision. Modulating the activity of integrins could serve as valuable therapeutic targets (Kapp et al. 2013). Several studies have revealed the expression pattern of integrins in the tissues of the eye during development and in pathology (see reviews, (Stepp 2006; Vigneault et al. 2007; Carter 2009)), however, very few studies reveal their actual function in the eye.

In the eye, conditional deletion of integrin β1 in the lens causes microphthalmia due to apoptosis of the lens epithelium and disintegration of the lens fibers (Simirskii et al. 2007). In a similar study, there was disrupted lens fiber morphology that suggested a role for integrin β1 in linking lens fibers to the surrounding ECM (Samuelsson et al. 2007). Also, deactivation of both α3 and α6 results in dysmorphogenesis of the developing lens (De Arcangelis et al. 1999). αvβ5 is the only integrin present at the retinal pigment epithelium-photoreceptor interface and its absence resulted in reduced retinal adhesion and age-related blindness as αvβ5 integrin in the retinal pigment epithelium is essential for phagocytic uptake of shed photoreceptor disks (Nandrot et al. 2004). On the other hand, polarized integrin localization is required for normal morphogenesis of vertebrate retinal pigment epithelium (Bogdanovic et al. 2012). α4β1 was found to be required for survival of developing retinal neurons (Leu et al. 2004). Blocking of β1 integrins significantly inhibited retinal ganglion cells migration from ventricular zone to the vitreal border in developing chick retina (Cann et al. 1996).

Alterations in integrin expression have alluded to their importance and role in vascular changes associated with diabetic retinopathy (Robbins et al. 1994; Ljubimov et al. 1998), in corneal disorders such as bullous keratopathy (Spirin et al. 1999; Ljubimov et al. 2001), in recurrent epithelial erosions (Pal-Ghosh et al. 2004), and in keratoconus (Bystrom et al. 2009). Integrins are also among various factors that cause retinal detachment in proliferative diabetic retinopathy and proliferative vitreoretinopathy (Kupper and Ferguson 1993; Guidry et al. 2003). In rats, blockade of α4 integrin diminished diabetes-induced increase in NF-kappaB activation, VEGF and TNF-alpha levels and significantly reduced leukocyte adhesion and vascular leakage (Iliaki et al. 2009). Synthetic peptide antagonists of integrin αvβ3 inhibited retinal neovascularization in a murine model when administered as intraperitonal or periocular injections (Luna et al. 1996), inhibition of αv integrins prevented basic fibroblast growth factor-induced neovascularization of cornea (Klotz et al. 2000), and inhibition of α5β1 inhibited and regressed corneal neovascularization after alkali-burns (Muether et al. 2007). Blockade of α4β1 decreased dry eye symptoms and inflammation significantly (Ecoiffier et al. 2008). Furthermore, integrin signaling and integrin-linked kinase activity could affect the organization and contractility of actin cytoskeleton in trabecular meshwork cells (Faralli et al. 2011), a factor known to modulate aqueous humor outflow.

Role of integrins in the cornea

Corneal epithelium is known to express β1, β4, β5, α2,α3, α4, α5, α6 and αv (Lauweryns et al. 1991; Stepp et al. 1993), with α5, α6, and β4 localizing specifically to the basal membrane of the basal cells (Stepp et al. 1993) while keratocytes are known to express α2β1, α3β1, α4β1, α6β1 and αvβ3 (Stepp 2006). Several studies have reported alterations in the distribution and expression of integrins during corneal wound healing (Grushkin-Lerner and Trinkaus-Randall 1991; Murakami et al. 1992; Stepp et al. 1993, 1996; Latvala et al. 1996; Hutcheon et al. 2005; Carter 2009) indicating an important role for integrins in corneal homeostasis. For example, elevated MMP9 activity correlated with cleavage of integrin β4 and recurrent corneal erosions (Pal-Ghosh et al. 2011). cDNA microarray studies indicated that pathogenesis of granular corneal dystrophy II may be due to alterations in transforming growth factor-β receptor- and integrin-mediated signaling pathways (Choi et al. 2010). In wound healing after corneal epithelial abrasion, integrin α6 was detected in migrating epithelial cells (Latvala et al. 1996); and in final stages of wound healing repression of α6 integrin is thought to contribute to the reduced proliferation and attachment of epithelium to the basal membrane (Gaudreault et al. 2007). In an indirect inference, mice lacking heparan sulfate proteoglycan, syndecan-1, showed slow cell migration and reduced expression of α9 integrin during corneal

epithelial wound closure (Stepp et al. 2002). In an in vitro study with implications for continuation of normal corneal health, αvβ6 was shown to have a role in the maintenance of corneal epithelial barrier (Guo et al. 2013). However, interpretation of the precise function of integrins in normal or wound healing cornea remains problematic as there is dearth of reports using integrin gene-knockout models. Thus, in a solitary study, mice lacking integrin β6 revealed that αvβ6 (which is upregulated in the corneal epithelium during wound healing (Hutcheon et al. 2005)) was essential for basement membrane zone regeneration during healing of keratectomy wounds (Blanco-Mezquita et al. 2011).

Integrin β1 and corneal structural integrity

It is in this scenario that keratocyte-integrin β1 (*Itgb1*) was found to have an important role in the maintenance of structural integrity of the normal adult cornea (Parapuram et al. 2011), with implications on corneal wound healing without scarring. Postnatal (at 23 to 26 days) deletion of *Itgb1* ($Itgb1^{-/-}$) in keratocytes caused considerable thinning of mice corneal stroma accompanied by loss of epithelial layers and other associated pathological changes similar to the pathology seen in the corneas of patients with keratoconus (Efron and Hollingsworth 2008). However, when *Itgb1* was deleted in keratocytes at postnatal day 40 the mouse cornea remained normal, unlike the corneas that developed keratoconus-like phenotype when *Itgb1* was deleted at postnatal days 23–26 (Parapuram et al. 2011). This implied that the deletion of *Itgb1* at days 23–26 after birth somehow affected the process of postnatal stromal maturation. It is known that corneal structural organization continues to mature after birth in cats, dogs and humans (Ehlers et al. 1976; Moodie et al. 2001; Montiani-Ferreira et al. 2003). In humans, adult corneal thickness is reached at about 3 years of age (Ehlers et al. 1976). In the mouse, even though the keratocyte cell division plateaus about postnatal day 21, the stroma continues to mature, to reach adult thickness only by postnatal day 30 (Song et al. 2003; Chakravarti et al. 2006; Jester et al. 2007). However, the specific molecular events that allow maturation of the stroma remain unknown.

Stromal ECM maturation

Several factors could influence the formation of the stromal matrix and its maturation. Thus, even though collagen I can self-assemble to form fibrils in vitro, several other molecules, including other collagens, fibronectin and integrins regulate collagen fibril formation in vivo (Kadler et al. 2008). Collagen type V is involved in initiating fibril assembly (Wenstrup et al. 2004, 2006). Antibody against α2β1 integrin inhibited collagen fibril assembly by vascular smooth muscle cells, while promoting the high-affinity binding state of α2β1 integrin enhanced the fibril assembly (Li et al. 2003). Collagen fibril diameter, on the other hand, is known to be determined by the core proteins of lumican and decorin (Rada et al. 1993), proteoglycans present in the corneal stroma, as well as by type V collagen (Birk et al. 1990).

The thinning of $Itgb1^{-/-}$ corneas was not accompanied by any change in stromal collagen fibril diameter or gross proteoglycan deposition (Parapuram et al. 2011). However, instances of reduction in space between collagen fibrils in the $Itgb1^{-/-}$ corneas (Parapuram et al. 2011), indicate the inability of the matrix to resist strain; repulsive charges of proteoglycans, for example, are known to resist compression (Buschmann and Grodzinsky 1995). These observations in the $Itgb1^{-/-}$ corneas support the prevalent hypothesis that thinning of keratoconus cornea is attributable to increased sliding of the collagen and not due to loss of tissue (Polack 1976; Edmund 1988). Consistent with this viewpoint is the recent report of decreased expression of lysyl oxidase (LOX), the enzyme that is involved in the cross-linking collagen molecules as well as elastin, in keratoconus corneas (Dudakova et al. 2012). Reducing the formation of cross-links in collagen by inhibiting LOX is known to reduce the mechanical stability of matrix and decreased adhesion signaling (Bruel et al. 1998; Levental et al. 2009). Conversely, LOX-induced collagen cross-linking and tissue stiffening promoted integrin clustering and increased adhesion signaling (Levental et al. 2009). Few studies have indicated that the interlamellar and probably intralamellar slippage in keratoconus stroma is due to loss of cohesion between collagen fibrils and non-collagenous matrix (proteoglycans) (Fullwood et al. 1992; Daxer and Fratzl 1997; Meek et al. 2005). It is possible that tractional forces generated by keratocytes (Petroll et al. 2003) through integrins could provide appropriate spatial conditions for cross-linking by LOX or for proteoglycan-collagen interaction, thus facilitating proper maturation of matrix. In this context, it has to be noted that decreased expression of integrins has been reported in keratoconus corneas (Vorkauf et al. 1995; Tuori et al. 1997). Deficiency of magnesium, which is required for activation of integrins, has also been reported in patients with keratoconus (Thalasselis et al. 1988). Thus, it is highly likely that the etiology of keratoconus could be multifactorial but converge on a common mechanism that prevents matrix maturation during corneal development resulting in gradual development of the disease. Similarly, in a healing cornea conditions for proper maturation of newly laid matrix may be deficient and is perhaps the reason for its disorganized deposition. Consequently, keratocyte-matrix interactions and integrin signaling during the entire course of corneal development and in wound healing warrants further study.

Furthermore, maturation of the stromal matrix is perhaps vital for its role as a load-bearing structure. Diseases such as keratoconus paradoxically allow appreciation of the mechanical role of matrix in a normal cornea. The stromal matrix can shield the keratocytes from stress; for any external stress applied (such as the intraocular pressure) the matrix typically bears most of the stress, resulting in almost no load on the cells (Pedersen and Swartz 2005). It is possible that in keratoconus as well as in the *Itgb1*$^{-/-}$ corneas there is increased stress on the keratocytes due to loss of integrity of the matrix, affecting the normal physiology of keratocytes. We can only speculate presently that such stress could disrupt the growth factor-mediated communications between the keratocyte and corneal epithelium (Wilson et al. 1999; Imanishi et al. 2000) and is a cause for the reduced cell division of basal epithelia in the *Itgb1*$^{-/-}$ corneas (Parapuram et al. 2011). The necessity of a mature matrix is again evident as the swelling pressure caused by hydrophilic glycosaminoglycans is counteracted by the lamellar tension and the cohesive forces between lamellae (Klyce et al. 1971; Smolek and McCarey 1990; Dupps and Wilson 2006). Disruption of the lamellar tension and the cohesive forces between lamellae is considered a cause of edema (Dupps and Wilson 2006) and could be one explanation for the occurrence of edema in *Itgb1*$^{-/-}$ corneas. In fact, activation of integrin β1 signaling is known to reduce edema in other systems (Rodt et al. 1994) and this may also have relevance in treating corneal edema. Also, cross-linking of collagen to strengthen the stroma, a therapeutic strategy for management of keratoconus (Wollensak et al. 2003), is thus a logical substitute for the role of integrins in the development of stromal matrix into a resilient load-bearing structure.

Integrin-mediated events that modulate stromal wound healing

The wound healing events are complex and often occur simultaneously and are influenced by several factors (Netto et al. 2005). In cutaneous wound healing, integrins are implicated in all phases of wound repair, including migration of various types of cells, matrix remodeling and in wound contraction (Larsen et al. 2006; Li et al. 2007). Deletion of integrin β1 in fibroblasts delayed cutaneous wound closure, less granulation tissue formation, reduced deposition of ECM and α-smooth muscle actin expression (Liu et al. 2010). Proteoglycans, such as lumican can promote skin wound healing by enhancing α2β1 integrin-mediated fibroblast contractility (Liu et al. 2013). Also, integrins are now considered to regulate the availability of active transforming growth factor-β (TGF-β), the most potent stimulator of fibrosis, at the site of injury by protease-dependent and protease-independent mechanisms (Margadant and Sonnenberg 2010). The protease-independent TGF-β activation occurs through integrin-mediated cell traction forces (Annes et al. 2004; Fontana et al. 2005; Wipff et al. 2007; Wipff and Hinz 2008) and is likely to influence the outcome of corneal wound healing, especially being a structure that experiences tension. Isoforms TGF-β2 and -β3 have been localized in the matrix of normal stroma, while isoforms TGF-β1, −β2 and -β3 were expressed in the stromal cells during wound healing (Saika et al. 2008). Such activation of TGF-β by αvβ3, αvβ5, αvβ6 and αvβ8, as well as by β1-integrin with a yet unidentified α-subunit, has been demonstrated in vitro (Wipff and Hinz 2008); αvβ3, as noted earlier, is expressed by keratocytes.

In corneal wound healing, stromal keratocytes are activated by factors released by the injured corneal epithelium, by the neutrophils and lymphocytes that reach the site of injury and from the tear film (Klenkler and Sheardown 2004). The migration of neutrophil in injured mouse cornea is integrin-dependent and these neutrophils interact with keratocytes and prefer to migrate along the keratocyte network (Petrescu et al. 2007; Hanlon et al. 2014). These activated keratocytes proliferate to repopulate the region of stromal injury in which keratocytes have disappeared due to apoptosis (Helena et al. 1998; Wilson 2002). At 1–2 weeks after injury myofibroblasts, mostly differentiating from bone marrow-derived cells (Barbosa et al. 2010; Wilson 2012), and less transparent than keratocytes (Jester et al. 1999; Piatigorsky 2000) can be detected (Jester et al. 1995). Myofibroblasts deposit large amounts of ECM (Klingberg et al. 2013) that contributes to stromal opacity (Wilson 2012) and their contraction is associated with wound closure, compaction of matrix (Tomasek et al. 2002; Gabbiani 2003) and activation of latent TGF-β (Wipff et al. 2007). Myofibroblast apoptosis (Wilson and Chaurasia 2007) results in their disappearance slowly over weeks following restoration of corneal epithelial basement membrane after injury (Stramer et al. 2003; Dupps and Wilson 2006); conversely, corneal haze due to fibrosis is associated with persistent presence of myofibroblasts (Netto et al. 2005). Stromal scar tissue had markedly decreased levels of lumican (Sundarraj et al. 1998) and this may be related to TGF-β-induced keratocyte-myofibroblast transition that is known to decrease the expression of normal stromal proteoglycans lumican, keratocan, mimecan and decorin, but also increase the expression of biglycan, a proteoglycan present in fibrotic tissue (Funderburgh et al. 2001). Stromal scar tissue may take several years to resolve and may involve remodeling of disorganized stromal collagen (Netto et al. 2005) by keratocytes that repopulate the stroma after the disappearance of myofibroblasts (Wilson 2012).

Fibrosis is associated with increased integrin-mediated adhesion signaling in the myofibroblasts (Hinz et al. 2007; Wong et al. 2011). Relatedly, conduciveness for growth of a particular type of cell can be determined by the stiffness of the matrix (Georges et al. 2006; Carracedo et al. 2010) and may explain the persistent presence of highly adhesive and

contractile myofibroblasts until remodeling of matrix occurs. In fibroblasts, blocking adhesion signaling through integrin β1 prevented an increase in alpha-smooth muscle actin positive stress fibers even in the presence TGF-β (Arora et al. 1999). Integrins, such as α11β1, α5β1, are known to regulate keratocyte to myofibroblast differentiation (Jester et al. 1994; Carracedo et al. 2010). Conversely, other studies have indicated that myofibroblastic differentiation required TGF-β and PDGF as well as synergistic signaling with integrins (Jester et al. 2002; Singh et al. 2011). Equally, autocrine TGF-β signaling is known to regulate conversion of keratocytes to myofibroblasts (Masur et al. 1996). On the other hand, overexpression of decorin in the keratocytes blocked TGF-β-mediated myofibroblast transformation of keratocytes (Mohan et al. 2010), while decorin gene therapy inhibited corneal scarring (Mohan et al. 2011). Also, decorin interacted with α2β1 in endothelial cells and modulated the collagen I binding activity of the integrin (Fiedler et al. 2008). Since α2β1 is also expressed in keratocytes, it should be interesting to know whether such integrin-decorin interaction has a part in stromal wound healing.

Non-structural matricellular proteins present in the ECM are known to influence corneal stromal wound healing. Secreted protein that is acidic and rich in cysteine (SPARC) is expressed by activated keratocytes adjacent to stromal wound and induced contraction of matrix by keratocytes (Mishima et al. 1998). Connective tissue growth factor (CTGF / CCN2) is also expressed by keratocytes and has a major role downstream of TGF-β in inducing fibrosis (Folger et al. 2001; Leask 2008). CTGF mediates its activity primarily through interaction with cell adhesion receptors, including integrins (e.g. α6β1, αvβ3) and heparan sulfate proteoglycans (Chen and Lau 2009) and also binds to mannose 6-phosphate / insulin-like growth factor 2 receptor on keratocytes (Blalock et al. 2012); IGF-2 has a major role in the development of fibrosis (Grotendorst et al. 2004). CCN3 (Nov) induced neovascularization when implanted in rat cornea and is a ligand of integrins αvβ3 and αvβ1 (Lin et al. 2003). Thrombospondins, usually produced by platelets during wound repair, are also produced in avascular cornea by keratocytes in a wound-repair phenotype (Hiscott et al. 1996; Armstrong et al. 2002). Thrombospondin-1 is implicated in the transformation of keratocytes into myofibroblasts via TGF-β (Matsuba et al. 2011) and is known to interact with different integrins to modulate inflammatory response, and cell migration (Li et al. 2002; Short et al. 2005; John et al. 2010). Thrombospondin-1 deficient mice have chronic edema and persistent opacity after penetrating corneal wounding (Blanco-Mezquita et al. 2013). Osteopontin (OPN) was also found to have a role in wound healing; wound healing was delayed and there were fewer myofibroblasts and TGF-β expression in the stromal wound of OPN knockout mice (Miyazaki et al. 2008). OPN is known to promote integrin activation in gastric cancer cells (Lee et al. 2007) and is upregulated along with αvβ3 during glial scar formation after focal stroke (Ellison et al. 1998). Hevin, though not expressed in normal cornea, is transiently expressed in early stages of stromal wound healing and excimer laser-induced irregular phototherapeutic keratectomy in hevin-null mice induced excessive apoptosis and aberrant wound healing, including early corneal haze, severe chronic inflammation and stromal fibrosis (Chaurasia et al. 2013). Tenascin was upregulated in the corneal stroma upon incision injury; delayed stromal wound healing was seen in tenascin C knockout mouse due to impaired keratocyte migration, myofibroblast differentiation and invasion of macrophages, along with reduced expression of TGF-β1 (Matsuda et al. 1999; Sumioka et al. 2013).

Hitherto, the importance of ECM components in maintaining the structural integrity of the cornea has been established unambiguously. In parallel, the current evidence also point to a fundamental role of keratocyte / integrin interactions with structural and non-structural matrix components in the development of normal corneal stroma and in regaining the integrity of the stroma after being affected by disease or injury. Keratocyte / myofibroblast and their integrins have a role in both normal wound healing as well as fibrosis, indicating that a delicate balance in their activity is required for normal healing to occur. Manipulations of integrin activity in keratocytes could be beneficial for regaining stromal integrity after being affected by disease or injury; the drug alpha-trinositol reduced edema due to acute inflammation after injury to skin by increasing integrin activity (Lund and Reed 1994; Rodt et al. 1994). Would enhancing integrin activity at crucial stages of corneal development increase the chance for normal maturation of matrix and prevent the development of keratoconus? A similar strategy may be employed for ordered matrix deposition during stromal wound healing; the exact role of integrins in normal stromal maturation will perhaps also reveal the elements required for transparency of newly deposited matrix. Further defining the events associated with integrin / keratocyte-mediated matrix maturation in the developing cornea as well as during wound healing thus remains a relevant strategy.

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