Mechanisms of Ageing and Development xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Protein modification and maintenance systems as biomarkers of ageing

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
Ageing
Biomarkers
Protein modification
Protein maintenance

ABSTRACT

Changes in the abundance and post-translational modification of proteins and accumulation of some covalently modified proteins have been proposed to represent hallmarks of biological ageing. Within the frame of the Mark-Age project, the workpackage dedicated to "markers based on proteins and their modifications" has been firstly focused on enzymatic and non-enzymatic post-translational modifications of serum proteins by carbohydrates. The second focus of the workpackage has been directed towards protein maintenance systems that are involved either in protein quality control (ApoJ/Clusterin) or in the removal of oxidatively damaged proteins through degradation and repair (proteasome and methionine sulfoxide reductase systems). This review describes the most relevant features of these protein modifications and maintenance systems, their fate during ageing and/or their implication in ageing and longevity.

1. Introduction

Changes in the abundance and post-translational modification of proteins and accumulation of some covalently modified proteins have been proposed to represent hallmarks of biological ageing (Friguet, 2002; Stadtman, 2006; Chondrogianni et al., 2014a). Within the frame of the Mark-Age project, the workpackage dedicated to "markers based on proteins and their modifications" has been firstly focused on enzymatic and non-enzymatic post-translational modifications of serum proteins by carbohydrates. Indeed, one important physiological post-translational modification of secreted proteins is addition of N-linked oligosaccharides (N-glycans). Since most N-glycans are on the outer surface of cellular and secreted macromolecules, they can modulate or mediate a wide variety of events in cell–cell and cell-matrix interactions crucial for the development and function of complex multicellular organisms (Ohtsubo and Marth, 2006). Because the biosynthesis of glycans is not controlled by interaction with a template but depends on the complicated concerted action of glycosyltransferases, the structures of glycans are much more variable than those of proteins and nucleic acids, and they can be easily altered by the physiological conditions of the cells. Accordingly, studying age-related alterations of the glycans could be relevant to understanding the complex physiological changes in ageing individuals (Dall'Olio et al., 2013). Non-enzymatic protein glycation is a common post-translational modification of proteins *in vivo*, resulting from reactions between glucose or its metabolites and amino groups on proteins, this process is termed "Maillard reaction" and

Abbreviations: AGEs, advanced glycation endproducts; ApoJ/CLU, Apolipoprotein J/Clusterin; ROS, reactive oxygen species; PBMC, peripheral blood mononuclear cells; Msr, methionine sulfoxide reductase; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; ER, endoplasmic reticulum; Ig, Immunoglobulins; MGO, methylglyoxal; GO, Glyoxal; 3-DG, deoxyglucosone; MRP, Maillard reaction products; CML, carboxymethyllysine; RAGE, receptor for advanced glycation end products; HMGB1, high mobility group box 1 protein; LPS, lipopolysaccharide; LC-MS/MS, liquid chromatography–mass spectrometry with tandem mass spectrometry; AF, autofluorescence; HDL, high-density lipoprotein; PA, proteasome activator.

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http://dx.doi.org/10.1016/j.mad.2015.03.009

Please cite this article in press as: Vanhooren, V., et al., Protein modification and maintenance systems as biomarkers of ageing. Mech. Ageing Dev. (2015), http://dx.doi.org/10.1016/j.mad.2015.03.009

leads to the formation of advanced glycation endproducts (AGEs) (Vlassara et al., 1984). During normal ageing, there is accumulation of AGEs of long-lived proteins such as collagens and several cartilage proteins. AGEs, either directly or through interactions with their receptors, are involved in the pathophysiology of numerous age-related diseases, such as cardiovascular and renal diseases and neurodegeneration (Sell and Monnier, 2012; Li et al., 2012; Simm, 2013).

The second focus of the workpackage has been directed towards protein maintenance systems that are involved either in protein quality control or in the removal of oxidatively damaged proteins through degradation and repair (Friguet, 2006; Breusing and Grune, 2008; Baraibar and Friguet, 2012; Chondrogianni et al., 2014a). Hence, the highly conserved multifunctional glycoprotein, Apolipoprotein J/Clusterin (ApoJ/CLU) has been analysed since, among its several physiological functions, this protein is a chaperone that stabilizes stressed proteins in a folding-competent state (Poon et al., 2000; Narayan et al., 2012). Moreover, previous work has shown that ApoJ/CLU is associated with human ageing and with ageing of human cells *in vitro*, and that its serum level is increased in patients with type II diabetes, coronary heart disease, and myocardial infarction (Trougakos and Gonos, 2006). Therefore ApoJ/CLU may represent a valuable ageing biomarker. Beside protein glycation, it is also well known that levels of oxidised proteins increase with age, due to increased protein damage induced by reactive oxygen species (ROS), decreased elimination of oxidized protein (*i.e.* repair and degradation), or a combination of both (Chondrogianni et al., 2014a). Since the proteasome is in charge of both general protein turnover and removal of oxidized protein, its fate during ageing has received considerable attention, and evidence has been provided for impairment of the proteasome function with age in different cellular systems, including human peripheral blood mononuclear cells (PBMC) (Friguet, 2006; Breusing and Grune, 2008; Baraibar and Friguet, 2012). In addition to being degraded, certain oxidised proteins can be repaired. However, repair is limited to the reversion of a few oxidative modifications of sulfur-containing amino acids, such as the reduction of methionine sulfoxide by the methionine sulfoxide reductase (Msr) system (Moskovitz, 2005). Evidence has been provided that Msr activity is impaired during ageing and replicative senescence (Petropoulos and Friguet, 2006). Thus, these protein maintenance systems may also be viewed as potential biomarkers of ageing.

This review article summarizes the most important features of the above mentioned protein modifications and maintenance systems relevant to ageing as well the current knowledge on their fate during ageing and/or the eventual effects of their modulation on ageing and longevity.

2. Protein modification by carbohydrates

2.1. Protein glycosylation

2.1.1. Glycosylation: an overview

The term "glycan" refers to all forms of mono-, oligo-, or polysaccharide, free or attached to another molecule, such as a protein or a lipid (Varki et al., 2009). The most common types of glycans found in eukaryotes are defined based on the nature of their linkage to the macromolecule. Glycoproteins are glycoconjugates in which a protein is covalently bound to one or more glycans. The binding usually occurs *via* N or O linkages (Spiro, 2002).

O-glycans (O-linked oligosaccharides) are mostly bindings between the polypeptide *via* a *N*-acetylgalactosamine (GalNAc) and a OH-group of a serine or a threonine residue of the protein, and can be extended into a variety of different structural core classes. N-glycans (N-linked oligosaccharide) are sugar chains covalently linked to asparagine residues of the protein, commonly involving a *N*-acetylglucosamine (GlcNAc) residue and the consensus peptide sequence: Asn-X-Ser/Thr (Varki et al., 2009). The focus in this review will be on N-glycans.

It is important to emphasize that glycan chain structures are not encoded directly in the genome and are secondary gene products. This is in contrast to protein (amino acid) sequences, which are encoded by the genes and are inherited. Glycans are built by glycosidases and glycosyltransferases, without an obvious encoded template (Kamerling et al., 2007; Kondo et al., 2006). A small percentage of genes in the human genome are dedicated to produce these enzymes and transporters responsible for the biosynthesis and assembly of glycan chains, in the endoplasmic reticulum (ER) and Golgi apparatus (Varki et al., 2009). The glycan chains themselves represent numerous combinatorial possibilities, generated by a variety of competing and sequentially acting glycosidases and glycosyltransferases (Lis and Sharon, 1993).

All N-glycans start with a common core sugar sequence, $(Man)_3(GlcNAc)_2$-Asn-peptide and are classified into three types: (1) "oligomannose", in which only mannose residues are attached to the core; (2) "complex", in which "antennae" initiated by *N*-acetylglucosaminyltransferases (GlcNAcTs) are attached to the core; and (3) "hybrid", in which only mannose residues are attached to the Manα1-6 arm of the core and one or two antennae are on the Manα1-3 arm (Varki et al., 2009).

N-glycans are found on many secreted and membrane-bound glycoproteins at Asn-X-Ser/Thr sequence. Analyses of protein sequence databases have revealed that about two thirds of the entries contain the consensus Asn-X-Ser/Thr sequence. It is estimated that at least two thirds of those sequences are likely to be N-glycosylated (von der Lieth et al., 2004). It is important to note that whereas, the presence of the Asn-X-Ser/Thr sequence is necessary for the receipt of an N-glycan, transfer of the N-glycan to this sequence does not always occur, due to conformational or other constraints during glycoprotein folding (Spiro, 2002).

Clearance of secreted glycoproteins can dependent on the composition of the glycan. Loss of sialic acid from glycoproteins triggers clearance by the Kupffer cells, specialised liver macrophages which carry receptors for asialoglycoproteins (Griffiths et al., 2014). The mannose receptor is an endocytic receptor for glycans expressed in a number of tissues, including the hepatic sinusoidal endothelium. It was shown that the mannose receptor is required for the rapid clearance of a subset of mannose and GlcNac bearing serum glycoproteins (Lee et al., 2002).

Along with glycosylation comes "microheterogeneity". This term indicates that at any given glycan attachment site on a certain protein synthesized by a particular cell type, a range of variations can be found in the structures of the attached glycan chain. The extent of this microheterogeneity can vary considerable from one glycosylation site to another, from glycoprotein to glycoprotein and from cell type to cell type. Thus a given protein originally encoded by a single gene can exist in numerous "glycoforms", each effectively a distinct molecular species (Varki et al., 2009).

Glycans can mediate a wide variety of biological roles by virtue of their mass, shape, charge or other physical properties. We refer to the review of Ohtsubo and Marth (2006), for details on the functions of glycans.

2.1.2. N-glycosylation profiling of serum proteins as marker of physiological age

Small changes in the environment can cause dramatic changes in glycans produced by a given cell therefore N-glycosylation can be seen as a mirror of the status of the cell. For example, during liver disease, hyperfucosylation, increased branching and bisecting GlcNAc are clearly observed on serum proteins (Blomme et al., 2009) and in rheumatoid arthritis patients a significant increase in serum

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