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A Liver Full of JNK: Signaling in Regulation of Cell Function and Disease Pathogenesis, and Clinical Approaches

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Abstract

c-Jun-N-terminal Kinase (JNK) is a mitogen-activated protein kinase (MAPK) family member that is activated by diverse stimuli, including cytokines (such as tumor necrosis factor and interleukin-1), reactive oxygen species (ROS), pathogens, toxins, drugs, endoplasmic reticulum stress, free fatty acids, and metabolic changes. Upon activation, JNK induces multiple biologic events through the transcription factor AP-1 and transcription-independent control of effector molecules. JNK isozymes regulate cell death and survival, differentiation, proliferation, ROS accumulation, metabolism, insulin signaling, and carcinogenesis in the liver. The biologic functions of JNK are isoform, cell-type, and context dependent. Recent studies using genetically engineered mice showed that loss or hyper-activation of the JNK pathway contributes to the development of inflammation, fibrosis, cancer growth, and metabolic diseases that include obesity, hepatic steatosis, and insulin resistance. We review the functions and pathways of JNK in liver physiology and pathology, and discuss findings from pre-clinical studies with JNK inhibitors.

Keywords

MAPK; hepatocellular carcinoma; insulin resistance; c-Jun; TNF; acetaminophen

Introduction

c-Jun-N-terminal Kinase (JNK) is a mitogen-activated protein kinase (MAPK) family member. There are 3 isoforms of JNK in mammals: JNK1, JNK2, and JNK3 (encoded by *MAPK8, MAPK9, and MAPK10*, respectively). JNK1 and JNK2 are expressed in almost all

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cells, including liver parenchymal cells, whereas JNK3 is mainly expressed in brain, heart, and testis[1,2]. At least 10 alternative splicing variants are known, which increase the diversity of JNK proteins, but their functional significance is unclear. The JNK proteins, including splicing variants, range from 46 kDa to 55 kDa in size. JNK's enzymatic activity is induced in response to diverse stimuli, such as cytokines (tumor necrosis factor [TNF], interleukin-1 [IL-1], transforming growth factor β [TGFβ], platelet-derived growth factor [PDGF], and epidermal growth factor [EGF]), intra- and extracellular pathogens (lipopolysaccharide [LPS], peptidoglycan, and bacterial unmethylated CpG-DNA that activates Toll-like receptors [TLRs]), reactive oxygen species (ROS), pathologic and environmental stress (ischemia, hypoxia, and ultraviolet and ionizing radiation), toxins, drugs, endoplasmic reticulum (ER) stress, and metabolic changes, including obesity and hyperlipidemia.

The JNKs are activated via 3-tiered signaling modules comprising MAP kinase kinase kinases (MAP3Ks or MKKKs), MAP kinase kinases (MAP2Ks or MKKs), and MAP kinases (MAPKs, in this case the JNKs). At least, 14 different MAP3Ks have been found to activate JNK (see Figure 1A). The best-characterized MAP3Ks are mixed-linage kinase 3 (MLK3), MEKK1, and TAK1. The 2 MAP2Ks (MKK4 and MKK7) phosphorylate JNKs at threonine and tyrosine residues within a conserved dual phosphorylation Thr-Pro-Tyr motif in their activation loop[3]. MKK7 primarily activates JNK, but MKK4 activates JNK and p38[4]. MKK7 is specifically associated with cytokine-induced JNK activation, through phosphorylation of the Thr residue of JNK[4]. The JNKs have a common substrate docking site in their C-terminus and a glutamateaspartate domain in their N-terminus that is the site of protein–protein interactions with MAP2Ks, phosphatases (e.g. JNK dual-specificity phosphatases), and substrates (Figure 1B)[3,4].

At least 50 proteins have been identified as JNK substrates. These proteins control multiple cellular processes, acting either as transcription factors or by controlling protein degradation, localization, and signaling. JNK substrates include c-Jun, JunB, JunD, activating transcription factor 2 (ATF2), p53, c-Myc, serum response factor (SRF), Itch, insulin receptor substrate-1 (IRS-1), JNK interacting protein 1 (JIP1), 14-3-3, Sab (SH3BP5), Bcl-2, Bcl-xL, Bid, Bim, Bad, Bax, and Mcl-1[1,5]. Among these substrates, c-Jun is a representative target of JNKs. c-Jun dimerizes with JunB, JunD, or Fos to form the transcription factor activator protein (AP)-1, whereas SRF controls expression of the Fos proteins that dimerize with the Jun proteins[6].

One of the best-studied pathways that leads to JNK activation is TNF signaling via TNF receptor1 (TNFR1) (Figure 2). Upon binding of a TNF trimer to trimerized TNFR1, the intracellular potion of TNFR1 recruits the adaptor TRADD through homotypic interaction between their death domains, leading to formation of an intracellular signaling complex that includes RIP1, cellular inhibitor of apoptosis 1 (cIAP1), cIAP2, and TRAF2, termed complex I[7,8]. With K63-polyubiquitination of RIP1 by cIAP1 and cIAP2, complex I recruits, phosphorylates, and ubiquitinates the MAP3Ks TAK1, MEKK1, or MLK3, which in turn activate MKK4 and MKK7, leading to activation of JNK and other downstream effectors including IκB kinase (IKK)[1,7]. In the second step, K63-polyubiquitination of RIP1 is removed by the deubiquitinases cylindromatosis (CYLD) or A20, leading to dissociation of the TRADD–RIP1–TRAF2 complex from TNFRI[8]. The dissociated cytosolic complex binds to FADD, caspase-8, RIP1, and RIP3 to form complex II, which contributes to programmed cell death, including apoptosis and necrosis[8–10]. In complex II, caspase-8 cleaves RIP1 and RIP3 to prevent necrotic death, promoting the induction of apoptosis[8–11]. When caspase-8 or FADD is inhibited, RIP1 and RIP3 prevent apoptotic death, shifting complex II activity towards necrosis[8,11]. TNF-induced ROS is generated by NADPH oxidase 1 (NOX1) and Rac1, which are recruited to complex I in a TRADD- and RIP1-dependent manner and by mitochondrial respiratory complex I. ROS accumulation promotes prolonged activation of

JNK by inactivating JNK phosphatases and TNF-mediated necrosis[12,13]. TNF-induced ROS accumulation and prolonged JNK activation are suppressed by nuclear factor (NF)-κB–mediated sequestration of ROS and induction of c-FLIP[12,14,15]. Initial TNFRI-mediated JNK activation is transient and associated with cell survival and proliferation through AP-1, whereas sustained JNK activation and ROS accumulation are associated with apoptotic and necrotic cell death[1,7,16,17]. Moreover, interactions among JNK, p38, and IKK–NF-κB pathways regulate transient and sustained activation of JNK. Upon inactivation of p38 or IKKs, TNF stimulation induces prolonged activation of JNK[17–21].

JNK signaling is associated with cell death, survival, differentiation, proliferation, and tumorigenesis in hepatocytes. In nonparenchymal liver cells, such as hepatic macrophages (Kupffer cells) and hepatic stellate cells (HSCs), JNK is involved in inflammation and fibrosis. Studies of cell-specific ablation (by the Cre/lox-P strategy) and bone marrow chimeras have identified the specific functions of JNK in distinct cell types and organs and in the interactions between the liver and other organs[22–24]. Moreover, the distinct functions of the isoforms JNK1 and JNK2 in pathogenesis of liver diseases have been characterized in *Jnk1*$^{-/-}$ and *Jnk2*$^{-/-}$ mice. We review recent advances in our understanding of the role of the JNK and/or c-Jun pathways in different types of liver injury (TNF-induced liver injury, fibrosis and carcinogenesis, and steatohepatitis) from studies of genetically engineered mice and human cells and tissues.

TNF-Mediated Hepatocyte Death and Liver Injury

In hepatocytes, TNF rapidly activates JNK, leading to phosphorylation and activation of AP-1/c-Jun[25]. Simultaneously, TNF activates NF-κB via the IKK complex and induces expression of anti-apoptotic genes that are regulated by NF-κB and block caspase-8–dependent cell death and prolonged activation of JNK[25]. Inhibition of de novo protein synthesis or NF-κB activation sensitizes hepatocytes to TNF-induced hepatocyte death as a result of sustained JNK activation[12,26]. Caspase or JNK inhibitors prevent TNF-mediated hepatocyte apoptosis[26]. Sustained JNK activation increases ROS accumulation, which further promotes JNK activation through oxidative inhibition of JNK dual-specificity phosphatases[12]. JNK-mediated apoptosis seems to depend on ROS production but is independent of AP-1[26]. Conversely, NF-κB activation inhibits ROS accumulation and excessive ROS attenuate NF-κB activation[12,14]. TNF-induced hepatocyte death is prevented by *Jnk1* disruption [15]. TNF-mediated activation of JNK1 phosphorylates and activates the E3 ubiquitin ligase Itch, which contributes to the K48-linked ubiquitination of c-FLIP, an NF-κB–induced anti-apoptotic molecule (an endogenous caspase-8 inhibitor), to promote activation of caspases-8 and -3 and apoptosis of hepatocytes (Figure 2). Loss of Itch prevents ubiquitination and degradation of inducible c-FLIP, which abrogates TNF-induced liver injury[15]. However, mice deficient in only JNK2 are resistant to D-galactosamine(GalN)/LPS or GalN/TNF-mediated liver injury, indicating that JNK2 activation is involved in caspase-8 activation, Bid cleavage, and mitochondrial cytochrome release in hepatocytes[27]. A subsequent study demonstrated that in *Jnk2*$^{-/-}$ hepatocytes, TNF overactivates JNK1, indicating that JNK1 activation protects *Jnk2*$^{-/-}$ mice from GalN/TNF-induced liver injury[28]. JNK1 activation in *Jnk2*$^{-/-}$ hepatocytes stabilizes the anti-apoptotic protein Mcl-1 to prevent its degradation and block TNF-mediated apoptosis[28]. The function of Mcl-1 in *Jnk2*$^{-/-}$ mice has been confirmed by induction of TNF-mediated liver injury in *Mcl-1*$^{-/-}$*Jnk2*$^{-/-}$ (double knockout) mice[28]. In wild-type hepatocytes, JNK1 is proapoptotic, but in *Jnk2*$^{-/-}$ cells, JNK1 might be anti-apoptotic, via stabilization of Mcl-1.

Recruitment of activated JNK to the outer membrane of mitochondria is another important step in induction of JNK-mediated hepatocyte death. Mitochondrial Bcl-X_L, Mcl-1, and Sab are substrates for JNK[29]. TNF induces recruitment of phosphorylated JNK and MKK4 and

the Bcl-2 family member Bax to mitochondrial outer membrane, which promotes generation of mitochondrial ROS and sustained activation of JNK, inducing hepatocyte death[29]. Knockdown of Sab initially activates JNK, but prevents accumulation of JNK, MKK4, and Bax at the mitochondrial outer membrane and sustained activation of JNK, thereby inhibiting TNF-induced hepatocyte death. These findings indicate the important roles of Sab and recruitment of JNK to mitochondrial outer membrane in sustaining activation of JNK[29]. They also indicate that a mitochondrial activation loop that produces ROS, rather than only continuous TNFR signaling, sustains JNK activation. Upon TNF stimulation, Bid, a BH3-only protein, is cleaved by caspase-8 in a JNK2-dependent manner[27,30]. The cleaved Bid translocates to the mitochondrial outer membrane to induce cytochrome-c release and caspase-9/caspase-3 activation, resulting in TNF-mediated hepatocyte death[27,31]. The requirement for Bid in hepatocyte death has been demonstrated by the resistance of *Bid*$^{-/-}$ mice to TNF-induced liver injury[30].

Although many biological functions induced by JNK are mediated through c-Jun activation, TNF-mediated hepatocyte apoptosis is JNK-dependent, but c-Jun- and transcription-independent[26,32]. c-Jun seems to protect against rather than induce cell death[33].

In concanavalin A (ConA)-induced liver injury, membrane bound TNF, rather than soluble TNF, is important. Membranous TNF binds to TNFRI and TNFRII, and, given the ability of TNFRII to activate JNK and not NF-κB, leads to strong activation of JNK, thereby promoting hepatocyte death. *Jnk1*$^{-/-}$ and *Jnk2*$^{-/-}$ mice are protected from ConA-induced liver injury[19]. Because IKK signaling to NF-κB prevents JNK activation, ConA-induced liver injury is exacerbated in mice that lack hepatocyte IKKβ (*Ikkβ*$^{\Delta hep}$), in which JNK activation is potentiated[19]. However, a recent study demonstrated that compound deletion of *Jnk1* and *Jnk2* from hepatocytes did not impair ConA- or LPS-induced liver injury[22]. Notably, hematopoietic deficiency in JNK1 and JNK2 prevented ConA-induced liver injury, suppressing TNF production[22]. These findings indicate that under certain conditions, JNK activation in hematopoietic cells is required for optimal production of TNF, which is essential for ConA-induced hepatitis. It is possible that JNK2 is required to protect *Jnk1*$^{-/-}$ hepatocytes from cell death, so hepatocytes with combined disruption of *Jnk1* and *Jnk2* are no longer protected. Interestingly, c-Jun is important in hepatocytes, rather than hematopoietic cells, where it negatively regulates ConA-induced hepatitis[34]. Loss of c-Jun aggravated ConA-induced liver injury and suppressed Nos2 expression. Liver-specific supplementation of NO attenuated overt ConA-induced liver injury in mice with hepatocyte-specific deletion of c-Jun (*c-Jun*$^{\Delta hep}$)[34]. *c-Jun*^{AA} mice, which have mutations in c-Jun at sites phosphorylated by JNK, did not have this phenotype, indicating that inducible expression of Nos2 requires c-Jun but does not require phosphorylation of c-Jun[34].

TRAIL induces cell death in cancer cells that express its receptor, and JNK inhibition sensitizes HCC cells to TRAIL-induced cell death[35]. In contrast, primary hepatocytes do not undergo apoptosis by TRAIL, but TRAIL amplifies Fas-induced death[36]. In mice, Fas-mediated hepatitis is associated with strong activation of JNK, leading to phosphorylation of Bim and its translocation to the mitochondrial outer membrane, causing release of cytochrome c and activation of the mitochondrial caspase cascade[36]. *Trail*$^{-/-}$ mice are protected from Fas-mediated fulminant hepatitis, and have reduced activation of JNK and no translocation of Bim to mitochondria[36]. Bim deficiency also prevented Fas-induced hepatitis[36]. These findings indicate that TRAIL is required for activation of JNK and Bim in Fas-mediated liver injury. In summary, JNK has dual roles in TRAIL signaling. In normal hepatocytes, JNK is required for TRAIL-mediated cell death. On the contrary, in HCC cells, JNK prevents TRAIL-mediated cell death.

Ischemia/Reperfusion (I/R) Liver Injury

I/R liver injury is a serious clinical complication following liver transplantation, surgical resection of liver tumors, and circulation shock. Hepatic I/R injury is characterized by hepatocyte necrosis and apoptosis induced by multiple mediators, including TNF, ROS, and intracellular signaling via JNK-dependent pathways. I/R liver injury causes JNK1 activation and subsequent increases in AP-1 activity during the reperfusion phase. Overexpression of superoxide dismutase 2 (SOD2) decreases AP-1 activity, indicating that I/R-mediated oxidative stress contributes to activation of JNK and AP-1 and I/R liver injury[37]. Specific inhibitors of JNK prevented c-Jun phosphorylation, AP-1 activation, Bak induction, Bid degradation, caspase-3 activation, and mitochondrial cytochrome c release, eventually attenuating hepatocyte necrosis and apoptosis after I/R or liver transplantation[38,39]. Absence of JunD, another AP-1 component, increased I/R liver injury, along with phosphorylation of c-Jun, activity of AP-1, and expression of NOX2 and NOX4, which was suppressed by overexpression of a dominant negative form of JNK1[40]. JunD therefore appears to regulate c-Jun activity and ROS generation in I/R liver injury; this pathway is controlled by JNK1. Moreover, *Jnk2*$^{-/-}$ mice had reduced I/R liver injury and increased expression of HO-1. Inhibition of HO-1 blocked the protective effect of *Jnk2* disruption, indicating a role for HO-1 in protecting *Jnk2*$^{-/-}$ mice from I/R injury[41]. JNK2 also increases the mitochondrial permeability transition, resulting in hepatocellular injury after I/R[42,43]. Interactions between the IKK–NF-κB and JNK–AP-1 pathways are also important in I/R injury. Hepatocyte-specific deficiency in IKKγ/NEMO (*Nemo*$^{\Delta hep}$ mice) have a greater level of I/R liver injury and stronger activation of JNK, indicating that lack of protective IKK–NF-κB signaling and increases in the apoptotic JNK pathway promote I/R liver injury[44].

Acetaminophen-Induced Liver Injury

Acetaminophen overdose is the most common cause of drug-induced acute liver failure in the United States. Acetaminophen is converted to N-acetyl-p-benzoquinone imine (NAPQI) by cytochrome P-450-CYP2E1, which is expressed in zone 3 hepatocytes. Metabolic inactivation of NAPQI requires its binding to glutathione. However, excess NAPQI generates ROS that further deplete glutathione and cause mitochondrial dysfunction, DNA damage, and large amounts of necrosis and apoptosis among hepatocytes.

Exposure of hepatocytes to acetaminophen activates JNK and leads to subsequent translocation of JNK and Bax to mitochondrial outer membrane, induction of the mitochondrial permeability transition, generation of ROS, and cell death (Figure 3)[45]. Disruption of *Jnk2*, but not *Jnk1*, prevented acetaminophen-induced liver injury, indicating the importance of JNK2 in this form of hepatoxicity[45]. JNK translocation to the mitochondria is required for acetaminophen-induced hepatotoxicity. Phosphorylated JNK and MKK4, but not apoptosis signal-regulating kinase 1 (ASK1) or MKK7, translocate to mitochondria when hepatocytes are exposed to acetaminophen [29]. Blocking this translocation by silencing Sab suppressed prolonged activation of JNK and acetaminophen-induced death of hepatocytes. Therefore, MKK4-mediated activation of JNK and Sab-mediated recruitment of JNK to the mitochondrial outer membrane (which sustains JNK activation) are required for acetaminophen-induced hepatocyte death (Figure 3)[29].

Bax$^{-/-}$ mice have reductions in only the early phase of acetaminophen-induced liver injury, indicating that additional factors are required for complete acetaminophen-induced liver injury[46]. Acetaminophen exposure induced JNK-dependent expression of the pro-apoptotic factor Bim in hepatocytes. *Bim*$^{-/-}$ mice are protected from acetaminophen liver injury[47]. Bim expression is amplified by TRAIL, and acetaminophen-induced liver injury is inhibited in *Trail*$^{-/-}$ mice, indicating a role for a TRAIL–JNK–Bim pathway in acetaminophen-

induced hepatocyte death[47]. Although JNK activation is required for mitochondrial dysfunction, the initial activation of JNK is induced by mitochondrial generation of ROS, which results from mitochondrial depletion of glutathione in response to acetaminophen[48]. Although ASK1 does not translocate to mitochondria, this factor is important for JNK activation in acetaminophen-induced liver injury[49]. ASK1 deficiency protected mice from acetaminophen-induced liver injury and reduced JNK activation[49], indicating that ASK1 acts upstream of MAP3K in this process.

The specific JNK inhibitors SP600125 and leflunomide rescued mice from acetaminophen-induced liver injury[45,50]. These reagents might therefore be developed for treatment of patients with acetaminophen-induced liver injury.

ER Stress and Hepatocyte Death

The ER is the major site of protein folding, maturation, and trafficking. When unfolded or misfolded proteins accumulate in the ER, the ER becomes stressed and the protective unfolded protein response (UPR) is activated. The UPR involves activation of 3 ER membrane sensors: ATF6α, inositol-requiring enzyme (IRE)1α, and PKR-like ER localized kinase (PERK)[51,52]. These sensors activate signaling pathways that induce molecules that reduce ER stress. Among the ER sensors, IRE-1α and the PERK-associated metaflammasome (which comprises PKR and eIF2) contribute to activation of JNK and IKK–NF-κB. IRE-1α interacts with TRAF2 to activate JNK through ASK1, which is implicated in ER stress-induced apoptosis[51, 52]. However, it is not clear whether ER stress-mediated activation of JNK is involved in hepatocyte death. In response to thapsigargin, an inducer of ER stress, *c-Jun*$^{-/-}$ hepatocytes had exacerbated and sustained ER stress, characterized by increased expression of CHOP, sXBP-1, and GRP78[53]. *c-Jun*$^{-/-}$ hepatocytes are more susceptible to ER-stress–induced cell death and have a defect in autophagy, indicating that c-Jun couples ER stress and autophagy to promote cell survival.

Distinct Roles of JNK1 and JNK2 in Liver Regeneration

The liver can regenerate to its original size after a substantial loss of its mass. The JNK isoforms and AP-1 are activated within 1 hour of partial hepatectomy. JNK activates AP-1, which promotes expression of cyclin D and initiates the G0–G1 transition[54,55]. In regenerating liver, JNK is activated by mediators such as TNF and EGF. Mice that lack TNFR1 and rats injected with an antibody against TNF have reduced liver regeneration and activation of JNK and AP-1[56–58]. Exogenous administration of ATP activates JNK and induces hepatocyte proliferation; this effect is potentiated by EGF[59]. Upon partial hepatectomy, pharmacologic inhibition of JNK blocks c-Jun phoshorylation, AP-1 activation, and cyclin D1 expression, delaying regeneration[58]. Consistently, *Jnk1*$^{-/-}$ mice have decreased liver regeneration following partial hepatectomy, with increased p21 and decreased c-Myc expression. Interestingly, p21 deficiency reversed defective regeneration in *Jnk1*$^{-/-}$ livers, indicating that JNK1 promotes hepatocyte proliferation by inhibiting p21[23,60]. The role of JNK2 in liver regeneration is less clear. One study demonstrated that the loss of JNK2 accelerated liver regeneration[61]. The authors of this study suggested that compensatory upregulation of JNK1 might accelerate regeneration in *Jnk2*$^{-/-}$ livers. Another reported no role for JNK2 in liver regeneration[23].

c-Jun$^{-/-}$ mice die during embryogenesis; the fetal livers have high levels of hepatocyte apoptosis[62]. Conditional or hepatocyte-specific deletion of *c-Jun* (*c-Jun*$^{\Delta hep}$) does not cause abnormalities in liver development, but causes a severe defect in liver regeneration after partial hepatectomy[63]. This defect results from increased accumulation of p21 protein that requires p53 and overactivation of p38[64]. Disruption of *p21* or *p53* rescued liver regeneration in *c-Jun*$^{\Delta hep}$ mice[64]. Interestingly, the high level of phosphorylation of p38

observed after partial hepatectomy in *c-Jun*$^{\Delta hep}$ mice was reduced by disruption of *p21* or *p53*[64]. Hepatic disruption of *p38α* also increased proliferation of hepatocytes, because it reduced expression of p21[64]. *Ikkβ*$^{\Delta hep}$ mice, however, have accelerated regeneration of liver, which correlates with increased activation of JNK[65].

In summary, the combined activities of JNK–AP-1, IKK–NF-κB, p38, and p53 signaling to p21 control liver regeneration. One study used *Jnk1*$^{\Delta hep}$*/JNK2*$^{-/-}$ mice to assess the specific roles of hepatocyte JNKs in liver regeneration and exclude the effects of compensatory activation of JNK2[23]. Although findings from the study agreed with those from previous reports of defects in liver regeneration in *Jnk1*$^{-/-}$ mice, it concluded that compensatory activation of JNK2 inhibits regeneration in livers of *Jnk1*$^{-/-}$ mice.

In contrast to the role of JNK in promoting liver regeneration, persistent JNK activation attenuates liver regeneration. Mice deficient in growth arrest and expression of the DNA-damage-inducible gene 45β (GADD45β) have reduced proliferation and increased death of hepatocytes, and sustained activation JNK following partial hepatectomy[66]. Disruption of *Jnk2* increased liver regeneration in *Gadd45β*$^{-/-}$ mice[66], indicating that the magnitude and duration of JNK activation is important.

Metabolic Syndrome and Hepatic Steatosis

Non-alcoholic fatty liver disease (NAFLD) is a hepatic manifestation of the metabolic syndrome, which is characterized by obesity and insulin resistance. The spectrum of NAFLD ranges from simple steatosis to steatosis with hepatic inflammation and fibrosis, known as non-alcoholic steatohepatitis (NASH). NASH is a high risk factor for cirrhosis. Excessive fat intake is believed to cause simple steatosis as a first hit, and then a second hit, such as oxidative stress, adipose tissue-derived cytokines, or translocation of endotoxin from the intestinal lumen, leads to hepatic inflammation. Alternatively, simple steatosis and NASH could have separate etiologies, such that steatosis is not a preliminary condition for NASH. Because obesity and insulin resistance are risk factors for NAFLD, activities of JNK in the liver, along with the adipose tissues and muscles, must be important in the development of NAFLD. Strong activation of JNK has been observed in the liver, fat, and muscle tissues in mice placed on a high fat diet (HFD) and genetically (*ob/ob*) obese mice[67,68]. We discuss how systemic activation of JNK affects development of hepatic steatosis and insulin resistance, based on the experiments in *Jnk1*$^{-/-}$ and *Jnk2*$^{-/-}$ mice.

The HFD causes obesity in wild-type, but not *Jnk1*$^{-/-}$ mice[67–69]. *Jnk1*$^{-/-}$ mice have decreased phosphorylation of IRS-1 at Ser 307 (an inhibitory site), compared with wild-type mice, and increased insulin-induced tyrosine phosphorylation of IRS-1, which ameliorates insulin resistance[67]. Moreover, HFD-induced hepatocyte injury and steatosis were suppressed in *Jnk1*$^{-/-}$ mice. *Jnk2*$^{-/-}$ mice placed on the HFD have a similar degree of hepatic steatosis as wild-type mice, but have increases in hepatocyte injury, obesity, and insulin resistance[69]. Interestingly, livers from *Jnk2*$^{-/-}$ mice have greater JNK activity, indicating that JNK1 overcompensation damages the liver and contributes to insulin resistance[69]. The effect of JNK1 overcompensation was confirmed in *Jnk2*$^{-/-}$ mice with *Jnk1* haploinsufficiency, which reduced obesity and hepatic steatosis and increased insulin sensitivity[70]. To avoid JNK1-mediated compensation, another study attempted to knockdown JNK1 or JNK2 in a model of established steatosis. Although acute knockdown of JNK1 or JNK2 increased insulin sensitivity, only JNK1 knockdown attenuated hepatocyte damage and steatosis. JNK2 knockdown actually increased liver injury, and increased levels of Bim, because its degradation is JNK2 dependent[69]. The effect of Bim accumulation in *Jnk2*$^{-/-}$ mice was demonstrated by a reduction in liver injury upon *Bim* ablation[69]. So,

JNK1 and JNK2 each participate in hepatic injury, steatosis, insulin resistance, and obesity (Figure 4).

The HFD and genetically induced obesity activate JNK in the liver. Hyperglycemia in mice with streptozotocin- or alloxan-induced, insulin-dependent diabetes does not activate hepatic JNK. Therefore, in obese mice, hyperglycemia is not a cause of hepatic JNK activation. However, other sugars, such as fructose, activate hepatic JNK and attenuate insulin signaling, by increasing phosphorylation at Ser307 and decreased tyrosine phosphorylation of IRS-1[71]. Treatment with saturated free fatty acids (FAA) activates JNK and leads to insulin resistance[68,72]. In adipocytes and fibroblasts, saturated FAA causes aggregation of the membrane-anchored tyrosine kinase c-Src within lipid rafts, leading to MLK3 activation and subsequent JNK activation[72]. However, this mechanism does not operate in hepatocytes[72]. JNK activation is also associated with ER stress-mediated insulin resistance. ER stress causes JNK activation through IRE1α, and insulin resistance occurs as a consequence of JNK-mediated IRS-1 phosphorylation[73]. In mice with mutations in XBP-1, abnormal ER functions cause insulin resistance through JNK activation[73]. However, mice with hepatocyte specific deficiency in XBP-1 did not develop insulin resistance, in spite of increased ER stress and JNK activation in the liver[74]. The ER stress induced by hepatic XBP-1 deficiency is therefore not associated with JNK-mediated insulin resistance.

JIP1 is a scaffold proteins involved in JNK activation that contributes to insulin resistance and hepatic steatosis[75]. Double-stranded RNA-dependent protein kinase (PKR) is another upstream regulator of JNK that has been associated with metabolic disease. PKR senses high levels of nutrients and obese states to activate JNK, which causes insulin resistance and hepatic steatosis[76].

Hepatocyte Lipoapoptosis and NASH

What are the specific roles of JNK in hepatocytes during lipoapoptosis and development of NASH? Saturated FFA cause apoptosis in hepatocytes—an effect known as lipoapoptosis. In hepatocytes, saturated FFA induce direct interaction between the small GTPase Cdc 42/Rac1 and MLK3 to activate JNK1, independent of the ER stress transducer IRE1α (Figure 4)[77]. This pathway promotes lipoapoptosis. GSK-3β is also involved in FFA-induced JNK activation, independently of FFA-induced ER stress responses[78]. The saturated FFA palmitate upregulates the p53-upregulated mediator of apoptosis (PUMA) and Bax in a JNK1-dependent manner (Figure 4)[79]. *Puma*$^{-/-}$ hepatocytes are resistance to FFA-induced lipoapoptosis, indicating the importance of this death regulation pathway. In addition, FFA cause degradation of Mcl-1 through JNK1, which contributes to lipoapoptosis[28,80]. Steatotic hepatocytes are more susceptible to TNF-induced apoptosis, which involves activation of ASK1 and JNK[81]. Moreover, TRAIL–DR5-mediated hepatocyte lipoapoptosis is mediated via JNK in steatotic hepatocytes[82]. However, these findings were all obtained through in vitro experiments with high concentrations of FFA, which causes high rates of cell death; the importance of lipoapoptosis and its relation to JNK activation in vivo are not clear.

The roles of JNK in NASH have been studied using mice placed on methione-choline deficient (MCD) diets. The MCD diet causes hepatic steatosis, injury, and inflammation, but does not induce extrahepatic metabolic features, such as obesity and insulin resistance. The MCD diet-induced model of NASH has been used to assess liver-specific functions of JNK. The diet was found to activate JNK and AP-1 in the liver[83]. JNK1 deficiency protected mice from the effects of the MCD diet, but JNK2 deficiency had no effect[83]. JNK1 therefore promotes induction of NASH by the MCD diet.

There has been debate over what liver cell type is involved in JNK-mediated metabolic effects. Studies in radiation chimeras showed that JNK1 functions within radiation-resistant

stroma cells and in radiation-sensitive hematopoietic cells. Activation of JNK1 in radiation-resistant stroma, including hepatocytes, adipocytes and muscles, is required for HFD-induced obesity[24]. Yet, JNK1 activation in hematopoietic cells, including tissue macrophages, is also involved in HFD-induced insulin resistance[24,84]. In myeloid cells exposed to FFA, JNK contributes to production of inflammatory cytokines, such as TNF, IL-1, and IL-6, which induce insulin resistance in other cells[24,84,85]. A study that used a choline-deficient amino acid-defined (CDAA) diet to induce NASH in mice found that activities of JNK1 in hematopoietic cells were more important in resident liver cells for induction of hepatocellular injury, inflammation, and fibrosis[86].

Intriguingly, hepatocyte-specific deletion of JNK1 does not protect mice from HFD-induced insulin resistance[87]. However, $Jnk1^{\Delta hep}$ mice have increased lipogenesis and attenuated insulin resistance on normal diets and importantly, spontaneously develop hepatic steatosis[87]. These findings indicate an anti-steatotic and anti-diabetic function of JNK1 in hepatocytes, and that the hepatic phenotypes of $Jnk1^{-/-}$ mice could result from JNK1 functions in non-parenchymal cells and extra-hepatic tissues (e.g. adipose tissues).

Disruption of the combination of *Jnk1* and *Jnk2* in adipocytes reduced obesity in mice placed on the HFD, along with hepatic steatosis and insulin resistance, indicating that adipocyte JNK affects metabolic states in other tissues, including liver and muscle[88]. Surprisingly, deletion of JNK1 from skeletal muscle worsened the metabolic state in liver and adipose tissues[89]. *Jnk1* ablation in the central nervous system reduced food intake and body weight gain in mice fed the HFD[90]. The main effects of JNK1 in the central nervous system are mediated through thyroid stimulating hormone and thyroid hormone[90].

Although JNK1 is important for FFA-induced lipoapoptosis and inhibition of insulin signaling by phosphorylation of IRS-1 at Ser 307 in hepatocytes, findings from studies in genetically engineered mice indicate that the activities of JNK1 in hematopoietic cells, adipocytes, and the nervous systems promote, (but in hepatocytes and muscles might prevent) NASH and metabolic syndrome. So, inhibitors of JNK1 might be used to treat patients with NASH or insulin resistance. BI-78D3, a small molecule JIP1 mimics that inhibits JNK activity, increased insulin sensitivity in mouse models of type 2 diabetes[91]. Cell- or tissue-specific delivery of JNK1 inhibitors might improve efficacy and reduce adverse effects.

HSC Activation and Fibrosis

Fibrosis is a wound-healing response following chronic liver damage caused by hepatitis B or C viruses, cholestatic liver inflammation, alcoholic steatohepatitis, NASH, and autoimmune hepatitis. The pathogenesis of liver fibrosis is characterized by excessive production and deposition of extracellular matrix proteins, including type I and III collagen, and requires activation of HSC. JNK is involved in HSC activation. Blocking JNK activity with SP600125 inhibited HSC activation, demonstrated by decreased expression of α smooth muscle actin (SMA), and reduced proliferation[92]. Interestingly, SP600125 increased production of collagen, indicating complexity in control of HSC activation, proliferation, and collagen production[92]. TGF-β and angiotensin II upregulate expression of αSMA through activation of JNK. PDGF-mediated HSC proliferation is also JNK dependent[93]. Both TGF-β and PDGF activate SMAD2 and SMAD3 through JNK, resulting in HSC migration[94]. TLR4 signaling potentiates TGF-β signaling by downregulating bone morphogenetic protein and activin membrane bound inhibitor (Bambi), which is partly dependent on JNK (Seki E, unpublished data)[95]. Endothelin promotes HSC activation in an autocrine manner. TNF also induces endothlin-1 production through JNK[96].

Among the AP-1 components, JunD is expressed and regulates TIMP-1 expression in activated HSCs[97]. However, JNK is dispensable for JunD-mediated TIMP-1 expression in HSCs, and JunD activation is mediated by ERK1/2[97]. JNK is strongly activated in HSCs following induction of liver fibrosis induced by bile duct ligation or chronic administration of carbon tetrachloride, and in patients with liver fibrosis from HCV infection or NASH[93]. *Jnk1*$^{-/-}$ mice are protected from liver fibrosis, but *Jnk2*$^{-/-}$ mice develop more liver fibrosis than wild-type mice—another example for the pathogenic effects of JNK1 overactivation in *Jnk2*$^{-/-}$ mice. Another study reported are requirement for hematopoietic JNK1, but not JNK2, in mice that develop liver fibrosis from the CDAA diet[86]. JNK therefore mediates many mechanisms that contribute to the pathogenesis of liver fibrosis.

Hepatocellular Carcinoma (HCC)

HCC is the third-leading cause of cancer-related deaths. HCC develops in individuals with liver diseases such as chronic HBV or HCV infection, NASH, or alcoholic cirrhosis. The incidence of HCC in the United States has doubled in recent decades, due to the increased prevalence of hepatitis C and NASH, which both involve JNK activity [98, 99]. Studies in mice with diethylnitrosamine (DEN)-induced HCC indicated a role for JNK1 in pathogenesis. A single injection of DEN to young mice induces HCC at the age of 8–10 months. JNK1 deficiency protects mice from DEN-induced hepatocyte death, resulting in reduced compensatory proliferation and HCC formation[100]. In a study that used the DEN-phenobarbital protocol (in which DEN is the initiator and phenobarbital is the promoter of hepatocarcinogenesis)[60], *Jnk1*$^{-/-}$ mice developed fewer and smaller liver tumors than wild-type or *Jnk2*$^{-/-}$ mice[60,100]. Livers from the *Jnk1*$^{-/-}$ had decreased levels of c-Myc and increased levels of p21; c-Myc overexpression or disruption of *p21* restored HCC development to levels observed in wild-type mice[60].

JNK1 downregulates transcription of *p21* via c-Myc but not p53. JNK1 regulates transcription of c-Myc expression, through phosphorylation of c-Jun and other mechanisms[60]. c-Jun is important in hepatocarcinogenesis[101]. Deletion of *c-Jun* from hepatocytes of mice reduced development of HCC following administration of DEN[33]. Disruption of *c-Jun* in hepatocytes increased their expression of p53 and sensitivity to TNF-induced death[33]. Interestingly, the activities of c-Jun effects in DEN-induced tumor formation do not require Jun phosphorylation by JNK, based on mutational analyses (in *c-Jun*^{AA} mice)[33].

JNK also phosphorylates SMAD3 in its linker region to antagonize TGF-β–induced carboxy-terminal phosphorylation of SMAD3, which would lead to p21 induction and tumor suppression[102]. JNK phosphorylation of SMAD3 therefore interferes with p21 upregulation by SMAD3 and c-Myc[102]. JNK also promotes apoptosis of HCC cells, as well as hepatocytes[103].

JNK1 therefore contributes to the initiation of HCC by inducing hepatocyte apoptosis and compensatory proliferation. JNK1-dependent downregulation of p21 is mediated by c-Myc upregulation, independently of c-Jun and its phosphorylation. JNK also regulates p21 expression by inhibiting TGF-β-induced activation of SMAD3. Independent of JNK, c-Jun is required for liver carcinogenesis, by suppressing p53 (Figure 5).

Inactivation of IKK and NF-κB promotes activation of JNK and increases hepatocarcinogenesis following administration of DEN[104]. In *Ikkβ*$^{\Delta hep}$ mice, disruption of *Jnk1* reduced development of HCC[100]. Increased JNK activation was also observed during spontaneous hepatocarcinogenesis in *Nemo*$^{\Delta hep}$ and *Tak1*$^{\Delta hep}$ mice, in which NF-κB is inactivated[105–107]. Administration of anti-oxidants to *Nemo*$^{\Delta hep}$ mice or disruption of *TnfrI* in *Tak1*$^{\Delta hep}$ mice prevented activation of JNK and development of HCC, indicating that

overactivation of JNK promotes HCC in these mice (Seki, unpublished data)[105,106]. Similarly, hepatocyte-specific disruption of *p38α* increased generation of ROS, activation of JNK, and hepatocarcinogenesis following a single injection of DEN or administration of DEN-phenobarbital. The increased formation of HCC was reversed when *c-Jun* was disrupted or JNK activity was inhibited with an anti-oxidant[18,108].

Mdr-2$^{-/-}$ mice develop inflammation-dependent spontaneous HCC; NF-κB and JNK are each induced through TNF overproduction[109]. In *Mdr-2*$^{-/-}$ mice, inactivation of NF-κB prevented formation of HCC, and antagonists of TNF also inhibited HCC development, by decreasing JNK phosphorylation[109]. TNF receptor signaling and ROS might be the major upstream mediators of JNK activation, whereas NF-κB and p38 suppress JNK activity, through inhibition of ROS. Importantly, JNK1 has a larger role than JNK2 in development of HCC.

The cell-specific functions of JNK in hepatocarcinogenesis were determined from studies of *Jnk1*$^{-/-}$*Jnk2*$^{-/-}$ mice and *Jnk1*$^{\Delta hep}$/*JNK2*$^{-/-}$ mice. Conditional *Jnk1*$^{-/-}$*Jnk2*$^{-/-}$ mice develop less numbers of HCCs, and have reduced activities of c-Myc and c-Jun and increased expression of p21[23]. Intriguingly, *Jnk1*$^{\Delta hep}$/*JNK2*$^{-/-}$ mice developed more HCCs, with increased c-Myc and c-Jun expression, hepatocyte apoptosis, and compensatory proliferation, but paradoxically increased p21 expression[23]. This puzzling finding indicates that HCC formation depends on c-Myc and c-Jun, but is independent of JNK. The carcinogenic effects of JNK might be restricted to non-parenchymal cells, rather than hepatocytes, and induce an inflammatory response, such as production of IL-6, TNF, and TGF-β. Alternatively, hepatocyte JNK might inhibit non-parenchymal, cell-mediated liver inflammation that contributes to hepatocarcinogenesis.

There are additional mechanisms for the paradoxical roles of JNK in HCC formation. Studies in different models of HCC could determine where JNK functions to promote hepatoccarcinogenesis, such as in non-parenchymal cells vs hepatocytes. Hui et al. studied induction of HCC with the DEN-phenobarbital protocol, whereas Das et al. study tumor formation following a single injection of DEN in young mice; *Tak1*$^{\Delta hep}$ mice develop spontaneous HCC[23,60,106,107]. Alternatively, deletion of a combination of JNK1 and JNK2 from hepatocytes increases their susceptibility to DEN-induced death and transformation; these effects are not observed in hepatocytes with disruption of only *Jnk1*. The presence or absence of JNK2 in hepatocytes could determine the contradictory functions of JNK1 in these cells. One study reported that *Ask1*$^{-/-}$ mice develop HCC following administration of DEN, with less activation of JNK than in other models[110]. Further investigations are required to determine the paradoxical roles of JNK in hepatocytes and hepatocarcinogenesis.

Studies support the importance of JNK in pathogenesis of human HCC. JNK1 and JNK2 are phosphorylated in primary HCC samples from patients; 50%–60% of HCC samples had higher levels of phospho-JNK1 than non-neoplastic lesions, whereas levels of phospho-JNK2 did not differ between HCC and non-tumor tissues[60,111]. JNK1 activation was correlated with tumor size, and knockdown of JNK1, but not JNK2, reduced proliferation of a human HCC cell line, indicating that JNK1 promotes proliferation of human HCCs[60,111]. Moreover, expression of dual-specific phosphatase (DUSP)1 is reduced in human HCCs, leading to overactivation of JNK[111]. In human HCCs, JNK1 upregulates c-Myc and downregulates p21; JNK1 also regulates expression of factors that control the cell cycle, proliferation and metabolism, through methylation of histone H3 lysine 4 and 9 by MLL3 and EZH2[60,111].

HCV is a major cause of HCC. The HCV core protein activates JNK through ROS[112]. Mice that express an HCV core transgene develop spontaneous HCC with high frequencies of p53

mutations, which are amplified by administration of DEN; hepatocyte-specific disruption of *c-Jun* reduces HCC in these mice[112]. The role of JNK in HCV-induced HCC development remains to be examined.

Reagents designed to target JNK1 might be developed to treat patients with HCC. D-JNKI1, a specific inhibitor of JNK1 and 2 (a dual inhibitor), suppressed JNK activity in HCC cells, decreasing levels of c-Myc and increasing levels of p21. This reagent suppressed growth of human HCC cells in culture and xenograft tumors in mice[1,60]. D-JNK1 also suppressed DEN-induced HCC in mice[60]. The JNK inhibitor SP600125 blocked HCC growth in rats with DEN-induced liver tumors[102,113]. The JNK inhibitor sensitizes HCC cells to TRAIL[35], indicating that JNK inhibitors might be given in combination with TRAIL as therapeutic agents.

Future Directions

The JNKs are important signaling molecules in multiple pathways in liver physiology and disease pathogenesis. JNKs regulate transcription by phosphorylating and activating transcription factors (such as cJun, JunB, and ATF2), or independently of transcription, by phosphorylating signaling molecules (such as IRS-1, Itch, Mcl-1, and Bid). When appropriately activated, JNKs regulate important biologic functions, such as liver regeneration. However, the same signaling pathway can also be detrimental, such in carcinogenesis. The liver expresses 2 JNK isoforms. Most pathologic processes are associated with JNK1; the severe phenotypes observed following deletion of JNK2 probably result from compensatory JNK1 activation.

The metabolic syndrome, which includes obesity, insulin resistance, and NASH is a significant public health issue worldwide. JNK activity regulates various pathophysiologic processes, including hepatocyte death, steatosis, inflammation, and insulin resistance, which are associated with NASH, fibrosis and HCC. Pre-clinical studies in animal models or human cells have indicated that reagents that inhibit JNK might be used to treat patients with liver diseases, including acute liver failure, I/R injury, fibrosis, HCC, and NASH[1,60,91]. Several JNK inhibitors, such as SP600125, D-JNKI1, and BI-78D3 have been tested in pre-clinical studies. CC-930 is currently being tested in Phase II clinical trial for idiopathic pulmonary fibrosis[114], and dual inhibitors of JIP and JNK have been identified[115]. To identify the most effective approach to block JNK signaling in patients with liver disease, in addition to the established reagents, new JNK inhibitors must also be developed and their specificity assessed for different JNK isoforms; the reagents must then be tested in different cells, tissues, and systems. Clinical trials are also required to assess new JNK inhibitors, alone or in combination with other therapeutics[116].

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Abbreviations

AP	activator protein
ASK	apoptosis signal-regulating kinase
ATF	activating transcription factor
Bambi	bone morphogenetic protein and activin membrane bound inhibitor
cDISC	cytosolic death-initiating signaling complex

cIAP	cellular inhibitor of apoptosis
ConA	concanavalin A
CYLD	cylindromatosis
EGF	epidermal growth factor
ER	endoplasmic reticulum
FAA	free fatty acid
GADD	growth arrest and DNA-damage-inducible gene
GalN	D-galactosamine
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HFD	high fat diet
HSC	hepatic stellate cells
IKK	IκB kinase
IL-1	interleukin-1
I/R	ischemia and reperfusion
IRE	inositol requiring enzyme
IRS	insulin receptor substrate
JIP	JNK interacting protein 1; c-Jun-N-terminal Kinase
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MCD	methione-choline deficient
MLK	mixed linage kinase
NAFLD	non-alcoholic fatty liver disease
NAPQI	N-acetyl-p-benzoquinone imine
NASH	non-alcoholic steatohepatitis
NOX	NADPH oxidase
PERK	PKR-like ER localized kinase
PDGF	platelet-derived growth factor
PKR	double-stranded RNA-dependent protein kinase
PUMA	p53-up-regulated mediator of apoptosis
ROS	reactive oxygen species
SMA	smooth muscle actin
SRF	serum response factor
SOD	superoxide dismutase
TGF	transforming growth factor
TLR	Toll-like receptor

TNF	tumor necrosis factor
UPR	unfolded protein response

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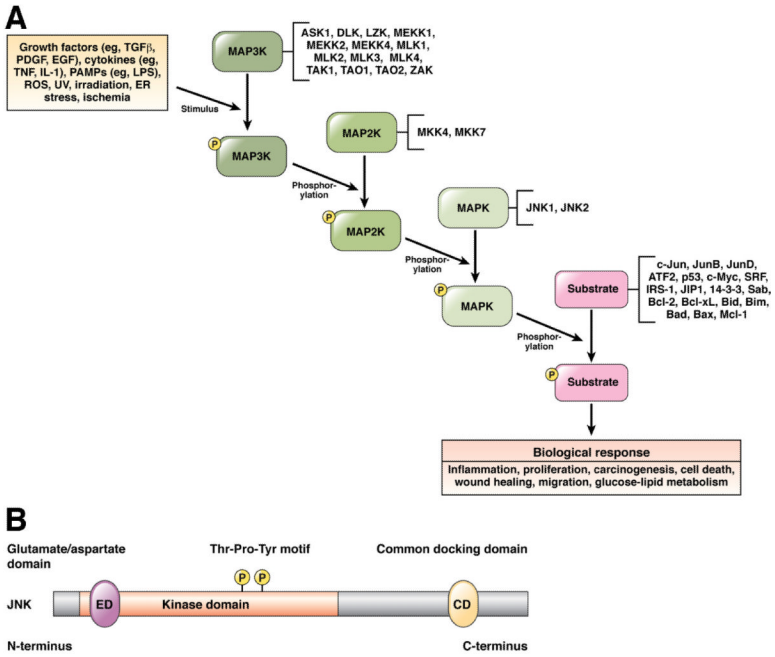

Figure 1. The JNK Activation Pathway and Domain Structure and

A. number of stimuli, including growth factors, cytokines, PAMPs, ROS, and environmental stresses activate the JNK signaling pathway through a 3-tier kinase cascade that includes MAP3Ks (ASK1, TAK1, MEKKs and MLKs), MAP2Ks (MKK4 and MKK7), and JNKs. Activated JNKs phosphorylate their substrates, which include transcription factors (c-Jun, JunB, JunD, p53, and c-Myc) and mitochondrial proteins (Bcl-2, Bid, Bim, Bax, and Mcl-1), to induce various biological responses.

B. The JNK protein contains a conserved dual phosphorylation Thr-Pro-Tyr motif in its activation loop and has a common substrate docking site in C-terminus and a glutamate/aspartate domain in its N-terminus to mediate interactions with MAP2Ks, phosphatases, and substrates.

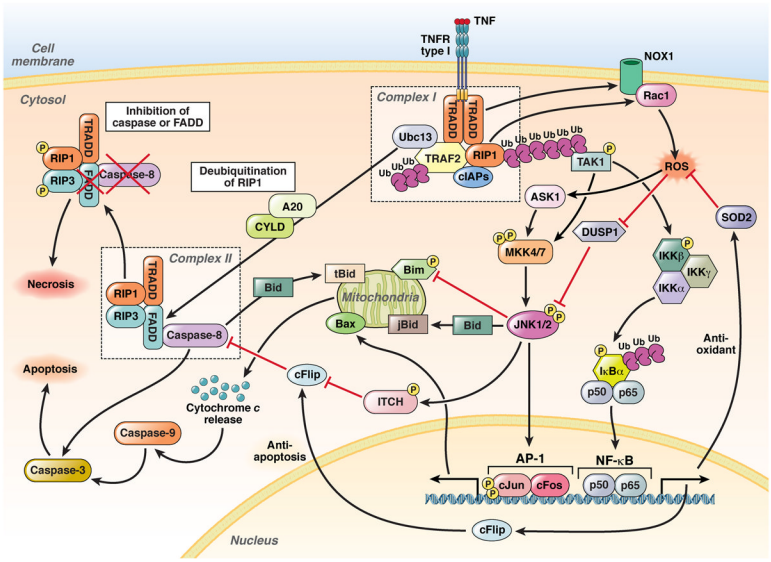

Figure 2. JNKs in TNF Signaling

(1) Binding of TNF to the TNFR type I leads to the rapid formation of complex I, comprising TRADD, RIP1, TRAF2, cIAP1, cIAP2, and Ubc13. cIAP-mediated K63-ubiquitination of RIP1 recruits and activates TAK1. (2) MAP3Ks (TAK1 and ASK1) activate JNK1 and 2 through MKK4 and 7. JNK activates AP-1, which comprises c-Jun and c-Fos. Simultaneously, JNK1 phosphorylates ITCH to ubiquitinate c-FLIP, which promotes caspase-8 dependent apoptosis. JNKs can also induce mitochondria-dependent apoptosis through Bax and degradation of Bim. (3) TAK1 phosphorylates and activates the IKK complex, which leads to phosphorylation, unbiquitination, and degradation of IκBα, resulting in nuclear translocation and activation of NF-κB, which is comprises the p50 and p65 subunits. NF-κB induces the transcription of SOD2 and c-FLIP to prevent ROS production and caspase-8 activation, respectively. (4) Complex I also contributes to ROS production through NOX1 and Rac1. (5) Following formation of complex I, RIP1 is deubiquitinated by CYLD or A20 to form complex II, comprising TRADD, FADD, RIP1, RIP3, and caspase-8. Normally, caspase-8 induces apoptosis. (6) However, if caspase-8 or FADD is blocked, RIP1 and RIP3 are phosphorylated and cause necrosis.

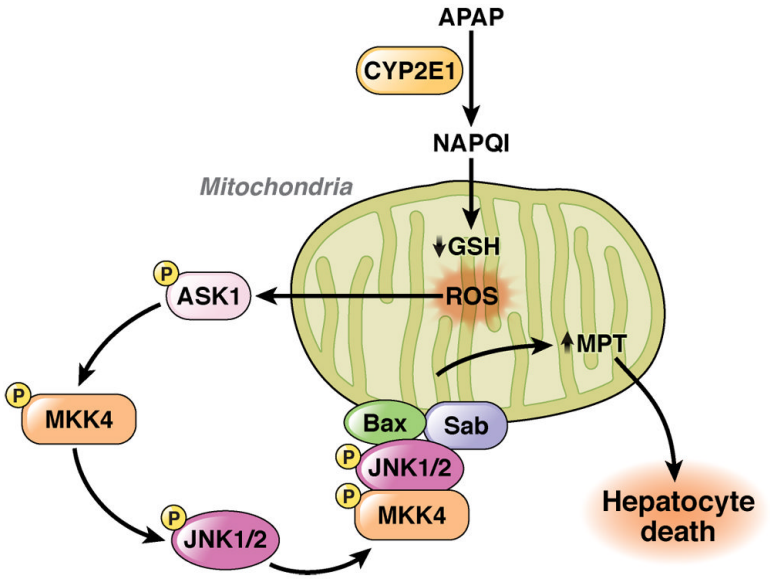

Figure 3. Role of JNK in Acetaminophen-Induced Liver Injury

Acetaminophen is metabolized to NAPQI through CYP2E1, which reduces glutathione levels in mitochondria. Excessive NAPQI induces ROS, which activates ASK1, MKK4, and JNK. JNK, MKK4 and Bax translocate to the outer membrane of mitochondria through binding of Sab to increase the mitochondrial permeability transition, resulting in induction of massive hepatocyte death.

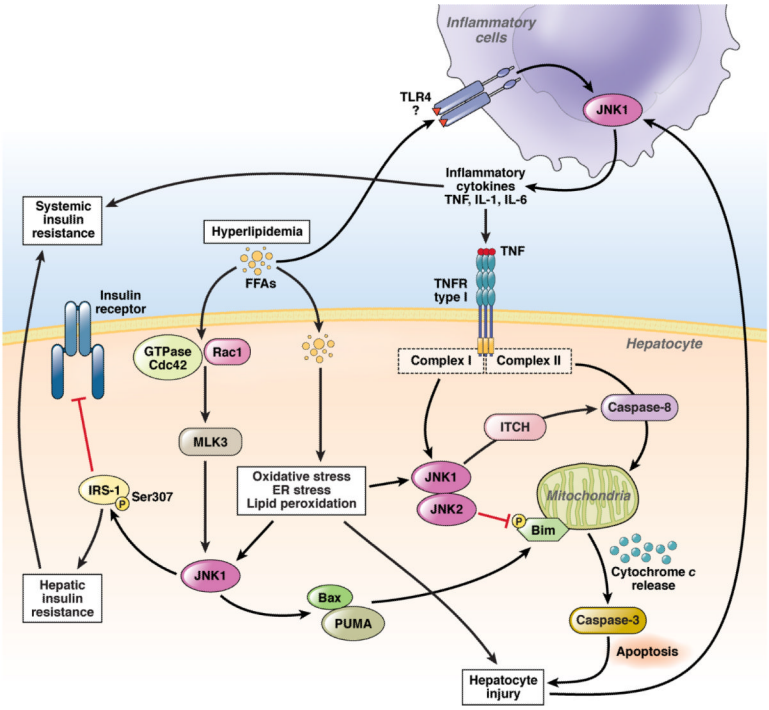

Figure 4. Roles of JNKs in the Pathogenesis of NAFLD

Obesity and hyperlipidemia increase plasma levels of FFA, which activate MLK3 and JNK in hepatocytes. JNK1 contributes to hepatic insulin resistance by phosphorylating IRS-1 at serine 307, and mitochondria-mediated hepatocyte death through Bax and PUMA. Saturated FFAs activate JNK through oxidative stress, ER stress, and lipid peroxidation in hepatocytes. Saturated FFAs also activate JNK in inflammatory cells to contribute to production of inflammatory cytokines. TLR4 on inflammatory cells might be involved in FFA-induced JNK activation.

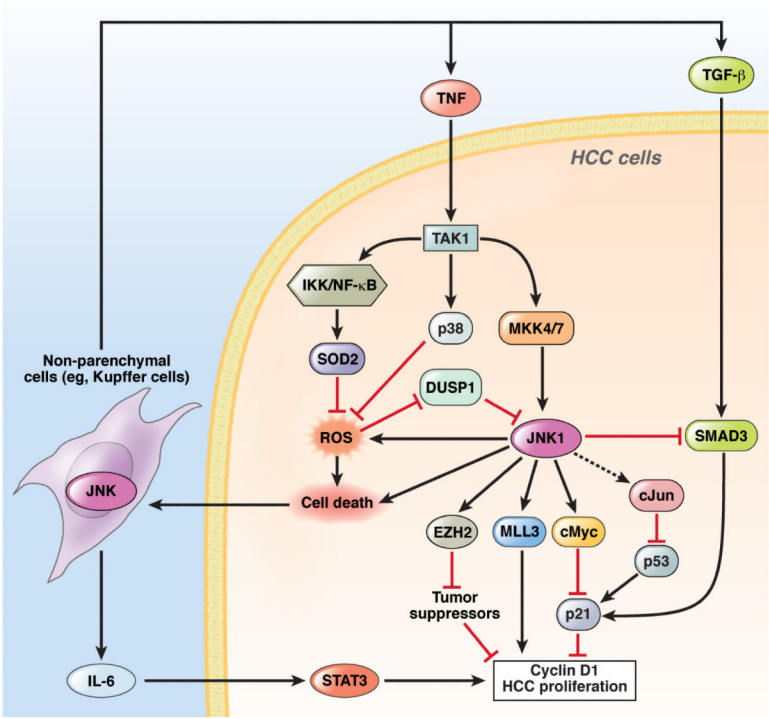

Figure 5. Functions of JNKs in HCC

In HCC, JNK1 is overactivated either by overactivation of the upstream kinases MKK4 and 7 or the inactivation of DUSP1. Activated JNK1 induces c-Myc, which suppresses p21 expression and promotes HCC proliferation via cyclin D1 expression. JNK1 also upregulates MLL3 and EZH2, 2 histone H3 methyl transferases to increase expression of the cell cycle-associated genes, such as cyclins, and inhibit the transcription of tumor suppressors, respectively. c-Jun inhibits p53 activity post-transcriptionally. JNK1 increase generation of ROS, which is inhibited by p38α and IKKβ activation of NF-κB, through induction of SOD2. JNK1 also inhibits TGF-β–induced Smad3 activation, thereby inducing p21 to suppress HCC promotion. All of these pathways regulate hepatocyte death, which results in the release of IL-6, TNF, and TGF-β through JNK1 in non-parenchymal cells, including Kupffer cells. IL-6 induces HCC proliferation via activation of STAT3in hepatocytes. JNK1 promotes (but TAK1, p38α, and IKKβ activation of NF-κB prevent) hepatocyte death and HCC formation.