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The Functional Contrariety of JNK

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Abstract

The JNK proteins are activated by multiple and diverse stimuli, leading to varied and seemingly contradictory cellular responses. In particular, JNKs have been reported to have a role in the induction of apoptosis, but have also been implicated in enhancing cell survival and proliferation. Thus the JNK proteins seem to represent an archetype of contrariety of intracellular signaling. The opposing roles of JNKs have been attributed to the observation that JNKs activate different substrates based on specific stimulus, cell type or temporal aspects. Because of their analogous expression in apparently almost every tissue, JNK1 and JNK2 have most often been considered to have overlapping or redundant functions. In spite of this assessment, research evidence suggests that the functions of JNKs should be addressed in a manner that differentiates between their precise contributions. Specifically in this review, we examine evidence regarding whether the JNKs proteins might play distinctive roles in cellular processes associated with carcinogenesis.

Keywords

JNK; MAP kinase; cancer; signal transduction

INTRODUCTION AND BACKGROUND

The c-Jun N-terminal kinases (JNKs) are recognized as members of the mitogen-activated protein (MAP) kinase family of proteins (reviewed in Reference [1]). The protein kinase kinases, mitogen-activated protein kinase kinase (MKK)4 and/or MKK7, are known to directly activate and phosphorylate JNKs. Complete activation of JNKs requires dual phosphorylation of threonine and tyrosine residues within a threonine/proline/tyrosine motif located in kinase domain VIII. MKK4 preferentially phosphorylates tyrosine 185, whereas MKK7 prefers threonine 183. MKK4 and MKK7 are also phosphorylated and activated by dual phosphorylation that is mediated by upstream MAPKK kinases (MAPKKKs; reviewed in Reference [1]). These protein kinases and their respective scaffolding proteins (reviewed in Reference [2]) are believed to form discrete signaling modules that mediate JNKs activation in response to distinct stimuli. Conversely, JNKs can be inactivated by serine and tyrosine phosphatases, including a family of dual specificity MAP kinase phosphatases (MKP; reviewed in Reference [1]).

The JNK proteins are encoded by three genes, *jnk1*, *jnk2*, *jnk3*, which are alternatively spliced to create at least 10 isoforms [3]. The protein products of *jnk1* and *jnk2* are believed to be expressed in every cell and tissue type, whereas the JNK3 protein is found primarily in brain and to a lesser extent in heart and testis. The JNK proteins seem to represent an archetype of contrariety of intracellular signaling because they are known to be activated by multiple and

diverse stimuli leading to varied and seemingly contradictory cellular responses. In particular, JNKs have a role in the induction of apoptosis, but have also been implicated in enhancing cell survival and proliferation. The opposing roles of JNKs have been attributed to the observation that JNKs activate a large number of different substrates based on specific stimulus, cell type, and temporal aspects. For example, JNKs seem to promote Bcr-Abl-induced lymphoma in B cells [4], but suppress *ras*-induced tumorigenesis in fibroblasts [5]. Furthermore, JNKs are known to phosphorylate various anti-apoptotic Bcl-2 family member proteins to promote apoptosis [6], but also can phosphorylate the pro-apoptotic BAD protein to inhibit apoptosis [7]. Recently, Ventura et al. [8] used a unique approach to show that the biological response of cells to JNKs activation is determined by both the physiological context and time course of JNKs signaling. They reported that the early transient phase of JNKs activation (<1 h) corresponds to cell survival, whereas the later and more sustained phase of JNKs activation (1–6 h) mediates pro-apoptotic signaling [8]. These types of studies strongly support the idea that cellular context determines response.

Because of its relative tissue specificity, JNK3 is believed to have functions mostly distinct from those of JNK1 or JNK2; whereas, because of their concurrent and seemingly ubiquitous expression, JNK1 and JNK2 have most often been considered to have overlapping or redundant functions. In spite of this assessment, research evidence seems to suggest that the functions of JNKs should be addressed in a manner that differentiates between their distinct contributions. Specifically in this review, we examine evidence focusing on whether the JNK proteins play distinctive or redundant roles in cellular processes associated with carcinogenesis.

JNK Substrates and Other Interacting Proteins

Depending on the stimulus and cell type, JNKs can phosphorylate a number of activator protein-1 (AP-1) components, including c-Jun [9], JunD, and activating transcription factor 2 (ATF2) [10]. Besides the AP-1 associated proteins, JNKs have been reported to phosphorylate Elk1 [10], c-Myc [11], p53 [12], MLK2 [13], NFATc2 [14], TIF-1A [15], 14-3-3 [16], the E3 ligase Itch [17], tau [18], histone H3 [19], histone H2AX [20], insulin receptor substrate 1 [21], JIP1 [22], and several members of the Bcl-2 family of apoptosis-related proteins [6,7]. More substrates are very likely yet to be discovered as protein technologies continue to improve.

In addition to their kinase activity, the JNK proteins also function to target their own various substrates, including c-Jun, ATF2 and p53, for degradation [10,23]. For instance, association of inactive JNKs with c-Jun or JunB marks these AP-1 complex proteins for ubiquitylation and degradation [10,24]. In a similar scenario, JNKs phosphorylation of ATF2 protects ATF2 from degradation, but ATF2's association with inactive JNKs targets ATF2 for degradation [10]. On the other hand, JNK phosphorylates Elk1, but does not protect it from degradation nor does inactive JNK1 associate with Elk1 or target it for ubiquitylation [10]. All three JNKs are known to regulate both the phosphorylation/activation and targeting of p53 for degradation [23]. Furthermore, under unstressed conditions, inactive JNK binds to p53 preferentially in the G0/G1 cell cycle phase and the interaction results in the ubiquitylation and degradation of p53 [23,25]. Conversely, under stress conditions, JNKs phosphorylate p53 (Thr81) resulting in p53 stabilization and activation [12]. Tafolla et al. [26] reported that p53 is differentially regulated by JNK1 and JNK2 in primary human fibroblasts. Inhibition of JNK2 resulted in a marked reduction of p53 protein levels, and cells deficient in both JNK1 and JNK2 did not express p53 [26]. In contrast, inhibition of JNK1 resulted in increased p53 protein levels, suggesting that JNK2 might be a positive regulator of p53, whereas JNK1 seems to negatively regulate p53 [26]. Therefore depending on the situation, JNKs can activate or deactivate critical signaling proteins resulting in a markedly different cellular response.

On the other hand, JNKs can themselves be differentially regulated by their interaction with JIPs [2] or other newly identified proteins. Notably, recent work has shown that the tumor suppressor p16^{INK4a} interacts with JNKs and inhibits UV-induced c-Jun phosphorylation by JNKs, but without affecting the phosphorylation of JNKs [27]. JNKs were also reported to phosphorylate histone H2AX (Ser139) and appears to be a major kinase in vivo to phosphorylate H2AX in response to UVA exposure [20]. Results of this study suggested that JNKs might also cooperate with activated caspases in the cellular apoptotic response by phosphorylating H2AX at a noncanonical site that is required for apoptotic DNA fragmentation [20]. The JNK proteins clearly interact with a multitude of various substrates, but the best characterized is probably c-Jun. As an important component of the AP-1 transcription factor complex, c-Jun plays a critical role in carcinogenesis, but whether it is regulated differentially by JNK1 and JNK2 is still not totally clear.

JNKs and c-Jun

Many studies have used c-Jun as the model JNK substrate to investigate JNK isoform functional specificity. As indicated earlier, JNKs have numerous and varied substrates and in many cases c-Jun has been presumed to be phosphorylated only by JNKs. Several reports indicate otherwise. For example, c-Jun has also been reported to be a substrate for cAbl [28], p34cdc2, extracellular signal-regulated kinases (ERKs), PKC [29], CKII [30], GSK-3 [31], DNA-PK [32], and CSK [33]. These data suggest that using c-Jun exclusively as an indication of JNKs activity may be somewhat misleading. However, in spite of this, c-Jun is clearly a major substrate for JNKs and its phosphorylation is closely tied to AP-1 activation. Even though all three MAP kinase cascades have been implicated in the activation of AP-1, JNKs may be most active for phosphorylating c-Jun in vivo. AP-1 activity has been reported to be directly increased by phosphorylation of c-Jun by JNKs, which increases stabilization and overall transcriptional activation.

AP-1 is a well-characterized transcription factor complex composed of homodimers and/or hetero-dimers of Jun and Fos proteins [34]. AP-1 regulates the transcription of various genes and is potently activated by a variety of stimuli, including tumor promoters. AP-1 activation can result in binding to the DNA of various genes involved in inflammation, proliferation and apoptosis [34], and in the skin, AP-1 is involved in carcinogenesis, the UV response, wound healing, photoaging, and keratinocyte differentiation (reviewed in Reference [35]). AP-1 plays an important role in preneoplastic-to-neoplastic transformation in cell and animal models and is involved in tumor promotion, progression and metastasis. Notably, blocking AP-1 activation inhibits neoplastic transformation [36]. However, AP-1 also has anti-cancer activities, which are likely related to the protein family composition of the AP-1 complex.

JNK1 and JNK2 appear to differ substantially in their ability to interact with c-Jun. Compared to JNK1, JNK2 seems to have a much greater affinity for binding with c-Jun. Affinity could account for the ability of JNK1 and JNK2 to specifically recognize different substrates. However, the suggestion has also been made that JNK1 may be more “efficient” in phosphorylating c-Jun [3]. In non-stimulated cells, JNK2 appears to function mainly to target c-Jun for ubiquitylation and degradation, whereas following stimulation, JNK1 phosphorylates, activates and stabilizes c-Jun leading to transcriptional activation [24]. In normal cells, c-Jun is essential for efficient transition of the G1-S phase of the cell cycle and cells lacking c-Jun have a marked proliferation defect [37]. Specifically, cells that are lacking *c-jun* appear to exhibit cell death and critical growth impairment. JNK2 knockout (JNK2^{-/-}) fibroblasts seem to grow slightly faster than or similar to wildtype (WT) cells, whereas JNK1 knockout (JNK1^{-/-}) fibroblasts appear to grow more slowly and less densely [38], suggesting that JNK1 rather than JNK2 may be more important for proliferation and also implicating JNK1 as a positive regulator of c-Jun. Tournier et al. [38] observed that compared to JNK2, disruption

of JNK1 resulted in much less overall JNKs activity. Sabapathy et al. [39] found that, compared to WT mouse fibroblasts or immortalized 3T3 fibroblasts, JNK1 deficiency caused a reduction in cumulative cell numbers, whereas JNK2 deficiency resulted in a larger number of cells. This group [39] further reported that in non-stimulated cells, JNK2 was closely associated with c-Jun, presumably contributing to c-Jun degradation. Following stimulation, c-Jun was preferentially associated with and phosphorylated by JNK1 resulting in c-Jun transcriptional activation. Furthermore, JNK2^{-/-} fibroblasts spent less time in G1 and entered S phase earlier than either WT or JNK1^{-/-} cells. JNK2^{-/-} fibroblasts also expressed high levels of cyclin D1 compared to either WT or JNK1^{-/-} cells, whereas JNK1^{-/-} cells expressed much lower levels compared to either WT or JNK2^{-/-}. Thus, these results suggested that JNK1 and JNK2 appear to have very distinct and opposite roles in proliferation. Similar results were observed in JNK1^{-/-} and JNK2^{-/-} erythroblasts stimulated by inducing anemia and in serum-starved and serum-re-stimulated 3T3 JNK1^{-/-} and JNK2^{-/-} fibroblasts [39]. In general, JNK2^{-/-} cells again exhibited increased proliferation, whereas JNK1^{-/-} showed little difference compared to WT. Furthermore, UV-induced c-Jun phosphorylation was reduced in JNK1^{-/-} cells, but increased in JNK2^{-/-} cells compared to WT cells [39]. Based on these results, Sabapathy et al. [39] concluded that JNK2 is a negative regulator of c-Jun, whereas JNK1 acts as a positive regulator of c-Jun, in vivo. Additional data [39] revealed that JNK1^{-/-} was associated with reduced AP-1 activity, but JNK2^{-/-} resulted in enhanced AP-1 activity, which appeared consistent with the phosphorylation profile observed for c-Jun.

All of these results have been questioned in recent work from the laboratory of Roger Davis [40]. These investigators [40] used a chemical genetic approach to examine the effect of specific and acute inhibition of JNK2 function. In these experiments, they used a JNK2 protein kinase mutant that was engineered to enlarge the ATP pocket. The mutant JNK2 appeared to maintain normal kinase activity toward c-Jun phosphorylation, but unlike WT cells, was sensitive to inactive derivatives of the general protein kinase inhibitor PP1 (1-naphthylmethyl-4-amino-1-*tert*-butyl-3-[*p*-methylphenyl]pyrazolo(3,4-*d*)pyrimidine [1NM-PP1]). In this report [40], WT murine embryonic fibroblasts (MEFs), MEFs expressing WT JNK1 and the mutant JNK2 (*J1*^{+/+}/*J2*^{MG/MG}), and MEFs without JNK1 but expressing the mutant JNK2 (*J1*^{-/-}/*J2*^{MG/MG}) were compared to delineate functional differences between JNK1 and JNK2. Results [40] indicated that pretreatment of *J1*^{-/-}/*J2*^{MG/MG} MEFs with 1NM-PP1 prior to UV caused a dose-dependent decrease in c-Jun phosphorylation (serine 63), but the drug had no effect on c-Jun or JNK phosphorylation in WT or, surprisingly, in *J1*^{+/+}/*J2*^{MG/MG} MEFs. This led to the conclusion that JNK2 caused phosphorylation of c-Jun, but that this function was mostly redundant with that of JNK1 [40]. Further studies with 1NM-PP1 and the three MEF lines revealed that JNK2 activity normally (1) increased the expression of c-Jun and JunD mRNA; (2) played a positive role in c-Jun expression; (3) increased cell proliferation; (4) regulated cell survival; and (5) contributed to in vitro wound repair and cell motility. However, all of these functions were concluded to be redundant with the respective functions of JNK1 [40]. The authors further contended that both JNK1 and JNK2 are positive regulators of c-Jun expression and proliferation and suggested that the opposite phenotypes reported for JNK1 and JNK2 are actually due to a compensatory increase of JNK1 in JNK2^{-/-} MEFs (or presumably, vice versa) compared to WT cells [40]. They [40] further showed that suppressing the activity of JNK1 prevented the increased c-Jun expression observed in JNK2^{-/-} MEFs. The major conclusion from these studies was that the phenotype of JNK2^{-/-} MEFs is misleading and that the increased expression of c-Jun and increased proliferation of JNK2^{-/-} MEFs is primarily due to compensatory increases in JNK1 function rather than negative regulation by JNK2. However, authors conceded that the expansion of the ATP pocket might confer a mutant phenotype in the absence of the inhibitor. Nonetheless, this study could definitely have an impact on the manner in which JNKs function might be viewed.

JNK Expression and Activity in Carcinogenesis

Each animal or cell model of JNK1 or JNK2 deficiency exhibits specific nonlethal phenotypes, suggesting that even though the JNKs may have common roles, they most likely also have unique roles in numerous cellular functions, such as survival, growth, development and apoptosis. Gupta et al. [3] were probably the first to recognize that individual JNK isoforms selectively targeted specific transcription factors in vivo. Mice deficient in *jnk1*, *jnk2*, or *jnk3* or combinations of *jnk1/jnk3* or *jnk2/jnk3* all survive normally, but notably, compound mutants lacking both *jnk1* and *jnk2* genes are embryonic lethal at about day 11 (E11) due to severe deregulation of apoptosis in the brain [41]. These results seem to support redundant roles for JNK1 and JNK2 in embryonic development. In addition, double mutant JNK1 and JNK2 MEFs were reported to exhibit no phosphorylation of c-Jun at serines 63 or 73, combined with a reduced c-Jun protein expression, low AP-1 transactivation and a profoundly impaired growth rate [42].

The recent work [40] described above suggested that long-term knockout of JNK1 or JNK2 might result in a compensatory change in the partner JNK MAP kinase and that normally both JNK1 and JNK2 are positive regulators of c-Jun with redundancy. One may now question the implication of these findings on whether JNKs might play distinctive roles as tumor suppressors or oncokinasases. However, several pieces of evidence suggest that JNK activity is chronically altered in various cancer types. Notably, many tumor cell lines have been reported to possess constitutively active JNKs, and JNK3 was found to be mutated in 10 out of 19 human brain tumors tested [43]. Although JNK1 or JNK2 mutations are not prevalent in cancer, the upstream MKK4 has been reported to harbor mutations in colorectal, prostate, breast, pancreatic and lung carcinomas [44]. MKK4 was also reported to be a metastasis suppressor and the loss of MKK4 activity was associated with decreased JNKs activation and tumor aggressiveness [45]. JNK activity has been reported to be elevated in some human cancers and loss of JNKs function can either inhibit or increase tumor growth in mice [46]. Wang et al. [47] investigated the expression and activity of MAP kinases in breast cancer and normal human breast tissue samples from 14 patients. Expression of p38 and ERK1/2 was substantially increased in cancerous tissue compared to control tissues obtained from the same patient. Overexpression of ERK1/2 has been observed by others in breast cancer tissue [48]. Notably, the expression of JNK1, but not JNK2, was increased markedly in breast cancer tissue compared to normal samples from all 14 patients. This finding supports the idea that JNK1 and JNK2 may be differentially regulated in cancer. Surprisingly, even though the expression was higher, the activity of JNKs toward phosphorylation of c-Jun was 30% lower in breast cancer tissue than that observed in corresponding normal tissue, which is in stark contrast to the markedly increased expression and corresponding activation of ERK1/2 observed in breast cancer [48]. However, further investigation revealed that the expression of the phosphoprotein phosphatases, MKP1 and MKP2, was significantly increased, which was suggested to account for the paradoxical observation of elevated JNK levels but reduced JNK activity [47]. These results continue to confirm the complexity of JNKs function and the interdependence of signaling pathways.

Several potential substrates of JNKs have also been reported to be up-regulated in mouse skin carcinogenesis [49]. However, even though elevated c-Jun and ATF2 expression corresponded with high expression levels of JNKs, whether the increased JNKs activity was directly responsible for the elevation is not clear. An increased expression of c-Jun has been found in bone marrow of acute myeloid leukemia patients [50] and JNKs activity was reported to be high in leukocytes isolated from patients with adult T-cell leukemia [51]. These studies all suggest that JNKs activity is deregulated in many cancer types and that chronic changes are not uncommon and are therefore relevant.

Tumor Suppressors or Oncokinases?

In cancer cells, one might expect an “oncokinase” to transduce signals leading to cell survival and proliferation, whereas a tumor suppressor kinase would mostly mediate signals leading to apoptosis of tumor cells. The JNKs pathway is well known to mediate both processes. For example, inhibition of JNKs activity has been shown to cause growth arrest or apoptosis in some tumor cells [52]. JNKs activation and c-Jun phosphorylation have been reported to be required for transformation induced by oncogenic *ras*, which is activated by mutation in about 30% of all human cancers. Fibroblasts containing mutant c-Jun proteins in which serines 63 and 73 were replaced with alanine exhibited an inability to cooperate with *ras* in promoting neoplastic transformation [53] and also decreased oncogene-induced tumor growth in nude mice [54]. Inactivation of the p16^{INK4a} tumor suppressor protein is a critical event in the development of human cancers, especially human melanoma skin cancer. The p16^{INK4a} protein was reported to suppress the activity of JNKs. Protein docking analysis predicted that p16^{INK4a} binds to the glycine-rich loop of the N-terminal domain of JNK1 or JNK3, but not JNK2 [27]. Although p16^{INK4a} did not affect the phosphorylation of JNKs, its interaction with JNKs inhibited c-Jun phosphorylation induced by UV exposure. This in turn interfered with cell transformation promoted by the H-Ras-JNK-c-Jun-AP-1 signaling axis [27]. In addition, Nateri et al. [55] reported that phosphorylation of c-Jun, presumably by JNKs, was required for intestinal cancer development in Apc^{Min/+} mice. These studies seem to suggest that JNKs might have an oncogenic role. On the other hand, double knockout of JNK (*Jnk1*^{-/-}/*Jnk2*^{-/-}) in fibroblasts was reported to cause marked increases in the number and growth of Ras-induced tumor nodules in vivo [5]. This study suggested that a tumor suppressor function of JNKs might be more prevalent than an oncogenic function.

The JNKs pathway can be activated by epidermal growth factor (EGF) in HeLa cells. Inhibition of JNK2, but not JNK1, protein expression in A549 human lung carcinoma cells was shown to inhibit EGF-induced doubling of growth and colony formation in soft agar [56] and suppressing JNK2 activity in human prostate carcinoma cells also inhibited their growth [57]. Tsui et al. [58] constructed GST fusion proteins for all 10 JNK isoforms and transiently transfected each into human glioblastoma U87MG cells to examine kinase activity with or without the activating upstream kinase. They [58] found that the JNK1 isoforms localized predominantly in the cytoplasm, whereas the JNK2 isoforms localized to the nucleus and were constitutively phosphorylated. JNK2 was further confirmed to be the main active JNK present in tumors, indicating that these constitutively active JNK2 isoforms appear to play a significant role in glial tumors [58]—data that suggests a role for JNK2 as a potential oncokinase. Du et al. [59] used antisense (AS)-JNK1 or AS-JNK2 to delineate a role for JNK1 and JNK2 in KB-3 human oral carcinoma cell proliferation. In this study [59], AS-JNK2, but not AS-JNK1, was reported to significantly inhibit growth of KB-3 human oral carcinoma cells, which further supports a role for JNK2 as an oncokinase. The use of small interfering RNA (siRNA) has replaced antisense as the state of the art technology for suppressing gene expression. MacCorkle and Tan [60] used siRNA to specifically block expression of JNK1 or JNK2 in human cervical carcinoma (HeLa) cells and human small lung carcinoma (Calu-1) cells. Their findings suggested that down-regulation of JNK2, but not JNK1, resulted in a twofold accumulation of viable cells with 4N DNA content, indicating that JNK2 is required for proper mitotic progression in these cells. Based on a variety of experiments examining the role of JNK in cell cycle, these investigators suggested that inhibition of JNK2 would be expected to reduce tumor growth, whereas blocking JNK1 would be predicted to be associated with an increased risk of tumorigenesis [60]. These results seem to have been confirmed using in vivo mouse models [46,61]. Tseng et al. [62] investigated the role of JNK1 and caspase 3 in tamoxifen-induced apoptosis of rat glioma cells. Tamoxifen was found to exert cytotoxic effects and induce apoptosis of glioma cells. These results corresponded with increased phosphorylation of JNK1 and increased caspase 3 activity. Notably, all of the tamoxifen-induced effects were blocked

by inhibition of JNK1 [62]. This suggests that JNK1 may play a key role in facilitating tamoxifen-induced apoptosis in these tumor cells, which would appear to support a role for JNK1 as a tumor suppressor. On the other hand, transformation of human small cell lung cells by *H-ras* required JNK1 [63], which suggests a role for JNK1 as a potential oncogene.

These cumulative data seem to support the idea that JNK1 and JNK2 can specifically function as either a tumor suppressor and as an “oncokinasase” and the distinct function might be cancer cell type specific. However, whether a protein can act as an “oncokinasase” or tumor suppressor might be more fully confirmed with more confidence in knockout mouse models. For example, increased or decreased cancer incidence in mice with targeted deletion of JNK1 or JNK2, respectively, could provide strong support of the ability of these proteins to act as a tumor suppressor or an “oncokinasase.”

In Vivo Animal Studies

A limited number of in vivo animal studies to differentiate the specific role of JNK1 and JNK2 have been performed. Hess et al. [4] reported that disruption of JNK1 in mice inhibited transformation of pre-B cells by Bcr-Abl. In addition, JNK1 was required for anchorage independent survival of transformed cells suggesting that in this model, JNK1 is a positive regulator of transformation, that is, an “oncokinasase.”

In contrast, we used the 12-dimethylbenz[A]anthracene (DMBA)/12-*O*-tetradecanoylphorbol-13-acetate (TPA) two-stage mouse skin carcinogenesis model to ascertain the role of JNK1 and JNK2 in carcinogenesis. In a comparison of JNK1-deficient (JNK1^{-/-}) and JNK2-deficient (JNK2^{-/-}) mice, the multiplicity of papillomas induced by the phorbol ester, TPA, was substantially lower in JNK2^{-/-} mice than that observed in WT mice [46]. In direct contrast, JNK1^{-/-} mice were more susceptible to TPA-induced skin tumor development than WT mice [61]. Papillomas on WT mice grew rapidly and were well vascularized compared with JNK2^{-/-} mice. On the other hand, the rate of tumor development in JNK1^{-/-} mice was significantly more rapid than that observed in WT or JNK2^{-/-} mice. Furthermore, JNK1^{-/-} mice developed more and larger tumors compared to WT but JNK2^{-/-} mice developed markedly fewer and smaller tumors compared to WT or JNK1^{-/-} mice. Analysis of skin samples revealed that TPA stimulated phosphorylation of ERKs and Akt and also increased AP-1 DNA binding in WT and JNK1^{-/-} mice [61], a result that was not found in JNK2^{-/-} mice [46]. Notably, these findings are supported by others who observed that JNK2 is constitutively activated in primary glial tumors [58] and JNK1 is inactivated in human breast cancer tumor samples [47]. In spite of this, results should be interpreted cautiously. Detection of a protein in a cancer cell does not unambiguously prove that it is involved in causing the cancer or is required for its persistence. On the other hand, the results from knockout mouse models strongly suggest that JNK1 and JNK2 might exhibit opposing roles in vivo.

In further support of this idea, Weston et al. [64] closely examined skin from JNK1^{-/-} and JNK2^{-/-} mice and found two very different phenotypes. In particular, JNK1^{-/-} had a very thin epidermis with fewer cell layers compared to WT mice. In addition, characteristic markers of epidermal differentiation were not found in JNK1^{-/-} mouse skin and the number of proliferating cells in the stratum basale was also substantially lower [64]. Skin from JNK2^{-/-} mice exhibited keratinocyte hyperplasia associated with an increased number of cell layers (i.e., thicker skin), p63 positive keratinocyte stem cells and an up-regulation of several differentiation markers. These data suggest that JNK1 normally might act as a positive regulator of keratinocyte proliferation in mouse skin, whereas JNK2 exhibits a negative or down-regulatory function. The investigators [64] suggested that the difference in epidermal structure between JNK1^{-/-} and JNK2^{-/-} mice may contribute to their opposing susceptibility to skin cancer [46,61], although relevance to human skin cancer is yet to be elucidated.

Gene Expression in JNK1^{-/-} and JNK2^{-/-} Cells

In primary embryo cells isolated from WT, JNK1^{-/-}, and JNK2^{-/-} mice, the patterns of gene expression was found to be very different [65]. After TPA treatment, the changes in the gene expression profiles in these three different kinds of cells appear to agree with the differences in susceptibility to tumorigenesis of each respective animal model. The results supported the idea that JNK1 and JNK2 proteins have distinct roles in modulating carcinogenesis [65]. WT cells exhibited high expression of genes normally associated with inhibition of cell growth and induction of differentiation or apoptosis. TPA treatment repressed expression of these genes possibly suggesting an increased susceptibility to tumor development. In JNK1^{-/-} cells treated with TPA, genes associated with apoptosis suppression were up-regulated, whereas in TPA-treated WT cells, these genes were not greatly affected. In contrast, JNK2^{-/-} cells treated with TPA expressed high levels of genes associated with tumor suppression and induction of cell differentiation, apoptosis, or cell growth arrest [65].

Potapova et al. [66] analyzed global gene expression changes in AS-JNK1 and AS-JNK2 human prostate carcinoma PC3 cells using SAGE. Overall, AS-JNK2 cells showed the most pronounced effects. AS-JNK1 or AS-JNK2 treated PC3 cells significantly decreased viability within 24 h and inhibition of growth was about twofold greater with AS-JNK2 compared with AS-JNK1. DNA synthesis was not affected. Both cell types showed accumulation of cells in S phase with AS-JNK2 being higher and exhibiting indications of apoptosis. AS-JNK2 expression corresponded to increased expression of transcription factors, stress-induced genes, and apoptosis-related genes whereas genes involved in DNA repair, mRNA turnover, and drug resistance were down-regulated by inhibition of JNK2 expression [66]. Overall these two genetic studies are consistent with one another and lend further support to the idea that JNK1 and JNK2 could have distinct albeit opposing roles in carcinogenesis.

CONCLUSIONS AND SUMMARY

Over the years, we have learned a great deal regarding the role of JNKs in normal physiology and pathologic conditions. Because of its relative tissue specificity, JNK3 is believed to have functions distinct from those of JNK1 or JNK2. On the other hand, because of their concurrent and ubiquitous expression, JNK1 and JNK2 have often been considered as having mostly overlapping or at least redundant functions. In spite of this assessment, most research evidence still suggests that the functions of JNKs need to be addressed in a way that clearly differentiates between the contributions of JNK1 and JNK2. Commonly used JNKs inhibitors, such as SP600125, do not differentially affect JNK1 or JNK2. On the other hand, dominant-negative mutants, siRNA and knockout mouse and cell models provide strong confirmation of distinct roles for the two isoforms but still have their limitations. This has been clearly pointed out by recent work using a chemical genetic approach. Specific and different small molecule inhibitors need to be developed for precise and effective treatment of various cancers. Some evidence seems to suggest that JNKs might be potential targets for the development of small inhibitory molecules for treating certain tumors. Distinctly different JNKs functions might occur through the specific substrate choice or might be related to temporal aspects. At present all known JNKs substrates are phosphorylated by all three JNK kinases but to different degrees, which may be a form of specificity. In spite of this, the different functions of JNKs are probably directly linked to substrate specificity based on stimulus, cell context, and temporal stimulation. However, specific substrates for JNK1, JNK2, or JNK3 are yet to be identified, but very likely exist. JNKs function may also differ by cancer type. For example, JNK1 may act as a tumor suppressor in skin cancer whereas JNK2 may function as a suppressor in other cancers, such as lymphomas. In addition, different systems may require different kinases for functional specificity. Based on in vitro and in vivo animal data, unambiguous targeting of JNK1 and JNK2 could produce more exact and effective therapies in addition to reducing undesirable or

toxic side effects that result from suppressing a cellular pathway unrelated to cancer progression.

Abbreviations

ATF2	activating transcription factor 2
AP-1	activator protein-1
BAD	Bcl-2-antagonist of cell death
Bcl-2	B-cell lymphoma 2
Bcr-Abl	breakpoint cluster region-Abelson
CKII	casein kinase II
CSK	COOH-terminal Src kinase
DNA-PK	DNA-dependent protein kinase
ERK	extracellular signal-regulated kinase
GSK-3	glycogen synthase kinase-3
GST	glutathione S-transferase
JIPs	JNK-interacting proteins
JNKs	c-Jun N-terminal kinases
MKK	mitogen-activated protein kinase kinase
MKP	MAP kinase phosphatases
AP-1	activator protein-1
ATF2	activating transcription factor 2
ERK	extracellular signal-regulated kinase.

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