Mechanisms of Ageing and Development 151 (2015) 71–84

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

Valerie Vanhooren[a,b], Alexander Navarrete Santos[c], Konstantinos Voutetakis[d], Isabelle Petropoulos[e,f,g], Claude Libert[a,b], Andreas Simm[c], Efstathios S. Gonos[d], Bertrand Friguet[e,f,g,*]

[a] VIB Inflammation Research Center, Technologiepark 927, B-9052 Ghent, Belgium
[b] Department of Molecular Biology, Ghent University, Technologiepark 927, B-9052 Ghent, Belgium
[c] Department of Cardiothoracic Surgery of the Martin Luther University Halle-Wittenberg, Ernst Grube Str. 40, 06120 Halle/Saale, Germany
[d] National Hellenic Research Foundation, Institute of Biology, Medicinal Chemistry and Biotechnology, 48 Vas. Constantinou Ave., Athens 11635, Greece
[e] Sorbonne Universités, UPMC University Paris 06, UMR 8256, Biological adaptation and ageing – IBPS, F-75005 Paris, France
[f] CNRS-UMR 8256, F-75005 Paris, France
[g] INSERM U1164, F-75005 Paris, France

ARTICLE INFO

*Article history:*
Available online 3 April 2015

*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.

* Corresponding author at: Sorbonne Universités, UPMC University Paris 06, UMR 8256, Biological adaptation and ageing – IBPS, F-75005 Paris, France.
Tel.: +33 1 44 27 37 02; fax: +33 1 44 27 51 40.
*E-mail address:* bertrand.friguet@upmc.fr (B. Friguet).

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

leads to the formation of advanced glycation end products (AGEs) (Vlassara et al., 1984). During normal ageing, there is accumulation of AGEs on 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 chapter 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 Man$\alpha$1-6 arm of the core and one or two antennae are on the Man$\alpha$1-3 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)

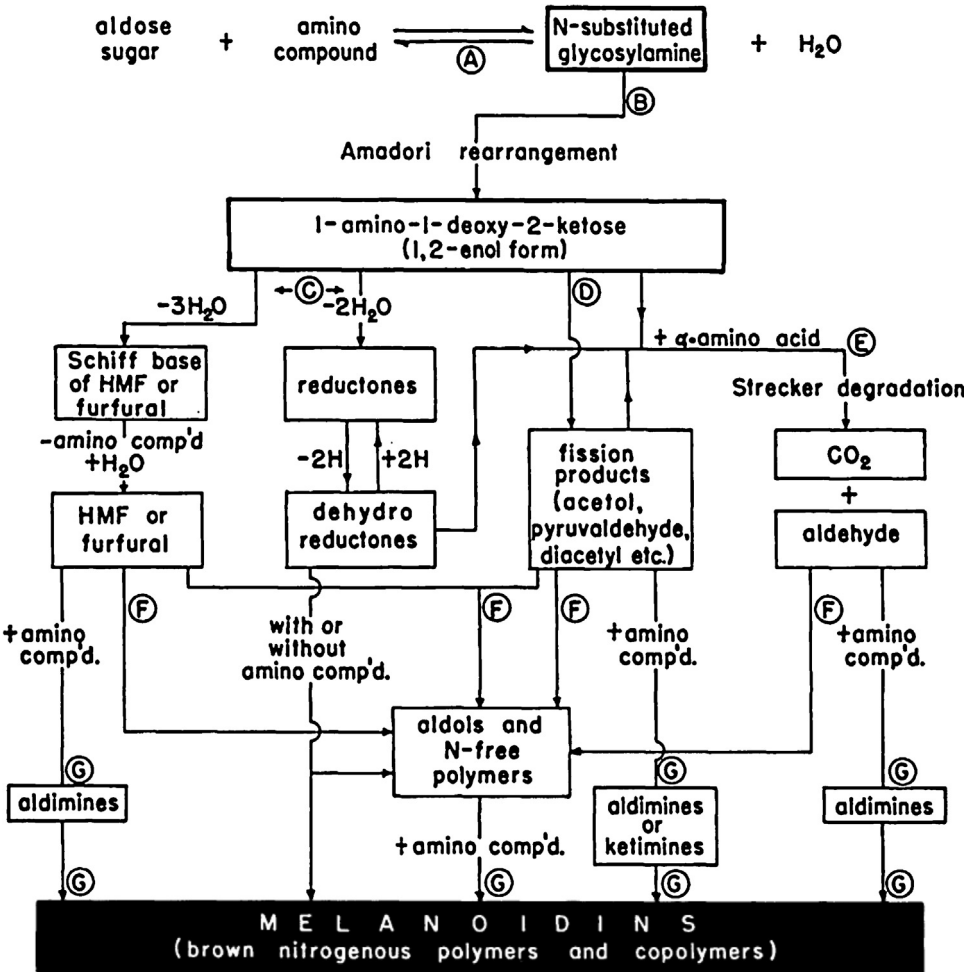


**Fig. 1.** Rearrangements of the Amadori products.
The different rearrangements of the Amadori products are shown according to the original description of Hodge in 1953 (Hodge, 1953) resulting in the formation of melanoidins (the name which is still taken in nutrition science, nowadays in the biomedicine context called advanced glycation endproducts, AGEs).

and in rheumatoid arthritis patients a significant increase in serum glycans devoid of galactose and terminating in GlcNac is observed (Van Beneden et al., 2009). The major sources of N-glycosylated proteins in serum are the B-cells (antibodies or immunoglobulins: Ig) and the liver, but also macrophages (cytokines) and other cell-types can contribute. Although many studies have shown the importance of structural changes of sugar chains of glycoproteins during development (Varki et al., 2009), limited data are available on the changes in the sugar chains that take place during ageing.

Parekh et al. (1988) were the first to investigate age-related N-glycosylation changes on human serum IgGs. Healthy males and females varying in age from 1 to 70 years old were studied. The relative incidence of agalactosyl (with both outer arms terminating in GlcNAc) N-glycans on total serum IgG decreased from birth to a minimum (at 25 years of age) and then increased with age. The relative incidence of digalactosyl structures varied inversely to the amount of agalactyl N-glycans and the relative incidence of monogalactosyl structures was constant. They concluded that galactosylation of the N-glycans on the human serum IgGs of normal individuals is an age-related molecular parameter (Parekh et al., 1988).

Ten years later a study similar to the one of Parekh et al. (1988) was performed by Shikata et al. (1998) on human serum IgGs from 80 men and women aged 18–73 years. The incidence of N-glycans with a bisecting GlcNAc increased with age in both male and female IgGs. It was also shown that the level of agalactosyl N-glycans in female IgG increases with age and that this change is caused by a decrease in digalactosylated glycans but not monogalactosylated glycans. A negative correlation between ageing and the incidence of monosialo glycans in female IgG was shown and this can be attributed to a low level of galactosylation, which is essential for sialylation of IgG glycans. In contrast, these age-related changes were not so clear in male IgG samples. Therefore, it was suggested that the decrease in galactosylation of IgG oligosaccharides with ageing is a female-specific phenomenon bearing in mind that the size of the cohort is small.

More recently, 3 studies were performed using cohorts from different European countries (study 1: Italian–Belgian subjects) (Vanhooren et al., 2007); study 2: Dutch subjects (Ruhaak et al., 2011); study 3: subjects from the Croatian Adriatic island of Vis (Knezevic et al., 2009, 2010). These were reviewed in depth by Dall'Olio et al. (2013). In these studies, not only serum Igs but also the full serum glycoproteome was analyzed. The general observation among all three studies is that serum proteins have reduced digalactosylated N-glycans and increased agalactosylated N-glycans with increasing age although the human serum glycome appears to be strongly influenced by genetic background. Therefore, a large study with standardized isolation methods combining subjects from different nationalities and genetic background is needed to study in depth the role of N-glycosylation of serum proteins as an ageing biomarker.

### 2.2. Protein glycation

#### 2.2.1. The Maillard reaction history

In 1912 the French chemist Louis-Maillard described a reaction nowadays known as the "*Maillard reaction or browning reaction*" (Maillard, 1912). He tried to explain the results of the interaction of amino-acids with sugars at high temperature. But only more than 40 years later in 1953 the Afro-American chemist John Edward Hodge published the mechanism of the Maillard reaction (Hodge, 1953). In 1979 the article published by Hodge in 1953 became a "Citation Classic" by the Science Citation Index giving Hodge popularity among the carbohydrate chemists.

#### 2.2.2. Mechanism of the Maillard reaction

The Maillard or browning reaction is the result of the non-enzymatic reaction of reduced sugars with the free amino group of nucleic acids, lipids and proteins. In the article published by Hodge (1953), the Maillard reaction was summarized as the result of seven different types of reactions that occur during the browning (Fig. 1). According to the three stage of development the reactions were classified:

I – Initial stage (colourless, no absorption in near UV)
A – Sugar-amine condensation
B – Amadori rearrangement
II – Intermediate stage (colourless or yellow, strong absorption in near UV)
C – Sugar dehydration
D – Sugar fragmentation
E – Amino acid degradation
III – Final stage (highly coloured)
F – Aldol condensation
G – Aldehyde-amine polymerization; formation of heterocyclic nitrogen compounds

#### 2.2.3. Introduction to the term: advanced glycation end products (AGEs)

In a manuscript by Vlassara et al. in 1984 (Vlassara et al., 1984) the Maillard reaction was described as follow: "*Short-term incubation of proteins with glucose results in the formation of ketoamine adducts (Amadori product) with protein amino groups. These adducts are reversible and reach equilibrium after several weeks. In addition to detaching from the protein, these glyco-adducts can undergo very slowly further rearrangements, dehydrations, and reactions to form a number of advanced glycosylation end-products (AGE), which are characteristically yellow-brown fluorescent chromophores that can cross-link proteins*".

This was the birth of the term advanced glycosylation end-products. However, in the same year the IUPAC–IUBMB (International Union of Pure and Applied Chemistry–International Union of Biochemistry and Molecular Biology) joint Commission on Biochemical Nomenclature and the Nomenclature Commission of IUBMB warned the improperly use of the terms glycosylated hemoglobin, protein glycosylation, etc. to refer to the products of the Maillard reaction. These compounds are not glycosides, but result from the formation of a Schiff's base followed by an Amadori rearrangement. The term "glycation" for any reaction that links a sugar to a protein, whether it is catalysed by an enzyme or not was suggested. Thus glycation includes glycosylation as a special case. However, commonly the term "advanced glycation end products" refers to the products of the non-enzymatic Maillard reaction.

#### 2.2.4. AGEs production

Outside of the body (exogenously) AGEs are formed during the food preparation. Temperatures at 121 °C or higher accelerate the reaction between sugars, protein and fat. Lower temperatures with longer cooking time also favour the AGEs production. Thus, foods like bread, roasted meat, French fries, etc., which during the preparation are exposed to high temperatures, contain high AGEs-levels. Among others detailed information about the AGEs-content in some foods was published by Goldberg et al. (2004), Uribarri et al. (2010) and Hull et al. (2012). The endogenous AGE-production (inside the body) is driven by the cellular metabolic processes. Reactive oxoaldehydes (dicarbonyls) such as Methylglyoxal (MGO) a spin-off product of the glycolysis (Fig. 2), Glyoxal (GO) formed among others by lipid peroxidation and deoxyglucosone (3-DG) formed after the decomposition of fructose 3-phosphate contribute to AGE-generation (Table 1).

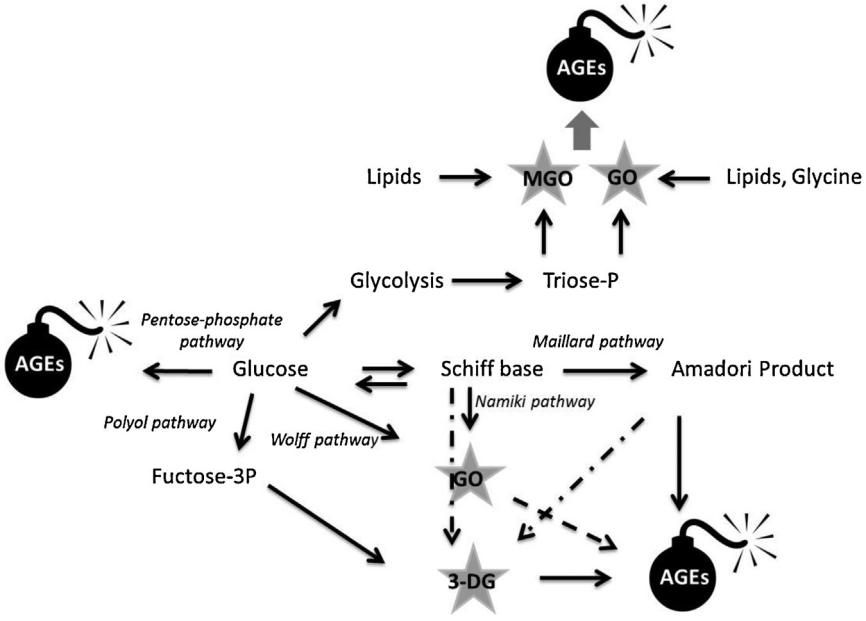


**Fig. 2.** Formation of MGO, GO and 3-DG. MGO, GO and 3-DG are formed as a result of the glucose metabolism.

**Table 1**
Involvement of the three most prominent dicarbonyls in the AGEs formation with the corresponding AGE.

| Dicarbonyl | AGE | Name |
|---|---|---|
| Glyoxal | G-H1 | $N_d$-(5-hydro-4-imidazolon-2-yl)ornithine |
| | GOLD | Glyoxal-derived lysine dimer |
| | CML | $N_e$-carboxymethyl-lysine |
| Methylglyoxal | MG-H1 | $N_d$-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine |
| | MOLD | methylglyoxal-derived lysine dimer |
| | CEL | $N_e$-(1-carboxyethyl)lysine |
| | Arg-pyr | Arg-pyrimidine |
| | THP | $N_d$-(4-carboxy-4,6-dimethyl-5,6-dihydroxy-1,4,5,6-tetrahydro-pyrimidin-2-yl)-ornithine |
| 3-Deoxyglucosone | 3DG-H | Hydroimidazolones derived from 3-deoxyglucosone |
| | DOLD | 3-Deoxyglucosone-derived lysine dimer |
| | Pyrraline | Pyrraline |

*2.2.5. Physical AGEs clearance*

The knowledge about the disposability of ingested AGEs is poor. In humans it was shown that about 3–10% of the consumed AGE (protein-bound fructose-lysine) is absorbed (Faist and Erbersdobler, 2001). Most recently the effect of Maillard reaction products- (MRP) high and MRP-low diet on carboxymethyllysine (CML) intake and excretion in 11–14 years adolescent males was studied (Delgado-Andrade et al., 2012). The consumption of the MRP-high diet led to a higher CML intake and absorption (11.28 vs. 5.36 mg/day CML for MRP-high and-low diet, respectively). In parallel, the faecal excretion was also greater (3.52 vs. 1.23 mg/day CML, respectively) and proportional to the dietary intake. The urinary elimination of CML was not increased significantly when the MRP-high diet was consumed compared to consumption of the MRP-low diet, and was not proportional to the dietary exposure of CML. The authors concluded that CML absorption and faecal excretion were highly influenced by dietary CML levels. The discordance between the CML intake and the CML amount found in the faecal excretion suggested that most of the ingested CML had an unknown destination in the body. Once in the gastrointestinal tract, the microbial activity will degrade a portion of AGEs and the rest could be absorbed or eliminated. A portion of the absorbed AGEs will reach the circulation intact and will be transported to different tissues/organs were metabolism can occur (Somoza et al., 2006).

*2.2.6. AGEs biological activity*

A vast amount of accumulated data ascribed AGEs a negative physiological role in the progress and complication of diseases such as diabetes mellitus, renal dysfunction, age-related neurodegenerative disorders (Alzheimer's, Parkinson's, and Huntington's diseases), cardiovascular diseases and many others (Sun et al., 2013; Chilelli et al., 2013; Nishizawa et al., 2012; Beisswenger et al., 2013; Fawver et al., 2012; Guerrero et al., 2013; Li et al., 2012; Simm, 2013). AGEs play a particular role in the process of arterial ageing (stiffness). In large arteries, a feature of ageing is the decreased turnover of collagen and elastin and the increased AGEs and cross-links. The lysis and disorganization of the elastic fibers is accompanied by their replacement by collagen and other matrix components. These events cause the loss of elasticity and induce stiffening (Sell and Monnier, 2012). A positive effect was observed by the use of aminoguanidine (blocker of the Maillard reaction) by reducing AGEs accumulation in tissues in experimental diabetes (Thomas et al., 2005). In other study aminoguanidine prevented the age-related increase in cardiac hypertrophy and arterial stiffening (Cantini et al., 2001). As for aminoguanidine side effects associated with its chronic administration during a human trial which included vasculitis and abnormalities in liver function was reported (Montagnani, 2008), its use in human is difficult (Sell and Monnier, 2012). Another group of compounds, the so called "AGEs and cross-links breakers" has been developed. The most studied is alagebrium (Sell and Monnier, 2012). However in a recent study the application of alagebrium to improve the excercise capacity and cardiac function in patients with systolic hear failure did not show any benefit (Hartog et al., 2011).

The cellular AGEs-action is mediated by receptors on the cell surface. At least six AGE-receptors are known and their expression is cell/tissue-type dependent (Table 2). The multi-ligand receptor for advanced glycation end products (RAGE) is the most studied AGE-receptor. Among the receptor's ligands are AGEs, amyloid $\beta$ peptide; S100/calgranulin protein, high mobility group box 1 protein (HMGB1) and lipopolysaccharide (LPS) (Rojas et al., 2013). By binding to the receptor these molecules can activate a series of signal pathways (Ott et al., 2014). In renal epithelial LLC-PKl cells, AGE-BSA stimulates the p42MAP kinase as well as the transcription factor AP1 and inhibits cell proliferation (Simm et al., 1997). AGE–RAGE interaction induces NADPH-oxidase activity, resulting in activation of Nf-κB and increased iNOS expression in rat vascular smooth muscle cells (San Martin et al., 2007). In human aortic smooth muscle cells, AGE–RAGE interaction activates alkaline phosphatase and thereby vascular calcification *via* the p38MAPK pathway (Tanikawa et al., 2009). In rat cardiac fibroblasts, AGE–BSA induces activation of ERK and p38-MAP kinases followd by NF-κB and ATF-2 transcription factor activation and expression of metalloproteinases MMP-2, MMP-9 and MMP-13 (Daoud et al., 2001). In the macrophage RAW cell line, AGEs stimulates Jak-2 and Stat-1 phosphorylation *via* RAGE and modify downstream the composition and activity of subunits of the immunoproteasome (Grimm et al., 2012). In human chondrocytes, AGE-RAGE interaction mediates inflammatory responses *via* JNK kinase, p38 MAP kinase, ERK, and NF-κB (Nah et al., 2008). In H9C2 cells (rat cardiac myofibroblasts) AGEs induces intracellular ROS production and the expression of inflammatory cytokines like TNF-α (Umadevi et al., 2013). In summary, in many cell types, AGEs modified proteins induces intracellular ROS, NF-κB induction resulting in the secretion of inflammatory cytokines (Fig. 3). In turn, the secretion of these molecules can yield to unwanted negative reactions (cell migration, cell activation, inflammatory response) resulting in cell and tissue damage.

*2.2.7. Methods for AGEs determination*

As fluorescent compounds AGEs can be measured by registering the AGEs-fluorescence. However, not all AGEs are fluorescent molecules or there exist molecules with the same range of fluorescence but are not AGEs. To the AGEs-fluorescent compounds belongs pentosidine, a ribose metabolite. One of the most studied AGE, carboxymethyllysine (CML) does not fluoresce. Regarding the fluorescence, AGEs were well characterized by Schmitt et al. (2005). Three different ranges of AGE-fluorescence were established (excitation/emission: 330/395, 365/440, 485/530 nm). Complementary the fluorescence of glycated tryptophan can be measured at an excitation of 280 nm and an emission of 350 nm.

**Table 2**
AGE-receptors (based on the review article of Ott et al., 2014).

| AGE-receptors | Cell types | Ligands | Function |
|---|---|---|---|
| RAGE | Monocytes, macrophages, T-lymphocytes, endothelial cells, mesangial cells, fibroblasts, smooth muscle cells, neuronal cells | S100 proteins, HMGB-1, β-amyloid, β-integrin Mac-1, AGEs | Endocytosis, signaling |
| AGR-R1/OST-48/p60 | Monocytes, macrophages, T-lymphocytes, endothelial cells, mesangial cells, fibroblasts, smooth muscle cells, neuronal cells | | Endocytic uptake and degradation of AGE-modified proteins, protective against oxidative stress |
| AGR-R2/80K-H/p90 | Monocytes, macrophages, T-lymphocytes, endothelial cells, mesangial cells, fibroblasts, smooth muscle cells, neuronal cells | AGEs | Signaling, regulatory subunit of glucosidase II |
| AGE-R3/galectin-3 | Monocytes, macrophages, T-lymphocytes, endothelial cells, mesangial cells, fibroblasts, smooth muscle cells, neuronal cells | IgE receptor, colon cancer mucin, CD66, LPS, Mac-1, Mac-3, LAMPs (1,2), AGEs, IgE, laminin, tenascin, fibronectin,collagen IV, gp90/Mac-2 binding protein | Signaling |
| SR-A (I/II) | Monocytes, macrophages, dendritic cells, endothelial cells | AcLDL, OxLDL, AGEs, β-amyloid, pathogen recognition | Endocytic uptake and degradation of modified LDL and AGE-modified proteins |
| SR-B/CD36 | Platelets, endothelial cells, epithelial cells, adipocytes, B-lymphocytes | AcLDL, OxLDL, HDL, LDL, VLDL, collagen, thrombospondin, long chain fatty acids, maleylated BSA, anionic phospholipids, AGE, apoptotic cells, pathogen recognition | Endocytic uptake and degradation of AGE-modified proteins, cell adhesion, regulator of fatty acid transport |
| SR-BI | Tissues which are active in selective uptake of HDL (liver, steroidogenic) | AcLDL, OxLDL, HDL, LDL, VLDL, maleylated BSA, anionic phospholipids, AGEs | Selective uptake of HDL, Endocytic uptake and degradation of AGEs |
| SR-E/LOX-1 | Endothelial cells, macrophages, smooth muscle cells | OxLDL, Hsp70, apoptotic cells, activated platelets, pathogen recognition | Endocytic uptake and degradation of modified oxLDL, signaling |
| FEEL-1/FEEL-2 | Endothelial cells, monocytes/macrophages | AcLDL, AGE, SPARC, hyaluronic acid, pathogen recognition | Endocytic uptake and degradation of AGEs hyaluronic acid and AcLDL |

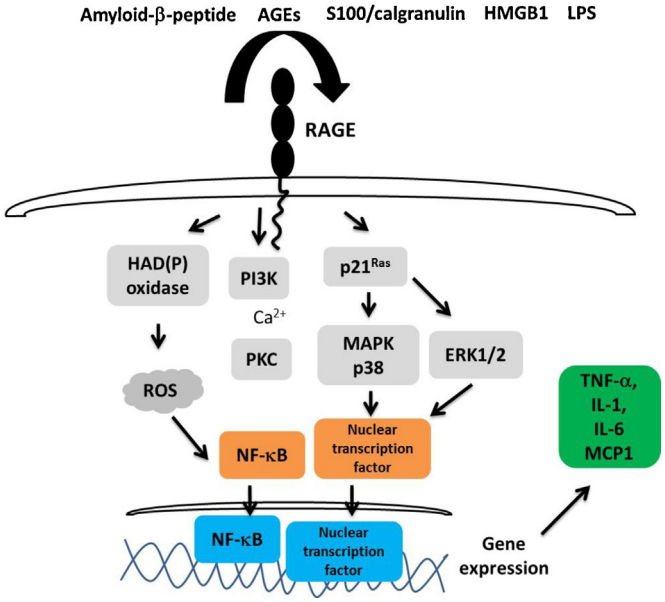


**Fig. 3.** Simplified RAGE signaling pathways.
The binding of the RAGE ligands to the receptor leads to the activation of different signaling cascades. In addition activated RAGE induces the generation of ROS. As a result of the activation nuclear transcription factors (NF-κB and others) translocate to the nucleus inducing among others the gene expression of inflammatory cytokines.

Other possibility to detect AGEs is the use of specific antibodies (ELISA, Western blot). However, the existence of commercially available antibodies for all of the known AGEs is limited. On the other hand this is one of the reasons why CML has been so well characterized in cells and tissues.

Although expensive, the most sensitive and accurate method for the AGEs-analysis is the liquid chromatography–mass spectrometry with tandem mass spectrometry (LC–MS/MS). Using this approach Thornalley et al. (2003) absolutely quantified AGEs in human plasma and urine and further found that AGEs content in cellular proteins was higher than in plasma protein (Thornalley et al., 2003).

#### 2.2.8. AGEs as biomarker

As AGEs accumulate during ageing their quantitation could be a good biomarker of age. With the development of the AGE-reader (DiagnOptics B.V., Groningen, The Netherlands) the measurement of the AGEs (their tissue accumulation) in the skin by mean of the skin autofluorescence (skin AF; sAF) is possible. We and others could show that indeed AGEs well correlate with the age and can be used as an age-biomarker (Simm et al., 2008). In addition, in the clinical routine, the percentage of glycated hemoglobin is widely used as biomarker in the diabetes mellitus diagnosis.

As a non-invasive method for the quantitation of AGEs, the AGE-reader actually finds an increasing propagation in the diagnosis and assessment of disease's complications. The below referred publications are only a short selection of recently published data on the very rapidly increasing field *"Quantitative-AGEs"* as a biomarker of age and diseases (Hofmann et al., 2013; Mulder et al., 2006; MacWay et al., 2014; Smit et al., 2013; Arsov et al., 2013).

## 3. Protein maintenance

### 3.1. Clusterin/Apolipoprotein J

#### 3.1.1. Clusterin/Apolipoprotein J: an overview

Clusterin/Apolipoprotein J (CLU) gene encodes for two protein isoforms; a highly conserved disulfide-linked heterodimeric secreted glycoprotein of ~75–80 kDa (sCLU) (de Silva et al., 1990a; Wong et al., 1994) was firstly identified in ram rete testis fluid (Blaschuk et al., 1983) and a truncated nuclear form (nCLU) (Leskov et al., 2003) was reported in human and murine cell lines exposed to various stress inducers (*e.g.* radiations, heat shock, drugs, etc.) as well as in carcinoma cells (Bettuzzi and Rizzi, 2009; Bhutia et al., 2012; Caccamo et al., 2003; Shannan et al., 2007, 2006). The sCLU has been found in most human tissues such as glands, kidney, placenta, liver, lymphoid tissues, epithelia, red blood cells and platelet alpha-granules (Antonelou et al., 2011 Aronow et al., 1993; de Silva et al., 1990a; Ekici et al., 2008; Gnatenko et al., 2003; Itahana et al.,

2007; Shin et al., 2008; Verbrugghe et al., 2008). It is a major protein in the serum and in most of the physiological fluids including cerebrospinal fluid, eye's aqueous, milk, urine and semen (Blaschuk et al., 1983; de Silva et al., 1990b; Dota et al., 1999; Jenne and Tschopp, 1989; Kapron et al., 1997; Kirszbaum et al., 1989; Nilselid et al., 2006). sCLU serves as apolipoprotein through its association with high-density lipoprotein (HDL) particles (de Silva et al., 1990a), dictating an important role in lipid metabolism and transport (Jenne et al., 1991; Kujiraoka et al., 2006). In addition sCLU also protects, in an ATP-independent manner, from protein aggregation and precipitation *via* its binding to hydrophobic regions of misfolding-prone proteins (Poon et al., 2000). Recently, sCLU has been classified as a functional homologue of the small heat shock proteins (Wyatt et al., 2009; Wyatt et al., 2011) due to its chaperone activity (Lakins et al., 2002; Materia et al., 2011; Narayan et al., 2012; Zoubeidi et al., 2010). With regards to its chaperone property sCLU is implicated in numerous physiological processes including development (Aronow et al., 1993), lipid transportation (de Silva et al., 1990b), differentiation (Trougakos et al., 2006), cellular senescence, *in vivo* ageing as well as in many age-related diseases including neurodegeneration (Charnay et al., 2012), vascular damage, diabetes and tumorigenesis (Trougakos and Gonos, 2006).

In clinical cancer research, clusterin has been established as a key therapeutic target as it has been found upregulated in various human malignancies promoting tumor growth, metastatic progression, cell survival to chemotherapy and ionizing radiation therapy (Criswell et al., 2003; Gleave and Jansen, 2003; Trougakos and Gonos, 2002).

#### 3.1.2. CLU association with stress and ageing

The impact of CLU on the regulation of ageing and stress response is highlighted not only by its induction in many pathological and age-related conditions including atherosclerosis, kidney degenerative diseases, carcinogenesis and several neurodegenerative disorders such as Scrapie, Alzheimer disease, epileptic foci and Pick's disease under increased oxidative stress and injury (Calero et al., 2000; Koch-Brandt and Morgans, 1996; Rosenberg and Silkensen, 1995) but also by its extracellular chaperone activity (Humphreys et al., 1999). CLU can interact with a few misfolded proteins in order to keep them soluble until the bound protein can be re-folded or degraded (Dabbs and Wilson, 2014; Wyatt et al., 2011). *In vivo*, complexes that were constructed between CLU and misfolded proteins in a rat model, were quickly cleared by hepatocytes and were degraded within lysosomes *via* receptor-mediated endocytosis by scavenger receptors (Wyatt et al., 2011). It has been suggested that CLU plays a key role in extracellular proteostasis because it is a member of extracellular chaperones that participate in the clearance of extracellular body fluids from misfolded proteins, inhibit them from forming toxic aggregates (Wyatt et al., 2013). Considering also that oxidants and oxidation injury modulate the activity of both the Activator Protein-1 (AP-1) and heat-shock transcription factor 1 (HSF1) (Martindale and Holbrook, 2002), the combined presence of both the AP-1 and "CLU-specific element" (CLE) regulatory elements in the CLU gene promoter (Jin and Howe, 1997; Michel et al., 1995) make CLU gene an extremely sensitive biosensor to environmental changes and particularly to the direct or indirect downstream effects of free radicals and their derivatives.

Recently, Riwanto et al. (2013) reported a higher prevalence of sCLU, a component of HDL, in healthy subjects compared to patients with coronary artery disease suggesting a protective role of sCLU in vascular disease progression (Riwanto et al., 2013). In line with these findings, reduced level of sCLU in high-density lipoproteins (HDL) has been correlated with increased insulin resistance, diabetes and obesity (Hoofnagle et al., 2010), while clusterin gene polymorphism was recently linked to type 2 diabetes mellitus (Daimon et al., 2011). Previous study had also shown that serum clusterin level increases significantly in diabetic type II patients and in patients with developing coronary heart disease or myocardial infarction (Trougakos et al., 2002).

During *in vivo* ageing CLU gene expression is significantly elevated from gestation to adults in humans (Mondello et al., 1999; Wong et al., 1994) whereas in fibroblast samples derived from donors of different ages, including centenarians, CLU expression was not positively correlated with donor age (Mondello et al., 1999). However, high levels of plasma clusterin were recently reported in healthy "supernormal" centenarians (older than 105 years), indicating a possible role of this protein in longevity (Miura et al., 2011; Shiota et al., 2011). Furthermore, various studies support a major impact of sCLU in the pathogenesis of Alzheimer disease (AD) revealing that allele variants of CLU are associated with increased risk of AD (Harold et al., 2009; Lambert et al., 2009) Finally, sCLU, among other apolipoproteins, has been identified as a potential plasma biomarker of cognitive decline in non-demented elderly individuals (Song et al., 2012). To conclude, the diverse functions of CLU and its pivotal action in the regulation of life expectancy and progression of age-related diseases emerge CLU as an excellent biomarker of cellular senescence and organismal ageing.

### 3.2. Proteasome

Protein homeostasis plays an important role for preserving biological integrity and fitness both at the cellular, tissular and organismal levels. The lysosomal and proteasomal pathways are the two main proteolytic machineries by which intracellular proteins are degraded. However, the proteasome is the main proteolytic system implicated in the turnover of cellular proteins through the targeted degradation of poly-ubiquitinated proteins as well as the removal of altered proteins. Impairment of proteasome function has been associated with ageing in a variety of mammalian tissues and cell types, hence contributing to the age-related accumulation of oxidatively proteins due to their decreased elimination. This chapter summarizes the most relevant features of the proteasomal system and the current knowledge on the impact of ageing on proteasome structure and function mostly in mammals but also on the modulation of proteasome activity and its effect on ageing and longevity.

#### 3.2.1. 20S and 26 proteasome structure and function

The 20S proteasome is a high molecular weight multicatalytic proteolytic complex found in Archae and Eukaryotes (Coux et al., 1996; Voges et al., 1999). In mammalian cells, in which it can represent up 1% of total proteins (Tanaka et al., 1986), the proteasome constitutes the main non lysosomal proteolytic system involved in protein degradation in the cytosol and in the nucleus. Besides acting as a housekeeping enzyme by eliminating abnormal and oxidized proteins (Davies, 2001; Grune et al., 1997), the proteasome is also implicated in a broad range of cellular functions through the selective degradation of ubiquitin-targeted regulatory proteins such as transcription factors, cyclins and rate-limiting enzymes in important metabolic pathways (Ciechanover and Iwai, 2004; Goldberg et al., 1997; King et al., 1996). The 20S proteasome is made up of four stacked rings of seven subunits classified as α or β subunits (Groll et al., 1997; Hegerl et al., 1991; Lowe et al., 1995). The eukaryotic proteasome carries three catalytically active β subunits : β1 for the peptidyl glutamylpeptide hydrolase (or caspase-like) activity, β2 for the trypsin-like activity and β5 for the chymotrypsin-like activity that cleave peptide bonds after an acidic, basic and hydrophobic aminoacid, respectively (Coux et al., 1996; Groll et al., 1997; Kisselev et al., 1999). When cells are exposed to such stimuli as IFNγ, TNFα or LPS the subunit composition of the

20S proteasome is modified, as inducible homologous subunits are incorporated in the structure upon *de novo* synthesis: the iβ1, iβ2 and iβ5 subunits, respectively replace their β1, β2 and β5 constitutive counterparts to form the immunoproteasome (Fruh et al., 1994; Gaczynska et al., 1993; Tanaka, 1994). Such replacement of proteasome subunits modify proteasome peptidase activities and lead to higher chymotrypsin-like and trypsin-like activities and lower peptidyl glutamylpeptide hydrolase activity, thus increasing production of peptides with higher affinity for MHC class I complex (Fruh et al., 1994; Gaczynska et al., 1993; Tanaka, 1994). In addition, the immunoproteasome has also been implicated in adaptation to oxidative stress by degrading oxidized proteins (Pickering et al., 2010), in longevity (Rodriguez et al., 2012) and in ageing as a response to chronic inflammation (Ferrington and Gregerson, 2012).

The 26S proteasome results from the ATP-dependent association with the PA700 or 19S regulator and is an essential component of the ubiquitin-proteasome degradation pathway of poly-ubiquitinated proteins. The association of PA28 or 11S regulator to the 20S proteasome is ATP-independent and results in an increase of proteasome peptidase activities while it does not improve protein degradation (Dubiel et al., 1992; Ma et al., 1992; Whitby et al., 2000). As for the immunoproteasome subunits, the expression of PA28 subunits is induced after treatment of cell by Interferon γ. In the cytosol PA28 is composed of two types of subunits α and β of about 28 kDa forming heptameric rings while in the nucleus PA28 is made of single type subunit γ (Knowlton et al., 1997; Mott et al., 1994). The 19S regulator is composed of eigthteen subunits belonging to either the « lid » or the « base » of the complex. Six of the nine subunits of the base are ATPases exhibiting a chaperone-like activity (Hershko and Ciechanover, 1998; Hershko and Ciechanover, 1998). The lid subunits are involved in the recognition of polyubiquitinated protein substrates and recycling the ubiquitin moiety through isopeptidase activity (Deveraux et al., 1994; Hershko and Ciechanover, 1998).

#### 3.2.2. Age-related impairment of proteasome function

Damage to macromolecules has been implicated in the cellular degeneration that occurs during ageing and accumulation of oxidized proteins represents a hallmark of cellular ageing (Beckman and Ames, 1998; Stadtman, 2006). Elimination of damaged protein and protein turnover is critical to preserve cell function and the main proteolytic system in charge of cytosolic protein degradation is the proteasome (Coux et al., 1996; Davies, 2001; Grune et al., 1997; Voges et al., 1999). Since accumulation of oxidatively damaged protein with age can be due to increased protein alteration, decreased elimination (*i.e.* repair and degradation) of damaged protein or the combination of both phenomenoms, one of the hypothesis that has been put forward to explain oxidized protein build-up is a decrease of proteasome activity with age (Friguet, 2006; Baraibar and Friguet, 2012).

During the past recent years several lines of evidence have indicated that proteasome function is impaired during the ageing process suggesting that this decreased activity might be causally related to ageing and age-associated diseases (Friguet, 2002; Breusing and Grune, 2008; Davies, 2001; Shringarpure and Davies, 2002), although some studies have shown that this decline may not be universal (Altun et al., 2010; Cook et al., 2009). Pioneering studies from our group and that of Walter Ward indicated that proteasome proteolytic activity is compromised with ageing (Conconi et al., 1996; Shibatani et al., 1996; Shibatani and Ward, 1996). A decrease in proteasome peptidase activity has been reported in aged tissues of other mammals (mouse, rat and bovine), like liver (Conconi et al., 1996; Shibatani et al., 1996; Shibatani and Ward, 1996; Hayashi and Goto, 1998), spinal cord (Keller et al., 2000), lens (Shang et al., 2001), heart (Bulteau et al., 2002) and retina (Louie et al., 2002). Furthermore, an age-related decline of proteasome activity has been also shown *ex vivo* in human lymphocytes (Ponnappan et al., 1999; Carrard et al., 2003), keratinocytes and fibroblasts (Petropoulos et al., 2000; Bulteau et al., 2000) and in human primary cell cultures undergoing replicative senescence (Merker and Grune, 2000; Sitte et al., 2000a,b; Chondrogianni et al., 2003). Impairment of proteasomal activity has also been reported during ageing in model organisms such as *Drosophila melanogaster* (Vernace et al., 2007; Tonoki et al., 2009) and *Caenorhabditis elegans* (Hamer et al., 2010). In contrast, centenarians who represent an interesting example of successful ageing, and the long-lived naked mole rats were found to exhibit elevated proteasome levels and activity (Chondrogianni et al., 2000; Perez et al., 2009).

In addition, recent studies have shown that the proteasome can be activated by genetic manipulations as well as by factors that affect either its conformation and stability or the expression of its subunits and the rate of proteasome assembly (Bulteau et al., 2006; Katsiki et al., 2007; Chondrogianni et al., 2014b). Indeed, over-expression of the 20S β5 subunit extended the replicative lifespan of cultured human fibroblasts (Chondrogianni et al., 2005). Interestingly, the restoration of normal level of proteasome activity upon lentiviral transfection of proteasome catalytic subunits was found to reduce ageing markers in dermal fibroblasts from elderly persons (Hwang et al., 2007). Concerning model organisms, overexpression of 19S proteasome activator subunit Rpn11 prolonged *D. melanogaster* lifespan (Tonoki et al., 2009), overexpression of the proteasome related transcription factor Rpn4 enhanced the replicative lifespan and resistance to proteotoxic stress of *Saccharomyces cerevisiae* (Kruegel et al., 2011; Yao et al., 2015), while overexpression of the 19S proteasome activator subunit rpn-6 and of the pbs-5 proteasome catalytic subunit also led to an extension of lifespan and resistance to proteotoxic stress (Vilchez et al., 2012; Chondrogianni et al., 2015)

### 3.3. Methionine sulfoxide reductase, an oxidized protein repair system

Most of the altered proteins are eliminated through degradation and very few repair systems relevant to protein oxidative damage have been documented. Indeed, only certain oxidation products of the sulfur-containing amino acids cysteine and methionine can be reversed back to their reduced forms within proteins (Petropoulos and Friguet, 2006; Chondrogianni et al., 2014a). The systems that have been implicated in reversing the oxidation of disulfide bridges and sulfenic acid, include the reduced forms of small proteins such as thioredoxin and glutaredoxin that get oxidized and are further recycled back to their reduced forms by thioredoxin reductase and glutathione/glutathione reductase, respectively. Conversely, methionine sulfoxide can be enzymatically reduced within proteins by the methionine sulfoxide reductase system that is further described below.

#### 3.3.1. Oxidized protein repair by the methionine sulfoxide reductases

Methionine sulfoxide reductases (Msr) are oxidoreductase enzymes that catalyze the reduction of methionine sulfoxide back to the reduced form of methionine within proteins. Oxidation of methionine most often lead to an impairment of protein function while reduction of methionine sulfoxide has been associated with its recovery. Thus, oxidation/reduction of methionine has been implicated in redox regulation of both protein function and protein-protein interaction (Moskovitz, 2005; Petropoulos and Friguet, 2006). It has also been proposed that Msr enzymes can act as effective antioxidant by scavenging ROS (Levine et al., 1996). Surface-exposed methionyl residues can be easily oxidized by almost all kinds of ROS and then further reduced by Msr through

successive oxidation/reduction reactions that are trapping ROS and preventing further protein irreversible oxidative modifications (Picot et al., 2005; Cabreiro et al., 2008). Hence, in the presence of active Msr surface-exposed methionyl residues appears as built-in protein antioxidants.

Methionine oxidation results in the formation of two diastereoisomers of methionine sulfoxide, Met-S(O) and Met-R(O), which can be further oxidized into methionine sulfone which is an irreversible oxidative modification. The two diastereoisomers of methionine sulfoxide, Met-S(O) and Met-R(O), are reduced back to methionine by Msr A and MsrB, respectively, that are then reduced by the thioredoxin/thioredoxine reductase system.

Msr genes are found in almost all organisms from bacteria to mammals (Delaye et al., 2007; Kryukov and Gladyshev, 2002). In mammals, one gene is coding for MsrA which exhibits different isoforms that are present both in the cytosol, in the nucleus and the mitochondrial matrix (Hansel et al., 2002; Vougier et al., 2003). In contrast, three distinct genes are encoding the different forms of MsrB in mammals. MsrB1, a selenoprotein, is present both in the nucleus and the cytosol while MsrB2 is present in the mitochondria. MsrB3 is localized in the endoplasmic reticulum and the mitochondria (Hansel et al., 2005; Kim and Gladyshev, 2004). All three MsrB are present in mammalian tissues, although at different levels (Hansel et al., 2005). Human MsrA mRNA was detected in all human tissues with highly varying expression levels. The maximum expression was found in kidney, cerebellum followed by liver, heart, bone marrow and hippocampus (Kuschel et al., 1999). Interestingly, FOXO3a was found to directly activate the human msrA promoter in a cell culture system, suggesting that this could be a conserved mechanism for MsrA regulation (Minniti et al., 2009). Although not present in certain leukemia cell lines, MsrA is expressed in peripheral blood lymphocytes (PBL), the highest expression being observed in the neutrophil granulocytes (Achilli et al., 2008).

#### 3.3.2. *Implication of Msr in resistance to oxidative stress, ageing and longevity*

Accumulation of damaged proteins has been proposed to play a crucial role in ageing and the expression of various age-related diseases (Stadtman, 2006) while failure of protein maintenance, i.e. degradation and repair, has been implicated in the age-related accumulation of damaged protein (Friguet, 2006; Petropoulos and Friguet, 2006). Hence, resistance to oxidative stress, decreased protein damage and improved protein maintenance are believed to be key elements for determining the lifespan and the rate of ageing of organisms.

The oxidized protein repair system Msr has been implicated in both cellular protection against oxidative stress and protein oxidative damage. Most of the evidence is coming from overexpression of either MsrA or MsrB in different eukaryotic cellular and organismal models ranking from yeast to *D. melanogaster*. Knock-out cellular and organismal models for Msr including *C. elegans* and mice, have also been the subject of studies addressing the role of Msr in resistance against oxidative stress and in longevity. In addition, the Msr system was shown to decline with age and has been recently associated with a number of human diseases (ref). Interestingly, age-related increase of methionine sulfoxide content has been documented in rat brain calmodulin and human skin collagen (Michaelis et al., 1996; Wells-Knecht et al., 1997).

We have first reported that MsrA expression is reduced during ageing in rat organs, *i.e.* liver, kidney and brain (Petropoulos et al., 2001). Conversely, MsrB1, but not MsrA activity, was reduced with age in mouse liver, suggesting that MsrB1 may account for the impaired methionine sulfoxide reduction in ageing mice liver (Novoselov et al., 2010). Moreover, both MsrA and MsrB2 expression were decreased exhibited a decrease in upon replicative senescence of WI-38 human fibroblasts which was concomitant with a decline of total Msr activity (Picot et al., 2004). Further studies have revealed that the decrease in Msr activity upon replicative senescence is more important in the mitochondria than in the cytosol (Ahmed et al., 2010). This decline of oxidized protein repair has been suggested to contribute to the age-associated accumulation of oxidatively damaged proteins.

Overexpression of MsrA predominantly in the nervous system of *D. melanogaster* resulted in a 70% increase in median survival and was shown to increase resistance to paraquat-induced oxidative stress (Ruan et al., 2002). However, ubiquitous overexpression of *Drosophila* MsrB or mouse MsrB1 or MsrB2 did not change the lifespan and oxidative stress resistance while ectopic expression of mouse MsrB1 in the nervous system led to an increased resistance against oxidative stress, but did not affect lifespan, revealing different effects of MsrA or MsrB on *Drosophila* ageing (Shchedrina et al., 2011; Shchedrina et al., 2009). In addition, both neuronal and ubiquitous expression of hMsrB3A rendered *Drosophila* resistant to oxidative stress and significantly extended their lifespan (Lim et al., 2012). It was initially reported that MsrA knock-out mice had a 40% reduced lifespan (Moskovitz et al., 2001) but more recent findings have shown that the lack of MsrA has no effect on mouse longevity when hosted in a proper environment in which wild-type lifespan was also increased as compared to the one observed in the previous study (Salmon et al., 2009). Nevertheless, MsrA knock-out mice were more sensitive to oxidative stress (Salmon et al., 2009) and presented a higher level of protein carbonyls than the wild type when exposed to hyperoxia (Moskovitz, 2007).

## 4. Conclusion

As described above, the candidate ageing biomarkers analysed within the workpackage dedicated to “markers based on proteins and their modifications” are focusing on enzymatic and non-enzymatic post-translational modifications of serum proteins by carbohydrates and on intracellular protein maintenance systems that are involved either in chaperoning or in the removal of oxidatively damaged proteins through degradation and repair. Indeed, changes in the abundance and post-translational modification of proteins and accumulation of modified proteins have been proposed to represent hallmarks of biological ageing while protein maintenance failure has been recognized as a major contributor to the age-associated accumulation of damaged proteins, including oxidized proteins that are analysed within the workpackage dedicated to “oxidative stress markers”. Thus, the markers based on proteins and their modifications represent a unique set of potential biomarkers to be further combined with the other biomarkers that will emerge from the complementary groups of classic and/or new markers reported in the other workpackages: “DNA-based markers”, “oxidative stress markers”, “clinical chemistry, hormones and markers of metabolism” and “immunological markers”. Indeed, it is expected that a combination of several biomarkers will provide a much better tool to measure biological age than any single biomarker in isolation. This combination is also aimed at optimising the weighting of the different markers since it is expected that not all biomarkers are of equal weight and just averageing all of them is most likely to be not appropriate.

For the most part, the markers based on proteins and their modifications that have been chosen are directly related with mechanistic aspects of the ageing process. Indeed, they are relevant to such important physiological features such as protein homeostasis and glycoprotein secretion that have been previously documented as being altered with age. Therefore, it is expected that they may be less influenced by other factors not necessarily related with ageing. Hence, The research tasks that have been specifically

addressed within the frame of the "markers based on proteins and their modifications" include: (1) the analysis of the *N*-glycomic changes in glycoproteins from donors blood, the urine glycoprotein changes being also studied in a subset of subjects, in Ghent, Belgium; (2) the monitoring of AGEs in plasma by fluorescence spectroscopy and by immunological analysis of specific AGEs such as carboxy-methyllysine, pentosidine, Arg-pyrimidine and imidazolone, in Halle, Germany; (3) the measurements of ApoJ/CLU levels in donors serum in Athens, Greece; (4) the measurements of proteasome and methionine sulfoxide reductase activities and the protein levels in donors PBMC lysates in Paris, France. These tasks have been achieved using biological samples (blood and urine) collected from the two recruited large groups of subjects from different European countries described earlier and referred as to: RASIG (for randomly recruited age-stratified individuals from the general population) and GEHA offspring (for subjects born from a long-living parent belonging to a family with long living sibling(s) already recruited in the framework of the GEHA project) and their spouses as controls.

## Acknowledgments

The authors thank all the partners of the MARK-AGE project (EU FP7 Integrated Project HEALTH-F4-2008-200880) and acknowledge the financial support from the European Union through this project.

## References

Achilli, C., Ciana, A., Rossi, A., Balduini, C., Minetti, G., 2008. Neutrophil granulocytes uniquely express, among human blood cells, high levels of Methionine-sulfoxide-reductase enzymes. J. Leukocyte Biol. 83, 181–189.

Ahmed, E.K., Rogowska-Wrzesinska, A., Roepstorff, P., Bulteau, A.L., Friguet, B., 2010. Protein modification and replicative senescence of WI-38 human embryonic fibroblasts. Aging Cell 9, 252–272.

Altun, M., Besche, H.C., Overkleeft, H.S., Piccirillo, R., Edelmann, M.J., Kessler, B.M., Goldberg, A.L., Ulfhake, B., 2010. Muscle wasting in aged, sarcopenic rats is associated with enhanced activity of the ubiquitin proteasome pathway. J. Biol. Chem. 285, 39597–39608.

Aronow, B.J., Lund, S.D., Brown, T.L., Harmony, J.A., Witte, D.P., 1993. Apolipoprotein J expression at fluid-tissue interfaces: potential role in barrier cytoprotection. Proc. Natl. Acad. Sci. U. S. A. 90, 725–729.

Arsov, S., Graaff, R., van Oeveren, W., Stegmayr, B., Sikole, A., Rakhorst, G., Smit, A.J., 2013. Advanced glycation end-products and skin autofluorescence in end-stage renal disease: a review. Clin. Chem. Lab. Med. 4, 1–10.

Baraibar, M.A., Friguet, B., 2012. Changes of the proteasomal system during the aging process. Prog. Mol. Biol. Transl. Sci. 109, 249–275.

Beckman, K.B., Ames, B.N., 1998. The free radical theory of aging matures. Physiol. Rev. 78, 547–581.

Beisswenger, P.J., Howell, S.K., Russell, G.B., Miller, M.E., Rich, S.S., Mauer, M., 2013. Early progression of diabetic nephropathy correlates with methylglyoxal-derived advanced glycation end products. Diabetes Care 36, 3234–3239.

Bettuzzi, S., Rizzi, F., 2009. Chapter 5: Nuclear CLU (nCLU) and the fate of the cell. Adv. Cancer Res 104, 59–88.

Bhutia, S.K., Das, S.K., Kegelman, T.P., Azab, B., Dash, R., Su, Z.Z., Wang, X.Y., Rizzi, F., Bettuzzi, S., Lee, S.G., Dent, P., Grant, S., Curiel, D.T., Sarkar, D., Fisher, P.B., 2012. mda-7/IL-24 differentially regulates soluble and nuclear clusterin in prostate cancer. J. Cell. Physiol. 227, 1805–1813.

Blaschuk, O., Burdzy, K., Fritz, I.B., 1983. Purification and characterization of a cell-aggregating factor (clusterin), the major glycoprotein in ram rete testis fluid. J. Biol. Chem. 258, 7714–7720.

Blomme, B., Van Steenkiste, C., Callewaert, N., Van Vlierberghe, H., 2009. Alteration of protein glycosylation in liver disease. J. Hepatol. 50, 592–603.

Bulteau, A.L., Petropoulos, I., Friguet, B., 2000. Age-related alterations of proteasome structure and function in aging epidermis. Exp. Gerontol. 35, 767–777.

Bulteau, A.L., Szweda, L.I., Friguet, B., 2002. Age-dependent declines in proteasome activity in the heart. Arch. Biochem. Biophys. 397, 298–304.

Bulteau, A.L., Moreau, M., Saunois, A., Nizard, C., Friguet, B., 2006. Algae extract-mediated stimulation and protection of proteasome activity within human keratinocytes exposed to UVA and UVB irradiation. Antioxid. Redox Signaling 8, 136–143.

Breusing, N., Grune, T., 2008. Regulation of proteasome-mediated protein degradation during oxidative stress and aging. Biol. Chem. 389, 203–209.

Cabreiro, F., Picot, C.R., Perichon, M., Castel, J., Friguet, B., Petropoulos, I., 2008. Overexpression of mitochondrial methionine sulfoxide reductase B2 protects leukemia cells from oxidative stress-induced cell death and protein damage. J. Biol. Chem. 283, 16673–16681.

Caccamo, A.E., Scaltriti, M., Caporali, A., D'Arca, D., Scorcioni, F., Candiano, G., Mangiola, M., Bettuzzi, S., 2003. Nuclear translocation of a clusterin isoform is associated with induction of anoikis in SV40-immortalized human prostate epithelial cells. Ann. N. Y. Acad. Sci. 1010, 514–519.

Calero, M., Rostagno, A., Matsubara, E., Zlokovic, B., Frangione, B., Ghiso, J., 2000. Apolipoprotein J (clusterin) and Alzheimer's disease. Microsc. Res. Tech. 50, 305–315.

Cantini, C., Kieffer, P., Corman, B., Liminana, P., Atkinson, J., Lartaud-Idjouadiene, I., 2001. Aminoguanidine and aortic wall mechanics, structure, and composition in aged rats. Hypertension 38, 943–948.

Carrard, G., Dieu, M., Raes, M., Toussaint, O., Friguet, B., 2003. Impact of ageing on proteasome structure and function in human lymphocytes. Int. J. Biochem. Cell Biol. 35, 728–739.

Charnay, Y., Imhof, A., Vallet, P.G., Kovari, E., Bouras, C., Giannakopoulos, P., 2012. Clusterin in neurological disorders: molecular perspectives and clinical relevance. Brain Res. Bull. 88, 434–443.

Chilelli, N.C., Burlina, S., Lapolla, A., 2013. AGEs, rather than hyperglycemia, are responsible for microvascular complications in diabetes: a glycoxidation-centric point of view. Nutr. Metab. Cardiovasc. Dis. 23, 913–919.

Chondrogianni, N., Petropoulos, I., Franceschi, C., Friguet, B., Gonos, E.S., 2000. Fibroblast cultures from healthy centenarians have an active proteasome. Exp. Gerontol. 35, 721–728.

Chondrogianni, N., Stratford, F.L., Trougakos, I.P., Friguet, B., Rivett, A.J., Gonos, E.S., 2003. Central role of the proteasome in senescence and survival of human fibroblasts: induction of a senescence-like phenotype upon its inhibition and resistance to stress upon its activation. J. Biol. Chem. 278, 28026–28037.

Chondrogianni, N., Tzavelas, C., Pemberton, A.J., Nezis, I.P., Rivett, A.J., Gonos, E.S., 2005. Overexpression of proteasome beta5 assembled subunit increases the amount of proteasome and confers ameliorated response to oxidative stress and higher survival rates. J. Biol. Chem. 280, 11840–11850.

Chondrogianni, N., Petropoulos, I., Grimm, S., Georgila, K., Catalgol, B., Friguet, B., Grune, T., Gonos, E.S., 2014a. Protein damage, repair and proteolysis. Mol. Aspect Med. 35, 1–71.

Chondrogianni, N., Sakellari, M., Lefaki, M., Papaevgeniou, N., Gonos, E.S., 2014b. Proteasome activation delays aging *in vitro* and *in vivo*. Free Radical Biol. Med. 71, 303–320.

Chondrogianni, N., Georgila, K., Kourtis, N., Tavernarakis, N., Gonos, E.S., 2015. 20S proteasome activation promotes lifespan extension and resistance to proteotoxicity in *C. elegans*. FASEB J. 29, 611–622.

Ciechanover, A., Iwai, K., 2004. The ubiquitin system: from basic mechanisms to the patient bed. IUBMB Life 56, 193–201.

Conconi, M., Szweda, L.I., Levine, R.L., Stadtman, E.R., Friguet, B., 1996. Age-related decline of rat liver multicatalytic proteinase activity and protection from oxidative inactivation by heat-shock protein 90. Arch. Biochem. Biophys. 331, 232–234.

Cook, C., Gass, J., Dunmore, J., Tong, J., Taylor, J., Eriksen, J., McGowan, E., Lewis, J., Johnston, J., Petrucelli, L., 2009. Aging is not associated with proteasome impairment in UPS reporter mice. PLoS One 4, e5888.

Coux, O., Tanaka, K., Goldberg, A.L., 1996. Structure and functions of the 20S and 26S proteasomes. Annu. Rev. Biochem. 65, 801–847.

Criswell, T., Klokov, D., Beman, M., Lavik, J.P., Boothman, D.A., 2003. Repression of IR-inducible clusterin expression by the p53 tumor suppressor protein. Cancer Biol. Ther. 2, 372–380.

Dabbs, R.A., Wilson, M.R., 2014. Expression and purification of chaperone–active recombinant clusterin. PLoS One 9, e86989.

Daimon, M., Oizumi, T., Karasawa, S., Kaino, W., Takase, K., Tada, K., Jimbu, Y., Wada, K., Kameda, W., Susa, S., Muramatsu, M., Kubota, I., Kawata, S., Kato, T., 2011. Association of the clusterin gene polymorphisms with type 2 diabetes mellitus. Metabolism 60, 815–822.

Dall'Olio, F., Vanhooren, V., Chen, C.C., Slagboom, P.E., Wuhrer, M., Franceschi, C., 2013. *N*-Glycomic biomarkers of biological aging and longevity: a link with inflammaging *N*-Glycomic biomarkers of biological aging and long evity: a link with inflammaging. Ageing Res. Rev. 12, 685–698.

Daoud, S., Schinzel, R., Neumann, A., Loske, C., Fraccarollo, D., Diez, C., Simm, A., 2001. Advanced glycation endproducts: activators of cardiac remodeling in primary fibroblasts from adult rat hearts. Mol. Med. 7, 543–551.

Davies, K.J., 2001. Degradation of oxidized proteins by the 20S proteasome. Biochimie 83, 301–310.

Delaye, L., Becerra, A., Orgel, L., Lazcano, A., 2007. Molecular evolution of peptide methionine sulfoxide reductases (MsrA and MsrB): on the early development of a mechanism that protects against oxidative damage. J. Mol. Evol. 64, 15–32.

Delgado-Andrade, C., Tessier, F.J., Niquet-Leridon, C., Seiquer, I., Pilar Navarro, M., 2012. Study of the urinary and faecal excretion of N(-carboxymethyllysine in young human volunteers. Amino Acids 43, 595–602.

de Silva, H.V., Harmony, J.A., Stuart, W.D., Gil, C.M., Robbins, J., 1990a. Apolipoprotein J: structure and tissue distribution. Biochemistry 29, 5380–5389.

de Silva, H.V., Stuart, W.D., Duvic, C.R., Wetterau, J.R., Ray, M.J., Ferguson, D.G., Albers, H.W., Smith, W.R., Harmony, J.A., 1990b. A 70 kDa apolipoprotein designated ApoJ is a marker for subclasses of human plasma high density lipoproteins. J. Biol. Chem. 265, 13240–13247.

Deveraux, Q., Ustrell, V., Pickart, C., Rechsteiner, M., 1994. A 26 S protease subunit that binds ubiquitin conjugates. J. Biol. Chem. 269, 7059–7061.

Dota, A., Nishida, K., Quantock, A.J., Kinoshita, S., 1999. Clusterin in human corneal endothelium and aqueous humor. Exp. Eye Res. 69, 705–708.
Dubiel, W., Pratt, G., Ferrell, K., Rechsteiner, M., 1992. Purification of an 11 S regulator of the multicatalytic protease. J. Biol. Chem. 267, 22369–22377.
Ekici, A.I., Eren, B., Turkmen, N., Comunoglu, N., Fedakar, R., 2008. Clusterin expression in non-neoplastic adenohypophyses and pituitary adenomas: cytoplasmic clusterin localization in adenohypophysis is related to aging. Endocr. Pathol. 19, 47–53.
Faist, V., Erbersdobler, H.F., 2001. Metabolic transit and *in vivo* effects of melanoidins and precursor compounds deriving from the Maillard reaction. Ann. Nutr. Metab. 45, 1–12.
Fawver, J.N., Schall, H.E., Petrofes Chapa, R.D., Zhu, X., Murray, I.V., 2012. Amyloid-β metabolite sensing: biochemical linking of glycation modification and misfolding. J. Alzheimers Dis. 30, 63–73.
Ferrington, D.A., Gregerson, D.S., 2012. Immunoproteasomes: structure, function, and antigen presentation. Prog. Mol. Biol. Transl. Sci. 109, 75–112.
Friguet, B., 2002. Aging of proteins and the proteasome. Prog. Mol. Subcell. Biol. 29, 17–33.
Friguet, B., 2006. Oxidized protein degradation and repair in ageing and oxidative stress. FEBS Lett. 580, 2910–2916.
Fruh, K., Gossen, M., Wang, K., Bujard, H., Peterson, P.A., Yang, Y., 1994. Displacement of housekeeping proteasome subunits by MHC-encoded LMPs: a newly discovered mechanism for modulating the multicatalytic proteinase complex. Embo J. 13, 3236–3244.
Gaczynska, M., Rock, K.L., Goldberg, A.L., 1993. Gamma-interferon and expression of MHC genes regulate peptide hydrolysis by proteasomes. Nature 365, 264–267.
Gleave, M., Jansen, B., 2003. Clusterin and IGFBPs as antisense targets in prostate cancer. Ann. N. Y. Acad. Sci. 1002, 95–104.
Gnatenko, D.V., Dunn, J.J., McCorkle, S.R., Weissmann, D., Perrotta, P.L., Bahou, W.F., 2003. Transcript profiling of human platelets using microarray and serial analysis of gene expression. Blood 101, 2285–2293.
Goldberg, A.L., Akopian, T.N., Kisselev, A.F., Lee, D.H., Rohrwild, M., 1997. New insights into the mechanisms and importance of the proteasome in intracellular protein degradation. Biol. Chem. 378, 131–140.
Goldberg, T., Cai, W., Peppa, M., Dardaine, V., Baliga, B.S., Uribarri, J., Vlassara, H., 2004. Advanced glycoxidation end products in commonly consumed foods. J. Am. Diet Assoc. 104, 1287–1291.
Griffiths, H.R., Dias, I.H., Willetts, R.S., Devitt, A., 2014. Redox regulation of protein damage in plasma. Redox Biol. 2, 430–435.
Grimm, S., Ott, C., Hörlacher, M., Weber, D., Höhn, A., Grune, T., 2012. Advanced-glycation-end-product-induced formation of immunoproteasomes: involvement of RAGE and Jak2/STAT1. Biochem. J. 448, 127–139.
Groll, M., Ditzel, L., Lowe, J., Stock, D., Bochtler, M., Bartunik, H.D., Huber, R., 1997. Structure of 20S proteasome from yeast at 2.4 A resolution. Nature 386, 463–471.
Grune, T., Reinheckel, T., Davies, K.J., 1997. Degradation of oxidized proteins in mammalian cells. FASEB J. 11, 526–534.
Guerrero, E., Vasudevaraju, P., Hegde, M.L., Britton, G.B., Rao, K.S., 2013. Recent advances in α-synuclein functions, advanced glycation, and toxicity: implications for Parkinson's disease. Mol. Neurobiol. 47, 525–536.
Hamer, G., Matilainen, O., Holmberg, C.I., 2010. A photoconvertible reporter of the ubiquitin-proteasome system *in vivo*. Nat. Methods 7, 473–478.
Hansel, A., Kuschel, L., Hehl, S., Lemke, C., Agricola, H.J., Hoshi, T., Heinemann, S.H., 2002. Mitochondrial targeting of the human peptide methionine sulfoxide reductase (MSRA): an enzyme involved in the repair of oxidized proteins. FASEB J. 16, 911–913.
Hansel, A., Heinemann, S.H., Hoshi, T., 2005. Heterogeneity and function of mammalian MSRs: enzymes for repair, protection and regulation. Biochim. Biophys. Acta 1703, 239–247.
Harold, D., Abraham, R., Hollingworth, P., Sims, R., Gerrish, A., Hamshere, M.L., Pahwa, J.S., Moskvina, V., Dowzell, K., Williams, A., Jones, N., Thomas, C., Stretton, A., Morgan, A.R., Lovestone, S., Powell, J., Proitsi, P., Lupton, M.K., Brayne, C., Rubinsztein, D.C., Gill, M., Lawlor, B., Lynch, A., Morgan, K., Brown, K.S., Passmore, P.A., Craig, D., McGuinness, B., Todd, S., Holmes, C., Mann, D., Smith, A.D., Love, S., Kehoe, P.G., Hardy, J., Mead, S., Fox, N., Rossor, M., Collinge, J., Maier, W., Jessen, F., Schurmann, B., Heun, R., van den Bussche, H., Heuser, I., Kornhuber, J., Wiltfang, J., Dichgans, M., Frolich, L., Hampel, H., Hull, M., Rujescu, D., Goate, A.M., Kauwe, J.S., Cruchaga, C., Nowotny, P., Morris, J.C., Mayo, K., Sleegers, K., Bettens, K., Engelborghs, S., De Deyn, P.P., Van Broeckhoven, C., Livingston, G., Bass, N.J., Gurling, H., McQuillin, A., Gwilliam, R., Deloukas, P., Al-Chalabi, A., Shaw, C.E., Tsolaki, M., Singleton, A.B., Guerreiro, R., Muhleisen, T.W., Nothen, M.M., Moebus, S., Jockel, K.H., Klopp, N., Wichmann, H.E., Carrasquillo, M.M., Pankratz, V.S., Younkin, S.G., Holmans, P.A., O'Donovan, M., Owen, M.J., Williams, J., 2009. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat. Genet. 41, 1088–1093.
Humphreys, D.T., Carver, J.A., Easterbrook-Smith, S.B., Wilson, M.R., 1999. Clusterin has chaperone-like activity similar to that of small heat shock proteins. J. Biol. Chem. 274, 6875–6881.
Hartog, J.W., Willemsen, S., van Veldhuisen, D.J., Posma, J.L., van Wijk, L.M., Hummel, Y.M., Hillege, H.L., Voors, A.A., 2011. BENEFICIAL investigators effects of alagebrium, an advanced glycation endproduct breaker, on exercise tolerance and cardiac function in patients with chronic heart failure. Eur. J. Heart Fail 13, 899–908.
Hayashi, T., Goto, S., 1998. Age-related changes in the 20S and 26S proteasome activities in the liver of male F344 rats. Mech. Ageing Dev. 102, 55–66.
Hegerl, R., Pfeifer, G., Puhler, G., Dahlmann, B., Baumeister, W., 1991. The three-dimensional structure of proteasomes from *Thermoplasma acidophilum* as determined by electron microscopy using random conical tilting. FEBS Lett. 283, 117–121.
Hershko, A., Ciechanover, A., 1998. The ubiquitin system. Annu. Rev. Biochem. 67, 425–479.
Hodge, J.E., 1953. Dehydrated foods, chemistry of browning reactions in model systems. J. Agric. Food Chem. 1, 928–943.
Hofmann, B., Adam, A.C., Jacobs, K., Riemer, M., Erbs, C., Bushnaq, H., Simm, A., Silber, R.E., Navarette Santos, A., 2013. Advanced glycation end product associated skin autofluorescence: a mirror of vascular function? Exp. Gerontol. 48, 38–44.
Hoofnagle, A.N., Wu, M., Gosmanova, A.K., Becker, J.O., Wijsman, E.M., Brunzell, J.D., Kahn, S.E., Knopp, R.H., Lyons, T.J., Heinecke, J.W., 2010. Low clusterin levels in high-density lipoprotein associate with insulin resistance, obesity, and dyslipoproteinemia. Arterioscler. Thromb. Vasc. Biol. 30, 2528–2534.
Hull, G.L.J., Woodside, J.V., Ames, J.M., Cuskelly, G.J., 2012. *N*(-(carboxymethyl) lysine content of foods commonly consumed in a Western style diet *N*(-(carboxymethyl) lysine content of foods commonly consumed in a Western style diet. Food Chem. 131, 170–174.
Hwang, J.S., Chang, I., Kim, S., 2007. Age-associated decrease in proteasome content and activities in human dermal fibroblasts: restoration of normal level of proteasome subunits reduces aging markers in fibroblasts from elderly persons. J. Gerontol. A Biol. Sci. Med. Sci. 62, 490–499.
Itahana, Y., Piens, M., Sumida, T., Fong, S., Muschler, J., Desprez, P.Y., 2007. Regulation of clusterin expression in mammary epithelial cells. Exp. Cell Res. 313, 943–951.
Jenne, D.E., Lowin, B., Peitsch, M.C., Bottcher, A., Schmitz, G., Tschopp, J., 1991. Clusterin (complement lysis inhibitor) forms a high density lipoprotein complex with apolipoprotein A-I in human plasma. J. Biol. Chem. 266, 11030–11036.
Jenne, D.E., Tschopp, J., 1989. Molecular structure and functional characterization of a human complement cytolysis inhibitor found in blood and seminal plasma: identity to sulfated glycoprotein 2, a constituent of rat testis fluid. Proc. Natl. Acad. Sci. U. S. A. 86, 7123–7127.
Jin, G., Howe, P.H., 1997. Regulation of clusterin gene expression by transforming growth factor beta. J. Biol. Chem. 272, 26620–26626.
Kamerling, J., Boons, G.-J., Lee, Y., Suzuki, A., Taniguchi, N., Voragen, A.G.J. (Eds.), 2007. Comprehensive Glycoscience, Vols. 1-4. Elsevier, Amsterdam, Boston, Heidelberg, London, New York.
Kapron, J.T., Hilliard, G.M., Lakins, J.N., Tenniswood, M.P., West, K.A., Carr, S.A., Crabb, J.W., 1997. Identification and characterization of glycosylation sites in human serum clusterin. Protein Sci. 6, 2120–2133.
Katsiki, M., Chondrogianni, N., Chinou, I., Rivett, A.J., Gonos, E.S., 2007. The olive constituent oleuropein exhibits proteasome stimulatory properties *in vitro* and confers life span extension of human embryonic fibroblasts. Rejuvenation Res. 10, 157–172.
Keller, J.N., Huang, F.F., Markesbery, W.R., 2000. Decreased levels of proteasome activity and proteasome expression in aging spinal cord. Neuroscience 98, 149–156.
Kim, H.Y., Gladyshev, V.N., 2004. Methionine sulfoxide reduction in mammals: characterization of methionine-R-sulfoxide reductases. Mol. Biol. Cell 15, 1055–1064.
King, R.W., Deshaies, R.J., Peters, J.M., Kirschner, M.W., 1996. How proteolysis drives the cell cycle. Science 274, 1652–1659.
Kirszbaum, L., Sharpe, J.A., Murphy, B., d'Apice, A.J., Classon, B., Hudson, P., Walker, I.D., 1989. Molecular cloning and characterization of the novel, human complement-associated protein, SP-40,40: a link between the complement and reproductive systems. EMBO J. 8, 711–718.
Kisselev, A.F., Akopian, T.N., Castillo, V., Goldberg, A.L., 1999. Proteasome active sites allosterically regulate each other, suggesting a cyclical bite-chew mechanism for protein breakdown. Mol. Cell 4, 395–402.
Knezevic, A., Polasek, O., Gornik, O., Rudan, I., Campbell, H., Hayward, C., Wright, A., Kolcic, I., O'Donoghue, N., Bones, J., Rudd, P.M., Lauc, G., 2009. Variability, heritability and environmental determinants of human plasma *N*-glycome. J. Proteome Res. 8, 694–701.
Knezevic, A., Gornik, O., Polasek, O., Pucic, M., Redzic, I., Novokmet, M., Rudd, P.M., Wright, A.F., Campbell, H., Rudan, I., Lauc, G., 2010. Effects of aging, body mass index, plasma lipid profiles, and smoking on human plasma N-glycans. Glycobiology 20, 959–969.
Knowlton, J.R., Johnston, S.C., Whitby, F.G., Realini, C., Zhang, Z., Rechsteiner, M., Hill, C.P., 1997. Structure of the proteasome activator REG alpha (PA28 alpha). Nature 390, 639–643.
Koch-Brandt, C., Morgans, C., 1996. Clusterin: a role in cell survival in the face of apoptosis? Prog. Mol. Subcell. Biol. 16, 130–149.
Kondo, A., Li, W., Nakagawa, T., Nakano, M., Koyama, N., Wang, X., Gu, J., Miyoshi, E., Taniguchi, N., 2006. From glycomics to functional glycomics of sugar chains: identification of target proteins with functional changes using gene targeting mice and knock down cells of FUT8 as examples. Biochim. Biophys. Acta 1764, 1881–1889.
Kruegel, U., Robison, B., Dange, T., Kahlert, G., Delaney, J.R., Kotireddy, S., Tsuchiya, M., Tsuchiyama, S., Murakami, C.J., Schleit, J., Sutphin, G., Carr, D., Tar, K., Dittmar, G., Kaeberlein, M., Kennedy, B.K., Schmidt, M., 2011. Elevated

proteasome capacity extends replicative lifespan in *Saccharomyces cerevisiae*. PLoS Genet. 7, e1002253.
Kryukov, G.V., Gladyshev, V.N., 2002. Mammalian selenoprotein gene signature: identification and functional analysis of selenoprotein genes using bioinformatics methods. Methods Enzymol. 347, 84–100.
Kujiraoka, T., Hattori, H., Miwa, Y., Ishihara, M., Ueno, T., Ishii, J., Tsuji, M., Iwasaki, T., Sasaguri, Y., Fujioka, T., Saito, S., Tsushima, M., Maruyama, T., Miller, I.P., Miller, N.E., Egashira, T., 2006. Serum apolipoprotein j in health, coronary heart disease and type 2 diabetes mellitus. J. Atheroscler. Thromb. 13, 314–322.
Kuschel, L., Hansel, A., Schonherr, R., Weissbach, H., Brot, N., Hoshi, T., Heinemann, S.H., 1999. Molecular cloning and functional expression of a human peptide methionine sulfoxide reductase (hMsrA). FEBS Lett. 456, 17–21.
Lakins, J.N., Poon, S., Easterbrook-Smith, S.B., Carver, J.A., Tenniswood, M.P., Wilson, M.R., 2002. Evidence that clusterin has discrete chaperone and ligand binding sites. Biochemistry 41, 282–291.
Lambert, J.C., Heath, S., Even, G., Campion, D., Sleegers, K., Hiltunen, M., Combarros, O., Zelenika, D., Bullido, M.J., Tavernier, B., Letenneur, L., Bettens, K., Berr, C., Pasquier, F., Fievet, N., Barberger-Gateau, P., Engelborghs, S., De Deyn, P., Mateo, I., Franck, A., Helisalmi, S., Porcellini, E., Hanon, O., de Pancorbo, M.M., Lendon, C., Dufouil, C., Jaillard, C., Leveillard, T., Alvarez, V., Bosco, P., Mancuso, M., Panza, F., Nacmias, B., Bossu, P., Piccardi, P., Annoni, G., Seripa, D., Galimberti, D., Hannequin, D., Licastro, F., Soininen, H., Ritchie, K., Blanche, H., Dartigues, J.F., Tzourio, C., Gut, I., Van Broeckhoven, C., Alperovitch, A., Lathrop, M., Amouyel, P., 2009. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat. Genet. 41, 1094–1099.
Lee, S.J., Evers, S., Roeder, D., Parlow, A.F., Risteli, J., Risteli, L., Lee, Y.C., Feizi, T., Langen, H., Nussenzweig, M.C., 2002. Mannose receptor mediated regulation of serum glycoprotein homeostasis. Science 295, 1898–1901.
Leskov, K.S., Klokov, D.Y., Li, J., Kinsella, T.J., Boothman, D.A., 2003. Synthesis and functional analyses of nuclear clusterin, a cell death protein. J. Biol. Chem. 278, 11590–11600.
Levine, R.L., Mosoni, L., Berlett, B.S., Stadtman, E.R., 1996. Methionine residues as endogenous antioxidants in proteins. Proc. Natl. Acad. Sci. U. S. A. 93, 15036–15040.
Li, J., Liu, D., Sun, L., Lu, Y., Zhang, Z., 2012. Advanced glycation end products and neurodegenerative diseases: mechanisms and perspective. J. Neurol. Sci. 317, 1–5.
Lim, D.H., Han, J.Y., Kim, J.R., Lee, Y.S., Kim, H.Y., 2012. Methionine sulfoxide reductase B in the endoplasmic reticulum is critical for stress resistance and aging in *Drosophila*. Biochem. Biophys. Res. Commun. 420, 130–135.
Lis, H., Sharon, N., 1993. Protein glycosylation: structural and functional aspects. Eur. J. Biochem. 218, 1–27.
Louie, J.L., Kapphahn, R.J., Ferrington, D.A., 2002. Proteasome function and protein oxidation in the aged retina. Exp. Eye Res. 75, 271–284.
Lowe, J., Stock, D., Jap, B., Zwickl, P., Baumeister, W., Huber, R., 1995. Crystal structure of the 20S proteasome from the archaeon *T. acidophilum* at 3.4 A resolution. Science 268, 533–539.
Ma, C.P., Slaughter, C.A., DeMartino, G.N., 1992. Identification, purification, and characterization of a protein activator (PA28) of the 20 S proteasome (macropain). J. Biol. Chem. 267, 10515–10523.
Mac-Way, F., Couture, V., Utescu, M.S., Ignace, S., De Serres, S.A., Loignon, R.C., Marquis, K., Larivière, R., Agharazii, M., 2014. Advanced glycation end products, aortic stiffness, and wave reflection in peritoneal dialysis as compared to hemodialysis. Int. Urol. Nephrol. 46, 817–824.
Maillard, L.C., 1912. Action of amino acids on sugars. Formation of melanoidins in a methodical may. Comptes Rendus 154, 66.
Martindale, J.L., Holbrook, N.J., 2002. Cellular response to oxidative stress: signaling for suicide and survival. J. Cell. Physiol. 192, 1–15.
Materia, S., Cater, M.A., Klomp, L.W., Mercer, J.F., La Fontaine, S., 2011. Clusterin (apolipoprotein J), a molecular chaperone that facilitates degradation of the copper-ATPases ATP7A and ATP7B. J. Biol. Chem. 286, 10073–10083.
Merker, K., Grune, T., 2000. Proteolysis of oxidised proteins and cellular senescence. Exp. Gerontol. 35, 779–786.
Michaelis, M.L., Bigelow, D.J., Schoneich, C., Williams, T.D., Ramonda, L., Yin, D., Huhmer, A.F., Yao, Y., Gao, J., Squier, T.C., 1996. Decreased plasma membrane calcium transport activity in aging brain. Life sci. 59, 405–412.
Michel, D., Chatelain, G., Herault, Y., Brun, G., 1995. The expression of the avian clusterin gene can be driven by two alternative promoters with distinct regulatory elements. Eur. J. Biochem. 229, 215–223.
Minniti, A.N., Cataldo, R., Trigo, C., Vasquez, L., Mujica, P., Leighton, F., Inestrosa, N.C., Aldunate, R., 2009. Methionine sulfoxide reductase A expression is regulated by the DAF-16/FOXO pathway in *Caenorhabditis elegans*. Aging Cell 8, 690–705.
Miura, Y., Sato, Y., Arai, Y., Abe, Y., Takayama, M., Toda, T., Hirose, N., Endo, T., 2011. Proteomic analysis of plasma proteins in Japanese semisuper centenarians. Exp. Gerontol. 46, 81–85.
Mondello, C., Petropoulou, C., Monti, D., Gonos, E.S., Franceschi, C., Nuzzo, F., 1999. Telomere length in fibroblasts and blood cells from healthy centenarians. Exp. Cell Res. 248, 234–242.
Montagnani, M., 2008. Diabetic cardiomyopathy: how much does it depend on AGE? Br. J. Pharmacol. 154, 725–726.
Moskovitz, J., Bar-Noy, S., Williams, W.M., Requena, J., Berlett, B.S., Stadtman, E.R., 2001. Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. Proc. Natl. Acad. Sci. U. S. A. 98, 12920–12925.
Moskovitz, J., 2005. Roles of methionine suldfoxide reductases in antioxidant defense, protein regulation and survival. Curr. Pharm. Des. 1, 1451–1457.
Moskovitz, J., 2007. Prolonged selenium-deficient diet in MsrA knockout mice causes enhanced oxidative modification to proteins and affects the levels of antioxidant enzymes in a tissue-specific manner. Free Radical Res. 41, 162–171.
Mott, J.D., Pramanik, B.C., Moomaw, C.R., Afendis, S.J., DeMartino, G.N., Slaughter, C.A., 1994. PA28, an activator of the 20 S proteasome, is composed of two nonidentical but homologous subunits. J. Biol. Chem. 269, 31466–31471.
Mulder, D.J., Van De Water, T., Lutgers, H.L., Graaff, R., Gans, R.O., Zijlstra, F., Smit, A.J., 2006. Skin autofluorescence, a novel marker for glycemic and oxidative stress-derived advanced glycation endproducts: an overview of current clinical studies, evidence, and limitations. Diabetes Technol. Ther. 8, 523–535.
Nah, S.S., Choi, I.Y., Lee, C.K., Oh, J.S., Kim, Y.G., Moon, H.B., Yoo, B., 2008. Effects of advanced glycation end products on the expression of COX-2, PGE2 and NO in human osteoarthritic chondrocytes. Rheumatology 47, 425–431.
Narayan, P., Orte, A., Clarke, R.W., Bolognesi, B., Hook, S., Ganzinger, K.A., Meehan, S., Wilson, M.R., Dobson, C.M., Klenerman, D., 2012. The extracellular chaperone clusterin sequesters oligomeric forms of the amyloid-beta(1-40) peptide. Nat. Struct. Mol. Biol. 19, 79–83.
Nilselid, A.M., Davidsson, P., Nagga, K., Andreasen, N., Fredman, P., Blennow, K., 2006. Clusterin in cerebrospinal fluid: analysis of carbohydrates and quantification of native and glycosylated forms. Neurochem. Int. 48, 718–728.
Nishizawa, Y., Koyama, H., Inaba, M., 2012. AGEs and cardiovascular diseases in patients with end-stage renal diseases. J. Ren. Nutr. 22, 128–133.
Novoselov, S.V., Kim, H.Y., Hua, D., Lee, B.C., Astle, C.M., Harrison, D.E., Friguet, B., Moustafa, M.E., Carlson, B.A., Hatfield, D.L., Gladyshev, V.N., 2010. Regulation of selenoproteins and methionine sulfoxide reductases A and B1 by age, calorie restriction, and dietary selenium in mice. Antioxid. Redox Signaling 12, 829–838.
Ohtsubo, K., Marth, J.D., 2006. Glycosylation in cellular mechanisms of health and disease. Cell 126, 855–867.
Ott, C., Jacobs, K., Haucke, E., Navarrete Santos, A., Grune, T., Simm, A., 2014. Role of advanced glycation end products in cellular signaling. Redox Biol. 2, 411–429.
Parekh, R., Roitt, I., Isenberg, D., Dwek, R., Rademacher, T., 1988. Age-related galactosylation of the N-linked oligosaccharides of human serum IgG. J. Exp. Med. 167, 1731–1736.
Perez, V.I., Buffenstein, R., Masamsetti, V., Leonard, S., Salmon, A.B., Mele, J., Andziak, B., Yang, T., Edrey, Y., Friguet, B., Ward, W., Richardson, A., Chaudhuri, A., 2009. Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. Proc. Natl. Acad. Sci. U. S. A. 106, 3059–3064.
Petropoulos, I., Conconi, M., Wang, X., Hoenel, B., Bregegere, F., Milner, Y., Friguet, B., 2000. Increase of oxidatively modified protein is associated with a decrease of proteasome activity and content in aging epidermal cells. J. Gerontol. A Biol. Sci. Med. Sci. 55, B220–227.
Petropoulos, I., Mary, J., Perichon, M., Friguet, B., 2001. Rat peptide methionine sulphoxide reductase: cloning of the cDNA, and down-regulation of gene expression and enzyme activity during aging. Biochem. J. 355, 819–825.
Petropoulos, I., Friguet, 2006. Maintenance of proteins and aging: the role of oxidized protein repair. Free Radical Res. 40, 1269–1276.
Pickering, A.M., Koop, A.L., Teoh, C.Y., Ermak, G., Grune, T., Davies, K.J., 2010. The immunoproteasome, the 20S proteasome and the PA28alphabeta proteasome regulator are oxidative-stress-adaptive proteolytic complexes. Biochem. J. 432, 585–594.
Picot, C.R., Perichon, M., Cintrat, J.C., Friguet, B., Petropoulos, I., 2004. The peptide methionine sulfoxide reductases, MsrA and MsrB (hCBS-1), are downregulated during replicative senescence of human WI-38 fibroblasts. FEBS Lett. 558, 74–78.
Picot, C.R., Petropoulos, I., Perichon, M., Moreau, M., Nizard, C., Friguet, B., 2005. Overexpression of MsrA protects WI-38 SV40 human fibroblasts against H2O2-mediated oxidative stress. Free Radical Biol. Med. 39, 1332–1341.
Ponnappan, U., Zhong, M., Trebilcock, G.U., 1999. Decreased proteasome-mediated degradation in T cells from the elderly: a role in immune senescence. Cell Immunol. 192, 167–174.
Poon, S., Easterbrook-Smith, S.B., Rybchyn, M.S., Carver, J.A., Wilson, M.R., 2000. Clusterin is an ATP-independent chaperone with very broad substrate specificity that stabilizes stressed proteins in a folding-competent state. Biochemistry 39, 15953–15960.
Riwanto, M., Rohrer, L., Roschitzki, B., Besler, C., Mocharla, P., Mueller, M., Perisa, D., Heinrich, K., Altwegg, L., von Eckardstein, A., Luscher, T.F., Landmesser, U., 2013. Altered activation of endothelial anti- and proapoptotic pathways by high-density lipoprotein from patients with coronary artery disease: role of high-density lipoprotein-proteome remodeling. Circulation 127, 891–904.
Rodriguez, K.A., Edrey, Y.H., Osmulski, P., Gaczynska, M., Buffenstein, R., 2012. Altered composition of liver proteasome assemblies contributes to enhanced proteasome activity in the exceptionally long-lived naked mole-rat. PLoS One 7, e35890.
Rojas, A., Delgado-López, F., González, I., Pérez-Castro, R., Romero, J., Rojas, I., 2013. The receptor for advanced glycation end-products: a complex signaling scenario for a promiscuous receptor. Cell Signaling 25, 609–614.
Rosenberg, M.E., Silkensen, J., 1995. Clusterin: physiologic and pathophysiologic considerations. Int. J. Biochem. Cell Biol. 27, 633–645.
Ruan, H., Tang, X.D., Chen, M.L., Joiner, M.L., Sun, G., Brot, N., Weissbach, H., Heinemann, S.H., Iverson, L., Wu, C.F., Hoshi, T., Joiner, M.A., 2002. High-quality life extension by the enzyme peptide methionine sulfoxide reductase. Proc. Natl. Acad. Sci. U. S. A. 99, 2748–2753.

Ruhaak, L.R., Uh, H.W., Beekman, M., Hokke, C.H., Westendorp, R.G., Houwing-Duistermaat, J., Wuhrer, M., Deelder, A.M., Slagboom, P.E., 2011. Plasma protein *N*-glycan profiles are associated with calendar age, familial longevity and health. J. Proteome Res. 10, 1667–1674.
Salmon, A.B., Perez, V.I., Bokov, A., Jernigan, A., Kim, G., Zhao, H., Levine, R.L., Richardson, A., 2009. Lack of methionine sulfoxide reductase A in mice increases sensitivity to oxidative stress but does not diminish life span. FASEB J. 23, 3601–3608.
San Martin, A., Foncea, R., Laurindo, F.R., Ebensperger, R., Griendling, K.K., Leighton, F., 2007. Nox1-based NADPH oxidase-derived superoxide is required for VSMC activation by advanced glycation end-products. Free Radical Biol. Med. 42, 1671–1679.
Schmitt, A., Schmitt, J., Münch, G., Gasic-Milencovic, J., 2005. Characterization of advanced glycation end products for biochemical studies: side chain modifications and fluorescence characteristics. Anal. Biochem. 338, 201–215.
Sell, D.R., Monnier, V.M., 2012. Molecular basis of arterial stiffening: role of glycation – a mini-review. Gerontology 58, 227–237.
Shang, F., Nowell Jr., T.R., Taylor, A., 2001. Removal of oxidatively damaged proteins from lens cells by the ubiquitin-proteasome pathway. Exp. Eye Res. 73, 229–238.
Shannan, B., Seifert, M., Boothman, D.A., Tilgen, W., Reichrath, J., 2007. Clusterin over-expression modulates proapoptotic and antiproliferative effects of 1,25(OH) 2D3 in prostate cancer cells *in vitro*. J. Steroid Biochem. Mol. Biol 103, 721–725.
Shannan, B., Seifert, M., Leskov, K., Willis, J., Boothman, D., Tilgen, W., Reichrath, J., 2006. Challenge and promise: roles for clusterin in pathogenesis, progression and therapy of cancer. Cell Death Differ. 13, 12–19.
Shchedrina, V.A., Vorbruggen, G., Lee, B.C., Kim, H.Y., Kabil, H., Harshman, L.G., Gladyshev, V.N., 2009. Overexpression of methionine-R-sulfoxide reductases has no influence on fruit fly aging. Mech. Ageing Dev. 130, 429–443.
Shchedrina, V.A., Kabil, H., Vorbruggen, G., Lee, B.C., Turanov, A.A., Hirosawa-Takamori, M., Kim, H.Y., Harshman, L.G., Hatfield, D.L., Gladyshev, V.N., 2011. Analyses of fruit flies that do not express selenoproteins or express the mouse selenoprotein, methionine sulfoxide reductase B1, reveal a role of selenoproteins in stress resistance. J. Biol. Chem. 286, 29449–29461.
Shibatani, T., Ward, W.F., 1996. Effect of age and food restriction on alkaline protease activity in rat liver. J. Gerontol. A Biol. Sci. Med. Sci. 51, B175–178.
Shibatani, T., Nazir, M., Ward, W.F., 1996. Alteration of rat liver 20S proteasome activities by age and food restriction. J. Gerontol. A Biol. Sci. Med. Sci. 51, B316–322.
Shikata, K., Yasuda, T., Takeuchi, F., Konishi, T., Nakata, M., Mizuochi, T., 1998. Structural changes in the oligosaccharide moiety of human IgG with aging. Glycoconjunate J. 15, 683–689.
Shin, J.K., Han, K.A., Kang, M.Y., Kim, Y.S., Park, J.K., Choi, W.J., Lee, S.A., Lee, J.H., Choi, W.S., Paik, W.Y., 2008. Expression of clusterin in normal and preeclamptic placentas. J. Obstet. Gynaecol. Res. 34, 473–479.
Shiota, M., Zoubeidi, A., Kumano, M., Beraldi, E., Naito, S., Nelson, C.C., Sorensen, P.H., Gleave, M.E., 2011. Clusterin is a critical downstream mediator of stress-induced YB-1 transactivation in prostate cancer. Mol. Cancer Res. 9, 1755–1766.
Shringarpure, R., Davies, K.J., 2002. Protein turnover by the proteasome in aging and disease. Free Radical Biol. Med. 32, 1084–1089.
Simm, A., Münch, G., Seif, F., Schenk, O., Heidland, A., Richter, H., Vamvakas, S., Schinzel, R., 1997. Advanced glycation endproducts stimulate the MAP-kinase pathway in tubulus cell line LLC-PK1. FEBS Lett. 410, 481–484.
Simm, A., 2013. Protein glycation during aging and in cardiovascular disease. J. Proteomics 92, 248–259.
Simm, A., Bartling, B., Navarrete-Santos, A., Friedrich, I., Scheubel, R.J., Hofmann, B., Bushnaq, H., Herrmann, M., Silber, R.E., Nass, N., 2008. Protein Glycation–a Biomarker of Ageing or More? In: Grune, T. (Ed.), Medimond International Proceedings-SFRR European meeting, 31–38.
Sitte, N., Merker, K., Von Zglinicki, T., Grune, T., Davies, K.J., 2000a. Protein oxidation and degradation during cellular senescence of human BJ fibroblasts: part I? effects of proliferative senescence. FASEB J. 14, 2495–2502.
Sitte, N., Merker, K., Von Zglinicki, T., Davies, K.J., Grune, T., 2000b. Protein oxidation and degradation during cellular senescence of human BJ fibroblasts: part II-aging of nondividing cells. FASEB J. 14, 2503–2510.
Smit, A.J., Smit, J.M., Botterblom, G.J., Mulder, D.J., 2013. Skin autofluorescence based decision tree in detection of impaired glucose tolerance and diabetes. PLoS One 8, e65592.
Somoza, V., Wenzel, E., Weib, C., Clawin-Rädecker, I., Grübel, N., Erberdobler, H.F., 2006. Dose-dependent utilisation of casein linked lysinoalanine, N(epsilon)-fructoselysine and N(epsilon) carboxymethyllysine in rats. Mol. Nutr. Food Res. 50, 833–841.
Song, F., Poljak, A., Crawford, J., Kochan, N.A., Wen, W., Cameron, B., Lux, O., Brodaty, H., Mather, K., Smythe, G.A., Sachdev, P.S., 2012. Plasma apolipoprotein levels are associated with cognitive status and decline in a community cohort of older individuals. PLoS One 7, e34078.
Spiro, R.G., 2002. Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. Glycobiology 12, 43R–56R.
Stadtman, E.R., 2006. Protein oxidation and aging. Free Radical Res. 40, 1250–1258.
Sun, Y.M., Su, Y., Li, J., Wang, L.F., 2013. Recent advances in understanding the biochemical and molecular mechanism of diabetic nephropathy. Biochem. Biophys. Res. Commun. 433, 359–361.
Tanaka, K., Yoshimura, T., Ichihara, A., Kameyama, K., Takagi, T., 1986. A high molecular weight protease in the cytosol of rat liver: II. Properties of the purified enzyme. J. Biol. Chem. 261, 15204–15207.
Tanaka, K., 1994. Role of proteasomes modified by interferon-gamma in antigen processing. J. Leukocyte Biol. 56, 571–575.
Tanikawa, T., Okada, Y., Tanikawa, R., Tanaka, Y., 2009. Advanced glycation end products induce calcification of vascular smooth muscle cells through RAGE/p38 MAPK. J. Vasc. Res. 46, 572–580.
Thomas, M.C., Baynes, J.W., Thorpe, S.R., Cooper, M.E., 2005. The role of AGEs and AGE inhibitors in diabetic cardiovascular disease. Curr. Drug Targets 6, 453–474.
Thornalley, P.J., Battah, S., Ahmed, N., Karachalias, N., Agalou, S., Babaei-Jadidi, R., Dawnay, A., 2003. Quantitative screening of advanced glycation endproducts in cellular and extracellular proteins by tandem mass spectrometry. Biochem. J. 375, 581–592.
Tonoki, A., Kuranaga, E., Tomioka, T., Hamazaki, J., Murata, S., Tanaka, K., Miura, M., 2009. Genetic evidence linking age-dependent attenuation of the 26S proteasome with the aging process. Mol. Cell Biol. 29, 1095–1106.
Trougakos, I.P., Gonos, E.S., 2002. Clusterin/apolipoprotein J in human aging and cancer. Int. J. Biochem. Cell Biol. 34, 1430–1448.
Trougakos, I.P., Gonos, E.S., 2006. Regulation of clusterin/apolipoprotein J, a functional homologue to the small heat shock proteins, by oxidative stress in ageing and age-related diseases. Free Radical Res. 40, 1324–1334.
Trougakos, I.P., Pawelec, G., Tzavelas, C., Ntouroupi, T., Gonos, E.S., 2006. Clusterin/Apolipoprotein J up-regulation after zinc exposure, replicative senescence or differentiation of human haematopoietic cells. Biogerontology 7, 375–382.
Trougakos, I.P., Poulakou, M., Stathatos, M., Chalikia, A., Melidonis, A., Gonos, E.S., 2002. Serum levels of the senescence biomarker clusterin/apolipoprotein: J increase significantly in diabetes type II and during development of coronary heart disease or at myocardial infarction. Exp. Gerontol. 37, 1175–1187.
Umadevi, S., Gopi, V., Vellaichamy, E., 2013. Inhibitory effect of gallic acid on advanced glycation end products induced up-regulation of inflammatory cytokines and matrix proteins in H9C2 (2-1) cells. Cardiovasc. Toxicol. 13, 396–405.
Uribarri, J., Woodruff, S., Goodman, S., Cai, W., Chen, X., Pyzik, R., Yong, A., Striker, G.E., Vlassara, H., 2010. Advanced glycation end products in foods and a practical guide to their reduction in the diet. J. Am. Diet Assoc. 110, 911–916.
Van Beneden, K., Coppieters, K., Laroy, W., De Keyser, F., Hoffman, I.E., Van den Bosch, F., Vander Cruyssen, B., Drennan, M., Jacques, P., Rottiers, P., Verbruggen, G., Contreras, R., Callewaert, N., Elewaut, D., 2009. Reversible changes in serum immunoglobulin galactosylation during the immune response and treatment of inflammatory autoimmune arthritis. Ann. Rheum Dis. 68, 1360–1365.
Vanhooren, V., Desmyter, L., Liu, X.E., Cardelli, M., Franceschi, C., Federico, A., Libert, C., Laroy, W., Dewaele, S., Contreras, R., Chen, C., 2007. *N*-glycomic changes in serum proteins during human aging *N*-glycomic changes in serum proteins during human aging. Rejuvenation Res. 10, 521–531a.
Varki, A., Cummings, R.D., Esko, J., Freeze, H., Stanley, P., Bertozzi, C., Hart, G., Etzler, M. (Eds.), 2009, 2nd edition. Cold Spring Harbor Laboratory Press, New York.
Verbrugghe, P., Kujala, P., Waelput, W., Peters, P.J., Cuvelier, C.A., 2008. Clusterin in human gut-associated lymphoid tissue, tonsils, and adenoids: localization to M cells and follicular dendritic cells. Histochem. Cell Biol. 129, 311–320.
Vernace, V.A., Arnaud, L., Schmidt-Glenewinkel, T., Figueiredo-Pereira, M.E., 2007. Aging perturbs 26S proteasome assembly in *Drosophila melanogaster*. FASEB J. 21, 2672–2682.
Vilchez, D., Morantte, I., Liu, Z., Douglas, P.M., Merkwirth, C., Rodrigues, A.P., Manning, G., Dillin, A., 2012. RPN-6 determines C. elegans longevity under proteotoxic stress conditions. Nature 489, 263–268.
Vlassara, H., Brownlee, M., Cerami, A., 1984. Accumulation of diabetic rat peripheral nerve myelin by macrophages increases with the presence of advanced glycosylation endproducts. J. Exp. Med. 160, 197–207.
Voges, D., Zwickl, P., Baumeister, W., 1999. The 26S proteasome: a molecular machine designed for controlled proteolysis. Annu. Rev. Biochem. 68, 1015–1068.
von der Lieth, C.W., Bohne-Lang, A., Lohmann, K.K., Frank, M., 2004. Bioinformatics for glycomics: status, methods, requirements and perspectives. Briefing Bioinf. 5, 164–178.
Vougier, S., Mary, J., Friguet, B., 2003. Subcellular localization of methionine sulphoxide reductase A (MsrA): evidence for mitochondrial and cytosolic isoforms in rat liver cells. Biochem. J. 373, 531–537.
Wells-Knecht, M.C., Lyons, T.J., McCance, D.R., Thorpe, S.R., Baynes, J.W., 1997. Age-dependent increase in ortho-tyrosine and methionine sulfoxide in human skin collagen is not accelerated in diabetes. Evidence against a generalized increase in oxidative stress in diabetes. J. Clin. Invest. 100, 839–846.
Whitby, F.G., Masters, E.I., Kramer, L., Knowlton, J.R., Yao, Y., Wang, C.C., Hill, C.P., 2000. Structural basis for the activation of 20S proteasomes by 11S regulators. Nature 408, 115–120.
Wong, P., Taillefer, D., Lakins, J., Pineault, J., Chader, G., Tenniswood, M., 1994. Molecular characterization of human TRPM-2/clusterin, a gene associated with sperm maturation, apoptosis and neurodegeneration. Eur. J. Biochem. 221, 917–925.
Wyatt, A., Yerbury, J., Poon, S., Dabbs, R., Wilson, M., 2009. Chapter 6: the chaperone action of clusterin and its putative role in quality control of extracellular protein folding. Adv. Cancer Res. 104, 89–114.

Wyatt, A.R., Yerbury, J.J., Berghofer, P., Greguric, I., Katsifis, A., Dobson, C.M., Wilson, M.R., 2011. Clusterin facilitates *in vivo* clearance of extracellular misfolded proteins. Cell. Mol. Life Sci. 68, 3919–3931.

Wyatt, A.R., Yerbury, J.J., Ecroyd, H., Wilson, M.R., 2013. Extracellular chaperones and proteostasis. Annu. Rev. Biochem. 82, 295–322.

Yao, Y., Tsuchiyama, S., Yang, C., Bulteau, A.L., He, C., Robison, B., Tsuchiya, M., Miller, D., Briones, V., Tar, K., Potrero, A., Friguet, B., Kennedy, B.K., Schmidt, M., 2015. Proteasomes, Sir2, and Hxk2 form an interconnected aging network that impinges on the AMPK/Snf1-regulated transcriptional repressor Mig1. PLoS Genet. 11, e1004968.

Zoubeidi, A., Chi, K., Gleave, M., 2010. Targeting the cytoprotective chaperone, clusterin, for treatment of advanced cancer. Clin. Cancer Res. 16, 1088–1093.