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Review Article

Redox regulation of antioxidants, autophagy, and the response to stress: Implications for electrophile therapeutics

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ABSTRACT

Redox networks in the cell integrate signaling pathways that control metabolism, energetics, cell survival, and death. The physiological second messengers that modulate these pathways include nitric oxide, hydrogen peroxide, and electrophiles. Electrophiles are produced in the cell via both enzymatic and nonenzymatic lipid peroxidation and are also relatively abundant constituents of the diet. These compounds bind covalently to families of cysteine-containing, redox-sensing proteins that constitute the electrophile-responsive proteome, the subproteomes of which are found in localized intracellular domains. These include those proteins controlling responses to oxidative stress in the cytosol—notably the Keap1-Nrf2 pathway, the autophagy-lysosomal pathway, and proteins in other compartments including mitochondria and endoplasmic reticulum. The signaling pathways through which electrophiles function have unique characteristics that could be exploited for novel therapeutic interventions; however, development of such therapeutic strategies has been challenging due to a lack of basic understanding of the mechanisms controlling this form of redox signaling. In this review, we discuss current knowledge of the basic mechanisms of thiol-electrophile signaling and its potential impact on the translation of this important field of redox biology to the clinic. Emerging understanding of thiol-electrophile interactions and redox signaling suggests replacement of the oxidative stress hypothesis with a new redox biology paradigm, which provides an exciting and influential framework for guiding translational research.

Contents

Abbreviations: ALDH2, aldehyde dehydrogenase 2; ANT, adenine nucleotide transportor; AR, aldose reductase; AMPK, AMP-activated protein kinase; ATF6, activating transcription factor 6; ATG, AuTophaGy; ATM, ataxia-telangiectasia mutate; COPD, chronic obstructive pulmonary disease; EMA, European Medicines Agency; ER, endoplasmic reticulum; ESR, electrophilic stress response; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; GRP78/BiP, 78-kDa glucose-regulated protein; GST, gluthione-*S*-transferase; HNE, 4-hydroxynonenal; HSE, heat shock element; HSF1, heat shock factor-1; HSP, heat shock protein; HSR, heat shock response; IRE1, inositol-requiring protein-1; ME, malic enzyme; mTOR, mammalian target of rapamycin; MS, multiple sclerosis; NNT, nicotinamide nucleotide transhydrogenase; NOS, nitric oxide synthase; Nox, NADPH oxidase; LKB1, liver kinase B1; LC3, microtubule-associated protein light chain 3; p62/SQSTM1, sequestosome-1; PERK, double-stranded RNA-dependent protein kinase (PKR)-like ER kinase; ROS, reactive oxygen species; UPR, unfolded protein response

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Introduction

In the field of free radical biology, the "oxidative stress paradigm" has been the central dogma that has provided the framework for understanding the mechanisms leading to the development of novel therapeutics. It is an attractive concept that simply postulates that there is a balance between free radicals or oxidants [commonly called reactive oxygen species (ROS) or reactive species] with antioxidants in normal physiology. Pathology occurs when reactive species are produced in excess of the endogenous antioxidants, and this leads to indiscriminate damage to cellular macromolecules (proteins, lipids, and DNA) and kills cells [1]. Interestingly, much of the evidence for this process occurring in health and disease is derived from the oxidative modifications of proteins by products of lipid peroxidation—the reactive lipid species [2–6]. Accordingly, the development of therapeutics initially focused on developing compounds that could terminate the lipid peroxidation chain reaction such as α-tocopherol or dietary-derived polyphenolics [7].

The oxidative stress paradigm resulted in the widespread notion that supplementation of dietary antioxidants that target lipid peroxidation will prevent many human diseases. Over time, the mechanistic basis of the concept was largely forgotten and instead of the oxidative stress hypothesis becoming more precise in terms of molecular targets and mechanism, it became diffuse and nonspecific. This has unfortunately resulted in the widely held belief that all ROS are extremely reactive and share common biophysical properties and that all antioxidants are then also capable of scavenging any reactive species irrespective of the biochemical mechanism. The antioxidants which have achieved most attention in this respect are those that intercept lipid radicals and include α-tocopherol (vitamin E), β-carotene, ascorbic acid (vitamin C), and the numerous natural polyphenolic compounds present in the diet [8–10]. However, despite excellent animal model studies, basic research, and epidemiological data that collectively show that oxidative protein modifications by reactive lipid species are increased in many chronic diseases, controlled clinical trials with lipid radical scavenging antioxidants have not yielded the anticipated benefits [6,11–19].

It is now clear that several critical predictions of the oxidative stress paradigm are not supported by experiment. Using advanced mass spectrometry techniques, it has become possible to measure both the frequency of modification of biomolecules by reactive species and their levels *in vivo*. In direct contrast to the predictions from the oxidative stress paradigm in oxidant-dependent pathologies, the relative levels of protein modification are extremely low, and antioxidants are still abundantly present in the cells and tissues [20,21]. In addition, the hypothesis predicts that exogenous oxidants should contribute to pathology. This is indeed the case, but the levels of exogenous oxidants needed to place the system out of balance *in vitro* and *in vivo* are orders of magnitude higher than the levels that can ever be produced in biology in either health or disease.

At the inception of the oxidative stress hypothesis, the concept that endogenous molecules such as nitric oxide or hydrogen peroxide played a role in cell signaling had not been developed. It is now clear that not only do low levels (typically 10–100 nM) of these compounds play a role in cell signaling, but, as with other signaling pathways, control is exerted in specific domains which are not in redox equilibrium with the rest of the cell. We have proposed that endogenous antioxidants serve as redox insulators of these cell signaling domains [22]. Because exogenous signaling molecules such as hydrogen peroxide must break down the redox insulation before an effect can be observed, high nonphysiological concentrations are often needed. Thus, the idea that "free radicals are bad and antioxidants are good" is clearly undergoing a critical and high-profile reappraisal [23]. As the field of redox biology has developed, it has become apparent that the major predictions of the oxidative stress paradigm do not effectively explain the biological actions of reactive species and are not supported by experimental evidence. In this review, we propose that the oxidative stress hypothesis has reached the limits of its utility and should be replaced with the "redox biology paradigm" in which antioxidants play the primary role of modulating the complex networks controlling cell signaling and metabolism.

While it is possible that the modifications of proteins by reactive species are an unimportant epiphenomenon, it is clear that this is not the case; reactive species (including nitric oxide, hydrogen peroxide, and reactive lipid species) are known to act as cell signaling molecules, supporting the need for a reevaluation of the oxidative stress paradigm [22,24–28]. With the discovery that nitric oxide is a signaling molecule, the field is now embracing the paradigm that reactive species play an essential role in biology and that antioxidants serve a regulatory, not a protective, function. An important example in the field has been the realization that one class of reaction products from both enzymatic and nonenzymatic lipid peroxidation is electrophilic and can selectively modify families of cysteine-containing proteins, or electrophile-responsive proteomes, so modulating cell function [22,29]. That these products are derived from lipid radical targets of α-tocopherol, vitamin C, and β-carotene likely explains the tight biological control of levels of these molecules in human subjects and the marginal beneficial effects of supplementation [30]. In this context, the role of radical scavenging antioxidants such as vitamin E is to control the domain and levels of reactive lipid species for normal redox cell signaling. The impact of these new concepts on the development of redox therapeutics is now emerging and is discussed below.

Electrophile signaling as the master regulator of cellular antioxidant regulation

Cells have developed intricate mechanisms by which they sense and adapt to oxidants and electrophiles that are either endogenous or environmental in origin. There are several stress-responsive signaling pathways that are activated by endogenously produced electrophiles or xenobiotics [22,29,31–34]. As with other