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Mini Review

Protein modification in aging: An update

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Abstract

Post-translational modifications of proteins are an important biologic tool for the production of various protein species from a single gene, which may vary in conformation, function, biologic half-life and complex formation with other proteins. The present minireview summarizes a few selected research observations important for the role of post-translational modifications in biologic aging and age-related diseases, including farnesylation, methylglyoxal-derivatization, transglutaminase pathways and the formation of 3-nitrotyrosine and 2-oxo-histidine *in vivo*.

Keywords: Aging; Protein; Post-translational modifications; Oxidation; Nitrotyrosine; Farnesylation; Transglutaminase; 2-Oxo-histidine

1. Introduction

Post-translational protein modifications play an important role in the regulation of protein function through the modulation of protein structure, activity, turnover, localization, and the nature of protein–protein complexes. Today more than 200 different post-translational modifications are known (Khidekel and Hsieh-Wilson, 2004), which are the result of both enzymatic and non-enzymatic processes. There is increasing evidence that post-translational modifications of specific proteins accompany pathologic processes and biological aging. An important goal of global and targeted proteomic experiments must, therefore, be the identification and functional characterization of post-translationally modified proteins *in vivo*, and to resolve the question whether such post-translational modifications are mechanistically related (in contrast to merely being associated with) to a disease process or a specific phenotype of aging. Such studies could eventually lead to the discovery of specific biomarkers for diagnostic purposes and/or the design of therapies or preventive measures. Recently, much emphasis has been placed on the search for biomarkers for aging and/or age-dependent pathologies. Such studies require a careful experimental design and accurate validation with respect to specificity and predictive value and few, if any, useful biomarkers have evolved (Ong and Mann, 2005; Gillette et al., 2005; LaBaer, 2005). An experimental limitation can the accessibility of biological material, for example, if the biomarker cannot be found in biological fluids. On the other hand, significantly more progress has been made in the mechanistic evaluation of post-translational protein modifications and their potential link to aging and age-dependent pathologies. Several selected examples of these will be presented below, covering enzymatic and non-enzymatic pathways, including protein modifications induced through oxidative stress. Rather comprehensive reviews on protein modifications during oxidative stress and aging have been published recently (Cloos and Christgau, 2004; Schöneich, 2005; Dalle-Donne et al., 2005, 2006). Therefore, this minireview shall be a true update focusing on a few selected areas rather than a comprehensive treatment.

2. Prelamin A farnesylation and Hutchinson–Gilford progeria syndrome

Mechanistic studies have recently provided a causal relationship between the etiology of Hutchinson–Gilford progeria syndrome (HGPS) and the aberrant processing of

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post-translationally modified prelamin A into mature lamin A (Mallampalli et al., 2005). HGPS represents a genetic disorder, which results in premature aging of children leading to death within their early teenage years. Prelamin A contains a C-terminal farnesylation motif (CSIM). In the general mechanism of farnesylation, farnesyl transferase catalyzes the reaction of the cysteine mercapto group with farnesyl diphosphate, an intermediate product of the cholesterol biosynthetic pathway (Liao, 2002; Bowers and Fierke, 2004; Greenwood et al., 2006). The natural processing of prelamin A involves farnesylation of the Cys residue within the CSIM sequence, followed by proteolytic removal of the SIM tag and methylation of the carboxylate group of the resulting C-terminal Cys residue. Subsequently, zinc metalloprotease Ste24 (Zmpste24) removes the C-terminal 15 amino acid residues, generating non-farnesylated lamin A. In a cell culture model, lamin A localizes to nuclear foci and the nucleoplasm. In HGPS, the most common mutation of the prelamin A gene, *LMNA*, leads to a deletion of 50 codons, which include the Zmpste24 processing site. In contrast, the farnesylation motif remains intact causing retention of the farnesyl chain in the protein, which is now termed progerin. The retention of the farnesyl chain leads to a primary localization of progerin at the nuclear envelope, different from the localization of wild-type lamin A to nuclear foci. Further evidence for a critical role of Zmpste24 in prelamin processing and farnesyl chain removal comes from several experimental mutants, lacking either the Zmpste24 cleavage site or the Cys residue of the C-terminal CSIM motiv (mutation to SSIM). While the former results in aberrant lamin A localization, the latter restores the correct localization to nuclear foci. Importantly, the aberrant cellular compartmentalization of progerin was also prevented pharmacologically through the exposure of cells in culture to farnesyl transferase inhibitors (FTI). More recently, similar experiments showed beneficial effects in a mouse model of HGPS, deficient in the gene for Zmpste24, *Zmpste24* (Fong et al., 2006), demonstrating that aberrant processing of a single farnesylation site in progerin may be critical for the pathogenesis of HGPS. It will be interesting to explore in the future whether low levels of farnesylated lamin A, increasing with age, may accompany also the slow process of normal aging.

3. Methylglyoxal and the regulation of gene expression

The reaction of methylglyoxal (MG) with proteins represents one pathway leading to the formation of advanced glycation end products (AGEs), prominent protein modifications accompanying, e.g., biological aging and diabetes. While increased levels of MG-derived protein modifications are generally observed under pathologic conditions, significant amounts are detectable also in healthy tissue (Ahmed et al., 2003). Proteomic studies identified a series of mitochondrial proteins as targets of MG-dependent modification in the diabetic rat kidney (Rosca et al., 2005). MG forms various stable adducts with arginine residues such as N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)ornithin (MG-H1), N^{δ}-[5-(2,3,4-trihydroxybutyl)-5-hydro-4- imidazolon-2-yl]ornithin and argpyrimidine (Ahmed et al., 2005). The inactivation of proteins through the accumulation of AGEs had been reported (Ahmed et al., 2005). Recent data (Yao et al., 2006) provide now evidence for a physiological role of MG (Ramasamy et al., 2006), where the modification of a corepressor, mSin3A, directly leads to an increased expression of angiopoietin-2. Usually, mSin3A is part of a protein complex with the repressor Sp3, which binds to the promoter region of angiopoetin-2. MG-modification of mSin3A results in the recruitment of O-GlcNAc transferase (OGT) to this complex and modification of Sp3 by OGT, followed by dissociation from the promoter region, which permits expression of angiopoietin-2. Considering the manifold of AGE structures formed through the reaction of reactive carbonyls with Arg and Lys, one should expect that future experiments will uncover additional mechanisms where AGE formation regulates specific biologic pathways. Here, the sensitivity of protein Arg residues towards MG *in vivo* may not only be defined by their chemical microenvironment (controlling accessibility and protonation equilibria) but also by the action of protein arginine methyl transferase (PRMT). The latter class of enzymes can transfer one and/or two methyl groups onto protein Arg residues, where dimethylation would prohibit the reaction with MG. Hence, dimethylation could protect protein Arg residues against the formation of MG-H1 (Fackelmayer, 2005), analogous to a mechanism characterized for human crystallins, where Cys methylation protects these proteins against oxidative modification/aggregation (Lapko et al., 2003).

4. Transglutaminase pathways of protein cross-linking and neurodegenerative diseases

Transglutaminases (TGs) are Ca^{2+}-dependent enzymes that catalyze the deamidation and transamination of protein glutamine residues. While deamidation results in the formation of glutamic acid, transamination usually leads to γ-glutamyl-ε-lysine (GGEL) cross-links. Increasing evidence is accumulating that TG-dependent protein cross-linking plays an important role in oxidative stress (Shin et al., 2004) and the pathologies of neurodegenerative diseases and aging. For example, the microtubule-associated protein tau represents a target for TG *in vitro* and *in vivo*, where, *in vitro*, TG-dependent cross-linking affects primarily protein domains located in close proximity to microtubule-binding domains (Tucholski et al., 1999). TG-dependent tau cross-linking is specifically increased in a transgenic mouse model with a higher tendency for the formation of neurofibrillary tangles, expressing the tau mutant P301L (Halverson et al., 2005). Increased TG activity appears to correlate with increased neuronal death in Alzheimer's disease (AD) (Bonelli et al., 2002) and Huntington's disease (HD), as crossing HD R6/1 transgenic