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Review Article

The role of c-Jun in the pathogenesis of multiple myeloma

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CONTENTS

Abstract

Multiple myeloma (MM) is characterized by clonal proliferation of malignant plasma cells in the bone marrow (BM), associated with bone loss, renal disease and immunodeficiency. The median survival of patients with MM is 4-5 years. Although primarily associated with tumor growth and survival, several studies have demonstrated that c-Jun also induces cell growth inhibition and apoptosis. Several compounds have been reported to induce c-Jun *N*-terminal kinase (JNK) activity in MM. However, effects on c-Jun, the most prominent molecule downstream of JNK, have only recently been investigated in more detail. For example, adaphostin and bortezomib trigger inhibition of MM cell proliferation and apoptosis, at least in part via robust upregulation of c-Jun and downstream caspase-dependent c-ABL cleavage. This mechanism may not be restricted to MM, but may also be important in a broad range of malignancies, including erythroleukemia and solid tumors. Moreover, c-Jun upregulation and subsequent c-ABL cleavage may not only be drug-induced, but may also represent a physiological defense mechanism of normal cells against malignant transformation.

Introduction

Multiple myeloma (MM), the second most common blood cancer in adults, is characterized by bone marrow plasmacytosis, monoclonal protein in blood and/or urine, bone lesions, renal compromise and immunodeficiency. The annual incidence of MM is about 3.8 per 100,000 population (approximately 14,400 new cases of MM/year) and the median age at diagnosis is approximately 62 years. Despite recent advances in our understanding of disease pathogenesis and treatment, the median overall survival of MM remains at only 3-5 years. The identification and validation of targets for novel therapeutic regimens are therefore urgently needed.

Molecular aspects of c-Jun in cell growth and survival

c-Jun belongs to the Jun subfamily (c-Jun, JunB, JunD) and is a central component of the activating protein-1 (AP-1) family of transcription factors, which are dimeric basic region-leucine zipper (bZIP) proteins. Specifically, c-Jun forms homodimers or heterodimers with Fos (v-Fos, c-Fos, FosB, FosL1, FosL2), activating transcription factor (ATF-2, LRF-1/ATF-3, B-ATF, JDP1, JDP2) (1-4) or musculoaponeurotic fibrosarcoma proteins (c-Maf, MafB, MafA, MafG/F/K and NRL). AP-1 controls the expression of genes, including the cyclin D1, WAF1, p53, INK4A, FAS, FASL, BIM, BCL3, proliferin and CD44 genes (5). Conversely, the activity of AP-1 and its components is triggered by growth factors, cytokines and extracellular matrix proteins, as well as physical and chemical stresses.

AP-1 functions in general and c-Jun functions in particular are dependent on: 1) homo- or heterodimerization among Jun, Fos, ATF and Maf family members; 2) mRNA and protein turnover; 3) post-translational modifications; and 4) a large number of ancillary proteins which interact with AP-1 components/c-Jun (2). Maf proteins homodimerize with each other or heterodimerize with Fos, but not with Jun (6-8). Jun heterodimerizes either with Fos or ATF family members, forming very stable complexes, or homodimerizes. Jun-Jun and Jun-Fos dimers preferentially bind to the phorbol 12-*O*-tetradecanoate-13-acetate (TPA)-responsive element (TRE), whereas Jun-ATF dimers preferentially bind to the cAMP-responsive element (CRE) (9, 10). Due to the complexity of possible combinations of AP-1 dimers involving c-Jun, the spectrum of transcriptional and DNA-binding activities, and thereby their role in physiological and pathophysiological

functions, is broad and diverse. In this way, different AP-1 factors regulate different target genes and thereby different biological functions. In addition to its role as a transcription factor, c-Jun interacts with and modulates the function of STAT3 (signal transducer and activator of transcription 3) and p53 (11, 12) molecules, which play an important role in the pathogenesis of MM.

AP-1 functions in general and c-Jun functions in particular are primarily associated with tumor growth and survival, with JunB as a negative regulator. Specifically, c-Jun-ATF-2 heterodimers induce autocrine growth and c-Jun-c-Fos heterodimers induce anchorage-independent growth (13). However, several recent studies demonstrate that AP-1 and c-Jun have truly double-edged activity, being either oncogenic or antioncogenic. Moreover, functional differences of c-Jun and JunB may be either cell-specific or dependent on the relative protein expression levels (14). For example, DNA-damaging short-wavelength UV light and H_2O_2 are even stronger activators of the c-Jun promoter than TPA, indicating that the response to these stressors is, at least in part, also independent of protein kinase C (PKC) signaling sequelae. In contrast to c-Jun and c-Fos, other *jun* and *fos* genes, as well as early response genes, are only marginally affected by these stressors (15-18). In addition to short-wavelength UV and H_2O_2, ionizing radiation, mitomycin C, hydroxyurea, aphidicolin and camptothecin also increase c-Jun expression (18-20), as well as growth factor deprivation-induced apoptosis, both in neuronal and lymphoid cell cultures (21-23). Cytokine- and genotoxic stress-induced AP-1 activation is mostly mediated via JNK and p38 MAPK (mitogen-activated protein kinase) signaling pathways (24). Specifically, when phosphorylated, they translocate to the nucleus and phosphorylate c-Jun, thereby enhancing its transcriptional activity. In addition, phosphorylated JNK and p38 can also directly activate ATF-2 (2, 25) and ATF-2, myocyte-specific enhancer factor (MEF2C) and ternary complex factors (TCFs), respectively (26).

Proapoptotic functions of c-Jun have been reported in neurons, fibroblasts and endothelial cells. These studies suggest that cell death is induced either indirectly via transcriptional regulation of survival/death genes, *e.g.,* Fas ligand (FasL), or directly via activation of the caspase cascade, which results in the cleavage of numerous molecules (*e.g.*, fodrin, PARP [poly(ADP-ribose) polymerase], DNA-PK [DNA-dependent protein kinase] and PKC) (21, 22, 27-31). In support of a proapoptotic role, c-Jun antisense oligonucleotides or neutralizing antibodies increase survival of growth factor-deprived lymphoid cells or nerve growth factor (NGF)-deprived neuronal cells (21-23). Importantly, JNK phosphorylation at c-Jun$^{Ala63/73}$ is required for efficient T-cell receptor (TCR)- and tumor necrosis factor-α (TNF-α)-induced thymocyte apoptosis. In contrast, JNK-induced nuclear factor of activated T-cells (NF-AT) DNA-binding activity, but not c-Jun phosphorylation, is required for T-cell proliferation or differentiation (32, 33). Moreover, c-Jun-mediated neuronal survival is dependent on both induced BIM expression and mitochondrial cytochrome *c* release, as well as JNK phosphorylation at c-Jun$^{Ala63/73}$ (32, 34, 35).

Thus, the double-edged activity of c-Jun is likely due to cell-, tissue- and stimulus-specific c-Jun-regulated expressional variations of pro- and antiproliferative and apoptotic target genes determining cell growth and survival *versus* cell death, respectively. Moreover, apart from its transcriptional activity, c-Jun also directly modulates the function of associated proteins. A deeper insight into the dynamics of c-Jun-maintaining complexes is still required to define derived pathophysiological functions in detail. An increase in our knowledge will likely result in new pharmacological treatment strategies in MM and other malignancies involving AP-1 in general, and c-Jun in particular.

JNK and c-Jun in multiple myeloma

The role of JNK and its downstream target c-Jun in the pathogenesis of MM has not yet been fully delineated. As in other model systems, studies in our laboratory and others show the double-edged activity of these molecules in MM. Depending on external stimuli or exposure to anti-MM agents, they induce either MM cell growth inhibition and death or proliferation and survival.

For example, IL-6-induced inhibition of MM cell apoptosis is, at least in part, mediated via inhibition of the JNK/SAPK (stress-activated protein kinase) pathway (36). Moreover, initial studies show that ionizing radiation, but not dexamethasone, induces JNK-dependent MM cell apoptosis (37). In addition, a variety of novel agents were shown to induce activation of JNK in MM, including adaphostin (38), JS-K (39), co-exposure to 7-hydroxystaurosporine (UCN-01) and either HMG-CoA reductase inhibitors (40) or the farnesyltransferase inhibitor L-744832 (41), monoclonal antibodies directed against β_2-microglobulin (42), PKC-412 (midostaurin) (43), arsenic trioxide (44, 45), perifosine (46), synthetic epothilone analogues (47), 2-methoxyestradiol (48), bortezomib (49) and lysophosphatidic acid acyltransferase-β inhibitors (50).

Importantly, although predominantly linked to stress-induced apoptosis, constitutive JNK activity in MM also triggers growth and survival, as demonstrated using the JNK-specific inhibitor SP-600125 (51).

A variety of effects downstream of JNK have been identified. For example, 2-methoxyestradiol triggers JNK activation, followed by the release of the mitochondrial protein second mitochondrial-derived activator of caspases (SMAC), thereby promoting apoptosis via activation of caspases (48). In addition, JNK-triggered downstream phosphorylation of c-Jun was reported upon treatment with epothilone analogues (47), bortezomib (49) and arsenic trioxide alone or when combined with Trolox (a hydrophilic vitamin E analogue) (52).

In addition to alterations in c-Jun phosphorylation, expression levels of c-Jun can also change. Specifically, similar to Fas-induced c-Jun upregulation (53), we and others have recently identified several compounds with

anti-MM activity which also trigger c-Jun upregulation, *e.g.,* adaphostin, bortezomib and PKC-412 (38, 43). However, the role of c-Jun upregulation in MM growth and survival still remained elusive. We have recently demonstrated that overexpression of c-Jun induces inhibition of MM cell growth and cell death, at least in part via adaphostin-induced JNK phosphorylation and c-ABL fragmentation (38). Although JNK is the best-known direct activator of c-Jun, it is likely that JNK-independent c-Jun activation pathways also exist in MM cells.

The potential pathophysiological significance of c-Jun in malignancies was also suggested by Oncomine analysis of 13 tumor data sets. This analysis showed a correlative trend of low c-Jun expression and poor survival in carcinomas of the liver, breast, prostate and lung, as well as in seminoma and hematological malignancies, *e.g.,* diffuse large B-cell lymphoma and MM (38). In addition to the above data, we recently showed a trend for shorter overall survival and event-free survival in 11 of 67 (17%) MM patients with low levels of c-Jun compared to patients with normal or high levels of c-Jun (54).

Ongoing studies are now delineating mechanisms that induce c-Jun upregulation and mediate the growth-inhibitory effects of the fragmented c-ABL protein. Moreover, larger studies to validate the potential positive prognostic value of c-Jun expression in some tumors, including MM, are ongoing.

Novel therapeutic agents inducing c-Jun upregulation in multiple myeloma cells

Adaphostin

The dihydroquinone derivative adaphostin (NSC-680410), a lipophilic ester of tyrophostin (AG-957), was first identified as an alternative to the 2-phenylaminopyrimidine derivate imatinib mesilate. It has undergone extensive preclinical testing as a potential anticancer drug (55). Importantly, responses were observed in patients with chronic myelogenous leukemia (CML), including those with BCR-ABL-positive, BCR-ABL-negative and BCR-ABL T315I mutant tumor cells resistant to both imatinib mesilate and the second-generation compounds BMS-354825 (dasatinib) and AMN-107 (nilotinib). Moreover, adaphostin demonstrated cytotoxicity against chronic lymphocytic leukemia (CLL) and acute myelogenous leukemia (AML) cells (56-62). In contrast to imatinib mesilate, adaphostin induced greater apoptosis, with an IC_{50} of 5-10 μM, which was associated with significant inhibition of BCR-ABL phosphorylation (59). In addition to hematological malignancies, adaphostin demonstrated activity against a variety of solid tumors, including carcinomas of the prostate, breast and colon (63).

Functionally, the generation and release of reactive oxygen species (ROS), cytochrome *c* and apoptosis-inhibiting factor (AIF), caspase cleavage, JNK activation, as well as inactivation of Raf-1, STAT3 and STAT5, have been proposed as the basis for its robust antitumor activity (60, 61, 64). Indeed, inhibition of complex III of the respiratory chain was recently identified as the source of adaphostin-induced ROS generation and cytotoxicity (65).

As these adaphostin-targeted proteins and associated pathways are relevant to MM pathogenesis (66), we sought to determine the potential molecular sequelae and therapeutic promise of adaphostin in MM. Adaphostin showed strong activity against both MM cells alone and in MM cell-BMSC (bone marrow stem cells) co-cultures. In addition to the above mechanisms, we recently demonstrated that adaphostin induced robust c-Jun upregulation even at the earliest point of microarray analysis (2 h), followed by c-ABL cleavage. Using caspase inhibitors, as well as caspase-resistant mutants of c-ABL (TM-c-ABL and D565A-ABL), we showed that c-ABL cleavage in MM cells requires caspase activity. Moreover, overexpression of the c-ABL fragment or c-Jun, as well as knockdown of c-ABL and c-Jun expression by siRNA, confirmed that adaphostin-induced c-Jun upregulation triggers downstream caspase-mediated c-ABL cleavage, inhibition of MM cell growth and apoptosis (38).

The exact mechanisms by which adaphostin induces c-Jun upregulation are still elusive, as are the downstream effects of fragmented c-ABL. Ongoing *in vivo* studies are evaluating the anti-MM activity of adaphostin in a mouse model.

Bortezomib

Based on preclinical and clinical studies, the proteasome inhibitor bortezomib was approved by the FDA in 2003 for the treatment of relapsed/refractory MM patients. However, the majority of patients with relapsed or refractory MM do not respond to bortezomib, and acquired resistance has already been observed (67). Although active by itself, the true strength of this compound lies in combination strategies. Indeed, combination of bortezomib with both novel and conventional agents can overcome bortezomib resistance (68). Ongoing preclinical studies are evaluating combinations of low-dose bortezomib together with conventional and novel therapies *in vitro* and *in vivo*. Clinical trials are evaluating bortezomib in combination with thalidomide, dexamethasone, melphalan, doxorubicin, liposomal doxorubicin, the p38 MAPK inhibitor SCIO-469, lenalidomide and the heat shock protein 90 (HSP90) inhibitor 17-AAG (tanespimycin).

Although bortezomib-triggered anti-MM effects have been investigated in great detail, the exact mechanisms whereby bortezomib induces MM cell death have not yet been elucidated. Specifically, we and others have shown that bortezomib induces apoptosis in drug-resistant MM cells and inhibits the binding of MM cells in the BM microenvironment, thereby decreasing the production and secretion of cytokines that mediate MM cell growth and survival. Moreover, bortezomib: 1) blocks IL-6-triggered activation of ERK (extracellular signal-regulated protein kinase), but not STAT3; 2) triggers phosphorylation of both p53 protein and JNK; and 3) induces cleav-

age of DNA-PKCs and ATM (ataxia telangiectasia mutated kinase), as well as caspase-dependent downregulation of gp130 (69, 70). The antiangiogenic effect of bortezomib (71, 72) is another potential mechanism of its anti-MM activity (73-75).

Similar to adaphostin, we recently demonstrated that bortezomib induces c-Jun expression and associated cleavage of c-ABL (38). Furthermore, we observed marked synergistic effects for adaphostin and bortezomib, associated with inhibition of the degradation of ubiquitinated proteins and the stabilization of cleaved c-ABL. Consistent with our own data in MM, the combination of adaphostin with the proteasome inhibitors MG-123 and bortezomib was also strongly synergistic in a variety of leukemia cell lines (62). In addition, c-ABL neutralizes the inhibitory effect of murine double minute 2 (Mdm2) on p53 (76), whereas bortezomib induces upregulation of p53 by inhibiting the ubiquitin-proteasome pathway (49). The complex formation of c-ABL with p53 may therefore be another mechanism for the synergistic effects on G1 arrest in MM cells (77).

PKC-412

PKC-412 (midostaurin), a derivative of the naturally occurring alkaloid staurosporine, inhibits both conventional Ca^{2+}-dependent (α, β_1, β_2, γ), as well as novel Ca^{2+}-independent (δ, ε, η), isoforms of PKC. Moreover, PKC-412 inhibits activation of the vascular endothelial growth factor (VEGF) receptors Flt-1 and mutant Flt-3, as well as of fibroblast growth factor receptor FGFR-1, mutant FGFR-3 and c-kit (78, 79). Antitumor activity for PKC-412 has been reported in multiple preclinical models of hematological malignancies (including B-cell chronic lymphocytic leukemia [B-CLL], AML, acute lymphoblastic leukemia [ALL], mast cell leukemia, pre-B-cell lymphoma and peripheral T-cell lymphoma) and solid tumors (non-small cell lung cancer [NSCLC]) (43, 80-87). Ongoing clinical studies are evaluating the therapeutic potential of PKC-412 in patients with aggressive systemic mastocytosis, mast cell leukemia and newly diagnosed AML (http://clinicaltrials.gov/ct). Moreover, significant activity for PKC-412 was observed in t(4;14)-positive MM cells carrying either the single activating kinase domain mutation K650E (OPM-1 cells) or the transmembrane mutation Y373C (KMS11 cells), as well as in the MM cell lines RPMI 8226, U266, MM.1S, MM.1R and NCI-H929 (43, 83, 88).

Functionally, the activity of PKC-412 in MM is associated with JNK-dependent upregulation of c-Jun and downregulation of c-Fos. Conversely, JNK inhibition abrogates both c-Jun activation and apoptosis.

Conclusions

Recent studies suggest a proapoptotic role for c-Jun expression and activity dependent on the cell type, tissue and stimulus. Specifically, adaphostin, bortezomib and PKC-412 trigger c-Jun upregulation in MM, similar to Fas stimulation. An important downstream target of c-Jun, at least in adaphostin-induced MM cell death, is c-ABL. However, mechanisms whereby these compounds upregulate c-Jun expression, as well as downstream events of adaphostin-induced c-Jun-mediated generation of a proapoptotic and antiproliferative c-ABL fragment, are unknown. Moreover, genetic analysis indicates that high levels of c-Jun expression may correlate with increased survival in a variety of tumors including MM. A deeper insight into the expressional regulation of c-Jun in MM and its functional effects is therefore of great interest and is very likely to result in new treatment strategies in MM to improve patient outcome.

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