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MAR 2013

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Idiopathic Pulmonary Fibrosis Drug Discovery using Fluorescent Fibronectin Proteins

Idiopathic Pulmonary Fibrosis (IPF) afflicts five million people worldwide with a median survival rate of 3-5 years[1,2,3]. This fibrotic disease is considered a chronic inflammatory tissue-repair response and is characterized by an excessive deposition of connective tissues that leads to failure in organ structure and function[4]. Fibronectin (FN) fibrillogenesis, a cell-mediated process that converts soluble plasma FN into insoluble FN, plays a central role in the development of this disease[5]. FN is a glycoprotein secreted as a dimer connected by two C-terminal disulfide bonds. It is comprised of multiple functional domains including the N-terminal 70 kDa domain, the 120 kDa central binding domain, and the heparin-binding domain HepII[4]. These domains interact with cell-surface receptors in a stepwise process that initially involves binding to integrin and heparin sulfate proteoglycan (HSPG) cell-surface receptors (see Fig. 1)[4]. This binding triggers actin cytoskeleton reorganization which generates cell tension, leading to clustering and translocation of the FN-bound receptors which unfolds the compact FN dimer into an extended structure that exposes binding sites for FN-FN interactions[4]. Although fibrillogenesis can lead to misregulated fibrosis and has been linked to several diseases including IPF and malignant, unchecked tissue growth[4,6], the insoluble cellular FN also plays a major role in cell adhesion, growth, migration, and differentiation which are important for restorative and developmental processes such as the deposition of connective tissue[4,7], wound healing, and embryonic development[7].

While there are no cures or direct treatments for IPF, there are several non-specific therapeutic options for IPF patients to help maintain their quality of life, including: the anti-inflammatory Pirfenidone, immune suppressors, corticosteroids and Azathioprine, antioxidant N-acetylcysteine, supplemental oxygen therapy, pulmonary rehabilitation, and surgery[3]. In addition, the anti-inflammatory agents Thalidomide and Macrolide have recently been suggested as a possible treatment for IPF[8,9]. Indeed, Macrolide antibiotic clinical trials have shown long term benefits for cystic fibrosis patients[9]. More direct treatment prospects include an anti-TGF-β compound or a FUD peptide[4]. Anti-TGF-β treatments in mice reduced skin and pulmonary fibrosis with sclerodermatous graft-versus-host disease[4]. However, anti-TGF-β treatments in human systemic sclerosis phase I/II trials were not successful. A residue sequence from the FUD domain of the F1 adhesin protein inhibits FN matrix assembly by binding to the N-terminal FNI_{1-5} domain of FN. This domain is crucial for FN binding to cell receptors[4] and demonstrates that FN binding domains could be promising drug targets for fibrosis disease treatments.

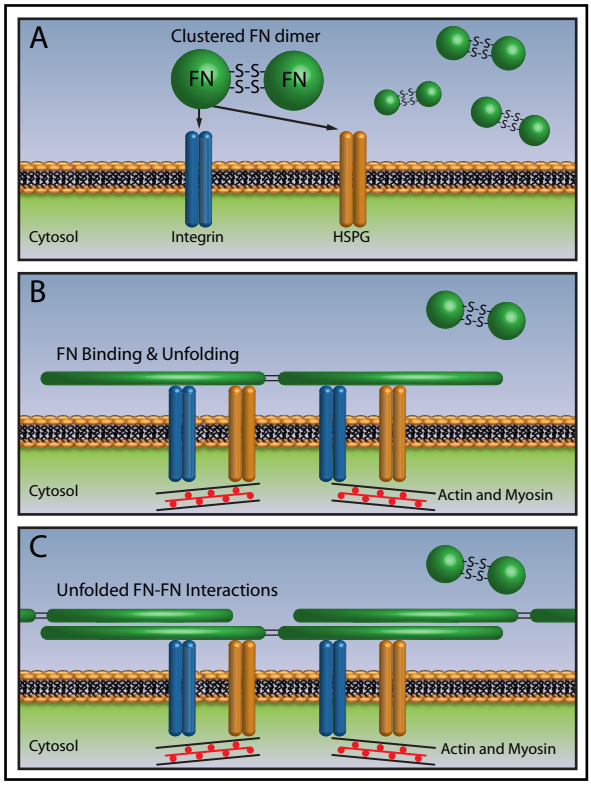

Fig. 1. Fibronectin matrix assembly (adapted from ref. 4). (A) Unbound compact FN dimer and cell membrane receptors. (B) FN binding induces cytoskeleton reorganization that generates cell tension, resulting in receptor clustering and unfolding of FN to expose FN binding sites. (C) Exposed FN binding sites allow for FN-FN interactions.

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An increased understanding of the mechanisms that regulate FN matrix assembly may aid in the discovery of better treatments for fibrotic diseases. Enhancing or suppressing the effects of FN *in vivo* via specific modulators is of particular interest in fibrosis research[6]. Recent advancements in high throughput screening (HTS) methods facilitated screening of small molecule libraries for their effects on FN fibrillogenesis[6]. An assay, as described by Tomasini-Hohansson et. al.[6], utilizes fluorescent FN to provide a read-out of FN fibrillogenesis with Z' values of >0.5. Tomasini-Hohansson et al.'s pilot experiment screened 4160 known bioactive compounds. As a result of this study, 9 compounds were found to inhibit FN assembly, including four kinase inhibitors (ML-9, HA-100, tryphostin, and imatinib mesylate), two cancer cell apoptosis promoters (piperlongumine and cantharidin), and three modulators of biogenic amine signaling (maprotiline, CGS12066B, and aposcopolamine). By utilizing fluorescent FN along with HTS techniques, additional modulators of FN fibrillogenesis may aid in the development of therapeutics for fibrotic diseases. Cytoskeleton, Inc. offers rhodamine, HiLyte488™, and biotinylated ECM proteins to study FN fibrillogenesis (See next right for available products).

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Fibronectin Research Tools

ECM Proteins

Fluorescently labeled and biotinylated fibronectins and laminins.

Fluorescent ECM	Source	Purity	Cat. #	Amount
Fibronectin Red fluorescent, rhodamine	Bovine serum	>80%	FNR01-A FNR01-B	5 x 20 µg 20 x 20 µg
Fibronectin Green fluorescent, HiLyte488™	Bovine serum	>80%	FNR02-A FNR02-B	5 x 20 µg 20 x 20 µg
Fibronectin Biotinylated	Bovine serum	>80%	FNR03-A FNR03-B	5 x 20 µg 20 x 20 µg
Laminin Red fluorescent, rhodamine	Engelbreth-Holm-Swarm mouse tumor	>90%	LMN01-A LMN02-B	5 x 20 µg 20 x 20 µg
Laminin Green fluorescent, HiLyte488™	Engelbreth-Holm-Swarm mouse tumor	>90%	LMN02-A LMN02-B	5 x 20 µg 20 x 20 µg
Laminin Biotinylated	Engelbreth-Holm-Swarm mouse tumor	>90%	LMN03-A LMN03-B	5 x 20 µg 20 x 20 µg

Small G-protein Activation Assays

Cytoskeleton's G-LISA offers a fast and sensitive way of performing small G-protein Activation Assays.

Small G-protein Activation Assays	Method	Cat. #	Amount
RhoA / Rac1 / Cdc42 Activation Assay Combo Biochem Kit™	Pull-down	BK030	3 x 10 assays
Arf1 G-LISA® Activation Assay, colorimetric	G-LISA®	BK132	96 assays
Arf1 Activation Assay Biochem Kit™	Pull-down	BK032-S	20 assays
Arf6 G-LISA® Activation Assay, colorimetric	G-LISA®	BK133	96 assays
Arf6 Activation Assay Biochem Kit™	Pull-down	BK033-S	20 assays
Cdc42 G-LISA® Activation Assay, colorimetric	G-LISA®	BK127	96 assays
Cdc42 Pull-down Activation Assay Biochem Kit™	Pull-down	BK034 BK034-S	50 assays 20 assays
Rac1,2,3 G-LISA® Activation Assay, colorimetric	G-LISA®	BK125	96 assays
Rac1 G-LISA® Activation Assay, colorimetric	G-LISA®	BK128	96 assays
Rac1 G-LISA® Activation Assay, luminescence	G-LISA®	BK126	96 assays
Rac1 Pull-down Activation Assay Biochem Kit™	Pull-down	BK035 BK035-S	50 assays 20 assays
RalA G-LISA® Activation Assay, colorimetric	G-LISA®	BK129	96 assays
RalA Pull-down Activation Assay Biochem Kit™	Pull-down	BK040	50 assays
Ras G-LISA® Activation Assay, colorimetric	G-LISA®	BK131	96 assays
Ras Pull-down Activation Assay Biochem Kit™	Pull-down	BK008 BK008-S	50 assays 20 assays
RhoA G-LISA® Activation Assay, colorimetric	G-LISA®	BK124	96 assays
RhoA G-LISA® Activation Assay, luminescence	G-LISA®	BK121	96 assays
RhoA Pull-down Activation Assay Biochem Kit™	Pull-down	BK036 BK036-S	80 assays 20 assays