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REVIEW ARTICLE

Role of C-Jun N-terminal Kinase in Hepatocellular Carcinoma Development

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Abstract Hepatocellular carcinoma (HCC) is among the most frequently occurring cancers and the leading causes of cancer mortality worldwide. Identification of the signaling pathways regulating liver carcinogenesis is critical for developing novel chemoprevention and targeted therapies. C-Jun N-terminal kinase (JNK) is a member of a larger group of serine/threonine (Ser/Thr) protein kinases known as the mitogen-activated protein kinase (MAPK) family. JNK is an important signaling component that converts external stimuli into a wide range of cellular responses, including cell proliferation, differentiation, survival, migration, invasion, and apoptosis, as well as the development of inflammation, fibrosis, cancer growth, and metabolic diseases. Because of the essential roles of JNK in these cellular functions, deregulated JNK is often found to contribute to the development of HCC. Recently, the functions and molecular mechanisms of JNK in HCC development have been addressed using mouse models and human HCC cell lines. Furthermore, recent studies demonstrate that the activation of JNK by oncogenes can promote the development of cancers by regulating the transforming growth factor (TGF)-β/Smad pathway, which makes the oncogenes/JNK/Smad signaling pathway an attractive target for cancer therapy. Additionally, JNK-targeted therapy has a broad potential for clinical applications. In summary, we are convinced that promising new avenues for the treatment of HCC by targeting JNK are on the horizon, which will undoubtedly lead to better, more effective, and faster therapies in the years to come.

Key Points

JNK signaling pathway plays an important role in the development of HCC.

Various molecules, especially oncogenes, promote the development of HCC by activating JNK signaling pathway.

JNK is an attractive target in HCC therapy.

1 Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related death [1]. The most prominent factors associated with HCC include chronic hepatitis B viral (HBV) and hepatitis C viral (HCV) infection, chronic alcohol consumption, aflatoxin-B1-contaminated food, and virtually all cirrhosis-inducing conditions [2–4]. Other etiological factors with lower frequencies have also been proposed for HCC, including long-term oral contraceptive use in women, certain metabolic disorders, diabetes, and non-alcoholic fatty liver disease (NAFLD) [5, 6]. In addition, gender can also influence the risk and behavior of HCC, because men account for a larger fraction of cases [2]. HBV or HCV infection-induced hepatitis is the most important risk factor for HCC development. In the mainland of China, more than 90 % of HCC patients are diagnosed with an HBV infection.

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The molecular analysis of human HCC has identified many genetic and epigenetic alterations that result in the deregulation of key oncogenes and tumor-suppressor genes, including transforming growth factor (TGF)-β, tumor protein P53 (TP53), β-cadherin-associated protein (β-catenin), ErbB receptor family members, Met and its ligand hepatocyte growth factor (HGF), p16 (INK4a), E-cadherin, and cyclooxygenase 2 (COX2) [2, 7]. Notably, many of these deregulated genes have been reported to be associated with an important signaling pathway, the c-Jun N-terminal kinase (JNK) pathway. In the present review, the roles of JNK in HCC development and the potential of targeting JNK for the treatment of HCC are discussed.

2 JNK Family Kinases

JNK is a member of a larger group of serine/threonine (Ser/Thr) protein kinases known as the mitogen-activated protein kinase (MAPK) family [8]. The JNK proteins are also known as stress-activated protein kinases (SAPKs), and their enzymatic activity is induced in response to diverse stimuli, such as cytokines (tumor necrosis factor [TNF], interleukin-1[IL-1], TGF-β, platelet-derived growth factor [PDGF], 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), pathological and environmental stress (ischemia, hypoxia, and ultraviolet and ionizing radiation), toxins, drugs, endoplasmic reticulum (ER) stress, and metabolic changes, including obesity and hyperlipidemia [Reviewed in 9].

A host of MAP kinase kinase kinases (MAP3Ks), such as members of the MEKK family, the mixed-lineage kinase family, the apoptosis signal-regulating kinase family, transforming growth factor β-activated kinase 1 (TAK1) and tumor progression locus 2 (TPL2), serve as proximal conduits for the diverse signals that activate the JNK pathway. These kinases phosphorylate and activate two distinct MAP kinase kinases (MAP2Ks), MKK4 and MKK7, which directly phosphorylate JNKs on threonine 183 (Thr183) and tyrosine (Tyr185) residues in a conserved tripeptide motif (Thr-Pro-Tyr) within their activation loop [10]. Moreover, the kinase activity of JNKs is regulated by interaction with scaffold proteins [11], as well as dual-specificity phosphatases [12] and nuclear factor-kB (NF-kB) transcription factors [13].

There are three isoforms of JNK in mammals: JNK1, JNK2, and JNK3 (encoded by *MAPK8*, *MAPK9*, and *MAPK10*, respectively). The JNK proteins, including splicing variants, range from 46 kDa to 55 kDa in size. Alternative splicing of these genes results in at least 10 different transcriptional isoforms [14]. Specifically, four splice forms (JNK1α1, JNK1β1, JNK1α2, JNK1β2) arise from the JNK1 gene, four (JNK2α1, JNK2β1, JNK2α2, JNK2β2) arise from the JNK2 gene, and two (JNK3α1, JNK3α2) arise from the JNK3 gene [15].

Upon activation by upstream MKK4/7, JNKs phosphorylate and activate a number of nuclear and non-nuclear proteins. To date, at least 50 proteins have been identified as JNK substrates. Phosphorylation can modulate the substrate protein activity in a positive (c-Jun, JunB, JunD, activating transcription factor 2 [ATF2], ets-like protein 1 [ElK1], c-Myc, p53, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent [NFATc]2, forkhead box O4 [FOXO4], signal transducer and activator of transcription 3 [STAT]3, STAT1, paired box 2 [Pax2], T-cell factor [TCF]-β1 and heterogeneous nuclear ribonucleoprotein K [hnRNP-K]), negative (Net, heat shock transcription factor 1 [HSF1], NFATc3, NFATc1α, peroxisome proliferator-activated receptor gamma 1 [PPARγ1], Glucocorticoid R, retinoic acid receptor alpha [RARα], Androgen R, nuclear receptor 77 [Nur77], transcription initiation factor IA [TIFIA], insulin receptor substrate 1 [IRS-1], B-cell cll/lymphoma 2 [Bcl-2], myeloid cell leukemia 1 [Mcl-1], Bcl-2-like 1 [Bcl-XL], Bcl-2-associated agonist of cell death [Bad], superior cervical ganglion-10 protein [SCG10], Tau and Kinesin), or modulating (retinoid x receptor α [RXRα], JNK-interacting protein 1 [JIP1], pol delta C subunit [p66] and 14-3-3) fashion. JNK binding can even modulate the activity of some target proteins in a phosphorylation-independent manner (e.g., degradation of c-Jun, ATF2, p53, and c-Myc) [Reviewed in 9, 15, 16]. Previous studies revealed that TGF-β and Ras differentially activated TGF-β type I receptor (TβRI) and JNK, which converted the common mediator Smad2/3 into two distinctive phospho-isoforms: C-terminally phosphorylated Smad2/3 (pSmad2/3C) and linker-phosphorylated Smad2/3 (pSmad2/3L). JNK is also responsible for the phosphorylation of Smad2/3L [17]. In some cases, the consequences of phosphorylation by JNK have not yet been defined (Jun dimerization protein 2 [JDP2], JNK-interacting protein 3 [JIP3], SH3BP5 [Sab], microtubule-associated protein 1B [MAP-1B], Keratin 8, and amyloid β-precursor) [Reviewed in 15] (Table 1). These proteins control multiple cellular processes, such as cell proliferation, apoptosis and survival, differentiation, acting as transcription factors, controlling protein degradation, localization, and signaling. Furthermore, recent studies using genetically engineered mice showed that the 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 [9] (Fig. 1).

Table 1 The summary of the effects of JNK on different substrates

Effects		Substrates
Activation		C-Jun, JunB, JunD, ATF2, ElK1, c-Myc, p53, NFATc2, FOXO4, STAT3, STAT1, Pax2, TCFβ1, hnRNP-k, Itch, Bcl-2, Bad, Bim, Bax, MK1, Akt, Paxillin, DCX, MAP-2, Smad2L, Smad3L
Inhibition	Phosphorylation-dependent manner	Net, HSF1, NFATc3, NFATc1α, PPARγ1, Glucocorticoid R, RARα, Androgen R, Nur77, TIFIA, IRS-1, Bcl-2, Mcl-1, Bcl-XL, Bad, SCG10, Tau, Kinesin
	Phosphorylation-independent manner	c-Jun, ATF2, p53, c-Myc
Modulation		RXRα, JIP1, p66, 14-3-3
Unknown or poorly characterised		JDP2, JIP3, Sab, MAP-1B, Keratin 8, amyloid β-precursor

Abbreviations: *JNK* c-Jun N-terminal kinase, *ATF2* activating transcription factor 2, *ElK1* ets-like protein 1, *NFATc2* nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2, *FOXO4* forkhead box O4, *STAT3* signal transducer and activator of transcription 3, *STAT1* signal transducer and activator of transcription 1, *Pax2* paired box 2, *TCF-β1* T-cell factor β1, *hnRNP-K* heterogeneous nuclear ribonucleoprotein K, *Bcl-2* B-cell cll/lymphoma 2, *Bad* Bcl-2-associated agonist of cell death, *MK1* potassium channel, voltage gated shaker related subfamily A, member 1, *Akt* protein kinase B, *DCX* doublecortin, *MAP-2* microtubule-associated protein 2, *Smad2L* linker region of Smad2, *Smad3L* linker region of Smad3, *HSF1* heat shock transcription factor 1, *NFATc3* nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3, *NFATc1α* nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1α, *PPARγ1* peroxisome proliferator-activated receptor gamma 1, *RARα* retinoic acid receptor α, *Nur77* nuclear receptor 77, *TIFIA* transcription initiation factor IA, *IRS-1* insulin receptor substrate 1, *Mcl-1* myeloid cell leukemia 1, *Bcl-XL* Bcl-2-like 1, *Bad* Bcl-2-associated agonist of cell death, *SCG10* superior cervical ganglion-10 protein, *RXRα* retinoid x receptor α, *JIP1* JNK-interacting protein 1, *p66* pol delta C subunit, *JDP2* Jun dimerization protein 2, *JIP3* JNK-interacting protein 3, *Sab* SH3BP5, *MAP-1B* microtubule-associated protein 1B

3 The Roles of JNK Pathway in Cancer Development

The transforming actions of several oncogenes such as Ras, c-fos, Met, Bcr-Abl, and mutant HER2 also could be JNK dependent [18–22], which suggests that JNK signaling contributes to the cellular transformation that supports the development of various cancers. A substantial body of evidence indicates that JNK activation is required for transformation induced by Ras, an oncogene that is activated by mutation in almost 30 % of human cancers, and N-Ras mutations were found in HCC, melanoma, and hematologic malignancies [23]. Ras induces phosphorylation of c-Jun on the same serine residues phosphorylated by JNK [18, 24], and acts cooperatively with c-Jun to enhance cellular transformation [25]. Moreover, fibroblasts from mice harboring a mutated c-Jun allele that lacks the JNK phosphorylation sites (JunAA) were resistant to transformation induced by activated Ras and Fos [21]. c-Fos induced osteosarcomas and skin tumors induced by chronic activation of the Ras pathway were reduced in JunAA mice [22]. In 1997, Rodrigues et al. found that the activation of the JNK pathway is essential for transformation by the Met oncogene [20]. Bcr-Abl, a leukemia oncogene, preferentially activated JNK, and also enhanced the activity of Jun-responsive promoters through a Ras- and JNK-dependent pathway, and dominant-negative mutants of Jun inhibit the transforming activity of Bcr-Abl [19]. Oncogenic HER2 and H-Ras could induce TGF-β secretion through the JNK/activator protein (AP)-1 pathway in mammary epithelial cells, which suggests that oncogenic HER2 and H-Ras promote the development of breast cancer by activating the JNK signaling pathway [26]. Differentiation and apoptosis mediated by the tumor-suppressive pSmad3C pathway are blocked by the oncogenic Ras/JNK/pSmad3L pathway [27]. This blockage is a frequent theme in the development of gastrointestinal malignancies [17]. Phospho-Smad3 signaling confers a selective advantage upon tumor cells by shifting from the tumor-suppressive TβRI/pSmad3C pathway to the oncogenic JNK/pSmad3L pathway during sporadic human colorectal carcinogenesis [28]. This observation has been extended to hepatic carcinogenesis [29, 30]. The phenotypes of benign tumors are dictated by genotype, and tumorigenic growth is essentially a cell-autonomous phenomenon that involves the constitutive shift induced by alterations in the Ras oncogene. Collectively, all these data indicate that JNK activity is necessary for efficient transformation and tumorigenesis by these oncogenes.

4 JNK1 Rather than JNK2 Is more Critical for the Development of HCC

In 2002, Tsutsumi et al. revealed that the development of HCV-associated HCC occurred through the activation of JNK and its downstream effector, AP-1 [31]. There were only a few scattered reports suggesting the potential involvement of JNK in HCC during the subsequent decade. Since that study, numerous studies have confirmed that JNK plays a key role in the progression and tumorigenesis of HCC by regulating various biological functions in cells. JNK1 and JNK2 are expressed in most tissues, whereas JNK3 is mainly expressed

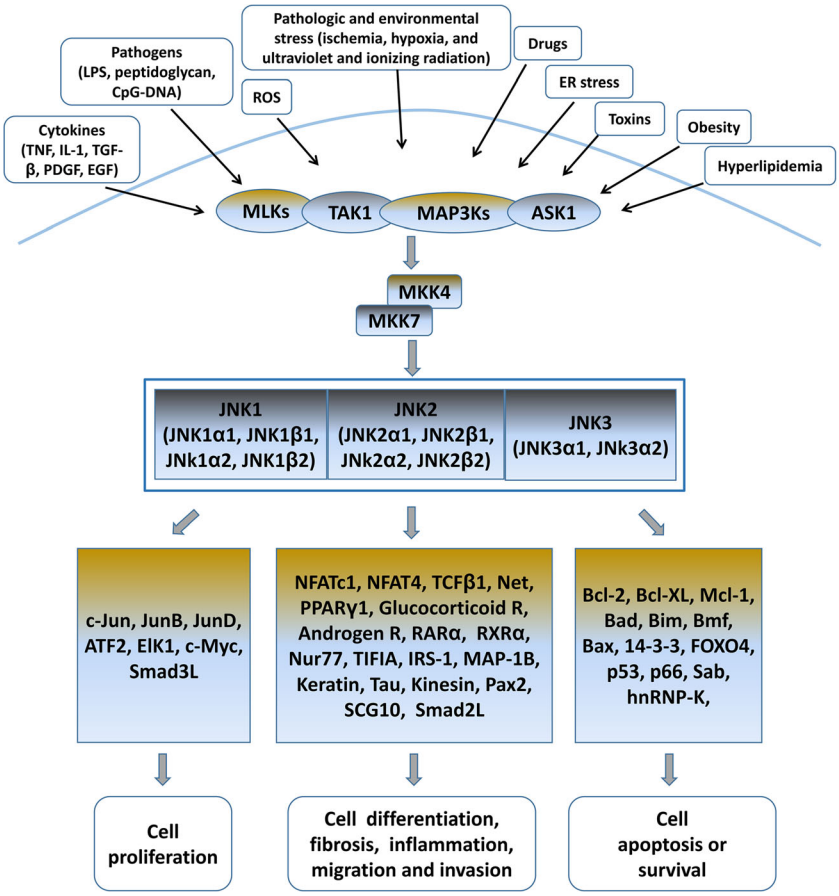

Fig. 1 Activation of JNK signaling pathways is involved in HCC tumorigenesis and tumor progression. Diverse stimuli can activate JNK through the upstream molecules, and the phosphorylated JNK activates a number of downstream nuclear and non-nuclear proteins involved in HCC cellular activities, including cell proliferation, differentiation, apoptosis, survival, migration, invasion, fibrosis, and inflammation. Abbreviations: JNK, c-Jun N-terminal kinase; HCC, Hepatocellular carcinoma; TNF, tumor necrosis factor; IL-1, interleukin-1; TGF-β, transforming growth factor β; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; LPS, lipopolysaccharide; ROS, reactive oxygen species; ER, endoplasmic reticulum; MLKs, mixed lineage kinases; TAK1, transforming growth factor β-activated kinase 1; MAP3Ks, MAP kinase kinase kinases; ASK1, apoptosis signal-regulating kinase 1; ATF2, activating transcription factor 2; ElK1, ets-like protein 1; Smad3L, linker region of Smad3; NFATc1, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1; NFAT4, nuclear factor of activated T-cells 4; TCF-β1, T-cell factor β1; PPARγ1, peroxisome proliferator-activated receptor gamma 1; RARα, retinoic acid receptor α; RXRα, retinoid x receptor α; Nur77, nuclear receptor 77; TIFIA; transcription initiation factor IA; IRS-1, insulin receptor substrate 1; MAP-1B, microtubule-associated protein 1B; Pax2, paired box 2; SCG10, superior cervical ganglion-10 protein; Smad2L, linker region of Smad2; Bcl-2, B-cell cll/lymphoma 2; Bcl-XL, Bcl-2-like 1; Mcl-1, myeloid cell leukemia 1; Bad, Bcl-2-associated agonist of cell death; FOXO4, forkhead box O4; p66, pol delta C subunit; Sab, SH3BP5; hnRNP-K, heterogeneous nuclear ribonucleoprotein K

in the brain, heart, and testes [10, 16]. This tissue-specific distribution, particularly for JNK3 expression, has led to the idea that different isoforms may perform different cellular roles. There are two isoforms of JNK expressed in HCC tissues: JNK1 and JNK2. Several biochemical experiments have revealed that JNK1, rather than JNK2, is the primary kinase in stress-induced phosphorylation of c-Jun, and several other studies also demonstrated JNK1 is more important than JNK2. In 2006, Sakurai et al. showed that in mice with DEN-induced HCC, NF-kB attenuates liver carcinogenesis by repressing JNK1 activation and that JNK1$^{-/-}$ mice exhibit reduced liver cancers [32]. A single injection of DEN to young mice induces HCC at the age of 8–10 months, while JNK1 deficiency protects mice from DEN-induced hepatocyte death, which resulted in reduced proliferation and HCC formation [32]. Later, in 2008, Hui et al. showed that activation of JNK1, but not JNK2, was increased in human primary HCC [33]. In both of the studies that used the DEN phenobarbital protocol, JNK1$^{-/-}$ mice developed fewer and smaller liver tumors than wildtype or JNK2$^{-/-}$ mice [32, 33]. Therefore, JNK1 was required for human HCC cell proliferation in vitro and tumorigenesis after xenotransplantation. In addition, in mouse models of liver carcinogenesis or regeneration, mice lacking JNK1 displayed decreased tumor cell proliferation [32, 33]. Consistent with the results of Hui et al., in 2009, Chang and colleagues found that enhanced JNK1 activation occurred in 17 out of 31 HCC

samples (55 %) relative to the corresponding adjacent non-cancerous tissues, whereas JNK2 activation was roughly equal between HCC and adjacent non-cancerous tissues [34]. The authors also showed that the activation of JNK1 led to an elevated expression of genes regulating cell growth and a decreased expression of the genes for cell differentiation in HCC [34]. The above data reveal a clear correlation between HCC and JNK1. Therefore, targeting JNK1 should be viewed as a new avenue for HCC therapy.

The progressive role of JNK in HCC has been confirmed by pharmacological inhibition of JNK and studies of human HCC cells. However, Davis and coworkers showed contradictory results. They reported an analysis of mice with tissue-specific deficiency of JNK and mice with a compound deficiency of both JNK1 and JNK2, and these analyses indicated that another mechanism must account for the protumorigenic effects of JNK in HCC development. Their results showed that compound JNK deficiency in hepatocytes did not reduce DEN-induced HCC, but compound JNK deficiency in hepatocytes, as well as nonparenchymal cells did reduce DEN-induced HCC. Their work provided important insight into the mechanism of JNK-promoted HCC development [35]. Additional studies by this group revealed that JNK in nonparenchymal cells promotes HCC development by providing an inflammatory environment that supports HCC, including the expression of the protumorigenic cytokines interleukin 6 (IL-6) and TNF-α that contribute to compensatory hepatocyte proliferation [35]. However, JNK in hepatocytes reduces HCC development by promoting hepatocyte survival, which decreases IL-1α release by necrotic hepatocytes and the activation of hepatic innate immune cells, including Kupffer cells, which express the protumorgenic cytokine IL-6 [35]. The study by Davis and coworkers demonstrates that JNK plays a complex role in the development of HCC. It is likely that previous studies using mice with a whole-body knockout of JNK1 or JNK2 reflected a composite phenotype derived from the cell type-specific functions of JNK in hepatocytes and nonparenchymal cells. In summary, all the studies have implications for a more general role for JNK in cancer. Both tumor promotion and inhibition by JNK may contribute to cancer development [10, 36]. These different outcomes complicate the analysis of JNK pathway mutations that have been identified in human cancers [37, 38]. Additionally, this dual role of JNK should be considered in the context of the potential uses for JNK as a therapeutic target for drug development and the treatment of human cancer.

5 Various Molecules Regulate HCC Development through JNK Signaling Pathways

As HBV or HCV infection, non-alcoholic steatohepatitis (NASH), or cirrhosis can develop into HCC, a number of reports suggested that the gene products of HBV and HCV, and NASH were involved in the activation of JNK. In 2005, Guo et al. used immunohistochemical and in situ hybridization techniques to study the role of JNK in HCC with or without HBV infection, and demonstrated positive staining in nuclei for the phosphorylation of JNK in 70 % of HCC tissues, but not in adjacent non-tumor tissues, which indicated that JNK activation may play an important role in the pathogenesis of HBV-associated HCC [39]. P21-activated protein kinase (Pak1), a main downstream effector of the small Rho GTPases Rac1 and cell division cycle 42 (Cdc42), plays an important role in the regulation of cell morphogenesis, motility, mitosis, and angiogenesis. A previous study documented that Pak1 was overexpressed in HCC and played an important role in the metastasis of HCC. Most importantly, the mechanism by which Pak1 induced cancer metastasis involved the activation of JNK [40]. In addition to HBV and Pak1, studies have shown that many factors such as HCV, acetaldehyde, alcohol, CD147, KITENIN, inhibit cytokine-dependent inducible nitric oxide synthase (iNOS), LPS, CYLD, and platelets could promote the proliferation, migration, invasion, or metastasis of HCC through the activation of JNK [41–47]. Furthermore, some other molecules, such as the aqueous extract of butea monosperma flowers, lipocalin 2 (Lcn2), isomalto oligosaccharide (IMOS), heat shock protein 20 (HSP20), Sorafenib, LK-A, and salinomycin, could reduce the development of HCC by inhibiting of JNK [48–54]. These studies suggest that the JNK pathway can regulate different cellular activities involved in HCC. LPS, one of the promoters of JNK activation, was well studied recently. While previous studies have shown that TLR4 is involved in hepatocarcinogenesis, the role of TLR4 in cancer cell survival and proliferation in HCC remains unclear. In 2013, Wang et al. revealed that LPS-induced activation of TLR4-JNK signaling pathway promoted the proliferation of HCC cells [45]. Furthermore, Li et al. and Dong et al. found that TLR4-JNK signaling was required for LPS-induced epithelial-mesenchymal transition (EMT), tumor cell invasion or metastasis, which ultimately provided molecular insights into LPS-related pathogenesis and a basis for developing new strategies against metastasis in HCC [55, 56].

6 MUC1 Promotes the Development of HCC by Activating the JNK/Smad Pathway

A recent study found that JNK was activated by oncoprotein Mucin 1 (MUC1), and could mediate autocrine TGF-β signaling in HCC cells [7]. MUC1 is a transmembrane glycoprotein that is expressed on the apical surface of epithelial cells and is aberrantly overexpressed in most epithelial malignant tumors and some hematological malignant tumors. MUC1 promotes the progression and tumorigenesis of many human adenocarcinomas [57–61]. Various studies have shown that

MUC1 is overexpressed in HCC cells and tissues [62, 63]. Previous results revealed that MUC1 gene silencing inhibited the growth of the SMMC-7721 HCC cell line in vivo and in vitro, which suggests that MUC1 plays a key role in HCC tumorigenesis. MUC1 is involved in many signaling pathways other than JNK [64], including Wnt/β-catenin [65], c-terminal Src kinase (c-Src) [66], growth factor receptor-bound protein 2 (Grb2)/son of sevenless (Sos) [67], PI3K/AKT [58], p53 [68], glycogen synthase kinase 3β (GSK3β) [69], epidermal growth factor receptor (EGFR) [70, 71], and NF-kB [72], to regulate the processes of cell survival, proliferation, and apoptosis. However, the downstream pathway by which activated JNK could regulate the biological function of HCC is still unclear. A series of studies has shown that JNK signaling communicates closely with TGF-β signaling to regulate hepatic carcinogenesis [29, 30, 73]. Reports showed that MUC1 overexpression in HCC cells reduced TGF-β-dependent tumor-suppressive activity by TβRI/pSmad3C/$p21^{(WAF1)}$, while promoted the proliferation of HCC cells by directly activated JNK/pSmad3L/c-Myc. Conversely, MUC1 gene silencing in MUC1 expressing HCC cells resulted in preserved tumor-suppressive functions via pSmad3C, while eliminated pSmad3L-mediated oncogenic activity both in vitro and in vivo. A high correlation between MUC1 and JNK/pSmad3L/c-Myc, but not TβRI/pSmad3C/$p21^{(WAF1)}$ expression was observed in HCC tissues from patients. Collectively, these results indicate that the activated JNK by MUC1 shifts Smad3 signaling from a tumor-suppressive pSmad3C/$p21^{(WAF1)}$ to an oncogenic pSmad3L/c-Myc pathway in HCC cells [74] (Fig. 2). Consistent with these studies, Nagata et al. also found that the JNK inhibitor SP600125 significantly prolonged the median survival time in rat model of DEN-induced HCC [73]. As JNK/pSmad3L/c-Myc was enhanced in the rat hepatocytes exposed to DEN, while TβRI/pSmad3C/$p21^{(WAF1)}$ was impaired as DEN-induced HCC developed and progressed, the specific inhibition of JNK activity by SP600125 suppressed pSmad3L/c-Myc in the damaged hepatocytes and enhanced pSmad3C/$p21^{(WAF1)}$ by acting as a tumor suppressor in normal hepatocytes [73]. Further investigation demonstrated that MUC1-mediated JNK activation not only enhanced the phosphorylation of Smad2 at C-terminal (Smad2C) through TGF-β/TβRI, but also directly enhanced the phosphorylation of Smad2 at linker region (Smad2L), and then both of them collaborate to upregulate matrix metalloproteinase (MMP)-9-mediated cell migration and invasion of HCC [75] (Fig. 2). Together, these results uncovered a new signaling pathway in which oncogenes are upstream of JNK and the TGF-β/Smad pathway is downstream of JNK, and oncogenes are the upstream switch of JNK activity in tumor cells. In summary, all of these recent studies demonstrate that oncogenic transformation by MUC1 is mediated by the activation of JNK in HCC. The findings from all these studies have led to the hypothesis that oncogenes could promote the development of various cancers by activating JNK signaling pathways, which makes JNK just as an attractive target as oncogenes for cancer therapy.

Clinical analyses of pSmad3L and pSmad3C in human tumor development have provided substantial insight into relevant mechanisms. For example, human livers infected by HCV progress from chronic hepatitis C through cirrhosis to HCC several decades later. Specimens from patients with chronic hepatitis C who develop HCC show abundant Smad3L but limited Smad3C phosphorylation in hepatocytic nuclei, while other patients with abundant hepatocytic pSmad3C but limited pSmad3L do not develop HCC [29]. The same relationships were observed in human HBV-related hepatocarcinogenesis [30]. These clinical observations point to roles for pSmad3C as a tumor suppressor and pSmad3L as a promoter during carcinogenesis. Therefore, pSmad3L and pSmad3C could function as biomarkers for the prediction of cancer risk in humans.

An improved understanding of Smad phospho-isoform signaling during human carcinogenesis suggests better ways to prevent human cancer development, exemplifying laboratory-driven translational research. A key question concerning the effectiveness of therapy for preventing liver HCC development is whether such a therapy still has value once preneoplastic hepatocytes have appeared. Molecular analyses of paired liver biopsy specimens enabled us to predict HCC risk after HCV clearance. Specimens from HCV-related chronic liver diseases can be divided into two subgroups based on phospho-Smad3 profiles [76]. One group carried a risk of HCC after HCV clearance, while another carried a lower risk of HCC occurrence. This grouping explains the observation that some patients with HCV-related liver disease respond effectively to antiviral therapy in terms of reversal from carcinogenic pSmad3L to tumor-suppressive pSmad3C signaling, while others do not. Irrespective of HCV clearance, patients with cirrhosis who maintain strong pSmad3L signaling in hepatocytic nuclei require continued close follow-up because the HCC risk is likely to persist. Deng et al. recently demonstrated reversibility of phospho-Smad3 signaling in stepwise human HBV-related carcinogenesis after anti-HBV therapy [77]. All the findings suggest that pSmad3L and pSmad3C are useful biomarkers for assessing the effectiveness of interventions aimed at reducing cancer risk in humans.

7 The Role of JNK Activation in HCC Apoptosis: A Double-Edged Sword

Apoptosis represents a physiological way to eliminate excess cells during both liver development and regeneration [78]. Apoptotic signaling within the cell is transduced mainly via two molecular pathways: the death receptor pathway and the mitochondrial pathway. Apoptotic events in hepatocytes can

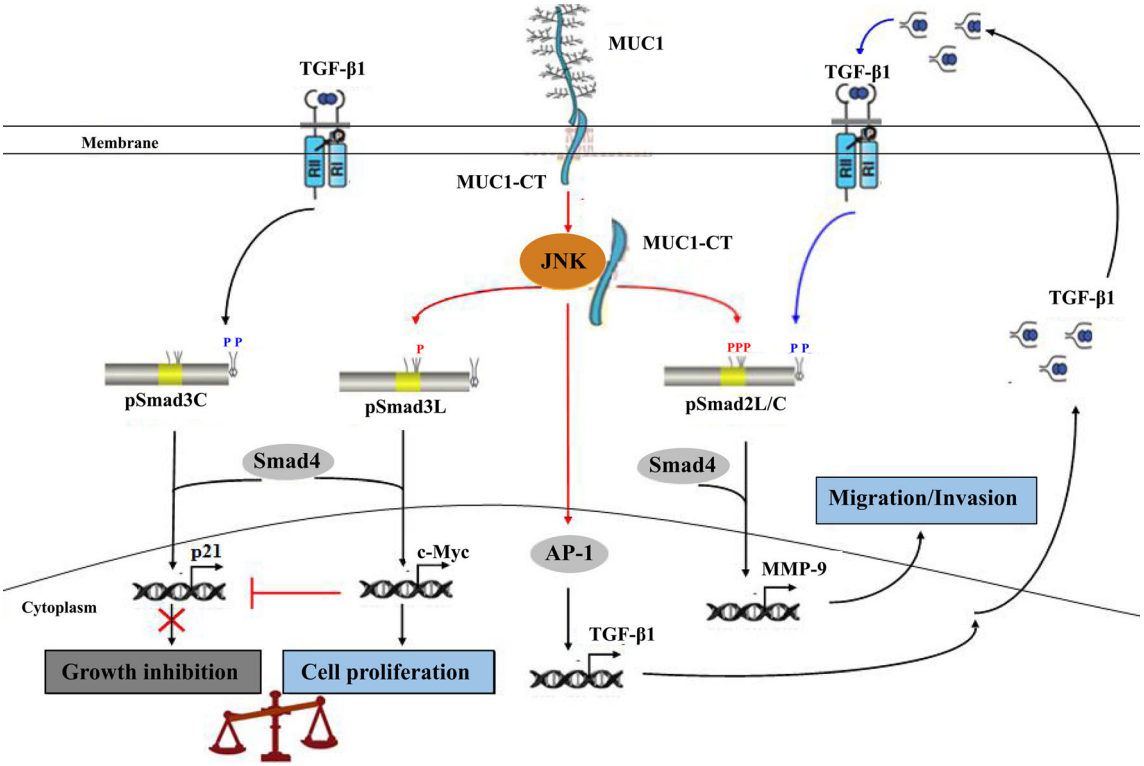

Fig. 2 MUC1 promotes the progression and tumorigenesis of HCC through the activation of JNK. MUC1 enhances the expression of the autocrine TGF-β1 by directly activating the JNK/AP-1 pathway in HCC cells, and MUC1-mediated JNK activation not only enhances the phosphorylation of Smad2C through TGF-β/TβRI, but also directly enhances the phosphorylation of Smad2L, and then pSmad2L/C collaborates to upregulate matrix metalloproteinase (MMP)-9-mediated cell migration and invasion of HCC. Furthermore, MUC1 directly activates JNK/pSmad3L/c-Myc, while suppressing TβRI/pSmad3C/$p21^{(WAF1)}$, which indicates that MUC1 can shift Smad3 signaling from a tumor-suppressive pSmad3C/$p21^{(WAF1)}$ to an oncogenic pSmad3L/c-Myc pathway by directly activating JNK in HCC cells. Abbreviations: MUC1, mucin 1; MUC1-CT, the cytoplasmic tail of MUC1; HCC, hepatocellular carcinoma; JNK, c-Jun N-terminal kinase; TGF-β1, transforming growth factor β1; AP-1, activator protein-1; TβRI, TGF-β type I receptor; Smad2C, the C-terminal of Smad2; Smad2L, the linker region of Smad2; pSmad2C, C-terminally phosphorylated Smad2; pSmad2L, linker-phosphorylated Smad2; MMP-9, matrix metalloproteinase-9; pSmad3L, linker-phosphorylated Smad3; pSmad3C, C-terminally phosphorylated Smad3

be regulated by different stimuli that bind to death receptors in the cell membrane, such as Fas ligand (FasL), TNF-α, or TNF-related apoptosis-inducing ligand (TRAIL) [79]. Furthermore, other factors, such as TGF-β, HGF, fibroblast growth factors (FGFs), EGF, heparin-binding EGF-like growth factor (HB-EGF), TNF-α, amphiregulin and others, do not bind to death receptors, but intracellular signals do couple to the apoptotic machinery through activation of the mitochondrial pathway [80]. Many pathways are involved in apoptosis, including p53, PI3K (phosphoinositide 3-kinase)/protein kinase B (AKT), extracellular regulated protein kinase (ERK), P38 MAPK, NF-kB, and TGF-β/Smad, among others [80]. Previous studies showed that both Aplidin and celastrol, which have anti-cancer activities, could induce apoptosis of human breast cancer and multiple myeloma cells through activation of the JNK signaling pathway [81, 82], which indicates that the JNK pathway is also involved in apoptosis. JNK phosphorylates and activates the BH3-only proteins Bim and Bad as well as the proapoptotic Bcl-2 protein Bax, which directly triggers the mitochondrial apoptotic pathway [83–85]. Finally, research has shown that JNK induces Fas and DR5 expression by sensitizing steatotic hepatocytes to circulating Fas or TRAIL-mediated toxicity [86, 87]. Indeed, insufficient apoptosis has been associated with the development and progression of HCC [80]. In 2003, Qi et al. showed that the activation of p53 and JNK signalings by Notch1 inhibited the growth of HCC through induction of the cell cycle arrest and apoptosis [88]. Since that study, JNK signaling-associated apoptosis in HCC was well studied. Sorafenib, as the first molecular targeted agent that showed survival benefit for patients with advanced HCC, could induce apoptosis of HCC cells through the activation of JNK signaling [89]. In addition, Adiponectin treatment resulted in increased apoptosis of HCC cells via the activation of caspase-3 and JNK, and inhibition of JNK-phosphorylation inhibited adiponectin-induced apoptosis and caspase-3 activation [90]. As ROS can induce the activation of JNK, several studies showed that activating the ROS/JNK signaling pathways could promote apoptosis in HCC induced by different factors, such as 8-Bromo-7-methoxychrysin, Longikaurin A (LK-A), and

Wentilactone B, and the applying of ROS and JNK inhibitors could markedly reverse the apoptosis [48, 91, 92]. Furthermore, TRAIL induces apoptosis in a wide range of malignant cells. However, several cancers, including HCC, exhibit a major resistance to TRAIL-induced cell death. Wang et al. found that in the presence of melittin, TRAIL-induced apoptosis is significantly increased in TRAIL-resistant HCC cells, which may be attributed to melittin-induced TAK1-JNK/p38 activation and melittin-mediated inhibition of IkappaBalpha kinase-NfkappaB [93]. Consistent with these results, Song et al. also found that inhibition of MKK7-JNK by the TOR signaling pathway regulator-like protein contributed to the resistance of HCC cells to TRAIL-induced apoptosis [94]. All these results reveal that the activation of JNK can promote apoptosis of HCC cells.

NAFLD is a group of syndromes ranging from hepatic steatosis to more severe forms, including NASH and cirrhosis, which may further progress to HCC [95]. NAFLD is strongly associated with an increased level of serum free fatty acids (FFAs), and report shows that the FFAs levels are increased in patients with NASH and correlate with disease severity [96]. Apoptosis or programmed cell death is a morphologic and pathogenic hallmark of NASH [97, 98], which in the context of NAFLD, and secondary to its association with excess lipid deposition, is referred to as lipoapoptosis. Lipoapoptosis is a key pathogenic process in NAFLD, and the severity of NAFLD correlates with the degree of hepatocyte lipoapoptosis [97]. Multiple studies suggest that an elevated concentration of FFAs in the circulation plays a key role in stimulating lipoapoptosis in liver cells [83, 99, 100]. Of the three mammalian JNK genes, only JNK1 and JNK2 are expressed in the liver [101]. JNK activity was increased in experimental murine dietary and genetic models of NASH [102–104] as well as in patients with NASH, and JNK activity correlates with the degree of apoptosis [105]. Activation of the JNK signaling pathway has been implicated as a central mediator of FFA-induced hepatocyte lipoapoptosis in both rodents and human steatohepatitis [103–105]. Alternatively, JNK can post-transcriptionally activate the pro-apoptotic members of the Bcl-2 family Bim, Bad, and Bax [84, 106, 107], or inactivate the anti-apoptotic members of this family Bcl-2 and Bcl-XL [108]. In addition, both JNK1 and JNK2 have been implicated in insulin resistance, although JNK1 is more strongly associated with steatohepatitis [103, 104]. Genetic deletion of JNK1 prevents FFA-mediated c-Jun activation and p53-upregulated modulator of apoptosis (PUMA) induction by FFA [109]. A study showed that SP600125, a JNK inhibitor, decreased lipoapoptosis in vitro [109, 110] by attenuating saturated FFA induced PUMA induction [109]. In summary, because lipoapoptosis is a key player in the progression of NAFLD, which is a high-risk factor for HCC, targeting the JNK signaling pathway would be useful to halt disease progression.

Nevertheless, the results from Mucha et al. were the opposite. They showed that inhibited JNK by SP600125 caused cell cycle arrest, enhanced caspase recruitment, and greatly sensitized HCC cells, but not normal hepatocytes, to TRAIL [111]. In 2008, Aderca et al. also showed that the JNK inhibitor SP600129 enhances apoptosis of HCC cells induced by the tumor suppressor WW domain containing oxidoreductase (WWOX) [112]. All these results indicate that the function of JNK in apoptosis is complex, and JNK may have a proapoptotic, antiapoptotic, or no role in the process. It is most likely that JNK activation modulates the apoptotic process in a cell type- and stimulus-dependent manner, and this work highlights the importance of understanding fully both the roles of JNK and the molecular basis for the distinct functions of JNK.

8 JNK Targeting for Potential Clinical Applications

The contributions of JNK in HCC pathogenesis strongly suggest that JNK signaling could be a promising target for developing novel chemoprevention and targeted therapies for HCC. To date, many small molecule inhibitors that might modulate specific components of JNK signaling have been developed. These inhibitors can be broadly classified into two categories: ATP-competitive inhibitors and ATP-non-competitive inhibitors (Tables 2 and 3).

8.1 ATP-Competitive Inhibitors

SP600125, one of the earliest and most commonly used ATP-competitive JNK inhibitors, is an anthrapyrazolone derivative that exhibited high efficacy in blocking the kinase activity of JNK1, JNK2, and JNK3 [157]. It was developed by Celgene for the treatment of auto-immune, inflammatory, and neurodegenerative diseases, and intriguing anticancer properties were recently described [157, 158]. SP600125 can inhibit the inflammatory response in vivo and showed promise as a potential therapeutic agent for rheumatoid arthritis and asthma in humans [113, 114]. Reports showed that SP600125 modified cell cycle progression induced cell apoptosis and cell cycle arrest and increased cell sensitivity to various antiproliferative drugs [48, 115–123]. When SP600125 was combined with some chemotherapy drugs, such as TRAIL and gartanin, cell apoptosis was increased in human HCC cultures [111, 124]. Furthermore, in a rat DEN-induced HCC model, the administration of SP600125 reduced the number and size of HCC tumors [73]. All the reported mechanisms of action make SP600125 an ideal candidate for developing new therapies against HCC. However, additional data showed that SP600125 binds to a range of kinases in phage interaction screening assays [159], which suggests there may be many additional kinase targets of SP600125. Despite concerns about the specificity of SP600125, it has good potential as an anti-

Table 2 The application of ATP-competitive JNK inhibitors

JNK inhibitor	Status	Experimental model	Findings	References
SP600125	Preclinical	Adjuvant-induced arthritis	Had a modest anti-inflammatory effect and a marked protective effect on joint destruction	[113]
		Ovalbumin sensitized asthma	Reduced inflammatory cell egress into the airway lumen after single allergen exposure	[114]
		Human lung carcinoma and human colon carcinoma in vitro	Modified cell cycle progression and causes endoreduplication with preferential activity against p53 null cells	[115–117]
		Human breast cancer and colon carcinoma in vitro	Induced p53-independent apoptosis and cell cycle arrest	[118, 119]
		Human uterine sarcoma, breast cancer, and oral squamous carcinoma in vitro	Increased cell sensitivity to various anti-proliferative drugs	[120, 121]
		Human HCC in vitro	Induced cell arrest in S phase and decreases G0/G1 phase; Inhibited HCC progression and LK-A-induced apoptosis	[48, 122, 123]
		Human HCC in vitro	In combination with TRAIL or gartanin, cell apoptosis was increased	[111, 124]
		DEN-induced HCC	Reduced the number and size of tumors	[73]
CC-401	Preclinical	Liver-transplantation	Decreased hepatic necrosis and apoptosis	[125]
		Human colon cancer	Resulted in greater DNA damage in the sensitive cells, the tumor growth was delayed greater in the presence of CC-401 combined with bevacizumab and oxaliplatin	[126]
		Liver injury	Improved rats survival rates	[126]
	Phase I (Terminated, NCT00126893)	Myeloid leukemia	Not clear	[127]
CC-930	Phase II (Terminated, NCT01203943)	Fibrosis	Prevented dermal thickening, myofibroblast differentiation, and the accumulation of collagen; Induced regression of established experimental fibrosis	[128–130]
	Phase II (Terminated, NCT01466725)	Lupus erythematosus	Not clear	[130]
AS601245	Preclinical	Ischemia injury	Protected neurons from ischemic injury both in two different models of cerebral ischemia	[131]
		Myocardial ischemia and reperfusion	Decreased cardiomyocyte apoptosis and infarct size	[132]
		Colon cancer in vitro	Reduced cell proliferation, cyclin D1 and PCNA expression, and induced apoptosis and differentiation	[133]
JNK-IN-8	Preclinical	Human head and neck squamous cell carcinoma in vitro	Alleviated AZD8055-induced cell death	[134]

Abbreviations: JNK C-Jun N-terminal kinase, *HCC* hepatocellular carcinoma, *LK-A* Longikaurin A, *TRAIL* TNF-related apoptosis-inducing ligand, *TSK1* tight skin 1, *PCNA* proliferating cell nuclear antigen

cancer therapeutic agent and will be further investigated because of its continued usefulness for in vivo studies with minimal toxicity or few undesirable side effects.

CC-401, a second generation of ATP-competitive anthrapyrazolone JNK inhibitor targeting JNK1/2/3, was developed by Celgene based on the chemistry of SP600125. Uehara et al. showed that CC-401 decreased hepatic necrosis and apoptosis after orthotopic liver transplantation, while Vasilevskaya et al. showed that CC-401 treatment resulted in greater DNA damage and delayed tumor growth when combined with bevacizumab and oxaliplatin in mouse xenografts [125, 126]. In addition, CC-401 has been used in models of liver injury and significantly improved rat survival rates from 40 % (vehicle) to 80 % (CC-401, 10 mg/kg) and 100 % (CC-401, 20 mg/kg) [160]. Celgene carried out a Phase I study to evaluate the safety, pharmacokinetics, and pharmacodynamics of CC-401 in subjects with refractory acute myelogenous leukemia, but the study was terminated [127]. Nevertheless, there are no reports yet of CC-401 being used in the therapy of HCC in humans. To confirm the benefits

Table 3 The application of peptide and small molecule ATP-non-competitive JNK inhibitors

JNK inhibitor	Status	Experimental model	Findings	References
D-JNKI-1 (AM-111, XG-102)	Phase III (Completed, NCT02508337 and NCT02235272)	Post-cataract surgery intraocular inflammation and pain	Safe and well tolerated, but the efficacy is unclear	[135, 136]
	Phase II (Completed, NCT00802425) Phase III (Recruiting participants, NCT02561091)	Acute sensorineural hearing loss	Showed statistically significant, clinically relevant, and persistent improvements in hearing and speech discrimination and higher tinnitus remission compared with placebo. The study drug and the intratympanic injections were well tolerated	[137, 138]
	Preclinical	Endotoxin-induced uveitis	Inhibited clinical signs of endotoxin-induced uveitis and reduced intraocular cell infiltration	[139, 140]
		Alzheimer's disease	Prevented synaptic dysfunction in TgCRND8 mice	[141, 142]
		Colitis	Resulted in a significant decrease in the disease activity index	[143, 144]
		Cochlear implantation	Local delivery of AM-111 provided a significant level of protection against EIT-induced hearing losses, HC losses, and damage to neural elements	[145]
		Spinal cord injury	Promoteed locomotor recovery and neuroprotection	[146]
		Arthritis	Dramatically reduced inflammation and joint destruction in WT mice	[147]
		Intracerebral hemorrhage	Significantly improved the neurological outcome and decreased the lesion volume	[148]
		Cerebral ischaemia	Reduced the total infarct area, diminished total infarct area, and improved the neurological function and reduced brain oedema	[149]
		Ischemic cochlear damage	Cochlear damage was significantly reduced	[150]
		Neonatal ischemic brain damage	Efficiently protects the neonatal brain against ischemic brain damage and subsequent cognitive and motor impairment	[151]
		Otitis media	Prevented hearing loss from semicircular canal injury	[152]
		Melanoma tumor growth in vivo and in vitro	Reduced melanoma cell growth	[153]
		Liver carcinogenesis in vivo	Reduced tumor size resulting from carcinogen injection; regulation of p21 and c-Myc expression in vitro	[33]
BI-78D3	Preclinical	α1-adrenoceptor-mediated prostate smooth muscle contraction	Reduced phenylephrine- and noradrenaline-induced contractions of human prostate strips	[154]
		Human osteosarcoma in vitro	BI-78D3 combined with doxorubicin increased the induction of apoptosis	[155]
		Liver injury	Blocked JNK dependent Con A-induced liver damage	[156]
		Type 2 diabetes	Restored insulin sensitivity	[156]

Abbreviations: JNK c-Jun N-terminal kinase, *EIT* electrode insertion trauma, *HC* hair cell, *WT* wild type

associated with CC-401 treatment in HCC, additional interventions directed towards JNK activity in vivo are needed. CC-930, which targets JNK1/2 as well as ERK1 and p38, is a JNK inhibitor discovered by and in clinical development with Celgene [128]. CC-930 is mainly being investigated for the prevention and treatment of fibrosis, including idiopathic pulmonary fibrosis and skin fibrosis [128, 129]. Two Phase II research studies to assess if CC-930 was safe for treating subjects with discoid lupus erythematosus, as well as to characterize the safety, PK, and biological activity of CC-930 in idiopathic pulmonary fibrosis have been terminated [130]. AS601245, which belongs to a new class of benzothiazole acetonitrile derivatives, proved to be a structurally unique JNK inhibitor. This inhibitor provided neuroprotective effects in rats with transient focal cerebral ischaemia and gerbils with transient global ischemia [131] and was effective in myocardial ischemia-reperfusion injury [132]. Moreover, Cerbone et al. showed that combined treatment of AS601245 with clofibrate synergistically reduced cell proliferation, cyclin D1 and proliferating cell nuclear antigen (PCNA) expression [133]. The

combination also induced apoptosis and differentiation in human colon cancer cells [133].

These inhibitors have poor kinase specificity because they target the highly conserved ATP-binding site. To overcome the specificity problem, Zhang et al. developed and characterized an inhibitor of all three JNK proteins, JNK-IN-8 [134]. JNK-IN-8 is a selective JNK inhibitor that inhibits phosphorylation of c-Jun in cells exposed to sub-micromolar drugs in a manner that depends on covalent modification of the conserved cysteine residue. Extensive biochemical, cellular, and pathway-based profiling established the selectivity of JNK-IN-8 for JNK and suggested that the compound will be broadly useful as a pharmacological probe for JNK-dependent signal transduction [134]. In 2013, Li et al. showed that JNK-IN-8 rescued head and neck squamous cell carcinoma cells from AZD8055-induced cytotoxicity by inhibiting JNK signals [161]. In summary, there has been significant progress in developing selective ATP-competitive inhibitors, and many of them have shown useful pharmacological effects in a variety of tumor cells, including HCC cells. However, the high homology of the ATP site for JNK isoforms has impeded the development of potent isoform selective JNK inhibitors. As a consequence, many JNK peptide and small molecule ATP-non-competitive inhibitors have also been developed.

8.2 ATP-Non-Competitive Inhibitors

The discovery of the JNK inhibitory properties of the JNK scaffold protein JIP1, followed by the identification of its minimum inhibitory sequence, has opened a new avenue in the use of JIP1-derived JNK inhibitory peptides. JIP1 is a scaffolding protein that enhances JNK signaling by creating a proximity effect between JNK and upstream kinases. D-JNKI-1 (also named as XG-102/AM-111) is a protease-resistant JNK-inhibiting peptide that is produced by linking the 20 amino acid terminal JNK-inhibitory sequence (JNK binding domain) of JIP1/IB1 to a 10-amino-acid TAT sequence of the HIV-TAT protein, which allows intracellular translocation [162]. D-JNKI-1 inhibits phosphorylation of the JNK substrate c-Jun and is more potent than the small-molecule inhibitor SP600125 [163]. A recent study showed that D-JNKI-1 crosses cellular membranes with fast kinetics through an active and passive mechanism. After acute intraperitoneal (ip) administration of D-JNKI-1 in mice, the peptide was found in the main organs, especially the liver and kidney, and could cross the blood brain barrier and reach the brain [164]. Peptidergic JNK inhibition using D-JNKI-1 has previously demonstrated benefits without undesirable side effects in various diseases, such as traumatic hearing loss, colitis, uveitis, diabetes, myocardial ischemia, acute inflammatory insult, Alzheimer's disease, antigen-induced arthritis, adult hemorrhage, and hepatic injury [Reviewed in 165]. Recently, the application of D-JNKI-1 in vivo achieved numerous new progresses in the therapy of those diseases [139–152]. Especially, the Phase III clinical trials to evaluate the efficacy and safety of XG-102 in the reduction of post-cataract surgery intraocular inflammation and pain have been completed [135, 136]. In addition, the Phase II clinical trial to evaluate the efficacy of AM-111 in patients with acute sensorineural hearing loss has been completed with good results, and the Phase III clinical trial of AM-111 in the treatment of acute inner ear hearing loss is currently recruiting participants [137, 138]. The range of therapeutic applications of D-JNKI-1 continues to expand, and it will be broadly applied in the treatment of cancers. In a mouse skin cancer pain model, repeated systemic injections of D-JNKI-1 not only produced accumulative inhibition of mechanical allodynia and heat hyperalgesia, but also suppressed tumor growth in vivo and melanoma cell proliferation in vitro [153]. In addition, D-JNKI-1 significantly reduced the development of chemical-induced mouse HCC or xenografted human HCC cells, which suggested there is great promise for this type of JNK inhibitor in HCC therapy [33]. Unlike D-JNKI-1, there have only been a few studies on other cell permeable JIP1-derived peptides, such as L-JNKI-1, TIJIP, or TAT-TIJIP (TAT-linked JIP-based Peptide), and the in vivo effect of these compounds in liver tumor suppression remains unknown [Reviewed in 165]. However, the efficacy of TAT-mediated drug delivery is still controversial. This controversy combined with poor cell permeability, peptide instability, and a short half-life in vivo has served to hinder the development of peptide-based inhibitors. Increased attention has been directed towards the discovery of small molecules that also could act in an analogous fashion, i.e., as ATP-noncompetitive inhibitors of JNK [166]. In 2008, Stebbins et al. reported a series of small molecule JIP1 mimics that function as substrate competitive inhibitors of JNK [156]. One such compound, BI-78D3 can inhibit human prostate smooth muscle contractions and increase apoptosis in vitro [154, 155]. In animal studies, BI-78D3 not only blocked JNK-dependent Con A-induced liver damage, but also restored insulin sensitivity in mouse models of type 2 diabetes [156]. It will be interesting to see how these inhibitors of JNK activity are broadly applied in the treatment of JNK-associated HCC and whether desired therapeutic outcomes would be achieved without unwanted side effects.

9 Conclusions and Perspectives

HCC is the third leading cause of cancer-related death worldwide. To date, numerous data have indicated that the JNK signaling pathway plays a key role in the progression and tumorigenesis of HCC, and JNK has increasingly been recognized as an attractive molecular target for HCC therapy. As we have reviewed in this article, molecular inhibitors that target JNK have been widely developed, and a new generation of

JNK inhibitors, such as small molecule ATP-non-competitive and peptide-based inhibitors, promise improved efficacy with the possibility of fewer off-target effects. In particular, the targeting of protein substrate docking domains provides a higher degree of specificity than previously achieved with ATP-competitive inhibitors. However, it is quite frustrating that many investigations have been discontinued or are still currently being clinically tested. To date, no clinical use of these molecules has been reported in the literature. Still, as the field of JNK inhibitors is rapidly moving, it is anticipated that several JNK targeted therapies with new drugs will be successfully developed and used in clinical settings in the near future. All in all, we are convinced that promising new avenues for the treatment of HCC are on the horizon, which will undoubtedly lead to better, more effective, and faster therapies in the years to come.

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Compliance with Ethical Standards

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