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Mini review

Oxidative proteome modifications target specific cellular pathways during oxidative stress, cellular senescence and aging

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ABSTRACT

Oxidatively modified proteins build-up with age results, at least in part, from the increase of reactive oxygen species and other toxic compounds originating from both cellular metabolism and external factors. Experimental evidence has also indicated that failure of protein maintenance is a major contributor to the age-associated accumulation of damaged proteins. We have previously shown that oxidized proteins as well as proteins modified by lipid peroxidation and glycoxidation adducts are accumulating in senescent human WI-38 fibroblasts and reported that proteins targeted by these modifications are mainly involved in protein maintenance, energy metabolism and cytoskeleton. Alterations in the proteome of human muscle adult stem cells upon oxidative stress have also been recently analyzed. The carbonylated proteins identified were also found to be involved in key cellular functions, such as carbohydrate metabolism, protein maintenance, cellular motility and protein homeostasis. More recently, we have built a database of proteins modified by carbonylation, glycation and lipid peroxidation products during aging and age-related diseases, such as neurodegenerative diseases. Common pathways evidenced by enzymes involved in intermediate metabolism were found targeted by these modifications, although different tissues have been examined. These results underscore the implication of potential deleterious effects of protein irreversible oxidative modifications in key cellular pathways during aging and in the pathogenesis of age-related diseases.

1. Introduction

Accumulation of damaged macromolecules, including oxidatively damaged proteins, is a hallmark of cellular and organismal aging. This accumulation has been viewed as the combined result of increased production of reactive oxygen species (ROS) and other toxic compounds coming from both cellular metabolism and external factors as well as the failure of protein maintenance (i.e. degradation and repair) with age. Protein oxidation is particularly detrimental as the resulting damages can render oxidized proteins inactive and lead to cellular functional abnormalities (Berlett and Stadtman, 1997). Various types of protein oxidative damage are induced either directly by ROS or indirectly by reactions with secondary products of oxidative stress such as reactive aldehydes as 4-hydroxy-2-nonenal and malondialdehyde. These aldehydes are produced by the peroxidation of polyunsaturated fatty acids of membrane lipids and can react with protein to form covalent Michael adducts with the side chains of cysteine, histidine and lysine residues (Esterbauer et al., 1991). Proteins can also be modified through the reaction of arginine and lysine side chains with reducing sugars or reactive dicarbonyl compounds such as glyoxal and methylglyoxal, based on the Maillard reaction (Thornalley et al., 2003). Formation of these lipid peroxidation and glycation adducts are found in many tissues and believed to contribute to a variety of age-associated diseases.

Although an increased load of oxidatively-modified proteins has been clearly associated with normal and pathological aging, in most cases the target proteins have not been identified and, only recently significant advances have been made towards the identification of proteins targeted by these modifications. Indeed, identification of these proteins, the modified forms of which are accumulating during aging or upon the development of an age-related disease, would be expected to give some insights into the mechanisms by which these damaged proteins would build-up and potentially affect protein function. Moreover, although the causative role of protein oxidative modifications has not yet been determined, the accumulation of oxidatively damaged proteins during aging and their particular increase in organs and tissues affected by age-related diseases imply that the restricted set of proteins targeted by damage may be a potential substratum for many of the observed cellular dysfunction.

In this mini-review, we will first briefly describe the most relevant protein oxidative and related modifications that have been previously documented to accumulate in senescent cells and aged tissues, and then address the intracellular fate of these modified proteins. Then, the recent identification of proteins preferentially targeted by oxidation within human myoblasts upon acute oxidative stress and of proteins

Abbreviations: ROS, Reactive Oxygen Species; SNO, s-nitrosothiol; ALE, advanced lipoperoxidation end products; AGE, advanced glycoxidation end products; Msr, methionine sulfoxide reductase; 2D-DIGE, 2 dimensional differential in gel electrophoresis; pI, isoelectric point; HNE, 4-hydroxy-2-nonenal; DNPH, 2,4-dinitrophenylhydrazine.

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accumulating as oxidized, glycated and conjugated with lipid peroxidation products in senescent human embryonic fibroblasts will be presented. Finally, we will elaborate on a newly created database aimed at inventorying all the mammalian proteins, mostly from brain origin, that have been shown to accumulate as oxidatively modified during normal and pathological aging. Indeed, over 180 proteins have been reported in the literature as increasingly modified. Interestingly, these proteins are involved in key cellular pathways such as inflammatory response, energy metabolism, protein homeostasis and antioxidant response, hence indicating the particular susceptibility of certain proteins for being prone for detrimental modifications and suggesting their likely implication in the underlying mechanisms of aging and age-related diseases.

2. Modification of proteins by oxidation and related pathways

Proteins represent the main targets for ROS mediated damage that occurs either directly or indirectly through their reaction with lipids and carbohydrates and the subsequent generation of oxidized products that can react with proteins. Protein oxidation by free radicals can be classified into those that oxidize and cleave the peptide bond and those that oxidize the side chains. Almost all amino acid side chains can react with the hydroxyl radical (OH^*), but certain amino acids are more sensitive to oxidation with such reactive oxygen species as hydrogen peroxide and superoxide. Indeed, the sulfur-containing amino acids methionine and cysteine are readily oxidized by all sorts of ROS, while aromatic amino acids and histidine are also prone to oxidation.

Oxidation products of cysteine include disulfide bridges and sulfenic acid that can be converted to disulfide bridges or further oxidized to sulfinic and then sulfonic acids. Both disulfide bridges and sulfenic acid can be enzymatically reduced while sulfinic acid reduction has so far been limited to oxidized cysteines within the active site of peroxiredoxins (Biteau et al., 2003). Cystein can also react with nitric oxide to produce S-nitrosothiol (SNO). Methionine oxidation leads to the formation of methionine sulfoxide and further oxidation of methionine sulfoxide leads to the irreversible formation of methionine sulfone. Some oxidative modifications are quite specific in terms of oxidized residues and products generated such as the oxidation of phenylalanine to tyrosine, which can be further converted to di-tyrosine (Giulivi et al., 2003). Moreover, tyrosine residues represent preferred targets for nitration by nitrogen dioxide and peroxynitrite and can be converted to nitrotyrosine.

Oxidation of several amino acid residues such as lysine, arginine, proline and threonine results in the formation of carbonyl groups (Berlett and Stadtman, 1997). Carbonyl derivatives can also originate from the fragmentation products of the peptide bond oxidative cleavage (Stadtman and Levine, 2003). Aminoadipic and glutamic semialdehydes resulting from the oxidation of lysine and arginine, respectively, are quantitatively important products of the carbonylation reaction. Protein carbonyls are the most commonly used marker of protein oxidation and different methods have been developed for the detection and quantification of carbonylated proteins. Most of these methods are based on immunochemical and/or spectrophotometric assays of protein carbonyls previously derivatized by 2-4-dinitrophenylhydrazine to form 2-4-dinitrophenylhydrazone protein adducts (Levine, 2002). Protein carbonylation has been considered as an indicator of severe oxidative damage as well as age- and disease-derived protein dysfunction since this modification often leads to a loss of protein function, as well as an increased thermosensitivity and hydrophobicity (Berlett and Stadtman, 1997).

In addition to direct oxidation of certain amino acid side chains, protein carbonyl derivatives can originate from the conjugation on cysteine, lysine and histidine residues of such aldehydes as malondialdehyde and 4-hydroxy-2-nonenal. Indeed, oxygen free radicals can attack cellular membranes and induce lipid peroxidation resulting in the production of these reactive aldehydes which are precursors of advanced lipid peroxidation end products (ALE) that have been found to accumulate on proteins during aging and certain age-related diseases (Sayre et al., 1997; Szweda et al., 2003). Moreover, sugar aldehydes or ketones can also react spontaneously with the amino groups of lysine and arginine through a Schiff base which is slowly rearranged to form an Amadori product (e.g. fructosamine when the reacting sugar is glucose). These products are referred as to early stage glycation adducts that are further modified to form stable end-stage products also called advanced glycation end products (AGE) through either rearrangement, oxidation, dehydration, fragmentation and/or cyclization. Deleterious effect on protein function is observed when the modification affects critical amino acids within the protein and many proteins, including intracellular proteins, accumulate with age as AGE-modified in vivo (Horiuchi and Araki, 1994).

3. Elimination of modified proteins by degradation and repair

In contrast to DNA, for which many repair enzymes and pathways have been described for oxidative and other insults, oxidized protein repair is limited to the reduction of certain oxidation products of the sulfur-containing amino acids, cysteine and methionine. Indeed, damaged intracellular proteins are mainly eliminated be degradation by the proteasomal and the lysosomal pathways. Major systems that have been implicated in oxidized protein repair include thiredoxin/thioredoxin reductase and the glutathione/glutathione reductase systems for the reduction of sulfenic acid and disulfide bridges, the sulfiredoxin and sestrin for the reduction of sulfinic acid when formed on the catalytic cysteine of peroxiredoxins, and the methionine sulfoxide reductases (Msr) for the reduction of methionine sulfoxide within proteins (Petropoulos and Friguet, 2005).

The Msr system is found in almost all organisms, from bacteria to mammals, and is composed of two enzyme families, MsrA and MsrB, that catalytically reverse the oxidation of the S-sulfoxide and R-sulfoxide diastereoisomeric forms of methionine sulfoxide, respectively (Boschi-Muller et al., 2008). Oxidized methionine sulfoxide reductases are then reduced by the thiredoxin/thioredoxin reductase system. Oxidation of methionine has been implicated in the impairment of protein structure and/or function while the reduction of methionine sulfoxide has been associated with the recovery of protein function. Hence, oxidation/reduction of methionine has been involved in redox regulation of protein function and protein–protein interactions. In combination with protein surface-exposed methionine residues, the Msr system has also been shown to be efficient as a built-in ROS scavenging system preventing further irreversible protein oxidation (Picot et al., 2005). Another protein repair system that has been described and that relates to modification of proteins by glycation, is fructosamine-3-kinase, an enzyme that has been involved in glycated protein repair since it phosphorylates fructosamines on proteins, making them unstable and causing them to detach from proteins, hence acting as a deglycating enzyme (Van Schaftingen et al., 2010).

Non-repairable protein alterations, which represent the majority of protein damage, are removed through degradation by the proteasomal or the lysosomal systems in the cytosol while oxidized proteins are degraded by the Lon protease in the mitochondrion (Ugarte et al., 2010). These proteolytic systems as well as the oxidized protein repair Msr system have been documented to decline with age and during replicative senescence, hence implicating protein maintenance failure in the age-associated build-up of damaged proteins (Baraibar and Friguet, 2012).

The proteasomal and lysosomal pathways are the two main proteolytic machineries by which intracellular proteins are degraded. Protein degradation by the proteasome is a key process for the maintenance of cellular protein homeostasis. In the cytosol and in the nucleus, the proteasome plays a key role in the elimination of altered proteins since moderately oxidized proteins are good substrates of