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Research article

The role of integrins in glaucoma

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ABSTRACT

Integrins are a family of heterodimeric transmembrane receptors that mediate adhesion to the extracellular matrix (ECM). In addition to their role as adhesion receptors, integrins can act as "bidirectional signal transducers" that coordinate a large number of cellular activities in response to the extracellular environment and intracellular signaling events. This bidirectional signaling helps maintain tissue homeostasis. Dysregulated bidirectional signaling, however, could trigger the propagation of feedback loops that can lead to the establishment of a disease state such as glaucoma. Here we discuss the role of integrins and bidirectional signaling as they relate to the glaucomatous phenotype with special emphasis on the αvβ3 integrin. We present evidence that this particular integrin may have a significant impact on the pathogenesis of glaucoma.

Glaucoma is a heterogeneous disease that is characterized by a number of changes in the trabecular meshwork and the optic nerve head (Morrison et al., 2005; Stamer and Acott, 2012; Braunger et al., 2015; Wallace et al., 2015; Downs, 2015; Schneider and Fuchshofer, 2016). The phenotypic changes most often associated with glaucoma involve the actomyosin-based contractile properties of the trabecular meshwork (TM), compliance of the extracellular matrix (ECM) and the types and amounts of proteins deposited in the ECM in both the TM and optic nerve head (ONH). In reality these changes are all interconnected and likely the result of bidirectional signaling between the ECM and cells. Bidirectional signaling involves the assessment of the tissue's microenvironment by its resident cells to maintain tissue homeostasis (Acott et al., 2014). When changes in the compliance or composition of the ECM in the microenvironment occur, these changes are sensed by the resident cells (Fig. 1). The resident cells then respond by activating signaling pathways that may lead to other changes in cell function, the ECM or expression of cell receptors, for example.

Bidirectional communication between cells and the extracellular environment is often directed by several classes of cell surface receptors. Perhaps the best characterized family of receptors involved in bidirectional communication is the integrins (Humphries et al., 2006; Barczyk et al., 2010; Gagen et al., 2014). How integrins respond to changes in the TM or the ONH is likely to contribute to the development of glaucoma and will be the subject of this review.

The review will emphasize the changes in integrin signaling induced by TGFβ2 and glucocorticoids. TGFβ2 levels are increased in most patients with primary open angle glaucoma (POAG) (Fuchshofer, 2011; Fuchshofer and Tamm, 2012; Wordinger et al., 2014) and treatments with glucocorticoids can lead to the development of ocular hypertension and an increased risk of developing POAG (Clark and Wordinger, 2009). Changes observed with glucocorticoid treatment also tend to mimic those observed in POAG patients and following TGFβ2 treatment. In addition, we will discuss the mechanosensitivity of integrins to mechanical forces that may be associated with glaucoma (Tamm, 2009; WuDunn, 2009; Tan et al., 2006).

1. Integrins

Integrins are a family of 24 transmembrane receptors (Humphries et al., 2006; Barczyk et al., 2010; Gagen et al., 2014). Each integrin is a heterodimer composed of an α- and a β-subunit and must be assembled as a heterodimer within the endoplasmic reticulum in order to be expressed on the cell surface (Bouvard et al., 2013). The individual α and β subunits are mixed and matched to form the 24 members of the integrin family each having tissue-specific biological properties. Three of the α-subunits and

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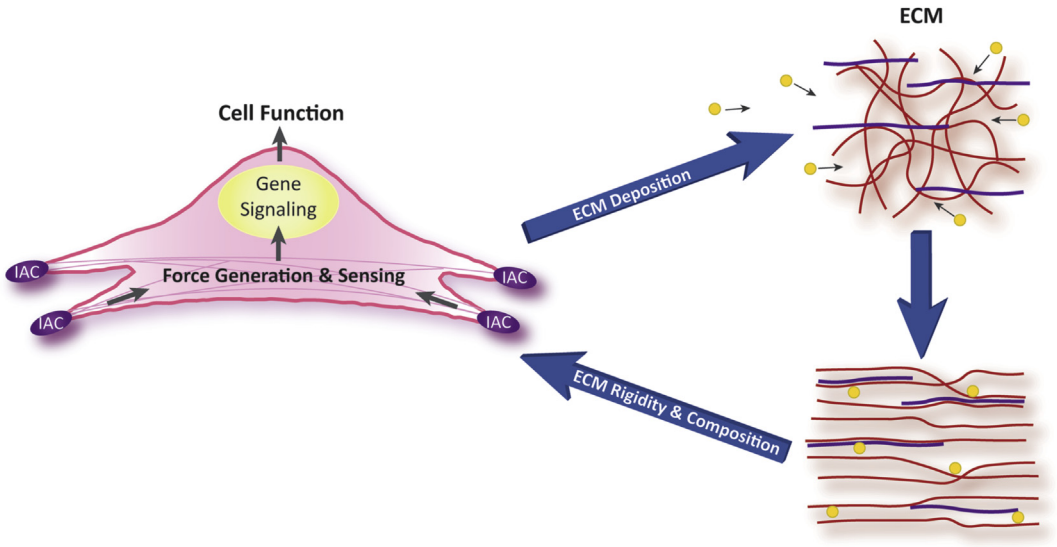

Fig. 1. Bidirectional signaling. The figure illustrates how changes made to the extracellular matrix (ECM) by the cell can in turn influence the signals perceived by the integrin adhesion complex (IAC) and alter the biological properties of the cell. Changes perceived by the IAC can be chemical in nature such as the expression of new ECM proteins (yellow circles) or physical due to a change in the 3-D architecture of the ECM or rigidity of the ECM. These physical changes can be triggered by contractile forces applied by cells through the IAC.

four of the β-subunits can be expressed as alternatively spliced variants giving rise to integrins with different biological properties (Van der Flier and Sonnenberg, 2001). Almost all cells express one or more members of the integrin family, although the repertoire of integrin expression can vary during development, ageing, and in response to environmental conditions.

The major ligands for integrins are ECM proteins. It is generally thought that integrins bind ECM proteins via an arginine-glycine-aspartic acid (RGD) sequence within the ligands. However, only a third of integrins are known to bind this sequence in native ECM molecules. In ECM proteins like collagen and laminin, these RGD sequences are buried and are only available following degradation or denaturation of their fibrillar structure which limits these ECM-integrin interactions to specific circumstances (Barczyk et al., 2010). To bind collagens assembled into native fibrils, integrins use the consensus sequence GFOGER in the triple helical molecule. Accessibility to this binding sequence, however, is also limited and dependent on the fibrillar structure *in vivo*. This suggests that the collagen binding integrins may have a limited role in tissue homeostasis. The recognition sequence used by integrins to bind native, non-denatured laminin is unknown.

An additional level of complexity with integrin binding to its ECM ligand comes from the fact that some integrins also rely on auxiliary binding sites within the ECM ligand (Mould et al., 1997; Redick et al., 2000; Adair et al., 2005). The best characterized examples of this are the α5β1 and α4β1 fibronectin binding integrins. As shown in Fig. 2, the α5β1 integrin uses both the canonical RGD site and an auxiliary site called the synergy site (PHSRN) within a neighboring FN III module to bind fibronectin (Obara et al., 1988; Aota et al., 1994) while the α4β1 integrin uses the RGD homologue, IDAPS, and an auxiliary PRARI sequence in the 13th and 14th FN III modules to bind fibronectin (Sharma et al., 1999).

Another factor affecting integrin binding is that several ECM proteins undergo alternative splicing which generates or removes integrin binding sites from the ECM. The best-known example of this is fibronectin which can bind 12 different integrins (Fig. 2). Fibronectin is a modular glycoprotein found in the TM (Acott and Kelley, 2008) and in ONH cells in culture (Zode et al., 2009). It contains two alternatively spliced sites, generated by exon splicing, called the extra domains A (EDA) and B (EDB). There is also a third site called the variable (V) region which undoes alternative splicing to generate five isoforms of the V region in humans (Hynes, 1990). The fibronectin isoform containing the EDA domain provides an additional binding site for α4β1, α4β7 and α9β1 integrins (Leiss et al., 2008; White and Muro, 2011). Both the EDA and EDB isoforms of fibronectin are detected in cultures of human TM cells (Medina-Ortiz et al., 2013) (Filla et al., manuscript in preparation). Both the EDA and EDB isoforms are upregulated in response to TGFβ2 (Medina-Ortiz et al., 2013) while dexamethasone upregulates the EDA isoform (Filla et al. manuscript in preparation). Alternative splicing of the V-region removes binding sites for α4β1 and α4β7 integrins (Leiss et al., 2008; White and Muro, 2011). In all these splice variants, the canonical RGD integrin binding site in fibronectin is still present, however, inclusion of the EDA and EDB domains have been reported to alter the cell-adhesive activity of α5β1 integrin to the RGD site. Presumably insertion of the EDA or EDB domains alters the spatial relationship between the RGD and synergy sites utilized by α5β1 integrin (Leahy et al., 1996; Manabe et al., 1997; Hashimoto-Uoshima et al., 1997).

Other ECM proteins can undergo alternative splicing as well. For example, tenascin C, collagen type XII, CD44 and versican expressed by TM cells are alternatively spliced in response to stretch (Keller et al., 2007). As shown in Fig. 2, alternative splicing of tenascin C would remove the α7β1 integrin binding site while leaving two other integrin binding sites capable of binding six separate integrins unaffected (Golledge et al., 2011; Yoshida et al., 2015). Alternative splicing of versican, on the other hand, would not affect its β1 integrin binding site (Wu et al., 2005), and to date no integrin binding sites have been reported in collagen type XII or CD44.

2. Integrins in the TM

At least eleven different integrins have been identified in the cells associated with the TM in the outflow pathway (Table 1). These integrins show a broad distribution and are found along the trabecular beams and in the juxtacanalicular tissue (JCT) demonstrating that multiple integrins are found on cells throughout the

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