Reversible Glutathiolation of Caspase-3 by Glutaredoxin as a Novel Redox Signaling Mechanism in Tumor Necrosis Factor-α–Induced Cell Death

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Atherosclerosis and hypertension are cardiovascular diseases that cause distinctive functional and structural changes in the vasculature. These pathological events have been intimately linked to modified cellular behavior in the vessel wall. Cell growth, endothelial apoptosis, smooth muscle migration, inflammation, fibrosis and matrix regulation are all known to contribute to plaque development and hypertension. Reactive oxygen species (ROS) produced by leukocytes, endothelial cells, vascular smooth muscle cells (VSMC), and adventitial fibroblasts play a key role in the initiation and progression of these cellular activities.

ROS have been implicated in numerous physiological and pathological processes depending on their concentrations and kinetic properties. Cells produce ROS such as $\text{O}_2^\cdot$, $\text{H}_2\text{O}_2$, and OH in response to growth factors, cytokines and shear stress. At relatively low concentrations, ROS produced by these external stimuli play critical roles in redox signaling and normal cell function. However, higher concentrations of ROS induce oxidative damage of DNA, proteins, carbohydrates and lipids. Glutathiolation has gained acclaim as an important cellular mechanism. Glutathiolation is the reversible formation of mixed disulfides between protein cysteines and glutathione (GSH). The exact mechanism regulating glutathiolation has not been clearly established, but a recent review by Ghezzi suggests that glutathiolation occurs mainly by thiol-disulfide exchange with GSSG (oxidized GSH). This indicates the importance of overall cellular GSH-GSSG ratio on glutathiolation of redox-sensitive proteins. Other mechanisms include direct oxidation by oxidants such as diamide, or via sulfenic acid intermediates, S-nitrosothiols, or thyl radicals (-S=O).

Glutathiolation has been recognized as critical machinery to quickly modify protein function and cellular response. Dominici and colleagues first demonstrated that protein thiolation could confer resistance to oxidative stress. Glutathiolation protects thiols during oxidative insult, stabilizes extracellular proteins, and regulates enzyme activity involved in metabolism and cell signaling. In addition to regulating ROS-mediated transcriptional control, protein synthesis, and ubiquitinylation, mixed disulfide formation has been closely linked to cellular functions associated with atherosclerosis and hypertension. In VSMC, glutathiolation of Ras was implicated in angiotensin II-dependent activation of p38 MAP kinase and Akt, as well as protein synthesis. Other studies also connected Ras glutathiolation to endothelial insulin resistance and myocyte hypertrophy. Moreover, extracellular matrix destruction and calcium-dependent VSMC relaxation, both induced by peroxynitrite formation, were related to oxidative modification of pro-MMPs and SERCA, respectively. Taken together, these studies suggest that glutathiolation acts as a common mechanism for ROS-induced cellular behavior and pathophysiology.

Reversibility, like that of phosphorylation and dephosphorylation events, offers acute regulation of cell signaling pathways. Similarly, deglutathiolation removes disulfide bonds from glutathiolated cysteines and this provides an acute redox-sensitive regulatory step. Much like glutathiolation, how deglutathiolation occurs is still elusive. In cells, deglutathiolation is achieved by members of the protein disulfide oxidoreductase family, thioredoxin (Trx), glutaredoxin (Grx) and protein disulfide isomerasers. Both Trx and Grx act as antioxidants, maintaining a reduced intracellular redox state via reduction of protein thiols. The major function of Grx is deglutathiolation of proteins back to their reduced states both intracellularly and in the extracellular space with concomitant release of GSH. Grx has been shown to protect against apoptosis and vascular hypertrophy. Its disruption may lead to oxidative damage associated with diseases such as atherosclerosis, hypertension, pulmonary diseases, and cancer.

In the current issue of *Circulation Research*, Pan and Berk identify a new role for Grx-dependent deglutathiolation in tumor necrosis factor-α (TNF-α)–induced apoptosis in endothelial cells. Their work demonstrated that caspase-3 activation by TNF-α correlates with its deglutathiolation status, and that this was regulated by Grx. The authors observed a significant increase in Grx activity with TNF-α treatment and noted that small interfering RNA targeted against Grx inhibited TNF-α–induced cell death. Grx depletion also resulted in reduced...
caspase-3 cleavage, with a concurrent increase in caspase-3 glutathiolation. Furthermore, overexpression of wild-type Grx increased caspase-3 activation, whereas thioltransferase-deficient Grx did not stimulate caspase-3 activity in response to TNF-α. Using procaspase-3 and recombinant caspase-8 in in vitro experiments, they showed evidence suggesting that glutathiolated pro-caspase-3 was a poor substrate of caspase-8. In contrast, deglutathiolated procaspase-3 was readily cleaved by caspase-8. Next, they attempted to identify which cysteine residues on procaspase-3 were targets for Grx-dependent deglutathiolation using 3 different Cys to Ser mutants. This study showed that Cys184 and Cys220, but not Cys163, are important in TNF-α-induced caspase-3 cleavage, suggesting that they are critical residues involved in Grx-dependent deglutathiolation of caspase-3. Additional studies showed that Grx is bound to caspase-3 under basal conditions, but dissociates on TNF-α stimulation. These studies, however, did not definitively identify which cysteine residues are glutathiolated in cells. In addition, it is not clear how glutathiolation of procaspase-3 under basal or stimulated conditions is regulated. Further effort is needed to resolve these issues and to determine whether other redox-sensitive proteins are regulated by similar glutathiolations.

This work portrays Grx as a mediator of apoptosis, but previous investigations have supported the role for Grx as an antiapoptotic protein. Murata et al reported that Grx protected cells against H2O2-induced apoptosis by regulating the redox state of Akt. Additionaly, Okuda et al found that H2O2 stimulated expression of Grx in cultured smooth muscle cells. These authors suggested that Grx was involved in a compensatory mechanism to protect against oxidative stress. The discrepancy between the previous notion of Grx as antiapoptotic mediator and the new report of Pan and Berk suggesting Grx as proapoptotic mediator may be because of different cell types studied and different experimental conditions.

In Figure 1, we attempt to incorporate the findings of Pan and Berk into the current understanding of TNF-α-dependent apoptotic pathways. TNF-α signaling is tightly regulated in various cell types and can induce or inhibit apoptosis. On TNF-α binding, the TNF receptors trimerize and form Complex I including TRADD, RIP, and TRAF2. Complex I activates 2 distinct pathways: 1) NFκB activation via MEKK3/IKK pathway; and 2) H2O2 production leading to JNK activation via...
ASK1/MEKK7 pathway. Subsequently, Complex I dissociates from the membrane and forms Complex II containing TRADD, RIP, FADD and caspase-8. TNF-α can induce apoptosis by coordinated effects of chronic JNK activation and caspase-8 activation. Chronic JNK activation leads to the formation of apoptosisomes and the activation of caspase-9. Both caspase-8 (activated in Complex II) and caspase-9 (activated in apoptosisome) cleave and activate caspase-3, which in turn executes apoptotic events (DNA fragmentation and protein cleavage). On the other hand, TNF-α can inhibit apoptosis if the NFκB pathway is activated. NFκB activation upregulates expression of antioxidant genes Mn-superoxide dismutase (Mn-SOD) and ferric heavy chain (FHC), which prevent ROS-induced apoptosis. In addition, nuclear factor-κB (NF-κB) also upregulates JNK phosphatase gene MKP-1, a redox-regulated phosphatase that prevents chronic JNK activation.

Many studies including those of Pan and Berk utilize cycloheximide treatment of cells to block the NF-κB–dependent protein synthesis that occurs in response to TNF-α. This allows TNF-α to induce apoptosis. The exciting contribution of Pan and Berk’s work is the identification of a redox-regulated phosphatase (activated in Complex II) and caspase-9 (activated in apoptosisome) cleave and activate caspase-3, which in turn executes apoptotic events (DNA fragmentation and protein cleavage). On the other hand, TNF-α can inhibit apoptosis if the NFκB pathway is activated. NFκB activation upregulates expression of antioxidant genes Mn-superoxide dismutase (Mn-SOD) and ferric heavy chain (FHC), which prevent ROS-induced apoptosis. In addition, nuclear factor-κB (NF-κB) also upregulates JNK phosphatase gene MKP-1, a redox-regulated phosphatase that prevents chronic JNK activation.

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References


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