Correction

CELL BIOLOGY

The authors note that Figs. 1C (Bottom), 2A, 2C, 3F, and 4E have been revised to include dividing lines between lanes to show where extraneous data have been removed. These changes do not affect the data presented nor the conclusions of the article. The changes were made to comply with the PNAS policy that requires dividing lines whenever entire nonessential lanes have been removed from a single original gel. The corrected figures appear below.

Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
TANK-binding kinase 1 (TBK1) controls cell survival through PAI-2/serpinB2 and transglutaminase 2

Mireille Delhase,b,c,d,1, Soo-Youl Kim,a,2, Ho Lee,a,2, Aya Naiki-Ito,g,h,2, Yi Chen,c,3, Eu-Ree Ahn,e,2, Kazuhiro Murata,d, Se-Jin Kim,a, Norman Lautsch,a,4, Koichi S. Kobayashi,b,h, Tomoyuki Shirai,i, Michael Karin,c,1,5, and Makoto Nakanishi,d,5

*Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA 02115; †Department of Pathology, Harvard Medical School, Boston, MA 02115; ‡Laboratory of Gene Regulation and Signal Transduction, Department of Pharmacology, School of Medicine, University of California at San Diego, La Jolla, CA 92039-0723; †Department of Cell Biology, Graduate School of Medical Sciences, Nagaoya City University, Nagaoya 467-8601, Japan; ‡Cancer Cell and Molecular Biology Branch and †Cancer Experimental Resources Branch, Division of Cancer Biology, Research Institute, National Cancer Center, Goyang 410-769, Republic of Korea; ‡Department of Experimental Pathology and Tumor Biology, Graduate School of Medical Sciences, Nagaoya City University, Nagaoya 467-8601, Japan; and †Department of Microbiology and Immunology, Harvard Medical School, Boston, MA 02115

AUTHOR SUMMARY

Apoptosis, the major form of programmed cell death in multicellular organisms, is a highly regulated process essential for proper cellular development and well being (1). Defective apoptosis is associated with multiple diseases, including immune disorders and cancer. The pleiotropic inflammatory mediator TNF-α binds to TNFR1, a receptor at the cell surface, to promote or prevent cell death, depending on the nature and relative strength of the signals that it elicits within the cell. TNF-induced apoptosis is orchestrated by the activation of a protease cascade (i.e., a sequence of protein activation that involves proteases, which function to cleave proteins) in which the protease caspase-8 acts upstream of caspase-3, an executioner caspase that destroys vital cellular components (Fig. P1). By contrast, the TNF-generated survival signal largely relies on activation of the IKK kinase complex (IKK), which regulates proteins by adding phosphate groups to specific sites (a process termed phosphorylation), and NF-κB, a protein that acts as a transcription factor by binding to gene sequences and by regulating their expression. Both IKK and NF-κB control the expression of antiapoptotic genes, the products of which attenuate activation of the caspase cascade (2). The balance between these two antagonistic signaling pathways ultimately determines whether a TNF-stimulated cell will survive or die. In the present study, we provide evidence for an additional survival pathway involving the IKK-related kinase, TANK-binding kinase 1 (TBK1), previously identified as an activator of NF-κB (3). We show that TBK1 triggers an antiapoptotic response by controlling the phosphorylation of RelA/p65, a subunit of NF-κB. Our results also show that the proteins plasminogen activator inhibitor-2 (PAI-2) and transglutaminase 2 (TG2) act as downstream mediators in the antiapoptotic response triggered upon TBK1 activation.

In the present study, we showed that, rather than being a general NF-κB activator, TBK1 exerts its survival function, at least in part, by modulating the transcriptional activity of the NF-κB subunit RelA/p65 through its specific phosphorylation by IKK. We then used TBK1-deficient cells (4), which are susceptible to TNF-induced apoptosis, to identify TNF-induced genes that are dependent on TBK1. This resulted in the identification of Pai-2 as a TBK1-dependent survival gene. The PAI-2 protein is a member of the serpin family, which includes a significant number of proteins involved in inhibiting proteases. Expression of the PAI-2 protein in TBK1-deficient cells protected these cells from TNF-induced apoptosis. PAI-2 expression prevented activation of caspase-3 and degradation of the protein modifier TG2. After the catalytic activity of TG2 is stimulated in response to TNF, TG2 cross-links inactive procaspase-3 (the precursor of caspase-3) into dimers, or protein pairs, that are targeted for degradation (Fig. P1).

Fig. P1. Schematic illustrating the signaling pathway through which TBK1 inhibits TNFα-induced apoptosis (red arrows) and the parallel pathway involving IKK-mediated NF-κB activation (black arrows). Engagement of TNFR1 triggers proapoptotic and survival signals through assembly of distinct multiprotein signaling platforms (DISK complexes). Heterodimeric cFLIP:caspase-8 complexes recruited to the DISK prevent cell death unless cFLIP is inactivated or degraded, allowing caspase-8 to induce apoptosis. The survival response is mediated through activation of TBK1 and IKK, both of which are required for NF-κB activation. Although TBK1 is dispensable for IkB phosphorylation and NF-κB-mediated induction of most antiapoptotic genes (i.e., TBK1-independent genes), it regulates the activation of Pai-2 and Tg2 (i.e., TBK1-dependent genes), whose expression requires RelA/p65 phosphorylation. Cytosolic PAI-2 associates with TG2, preventing its degradation. Calcium (Ca2+) -activated TG2 accumulates in the cytosol and cross-links procaspase-3 into inactive dimers that are degraded. This process inhibits apoptosis by depleting the pool of procaspase-3 available for activation by caspase-8.


The authors declare no conflict of interest.

This is a Contributed submission.

To whom correspondence may be addressed. E-mail: mdelhase@gmail.com or karinoffice@ucsd.edu.

1Present address: Shanghai Kanda Biotechnology, Shanghai 201203, China.

2Present address: Department of Integrative Physiology and Metabolism, Joslin Diabetes Center, Boston, MA 02215.

3M.K. and M.N. contributed equally to this work.

See full research article on page 177 of www.pnas.org.

Cite this Author Summary as: PNAS 10.1073/pnas.1119296109.

www.pnas.org/cgi/doi/10.1073/pnas.1119296109

PNAS | January 24, 2012 | vol. 109 | no. 4 | 1005–1006
This reduces the pool of procaspase-3 available for activation, thereby preventing cell death. To validate the antiapoptotic function of TG2 in vivo, we injected TNF and actinomycin D (a transcriptional inhibitor) into WT and TG2-deficient mice and examined the level of apoptosis in the liver, an organ whose survival depends on NF-κB, IKK, and TBK1 signaling. TG2-deficient mice exhibited massive TNF-induced liver destruction as a result of apoptosis, supporting the importance of the TBK1–PAI-2–TG2 survival pathway. TG2 was also found to protect the liver from apoptosis induced by engagement of CD95 (Fas), another member of the TNF receptor family (5). This suggests that the TBK1–PAI-2–TG2 survival pathway might also inhibit other apoptotic responses.

Although numerous NF-κB–dependent antiapoptotic genes have been identified and their specific prosurvival functions characterized, a remaining challenge is the identification of a minimal set of survival genes that need to be expressed in a particular cell type under given environmental conditions to suppress specific cell death triggers. Our findings add additional components to the intricate regulatory network that protects cells from death induced by TNF and related proteins, and demonstrate that activation of these components depends on a specific modification of one NF-κB protein, RelA/p65.

In light of our work, it is likely that targeting TBK1 or its downstream targets by using specific inhibitors may constitute an attractive approach to modulate the balance between life and death, not only in normal cells but also in cancer cells developing in the context of chronic inflammation.