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# TNFα-Mediated Cytotoxic Responses to IAP Inhibition Are Limited by the p38α MAPK Pathway

Nomeda Girnius[1] and Roger J. Davis[1,2,*]
[1]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605, USA
[2]Howard Hughes Medical Institute
*Correspondence: roger.davis@umassmed.edu
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**Smac mimetics (SMs), a class of drugs that can promote tumor cell death, represent a potential therapeutic strategy for the treatment of cancer. In this issue of *Cancer Cell*, Lalaoui et al. (2016) report that SM efficacy can be potently increased by inhibition of the p38α MAPK/MK2 signaling pathway.**

Smac mimetics (SMs) bind inhibitor of apoptosis proteins (cIAP1, cIAP2, and XIAP) and promote either apoptosis or necroptosis in target cells by inducing autocrine tumor necrosis factor α (TNFα) signaling (Varfolomeev et al., 2007; Vince et al., 2007). This effect of IAP inhibition suggests that SM-like drugs may act as powerful anti-tumor agents (LaCasse et al., 2008). Indeed, clinical trials using SM to treat acute myeloid leukemia (AML) are in progress (https://clinicaltrials.gov).

The mechanism that accounts for SM-stimulated TNFα expression is unclear, but it appears to involve activation of canonical and non-canonical nuclear factor of kappa light polypeptide gene enhancer of B cells (NF-κB) signaling pathways and receptor-interacting serine/threonine protein kinases 1 and 3 (RIPK1/3) (LaCasse et al., 2008). A detailed understanding of the mechanism of SM-stimulated TNFα expression would facilitate the development of more-potent SM therapeutic strategies.

Lalaoui et al. (2016) conducted an elegant screen using small molecule protein kinase inhibitors to identify drugs that increase SM-stimulated TNFα expression. This analysis demonstrated that inhibiting the p38α mitogen-activated protein kinase (MAPK)/MAPK-activated protein kinase 2 (MK2) signaling pathway potently increased SM-stimulated TNFα expression and cell death. Moreover, this inhibition increased SM-induced cytotoxicity in mouse models of leukemia and primary human AML cells. Drugs targeting p38α/MK2 may therefore be therapeutically beneficial during SM-based therapy of patients with AML (Figure 1).

The identification of the p38α/MK2 pathway as a repressor of SM-stimulated TNFα expression was unexpected because prior studies have firmly established that the p38α/MK2 signaling pathway is essential for endotoxin-stimulated TNFα expression by both transcriptional and post-transcriptional mechanisms (Sabio and Davis, 2014). This differential role of p38α/MK2 signaling in TNFα expression by SM-treated and endotoxin-treated cells is difficult to rationalize based on current knowledge. Clearly, more detailed mechanistic studies of p38α/MK2 signaling in SM-treated cells are required to gain a full understanding of this very surprising finding. In addition, roles for other members of the p38 MAPK family need to be clarified. For example, p38γ/p38δ MAPKs promote endotoxin-stimulated TNFα expression by increasing TNFα mRNA translation elongation by a eukaryotic elongation factor 2 (eEF2) kinase-dependent mechanism (González-Terán et al., 2013) and by stabilizing the MAP3K isoform TPL2 (Risco et al., 2012), but whether p38γ/p38δ MAPKs affect SM-stimulated TNFα expression is not known. Similarly, it is established that SM treatment causes NF-κB activation (LaCasse et al., 2008), but it is not known whether inhibiting p38α MAPK increases SM-induced NF-κB activity that may influence cell fate. These questions need to be addressed by future studies.

How might the p38α/MK2 pathway suppress SM-induced TNFα expression? Inhibitory p38α MAPK-mediated phosphorylation of a subunit of the TNFα-stimulated MAP3K transforming growth factor-β activated kinase 1 (TAK1), known as TAK1 binding protein 1 (TAB1), may be a contributing factor (Cheung et al., 2003). However, this mechanism does not account for the role of the p38α MAPK target MK2. A possible mechanism that may also contribute to negative feedback regulation by p38α/MK2 pathway is represented by increased MAPK phosphatase (MKP) expression (Breitwieser et al., 2007); this remains to be tested. The relative roles of these negative regulatory mechanisms (TAB1 and MKP) are unclear. Nevertheless, both of these mechanisms would be anticipated to promote TNFα signaling (Wagner and Nebreda, 2009). Indeed, p38α/MK2 inhibition in SM-treated cells caused increased activation of other MAPKs, including members of the c-Jun $NH_2$-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) families. These changes in JNK and ERK activity are functionally important because these MAPKs are required for the effects of p38α/MK2 pathway inhibition to promote SM-induced TNFα expression and cell death (Lalaoui et al., 2016). Similarly, JNK and ERK are essential for TNFα expression by endotoxin-treated macrophages (Sabio and Davis, 2014).

The requirement of ERK, JNK, and p38α MAPKs for endotoxin-induced TNFα expression contrasts with the negative role of p38α MAPK and positive roles of ERK and JNK in SM-induced TNFα expression. These contradictory observations suggest that SM treatment might partially rewire signaling pathways. Systems-based comparative analysis of SM, TNFα, and endotoxin-treated cells may provide significant insight into the molecular mechanisms that mediate these effects of SM. Such insight is needed because Lalaoui et al. (2016) show that p38α/MK2 inhibition only increases cell death in some subtypes of AML. Thus,

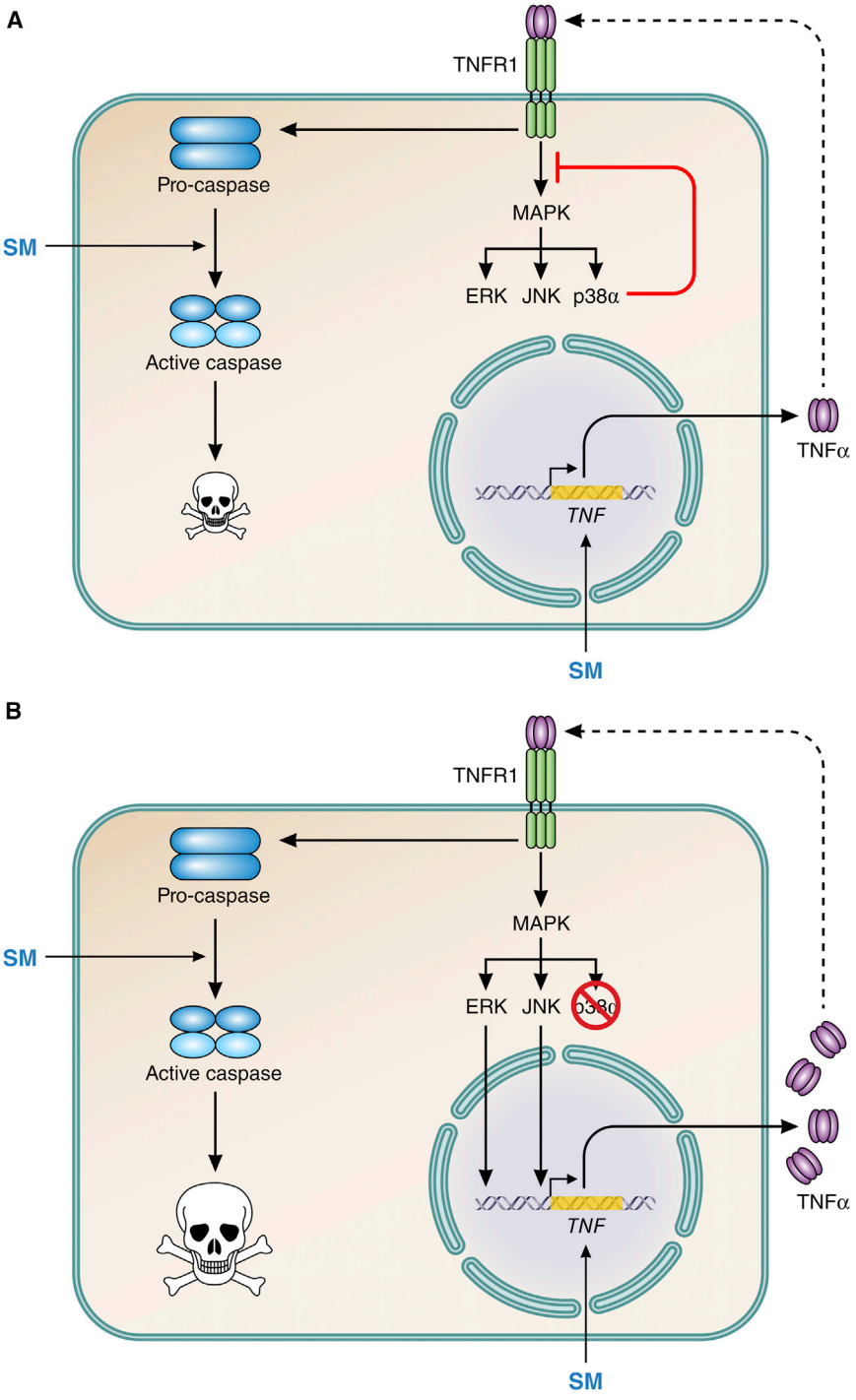


**Figure 1. The p38α MAPK/MK2 Pathway Suppresses Autocrine TNFα Signaling in SM-Treated Cells**

(A) Smac mimetics (SMs) induce expression of TNFα, promote caspase activation, and cause TNFR1-dependent cell death. The cytotoxic response is limited by a negative feedback mechanism mediated by the p38α MAPK/MK2 pathway.

(B) Blocking the p38α MAPK/MK2 pathway increases TNFR1 signaling and increases the activation of the ERK and JNK groups of MAPK that promote TNFα expression and autocrine signaling, leading to cell death.

p38α/MK2 inhibition sensitized MLL-ENL, MLL-AF9, NUP98-HoxA9, and HoxA9/Meis1 leukemic cells to SM-induced cell death by a TNFR1-dependent mechanism (Lalaoui et al., 2016). In contrast, SM-treated CBFβ-MYH11 and AML-ETO9a leukemic cells were unresponsive to p38α/MK2 inhibition (Lalaoui et al., 2016). Mechanisms that contribute to these differential responses are unclear, but it is possible that the alternative functional roles of TAK1 in different myeloid cell types (Sabio and Davis, 2014) may contribute to the selective potency of p38α/MK2 inhibition. Importantly, a molecular explanation for the differential effect of p38α/MK2 inhibition on SM-induced leukemic cell death would help identify appropriate patients for potential combination therapies with SM plus p38α/MK2 inhibition. Furthermore, knowledge of mechanism may provide clues to alternative combination therapies when p38α/MK2 inhibition is not appropriate.

In conclusion, the exciting study reported by Lalaoui et al. (2016) provides significant new insight into the use of SM for the treatment of cancer. The surprising role of the p38α/MK2 pathway in SM-treated cells represents a very interesting basic science challenge to our current understanding of mechanisms that regulate TNFα expression. Importantly, the study of mouse leukemia models and primary human leukemia cells by Lalaoui et al. (2016) establishes a novel drug combination that may increase the therapeutic efficacy of SM and might overcome resistance to other forms of therapy. These aspects of the report by Lalaoui et al. (2016) represent a major conceptual advance in current knowledge.

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# miR-126 Drives Quiescence and Self-Renewal in Leukemic Stem Cells

**Simon Raffel[1,2] and Andreas Trumpp[1,2,*]**
[1]Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH), 69120 Heidelberg, Germany
[2]Division of Stem Cells and Cancer, Deutsches Krebsforschungszentrum (DKFZ), 69120 Heidelberg, Germany
*Correspondence: a.trumpp@dkfz.de
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**Leukemic stem cells (LSCs) are resistant to conventional chemotherapy and promote acute myeloid leukemia (AML) progression and recurrence. In this issue of *Cancer Cell*, Lechman and colleagues (2016) identify the microRNA miR-126 as a regulator of PI3K-AKT-mTOR and CDK3 signaling driving LSC self-renewal and chemotherapy resistance.**

Similar to the normal hematopoietic system, acute myeloid leukemias (AMLs) are hierarchically organized, with leukemic stem cells (LSCs) at the top. Human LSCs are functionally defined by their capacity to re-initiate AML after transplantation into immuno-compromised mice. The biological properties of LSCs differ from the remainder of leukemic cells by their capacity for long-term self-renewal and by a transient status of cell-cycle quiescence or even dormancy. Both features have been linked to therapy resistance and disease recurrence but are also characteristic of normal hematopoietic stem cells (HSCs). In agreement with these similarities, recent data suggest that the leukemia cell of origin is a multipotent cell, possibly the HSC itself (Jan et al., 2012). Thus, specific targeting of LSCs without harming normal HSCs remains a challenging task (Trumpp et al., 2010). Although the patient-specific mutational landscape is the driver of disease development and progression, the epigenetic hierarchy of LSCs and blasts within each AML further contributes to intra-tumor heterogeneity. LSC expression signatures are highly prognostic for patient survival, providing further evidence for their crucial role in leukemia progression and recurrence (Eppert et al., 2011).

Lechman and colleagues now interrogate whether miRNAs are involved in human LSC function (Lechman et al., 2016). The authors fractionated 16 AML patient samples into four populations based on the expression of the surface markers CD34 and CD38, determined their leukemia-initiating capacity, and subjected them to global miRNA profiling. This revealed an LSC-associated miRNA signature that was further optimized to predict clinical outcome. The optimized signature was highly prognostic of overall survival in both univariate and multivariate analyses in an independent cohort. Further analyses were focused on the signature member miR-126, a miRNA already known to display regulatory roles in HSCs and LSCs, as well as in other cell types (de Leeuw et al., 2014; Lechman et al., 2012). Indeed, high expression levels of miR-126 alone were associated with poor prognosis. In agreement, another recent study showed that older AML patients expressed higher levels of miR-126, which correlated with poor overall survival in older, but not younger, patients (Dorrance et al., 2015). Thus, miRNA expression, including miR-126 in LSCs, impacts on the clinical outcome of AML patients and is likely important for LSC biology.

To elucidate the mechanism of miR-126 function, Lechman and colleagues used several approaches. Moreover, a hierarchically organized AML cell line from a relapse patient sample with both LSCs and more differentiated blast fractions was generated in order to overcome technical limitations related to low LSC frequencies in primary samples. In this cell culture system, miR-126 overexpression increased the fraction of primitive quiescent AML cells—thereby decreasing their overall proliferative output—but, importantly, attenuated their differentiation toward AML blasts. In contrast, miR-126 knockdown promoted the exit of primitive AML cells from their quiescent stem-like state into a more committed population of progenitors with decreased self-renewing capacity. The role of miR-126 in primary AML samples was then investigated using xenotransplantation assays. Interestingly, neither overexpression nor knockdown of miR-126 generated an overt phenotype in primary transplants. However, quantification of primitive $CD117^+$ and $CD34^+$ leukemic cells revealed an increase of putative LSCs after miR-126 overexpression in the majority of the cases. When transplanted into