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News and Commentary

JNK: a killer on a transcriptional leash

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Whether to live or die is arguably the most important decision a cell has to make, and the NF-κB/Rel group of transcription factors is a key element in this decision. Since the discovery of the Anti-Apoptotic Function of NF-κB in 1996, the number of publications dealing with the control of apoptosis by NF-κB has increased at an astonishing pace – over 600 papers were published in the last 18 months alone! This plethora of publications underscores the crucial role that this particular biological activity of NF-κB plays in widespread human diseases, including cancer; and indeed, blockers of NF-κB have already been used successfully to treat otherwise recalcitrant malignancies. As with other functions of NF-κB, the suppression of apoptosis involves activation of a program of gene expression. While progress in understanding this program has undoubtedly been made, it remains unclear as to which genes are most critical to this activity of NF-κB, and ultimately, by which mechanisms their products inhibit the apoptotic signaling cascade. Recently, an important piece of this puzzle – pertaining to the control of tumor necrosis factor (TNF)α-induced apoptosis – has been found, with the demonstration that activation of NF-κB shuts down the c-Jun-N-terminal kinase (JNK) cascade[1–4] (Figure 1).

NF-κB is widely utilized by nature to marshal rapid cellular and organismal responses to environmental challenges.[5] Normally, ubiquitous NF-κB dimers lie dormant in the cytoplasm, retained there by inhibitory proteins known as IκBs, and can be activated rapidly by numerous signals, among which TNFα stands out as one of the most potent. Compelling evidence – substantiated by analyses of various knockout models – shows that activation of NF-κB is crucial to antagonize killing by TNFα.[6] In fact, most cells are completely refractory to TNFα cytotoxicity, unless NF-κB activation or protein synthesis is blocked.[6] Interestingly, the potent prosurvival effects of NF-κB are not limited to TNF-R signaling, but serve a wide range of biological processes, including oncogenic transformation and chemo- and radioresistance in cancer.[7]

As it turns out, recent reports from several groups, including ours, have shown that the JNK and NF-κB pathways – almost invariably coactivated by cytokines and stress – are intimately linked.[1–4] JNKs are the downstream component of one of the main mitogen-activated protein kinase (MAPK) cascades found in mammalian cells.[8] Primarily, activation of this cascade is associated with the induction of apoptosis,[8] as best documented by analyses of mice with targeted disruptions of *jnk* genes. Fibroblasts lacking both JNK1 and JNK2 are completely resistant to apoptosis by various stress stimuli,[9] and *jnk3*$^{-/-}$ neurons exhibit a severe defect in the apoptotic response to excitotoxins.[10] Remarkably, the blocking of NF-κB activation by either the ablation of RelA or IKKβ or expression of IκBαM – a superactive variant of the IκBα inhibitor – hampers the normal shut down of JNK induction by TNF-R.[1–4] This targeting of the JNK cascade by NF-κB is crucial to the suppression of TNFα-induced apoptosis, as inhibition of JNK signaling by various means effectively rescues NF-κB-deficient cells from death.[1–4] Of note, while caspases have the ability to activate JNK, in cells lacking NF-κB, JNK activation remains sustained even after protective treatment with caspase inhibitors,[1,3,11] indicating that the effects of NF-κB on JNK are not a secondary consequence of caspase inhibition.

NF-κB-inducible blockers of the JNK cascade have been identified. *gadd45*β – a member of the *gadd45* family of inducible genes – was isolated using an unbiased screen for cDNAs capable of blocking TNFα-induced apoptosis in RelA$^{-/-}$ fibroblasts.[1] Expression of Gadd45β in cells lacking NF-κB completely abrogated the JNK activation response to TNFα, and most importantly, inhibition of endogenous proteins by antisense *gadd45*β hindered the termination of this response. Gadd45β also suppressed the caspase-independent phase of JNK induction by TNFα, and hence, is a *bonafide* inhibitor of the JNK cascade. Interestingly, the c-IAP-like caspase inhibitor, XIAP, was also proposed to mediate the suppression of JNK by NF-κB.[2] XIAP induction by TNFα was slightly reduced in RelA$^{-/-}$ cells, and its overexpression diminished JNK activation by the cytokine. The mechanisms by which these factors, and in fact NF-κB itself, blunt activation of JNK remain unclear. However, since neither NF-κB nor Gadd45β affect ERK or p38 activation,[1,2] these latter factors likely block JNK signaling downstream of the MAP3K module.

The above findings with NF-κB-deficient cells establish a role for JNK in the apoptotic response to TNFα. Despite these findings, however, the relevance of JNK to TNFα-induced apoptosis is still a controversial issue. The notion that JNK is not a critical mediator of TNF-R-induced killing is largely based on the early observation that, during challenge with TNFα, inhibition of JNK activation by dominant-negative (DN) MEKK1 mutants has no effect on cell survival.[12] In support of this view, it was also noted[6] that despite their resistance to stress-induced killing, JNK null fibroblasts are sensitive to apoptosis by Fas[9] – a relative of TNF-R1.[13] Curiously, another early study using DN variants of the JNK kinase, MKK4/SEK1, had instead indicated an important role for JNK in proapoptotic signaling by TNF-R.[14] The issue has now been clarified by

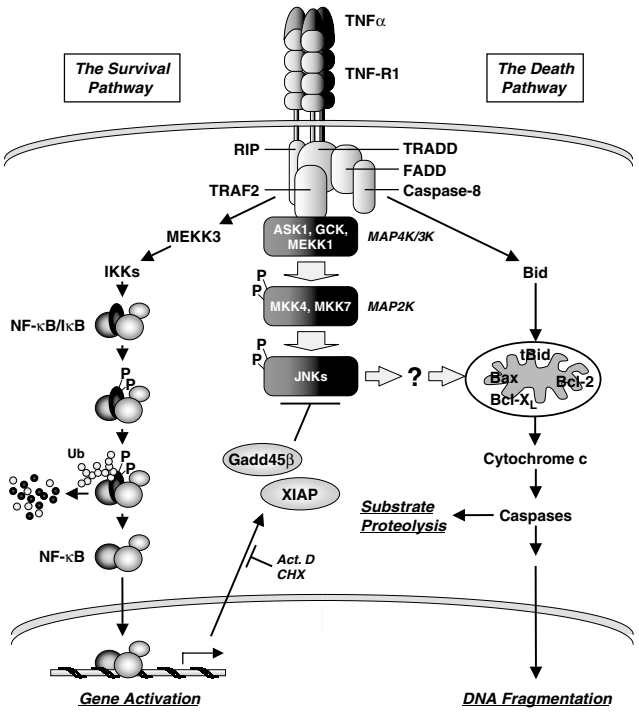

Figure 1 Schematic representation of TNF-R1-induced pathways modulating apoptosis. Previous studies have shown that the blocking of the NF-κB-dependent pathway by either knockout mutations of IKKβ or RelA, expression of IκBαM proteins or antisense *gadd45*β plasmids, or treatment with CHX leads to sustained JNK activation and apoptosis.[1–4] Conversely, the blocking of the TNFα-induced JNK pathway by either JNK or ASK1 null mutations, expression of DN-MKK7 proteins, or treatment with well-characterized pharmacological blockers promotes cell survival, even in the absence of NF-κB.[1–4] Most likely, during challenge with TNFα, apoptosis by JNK does not involve modulation of gene expression. Rather, JNK might induce death by triggering mitochondrial events,[8,18] either directly or indirectly. Its targets, however, remain unknown

further analyses of mice lacking nonredundant activators of JNK. It was shown that mouse embryonic fibroblasts (MEFs) lacking ASK1 – an essential component of the TNF-R pathway signaling to JNK (and p38) – are resistant to killing by TNFα.[15] Furthermore, *jnk1*$^{-/-}$ and *jnk2*$^{-/-}$ MEFs exhibit a profound – albeit not absolute – defect in the apoptotic response to combined TNFα and cycloheximide (CHX) treatment (FZ and GF, unpublished observations). Like JNK null cells,[9] *ask1*$^{-/-}$ fibroblasts retain normal sensitivity to Fas-induced apoptosis,[15] thereby highlighting a fundamental difference between the apoptotic mechanisms triggered by Fas and TNF-R. Interestingly, with regard to the role of JNK in apoptosis signaling by TNF-R, it was recently shown that the TNFα homolog of *Drosophila*, Eiger, completely depends on JNK to induce death, whereas it does not require the caspase-8 homolog, DREDD.[16] Thus, the connection to JNK appears to be a vestigial remnant of a primordial apoptotic mechanism engaged by TNFα, which only later in evolution began to exploit the FADD-dependent pathway to activate caspases.

Of course, there are JNK-independent means by which TNF-R can induce death. In NF-κB-deficient cells, as well as in JNK null MEFs, protection by JNK inhibition is not complete.[1–4] Nevertheless, findings with these model systems conclusively demonstrate that JNK activation is obligatory for efficient killing by TNFα. How can this notion be reconciled with some of the early observations? Most likely, the key lies in the kinetics of JNK induction by TNF-Rs. Indeed, apoptosis has been previously linked to persistent, but not transient, JNK activity.[8,17] This view is supported by the recent discovery that JNK activation is apoptogenic on its own – elegantly demonstrated by the use of MKK7-JNK fusion proteins, which result in constitutively active JNK in the absence of extrinsic cell stimulation.[18] Unlike UV and other forms of stress, TNFα causes only transient induction of JNK[17] and, in fact, this induction normally occurs without significant cell death, which might explain the early findings with DN-MEKK1.[12] Conversely, JNK proapoptotic activity is unmasked when the kinase is allowed to signal chronically, for instance, by the inhibition of NF-κB.[1–4]

The exact mechanism by which JNK promotes apoptosis is not known. While in some circumstances JNK-mediated killing involves modulation of gene expression,[8] during challenge with stress, and most likely with TNFα, the targets of JNK proapoptotic signaling appear to be already present in the cell.[8,9] Killing by MKK7-JNK proteins was shown to require Bax-like factors of the Bcl-2 group;[18] however, it is not clear that these factors are direct targets of JNK or that they mediate JNK cytotoxicity during TNF-R signaling. It is also unclear as to why only prolonged activation of JNK may lead to cell death. It is possible that critical effectors of JNK proapoptotic signaling become available within cells only with time after TNFα stimulation, or that upon phosphorylation, these effectors need to accumulate to induce apoptosis. Another possibility is that JNK apoptotic signaling is counteracted temporarily by concomitant activation of prosurvival pathways,[8,18] such as the ERK and Akt/PKB pathways, which might subsequently fade away. Indeed, the actual biological response to JNK activation also depends upon context- and tissue-specific elements.

It is conceivable that the relevance of the targeting of the JNK cascade by NF-κB extends to other biological processes, including oncogenesis and cancer chemoresistance. With regard to this, it is intriguing that while NF-κB activation is required to suppress transformation-associated apoptosis,[7] nonredundant activators of the JNK cascade (e.g. MKK4 and BRCA1) have been identified as tumor suppressors.[8,19–22] Moreover, apoptosis by genotoxic agents – a desirable effect of certain anticancer treatments – requires JNK,[8,9] whereas NF-κB activation antagonizes it.[7] An important future challenge will be to determine the targets of JNK in apoptosis signaling and the precise mechanisms by which NF-κB blocks the JNK cascade. Indeed, this might lead to the generation of pharmacological compounds that specifically interfere with the ability of NF-κB to suppress JNK activation, and so uncouple the antiapoptotic and proinflammatory functions of the transcription factor. Unlike global blockers of NF-κB, such compounds might lack immunosuppressive effects, and thereby represent a promising new tool in cancer therapy. A suitable therapeutic target might be Gadd45β itself, since this factor is capable of inhibiting apoptosis by chemotherapeutic drugs, and its induction by these drugs depends on NF-κB.[1]

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