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Review

Mechanical interactions and crosstalk between corneal keratocytes and the extracellular matrix

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ABSTRACT

The generation of cellular forces and the application of these physical forces to the ECM play a central role in mediating matrix patterning and remodeling during fundamental processes such as developmental morphogenesis and wound healing. In addition to growth factors and other biochemical factors that can modulate the keratocyte mechanical phenotype, another key player in the regulation of cell-induced ECM patterning is the mechanical state of the ECM itself. In this review we provide an overview of the biochemical and biophysical factors regulating the mechanical interactions between corneal keratocytes and the stromal ECM at the cellular level. We first provide an overview of how Rho GTPases regulate the sub-cellular pattern of force generation by corneal keratocytes, and the impact these forces have on the surrounding ECM. We next review how feedback from local matrix structural and mechanical properties can modulate keratocyte phenotype and mechanical activity. Throughout this review, we provide examples of how these biophysical interactions may contribute to clinical outcomes, with a focus on corneal wound healing.

1. Introduction

The cornea is an optically clear tissue that forms the front surface of the eye, and accounts for nearly two-thirds of its refractive power. The corneal stroma, which makes up 90% of corneal thickness, is a highly ordered structure consisting of approximately 200 collagen lamellae (Pepose and Ubels, 1992). Corneal stromal cells (keratocytes) reside between the collagen lamellae, and are responsible for secreting extracellular matrix (ECM) components required to develop and maintain normal corneal structure and function (Chakravarti et al., 2000; Funderburgh et al., 2003; Hassell and Birk, 2010). In addition to their role in matrix synthesis, the mechanical activity of corneal keratocytes (i.e. the generation of cellular forces and the application of these physical forces to the ECM) plays a central role in mediating fundamental processes such as developmental morphogenesis and wound healing. In general, cellular forces organize ECM into tissue-specific patterns during embryonic development, and feedback between cell and matrix mechanics is a key factor regulating this process (Bard and Higginson, 1977; Engler et al., 2006; Krieg et al., 2008; Stopak and Harris, 1982; Stopak et al., 1985).

As in development, corneal wound healing following lacerating injury, penetrating keratoplasty or refractive surgery involves an ordered sequence of cell-matrix mechanical interactions (Jester et al., 1999b; Netto et al., 2005; Stepp et al., 2014; Wilson, 2012). In the corneal stroma, quiescent keratocytes normally have a dendritic morphology and a cortical distribution of f-actin (Jester et al., 1994). From a mechanical standpoint, resting keratocytes are considered quiescent; they do not express stress fibers or generate substantial contractile forces (Jester et al., 1994; Lakshman et al., 2010). Following injury or surgery, corneal keratocytes surrounding the area of injury generally become activated by cytokines present in the wound environment, and transform into a fibroblastic repair phenotype (Garana et al., 1992; Moller-Pedersen et al., 1998b; Stramer et al., 2003). Corneal fibroblasts proliferate, develop intracellular stress fibers, migrate into the wound and reorganize the ECM through the application of mechanical forces.

In certain wound types, the presence of transforming growth factor beta (TGFβ) in the wound induces transformation of corneal fibroblasts to a myofibroblasts phenotype. Corneal myofibroblasts express α-smooth muscle actin, generate even stronger forces on the matrix and synthesize a disorganized fibrotic ECM (Blalock et al., 2003; Jester et al., 1999a). Together these processes can alter corneal shape both through the addition of tissue (Moller-Pedersen et al., 2000), as well as the redistribution of mechanical

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load bearing and tension within the stroma (Dupps and Wilson, 2006; Ruberti et al., 2011). Fibrotic wound healing can also reduce transparency due to increased light scattering by both cells and the newly synthesized ECM (Boote et al., 2012; Jester et al., 2012; Moller-Pedersen et al., 1998a). Overall, a better understanding of the underlying cellular and molecular mechanisms that regulate the biomechanical activities of corneal fibroblasts could ultimately lead to more effective approaches to modulating the wound healing response *in vivo*.

The Rho-family of small GTPases such as Rho, Rac, and Cdc42 play a central role in mediating changes in cell mechanical activity in response to growth factors and other cytokines in a variety of cell types (Amano et al., 2010; Hall, 2005; Jaffe and Hall, 2005; Wang et al., 2013). These GTP binding proteins function as molecular switches; alternating between the active GTP-bound state and the inactive GDP-bound state. In fibroblasts on rigid 2-D substrates, activated Rho stimulates the formation of stress fibers and the development of focal contacts (Anderson et al., 2004; Parizi et al., 2000; Rottner et al., 1999; Sander et al., 1999; Totsukawa et al., 2000), and these cytoskeletal changes are dependent on actomyosin contraction (Jester and Chang, 2003; Rottner et al., 1999). Activated Rho binds to and activates Rho kinase, which inhibits myosin light chain (MLC) phosphatase, resulting in elevated MLC phosphorylation and increased cell contractility (Amano et al., 1998, 1996; Chrzanowska-Wodnicka and Burridge, 1994; Kimura et al., 1996; Kolodney and Elson, 1993; Parizi et al., 2000; Rayan et al., 1996; Sanderson et al., 1992; Tomasek et al., 1992). In contrast to Rho, Rac induces cell spreading, via the creation of smaller focal complexes and actin polymerization (Demali and Burridge, 2003; Rottner et al., 1999; Sander et al., 1999; Svitkina and Borisy, 1999; Totsukawa et al., 2000). Rac activation enhances both cell spreading and migration within 3-D collagen matrices (Andresen et al., 1997; Grinnell et al., 2006).

In addition to growth factors and other biochemical factors that can modulate the keratocyte mechanical phenotype, another key player in the regulation of cell-induced ECM patterning is the mechanical state of the ECM itself. In the cornea, large shifts in the global distribution of ECM tension can be induced by lacerating injury, penetrating keratoplasty, or refractive surgery (Dupps and Wilson, 2006; Ruberti et al., 2011). In addition, the provisional matrix produced during wound healing is generally less dense and more compliant than normal corneal tissue. Diseases such as keratoconus can also produce changes in stromal structural and mechanical properties. Keratoconus corneas generally have reduced mechanical stiffness (Ali et al., 2014; Andreassen et al., 1980; Edmund, 1989; Morishige et al., 2007), and thinning of the central cornea in keratoconus patients induces a redistribution of tension within the stromal ECM (Ambekar et al., 2011). In contrast, treatment of keratoconus with UV cross-linking increases corneal stromal rigidity (Beshtawi et al., 2013). Overall, changes in ECM structure, stress and elasticity have the potential modulate both the acute and long-term responses of corneal keratocytes to a range of clinical conditions and treatments (Winkler et al., 2011).

In this review we provide an overview of the biochemical and biophysical factors regulating mechanical interactions between corneal keratocytes and the stromal ECM at the cellular level. We first review how Rho GTPases regulate the sub-cellular pattern of force generation by corneal keratocytes, and the impact these forces have on the surrounding ECM. We next review how feedback from local matrix structural and mechanical properties can modulate keratocyte mechanical activity. Throughout this article, we provide examples of how these biophysical interactions may contribute to clinical outcomes, with a focus on corneal wound healing.

2. Modulation of corneal keratocyte mechanical behavior by Rho and Rac

2.1. Regulation of keratocyte contractility and matrix reorganization by Rho and Rho kinase

The Rho GTPases are prime candidates for regulating the mechanical interactions between corneal keratocytes and the stromal ECM during various phases of wound healing. In corneal fibroblasts, time-lapse imaging of cells plated on top or within restrained 3-D collagen matrices have shown that activation of Rho using lysophosphatidic acid (LPA), induces retraction of cell processes and a corresponding pulling in of the surrounding ECM (Petroll et al., 2008b; Roy et al., 1999). In contrast, inhibiting Rho kinase with the inhibitor Y-27632 induces rapid cell body elongation, formation and extension of dendritic cell processes, and a corresponding relaxation of cell-induced tension on the matrix (Fig. 1) (Vishwanath et al., 2003). Static imaging of collagen fibril organization surrounding isolated corneal fibroblasts in 3-D culture has directly demonstrated increased ECM compaction and alignment when Rho is activated by serum (Hay, 1985; Kim et al., 2006; Tomasek et al., 1982). When Rho kinase is inhibited, this cell-induced matrix reorganization is significantly reduced (Kim et al., 2006). In order to quantify cell-mediated changes in matrix tension, Brown and coworkers developed a culture force monitor system in which cell-seeded collagen matrices are suspended between a fixed bar and a force transducer (Eastwood et al., 1994). A similar system was recently used to measure cellular force generation by corneal fibroblasts. Culture in serum induced a gradual rise in cell-induced matrix tension, which reached a plateau after 24 h. Subsequent addition of Y-27632 produced a 64% decrease in the measured force produced by corneal fibroblasts (Zhou, 2014). Furthermore, pre-incubation with Y-27632 blocked the initial rise in matrix tension.

In addition to serum and LPA, Rho kinase has also been shown to mediate fibroblastic transformation of keratocytes in response to basic fibroblast growth factor (FGF2) treatment, as well as myofibroblast transformation in response to TGFβ (Chen et al., 2009; Lakshman and Petroll, 2012; Yamamoto et al., 2012). Specifically, treatment with Y-27632 inhibits stress fiber formation, and blocks the induction of α-SM-actin expression and the increase in cell-induced matrix remodeling normally induced by TGFβ. Inhibition of Rho/Rho kinase has also been shown to block the decrease in keratin sulfate proteoglycan synthesis normally associated with corneal myofibroblast transformation, suggesting a linkage between increased cell contractility and altered ECM synthesis (Chen et al., 2009).

The role of Rho kinase in regulating corneal fibroblast migration mechanics has also been investigated, using a nested 3-D collagen matrix model that facilitates dynamic imaging of cell-matrix interactions during cell translocation. In this model, corneal fibroblasts cultured in serum-containing media generate significant tractional forces on the matrix during migration, as indicated by inward displacement and reorganization of collagen in front of cells (Karamichos et al., 2009; Kim et al., 2010; Zhou and Petroll, 2010). When Rho kinase is inhibited, cells become more elongated and form dendritic cell processes, and the rate of cell migration is significantly reduced. Interestingly, these dendritic cells are still able to generate tractional forces at the leading edge. Overall, these data suggests that Rho kinase impacts corneal fibroblast migration by affecting morphology, polarization, and mechanical coordination between the leading and trailing edges of cells (Amano et al., 2010; Zhou and Petroll, 2010).

Taken together, previous studies demonstrate that Rho kinase plays a central role in regulating corneal fibroblast contractility, migration and matrix remodeling *in vitro* (Anderson et al., 2004; Kim et al., 2006; Petroll et al., 2008b; Roy et al., 1999;