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A Five-Year Itch in TNF-α Cytotoxicity: The Time Factor Determines JNK Action

The intricate interplay between NF-κB and JNK determines TNF-α cytotoxicity. In a recent *Cell* paper, Chang et al. (2006) report that prolonged JNK1 activation promotes TNF-α killing via E3 ligase-mediated degradation of the caspase 8 inhibitor cFLIP$_L$. On the other hand, Ventura et al. (2006) show in their recent *Molecular Cell* paper that transient JNK activation suppresses TNF-α-induced cell death.

The proinflammatory cytokine tumor necrosis factor (TNF-α) regulates a variety of biological activities, including immune responses, inflammation, proliferation, and programmed cell death. TNF-α exerts its biological functions via activation of multiple downstream effectors, such as NF-κB, JNK, and caspases. TNF-α activates NF-κB and JNK via the signaling Complex I, which is composed of TNFR1, TRADD, TRAF2, RIP1, cIAP1 and cIAP2. TNF-α is also able to activate the cell death pathway through the signaling Complex II, which consists of TRADD, TRAF2, RIP1, FADD, and caspase 8. Yet, TNF-α does not typically induce cell death unless NF-κB activation is blocked. This is because NF-κB induces expression of various inhibitors of apoptosis including cFLIP$_L$, which specifically inhibits the key initiation caspase 8 and thereby blocks TNF-α-induced programmed cell death.

For a long time, the role of JNK activation in TNF-α-induced cell death has been highly controversial (Lin, 2003). Recent studies demonstrate that in addition to inhibition of caspases, NF-κB inhibits JNK activation to suppress TNF-α-induced cell death. In the presence of simultaneous activation of NF-κB, TNF-α induces transient JNK activation. When NF-κB activation is abrogated, TNF-α induces prolonged JNK activation, which is required for TNF-α-induced cell death (De Smaele et al., 2001; Tang et al., 2001). NF-κB inhibits JNK activation via several mechanisms, including the induction of antioxidants that prevent the oxidation-mediated inhibition of MAPK phosphatases by reactive oxygen species (ROS) (Kamata et al., 2004). The negative regulation of JNK activation by NF-κB for cell survival appears to be TNF-α specific, as NF-κB does not affect IL-1-induced JNK activation (Tang et al., 2001; Chang et al., 2006), or even positively regulates UV-induced JNK activation for cell death (Liu et al., 2006).

Despite its ability to promote TNF-α-induced cell death, prolonged JNK1 activation is insufficient to induce cell death even in the absence of NF-κB activation (Tang et al., 2001; Chang et al., 2006). Thus, it has been hypothesized that TNF-α-induced prolonged JNK1 activation may eliminate inhibitors of apoptosis to promote, but not initiate, cell death, i.e., "breaking the brake on apoptosis" (Lin, 2003). Chang et al. (2006) convincingly show that in the presence of the protein synthesis inhibitor cycloheximide or the superrepressor IκBα-SR, both of which inhibit the antiapoptotic functions of NF-κB, TNF-α induces proteosome-mediated degradation of cFLIP$_L$ and subsequent apoptosis in wild-type, but not *Jnk1*$^{-/-}$ hepatocytes. The authors elegantly demonstrate that the link between prolonged JNK1 activation and proteosome-mediated degradation of cFLIP$_L$ is the E3 ligase Itch, a newly identified JNK1 substrate (Gao et al., 2004). According to the model proposed by the authors, prolonged JNK1 activation promotes cell death by targeting the same caspase 8 inhibitor cFLIP$_L$ that is induced by NF-κB for cell survival. Phosphorylation and activation of Itch by JNK1 is required for selective ubiquitination of cFLIP$_L$, a requisite for its degradation by the proteosome. The authors suggest that prolonged, but not transient, JNK1 activation is required for sustained activation of Itch so that cFILP$_L$ can be fully eliminated by the proteosome. Furthermore, the authors provide strong evidence that the JNK1-Itch-cFLIP$_L$ pathway promotes TNF-α-induced toxicity in two mouse model systems, i.e., Con-A- or LPS/GlaN-induced acute liver failure. Since liver hepatocytes are one of the major targets of TNF-α-induced cell death in vivo, the finding by Chang et al. provides a novel mechanism by which prolonged JNK1 activation promotes TNF-α killing in a pathophysiological setting.

Unlike the well-known proapoptotic function of prolonged JNK1 activation in TNF-α-induced cell death, the role of transient JNK activation is less understood. Earlier studies show that JNK suppresses TNF-α-induced apoptosis through activation of JunD, which collaborates with NF-κB to induce anti-apoptotic genes expression (Lamb et al., 2003). Ventura et al. (2006) elegantly generate a JNK mutant with the approach of chemical genetics. The JNK mutant has an enlarged ATP binding pocket so it can be selectively inhibited by 1NM-PP1, a bulk inhibitor that can fit into the enlarged ATP binding pocket to compete with ATP. Using primary *Jnk1*$^{-/-}$*Jnk2*$^{-/-}$ murine embryonic fibroblasts (MEFs) expressing the JNK mutant as a model system, the authors provide strong evidence that in the presence of NF-κB activation, both transient early phase and sustained late phase of JNK activation contribute to TNF-α-induced gene expression. The sustained late phase JNK activation is very weak and is different from the prolonged strong JNK activation when NF-κB activation is blocked. The authors clearly show that when NF-κB activation is abrogated, addition of INM-PP1 prior to TNF-α stimulation, which abolishes both transient and prolonged JNK activation, enhances cell death. By contrast, addition of INM-PP1 after TNF-α stimulation, which only inhibits the prolonged JNK activation, suppresses cell death. The authors suggest that in primary MEFs, transient JNK activation inhibits TNF-α-induced cell death, whereas prolonged JNK activation promotes TNF-α killing. According to the model proposed by the authors, transient JNK activation is required for TNF-α-induced expression of antiapoptotic

genes, including the ubiquitin ligase cIAP2. These results provide an important step toward understanding how the timing plays a part in the functional output of JNK activation.

While the findings by Chang et al. (2006) and Ventura et al. (2006) provide insight into the molecular mechanism by which JNK regulates TNF-α-induced cell death, many questions still remain to be answered. How is the inactivation of JNK1-activated Itch regulated? Is there any other Itch target in the TNF-α-induced death pathway? How does prolonged JNK1 promote TNF-α-induced cell death in cells where $cFLIP_L$ may not be the major inhibitor of caspase 8? What is the relation between Itch-triggered and caspase 8-mediated degradation of $cFLIP_L$? Does transient JNK activation inhibit TNF-α-induced cell death in a pathophysiological setting? Is transient JNK activation required for suppression of TNF-α-induced cell death in other cell types? How important is the induction of cIAP2 in mediating the antiapoptotic function of transient JNK activation? Since JNK is a potential therapeutic target for various human diseases and cancer, future studies that uncover the molecular mechanisms underlying the functions of JNK will not only shed light on the JNK biology, but may also provide novel strategies in disease prevention and treatment.

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Selected Reading

Chang, L., Kamata, H., Solinas, G., Luo, J.-L., Maeda, S., Venuprasad, K., Liu, Y.-C., and Karin, M. (2006). Cell *126*, 601–613.

De Smaele, E., Zazzeroni, F., Papa, S., Nguyen, D.U., Jin, R., Jones, J., Cong, R., and Franzoso, G. (2001). Nature *414*, 308–313.

Gao, M., Labuda, T., Xia, Y., Gallagher, E., Fang, D., Liu, Y.C., and Karin, M. (2004). Science *306*, 271–275.

Kamata, H., Honda, S., Maeda, S., Chang, L., Hirata, H., and Karin, M. (2004). Cell *120*, 649–661.

Lamb, J.A., Ventrue, J.-J., Hess, P., Flavell, R.A., and Davis, R.J. (2003). Mol. Cell *11*, 1479–1489.

Lin, A. (2003). Bioessays *25*, 17–24.

Liu, J., Yang, D., Minemoto, Y., Leitges, M., Marsha, R., Rosner, M.R., and Lin, A. (2006). Mol. Cell *21*, 467–480.

Tang, G., Minemoto, Y., Dibling, B., Purcell, N., Li, Z., Karin, M., and Lin, A. (2001). Nature *414*, 313–317.

Ventura, J.-J., Hubner, A., Zhang, C., Flavell, R.A., Shokat, K.M., and Davis, R.J. (2006). Mol. Cell *21*, 701–710.

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No ESCRT to the Melanosome: MVB Sorting without Ubiquitin

Multivesicular bodies (MVBs) are critical for a variety of cellular functions ranging from lysosomal degradation to the budding of HIV. To date, delivery into MVBs has been dependent on the ESCRT machinery. However, analysis of a melanosomal protein has uncovered an alternative pathway for MVB sorting.

The delivery of transmembrane precursors and receptors into the lumen of lysosome and lysosome-related organelles requires the sorting of these proteins into intralumenal vesicles (ILV) in an endosomal compartment, giving rise to the multivesicular endosome or body (MVE or MVB). However, the mechanism of discrete sorting of ILV cargoes between the lysosome and nondegradative lysosome-related organelles, such as the melanosome, has been a mystery. Theos et al. (2006) have now shed light on this problem through identifying the ILV sorting determinants of the melanosomal protein Pmel17. In contrast to the previously characterized cytoplasmic ubiquitin signal that directs sorting into MVBs destined for the lysosome, the sorting of Pmel17 occurs through a lumenal signal that is independent of the ESCRT (endosomal sorting complex required for transport) machinery. These findings establish a novel paradigm for sorting into MVBs.

The endosomal system is a dynamic site of protein sorting that coordinates protein trafficking between the Golgi, plasma membrane, lysosome, and specialized lysosome-related organelles including melanosomes. Endosomal cargoes must be directed to these subcellular compartments for a variety of purposes including recycling, degradation, or biological function. An elaborate system of *cis*- and *trans*-acting factors ensure that these sorting reactions are executed properly, as aberrant sorting can negatively impact a variety of cellular functions. For example, inappropriate lysosomal degradation of cell surface receptors prevents appropriate environmental sensing and uptake of nutrients. An additional level of endosomal sorting occurs through the formation of MVBs. MVB formation occurs when selected lipid and protein cargoes are actively partitioned into membrane domains that will invaginate and bud into the lumen of the endosome, giving rise to its characteristic "multivesicular" appearance. MVB formation is critical for a variety of cellular functions including protein degradation via the lysosome, but also nondegradative functions including exosome release, formation of lysosome-related organelles, and retroviral budding.