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Redox control of caspases

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Abstract

Caspases are critical mediators of apoptotic cell death. All members of the caspase family contain the sequence QA**C**X**G** which contains the active site cysteine. The putative active site of caspase 3 contains a cysteine residue that is subject to redox control. Both thioredoxin and glutathione have been shown to be required for caspase-3 activity to induce apoptosis. The regulation of inducible caspase 3 activity by oxidation–reduction (redox) dependent mechanisms is reviewed. Up until a few years ago, reactive oxygen species (ROS) research mostly focussed on oxidative damage and ROS were thought to be a key trigger for cell death. This view has been refined, leading to the understanding that the biological function of ROS is determined by numerous variables such as concentration, chemical type and cellular localization. For example, ROS and reactive nitrogen species may intercept inducible cell death under certain circumstances via the redox regulation of inducible caspase activity and/or by depleting cellular energy stores. Likewise, death of unwanted diseased or degenerative cells may be facilitated by pharmacologically enhancing the thiol status of such cells using redox-active α-lipoic acid.

Keywords: Antioxidant; Thiol; Cell death; Nutrition; Lipoic acid; Signal transduction

1. Introduction

Caspases are critical mediators of apoptotic cell death. The activation of these otherwise latent intracellular proteases is implicated in the execution of most, if not all apoptosis in mammals. Caspases are also implicated in various non-apoptotic aspects of cellular physiology (Zeuner et al., 1999), such as cytokine processing during inflammation, differentiation of progenitor cells during erythropoiesis and lens fiber development, and proliferation of T lymphocytes, thus attesting to the pleiotropic functions of these proteases (Fadeel et al., 2000). The caspase family is diversified by the presence of over a dozen members. Caspase-1 was discovered first. The finding was based on the sequence similarity to the *Caenorhabditis elegans* death gene, ced-3. Caspase-1 was originally labeled ICE (for interleukin-1-beta-converting enzyme). Subsequently, the entire gene family was unveiled. The term *caspase* was created to denote the Cysteine requiring ASPartate proteASE activity of these enzymes. The protease activity of the caspase family is unique in that they cleave following (C-terminal to) aspartate residues (Asp-X), a property shared only by the cytotoxic lymphocyte serine protease, granzyme B.

Caspases exist as inactive proenzymes made of a prodomain, a large subunit, and a small subunit which must be cleaved at caspase recognition sequences (Asp-X) to form the active enzyme (Fig. 1). This means that active caspases can autoactivate other caspases following an initial activating stimulus. Active caspases are tetramers consisting of two large and two small subunits from the cleaved proezymes. Each caspase cascade has two types of caspases involved, the upstream or class I caspases, and the downstream or class II caspases. Class I caspases are characterized by long amino-terminal prodomains that carry specific protein–protein interaction domains which mediate oligomerisation of caspases, often assisted by specific adaptor molecules (Salvesen and Dixit, 1999). Oligomerisation appears to be sufficient for autocatalytic activation of class I caspases. Once the first caspase in the pathway has been activated, it processes downstream caspases, initiating a cascade of amplifying events that lead to the apoptotic death of a cell. Among members of the

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caspase family, caspase-3 was shown to have the highest similarity to ced-3. All members of the caspase family contain the sequence **QACXG** that contains the active site cysteine. The putative active site of caspase 3 (CPP32, apopain, YAMA) contains a cysteine residue (Fernandes-Alnemri et al., 1994) that is subject to redox control (Sen et al., 1999c). Both thioredoxin and glutathione have been shown to be required for caspase-3 activity to induce apoptosis (Ueda et al., 1998). The regulation of inducible caspase 3 activity by oxidation–reduction (redox) dependent mechanisms is reviewed.

Redox based regulation of signal transduction and gene expression is emerging as a fundamental regulatory mechanism in cell biology (Sen and Packer, 1996; Sen, 1998, 2000). Electron flow through side chain functional CH_2–SH groups of conserved cysteinyl residues in proteins accounts for their redox-sensing properties. In most intracellular proteins thiol groups are strongly 'buffered' against oxidation by the highly-reduced environment inside the cell, therefore, only accessible protein thiol groups with high thiol-disulfide oxidation potentials are likely to be redox-sensitive. The list of redox-sensitive signal transduction pathways is steadily growing (Sen et al., 2001, 2000; Sen and Packer, 2002). Up until a few years ago, ROS research mostly focussed on oxidative damage and these species were thought to be a key trigger for cell death (Slater et al., 1995). This view has been refined over time and has led to our current understanding that the biological function of ROS is determined by numerous variables such as concentration, chemical type and cellular localisation (Irani, 2000). Indeed, a critical consideration of these variables is of vital importance because the net impact on overall cellular responses may even be reversed (Hampton and Orrenius, 1997). For example, as described below, ROS and reactive nitrogen species (RNS) may intercept inducible cell death under certain circumstances via the redox regulation of inducible caspase activity and/or by depleting cellular energy stores (Hampton and Orrenius, 1997; Lee and Shacter, 1999). Likewise, death of unwanted diseased or degenerative cells may be facilitated by pharmacologically enhancing the thiol redox status of such cells (Sen et al., 1999c).

2. Oxidant-dependent inhibition of inducible caspase activity

Programmed cell death, or apoptosis, represents a highly controlled form of cell death in which single cells are selectively eliminated without release of cellular debris and perturbation of neighboring tissues (Ashkenazi and Dixit, 1998; Granville et al., 1998; Green,

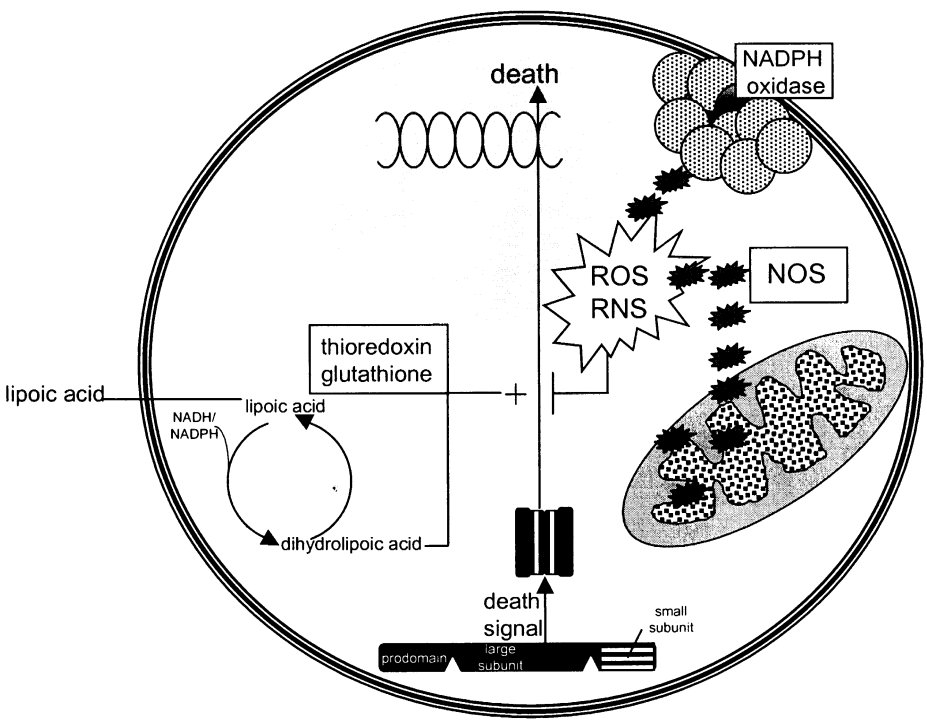

Fig. 1. Redox regulation of inducible caspase activity. Death signal cleaves caspase pro-enzyme to form active tetrameric caspase. Under certain conditions reactive oxygen and nitrogen species (ROS/RNS) may inhibit inducible caspase function and therefore counteract the execution of death. Cellular thiols such as glutathione and thioredoxin support inducible caspase activity. Unwanted harmful cells may be sensitized to inducible death using redox-active nutrients such as α-lipoic acid. Lipoic acid enters the cell and is reduced to dihydrolipoic acid, a potent reductant capable of regulating inducible caspase 3 activity. NOS, nitric oxide synthase.

1998). Apoptosis regulates several key physiological functions. For example, developing lymphocytes undergo extensive cell death during selection of the immune repertoire. Understanding the fundamental mechanism of apoptosis is crucial in developing therapeutic strategies for controlling apoptosis in diseased tissues (Holzman, 1997). Such information may be used to protect healthy cells against apoptosis, and also to selectively kill diseased cells. For example, inhibitors of apoptosis may be utilized to induce resistance to chemotherapeutic drugs and irradiation, whereas, inducers of apoptosis may be used to control neoplastic events that result from uncontrolled cell proliferation. Caspase 3 has been investigated as a therapeutic target to induce death of cancer cells (Martinez-Lorenzo et al., 1998; Yamabe et al., 1999; Shinoura et al., 2000). Under certain conditions, ROS and RNS have been shown to intercept caspase 3 mediated death. A physiological example of this is the NADPH oxidase-derived oxidants generated by stimulated neutrophils that prevent caspase activation in these cells. Human neutrophils have a short half-life and are believed to die by apoptosis or programmed cell death both in vivo and in vitro. It has been observed that caspases are activated in a time-dependent manner in neutrophils undergoing spontaneous apoptosis. However, in cells treated with the potent neutrophil activator phorbol 12-myristate 13-acetate (PMA), caspase activity was only evident after pharmacologic inhibition of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Similarily, inhibition of the NADPH oxidase in constitutive as well as Fas/APO-1-triggered apoptosis resulted in increased rather than suppressed levels of caspase activity, suggesting that ROS may prevent caspases from functioning optimally in these cells. Thus, caspases are an important component of constitutive and Fas/APO-1-triggered neutrophil apoptosis. However, these redox-sensitive death-executing enzymes are suppressed in activated neutrophils, and an alternate oxidant-dependent pathway is used to mediate neutrophil clearance under these conditions (Fadeel et al., 1998). Impairment of caspase-mediated death is also observed under conditions of GSH deficiency (Boggs et al., 1998). Other thiol blocking agents such as N-ethylmaleimide, or iodoacetamide has been shown to impair caspase function as well (Mohr et al., 1997). Enzymatic activity of caspase 3-like protease in cell lysates of UVB-exposed cells was repressed in vitro by the presence of selenite via an oxidant-dependent mechanism. Selenite also inhibited the in vitro activity of purified recombinant caspase-3. The inhibitory action of selenite on a recombinant active caspase-3 was reversed by sulfhydryl reducing agents, such as dithiothreitol and β-mercaptoethanol (Park et al., 2000).

The transmembrane Fas Ag is a member of the tumor necrosis factor/nerve growth factor receptor family, which can trigger apoptosis. Interaction between Fas–Fas ligand (FasL) transduces apoptotic signals in sensitive target cells. This pathway to induce programmed cell death has been suggested to be of potential use in cancer treatment. Treatment with anti-Fas Ab has been shown to suppress the growth of Fas bearing (Fas+) tumor cells (Shimizu et al., 1996). Also, malignant glioma cells are susceptible to Fas mediated apoptosis triggered by agonistic Ab (Roth et al., 1997). The killing of myelogenous leukemia cells by the Fas/FasL pathway has the remarkable potential of serving as a novel and effective approach for leukemia immunotherapy (Komada and Sakurai, 1997). Proliferation of vascular smooth muscle cells in response to injury plays a central role in the pathogenesis of vascular disorders. FasL gene transfer to the wall of blood vessel-induced apoptosis of Fas+ vascular smooth muscle cells and inhibited neointima formation in injured rat carotid artery (Sata et al., 1998). Thus, Fas-mediated apoptosis is expected to have therapeutic potential in certain disorders, especially cancer treatment. ROS and RNS have been observed to confer resistance to Fas-mediated apoptosis by inhibiting inducible caspase 3 activity. Tumor cells are usually rich in ROS and also resistant to oxidative damage. Clement and Stamenkovic first observed that increased intracellular superoxide anion concentration ($[O_2^{\bullet -}]i$) can abrogate Fas-mediated apoptosis in cells that are constitutively sensitive to Fas. Conversely, decreased $[O_2^{\bullet -}]i$ sensitized cells that are naturally resistant to Fas signals (Clement and Stamenkovic, 1996). A similar situation was evident in the study of virus-induced cell death. Elevated $[O_2^{\bullet -}]i$ served as a survival signal and strategies to lower $[O_2^{\bullet -}]i$ favored execution of death (Lin et al., 1999). These observations led to the hypothesis that manipulation of cellular redox state may serve as a productive strategy to modulate caspase-mediated death. It is thought that intracellular redox status may either trigger or block the apoptotic death program, depending on the severity of the oxidative stress (Hampton and Orrenius, 1998). While excessive oxidative stress is likely to trigger necrosis and levels of ROS much lower than that required to inflict oxidative damage may trigger death-signaling (Lieberthal et al., 1998; Turner et al., 1998; Li et al., 1999; Olejnicka et al., 1999; Kim et al., 2000), intermediary levels of ROS in a cell interrupt programmed cell death by intercepting caspase function.

S-nitrosylation (transfer of the NO group to a cysteine sulfhydryl {thiolate anion} to form an RS-NO; further oxidation of critical thiols can possibly form disulfide bonds) and oxidation of members of the caspase family by RNS accounts for their anti-death property (Haendeler et al., 1997; Mohr et al., 1997; Lipton, 1999; Stefanelli et al., 1999; Kolb, 2000; Li and Billiar, 2000). NO-mediated enzyme inhibition was fully re-

versible upon the addition of dithiothreitol (Mohr et al., 1997). NO supplied by exogenous NO donors serves in vivo as an anti-apoptotic regulator of caspase activity via S-nitrosylation of the Cys-163 residue of caspase-3 (Rossig et al., 1999). Inhaled NO, frequently administered in combination with hyperoxic gas mixtures, has been shown to protect against the injurious consequences of prolonged hyperoxia by impairing caspase function (Howlett et al., 1999). Denitrosylation of caspase 3 has been shown to be one mechanism that mediates Fas-induced death. Decreased caspase-3 S-nitrosylation in Fas-activated cells was associated with an increase in intracellular caspase activity (Mannick et al., 1999).

3. Thiol-dependent facilitation of inducible caspase activity

Given the central role of biological thiols in regulating numerous key cell regulatory processes, the focus has been turned toward identifying clinically relevant modulators of cellular thiol status (Sen, 1998, 2000; Sen et al., 2000; Sen and Packer, 2002). Among the several candidates that have emerged, *N*-acetyl-L-cysteine and α-lipoic acid represent the two most potent agents (Sen, 1997). Because α-lipoic acid can be reduced by NADH- and NADPH-dependent cellular enzymes, it has an advantage over *N*-acetyl-L-cysteine (Sen, 1997; Sen and Packer, 2000). Lipoic acid is present in trace amounts in vegetables. When added to cells it is promptly taken up by cells and reduced enzymatically to the corresponding reduced form, dihydrolipoic acid. Dihydrolipoic acid is a potent stimulator of cellular GSH synthesis and enhances overall cellular reduced thiol status (Sen et al., 1997, 1999a,b). The lipoic/dihydrolipoic acid redox-couple has a strong reducing power with -0.32 V as the reduction potential. Reduction potential of this redox couple is stronger than all other endogenous redox couples such as GSH/GSSG or $NADH/NAD^+$ (Jocelyn, 1967). The ability of dihydrolipoic acid to reduce protein thiols, e.g. thioredoxin has been evident (Packer et al., 1997).

Lipoic acid remarkably potentiated Fas mediated cell death in leukemic Jurkat cells, but not in healthy peripheral blood lymphocytes (Sen et al., 1999c). Previously chemotherapeutic agents such as doxorubicin, vincristine, and the alkaloid taxol have been shown to facilitate Fas-mediated cell death (Roth et al., 1997, 1998). Doxorubicin, vincristine and taxol are anti-tumor drugs that are used for cancer therapy. At high concentrations, these agents per se are toxic to cells. In contrast, lipoic acid is a safe nutrient, mostly known for its ability to bolster cellular glutathione levels, alter intracellular redox state and help protect against diabetic complications (Packer et al., 1997; Khanna et al., 1999a,b). This work presented first evidence showing that a redox active agent, lipoic acid, may potentiate Fas-mediated death in leukemic Jurkat cells. This observation is consistent with a previous report showing that ROS such as superoxide anion function as a natural inhibitor of Fas-mediated cell death (Clement and Stamenkovic, 1996), since dihydrolipoic acid is known to quench superoxide anions (Packer et al., 1997).

Because the potentiating effect of lipoic acid treatment on Fas-mediated apoptosis was observed in one of the earliest markers of apoptosis (externalization of membrane phosphatidyl serine), it was suspected that lipoic acid regulates one or more early intracellular events. Expression of the Fas receptor was not influenced by lipoic acid treatment, suggesting that intracellular events signaling for apoptosis may have been influenced. An early event in Fas-mediated apoptosis that was strikingly influenced by lipoic acid treatment was the activation of the caspase 3. The potentiating effect of lipoic acid treatment on Fas-mediated apoptosis of Jurkat cells was markedly decreased by a caspase 3 inhibitor, indicating that indeed increased caspase 3 activity in lipoic acid treated Fas-activated cells played a significant role in potentiating cell death. As described in a previous section, low concentration of hydrogen peroxide inhibits caspase activity in Jurkat cells (Hampton and Orrenius, 1997). Consistently we observed that the activity of purified caspase 3 protein was inhibited by hydrogen peroxide. Our report provided first evidence showing that indeed caspase 3 activity may be also potentiated by intracellular reducing agents such as dihydrolipoic acid demonstrating that inducible caspase 3 activity may be up-regulated pharmacologically. In a later study, Baker et al. provided further support to the contention that reductants may facilitate caspase 3 activity (Baker et al., 2000). They examined the ability of various recombinant human thioredoxins to activate caspase 3. The EC_{50} for caspase 3 activation by reduced thioredoxin-1 was 2.5 μM, by reduced glutathione 1.0 mM and by the bench-top reductant dithiothreitol 3.5 mM. A catalytic site redox-inactive mutant thioredoxin-1 was almost as active as thioredoxin-1 in activating caspase-3. Caspase activation was shown to correlate with the number of reduced cysteine residues in the thioredoxins. Reduced insulin and serum albumin were as effective on a molar basis as thioredoxin-1 in activating caspase-3 (Baker et al., 2000). Dithothreitol has been observed to enhance the caspase-dependent toxicity of As_2O_3. While the mechanism of such action of the dithiol was not clearly delineated in the study, the effect may be related to facilitation of inducible caspase activity by the reductant (Gurr et al., 1999).

Oxidants may trigger, facilitate or even prevent death depending on specific cellular conditions. Inducible caspase activity is sensitive to the cellular redox state (Fig.

1). The nature of this control is dependent on several factors such as the amount of oxidant produced and the kinetics of its generation. In addition to the well-established fact that oxidants may have cytotoxic properties, it is now clear that oxidants may prevent death by inhibiting inducible caspase activity. Unwanted harmful cells may be sensitized to inducible death by the use of redox-active nutrients such as α-lipoic acid.

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