Combatting Fibrosis: Exosome-Based Therapies in the Regression of Liver Fibrosis

Li Chen,[1] David A. Brenner,[1] and Tatiana Kisseleva[2]

Hepatic fibrosis results from chronic injury and inflammation in the liver and leads to cirrhosis, liver failure, and portal hypertension. Understanding the molecular mechanisms underlying hepatic fibrosis has advanced the prospect of developing therapies for regression of the disease. Resolution of fibrosis requires a reduction of proinflammatory and fibrogenic cytokines, a decrease in extracellular matrix (ECM) protein production, an increase in collagenase activity, and finally, a disappearance of activated myofibroblasts. Exosomes are nanovesicles of endocytic origin secreted by most cell types. They epigenetically reprogram and alter the phenotype of their recipient cells and hold great promise for the reversal of fibrosis. Recent studies have shown that exosomes function as conduits for intercellular transfer and contain all the necessary components to induce resolution of fibrosis, including the ability to (1) inhibit macrophage activation and cytokine secretion, (2) remodel ECM production and decrease fibrous scars, and (3) inactivate hepatic stellate cells, a major myofibroblast population. Here, we discuss the research involving the regression of hepatic fibrosis. We focus on the newly discovered roles of exosomes during fibrogenesis and as a therapy for fibrosis reversal. We also emphasize the novel discoveries of exosome-based antifibrotic treatments *in vitro* and *in vivo*. (*Hepatology Communications* 2019;3:180-192).

Hepatic fibrosis is caused by the excessive production and accumulation of insoluble collagen and extracellular matrix (ECM) components following sustained chronic injury in the liver. Various chronic liver diseases, such as hepatitis B virus, hepatitis C virus, alcoholic liver disease, and nonalcoholic steatohepatitis (NASH), result in fibrosis.[1] If the death rate from cirrhosis continues to increase as projected, cirrhosis will become the twelfth leading cause of death by 2020.[2]

Essential mechanisms have been identified for the circuitous nature of the pathogenesis and resolution of hepatic fibrosis due to chronic liver disease. Transforming growth factor β (TGF-β) is a central regulator in chronic liver disease; it contributes to all stages of disease progression from initial liver injury through inflammation and fibrosis.[3] Liver damage-induced levels of active TGF-β stimulate an increase in expression levels of many growth factors and cytokines involved in fibrogenesis, including platelet-derived

Abbreviations: α-SMA, α-smooth muscle actin; CCN2, connective tissue growth factor; CD, clusters of differentiation; Colα1(I), collagen α1(I); DC, dendritic cell; ECM, extracellular matrix; Grp78, glucose-regulated protein 78; HSC, hepatic stellate cell; HSP, heat shock protein; IL, interleukin; LOXL2, lysyl oxidase-like 2; miRNA or miR, microRNA; MMP, matrix metalloproteinase; mRNA, messenger RNA; MSC, mesenchymal stem cell; MT1, membrane-type 1; mtPmp70, mitochondria peroxisomal membrane protein 70 kDa; NK, natural killer; PAMP, pathogen-associated molecular pattern; PDGF, platelet-derived growth factor; PF, portal fibroblast; Pmp70, peroxisomal membrane protein 70 kDa; STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor β; TIMP, tissue inhibitors of metalloproteinase; TNF-α, tumor necrosis factor α; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

Received August 7, 2018; accepted October 24, 2018.

Supported by the National Institutes of Health (awards R01 DK099205-01A1 to T.K. and P50AA011999 to D.A.B.

View this article online at wileyonlinelibrary.com

DOI 10.1002/hep4.1290

Potential conflict of interest: Nothing to report.

growth factor (PDGF), connective tissue growth factor (CCN2), interleukins (ILs [IL-1α, IL-β, and IL-6]), and tumor necrosis factor α (TNF-α).[4-8] Increased levels of active TGF-β enhance hepatocyte destruction and mediate hepatic stellate cell (HSC) and fibroblast activation, resulting in a wound-healing response that includes myofibroblast generation and ECM deposition.[3] Overexpression of CCN2 in concert with signaling pathways associated with development of liver fibrosing injury can lead to the initiation or exacerbation of fibrosis.[8] IL-1β exerts a stimulatory effect on the synthesis of ECMs,[9] IL-6 induces hepatic inflammation and collagen synthesis,[10] and TNF-α is required for cholestasis-induced liver fibrosis.[11]

A key event during liver fibrosis is the activation of myofibroblasts, which originate from fibroblasts, including HSCs, portal fibroblasts (PFs), and fibrocytes. Depending on the ECM composition, fibroblasts maintain quiescence or activate into myofibroblasts.[12] Due to chronic insult, fibroblasts subjected to extracellular stress caused by abnormal ECM (e.g., fibronectin, collagen type I and III) proliferate and obtain a myofibroblast-like phenotype. Activated myofibroblasts secrete ECM and form stress fiber-induced cell-matrix junctions, which further facilitate ECM remodeling. Excessive ECM deposition and significant changes in topographic distribution of ECM components increase expression of tissue inhibitors of metalloproteinases (TIMPs).[13] Following resolution of the injury, liver fibrosis can be reversed after the withdrawal of the underlying cause of disease. This is associated with a significant reduction of myofibroblasts due to apoptosis, induction of senescence and killing apoptosis of senescent HSCs by natural killer (NK) cells, or phenotypic reversion to the quiescent-like phenotype. Meanwhile, a reduction in collagen production as well as decreased TIMP-1 expression and an increase in hepatic collagenase and elastase activity result in ECM degradation and remodeling.[14-18]

Over the past 3 decades, the drive to discover the mechanisms underlying the critical events during fibrogenesis has been fundamentally relevant to the development of antifibrotic strategies. However, even with this push to understand the mechanisms of disease progression, there are currently no antifibrotic treatments. Therefore, the need to continue to uncover the mechanisms of fibrogenesis and discover potential targets for treatment is essential in drug development.

Exosomes are cell-derived vesicles that are present in eukaryotic fluids. They are either released directly from the plasma membrane or from the cell when multivesicular bodies fuse with the plasma membrane. They contain proteins and other molecules that reflect the transcriptional and/or translational activity of the cell of origin.[19] The differential contents of RNAs, proteins, lipids, and metabolites in exosomes are distinct to the cell type of origin. Following their release into the intercellular space, exosomes bind to recipient cells and deliver their informative cargo. The recipient cells may then undergo epigenetic reprogramming and subsequent phenotypic alterations according to the molecular information received. The presence of specific components (protein, microRNA [miRNA or miR], or messenger RNA [mRNA]) in different types of exosomes results in different functional properties in the recipient cells. For instance, lipotoxic

ARTICLE INFORMATION:

From the [1]Department of Medicine; [2]Department of Surgery, University of California San Diego, La Jolla, CA.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Tatiana Kisseleva, M.D., Ph.D.
Department of Surgery, University of California San Diego
5217 BRFII
9500 Gilman Drive
La Jolla, CA 92093-0063
E-mail: tkisseleva@mail.ucsd.edu
Tel.: +1-858-822-5339
or

Li Chen, Ph.D.
Department of Medicine, University of California San Diego
5217 BRFII
9500 Gilman Drive
La Jolla, CA 92093-0063
E-mail: l3chen@ucsd.edu
Tel.: +1-858-822-5339

fatty acid-injured hepatocytes produce exosome-like vesicles, which are then taken up by HSCs, leading to fibrogenic activation.[20,21] Additionally, the use of human mesenchymal stem cell (MSC)-derived exosomes allows an MSC-like therapeutic payload to be delivered to the liver, followed by a subsequent reduction of liver fibrosis, thus protecting hepatocytes.[22] Further, fibrogenic signaling in HSCs is suppressed by exosomes shuttled between quiescent and activated HSCs.[23-25]

This review covers some of the most important functions of different exosomes during liver fibrogenesis and the regression of liver fibrosis. We emphasize both the established mechanism of regression of liver fibrosis and the new developments in novel exosome-based antifibrotic strategies. We also highlight the emerging consensus about rodent models of fibrosis regression, which demonstrate a return of normal or near-normal liver histology and function.

Exosomes in Liver Fibrosis

THE PATHOGENESIS OF EPITHELIAL INJURY

Recurrent epithelial injury is a prominent driving factor in the pathogenesis of progressive fibrosis[26] and results in hepatocyte dysfunction, which can occur through apoptosis.[26,27] Hepatocytes, in response to the hostile environment, undergo apoptosis through an extrinsic death receptor-mediated pathway, or alternatively, intracellular stress can activate the intrinsic pathway of apoptosis. Both pathways target the mitochondria, and mitochondrial dysfunction is a prerequisite for hepatocyte apoptosis.[28]

Hepatocytes can produce exosomes that contain caveolae (Caveolin-1), early endosome (Eaa1), endoplasmic reticulum (glucose-regulated protein 78 [Grp78]), peroxisome (peroxisomal membrane protein 70 kDa [Pmp70]), or mitochondria (Prohibitin1 and mtPmp70),[29] and these exosomes are able to communicate with other hepatocytes or other cell types throughout the body. Interestingly, the proteins that are found in hepatic-derived exosomes have been demonstrated to play a role in metabolizing lipoproteins, endogenous compounds, and xenobiotics. Further, exosomes derived from injured hepatocytes are enriched with cytochrome P450s that serve important roles in the cellular detoxification of endogenous toxic substances.[29] Cytochrome P450 2E1 (CYP2E1) generates reactive oxygen species that can produce superoxide anion radicals, hydrogen peroxide, and powerful oxidants, such as the hydroxyl radical, in the presence of iron catalysts. Elevated levels of CYP2E1 under a variety of pathophysiologic conditions lead to hepatic apoptosis through mechanisms of oxidative stress.[30] Therefore, it is speculated that injured hepatocyte-derived exosomes containing P450s participate in the development of steatosis, increased fibronectin expression, and hepatocyte apoptosis.

In response to lipid injury, hepatocytes release exosome-like vesicles containing tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and clusters of differentiation (CD)40 ligand, inducing the production of inflammatory-type macrophages.[31] Hepatocytes injured by lipotoxic fatty acids produce exosome-like vesicles enriched in miR17-92 clusters, which are taken up by HSCs, leading to fibrogenic activation.[20,21]

The exosomes derived from CCl_4-treated hepatocytes include diverse types of self-RNAs and recognize an activator of toll-like receptor 3, which increases the production of IL-17A production in hepatic γδ T cells. The increased levels of proinflammatory cytokines are tightly associated with HSC activation.[32] In agreement, Il-17-produced T cells regulate production of TGF-β1 in Kupffer cells and can directly activate collagen type I production by HSCs, the major source of fibrogenic myofibroblasts in fibrotic liver.[33]

Exosomes released from epithelial cells carry information that can activate fibroblasts and initiate and perpetuate fibrosis. For example, injured epithelial cells produce increased numbers of exosomes containing information sufficient to activate fibroblasts. When released by injured epithelial cells, these exosomes are taken up by neighboring fibroblasts, resulting in their increased production of α-smooth muscle actin (α-SMA) and type I collagen, thus driving liver fibrosis.[34]

EXCESSIVE DEPOSITION OF ECM DURING DEVELOPMENT OF LIVER FIBROSIS

The ECM represents a noncellular component in the liver that is mainly composed of proteins and proteoglycans.[35] It forms an intricate network that provides a physical scaffold for cellular support

while allowing unimpeded transport of solutes and growth factors.[36] The ECM undergoes continuous remodeling, particularly during injury and wound healing.[37] In response to chronic liver injury, the secretion of types of ECM proteins alters dramatically, resulting in abundant production of type I and III collagens, increased deposition of fibronectin and proteoglycans, as well as other subtypes of collagens.[36] During this process, lysyl oxidase-like 2 (LOXL2) facilitates crosslinking of collagens and elastin by catalyzing oxidative deamination of lysine residues.[38] As a consequence, tissue stiffness is also increased. This heavily crosslinked collagen network replaces normal tissue structure and results in a change in the phenotype of normal resident cells as well as pathologic myofibroblasts.

Exosomes play an important role in ECM crosslinking, thus affecting processes such as angiogenesis, fibroblast activation, and premetastatic niche formation.[39] LOXL2 has been detected on the exterior of endothelial cell-derived exosomes, placing it in the direct vicinity of the ECM. Increased LOXL2 levels in both endothelial cells and endothelial cell-derived exosomes enhance the activity of collagen gel contraction. However, knockdown of LOXL2 in exosome-producing endothelial cells in both normal and hypoxic conditions reduces exosome activity.[40] Thus, ECM crosslinking by endothelial cell-derived exosomes is mediated by LOXL2.

THE ORIGIN OF MYOFIBROBLASTS IN FIBROTIC LIVER

Hepatic fibrosis is accompanied by the accumulation of increased numbers of myofibroblasts in the liver.[41,42] These myofibroblasts are the source of ECM components necessary for building the fibrous scar tissue surrounding the wound. The production of these stress fibers makes myofibroblasts highly contractible and mobile, enabling their migration throughout the injured tissue and their further secretion of ECM components. Therefore, activation of myofibroblasts is a key mechanism in the development of liver fibrosis.

The origin of myofibroblasts has been well studied. HSCs are the major source of myofibroblasts.[1] Hepatic myofibroblasts also originate from PFs and fibrocytes.[43] Despite earlier studies,[44,45] recent reports have demonstrated that myofibroblasts do not originate from epithelial cells undergoing an epithelial-to-mesenchymal transition.[46,47]

HSCs

HSCs are perisinusoidal cells that reside between the hepatocytes and small blood vessels in the liver. They are characterized by the presence of numerous retinoid and lipid droplets[48,49] where they store vitamin A.[50] A critical feature of the wound-healing response during liver injury is the differentiation of HSCs from a "quiescent" state in the normal liver to an "activated" state in the injured liver.[51] This transition is characterized by both morphologic and functional changes, including down-regulation of vitamin A expression; production of α-SMA, which confers contractility and promotes wound closure; and ECM synthesis.[51,52]

TGF-β is a potent cytokine that activates HSCs into myofibroblasts followed by increased expression of α-SMA, PDGF, CCN2, type I collagen, and TIMP1,[53-55] all of which result in a wound-healing response, including myofibroblast generation and ECM deposition.[3] CCN2, a fibrogenic molecule synthesized downstream of TGF-β, is tightly associated with fibrogenic pathways in activated HSCs.[56] It has recently been found that activated HSC-derived exosomes contain CCN2 or CCN2 mRNA, each of which increases in concentration during HSC activation and amplified fibrogenic signaling.[57] The induction of CCN2 expression in activated HSCs is due to decreased expression of miR-214, which otherwise inhibits CCN2 expression by directly binding to the CCN2 3′-untranslated region.[23,57] Further, miR-214 can be exported from HSCs through exosomes to neighboring cells, leading to regulation of miR-214 target genes.[23] The dynamic expression of miR-214 in HSCs is the result of its transcriptional regulation by Twist-1, which is also exosomally transferred between HSCs where it maintains its ability to induce miR-214 in recipient cells.[25] Thus, a Twist1–miR-214–CCN2 axis is exosomally shuttled to activate additional HSCs in which fibrogenic signaling is then modulated.

Fibrocytes

Although HSCs are believed to be a major source of myofibroblasts (which produce collagen type I in the fibrotic liver), bone marrow-derived fibrocytes,

as defined by their simultaneous expression of CD45 and collagen type I, are also a potential source of myofibroblasts and are implicated in the pathogenesis of liver fibrosis.[58] Fibrocytes also express CD34 and major histocompatibility complex II and secrete TGF-β, promoting the deposition of ECM.[59,60]

Exosomes released from fibrocytes have a concentration-dependent proangiogenic activity. The recipient cells of fibrocyte-derived exosomes demonstrated a dose-dependent increase in the expression of collagen α1(I) [Colα1(I)] and α-SMA.[61] Heat shock protein (HSP)-90a and total activated signal transducer and activator of transcription 3 (STAT3) are important components of the fibrocyte exosome cargo. Fibrocyte-derived exosomes are also enriched with miR-21, miR-142a, miR-125b, miR-126, miR-130a, and miR-132, all of which work in tandem to modulate collagen production, resulting in enhanced deposition of mature collagen fibrils in the wound and promotion of wound contraction at an early stage in the wound-healing process.[62]

PFs

PFs normally comprise a small population of the fibroblastic cells that surround the portal vein to maintain integrity of the portal tract. They are associated with the pathogenesis of cholestatic liver injury.[63] It has been demonstrated that PFs are a major source of myofibroblasts in cholestatic liver injury, contributing to greater than 70% of myofibroblasts at the onset of injury (5 days after bile duct ligation).[64] PFs respond rapidly to TGF-β1, as demonstrated by up-regulation of Colα1(I), α-SMA, TIMP1, TGF-β2, plasminogen activator inhibitor 1, elastin, fibronectin, and CD73 ecto-enzyme.[65-68] However, unlike HSCs, PFs respond to stimulation with taurocholic acid and IL-25, leading to an induction of Colα1(I) and IL-13, respectively.[64]

Although PFs play a critical role in the pathogenesis of cholestatic liver fibrosis, functional properties of PFs and the mechanism by which PFs contribute to cholestatic fibrosis are not well understood. Additionally, exosomes originated from PFs have not yet been reported.

MACROPHAGES AND IMMUNE CELLS

Chronic inflammation and fibrosis are inextricably linked through the interactions among immune cells.[69-72] Macrophages play an important role in inflammation and subsequent fibrogenesis.[73-77] To promote fibrosis, macrophages produce specific matrix metalloproteinases (MMPs), such as MMP9, that degrade the basement membrane and allow inflammatory cells and recruited fibroblasts to enter sites of injury. They secrete a variety of profibrotic mediators, including TGF-β1, PDGF, and many chemokines, that recruit and activate inflammatory cells.[78,79] Macrophages are also tightly associated with collagen-producing myofibroblasts *in vivo* and produce cytokines and growth factors that modulate myofibroblast activity. Various types of macrophages, such as M1 (inflammatory), M2a-like (profibrotic), and $M_{regulatory}$/M2c-like (regulatory),[73,75-77,80,81] are recruited during fibrogenesis, resulting in re-epithelization, healing, or pathologic scarring. In contrast, macrophages also play a distinct role in the resolution of fibrosis. Macrophages may activate additional stem cell and local progenitor cell populations that participate in repair; thus, macrophages that exhibit an anti-inflammatory phenotype become the dominant population.[16] These macrophages respond to IL-10 and other inhibitory mediators and secrete a variety of anti-inflammatory mediators, such as IL-10 and TGF-β1, that play major roles in suppressing the immune system and quieting the inflammation.[82-85] Macrophages can also induce myofibroblast apoptosis, remove cellular debris, and stimulate the production of collagen-degrading MMPs in myofibroblasts.[86] Therefore, different phenotypes of macrophages play unique and crucial roles at different stages of tissue repair.

Macrophages can reportedly release exosomes, which contain pathogen-associated molecular patterns (PAMPs), that lead to the activation of naive recipient immune cells.[87] Moreover, these macrophage exosomes can be actively endocytosed into placenta tissue and drive cytokine release.[87] Thus, the macrophage-immune cell exosome pathway represents a novel non–cell-associated mechanism of antigen transfer between immune cells; this can exert varying effects on naive cells. The uptake of macrophage-derived exosomes into neighboring immune cells could then result in immunomodulation and alteration of subsequent inflammatory stimuli. However, functional properties of macrophage exosomes in the resolution of fibrosis have not been reported, although it is clear that macrophages exhibit an important role in the mechanisms of fibrosis regression.

NK cells and NKT cells provide the initial defense, invading infectious microbes and neoplastic cells.[88] Dendritic cells (DCs) are central to the processes that modulate liver immunity,[89] whereas regulation of T cells mediates immune tolerance.[90] The role of B cells in the pathogenesis of fibrosis was identified by the reduction in collagen deposition observed in CCl_4-induced fibrosis in B-cell-deficient mice.[72] It has been shown that DC-derived exosome-like vesicles can enhance the antigen-specific responses of $CD4^+$ and $CD8^+$ T cells and participate in the activation of NK cells.[91] Exosomes from IL-10-treated DCs suppressed inflammation and collagen-induced arthritis in mice.[92] In addition, miRNAs released from T-cell exosomes are transferred into DCs in an antigen-specific manner.[93] However, the mechanisms of immune cell-derived exosomes during liver fibrogenesis are still under investigation. The actions and roles of exosomes in liver fibrosis are summarized in Figure 1 and Table 1.

Exosomes in the Regression of Hepatic Fibrosis

Hepatic fibrosis was considered to be irreversible.[94] However, numerous studies have demonstrated that regression of hepatic fibrosis is possible and is dependent on an increase in collagenase activity, a disappearance of activated myofibroblasts, and a suppression of proinflammatory and fibrogenic cytokines, subsequently resulting in a decrease in ECM production and increased ECM degradation.

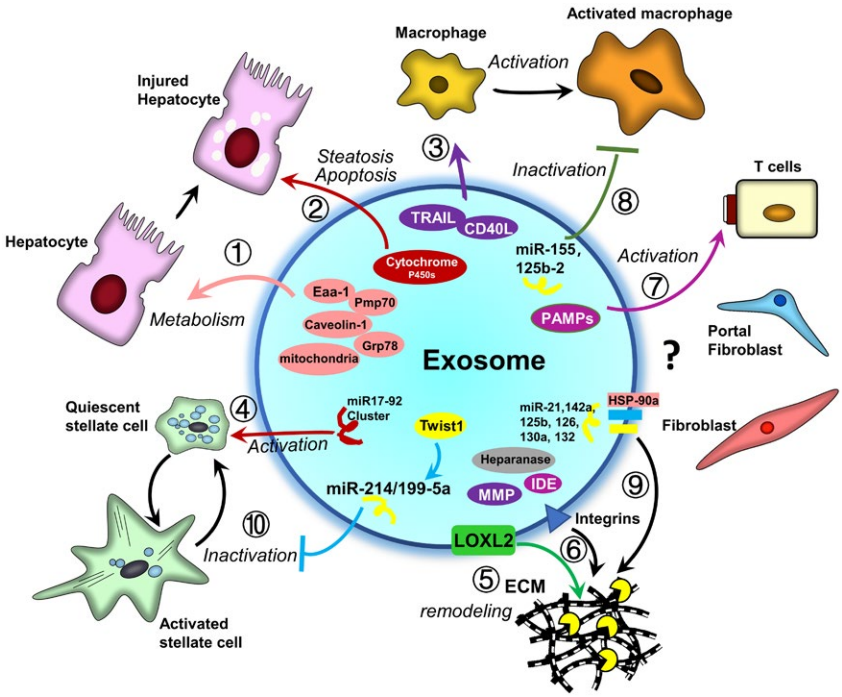

FIG. 1. Exosomes regulate cell functions. 1, Hepatocytes produce exosomes enriched in Caveolin-1, early endosome (Eaa-1), endoplasmic reticulum (Grp78), peroxisome (Pmp70), and mitochondria (Prohibitin 1 and mtPmp70), participating in hepatocyte metabolism. 2, Injured hepatocytes enriched in cytochrome P450s promote hepatocyte steatosis and apoptosis. 3, Lipid-induced injury of hepatocytes enriched in TRAIL and CD40 ligand promote activation of macrophages and HSCs. 4, Injured hepatocytes by lipotoxic fatty acids produce exosomes enriched in miR17-92 clusters, promoting HSC activation. 5, Endothelial cells release exosomes enriched in LOXL2, enhancing the activity of collagen contraction. 6, Fibrocytes release exosomes enriched in HSP-90a, activated STAT3, and miRs (21, 142a, 125b, 126, 130a, and 132), participating in ECM remodeling. 7, Activated macrophages produce exosomes enriched in PAMPs, leading to the activation of naive recipient immune cells. 8, miR155- and miR125b-enriched exosomes promote differentiation of M1 macrophages over M2 macrophages. 9, Exosomes enriched with MT1-MMP, IDE, heparanase, integrins, and LOXL2 lead to collagen cleavage and degradation. 10, HSCs release exosome-enriched Twist1 and miR214/199-5a clusters, reducing CCN2 expression in activated HSCs. Abbreviations: IDE, insulin-degrading enzyme; MT1-MMP, membrane-type 1 MMP.

TABLE 1. EXOSOMES REGULATE CELL FUNCTIONS

Donor Cells	Exosomal Cargo	Target Cells	Outcomes
Normal hepatocytes[36]	Caveolin-1, Eaa-1, Grp78, Pmp70, and mitochondria (Prohibitin 1 and mtPmp70)	Hepatocytes	Participate in hepatocyte metabolism
Injured hepatocytes[37]	Cytochrome P450s	Hepatocytes	Promote hepatocyte steatosis and apoptosis
Steatotic hepatocytes[38]	TRAIL and CD40 ligand	Macrophages	Promote activation of macrophages
Lipotoxic fatty acid-induced injury of hepatocytes[27,28]	miR17-92 clusters	HSC	Promote HSC activation
Endothelial cell[44,45]	LOXL2	ECM	Enhance activity of collagen contraction
Fibrocytes[67]	HSP-90a, activated STAT3, and miRs (21, 142a, 125b, 126, 130a, and 132)	ECM	Participate in ECM remodeling
Activated macrophages[84]	PAMPs	Immune cells	Lead to activation of naive recipient immune cells
Activated HSC[58]	CCN2	HSC	HSC activation
Normal HSC[30-32]	Twist-1 and miR214/miR199-5a	Activated HSC	Reduce CCN2 expression in activated HSC

The emerging field of exosome biology has identified several novel pathways of exosome-dependent intercellular transfer of biologically active materials that not only facilitate the development of liver fibrosis but can also initiate fibrosis resolution. Exosomes from healthy subjects can transport biologically active antifibrotic molecules, including proteins and nucleic acids, that in turn regulate gene expression and cellular function in target cells. For example, DC-derived exosomes from mice subjected to immunosuppressive treatments or modified to express immunosuppressive cytokines promoted tolerogenic immune responses, leading to amelioration of inflammatory responses in mice,[95] and mRNA-155- and miRNA-125b-enriched exosomes promoted differentiation of M1 macrophages over M2 macrophages.[96] (Figure 1) Human amnion epithelial cell-derived exosomes significantly reduced the number of macrophages and macrophage infiltration during liver fibrosis.[97]

In addition, exosomes from healthy subjects also contain a variety of molecules capable of interacting with and altering ECM components, including enzymes, such as membrane-type 1 (MT1) MMP, insulin-degrading enzyme, and heparanase, as well as integrins and LOXL2.[39] These enzymes could potentially localize on the surface of exosomes and through their contact with molecules in the ECM and could lead to cleavage of a wide range of substrates, such as collagen, a step necessary for collagen degradation. For example, MT1-MMP derived from exosomes has been demonstrated to target the ECM and degrade fibrillar collagen (type I, II, and III) as well as other matrix components, including fibronectin and vitronectin, promoting cell migration.[98] On the other hand, fibronectin-enriched exosomes interact with integrins to promote adhesion formation of cells.[39] Thus, a dynamic mechanism of forming adhesion interactions and breaking interactions by exosomal enzymes and components of the ECM is involved in wound healing and inflammation. Another enzyme, heparanase, is expressed on the surface of exosomes and has been shown to degrade heparan sulfate within the ECM and to participate in an inflammatory response.[99] Further, exosomes from hypoxia-induced endothelial cells have increased collagen crosslinking activity in the ECM through upregulation of LOXL2, whereas knockdown of LOXL2 in endothelial-derived exosomes in both normal and hypoxic conditions reduced activity of exosomes.[40] (Figure 1 & Table 1) Therefore, exosomes have been implicated in the regulation of both inflammation and ECM remodeling.

Studies to elucidate the signaling molecules that contribute to the activated HSC phenotype have identified potential therapeutic targets for antifibrotic therapy.[56,100] One potential target is CCN2, a profibrotic factor that is produced in fibrosing liver tissue. CCN2, a cysteine-rich matricellular protein, interacts with integrins, low-density lipoprotein receptor-related proteins, and heparan sulfate proteoglycan coreceptors,[101,102] thus stimulating adhesion, migration, proliferation, survival, and differentiation of HSCs. CCN2 exhibits strong profibrogenic properties. Overexpression of CCN2 promotes ECM deposition and development of fibrotic lesions. Hepatic levels of CCN2 correlate with the severity of liver disease in patients with liver fibrosis.[103,104] Additionally, overexpression of CCN2 mediates TGF-β1-dependent

fibrotic pathways in HSCs,[105,106] and TGF-β1 mRNA transported by injured epithelial-derived exosomes results in a rapid initiation of activation of myofibroblasts.[34] In the exosome-mediated transfer of activated HSC-derived exosomes to quiescent HSCs, CCN2 was directly targeted through modulation of a Twist–miR-214/199 axis, resulting in HSC activation.[23-25]

Quiescent HSCs produce exosomes that inhibit activation of HSCs and attenuate pathways of fibrogenesis.[23-25] This results in an exosomal transfer of miR-214, miR-199a-5p, or Twist-1 into the recipient HSCs and directly inhibits transcription of CCN2, thus suppressing downstream collagen production and reverting HSCs to a more quiescent phenotype (Figure 1 & Table 1). Hepatocytes have also been demonstrated to produce exosomes that cause a reversal of fibrosis-associated gene expression and ethanol-induced damage in hepatocytes.[107] Hepatocyte-derived exosomes can bind to activated HSCs or injured hepatocytes through mechanisms that involve heparin-like molecules and cellular integrin subunits αv or β1, thus mediating therapeutic changes.[107] Therefore, hepatic fibrosis is amendable to therapy. Exosomes produced either from quiescent HSCs or normal hepatocytes may be important for a reduction in the progression of fibrosis and therefore have serious potential as antifibrotic therapies.

The Role of Exosomes as a Biomarker of Liver Fibrosis

Clinically, patient management decisions depend on the accurate assessment of the severity and progression of liver fibrosis. Liver biopsy is the "gold standard" and is invasive, expensive, and risky to patients. Because the components of exosomes are a "fingerprint" of the dynamic status of the underlying pathologic condition in patients, they might represent a new biomarker for identifying and assessing molecular signatures associated with liver fibrosis. In addition, exosomal components are protected from proteinase-dependent degradation and thus can be stably detected in the circulating plasma and serum, making them ideal biomarkers for a number of clinical applications.[108,109] Increased levels of CD10 protein in urinary exosomes from glycine N-methyltransferase knockout mice have been associated with steatosis, fibrosis, and hepatocellular carcinoma.[110] CD81-enriched serum exosomes of patients with chronic HCV was associated with inflammation and severity of fibrosis.[111] Decreased levels of miRNAs (miR-34c, miR-151-3p, miR-483-5p, or miR-532-5p) were detected in serum exosomes of CCl_4-induced mice or human patients with F3/4 fibrosis.[97]

MSC-Derived Exosomes or Other Exosomes as a New Therapeutic Strategy for Experimental Fibrosis Models

The transfer of MSCs has been proposed as a potential therapeutic strategy for the treatment of various diseases and immune disorders, mostly due to their immunoregulatory properties.[112] For example, MSCs secrete several antifibrotic molecules, such as hepatocyte growth factor, fibroblast growth factor, epidermal growth factor, insulin, and dexamethasone. They were also reported to mediate cytoprotective, anti-angiogenic, and regenerative effects in damaged liver.[113] Although the exact mechanism remains unknown, MSCs were demonstrated to attenuate liver fibrosis by suppressing activation of T helper 17-positive immune cells in fibrotic liver.[114] A similar effect can be achieved using adoptive transfer of MSC-derived exosomes to mice with liver fibrosis.[115,116]

Exosomes are emerging as effective therapeutic tools for different diseases because these particles can bypass biological barriers and can serve as powerful drug and gene therapy transporters, raising the exciting prospect of "cell therapy without the cells."[117-119] The administration of MSC-derived exosomes is a potential strategy for treating liver disease.[22,120-122] The safety and feasibility observed in early clinical trials using MSCs has resulted in increased interest in the translation of the use of these cells to the clinic.[123,124] Likewise, increasing evidence suggests that MSC-derived therapeutic effects are mainly mediated in a paracrine manner by extracellular vehicles, such as exosomes.[125,126] In this regard, use of MSC-derived exosomes will allow the delivery of

anti-inflammatory cytokines and other biologically active proteins to injured livers without administration of heterologous divergent cells. For example, by using a CCl_4-induced liver injury model in Kunming mice, delivery of human umbilical cord MSC-derived exosomes reduced hepatic fibrosis through inhibition of collagen production.[122] Furthermore, the delivered exosomes migrated to the liver, resulting in a significant suppression of the TGF-β1/Smad pathway and subsequent down-regulation of collagen type I/III and TGF-β1 in these mice.[22] Moreover, it has been demonstrated that exosomes released from adipose tissue-derived MSCs inhibited the proliferation and activation of the human LX-2 cell line as well as primary HSCs from male Sprague Dawley rats.[121]

In addition to MSC-derived exosomes, exosomes isolated from serum have also been shown to have a therapeutic effect in mice with liver fibrosis. For instance, hepatic fibrosis was decreased in CCl_4-injured or thioacetic acid-injured mice treated with exosomes derived from the serum of healthy mice (but not from fibrotic mice)[127]; mice showed improved liver function, reduced apoptosis of hepatocytes, suppression of an inflammatory response in the injured liver, reduced release of hepatic or circulating proinflammatory cytokines (IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, interferon-gamma, and TNF-α), reduced inflammatory infiltration, and reduced circulating aspartate aminotransferase/alanine aminotransferase levels.[127]

Overall, adoptive transfer of exosomes from normal healthy individuals may be beneficial for patients with liver fibrosis. The main mechanism by which "healthy exosomes" support the repair of injured livers may be the release of paracrine factors.[128] These exosomes shuttle across the intercellular space and deliver "therapeutic molecules" between different liver cells; this results in the elevation of collagenase activity, the abortion of activated myofibroblasts, and the reduction in proinflammatory and fibrogenic cytokines, which finally results in a decrease in ECM production and regression of liver fibrosis.

Summary

Decades of basic and translational research have brought us to a new era where promising therapies for liver inflammation and fibrosis are being developed. Hepatic fibrosis has been shown to be reversible in patients with chronic liver disease and in experimental models, as demonstrated by a reduction of proinflammatory and fibrogenic cytokines, remodeling of ECM production and decreased fibrous scar, and the inactivation or disappearance of myofibroblast populations. Because exosomes function as conduits for intercellular transfer and have been demonstrated to be sufficient to inhibit liver fibrosis in rodent models, the administration of exosomes as a therapy for hepatic fibrosis in humans holds great promise.

Exosomes can remain hidden in the bloodstream, carry multiple doses, specifically target the cells, and store and administer treatment, and their small size allows them to cross barriers that cells cannot. However, there are some open questions remaining that limit the application of exosome therapy: (1) How should the the characterization and quantification of exosomes be standardized? More effective methods and techniques for large-scale exosome production are needed; (2) What is the proper source of exosomes for therapy? Careful analysis of the complex information in cell-derived exosomes or circulating exosomes may result in the identification of unique "molecular signatures" for exosome therapy; (3) How should dosing of exosomes be evaluated? Exosome-regulated signaling pathways are dose dependent.[129] Therefore, the tuning of exosome dose may enable the balancing of potential deleterious and therapeutic effects of exosome administration.

REFERENCES

1) Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005;115:209-218.
2) Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. Lancet 1997;349:1498-1504.
3) Dooley S, ten Dijke P. TGF-beta in progression of liver disease. Cell Tissue Res 2012;347:245-256.
4) Brenner DA. Transforming growth factor B and hepatic fibrosis: cause or effect? Hepatology 1991;14:740-742.
5) Tsukamoto H. Cytokine regulation of hepatic stellate cells in liver fibrosis. Alcohol Clin Exp Res 1999;23:911-916.
6) Chen MH, Chen JC, Tsai CC, Wang WC, Chang DC, Tu DG, et al. The role of TGF-beta 1 and cytokines in the modulation of liver fibrosis by Sho-saiko-to in rat's bile duct ligated model. J Ethnopharmacol 2005;97:7-13.
7) Tong Z, Chen R, Alt DS, Kemper S, Perbal B, Brigstock DR. Susceptibility to liver fibrosis in mice expressing a connective tissue growth factor transgene in hepatocytes. Hepatology 2009;50:939-947.

8) Brigstock DR. Connective tissue growth factor (CCN2, CTGF) and organ fibrosis: lessons from transgenic animals. J Cell Commun Signal 2010;4:1-4.
9) Armendariz-Borunda J, Katayama K, Seyer JM. Transcriptional mechanisms of type I collagen gene expression are differentially regulated by interleukin-1 beta, tumor necrosis factor alpha, and transforming growth factor beta in Ito cells. J Biol Chem 1992;267:14316-14321.
10) Choi I, Kang HS, Yang Y, Pyun KH. IL-6 induces hepatic inflammation and collagen synthesis in vivo. Clin Exp Immunol 1994;95:530-535.
11) Gabele E, Froh M, Arteel GE, Uesugi T, Hellerbrand C, Scholmerich J, et al. TNFalpha is required for cholestasis-induced liver fibrosis in the mouse. Biochem Biophys Res Commun 2009;378:348-353.
12) Brenner DA, Kisseleva T, Scholten D, Paik YH, Iwaisako K, Inokuchi S, et al. Origin of myofibroblasts in liver fibrosis. Fibrogenesis Tissue Repair 2012;5(Suppl. 1):S17.
13) Iredale JP, Thompson A, Henderson NC. Extracellular matrix degradation in liver fibrosis: biochemistry and regulation. Biochim Biophys Acta 2013;1832:876-883.
14) Pellicoro A, Ramachandran P, Iredale JP, Fallowfield JA. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. Nat Rev Immunol 2014;14:181-194.
15) Lee YA, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: a translational success story. Gut 2015;64:830-841.
16) Ramachandran P, Iredale JP, Fallowfield JA. Resolution of liver fibrosis: basic mechanisms and clinical relevance. Semin Liver Dis 2015;35:119-131.
17) Campana L, Iredale JP. Regression of liver fibrosis. Semin Liver Dis 2017;37:1-10.
18) Sun M, Kisseleva T. Reversibility of liver fibrosis. Clin Res Hepatol Gastroenterol 2015;39(Suppl. 1):S60-S63.
19) Tkach M, Thery C. Communication by extracellular vesicles: where we are and where we need to go. Cell 2016;164:1226-1232.
20) Ban LA, Shackel NA, McLennan SV. Extracellular vesicles: a new frontier in biomarker discovery for non-alcoholic fatty liver disease. Int J Mol Sci 2016;17:376.
21) Povero D, Panera N, Eguchi A, Johnson CD, Papouchado BG, de Araujo Horcel L, et al. Lipid-induced hepatocyte-derived extracellular vesicles regulate hepatic stellate cell via microRNAs targeting PPAR-gamma. Cell Mol Gastroenterol Hepatol 2015;1:646-663.e644.
22) Li T, Yan Y, Wang B, Qian H, Zhang X, Shen L, et al. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. Stem Cells Dev 2013;22:845-854.
23) Chen L, Charrier A, Zhou Y, Chen R, Yu B, Agarwal K, et al. Epigenetic regulation of connective tissue growth factor by MicroRNA-214 delivery in exosomes from mouse or human hepatic stellate cells. Hepatology 2014;59:1118-1129.
24) Chen L, Chen R, Velazquez VM, Brigstock DR. Fibrogenic signaling is suppressed in hepatic stellate cells through targeting of connective tissue growth factor (CCN2) by cellular or exosomal microRNA-199a-5p. Am J Pathol 2016;186:2921-2933.
25) Chen L, Chen R, Kemper S, Charrier A, Brigstock DR. Suppression of fibrogenic signaling in hepatic stellate cells by Twist1-dependent microRNA-214 expression: role of exosomes in horizontal transfer of Twist1. Am J Physiol Gastrointest Liver Physiol 2015;309:G491-G499.
26) Feldstein AE, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD, et al. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology 2003;125:437-443.
27) Ribeiro PS, Cortez-Pinto H, Sola S, Castro RE, Ramalho RM, Baptista A, et al. Hepatocyte apoptosis, expression of death receptors, and activation of NF-kappaB in the liver of nonalcoholic and alcoholic steatohepatitis patients. Am J Gastroenterol 2004;99:1708-1717.
28) Malhi H, Gores GJ. Cellular and molecular mechanisms of liver injury. Gastroenterology 2008;134:1641-1654.
29) Conde-Vancells J, Rodriguez-Suarez E, Embade N, Gil D, Matthiesen R, Valle M, et al. Characterization and comprehensive proteome profiling of exosomes secreted by hepatocytes. J Proteome Res 2008;7:5157-5166.
30) Cho EY, Yun CH, Chae HZ, Chae HJ, Ahn T. Anionic phospholipid-induced regulation of reactive oxygen species production by human cytochrome P450 2E1. FEBS Lett 2008;582:1771-1776.
31) Hirsova P, Ibrahim SH, Verma VK, Morton LA, Shah VH, LaRusso NF, et al. Extracellular vesicles in liver pathobiology: small particles with big impact. Hepatology 2016;64:2219-2233.
32) Seo W, Eun HS, Kim SY, Yi HS, Lee YS, Park SH, et al. Exosome-mediated activation of toll-like receptor 3 in stellate cells stimulates interleukin-17 production by gammadelta T cells in liver fibrosis. Hepatology 2016;64:616-631.
33) Ma HY, Xu J, Liu X, Zhu Y, Gao B, Karin M, et al. The role of IL-17 signaling in regulation of the liver-brain axis and intestinal permeability in alcoholic liver disease. Curr Pathobiol Rep 2016;4:27-35.
34) Borges FT, Melo SA, Ozdemir BC, Kato N, Revuelta I, Miller CA, et al. TGF-beta1-containing exosomes from injured epithelial cells activate fibroblasts to initiate tissue regenerative responses and fibrosis. J Am Soc Nephrol 2013;24:385-392.
35) Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. J Cell Sci 2010;123:4195-4200.
36) Friedman SL, Sheppard D, Duffield JS, Violette S. Therapy for fibrotic diseases: nearing the starting line. Sci Transl Med 2013;5:167sr161.
37) Arriazu E, Ruiz de Galarreta M, Cubero FJ, Varela-Rey M, Perez de Obanos MP, Leung TM, et al. Extracellular matrix and liver disease. Antioxid Redox Signal 2014;21:1078-1097.
38) Hollosi P, Yakushiji JK, Fong KS, Csiszar K, Fong SF. Lysyl oxidase-like 2 promotes migration in noninvasive breast cancer cells but not in normal breast epithelial cells. Int J Cancer 2009;125:318-327.
39) Shimoda M, Khokha R. Proteolytic factors in exosomes. Proteomics 2013;13:1624-1636.
40) de Jong OG, van Balkom BW, Gremmels H, Verhaar MC. Exosomes from hypoxic endothelial cells have increased collagen crosslinking activity through up-regulation of lysyl oxidase-like 2. J Cell Mol Med 2016;20:342-350.
41) Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest 2003;112:1776-1784.
42) Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008;134:1655-1669.
43) Kisseleva T, Brenner DA. Fibrogenesis of parenchymal organs. Proc Am Thorac Soc 2008;5:338-342.
44) Zeisberg EM, Potenta SE, Sugimoto H, Zeisberg M, Kalluri R. Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. J Am Soc Nephrol 2008;19:2282-2287.
45) Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nat Med 2007;13:952-961.
46) Scholten D, Osterreicher CH, Scholten A, Iwaisako K, Gu G, Brenner DA, et al. Genetic labeling does not detect epithelial-to-mesenchymal transition of cholangiocytes in liver fibrosis in mice. Gastroenterology 2010;139:987-998.
47) Chu AS, Diaz R, Hui JJ, Yanger K, Zong Y, Alpini G, et al. Lineage tracing demonstrates no evidence of cholangiocyte

epithelial-to-mesenchymal transition in murine models of hepatic fibrosis. Hepatology 2011;53:1685-1695.
48) Iredale JP. Models of liver fibrosis: exploring the dynamic nature of inflammation and repair in a solid organ. J Clin Invest 2007;117:539-548.
49) Geerts A. History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells. Semin Liver Dis 2001;21:311-335.
50) Senoo H, Kojima N, Sato M. Vitamin A-storing cells (stellate cells). Vitam Horm 2007;75:131-159.
51) Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 2000;275:2247-2250.
52) Gaca MD, Zhou X, Benyon RC. Regulation of hepatic stellate cell proliferation and collagen synthesis by proteinase-activated receptors. J Hepatol 2002;36:362-369.
53) Arias M, Lahme B, Van de Leur E, Gressner AM, Weiskirchen R. Adenoviral delivery of an antisense RNA complementary to the 3' coding sequence of transforming growth factor-beta1 inhibits fibrogenic activities of hepatic stellate cells. Cell Growth Differ 2002;13:265-273.
54) Knittel T, Mehde M, Kobold D, Saile B, Dinter C, Ramadori G. Expression patterns of matrix metalloproteinases and their inhibitors in parenchymal and non-parenchymal cells of rat liver: regulation by TNF-alpha and TGF-beta1. J Hepatol 1999;30:48-60.
55) Sysa P, Potter JJ, Liu X, Mezey E. Transforming growth factor-beta1 up-regulation of human alpha(1)(I) collagen is mediated by Sp1 and Smad2 transacting factors. DNA Cell Biol 2009;28:425-434.
56) Huang G, Brigstock DR. Regulation of hepatic stellate cells by connective tissue growth factor. Front Biosci (Landmark Ed) 2012;17:2495-2507.
57) Charrier A, Chen R, Chen L, Kemper S, Hattori T, Takigawa M, et al. Exosomes mediate intercellular transfer of pro-fibrogenic connective tissue growth factor (CCN2) between hepatic stellate cells, the principal fibrotic cells in the liver. Surgery 2014;156:548-555.
58) Kisseleva T, Uchinami H, Feirt N, Quintana-Bustamante O, Segovia JC, Schwabe RF, et al. Bone marrow-derived fibrocytes participate in pathogenesis of liver fibrosis. J Hepatol 2006;45:429-438.
59) Quan TE, Cowper S, Wu SP, Bockenstedt LK, Bucala R. Circulating fibrocytes: collagen-secreting cells of the peripheral blood. Int J Biochem Cell Biol 2004;36:598-606.
60) Bellini A, Mattoli S. The role of the fibrocyte, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses. Lab Invest 2007;87:858-870.
61) Geiger A, Walker A, Nissen E. Human fibrocyte-derived exosomes accelerate wound healing in genetically diabetic mice. Biochem Biophys Res Commun 2015;467:303-309.
62) Wang T, Feng Y, Sun H, Zhang L, Hao L, Shi C, et al. miR-21 regulates skin wound healing by targeting multiple aspects of the healing process. Am J Pathol 2012;181: 1911-1920.
63) Beaussier M, Wendum D, Schiffer E, Dumont S, Rey C, Lienhart A, et al. Prominent contribution of portal mesenchymal cells to liver fibrosis in ischemic and obstructive cholestatic injuries. Lab Invest 2007;87:292-303.
64) Iwaisako K, Jiang C, Zhang M, Cong M, Moore-Morris TJ, Park TJ, et al. Origin of myofibroblasts in the fibrotic liver in mice. Proc Natl Acad Sci U S A 2014;111:E3297-E3305.
65) Dranoff JA, Wells RG. Portal fibroblasts: underappreciated mediators of biliary fibrosis. Hepatology 2010;51:1438-1444.
66) Fausther M, Sheung N, Saiman Y, Bansal MB, Dranoff JA. Activated hepatic stellate cells upregulate transcription of ecto-5'-nucleotidase/CD73 via specific SP1 and SMAD promoter elements. Am J Physiol Gastrointest Liver Physiol 2012;303:G904-G914.
67) Knittel T, Kobold D, Saile B, Grundmann A, Neubauer K, Piscaglia F, et al. Rat liver myofibroblasts and hepatic stellate cells: different cell populations of the fibroblast lineage with fibrogenic potential. Gastroenterology 1999;117:1205-1221.
68) Li Z, Dranoff JA, Chan EP, Uemura M, Sevigny J, Wells RG. Transforming growth factor-beta and substrate stiffness regulate portal fibroblast activation in culture. Hepatology 2007;46:1246-1256.
69) Radaeva S, Sun R, Jaruga B, Nguyen VT, Tian Z, Gao B. Natural killer cells ameliorate liver fibrosis by killing activated stellate cells in NKG2D-dependent and tumor necrosis factor-related apoptosis-inducing ligand-dependent manners. Gastroenterology 2006;130:435-452.
70) Cheever AW, Williams ME, Wynn TA, Finkelman FD, Seder RA, Cox TM, et al. Anti-IL-4 treatment of Schistosoma mansoni-infected mice inhibits development of T cells and non-B, non-T cells expressing Th2 cytokines while decreasing egg-induced hepatic fibrosis. J Immunol 1994;153:753-759.
71) Chiaramonte MG, Donaldson DD, Cheever AW, Wynn TA. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. J Clin Invest 1999;104:777-785.
72) Novobrantseva TI, Majeau GR, Amatucci A, Kogan S, Brenner I, Casola S, et al. Attenuated liver fibrosis in the absence of B cells. J Clin Invest 2005;115:3072-3082.
73) Wynn TA, Barron L. Macrophages: master regulators of inflammation and fibrosis. Semin Liver Dis 2010;30:245-257.
74) Lupher ML Jr, Gallatin WM. Regulation of fibrosis by the immune system. Adv Immunol 2006;89:245-288.
75) Anders HJ, Ryu M. Renal microenvironments and macrophage phenotypes determine progression or resolution of renal inflammation and fibrosis. Kidney Int 2011;80:915-925.
76) Fallowfield JA, Mizuno M, Kendall TJ, Constandinou CM, Benyon RC, Duffield JS, et al. Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. J Immunol 2007;178:5288-5295.
77) Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, et al. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J Clin Invest 2005;115:56-65.
78) Kaviratne M, Hesse M, Leusink M, Cheever AW, Davies SJ, McKerrow JH, et al. IL-13 activates a mechanism of tissue fibrosis that is completely TGF-beta independent. J Immunol 2004;173:4020-4029.
79) Olman MA. Beyond TGF-beta: a prostaglandin promotes fibrosis. Nat Med 2009;15:1360-1361.
80) Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008;8:958-969.
81) Mantovani A, Sica A, Locati M. Macrophage polarization comes of age. Immunity 2005;23:344-346.
82) Khalil N, Bereznay O, Sporn M, Greenberg AH. Macrophage production of transforming growth factor beta and fibroblast collagen synthesis in chronic pulmonary inflammation. J Exp Med 1989;170:727-737.
83) Said EA, Dupuy FP, Trautmann L, Zhang Y, Shi Y, El-Far M, et al. Programmed death-1-induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection. Nat Med 2010;16:452-459.

84) Shouval DS, Biswas A, Goettel JA, McCann K, Conaway E, Redhu NS, et al. Interleukin-10 receptor signaling in innate immune cells regulates mucosal immune tolerance and anti-inflammatory macrophage function. Immunity 2014;40:706-719.
85) Zigmond E, Bernshtein B, Friedlander G, Walker CR, Yona S, Kim KW, et al. Macrophage-restricted interleukin-10 receptor deficiency, but not IL-10 deficiency, causes severe spontaneous colitis. Immunity 2014;40:720-733.
86) Issa R, Zhou X, Trim N, Millward-Sadler H, Krane S, Benyon C, et al. Mutation in collagen-1 that confers resistance to the action of collagenase results in failure of recovery from CCl4-induced liver fibrosis, persistence of activated hepatic stellate cells, and diminished hepatocyte regeneration. FASEB J 2003;17:47-49.
87) Bhatnagar S, Shinagawa K, Castellino FJ, Schorey JS. Exosomes released from macrophages infected with intracellular pathogens stimulate a proinflammatory response in vitro and in vivo. Blood 2007;110:3234-3244.
88) Xu R, Zhang Z, Wang FS. Liver fibrosis: mechanisms of immune-mediated liver injury. Cell Mol Immunol 2012;9:296-301.
89) Connolly MK, Bedrosian AS, Mallen-St Clair J, Mitchell AP, Ibrahim J, Stroud A, et al. In liver fibrosis, dendritic cells govern hepatic inflammation in mice via TNF-alpha. J Clin Invest 2009;119:3213-3225.
90) Zhang X, Lou J, Bai L, Chen Y, Zheng S, Duan Z. Immune regulation of intrahepatic regulatory T cells in fibrotic livers of mice. Med Sci Monit 2017;23:1009-1016.
91) Greening DW, Gopal SK, Xu R, Simpson RJ, Chen W. Exosomes and their roles in immune regulation and cancer. Semin Cell Dev Biol 2015;40:72-81.
92) Kim SH, Lechman ER, Bianco N, Menon R, Keravala A, Nash J, et al. Exosomes derived from IL-10-treated dendritic cells can suppress inflammation and collagen-induced arthritis. J Immunol 2005;174:6440-6448.
93) Mittelbrunn M, Gutierrez-Vazquez C, Villarroya-Beltri C, Gonzalez S, Sanchez-Cabo F, Gonzalez MA, et al. Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. Nat Commun 2011;2:282.
94) Ramachandran P, Iredale JP. Reversibility of liver fibrosis. Ann Hepatol 2009;8:283-291.
95) Chaput N, Thery C. Exosomes: immune properties and potential clinical implementations. Semin Immunopathol 2011;33:419-440.
96) Su MJ, Aldawsari H, Amiji M. Pancreatic cancer cell exosome-mediated macrophage reprogramming and the role of microRNAs 155 and 125b2 transfection using nanoparticle delivery systems. Sci Rep 2016;6:30110.
97) Alhomrani M, Correia J, Zavou M, Leaw B, Kuk N, Xu R, et al. The human amnion epithelial cell secretome decreases hepatic fibrosis in mice with chronic liver fibrosis. Front Pharmacol 2017;8:748.
98) Itoh Y. Membrane-type matrix metalloproteinases: their functions and regulations. Matrix Biol 2015;44–46:207-223.
99) Bandari SK, Purushothaman A, Ramani VC, Brinkley GJ, Chandrashekar DS, Varambally S, et al. Chemotherapy induces secretion of exosomes loaded with heparanase that degrades extracellular matrix and impacts tumor and host cell behavior. Matrix Biol 2018;65:104-118.
100) Puche JE, Saiman Y, Friedman SL. Hepatic stellate cells and liver fibrosis. Compr Physiol 2013;3:1473-1492.
101) Brigstock DR. The connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed (CCN) family. Endocr Rev 1999;20:189-206.
102) Leask A, Abraham DJ. All in the CCN family: essential matricellular signaling modulators emerge from the bunker. J Cell Sci 2006;119:4803-4810.
103) Blom IE, Goldschmeding R, Leask A. Gene regulation of connective tissue growth factor: new targets for antifibrotic therapy? Matrix Biol 2002;21:473-482.
104) Dziadzio M, Usinger W, Leask A, Abraham D, Black CM, Denton C, et al. N-terminal connective tissue growth factor is a marker of the fibrotic phenotype in scleroderma. QJM 2005;98:485-492.
105) Paradis V, Dargere D, Vidaud M, De Gouville AC, Huet S, Martinez V, et al. Expression of connective tissue growth factor in experimental rat and human liver fibrosis. Hepatology 1999;30:968-976.
106) Williams EJ, Gaca MD, Brigstock DR, Arthur MJ, Benyon RC. Increased expression of connective tissue growth factor in fibrotic human liver and in activated hepatic stellate cells. J Hepatol 2000;32:754-761.
107) Chen L, Chen R, Kemper S, Brigstock DR. Pathways of production and delivery of hepatocyte exosomes. J Cell Commun Signal 2018;12:343-357.
108) Lin J, Li J, Huang B, Liu J, Chen X, Chen XM, et al. Exosomes: novel biomarkers for clinical diagnosis. ScientificWorldJournal 2015;2015:657086.
109) Taverna S, Giallombardo M, Gil-Bazo I, Carreca AP, Castiglia M, Chacartegui J, et al. Exosomes isolation and characterization in serum is feasible in non-small cell lung cancer patients: critical analysis of evidence and potential role in clinical practice. Oncotarget 2016;7:28748-28760.
110) Conde-Vancells J, Rodriguez-Suarez E, Gonzalez E, Berisa A, Gil D, Embade N, et al. Candidate biomarkers in exosome-like vesicles purified from rat and mouse urine samples. Proteomics Clin Appl 2010;4:416-425.
111) Welker MW, Reichert D, Susser S, Sarrazin C, Martinez Y, Herrmann E, et al. Soluble serum CD81 is elevated in patients with chronic hepatitis C and correlates with alanine aminotransferase serum activity. PLoS ONE 2012;7:e30796.
112) Ren G, Chen X, Dong F, Li W, Ren X, Zhang Y, et al. Concise review: mesenchymal stem cells and translational medicine: emerging issues. Stem Cells Transl Med 2012;1:51-58.
113) Berardis S, Lombard C, Evraerts J, El Taghdouini A, Rosseels V, Sancho-Bru P, et al. Gene expression profiling and secretome analysis differentiate adult-derived human liver stem/progenitor cells and human hepatic stellate cells. PLoS ONE 2014;9:e86137.
114) Milosavljevic N, Gazdic M, Simovic Markovic B, Arsenijevic A, Nurkovic J, Dolicanin Z, et al. Mesenchymal stem cells attenuate liver fibrosis by suppressing Th17 cells - an experimental study. Transpl Int 2018;31:102-115.
115) Baglio SR, Pegtel DM, Baldini N. Mesenchymal stem cell secreted vesicles provide novel opportunities in (stem) cell-free therapy. Front Physiol 2012;3:359.
116) Barile L, Lionetti V, Cervio E, Matteucci M, Gherghiceanu M, Popescu LM, et al. Extracellular vesicles from human cardiac progenitor cells inhibit cardiomyocyte apoptosis and improve cardiac function after myocardial infarction. Cardiovasc Res 2014;103:530-541.
117) Haney MJ, Klyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z, et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy. J Control Release 2015;207:18-30.
118) Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, et al. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. Mol Ther 2010;18:1606-1614.
119) Johnsen KB, Gudbergsson JM, Skov MN, Pilgaard L, Moos T, Duroux M. A comprehensive overview of exosomes as drug delivery vehicles - endogenous nanocarriers for targeted cancer therapy. Biochim Biophys Acta 2014;1846:75-87.

120) Hyun J, Wang S, Kim J, Kim GJ, Jung Y. MicroRNA125b-mediated Hedgehog signaling influences liver regeneration by chorionic plate-derived mesenchymal stem cells. Sci Rep 2015;5:14135.
121) Li J, Ghazwani M, Zhang Y, Lu J, Li J, Fan J, et al. miR-122 regulates collagen production via targeting hepatic stellate cells and suppressing P4HA1 expression. J Hepatol 2013;58: 522-528.
122) Lou G, Chen Z, Zheng M, Liu Y. Mesenchymal stem cell-derived exosomes as a new therapeutic strategy for liver diseases. Exp Mol Med 2017;49:e346.
123) Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: an update. Cell Transplant 2016;25: 829-848.
124) Mobasheri A, Kalamegam G, Musumeci G, Batt ME. Chondrocyte and mesenchymal stem cell-based therapies for cartilage repair in osteoarthritis and related orthopaedic conditions. Maturitas 2014;78:188-198.
125) Yu B, Zhang X, Li X. Exosomes derived from mesenchymal stem cells. Int J Mol Sci 2014;15:4142-4157.
126) Camussi G, Deregibus MC, Cantaluppi V. Role of stem-cell-derived microvesicles in the paracrine action of stem cells. Biochem Soc Trans 2013;41:283-287.
127) Chen L, Chen R, Kemper S, Cong M, You H, Brigstock DR. Therapeutic effects of serum extracellular vesicles in liver fibrosis. J Extracell Vesicles 2018;7:1461505.
128) Fiore EJ, Mazzolini G, Aquino JB. Mesenchymal stem/stromal cells in liver fibrosis: recent findings, old/new caveats and future perspectives. Stem Cell Rev 2015;11:586-597.
129) Yu S, Liu C, Su K, Wang J, Liu Y, Zhang L, et al. Tumor exosomes inhibit differentiation of bone marrow dendritic cells. J Immunol 2007;178:6867-6875.