CHAPTER

4

The JNK Pathway in Drug Resistance

*Lanlin Hu**, *Fangdong Zou**, *Jennifer R. Grandis***, *Daniel E. Johnson****

Sichuan University, Chengdu, Sichuan, PR China

****Department of Otolaryngology – Head and Neck Surgery, University of California at San Francisco, San Francisco, CA, United States**

Abstract

The c-JUN NH2-terminal kinase (JNK) pathway plays key roles in cellular proliferation and survival. Aberrant activation of the pathway occurs in multiple malignancies, where it contributes primarily to tumor growth. In addition, JNK pathway activation has been reported to promote resistance to anticancer agents. In this chapter, we will review current understanding of the involvement of the JNK pathway in tumor development and drug resistance. The identification and characterization of JNK pathway inhibitors will be described. Lastly, we will discuss the potential application of JNK inhibitors in human malignancies as a means of enhancing the effects of conventional chemotherapy drugs or overcoming resistance to these agents.

ABBREVIATIONS

ASK1	Apoptosis signal-regulating kinase 1
DMBA	7,12-Dimethylbenz[α]anthracene
DUSP	Dual-specificity phosphatase
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
GPCR	G-protein coupled receptor
GRASP-1	GRIP1-associated protein 1
HCC	Hepatocellular carcinoma
HNSCC	Head and neck squamous cell carcinoma
HPK1	Hematopoietic progenitor kinase 1
JAK	Janus kinase
JIP	c-JUN NH2-terminal kinase-interacting protein 1
JNK	c-JUN NH2-terminal kinase
MAPK	Mitogen-activated protein kinase
MAPKK	Mitogen-activated protein kinase kinase
MAP2K	Mitogen-activated protein kinase kinase
MAPKKK	Mitogen-activated protein kinase kinase kinase

Targeting Cell Survival Pathways to Enhance Response to Chemotherapy. http://dx.doi.org/10.1016/B978-0-12-813753-6.00004-4

MAP3K	Mitogen-activated protein kinase kinase kinase
MKP	Mitogen-activated protein kinase phosphatase
MLK	Mixed-lineage kinase
POSH	Plenty of SH3s
RACK1	Receptor for activated C kinase 1
SAPK1	Stress-activated mitogen-activated protein kinase
STAT3	Signal transducer and activator of transcription 3
TAK1	Transforming growth factor-β-activated kinase 1
T-ALL	T-cell acute lymphoblastic leukemia
TGF-β	Transforming growth factor-β
TNF	Tumor necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
VEGF	Vascular endothelial growth factor
WDR62	WD repeat domain 62

4.1 INTRODUCTION

Cells respond to changes in the environment, including stimulation with cytokines, growth factors, and stresses, by activating intracellular signal transduction pathways. A key signaling pathway is the mitogen-activated protein kinase (MAPK) pathway, which relays, amplifies, and integrates signals from various stimuli and regulates several important physiological processes, including inflammation, proliferation, differentiation, and cell death [1]. As a ubiquitous and highly conserved signaling mechanism, the MAPK pathway consists of three classic tiers of kinase families, MAPKs, MAPK kinases (MAPKK or MAP2K), and MAPKK kinases (MAPKKK or MAP3K) (Fig. 4.1). Within the distal MAPK family, four subfamilies have been identified in eukaryotic cells, including the c-JUN NH2-terminal kinase (JNK) subfamily, the extracellular signal-related kinases (ERK) 1/2 subfamily, p38, and ERK5 [1,2]. Each of these MAPKs is activated by tyrosine and threonine phosphorylation catalyzed by upstream MAPKKs (Fig. 4.1). The ERK proteins contain classical dual phosphorylation motifs, Thr-Glu-Tyr, and are typically activated following stimulation with mitogens [3]. JNK and p38 contain the dual phosphorylation motifs Thr-Pro-Tyr and Thr-Gly-Tyr, respectively, which commonly are phosphorylated in response to cellular stresses or stimulation with proinflammatory cytokines. The dual phosphorylation of MAPKs is mediated by MAP2Ks, which are themselves activated by serine/threonine phosphorylation by the upstream MAP3Ks.

Early studies demonstrated that JNK, also known as stress-activated MAP kinase (SAPK), binds to the N-terminal activation domain of the transcription factor c-JUN [4]. JNK phosphorylates c-JUN on Ser-63 and Ser-73, promoting the transcriptional activity of the protein [5]. The JNK pathway has been intensively investigated for more than three decades, but its perplexing complexity is still not fully understood. The diverse contributions of the JNK pathway to processes such as proliferation and differentiation give rise to complex roles in cancer, where both oncogenic and tumor suppressor roles have been described. Additionally, a considerable body of evidence has emerged, implicating JNK pathway activation in the development of resistance to anticancer drugs [6]. Thus, considerable effort has been invested by academic and pharmaceutical teams to screen chemical libraries or pursue rational drug design as a means of identifying inhibitors of the

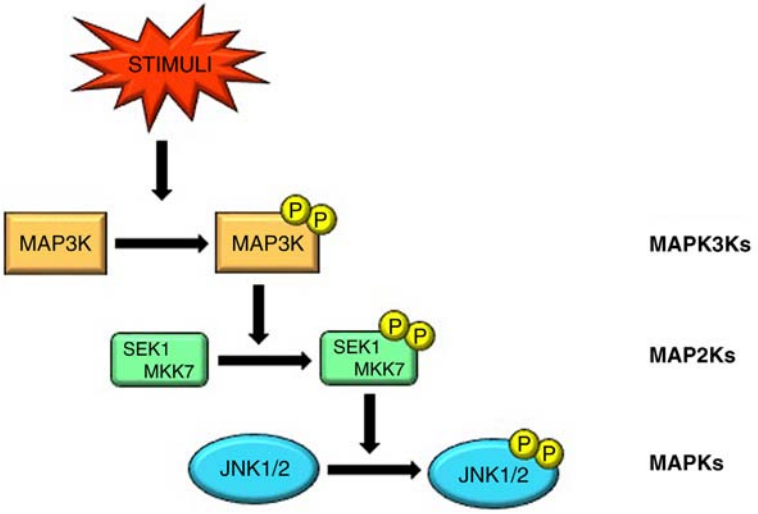


FIG. 4.1 **The MAPK signaling cascade.** In response to cellular stimuli, MAP3Ks become activated via phosphorylation. The phosphorylated/activated MAP3Ks phosphorylate and activate MAP2Ks, which subsequently phosphorylate and activate MAPKs (including JNK1/2).

JNK pathway. In this chapter, we will describe the JNK pathway and discuss its role in the development of cancer and drug resistance. The potential for therapeutic targeting of the JNK pathway will also be discussed.

4.2 THE JNK PATHWAY

4.2.1 Structure and Isoforms

The JNK family comprises three kinases, JNK1, JNK2, and JNK3, encoded by the *jnk1*, *jnk2*, and *jnk3* genes, respectively. The general structure of the JNK proteins consists of N- and C-terminal lobes (Fig. 4.2). The docking site domain, which is comprised of the negatively charged common docking (CD) region and the hydrophobic docking groove, plays a crucial role in physical interactions with binding partners and substrate recruitment. The activation loop (A-loop) contains the Thr-Pro-Tyr motif [7–11]. *Jnk1* and *Jnk2* are ubiquitously expressed, whereas *Jnk3* expression is restricted to brain, heart, and testis [7]. The three *Jnk* genes are also subject to alternative splicing, leading to the production of different isoforms. One alternative splice site lies within the sequence coding for the C-terminal lobe. The use of alternate internal coding exons leads to the generation of two similar isoforms, the α- and β-isoforms. In certain tissues, the expression of tissue-specific splicing factors, such as the Nova family of neuronal splicing factors, results in expression of tissue-specific JNK isoforms. Additionally, alternative splicing in the final coding exon results in a 5-nucleotide shift and the use of different reading frames, producing isoforms with distinct lengths and C-termini [9]. JNK isoforms with different C-termini resulting from frameshift can exhibit distinct cellular

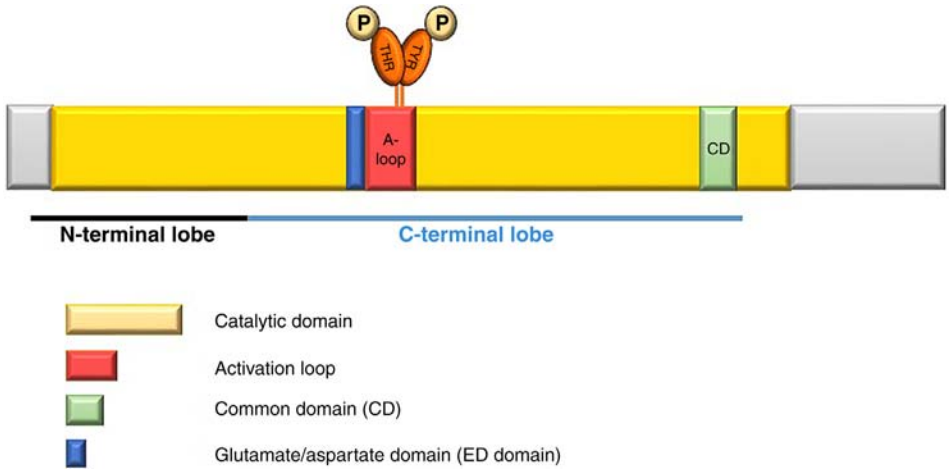


FIG. 4.2 **JNK schematic representation.**

activities. For example, the short isoform JNK1-α1 has an anti-apoptotic function in Colo205 colon cancer cells, whereas the long isoforms JNK1-α2 and -β2 promote apoptosis in these cells [12].

4.2.2 Upstream Activation of the JNK Signaling Pathway

JNK pathways are initiated by an assortment of extracellular and intracellular stimuli, including hormones, pathogens, cytokines, UV radiation, oxidative stress, and DNA damage. Exposure to these stimuli can lead to activation of a broad variety of upstream signaling components, including G-protein coupled receptors (GPCRs), Wnt receptors, transforming growth factor-β (TGF-β) receptors, tumor necrosis factor (TNF) receptors, and the Toll-like receptor complex [9]. Consequently, cascades of kinase signaling are activated that converge on JNKs.

JNKs are activated by phosphorylation of A-loop Thr and Tyr residues by two MAP2ks, MKK7 and SEK1 (also known as MKK4). SEK1 and MKK7 preferentially phosphorylate JNK on Tyr and Thr residues, respectively [13]. In stress-stimulated mouse embryonic stem (ES) cells, JNK1 is first phosphorylated by SEK1 (on Tyr), followed by phosphorylation by MKK7 (on Thr) [14]. Thus, SEK1 and MKK7 appear to cooperate in the activation of JNKs. SEK1 and MMK7 become activated following dual phosphorylation by MAP3Ks. Several MAP3Ks capable of activating the JNK pathway have been identified, including MEKK1 [15], mixed-lineage kinases (MLKs) [16], apoptosis signal-regulating kinase 1 (ASK1) [17], and transforming growth factor β-activated kinase 1 (TAK1) [18].

4.2.3 The Role of Scaffold Proteins

Scaffold proteins play key roles in providing a platform for signaling molecules to assemble, promoting the localization of signaling molecules at specific sites and coordinating

positive and negative feedback signals for pathway regulation. Several scaffold proteins have been identified that bind to JNKs and upstream activators. In melanoma cells, filamin was found to interact with SEK1 as a scaffold protein, in response to TNF-α stimulation [19]. The scaffold proteins POSH (plenty of SH3s) promote JNK pathway activation by directly interacting with GTP-bound RAC1 [20]. POSH complexes with and sequentially stimulates RAC1, MLKs, SEK1, MKK7, and JNKs [21]. Scaffolds JIP1 (JNK-interacting protein 1) and JIP3 recruit several different activators and JNKs. JIP1 recruits DLK, MKK7, and JNK as a complex [22], whereas JIP3 binds SEK1 and MEKK1 to stimulate SEK1, leading to JNK3 activation [23]. β-Arrestin-2 contains a MAP kinase docking site and mainly functions as a scaffold protein in activation of JNK3, by tethering ASK1, SEK1, and JNK3 [24]. Crk II activates HPK1 (hematopoietic progenitor kinase 1) and SEK1-dependent activation of the JNK pathway, by recruiting JNK1 to a p130Cas multiprotein complex [25].

DUSP19 (dual-specificity phosphatase 19) is the first MAPK phosphatase (MKP) identified as a scaffold protein. DUSP19 physically interacts with MKK7 and indirectly regulates JNK [26]. In 2016, another MKP, DUSP22, was reported to interact with ASK1, MKK7, and JNK1 to promote JNK activation independent of its phosphatase activity [27]. In neurons, GRASP-1 (GRIP1-associated protein 1) binds both JNK1 and MEKK1. Following cleavage by caspase-3, the C-terminal domain of GRASP1 is released to promote activation of JNK [28]. In response to stress resulting from X-rays and genotoxic drugs, RACK1 (Receptor for Activated C Kinase 1) has been shown to bind and activate JNK1 and MTK1 [29]. Additionally, WDR62 (WD repeat domain 62) has been implicated in noncanonical activation of JNK via association with JNK and MKK7 [30]. Additional scaffold proteins have been identified that participate in the activation of p38 and ERKs. However, the mechanistic details whereby many of the scaffold proteins promote pathway activation remain to be elucidated.

4.2.4 Substrates of JNK

The JNKs were initially described as kinases responsible for the phosphorylation of c-JUN transcription factor. Subsequently, over 100 proteins have been identified as JNK substrates, including several transcription factors and nuclear hormone receptors, as well as proteins that are not transcription factors. JNK1 binds to the activation domain of c-JUN, specifically phosphorylating Ser63 and Ser73 residues near the N-terminus. JNK1-mediated phosphorylation of c-JUN inhibits the ubiquitination and proteasomal degradation of c-JUN, leading to enhanced transcriptional activity of the AP-1 complex containing c-JUN and c-FOS, and subsequently leading to the induction of AP-1 target genes [1]. The impact of JNK on c-JUN function has been explored extensively and is well documented [30]. Below, we will describe the influence of JNKs on three less characterized, albeit key, substrates, the transcription factor STAT3, the anti-apoptotic protein Bcl-2, and the pro-apoptotic protein Bax.

STAT3, or signal transducer and activator of transcription 3, exists in the cytoplasm as an inactive monomer. When cells are stimulated by cytokines or growth factors, STAT3 becomes phosphorylated on Tyr705 by activated tyrosine kinases called JAK enzymes. Phosphorylation of Tyr705 leads to dimerization of STAT3 and translocation to the nucleus, where the active STAT3 dimer induces the expression of a number of target genes that promote cellular proliferation and survival. It has also been reported that JNK1/2 directly phosphorylates Ser727 on STAT3 following UVA irradiation, enhancing the DNA binding activity of STAT3 in epidermal

JB6 cells [31,32]. In BEAS-2B cells, a human bronchial epithelial cell line, carcinogenic arsenic also activates JNK to promote STAT3 Ser727 phosphorylation, followed by STAT3-mediated induction of vascular endothelial growth factor (VEGF) and cell migration [33]. By contrast, in head and neck squamous cell carcinoma (HNSCC) cell lines, JNK phosphorylation of Ser727 has been reported to reduce phosphorylation of Tyr705, leading to inhibition of STAT3 transcriptional activity, decreased cyclin D1 expression, and reduced cellular proliferation and viability [34]. These somewhat discordant findings have raised controversy surrounding the role of JNK-dependent Ser727 phosphorylation. It is possible that the effects of Ser727 phosphorylation on STAT3 functional activity may be dictated by the repertoire of accessory transcriptional modulators expressed by the cell and, hence, are cell-type specific.

Bcl-2 is an anti-apoptotic protein that resides primarily in the mitochondria. Under healthy growing conditions, Bcl-2 prevents apoptosis by binding to the pro-apoptotic Bcl-2 family members Bax and Bak. Additionally, Bcl-2 prevents induction of autophagy by binding to Beclin-1. When cells are subjected to stress conditions, such as nutrient deprivation, JNK1 becomes activated and phosphorylates Ser70, Ser87, and Thr69 in the unstructured loop region of Bcl-2 [35–38]. Phosphorylation of Bcl-2 on these residues causes dissociation from Beclin-1, as well as Bax (Fig. 4.3). Initially, sufficient levels of Beclin-1 are released to trigger induction of autophagy and the cells undergo characteristic biochemical and morphologic features of the autophagy process. If the stress is not removed, and JNK1 remains active, more Bcl-2 is phosphorylated and the amount of released Bax rises to a level that leads to induction of apoptosis and the ultimate death of the cell. Additional mechanisms of Bax regulation by the JNK pathway have also been reported (Fig. 4.3). In one mechanism, activated JNK directly

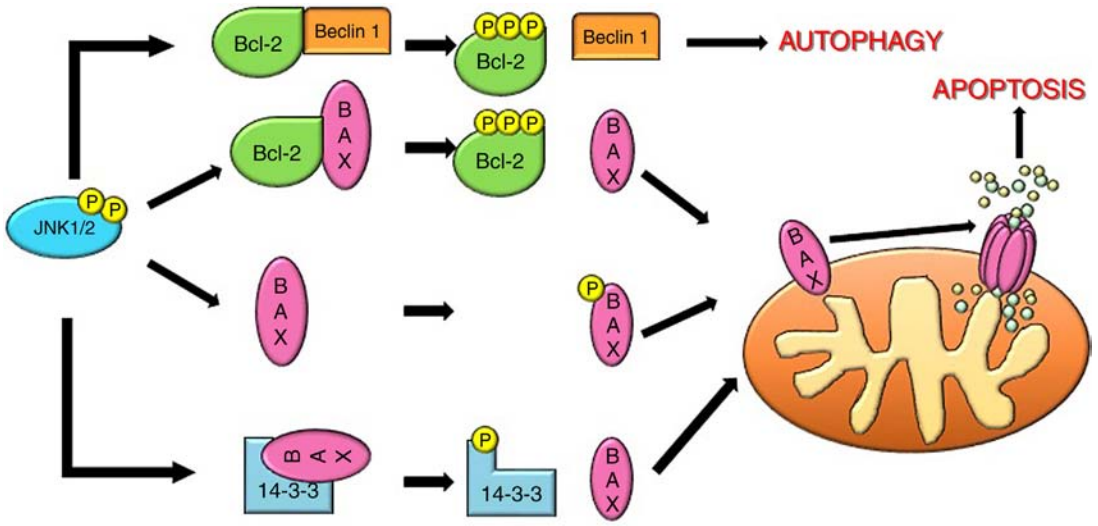


FIG. 4.3 **Induction of apoptosis and autophagy by JNKs.** In response to cellular stimuli, activated JNK1/2: (1) phosphorylate 14-3-3 on serine, causing release of bound Bax, (2) directly phosphorylate Bax on Thr167, and (3) phosphorylate Bcl-2 on Thr69, Ser70, and Ser87, causing the release of Bax (promotes apoptosis) and Beclin-1 (promotes autophagy) from the Bcl-2 protein.

phosphorylates cytosolic Bax on Thr167. This stimulates translocation of Bax to the outer mitochondrial membrane where it becomes available for homo- or hetero-oligomerization with another Bcl-2 family member [38]. In another described mechanism, JNK acts to release cytosolic Bax that is complexed with 14-3-3 protein. Here, Bax is bound by 14-3-3 in a phosphorylation-independent manner, and activated JNK directly phosphorylates the 14-3-3 protein. This allows release of Bax and translocation of Bax to the mitochondria, promoting cytochrome c release and induction of apoptosis [39,40].

4.3 JNK PATHWAY IN CANCER

The roles of JNK enzymes in cancer have been extensively investigated and reported in a large body of scientific literature. While a majority of studies implicate JNKs in promoting cancer, tumor suppressor roles have also been described. It appears that the precise roles of JNKs are context dependent. Thus, it is particularly important to understand the specifics of the cancer model being studied when investigating the functional consequences of JNK activation.

4.3.1 Tumor Suppressive Role

Tumor suppressive roles for JNKs have become readily apparent through the study of knockout mice. Treatment of *JNK1*-deficient mice with 7,12-dimethylbenz[α]anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) results in enhanced papilloma development relative to treatment of wild-type mice [41]. JNK1 knockout models are also prone to spontaneous development of intestinal tumors [42]. Further studies have shown that the JNK pathway can act to suppress the oncogenic properties of RAS and inhibit the development of tumors of the breast, prostate, and colon [43–47]. The tumor suppressive function of JNKs is likely related to their role in regulating cell death/cell survival processes, including apoptosis and autophagy. For example, when multiple myeloma cells are subjected to stress, JNK activation stimulates the release of pro-apoptotic SMAC from the mitochondria, leading to induction of apoptotic cell death [48]. In addition, treatment with cytotoxic drugs leads to JNK activation in numerous types of cancer cells. In this scenario, the activated JNKs promote apoptosis by phosphorylating transcription factors such as c-JUN, ATF2, or p53, or by phosphorylating anti-apoptotic Bcl-2, causing the release and activation of pro-apoptotic Bax [7,49,50].

4.3.2 Role in Oncogenesis

Emerging evidence indicates that the JNK signaling pathway plays an important role in the transformation of cells, as well as the processes of migration and invasion. Signaling via JNKs has been shown to be important for the transforming activities of several different oncogenes, including the genes encoding RAS, c-FOS, c-MET, and BCR-ABL [51,52]. Interestingly, studies in liver biology have shown that JNK1$^{-/-}$ and JNK2$^{-/-}$ mice exhibit resistance to hepatitis, with JNK1 playing a primary role in the development of hepatitis [53,54]. Additional studies have revealed that JNK1 promotes the development of hepatocellular carcinoma (HCC) [55,56]. The ability of JNK1 signaling to stimulate inflammation may underlie its important

role in both hepatitis and HCC [57,58]. Like JNK1, JNK2 has been strongly implicated in promoting the growth of a variety of tumor types, including cancers of the lung, brain, prostate, and skin, as well as multiple myeloma [59–63]. Chemical carcinogenesis in skin caused by the application of DMBA/TPA is significantly reduced in mice lacking normal expression of JNK2 [64]. In the lung, JNK1 and JNK2 have been shown to mediate important oncogenic properties of K-RAS [65–67]. These findings are particularly important in view of the fact that roughly 36% of lung adenocarcinomas exhibit genetic alterations in *K-Ras*. In sum, JNK activation plays a key role in multiple cancers, underscoring the potential value of identifying strategies and agents that can be used to modulate the activity or expression of components of the JNK signaling pathway.

4.4 THE JNK PATHWAY IN DRUG RESISTANCE

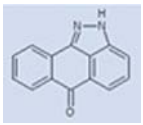
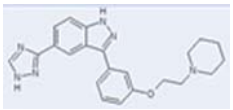
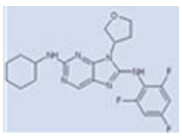
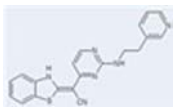
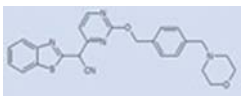
As discussed in the last section, the JNK pathway plays an important role in tumor development. Multiple studies have also documented a role for the JNK pathway in mediating resistance to chemotherapy drugs. However, similar to what is seen in tumor development or suppression, roles for the JNK pathway in cellular responses to chemotherapy differ depending on the cancer cell type. Clear roles for the JNK pathway in drug resistance have been demonstrated in several cancer models, including lung adenocarcinoma, squamous cell carcinoma, melanoma, T-cell acute lymphoblastic leukemia (T-ALL), HCC, and colon cancer [68–74]. In breast cancer cell lines, the JNK pathway has been shown to mediate acquired resistance to EGFR/HER2-targeted therapies [73]. In tetrandrine-resistant Jurkat T leukemic cells, AP-1 activity has been shown to be elevated [75]. In lung cancer cells, the JNK pathway mediates cisplatin induction of drug resistance genes [76]. By contrast, inhibition of the JNK pathway in HCC cell lines leads to enhanced resistance to TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) [77]. Despite these divergent roles, on the whole, activation of the JNK is more commonly associated with drug resistance than drug sensitization. Thus, it is not surprising that considerable effort has been invested in developing inhibitors of the JNK pathway. In the next section, we describe several of these inhibitors, including ATP-competitive inhibitors and noncompetitive peptide inhibitors.

4.5 INHIBITORS OF THE JNK PATHWAY

4.5.1 ATP-Competitive Inhibitors in Clinical Trials

First described in 2001, SP600125, an anthrapyrazolone inhibitor of JNK1/2/3 has been used extensively to investigate *in vitro* and *in vivo* roles of the JNK pathway (Table 4.1) [78]. SP600125 is a reversible ATP-competitive inhibitor with >300-fold selectivity for JNKs over the related MAPKs, ERK1, and p38-2 [78]. SP600125 inhibits phosphorylation of c-JUN in a dose-dependent fashion and reduces the expression of pro-inflammatory genes [78]. In some disease models, treatment with SP600125 has been shown to control disease progression [79], whereas in other disease models the inhibitor failed to impact tumor growth and was found to induce significant toxicities [80]. CC-401 is an ATP-competitive inhibitor based

TABLE 4.1 JNK Inhibitors

Name	Structure or sequence	Target	Clinical trials	References
SP600125		JNK1/2/3 MKK3/4/6 COX-2 IL-2 IFN-y TNF-a	None	[78–80]
CC-401		JNK1/2/3	NCT00126893	[69,81,82]
CC-930 (Tanzisertib)		JNK1/2/3	NCT01466725 NCT01203943	[83,84]
AS601245		JNK1/2/3		[85,86]
AS602801 (PGL-5001 or Bentamapimod)		JNK1/2/3	NCT01630252	[87]
D-JNKI (XG-102 or AM-111)	DQSRPVQPFLNLTTPRKP RPPRRRQRRKKRG	JNK1/2/3	NCT01570205 NCT00802425 NCT02235272 NCT02508337 NCT02809118 NCT02561091	[88–95]

on SP600125 that potently inhibits all three JNKs (JNK1/2/3) and exhibits greater than 40-fold selectivity for JNKs compared to other related kinases [69]. CC-401 has shown efficacy in sensitizing colon cancer cells to DNA-damaging agents and also exhibits activity in experimental models of unilateral ureteral obstruction and antiglomerular basement membrane disease [69,81,82]. CC-401 has been investigated in a Phase I clinical trial of high-risk myeloid leukemia patients (NCT00126893; clinicaltrials.gov). Another SP600125-derived JNK inhibitor is CC-930 (Tanzisertib), an orally active aminopurine compound [83]. Phase I evaluation of CC-930 has demonstrated a decrease in UV-induced phosphorylation of c-JUN in the skin of treated patients [84]. However, two Phase II clinical trials of CC-930 in discoid lupus erythematosus (NCT01466725) and idiopathic pulmonary fibrosis (NCT01203943) were prematurely terminated due to a poor benefit–risk profile. AS601245 is a structurally unique ATP-competitive JNK inhibitor with anti-inflammatory and neuroprotective properties [85].

AS601245 inhibits the proliferation of colon cancer cells and has been reported to protect the brain from ischemic injury [85,86]. Phase II testing of AS602801 (also known as PGL-5001 or Bentamapimod), a related orally active inhibitor, has recently concluded in patients with inflammatory endometriosis (NCT01630252). AS602801 has been reported to inhibit pancreatic cancer stem cells *in vitro* and *in vivo* [87]. The structures, activities, and mechanisms of additional ATP-competitive inhibitors of JNKs are described elsewhere [88,89].

4.5.2 Peptide Inhibitors of the JNK Signaling Pathway

The vast majority of inhibitors of protein kinases are directed against the kinase ATP binding site. However, since all kinases contain ATP binding sites, these inhibitors often lack specificity and inhibit multiple different kinases. Thus, it is beneficial to design or screen for noncompetitive inhibitors, including small molecules and peptides that act to disrupt key protein–protein interactions. A cell-permeable peptide encompassing the JNK-binding site of c-JUN has been reported to specifically and efficiently disrupt the c-JUN/JNK complex *in vitro* and *in vivo* [90]. An additional peptide inhibitor, D-JNKI (also known as XG-102, AM-111), interferes with JIP1/JNK interactions (Table 4.1) [91]. This D-amino acid-containing, retro-inverso peptide demonstrates impressive stability in cells and significant biological activity in a variety of *in vivo* models, including preclinical models of cerebral ischemia, hearing loss, liver injury, and cancer [92–95]. Phase I testing has been completed examining the tolerability and pharmacokinetics of a single intravenous infusion of D-JNKI in healthy volunteers (NCT01570205). Clinical trials are currently ongoing, examining the impact of D-JNKI in patients with acute sensorineural hearing loss (NCT00802425, NCT02809118, NCT02561091). In addition, a Phase III clinical trial is investigating the efficacy of D-JNKI in reducing inflammation and pain following cataract surgery (NCT02235272, NCT02508337). In view of the antiproliferative activity of D-JNKI against melanoma cells and its relatively positive safety profile in humans, testing of this novel compound in cancer patients appears warranted [91].

4.6 CONCLUSIONS

To date, the evaluation of JNK inhibitors in cancer clinical trials has been limited, despite ongoing testing in other diseases. The ability to deliver tolerable doses over extended periods of time remains a significant challenge. Moreover, it is doubtful that JNK inhibitors will prove to be highly effective as single agents. The identification of additional, well-tolerated inhibitors and the identification of combinatorial targeting strategies, preferably those that generate synergistic antitumor responses or overcome resistance to conventional anticancer agents, should be key goals of future investigations

Acknowledgments

This work was supported by National Institutes of Health grants R01 DE24728 (DEJ), P50CA097190 (DEJ and JRG), and R01 DE023685 (JRG).

Conflict of Interest: No potential conflicts of interest were disclosed.

References

[1] Weston CR, Davis RJ. The JNK signal transduction pathway. Curr Opin Genet Dev 2002;12(1):14–21.
[2] Lee JD, Ulevitch RJ, Han JH. Primary structure of Bmk1—a new mammalian map kinase. Biochem Biophys Res Commun 1995;213(2):715–24.
[3] Kamakura S, Moriguchi T, Nishida E. Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases—identification and characterization of a signaling pathway to the nucleus. J Biol Chem 1999;274(37):26563–71.
[4] Adler V, Polotskaya A, Wagner F, Kraft AS. Affinity-purified C-Jun amino-terminal protein-kinase requires serine threonine phosphorylation for activity. J Biol Chem 1992;267(24):17001–5.
[5] Pulverer BJ, Kyriakis JM, Avruch J, Nikolakaki E, Woodgett JR. Phosphorylation of C-Jun mediated by Map kinases. Nature 1991;353(6345):670–4.
[6] Vasilevskaya I, O'Dwyer PJ. Role of Jun and Jun kinase in resistance of cancer cells to therapy. Drug Resist Update 2003;6(3):147–56.
[7] Davis RJ. Signal transduction by the JNK group of MAP kinases. Cell 2000;103(2):239–52.
[8] Xie XL, Gu Y, Fox T, Coll JT, Fleming MA, Markland W, et al. Crystal structure of JNK3: a kinase implicated in neuronal apoptosis. Struct Fold Des 1998;6(8):983–91.
[9] Zeke A, Misheva M, Remenyi A, Bogoyevitch MA. JNK signaling: regulation and functions based on complex protein–protein partnerships. Microbiol Mol Biol Rev 2016;80(3):793–835.
[10] Shaw D, Wang SM, Villasenor AG, Tsing S, Walter D, Browner MF, et al. The crystal structure of JNK2 reveals conformational flexibility in the MAP kinase insert and indicates its involvement in the regulation of catalytic activity. J Mol Biol 2008;383(4):885–93.
[11] Heo JS, Kim SK, Seo CI, Kim YK, Sung BJ, Lee HS, et al. Structural basis for the selective inhibition of JNK1 by the scaffolding protein JIP1 and SP600125. EMBO J 2004;23(11):2185–95.
[12] Mahalingam D, Keane M, Pirianov G, Mehmet H, Samali A, Szegezdi E. Differential activation of JNK1 isoforms by TRAIL receptors modulate apoptosis of colon cancer cell lines. Br J Cancer 2009;100(9):1415–24.
[13] Lawler S, Fleming Y, Goedert M, Cohen P. Synergistic activation of SAPK1/JNK1 by two MAP kinase kinases *in vitro*. Curr Biol 1998;8(25):1387–90.
[14] Kishimoto H, Nakagawa K, Watanabe T, Kitagawa D, Momose H, Seo J, et al. Different properties of SEK1 and MKK7 in dual phosphorylation of stress-induced activated protein kinase SAPK/JNK in embryonic stem cells. J Biol Chem 2003;278(19):16595–601.
[15] Charlaftis N, Suddason T, Wu XF, Anwar S, Karin M, Gallagher E. The MEKK1 PHD ubiquitinates TAB1 to activate MAPKs in response to cytokines. EMBO J 2014;33(21):2581–96.
[16] Gallo KA, Johnson GL. Mixed-lineage kinase control of JNK and p38 MAPK pathways. Nat Rev Mol Cell Biol 2002;3(9):663–72.
[17] Takeda K, Matsuzawa A, Nishitoh H, Ichijo H. Roles of MAPKKK ASK1 in stress-induced cell death. Cell Struct Funct 2003;28(1):23–9.
[18] Brown K, Vial SCM, Dedi N, Long JM, Dunster NJ, Cheetham GMT. Structural basis for the interaction of TAK1 kinase with its activating protein TAB1. J Mol Biol 2005;354(5):1013–20.
[19] Marti A, Luo Z, Cunningham C, Ohta Y, Hartwig J, Stossel TP, et al. Actin-binding protein-280 binds the stress-activated protein kinase (SAPK) activator SEK-1 and is required for tumor necrosis factor-α activation of SAPK in melanoma cells. J Biol Chem 1997;272(5):2620–8.
[20] Tapon N, Nagata K, Lamarche N, Hall A. A new Rac target POSH is an SH3-containing scaffold protein involved in the JNK and NF-κB signalling pathways. EMBO J 1998;17(5):1395–404.
[21] Xu Z, Kukekov NV, Greene LA. POSH acts as a scaffold for a multiprotein complex that mediates JNK activation in apoptosis. EMBO J 2003;22(2):252–61.
[22] Nihalani D, Wong HN, Holzman LB. Recruitment of JNK to JIP1 and JNK-dependent JIP1 phosphorylation regulates JNK module dynamics and activation. J Biol Chem 2003;278(31):28694–702.
[23] Ito M, Yoshioka K, Akechi M, Yamashita S, Takamatsu N, Sugiyama K, et al. JSAP1, a novel jun N-terminal protein kinase (JNK)-binding protein that functions as a Scaffold factor in the JNK signaling pathway. Mol Cell Biol 1999;19(11):7539–48.
[24] Miller WE, Lefkowitz RJ. Expanding roles for β-arrestins as scaffolds and adapters in GPCR signaling and trafficking. Curr Opin Cell Biol 2001;13(2):139–45.
[25] Girardin SE, Yaniv M. A direct interaction between JNK1 and CrkII is critical for Rac1-induced JNK activation. EMBO J 2001;20(13):3437–46.

[26] Zama T, Aoki R, Kamimoto T, Inoue K, Ikeda Y, Hagiwara M. A novel dual specificity phosphatase SKRP1 interacts with the MAPK kinase MKK7 and inactivates the JNK MAPK pathway implication for the precise regulation of the particular MAPK pathway. J Biol Chem 2002;277(26):23909–18.
[27] Ju A, Cho Y-C, Kim BR, Park SG, Kim J-H, Kim K, et al. Scaffold role of DUSP22 in ASK1-MKK7-JNK signaling pathway. PLoS ONE 2016;11(10):e0164259.
[28] Ye B, Yu W-P, Thomas GM, Huganir RL. GRASP-1 is a neuronal scaffold protein for the JNK signaling pathway. FEBS Lett 2007;581(23):4403–10.
[29] Arimoto K, Fukuda H, Imajoh-Ohmi S, Saito H, Takekawa M. Formation of stress granules inhibits apoptosis by suppressing stress-responsive MAPK pathways. Nat Cell Biol 2008;10(11):1324.
[30] Bogoyevitch MA, Kobe B. Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. Microbiol Mol Biol Rev 2006;70(4):1061–95.
[31] Zhang Y, Liu G, Dong Z. MSK1 and JNKs mediate phosphorylation of STAT3 in UVA-irradiated mouse epidermal JB6 cells. J Biol Chem 2001;276(45):42534–42.
[32] Liu J, Chen B, Lu Y, Guan Y, Chen F. JNK-dependent Stat3 phosphorylation contributes to Akt activation in response to arsenic exposure. Toxicol Sci 2012;129(2):363–71.
[33] Sun J, Yu M, Lu Y, Thakur C, Chen B, Qiu P, et al. Carcinogenic metalloid arsenic induces expression of mdig oncogene through JNK and STAT3 activation. Cancer Lett 2014;346(2):257–63.
[34] Gkouveris I, Nikitakis N, Karanikou M, Rassidakis G, Sklavounou A. JNK1/2 expression and modulation of STAT3 signaling in oral cancer. Oncol Lett 2016;12(1):699–706.
[35] Maundrell K, Antonsson B, Magnenat E, Camps M, Muda M, Chabert C, et al. Bcl-2 undergoes phosphorylation by c-Jun N-terminal kinase/stress-activated protein kinases in the presence of the constitutively active GTP-binding protein Rac1. J Biol Chem 1997;272(40):25238–42.
[36] Yamamoto K, Ichijo H, Korsmeyer SJ. BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G2/M. Mol Cell Biol 1999;19(12):8469–78.
[37] Wei Y, Pattingre S, Sinha S, Bassik M, Levine B. JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. Mol Cell 2008;30(6):678–88.
[38] Kim B-J, Ryu S-W, Song B-J. JNK-and p38 kinase-mediated phosphorylation of Bax leads to its activation and mitochondrial translocation and to apoptosis of human hepatoma HepG2 cells. J Biol Chem 2006;281(30):21256–65.
[39] Tsuruta F, Sunayama J, Mori Y, Hattori S, Shimizu S, Tsujimoto Y, et al. JNK promotes Bax translocation to mitochondria through phosphorylation of 14-3-3 proteins. EMBO J 2004;23(8):1889–99.
[40] Muscarella DE, Bloom SE. The contribution of c-Jun N-terminal kinase activation and subsequent Bcl-2 phosphorylation to apoptosis induction in human B-cells is dependent on the mode of action of specific stresses. Toxicol Appl Pharmacol 2008;228(1):93–104.
[41] She Q-B′, Chen N, Bode AM, Flavell RA, Dong Z. Deficiency of c-Jun-NH2-terminal kinase-1 in mice enhances skin tumor development by 12-*O*-tetradecanoylphorbol-13-acetate. Cancer Res 2002;62(5):1343–8.
[42] Tong C, Yin Z, Song Z, Dockendorff A, Huang C, Mariadason J, et al. c-J un NH 2-terminal kinase 1 plays a critical role in intestinal homeostasis and tumor suppression. Am J Pathol 2007;171(1):297–303.
[43] Kennedy NJ, Sluss HK, Jones SN, Bar-Sagi D, Flavell RA, Davis RJ. Suppression of Ras-stimulated transformation by the JNK signal transduction pathway. Genes Dev 2003;17(5):629–37.
[44] Cellurale C, Weston CR, Reilly J, Garlick DS, Jerry DJ, Sluss HK, et al. Role of JNK in a Trp53-dependent mouse model of breast cancer. PLoS ONE 2010;5(8):e12469.
[45] Cellurale C, Girnius N, Jiang F, Cavanagh-Kyros J, Lu S, Garlick DS, et al. Role of JNK in mammary gland development and breast cancer. Cancer Res 2012;72(2):472–81.
[46] Hübner A, Mulholland DJ, Standen CL, Karasarides M, Cavanagh-Kyros J, Barrett T, et al. JNK and PTEN cooperatively control the development of invasive adenocarcinoma of the prostate. Proc Natl Acad Sci 2012;109(30):12046–51.
[47] Marusiak AA, Stephenson NL, Baik H, Trotter EW, Li Y, Blyth K, et al. Recurrent MLK4 loss-of-function mutations suppress JNK signaling to promote colon tumorigenesis. Cancer Res 2016;76(3):724–35.
[48] Chauhan D, Li G, Hideshima T, Podar K, Mitsiades C, Mitsiades N, et al. JNK-dependent release of mitochondrial protein, Smac, during apoptosis in multiple myeloma (MM) cells. J Biol Chem 2003;278(20):17593–6.
[49] Hayakawa J, Depatie C, Ohmichi M, Mercola D. The activation of c-Jun NH2-terminal kinase (JNK) by DNA-damaging agents serves to promote drug resistance via activating transcription factor 2 (ATF2)-dependent enhanced DNA repair. J Biol Chem 2003;278(23):20582–92.

[50] Oleinik N, Krupenko N, Krupenko S. Cooperation between JNK1 and JNK2 in activation of p53 apoptotic pathway. Oncogene 2007;26(51):7222.
[51] Behrens A, Jochum W, Sibilia M, Wagner EF. Oncogenic transformation by ras and fos is mediated by c-Jun N-terminal phosphorylation. Oncogene 2000;19(22):2657.
[52] Manning AM, Davis RJ. Targeting JNK for therapeutic benefit: from junk to gold? Nat Rev Drug Discov 2003;2(7):554.
[53] Maeda S, Chang L, Li Z-W, Luo J-L, Leffert H, Karin M. IKKβ is required for prevention of apoptosis mediated by cell-bound but not by circulating TNFα. Immunity 2003;19(5):725–37.
[54] Kamata H, Honda S-I, Maeda S, Chang L, Hirata H, Karin M. Reactive oxygen species promote TNFα-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. Cell 2005;120(5):649–61.
[55] Sakurai T, Maeda S, Chang L, Karin M. Loss of hepatic NF-κB activity enhances chemical hepatocarcinogenesis through sustained c-Jun N-terminal kinase 1 activation. Proc Natl Acad Sci 2006;103(28):10544–51.
[56] 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 downregulation. J Clin Invest 2008;118(12):3943.
[57] Das M, Garlick DS, Greiner DL, Davis RJ. The role of JNK in the development of hepatocellular carcinoma. Genes Dev 2011;25(6):634–45.
[58] Han MS, Barrett T, Brehm MA, Davis RJ. Inflammation mediated by JNK in myeloid cells promotes the development of hepatitis and hepatocellular carcinoma. Cell Rep 2016;15(1):19–26.
[59] Nitta R, Del Vecchio C, Chu A, Mitra S, Godwin A, Wong A. The role of the c-Jun N-terminal kinase 2-[alpha]-isoform in non-small cell lung carcinoma tumorigenesis. Oncogene 2011;30(2):234.
[60] Cui J, Han S-Y, Wang C, Su W, Harshyne L, Holgado-Madruga M, et al. c-Jun NH2-terminal kinase 2α2 promotes the tumorigenicity of human glioblastoma cells. Cancer Res 2006;66(20):10024–31.
[61] Yang Y-M, Bost F, Charbono W, Dean N, McKay R, Rhim JS, et al. C-Jun NH2-terminal kinase mediates proliferation and tumor growth of human prostate carcinoma. Clin Cancer Res 2003;9(1):391–401.
[62] Ke H, Harris R, Coloff JL, Jin JY, Leshin B, de Marval PM, et al. The c-Jun NH2-terminal kinase 2 plays a dominant role in human epidermal neoplasia. Cancer Res 2010;70(8):3080–8.
[63] Barbarulo A, Iansante V, Chaidos A, Naresh K, Rahemtulla A, Franzoso G, et al. Poly(ADP-ribose) polymerase family member 14 (PARP14) is a novel effector of the JNK2-dependent pro-survival signal in multiple myeloma. Oncogene 2013;32(36):4231.
[64] Chen N, Nomura M, She Q-B, Ma W-Y, Bode AM, Wang L, et al. Suppression of skin tumorigenesis in c-Jun NH2-terminal kinase-2-deficient mice. Cancer Res 2001;61(10):3908–12.
[65] Cellurale C, Sabio G, Kennedy NJ, Das M, Barlow M, Sandy P, et al. Requirement of c-Jun NH2-terminal kinase for Ras-initiated tumor formation. Mol Cell Biol 2011;31(7):1565–76.
[66] Taylor E, Alqadri N, Dodgson L, Mason D, Lyulcheva E, Messina G, et al. MRL proteins cooperate with activated Ras in glia to drive distinct oncogenic outcomes. Oncogene 2017;36(30):4311.
[67] Kim H, Hwang H, Lee H, Hong HJ. L1 cell adhesion molecule promotes migration and invasion via JNK activation in extrahepatic cholangiocarcinoma cells with activating KRAS mutation. Mol Cells 2017;40(5):363.
[68] Chen R, Khatri P, Mazur PK, Polin M, Zheng Y, Vaka D, et al. A meta-analysis of lung cancer gene expression identifies PTK7 as a survival gene in lung adenocarcinoma. Cancer Res 2014;74(10):2892–902.
[69] Vasilevskaya IA, Selvakumaran M, Hierro LC, Goldstein SR, Winkler JD, O'Dwyer PJ. Inhibition of JNK sensitizes hypoxic colon cancer cells to DNA-damaging agents. Clin Cancer Res 2015;21(18):4143–52.
[70] Fallahi-Sichani M, Moerke NJ, Niepel M, Zhang T, Gray NS, Sorger PK. Systematic analysis of BRAF V 600E melanomas reveals a role for JNK/c-Jun pathway in adaptive resistance to drug-induced apoptosis. Mol Syst Biol 2015;11(3):797.
[71] Lim S-C, Jeon HJ, Kee KH, Lee MJ, Hong R, Han SI. Involvement of DR4/JNK pathway-mediated autophagy in acquired TRAIL resistance in HepG2 cells. Int J Oncol 2016;49(5):1983–90.
[72] Lin Y-T, Liu Y-C, Chao CC-K. Inhibition of JNK and prothymosin-alpha sensitizes hepatocellular carcinoma cells to cisplatin. Biochem Pharmacol 2016;122:80–9.
[73] Manole S, Richards EJ, Meyer AS. JNK pathway activation modulates acquired resistance to EGFR/HER2 targeted therapies. Cancer Res 2016;76(18):5219–28.
[74] Yang S, Qiang L, Sample A, Shah P, He Y-Y. NF-κB signaling activation induced by chloroquine requires autophagosome, p62 protein, and c-Jun N-terminal kinase (JNK) signaling and promotes tumor cell resistance. J Biol Chem 2017;292(8):3379–88.

[75] Liou J-T, Lin C-S, Liao Y-C, Ho L-J, Yang S-P, Lai J-H. JNK/AP-1 activation contributes to tetrandrine resistance in T-cell acute lymphoblastic leukaemia. Acta Pharmacol Sin 2017;38(8):1171.
[76] Xu L, Fu Y, Li Y, Han X. Cisplatin induces expression of drug resistance-related genes through c-jun N-terminal kinase pathway in human lung cancer cells. Cancer Chemother Pharmacol 2017;80(2):235–42.
[77] Song IS, Jun SY, Na HJ, Kim HT, Jung SY, Ha GH, et al. Inhibition of MKK7–JNK by the TOR signaling pathway regulator-like protein contributes to resistance of HCC cells to TRAIL-induced apoptosis. Gastroenterology 2012;143(5):1341–51.
[78] Bennett BL, Sasaki DT, Murray BW, O'Leary EC, Sakata ST, Xu W, et al. SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. Proc Natl Acad Sci 2001;98(24):13681–6.
[79] Gross ND, Boyle JO, Du B, Kekatpure VD, Lantowski A, Thaler HT, et al. Inhibition of Jun NH2-terminal kinases suppresses the growth of experimental head and neck squamous cell carcinoma. Clin Cancer Res 2007;13(19):5910–7.
[80] Tanemura S, Momose H, Shimizu N, Kitagawa D, Seo J, Yamasaki T, et al. Blockage by SP600125 of Fcε receptor-induced degranulation and cytokine gene expression in mast cells is mediated through inhibition of phosphatidylinositol 3-kinase signalling pathway. J Biochem 2008;145(3):345–54.
[81] Flanc R, Ma F, Tesch G, Han Y, Atkins R, Bennett B, et al. A pathogenic role for JNK signaling in experimental anti-GBM glomerulonephritis. Kidney Int 2007;72(6):698–708.
[82] Ma FY, Flanc RS, Tesch GH, Han Y, Atkins RC, Bennett BL, et al. A pathogenic role for c-Jun amino-terminal kinase signaling in renal fibrosis and tubular cell apoptosis. J Am Soc Nephrol 2007;18(2):472–84.
[83] Krenitsky VP, Nadolny L, Delgado M, Ayala L, Clareen SS, Hilgraf R, et al. Discovery of CC-930, an orally active anti-fibrotic JNK inhibitor. Bioorg Med Chem Lett 2012;22(3):1433–8.
[84] van der Velden JL, Ye Y, Nolin JD, Hoffman SM, Chapman DG, Lahue KG, et al. JNK inhibition reduces lung remodeling and pulmonary fibrotic systemic markers. Clin Transl Med 2016;5(1):36.
[85] Cerbone A, Toaldo C, Pizzimenti S, Pettazzoni P, Dianzani C, Minelli R, et al. AS601245, an anti-inflammatory JNK inhibitor, and clofibrate have a synergistic effect in inducing cell responses and in affecting the gene expression profile in CaCo-2 colon cancer cells. PPAR Res 2012;2012. 269751.
[86] Gaillard P, Jeanclaude-Etter I, Ardissone V, Arkinstall S, Cambet Y, Camps M, et al. Design and synthesis of the first generation of novel potent, selective, and *in vivo* active (benzothiazol-2-yl) acetonitrile inhibitors of the c-Jun N-terminal kinase. J Med Chem 2005;48(14):4596–607.
[87] Okada M, Kuramoto K, Takeda H, Watarai H, Sakaki H, Seino S, et al. The novel JNK inhibitor AS602801 inhibits cancer stem cells *in vitro* and *in vivo*. Oncotarget 2016;7(19):27021.
[88] Bogoyevitch MA, Arthur PG. Inhibitors of c-Jun N-terminal kinases—JuNK no more? Biochim Biophys Acta 2008;1784(1):76–93.
[89] Messoussi A, Feneyrolles C, Bros A, Deroide A, Daydé-Cazals B, Chevé G, et al. Recent progress in the design, study, and development of c-Jun N-terminal kinase inhibitors as anticancer agents. Chem Biol 2014;21(11):1433–43.
[90] Holzberg D, Knight CG, Dittrich-Breiholz O, Schneider H, Dörrie A, Hoffmann E, et al. Disruption of the c-JUN-JNK complex by a cell-permeable peptide containing the c-JUN δ domain induces apoptosis and affects a distinct set of interleukin-1-induced inflammatory genes. J Biol Chem 2003;278(41):40213–23.
[91] Barr RK, Kendrick TS, Bogoyevitch MA. Identification of the critical features of a small peptide inhibitor of JNK activity. J Biol Chem 2002;277(13):10987–97.
[92] Borsello T, Clarke PG, Hirt L, Vercelli A, Repici M, Schorderet DF, et al. A peptide inhibitor of c-Jun N-terminal kinase protects against excitotoxicity and cerebral ischemia. Nat Med 2003;9(9):1180–6.
[93] Eshraghi AA, Wang J, Adil E, He J, Zine A, Bublik M, et al. Blocking c-Jun-N-terminal kinase signaling can prevent hearing loss induced by both electrode insertion trauma and neomycin ototoxicity. Hearing Res 2007;226(1):168–77.
[94] Gao Y-J, Cheng J-K, Zeng Q, Xu Z-Z, Decosterd I, Xu X, et al. Selective inhibition of JNK with a peptide inhibitor attenuates pain hypersensitivity and tumor growth in a mouse skin cancer pain model. Exp Neurol 2009;219(1):146–55.
[95] Lehnert M, Relja B, Lee VS-Y, Schwestka B, Henrich D, Czerny C, et al. A peptide inhibitor of C-jun N-terminal kinase modulates hepatic damage and the inflammatory response after hemorrhagic shock and resuscitation. Shock 2008;30(2):159–65.