Adipocytokines and Hepatic Fibrosis

Neeraj K. Saxena, Ph.D.[1] and **Frank A. Anania, M.D., F.A.C.P., A.G.A.F.**[2]

[1]University of Maryland School of Medicine, Department of Medicine, Division of Gastroenterology and Hepatology, Howard Hall, Room 301, 660 W. Redwood Street, Baltimore, MD 21201

[2]Emory University School of Medicine, Division of Digestive Diseases, Suite 201, 615 Michael Street, NE, Atlanta, GA 30322

Abstract

Obesity and metabolic syndrome pose significant risk for progression of many types of chronic illnesses, including liver disease. Hormones released from adipocytes, adipocytokines, associated with obesity and metabolic syndrome, have been shown to control hepatic inflammation and fibrosis. Hepatic fibrosis is the final common pathway that can result in cirrhosis, and can ultimately require liver transplantation. Initially, two key adipocytokines, leptin and adiponectin, appeared to control many fundamental aspects of the cell and molecular biology related to hepatic fibrosis and its resolution. Leptin appears to act as a profibrogenic molecule while adiponectin possesses strong-anti-fibrotic properties. In this review, we emphasize pertinent data associated with these, and recently discovered, adipocytokines that may drive or halt the fibrogenic response in the liver.

Keywords

Adiponectin; leptin; liver fibrosis; adipocytokines; hepatic stellate cells

Focusing on Fibrosis Fat—a connection derived from endocrine function and hepatic dysfunction

Liver fibrosis occurs as a consequence of acute or chronic liver injury. While hepatic fibrosis is reversible, in many chronic liver diseases, deposition of dense extracellular matrix (ECM) is progressive. The consequence of progressive ECM deposition leads to histological cirrhosis in which swirls of collagen and other ECM proteins surround functioning

Address all correspondence to: Anania, F.A., R. Bruce Logue Chair in Digestive Health, Professor of Medicine and Director, Division of Digestive Diseases, 615 Michael Street, Suite 201, Atlanta, GA 30322 USA, fanania@emory.edu, 404-712-2867, Facsimile 404-727-57670. Saxena, N.K., Assistant Professor of Medicine, Division of Gastroenterology and Hepatology, N3W50, Department of Medicine, Room 301, Howard Hall, 660 W Redwood Street, Baltimore, MD 21201, Phone: 410-706-6949, nsaxena@medicine.umaryland.edu.

hepatoctyes. Functionally liver has extensive reserve however, when normal hepatocyte function is significantly impaired (usually less than twenty percent), clinical cirrhosis, or end-stage liver disease (ESLD) can ensue—leading to portal hypertension and other clinical complications [1]. Cirrhosis places patients at considerable risk for development of hepatocellular carcinoma (HCC) [2]. Both palliative and supportive treatment are widely available, definitive treatment of ESLD and many cases of HCC remains limited to liver transplantation (LT).

The primary cell associated with liver fibrosis is the hepatic stellate cell (HSC). In the early years of fibrosis research conventional thought was that these cells originated from embryologic mesoderm[3]. Today, there is some degree of controversy not only concerning the origin of these cells, but also whether they are the result of epithelial-mesenchymal transition (EMT)[4], and whether there are subsets of these cells[5–7]. There are three broad components to the life cycle of the activated HSC—quiescence, activation, and perpetuation. The reader is referred to Figure 1A which includes a scheme outlining these major life cycle events.

In quiescence, the HSC is a storage depot for retinyl esters, has a low mitotic index, and lacks key proteins associated with cytoskeletal contraction. During the process of 'activation' a loosely defined term, the stellate cell becomes a myofibroblast-like cell. Characteristics of activated HSCs include a marked increase in the mitotic rate, the loss of retinyl ester stores, and a spindle-like appearance in light microscopy[8]. The change in microscopic appearance is a consequence of marked increase in both transcriptional and translational activation of cytoskeletal proteins, e.g. alpha smooth muscle actin (αSMA), and intermediate filaments, e.g. desmin[9].

Fibrosis can be a perpetual pathophysiologic condition, as activated HSCs present cell surface receptors for key cytokines including platelet-derived growth factor (PDGF), and transforming growth factor beta one (TGFβ1)[10]. Both by autocrine and paracrine stimulation, the activated HSC continues to proliferate and becomes characteristically resistant to apoptosis. These derangements best define the perpetuation phase. Activated HSCs can partially reverse phenotype, termed 'reversion'[11]. Activated HSCs may undergo senescence, and others may fully revert back to a retinyl ester storage depot. Taken together these fundamental changes in HSC biology, along with sensitization to apoptosis, and increased destruction of ECM by matrix metalloproteinases (MMPs) is collectively termed 'resolution' of fibrosis [12] (**cf.** Fig. 1). Contributions to research advanced in liver fibrosis research are in part due to the discovery of adipocytokines. Derived from white adipose tissue (WAT), adipocytokines, or adipokines, are secreted molecules with diverse biological functions. WAT has far reaching effects in liver as well as the immune and central nervous systems. In the nearly two decades since leptin was discovered[13], the liver investigators have concluded adipokines play a dynamic role in modulating control of ECM in liver[14, 15]. Initially these observations had little to do with non-alcoholic fatty liver disease (NAFLD). Rather, the discovery—that leptin, and adiponectin in particular, were critical to fibrosis development and resolution, respectively, were observed in models designed to study liver fibrosis.

In this concise review the reader will gain insight into the primary adipocytokines—leptin, adiponectin, plasminogen activator I (PAI-1)—that have established, well-defined roles in the pathophysiology of fibrosis and NAFLD-related pathology [16]. A brief review of newly-discovered adipocytokines is also presented. Readers will find that Table I provides a succinct review of major adipokines with their currently held respective contributions to hepatic fibrosis, while Table II provides take-home points related to established adipocytokines associated with liver fibrosis. In the broad field of Digestive Diseases, NAFLD, its complications and associated risk for all-cause mortality, is now the commonest reason patients come to a gastroenterologist's attention. Importantly, NAFLD-related cirrhosis is estimated to become the leading indication for LT by 2020[17]. Upon studying this succinct review readers are encouraged to consider the critical importance of potential research collaboration for fibrosis and NAFLD therapies, biomarker development, as well as integrated clinical care of patients.

The biology of leptin and its relationship to chronic liver disease

Leptin was the first adipocytokine associated directly with hepatic fibrosis [18–21]. Leptin is increased in mammalian circulation coincident with excess subcutaneous and visceral fat accumulation [22]. Leptin is a 16 kDa hormone, or cytokine, secreted by WAT. Like insulin, leptin resistance plays a critical role in metabolic syndrome. Genetic leptin deficiency is rare [23]; conversely, circulating serum leptin levels are positively correlated with obesity. Leptin has cognate receptors called Ob-R in many tissues including hypothalamus, liver, and fat [24]. Currently five leptin receptor isoforms exist and result from multiple gene splicing events at the transcriptional level [25]. The critical biologically active isoform is the long form, or Ob-Rb [25]. Upon leptin binding to Ob-Rb, the Janus kinase 2 (Jak2) moiety of the receptor phosphorylates Signal Transduction and Transcription Factor 3 (Stat3) [15]. In turn pStat3 translocates to the nucleus whereby it conveys leptin modulation of gene transcription [15]. A short form of the leptin receptor also exists called Ob-Ra [25]. Ob-Ra is a truncated version of Ob-Rb lacking Jak2/Stat3 signaling capability [25]. In addition to its secretion from WAT, leptin is also secreted by activated hepatic stellate cells HSCs (Box 1). In liver, leptin has been shown to convey profibrogenic properties via its interaction with the activated HSC [26] with signaling capability via Ob-Rb [27, 28].

BOX 1

The Hepatic Stellate Cells (HSCs) and Myofibroblasts

The quiescent HSC in mammals is a non-dividing cell that resides in the Space of Disse in the healthy liver. Its principle function is to serve as a depot for vitamin A storage in the form of retinyl esters. Following either acute or chronic liver injury, the quiescent HSC becomes 'activated' and loses its vitamin A storage capacity. The HSC then enters the cell cycle and rapidly proliferates, taking on a myofibroblast-like phenotype with extensions and protrusions that resemble a star, hence the term stellate. A major molecular marker of activation includes the appearance of the cytoskeletal proteins — smooth muscle alpha actin (αSMA), and desmin. We now know that portal myofibroblasts, following liver injury, behave in the same way, but differ in that they are neither located in the neither Space of Disse, nor do they share the same lineage tracing

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as do the quiescent HSCs. The activated HSC stains positively for desmin, while the portal myofibroblast does not. Furthermore, the portal myofibroblast appears to be closely associated with fibrotic diseases related to cholestatic and biliary tract injury [91–95]. Following activation into myofibroblasts, HSCs constitute approximately 10–15% of liver mass, and function to promote the net accumulation of dense extracellular matrix (ECM). The myofibroblasts (activated HSC) synthesizes excess amounts of type I, or fibrillar collagen, are resistant to apoptosis, and impede fibrosis resolution by the active secretion of tissue inhibitor of metalloproteinase I (TIMP-1), which in turn prohibits activity of the matrix metalloproteinases (MMPs). Thus, the myofibroblast not only results in net ECM production, but also prevents fibrosis resolution by failing to activate MMPs. In addition, the myofibroblast is resistant to apoptosis driven by up-regulated TIMP-1 activity [19].

Liver myofibroblasts have other unique signaling capabilities, including the ability to respond to transforming growth factor beta one (TGFβ1) as well as plate-derived growth factor (PDGF), the most potent HSC mitogen. The activated HSC also possesses receptors for CCL2, or MCP-1. The myofibroblast, enabled by autocrine and paracrine stimuli, can result in significant injury to the microenvironment of the hepatic sinusoid with excessive ECM deposition resulting in architectural distortion and loss of normal function in liver. A key physiological finding includes profound circulatory derangement of normal portal blood flow which portends development of portal hypertension [96].

Profibrogenic properties of leptin in liver

Leptin is secreted by activated HSCs, but not quiescent cells [20], hence activated HSC-synthesized leptin can function in an autocrine feedback loop. Alternatively, leptin can be secreted via Kupffer cells, other non-parenchymal cells in the liver sinusoid, and trigger HSC signal transduction [29]. Leptin affects the perpetuation phase of the activated HSC life cycle. It also sharply impairs hepatic fibrosis resolution [30] (Box 1). Leptin first is a potent mitogen for HSCs. In both *in vivo* and *in vitro* studies leptin promotes HSCs into the M phase of the cell cycle; and, is nearly as potent a mitogen as PDGF (Fig. 1A). Leptin is also a powerful stimulus to the transcriptional activation of both the $\alpha_1(I)$ and $\alpha_2(I)$ fibrils that are major components of dense, fibrotic ECM (Fig. 1A). It stimulates the transcriptional activation of TIMP-1; and, is chiefly responsible for *de novo* mRNA synthesis of matrix metalloproteinase-2 (MMP-2), tissue inhibitor of metalloproteinase-1 (TIMP-1), TIMP-2, and α-SMA transcripts (Fig. 1A)—all central actors in the pathogenesis of liver fibrosis [31, 32]. Finally, leptin provokes additional HSC protection against apoptosis *in vivo* as assessed by TUNEL staining. *In vitro* assays reveal while tumor necrosis factor alpha apoptosis inducing ligand (TRAIL) can selectively target activated HSCs for apoptosis, leptin renders activated HSCs impervious to TRIAL-mediated apoptosis.

In vivo, ob/ob mice fail to produce leptin but clarify the significance of leptin in hepatic fibrosis development

Lean wild- type mice, compared to *ob/ob* littermates, develop hepatic fibrosis following repeated low dose carbon tetrachloride (CCl$_4$) administration [33, 34]. Leptin deficient

ob/ob mice are resistant to fibrosis development following administration of carbon tetrachloride (CCl$_4$), thus indicating leptin is a requirement for liver fibrosis [31]. As proof-of- concept, when leptin was administered to *ob/ob* mice gavaged with CCl$_4$, *ob/ob* mice were capable of developing liver fibrosis.

Ob/ob mice are a reasonable model of NAFLD and one would think that such mice should be highly sensitive to a fibrotic stimulus such as CCl$_4$. However, bland steatosis—which is a typical finding in most fatty livers in humans, and arguably in *ob/ob* mice, does not develop to fibrosis under basal conditions. The reasons for each are entirely different—not entirely known in humans but can be better explained in the mice since they lack leptin. It is well-known that despite the NAFLD epidemic, only 3–5% of all people afflicted go on to develop significant liver disease due to hepatic fibrosis. In retrospect, early mouse studies demonstrate that leptin is necessary for fibrosis—since *ob/ob* mice do not synthesize leptin —but there are also multiple other factors at play in the genesis of hepatic fibrosis. Adipocytokines, therefore, are not alone in modulating fibrosis, and this is also true in NASH-related cirrhosis.

Additional considerations of leptin as a pro-fibrogenic cytokine

Leptin signaling during liver injury also includes increased release of TGF-β1 from Kupffer cells, macrophages, and endothelial cells, influencing the sinusoidal microenvironment of liver [35, 36]. Leptin down-regulates nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ) [37], which is an anti-fibrogenic nuclear receptor, and has been shown to reverse HSC activation to quiescence [38] (Fig. 1A).

Indirectly, leptin deficiency has been shown to reduce fibrogenesis by decreasing the activity of norepinephrine, in turn leading to decreased activity of natural killer (NK) cells [39]. Decreased NK cell activity is correlated with increased release of profibrogenic cytokines, which would serve to ECM production [40]. Leptin has been shown to inhibit sterol regulatory element binding protein-1 (SREBP-1) expression in vivo and in vitro, resulting in an increase in the expression of alpha (α) collagen in HSCs [41, 42]. Finally, leptin also activates the sonic-Hedgehog pathway and promote HSC activation [27].

In summary, leptin promotes myofibroblast proliferation, migration, vasoconstriction, secretion of ECM molecules, augments actions of key profibrogenic cytokines such as TGFβ1, and up-regulates TIMP-1, both *in vivo* and *in vitro*. Readers should know, however, that leptin is not the only pro-fibrogenic substance that possesses such properties.

Adiponectin as an anti-fibrogenic cytokine and adiponectin biology

Adiponectin is a 28-kDa protein composed of 274 amino acids encoded by the AdipoQ gene. Adiponectin represents approximately 0.05% of the total plasma protein in humans, making it the most abundant adipocytokine synthesized by WAT [43]. Although adiponectin is not secreted by hepatocytes, or hepatic non-parenchymal cells, the two adiponectin receptors (AdipoR1 and 2) are expressed in liver, a major target for adiponectin. Down regulation of adiponectin plasma-levels can predispose the liver to various pathologic processes including steatosis, inflammation, and fibrosis [44]. Compared to other

adipocytokines, serum adiponectin levels are decreased in obesity as well as in fibrosis in patients with NAFLD [45]. Hypo-adiponectinemia is much more likely to be responsible for obesity related NAFLD progression, fibrosis, and potential for treatment[46].

Upstream elements of Adiponectin Signal Transduction

Adiponectin exists in three oligomeric isoforms: low molecular weight (LMW) monomers; hexamers, or middle molecular weight monomers; and the high molecular weight (HMW) oligomeric species which consists of 18 monomers in human circulation [47, 48]. HMW adiponectin is the major bioactive form of adiponectin in mammals and is inversely correlated with hepatic alanine aminotransferase levels [49]. As mentioned above, and shown in Fig. 2, adiponectin conveys biological action in activated HSCs via its own cognate receptors—AdipoR1 and AdipoR2. AdipoR1 is primarily expressed in skeletal muscle and activated HSCs, while AdipoR2 is primarily expressed in other liver cells [31]. AdipoR activation requires downstream activation of adenosine monophosphate kinase (AMPK). It is worth noting however with respect to hepatic fibrosis, the AdipoR1, and its respective adaptor protein phosphotyrosine interaction PH domain and Leucine zipper containing 1 (APPL1) has been shown to play a critical role in the anti-fibrotic actions of adiponectin via a SHP-2 dependent mechanism [50, 51].

Anti-fibrogenic properties of adiponectin

In vitro studies with recombinant adiponectin, adiponectin over-expression in activated HSCs, and *in vivo* studies in adiponectin global knock-out (KO) mice have contributed a detailed understanding of the major anti-fibrotic mechanisms, depicted in Figure 1B. Adiponectin has been shown to reduce HSC activation and proliferation. Additional data demonstrate that adiponectin favors matrix degradation by changing the molecular ratio of MMP-1 to TIMP-1. Finally, both *in vivo* and *in vitro* assays reveal that adiponectin can sensitize activated HSCs to apoptosis. While Fig. 1B demonstrates the potential for adiponectin inducing reversion to HSC quiescence, and senescence, concepts at present of active investigation, however, a role for p53 may be also critical to activated HSC quiescence [52].

Recent findings associate with adiponectin—focal adhesions and integrins

It was recently shown that adiponectin mediates the dephosphorylation of focal adhesion kinase (FAK) through an AdipoR1-pAPPL1 dependent mechanism [53]. In turn, by deactivating FAK, focal adhesions (FAs), critical to maintaining the myofibroblast phenotype, and serving to promote fibrogenesis—are disassembled (Fig. 1B). FAs, juxtaposed to key β integrin proteins, spanning the cell membrane and transmitting mechanical information from the ECM to the HSC signaling systems, consequently become unable to serve as transformers for molecular information and liver stiffness[53]. The role of biomechanics and fibrosis is beyond the scope of this review, but readers are referred to a thorough discussion elsewhere [54]; however mechanical sensing via integrin clustering and FA assembly is relevant to key properties of activated HSCs, which are capable of stretch, and possess contractile properties associated with development of portal hypertension. *In vitro* adiponectin—on increasingly stiffer matrices *in vitro*—has the capacity to prevent

FAK activation [55]. These data were corroborated *in vivo* using adiponectin global KO mice injected with carbon tetrachloride (CCl_4); adiponectin rescue suppressed FA assembly in spite of the CCl_4 stimulus[53].

Interplay in signal transduction between leptin and adiponectin

Figure 2 summarizes the interplay in the signaling cascades between leptin and adiponectin; and, importantly, how adiponectin elicits an anti-fibrogenic response by inhibiting leptin signal transduction. Adiponectin inhibits the leptin-mediated activation of the Jak2/Stat3 pathway via suppressors of cytokine 3 (SOCS3) which serves to deactivate the leptin signaling pathway by preventing phosphorylation of two key tyrosine moieties on OB-Rb (Y985 and Y1138). [31]. Adiponectin, via protein tyrosine phosphatase 1B (PTP1B), dephosphorylates Jak2, the most proximal element in leptin/Ob-Rb signal transduction. Adiponectin also prevents phosphorylation of Stat3. Adiponectin-mediated inhibition of leptin signaling results in down-regulation of TIMP-1 transcription, and TIMP-1 activity. Adiponectin is hypothesized to increase transcriptional activation of MMP-1 and increase MMP-1 protease activity [31, 56]. In short, adiponectin antagonizes the leptin mediated signaling in hepatic fibrosis.

Synthetic adiponectin analogues and nanotechnology, and other recent findings

Recent reports of small adiponectin-like peptides with effective physiologic actions similar to those of naturally occurring adiponectin polymers, are attractive anti-fibrotic agents and currently in pre-clinical and phase I clinical studies. Since adiponectin monomers are 30 kDa, and HMW species can be greater than 300 kDa, the advent of synthetic peptides with *in vivo* therapeutic effects is a promising break through. As recently reported [57], an orally active peptide particle has been produced that has equal binding capacity to AdipoR1 and AdipoR2. This oral agent significantly improved glucose tolerance and insulin resistance in mice fed a high-fat diet; and these beneficial effects were blunted in mice lacking both the AdipoR1 and AdipoR2. Pepping and colleagues used the peptide ADP355 to demonstrate that injection of ADP355 reversed the untoward effects of protease inhibitors for human immunodeficiency virus (HIV)— lipodystrophy [58]. Intra-peritoneal injection of ADP355 peptide conjugate gold (Au) nanoparticles ameliorates hepatic fibrosis in adiponectin KO mice gavaged with carbon tetrachloride [50]. ADP355 was described by Otvos and Surmacz in 2011 [59], and future studies will likely show that small peptides, particularly those which can be ingested, will have a significant impact in anti-fibrosis therapy. Similarly, as an anti-inflammatory molecule, adiponectin has potentially other attractive properties in combating metabolic syndrome disease endpoints [51, 60]. Taken together, adiponectin, and newly related peptide analogues, have been shown to possess anti-fibrotic properties not only in liver but also in the heart, kidney, and skin [61–63]. Phase I clinical studies with adiponectin-like small molecules with highly specific activity may be on the near horizon as anti-fibrosis therapy.

Other adipocytokines relevant to hepatic fibrosis

Plasminogen activator inhibitor-1

Plasminogen activator inhibitor-1 (PAI-1) is a member of the serine protease inhibitor family, which blocks serine protease function of urokinase-type plasminogen activator (uPA), and tissue-type plasminogen activator (tPA). PAI-1 action results in the inhibition of plasminogen-to-plasmin conversion and plasmin-dependent MMP activation. A major function of PAI-1 is to regulate net ECM deposition reflecting a balance between collagen production and degradation [64]. Proteolytic activities are known to be inhibited during liver fibrosis, due to elevated PAI-1 levels that ultimately leads to decreased rates of collagen degradation and enhanced matrix deposition[65]. PAI-1 activity is also regulated by a variety of cytokines and growth factors including TGF-β, IL-1, epidermal growth factor (EGF), and insulin [66]. Therefore PAI-1 KO mice have improved liver histology with less hepatic fibrosis in the bile-duct ligation fibrosis model. Recent studies have shown that down regulation of PAI-1 by use of a PPARα agonist results in the resolution of liver fibrosis via activation of AMPK signaling of the nuclear receptor small heterodimer partner (SHP). [67].

Monocyte chemoattractant protein-1 and Retinol binding protein 4

Monocyte chemoattractant protein-1 (MCP-1) is the best characterized chemoattractant, also known as chemokine 2 (CCL2), that is associated with HSC activation (Box 1), and blockade of the CCL2 receptor conversely results in reduction of liver scarring [68]. CCL2 receptors are known to be present on HSCs and can trigger pro-fibrotic responses in various types of liver injury [69]. A recent, and thorough, review of chemokines and liver injury was published [70]. Retinol binding protein 4 (RBP4) is also produced by adipocytes and has been categorized as an adipokine, though the primary cell for synthesis and secretion is the hepatocyte[71]. RBP4 is known to be a key regulator in hepatic lipogenesis [72], but there is no supporting literature for a role for RBP4 directly triggering liver fibrosis.

Resistin

Resistin, a 12.5 kDa peptide produced by adipose tissue in rodents, possesses metabolic properties related to glucose handling and insulin resistance [73]; however, the source in humans is not clearly known. Resistin is secreted by WAT in rodents but sources for resistin may include bone marrow derived cells and macrophages [74]. A recent paper by Nobili and colleagues indicates that in pediatric NAFLD [75], hepatic progenitor cells were expanded in children with biopsy-proven NASH. Moreover, resistin expression was significantly increased in hepatic progenitor cells, and this increase was highly correlated with the degree of fibrosis.

Recent, *in vitro* and *in vivo* data implicate resistin in the activation of HSCs into myofibroblasts in rats that underwent bile duct ligation, resulting in increased expression of pro-inflammatory adipocytokines, including MCP-1[76]. Investigators also report that resistin has a direct effect on HSCs, demonstrating enhanced Kupffer cell production of TGFβ1 and, in turn, resulting in increased type I collagen [77]. Finally, data in obese adults with known NASH on liver biopsy revealed a significant correlation between resistin

expression in Kupffer cells and HSCs with the patient's obesity. Importantly, this study revealed a strong correlation between hepatic resistin with the degree of fibrosis [78]. These data suggest that resistin may be an independent pro-fibrogenic mediator in hepatic fibrosis secondary to NASH, although the data were derived from small clinical cohorts. More robust molecular explanations relating resistin to hepatic fibrosis need to be demonstrated, including signal transduction events. Importantly, in humans the source of resistin would need to be clarified. Resistin could be an attractive biomarker for patients with NASH who may be likely to have progressive liver disease.

Apelin

Apelin is an adipocytokine encoded by the APLN gene and is a ligand for the G-protein coupled receptor APJ, present on various organs including liver. Levels of apelin and its cognate receptor increase significantly in rat and human cirrhotic liver [79, 80] and appear to be profibrogenic. Importantly, activated HSCs have been shown to be the source for apelin; whereas its receptor is over-expressed on hepatocytes in cirrhotic livers. Overall, apelin and its signaling apparatus appear to be activated in the cirrhotic liver; and, in pre-clinical studies targeting the apelin receptor with the receptor antagonist F13A, resulted in significantly decreased liver fibrosis in rats [81].

Visfatin

Visfatin is an adipocytokine isolated and discovered by Iichiro and colleagues in 2004 [82]. Two recent reports indicate a potential role for visfatin in hepatic fibrogenesis. Nan and colleagues reported that ethanol and CCl_4 treated mice had increased whole liver visfatin mRNA expression, which was suppressed by the PPARα agonist WY14643, and resulted in reduced collagen deposition and αSMA[83]. Finally, *in vitro* studies using cardiac myofibroblasts exposed to excess glucose resulted in increased visfatin mRNA and protein expression that was attenuated with a Rho-associated protein kinase inhibitor. While neither study mechanistically linked visfatin with increased fibrogenesis, the relationship of visfatin to glucose handling and fatty acid metabolism may be crucial in identifying a molecular basis for fibrosis progression in NASH, and be a potential biomarker for NASH disease progression in humans [84].

Chemerin

Chemerin is also known as retinoic receptor responder protein (RARRES2), tazarotene-induced gene 2 protein (TIG2), or RAR-responsive protein TIG2. In humans, mRNA is highly expressed in WAT and liver, but to date its cognate receptor—a G protein coupled receptor—is expressed only on adipocytes and inflammatory cells [85]. While chemerin may be an important future biomarker in human NASH, there is no evidence in the literature that it is directly associated with fibrogenic mechanisms in chronic liver disease, although a recent review indicates that chemerin can be responsible for increased TGFβ1 synthesis [39]. This finding merits further study, however, a human cross sectional study also found that hepatic chemerin mRNA levels were correlated with NAFLD activity histological scores (NAS) on liver biopsy. Furthermore, chemerin mRNA levels were significantly

elevated in patients with definitive NASH; and, linear regression analysis revealed chemerin mRNA levels strongly associated with hepatic fibrosis [86].

Visceral adipose tissue-derived serine protease inhibitor

Visceral adipose tissue-derived serine protease inhibitor (Vaspin) has no direct relationship with hepatic fibrosis to date. Vaspin is a member of the serine protease inhibitor family (serpin) and a recently discovered adipokine. [87]. Because of its recent discovery however, there are discrepancies reported regarding the potential for vaspin in hepatic fibrosis or NASH-related disease progression. One recent review provides evidence that vaspin suppresses leptin and TNFα production [88], which could be beneficial in treating NASH-related fibrosis. However it is premature to conclude that vaspin has a specific role in hepatic fibrosis or mediates NASH pathology.

Concluding remarks and future perspectives

Leptin, adiponectin, MCP-1, PAI-I, and to a lesser extent-apelin and resistin (Table II) were cited in some detail here based on substantial evidence in recently published findings related to respective roles in hepatic fibrogenesis. The newly identified adipocytokines—vaspin, chemerin, and visfatin—are not known yet to be directly associated with mechanisms of mammalian fibrosis. IL-6 and TNF α are, in the strictest sense, not directly involved in modulating hepatic fibrosis either; however, not unlike the new players discussed—visfatin and vaspin—all offer new potential targets in NAFLD. While many patients store hepatic fat, only a small fraction (10–15%) are likely to develop more aggressive hepatic lesions, e.g. steatohepatitis, or NASH. A smaller proportion of NASH patients will go on to develop cirrhosis, and ultimately need liver transplantation. All of these patients are at higher risk for cardiovascular and all-cause mortality [89].

Newly discovered adipokines that promote inflammation will undoubtedly help elucidate the pathogenesis of the NAFLD spectrum since each of these molecules may have an indirect effect on chronic liver injury and fibrosis by influencing the innate and acquired immune systems. Presently, clinicians and biomedical researchers cannot identify patients with NAFLD who are likely to have progressive cardio-metabolic disease, risk for cirrhosis or risk for hepatocellular carcinoma (HCC). Tools including biomarkers and imaging are not yet available to offer predictive signatures of patients that clinicians should be following as opposed to those who have lower risk for adverse outcomes. The liver community has not yet identified a safe, effective, and tolerable anti-fibrotic—which is all the more pressing given the statistics about NASH-related cirrhosis. Development of adiponectin like small peptides packaged in nanoparticles could offer safe and effective anti-fibrotic therapy. In the next several decades, NAFLD progression will compel intensive clinical and basic investigation of anti-fibrosis therapy in an emerging cross-disciplinary approach. The clinical burdens associated with NAFLD and NASH-related fibrosis will only add to the significant morbidity, mortality, and expense burdens already resulting from metabolic syndrome [90].

Acknowledgments

Glossary

Autocrine	the cell is capable of not only secretion of the agonist but has receptors for the same and is thus responsive to cytokines it synthesizes.
Hepatic stellate cell (HSC)	the principle cell that following activation is responsible for laying down extracellular matrix (ECM) in the Space of Disse in the liver. The primary molecule is fibrillar collagen, or type I collagen.. The activated HSC is also called a myofibroblast because its cytoskeleton has contractile (myo-) properties. A key marker of activation is detection of alpha smooth muscle actin. In cirrhosis these contractile properties are important for the development of portal hypertension.
Liver fibrosis	the excessive accumulation of extracellular matrix proteins, including type I collagen that occurs as a result of chronic liver injury. Activated hepatic stellate cells, portal fibroblasts, and myofibroblasts have been identified as major collagen-producing cells in the injured liver. These cells are activated by fibrogenic cytokines. Advanced liver fibrosis results in cirrhosis, liver failure, and portal hypertension, and often requires liver transplantation.
Myofibroblast	a term used to describe either the activated stellate cell, or cells found in the portal areas of the liver. Portal fibroblasts have similar function but may be more involved in biliary tract diseases—they also do not express the protein desmin but do express alpha smooth muscle actin.
Non alcoholic fatty liver disease (NAFLD)	a spectrum of diseases that is characterized by the storage of free fatty acids, or triglyceride in hepatocytes. This is consistent with 'bland steatosis' meaning that fat-storage does not induce inflammation or death.
Non-alcoholic steatohepatitis (NASH)	a disease that is defined by progressive inflammation and injury to hepatocytes, as well as pericellular fibrosis, or 'chicken wiring', this seems to be a significant risk factor developing cirrhosis and is associated in humans with higher cardiovascular risk.
Paracrine	the idea that in liver Kupffer cells, resident macrophages in the liver secrete cytokines that affect nearby activated myofibroblasts to set off biochemical and cellular response, in this case in myofibroblasts.

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Box 2

Outstanding Questions

- What aspects of innate immune function would newly discovered adipocytokines likely play a role? How could deleterious innate immune function, e.g., hepatic NK cells are thought to be protective against fibrosis, but are lost in chronic liver disease be modulated?

- Are adipokines responsible for epigenetic changes that drive organ fibrosis?

- How would a clinical metabolic center (e.g. with endocrinologists, hepatologists, and cardiologists) enhance our ability to identify at-risk patients for NAFLD disease progression and associated risks?

Highlights

- Hepatic fibrosis, though reversible, can if unchecked, lead to cirrhosis.

- Clinical therapy to prevent liver fibrosis progression is not available.

- Cirrhosis poses a significant increased risk for development of hepatocellular carcinoma (HCC.

- Measurements of key cytokines could prove useful as biomarkers of predicting which NAFLD patients are likely to have disease progression.
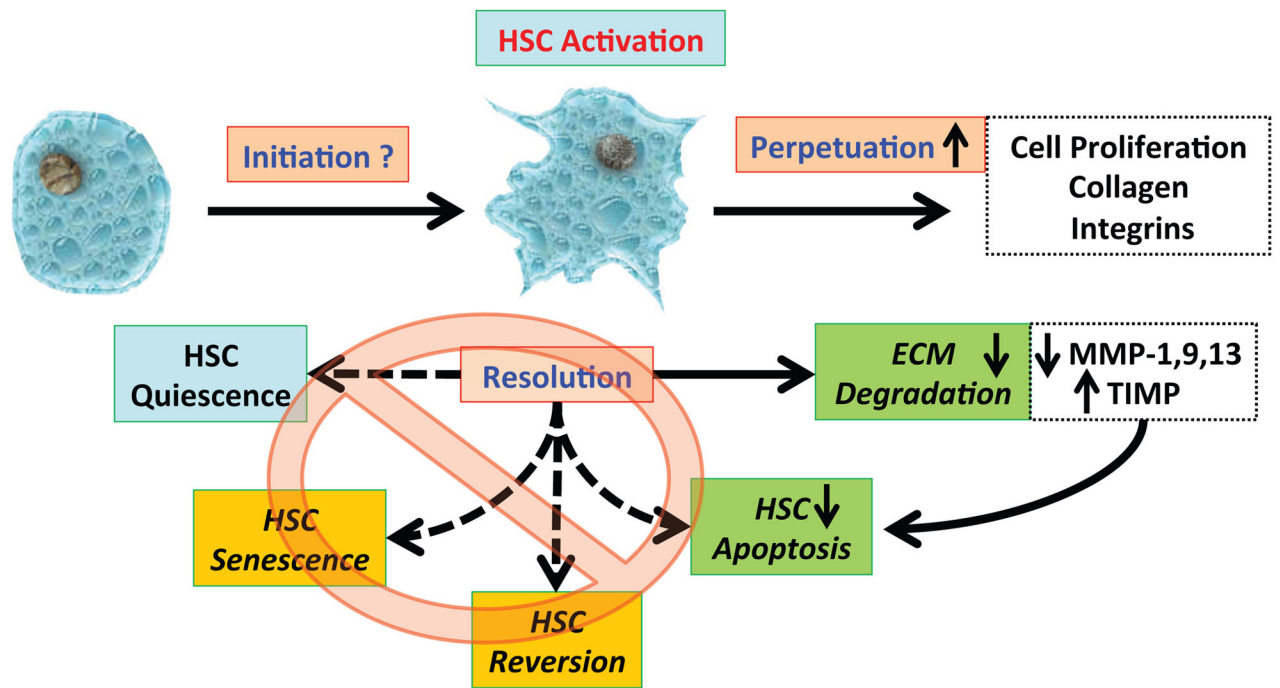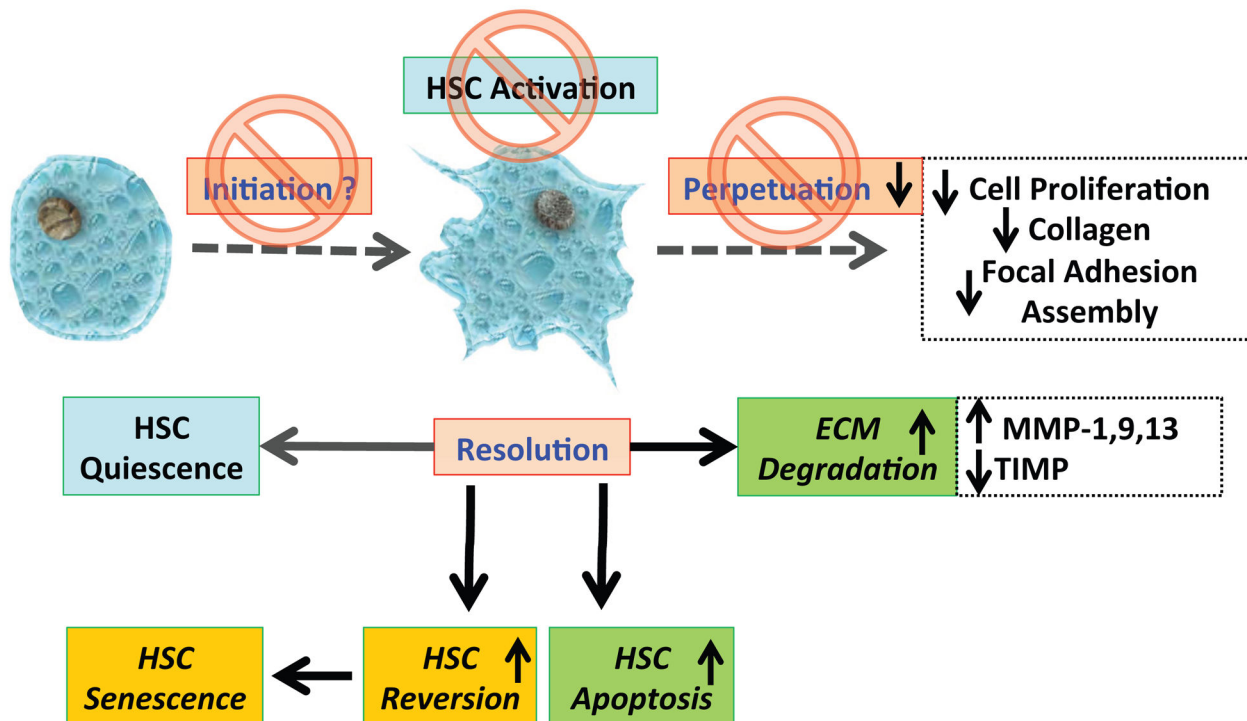
FIG. 1A

FIG. 1B

Figure 1. Role of Leptin and adiponectin in liver fibrosis

A) Leptin acts as a profibrotic adipocytokine in liver fibrosis. The pro-fibrotic role of leptin is mediated via central effector cells of the liver, the hepatic stellate cells (HSCs). All the stages of liver fibrosis; initiation, perpetuation and resolution, are impacted by leptin. Leptin modulates initiation of liver fibrosis by priming quiescent HSCs and transforming them to activated HSCs. In the later stage known as perpetuation, leptin maintains the activated HSC phenotype and increases HSCs proliferation, impedes TRAIL-induced HSC apoptosis, and creates a molecular environment favorable for the net production of extra cellular matrix (ECM). Leptin has also been attributed to inhibit the final stage of liver fibrosis; resolution. Leptin is known to inhibit the expression of matrix metalloproteinase 1 (MMP-1) and increases the expression and activity of tissue inhibitor of metalloproteinase I (TIMP-1) —thereby inhibiting ECM degradation. Finally, leptin prohibits HSC phenotypic reversal or death.

B) Adiponectin is an anti-fibrogenic adipocytokine in liver fibrosis. Adiponectin can block leptin activity by inducing suppressors of cytokine signaling 3 (SOCS3), however, adiponectin has several properties that disengage the HSC and the fibrosis process, independent of other molecules. Adiponectin can induce HSC apoptosis, and results in the loss of alpha smooth muscle (αSMA) proteins in HSCs. Still unknown is whether adiponectin pushes HSCs to partial reversion, or inactivation, or to senescence via a p53 mechanism. This mechanism has been reported to be critical to the resolution and inhibition of hepatic fibrosis from the HSC. Adiponectin also inhibits HSC proliferation and

suppresses alpha collagen biosynthesis. Importantly adiponectin inhibits the transcription of tissue inhibitor of metalloproteinase I (TIMP-1); and inhibits TIMP-1 activity. Conversely adiponectin increases transcription of matrix metalloproteinase (MMP) mRNA as well as increases, *in vitro*, the ability of MMP-1 to degrade fibrillar collagen in matrix. Adiponectin inhibits focal adhesion kinase (FAK) activity and disrupts formation of mature focal adhesions (FA).
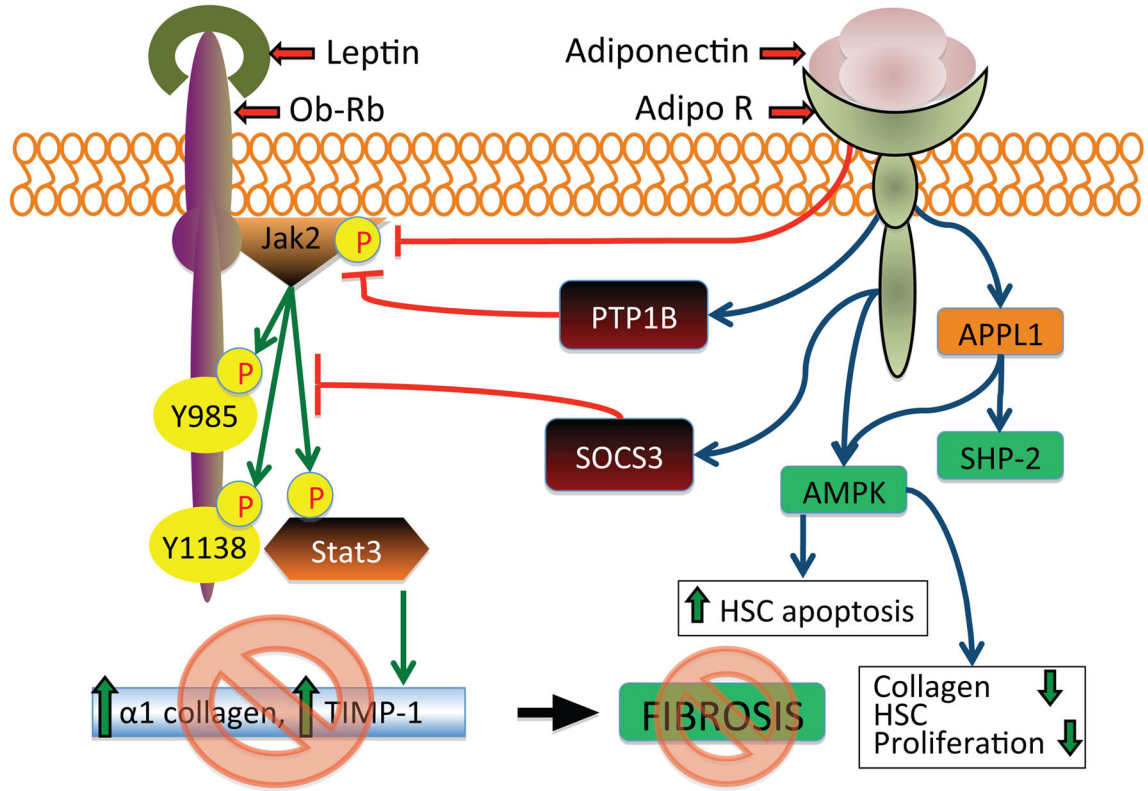
Figure 2. Interplay in signal transduction between leptin and adiponectin
The profibrogenic role of leptin requires leptin-signaling via activation of the long form of leptin receptor (Ob-Rb) and the downstream Jak/Stat pathway. Conversely, adiponectin inhibits leptin signaling via activating protein tyrosine phosphate 1B (PTP1B) and suppressors of cytokine 3 (SOCS3), via the AdipoR2. Adiponectin opposes leptin signaling during the perpetuation stage of liver fibrosis by down regulating tissue inhibitor of metalloproteinase I (TIMP-1) expression and activity, along with concomitant increases in both transcriptional activation of matrix metalloproteinase 1 (MMP-1) and MMP-1 protease activity.

Table 1

Major Adipocytokines and Current Relevance to Hepatic Fibrosis

Adipocytokine	Relevance to Liver Fibrosis	References
Leptin	+ + + + +	1–6, 10–12, 15–28, 40
Adiponectin	+ + + + +	17, 29–31, 35–37, 40–44
Plasminogen Activator I (PAI-1)	+ +	48–51
Monocyte chemoattractant protein-1 (MCP-1)	+	52–53, 60
Retinol Binding Protein 4 (RBP4)	+/−	55, 56
Resistin	+	57–62
Apelin	+	63–65
Visfatin	+/−	66–68
Chemerin	+/−	25, 69, 70
Vaspin	Unknown	71, 72

Table 2

Major Functions of Key Adipocytokines involved in Hepatic Fibrosis

Adipocytokine	Functions in Liver Fibrosis	References
Leptin	Activation of HSCs, Profibrogenic; Increases Proliferation of HSCs and ECM, Activates Kupffer cells, macrophages, endothelial cells.	1–6, 10–12, 15–28, 40
Adiponectin	Inhibits liver fibrogenesis, Impedes activation of HSCs, Anti-fibrogenic; Induces apoptosis of HSCs and degrades ECM. ADP 355 (Adiponectin analogous-synthetic peptide) ameliorates hepatic fibrosis.	17, 29–31, 35–37, 40–44
PAI-1	Profibrogenic as a potent inhibitor of fibrinolytic activity in liver after BDL, protective in CCL4 induced fibrosis.	48–51
MCP-1	Profibrogenic chemokine, recruitment of macrophages, Kupffer cells and modulates HSC activation.	52–53, 60
Resistin	Modulates degree of fibrosis, over expressed in hepatic progenitor cells.	57–62
Apelin	Profibrogenic and pro-inflammatory, over expressed in HSCs of fibrotic livers modulates ECM	63–65