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The 2 Faces of JNK Signaling in Cancer

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Abstract

c-Jun NH_2-terminal kinase (JNK) was discovered almost 20 years ago as the protein kinase responsible for phosphorylating c-Jun at Ser-63 and Ser-73. These sites had previously been demonstrated to be essential for the stimulation of c-Jun activity and for cooperation with Ha-*ras* in oncogenic transformation. This led to the idea that JNK was a positive regulator of cellular transformation. However, the analysis of *jnk* gene deletion in various mouse models of cancer has produced conflicting findings, with some studies supporting the pro-oncogenic function of JNK and others providing evidence that JNK acts as a tumor suppressor. This review will discuss how these unexpected findings have increased our understanding of the role of JNK signaling in cancer and have provided a source of new working hypotheses.

Keywords: MAPK, JNK, MKK, c-Jun, cancer, Ras

Introduction

The proto-oncogene *c-jun* is a component of the activating protein 1 (AP-1) transcription factor family capable of the malignant transformation of primary rat embryonic fibroblasts (REFs) in cooperation with activated Ha-Ras.[1] Site-directed mutagenesis established that this process required increased c-Jun activity upon phosphorylation at Ser-63 and Ser-73.[2-4] The same changes in c-Jun phosphorylation were elicited by a variety of other transforming oncoproteins including v-Src and c-Raf, supporting the idea that the stimulation of AP-1 activity accounted for the cooperation between c-Jun and oncogenes in the neoplastic process.[5] Consequently, the identification of c-Jun NH_2-terminal kinase (JNK) as the mitogen-activated protein kinase (MAPK) responsible for phosphorylating c-Jun at Ser-63 and Ser-73 in cells exposed to ultraviolet C radiation or expressing oncogenic Ha-*ras* was a major breakthrough in our molecular understanding of cellular transformation.[6,7] By increasing c-Jun activity, it was possible that JNK positively controlled cell proliferation. However, this hypothesis could not apply to chicken embryonic fibroblasts (CEFs) in which the transforming activity of c-Jun inversely correlated with its transcriptional activity.[8,9] Based on this finding, Håvarstein and colleagues[9] proposed that the oncogenic activity of v-Jun could result from its failure to activate the transcription of growth-attenuating genes that require high c-Jun activity. The JNK binding site encompasses the δ region of c-Jun, which is deleted in v-Jun.[6,10] Consequently, v-Jun is defective in JNK binding and is a poor JNK substrate.[6] Therefore, by increasing c-Jun activity, JNK was more likely to negatively control the proliferation of CEFs. Evidence that *c-jun* was a much more potent oncogene than *v-jun* in REFs in which the inverse correlation between the transcriptional activity of c-Jun and its transforming activity did not apply[11] provided some explanation for the predicted opposite role of JNK in cellular transformation. This review will highlight key experiments that have since contributed to a more complete understanding of the function of JNK in oncogenic signaling.

JNK Signaling Cascade

Since its discovery, a large amount of effort has been spent to delineate the molecular complexity of the JNK signaling pathway. JNK is mainly activated in response to stress and proinflammatory cytokines, including tumor necrosis factor α (TNF-α) and interleukin 1 (IL-1). Three genes, *jnk1, jnk2*, and *jnk3*, encoding 10 JNK isoforms, have been identified.[12] Whereas JNK1 and JNK2 expression is ubiquitous, JNK3 is predominantly expressed in the brain, testis, and heart. Like other MAPKs, increased JNK activity requires its dual phosphorylation at Tyr and Thr residues by a MAPK kinase, namely MKK4 and MKK7, which consist of a group of protein kinases with different biochemical properties.[13,14] For example, whereas MKK4 can also activate p38 MAPK, MKK7 functions as a specific activator of JNK. Furthermore, MKK4 and MKK7 are selectively regulated by extracellular stimuli and have a distinct affinity for JNK, with MKK4 and MKK7 preferentially phosphorylating JNK on Tyr and Thr residues, respectively.[15] Similar to the early embryonic death caused by the targeted deletion of both *jnk1* and *jnk2* genes,[16] mice lacking *mkk4* or *mkk7* die before birth, indicating that MKK4 and MKK7 function in a nonredundant manner *in vivo*.[15] Active JNK regulates by phosphorylation the activity of c-Jun and activating transcription factor 2 (ATF-2), 2 transcription factors of the AP-1

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family.[17] Transcriptional regulation of genes via AP-1 activity is one mechanism by which the JNK signaling pathway regulates cellular functions.

JNK Signaling and Regulation of Cell Death

Analyses of loss-of-function mutations in mice genetically established the requirement of JNK for promoting neuronal apoptosis during brain development[16] and in response to excitotoxic stress,[18] in part by up-regulating the expression of proapoptotic genes.[19] Apoptosis is a well-characterized biological process by which cells undergo a programmed death that is distinct from death by necrosis. Similar to JNK, MKK4 and MKK7 have both been implicated in mediating the apoptotic response of neurons to stress *in vitro*.[15] In addition, neuronal JNK can suppress autophagy by blocking FOXO1-mediated transcriptional activation of Bnip3, thereby preventing the release of the autophagic effector Beclin-1 from Bcl-x_L complexes.[20] Autophagy is a highly conserved physiological process of self-digestion implicated in maintaining energy homeostasis to promote cell survival during starvation.[21] Therefore, it is possible that increased autophagy associated with the loss of JNK contributes to extending the life span of neurons in the developing brain.

JNK is also an essential component of stress-induced apoptosis in mouse embryonic fibroblasts (MEFs).[22] However, unlike in neurons, JNK-induced mitochondrial release of cytochrome c in MEFs is independent of new gene expression. Furthermore, JNK acts as a positive regulator of autophagy in nonneuronal cells via the phosphorylation of Bcl-2 and the subsequent release of Beclin-1 following starvation in HBSS medium.[20,23] Interestingly, phosphorylation of Bcl-2 had previously been shown to inhibit its ability to bind proapoptotic members of the Bcl-2 family, thereby promoting apoptosis.[24] Careful kinetics analyses indicated that Bcl-2 dissociation from Beclin-1 occurs prior to its dissociation from Bax in starved cells.[25] Assuming that Bcl-2 associated with Beclin-1 is more readily phosphorylated by JNK than when it is in complex with Bax, these studies provide a mechanism by which JNK switches from a proautophagic/prosurvival to a proapoptotic/prodeath function in nonneuronal cells depending on the intensity and kinetics of the signal. Accordingly, transient JNK activation promotes cell survival, while prolonged JNK activation induces cellular apoptosis.[26] Furthermore, JNK prevents early senescence by negatively regulating the p53 tumor suppressor via c-Jun and JunD.[27] The premature senescence caused by JNK deficiency is consistent with the reduced proliferation of *Jnk*-null MEFs.[22] Likewise, late-passage primary *mkk7*$^{-/-}$ MEFs displayed premature senescence and defective proliferation caused by the reduced expression of the G2/M cell cycle kinase CDC2.[28]

JNK Signaling and Cellular Transformation

Apoptosis, autophagy, and senescence have all been functionally linked to Ras signaling. Together with the demonstration that the stimulation of c-Jun activity was essential for cooperation with Ha*ras* in the oncogenic transformation of normal mammalian cells, JNK was predicted to be an important component of signal transduction in *ras*-mediated oncogenesis. This idea was further supported by direct genetic evidence that the expression of a c-Jun mutant protein containing alanine substitutions at residues 63 and 73 (c-JunAA) could not rescue the ability of *ras*-expressing *c-jun*–null fibroblasts to form tumors in nude mice.[29] Consistently, tumor formation caused by the constitutive activation of Ras following overexpression of a dominant form of Son of Sevenless (SOS) was delayed in mice harboring the mutated *c-junAA* allele.[30] However, genetic analyses of JNK deficiency led to conflicting findings, with results indicating that JNK was required for mediating Ras-induced transformation[31,32] and others showing that Ras-transformed *jnk*-null MEFs displayed an enhanced tumorigenic potential compared to wild-type cells.[33] In this model of lung tumor metastasis, JNK might have restricted the tumor burden by promoting Ras-induced apoptosis. The tumor-suppressive function of JNK was consistent with previous evidence that loss-of-function mutations in the *mkk4* gene correlated with aggressive tumor development and metastasis in human cancer.[34] Overall, these studies suggested that analysis of JNK deficiency in mouse models of human cancer was essential to clarify the complex link between JNK, tumor growth, and malignancy and determine the extent to which JNK contributed to mediating oncogenic transformation.

Genetic Analysis of JNK Signaling in Cancer

One of the oldest and probably best analyzed mouse models of cancer is the classic 2-stage chemical carcinogenesis protocol that gives rise to benign papillomas with a high incidence of *ras* mutation.[35] In this model, a strong inflammatory reaction that is induced following the repeated treatment of the mouse skin with 12-O-tetradecanoylphorbol-13-acetate (TPA) is required to support the hyperproliferation of keratinocytes harboring the Ha*ras* mutation caused by a single application of the genotoxic carcinogen 7,12-dimethylbenzanthracene (DMBA) to the skin. Interestingly, *jnk1*-null mice displayed an increased susceptibility to skin papillomas induced by DMBA/TPA.[36] Evidence that *jnk1*$^{+/-}$*jnk2*$^{-/-}$ mice or mice with a specific loss of *mkk4* or *mkk7* gene expression in the bronchial epithelium developed $Kras^{G12D}$-induced lung tumors earlier than their control littermates provided further support for a tumor-suppressive function of JNK downstream of oncogenic Ras.[37,38]

Similarly, the loss of JNK1 or JNK2 enhanced mammary tumor development in mouse models of breast cancer driven by the decreased expression of p53 or ectopic expression of the polyoma middle T antigen transgene.[39-41] Consistently, conditional inactivation of

the *mkk7* gene in mammary epithelial cells increased tumor formation induced by the NeuT oncogene.[37] Likewise, the absence of JNK1 in the prostate epithelium of *jnk2*$^{-/-}$ mice accelerated the development of large prostate tumors in the conditional *tumor suppressor phosphatase and tensin homolog* (*Pten*) gene deletion mouse model.[42] Interestingly, the Δ*Jnk*Δ*Pten* tumors were unresponsive to androgen withdrawal and highly metastatic. Invasive adenocarcinoma was also detected following the functional inactivation of the *mkk4* and *mkk7* genes in the Δ*Pten* mouse model.[42] Increased p53 protein stability upon phosphorylation by JNK may be one important mechanism by which JNK signaling contributes to mediating oncogene-induced senescence and cell cycle arrest as a means to maintain genomic stability and to suppress tumorigenesis.[37,41]

However, unlike *jnk1*$^{-/-}$ mice, *jnk2*$^{-/-}$ mice and mice harboring a specific deletion of *mkk4* in keratinocytes were resistant to the 2-stage chemical carcinogenesis protocol.[43,44] Similarly, mice lacking JNK1 exhibited a marked decrease in gastric carcinogenesis induced by N-methyl-N-nitrosourea compared to their wild-type counterparts.[45] Likewise, *jnk1*$^{-/-}$ mice or *jnk2*$^{-/-}$ mice with a compound deficiency of JNK1 in the liver were less susceptible than wild-type animals to hepatocellular carcinoma (HCC) following the diethylnitrosamine-phenobarbital (DEN) protocol.[46-48] Reduced liver cancer development associated with the genetic inactivation of *jnk1* could be a consequence of decreased tumor cell proliferation caused by the down-regulation of c-Myc and cyclin D1 expression.[47,48] In addition, JNK-deficient mice treated by DEN displayed a lower level of hepatic expression of protumorigenic cytokines compared to wild-type animals.[48] In particular, IL-6–stimulated STAT3 signaling in the liver lacking JNK1 was impaired, as demonstrated by a specific defect in DEN-induced increased *Socs3* and *miR-21* gene expression.[48] Together with evidence that human HCC displayed a high level of active JNK and that inhibiting JNK activity using the D-JNKI1 peptide reduced DEN-induced HCC in mice,[47] these studies strongly supported the idea of using JNK1 as a target for liver cancer therapy. However, in contrast to expectations, JNK acted to suppress hepatocyte death in the DEN model.[48] As a result, the liver of DEN-treated *jnk2*$^{-/-}$ mice with hepatocyte-specific compound JNK1 deficiency displayed increased compensatory cell proliferation and increased expression of inflammatory cytokines, leading to the development of HCC.[48] This indicated that JNK could both inhibit and promote tumor formation in the DEN model of HCC.

Conclusion

The physiological and pathological functions of JNK signaling have been very difficult to predict because of the seemingly contradictory role of JNK in promoting cell survival and proliferation on one hand and cell death on the other. Accordingly, studies in mice have demonstrated that the contribution of JNK is cell type and isoform specific. For example, Purkinje cells are refractory to the proautophagy JNK1 signaling pathway identified in nonneuronal cells.[20,23] The ability of JNK1 to suppress the expression of antiapoptotic genes, while JNK2 negatively regulates the activity of genes related to tumor suppression and the induction of cell differentiation, apoptosis, or cell growth, may reflect the distinct function of JNK isoforms in the skin.[49] These findings have exemplified the limitation of studies using mice with whole body knockout and supported the development of more specific transgenic lines that enable the temporal loss of JNK signaling in specific tissues. However, in spite of these latest efforts, there is still no consensus on whether components of the JNK signaling pathway are suitable drug targets for cancer therapy. The unexpected contribution of JNK in both tumor promotion and inhibition may reflect our little understanding of the role of JNK in the tumor microenvironment. This idea rests on evidence that, in addition to controlling cell-autonomous functions, JNK can drive the expression of cytokines that can act in a paracrine manner to sustain the proliferation of cancer cells.[48] Therefore, the paradoxical role of JNK in cancer may be unraveled in future studies aimed at elucidating the impact of JNK signaling in inflammation that operates downstream of oncogenic mutations. Furthermore, the sequencing analyses of cancer genomes have revealed a clustering of mutations in multiple genes of the JNK pathway (*jnk1, jnk2, mkk4*, and *mkk7*) in various human cancers.[50,51] Consequently, the phenotypic analysis of novel transgenic mouse lines harboring similar mutations may also be essential to draw a general conclusion regarding JNK and oncogenesis. In parallel, potent and selective covalent inhibitors of JNK will provide a fantastic opportunity to directly test the therapeutic implication of blocking JNK signaling in animal models of cancer directly relevant to human disease.[52]

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