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TNFα in liver fibrosis

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Abstract

Hepatocyte death, inflammation, and liver fibrosis are the hallmarks of chronic liver disease. Tumor necrosis factor-α (TNFα) is an inflammatory cytokine involved in liver inflammation and sustained liver inflammation leads to liver fibrosis. TNFα exerts inflammation, proliferation, and apoptosis. However, the role of TNFα signaling in liver fibrosis is not fully understood. This review highlights the recent findings demonstrating the molecular mechanisms of TNFα and its downstream signaling in liver fibrosis. During the progression of liver fibrosis, hepatic stellate cells play a pivotal role in a dynamic process of production of extracellular matrix proteins and modulation of immune response. Hepatic stellate cells transdifferentiate into activated myofibroblasts in response to damaged hepatocyte-derived mediators and immune cell-derived cytokines/chemokines. Here, we will discuss the role of TNFα in hepatic stellate cell survival and activation and the crosstalk between hepatic stellate cells and hepatocytes or other immune cells, such as macrophages, dendritic cells, and B cells in the development of liver fibrosis.

Keywords

Liver fibrosis; TNFα; hepatic stellate cell; hepatocyte; macrophage

Introduction

Cirrhosis is the advanced stage of liver fibrosis. It causes over 1 million deaths per year, being the 14th leading cause of death worldwide [1]. Liver fibrosis is characterized by the excessive deposition of extracellular matrix (ECM) proteins (e.g. collagen, elastin, and fibronectin), which is a result of hepatocyte death and subsequent liver inflammation [2]. The pathogenesis of liver fibrosis orchestrates the complex interplay of various hepatic cells including hepatocytes, Kupffer cells and hepatic stellate cells (HSCs). HSCs and portal fibroblasts are the major sources of myofibroblasts in the liver, which are involved in production of ECM proteins, ECM degradation, hepatic wound healing, tissue scarring,

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Conflict of Interest

Yoon Mee Yang and Ekihiro Seki declare that they have no conflict of interest.

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fibrosis, and remodeling [3]. A recent fate-tracing study demonstrated that HSCs are the major contributors to liver fibrosis among candidate cellular sources for hepatic myofibroblasts [4]. Hepatocyte death is associated with the development of liver fibrosis, as proven by the fact that mice with specific deletion of anti-apoptotic genes, such as Bcl-xl or Mcl-1, in hepatocytes developed spontaneous hepatocyte apoptosis and liver fibrosis [5, 6]. The apoptosis and/or necrosis of the parenchymal cells may contribute to the activation of HSCs as well as liver macrophages. Activated HSCs and liver resident macrophages recruit other immune cells such as circulating monocytes, T cells and neutrophils by secretion of pro-inflammatory mediators [7].

Tumor necrosis factor-α (TNFα) is a pleiotropic cytokine produced by a variety of immune cells including macrophages/monocytes. TNFα can trigger multiple signaling pathways involved in inflammation, proliferation, and apoptosis. Although TNFα has been implicated in the pathogenesis of chronic liver inflammation that leads to liver fibrosis, the role of TNFα in liver fibrosis has not been fully characterized. The inflammatory phase is perpetuated by TNFα production, which results in the activation of resident HSCs into fibrogenic myofibroblasts. The TNF receptor 1 (TNFR1) knockout mice showed reduced carbon tetrachloride (CCl_4)-induced liver fibrosis [8]. It is no doubt that TNFα can promote fibrosis. However, TNF α is also known to suppress collagen α1(I) gene expression in culture fibroblasts [9]. This review will summarize the current available knowledge of the role of TNFα signaling in liver fibrosis.

TNFα Signaling

TNFα is synthesized as a 26 kDa membrane bound precursor form which is cleaved into its soluble mature form (17 kDa) by TNFα-converting enzyme (TACE) [10]. Both precursor and soluble form of TNFα can transmit their downstream signal through binding to TNFR1 while TNFR2 is preferentially activated by the precursor form [11]. These two types of TNF receptor exert separate or overlapping downstream signal cascades. TNFR1 contains a death domain in the cytoplasmic tail whereas TNFR2 lacks this motif [12, 13]. Trimeric TNFα binding to TNFR1 leads to the receptor trimerization that forms Complex I by recruiting TNFR1-associated death domain protein (TRADD), receptor interacting kinase 1 (RIP1), and several E3 ligases, such as TNF-receptor-associated factor 2 and 5 (TRAF2/5), cellular inhibitor of apoptosis 1/2 (cIAP1/2), and dimeric linear ubiquitin chain assembly complex (LUBAC) [14, 15]. In the Complex I, the Lys63 (K63)-linked polyubiquitination of RIP1 by cIAPs induces the recruitment of the transforming growth factor β (TGF-β)-activated kinase 1 (TAK1)-TAK1-binding protein 1 (TAB1)-TAB2/TAB3 complex. TAK1 binds to RIP1 polyubiquitin chains through TAB2 [16], which is required to recruit the IκB kinase (IKK) complex containing two catalytic α and β subunits, and a regulatory γ subunit, also known as NF-κB essential modulator (NEMO) [17]. Activated TAK1 phosphorylates IKKβ, which in turn leads to phosphorylation and degradation of IκBα. Ultimately, NF-κB homo- or heterodimers released from NF-κB-IκBα complex is translocated into nucleus, followed by the regulation of gene transcription [18]. Phosphorylated TAK1 also activates MAPK pathways by the phosphorylation-dependent manner [19, 20]. In addition, TRAF2 and RIP1 are required for TNFR1-mediated c-Jun N-terminal Kinase (JNK) activation [21]. A20- and cylindromatosis (CYLD)-mediated deubiquitylation of Complex I components induces

disassembly of Complex I. Subsequently either cytosolic Complex IIa or Complex IIb is formed [22]. Complex IIa comprises TRADD, Fas-associated death domain protein (FADD), caspase-8 and RIP1, which mediates activation of caspase cascades, leading to apoptosis [23]. When caspase-8 or FADD is inhibited, Complex IIb consisting of RIP1 kinase and RIP3 is formed, which mediates necroptosis (Figure 1) [24–26]. TNFR2 does not bind to TRADD and FADD, but directly interacts with TRAF2, which in turn recruits TRAF1 [14].

TNFα Signaling in Liver Fibrosis

TACE

TACE cleaves pro-TNFα between Ala76 and Val77, which releases a bioactive soluble 17kDa TNFα. In the diet-induced non-alcoholic steatohepatitis (NASH) models (choline-deficient L-amino acid defined [CDAA] diet and western diet supplemented with high fructose), TACE activity is increased with a concomitant increase in TNFα production and fibrogenic transcripts, such as collagen, α-smooth muscle actin (α-SMA) and TGF-β [27]. Elevated advanced glycation endproducts in diabetic patients may upregulate TACE activity, leading to the progression of NASH through TNFα as well as its multiple target cytokines/chemokines [27].

TNFα

Macrophages play a crucial role in the development of liver fibrosis. HSCs cocultured with hepatic macrophages showed a similar gene expression pattern to *in vivo*-activated HSCs isolated either from bile duct-ligated or CCl_4-treated mice [28]. Macrophage depletion resulted in the attenuation of liver fibrosis [29–31]. Hepatic macrophages profoundly activated NF-κB signaling pathways in HSCs as mediated by TNFα and interleukin-1 (IL-1) [28]. Macrophage-derived TNFα and IL-1 enhanced the survival of HSCs, but had no effect on HSC activation [28].

TNF Receptors

To evaluate the specific involvement of TNF receptors, TNFR1 and TNFR2, in liver fibrosis, genetic modified animals were used [32]. During the 7-day culture activation, the induction of procollagen-α1(I) mRNA levels were inhibited in HSCs isolated from TNFR1 knockout or TNFR1/R2 double knockout (TNFR-DKO) mice, but not from TNFR2 knockouts, indicating that TNFR1, but not TNFR2, is involved in HSC activation [32]. TNFR1 also participates in HSC proliferation elicited by platelet-derived growth factor (PDGF) [32]. Given that PDGF-induced Akt phosphorylation is diminished by knockdown of the p65 subunit of NF-κB (a downstream of TNFR1), a crosstalk between PDGF and TNFR1 receptors may play a role [32]. TNFα can induce matrix metalloproteinase-9 (MMP9) production through TNFR1 in an NF-κB-dependent manner, which may contribute to remodeling of ECM [32]. In mouse liver fibrosis model induced by CCl_4, dimethylnitrosamine, or bile duct ligation, TNFR1 knockout mice showed reduced liver damage and fibrosis, demonstrating the contribution of TNFR1 in liver fibrosis [32–34]. In addition, TNFR-DKO mice had attenuated liver fibrosis in NASH induced by methione-

choline-deficient (MCD) diet [35]. Taken together, TNFR1, but not TNFR2, is important for liver fibrosis development.

Alcoholic hepatitis is characterized by the presence of hepatocellular damage, steatosis and pericellular fibrosis. The microarray transcriptomic analysis of patients with alcoholic hepatitis compared to healthy individuals showed that several TNF superfamily receptors, but not ligands, were upregulated in alcoholic hepatitis patients [36]. Among them, Fn14, the receptor for TNF-like weak inducer of apoptosis (TWEAK), is solely overexpressed in alcoholic hepatitis patients, but not in patients with chronic hepatitis C or NASH [36]. Fn14 is mainly expressed in the parenchymal cells around the areas of fibrosis in patients with alcoholic hepatitis [36]. When high precision-cut mouse liver slices were treated with TGF-β, Fn14 expression was enhanced [36]. These findings suggest that a TNF family member TWEAK and its receptor Fn14 may participate in alcohol-induced liver fibrosis.

TAK1

TAK1, a MAP3K family member, is activated *via* TNFR1 as well as IL-1, TGF-β, and Toll-like receptors (TLRs) [37]. Mice with deletion of TAK1 in hepatocytes developed spontaneous hepatocyte death, inflammation, and liver fibrosis, starting as early as the age of 1 month [38]. TNFα-induced JNK and NF-κB activation is diminished in primary hepatocytes from Tak1$^{flox/flox}$/Alb-Cre (*Tak1*Δ*HEP*) mice [38]. Deletion of TNFRI or TGF-βRII in *Tak1*Δ*HEP* mice ameliorated liver fibrosis, indicating that TNFα and TGF-β receptor signaling drive spontaneous liver fibrosis in the setting of TAK1 inactivation [38, 39]. Spontaneous liver injury and subsequent inflammation in *Tak1*Δ*HEP* mice were caused by Kupffer cell-derived TNFα and TGF-β1, leading to HSC activation and liver fibrosis [38]. TNFα and TGF-β-mediated hepatocyte damage further activated Kupffer cell/macrophages and induced the inflammatory cytokine production in *Tak1*Δ*HEP* mice [39].

CYLD

The tumor suppressor CYLD, a deubiquitinating enzyme, deactivates TNFα-induced NF-κB or JNK signaling by TAK1 inactivation through cleavage of K63-linked polyubiquitin chains [40–45]. Deletion of CYLD exon 9 causes the truncation and inactivation of the carboxyl-terminal deubiquitinating domain of CYLD, which were found in human skin appendage tumors [46]. Liver-specific deletion of CYLD exon9 (CYLD$^{lox/lox}$/ALFP-Cre) induces spontaneous apoptosis of periportal hepatocyte, and subsequent HSC and Kupffer cell activation, thereby triggering fibrosis, inflammation, and TNFα production [46, 47]. In these mice, TNFR1 is required for the expansion of hepatocyte death toward central veins [47]. The expression of the naturally occurring short-CYLD splicing variant is increased in the mice with liver-specific deletion of CYLD exon7/8 (CYLD$^{flox/flox}$/Alb-Cre) [48]. These mice developed severe biliary damage, cholestatic liver fibrosis, and ultimately liver cancer [48].

In fibrotic septa of cirrhotic human liver tissues [49], co-localization of CYLD with α-SMA was observed. During *in vitro* HSC activation, CYLD expression is increased, which is further upregulated in response to TNFα [49]. In addition to TNFα, CYLD also plays an important role in regulation of hepatocyte growth factor (HGF) expression. CYLD can

interact with HDAC7 in the cytoplasm of HSC, which enhances removal of HDAC7 from the HGF promoter to increase HGF gene transcription. This transcriptional regulation of HGF is independent of the deubiquitinating activity of CYLD [49]. Thus, CYLD also contributes to amelioration of liver injury and fibrosis through HGF transcriptional regulation [49]. These findings all support the importance of CYLD in chronic liver disease.

Liver biopsy specimens from cirrhotic patients showed that, in $CD14^+$ macrophages, CYLD levels negatively correlated with the levels of the cytoplasmic Notch intracellular domain (NICD), a marker for Notch activation [50]. In macrophages, CYLD expression is negatively regulated by recombinant signal binding protein Jκ (RBP-J), the main transcription factor of Notch receptor signaling. CYLD plays a role in Notch-RBP-J-mediated liver fibrosis by inhibiting NF-κB activation [50].

JNK

JNK pathway has been implicated in TNFα-mediated liver injury [51]. Recent studies have shown the cell-type-specific functions of JNK in liver pathophysiology. Hepatocyte-derived JNK1 is believed crucial for TNFα-induced hepatocyte apoptosis [52], but is less important in liver fibrosis [53]. JNK activation was observed in the myofibroblasts of human fibrotic/cirrhotic livers [53]. Disruption of JNK1 in primary HSCs inhibited their transdifferentiation into myofibroblasts but induced cell death [53]. The *in vivo* role of HSC-derived JNK1 in the development of liver fibrosis should be reevaluated by using Lrat-Cre mice. In non-stress condition, JNK1 and JNK2 upregulate α-SMA levels in HSCs, but only JNK1 participates in α-SMA upregulation under stress condition induced by TGF-β during liver fibrosis [54]. JNK1 in bone marrow-derived cells contributes to the development of chronic liver inflammation and carcinogenesis [55]. Intriguingly, JNK1 in Kupffer cells promotes CDAA diet-induced liver fibrosis by inducing inflammatory and fibrogenic mediators [56]. However, in CCl_4 and bile duct ligation-induced mouse liver fibrosis, JNK1 in bone marrow-derived cells does not play a role [53].

NF-κB

An NF-κB decoy, a synthetic oligodeoxynucleotide imitating the NF-κB binding site, has potential to suppress CCl_4-induced liver fibrosis, through its anti-inflammatory effects on hepatic macrophages [57]. In primary rat hepatocytes, IKKβ is mainly involved in TNFα-induced NF-κB activation whereas IKKα has a minimal effect. Surprisingly, hepatocyte-specific IKKβ knockout mice did not show the high susceptibility to lipopolysaccharide (LPS)-induced fulminant hepatitis, even though LPS is a strong inducer of TNFα [58]. However, these mice were highly susceptible to concanavalin A-induced liver injury through the membrane-bound form of TNFα, but not by the circulating free TNFα [58]. Hepatocyte-specific deletion of RelA/p65 or NEMO sensitized hepatocytes to TNFα-induced apoptosis [59, 60]. Interestingly, mice with hepatocyte-specific deletion of NEMO developed spontaneous chronic hepatitis, steatosis, liver fibrosis, and HCC [60]. Crossing hepatocyte-specific NEMO knockout mice with $TNFR1^{-/-}$ mice decreased liver fibrogenesis, whereas deletion of TNFR1 in hematopoietic cells enhanced acute and chronic liver injury, suggesting that TNFR1 in hepatocytes and immune cells have opposite roles in liver injury in hepatocyte-specific NEMO knockout mice [61].

RIP1 and RIP3

TNFα-mediated rapid formation of Complex I participates in proinflammatory and anti-apoptotic NF-κB pathways. Subsequently, the Complex IIa (consisting of TRADD, FADD and pro-caspase-8) is formed and mediates TNFα-mediated apoptosis. Alternatively, necroptosis, a programmed necrosis regulated by RIP1 and RIP3 downstream of TNF receptor, is induced through formation of Complex IIb [24–26] when caspase-8 or FADD is inhibited [62]. The treatment of concanavalin A that induces T cell-mediated liver injury increased necrotic liver injury in hepatocyte-specific caspase-8 knockout mice [62]. Hepatocyte-specific caspase-8 and NEMO double-knockout mice showed massive liver necrosis and cholestasis [62]. Caspase-8 deletion inhibited proteolytic cleavage of RIP1, resulting in the formation of FADD-RIP1–RIP3 kinase complexes required for the programmed necrosis and premature activation of NF-κB and JNK [62, 63]. RIP3 is overexpressed in the livers of human NASH patients and MCD diet-fed mice [64]. RIP3-dependent necroptosis accelerates NASH-induced liver fibrosis through a positive feedback loop between RIP3 and JNK, but it has no effect on CCl_4-induced liver fibrosis [64]. Thus, RIP1- or RIP3-dependent necroptosis may promote non-apoptotic liver injury and may contribute to the transition from simple fatty liver to NASH, but does not play a role in toxin-induced liver fibrosis [62, 64].

Role of TNFα in HSCs and other liver cells

HSCs

HSCs constitute ~15% of the resident cell population in normal liver [65]. A novel fate-tracing strategy suggested that HSCs are the primary cells that contribute to liver fibrosis among the various cellular sources that differentiate into myofibroblasts [4]. HSCs can be activated by stimulation with inflammatory and fibrogenic cytokines. The contribution of TNFα in HSC activation and liver fibrosis has been reported in several papers [9, 66–68]. However, their conclusions were mixed. One hand, TNFα treatment suppressed collagen α1 gene expression, apoptosis, and proliferation in activated HSC *in vitro* [9, 66]. On the other hand, TNFα upregulated both matrix metalloproteinases and a tissue inhibitor of metalloproteinase-1 (TIMP1) expression of HSCs [67]. Cholestasis-induced TNFα production might be involved in liver fibrosis progression through TIMP1 induction from HSCs [68]. Furthermore, TNFR1 deficiency inhibited PDGF-induced HSC proliferation, procollagen-α1 expression, and TNFα-induced MMP9 and TIMP1 expression [32]. A recent study demonstrated that TNFα and IL-1 contribute to liver fibrosis through NF-κB-dependent HSC survival rather than myofibroblastic activation [28]. TNFα has pleiotropic effects on HSCs, but overall TNFα is suggested to promote liver fibrosis through its pro-survival effect.

Hepatocytes

Hepatocyte death is the initial event to drive liver inflammation and fibrosis [69]. The proapoptotic stimuli, such as FasL and TRAIL, induce hepatocyte apoptosis. Apoptotic bodies are engulfed by HSC and Kupffer cells, which induce the upregulation of profibrogenic factors (e.g., TGF-β) and death ligands (e.g., TNFα) [70]. Chronic activation of HSC and Kupffer cells further accelerates hepatocyte death and hepatic inflammation,

creating a feedforward loop [70, 71]. TNFα triggers the extrinsic cell death pathway *via* the caspase cascade, but it also affects survival pathway *via* NF-κB activation [69]. TNFα injection alone does not induce hepatocyte death *in vivo*. However, in combination with the inhibition of canonical NF-κB signaling, TNFα induces hepatocyte death and acute liver failure [60, 72]. Hepatitis C virus infection also triggers TNFα-induced cell death through NF-κB inhibition [73]. In addition, LPS injection potentiates TNFα production and accelerates TNFα-induced hepatocyte apoptosis in fatty liver mice induced by MCD diet feeding [74]. Multiple LPS injection also enhances the development of liver fibrosis in MCD-fed mice [74]. Although the underlying mechanism remains to be elucidated, enhanced TNFα-induced hepatocyte apoptosis may be involved in LPS-mediated liver fibrosis in fatty liver disease.

Hence, the dynamic interplay between hepatocytes and activated HSCs through secreted mediators may be involved in the development of liver fibrosis. TNFα treatment induces production of periostin, a secretory profibrogenic protein, in HepG2 cells [75]. Fibroblasts treated with a supernatant collected from TNFα-treated HepG2 cells have increased type 1 collagen expression, which may be through periostin. Moreover, TNFα and IL-17 co-treatment synergistically increase periostin through c-Jun and STAT-3, respectively. Recently, a novel cytokine IL-32 was identified to play a role in HCV-related liver inflammation and fibrosis [76]. The study found a positive correlation between TNFα and IL-32 in hepatitis C patients and increased IL-32 expression in HCV replicating Huh7.5 cells treated with TNFα. These data support the role of TNFα in IL-32-associated HCV infection and its related fibrosis [76].

Macrophages

Activation of hepatic macrophages promotes the development of liver fibrosis through secretion of proinflammatory cytokines and chemokines that activate HSC. $CD68^+$ cells (abundantly expressed in monocytes and macrophages including hepatic resident Kupffer cells) undergo apoptosis in dimethylnitrosamine-induced liver fibrosis in rats [77]. $CD68^+$/$TNFα^+$ cells reside close to $α\text{-}SMA^+$ HSCs in hepatic lobules [77]. Hepatic macrophage-derived TNFα contributes to NF-κB-dependent HSC survival, thereby promoting liver fibrosis [28]. However, hepatic macrophages seem no effect on myofibroblastic activation in HSC [28].

It has been reported that chemokines and their receptors participate in the development of liver fibrosis. For example, C-C motif chemokine receptor (CCR) 9^+ macrophages play an important role in the pathogenesis of liver fibrosis [78]. Among mononuclear liver cells, TNFα-producing $CCR9^+$ cells are infiltrated in acute liver injury induced by CCl_4. CCR9 knockout mice showed protection against CCl_4-induced liver fibrosis. TNFα neutralization using anti-TNFα antibody inhibited HSC activation mediated by $CCR9^+$ macrophages [78], indicating that TNFα is a key cytokine in CCR9-mediated liver fibrosis.

Dendritic cells

Hepatic dendritic cells represent one fourth of the fibrotic leukocytes and TNFα dramatically induce proinflammatory and immunogenic activity of dentritic cells in liver

fibrosis [79]. Fibrotic liver-derived dendritic cells co-cultured with normal liver-derived HSCs induced various cytokines (e.g., IL-1α, IL-6, G-CSF, GM-CSF, IL-13, LIF, and MCP-1) and chemokines (e.g., MIP-1α, MIP-1β, MIP-2, KC, and MIG) and TNFα blockade partially blocked the production of these cytokines and chemokines [79]. However, $CD11c^+$ dendritic cells modestly activated NF-κB in HSCs, and depletion of dendritic cells had no significant effect on BDL- and CCl_4-induced liver fibrosis [28]. This study suggests the minor role of dendritic cells in liver fibrosis.

B cells

Hepatic $CD19^+$ B cells are increased in CCl_4-treated mouse livers [80]. HSC-derived retinoic acids are essential for B cell survival. B cell-deficient mice (μMT mice) protected against CCl_4-induced liver HSC activation and fibrosis. Hepatic B cells from fibrotic mice can stimulate the production of inflammatory cytokines and chemoattractants, including TNFα. *In vivo* roles of MyD88-mediated innate immune signaling in B cells were examined by generating B cell specific-MyD88-deficient mice. After 6 weeks of CCl_4 treatment, these mice showed a significant reduction of liver fibrosis. Moreover, liver B cells from fibrotic B cell specific-MyD88-deficient mice produced less proinflammatory cytokines (e.g., TNFα). This study demonstrated that hepatic B cells can amplify the fibrogenic response by secretion of inflammatory cytokines and chemoattractants through intrinsic MyD88 signaling [80].

Clinical Implications and Conclusions

TNFα plays an important role in proinflammatory response and cell-to-cell communication. TNF signaling is closely associated with various autoimmune and inflammatory diseases. Until now, five drugs targeting TNF have been developed: infliximab, etanercept, adalimumab, glomumab, and certolizumab pegol [81]. The indications of these TNF-targeting drugs have been approved for rheumatoid arthritis, psoriatic arthritis, psoriasis, ankylosing spondylitis, juvenile idiopathic arthritis, Crohn's disease, and ulcerative colitis [82]. Owing to the remarkable successes, the pharmaceutical industries are undergoing to develop new drugs targeting TNFR signaling, and these drugs have been tested in clinical trials in various diseases.

Liver fibrosis is a consequence of chronic liver injury and inflammation. TNFα is a key player involved in both chronic liver injury and inflammation, but the role of TNFα in liver fibrosis is complicated and the conclusions were still controversial. Recent studies have provided a better understanding of TNFα signaling in liver fibrosis. TNFα enhances HSC survival, hepatocyte death, and immune cell activation, which are associated with enhanced liver fibrosis (Figure 2). There is currently no ongoing clinical trial of TNF inhibitors for the treatment of liver fibrosis. Due to its pleiotropic effect, TNF/TNFR-targeted drugs occasionally have serious adverse events, such as lymphomas, lupus-like syndrome, and cutaneous or systemic vasculitis [82]. Importantly, the clinical trials using anti-TNFα antibody have been failed in alcoholic hepatitis [83]. Therefore, targeting of specific TNFα signaling pathways should be carefully considered as new therapeutic approach for the liver fibrosis.

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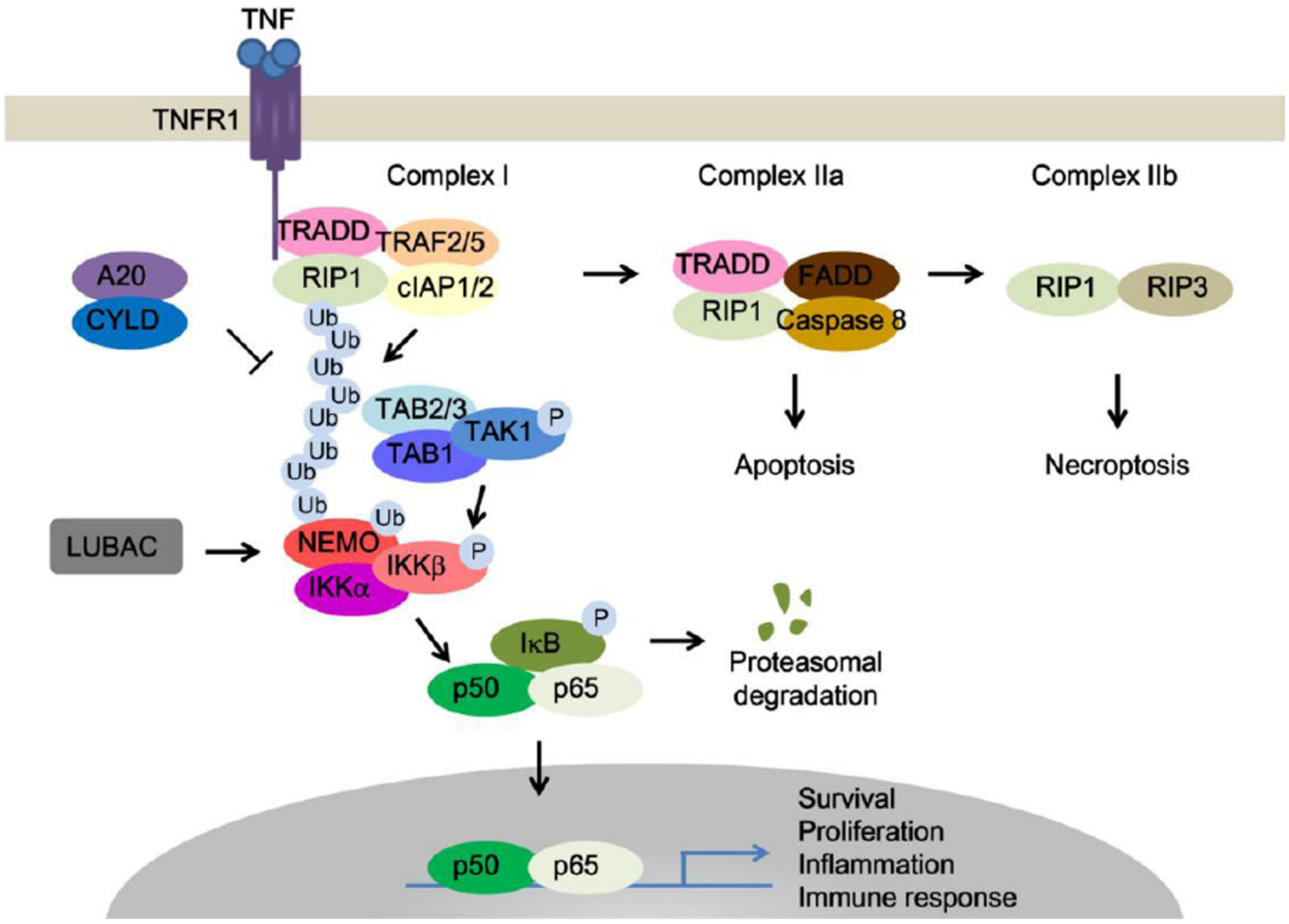

Figure 1. Activation of TNF signaling

TNFα binding to TNFR1 causes Complex I formation by recruitment of TRADD, RIP1, TRAF2/5, cIAP1/2, and LUBAC. cIAP1/2 promotes K63-linked polyubiquitination of RIP1, whereas A20 and CYLD deubiquitinates RIP1. In the Complex I, K63-linked polyubiquitination of RIP1 mediates TAK1-TAB1-TAB2/3 complex recruitment. TAK1 is responsible for the phosphorylation and activation of IKK complex, resulting in IκBα degradation and NF-κB-mediated gene transcription. Inactivation of cIAP1 or deubiquitination of RIP1 by CYLD facilitates Complex I transition to Complex II. Complex IIa consists of TRADD, RIP1, FADD and caspase-8, which induces apoptosis. Inhibition of caspase-8 or FADD causes the formation of Complex IIb, ultimately leading to necroptosis.

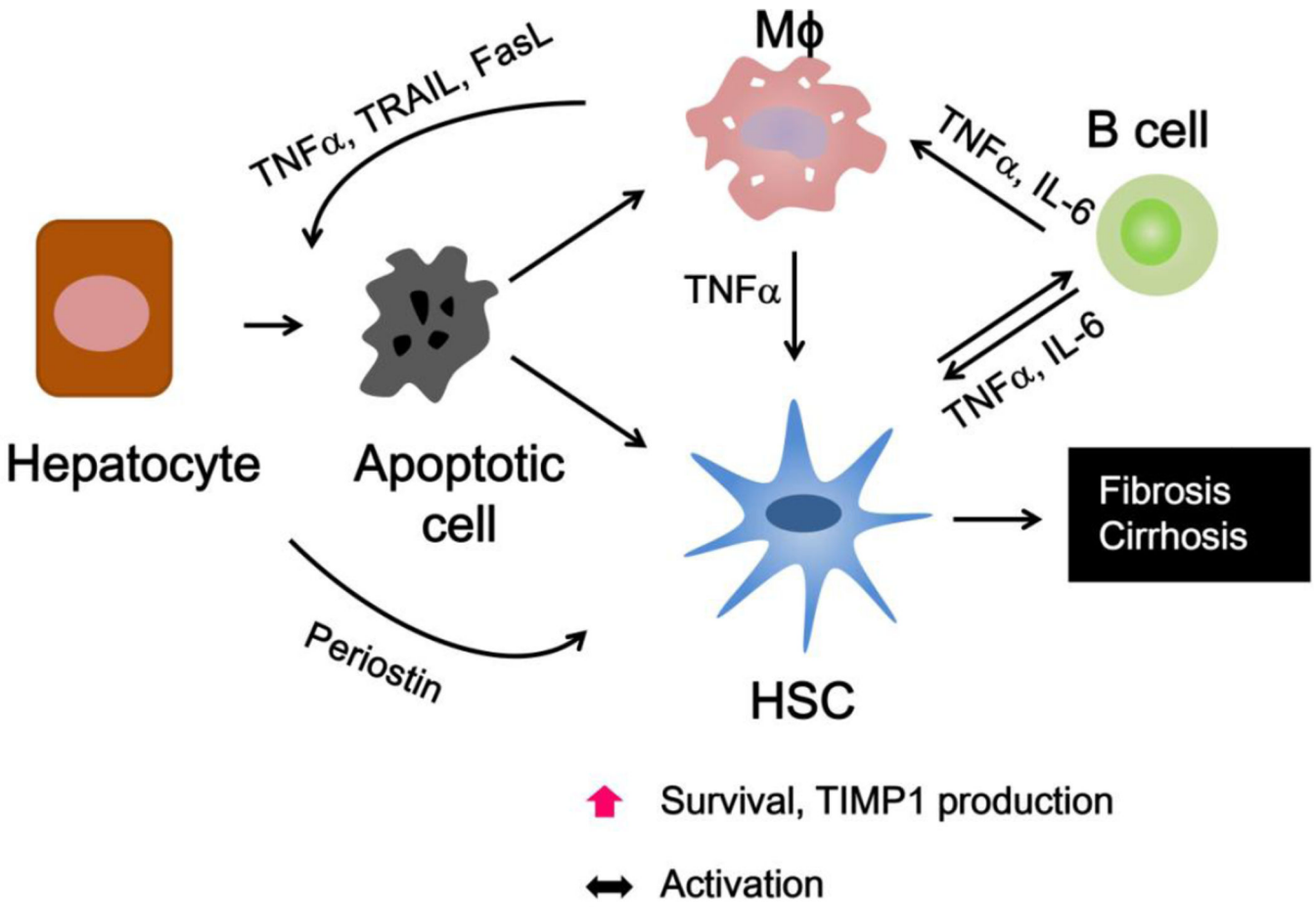

Figure 2. Overview of TNFα-mediated liver fibrosis

TNFα augments HSC survival, but not activation. Hepatocyte apoptosis results in the engulfment of apoptotic bodies by macrophages and HSCs. It enhances the production of death ligands (e.g., TNFα, TRAIL and FasL) by macrophages, which further stimulates hepatocyte death. Engulfment of apoptotic bodies by HSCs increases the profibrogenic responses. TNFα-treated hepatocytes produce periostin, which can mediate collagen production in HSCs. HSCs also promote B cell survival. In fibrotic liver, B cells produce proinflammatory cytokines and chemoattractants (e.g., TNFα, IL-6, MCP-1, and MIP-1α), which can accelerate liver fibrosis.