Preventing Apoptosis With Thioredoxin
ASK Me How
Nanette H. Bishopric, Keith A. Webster

Reactive oxygen species (ROS) are critical signaling molecules in eukaryotic cells, regulating growth, survival, and death pathways. High concentrations of ROS are cytotoxic, but low concentrations can promote growth and activate critical signal pathways in many cell types. In the cardiovascular system, significant amounts of ROS are generated as byproducts of mitochondrial metabolism and may increase to toxic levels in the myocardium during and after periods of reduced blood flow. In addition, a number of extracellular peptide hormones are able to increase the production of ROS through receptor-mediated stimulation of membrane-bound NADPH oxidase. The hypertrophic effects of angiotensin II and more recently other G protein-coupled receptor (GPCR) agonists have been shown to involve the generation of ROS through this mechanism. In addition, many apoptotic stresses in the cardiac myocyte, including ischemia-reperfusion and high concentrations of NO, signal through ROS. Oxidative stress is a central component of the mitochondrial permeability transition in cardiac myocytes, and the latter seems to underlie most pathologically relevant examples of cardiac myocyte apoptosis in vivo. Consequently, a clear understanding of the relationship between myocardial growth and apoptosis signaling will require a better understanding of the intracellular signals that respond to ROS.

In this issue of Circulation Research, Liu and Min shed light on a fascinating redox-sensitive mechanism that can generate both apoptotic and hypertrophic signals in the cardiovascular system. Apoptosis signal-regulating kinase 1 (ASK1), a member of the mitogen-activated protein kinase kinase (MAP3K) family, is an upstream activator of p38 MAP kinases and c-Jun N-terminal kinases (JNKs) through MKK3/6 and MKK7, respectively. ASK1 itself is activated in cells undergoing various types of stress, including oxidant and cytokine exposure. ASK1 associates with TRAF2 during tumor necrosis factor-α (TNF-α) signaling and is required both for TNF-α-induced apoptosis and for TRAF2-mediated activation of JNK. Cells deficient in ASK1 cannot induce a sustained activation of JNK in response to TNF-α and H2O2, although transient JNK activation still occurs. Overexpression of wild-type ASK induces apoptosis in a number of cell types. ASK1 thus seems to play a key role in cytokine and oxidative stress–induced apoptosis. On the other hand, endogenous cardiac myocyte ASK1 was recently shown to be activated by GPCR-induced ROS and to mediate the hypertrophic effects of various vasoactive peptides that work through these receptors. Titration of ASK1 activity may therefore determine whether oxidative stress is interpreted as a survival or death signal.

Under resting conditions, ASK1 exists in the cytosol bound to a small redox-sensitive protein, thioredoxin (Trx). Although there are several negative regulators of ASK1, including 14-3-3 proteins and protein phosphatase 5, Trx seems to play a critical role in the tonic inhibition of ASK1. In support of this, the human immunodeficiency virus Nef protein was recently shown to inhibit ASK1 activity by preventing the dissociation of Trx and ASK1.

Trx is an ancient molecule, conserved in evolution from archaebacteria to plants and mammals. Trx modulates a variety of oxidation-reduction reactions within the cell that regulate cell growth and survival decisions, for example, the redox state of zinc finger transcription factors, and the potency of some (but not all) growth factors. The transcriptional activity of estrogen and glucocorticoid hormone receptors is mediated by cysteine residues within the DNA binding domain; when these cysteines are oxidized, both ligand binding and DNA interaction are reduced. Trx seems to play a critical role in the control of the immune and inflammatory systems. Transient and stable transfection of cells with Trx promotes NF-κB reporter gene activity in some cell types but inhibits it in others, consistent with the observation that NF-κB is redox sensitive in many but not all systems. Trx is upregulated in a number of tumor cell lines, and overexpression of Trx confers survival and growth advantages on cell lines transplanted into SCID mice. Trx thus generally functions as a progrowth, prosurvival antioxidant molecule with some functional similarities to Bcl-2. This similarity is made more striking by the recent identification of a second Trx species, Trx-2, that resides specifically in the mitochondria and is required for inhibition of mitochondrial apoptosis signaling.

Interaction of Trx with its cellular targets is often, but not always, redox sensitive. In contrast, the interaction between Trx and the amino terminus of ASK1 is highly redox sensitive and invariably reduces both apoptotic signaling and

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constitutive inhibitors of ASK1-mediated apoptosis and JNK activation.

The concept of a redox-sensitive dissociation is both elegant and simple. It seems that the association of ASK with Trx can utilize either of the two catalytic cysteines, and that only removal of both, either by mutagenesis or (oxidative) intramolecular disulfide bond formation, can prevent this interaction. However, as with any important result, this finding leaves additional questions. Does mutagenesis of Trx affect its site of interaction with ASK1? The authors demonstrate that the amino terminus of ASK1 is required, as with the wild-type Trx, but it is at least theoretically possible that subtle changes in the association have occurred. How does oxidation and Cys32-S–Cys35 bond formation affect Trx interaction with other substrates, such as NF-κB, or its regulatory enzyme Trx reductase? What about the functional impact on other substrates binding to either Cys32 or Cys35?

A related question is how irreversible targeting of Trx to ASK1 might affect other signal transduction pathways, including GPCR-mediated NF-κB activation. Because Trx targets ASK1 for ubiquitination, it is likely that ASK1 levels will be significantly reduced by constitutively bound Trx, but whether ASK1 functions will be completely eliminated is unclear. The pathway by which ASK1 signals to NF-κB activation has not been worked out, but it does not seem to involve activation of IκB kinases (IKKs) or degradation of IκB. NF-κB DNA binding and transcriptional activity have been shown to be modulated by phosphorylation in an IκB–independent manner. It is possible that ASK1, or a protein kinase target of ASK1, could be involved in this regulation. It will be interesting to determine whether redox-insensitive binding of ASK1 by Trx affects either the activation of NF-κB or the hypertrophic response to GPCR agonists such as endothelin and angiotensin II.

Ultimately, what, if anything is special about the interaction between Trx and this particular substrate, ASK1? The question has bearing on whether these Trx mutants could realistically be used as antiapoptotic agents. As with all interventions in cell survival, tissue and pathway specificity are likely to be critical. Nature has provided few examples of spontaneous mutation of mammalian Trx that might be informatively. In addition to the 12-kDa Trx-1, variant forms of Trx, including Trx-2 and SpTrx, are selectively localized in mitochondria and sperm, respectively. However, all of these variants have the tightly conserved Cys-Gly-Pro-Cys active site motif, and there have been no reports of germ-line mutations at this site. A truncated form of Trx, Trx80, is secreted from monocytes and lymphocytes, but it has no ability to reduce protein disulfides. A mutant form of human thioredoxin was cloned from an Epstein-Barr virus–transformed B-cell line that had predicted amino acid substitutions of Lys39-Asn and Met74-Thr; these mutations lie within the homodimerization domain and might affect the biological if not the catalytic properties of Trx. Given the lack of spontaneous variants, study of these novel single cysteine Trx mutants will be very useful in clarifying the nature of Trx interactions with other known targets. It should be possible to determine which of the other functions of Trx are regulated by the same redox-sensitive dissociation and which are not.
As a clinical issue, it will be particularly important to learn whether regulation of estrogen receptor transcription is also irreversibly activated by these mutants. If the effects of Trx modification can be directed to cardiovascular targets and away from its potentially tumor-promoting properties, these variant proteins may point the way to a new treatment strategy in myocardial and vascular protection.

References


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