

## Inflammation- and stress-related signaling pathways in hepatocarcinogenesis

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### Abstract

It has been established that cancer can be promoted and exacerbated by inflammation. Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, and its long-term prognosis remains poor. Although HCC is a complex and heterogeneous tumor with several genomic mutations, it usually develops in the context of chronic liver damage and inflammation, suggesting that understanding the mechanism(s) of inflammation-mediated hepatocarcinogenesis is essential for the treatment and prevention of HCC. Chronic liver damage induces a persistent cycle of necroinflammation and hepatocyte regeneration, resulting in genetic mutations in hepatocytes and expansion of initiated cells, eventually leading to HCC development. Recently, several inflammation- and stress-related signaling pathways have been identified as key players in these processes, which include the nuclear factor- $\kappa$ B, signal transducer and activator of transcription, and stress-activated mitogen-activated protein kinase pathways. Although these pathways may suggest potential therapeutic targets, they have a wide range of functions and complex crosstalk occurs among them.

This review focuses on recent advances in our understanding of the roles of these signaling pathways in hepatocarcinogenesis.

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**Key words:** Hepatocellular carcinoma; Inflammation; Nuclear factor- $\kappa$ B; Mitogen-activated protein kinase; Signal transducer and activator of transcription; c-Jun NH<sub>2</sub>-terminal kinase; p38; Transforming growth factor-activated kinase 1; Apoptosis signal-regulating kinase 1

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### INTRODUCTION

Various types of cancer arise in the setting of chronic inflammation, suggesting a strong link between inflammation and carcinogenesis<sup>[1,2]</sup>. Although Virchow first suggested this relationship in the 19th century, clear evidence for it has been obtained only during the last decade<sup>[3]</sup>. The development of hepatocellular carcinoma (HCC) is one of the most extensively investigated inflammation-based carcinogenic processes because more than 90% of HCCs develop in the context of chronic liver damage and inflammation.

HCC is diagnosed in more than half a million people each year and is the third most common cause of cancer mortality worldwide<sup>[4]</sup>. The short-term prognosis of patients with HCC has improved recently due to advances in early diagnosis and treatment, but long-term prognosis remains unsatisfactory, as indicated by the low overall survival of 22%-35% at 10 years after curative treatment<sup>[5,6]</sup>. Thus, understanding the molecular carcinogenic mechanisms and the unique pathogenic biology of HCC has become an important issue worldwide.

HCC is a complex and heterogeneous tumor with several genomic mutations, but even the most frequent genetic mutations, such as those in p53 and  $\beta$ -catenin, are seen in 30%-50% of HCC cases at most<sup>[7]</sup>. On the other hand, as mentioned above, more than 90% of HCC develops based on chronic inflammation, indicating that understanding the mechanism(s) of inflammation-mediated hepatocarcinogenesis is necessary for the treatment and prevention of HCC.

The main cause of HCC is viral hepatitis caused by the hepatitis B virus (HBV) or hepatitis C virus (HCV); other major etiologies include hemochromatosis, alcoholic hepatitis, and non-alcoholic steatohepatitis (NASH)<sup>[7]</sup>. Most of these diseases are known to cause chronic inflammation in the liver, which plays a critical role in hepatocarcinogenesis. For example, in chronic viral hepatitis, the host immune responses to HBV or HCV are often insufficiently strong to completely clear the infection, resulting in chronic stimulation of an antigen-specific immune response<sup>[8]</sup>. Virus-infected hepatocytes are killed by host immune cells as well as the intrinsic cytopathic effects of the hepatitis viruses, triggering the production of various cytokines and growth factors and subsequently inducing compensatory hepatocyte regeneration. This persistent cycle of necro-inflammation and hepatocyte regeneration is thought to increase the risk of genetic mutation in hepatocytes, and, furthermore, to promote survival and expansion of initiated cells<sup>[9-11]</sup>. Additionally, reactive oxygen species (ROS) and nitrogen oxygen species, generated by both initiated cells and inflammatory cells, could accelerate hepatocarcinogenesis through several mechanisms, such as the induction of oxidative DNA damage, DNA methylation, and hepatocyte injury<sup>[11]</sup>.

Multiple signaling pathways are involved in these processes. Among them, recent *in vivo* studies have shown that several inflammation- and stress-related signaling pathways are key players in hepatocarcinogenesis, including the nuclear factor- $\kappa$ B (NF- $\kappa$ B), signal transducer and activator of transcription (STAT), and stress-activated mitogen-activated protein kinase (MAPK) pathways. Mutations in genes involved in these signaling pathways are currently thought to be rare. Nevertheless, constitutive activation of these pathways is frequently seen in the tumor and surrounding liver tissues, and may be due to the inflammatory microenvironment. Interestingly, these signaling pathways do not act independently, but are linked through extensive crosstalk. This review highlights

advances in the understanding of these interesting but complex signaling pathways in hepatocarcinogenesis.

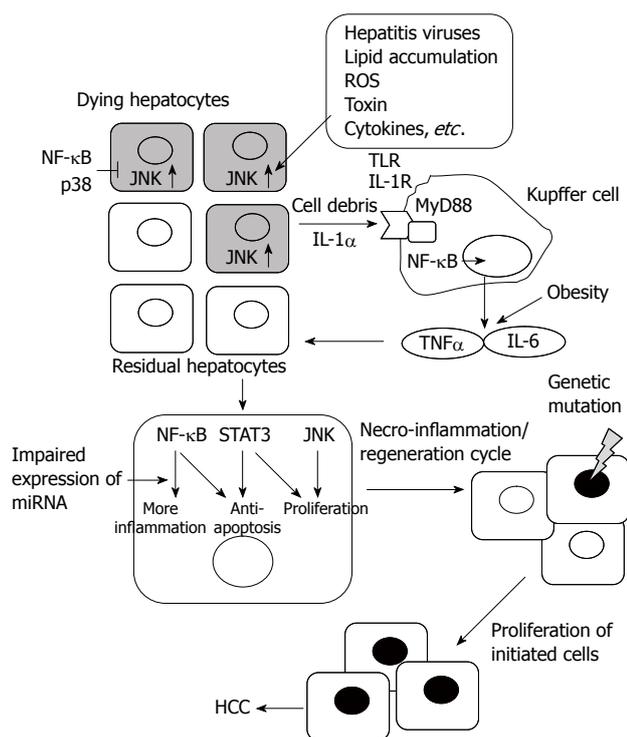
## INFLAMMATION-RELATED SIGNALING IN HEPATOCARCINOGENESIS

### *Role of the I $\kappa$ B kinase/NF- $\kappa$ B pathway in hepatocytes*

The NF- $\kappa$ B family of transcription factors consists of five members: p65/RelA, c-Rel, RelB, p50/NF- $\kappa$ B1, and p52/NF- $\kappa$ B2. Two of the five members dimerize and are held in the cytoplasm by the inhibitor of NF- $\kappa$ B (I $\kappa$ B) proteins<sup>[12]</sup>. In response to many kinds of proinflammatory stimuli, the I $\kappa$ B kinase (IKK) complex, which consists of two catalytic subunits, IKK $\alpha$  and IKK $\beta$ , and a regulatory component, IKK $\gamma$ /I $\kappa$ B kinase (NEMO), phosphorylates I $\kappa$ B and subsequently induces degradation of it. Once activated, NF- $\kappa$ B dimers translocate into the nucleus and stimulate the transcription of various genes, such as those encoding cytokines and anti-apoptotic factors<sup>[13]</sup>. Mice lacking RelA, IKK $\beta$ , or NEMO reveal embryonic lethality with extensive liver apoptosis and degeneration<sup>[14-16]</sup>. This liver apoptosis is induced by tumor necrosis factor (TNF)- $\alpha$ , and intercrossing with TNF receptor 1 knockout mice prevents liver damage and the lethal phenotype<sup>[14,17,18]</sup>. Furthermore, hepatocyte-specific IKK $\beta$  or NEMO knockout mice are not embryonic-lethal, but are more sensitive to TNF- $\alpha$ -mediated liver injury<sup>[19,20]</sup>. Thus, NF- $\kappa$ B plays a key role in liver homeostasis by preventing hepatocyte death.

The role of IKK $\beta$  in hepatocarcinogenesis has been examined using the diethylnitrosamine (DEN)-induced mouse HCC model<sup>[21]</sup>. DEN is the most commonly used genotoxic chemical carcinogen to investigate the mechanism of hepatocarcinogenesis, because it is easy to induce HCC and DEN-induced HCC shows histology and gene expression similar to human HCC, especially with a poor prognosis<sup>[22,23]</sup>. A single dose of DEN given to 2-wk-old male mice is sufficient to induce HCC. However, when DEN is administered to mice older than 4 wk of age, it cannot induce HCC and requires assistance from tumor promoters, such as phenobarbital, because hepatocyte proliferation is rare in adult mice<sup>[24]</sup>. Thus, some stimulation that induces hepatocyte proliferation is indispensable as a tumor promoter in this model.

Strikingly, DEN-induced HCC was markedly increased in hepatocyte-specific IKK $\beta$  knockout mice<sup>[21]</sup>. Hepatocyte-specific knockout of IKK $\beta$  induced a greater extent of hepatocyte death with ROS accumulation after DEN administration, because NF- $\kappa$ B activation is required for the up-regulation of antioxidative genes, such as ferritin heavy chain and manganese-dependent superoxide dismutase. Excess ROS accumulation promotes cell death through various mechanisms, including prolonged c-Jun NH<sub>2</sub> terminal kinase (JNK) activation<sup>[25]</sup>. Cell death is accompanied by an inflammatory reaction, and the elevated hepatocyte death rate enhances compensatory proliferation. Thus, the hepatocyte-specific deletion of



**Figure 1** Role of inflammation- and stress-related signaling pathways in hepatocarcinogenesis. Chronic liver damage induces a persistent cycle of necro-inflammation and hepatocyte regeneration, resulting in genetic mutations in hepatocytes and expansion of initiated cells, eventually leading to hepatocellular carcinoma (HCC) development. As shown in the figure, nuclear factor- $\kappa$ B, signal transducer and activator of transcription 3, and stress-activated mitogen-activated protein kinase pathways play critical roles in these processes. Furthermore, other factors, such as obesity and impaired expression of microRNA, can modify these inflammatory processes and accelerate HCC development. ROS: Reactive oxygen species; TLR: Toll-like receptor; NF- $\kappa$ B: Nuclear factor- $\kappa$ B; TNF: Tumor necrosis factor; IL: Interleukin; JNK: c-Jun NH<sub>2</sub> terminal kinase; STAT: Signal transducer and activator of transcription.

IKK $\beta$  augments DEN-induced hepatocyte death and cytokine-driven compensatory proliferation, which acts as a tumor promoter and eventually leads to increased HCC development (Figure 1). Similar findings were obtained in mice lacking IKK $\gamma$ /NEMO, the hepatocyte-specific deletion of which results in spontaneous liver damage, hepatosteatosis, fibrosis, and HCC development<sup>[20]</sup>.

Although the experiments described above demonstrate a tumor-suppressive role of NF- $\kappa$ B in the hepatocyte, hepatocyte NF- $\kappa$ B has been also identified to have tumor-promoting roles in other HCC models that depend on chronic inflammation rather than liver damage and death-driven compensatory proliferation. Experiments crossing transgenic mice expressing a non-degradable I $\kappa$ B $\alpha$  mutant in hepatocytes with *MDR2* knockout mice, which show low-grade chronic inflammation in the portal area and subsequent cancer development, revealed that the inhibition of NF- $\kappa$ B activation resulted in reduced HCC development<sup>[26]</sup>. In this model, NF- $\kappa$ B activation in the hepatocyte promoted a low degree of TNF- $\alpha$  production and paracrine TNF- $\alpha$  signaling maintained NF- $\kappa$ B activation in the malignant cells, leading to

the expression of anti-apoptotic genes and the survival of malignant cells. Notably, NF- $\kappa$ B activation is more important in the progression of hepatocarcinogenesis than in the initiation step in this model. A similar tumor-promoting role of NF- $\kappa$ B in the hepatocyte has been reported in hepatocyte-specific lymphotoxin  $\alpha\beta$  transgenic mice<sup>[27]</sup>. Lymphotoxin  $\alpha\beta$  transgenic hepatocytes produce chemokines, such as CCL2, CCL7, CXCL1, and CXCL10, in an IKK $\beta$ -dependent manner, recruiting inflammatory cells into the liver and inducing spontaneous chronic inflammation and the subsequent development of HCC. These findings suggest that NF- $\kappa$ B activation in the hepatocyte is involved in the production of cytokines and chemokines that maintain the inflammatory environment as a tumor promoter. Thus, the NF- $\kappa$ B pathway in the hepatocyte plays dual roles in hepatocarcinogenesis, according to the disease model and the carcinogenesis stage.

### Role of the IKK/NF- $\kappa$ B pathway in myeloid cells

The role of the NF- $\kappa$ B pathway in myeloid cells has also been investigated using a DEN-induced HCC model<sup>[21]</sup>. In contrast to hepatocyte-specific IKK $\beta$  knockout mice, deletion of IKK $\beta$  in both hepatocytes and myeloid cells, including Kupffer cells, strongly inhibited DEN-induced HCC development. This phenotype was derived from the markedly reduced production of cytokines, such as TNF- $\alpha$ , interleukin-6 (IL-6), and hepatocyte growth factor, which are secreted by non-parenchymal cells in response to dying hepatocytes and induce compensatory proliferation of residual hepatocytes. Thus, IKK $\beta$ /NF- $\kappa$ B in myeloid cells is required for the production of liver growth factors and subsequent hepatocarcinogenesis. Furthermore, IKK $\beta$  in myeloid cells, especially in Kupffer cells, has also been implicated in the development of metastatic liver tumors through IL-6 production<sup>[28]</sup>. Thus, the IKK $\beta$ /NF- $\kappa$ B pathway orchestrates inflammatory crosstalk between hepatocytes and myeloid cells in liver cancer development (Figure 1).

### Role of inflammatory cytokines in hepatocarcinogenesis

As mentioned above, NF- $\kappa$ B activation-mediated production of inflammatory cytokines plays an important role in the inflammation-carcinogenesis axis of the liver. Various inflammatory cytokines, including TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8, have been implicated in chronic liver inflammation, among which IL-6 is thought to be one of the most important<sup>[8,29,30]</sup>. In chronic hepatitis, IL-6 is considered to be produced mainly by activated Kupffer cells and to intensify local inflammatory responses, and then induce compensatory hepatocyte proliferation, facilitating malignant transformation of hepatocytes<sup>[30]</sup>. Hepatocytes express high amounts of the IL-6 receptor and a signal-transducing element (gp130) that, upon IL-6 binding, activates two signaling pathways, Janus activated kinase (JAK)-STAT and MAPK, which are important in the regulation of cell survival and proliferation<sup>[31]</sup>. In fact, serum IL-6 levels are elevated in patients with chronic liv-

er diseases, including alcoholic hepatitis, HBV and HCV infections, and NASH<sup>[32-34]</sup>. Additionally, a higher serum IL-6 level correlates with future HCC development in patients with chronic hepatitis B or C<sup>[35,36]</sup>. These findings suggest that IL-6 plays a role linking chronic inflammation and hepatocarcinogenesis in humans.

In a mouse HCC model, IL-6 knockout mice showed a marked reduction in DEN-induced HCC development, indicating that IL-6 signaling is directly involved in hepatocarcinogenesis<sup>[37]</sup>. This study also demonstrated the key role played by the toll-like receptor (TLR) adapter protein MyD88. Necrotic hepatocyte-induced IL-6 production was reduced significantly in MyD88-deficient Kupffer cells. Furthermore, deletion of MyD88 suppresses DEN-induced carcinogenesis, indicating that IL-6 production through the TLR/MyD88/NF- $\kappa$ B pathway in Kupffer cells is essential for HCC development. Another study showed that the DEN-induced acute inflammatory response is triggered by IL-1 $\alpha$  release from necrotic hepatocytes, and IL-1 $\alpha$  subsequently induces IL-6 production by Kupffer cells<sup>[38]</sup>. Indeed, IL-1 receptor knockout mice showed significantly reduced DEN-induced IL-6 production and subsequent HCC development<sup>[38]</sup>. Of note, a clinical study revealed that higher serum IL-6 levels correlated with higher aspartate aminotransferase levels in chronic hepatitis C, suggesting that IL-6 may be produced in response to HCV-induced hepatocyte injury<sup>[36]</sup>.

HCC develops much more frequently in males than in females in almost all populations, with a male-to-female ratio of 2:1-4:1<sup>[39]</sup>. Interestingly, although this sex disparity is also found in this DEN-induced HCC model, ablation of IL-6 abolished the sex differences in hepatocarcinogenesis<sup>[37]</sup>. However, ovariectomized female mice revealed enhanced IL-6 production and aggravated HCC development. Furthermore, estrogen administration to Kupffer cells inhibits necrotic hepatocyte-induced IL-6 production. These results suggest that estrogen-mediated down-regulation of IL-6 may partly explain the sex disparity in HCC development. However, more recently, Li *et al*<sup>[40]</sup> reported that transcription factors Foxa1 and Foxa2 in the hepatocyte played important roles in the sex disparity in hepatocarcinogenesis through an interaction with the estrogen and androgen receptors, independent of IL-6 signaling. Thus, the sex disparity in hepatocarcinogenesis may have several causes in addition to estrogen-mediated down-regulation of IL-6.

Several epidemiologic studies have shown that obesity and metabolic syndrome increase the risk of HCC<sup>[41-43]</sup>. Although the mechanism by which obesity and metabolic syndrome promote hepatocarcinogenesis is not fully understood, it seems likely to be mediated, in part, by a state of chronic inflammation. A recent report by Park *et al*<sup>[44]</sup> demonstrated that dietary- or genetically induced obesity promoted DEN-induced HCC along with low-grade inflammation, and ablation of IL-6 or the TNF receptor 1 abrogated their tumor-promoting effects, suggesting that IL-6 and TNF- $\alpha$  are required for the promotion of obesity-associated HCC. IL-6 and TNF- $\alpha$  produced by

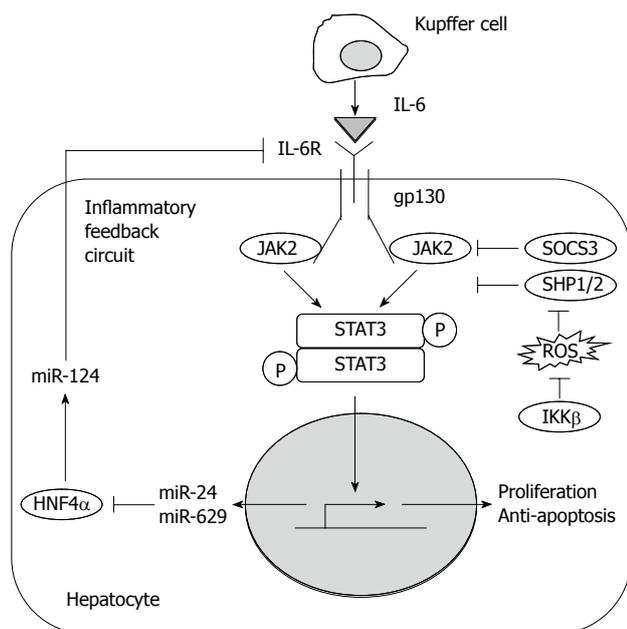
adipose tissue or Kupffer cells activate hepatocyte STAT3 and NF- $\kappa$ B, respectively, promoting cell proliferation and survival of initiated hepatocytes. In fact, recent clinical studies have suggested that visceral fat accumulation, insulin resistance, and dysregulation of adipokines, which induce the activation of the inflammatory response, play important roles in hepatocarcinogenesis<sup>[45-47]</sup>. As the incidence rate of such obesity-associated hepatocarcinogenesis is likely to increase in the near future, inflammatory cytokines have also attracted considerable attention as a mediator of the association between obesity and hepatocarcinogenesis<sup>[48]</sup>.

### Role of JAK-STAT signaling

JAK/STAT signaling pathways are important components of many cytokine receptor systems. Cytokines function by specifically recognizing their receptors, which, as a result of binding to their ligand, undergo conformational changes, resulting in the displacement of JAKs, and subsequently JAKs phosphorylate and activate STATs. The STAT protein family consists of seven members encoded by distinct genes. Among them, STAT3 is the most important IL-6 signaling pathway molecule and is recognized as a key player linking inflammation and cancer<sup>[49,50]</sup>. In response to IL-6 signaling through gp130/JAK, STAT3 forms homodimers that translocate to the nucleus. STAT3 is constitutively active in many tumor cells, but not in normal cells, and regulates the expression of genes involved in tumor progression, such as cell survival, proliferation, invasion, and angiogenesis<sup>[51]</sup>.

Clinical and experimental evidence suggest the involvement of the STAT3 signaling pathway in hepatocarcinogenesis. Activated nuclear STAT3 is found in 60% of HCC and is more pronounced in aggressive tumors<sup>[52]</sup>. In contrast, suppressors of this pathway, such as suppressor of cytokine signaling 3 (SOCS3), are down-regulated in HCC<sup>[53]</sup>. In a mouse model, hepatocyte-specific STAT3 ablation prevented DEN-induced HCC development<sup>[52]</sup>, whereas hepatocyte-specific SOCS3 knockout mice were susceptible to HCC development through the enhanced activation of JAK/STAT and MAPK signaling<sup>[53]</sup>. Hepatocyte-specific IL-6 and IL-6R transgenic mice spontaneously develop hepatocellular hyperplasia and adenomas, which are considered precancerous lesions in humans, accompanying STAT3 activation<sup>[54]</sup>. Furthermore, in a human study, gain-of-function mutations in gp130 have been identified in 60% of benign hepatocellular adenomas with an inflammatory phenotype, and when combined with  $\beta$ -catenin-activating mutation, lead to HCC development<sup>[55]</sup>. Thus, the IL-6-gp130-JAK-STAT3 signaling axis is an important contributor to HCC development, making it an attractive target for the treatment and/or prevention of hepatocarcinogenesis.

Interaction between STAT3 and NF- $\kappa$ B has been reported at several levels in tumors. Some studies showed that STAT3 and NF- $\kappa$ B co-regulate numerous oncogenic and inflammatory genes<sup>[50]</sup>. Additionally, STAT3 directly interacts with RelA, trapping it in the nucleus, thereby



**Figure 2** Implications and regulatory system of interleukin-6/gp130/janus activated kinase/signal transducer and activator of transcription 3 signaling in hepatocarcinogenesis. Interleukin (IL)-6 secreted by Kupffer cells activates signal transducer and activator of transcription (STAT) 3, which promotes the proliferation and survival of initiated hepatocytes. STAT3 activation is suppressed by I $\kappa$ B kinase  $\beta$  through the prevention of reactive oxygen species. However, once STAT3 is activated, STAT3 activation becomes sustained through a microRNA feedback inflammatory loop. JAK: Janus activated kinase; SOCS3: Suppressor of cytokine signaling 3; IKK: I $\kappa$ B kinase; HNF: Hepatocyte nuclear factor; ROS: Reactive oxygen species.

contributing to constitutive NF- $\kappa$ B activation<sup>[56]</sup>. On the other hand, a recent *in vivo* study revealed that IKK $\beta$ /NF- $\kappa$ B in the hepatocyte negatively regulated STAT3 activation in a DEN-induced HCC model<sup>[52]</sup>. Inactivation of IKK $\beta$  caused ROS accumulation and ROS were found to oxidize protein tyrosine phosphatases, including SHP1 and SHP2, which dephosphorylated JAK2 and STAT3. Oxidation of SHP1 and SHP2 results in the loss of their catalytic activity and the constitutive activation of STAT3. In fact, an inverse correlation between NF- $\kappa$ B and STAT3 has been found in human HCC samples. This crosstalk may be a reason for the aggravation of DEN-induced HCC in hepatocyte-specific IKK $\beta$  knockout mice. Furthermore, Bard-Chapeau *et al.*<sup>[57]</sup> showed that hepatocyte-specific SHP2 knockout mice revealed spontaneous liver inflammation and tumorigenesis, accompanied by STAT3 activation. These mice also showed enhanced DEN-induced HCC, which was decreased significantly by intercrossing with hepatocyte-specific STAT3 knockout mice. These results suggest that the inhibition of STAT3 activation by SHP2 plays an important tumor-suppressing role in hepatocarcinogenesis (Figure 2).

#### Implications of microRNA in inflammatory signaling

MicroRNAs (miRNAs) are endogenous 20-23-nucleotide RNAs that play important gene-regulatory roles by pairing with the messenger RNAs of protein-coding genes to

direct their post-translational repression<sup>[58]</sup>. Recently, miRNAs have been reported to be implicated in hepatocarcinogenesis through modulating inflammatory signaling pathways. Ji *et al.*<sup>[59]</sup> found that miR-26 was significantly reduced in human HCC tissues, compared with surrounding non-tumor tissues, and a gene network analysis revealed that miR-26 expression was inversely correlated with the activation of NF- $\kappa$ B and IL-6 signaling pathways. Although a causal relationship between miR-26 and hepatocarcinogenesis could not be evaluated in this study, Kota *et al.*<sup>[60]</sup> showed that low miR-26 expression played a causal role in hepatocarcinogenesis using a myc-induced mouse HCC model, and induction of miR-26 by gene therapy suppressed HCC development.

More recently, hepatocyte nuclear factor 4 $\alpha$  (HNF4 $\alpha$ ), a transcription factor that is essential for the development of hepatocytes, was reported to play a key role in hepatocarcinogenesis, linking miRNAs and inflammatory signaling pathways<sup>[61]</sup>. Transient suppression of HNF4 $\alpha$  induces decreased miR-124 expression, leading to increased IL-6R expression and subsequent STAT3 activation. STAT3 activation not only plays the tumor-promoting roles described above, but also up-regulates HNF4 $\alpha$ -targeting miRNAs, miR-24 and miR-629, resulting in continued suppression of HNF4 $\alpha$ . Thus, transient suppression of HNF4 $\alpha$  initiates an IL-6/STAT3-mediated hepatocarcinogenesis process through a miRNA feedback loop circuit (Figure 2). The authors also showed that systemic administration of miR-124 prevented hepatocarcinogenesis by inhibiting the feedback loop and inducing tumor-specific apoptosis in mice. Thus, a therapeutic strategy targeting miRNA may be useful for the prevention and treatment of HCC.

## STRESS-RELATED SIGNALING IN HEPATOCARCINOGENESIS

### Stress-activated MAPK

MAPK cascades are signaling systems that transmit intracellular signals initiated by extracellular stimuli to the nucleus<sup>[62,63]</sup>. The MAPK family consists of three major MAPK cascades, converging on extracellular signal-regulated kinases (ERKs), JNKs, and p38 MAPKs. Each MAPK signaling system comprises at least three components: MAPK, MAPK kinase (MAPKK), and MAPKK kinase (MAP3K). MAP3K phosphorylates, and thereby activates, MAPKK, and activated MAPKK, in turn, phosphorylates and activates MAPK. Among the three MAPKs, ERKs are activated predominantly by growth factors, whereas JNKs and p38 MAPKs, also called stress-activated MAPKs, are activated by stresses. We discuss the function and regulation of the stress-activated MAPKs in hepatocarcinogenesis in detail below.

### Role of JNK signaling

JNK has three isoforms (JNK1, JNK2, JNK3) encoded by three loci. JNK1 and JNK2 are expressed ubiquitously, including in the liver, whereas JNK3 is expressed primar-

ily in the brain<sup>[64]</sup>. JNK is phosphorylated and activated by two MAPKs, MKK4 and MKK7, and subsequently phosphorylates transcription factors, such as c-Jun and JunD, which compose the AP-1 complex<sup>[65]</sup>. Additionally, JNK phosphorylates other proteins, such as Bcl-2 family members, and exerts various kinds of functions, depending on the cell type and stimuli<sup>[66]</sup>. Furthermore, although JNK1 and JNK2 isoforms play redundant roles in many physiological processes, they also have distinct biological activities in some situations<sup>[67,68]</sup>.

The major functions of JNK in the liver are thought to be the induction of hepatocyte proliferation and cell death. JNK is involved in cell cycle progression, mostly through the activation of c-Jun. In this function, JNK1 is considered to be more important than JNK2, because proliferation of hepatocytes after partial hepatectomy is significantly impaired in JNK1 knockout mice, but not in JNK2 knockout mice<sup>[69]</sup>. Additionally, hepatocyte death due to TNF- $\alpha$ , lipotoxicity, ER stress, ischemia-reperfusion, and drug toxicity, such as that from acetaminophen, are also considered to be JNK-dependent<sup>[19,70-73]</sup>. JNK1 and JNK2 are, to some extent, redundant in this function. Although the downstream targets of JNK are not fully understood, most studies have demonstrated that JNK is required for the activation of the mitochondrial apoptotic pathway, through the activation of pro-apoptotic Bcl-2 family members<sup>[74,75]</sup>.

The role of JNK in hepatocarcinogenesis has been investigated using the DEN-induced HCC model<sup>[76]</sup>. As mentioned above, hepatocyte-specific knockout of IKK $\beta$  markedly promotes DEN-induced HCC, through enhanced hepatocyte death and compensatory proliferation<sup>[21]</sup>. These phenomena can be partly explained by enhanced JNK activation in the setting of IKK $\beta$  depletion. Because prolonged JNK activation is closely related to cell death, systems for the regulation of JNK activity are needed for tissue homeostasis. In this regard, NF- $\kappa$ B plays an important role. Although several mechanisms have been proposed in the NF- $\kappa$ B-mediated inhibition of JNK activation, ROS is one of the most important mediators<sup>[25,77-79]</sup>. ROS accumulation, caused by the reduced expression of NF- $\kappa$ B-dependent antioxidative enzymes, extends JNK activation by inactivating MAPK phosphatases that are essential for the dephosphorylation of activated JNK<sup>[25]</sup>. In fact, the administration of antioxidants to hepatocyte-specific IKK $\beta$  knockout mice decreased sustained JNK activation and hepatocyte death after DEN injection, and furthermore, intercrossing hepatocyte-specific IKK $\beta$  knockout mice with JNK1 knockout mice significantly reduced DEN-induced hepatocyte death and compensatory proliferation, eventually suppressing HCC development<sup>[21,76]</sup>. Additionally, JNK1 knockout mice showed a significant reduction of DEN-induced HCC, compared with wild-type controls. Thus, JNK1 is involved in hepatocarcinogenesis through hepatocyte death and proliferation, which are key components of necro-inflammatory cycles (Figure 1). Furthermore, in addition to the initial phase, JNK1 plays a tumor-

promoting role by enhancing cancer cell proliferation and neovascularization through the increased expression of cyclin D1 and vascular endothelial growth factor, respectively<sup>[76]</sup>. Another study showed that JNK1 promoted HCC cell proliferation *in vivo* through the up-regulation of c-myc expression and the down-regulation of p21 expression<sup>[69]</sup>. This study, however, also showed that JNK2 was not involved in hepatocarcinogenesis. In fact, JNK1, but not JNK2, is activated in approximately half of human HCC tissues, compared with adjacent non-tumor tissues<sup>[69,80]</sup>. These results suggest that JNKs, especially JNK1, play an important role in the development of HCC. Notably, the pharmacological inhibition of JNK suppressed DEN-induced HCC and the growth of xenografted human HCC cells, suggesting that JNK may be a promising therapeutic target for HCC<sup>[69]</sup>.

On the other hand, a recent study using conditional JNK knockout mice showed that the ablation of both JNK isoforms, JNK1 and JNK2, in hepatocytes increased DEN-induced HCC, whereas the ablation of JNK1 and JNK2 in both hepatocytes and myeloid cells reduced hepatic inflammation and the development of HCC, indicating that JNK plays dual roles in hepatocarcinogenesis, depending on cell type and carcinogenesis stage<sup>[81]</sup>.

JNK plays a pivotal role in the development of metabolic syndrome-related disorders, including NASH<sup>[41]</sup>. Inflammatory cytokines and ROS accumulation in the liver caused by obesity and fatty liver disease induce JNK activation, leading to insulin resistance by increasing inhibitory insulin receptor substrate 1 ser307 phosphorylation<sup>[82]</sup>. As a clinical study showed that insulin resistance was a major contributor to obesity-mediated hepatocarcinogenesis, JNK may be a candidate therapeutic target in such situations<sup>[83]</sup>. Furthermore, ROS-mediated JNK activation in the liver is linked not only to liver disease, but also to systemic disorders, such as atherosclerotic cerebrovascular diseases; thus, further elucidation of this process is important<sup>[84,85]</sup>.

### Role of p38 signaling

The p38 MAPK family consists of four members: p38 $\alpha$ , p38 $\beta$ , p38 $\gamma$ , and p38 $\delta$ <sup>[86]</sup>. Among them, p38 $\alpha$  is abundant in most cell types, and its function has been investigated in most published studies of p38 MAPKs. p38 is activated through phosphorylation, primarily by MKK3 and MKK6, but phosphorylation by MKK4 and autophosphorylation are also involved in some stimuli<sup>[87]</sup>. p38 can activate not only transcription factors, such as ATF2, p53, and Mitf, but also protein kinases, such as MAPKAP kinase 2 (MK2) and MK5<sup>[88]</sup>. Although p38 was initially discovered as a regulator of inflammatory cytokine production, recent studies have revealed that it has tumor-suppressing properties. p38 inhibits tumorigenesis by the down-regulation of cyclins, up-regulation of cyclin-dependent kinase inhibitors, and modulation of the tumor suppressor p53, resulting in cell cycle arrest, oncogene-induced senescence, apoptosis induction, and contact inhibition<sup>[86]</sup>.

Although the roles of p38 in the liver have yet to be clarified, compared with JNK, major roles reported to date are the inhibition of hepatocyte death and proliferation. These effects of p38 are partially mediated by negative regulation of the JNK/c-Jun pathway. For example, hepatocyte-specific p38 $\alpha$  knockout mice showed much stronger lipopolysaccharide (LPS)-induced JNK activation in the liver, and intercrossing with hepatocyte specific IKK $\beta$  knockout mice induced severe liver injury after LPS administration, suggesting that p38 $\alpha$  and IKK $\beta$  act synergistically to protect the liver from TNF- $\alpha$ -induced hepatocyte death<sup>[89]</sup>.

The role of p38 in hepatocarcinogenesis has also been investigated using the DEN-induced HCC model<sup>[38]</sup>. Similar to hepatocyte-specific IKK $\beta$  knockout mice, hepatocyte-specific p38 $\alpha$  knockout mice showed enhancement of DEN-induced ROS accumulation, JNK activation, liver damage, and compensatory hepatocyte proliferation, eventually resulting in enhanced carcinogenesis. Another study showed the tumor-suppressing role of p38 through interaction with the JNK/c-Jun pathway by focusing on the antiproliferative effects in the advanced stage<sup>[90]</sup>. However, in contrast to IKK $\beta$  knockout mice, enhanced activation of the JNK pathway in p38 $\alpha$  knockout mice was accompanied by MAPKK activation, suggesting that the targets of p38 $\alpha$  may be upstream of JNK, such as MAPKKs and MAP3Ks<sup>[38]</sup>. Consistent with these animal experiments, in human samples, the activity of the MKK6/p38 pathway is decreased in HCC tissues, compared with adjacent non-tumor tissues, and is significantly lower in larger HCC tissues<sup>[91]</sup>. These findings suggest that the p38 pathway may play an anti-proliferative role in human HCC.

### **Regulatory system of stress-activated MAPK signaling by MAP3Ks**

The evidence presented above suggests that JNK acts generally as a tumor promoter and p38 acts generally as a tumor suppressor in hepatocarcinogenesis, but some studies have shown opposite roles in HCC and other cancers. For example, JNK plays tumor-suppressing roles in mouse skin cancer and mammalian cancer models<sup>[92,93]</sup>. Additionally, JNK has been reported to act as a tumor suppressor by inducing cancer cell apoptosis in HCC<sup>[94]</sup>. As mentioned above, JNK was also reported to play dual roles in hepatocarcinogenesis, depending on cell type and carcinogenesis stage<sup>[81]</sup>. p38 may also have oncogenic effects, facilitating cell invasion, inflammation, and angiogenesis<sup>[95,96]</sup>. Furthermore, crosstalk among JNK, p38, and molecules involved in other signaling pathways, such as NF- $\kappa$ B, further complicates their roles<sup>[97]</sup>. Thus, understanding of the regulatory system of stress-activated MAPK signaling is necessary for the potential use of these molecules as therapeutic targets. Importantly, only two molecules, JNK and p38, are downstream in this pathway, whereas more than 10 molecules have been identified for upstream MAP3Ks<sup>[98]</sup>. Each MAP3K is activated by several different kinds of stimuli, and integrated

into a unique pattern of MAPK activation and substrate phosphorylation, leading to a specific cellular response to the stimulus. Thus, the activities of JNK and p38 are tightly regulated by MAP3Ks. Several recent studies have uncovered roles of MAP3Ks in the regulation of stress-activated MAPK signaling in hepatocarcinogenesis.

### **Role of apoptosis signal-regulating kinase 1**

Apoptosis signal-regulating kinase 1 (ASK1), one of the most important MAP3Ks, selectively activates JNK and p38 signaling in response to a variety of stimuli, including ROS and cytokines<sup>[99]</sup>. In particular, ASK1 plays a key role in oxidative stress-induced cell death. In the absence of oxidative stress, thioredoxin (Trx), a reduction/oxidation regulatory protein, inhibits ASK1 kinase activity *via* direct binding to the N-terminal region of ASK1. However, once oxidative stress occurs in the cell, Trx is converted to its oxidized form and dissociates from ASK1, resulting in ASK1 kinase activation<sup>[100]</sup>. ASK1 is considered to induce cell death through stress-activated MAPK-mediated activation of the mitochondrial cell death pathway<sup>[101]</sup>. In fact, ASK1 is involved in acetaminophen-induced hepatocyte death, a typical ROS-mediated liver injury, through mechanisms involving Trx-ASK1 dissociation<sup>[102]</sup>. Furthermore, ASK1 is involved in hepatocyte death mediated by death receptors, such as TNF-R and Fas<sup>[75]</sup>.

ASK1 knockout mice showed significantly increased DEN-induced HCC, suggesting that ASK1 plays tumor-suppressing roles in hepatocarcinogenesis in this model<sup>[75]</sup>. Activation of JNK and the pro-apoptotic Bcl-2 family member Bim, which are required for death receptor-mediated apoptosis, are attenuated in ASK1 knockout HCC, resulting in decreased cancer cell apoptosis. On the other hand, ASK1 plays a minor role in the tumor-promoting effects of JNK, such as the DEN-induced acute phase reaction, cancer cell proliferation, and neovascularization. Thus, ASK1 is considered to play major roles in the tumor-suppressing part of JNK activity in hepatocarcinogenesis. Furthermore, DNA damage-induced p38 activation and subsequent p21 up-regulation is impaired in ASK1 knockout mice. Thus, ASK1 controls the tumor-suppressing function in stress-activated MAPK signaling through the induction of apoptosis and the DNA damage response.

Another study indicated that ASK1 and Bim are also required for sorafenib-induced apoptosis in HCC cells<sup>[103]</sup>. Sorafenib is a small-molecule multikinase inhibitor that is currently the sole therapeutic drug effective for the treatment of HCC<sup>[104]</sup>. Most recently, somatic mutations in the ASK1 gene, which reduce the kinase activity of ASK1, have been identified in melanoma<sup>[105]</sup>. Thus, it may be important to clarify whether similar mutations are found in HCC, from the point of view of not only the carcinogenesis mechanism, but also possible therapeutic effects of anticancer drugs.

### **Role of transforming growth factor $\beta$ -activated kinase 1**

Another major MAP3K, activated kinase 1 (TAK1), is

activated through TNF receptor, TLR, IL-1 receptor, and transforming growth factor  $\beta$  receptor signaling, and then activates the JNK and NF- $\kappa$ B pathways, which play opposing roles in cell death<sup>[106]</sup>. Interestingly, hepatocyte-specific TAK1 knockout mice show spontaneous hepatocyte death, and this phenotype is partially rescued by crossing with TNF receptor 1 knockout mice, suggesting that TAK1 knockout hepatocytes are highly sensitive to endogenous TNF- $\alpha$ -induced apoptosis<sup>[107]</sup>. This spontaneous cell death subsequently causes compensatory hepatocyte proliferation, inflammation, fibrosis, and the eventual development of HCC in aged mice<sup>[107,108]</sup>. These phenomena resemble the phenotype observed in hepatocyte-specific NF- $\kappa$ B knockout mice. Furthermore, JNK activation is rather enhanced in TAK1 knockout mice, indicating that TAK1 in the hepatocytes acts as a tumor suppressor, mainly by regulating the activation of the NF- $\kappa$ B pathway. However, enhanced JNK activation in TAK1 knockout mice occurs partially through the activation of another MAP3K, TAO2, suggesting that TAK1 may interact with other MAP3Ks<sup>[108]</sup>. Interestingly, crossing hepatocyte-specific TAK1 knockout mice with NEMO knockout mice attenuated JNK activation and prevented hepatocyte death and the development of HCC, suggesting that NEMO has a tumor-promoting function in the setting of TAK1 deletion<sup>[108]</sup>. Additionally, this function of NEMO is considered to be independent of NF- $\kappa$ B. Furthermore, a recent study showed that TAK1 inhibits ASK1-mediated apoptosis through a direct interaction between the C-terminal domain of TAK1 and the N-terminal or C-terminal domain of ASK1 in HEK 293 cells<sup>[109]</sup>. Thus, in the setting of TAK1 deletion, ASK1 may play a tumor-promoting role by accelerating hepatocyte apoptosis and subsequent inflammation. Because crosstalk among MAP3Ks is less well understood, further studies are needed to clarify the whole picture of stress-activated MAPK signaling pathways.

## CONCLUSION

One of the most important reasons for the poor prognosis of HCC is its frequent recurrence. Once HCC has developed, the recurrence rate does not decline with time, suggesting that most cases of late-phase recurrence are due to metachronous multicentric carcinogenesis caused by persistent chronic inflammation<sup>[110]</sup>. Thus, determining the molecular mechanism(s) of inflammation-mediated hepatocarcinogenesis is important in preventing not only the occurrence, but also the recurrence, of HCC. As discussed in this review, recent studies have indicated that NF- $\kappa$ B, STAT3, and stress-activated MAPK signaling pathways play key roles in inflammation-mediated hepatocarcinogenesis. These findings may prompt their introduction into the clinical setting as therapeutic targets. However, these pathways have a wide range of functions and exhibit complex crosstalk, and furthermore, may play opposing roles, depending on the cell type and carcinogenesis stage. Thus, alternative strategies, such as

targeting particular isoforms, including JNK1; upstream regulators, including MAP3K; and other modulators, including miRNA; may be more beneficial than targeting the entire pathway. In this regard, further studies clarifying the entire picture of the signaling network are needed to translate these signaling pathways into clinical practice.

## REFERENCES

- 1 **Grivennikov SI**, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010; **140**: 883-899
- 2 **Maeda S**, Omata M. Inflammation and cancer: role of nuclear factor-kappaB activation. *Cancer Sci* 2008; **99**: 836-842
- 3 **Balkwill F**, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539-545
- 4 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127
- 5 **Masuzaki R**, Yoshida H, Tateishi R, Shiina S, Omata M. Hepatocellular carcinoma in viral hepatitis: improving standard therapy. *Best Pract Res Clin Gastroenterol* 2008; **22**: 1137-1151
- 6 **Shiina S**, Tateishi R, Arano T, Uchino K, Enooku K, Nakagawa H, Asaoka Y, Sato T, Masuzaki R, Kondo Y, Goto T, Yoshida H, Omata M, Koike K. Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. *Am J Gastroenterol* 2012; **107**: 569-577; quiz 578
- 7 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576
- 8 **Budhu A**, Wang XW. The role of cytokines in hepatocellular carcinoma. *J Leukoc Biol* 2006; **80**: 1197-1213
- 9 **Levrero M**. Viral hepatitis and liver cancer: the case of hepatitis C. *Oncogene* 2006; **25**: 3834-3847
- 10 **Maeda S**. NF- $\kappa$ B, JNK, and TLR Signaling Pathways in Hepatocarcinogenesis. *Gastroenterol Res Pract* 2010; **2010**: 367694
- 11 **He G**, Karin M. NF- $\kappa$ B and STAT3 - key players in liver inflammation and cancer. *Cell Res* 2011; **21**: 159-168
- 12 **Hoffmann A**, Baltimore D. Circuitry of nuclear factor kappaB signaling. *Immunol Rev* 2006; **210**: 171-186
- 13 **Ghosh S**, Karin M. Missing pieces in the NF-kappaB puzzle. *Cell* 2002; **109** Suppl: S81-S96
- 14 **Beg AA**, Sha WC, Bronson RT, Ghosh S, Baltimore D. Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. *Nature* 1995; **376**: 167-170
- 15 **Li Q**, Van Antwerp D, Mercurio F, Lee KF, Verma IM. Severe liver degeneration in mice lacking the IkappaB kinase 2 gene. *Science* 1999; **284**: 321-325
- 16 **Rudolph D**, Yeh WC, Wakeham A, Rudolph B, Nallainathan D, Potter J, Elia AJ, Mak TW. Severe liver degeneration and lack of NF-kappaB activation in NEMO/IKKgamma-deficient mice. *Genes Dev* 2000; **14**: 854-862
- 17 **Doi TS**, Marino MW, Takahashi T, Yoshida T, Sakakura T, Old LJ, Obata Y. Absence of tumor necrosis factor rescues RelA-deficient mice from embryonic lethality. *Proc Natl Acad Sci USA* 1999; **96**: 2994-2999
- 18 **Rosenfeld ME**, Prichard L, Shiojiri N, Fausto N. Prevention of hepatic apoptosis and embryonic lethality in RelA/TNFR-1 double knockout mice. *Am J Pathol* 2000; **156**: 997-1007
- 19 **Maeda S**, Chang L, Li ZW, Luo JL, Leffert H, Karin M. IKKbeta is required for prevention of apoptosis mediated by cell-bound but not by circulating TNFalpha. *Immunity* 2003; **19**: 725-737
- 20 **Luedde T**, Beraza N, Kotsikoris V, van Loo G, Nenci A, De Vos R, Roskams T, Trautwein C, Pasparakis M. Deletion of NEMO/IKKgamma in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. *Cancer Cell* 2007; **11**: 119-132
- 21 **Maeda S**, Kamata H, Luo JL, Leffert H, Karin M. IKKbeta

- couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. *Cell* 2005; **121**: 977-990
- 22 **Lee JS**, Chu IS, Mikaelyan A, Calvisi DF, Heo J, Reddy JK, Thorgeirsson SS. Application of comparative functional genomics to identify best-fit mouse models to study human cancer. *Nat Genet* 2004; **36**: 1306-1311
- 23 **Leenders MW**, Nijkamp MW, Borel Rinkes IH. Mouse models in liver cancer research: a review of current literature. *World J Gastroenterol* 2008; **14**: 6915-6923
- 24 **Fausto N**. Mouse liver tumorigenesis: models, mechanisms, and relevance to human disease. *Semin Liver Dis* 1999; **19**: 243-252
- 25 **Kamata H**, Honda S, Maeda S, Chang L, Hirata H, Karin M. Reactive oxygen species promote TNF $\alpha$ -induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* 2005; **120**: 649-661
- 26 **Pikarsky E**, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gulkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y. NF- $\kappa$ B functions as a tumour promoter in inflammation-associated cancer. *Nature* 2004; **431**: 461-466
- 27 **Haybaeck J**, Zeller N, Wolf MJ, Weber A, Wagner U, Kurrer MO, Bremer J, Iezzi G, Graf R, Clavien PA, Thimme R, Blum H, Nedospasov SA, Zatloukal K, Ramzan M, Ciesek S, Pietschmann T, Marche PN, Karin M, Kopf M, Browning JL, Aguzzi A, Heikenwalder M. A lymphotoxin-driven pathway to hepatocellular carcinoma. *Cancer Cell* 2009; **16**: 295-308
- 28 **Maeda S**, Hikiba Y, Sakamoto K, Nakagawa H, Hirata Y, Hayakawa Y, Yanai A, Ogura K, Karin M, Omata M. Ikappa B kinasebeta/nuclear factor-kappaB activation controls the development of liver metastasis by way of interleukin-6 expression. *Hepatology* 2009; **50**: 1851-1860
- 29 **Berasain C**, Castillo J, Perugorria MJ, Latasa MU, Prieto J, Avila MA. Inflammation and liver cancer: new molecular links. *Ann N Y Acad Sci* 2009; **1155**: 206-221
- 30 **Naugler WE**, Karin M. The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. *Trends Mol Med* 2008; **14**: 109-119
- 31 **Gao B**. Cytokines, STATs and liver disease. *Cell Mol Immunol* 2005; **2**: 92-100
- 32 **Devriere J**, Content J, Denys C, Vandebussche P, Schandene L, Wybran J, Dupont E. High interleukin-6 serum levels and increased production by leucocytes in alcoholic liver cirrhosis. Correlation with IgA serum levels and lymphokines production. *Clin Exp Immunol* 1989; **77**: 221-225
- 33 **Lee Y**, Park US, Choi I, Yoon SK, Park YM, Lee YI. Human interleukin 6 gene is activated by hepatitis B virus-X protein in human hepatoma cells. *Clin Cancer Res* 1998; **4**: 1711-1717
- 34 **Wieckowska A**, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol* 2008; **103**: 1372-1379
- 35 **Wong VW**, Yu J, Cheng AS, Wong GL, Chan HY, Chu ES, Ng EK, Chan FK, Sung JJ, Chan HL. High serum interleukin-6 level predicts future hepatocellular carcinoma development in patients with chronic hepatitis B. *Int J Cancer* 2009; **124**: 2766-2770
- 36 **Nakagawa H**, Maeda S, Yoshida H, Tateishi R, Masuzaki R, Ohki T, Hayakawa Y, Kinoshita H, Yamakado M, Kato N, Shiina S, Omata M. Serum IL-6 levels and the risk for hepatocarcinogenesis in chronic hepatitis C patients: an analysis based on gender differences. *Int J Cancer* 2009; **125**: 2264-2269
- 37 **Naugler WE**, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 2007; **317**: 121-124
- 38 **Sakurai T**, He G, Matsuzawa A, Yu GY, Maeda S, Hardiman G, Karin M. Hepatocyte necrosis induced by oxidative stress and IL-1 alpha release mediate carcinogen-induced compensatory proliferation and liver tumorigenesis. *Cancer Cell* 2008; **14**: 156-165
- 39 **Ruggieri A**, Barbati C, Malorni W. Cellular and molecular mechanisms involved in hepatocellular carcinoma gender disparity. *Int J Cancer* 2010; **127**: 499-504
- 40 **Li Z**, Tuteja G, Schug J, Kaestner KH. Foxa1 and Foxa2 are essential for sexual dimorphism in liver cancer. *Cell* 2012; **148**: 72-83
- 41 **Gregor MF**, Hotamisligil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol* 2011; **29**: 415-445
- 42 **Muto Y**, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, Kato M, Nakamura T, Higuchi K, Nishiguchi S, Kumada H, Ohashi Y. Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. *Hepatology* 2006; **35**: 204-214
- 43 **Ioannou GN**, Splan MF, Weiss NS, McDonald GB, Beretta L, Lee SP. Incidence and predictors of hepatocellular carcinoma in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2007; **5**: 938-945, 945.e1-4
- 44 **Park EJ**, Lee JH, Yu GY, He G, Ali SR, Holzer RG, Osterreicher CH, Takahashi H, Karin M. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 2010; **140**: 197-208
- 45 **Ohki T**, Tateishi R, Shiina S, Goto E, Sato T, Nakagawa H, Masuzaki R, Goto T, Hamamura K, Kanai F, Yoshida H, Kawabe T, Omata M. Visceral fat accumulation is an independent risk factor for hepatocellular carcinoma recurrence after curative treatment in patients with suspected NASH. *Gut* 2009; **58**: 839-844
- 46 **Marra F**, Bertolani C. Adipokines in liver diseases. *Hepatology* 2009; **50**: 957-969
- 47 **Arano T**, Nakagawa H, Tateishi R, Ikeda H, Uchino K, Enooku K, Goto E, Masuzaki R, Asaoka Y, Kondo Y, Goto T, Shiina S, Omata M, Yoshida H, Koike K. Serum level of adiponectin and the risk of liver cancer development in chronic hepatitis C patients. *Int J Cancer* 2011; **129**: 2226-2235
- 48 **Toffanin S**, Friedman SL, Llovet JM. Obesity, inflammatory signaling, and hepatocellular carcinoma-an enlarging link. *Cancer Cell* 2010; **17**: 115-117
- 49 **Yoshimura A**. Signal transduction of inflammatory cytokines and tumor development. *Cancer Sci* 2006; **97**: 439-447
- 50 **Yu H**, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer* 2009; **9**: 798-809
- 51 **Aggarwal BB**, Kunnumakkara AB, Harikumar KB, Gupta SR, Tharakan ST, Koca C, Dey S, Sung B. Signal transducer and activator of transcription-3, inflammation, and cancer: how intimate is the relationship? *Ann N Y Acad Sci* 2009; **1171**: 59-76
- 52 **He G**, Yu GY, Temkin V, Ogata H, Kuntzen C, Sakurai T, Sieghart W, Peck-Radosavljevic M, Leffert HL, Karin M. Hepatocyte IKKbeta/NF-kappaB inhibits tumor promotion and progression by preventing oxidative stress-driven STAT3 activation. *Cancer Cell* 2010; **17**: 286-297
- 53 **Ogata H**, Kobayashi T, Chinen T, Takaki H, Sanada T, Minoda Y, Koga K, Takaesu G, Maehara Y, Iida M, Yoshimura A. Deletion of the SOCS3 gene in liver parenchymal cells promotes hepatitis-induced hepatocarcinogenesis. *Gastroenterology* 2006; **131**: 179-193
- 54 **Maione D**, Di Carlo E, Li W, Musiani P, Modesti A, Peters M, Rose-John S, Della Rocca C, Tripodi M, Lazzaro D, Taub R, Savino R, Ciliberto G. Coexpression of IL-6 and soluble IL-6R causes nodular regenerative hyperplasia and adenomas of the liver. *EMBO J* 1998; **17**: 5588-5597
- 55 **Rebouisson S**, Amessou M, Couchy G, Poussin K, Imbeaud S, Pilati C, Izard T, Balabaud C, Bioulac-Sage P, Zucman-Rossi J. Frequent in-frame somatic deletions activate gp130 in inflammatory hepatocellular tumours. *Nature* 2009; **457**: 200-204

- 56 **Lee H**, Herrmann A, Deng JH, Kujawski M, Niu G, Li Z, Forman S, Jove R, Pardoll DM, Yu H. Persistently activated Stat3 maintains constitutive NF-kappaB activity in tumors. *Cancer Cell* 2009; **15**: 283-293
- 57 **Bard-Chapeau EA**, Li S, Ding J, Zhang SS, Zhu HH, Princen F, Fang DD, Han T, Bailly-Maitre B, Poli V, Varki NM, Wang H, Feng GS. Ptpn11/Shp2 acts as a tumor suppressor in hepatocellular carcinogenesis. *Cancer Cell* 2011; **19**: 629-639
- 58 **Bartel DP**. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; **136**: 215-233
- 59 **Ji J**, Shi J, Budhu A, Yu Z, Forgues M, Roessler S, Ambs S, Chen Y, Meltzer PS, Croce CM, Qin LX, Man K, Lo CM, Lee J, Ng IO, Fan J, Tang ZY, Sun HC, Wang XW. MicroRNA expression, survival, and response to interferon in liver cancer. *N Engl J Med* 2009; **361**: 1437-1447
- 60 **Kota J**, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, Chang TC, Vivekanandan P, Torbenson M, Clark KR, Mendell JR, Mendell JT. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* 2009; **137**: 1005-1017
- 61 **Hatziaepostolou M**, Polytaichou C, Aggelidou E, Drakaki A, Poultsides GA, Jaeger SA, Ogata H, Karin M, Struhl K, Hadzopoulou-Cladaras M, Iliopoulos D. An HNF4c-miRNA inflammatory feedback circuit regulates hepatocellular oncogenesis. *Cell* 2011; **147**: 1233-1247
- 62 **Kyriakis JM**, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* 2001; **81**: 807-869
- 63 **Chang L**, Karin M. Mammalian MAP kinase signalling cascades. *Nature* 2001; **410**: 37-40
- 64 **Davis RJ**. Signal transduction by the JNK group of MAP kinases. *Cell* 2000; **103**: 239-252
- 65 **Shaulian E**, Karin M. AP-1 as a regulator of cell life and death. *Nat Cell Biol* 2002; **4**: E131-E136
- 66 **Weston CR**, Davis RJ. The JNK signal transduction pathway. *Curr Opin Genet Dev* 2002; **12**: 14-21
- 67 **Sabapathy K**, Hochedlinger K, Nam SY, Bauer A, Karin M, Wagner EF. Distinct roles for JNK1 and JNK2 in regulating JNK activity and c-Jun-dependent cell proliferation. *Mol Cell* 2004; **15**: 713-725
- 68 **Shen HM**, Liu ZG. JNK signaling pathway is a key modulator in cell death mediated by reactive oxygen and nitrogen species. *Free Radic Biol Med* 2006; **40**: 928-939
- 69 **Hui L**, Zatloukal K, Scheuch H, Stepniak E, Wagner EF. Proliferation of human HCC cells and chemically induced mouse liver cancers requires JNK1-dependent p21 down-regulation. *J Clin Invest* 2008; **118**: 3943-3953
- 70 **Malhi H**, Bronk SF, Werneburg NW, Gores GJ. Free fatty acids induce JNK-dependent hepatocyte lipoapoptosis. *J Biol Chem* 2006; **281**: 12093-12101
- 71 **Malhi H**, Gores GJ. Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. *Semin Liver Dis* 2008; **28**: 360-369
- 72 **Uehara T**, Bennett B, Sakata ST, Satoh Y, Bilter GK, Westwick JK, Brenner DA. JNK mediates hepatic ischemia reperfusion injury. *J Hepatol* 2005; **42**: 850-859
- 73 **Gunawan BK**, Liu ZX, Han D, Hanawa N, Gaarde WA, Kaplowitz N. c-Jun N-terminal kinase plays a major role in murine acetaminophen hepatotoxicity. *Gastroenterology* 2006; **131**: 165-178
- 74 **Kaufmann T**, Jost PJ, Pellegrini M, Puthalakath H, Gugasyan R, Gerondakis S, Cretney E, Smyth MJ, Silke J, Hakem R, Bouillet P, Mak TW, Dixit VM, Strasser A. Fatal hepatitis mediated by tumor necrosis factor TNFalpha requires caspase-8 and involves the BH3-only proteins Bid and Bim. *Immunity* 2009; **30**: 56-66
- 75 **Nakagawa H**, Hirata Y, Takeda K, Hayakawa Y, Sato T, Kinoshita H, Sakamoto K, Nakata W, Hikiba Y, Omata M, Yoshida H, Koike K, Ichijo H, Maeda S. Apoptosis signal-regulating kinase 1 inhibits hepatocarcinogenesis by controlling the tumor-suppressing function of stress-activated mitogen-activated protein kinase. *Hepatology* 2011; **54**: 185-195
- 76 **Sakurai T**, Maeda S, Chang L, Karin M. Loss of hepatic NF-kappa B activity enhances chemical hepatocarcinogenesis through sustained c-Jun N-terminal kinase 1 activation. *Proc Natl Acad Sci USA* 2006; **103**: 10544-10551
- 77 **De Smaele E**, Zazzeroni F, Papa S, Nguyen DU, Jin R, Jones J, Cong R, Franzoso G. Induction of gadd45beta by NF-kappaB downregulates pro-apoptotic JNK signalling. *Nature* 2001; **414**: 308-313
- 78 **Kaur S**, Wang F, Venkatraman M, Arsuru M. X-linked inhibitor of apoptosis (XIAP) inhibits c-Jun N-terminal kinase 1 (JNK1) activation by transforming growth factor beta1 (TGF-beta1) through ubiquitin-mediated proteosomal degradation of the TGF-beta1-activated kinase 1 (TAK1). *J Biol Chem* 2005; **280**: 38599-38608
- 79 **Lee EG**, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A. Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* 2000; **289**: 2350-2354
- 80 **Chang Q**, Zhang Y, Beezhold KJ, Bhatia D, Zhao H, Chen J, Castranova V, Shi X, Chen F. Sustained JNK1 activation is associated with altered histone H3 methylations in human liver cancer. *J Hepatol* 2009; **50**: 323-333
- 81 **Das M**, Garlick DS, Greiner DL, Davis RJ. The role of JNK in the development of hepatocellular carcinoma. *Genes Dev* 2011; **25**: 634-645
- 82 **Hirosumi J**, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. *Nature* 2002; **420**: 333-336
- 83 **Nkontchou G**, Bastard JP, Ziol M, Aout M, Cosson E, Ganne-Carrie N, Grando-Lemaire V, Roulot D, Capeau J, Trinchet JC, Vicaute E, Beaugrand M. Insulin resistance, serum leptin, and adiponectin levels and outcomes of viral hepatitis C cirrhosis. *J Hepatol* 2010; **53**: 827-833
- 84 **Kaneto H**, Katakami N, Matsuhisa M, Matsuoka TA. Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. *Mediators Inflamm* 2010; **2010**: 453892
- 85 **Nakagawa H**, Isogawa A, Tateishi R, Tani M, Yoshida H, Yamakado M, Koike K. Serum gamma-glutamyltransferase level is associated with serum superoxide dismutase activity and metabolic syndrome in a Japanese population. *J Gastroenterol* 2011; Epub ahead of print
- 86 **Han J**, Sun P. The pathways to tumor suppression via route p38. *Trends Biochem Sci* 2007; **32**: 364-371
- 87 **Ge B**, Gram H, Di Padova F, Huang B, New L, Ulevitch RJ, Luo Y, Han J. MAPKK-independent activation of p38alpha mediated by TAB1-dependent autophosphorylation of p38alpha. *Science* 2002; **295**: 1291-1294
- 88 **Hui L**, Bakiri L, Stepniak E, Wagner EF. p38alpha: a suppressor of cell proliferation and tumorigenesis. *Cell Cycle* 2007; **6**: 2429-2433
- 89 **Heinrichsdorff J**, Luedde T, Perdiguero E, Nebreda AR, Pasparakis M. p38 alpha MAPK inhibits JNK activation and collaborates with IkappaB kinase 2 to prevent endotoxin-induced liver failure. *EMBO Rep* 2008; **9**: 1048-1054
- 90 **Hui L**, Bakiri L, Mairhorfer A, Schweifer N, Haslinger C, Kenner L, Komnenovic V, Scheuch H, Beug H, Wagner EF. p38alpha suppresses normal and cancer cell proliferation by antagonizing the JNK-c-Jun pathway. *Nat Genet* 2007; **39**: 741-749
- 91 **Iyoda K**, Sasaki Y, Horimoto M, Toyama T, Yakushiji T, Sakakibara M, Takehara T, Fujimoto J, Hori M, Wands JR, Hayashi N. Involvement of the p38 mitogen-activated protein kinase cascade in hepatocellular carcinoma. *Cancer* 2003; **97**: 3017-3026
- 92 **She QB**, Chen N, Bode AM, Flavell RA, Dong Z. Deficiency of c-Jun-NH(2)-terminal kinase-1 in mice enhances skin tumor development by 12-O-tetradecanoylphorbol-13-acetate. *Cancer Res* 2002; **62**: 1343-1348

- 93 **Cellurale C**, Weston CR, Reilly J, Garlick DS, Jerry DJ, Sluss HK, Davis RJ. Role of JNK in a Trp53-dependent mouse model of breast cancer. *PLoS One* 2010; **5**: e12469
- 94 **Saxena NK**, Fu PP, Nagalingam A, Wang J, Handy J, Cohen C, Tighiouart M, Sharma D, Anania FA. Adiponectin modulates C-jun N-terminal kinase and mammalian target of rapamycin and inhibits hepatocellular carcinoma. *Gastroenterology* 2010; **139**: 1762-1763
- 95 **Hsieh YH**, Wu TT, Huang CY, Hsieh YS, Hwang JM, Liu JY. p38 mitogen-activated protein kinase pathway is involved in protein kinase C $\alpha$ -regulated invasion in human hepatocellular carcinoma cells. *Cancer Res* 2007; **67**: 4320-4327
- 96 **Kim MS**, Lee EJ, Kim HR, Moon A. p38 kinase is a key signaling molecule for H-Ras-induced cell motility and invasive phenotype in human breast epithelial cells. *Cancer Res* 2003; **63**: 5454-5461
- 97 **Wagner EF**, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. *Nat Rev Cancer* 2009; **9**: 537-549
- 98 **Runchel C**, Matsuzawa A, Ichijo H. Mitogen-activated protein kinases in mammalian oxidative stress responses. *Antioxid Redox Signal* 2011; **15**: 205-218
- 99 **Ichijo H**, Nishida E, Irie K, ten Dijke P, Saitoh M, Moriguchi T, Takagi M, Matsumoto K, Miyazono K, Gotoh Y. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 1997; **275**: 90-94
- 100 **Saitoh M**, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 1998; **17**: 2596-2606
- 101 **Hatai T**, Matsuzawa A, Inoshita S, Mochida Y, Kuroda T, Sakamaki K, Kuida K, Yonehara S, Ichijo H, Takeda K. Execution of apoptosis signal-regulating kinase 1 (ASK1)-induced apoptosis by the mitochondria-dependent caspase activation. *J Biol Chem* 2000; **275**: 26576-26581
- 102 **Nakagawa H**, Maeda S, Hikiba Y, Ohmae T, Shibata W, Yanai A, Sakamoto K, Ogura K, Noguchi T, Karin M, Ichijo H, Omata M. Deletion of apoptosis signal-regulating kinase 1 attenuates acetaminophen-induced liver injury by inhibiting c-Jun N-terminal kinase activation. *Gastroenterology* 2008; **135**: 1311-1321
- 103 **Huynh H**, Choo SP, Toh HC, Tai WM, Chung AY, Chow PK, Ong R, Soo KC. Comparing the efficacy of sunitinib with sorafenib in xenograft models of human hepatocellular carcinoma: mechanistic explanation. *Curr Cancer Drug Targets* 2011; **11**: 944-953
- 104 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390
- 105 **Stark MS**, Woods SL, Gartside MG, Bonazzi VF, Dutton-Regester K, Aoude LG, Chow D, Sereduk C, Niemi NM, Tang N, Ellis JJ, Reid J, Zismann V, Tyagi S, Muzny D, Newsham I, Wu Y, Palmer JM, Pollak T, Youngkin D, Brooks BR, Lanagan C, Schmidt CW, Kobe B, MacKeigan JP, Yin H, Brown KM, Gibbs R, Trent J, Hayward NK. Frequent somatic mutations in MAP3K5 and MAP3K9 in metastatic melanoma identified by exome sequencing. *Nat Genet* 2012; **44**: 165-169
- 106 **Rincón M**, Davis RJ. Regulation of the immune response by stress-activated protein kinases. *Immunol Rev* 2009; **228**: 212-224
- 107 **Inokuchi S**, Aoyama T, Miura K, Osterreicher CH, Kodama Y, Miyai K, Akira S, Brenner DA, Seki E. Disruption of TAK1 in hepatocytes causes hepatic injury, inflammation, fibrosis, and carcinogenesis. *Proc Natl Acad Sci USA* 2010; **107**: 844-849
- 108 **Bettermann K**, Vucur M, Haybaeck J, Koppe C, Janssen J, Heymann F, Weber A, Weiskirchen R, Liedtke C, Gassler N, Müller M, de Vos R, Wolf MJ, Boege Y, Seleznik GM, Zeller N, Erny D, Fuchs T, Zoller S, Cairo S, Buendia MA, Prinz M, Akira S, Tacke F, Heikenwalder M, Trautwein C, Luedde T. TAK1 suppresses a NEMO-dependent but NF- $\kappa$ B-independent pathway to liver cancer. *Cancer Cell* 2010; **17**: 481-496
- 109 **Kim SY**, Shim JH, Chun E, Lee KY. Reciprocal inhibition between the transforming growth factor- $\beta$ -activated kinase 1 (TAK1) and apoptosis signal-regulating kinase 1 (ASK1) mitogen-activated protein kinase kinases and its suppression by TAK1-binding protein 2 (TAB2), an adapter protein for TAK1. *J Biol Chem* 2012; **287**: 3381-3391
- 110 **Masuzaki R**, Yoshida H, Omata M. Does chemotherapy prevent HCV-related hepatocellular carcinoma? *Pros. Dig Liver Dis* 2010; **42** Suppl 3: S281-S286

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