Translational control of the fibroblast-extracellular matrix association
An application to pulmonary fibrosis

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Pulmonary fibrosis is a severe lung disease characterized by sustained propagation of lung fibroblasts and relentless accumulation of extracellular matrix (ECM). Idiopathic pulmonary fibrosis (IPF) is the most severe chronic form of pulmonary fibrosis and results both in the gradual exchange of normal lung parenchyma with fibrotic tissue and in the irreversible impairment of gas exchange in the lung. Despite the urgency for novel therapies in IPF treatment, there is no effective and proven medical therapy available. Molecular mechanisms underlying IPF pathogenesis include aberrant ECM signaling through the canonical integrin/PI3K/Akt/mTORC1 signal transduction pathway. One important and well-characterized downstream effector of this pathway is the cellular protein synthesis machinery. Here we will review the recent advances in our understanding of the function of ECM and integrin receptor signaling in development of IPF and will present evidence indicating that the dysregulation of the eIF4F-mediated translational apparatus is an important factor in the development and progression of IPF and other fibrotic disorders. We further discuss the perspectives and challenges to curbing this deadly disease by targeting aberrant translation.

Etiology and Pathogenesis of IPF

By definition, etiology and genesis of IPF is unknown and the mechanisms underlying its pathogenesis remain poorly understood. The current concept for the development of pulmonary fibrosis, including IPF, is that at least three physiologically balanced processes implicated in the maintenance of lung fibroblast populations: proliferation, apoptosis of (myo)fibroblasts and production of ECM—are disturbed. Unlike normal tissue repair, where (myo)fibroblast proliferation is self-limited and cells are eliminated by apoptosis upon completion of repair, in IPF there is a relentless accumulation of fibroblasts and ECM due to evading apoptosis and sustained cell proliferation. Morphological studies have demonstrated that the sentinel morphological lesion of IPF is the sub-epithelial accumulation of fibroblasts termed the “fibroblastic focus”. The origin of pathological fibroblast foci remains puzzling. Possibilities include abnormal self-renewal and differentiation of resident mesenchymal stem/progenitor cells, recruitment of circulating fibroblast progenitors and transdifferentiation of epithelial cells into the pathological fibroblast phenotypes (epithelial-to-mesenchymal transition, EMT). Ultrastructural analysis of the fibroblastic focus has further revealed that it is composed of myofibroblasts which express the specific marker α-smooth muscle actin and which are enmeshed in a matrix rich in polymerized type I collagen.

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IPF Cells can Propagate on ECM that does not Support Normal Lung Fibroblasts

Continuing propagation of myofibroblasts in fibroblastic foci and specific alterations in the ECM structure due to extensive deposition of type I collagen lead to permanently scarred and functionally disabled alveoli. ECM is composed of collagens, elastin, proteoglycans (including hyaluronan) and noncollagenous glycoproteins and forms a complex, three dimensional network among cells of different tissues in an organ-specific manner and reciprocally influences cellular function to modulate diverse fundamental aspects of cell biology. ECM components are classified as fiber forming and non-fiber forming (interfibrillar) molecules. ECM constitutes the cellular microenvironment for all cells outside the circulation and its composition profoundly affects cell proliferation, adhesion, migration, differentiation and viability. Several essential ECM components such as proteoglycans, fibronectin, elastin and fibrillins form its macromolecular structures. In animal tissues, the most abundant matrix components are the collagens that form a super-family of 27 different members which are divided into different subgroups. The fibrillar collagens, types I, III and V, the FACIT collagens, types XII, XIV and XVI, and collagen VI are all expressed in the collagen-rich dermis. About one-third of the 19 collagens—types I, II, III, V, and XI—are called fibrillar collagens, because they are found in tissues as long, highly ordered fibrils with a characteristic banding pattern readily detected by electron microscopy. Among them, type I collagen is the major component of ECM in skin, bone, ligaments, etc., is composed of glycin- and proline rich two-α(1) (I) and one-α(2) (I) chains. The pro-COL1A1 and COL1A2 polypeptide chains are synthesized by fibroblasts, osteoblasts, or odontoblasts. Normal structural and functional type I collagen production and deposition in ECM to make normal physiological connective tissue needs regulation at several steps. Studies showed abnormality in any step may cause hypo- or hyper- or defective synthesis and accumulation of collagen in ECM, which in turn causes different diseases in humans such as osteogenesis imperfecta, scurvy, scleroderma or systemic sclerosis, keloids, lung fibrosis, liver fibrosis, etc. Inactivation of the collagen α1(I) gene in mice results in embryonic lethality.

ECM dynamics result from altered synthesis or degradation of one or more ECM components. Recent findings indicate that there is a sharp difference in proliferation profiles of human lung fibroblasts when they are cultured on 2D and 3D type 1 collagen matrices. Specifically, tissue culture plates coated with 2D monomeric collagen provide a proliferation-permissive environment for normal and IPF lung fibroblasts. 3D matrix, which closely imitates the physiological forms of ECM, is composed of polymerized collagen (fibrillar collagen). In sharp contrast to the 2D matrix, 3D polymerized collagen does not support proliferation and viability of normal lung fibroblasts, whereas IPF fibroblasts survive and propagate. Furthermore, immunohistochemical analysis revealed that α-smooth muscle actin-expressing myofibroblasts are enmeshed in a type I collagen-rich 3D matrix. These findings show that the composition and architecture of ECM govern a category of signals required for viability and proliferation of lung fibroblasts and that IPF cells harbor an intrinsic program(s) allowing them to survive and propagate under the ECM conditions that do not support growth and viability of normal fibroblasts.

IPF Cells Manifest Sustained Activity of the PI3K/Akt/mTORC1 Signaling Pathway

Several cell surface receptors including integrins, discoidin domain receptors (DDRs), syndecans and CD44 have been identified as components of a complex system responsible for cell immobilization on normal ECM. Integrins are transmembrane molecules, which primarily mediate cell-to-cell and cell-to-ECM adhesion and are composed of non-covalently associated α and β chains which form heterodimeric receptor complexes. Eighteen α subunits and 8 β subunits of integrin have been identified that associate to form 24 known types of integrins. The α and β chains of integrins cooperate in a specific mode in which the extracellular portion of the α chain is responsible for the ligand-binding specificity of the complex whereas the intracellular domain of the β chain is associated with the induction of intracellular signaling cascades. Among them, β1 integrin is the major integrin subunit that mediates attachment of fibroblasts to ECM by associating with several α subunits (Table 1). For example, α2β1 integrin predominantly binds to collagen while α5β1 integrin binds to fibronectin. ανβ3, α6β1 are receptors known to bind to vitronectin, laminin, respectively.

Beyond these proximal events lie several critical intracellular decision points within cellular signaling cascades regulating cell viability and proliferation. Recent studies demonstrated that lung fibroblasts utilize the PTEN-regulated PI3K/Akt-dependent signaling pathway to curb apoptosis and stimulate cell proliferation. When normal fibroblasts attach to monomeric type I collagen, integrin receptors signal the downregulation of PTEN and activation of the PI3K/Akt protein phosphorylation cascade. In contrast, interactions with polymerized collagen do not signal decreasing PTEN and the activity of PI3K/Akt remains low. The integrin-mediated signaling is partly modulated by the membrane protein caveolin-1 (cav-1), which regulates integrin turnover and modifies a variety of cellular processes including cell proliferation and apoptosis operating mainly through the PI3K/Akt/mTORC1 signal pathway. Cav-1 can bind to PTEN using the consensus binding sequence (φXbXXXφ corresponding to amino acids 271–278 (FHFWVNTF, φ = aromatic amino acid phenylalanine)). Binding to cav-1 increases the ability of PTEN to antagonize PI3K activity. Normal lung fibroblasts express a relatively high level of cav-1, which interacts with PTEN to suppress PI3K/Akt/mTORC1 signaling on polymerized collagen. Conversely, IPF cells express aberrantly low levels of α2β1 integrin, cav-1 and PP2A. As a result, IPF fibroblasts reveal sustained PI3K/Akt/mTORC1 signaling and propagate under conditions when normal fibroblasts undergo apoptotic death.
Translational Control in Fibrotic Disease

The eIF4F-mediated translational apparatus is a key regulatory hub in the cancer signaling circuitry. The PI3K/Akt/mTORC1-mediated signaling pathway senses and integrates a variety of environmental cues to regulate cell homeostasis, viability and propagation. One important downstream effector of mTORC1 signaling is the translational apparatus. Translational regulation primarily occurs at the mRNA recognition step. In eukaryotes, this step is facilitated by the mRNA 5’ cap structure (7-methyl-G(5’)ppp(5’)N where N is any nucleotide). The heterotrimeric translation initiation complex eIF4F functions to recognize capped mRNA and recruit these transcripts to the 40S ribosome subunit with subsequent ribosome scanning toward the initiation codon. It consists of the translational factors eIF4E, eIF4G and eIF4A. A 25 kDa phosphoprotein eIF4E directly associates with the cap structure and is rate-limiting for translation initiation. eIF4GI and II serve a docking function, binding to eIF4E and eIF4A. The eIF4AI and eIF4AII isoforms function as an ATP-requiring helicase, which unwinds the 5’ region of the mRNA. The primary regulation of eIF4F integrity is exerted by three repressor proteins, designated 4E binding protein (4E-BP) 1, -BP2 and -BP3. When hypophosphorylated, 4E-BPs avidly bind with eIF4E thereby sequestering it into a translationally inactive complex. A wide variety of extracellular regulatory cues including growth factors, hormones and components of ECM stimulate eIF4F assembly and cap-dependent translation by signaling phosphorylation of the 4E-BPs on four serine/threonine sites important for their association with eIF4E through the PI3K/Akt/mTORC1 kinase cascade, which is positively regulated by Ras. Ras can activate eIF4F-driven translation operating through the canonical MAPK/ERK pathway leading to phosphorylation of eIF4E by protein kinases MNK1 and MNK2. Deregulation of cap-dependent translation consists of sustained, constitutive activation of eIF4F by a vast array of upregulated upstream signals including the Myc, Ras and PI3K/AKT/mTORC1 pathways as well as by genetic alterations in components of the translational apparatus. There is now strong support for the idea that eIF4F is an obligatory regulatory hub in most - if not all - human malignancies. More recently, gene analysis revealed derangement of translational control is also an important pathological mechanism of IPF.

| β subunit | α subunit |
|-----------|-----------|
| β1        | α1        |
| β2        | αL        |
| β3        | αV        |
| β4        | α6        |
| β5        | αV        |
| β6        | αV        |
| β7        | α4        |
| β8        | αV        |

Translational control in IPF fibroblasts on ECM. Emerging pieces of evidence demonstrate that extracellular matrix greatly regulates PI3K/Akt/mTORC1 via the integrin/PTEN axis. Because aberrant translation is the ultimate step for producing proteins driving abnormal cell proliferation, motility and microenvironment in cancer and other proliferative disorders, it is reasonable to propose that translational control can be a crucial step in the interactions of fibroblasts with matrix constituents and that its dysregulation may be involved in the onset and progression of IPF. Indeed, fibroblast adhesion to the major matrix protein fibronectin activates eIF4F-dependent translation through a PI3K-mediated pathway in a manner that is independent on mTORC1 activity and that can be impaired by blocking the β1 integrin engagement. Integrin-induced activation of the translational machinery may be selective for changes in translational efficiency of some subsets of mRNAs. It has been shown that α6β4 integrin specifically stimulates recruitment of ribosomes to mRNAs encoding the Ras, ErbB2 and VEGF oncoproteins in breast carcinoma cells and that this activity is mediated through the Ras/MAPK/ERK signaling pathways leading to MNK1-mediated phosphorylation of eIF4E. In line with this, downregulation of the αVβ3 integrin-dependent PI3K/Akt/mTORC1 signaling axis suppresses eIF4F-mediated translation and promotes apoptotic death of endothelial cells by stimulating the sequestration of eIF4E by hypophosphorylated 4E-BP1. In the course of interactions between normal lung fibroblasts and collagen matrix, mTORC1 kinase is upregulated as a result of signaling from the PI3K/Akt pathway, thereby affecting the activity of the translational machinery by hyperphosphorylation of 4E-BP1.
The activation of PP2A. Moreover, active proliferation of fibroblasts signaled by β1 integrin receptors is associated with profound reduction of 4E-BP1 expression and subsequent dissociation of the translationally-inactive 4E-BP1/eIF4E complex. These studies suggest that permissive ECM signals proliferation and viability of normal fibroblasts through activation of multiple pathways leading to regulation of eIF4F. In sharp contrast, the rescue of IPF fibroblasts from apoptosis and the maintenance of their proliferation on 3D matrix is associated with sustained PI3K/Akt/mTORC1- and PP2A-mediated activation of eIF4F.

PP2A is an important phosphatase that regulates the activity of many proteins via dephosphorylation and is thought to regulate translational activity. Studies also showed that Src-dependent suppression of PP2A activity stimulates eIF4F-mediated translation in lung fibroblasts cultivated on monomeric collagen (2D matrix). These observations support the notion that the eIF4F integrity and its activity can be curbed by activation of PP2A. Moreover, active proliferation of fibroblasts signaled by β1 integrin receptors is associated with profound reduction of 4E-BP1 expression and subsequent dissociation of the translationally-inactive 4E-BP1/eIF4E complex. These studies suggest that permissive ECM signals proliferation and viability of normal fibroblasts through activation of multiple pathways leading to regulation of eIF4F. In sharp contrast, the rescue of IPF fibroblasts from apoptosis and the maintenance of their proliferation on 3D matrix is associated with sustained PI3K/Akt/mTORC1- and PP2A-mediated activation of eIF4F-mediated translation (Fig. 1).
In response to injury, normal interstitial fibroblasts differentiate into myofibroblasts which synthesize and deposit type I collagen into the wound provisional matrix and contract this matrix, thereby facilitating wound closure.\(^4\) Under normal physiological conditions, contraction of matrix results in myofibroblast apoptosis to prevent hyperproduction of granulation tissue during the wound healing process.\(^4\) Analysis of the translational landscape in myofibroblasts growing on non-contractile or on contractile collagen matrices using microarray technology\(^3\) identified a substantial subset of mRNAs that exhibit differential patterns of ribosomal recruitment in IPF and normal myofibroblasts in a manner regulated by the state of the collagen matrix. Specifically, it was shown that mTORC1 and keratin 18 are translationally activated in IPF. The other mRNAs differentially recruited into and out of ribosomes are those encoding proteins involved in regulation of cell cycle transition, apoptosis, cytoskeleton and cell motility.\(^2\,\,4\) Pathological studies suggest an epithelial origin for IPF myofibroblasts through the epithelial to mesenchymal transition (EMT). In accordance with this, systems-level indications for TGF-\(\beta\)-driven EMT as one source of IPF myofibroblasts were found.\(^4\) Collectively, these findings strongly suggest that survival and propagation of IPF fibroblasts, under conditions that are non-permissive for viability of normal fibroblasts, are associated with an intrinsic capability to activate the integrin-dependent signaling cascades, leading to increased translational apparatus.

**Conclusion and Future Challenges**

IPF fibroblasts reveal high proliferative and anti-apoptotic capabilities under conditions that are non-permissive for viability and growth of normal fibroblasts. Available data show that these propensities are mediated by a constellation of signaling pathways leading to the eIF4F-driven translational apparatus. Although there has been significant progress in understanding the molecular mechanisms of translational control, particularly in cancer models, there are still gaps in understanding the function of translational control in the pathologic nature of IPF fibroblasts for fibrotic progression and the physiologic function of myofibroblasts for normal lung repair. In order to find potential therapeutic targets that are effective to fibrotic diseases, the nature of cellular pathways by which collagen matrix signals normal or aberrant cell proliferation and viability must particularly be considered. Because patients come to medical attention with some degree of established fibrosis, the present therapeutic strategies predominantly serve to prevent the fibrotic obliteration of additional airspaces, but not to reverse what has already occurred. Rational therapy, however, could also target established fibrotic lesions, creating a state of vulnerability in fibrotic fibroblasts. The findings reviewed here suggest that the aberrant translational apparatus and the components of ECM regulating their activity can be a target for therapeutic interventions. Thus we propose that eIF4E target approach using small peptides or others may be more effective for limiting progression of IPF. This central integrative role of pathologically activated eIF4E in the oncogenic circuitry motivated the development of small molecule inhibitors of eIF4F as potential cancer thera-

ds.\(^2\,\,6\,\,4\) For example, targeting eIF4E in eIF4F complex is a promising approach for the limiting fibrotic progression. This approach has already been validated in cancer models. Graff and his coworkers reported their findings that eIF4E targeting using eIF4E specific anti-sense oligonucleotide reduces tumor size without toxicity.\(^5\) Specifically this and other eIF4E targeting approaches might be effective in suppression of growth of breast cancer cells.\(^6\)\(^,\)\(^7\) We propose that similar approaches can be applied to normalize aberrant translation in IPF fibroblasts and can be considered as a potential therapeutic intervention to limit the progression of IPF.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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