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Fibroblast—Extracellular Matrix Interactions in Tissue Fibrosis

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Abstract

Activated myofibroblasts are key effector cells in tissue fibrosis. Emerging evidence suggests that myofibroblasts infiltrating fibrotic tissues originate predominantly from local mesenchyme-derived populations. Alterations in the extracellular matrix network play an important role in modulating fibroblast phenotype and function. In a pro-inflammatory environment, generation of matrix fragments may induce a matrix-degrading fibroblast phenotype. Deposition of ED-A fibronectin plays an important role in myofibroblast transdifferentiation. In fibrotic tissues, the matrix is enriched with matricellular macromolecules that regulate growth factor-mediated responses and modulate protease activation. This manuscript discusses emerging concepts on the role of the extracellular matrix in regulation of fibroblast behavior.

Keywords

Fibroblast; Pericyte; Myofibroblast; Extracellular matrix; Matricellular protein; Thrombospondin

Introduction

Fibrosis is defined as the accumulation of extracellular matrix proteins in tissue and is a common pathophysiologic companion of many pathologic conditions [1]. Expansion of the extracellular matrix network is an important part of the reparative response following tissue injury. Cell necrosis triggers an inflammatory reaction, ultimately activating fibrogenic pathways and stimulating deposition of matrix proteins. Whether the reparative fibrotic response is a reversible component of wound healing, or results in permanent disruption of organ function, is dependent on the regenerative capacity of the tissue, and the type and extent of the initial injury. For example, skeletal muscle injury heals through activation of a robust regenerative response triggered by myogenic precursor cells [2]. In contrast, the myocardium and the central nervous system exhibit negligible regenerative capacity; as a result, cellular loss in these organs is repaired through the formation of a collagen-based scar [3]. In all cases, irreversible fibrosis disrupts the normal architecture of the tissue and causes

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Compliance with Ethics Guidelines

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organ dysfunction. A wide range of diseases are associated with fibrosis, including hepatic cirrhosis, chronic renal disease, idiopathic pulmonary fibrosis, and heart failure [1, 4•, 5, 6]. Moreover, metabolic conditions (such as diabetes, obesity, and the metabolic syndrome), and disorders associated with activation or dysregulation of the immune system (such as scleroderma) cause fibrotic remodeling in many tissues [7–9]. Considering the involvement of fibrosis in renal failure, end-stage liver disease, and in the pathogenesis of heart failure and cardiac arrhythmias, fibrotic remodeling is implicated, directly or indirectly, in a substantial number of deaths worldwide.

Fibroblasts are the main effector cells in tissue fibrosis, capable of synthesizing and remodeling the extracellular matrix. Most tissues contain large numbers of fibroblasts that can be activated by a wide range of stimuli, such as pathogens, mediators released by necrotic cells, toxins, and leukocyte-derived products. Conversion of quiescent fibroblasts into activated matrix-producing myofibroblasts (Fig. 1) [10] is a key event in tissue fibrosis [11, 12]. Moreover, a growing body of evidence suggests that in injured and remodeling tissues, other resident or newly recruited cell types may transdifferentiate into myofibroblasts, expanding the pool of cells with fibrogenic potential. Despite the critical role of activated fibroblasts in the fibrotic process, the cell biology and molecular mechanisms of fibroblast activation remain mysterious. Traditionally defined by their morphology and capacity to secrete matrix proteins, fibroblasts exhibit significant heterogeneity and plasticity. The features that characterize various fibroblast subpopulations have not been systematically defined; the molecular signals implicated in fibroblast activation are poorly understood. The current manuscript reviews recent advances on the cellular origin of fibroblasts in injured tissues. Because fibroblast phenotype is critically modulated by alterations in the extracellular matrix environment, we focus specifically on the effects of matrix macromolecules in fibroblast activity and function.

Origin of Fibroblasts in Fibrotic Tissues: The Concept of Cellular Plasticity

A wide range of injurious stimuli can cause expansion and activation of fibroblast populations in affected tissues. The origin of these activated fibroblasts remains a hotly debated question. More than a decade ago, a large body of evidence suggested that a wide range of cell types, including epithelial cells, vascular endothelial cells, and pericytes, exhibit a remarkable plasticity and can differentiate into fibroblasts in response to microenvironmental cues [13, 14]. In the pressure-overloaded myocardium, endothelial-to-mesenchymal transdifferentiation was implicated in generation of abundant myofibroblasts [15]. Moreover, hematopoietic progenitors were proposed as an additional source of fibroblasts in injured tissues [16–19]. Unfortunately, the lack of reliable and specific markers to identify fibroblasts has hampered efforts to understand the origin of fibroblasts in health and disease. Many studies attempting to trace fibroblast lineages have used non-specific markers, such as fibroblast-specific protein (FSP)-1/S100A4 [15, 20], a calcium-binding protein that is also abundantly expressed by leukocytes and vascular cells [21, 22]. Moreover, the origin of activated fibroblasts may depend on the tissue studied and on the type of injury. For example, injurious responses that cause cellular necrosis are often associated with robust upregulation of chemokines [23] and recruitment of abundant circulating cells. In this context, the contribution of circulating fibrocytes may be

accentuated, due to increased chemoattraction of fibroblast progenitors through chemokine-mediated pathways [24, 25].

In recent years, experimental studies using fate-mapping strategies have produced an explosion in our knowledge on the origin of fibroblasts in fibrotic tissues. A growing body of evidence suggests that in most tissues, local mesenchyme-derived subpopulations of fibroblasts may act as the main cellular effectors of fibrotic responses. Although small populations of blood-derived fibroblasts have been identified in the fibrotic kidney and liver [26, 27], their relative contribution remains unknown. Several seminal investigations have identified local interstitial cell subsets as important sources of activated fibroblasts in various organs.

In normal and lesional mouse skin, lineage tracing approaches demonstrated that the *Engrailed-1* lineage is the main source of matrix-secreting fibroblasts in normal skin, in healing cutaneous wounds, in radiation injury, and in the stroma of melanomas [28•]. Ablation of these highly fibrogenic cells that expressed CD26 significantly reduced scarring following cutaneous injury and delayed growth of melanomas.

In several different organs, perivascular cells serve as important sources of activated myofibroblasts [26, 29, 30]. Pericytes are defined as perivascular cells of mesenchymal origin, which are sheathed with a basement membrane, and directly communicate with endothelial cells [31]. Studies in experimental models of kidney fibrosis have suggested that pericytes may be a major source of myofibroblasts [26, 30]. Recent investigations have indicated a broader role for perivascular cells in the pathogenesis of tissue fibrosis. Expression of Gli1, a transcription factor that mediates hedgehog signaling, marks a population of tissue-resident perivascular cells that transdifferentiate into myofibroblasts in the heart, kidney, lung, and liver [32•].

Local fibroblast progenitor populations may also be crucial contributors in the pathogenesis of fibrosis. The adult mammalian myocardium contains a large number of poorly defined fibroblast-like cells that may outnumber cardiomyocytes [33, 34] and, upon activation, may generate myofibroblasts. Lineage tracing studies suggested that, in the pressure-overloaded heart, the majority of activated myofibroblasts originate from proliferation and activation of resident fibroblast lineages, rather than from hematopoietic or endothelial cells [35•, 36]. In the infarcted myocardium, an epicardial-derived subset of cardiac fibroblasts appears to contribute to the reparative/fibrotic response [37].

Role of the Extracellular Matrix in Fibroblast Activation

An important question in understanding both the reparative process and the fibrotic response is whether fibroblasts become intrinsically activated, or simply respond to alterations in their matrix environment. In tissue repair, fibroblasts appear to be highly dynamic cells that can acquire distinct phenotypes depending on changes in their environment [38]. In healing myocardial infarcts, fibroblasts during the early inflammatory phase of cardiac repair exhibit high expression of matrix metalloproteinases (MMPs) [39] and may act as inflammatory cells, activating the inflammasome [40], and producing pro-inflammatory cytokines and

chemokines. As the inflammatory response is suppressed, fibroblasts proliferate, transdifferentiate into myofibroblasts, and secrete large amounts of extracellular matrix proteins [41–43]. Transforming growth factor (TGF)- β -mediated Smad-dependent signaling plays an important role in activation of a matrix-synthetic phenotype in infarct myofibroblasts [44]. As the scar matures, myofibroblasts become quiescent or undergo apoptosis. Alterations in the environmental conditions regulate modulation of fibroblast phenotype. Secreted mediators such as angiotensin II, TGF- β , the mast cell proteases tryptase and chymase, endothelin-1, and platelet-derived growth factors (PDGFs) participate in regulation of fibroblast activation and gene expression [5, 45–48]. The composition of the extracellular matrix also critically regulates fibroblast phenotype in fibrotic tissues [49]. The importance of the extracellular matrix in directing fibroblast phenotype is suggested by experimental studies using cells and decellularized matrix isolated from patients with idiopathic pulmonary fibrosis. These experiments suggested that the fibrotic matrix accentuates fibroblast-derived matrix synthesis, driving a highly fibrogenic phenotype [50•].

Matrix Degradation Products May Activate a Pro-inflammatory Fibroblast Phenotype

In an inflammatory environment, fibroblasts acquire a pro-inflammatory and matrix-degrading phenotype [51]. Pro-inflammatory cytokines, such as interleukin-1, delay myofibroblast conversion and induce chemokine and MMP synthesis by tissue fibroblasts [39, 52]. Inflammatory activation of fibroblasts may also be driven by changes in the extracellular matrix. Inflammation is associated with protease activation and generation of matrix fragments with pro-inflammatory properties; these fragments serve as “danger signals” that trigger inflammation (Fig. 2a). Fibronectin fragments are rapidly released in the extracellular space following injury and activate pro-inflammatory signaling. In contrast to the intact fibronectin molecule, the 120 kDa cell-binding fibronectin fragment (Fn120) potently stimulates monocyte chemotaxis [53]. Both 120 and 45 kDa fibronectin fragments have been shown to activate a matrix-degrading program in human fibroblasts, stimulating MMP expression and activity [54–56]. In vitro studies suggest that hyaluronan fragments may also exert potent pro-inflammatory actions. In synovial fibroblasts, hyaluronan degradation products exerted pro-inflammatory actions activating CD44 and toll-like receptor (TLR)-4 signaling [57]. In human dermal fibroblasts, hyaluronan fragments increased MMP expression [58]. Support for the pro-inflammatory actions of matrix degradation products on cardiac fibroblasts is predominantly derived from in vitro studies. In vivo evidence on the role of these actions in regulating fibroblast phenotype is lacking.

The Provisional Matrix Provides a Conduit for Fibroblast Migration in Repair and in Fibrosis

In tissue repair, matrix degradation during the inflammatory phase is accompanied by extravasation of plasma proteins through hyperpermeable vessels, resulting in the formation of a provisional matrix network that comprises predominantly fibrin (derived from extravasated fibrinogen) and plasma fibronectin [59]. The fibrin-rich provisional matrix is later lysed by proteolytic enzymes, and replaced by a cell-derived “second order”

provisional matrix that contains cellular fibronectin and hyaluronan [60]. Fibronectin and fibrin are also deposited in the extracellular matrix in chronic fibrotic conditions [61]. The provisional matrix plays a critical role in regulating the orchestrated movement of leukocytes, fibroblasts, and vascular cells in healing and fibrotic tissues, serving as a conduit for cell migration [62]. In addition to its effects on fibroblast migration, fibronectin also activates fibroblast proliferation [63] and modulates fibroblast gene expression, acting through pathways involving $\alpha 5\beta 1$ and $\alpha v\beta 3$ integrins, and syndecans [64–67]. Cell adhesion to the central cell-binding domain of fibronectin, and activation of growth factor responses facilitated by fibronectin are essential for fibroblast survival [68].

The Matrix Regulates Myofibroblast Conversion

Modulation of the extracellular matrix critically regulates myofibroblast transdifferentiation, the key step in tissue fibrosis. The ED-A variant of fibronectin, non-fibrillar collagens, and hyaluronan have been implicated in myofibroblast conversion. ED-A fibronectin expression precedes myofibroblast transdifferentiation in healing and fibrotic tissues and is essential for TGF- β 1-mediated myofibroblast conversion [69]. Non-fibrillar collagens (such as collagen VI) have been reported to stimulate myofibroblast transdifferentiation, both in vitro and in vivo [70, 71]. Moreover, deposition of pericellular hyaluronan may also facilitate and maintain myofibroblast transdifferentiation in response to TGF- β [72–74]. Activation of CD44 signaling by hyaluronan may accentuate the TGF- β /Smad signaling cascade [75]. In vitro experiments demonstrated that hyperglycemia-mediated collagen glycation may promote myofibroblast transdifferentiation, suggesting a matrix-dependent pathway that may be involved in diabetes-associated tissue fibrosis [76].

Matricellular Proteins Regulate Fibroblast Phenotype and Function

The term “matricellular protein” was coined to describe a family of structurally unrelated extracellular macromolecules that do not serve a structural role, but bind to matrix proteins, growth factors, and cell surface receptors, transducing signaling cascades [77, 78]. The family includes the thrombospondins (TSPs), SPARC (secreted protein acidic and rich in cysteine), osteopontin (OPN), tenascin-C, periostin, and the members of the CCN (Cyr61/CTGF/NOV) family. Most matricellular proteins are not expressed in normal tissues, but enrich the extracellular matrix following injury and participate in reparative and fibrotic responses.

The prototypical matricellular protein TSP-1 is consistently upregulated in fibrotic tissues [79] and regulates fibroblast function through several distinct mechanisms (Fig. 2b). First, TSP-1 may promote myofibroblast transdifferentiation through activation of TGF- β [80, 81]. TSP-1 binds to the latency-associated peptide (LAP) and promotes the release of the active TGF- β dimer from the latent complex [82]. Second, TSP-1 may exert direct actions on cardiac fibroblasts (possibly mediated through CD36 activation), stimulating an activated matrix-synthetic phenotype [83]. Third, TSP-1 may exert direct matrix-stabilizing actions by inhibiting protease activation [84, 85]. Tenascin-C is also markedly upregulated in injured, healing, and remodeling tissues and may contribute to the pathogenesis of fibrosis through actions on fibroblasts and macrophages [86, 87]. Tenascin-C has broad actions on fibroblast

phenotype, stimulating migration and accelerating myofibroblast conversion [88]. Tenascin-C binds to a wide range of growth factors, including TGF- β s, PDGFs, and fibroblast growth factors (FGFs) [89] and may regulate their actions on cardiac fibroblasts. SPARC expression is also upregulated in fibrotic tissues and has been reported to modulate growth factor-mediated responses [90]. OPN may act both as a cytokine (when secreted in a soluble form), and as a matricellular protein (when bound to the extracellular matrix). A large body of in vitro and in vivo evidence suggests that OPN plays an important role in activation of fibroblasts in several different tissues [91–93]. OPN may act by stimulating fibroblast migration [93], by increasing fibroblast survival [94], and by promoting proliferation [95] through effects involving CD44 and $\alpha\beta$ 3 integrin signaling. Periostin is consistently induced in activated myofibroblasts and critically regulates their matrix-synthetic function [96]. Periostin potently stimulates fibroblast proliferation and myofibroblast persistence [97]; whether its stimulatory actions involve intracellular or matricellular effects remains unknown.

Several members of the CCN matricellular family have also been implicated in the pathogenesis of fibrotic diseases. CCN2 is a fibrogenic mediator that potentiates fibroblast adhesion to fibronectin [98], induces matrix protein synthesis [99], and stimulates fibroblast proliferation [100]. In vivo, CCN2 has been implicated in the pathogenesis of fibrosis in several distinct models of fibrosis [101, 102]. In contrast, CCN1 and CCN5 exert anti-fibrotic actions. In the liver, CCN1 is upregulated following injury and suppresses fibrosis [103], attenuating TGF- β signaling and accentuating apoptosis [104]. CCN5 also reduces fibrosis, attenuating TGF- β /Smad responses [105].

Conclusions

The extracellular matrix actively participates in the pathogenesis of fibrosis. Over the last 10 years, an impressive body of evidence has documented critical effects of matrix macromolecules on fibroblast phenotype and function. Important questions remain to be explored. Are matricellular actions involved in the plasticity of interstitial cells in fibrotic tissues? Do fibroblast subsets have distinct responses to alterations in their matrix environment? Can we modulate the extracellular matrix to restrain fibrosis? Understanding the molecular basis for matrix-dependent actions on fibroblast activation holds therapeutic promise. Identification of specific functional domains responsible for the effects of matrix macromolecules may lead to design of novel therapeutic strategies using peptides that selectively reproduce or inhibit specific matrix-dependent actions.

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Fig. 1. Activated myofibroblasts are the main effector cells in tissue fibrosis. *Left* Immunofluorescent staining for α -smooth muscle actin identifies myofibroblasts in the infarcted heart. These cells are localized in the infarct border zone (*arrows*) and produce large amounts of extracellular matrix proteins. *Right* Myofibroblast conversion is mediated through activation of TGF- β signaling cascades and involves components of the extracellular matrix (ED-A fibronectin, hyaluronan, non-fibrillar collagens, etc.)

Fig. 2.

Alterations in the extracellular matrix network modulate fibroblast phenotype. **a** In a pro-inflammatory environment, collagen (Col), fibronectin (Fn), and low-molecular weight hyaluronan (LMWH) fragments stimulate a matrix-degrading fibroblast phenotype and may induce expression of pro-inflammatory mediators. **b** In fibrotic tissues, the extracellular matrix is enriched with matricellular proteins. These macromolecules do not play a structural role, but bind to growth factors and cell surface receptors and transduce signaling responses. For example, the prototypical matricellular protein thrombospondin (TSP)-1 is deposited into the matrix and modulates fibroblast function by facilitating the release of bioactive TGF- β (through a direct interaction with the latency-associated peptide/LAP), and by modulating growth factor (GF)- and integrin (ITG)-mediated responses. Some of the effects of TSP-1 may be mediated through activation of CD36 signaling