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Invited Commentary

Reactive Oxygen Intermediates Regulate Cellular Response to Apoptotic Stimuli: An Hypothesis

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Production of reactive oxygen intermediates (ROI) has been thought for a long time to adversely affect the physiology and survival of a cell. There is now a growing body of evidence to suggest that ROI such as superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) can influence the growth, as well as death, of animal cells *in vitro*. The observation that cells release $O_2^{\bullet -}$ or its dismutation product H_2O_2, either constitutively in the case of tumor cells or following cytokine stimulation, has led to the speculation that they might possibly serve as intercellular messengers to stimulate proliferation via mechanisms common to natural growth factors. However, as the balance between cell populations in an organism is tightly controlled by the rate of proliferation and death of constituent cells, an increase in cell numbers could reciprocally be viewed as deregulation of cell death. Hence, it is equally important to decipher how ROI influence the response of cells to signals that activate cell death pathway(s). We propose that ROI not only regulate proliferation but also affect cell sensitivity to triggers which activate the cellular suicide program (apoptosis) versus those that cause accidental (necrotic) cell death.

Keywords: Apoptosis, superoxide, hydrogen peroxide, reduction

INTRODUCTION

Reactive oxygen intermediates (ROI) are produced in all mammalian cells, partly as a result of normal cellular metabolism, and partly due to activation of a variety of ROI-producing enzymes in response to exogenous stimuli. The principal intermediates are $O_2^{\bullet -}$ and H_2O_2.[1–4] These species are only moderately reactive with other biological molecules, but can sometimes give rise to the highly reactive hydroxyl radical ($^{\bullet}OH$) which might be directly responsible for much of the oxidative damage attributed to ROI in biological systems.[3–6]

Historically, ROI have been thought to be harmful to the cell.[4,6–8] This idea is supported by the fact that levels of ROI are tightly regulated by multiple defence mechanisms involving small anti-oxidant molecules, which often contain sulphydryl groups, and ROI-scavenging enzymes,

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such as superoxide dismutase (SOD), catalase, and glutathione peroxidase.[4,9] Alterations of any of these components of the oxidant defence system modulate the fate of ROI in the cell. However, it has been suggested that ROI could also have physiological roles, such as stimulation of cell division, and there is substantial experimental evidence to propose that ROI like $O_2^{\bullet -}$ and H_2O_2 constitute a novel class of cellular second messengers that appear to be involved in mitogenic stimulation of cultured cells.[10,11,39] Moreover, cellular pro-oxidant states where the intracellular concentrations of activated forms of oxygen are increased due to either overproduction or deficient antioxidant defence systems, can modulate expression of early growth-related genes like *c-fos* and *c-jun*[11] leading in some cases to aberrant expansion of rapidly growing clones and formation of tumors. Indeed, one characteristic of tumor cells with respect to normal cells is an increase in metabolic rate, ROI generation and decreased elimination of ROI, which may provide them with a survival advantage over their normal counterparts.[12] However, as an increase in cell numbers could either be due to induction of proliferation or a reciprocal defect in cell elimination, it is logical to propose that the survival advantage that a prooxidant state provides to tumor cells, could also be through creating an environment inhibitory to pathways required for efficiently carrying out the cell death program.

ROI PRODUCTION AND CELL DEATH

Based on morphologic and pathologic criteria, cell death is currently subdivided into two categories, necrosis (accidental cell death) and apoptosis (programmed cell death).[13,14] Aside from the phenotypic features that separate apoptotic cell death from necrotic cell death, apoptosis is an active process that involves activation of the caspases family of proteases. Activation of one or the other caspase members triggers a cascade of events culminating in specific morphological and biochemical changes leading to cell death[15–21] via proteolytic degradation of a number of cellular proteins, e.g. lamin, actin, and poly(ADP-ribose) polymerase.[22–26] On the contrary, necrotic cell death is a passive process, which results in disruption of the cell membrane, and death of the cell without a series of programmed events. While the role of ROI in necrotic cell death has been attributed to their ability to form highly reactive intermediates such as hydroxyl radical ($^{\bullet}OH$), evidence supporting a role for ROI in the apoptotic process is scant, and as yet controversial. A host of agents that induce apoptosis, such as TNFα, C2 ceramide, anti-IgM antibody, dexamethasone, irradiation and numerous anticancer drugs stimulate intracellular production of ROI, leading most frequently to an accumulation of H_2O_2. Similarly, many known inhibitors of apoptosis have antioxidant properties, or enhance the cellular antioxidant defence mechanism.[27,28] A direct cause and effect relationship between ROI and apoptosis has also been reported by direct treatment of tumor cells with low doses of H_2O_2.[29–31] Although the evidence that H_2O_2 can activate the apoptotic pathway is compelling, it has to be reconciled with other observations that cells can undergo spontaneous, as well as triggered apoptosis even in the absence of ROI, as shown in near anaerobic conditions.[32,33] Moreover, recent data demonstrate that scavenging or inhibiting intracellular ROI facilitates the execution of the cell death program.[34,35] Thus, whereas under certain circumstances ROI can efficiently activate apoptosis, it is clear that they are not necessary in the execution of the apoptotic program.[28] Hence, we propose that in addition to activate apoptosis, intracellular ROI are also important regulators of the cellular response to apoptotic stimuli.

ROI REGULATE RESPONSE TO APOPTOTIC STIMULI

Increased intracellular ROI concentration is an accepted characteristic of tumor cells, and has

been linked to a deficient intracellular antioxidant system.[36] There is evidence linking antioxidant enzymes, specifically superoxide dismutase (SOD), with cell differentiation[37,38] and density limitation of growth.[36] More recent findings demonstrate that a mild increase in intracellular $O_2^{\bullet-}$ concentration can affect cell response to apoptosis induced by either the cell surface receptor CD95,[35] anticancer agents [34] or other inducers of apoptosis, such as C2 ceramide and staurosporine (Clement and Pervaiz, unpublished data). An increase in intracellular $O_2^{\bullet-}$ concentration achieved by either its overproduction (direct or drug-induced) or as a result of an inhibition of Cu/Zn SOD, affects tumor cell apoptosis triggered by anticancer drugs, by interfering either directly or indirectly with the caspase activation pathway. For example, significantly lower caspase 3 activation is detected following induction of apoptosis by etoposide or daunorubicin in a melanoma cell line tailored to possess an increased intracellular $O_2^{\bullet-}$ concentration by expression of an antisense SOD mRNA, compared to the control cell line.[34] Similarly, low concentrations of H_2O_2 ($< 200\ \mu M$) effectively activate caspase 3 and induce apoptosis, whereas higher concentrations inhibit protease activity and the cells eventually succumb to this overwhelming increase in intracellular ROI and undergo necrotic instead of apoptotic cell death.[30] Thus, we propose that the survival advantage provided by a pro-oxidant environment in tumor cells may well be a function not only of increased cell proliferation[11,39] but also of resistance to apoptotic signals through a direct or indirect inhibition of the caspase pathway.

Oxidation–reduction state of the cell, better known as the redox state has been shown to influence the physiology of the cell. For example, changes in redox state influence transcription factor activation,[40] protein conformation and phosphorylation states, as well as cytosolic Ca^{2+} metabolism.[6] Any of these events may potentially influence caspase activity. Moreover, recent findings have shown that intracellular acidification induces apoptosis by directly stimulating caspase activity.[41] Therefore, it has been proposed that due to the nature of the caspases as cysteine proteases, cells may need to maintain some degree of reduced environment for effective activation of these enzymes and successful execution of the apoptotic program[30,42] reinforcing the notion that oxidative stress would inhibit rather than activate the apoptotic machinery.

Now that we have established that a mild oxidative stress can inhibit induction of apoptosis, we have to explain these findings in the light of the age-old dogma in free radical biology that oxidative stress induces apoptosis. Recent observations from our laboratory may help to propose an explanation for this apparent conflict. We show that apoptosis induced by low doses of H_2O_2 exhibits two major characteristics, namely, a decrease in intracellular $O_2^{\bullet-}$ concentration and acidification of the intracellular milieu which suggests a trend toward reduction as opposed to oxidation of the intracellular environment.[29] Thus, similar to apoptosis induced in the absence of ROI, a relatively more reduced (decreased $O_2^{\bullet-}$ concentration and low pH) intracellular state facilitates H_2O_2-induced apoptotic cell death.[29,43] By contrast, cell death triggered by H_2O_2 concentrations that induce an overwhelming oxidative stress, as measured by a dramatic drop in the total intracellular glutathione content, is of the necrotic nature.[29] These observations suggest that a decrease in $O_2^{\bullet-}$ concentration, coupled with an increase in intracellular H^+ ion concentration (reduced intracellular milieu) may represent a common mechanism for triggering apoptosis by stimuli which, either induce generation of low levels of H_2O_2 or directly inhibit intracellular $O_2^{\bullet-}$ production. We, therefore propose to refer to the mechanism of apoptosis induced by such stimuli as "reductive stress" as opposed to "oxidative stress" which, should be reserved for production of intracellular ROI that induces necrotic as opposed to apoptotic cell death.

CONCLUSION

In order to survive, all cells depend on a constant repression of their intrinsic suicide program by signals from surrounding cells and the extracellular matrix.[44] An important effect of extracellular survival signals may therefore be to maintain a critical cellular redox equilibrium, based, at least in part, on adequate intracellular ROI production. Thus, an oxidative shift in the cellular redox state may be critical in directly inducing or regulating tumor cell response to apoptotic stimuli. Whereas a high concentration of intracellular ROI (oxidative stress) provides a direct effector mechanism for necrotic cell death, a mild increase of ROI (pro-oxidant state) could provide protection against apoptosis. By contrast, low intracellular levels of ROI which favor reduction of the intracellular milieu (reduced state) will sensitize cells to apoptotic triggers, which eventually could lead to spontaneous apoptosis as seen under hypoxic conditions (Figure 1). Moreover, because much of the H_2O_2 produced in the cell is due to $O_2^{\bullet -}$ dismutation by SOD, we propose that the regulation of cell sensitivity to apoptotic stimuli by ROI is mainly a function of intracellular $O_2^{\bullet -}$ levels. Thus, protection against apoptosis can be attributed to an intracellular increase in $O_2^{\bullet -}$ concentration, but in contrast if intracellular $O_2^{\bullet -}$ levels fall toward the apoptotic threshold (reduced state) the cell will be highly responsive to any apoptotic trigger or even undergo spontaneous apoptosis. Understanding the precise mechanism by which such regulation occurs will surely represent the next challenge in the fields of free radical and apoptosis research.

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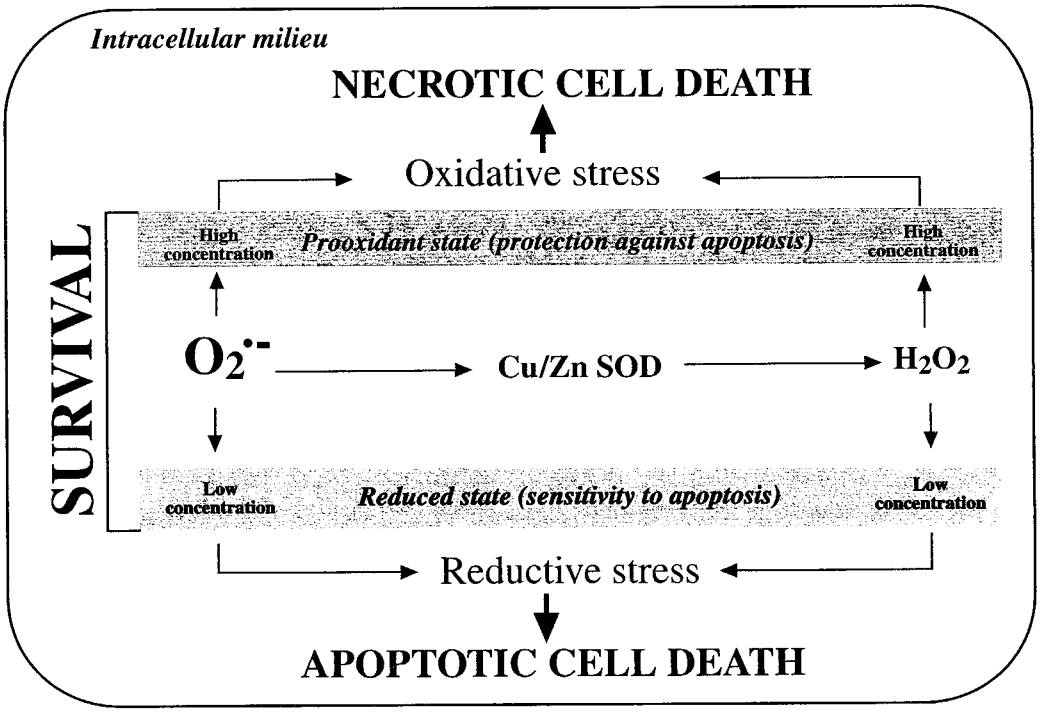

FIGURE 1 Regulation of cell sensitivity to apoptosis by intracellular ROI.

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