Fibrogenesis
I. New insights into hepatic stellate cell activation: the simple becomes complex

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Eng, Francis J., and Scott L. Friedman. Fibrogenesis. I. New insights into hepatic stellate cell activation: the simple becomes complex. Am J Physiol Gastrointest Liver Physiol 279: G7–G11, 2000.—Hepatic stellate cell activation is a complex process. Paradoxes and controversies include the origin(s) of hepatic stellate cells, the regulation of membrane receptor signaling and transcription, and the fate of the cells once liver injury resolves. Major themes have emerged, including the dominance of autocrine signaling and the identification of counterregulatory stimuli that oppose key features of activated cells. Advances in analytical methods including proteomics and gene array, coupled with powerful bioinformatic methods, promise to revolutionize how we view cellular responses. Our understanding of stellate cell activation is likely to benefit from these advances, unearthing modes of regulating cellular behavior that are not even conceivable on the basis of current paradigms.

signaling; transcription; extracellular matrix; receptors; hepatic fibrosis

ACTIVATED HEPATIC STELLATE cells (HSCs) have been established as the unequivocal source of extracellular matrix in liver injury, regardless of the underlying disease (see Ref. 6 for review). The initial, simple paradigm of stellate cell activation first envisioned a one-way pathway from cellular quiescence to activation in early injury, with upregulation of key genes and mediators. But things are not so simple. As our understanding of stellate cell activation has advanced, subtle complexities have begun to emerge with respect to cell lineage, membrane and intracellular signaling, and transcriptional regulation of gene expression. Simple paradigms have yielded to more complex and sometimes contradictory modes of regulation in this cell’s response to liver injury. This themes article will highlight recent insights into this fascinating cell type, with particular emphasis on paradoxes and controversies as the biology of stellate cell activation continues to unfold. For more comprehensive, conventional reviews the reader is referred to several recent citations (6, 16, 23).

WHERE DO ACTIVATED STELLATE CELLS COME FROM AND WHERE DO THEY GO?

The original view that all stellate cells are simply desmin-positive cells with perinuclear vitamin A droplets is no longer tenable. Evidence has mounted that a remarkably diverse population of mesenchymal cells exists in both normal and injured liver, with differing content of vitamin A and variable expression of intermediate filaments characteristic of myogenic and/or neural crest cells. In particular, a growing list of neural markers has been identified in stellate cells, including RhoN, glial fibrillary acidic protein, nestin, and neurotrophin receptors. As a result of this heterogeneity, it is uncertain whether all the liver’s mesenchymal cells derive from the same embryonic source. It is a semantic issue as to whether they all should be termed “stellate cells.” A more important question, however, is whether all harbor the same capacity to undergo either activation or apoptosis. For example, an outgrowth from primary rat stellate cells was recently characterized that was relatively resistant to apoptosis and expressed some genes that were not found in early stellate cell cultures such as the matrix glycoprotein fibulin-2 (14). Such fibulin-2 cells can also be found in vivo, but we do not know to what extent this subpopulation comprises the source of extracellular matrix in liver injury. Nonetheless, it seems likely that a greater appreciation for the functional heterogeneity of stellate cells will develop, much as we already appreciate their morphological and cytoskeletal heterogeneity.

Even more intriguing is the recognition that cellular plasticity may not be confined within the mesenchymal lineage but, rather, “transdifferentiation,” or conversion from one cell lineage to another, may be possible in adult tissues. For example, in adult kidney a population of interstitial fibroblasts can develop from epithe-
cellular cells under the influence of fibroblast-specific protein-1 (29). Advances such as this one documenting the pluri-potentiality of adult cell types force us to reexamine our notions of cell lineage commitment and could lead to fundamental new therapeutic approaches to cellular reconstitution in liver. Progress is also likely to accelerate as gene array techniques are applied to stellate cell systems to characterize broad changes in gene expression profiles, complemented by the impending sequencing of the entire human genome.

Neither the half-life of stellate cells in normal or injured liver nor their ultimate fate has been established with certainty. If liver injury persists, they might be replaced by other activated cells or perhaps by quiescent cells that have not yet activated. If liver injury resolves, there must be mechanisms whereby the number of activated, fibrogenic cells is diminished. Apoptosis is one potential way in which activated cells are cleared during resolution of liver injury (11); another is reversion to a quiescent phenotype. It is uncertain how either of these fates is determined; perhaps there is a “point of no return,” where an activated cell can no longer be reverted to a quiescent state and instead must undergo apoptosis as injury resolves. If so, the signals marking this event could have major importance in devising new antifibrotic therapies.

**MEMBRANE RECEPTORS DURING STELLATE CELL ACTIVATION—UP, DOWN, AND ALL AROUND**

An emphasis on autocrine signaling has helped clarify our understanding of ligand receptor interactions in activated stellate cells. Examples where both ligand and receptor are expressed locally include platelet-derived growth factor (PDGF), endothelin-1 (ET-1), fibroblast growth factor, vascular endothelial growth factor (VEGF), and transforming growth factor-β1 (TGF-β1). For example, injury is associated with both upregulation of the PDGF receptor and increased autocrine PDGF production (23). Activated PDGF receptor recruits the signaling molecule Ras, followed by activation of the extracellular signal-related kinase/mitogen-activated protein kinase (ERK/MAPK) pathway and of focal adhesion kinase (FAK) (4, 19). Additionally, activation of phosphoinositol 3-kinase is necessary for both mitogenesis and chemotaxis by pathways largely independent of ERK activation (21).

Receptors previously thought to be confined to sinusoidal endothelial cells have now been identified on activated stellate cells as well. For example, two VEGF receptors, Flt-1 and Flk-1, are upregulated after injury in both sinusoidal endothelial cells and stellate cells (2). Stimulation of these receptor tyrosine kinases induces cell proliferation and inhibits stellate cell contraction (22). In hypoxic conditions, both VEGF and Flt-1 mRNAs are rapidly induced in stellate cells, establishing an autocrine and paracrine loop supporting angiogenesis (3).

In some cases, receptors are not upregulated during activation but their subtypes change, with important functional consequences. For example, two G protein-coupled ET-1 receptors (ET<sub>A</sub> and ET<sub>B</sub>) are expressed by both quiescent and activated stellate cells. However, the amount and relative prevalence of these receptors changes with cellular activation, and each mediates divergent responses (24). In cell culture, ET-1 stimulates proliferation of quiescent stellate cells but is growth inhibitory toward activated cells. Proliferative responses in quiescent cells are attributed to the ET<sub>A</sub> receptor and correlate with activation of MAPK and a rapid increase in intracellular Ca<sup>2+</sup> (24). In contrast, growth inhibition by ET-1 toward activated stellate cells is mediated by the ET<sub>B</sub> receptor and correlates with increased prostaglandin (PG) synthesis and a rise in intracellular cAMP levels, which reduces activation of ETB receptor and correlates with activation of MAPK and an increase in intracellular Ca<sup>2+</sup> (24).

Signals that inhibit activation are increasingly recognized even in early stellate cell activation. These may provide an important “brake” on the cascade of activation, limiting the cellular response to an injury if it is brief and self-limited. For example, although mitogen stimulation with either PDGF or thrombin on activated human stellate cells leads to increased cell proliferation, it also elevates PG and cAMP that in turn upregulates ET<sub>B</sub> receptors. Thus the overall proliferative effect of PDGF on activated stellate cells in vitro is counterbalanced by the opposing growth inhibitory effects of ET<sub>B</sub> receptor stimulation (17). A recent in vivo study demonstrated that treatment of cirrhotic rats with a mixed ET<sub>AB</sub> receptor antagonist led to increased collagen deposition (25). This finding supports the concept that the ET<sub>B</sub> receptor may play a role in an autocrine loop that counteracts fibrogenesis through its growth-inhibitory effects. It has not yet been determined whether specific ET<sub>A</sub> receptor antagonists modulate fibrosis.

The regulation of vascular tone by hepatic stellate cells reflects the balance between contractility and vasodilation. Several receptors mediating contractility have been identified recently in addition to endothelin receptors. These include receptors for arginine vasopressin, angiotensin II, thrombin, and thromboxane. Counteracting these contractile stimuli is not only the well-characterized vasodilator nitric oxide but also two natriuretic peptide receptors, NPR-A and NPR-B, that bind the circulating vasodilators atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) (9, 30). Both are guanylate cyclase receptors that, on stimulation, lead to an increase in the intracellular second messenger cGMP. Activation of either receptor blunts the contractile responses elicited by ET-1 or thrombin. This relaxation correlates with diminished influx of intracellular Ca<sup>2+</sup>. CNP also inhibits stellate cell growth and is associated with a reduction of ERK and JNK activity and diminished DNA binding by the transcription factor AP-1. This suggests that CNP activation of NPR-B receptors may counteract both fibrogenesis and contractile stimuli that lead to portal hypertension.
Stellate cell receptors signal in response to more than cytokines. Signals from the extracellular matrix also play a critical role in cellular quiescence and activation. Integrins, heterodimeric proteins consisting of an α- and a β-chain, interact with extracellular matrix molecules; their specificity is defined by their subunit composition. Several integrins and their downstream effectors have been identified in stellate cells, including α1β1, α2β1, α4β1, and α6β4 (23).

In addition to the classic integrin matrix receptors, a new paradigm has been uncovered in which a tyrosine kinase receptor mediates stellate cell interactions with the extracellular matrix. The discoid domain receptor 2 (DDR2) has been cloned from activated stellate cells and interacts with fibrillar collagens (2, 28). The identification of DDR2 mRNA and protein in activated stellate cells suggests that it may be a key receptor in hepatic fibrosis. Because fibrillar collagens are produced by activated stellate cells, the DDR2 receptor signals in response to an autocrine stimulus.

There are likely to be still more extracellular matrix receptors discovered with important roles in stellate cell quiescence and activation. For example, a splice variant of fibronectin containing the EIIIA region activates stellate cells in early liver injury (12), but its receptor has not been characterized. Identification of a putative EIIIA Fn receptor could provide a key target for antifibrotic therapy.

Stellate cells also express receptors that modulate the local inflammatory milieu. They express cell adhesion molecules (CAM) that recruit immune cells during tissue repair. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are rapidly induced after CCl4 administration in rodents, which precedes mononuclear cell infiltration (13). The inflammatory cytokine tumor necrosis factor-α (TNF-α) induces ICAM-1 and VCAM-1 expression severalfold, whereas TGF-β1 reduces their expression. The recruitment of inflammatory cells that express ICAM-1 and VCAM-1 ligand may have two important consequences, 1) neutralization of toxic stimuli and 2) paracrine stimulation of stellate cell activation and fibrogenesis.

**TRANSCRIPTION FACTORS—THE KEYS TO THE KINGDOM?**

Control of gene regulation by transcription is an essential mode of determining cell fate and activity. To date, the major insights into stellate cell biology have been in understanding the production of extracellular matrix and the roles of cytokines and their receptors. Yet it is the complex system of tissue-specific gene expression that holds the key to biological activity in virtually all mammalian systems. Thus far, however, no unique modes of gene regulation have been uncovered in stellate cells. Instead, progress in understanding transcriptional regulation in this cell type has been marked by incremental advances and by revealing subtle variations of regulatory mechanisms already unearthed in other cell types.

Nuclear factor-κB (NF-κB) has been the most extensively studied transcription factor to date in stellate cells, owing to the variety of tools available to inhibit its activity and the rapid advances in understanding this factor in other cell types. The complexity of its regulation provides a cautionary tale for trying to develop simplistic paradigms to understand transcription in stellate cells. The NF-κB family of transcription factors are related by their Rel homology domain (RHD) and comprise at least five members, including p65 (RelA), p50, p52, RelB, and c-Rel, that form homo- or heterodimers that bind to DNA. In most cell types, NF-κB is found in the cytoplasm as an inactive dimer bound to one of the IκB inhibitory proteins (IκBα or IκBβ) that mask its nuclear localization signal (NLS). Phosphorylation of IκBα leads to its ubiquitination and degradation by the proteasome. The active NF-κB dimer then translocates into the nucleus. Reagents that block IκBα degradation have been used to inhibit NF-κB activity to study its functional role.

The NF-κB dimers p65/p50 and p50/p55 have been identified in stellate cells. Recently, an NF-κB DNA binding complex consisting of potentially novel Rel-like factors has been detected (5). This complex is maintained at basal levels by an autocrine-derived factor from activated cells and is upregulated by TNF-α. The physiological significance of these different NF-κB dimers is not yet clear. IκBα expression is reduced during cellular activation consistent with an increase NF-κB activity. IκBβ expression is transiently reduced but later replenished with a putative hypophosphorylated form that, rather than inhibiting NF-κB activity, shields it from IκBα interaction. Additionally, Bcl 3, a member of the IκB family that like IκBβ can function as a positive regulator, is also upregulated in activated cells. Thus both IκBβ and Bcl 3 may sustain the basal activation of NF-κB.

NF-κB activity can be further induced by cytokines in activated but not quiescent HSCs. Induction by TNF-α and interleukin (IL)-1β leads to the expression of ICAM-1, IL-6, and macrophage inflammatory protein-2 (MIP-2) in rats (10). In human HSCs, activation of NF-κB by ET-1 or TNF-α leads to increased COX-2, which blocks cell proliferation (8). NF-κB activity also protects HSCs and other cell types against apoptosis through as yet uncertain mechanisms. Finally, NF-κB activation by TNF-α also inhibits expression of the α1(I) collagen gene (27). Collectively, these findings document a persistent level of NF-κB maintained by an autocrine factor in activated HSCs that can be further upregulated by cytokines. Thus extracellular factors determine the state of NF-κB activation, the extent of which dictates the physiological response.

Exciting advances have been made in understanding peroxisome proliferator-activated receptors (PPARs), a family of transcription factors belonging to the nuclear receptor superfamily. For example, PPAR-γ is predominantly expressed in adipose tissue, where it regulates lipid metabolism and adipocyte differentiation. Yet PPAR-γ is also expressed in human HSCs, and its activity is reduced during activation in culture (7).
PPAR-γ functions as a heterodimer with another nuclear receptor, 9-cis retinoic acid (9-cisRA) receptor (RXR). PPAR-γ ligands 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2) and ciglitizone decrease PDGF-induced proliferation of activated HSCs and inhibit α-smooth muscle actin expression during HSC activation (20). This suggests that reduced transcriptional activity of PPAR-γ might augment HSC activation and modulate mitogen-induced proliferation in activated cells. Furthermore, PGs produced by stellate cells through the upregulation of COX-2 expression may exert autocrine effects through PPAR-γ.

We have recently cloned a novel Krüppel-like factor (KLF), Zf9/COPEB/GBF (recently renamed KLF6), as an immediate-early gene induced in stellate cells after acute liver injury (26). This zinc finger transcription factor has a number of potential transcriptional targets including collagen α1(1), TGF-β1 and its receptors, as well as urokinase type plasminogen activator (uPA). Most recently, however, an antiproliferative activity of this factor has been described (15). Why would a gene upregulated in the midst of stellate cell activation inhibit proliferation? Is this another example of a counterregulatory “brake” to limit the extent of activation? The answer is not clear, but this paradox further underscores the complexity of gene regulation in stellate cell activation and hints at the challenge in dissecting modes of cellular regulation.

WHITHER THE FUTURE?

As the complexity of stellate cell activation becomes increasingly apparent, the tools available to clarify its regulation have also advanced. With the availability of proteomics, gene array, and bioinformatics analyses, biological processes such as stellate cell activation need no longer be viewed as isolated, parallel pathways but rather as highly integrated events in which many genes are regulated and proteins are modified simultaneously and interdependently. Gene array technology has uncovered patterns of disease in which hundreds of genes are jointly regulated, for example, in patients with subtypes of B cell lymphoma (1). Approaches like this promise to revolutionize the way we view cellular responses and to create new paradigms of regulation that are not even conceivable based on current knowledge. Those who study stellate cell activation are likely to be major beneficiaries of such advances in the coming years.

REFERENCES


