Proteome Oxidative Modifications and Impairment of Specific Metabolic Pathways During Cellular Senescence and Aging

Marie-Paule Hamon, Emad K. Ahmed, Martín A. Baraibar, and Bertrand Friguet*

Accumulation of oxidatively modified proteins is a hallmark of organismal aging in vivo and of cellular replicative senescence in vitro. Failure of protein maintenance is a major contributor to the age-associated accumulation of damaged proteins that is believed to participate to the age-related decline in cellular function. In this context, quantitative proteomics approaches, including 2-D gel electrophoresis (2-DE)-based methods, represent powerful tools for monitoring the extent of protein oxidative modifications at the proteome level and for identifying the targeted proteins, also referred as to the "oxi-proteome." Previous studies have identified proteins targeted by oxidative modifications during replicative senescence of human WI-38 fibroblasts and myoblasts and have been shown to represent a restricted set within the total cellular proteome that fall in key functional categories, such as energy metabolism, protein quality control, and cellular morphology. To provide mechanistic support into the role of oxidized proteins in the development of the senescent phenotype, untargeted metabolomic profiling is also performed for young and senescent myoblasts and fibroblasts. Metabolomic profiling is indicative of energy metabolism impairment in both senescent myoblasts and fibroblasts, suggesting a link between oxidative protein modifications and the altered cellular metabolism associated with the senescent phenotype of human myoblasts and fibroblasts.

1. Introduction

Among the main features of aging are failures in proteome homeostasis resulting in the accumulation of abnormal proteins such as oxidatively modified proteins. These proteostasis impairments are due to a decreased efficiency of systems involved in protein quality control and stress response together with an increased occurrence of damaging posttranslational protein modifications.[1–3]

Protein posttranslational modifications are numerous and various.[4] Some are helpful and others are deleterious for the cell, particularly the irreversible ones that are often associated with diseases and aging. By altering protein structure and function, and consequently cell homeostasis, these modifications are even more harmful in the aging process as they occur in a context of maintenance systems displaying declining abilities.[5,6]

Oxidation is one of the most common posttranslational modifications of proteins. This phenomenon is due to reaction of protein with reactive oxygen species (ROS). Unless repaired or removed from cells, oxidized proteins accumulate and threaten cell viability. The important role of protein oxidation in aging relies on the fact that increase in oxidized proteins levels is observed during the aging process.[7,8] In this case, as in a number of age related diseases, ROS reach levels overwhelming cellular defenses capacities creating an oxidative stress and triggering proteome damage. Enabling protein homeostasis to cope with oxidative stress, particularly at the level of protein quality control, could be one of the major ways to fight against the senescence-associated oxidative damages.[9]

The irreversible oxidative damages to proteins is manifested mainly by the addition of carbonyl groups, either by direct oxidation of amino acids, by conjugation with lipid peroxidation products such as 4-hydroxyl-2-nonenal (HNE), or by glycoxidation generating AGE (advanced glycation end-product).[10,11] The carbonylation of proteins is the result of a process by which aldehydes or ketones are introduced by oxidation. It occurs on the polypeptide chain and on the nucleophilic or redox sensitive side chains of amino acids. Because of their constitution, some amino acids are more prone to oxidative carbonylation than others. This is the case of proline, arginine, lysine, threonine, and histidine.[12,13]

Lipid peroxidation is another protein damaging modification inducer that refers to the oxidation of polyunsaturated lipids following a radical chain reaction process.[14] This results initially

Dr. M.-P. Hamon, Prof. B. Friguet
Sorbonne Université, CNRS, INSERM
Institut de Biologie Paris-Seine
Biological Adaptation and Ageing
B2A-IBPS
F-75005 Paris, France
E-mail: bertrand.friguet@sorbonne-universite.fr
Dr. E. K. Ahmed
Biochemistry Department
Faculty of Science
Ain Shams University
Cairo, Egypt
Dr. M. A. Baraibar
OxiProteomics SAS
F-75005 Paris, France

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/pmic.201800421

**ADVANCED
SCIENCE NEWS**

www.advancedsciencenews.com

Proteomics
Proteomics and Systems Biology

www.proteomics-journal.com

in a wide range of oxidized products, including lipid hydroperoxides, which will then give rise to various aldehydes such as HNE and MDA (malondialdehyde). The aldehydes resulting from this oxidative mechanism are stable products capable of attacking protein targets relatively far from their place of formation, on which they form Michael adducts. Particularly deleterious, these carbonyl adducts are formed on histidine, cysteine, and lysine residues.

The modification of the proteins can also result from protein glycoxidation occurring on a previously glycated protein.[15] This cascade of events begins with a process of glycation, namely the formation of a Schiff base by interaction between the aldehyde of a glucose and the free amino function of an amino acid, usually a lysine or arginine. Until then the process is reversible. However, if it continues, the Schiff base undergoes an Amadori rearrangement that stabilizes the condensation of the saccharide residue on the protein chain. These Amadori products can then be the target of glycoxidation reactions responsible for the formation of protein carbonyls and/or for the constitution of advanced glycation end products, i.e., the deleterious AGEs.[16] In addition, protein can also be modified by reaction of arginine or lysine amino groups with reactive dicarbonyl compounds as glyoxal and methylglyoxal that gives rise to the formation of such end products as carboxymethyllysine, carboxyethyllysine, or imidazolone derivatives.

Accumulation of carbonylated proteins is observed during various oxidative stresses, during aging and in many pathologies. So much so that carbonylation can be considered as a hallmark of oxidative stress but the proteins that are targeted by this or other damaging modifications have not been identified in most cases. Indeed, the identification of damaged protein targets is expected not only to define potential biomarkers but also to give insight into the mechanisms by which these damaged proteins accumulate and may contribute to cellular dysfunction. Interestingly, the identification of proteins increasingly carbonylated or modified by AGE or HNE during aging, age-related diseases, and cellular senescence has shown that they represent a restricted set of the cellular proteome that fall in key functional categories, such as protein quality control, cellular morphology, and energy metabolism.[17] Several proteins were recently identified in a proteomic study demonstrating in WI-38 human fibroblasts a strong interplay between replicative senescence, selenium, and selenoproteins, which are known to play a role in antioxidant defense and redox homeostasis.[18] Of note, the restricted subsets of modified proteins in senescent fibroblasts and myoblasts are different, although an overlap of four proteins, involved in protein quality control, cellular morphology, and energy metabolism, was found for both cell types.[19,20] In this paper, these findings are described and further discussed in view of the potential impact of oxidatively modified proteins on the altered metabolism of both senescent myoblasts and fibroblasts, with a specific focus on energy metabolism. Since senescence and also aging have to be approached with a multimarker process,[21] this issue was addressed at both proteomic and metabolomic levels in these two cell types. Indeed, while informative publications studied replicative senescence in fibroblasts with either a proteomic [22] or a metabolomic [23,24] points of view, here, we used both approaches to analyze the effects of replicative senescence in human fibroblasts and myoblasts.

Marie-Paule Hamon entered the world of mitochondria with a master's degree devoted to the study of the mitochondrial network dynamics and more particularly to the effect of a knockdown of the mitochondrial Lon protease on this network. She went on with a Ph.D. in biology of aging obtained in 2018. Since Lon protease is specialized in oxidized proteins degradation and is impaired with age, her thesis, titled "Importance of mitochondrial Lon protease in the maintenance of protein homeostasis and of mitochondrial function," relies notably on the study of protein oxidation and on the consequences of this phenomenon on metabolism.

Bertrand Friguet is a professor of physiology at Sorbonne Université, Paris, France, since 2007 and he is heading the Department of Biological Adaptation and Aging at IBPS, UMR SU-CNRS 8256, INSERM U1164. He got his Ph.D. in 1987. The major focus of his research is to elucidate the role of protein oxidative modification and redox homeostasis as well as oxidized protein degradation and repair during cellular aging and in oxidative stress situations leading to accelerated aging with relevant human cellular models.

2. Protein Oxidative Modifications in Senescent Fibroblasts and Myoblasts are Mainly Observed for Protein Involved in Energy Metabolism, Quality Control, Stress Response, and Cytoskeleton Organization

Since replicative senescence has proven to be a model of cellular aging in vitro,[17] we performed studies using young and senescent fibroblasts and myoblasts in order to address the role of protein oxidative modifications during cellular aging.[19–21,25] These studies were particularly focused on highlighting the affected cellular pathways through the identification of proteins presenting increased oxidative modification levels during replicative senescence.

Immunodetection of carbonylated, HNE- and AGE- modified proteins was performed after 1-DE and 2-DE on young and senescent human WI-38 fibroblasts and skeletal muscle satellite cells.[19,20] Increased levels of these oxidative modifications were observed during replicative senescence in both cell types, encouraging the identification of the proteins targeted by these

ADVANCED
SCIENCE NEWS
www.advancedsciencenews.com

Proteomics
Proteomics and Systems Biology
www.proteomics-journal.com

Table 1. Carbonylated, HNE-modified, and AGE-modified proteins in senescent fibroblasts.

Identified protein name	Protein full name	Carbonylated	HNE-modified	AGE-modified	Localization
ACTB	Actin, cytoplasmic 1		X		Cytoplasm
ALDH4A1	Delta-1-pyrroline-5-carboxylate dehydrogenase		X		Mitochondria
ALDH2	Aldehyde dehydrogenase		X		Mitochondria
ANXA5	Annexin A5	X			Cytoplasm
ATP5F1A	ATP synthase subunit alpha		X		Mitochondria
CALR	Calreticulin	X			Cytoplasm
C19orf10	Myeloid-derived growth factor		X		Extracellular
SDHA	Succinate dehydrogenase			X	Mitochondria
DLD	Dihydrolipoyl dehydrogenase			X	Mitochondria
TUFM	Elongation factor Tu	X		X	Mitochondria
ERP29	Endoplasmic reticulum resident protein 29	X			Endoplasmic reticulum
ETHE1	Persulfide dioxygenase ETHE1	X			Mitochondria
FLNA	Filamin-A			X	Cytoplasm–nucleus
G6PD	Glucose-6-phosphate dehydrogenase			X	Cytoplasm
GANAB	Neutral alpha-glucosidase AB		X		Endoplasmic reticulum–Golgi
HSD17B10	3-Hydroxyacyl-CoA dehydrogenase type-2	X			Mitochondria
HSPA8	Heat shock cognate 71 kDa protein		X		Cytoplasm–nucleus
IMMT	MICOS complex subunit MIC60		X	X	Mitochondria
IVD	Isovaleryl-CoA dehydrogenase		X		Mitochondria
MDH2	Malate dehydrogenase	X			Mitochondria
NDUFS2	NADH dehydrogenase [36] iron-sulfur protein 2		X		Mitochondria
OAT	Ornithine aminotransferase			X	Mitochondria
OGDH	2-Oxoglutarate dehydrogenase		X		Mitochondria
PSMA2	Proteasome subunit alpha type-2	X			Nucleus
UQCRC1	Cytochrome b-c1 complex subunit 1	X			Mitochondria
OXCT1	Succinyl-CoA:3-ketoacid coenzyme A			X	Mitochondria
WARS	Tryptophan–tRNA ligase			X	Cytoplasm
TUBB	Tubulin beta chain		X		Cytoplasm
CCT6A	T-complex protein 1 subunit zeta			X	Cytoplasm
VIM	Vimentin	X	X	X	Cytoplasm–nucleus

Increasingly modified proteins with RMI (Relative Modification Index) ≥1.3 identified after the experimental procedures described in ref. [19].

modifications and, hence, the potential mechanisms by which this accumulation of modified proteins could affect cellular functions.

For this purpose, total protein extracts from young and senescent cells were resolved by bidimensional polycrylamide gel electrophresis (2DPAGE). For each sample, two gel series were prepared in parallel, one for immunodetection of carbonylated, AGE- or HNE-modified proteins and the second for silver nitrate or Coomassie colloidal blue staining to visualize the total protein profile, equal loading control, and subsequent identification by mass spectrometry (MS) analysis. Interestingly, the pattern of the modified proteins was not superimposable with the pattern obtained for the total protein staining, indicating that certain proteins represent preferential targets for these detrimental modifications.

According to these analyses, 30 and 28 protein spots were respectively found increasingly modified during replicative senescence in fibroblast and myoblasts (**Tables 1** and **2**). All the

proteins identified and listed in the Tables 1 and 2 have been assigned a Mascot score greater than 56, i.e., significant at $p < 0.05$. In senescent fibroblasts, about half of the modified proteins are localized in mitochondria while, in senescent myoblasts, only one modified protein belongs to this organelle. Hence, protein oxidation accompanying replicative senescence is impacting differently the various cellular compartments depending on the cell type. In senescent fibroblasts, although the most important modification process is lipid peroxidation through the formation of HNE adducts, the number of protein targets for one or more of the three ways of oxidative modification is quite the same. In contrast, it is the lipid peroxidation that has the lowest impact in myoblasts.

In both cell types, most of the oxidatively modified proteins identified are found to fall into three main categories: energy metabolism, quality control-stress response, and cytoskeleton organization (**Figure 1**A,B). Regarding quality control and stress response, about 40% of the spotted proteins act interestingly on

Table 2. Carbonylated, HNE-modified, and AGE-modified proteins in senescent myoblasts.

Identified protein name	Protein full name	Carbonylated	HNE-modified	AGE-modified	Localization
ALDH1A1	Retinal dehydrogenase 1	X		X	Cytoplasm
ALDOA	Fructose-bisphosphate aldolase A	X	X	X	Cytoplasm
ANXA2	Annexin A2	X	X		Cytoplasm–extracellular
CALD1	Caldesmon	X		X	Cytoplasm
CAP1	Adenylyl cyclase-associated protein 1	X			Plasma membrane
DES	Desmin			X	Plasma membrane–nucleus
DPYSL2	Dihydropyrimidinase-related protein 2			X	Cytoplasm
ENO1	Alpha-enolase	X	X	X	Cytoplasm–nucleus
HSP90B1	Heat shock protein 90 kDa beta member 1			X	Endoplasmic reticulum
FSCN1	Fascin	X		X	Cytoplasm
G6PD	Glucose-6-phosphate dehydrogenase	X			Cytoplasm
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	X		X	Cytoplasm–nucleus
GNB2	G protein subunit beta-2	X			Cytoplasm–perinuclear
GSN	Gelsolin	X		X	Cytoplasm–extracellular
HSPA8	Heat shock cognate 71 kDa protein	X			Cytoplasm–nucleus
LMNA	Prelamin-A/C			X	Nucleus
PGAM1	Phosphoglycerate mutase 1		X		cytoplasm–extracellular
PDK1	Pyruvate dehydrogenase kinase isozyme 1	X			Mitochondria
PLOD2	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	X			Endoplasmic reticulum
PLOD3	Multifunctional procollagen lysine hydroxylase and glycosyltransferase LH3	X			Endoplasmic reticulum–extracellular
PRDX6	Peroxiredoxin-6		X		Cytoplasm
PSMC6	26S proteasome regulatory subunit 10B		X	X	Cytoplasm–nucleus
ATIC	Bifunctional purine biosynthesis protein PURH			X	Cytoplasm–extracellular
STIP1	Stress-induced-phosphoprotein 1			X	Cytoplasm
CCT3	T-complex protein 1 subunit gamma			X	Cytoplasm
CCT6A	T-complex protein 1 subunit zeta			X	Cytoplasm
TPI1	Triosephosphate isomerase		X		Cytoplasm
VIM	Vimentin			X	Cytoplasm–nucleus

Increasingly modified proteins with RMI (Relative Modification Index) ≥1.3 after the experimental procedures described in ref. [20].

oxidative stress. However, the similarities stop here. Only four modified proteins are common to senescent fibroblasts and myoblasts, i.e., G6PD, HSPA8, CCT6A, and vimentin (**Figure 2**A,B) and, except for CCT6A, they are not even affected in the same way.

For instance, in fibroblasts, vimentin is particularly affected during senescence as it simultaneously suffers from direct carbonylation, as well as HNE and AGE adductions. Vimentin has been shown to be prone to binding of HNE[26] and it has been observed that senescent primary fibroblasts express on their cell surface vimentin presenting lipid peroxidation adducts.[27] Indeed vimentin is subject to various nonenzymatic modifications, mostly oxidative in nature, which may be involved in the regulation of the vimentin network under stress conditions. These modifications may become particularly influential in senescent fibroblasts.[28] Interestingly, it has been shown that vimentin delays senescence and promote immortalization in primary mouse embryo fibroblasts.[29]

Upon examination of the oxidatively modified proteins detected in the energy metabolism pathways, quality control–

stress response, and cytoskeleton of senescent fibroblasts and myoblasts, we noted that they are not involved in the same mechanisms of these three cellular functions (**Figure 3**A,B). Regarding energy metabolism of senescent fibroblasts, oxidatively modified proteins are essentially associated with the TCA (tricarboxylic acid) cycle (SDHA, DLD, OGDH, MDH2 proteins), the mitochondrial function (HSD17B10, SDHA, NDUFS2, UQCRC1, OGDH proteins), the oxidative phosphorylation (SDHA, NDUFS2, UQCRC1 proteins), and the fatty acid β oxidation (ALDH2, ALDH1A1 proteins). This result is consistent with the important proportion of oxidized proteins found in mitochondria of senescent fibroblasts. In senescent myoblast, energy metabolism is mainly affected by oxidative modifications when it comes to glycolysis (ENO1, TPI1, PGAM1, ALDOA, GAPDH proteins).

Concerning the quality control and stress response, although the modified proteins are not the same in senescent fibroblast compared to senescent myoblasts, the impacted pathways are more similar, that is, the unfolded protein response (HSPA8,
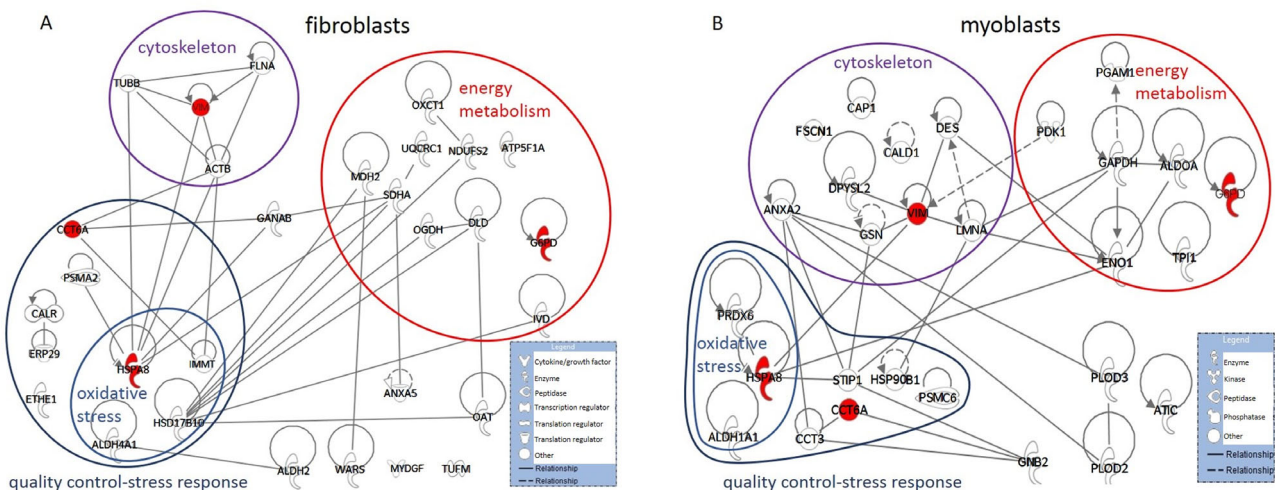
ADVANCED
SCIENCE NEWS

www.advancedsciencenews.com

Proteomics
Proteomics and Systems Biology

www.proteomics-journal.com

Figure 1. Network analysis of modified proteins in fibroblasts and myoblasts. Networks obtained using Ingenuity Pathways Analysis (Ingenuity Systems, http://www.ingenuity.com/) for proteins reported to be significantly more oxidized in senescent cells compared to young cells. A) Analysis of modified proteins in fibroblasts. B) Analysis of modified proteins in myoblasts. Proteins in red correspond to proteins that are common to fibroblasts and myoblasts. Information about the analysis of biological functions and pathways as well as network interactions is available at the Ingenuity Pathway Analysis website. A line denotes binding of proteins, whereas a line with an arrow denotes "acts on." A dotted line denotes an indirect interaction.
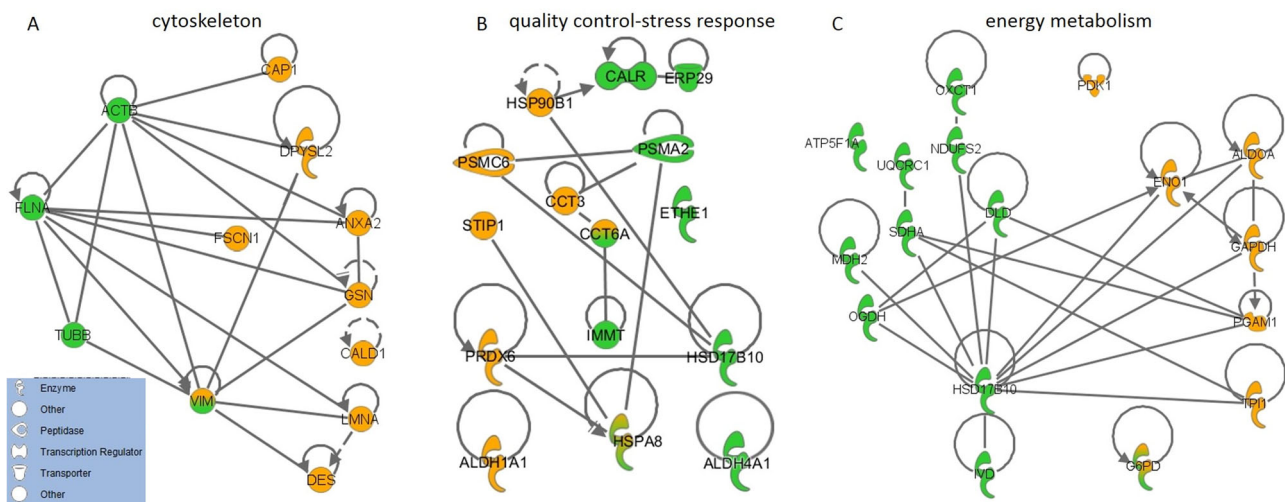
Figure 2. Network analysis of modified proteins acting in cytoskeleton organization, quality control–stress response, and energy metabolism. Networks obtained using Ingenuity Pathways Analysis (Ingenuity Systems, http://www.ingenuity.com/) for proteins reported to be significantly more oxidized in senescent cells compared to young cells in both fibroblasts and myoblasts. A) Analysis of modified proteins acting in cytoskeleton. B) Analysis of modified proteins acting in quality control and stress response. C) Analysis of modified proteins acting in energy metabolism. Proteins in green correspond to proteins identified in fibroblasts and proteins in orange correspond to proteins identified in myoblasts. Information about the analysis of biological functions and pathways as well as network interactions is available at the Ingenuity Pathway Analysis website. A line denotes binding of proteins, whereas a line with an arrow denotes "acts on." A dotted line denotes an indirect interaction.

CALR in fibroblasts; HSPA8, HSP90B1 in myoblasts), the sirtuin signaling pathway (SDHA, G6PD, NDUFS2 in fibroblasts; PGAM1, G6PD, PDK1 in myoblasts), and the endoplasmic reticulum stress pathway (CALR in fibroblasts; HSP90B1 in myoblast). However, senescent fibroblasts present a greatest effect on proteins associated with the NRF-2 (Nuclear factor (erythroid-derived 2)-like 2)-mediated oxidative stress response (ERP29, ACTB in fibroblasts; STIP1 in myoblasts) while in senescent myoblasts oxidized proteins are more highlighting the pro-

tein ubiquitination pathway (HSPA8, HSP90B1, PSMC6 in myoblasts; HSPA8, PSMA2 in fibroblast) and also the glutathione redox reactions I (PRDX6) that is only emerging in this cell type.

In fibroblasts like in myoblasts, replicative senescence is associated with the oxidative modification of a number of proteins mostly belonging to the same main cellular functions. However, within these three functions (cytoskeleton organization, quality control–stress response, and energy metabolism), it is not
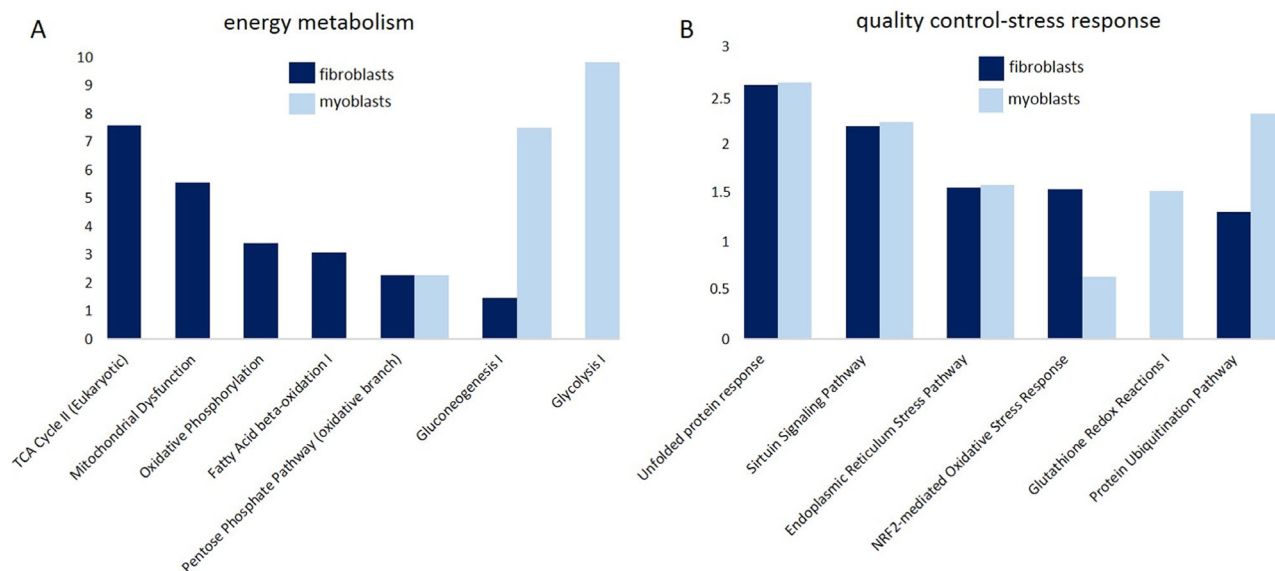
ADVANCED
SCIENCE NEWS
www.advancedsciencenews.com

Proteomics
Proteomics and Systems Biology
www.proteomics-journal.com

Figure 3. Canonical pathways associated with modified proteins acting in energy metabolism, quality control and stress response. Modified proteins were grouped by canonical pathways through the use of Ingenuity Pathways Analysis. The bars represent the canonical pathways identified, named in the x-axis. The y-axis shows the −log of the p-value calculated based on Fisher's exact test. A) Canonical pathways associated with modified proteins acting in energy metabolism. B) Canonical pathways associated with modified proteins acting in quality control and stress response.

the same pathways that are targeted by the oxidative protein modifications observed in fibroblasts and myoblasts. Therefore, it can be expected that the oxidatively modified protein accumulation accompanying replicative senescence may differently affect fibroblasts and myoblasts.

Regarding cytoskeleton organization, senescent fibroblasts notably exhibit HNE adducts on actin and tubulin (Table 1). Oxidation of both proteins have yet been highlighted with an ensuing cytoskeletal disruption [30] and inducing damages in the nervous system cytoskeleton.[31] In contrast, protein oxidative modifications observed in senescent myoblasts are not directly acting on actin and tubulin but on proteins such as desmin, caldesmon, gelsolin, lamin A, and fascin (Figure 2B) known to regulate three major components of the cytoskeleton architecture, i.e., actin, tubulin, and intermediate vimentin filaments.[32]

Concerning the quality control and stress response, the pathways using proteins affected by oxidative modifications during senescence of fibroblasts and myoblasts are quite similar (Figure 3). However, we noted that the protein oxidation observed in the protein ubiquitination pathway seems more pronounced in myoblasts. Ubiquitinated proteins are intended for degradation by the proteasome and several studies suggest that oxidation products could inhibit the proteasome through a blockage of substrates accessibility.[33,34]

Detoxification systems are other defense mechanisms used by cells against oxidative stress. According to Ingenuity Pathway Analyses (Figure 3), the "Glutathione Redox Reactions" canonical pathway seems only affected in senescent myoblasts with PRDX6 being HNE-modified. Conversely, the "NRF2-mediated oxidative stress response" canonical pathway seems more affected in senescent fibroblasts than in senescent myoblasts with ERP29 and ACTB versus STIP1 being oxidatively modified, respectively. This observation may be linked with the

high number of oxidized proteins found in the mitochondria of senescent fibroblasts since NRF-2 is also involved in the regulation of mitochondrial biogenesis.[35]

However, it is at the energy metabolism level that the disparities between senescent fibroblasts and myoblasts are the most obvious (Figure 3). Oxidatively modified proteins identified in senescent fibroblasts are principally acting in metabolic processes localized in mitochondria, i.e., TCA cycle, oxidative phosphorylation, and fatty acid β-oxidation. By altering the function of proteins targeted by oxidative modifications, these modifications might have caused a dysfunction of the quality control systems and the mitochondrial energetic metabolism that could result in a stimulation of the ROS production thus being able to induce a vicious circle. Such an effect has already been observed for the complex I of the respiratory chain.[36] In contrast, it has been shown that fibroblasts adopt a more glycolytic state when they become senescent.[37]

On the contrary, glycolysis is particularly impacted by protein oxidative modifications in senescent myoblasts. Indeed five enzymes belonging to this pathway are modified and, among them, two are notably affected, namely ALDOA and ENO1 that present the three forms of oxidative modifications (Table 2). Thus, in the case of myoblasts, senescence appears to impair glucose oxidation at the level of anaerobic glycolysis.

Interestingly, G6PD was found oxidatively modified in both senescent fibroblasts and myoblasts (Figure 2). G6PD is known to supply reducing equivalents by regenerating NADPH (nicotinamide adenine dinucleotide phosphate) levels as well as pentose phosphates for fatty acid and nucleic acid synthesis. Acting in the oxidative branch of the pentose phosphate pathway, G6PD is a major source of NADPH and therefore a key enzyme for maintaining redox homeostasis.[38] The protective role of G6PD against the deleterious effects of ROS has been observed on
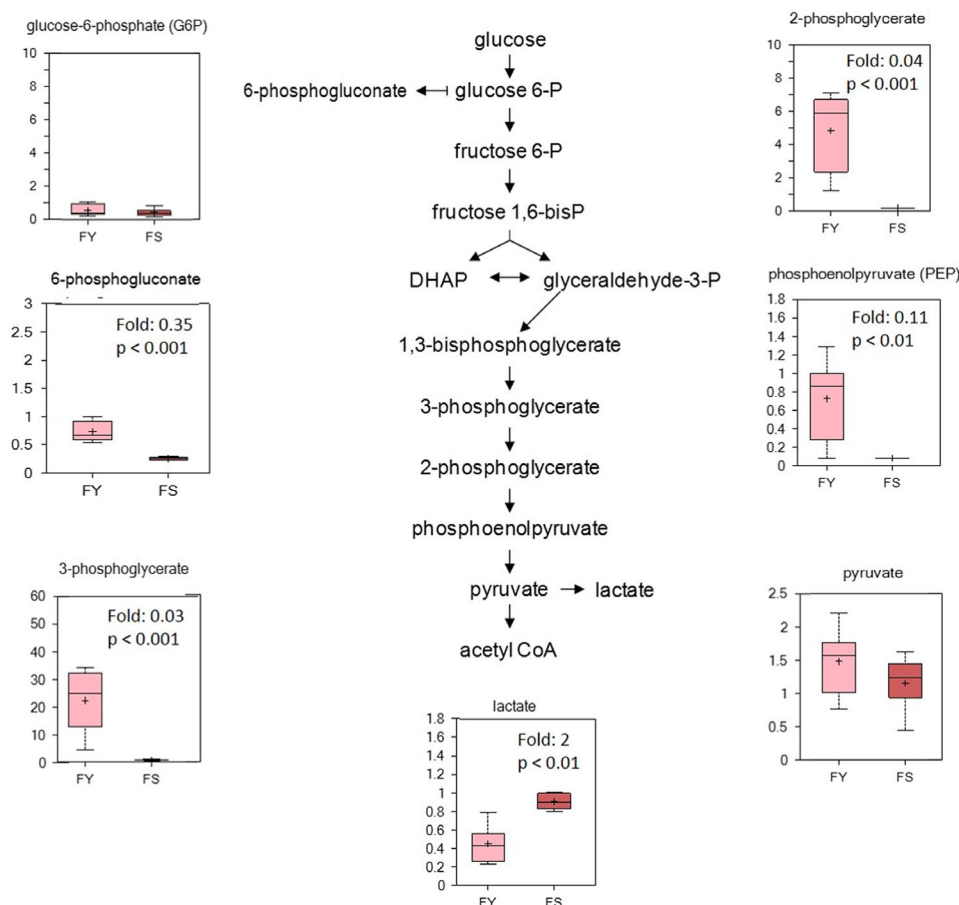
ADVANCED
SCIENCE NEWS

www.advancedsciencenews.com

Proteomics
Proteomics and Systems Biology

www.proteomics-journal.com

Figure 4. Glycolysis pathway related metabolites alterations in senescent fibroblasts. Glycolysis pathway related metabolites profiling in young (FY) and senescent WI-38 fibroblasts (FS). For the box plots, the top and bottom of the boxes represent the 75th and 25th percentile, respectively. The solid bar across the box represents the median value, while the "+" is the mean. The Y axis is the median scaled value (relative level). The fold change and the corresponding p-value in senescent cells relative to their young counterpart is indicated in each plot.

several occasions.[38,39] Thus, with the oxidative modification of this enzyme, senescent fibroblasts such as senescent myoblasts could lose here another way to deal with the age-associated increase of oxidative stress.

We have also noted that many mitochondrial dehydrogenases were found oxidized in senescent fibroblasts, namely ALDH4A1, ALDH2, HSD17B10, MDH2, SDHA, DLD, OGDH, NDUFS2, and IVD (Table 1). The oxidative modification of these enzymes may also have an impact on their functioning. For example, ALDH4A1 and ALDH2 are respectively involved in acetaldehyde and glutamate semi-aldehyde detoxification and their adduction with lipid peroxidation products could increase the load of reactive aldehyde in senescent fibroblasts. In addition, HSD17B, DLD, and NDUFS2, as component of NADH dehydrogenase, complex I of the respiratory chain, can influence the redox homeostasis through the dysregulation of the couple NADH/NAD$^+$ as observed for both myoblasts[20] and fibroblasts (**Figure 5**) although in opposite directions. Indeed, changes of the NAD$^+$/NADH ratio can modulate mitochondrial quantity and quality.[40] Moreover, NAD$^+$ is a co-substrate for sirtuins and a critical molecule in metabolism and redox signaling.[41]

3. Metabolome Analyses Show Differential Effects on Energy Metabolism in Senescent Myoblasts and Fibroblasts

Metabolites profiling analysis was performed by Metabolon, Inc. as previously described.[42] Cellular pellets (10^6 cells) from six different batches from young and senescent fibroblasts were accessioned into the Metabolon LIMS system. Raw data was extracted, peak-identified, and QC processed using Metabolon's hardware and software.[43] Welch's two-sample t-test was used to identify biochemicals that differed significantly between experimental groups.

Differential effects on energy metabolism were observed in myoblasts and fibroblasts undergoing replicative senescence. As previously reported,[20] glycolytic intermediates such as glucose-6-phosphate, fructose-6-phosphate, and the isobar containing fructose 1,6-diphosphate, glucose 1,6-diphosphate, and myo-inositol 1,4 or 1,3-diphosphate were decreased in senescent myoblasts, which is indicative of a decreased metabolism of glucose via glycolysis. Interestingly, six enzymes involved in glycolysis were found to be increasingly modified in senescent myoblasts: aldolase, triosephosphate isomerase, glyceraldehyde
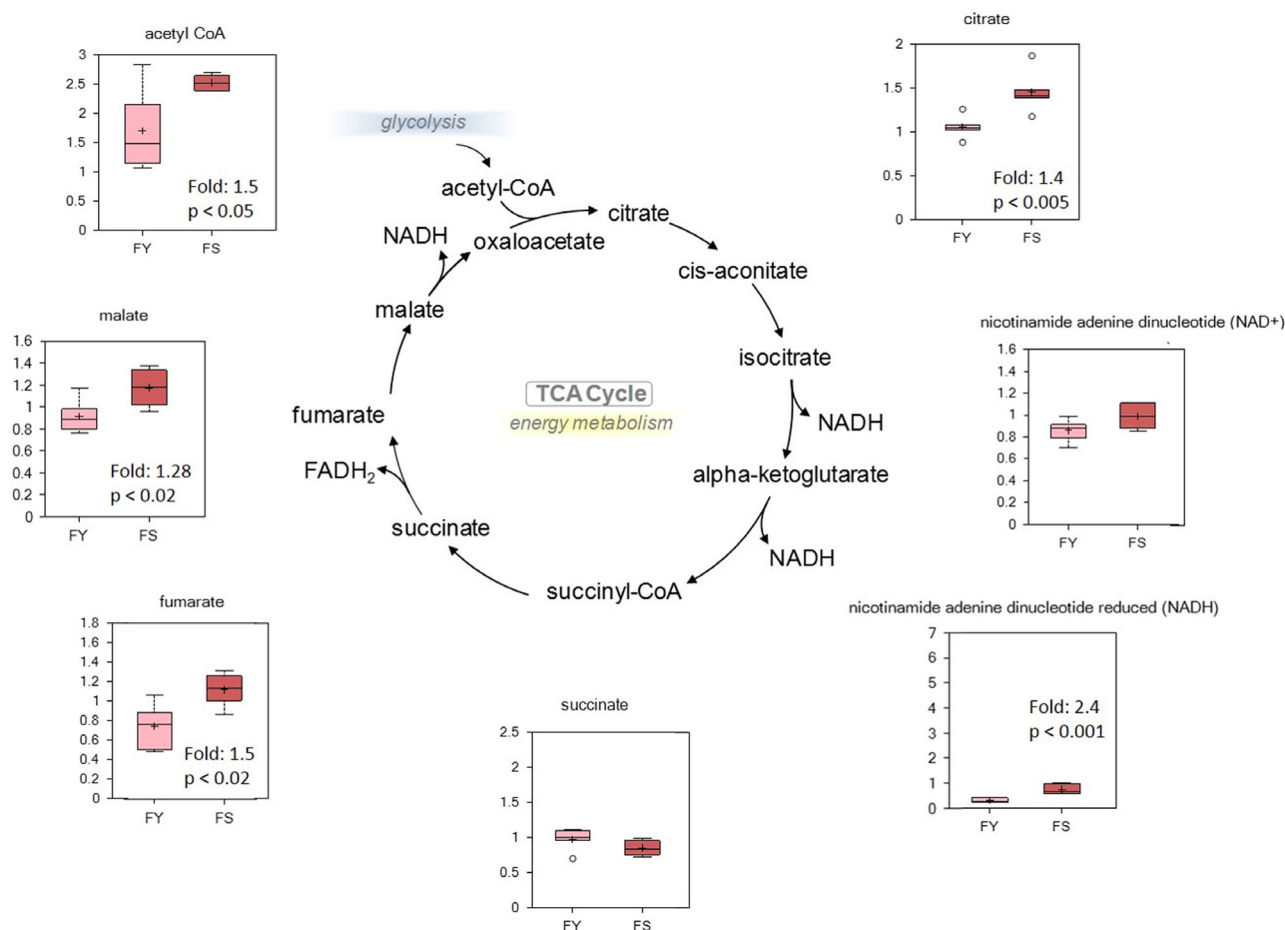
ADVANCED
SCIENCE NEWS
www.advancedsciencenews.com

Proteomics
Proteomics and Systems Biology
www.proteomics-journal.com

Figure 5. Tricarboxylic acid (TCA) cycle related metabolites alterations in senescent fibroblasts. Tricarboxylic acid (TCA) cycle related metabolites profiling in young (FY) and senescent WI-38 fibroblasts (FS). For the box plots, the top and bottom of the boxes represent the 75th and 25th percentile, respectively. The solid bar across the box represents the median value, while the "+" is the mean. Statistical outlier is represented by a circle. The y-axis is the median scaled value (relative level). The fold change and the corresponding p-value in senescent cells relative to their young counterpart is indicated in each plot.

3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, and pyruvate kinase.[20] The oxidative modification of these enzymes is concomitant with a decreased glucose oxidation and an increased NAD^+/NADH ratio, reflecting a decreased cellular reducing potential.[20] Since the functionality of the mitochondrial respiratory chain was not affected in human myoblasts during replicative senescence, the decreased glucose oxidation we observed is most likely due to an impairment in glycolysis and/or TCA cycle. Oxidative modifications of enzymes involved in glycolysis and the TCA cycle have also been suggested to be an important pathophysiological factor in age-related diseases, such as neurodegenerative diseases.[44] Other studies have shown that the inhibition of glycolytic enzymes, such as GAPDH and PGAM by siRNA can induce premature senescence.[45]

In contrast to senescent myoblasts, the glycolytic intermediates glucose-6-phosphate (**Figure 4**), fructose-6-phosphate, and the isobar containing fructose 1,6-diphosphate, glucose 1,6-diphosphate, and myo-inositol 1,4 or 1,3-diphosphate (data not shown) were found relatively unchanged in senescent fibroblasts while the downstream intermediates 2-phosphoglycerate, 3-phosphoglycerate, and phosphoenolpyruvate are dramatically depleted as compared to young fibroblasts (Figure 4). Together with the observed increase in lactate in senescent fibroblasts, this result suggests an increased glycolytic activity in these cells. Depletion of the pentose phosphate pathway intermediate 6-phosphogluconate also suggests a modified activity of this pathway. Consistent with increased glycolysis, acetyl-CoA (acetyl coenzyme A) levels are elevated in fibroblasts during replicative senescence that likely accounts for the increased TCA intermediates citrate, fumarate, and malate and may indicate a decreased efficiency of the TCA cycle (Figure 5). Furthermore, the increased levels of NADH together with a NAD^+ level not significantly modified suggest an impairment of the mitochondrial respiratory chain since NADH produced by the TCA cycle in the mitochondria is utilized by the electron transport chain for ATP generation by oxidative phosphorylation.

4. Concluding Remarks

Identification of proteins targeted by oxidative modification during replicative senescence of both human fibroblasts and

myoblasts showed that they represent a restricted set within the total cellular proteome that fall in key functional categories, such as energy metabolism, quality control–stress response, and cellular morphology. A total of 30 and 28 protein spots were respectively found increasingly modified during replicative senescence of fibroblasts and myoblasts with about half of the modified proteins localized in mitochondria in senescent fibroblasts while only one modified protein belongs to this organelle in senescent myoblasts. Of note, about 35% of these proteins that are accumulating as oxidatively modified in senescent fibroblasts and myoblasts have been previously shown to accumulate during aging and age-related diseases.[17] During replicative senescence of myoblasts, proteins involved in protein quality control and glycolytic enzymes were the main targets of oxidation and modification with advanced glycation/lipid peroxidation end products. These studies underscore the importance of performing proteomic analyses addressing different aspects, such as expression levels and modifications by carbonylation or glycoxidation, to have a broader view of the age-related changes affecting the cellular proteome. Moreover, for both human fibroblasts and myoblasts, the metabolome analyses are indicative of an energy metabolic switch that is associated with replicative senescence. Interestingly, this energy metabolic switch is different depending on cell type: from glycolysis to fatty acid β oxidation in senescent myoblasts and from TCA cycle/oxidative phosphorylation to glycolysis in senescent fibroblasts. Taken together with the proteomic modification analyses showing that oxidatively modified proteins are mainly linked with the TCA cycle and oxidative phosphorylation in senescent fibroblasts while mainly linked with glycolysis in senescent myoblasts, these results suggest that the observed increase of oxidative damage to specific protein targets may have indeed contributed to the senescent associated impairment of energy metabolism specific pathways in both cell types. This was further investigated in senescent myoblasts for which glycolytic flux analyses aimed at monitoring glucose oxidation to CO_2 have shown the glycolytic pathway is indeed decreased by about 40% while the mitochondrial respiration capacity remained the same as compared with young myoblasts.[20] Furthermore, the rationale behind the cell type-dependent accumulation of oxidatively modified proteins in specific metabolic pathways during cellular senescence remains to be elucidated. Indeed, beside protein localization and intrinsic susceptibility to oxidation, it may depend on such cell specific features as differences in basal energy metabolism and/or increased susceptibility of catalytically active metabolic enzymes to oxidative modifications. Finally, since more than one third of those proteins that are accumulating as oxidatively modified during cellular senescence have also been shown to accumulate during aging and age-related diseases, whether this buildup of specific oxidatively modified proteins is also resulting in the impairment of such metabolic pathways as those evidenced in senescent cells remains to be investigated.

Conflict of Interest

The authors declare no conflict of interest.

[1] M. A. Baraibar, B. Friguet, *Exp. Gerontol.* **2013**, *48*, 620.
[2] N. Chondrogianni, K. Georgila, N. Kourtis, N. Tavernarakis, E. S. Gonos, *Free Radic. Biol. Med.* **2014**, *75*, S18.
[3] M. P. Hamon, A. L. Bulteau, B. Friguet, *Ageing Res. Rev.* **2015**, *23*, 56.
[4] C. T. Walsh, S. Garneau-Tsodikova, G. J. Gatto, Jr, *Angew. Chem.* **2005**, *44*, 7342.
[5] V. N. S. Vanhooren, A. Navarrete Santos, K. Voutetakis, I. Petropoulos, C. Libert, A. Simm, E. S. Gonos, B. Friguet, *Mech. Ageing Dev.* **2015**, *151*, 71.
[6] K. A. Liddy, M. Y. White, S. J. Cordwell, *Genome Med.* **2013**, *5*, 20.
[7] E. R. Stadtman, *Free Radic. Res.* **2006**, *40*, 1250.
[8] S. Reeg, T. Grune, *Antioxid. Redox. Signal.* **2015**, *23*, 239.
[9] D. Reichmann, W. Voth, U. Jakob, *Mol. Cell.* **2018**, *69*, 203.
[10] M. Le Boulch, E. K. Ahmed, A. Rogowska-Wrzesinska, M. A. Baraibar, B. Friguet, *Mech. Ageing Dev.* **2018**, *170*, 59.
[11] H. Bakala, E. Delaval, M. Hamelin, J. Bismuth, C. Borot-Laloi, B. Corman, B. Friguet. *Eur. J. Biochem.* **2003**, *270*, 2295.
[12] C. M. Wong, G. Bansal, L. Marcocci, Y. J. Suzuki, *Redox Rep.* **2012**, *17*, 90.
[13] M. Cattaruzza, M. Hecker, *Circ. Res.* **2008**, *102*, 273.
[14] A. K. Hauck, D. A. Bernlohr, *J. Lipid Res.* **2016**, *57*, 1976.
[15] G. Vistoli, D. De Maddis, A. Cipak, N. Zarkovic, M. Carini, G. Aldini, *Free Radic. Res.* **2013**, *47*, 3.
[16] K. Byun, Y. Yoo, M. Son, J. Lee, G. B. Jeong, Y. M. Park, G. H. Salekdeh, B. Lee, *Pharmacol. Ther.* **2017**, *177*, 44.
[17] M. A. Baraibar, L. Liu, E. K. Ahmed, B. Friguet, *Oxid. Med. Cell Longev.* **2012**, *2012*, 919832.
[18] G. Hammad, Y. Legrain, Z. Touat-Hamici, S. Duhieu, D. Cornu, A. L. Bulteau, L. Chavatte, *Antioxidants* **2018**, *7*.
[19] E. K. Ahmed, A. Rogowska-Wrzesinska, P. Roepstorff, A. L. Bulteau, B. Friguet, *Aging Cell* **2010**, *9*, 252.
[20] M. A. Baraibar, J. Hyzewicz, A. Rogowska-Wrzesinska, A. L. Bulteau, C. Prip-Buus, G. Butler-Browne, B. Friguet, *Aging* **2016**, *8*, 3375.
[21] M. Ogrodnik, H. Salmonowicz, V. N. Gladyshev, *Aging Cell* **2019**, *18*, e12841.
[22] X. Zhang, S. Zhang, X. Liu, Y. Wang, J. Chang, X. Zhang, S. G. Mackintosh, A. J. Tackett, Y. He, D. Lv, R. M. Laberge, J. Campisi, J. Wang, G. Zheng, D. Zhou, *Aging Cell* **2018**, *17*, e12780.
[23] E. L. James, J. A. Lane, R. D. Michalek, E. D. Karoly, E. K. Parkinson, *Sci. Rep.* **2016**, *6*, 38489.
[24] E. L. James, R. D. Michalek, G. N. Pitiyage, A. M. de Castro, K. S. Vignola, J. Jones, R. P. Mohney, E. D. Karoly, S. S. Prime, E. K. Parkinson, *J. Proteome Res.* **2015**, *14*, 1854.

[25] E. K. Ahmed, C. R. Picot, A. L. Bulteau, B. Friguet, *Ann. N. Y. Acad. Sci.* **2007**, *1119*, 88.

[26] A. Monico, S. Duarte, M. A. Pajares, D. Perez-Sala, *Redox Biol*. **2019**, https://doi.org/10.1016/j.redox.2019.101098.

[27] D. Frescas, C. M. Roux, S. Aygun-Sunar, A. S. Gleiberman, P. Krasnov, O. V. Kurnasov, E. Strom, L. P. Virtuoso, M. Wrobel, A. L. Osterman, M. P. Antoch, V. Mett, O. B. Chernova, A. V. Gudkov, *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E1668.

[28] D. Perez-Sala, C. L. Oeste, A. E. Martinez, M. J. Carrasco, B. Garzon, F. J. Canada, *Nat. Commun.* **2015**, *6*, 7287.

[29] G. V. Tolstonog, R. L. Shoeman, U. Traub, P. Traub, *DNA Cell Biol*. **2001**, *20*, 509.

[30] A. Keshavarzian, A. Banan, A. Farhadi, S. Komanduri, E. Mutlu, Y. Zhang, J. Z. Fields, *Gut* **2003**, *52*, 720.

[31] J. Gardiner, R. Overall, J. Marc, *Diseases* **2013**, *1*, 36.

[32] D. D. Tang, B. D. Gerlach, *Respir. Res.* **2017**, *18*, 54.

[33] B. Friguet, L. I. Szweda, *FEBS Lett.* **1997**, *405*, 21.

[34] A. L. Bulteau, P. Verbeke, I. Petropoulos, A. F. Chaffotte, B. Friguet, *J. Biol. Chem.* **2001**, *276*, 45662.

[35] R. A. Gottlieb, D. Bernstein, *Cell Calcium* **2016**, *60*, 88.

[36] L. K. Sharma, J. Lu, Y. Bai, *Curr. Med. Chem*. **2009**, *16*, 1266.

[37] C. D. Wiley, J. Campisi, *Cell Metab*. **2016**, *23*, 1013.

[38] A. Stincone, A. Prigione, T. Cramer, M. M. Wamelink, K. Campbell, E. Cheung, V. Olin-Sandoval, N. M. Gruning, A. Kruger, M. Tauqeer Alam, M. A. Keller, M. Breitenbach, K. M. Brindle, J. D. Rabinowitz, M. Ralser, *Biol. Rev. Camb. Philos. Soc.* **2015**, *90*, 927.

[39] S. Nobrega-Pereira, P. J. Fernandez-Marcos, T. Brioche, M. C. Gomez-Cabrera, A. Salvador-Pascual, J. M. Flores, J. Vina, M. Serrano, *Nat. Commun.* **2016**, *7*, 10894.

[40] S. Y. Jang, H. T. Kang, E. S. Hwang, *J. Biol. Chem*. **2012**, *287*, 19304.

[41] J. M. Berthiaume, J. G. Kurdys, D. M. Muntean, M. G. Rosca. *Antioxid Redox Signal*. **2017**, *30*, 375.

[42] Z. J. Reitman, G. Jin, E. D. Karoly, I. Spasojevic, J. Yang, K. W. Kinzler, Y. He, D. D. Bigner, B. Vogelstein, H. Yan, *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 3270.

[43] C. D. Dehaven, A. M. Evans, H. Dai, K. A. Lawton, *J. Cheminformatics* **2010**, *2*, 9.

[44] A. Martinez, M. Portero-Otin, R. Pamplona, I. Ferrer, *Brain Pathol.* **2010**, *20*, 281.

[45] H. Kondoh, M. E. Lleonart, J. Gil, J. Wang, P. Degan, G. Peters, D. Martinez, A. Carnero, D. Beach, *Cancer Res.* **2005**, *65*, 177.