

Review Article

The role of the c-Jun N-terminal Kinase signaling pathway in skin cancer

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Abstract: The c-Jun N-terminal Kinases (JNK), along with Erk and p38, constitute the principle members of the mitogen-activated protein kinase (MAPK) family. JNK functions primarily through AP1 family transcription factors to regulate a plethora of cellular processes, including cell proliferation, differentiation, survival and migration. It also cross-talks and integrates with other signaling pathways in a cell context-specific and cell type-specific manner. The current views of JNK function in various skin cancers and the need of developing JNK subunit-specific inhibitors for cancer type-specific applications have been summarized in this review.

Keywords: JNK, skin cancer, squamous cell carcinoma, basal cell carcinoma, cylindroma

Introduction

The c-Jun N-terminal Kinases (JNK), along with Erk and p38, constitute the principle members of the mitogen-activated protein kinase (MAPK) family [1, 2]. JNK functions primarily through AP1 family transcription factors to regulate a plethora of cellular processes, including cell proliferation, differentiation, survival and migration. It also cross-talks and integrates with other signaling pathways in a cell context-specific and cell type-specific manner [2]. This review is focused on addressing the current views of JNK function in various skin cancers and the need of developing JNK subunit-specific inhibitors for cancer type-specific applications.

The cost of skin cancer

Skin represents the largest and outermost organ of the human body. It is constantly challenged by a myriad of environmental insults. As a result, skin cancers are estimated to exceed 1 million new cases per year in the US, roughly 10-20 times more prevalent than any other cancers combined [3]. Among the most common types of skin cancers are basal cell carcinoma (BCC), squamous cell carcinoma (SCC)

and melanoma accounting for about 80%, 15% and 5%, respectively. BCC is mostly a local neoplastic process that rarely invades to other parts of the body. In contrast, SCC and melanoma can be invasive and are responsible for an annual death of 2,000 and 8,700, respectively (<http://www.cancer.org>, 2010; <http://www.cancer.gov/cancertopics>, 2010) [3, 4]. There are other less common epithelial tumors arising from cutaneous adnexal structures that are part of syndromes such as the case of familial cylindromatosis, Brooke-Speigler syndrome and multiple familial trichoepithelioma. Although benign in most cases, these tumors are often disfiguring and can become metastatic over time [5, 12]. The average treatment cost of skin cancer for each patient is significantly (11-19 times) lower than other cancers. However, due to the high incidence, skin cancer represents the 5th most costly cancer immediately following lung/bronchus, prostate, colon/rectum, and breast cancers [13]. To date, surgery is the most effective treatment option for BCC, early stage SCC and melanoma. Chemotherapeutics targeting the SHH-pathway and COX inhibitors have produced promising results for BCC [14, 15]. In contrast, treatment

options for late stage SCC and melanoma are limited and the outcome is far from satisfactory. Thus, novel target treatment strategies are clearly needed in order to reduce the financial burden of the health system and improve clinical outcome.

The c-Jun N-terminal Kinase (JNK) signaling pathway in human disease

JNK proteins, also known as SAPKs (stress activated protein kinases), are first discovered in early 1990s [16, 17]. They are encoded by three different genes, *jnk1* (MAPK8), *jnk2* (MAPK9) and *jnk3* (MAPK10), the encoded mRNAs undergo differential splicing giving rise to 10 different isoforms [18]. To date, nearly 22,500 papers have been published to directly or indirectly address the role of JNK in tissue homeostasis, cellular metabolism, inflammation and carcinogenesis. Studies using animal models have established the essential roles for JNK proteins in a number of pathological conditions, including but not limited to neurodegenerative disorders, diabetes, arthritis, atherosclerosis and skin cancer [19-23]. In parallel, studies with human tissues have demonstrated the relevance of JNK activation to not only the above mentioned diseases but also to human cancers, including glioma, prostate carcinoma, osteosarcoma and squamous cell carcinoma (SCC) [24-28]. Accordingly, a number of great review papers have been published to describe the role of JNK in cell death and survival and tissue pathogenesis, eluding that the JNK signaling pathway is a goldmine for pharmacological targeting [19, 29, 30]. This paper intends to provide a focused review of JNK in skin cancer and discusses the possibilities of therapeutic targeting of this pathway.

The upstream and downstream targets of JNK

JNK is highly responsive to a variety of extracellular stimuli, including inflammatory cytokines and UV irradiation [31]. Signals transmitted from membrane receptors travel through TRAF2/6 protein complexes to activate mitogen-activated protein kinase (MAPK) kinases [32, 33], including MKK4 and MKK7. MKK4/7 then acts synergistically to activate JNK via dual phosphorylation of the ThrProTyr (TPY) motif [34, 35]. MKK7 is an essential component of the JNK signal transduction pathway activated by proinflammatory cytokines and

demonstrates great specificity to JNK, whereas MKK4 activates both JNK and p38 [36]. MKK4 and MKK7 are both involved in embryonic development, as disrupting either leads to early embryonic lethality [37, 38]. Genetic deletion of either *Jnk1* or *Jnk2* alone or together with *Jnk3* produces viable mice, while compound deletion of *Jnk1* and *Jnk2* leads to early embryonic lethality, a stage too early for epidermal phenotypic assessment [39, 40].

The major downstream targets of the JNK cascade are members of the activator protein 1 family (AP-1) transcription factors, including Jun and Fos family members that function as hetero- or homo-dimers to regulate gene transcription [41-43]. c-Jun and c-Fos are the mammalian counterparts of v-Jun and v-fos retroviral oncogenes, respectively, and are recognized as proto-oncogenes in various mammalian cancers. In particular, c-Jun as a predominant JNK-target is responsible for induction of a plethora of target genes that are involved in regulating cell cycle progression, migration and survival. In addition, JNK mediates phosphorylation and subsequent downregulation of p53 tumor suppressor [44, 45], and therefore suppresses p53-mediated cell senescence [46].

JNK function in animal and human models of SCC

JNK function has been explored in both animal and human tissue models of SCC. In animal studies, skin tumors are often induced by either ultraviolet radiation (UV) or a two-stage chemical tumorigenesis protocol with one dose of topical 7,12-dimethylbenzanthracene (DMBA) followed by biweekly 12-O-tetradecanoylphorbol-13-acetate (TPA). *Jnk1*^{-/-} mice were highly susceptible to DMBA/TPA-induced skin carcinogenesis as indicated by the increased rates of tumor growth kinetic and progression into carcinomas as compared to the WT counterparts [47]. The enhanced tumor growth phenotype in *Jnk1*^{-/-} mice was attributed to the increased level of TPA-induced AP-1 DNA binding activity and phosphorylation of extracellular signal-regulated kinases and Akt. In agreement with these data, suppression of JNK1 by Serpin SCC antigen (SCCA1) prevents UV-induced epidermal cell death and consequently promotes tumorigenesis [48]. In contrast, JNK2-deficient (*Jnk2*^{-/-}) mice were resistant to tumor induction as indicated by the reduced number of papillo-

ma developed following DMBA/TPA chemical challenge as compared to the WT mice [23]. Furthermore, the *Jnk2*^{-/-} papillomas were not prone to progression into carcinoma and expressed significantly reduced levels of AP-1 and Erk activity. This constellation of findings indicate that JNK1 and JNK2 have opposite effects on skin carcinogenesis with JNK2 being a tumor promoter.

The oncogenic effects of JNK2 were also observed in the regenerated human SCC model, in which primary human keratinocytes were subject to multiplex gene transduction for expression of genes under investigation and then used for skin regeneration on immunodeficient mice [49, 50]. By using this model, we have demonstrated that JNK2 and c-Jun are essential for the invasive human epidermal neoplasia triggered by NF- κ B blockade and oncogenic Ras [27, 51]. Moreover, expression of constitutively active mutants of either MKK7, JNK2 (MKK7-JNK2 fusion) or c-Jun is sufficient to couple with oncogenic Ras to drive normal human epidermal cells into malignancy [27, 28, 52]. In contrast, expression of active MKK7-JNK1 fusion protein is not sufficient in promoting Ras-driven human epidermal malignancy. Consistent with the findings obtained with animal models, JNK2 and c-Jun but not JNK1 are highly activated in human SCC, confirming that these molecules are clinically relevant [28, 52]. Of further interest, JNK2 but not JNK1 potentiates Ras-induction of glycolysis [28], an energy producing process commonly utilized by cancer cells and also known as the Warburg effect [53]. On the other hand, JNK2 blocks Ras-induced NF- κ B activation [28], an activity previously reported to induce human epidermal cell senescence and growth arrest [49]. Thus, coactivation of JNK2 and Ras produces an optimal molecular and metabolic environment required for tumorigenesis.

JNK downstream target AP1 proteins in SCC

Direct AP1 inhibition by expression of the dominant-negative mutant of c-Jun (DNc-Jun, also known as TAM67) inhibits tumorigenesis of murine SCC cell lines both *in vitro* and *in vivo* [54]. Consistently, epidermal deletion of *c-Jun* or *K14*-driven expression of TAM67 suppresses murine skin carcinogenesis induced by chemicals, UV radiation or papilloma viral oncogene [42, 43, 55-58]. In addition, mice defi-

cient in *c-fos* are resistant to malignant progression of skin tumors induced by Ras [59, 60]. These findings underscore an important role for AP1 in skin tumorigenesis. However, AP1 function is rather complex such that different AP1 subunits are differentially involved in various cellular processes. For example, overexpression of JunB enhances the malignant phenotype of transformed rat keratinocytes *in vitro* [61], suggesting that JunB might be pro-tumor growth. On the other hand, JunB is responsible for the resistance of the JB6(P-) SCC cells to tumor promotion, as well as the suppression of cell proliferation and epithelial-to-mesenchymal transition (EMT) of multiple SCC cell lines [62, 63], indicating that JunB suppresses tumorigenesis. In agreement with these latter findings, our recent studies have shown that the nuclear level of JunB is reduced in spontaneous human SCC, and that exogenous expression of JunB inhibits epidermal neoplasia induced by coexpression of MKK7 and Ras oncogene [52]. These results highlight opposite functions of JunB and c-Jun in epidermal growth and neoplasia.

JNK function in BCC

JNK function has also been recently implicated in BCC. The JNK target c-Jun is highly activated in human BCC samples [64]. In addition, Gli-mediated cell cycle promotion and target gene induction is abolished by the presence of the pharmacological JNK inhibitor SP600125 or by siRNA-mediated gene silencing of c-Jun [64]. These findings indicate that JNK and c-Jun are important for the oncogenic activity of Hedgehog/Gli proteins in BCC. Moreover, the Hedgehog/Gli signaling pathway is found to act in synergy with the epidermal growth factor receptor signaling pathway to drive oncogenesis of a mouse BCC cell line [65]. In this case, Gli-driven tumorigenesis requires c-Jun activation by MEK/ERK but not JNK. Taken together, JNK is involved in BCC in a cell-context dependent manner. It is not clear whether JNK subunits are differentially involved in the tumorigenesis of BCC.

JNK function in cylindromas and other hair follicle derived tumors

Genetic mutation of the cylindromatosis gene (*Cyld*) predispose patients to not only cylindroma but also other skin tumors derived from hair

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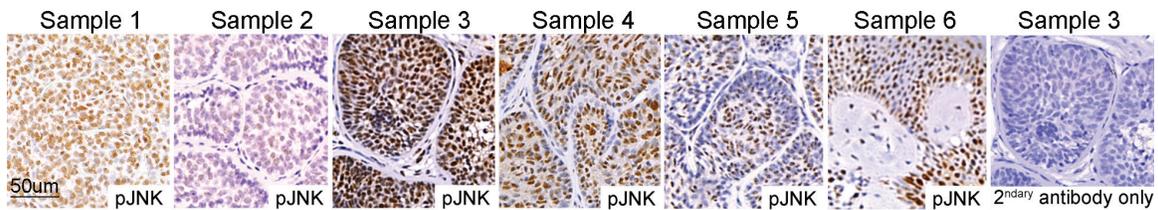


Figure 1. JNK is activated in cylindroma. A panel of paraffin sections of cylindromas tissues derived from different patients were obtained from Duke Pathology lab in accordance with an IRB protocol approved by Duke University Human Subject Use committee. Tissue sections were antigen unmasked and undergone immunoperoxidase staining with a rabbit antibody against pJNK (Promega) followed by peroxidase-conjugated secondary antibody. 100% of the 11 patient samples examined displayed positive staining for pJNK [brown], however different degree of protein expression was detected in these 6 representative patients. Sections were counter-stained with hematoxylin [Nuclei, blue]; Scale bar=50 μ m. Negative control was shown by 2^{ndary} antibody only.

follicles, including Brooke-Speigler syndrome and multiple familial trichoepithelioma [5-12, 66]. CYLD is a deubiquitinase that specifically removes K63-ubiquitin from target proteins to inhibit signal transduction to multiple signaling pathways including NF- κ B and JNK [67, 68]. To examine the relevance of JNK to cylindroma, we performed immune peroxidase staining of a panel of human cylindroma tissues for pJNK. We found that nuclei presence of pJNK was detected in the tumor cells of 100% of samples examined (n=11) (**Figure 1**), indicating that JNK is activated in cylindroma. Increased JNK activation was also observed in skin cancers chemically induced in transgenic animals with K14-driven expression of a patient-relevant CYLD mutant [69]. Topical JNK inhibition markedly reduced tumor formation and malignant progression in these animals, suggesting that JNK plays an important role in epidermal tumorigenesis associated with CYLD loss-of-function.

JNK function in melanoma

The JNK signaling pathway is known to display functional dichotomy in cell growth and survival. Such dichotomy is reflected on the controversial roles of JNK/AP1 proteins in melanoma. JNK activation mediates aspirin-induced suppression of B16 melanoma cellular proliferation [70]. Expression of dominant negative mutants of c-Jun or c-Fos increases growth and soft agar colony formation of human and mouse melanoma cell lines, respectively [71, 72], indicating that AP1 is inhibitory to melanoma growth. In contrast to these findings, recent studies have pinpointed an important role of the JNK signaling axis in melanoma. Activation of JNK and c-Jun by the constitutively active

MEK-ERK signaling axis is a central process in melanoma tumorigenesis [73]. ERK increases c-Jun transcription and stability, which subsequently increases transcription of target genes such as cyclinD1 and RACK1. RACK1 in turn enables PKC to phosphorylate and enhance JNK activity, enforcing a feed-forward mechanism of the JNK-AP1 pathway [73]. In agreement with these findings, our recent studies have shown that JNK activation together with CYLD loss-of-function occurs in human melanoma. Exogenous expression of MKK7 or c-Jun prevents CYLD-induced inhibition of melanoma growth and metastasis as assessed by intravenous tumor growth analysis in mice [74]. Conversely, JNK inhibition with the small molecule inhibitor SP600125 induces melanoma cell growth arrest or apoptosis through p53-dependent induction of p21 cell cycle inhibitor and induction of p53, Bad and Bax apoptotic molecules, as shown in 1205Lu and WM983B melanoma cells, respectively [75]. In addition, targeted gene silencing of JNK1 but not JNK2 impairs melanoma cell growth and survival [75]. Taken together, these findings underscore that the JNK1-AP1 signaling pathway has an important role in melanoma tumorigenesis.

Future perspectives

The JNK signaling pathway has long been recognized as a gold mine for therapeutic targeting [76, 77]. However, strategies targeting the JNK pathway have not been translated into clinical use thus far. Presumably, isoform specific inhibition is pivotal for clinical applications, which has not been achieved with the current JNK inhibitors, including SP600125, BI-78D3,

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JNK1, CEB-1347 and CC-930 [78-82]. Isoform specific inhibitors are under active development, are expected to emerge in the next few years. A JNK1-specific inhibitor AV-7 has been recently characterized via *in vitro* studies [83]. It will be interesting to see the *in vivo* effects of AV-7. Overall, further efforts are required to develop JNK isoform specific inhibitors, and topical application of such agents represents a promising strategy for skin cancer prevention and treatment.

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