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Age-related decline in cellular response to oxidative stress: links to growth factor signaling pathways with common defects

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Abstract

Accumulation of oxidative damage is believed to be a major contributor to the decline in physiologic function that characterizes mammalian aging, and recent studies suggest that how well you respond to acute oxidative stress is an important factor in determining longevity. Oxidant injury elicits a wide spectrum of responses ranging from proliferation to cell death. The particular outcome observed largely reflects the severity of the stress encountered and the relative degree of activation of various signal transduction pathways aimed at enhancing survival or inducing cell death. Herein we examine the relationship between pathways important in supporting cell survival in response to oxidant injury and those involved in regulating proliferation. We review evidence indicating that [Curr. Opin. Cell Biol. 10 (1998) 248] common pathways are indeed involved in regulating these responses, and [Physiol. Rev. 82 (2002) 47] alterations in shared signaling events likely account for the age-related decline in the ability of cells to respond to both proliferative signals and oxidant stimuli. Published by Elsevier Science Inc.

Keywords: Reactive oxygen species; Aging; Calorie restriction; Mitogen-activated protein kinase; PI3-K/Akt; Proliferation; Growth factor signaling

1. Introduction

A decline in physiologic function constitutes a hallmark of mammalian aging. Two particular features that have been associated with this reduced physiologic function are a reduction in proliferative capacity following mitogenic stimulation and reduced tolerance to stress. The question we would like to pose in the following discussion is, are these features related, and if so, how? The answers we hope to convince you of are, yes, by the signaling pathways that serve to regulate them. Focusing on the cellular response to oxidative stress, we will review evidence showing that signal transduction pathways important in promoting survival following oxidant injury are intimately linked to those regulating proliferation. Further, drawing from our own studies with cultured primary hepatocytes derived from young adult vs. aged rats, we will summarize findings indicating that the ability of cells to respond to both proliferative signals and oxidative damage is diminished with aging, in a manner consistent with common signaling defects. Finally, we will provide evidence that an inability of aged cells to mount host defenses to oxidant injury compromises cellular homeostasis and increases their vulnerability to oxidative stress.

2. Role of oxidants and oxidative stress in aging and longevity

The term oxidant (used synonymously with oxidizing agent) refers to a molecule capable of serving as an electron acceptor in an oxidation-reduction reaction. Molecular oxygen possesses strong oxidizing capacity and as such interacts with a variety of other molecules resulting in the generation of partially reduced metabolites of oxygen possessing higher reactivities than molecular oxygen. So-called reactive oxygen species (ROS), these diverse molecules include superoxide anions, hydroxyl radicals and hydrogen peroxide. Exposure to ROS is unavoidable for cells living in an aerobic environment, as ROS are continually generated within the cell both during normal

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Abbreviations: ROS, reactive oxygen species; ERK, extracellular signal-regulated kinase; PI3-K, phosphoinositide 3-kinase; PLC-γ1, phospholipase C-γ1; JNK, c-*jun* N-terminal kinase; NF-κB, nuclear factor-kappa B; JAK/STAT, janus protein tyrosine kinase/signal transducers and activators of transcription; EGF, epidermal growth factor; PDGF, platelet-derived growth factor.

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metabolic activity and as a consequence of environmental stimuli. They can also be taken up directly by cells from the extracellular milieu. ROS serve as specific signaling molecules under both physiological and pathophysiologic conditions, and the transient generation of ROS, within boundaries, is essential to maintain homeostasis [1,2]. For example, ROS are generated transiently in response to growth factor stimulation and serve important second messenger functions in regulating proliferation. However, when present at high and/or sustained levels, ROS can cause severe damage to DNA, protein and lipids. Cells possess a host of antioxidant defenses aimed at controlling the level of ROS, but these mechanisms are not always sufficient to counteract the production of ROS, resulting in what is termed a state of oxidative stress.

The idea that ROS play an important role in aging was first advanced nearly half a century ago in the ''free radical theory of aging'' [3]. Research over the past few decades has provided strong evidence that oxidative stress is an important factor in the development of many age-associated diseases and oxidative damage is believed to contribute to the general decline in physiologic function that occurs during normal aging [4]. In accordance with this view, mammalian aging is associated with the accumulation of proteins, lipids and DNA that have been modified by oxidative damage [5,6]. Calorie restriction, the only intervention known to delay the onset of age-related deficits and increase lifespan of mammals, reduces the levels of such modified molecules [7], improves thermotolerance and reduces heat-induced oxidative damage in aged rats [8]. Strong genetic links between resistance to oxidative stress and longevity have been established for a number of species including yeast, *Caenorhabditis elegans*, *Drosophila* and mice. A number of specific mutations resulting in increased longevity have been shown to confer tolerance to acute oxidative insults [9–14]. Conversely, mutations associated with reduced longevity have been linked to reduced stress tolerance [15]. Finally, approaches capable of increasing resistance to oxidative stress, such as transgenic expression of antioxidant proteins or treatment with antioxidant mimetics, in some instances lead to increased lifespan [16–18]. Such findings argue strongly that not only is oxidative damage an important contributor to aging, but how well we respond to oxidative insults is likely to be a key factor in combating age-related declines in physiologic function. We propose that the ability to mount these host defenses to acute oxidative injury are likewise diminished as a function of aging and this contributes to the decline in stress tolerance.

3. Role of growth factor receptor pathways in mediating signaling and survival following exposure to oxidants

At the cellular level, oxidant exposure can elicit a wide spectrum of phenotypic responses ranging from proliferation to growth arrest, to senescence or cell death. The particular response seen varies widely dependent on the nature and severity of the stimulus, as well as the cell type examined. Whatever the outcome, it largely reflects the balance between a variety of intracellular stress signaling pathways that are activated in response to the stress [19]. Among the major signaling pathways known to be activated in response to oxidant injury are the extracellular signal-regulated kinase (ERK), c-*jun* N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) signaling cascades, the phosphoinositide 3-kinase (PI3-K)/Akt pathway, nuclear factor (NF)-κB, p53, phospholipase C-γ1 (PLC-γ1), janus protein tyrosine kinase (JAK)/signal transducers and activators of transcription (STAT) pathway, and the heat shock response. These pathways exert their phenotypic effects largely by modulating the activities of transcription factors, which, in turn, lead to alterations in the pattern of gene expression. In general, ERK, PI3-K/Akt, PLC-γ1, JAK/STAT and the heat shock response exert a prosurvival influence during oxidant injury, whereas activation of p53, JNK and p38 are more commonly linked to apoptosis. However, it is important to emphasize that many exceptions to these generalities can be found.

Of particular relevance to the present discussion, three of the pathways shown to support survival following oxidant injury are known to play important roles in regulating growth and differentiation through transduction of proliferative signals to the nucleus. These include ERK, Akt and PLC-γ1. The signaling cascade leading to ERK activation following growth factor stimulation is well established [20]. In brief, ligand-mediated dimerization of growth factor receptors stimulates intrinsic tyrosine kinase activities leading to autophosphorylation of receptor tyrosine residues, which serve as docking sites for the recruitment of downstream signaling mediators necessary for activation of membrane-localized Ras. Ras in turn activates Raf, marking the start of the sequential phosphorylation cascade in which Raf phosphorylates and activates MEK, which in turn phosphorylates and activates ERK. Akt (also known as protein kinase B) is activated following growth factor stimulation *via* a PI3-K dependent pathway in which PI3-K-mediated generation of 3′-phosphorylated phosphoinositides leads to the recruitment of Akt to the cell membrane where it undergoes phosphorylation by phosphoinositide-dependent kinases [21]. PLC-γ1 is an essential component of a third growth factor receptor signaling pathway that can be activated by oxidant injury. Present in the cytoplasm of unstimulated cells, growth factor stimulation results in the translocation of PLC-γ1 to the membrane allowing its phosphorylation by receptor and nonreceptor tyrosine kinases. Activated PLC-γ1 then catalyzes the hydrolysis of phosphatidylinositol 4,5-P2 to inositol 1,4,5-triphosphate and diacylglycerol, which act as second messengers to provoke the mobilization of Ca^{2+} and activation of protein kinase C, respectively [22].

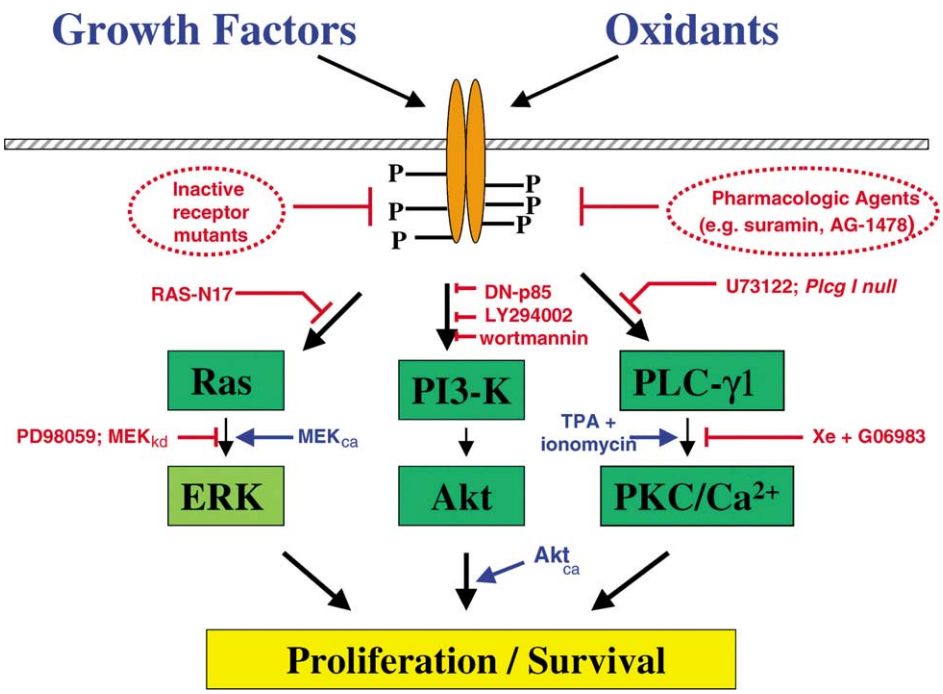

Fig. 1. Proliferation-associated pathways activated by oxidants and important for cell survival. Growth factors as well as oxidants result in receptor phosphorylation leading to the activation of ERK, Akt and PLC-γ1. Inhibition of growth factor receptor phosphorylation can prevent activation of the pathways in response to oxidative insults. Other pharmacologic and genetic approaches can be used to either inhibit or enhance the activities of the respective pathways by oxidant signals. Inhibition of the responses compromises survival, while enhancement of their activities improves survival.

In all three cases, oxidants seem to activate these pathways largely through stimulation of growth factor receptors, mimicking the actions of normal ligands (Fig. 1). Many growth factor receptors including the epidermal growth factor (EGF) receptor, platelet-derived growth factor (PDGF) receptor, and the T-cell receptor complex have been shown to undergo enhanced phosphorylation in response to oxidative insults such as hydrogen peroxide, asbestos, short wave length ultraviolet radiation, and sodium arsenite [23–29]. Interference with the phosphorylation of these receptors achieved by treatment with either broad spectrum inhibitors such as suramin, or selective inhibitors of specific receptors such as the EGF receptor in many cell types, has been shown to attenuate ERK activation in response to oxidant exposure [23–29]. Likewise, expression of inactive mutant forms of various growth factor receptors reduces activation of ERK by oxidative stress [23,24]. In contrast, overexpression of normal growth factor receptors, such as the Trk receptor for nerve growth factor in rat PC12 cells, results in enhanced activation of ERK by hydrogen peroxide [32]. EGF receptor-mediated signaling may play a particularly important role in the response of oxidants, as interference with its activation also attenuates hydrogen peroxide-induced activation of PI3-K/Akt and PLC-γ1 [29,33]. PDGF receptor inhibition has likewise been shown to prohibit Akt activation in response to certain oxidants [28].

Various strategies have been used to examine the importance of these three pathways in influencing cell survival following oxidant injury (see Fig. 1). While some exceptions certainly exist [34–36], most studies have documented that, in general, pharmacologic agents (including inhibitors of EGF receptor phosphorylation) as well as molecular alterations resulting in reduced ERK activation, lead to senstitization of cells to hydrogen peroxide, while molecular strategies resulting in elevated ERK activation enhance survival of cells treated with the oxidant [30,31,37–40]. Similarly, pharmacologic agents that inhibit PI3-K/Akt activity lead to increased sensitivity of cells to hydrogen peroxide, while expression of constitutively active mutant forms of Akt enhances survival [29,41–44]. Finally, both pharmacologic and genetic approaches have demonstrated that cells rendered deficient in PLC-γ1 activity display heightened sensitivity to toxic effects of hydrogen peroxide [33,45]. Taken together, the above evidence indicates that growth factor receptor signaling pathways contribute to the activation of ERK, Akt and PLC-γ1 by oxidant injury, and that the activation of the three pathways during oxidative stress promotes cell survival.

4. Pathways contributing to age-related decline in proliferative capacity

The proliferative capacity of many different cell types has been shown to decline with aging [46]. This has been extensively studied in T lymphocytes, for which many

age-related alterations in T cell signaling have been identified and associated with the reduced mitogenic response [47,48]. In human, rat and mouse cells alike, it has been found that mitogenic stimulation results in lower ERK activation in aged T cells relative to young T cells [49–51]. Caloric restriction largely prevents the age-related loss in ERK activity and inhibits the decline in proliferative capacity [52]. In all cases, the 'defect' in aged cells appears to lie at an early step in the signaling pathway at or around the level of the T cell receptor complex.

Hepatocytes also show a reduction in proliferative capacity as a function of aging, both *in vivo* and *in vitro*. Following partial hepatectomy, DNA synthesis is delayed and reduced in magnitude in aged rats [53]. Likewise, EGF-stimulated DNA synthesis is markedly lower in cultured primary hepatocytes obtained from aged donors compared to similarly treated cells of young donors [54,55]. We and others have demonstrated that this reflects an age-related reduction in EGF-stimulated ERK activation [55,56]. The defect in aged cells is believed to lie at the level of the EGF receptor, and perturbations in its interaction with Shc adaptor proteins [56,57]. Although overall phosphorylation patterns do not differ between young and old cells, it has been suggested that a specific reduction in EGF-stimulated phosphorylation of tyrosine residue 1173 (which lies within the Shc-binding domain) accounts for the age-related defect in ERK signaling in response to EGF [56]. More recently, we have shown that EGF-stimulated Akt activation is also lower in aged hepatocytes (unpublished findings), but the mechanism remains unclear.

5. Pathways contributing to age-related decline in oxidative stress tolerance

Based on our knowledge concerning the intimate links between signaling pathways involved in regulating proliferation and oxidative stress responsiveness, and prior findings indicating that EGF-induced ERK and Akt activations are reduced with aging in rat hepatocytes, we hypothesized that aged hepatocytes would show diminished responsiveness to oxidant stimulation and that this would be associated with greater sensitivity to the stress. Examining the responses of young and old hepatocytes to a wide range of hydrogen peroxide concentrations, we have found that low concentrations of the oxidant (50–100 μM) are mitogenic for hepatocytes, while higher doses (300–1000 μM) inhibit proliferation and result in the induction of apoptosis in a dose-dependent fashion. Such a mitogenic effect of hydrogen peroxide has been described by others [58]. Concentrating first on the response to the higher doses of hydrogen peroxide (300–1000 μM), we observed that hepatocytes derived from aged hosts (24–26 months old) were much more sensitive to hydrogen peroxide-induced apoptosis than those of young adult rats (4–6 months) [40]. Importantly, this was associated with reduced activations of both ERK and Akt. In keeping with findings in other cell types, we found that inhibiting the activities of ERK and Akt in young hepatocytes with pharmacologic agents, reduced their survival to a level similar to that seen in old cells. Consistent with the diminished responsiveness of aged cells to EGF stimulation, the mitogenic response of hepatocytes to low doses of hydrogen peroxide is also greatly attenuated in aged cells. Again, this correlates with reduced activation of ERK and Akt (unpublished findings).

6. Calorie restriction reduces the age-related declines in proliferation and oxidative stress responsiveness

Calorie restriction, or the limiting of food intake without deprivation of essential nutrients, has been shown to extend lifespan in a wide range of species [7]. In rodents, in particular, it has been shown to prevent or slow the progression of many age-related diseases as well as attenuate the declines in physiologic function that occur with normal aging. Based on this information, we hypothesized that calorie restriction would prevent the age-related loss in ERK and Akt activation in response to growth factor treatment and oxidant stimulation, and thereby enhance proliferation and improve stress tolerance of old rats. Comparing the responsiveness of hepatocytes derived from aged animals subjected to *ad lib.* feeding conditions with those from animals placed on calorie restriction we have obtained evidence supportive of this view. Indeed, both EGF- and low dose hydrogen peroxide-stimulated DNA synthesis and resistance to toxic concentrations of hydrogen peroxide are improved in cells of rats maintained on long term calorie restriction ([40] and unpublished findings), and both effects are likewise correlated with higher activation of ERK and Akt in response to the stimuli. Curiously, however, we have observed that the ability of calorie restriction to enhance responsiveness to stress signals is much greater than its ability to enhance proliferation. That is, while ERK activation and tolerance to oxidative stress in aged calorie restricted rats approaches that seen in young animals (both *ad lib.* and calorie restricted groups), the proliferative response to EGF and mitogenic concentrations of hydrogen peroxide remain significantly lower than that seen in young *ad lib.* fed rats. This may reflect the fact that calorie restriction itself results in an attenuated response to proliferative signals, even in young animals. This finding, though initially surprising to us, is consistent with other reports in the literature indicating that restricting calorie intake attenuates the regenerative process in liver following partial hepatectomy [59,60], and raises the possibility that perhaps it is the limitation of this response early on by calorie restriction that preserves the capacity to proliferate over the long term.

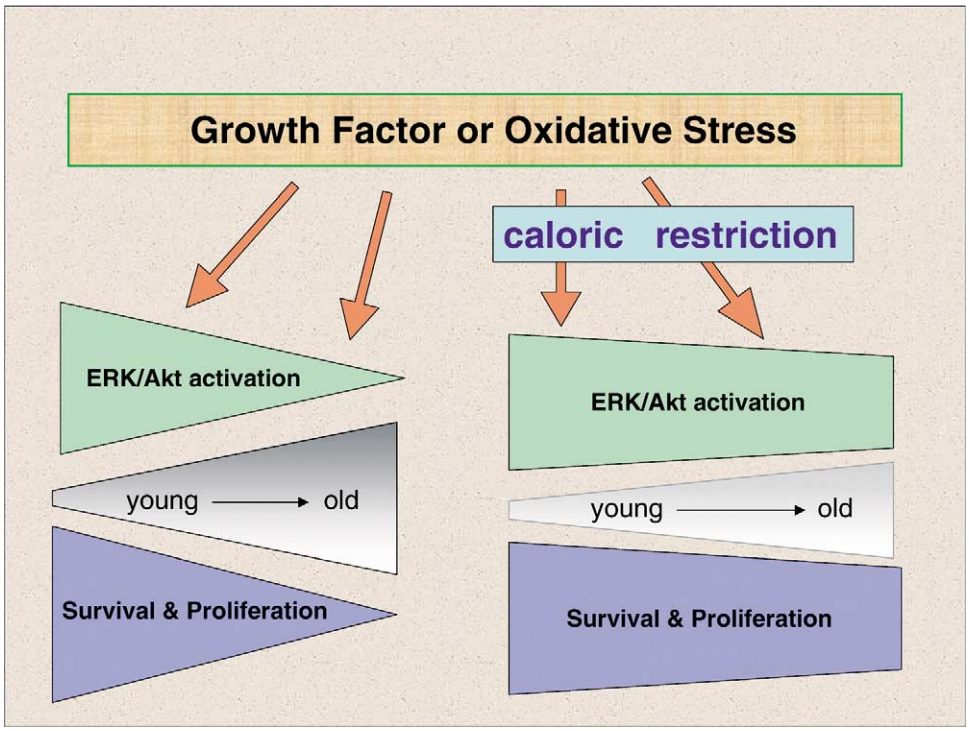

Fig. 2. Model summarizing age-related changes in the hepatocyte's response to growth factor and oxidative stress signals and the influence of CR on these processes. Activation of ERK and Akt are attenuated with aging, leading to reduced proliferation and a decline in oxidative stress tolerance. CR acts to prevent the loss in ERK and Akt activities, preserving proliferative capacity and improving survival of cells exposed to oxidative stress.

7. Summary and conclusions

There is much anecdotal support for the notion that mammalian aging is associated with a reduced ability to withstand environmental insults, but experimental evidence linking a decline in stress tolerance to alterations in specific pathways known to be involved in regulating stress responsiveness at the cellular level has been lacking. As discussed here, the cell's ability to activate signaling pathways important for survival following oxidative stress is intimately linked to its ability to respond to proliferative signals. As depicted in Fig. 2, both responses decline as a function of normal aging, but can be largely preserved in animals maintained on calorie restricted diets, and even restored to a substantial degree in aged animals by short term calorie restriction. These findings suggest the likelihood of a common defect as a mediator of both effects. Given, that growth factor receptors play an important role in transducing signals to multiple pathways involved in both responses, it is likely that the defect lies at this level. Better understanding of the nature of the signaling alterations that occur with aging and how calorie restriction works to prevent the changes could lead to the development of strategies to boost these responses in the aged cell. Such strategies could offer significant therapeutic benefits for the aged host.

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