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Functional Properties of Fibronectin in the Trabecular Meshwork

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Abstract

Fibronectin plays a number of important roles in the extracellular matrix (ECM) including providing structural support and signaling cues for cell survival, migration, differentiation, gene expression, growth factor signaling, and cell contractility. In this review, we examine recent findings about the biological and structural properties of fibronectin and discuss how these properties could contribute to the regulation of aqueous humor (AH) outflow in the trabecular meshwork (TM).

Keywords

Fibronectin; Trabecular Meshwork; Integrins; HepII Domain

Introduction

Regulation of AH through the TM is thought to involve the ECM of the juxtacanalicular tissue (JCT). How the ECM in the JCT restricts the movement of AH outflow is unknown. One theory suggests that it acts as a filter that restricts outflow across the TM by providing physical resistance to the movement of AH. Increases in matrix deposition, as are often observed in glaucomatous patients, would clog the filter and restrict AH movement. The ECM, however, is not a static scaffold. It is a dynamic structure composed of a number of different matrix proteins (Acott and Kelley, 2008) that is constantly undergoing change by proteolysis. Physical forces created by the contractile properties of the TM could further modify the structural features of the ECM. This continuous remodeling of the ECM would create a dialogue between JCT cells and the ECM that could be capable of mediating AH outflow. Hence, changes in the composition of the ECM would be expected to have a profound influence on the biological activity of the JCT by altering this interaction. This review focuses on how the functional properties of fibronectin could contribute to such an interchange.

Fibronectin Localization and Expression

Fibronectin is one of the major ECM proteins in the TM (Hann, et al., 2001). It is found in the sheath material surrounding the elastin tendons, in the amorphous fibrogranular material in the JCT and scattered along the basement membranes of the inner wall of Schlemm's canal and

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the trabecular beams. It is also found in the core of the trabecular beams and as a soluble protein in AH (Reid, et al., 1982).

Increases in fibronectin expression have sometimes, but not always, been observed during aging and in glaucomatous patients (Floyd, et al., 1985, Babizhayev and Brodskaya, 1989, Tripathi, et al., 1997, Hann, et al., 2001). Factors that regulate fibronectin expression in the TM and hence could cause an increase in outflow resistance include glucose, glucocorticoids, ascorbic acid, and TGF- β 2 (Steely, et al., 1992, Zhou, et al., 1998, Zhou, et al., 1998, Li, et al., 2000, Filla, et al., 2002, Sato and Roy, 2002). TGF- β 2 also increases cross-linking of fibronectin to itself and the surrounding ECM through the action of tissue transglutaminase (tTG)(Welge-Lussen, et al., 2000). This may promote the deposition and retention of fibronectin in the ECM, since the tTG-fibronectin complex is more resistant to degradation. Interestingly, the tTG-fibronectin complex has been shown to amplify signaling to Rho GTPases(Janik, et al., 2006, Telci, et al., 2008). A major implication of this is that the contractile properties of the TM could be potentially increased by the deposition of a tTG-fibronectin rich matrix, thereby contributing to a reduction in outflow facility.

Little is known about the activity of fibronectin in the TM and how it regulates outflow resistance in response to changes in intraocular pressure (IOP). Extensive literature from other cell types suggests that fibronectin and its receptors provide mechanical support for cell attachment and could regulate many of the biological processes involved in modulating outflow resistance, including matrix production, ECM turnover, gene expression, growth factor signaling, and cytoskeletal organization (Ivaska and Heino, 2000, Schwartz and Assoian, 2001, Lee and Juliano, 2004, Calderwood, 2004, Morgan, et al., 2007). Furthermore, fibronectin and its receptors modulate cellular mechanoresponsiveness to physical forces such as stretch (Katsumi, et al., 2005).

Fibronectin Receptors

Many of the biological activities of fibronectin are mediated via interactions with integrins. Integrins are heterodimeric transmembrane receptors composed of a α - and β -subunit. There are at least 8 integrins that bind fibronectin in the TM, including α 3 β 1, α v β i, α 5 β 1, α v β 3, α v β 5, α v β 6, α 4 β 1 and α 4 β 7 (Zhou, et al., 1999). These integrins are distributed throughout the TM with the heaviest localization observed along cells on the beams.

The major integrin binding site in fibronectin for all integrins, except α 4 β 1, is the RGD sequence in the III10 repeat (Pankov and Yamada, 2002). The α 4 β 1 integrin binds RGD homologues found in five different regions of fibronectin. They are: the KLDAPT sequence in the III5 repeat, the EDGIHEL sequence in EDA, the IDAPS sequence in III14 repeat of the HepII domain and the LDV and REDV sequences in the V region of fibronectin (Figure 1). The integrin signaling response triggered by fibronectin is complex and controlled, in part, by multiple integrins binding fibronectin and by other sequences in fibronectin. For example, signaling from α 5 β 1 integrin is enhanced by the PHSRN sequence in the III9 repeat which interacts with the α 5-subunit (Obara, et al., 1988). Signaling specificity is also controlled by various molecules such as talin and paxillin that associate with the cytoplasmic tails of the α - and β -subunits (Schwartz and Ginsberg 2002, Calderwood, 2004, Rose, et al., 2007).

Integrin signaling in the TM is affected by the presence of other receptors and matrix proteins which “co-direct” signaling events with integrins (Muller, et al., 2008). This process, referred to as “cross-talk”, can be either cooperative or antagonist. For example, cooperative signaling between β 1 and β 3 integrins enhances the formation of cross-linked actin networks in TM cells (Filla, et al., 2006) while the matrix protein, myocilin downregulates the assembly of actin networks formed when TM cells, or fibroblasts, are plated on fibronectin (Peters, et al., 2005, Shen, et al., 2008). In subconfluent TM cultures plated on fibronectin, activation of

$\alpha 4\beta 1$ integrins by the HepII domain triggers crosstalk with $\alpha 5\beta 1$ integrins bound to fibronectin (Peterson, et al., 2004). This cross-talk enhances stress fiber formation, cell spreading, and FAK phosphorylation (Figure 2B). However, in confluent cultures when signaling from other receptors (i.e. cadherins in cell-cell contacts or integrins bound to collagen) is also present (Figure 2C), the HepII domain triggers the opposite effect (Peterson, et al., 2004, Gonzalez, et al., 2006).

The other major fibronectin receptors in the TM are members of the syndecan family (Alexopoulou, et al., 2007). Syndecans are transmembrane HSPGs that typically interact with the heparin binding domains of fibronectin via their heparan sulfate chains. All four members of the syndecan family are found in the TM, but only syndecans-1, -2, and -4 bind to fibronectin (Filla, et al., 2004, Alexopoulou, et al., 2007). Syndecan-4 which is the most prominent syndecan in the TM (Filla, et al., 2004) is best known for its role in focal adhesion formation and organization of the actin cytoskeleton (Alexopoulou, et al., 2007).

Integrins and syndecans are ideal candidates to regulate outflow facility. Besides controlling cell adhesion, growth factor signaling, and assembly and turnover of extracellular matrices (Schwartz and Ginsberg 2002, Alexopoulou, et al., 2007, Morgan, et al., 2007), they also activate members of the Rho GTPase family which control the contractility of the actomyosin network and play a critical role in regulating AH outflow (Rao and Epstein, 2007). In addition, integrin and syndecan signaling is responsive to stretch, steroids, and TGF- β ; all factors that affect IOP. In the TM, stretch upregulates the expression of αv , $\alpha 5$, and $\beta 1$ integrins (Rose, et al., 2001). Steroids, on the other hand, downregulate the expression of αv integrins in TM cells and upregulate the expression of the $\alpha 5$ integrins (Dickerson, et al., 1998). To date, it is not known if TGF- β affects integrin and syndecan expression in the TM. In other cell types, TGF- β increases the expression of $\alpha 5$, αv , $\beta 1$ and $\beta 3$ integrins (Kim and Yamada, 1997) and syndecan-1 (Li and Chaikof, 2002). In some instances, integrin expression may not be affected by these factors, but its activity is (Calderwood, 2004). For example, $\alpha 4\beta 1$, $\alpha 5\beta 1$, $\alpha 2\beta 1$ and $\alpha v\beta 3$ integrins can switch from an inactive (low affinity) state to an active (high affinity) state in response to stretch and external stimuli.

Structure-Function of Fibronectin

A key feature of fibronectin's biological activity is its modular and flexible structure (Hynes, 1990, Mao and Schwarzbauer, 2005). As a soluble protein in AH, fibronectin's tertiary structure is probably similar to that of plasma fibronectin and would exist as a compact protein with many of its biological domains inaccessible. The active form of fibronectin is generally thought to be an extended protein assembled into an insoluble fibril. This transition into a fibril is a highly controlled cell-mediated process involving integrins (especially $\alpha 5\beta 1$) and cell contractility (Mao and Schwarzbauer, 2005). Changes in this process are, therefore, just as likely to affect outflow resistance as changes in the levels of fibronectin expression.

Human fibronectin is a dimer composed of two nonidentical polypeptide chains disulfide bonded at their carboxyl termini (Figure 1). Each chain contains homologous repeating units termed types I, II, and III. The type III repeats form the majority of each chain and therefore play a critical role in determining the activity of fibronectin. Each type III repeat consists of seven β -strands packed into a β -sandwich (Main, et al., 1992). They lack intrachain disulfide bonds, making their conformation sensitive to proteolysis or mechanical perturbations which expose novel "cryptic sites" with unique biological activities. For example, applying a 30-35% stretch to immobilized fibronectin reveals a cryptic site in the III1 repeat that is involved in promoting the assembly of fibronectin fibrils (Zhong, et al., 1998) and stimulating cell growth and contractility (Hocking and Kowalski, 2002).

The extensibility of these repeats is retained even after fibronectin has been assembled into a fibril (Mao and Schwarzbauer, 2005), giving fibronectin fibrils an elasticity capable of stretching about four times its original length. The force required to unfold these repeats and stretch a fibronectin fibril can be generated by the actomyosin network (Abu-Lail, et al., 2005). This suggests that the biological activity of fibronectin fibrils can be altered as changes in contractility or IOP “stretch” the TM. Interestingly, not all fibrils exhibit the same level of “stretch”. This could explain why alternative conformations of fibronectin fibrils are observed (Chen, et al., 1997) and would suggest that certain regions of the JCT may have different activities.

Fibronectin is a highly conserved protein with greater than 90% homology among mammals and greater than 80% homology between chickens and mammals. As many as 20 forms of fibronectin are derived from a single gene (Hynes, 1990). These forms arise from alternative splicing of 3 exons called EDA (or EIIIA), EDB (or EIIIB) and IIICS (or V). These exons are either spliced out entirely or, in the case of the V exon, undergo exon subdivision to generate a V1, V2 or V3 region (Figure 1).

These splice variants are responsible for altering the biological activity of fibronectin. For example, the V1 and V3 regions are exclusively expressed in stretched porcine TM cells, (Vittal, et al., 2005), but the V2 region, which is encoded in 70% of the transcripts in nonstretched cells, is not. Alternative splicing of this region in stretched TM cells could conceivably affect cell contractility, since the V1 and V3 regions contain binding sites for $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrins (Mould, et al., 1990, Mould, et al., 1991, Rose, et al., 2007) which when activated decrease cell contractility in leukocytes, melanoma cells, and peripheral neurons (Rose, et al., 2007). Alternative splicing of the V region could also modify the adhesive properties of the HepII domain, switching it from a HSPG-dependent binding domain to a HSPG-independent binding domain (Santas, et al., 2002) as well as modulate the ability of the HepII domain to regulate MMP expression and cell survival (Huhtala, et al., 1995, Kapila, et al., 1999).

Alternative splicing of the EDA and EDB exons also occurs in TM cells. In primary porcine TM cells, fibronectin mRNA lacks both these exons which is consistent with the observation that these exons are only expressed in embryonic tissues or in some disease states (ffrench-Constant, 1995). Stretch does not significantly affect expression of these exons (Vittal, et al., 2005) since at least 90% of the transcripts from stretched porcine TM cells lack the EDB exon and none of the transcripts contain the EDA exon. Alternative splicing of the EDA and EDB exons in the TM, however, can be upregulated by TGF- $\beta 1$ or TGF- $\beta 2$. Thus, patients with primary open angle glaucoma in which the levels of TGF- $\beta 2$ are elevated are more likely to express EDA and EDB-containing fibronectin.

The influence of the EDA insertion on AH outflow is a complicated issue, as suggested by conflicting reports. Studies in rabbit synovial cells suggest that EDA domain may increase outflow facility, because it triggers the reorganization of the actin stress fibers into cortical actin filaments and the induction of MMP-1, MMP-3 and MMP-9 via an interleukin-1-dependent mechanism (Saito, et al., 1999). However, in CHO cells inclusion of the EDA domain in fibronectin promotes the assembly of fibronectin matrices, cell cycle progression and mitogenic transduction (Manabe, et al., 1997, Manabe, et al., 1999). The enhanced assembly of fibronectin into the ECM would suggest that expression of fibronectin with an EDA domain may decrease outflow facility. Such a decrease is consistent with the finding that TGF- $\beta 2$ decreases outflow facility when perfused into cultured human anterior segments (Gottanka, et al., 2004).

Fibronectin is a multi-domain protein with each domain exhibiting a remarkable number of biological activities (Hynes, 1990). Many of the domains are proteolytically resistant and can be isolated without loss of activity. This means that small bioactive domains of fibronectin could be available in the TM in vivo during ECM turnover. One such domain that is relevant to the regulation of outflow resistance is the HepII domain which increases outflow facility in both human and monkey cultured anterior segments (Santas, et al., 2003, Gonzalez, et al., submitted). This domain consists of the 12th-14th type III repeats (Figure 1) and can bind HSPGs either on the cell surface or in the TM. It also binds myocilin (Filla, et al., 2002), a glucocorticoid response protein associated with glaucoma and VEGF (Wijelath, et al., 2006), which is present in AH (Hu, et al., 2002). VEGF can regulate metalloproteases (MMPs) activity in the TM (Alexander, et al., 1998) and endothelial cell permeability (Gavard and Gutkind, 2006). Thus, the HepII domain could be an important matrix reservoir for VEGF to help modulate the permeability of Schlemm's canal or control the localized activity of MMPs.

The most likely role for the HepII domain in the JCT is to modify signaling events from fibronectin or other matrix components, either by strengthening them, or weakening them (Figure 2). For example in proliferating TM cells, the HepII domain activates a $\alpha 4 \beta 1$ integrin signaling pathway which enhances cell attachment to $\alpha 5 \beta 1$ integrins (Peterson, et al., 2004) by increasing actin stress fiber formation. In other cell types, it regulates MMP activity, especially MMP-3 and MMP-9, and mediates cell survival (Huhtala, et al., 1995, Alexopoulou, et al., 2007). All of these events involve using either $\alpha 4 \beta 1$ integrins or syndecans bound to the HepII domain to modify signals from other integrins (Morgan, et al., 2007). By itself, the HepII domain does not mediate cell survival, actin polymerization, MMP expression or apoptosis. Nor can it function as a major attachment site for TM cells in the JCT. TM cells attach poorly to the isolated HepII domain and do not form actin stress fibers when plated on this domain (Filla, et al., 2006).

In summary, the modular and flexible structure of fibronectin provides a unique mechanochemical responsive mechanism by which the biological activity of fibronectin fibrils in the ECM can be modulated. This is likely to provide critical signals capable of mediating AH outflow.

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References

- Abu-Lail NI, Ohashi T, Clark RL, Erickson HP, Zauscher S. Understanding the elasticity of fibronectin fibrils: unfolding strengths of FN-III and GFP domains measured by single molecule force spectroscopy. *Matrix Biology* 2005;25:175–184. [PubMed: 16343877]
- Acott TS, Kelley MJ. Extracellular matrix in the trabecular meshwork. *Exp Eye Res* 2008;86:543–561. [PubMed: 18313051]
- Alexander JP, Samples JR, Acott TS. Growth factor and cytokine modulation of trabecular meshwork matrix metalloproteinase and TIMP expression. *Current Eye Research* 1998;17:276–285. [PubMed: 9543636]
- Alexopoulou AN, Mulhaupt HAB, Couchman JR. Syndecans in wound healing, inflammation and vascular biology. *Int J Biochem Cell Biol* 2007;39:505–528. [PubMed: 17097330]
- Babizhayev MA, Brodskaya MW. Fibronectin detection in drainage outflow system of human eyes in aging and progression of open-angle glaucoma. *Mech Ageing Dev* 1989;47:145–157. [PubMed: 2654504]
- Calderwood DA. Integrin Activation. *J Cell Sci* 2004;117:657–666. [PubMed: 14754902]
- Chen Y, Zardi L, Peters DM. High-resolution cryo-scanning electron microscopy study of the macromolecular structure of fibronectin fibrils. *Scanning* 1997;19:349–355. [PubMed: 9262019]

- Dickerson JE Jr, Steely HT Jr, English-Wright SL, Clark AF. The effect of dexamethasone on integrin and laminin expression in cultured human trabecular meshwork cells. *Exp Eye Res* 1998;66:731–738. [PubMed: 9657905]
- French-Constant C. Alternative splicing of fibronectin-many different proteins but few different functions. *Exp Cell Res* 1995;221:261–271. [PubMed: 7493623]
- Filla M, David G, Weinreb RN, Kaufman PL, Peters DM. Distribution of syndecans 1–4 within the anterior segment of the human eye: expression of a variant syndecan-3 and matrix-associated syndecan-2. *Exp Eye Res* 2004;79:61–74. [PubMed: 15183101]
- Filla M, Woods A, Kaufman PL, Peters DMP. β 1 and β 3 integrins cooperate to induce syndecan-4 containing cross-linked actin networks (CLANs) in human trabecular meshwork (HTM) cells. *Invest Ophthalmol Vis Sci* 2006;47:1956–1967. [PubMed: 16639003]
- Filla MS, Lui X, Nguyen TD, Polansky JR, Brandt BR, Kaufman PL, Peters DM. In vitro localization of TIGR/MYOC in trabecular meshwork extracellular matrix and binding to fibronectin. *Invest Ophthalmol Vis Sci* 2002;43:151–161. [PubMed: 11773026]
- Floyd BB, Cleveland PH, Worthen DM. Fibronectin in human trabecular drainage channels. *Invest Ophthalmol Vis Sci* 1985;26:797–804. [PubMed: 3891665]
- Gavard J, Gutkind JS. VEGF controls endothelial-cell permeability by prompting the β -arrestin-dependent endocytosis of VE-cadherin. *Nat Cell Biol* 2006;8:1223–1233. [PubMed: 17060906]
- Gonzalez JMJ, Hu Y, Gabelt B, Kaufman PL, Peters DM. Identification of the active site in the Heparin II domain of fibronectin that increases outflow facility in cultured monkey anterior segments. *Invest Ophthalmol Vis Sci*. submitted
- Gonzalez JMJ, Peterson JA, Peters JM, Newman J, Peters DM. Effect of Heparin II domain of fibronectin on actin cytoskeleton and adherens junctions in human trabecular meshwork cultures. *Invest Ophthalmol Vis Sci* 2006;47:2924–2931. [PubMed: 16799035]
- Gottanka J, Chan D, Eichhorn M, Lutjen-Drecoll E, Ethier CR. Effects of TGF- β 2 in Perfused Eyes. *Invest Ophthalmol Vis Sci* 2004;45:153–158. [PubMed: 14691167]
- Hann CR, Springett MJ, Wang X, Johnson DH. Ultrastructural localization of collagen IV, fibronectin, and laminin in the trabecular meshwork of normal and glaucomatous eyes. *Ophthalmic Res* 2001;33:314–324. [PubMed: 11721183]
- Hocking DC, Kowalski K. A cryptic fragment from fibronectin's III1 module localizes to lipid rafts and stimulates cell growth and contractility. *J Cell Biol* 2002;158:175–184. [PubMed: 12105189]
- Hu DN, Ritch R, Liebmann J, Liu Y, Cheng B, Hu MS. Vascular endothelial growth factor is increased in aqueous humor of glaucomatous eyes. *J Glauc* 2002;11:406–410.
- Huhtala P, Humphries MJ, McCarthy JB, Tremble PM, Werb Z, Damsky CH. Cooperative signaling by α 5 β 1 and α 4 β 1 integrins regulates metalloproteinase gene expression in fibroblasts adhering to fibronectin. *J Cell Biol* 1995;129:867–879. [PubMed: 7537277]
- Ivaska J, Heino J. Adhesion receptors and cell invasion: mechanisms of integrin-guided degradation of extracellular matrix. *Cell Mol Life Sci* 2000;57:16–24. [PubMed: 10949578]
- Janik A, Zemskov EA, Belkin AM. Cell surface transglutaminase promotes RhoA activation via integrin clustering and suppression of the Src-p190RhoGAP signaling pathway. *Mol Biol Cell* 2006;17:1606–1619. [PubMed: 16452636]
- Kapila YL, Wang S, Johnson PW. Mutations in the heparin binding domain of fibronectin in cooperation with the V region induce decreases in pp125(FAK) levels plus proteoglycan-mediated apoptosis via caspases. *J Biol Chem* 1999;274:30906–30913. [PubMed: 10521484]
- Katsumi A, Naoe T, Matsushita T, Kaibuchi K, Schwartz MA. Integrin Activation and Matrix Binding Mediate Cellular Responses to Mechanical Stretch. *J Biol Chem* 2005;280:16546–16549. [PubMed: 15760908]
- Kim LT, Yamada KM. The regulation of expression of integrin receptors. *Proc Soc Exp Biol Med* 1997;214:123–131. [PubMed: 9034129]
- Lee JW, Juliano RL. Mitogenic signal transduction by integrin-and growth factor receptor mediated pathways. *Mol Cells* 2004;17:188–202. [PubMed: 15179030]
- Li J, Tripathi BJ, Tripathi RC. Modulation of pre-mRNA splicing and protein production of fibronectin by TGF- β 2 in porcine trabecular cells. *Invest Ophthalmol Vis Sci* 2000;41:3437–3443. [PubMed: 11006236]

- Li L, Chaikof EL. Mechanical stress regulates syndecan-4 expression and redistribution in vascular smooth muscle cells. *Arteriosclerosis, Thrombosis & Vascular Biology* 2002;22:61–68.
- Main AL, Harvey TS, Baron M, BOyd J, Campbell ID. The three dimensional structure of the tenth type III module of fibronectin: An insight into RGD-mediated interactions. *Cell* 1992;71:671–678. [PubMed: 1423622]
- Manabe R, Oh-e N, Maeda T, Fukuda T, Sekiguchi K. Modulation of Cell-adhesive activity of fibronectin by the alternatively spliced EDA segment. *J Cell Biol* 1997;139:295–307. [PubMed: 9314547]
- Manabe R, Oh-e N, Sekiguchi K. Alternatively spliced EDA segment regulates fibronectin-dependent cell cycle progression and mitogenic signal transduction. *J Biol Chem* 1999;274:5919–5924. [PubMed: 10026216]
- Mao Y, Schwarzbauer J. Fibronectin fibrillogenesis, a cell-mediated matrix assembly process. *Matrix Biology* 2005;24:389–399. [PubMed: 16061370]
- Morgan MR, Humphries MJ, Bass MD. Synergistic control of cell adhesion by integrins and syndecans. *Nat Rev Cell Biol* 2007;8:957–969.
- Mould AP, Komoriya A, Yamada KM, Humphries MJ. The CS5 peptide is a second site in the IIICS region of fibronectin recognized by the integrin $\alpha 4 \beta 1$. Inhibition of $\alpha 4 \beta 1$ function by RGD peptide homologues. *J Biol Chem* 1991;266:3579–3585. [PubMed: 1750929]
- Mould AP, Wheldon LA, Komoriya A, Wayner EA, Yamada KM, Humphries MJ. Affinity chromatographic isolation of the melanoma adhesion receptor for the IIICS region of fibronectin and its identification as the integrin $\alpha 4 \beta 1$. *J Biol Chem* 1990;265:4020–4024. [PubMed: 2137460]
- Muller EJ, Williamson L, Kolly C, Suter MM. Outside-in signaling through integrins and cadherins: a central mechanism to control epidermal growth and differentiation? *J Invest Dermatol* 2008;128:501–516. [PubMed: 18268536]
- Obara M, Kang MS, Yamada KM. Site-directed mutagenesis of the cell-binding domain of human fibronectin: separable, synergistic sites mediate adhesive function. *Cell* 1988;53:649–657. [PubMed: 3286012]
- Pankov R, Yamada KM. Fibronectin at a glance. *J Cell Sci* 2002;115:3861–3863. [PubMed: 12244123]
- Peters DM, Herbert K, Biddick B, Peterson JA. Myocilin binding to Hep II domain of fibronectin inhibits cell spreading and incorporation of paxillin into focal adhesions. *Exp Cell Res* 2005;303:218–228. [PubMed: 15652337]
- Peterson JA, Sheibani N, David G, Garcia-Pardo A, Peters DM. Heparin II domain of fibronectin uses $\alpha 4 \beta 1$ integrin to control focal adhesion and stress fiber formation, independent of syndecan-4. *Journal Biological Chemistry* 2004;280:6915–6922.
- Rao VP, Epstein DL. Rho GTPase/Rho kinase inhibition as a novel target for the treatment of glaucoma. *Biodrugs* 2007;21:167–171. [PubMed: 17516712]
- Reid T, Kenney C, Waring GO. Isolation and characterization of fibronectin from bovine aqueous humor. *Invest Ophthal Vis Sci* 1982;22:57–61. [PubMed: 7056625]
- Rose A, Radecki M, Hernandez NI, Acott TS, Kelley MJ. Changes in protein associations and phosphorylation with mechanical stretch in trabecular meshwork cells. *ARVO*. 2001Abstract. #754
- Rose DM, Alon R, Ginsberg MH. Integrin modulation and signaling in leukocyte adhesion and migration. *Immunol Rev* 2007;217
- Saito S, Yamaji N, Yasunaga K, Saito T, Matsumoto Si, Katoh M, Kobayashi S, Masuho Y. The fibronectin extra domain A activates matrix metalloproteinase gene expression by an interleukin-1-dependent mechanism. *J Biol Chem* 1999;274:30756–30763. [PubMed: 10521465]
- Santas AJ, Bahler C, Peterson JA, Filla MS, Kaufman PL, Tamm ER, Johnson D, Peters DP. Effect of Heparin II domain of fibronectin on aqueous outflow in cultured anterior segments of human eyes. *Invest Ophthal Vis Sci* 2003;44:4796–4801. [PubMed: 14578401]
- Santas AJ, Peterson JA, Halbleib JL, Craig SE, Humphries MJ, Peters DMP. Alternative splicing of the IIICS domain in fibronectin governs the role of the Heparin II domain in fibrillogenesis and cell spreading. *J Biol Chem* 2002;277:13650–13658. [PubMed: 11832485]
- Sato T, Roy S. Effect of high glucose on fibronectin expression and cell proliferation in trabecular meshwork cells. *Invest Ophthal Vis Sci* 2002;43:170–175. [PubMed: 11773028]
- Schwartz MA, Assoian RK. Integrins and cell proliferation: regulation of cyclin-dependent kinases via cytoplasmic signaling pathways. *J Cell Sci* 2001;114:2553–2560. [PubMed: 11683383]

- Schwartz MA, Ginsberg MH. Networks and crosstalk: integrin signalling spreads. *Nat Cell Biol* 2002;4:E65–E68. [PubMed: 11944032]
- Shen X, Koga T, Park BC, SundarRaj N, Yue BYJT. Rho GTPase and cAMP/Protein Kinase A signaling mediates myocilin-induced alterations in cultured human trabecular meshwork cells. *J Biol Chem* 2008;283:603–612. [PubMed: 17984096]
- Steely HT, Browder SL, Julian MB, Miggans ST, Wilson KL, Clark AF. The effects of dexamethasone on fibronectin expression in cultured human trabecular meshwork cells. *Invest Ophthalmol Vis Sci* 1992;33:2242–2250. [PubMed: 1607235]
- Telci D, Wang Z, Li X, Verderio EAM, Humphries MJ, Baccarini M, Basaga H, Griffin M. Fibronectin-tissue transglutaminase matrix rescues RGD-impaired cell adhesion through syndecan-4 and $\beta 1$ integrin co-signaling. *J Biol Chem* 2008;283:20937–20947. [PubMed: 18499669]
- Tripathi BJ, Li T, Li J, Tran L, Tripathi RC. Age-related Changes in Trabecular Cells *in vitro*. *Exp Eye Res* 1997;64:57–65. [PubMed: 9093021]
- Vittal V, Rose A, Gregory KE, Kelley MJ, Acott TS. Changes in gene expression by trabecular meshwork cells in response to mechanical stretching. *Invest Ophthalmol Vis Sci* 2005;46:2857–2868. [PubMed: 16043860]
- Welge-Lussen U, May CA, Lutjen-Drecoll E. Induction of tissue transglutaminase in the trabecular meshwork by TGF- $\beta 1$ and TGF- $\beta 2$. *Invest Ophthalmol Vis Sci* 2000;2000:2229–2238. [PubMed: 10892867]
- Wijelath ES, Rahman S, Namekata M, Murray J, Nishimura T, Mostafavi-Pour Z, Patel Y, Suda Y, Humphries MJ, Sobel M. Heparin-II domain of fibronectin is a vascular endothelial growth factor-binding domain: enhancement of VEGF biological activity by a singular growth factor/matrix protein synergism. *Circ Res* 2006;99:853–860. [PubMed: 17008606]
- Zhong C, Chrzanowska Wodnicka M, Brown J, Shaub A, Belkin AM, Burridge K. Rho-mediated contractility exposes a cryptic site in fibronectin and induces fibronectin matrix assembly. *J Cell Biol* 1998;141:539–551. [PubMed: 9548730]
- Zhou L, Higginbotham EJ, Yue BYJT. Effects of ascorbic acid on levels of fibronectin, laminin and collagen type I in bovine trabecular meshwork in organ culture. *Curr Eye Res* 1998;17:211–217. [PubMed: 9523101]
- Zhou L, Li Y, Yue BY. Glucocorticoids effects on extracellular matrix proteins and integrins in bovine trabecular meshwork cells in relation to glaucoma. *Int J Mol Med* 1998;1:339–346. [PubMed: 9852235]
- Zhou LL, Maruyama I, Li YH, Cheng EL, Yue B. Expression of integrin receptors in the human trabecular meshwork. *Curr Eye Res* 1999;19:395–402. [PubMed: 10520215]

Figure 1. Diagram of Dimeric Fibronectin

The disulfide bonded nonidentical polypeptide chains consist of homologous type I (circles), II (hexagons), and III (squares) repeats. The RGD containing cell binding domain (CBD) and the HepII domain are indicated by shaded squares. Alternatively spliced exons (EDA, EDB, and V) contribute to the various isoforms of fibronectin. Exon subdivision of the V exon is indicated in the box.

Figure 2. Model of Fibronectin (FN) Signaling in the TM

(A) HTM cells bound to fibronectin via $\alpha5\beta1$ integrins spread and form a few actin stress fibers (Peterson, et al., 2004). (B) In subconfluent cells, engagement of $\alpha4\beta1$ integrin by the HepII domain initiates co-signaling with $\alpha5\beta1$ which enhances actin stress fiber and focal adhesion (FA) formation. This co-signaling could also trigger changes in gene transcription and levels of MMPs resulting in less ECM. Stretch or degradation of ECM would be one way exposure to the HepII domain is controlled in the TM. (C) In confluent HTM monolayer, signaling from the HepII domain alters cross-talk between collagen engaged integrins (i.e. $\alpha2\beta1$) and cell-cell contacts resulting in stress fiber disassembly.