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Forum Editorial

Redox Control of Apoptosis

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APOPTOSIS is a genetically encoded form of cell death that is central to the development and homeostasis of multicellular organisms, designed to rapidly remove unwanted surplus cells or potentially dangerous cells. The inappropriate regulation of apoptosis associated with a variety of diseases, including cancer, AIDS, neurodegenerative diseases, and ischemic stroke (28). The existence of a cell-suicide program was first proposed based on the observations of typical and common morphological changes characterizing apoptotic cell consisting of plasma membrane blebs, cell shrinkage, chromatin condensation, protein aggregates, and apoptotic body formation. Apoptosis is a complex process that proceeds through at least two main pathways regulated at multiple levels: extrinsic and intrinsic. The extrinsic apoptotic pathway is mediated by the so-called "death receptors," including tumor necrosis factor (TNF), Fas (APO1/CD95), and TNF-related apoptosis-inducing ligand (TRAIL). This pathway includes inhibitory counterparts and receptor-associated cytoplasmic proteins required for procaspase activation upon ligand binding (23). Shakibaei *et al.* review the redox regulation of apoptosis by members of the TNF superfamily in this Forum issue (26). The intrinsic pathway centers on the mitochondria. Mitochondria have, in addition to their function in respiration, an important role in the apoptotic-signaling pathway. Malfunctioning at any level of the cell is eventually translated into the release of apoptogenic factors from the mitochondrial intermembrane space, resulting in the organized demise of the cell (16). In particular, cytochrome *c,* beside its key role in oxidative phosphorylation as electrons shuttle between complex III and IV, can be released, under a variety of stress conditions, from the intermembrane space into the cytosol. Here it triggers the assembly of a high-molecular-weight complex termed apoptosome, which eventually leads to caspase activation (18). In fact, the most important event associated with apoptosis is uncoupling of the mitochondrial oxidative phosphorylation that gives rise to the dissipation of the transmembrane potential ($\Delta\psi_m$), a decrease in ATP formation, and an increase in reactive oxygen species (ROS) production (16). This condition has often been associated with the opening of existing pores, functioning within the context of energy metabolism, such as the mitochondrial permeability transition pore or megachannel, which could be responsible for the release of cytochrome *c*. However, there are also numerous reports that have described the release of cytochrome *c* without a detectable decrease in the transmembrane potential. A growing body of evidence now suggests a direct pore-forming property of proapoptotic members of the Bcl-X_L family protein, some of which prevent apoptosis (such as BCl-X_L and Bcl-2), whereas others induce cell death (such as Bax and Bid) (2). Kowaltowski and Fiskum review the role of cytoprotection by Bcl-2 in this Forum issue (15).

In this context, a number of other factors contribute to the redox regulation of apoptosis. Heat shock proteins (Hsps) have recently been found to function at key regulatory points in the control of the apoptotic machinery by directly interacting with different apoptotic proteins. Hsps have cytoprotective functions: they allow the cells to adapt to gradual changes in their environment and to survive in otherwise lethal conditions. The inducible synthesis of Hsps is known to be tightly controlled by heat shock transcription factors, among which only HSF1 is essential for the transcriptional activation in mammalian organisms (24, 30). Several apoptotic stimuli induce overexpression of Hsp27, which counteracts cell death by various distinct mechanisms (22): it could increase the antioxidant defense of cells by decreasing ROS content and neutralizing the toxic effects of oxidized proteins; it interferes with caspase activation upstream of the mitochondria involvement; and it prevents the formation of apoptosome by directly sequestering cytochrome *c* once released into the cytosol. The last activity involves the formation of large nonphosphorylated oligomers of Hsp27 that are essential for the protective effect of this family of stress proteins (5, 22). The capability of Hsp70 to interact and recruit apoptosis protease activating factor-1 (Apaf-1), in order to prevent apoptosome oligomerization, has also been demonstrated, as well as its ability to inhibit caspase-independent cell death. It has been suggested that Hsp90 and Hsp60 also play an ambiguous role in the control of cell death, depending on the apoptotic stimulus and/or cell type (22).

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The redox environment of the cell is now thought to be extremely important to control the apoptotic machinery as many redox-sensitive proteins are involved in this network. It is now widely accepted that free radicals not only are dangerous species, but they have also been designed by evolution to participate, when produced at low concentration, in the maintenance of cellular redox (reduction/oxidation) homeostasis. This notion derives from the evidence that cells constantly generate free radicals both as waste products of aerobic metabolism and in response to a large variety of stimuli. Free radicals, once produced, provoke cellular responses (redox regulation) against oxidative stress, transducing the signals to maintain the cellular redox balance (12). Among radicals, those derived from O_2 are the most frequent reactive species that are physiologically produced. Due to the triplet ground state, O_2 is preferentially reduced by subsequent monoelectronic addition, resulting in intermediate ROS such as superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). The major physiological source of ROS is represented by mitochondria during the phosphorylative process, where O_2 is fully reduced to H_2O. About 2% of electrons carried by the mitochondrial electron transport chain can escape from this pathway and react with O_2 directly generating $O_2^{\bullet -}$, probably by a nonenzymatic mechanism at the level of complex I and complex III. Minor, but not less important, sources of ROS exist intracellularly that include a variety of cellular enzymes. Another radical species is represented by nitric oxide (NO), which is endogenously formed by a family of NADPH-dependent enzymes, the NO synthases. They function as homodimers and catalyze a two-step oxidation of the amino acid L-arginine to citrulline and NO via the intermediate compound *N*-hydroxyl-L-arginine (20). Being highly reactive, ROS and NO-derived species (RNS), when produced at high rate, can interact in a nonspecific way with a wide range of macromolecules, including proteins, carbohydrates, lipids, and DNA, thus leading to the alteration and impairment of function of all cellular components (27).

Growing evidence suggests that, in many instances, the production of radical species is tightly regulated and their downstream targets very specific, indicating that ROS and RNS actively participate in several cell-signaling pathways as physiological "second messengers" in cell growth, differentiation, and apoptosis. The importance of ROS in the activation of the cell death program of apoptosis was understood from the discovery that they were produced in large amounts after TNF-α-induced cell killing (17). During this process, ROS accumulation was found to precede all the morphological and biochemical alterations typical of apoptosis. However, in other cases, ROS increase may be a side effect rather than a causal effect of the phenomena accompanying the apoptotic process. For example, release of cytochrome *c* in cytosol causes the impairment of the mitochondrial respiratory chain, thus leading to ROS accumulation and setup of the oxidation state of the apoptotic cells. In most cases, ROS trigger the apoptotic program, oxidatively altering cellular components and/or activating the mitochrondrial pathway (4, 7). Differently from ROS, which are primarily considered proapoptotic molecules, NO has a dual role in the apoptotic process that strongly depends on its concentration (13). When produced at nonphysiological concentrations, NO may induce cell death by apoptosis, as well as drive an apoptotic response into a necrotic one. The cytotoxicity of NO mostly derives from its encounter with $O_2^{\bullet -}$ to form peroxynitrite ($ONOO^-$), but it may be responsible for cell damage by other pathways involving the production of reactive oxides of nitrogen, which may be formed preferentially within lipid membranes. However, NO at low concentration exerts an inhibitory action at different levels of the apoptotic machinery. Due to its reactivity with thiol groups, it can block the catalytic cysteine residue of caspases (29). Moreover, NO donors raised Bcl-2 protein expression, preventing apoptotic cell death. Brüne reviews the relationship between NO and $O_2^{\bullet -}$ in regulating apoptosis or cell survival in this Forum issue (3).

A way in which ROS might act on the redox status of the cells is oxidative modifications of cysteine residue that are important for the regulation of several protein functions. Protein sulfhydryls can be oxidized to protein disulfides and sulfenic acids, as well as more highly oxidized states, such as the sulfinic and sulfonic acid forms of protein cysteines. Under nonstressed conditions, disulfide bond formation occurs primarily in the oxidizing environment of the endoplasmic reticulum in eukaryotic cells. The sulfhydryl groups, in the vast majority of protein cysteine residues, in the reducing environment of cytoplasm, remain protonated at physiological pH. Thus, cytoplasmic proteins, in general, do not contain disulfide bonds. However, certain redox-sensitive proteins possess cysteine residues that exist as thiolate anions ("reactive cysteines") at neutral pH due to charge interactions with neighboring amino acid residues, and therefore are more vulnerable to oxidation (10). Oxidized cysteines can be either re-reduced to a thiol form or transformed into thiol derivatives, such as nitrosylated, glutathiolated, or disulfide bridges. These chemical species can be reduced back through the reaction catalyzed by thioredoxin (Trx) and glutaredoxin. Cysteine redox state influences the structure of sensitive proteins, leading to "gain" or "loss" of function. In particular, it has been suggested that, whereas intramolecular disulfide bond formation results in alteration of protein three-dimensional conformation, the intermolecular disulfide bridge formation could lead to protein arrangement in dimers and/or multimers. The latter process could influence the association between different cellular proteins and be implicated in regulatory pathways. Antioxidants, such as superoxide dismutase, catalase, and gluthathione-dependent enzymes, by scavenging ROS, also regulate the intracellular redox balance. However, in the context of redox regulation, molecules able to cycle from reduced into oxidized form are the most important systems in maintaining the intracellular redox environment, *e.g.,* the redox couples $NADP^+$/NADPH, $TrxSS/Trx(SH)_2$, and glutathione (GSSG/GSH). NADPH is the major source of electrons for bioreductive biosynthesis; furthermore, it limits the action of freely diffusing oxidizing radicals, which alter redox environment, by scavenging them or re-reducing the oxidized biomolecules directly operating as an antioxidant. Besides such a cofactor role, recently, a direct action on reduction reactions has been suggested, especially in the mitochondrial compartment where NADPH can scavenge toxic free radicals and repair biomolecule-derived radicals (14). Mammalian and prokaryotic Trxs are proteins with oxidoreductase activity. They contain a highly conserved -Cys-Gly-Pro-Cys- active site, essential for the function as a general and potent protein disulfide oxidoreduc-

tase. Specific protein disulfide targets for reduction by Trx are ribonucleotide reductase, protein disulfide isomerase, and several transcription factors, including p53, nuclear factor-κB, and activator protein-1 (21). Moreover, these redox couples are strictly related by chemical reactions in maintaining a GSH/GSSG proper ratio. In fact, both Trx and NADPH are necessary to allow the re-reduction of oxidized species of glutathione. Glutathione represents the most prevalent low-molecular-weight thiol with several fundamental functions ranging from detoxification to messenger molecule. It exists as a reduced predominant form (GSH), as a disulfide form (GSSG), or as mixed disulfide with protein thiols (GS-R). As GSH concentration is 100–1,000-fold higher than the reduced form of the other couples, glutathione redox state usually reflects the status of intracellular redox environment (6). The concentration of GSH and/or the ratio GSH/GSSG are fundamental in the processes leading to execution of apoptosis, especially when a redox-related stimulus is involved. A strictly dependent correlation has been demonstrated between GSH decrement and induction of apoptosis; it seems that the cell has to be void of the tripeptide to orderly execute the death program. In particular, the release of cytochrome *c* from mitochrondria is profoundly affected by GSH decrease, as the process occurs even in the absence of cell commitment to apoptosis (9, 11).

Among proteins that are responsive to the alteration of the redox state that operate in crucial cross-points in the processes regulating cell survival or apoptosis are well established examples. Trx functions as a regulatory protein interacting with apoptosis signal-regulating kinase (ASK-1) (25). The Trx/ASK-1 association gives rise to the inactivation of kinase activity of ASK-1; moreover, this interaction is found only in nonstressed cells and seems to be modulated by intracellular ROS levels. In particular, an increase in ROS concentration causes disulfide bridge formation between or within Trx molecules and ASK-1 activation. C-Jun NH_2-terminal kinase (JNK) is maintained at low levels inside unstressed cells, even in the presence of high concentrations of growth factors; its activity seems to be inhibited by the interaction with glutathione-*S*-transferase-π1 (GST-π1) (1). Under H_2O_2 or UV treatment, the GST-π1/JNK complex dissociates, leading to GST-π1 dimer and/or multimer structures formation and JNK activation. The identification of Trx and GST-π1 proteins as modulators of ASK-1 and JNK activities provides a mechanistic model of how redox-mediated signaling events could be translated into downstream processes mediated by protein kinase activation and, in some cases, induction of apoptosis. Moreover, thiol compounds that do not necessarily produce ROS by increasing the disulfide power of the cell are able to induce apoptosis by specifically activating different stress-responsive protein kinases (8). Matsuzawa and Ichijo review the role of stress-responsive protein kinases in redox-regulated apoptosis signaling in this Forum issue (19).

Finally, it is noteworthy to mention that the first evidence of a regulatory role of ROS was associated with the function of nuclear transcription factors, as the activity of several transcription factors seems to be modulated by redox changes. A bulky amount of data is reported in the literature, and several reviews deal with this topic, which is outside the focus of this forum.

Although incomplete, our current understanding of redox regulation illustrates how apoptosis can be integrated into a larger network controlled by different signals. Understanding this network in more detail will provide insights into cancer and other diseases and will identify strategies to improve their therapeutic treatment.

The review articles and original articles that are presented in this Forum issue will cover some crucial aspects of the most updated research in the field of redox regulation of apoptosis.

ABBREVIATIONS

ASK-1, apoptosis signal-regulating kinase; GSH, glutathione; GSSG, glutathione disulfide; GST-π1, glutathione-*S*-transferase-π1; H_2O_2, hydrogen peroxide; Hsp, heat shock protein; JNK, c-Jun NH_2-terminal kinase; NO, nitric oxide; $O_2^{\bullet -}$, superoxide radical; RNS, NO-derived species; ROS, reactive oxygen species; TNF, tumor necrosis factor; Trx, thioredoxin.

REFERENCES

1. Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew KD, Pincus MR, Sardana M, Henderson CJ, Wolf CR, Davis RJ, and Ronai Z. Regulation of JNK signaling by GSTπ. *EMBO J* 18: 1321–1334, 1999.
2. Borner C. The Bcl-2 protein family: sensors and checkpoints for life-or-death decisions. *Mol Immunol* 39: 615–647, 2003.
3. Brüne B. The intimate relation between nitric oxide and superoxide in apoptosis and cell survival. *Antioxid Redox Signal* 7: 497–507, 2005.
4. Clément M-V, Sivarajah S, and Pervaiz S. Production of intracellular superoxide mediates dithiothreitol-dependent inhibition of apoptotic cell death. *Antioxid Redox Signal* 7: 456–464, 2005.
5. Diaz-Latoud C, Buache E, Javouhey E, and Arrigo A-P. Substitution of the unique cysteine residue of murine Hsp25 interferes with the protective activity of this stress protein through inhibition of dimer formation. *Antioxid Redox Signal* 7: 436–445, 2005.
6. Filomeni G, Rotilio G, and Ciriolo MR. Cell signalling and the glutathione redox system. *Biochem Pharmacol* 64: 1057–1064, 2002.
7. Filomeni G, Aquilano K, Rotilio G, and Ciriolo MR. Reactive oxygen species-dependent c-Jun NH_2-terminal kinase/c-Jun signaling cascade mediates neuroblastoma cell death induced by diallyl disulfide. *Cancer Res* 63: 5940–5949, 2003.
8. Filomeni G, Rotilio G, and Ciriolo MR. Glutathione disulfide induces apoptosis in U937 cells by a redox-mediated p38 MAP kinase pathway. *FASEB J* 17: 64–66, 2003.
9. Filomeni G, Aquilano K, Rotilio G, and Ciriolo MR. Anti-apoptotic response to induced GSH depletion: involvement of heat shock proteins and NF-κB activation. *Antioxid Redox Signal* 7: 446–455, 2005.
10. Finkel T. Redox-dependent signal transduction. *FEBS Lett* 476: 52–54, 2000.

11. Ghibelli L, Coppola S, Fanelli C, Rotilio G, Civitareale P, Scovassi AI, and Ciriolo MR. Glutathione depletion causes cytochrome *c* release even in the absence of cell commitment to apoptosis. *FASEB J* 13: 2031–2036, 1999.
12. Haddad JJ. Redox and oxidant-mediated regulation of apoptosis signaling pathways: immuno-pharmaco-redox conception of oxidative seige versus cell death commitment. *Int Immunopharmacol* 4: 475–493, 2004.
13. Kim YM, Bombeck CA, and Billiar TR. Nitric oxide as a bifunctional regulator of apoptosis. *Circ Res* 84: 253–256, 1999.
14. Kirsgh M and De Groot H. NAD(P)H, a directly operating antioxidant? *FASEB J* 15: 1569–1574, 2001.
15. Kowaltowski AJ and Fiskum G. Redox mechanisms of cytoprotection by Bcl-2. *Antioxid Redox Signal* 7: 508–514, 2005.
16. Kroemer G and Reed JC. Mitochondrial control of cell death. *Nat Med* 6: 513–519, 2000.
17. Larrick JW and Wright SC. Cytotoxic mechanism of tumor necrosis factor-alpha. *FASEB J* 4: 3215–3223, 1990.
18. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, and Wang X. Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91: 479–489, 1997.
19. Matsuzawa A and Ichijo H. Stress-responsive protein kinases in redox-regulated apoptosis signaling. *Antioxid Redox Signal* 7: 472–481, 2005.
20. Moncada S, Palmer RM, and Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 43: 109–142, 1991.
21. Nordberg J and Arner SJ. Reactive oxygen species, antioxidants and mammalian thioredoxin system. *Free Radic Biol Med* 31: 1287–1312, 2001.
22. Parcellier A, Gurbuxani S, Schmitt E, Solary E, and Garrido C. Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. *Biochem Biophys Res Commun* 304: 505–512, 2003.
23. Peter ME and Krammer PH. The CD95(APO-1/Fas) DISC and beyond. *Cell Death Differ* 10: 26–35, 2003.
24. Pirkkala L, Nykanen P, and Sistonen L. Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. *FASEB J* 15: 1118–1131, 2001.
25. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, and Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17: 2596–2606, 1998.
26. Shakibaei M, Schulze-Tanzil G, Takada Y, and Aggarwal BB. Redox regulation of apoptosis by members of the TNF superfamily. *Antioxid Redox Signal* 7: 482–496, 2005.
27. Stamler JS and Hausladen A. Oxidative modifications in nitrosative stress. *Nat Struct Biol* 5: 247–249, 1998.
28. Strasser A, O'Connor L, and Dixit VM. Apoptosis signaling. *Annu Rev Biochem* 69: 217–245, 2000.
29. Torok NJ, Higuchi H, Bronk S, and Gores GJ. Nitric oxide inhibits apoptosis downstream of cytochrome C release by nitrosylating caspase 9. *Cancer Res* 62: 1648–1653, 2002.
30. Yan L-J, Rajasekaran NS, Sathyanarayanan S, and Benjamin IJ. Mouse HSF1 disruption perturbs redox state and increases mitochondrial oxidative stress in kidney. *Antioxid Redox Signal* 7: 465–471, 2005.

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2. Phil-Sun Oh, Kye-Taek Lim. 2008. Protective activity of 30kDa phytoglycoprotein from glucose/glucose oxidase-induced cell death in primary cultured mouse thymocytes. *Environmental Toxicology and Pharmacology* **25**:1, 114-120. [CrossRef]
3. M MISHRA, P KOLI, S BHOWMICK, A BASU. 2007. Neuroprotection conferred by astrocytes is insufficient to protect animals from succumbing to Japanese encephalitis. *Neurochemistry International* **50**:5, 764-773. [CrossRef]