

TNF-R1 Signaling: A Beautiful Pathway

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Tumor necrosis factor (TNF) is a major mediator of apoptosis as well as inflammation and immunity, and it has been implicated in the pathogenesis of a wide spectrum of human diseases, including sepsis, diabetes, cancer, osteoporosis, multiple sclerosis, rheumatoid arthritis, and inflammatory bowel diseases. The interaction of TNF with TNF receptor-1 (TNF-R1) activates several signal transduction pathways. A common feature of each pathway is the TNF-induced formation of a multiprotein signaling complex at the cell membrane. Over the past decade, many of the components and mechanisms of these signaling pathways have been elucidated. We provide an overview of current knowledge of TNF signaling and introduce an STKE Connections Map that depicts a canonical view of this process.

The anticancer activity now known as tumor necrosis factor (TNF) was first described more than a century ago. However, it wasn't until 1984 that human TNF was purified and its encoding cDNA was cloned and expressed. The subsequent availability of recombinant TNF led to a rapid cataloging of TNF's pleiotropic activities.

In addition to triggering apoptosis of certain tumor cells, TNF mediates the inflammatory response and regulates immune function. Inappropriate production of TNF or sustained activation of TNF signaling has been implicated in the pathogenesis of a wide spectrum of human diseases, including sepsis, cerebral malaria, diabetes, cancer, osteoporosis, allograft rejection, and autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and inflammatory bowel diseases.

In the past dozen years, the molecular details of signal transduction by TNF gradually have been unveiled. The TNF Pathway Connections Map (http://stke.sciencemag.org/cgi/cm/CMP_7107) at *Science's* Signal Transduction Knowledge Environment presents current knowledge of the pathway's components and connections between them (1). Today, our understanding of the TNF signaling network provides a paradigm for elucidating the signaling pathways utilized by other TNF-related proteins and their receptors (2). This understanding has also led to the development of novel therapies that neutralize the deleterious effects of TNF for treatment of recalcitrant inflammatory conditions such as rheumatoid arthritis (3).

TNF is a homotrimer of 157 amino acid subunits primarily produced by activated macrophages. TNF signals through two distinct cell surface receptors, TNF-R1 and TNF-R2. Multiple experimental approaches have revealed that TNF-R1 initiates the majority of TNF's biolog-

ical activities. The binding of TNF to TNF-R1 triggers a series of intracellular events that ultimately result in the activation of two major transcription factors, nuclear factor κ B (NF- κ B) and c-Jun. These transcription factors are responsible for the inducible expression of genes important for diverse biological processes, including cell growth and death, development, oncogenesis, and immune, inflammatory, and stress responses.

The initial step in TNF signaling (1) involves the binding of the TNF trimer to the extracellular domain of TNF-R1 and the release of the inhibitory protein silencer of death domains (SODD) from TNF-R1's intracellular domain (ICD). The resulting aggregated TNF-R1 ICD is recognized by the adaptor protein TNF receptor-associated death domain (TRADD), which recruits additional adaptor proteins receptor-interacting protein (RIP), TNF-R-associated factor 2 (TRAF2), and Fas-associated death domain (FADD). These latter proteins recruit key enzymes to TNF-R1 that are responsible for initiating signaling events (Fig. 1). For instance, caspase-8 is recruited by FADD to the TNF-R1 complex, where it becomes activated, presumably by self-cleavage, and initiates a protease cascade that leads to apoptosis. TRAF2 recruits cellular inhibitor of apoptosis protein-1 (cIAP-1) and cIAP-2, two anti-apoptosis proteins that also have ubiquitin protein ligase activity. TRAF2 is also thought to activate a mitogen-activated protein kinase kinase (MAPKKK), such as

extracellular signal-regulated kinase kinase kinase 1 (MEKK1) or apoptosis-stimulated kinase 1 (ASK1), in a complex at or near the receptor, thereby activating a cascade of kinases resulting in the activation of c-Jun NH₂-terminal kinase (JNK), a kinase that phosphorylates c-Jun and increases its transcriptional activity. Finally, the protein kinase RIP is critical to the functioning of a third arm of the TNF signaling network, the activation of the transcription factor NF- κ B. However, the enzymatic activity of RIP is not required for TNF-induced activation of NF- κ B.

TNF-induced activation of NF- κ B relies on phosphorylation-dependent ubiquitination and degradation of inhibitor of κ B (I κ B) proteins,

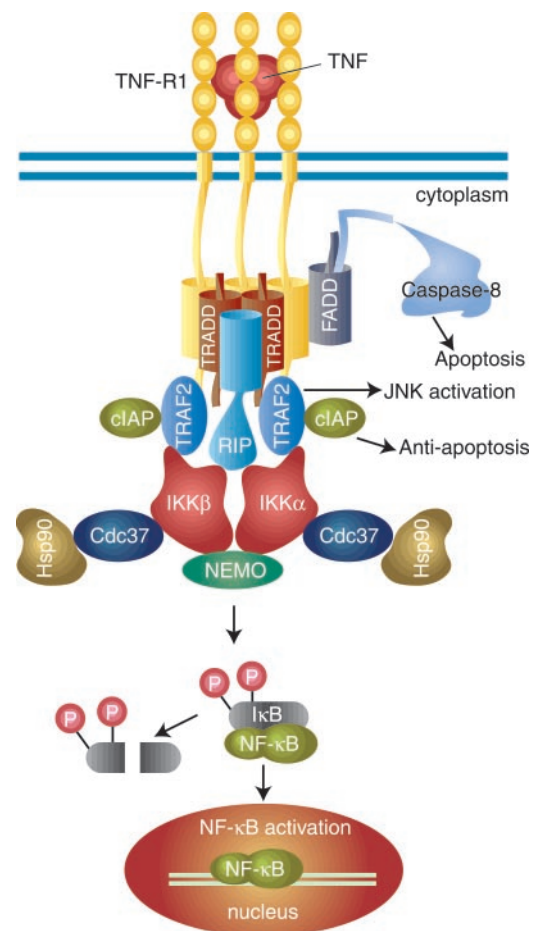


Fig. 1. TNF signal transduction pathway. Engagement of TNF with its cognate receptor TNF-R1 results in the release of SODD and formation of a receptor-proximal complex containing the important adaptor proteins TRADD, TRAF2, RIP, and FADD. These adaptor proteins in turn recruit additional key pathway-specific enzymes (for example, caspase-8 and IKK β) to the TNF-R1 complex, where they become activated and initiate downstream events leading to apoptosis, NF- κ B activation, and JNK activation.

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which normally retain NF- κ B within the cytoplasm of unstimulated cells. The past 5 years have witnessed tremendous advances in our understanding of this branch of the TNF signaling network. Especially noteworthy was the identification of the multiprotein I κ B kinase (IKK) complex that mediates phosphorylation of I κ B in a TNF-dependent manner (4). The core of the IKK complex consists of two catalytic subunits, IKK α and IKK β , and a regulatory subunit, NF- κ B essential modulator (NEMO, or IKK γ). In addition, the IKK complex contains a kinase-specific chaperone consisting of Cdc37 and Hsp90 that plays a role in shuttling the complex from the cytoplasm to the membrane. The IKK complex is also recruited to TNF-R1, where it becomes activated within minutes of TNF treatment. This activation depends on RIP, indicating that the IKK activation within the receptor complex likely occurs through a RIP-dependent intermediate factor, perhaps a kinase. Gene knockout studies in mice have established essential roles for IKK β in TNF-induced activation of NF- κ B, and for NEMO in regulation of IKK complex activation in response to numerous upstream signals. In contrast, IKK α plays only a

minor role in TNF-induced activation of NF- κ B, but it has other important functions, such as serving as a NF- κ B2/p100 kinase in B cells.

An interesting feature of the TNF signaling network is the existence of extensive cross talk between the apoptosis, NF- κ B, and JNK signaling pathways that emanate from TNF-R1. In the absence of NF- κ B activity, cellular susceptibility to TNF-induced apoptosis increases, whereas enforced activation of NF- κ B protects against apoptosis. Similarly, TNF-induced JNK activation is stronger and more prolonged in cells lacking NF- κ B, and the products of several NF- κ B-activated genes inhibit activation of JNK by TNF. Moreover, NF- κ B activation prompts the resynthesis of I κ B and other inhibitory molecules, such as the IAPs, thereby adding another layer of regulation of the duration and amplitude of TNF signaling.

To date, most of the players in the TNF pathway have been validated by both biochemical and genetic means, thus providing a rich source of potential drug targets for the development of a new generation of anti-inflammatory agents. However, many questions remain unanswered. For example, what MAPKKK initiates

the kinase cascade that activates JNK, and how is this kinase recruited to TNF-R1 and activated within the receptor complex in response to TNF? In the case of IKK complex activation, the possibility remains that an intermediate factor or kinase is required between RIP and NEMO. Unraveling the molecular details of how the enzymes like caspase-8 and the IKK complex become activated within the TNF-R1 complex will be key to a full understanding of the dynamic nature of TNF signaling. Finally, the molecular basis for cross talk between TNF-mediated apoptosis, NF- κ B, and JNK signaling pathways is not well understood. Deciphering these puzzles will greatly help interpret how a specific outcome of TNF signaling is achieved in distinct biological contexts.

References

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VIEWPOINT

The Fas Signaling Pathway: More Than a Paradigm

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Apoptosis and related forms of cell death have central importance in development, homeostasis, tumor surveillance, and the function of the immune system. Apoptosis is initiated by two principal pathways. The intrinsic pathway emerges from mitochondria, whereas the extrinsic pathway is activated by the ligation of death receptors. This Viewpoint introduces the basic mechanisms of the extrinsic pathway, using the example of the prototypical death receptor Fas and its role in apoptosis, but it also points out the increasingly understood importance of this receptor as a non-apoptotic signal transducer.

Fas (also called Apo-1 or CD95) is a death domain-containing member of the tumor necrosis factor receptor (TNFR) superfamily. It has a central role in the physiological regulation of programmed cell death and has been implicated in the pathogenesis of various malignancies and diseases of the immune system (1, 2) [see Fas Signaling Pathway, http://stke.sciencemag.org/cgi/cm/CMP_7966 (3) and Fas Signaling Pathway in Cardiomyocytes, http://stke.sciencemag.org/cgi/cm/CMP_9993 (4)]. Although the Fas ligand (FasL)-Fas system has been appreciated mainly with respect to its death-inducing function, it also transduces proliferative and activating signals through pathways that are still poorly defined (1, 2).

In the absence of membrane-bound ligand, inactive complexes of Fas are formed by the pre-ligand-binding assembly domain of the molecule (2). Interaction with membrane-bound FasL (or agonistic antibodies) reorganizes these complexes and allows the formation of a death-inducing signaling complex (DISC). The Fas DISC contains the adaptor protein Fas-associated death domain protein (FADD) and caspases 8 and 10, which can initiate the process of apoptosis. FasL-induced clustering of Fas, FADD, and caspase-8 or -10 within the DISC results in autoproteolytic processing of these caspases by induced proximity and in release of the processed active proteases (Fig. 1). In type I cells, processed caspase-8 is sufficient to directly activate other members of the caspase family, whose action on defined substrates paves the way to the execution phase of apoptosis (1). In type II cells, proper activation of effector

caspases by Fas depends on an amplification loop that relies on caspase-8-mediated cleavage of the pro-apoptotic Bcl-2 family member Bid and subsequent release of mitochondrial pro-apoptotic factors [for example, cytochrome c and second mitochondria-derived activator of caspases (SMAC, also called Diablo)] to drive the formation of the caspase-9-activating aptosome. Active caspase-9 activates the executioner caspase-3, which in turn activates caspase-8 outside the Fas DISC, thereby completing a positive feedback loop (1).

Each step in Fas-mediated apoptosis can be a target of regulatory mechanisms enabling cells to show flexible responses to stimulation by Fas. Corresponding to the hierarchy of events in Fas-mediated apoptosis, these regulatory mechanisms can be specific for Fas or common to death receptors, or they can affect the apoptotic core machinery of the cell. The FasL gene is transcriptionally inactive in most cells. Thus, regulation of FasL expression itself, for example, by the transcription factors nuclear factor kappa B (NF- κ B), activating protein 1 (AP1), or nuclear factor of activated T cells (NF-AT), regulates FasL/Fas-mediated effects, such as those of activation-induced cell death of CD4⁺ T cells (5). To a lesser extent, regulation of Fas expression is also used to control Fas responses, for example,

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