Chapter 3

Interplay Between Mitochondrial Proteins and Age-Associated Risk of Cardiovascular Diseases

Zuzana Tatarkova, Martin Kolisek, Ivana Pilchova, Peter Racay and Peter Kaplan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71789

Abstract

Normal functioning of mitochondria is crucial for cardiac performance. Mitochondria undergo mitophagy (mitochondrial autophagy) and biogenesis, and mitochondrial proteins are subject to extensive post-translational modifications (PTMs). The state of mitochondrial homeostasis reflects overall cellular fitness and longevity. Perturbed mitochondria produce less adenosine triphosphate (ATP), release greater amounts of reactive molecules, and are more prone to apoptosis. Therefore mitochondrial turnover is an integral aspect of quality control in which dysfunctional mitochondria are selectively eliminated through mitophagy. Currently, the progressive deterioration of physiological functions is seen as accumulation of modified/damaged proteins with limiting regenerative ability throughout aging in myocardial cells. Mitochondrial stress response to reactive species was evaluated as electron transport chain (ETC) complexes, redox-active molecules, and their possible communication. Protein-protein interactions revealed a strong linkage between age and ETC protein subunits. Redox state was strongly affected in senescent mitochondria with shift in favor of more pro-oxidizing condition within cardiomyocytes. Assume all together, dysfunctional proteostasis can play a causative role in aging and restoration of protein homeostasis machinery is protective against aging and possibly age-related disorders.

Keywords: aging, heart, mitochondria, protein-protein interactions, redox homeostasis

1. Introduction

The world population ages rapidly, mostly because of increasing longevity and declining fertility [1]. The average lifespan of the human populations is increasing worldwide and it is predicted that in 2035, nearly every fourth individual will be 65 years or older [2]. Aging

is a complex phenomenon with a large impact on society. Aging is recently an emerging topic since the life expectancy is rising and because aging itself is the basis for the development of age-related diseases such as cardiovascular disease (CVD), cancer, neurodegenerative diseases and degenerative metabolic diseases (e.g. *diabetes mellitus* type 2) [3]. Since age is the largest risk factor for CVD, the prevalence of these ailments increases dramatically with increasing age. Over 80% of all cases of coronary artery disease and more than 75% of those of congestive heart failure are observed in elderly patients [4]. In order to improve prevention and care for patients, it is important to analyze processes linked with cardiac aging. Prevalence of high blood pressure, obesity and metabolic syndrome correlate with age, and all these conditions facilitate the development of cardiomyopathies that are the major cause of chronic disability, morbidity and mortality in the elderly. Although the long-term exposure to cardiovascular risk factors plays a major role in the etiopathogenesis of CVD and neurodegeneration, intrinsic alterations in the heart and the vascular system occur during lifespan and render the cardiovascular system more vulnerable during senescence. The possible link between aging and senescence was first described in 1961 [5] and it based on the inability of telomeres to sustain their lengths. In addition to this, other events and signals were identified including non-telomeric/genotoxic stress generated by various signals, such as mitochondrial deterioration, oxidative stress, DNA-replication "stress" or activated oncogenes [6]. Several lines of evidence indicate mitochondrial dysfunction to be a major contributor to cardiovascular senescence. Damaged mitochondria are bioenergetically less efficient and they are producing excessive amounts of reactive oxygen species (ROS) with detrimental structural and functional consequences [7]. The ROS impair excitation-contraction coupling, cause arrhythmias, and contribute to cardiac remodeling by inducing cardiac hypertrophy, apoptosis, necrosis, and fibrosis [8]. However, antioxidant interventions in patients with CVD yielded only disappointing results so far [9].

The accumulation of abnormal/dysfunctional mitochondria is usually a consequence of impaired clearance of damaged organelles by autophagy and inadequate replenishment of the cellular mitochondrial pool by mitochondriogenesis [7]. Autophagic flux is generally decreased in aging hearts. Murine loss-of-function models for autophagy develop exacerbated cardiac dysfunction that is accompanied by accumulation of misfolded proteins and dysfunctional organelles. On the other hand, stimulation of autophagy in mouse models improves cardiac function and enables to study a protein aggregation by removing accumulated misfolded proteins, dysfunctional mitochondria, and damaged DNA, thereby alleviating aging-associated pathology in the heart. Multiple lines of evidence suggest that autophagy is required for many mechanisms that mediate lifespan extension, such as caloric restriction. These results are pointing out the possibility that autophagy may play an important role in combating the adverse effects of aging in the heart [10]. At the molecular level, the aging process is associated with accumulation of damaged proteins and organelles, partially due to defects in protein quality control systems. Since most cellular functions are performed by proteins, aging may be, in part, the consequence of a deregulation or malfunction of the cellular proteome [11]. Modern techniques enabled the investigation of the internal structure and morphology of mitochondria and revealed a highly complex compartmentalization [12]

with the challenge to dissect the communication and maintenance of the individual compartments. Part of this is to ensure proteostasis (folding, unfolding and degradation) to generate a homeostasis of the functional proteome and to clear mistargeted/damaged proteins. It is not easy, because every submitochondrial compartment needs to control its redox milieu, which is interestingly highly different, e.g. the inner membrane separates the reducing matrix from the more oxidizing intermembrane space [13]. The complexity of the proteome supersedes that of the genome due to alternative splicing events and post-translational modifications (PTMs). Specific position has the mitochondrial intermembrane space (MIMS) with its role in protein and lipid transport, regulation and assembly of the respiratory transport system, regulation of redox processes, coordination of apoptosis and metal ion homeostasis [14]. One of the big challenges of future research will be to investigate how the mitochondrion communicates with the cytosol and the nucleus. The MIMS exhibits specific redox environment and controlled porine-facilitated leakiness through the outer membrane allowing the free diffusion of small molecules (less than 5 kDa) that might harbor candidates mediating the communication from signaling pathways occurring inside the mitochondria toward other organelles [15].

Mitochondrial homeostasis is associated with overall cellular fitness and cellular longevity. Therefore, it determines also normal physiology of organ systems and performance of the body. Recent studies suggest that restoration of mitochondrial dynamics and mitophagy could delay organ senescence and prevent age-associated cardiac diseases. Here, we discuss the current understanding of mitochondria with particular focus on the heart, specifically the close relationship between mitochondrial dynamics or mitophagy suggesting a possible link to the regulation of redox metabolism, and intercellular protein communication.

2. Cellular senescence and mitochondria

Cells undergo widespread changes and develop specific characteristics during senescence that are considered as senescence markers. However, no individual marker has been so far identified as entirely selective parameter for cellular senescence. Nevertheless, a combination of several markers might be evaluated and can help to define the current stage of senescence. Phenotypically, the increase in size and protein content was reported in senescent cells [16, 17] which is in agreement with our results. The data in **Table 1** show that the total protein concentration was elevated in senescent (27 months old) rat mitochondria by 40% ($p < 0.01$).

Protein concentration (mg/ml)	**Age (months)**		
	6	**14**	**27**
Homogenate	20.87 ± 2.50	23.96 ± 1.46	19.19 ± 2.96
Mitochondria	5.69 ± 0.61	5.38 ± 0.49	9.56 ± 0.05**

Values are expressed as Mean ± SEM of 5 individual experiments, **p<0.01; significantly different in comparison to 6 months old rats.

Table 1. Protein concentration in homogenate and mitochondria during aging in heart (yet unpublished data).

However, the protein content in homogenates as well as in isolated mitochondrial fraction was maintained during overall aging process comparing the samples from old (14 months old) and adult (6 months old) rat hearts.

Further, senescent cells exhibit enlarged nuclei and lysosomes, which possess elevated senescence-associated β-galactosidase activity (SA-β-Gal), the most widely used marker [17]. They also enter a proliferative arrest state, detected by cell cycle inhibitor levels such as p53/p21, tumor suppressor $p16^{INK4a}$ [18, 19] and markers of proliferation like Ki-67 and 5-bromodeoxyuridine [20]. Other factors secreted during senescence are cytokines, chemokines, growth factors, proteases, fibronectin as well as ROS and reactive nitrogen species (RNS). Additionally, proteostatic changes during senescence accompanied by an increase in modified proteins, accumulation of protein aggregates and reduced functionality of the proteasomal and autophagy systems [3] will be discussed in following chapters. Only two parameters currently correlate with species longevity in the right sense: the mitochondrial rate of ROS production and the degree of fatty acid unsaturation of tissue membranes. Their basal level is in both cases low in long-lived animals. In addition, the best-known manipulation that extends longevity, dietary restriction, also decreases the rate of mitochondrial ROS production and oxidative damage to mtDNA [21]. The available information supports a mitochondrial free radical theory of aging focused on low generation of endogenous damage and low sensitivity of membranes to oxidation in long-lived animals.

2.1. Mitochondrion – organelle with two faces

According to mitochondrial theory of aging, mitochondria are both the main source and targets of detrimental reactions initiated in association with age-dependent deterioration of the cellular functions. Reactions leading to increased ROS generation, mtDNA mutations, oxidation of mitochondrial proteins and lipids result in subsequent induction of apoptotic events, impaired oxidative phosphorylation capacity, mitochondrial dynamics, and autophagy [22]. In addition, mitochondrial function may be affected by subject parameters like physical activity history [23], caloric restriction [21], drugs [24] and various comorbidities including obesity [25], insulin resistance and hypertension [26]. The primary function of mitochondria is to produce adenosine triphosphate (ATP) by the process of oxidative phosphorylation. In fact, about 90–95% of cellular oxygen is used up in oxidative phosphorylation and 3% from that pool can be converted to superoxide anion radical ($O_2^{\bullet -}$). This is a very strong argument to mitochondria as a main source of this oxygen radical [27]. Among the most relevant ROS sources in heart belong NADPH oxidases (NOX) and mitochondria [28]. Recent studies demonstrate that mitochondrial ROS play a critical role in mediating the cellular effects of angiotensin II in the cardiovascular system [29]. Angiotensin II binds to angiotensin receptor 1, thereby activating NOX isoform 2 and 4 leading to increased mitochondrial ROS production in vascular endothelial cells as well as in cardiac myocytes [30].

Two principal scenarios can be envisioned that favor increased mitochondrial ROS-formation: increased formation of $O_2^{\bullet -}$ at the electron transport chain (ETC) and decreased elimination of $O_2^{\bullet -}$ or hydrogen peroxide (H_2O_2) in the mitochondrial matrix. In heart failure, the first scenario occurs when modifications of ETC complexes like disturbed stoichiometry and

PTMs hamper electron flux along the ETC to provoke excessive $O_2^{\bullet-}$ formation [31] mostly by NADH dehydrogenase (complex I) and Cytochrome c reductase (complex III) causing functional uncoupling of the respiratory chain. The extent and way of individual ETC complexes inhibition is different. According to **Figure 1**, the decline in activity of complex I and cytochrome c oxidase (complex IV) was more obvious during aging when compared to the succinate dehydrogenase (complex II) and complex III activities. Literature data are inconsistent among the studies, mainly due to differences in the experimental age groups and animal models, isolation/purity of mitochondria or enzyme substrate/inhibitor used for study. Complex I is considered to be the most important player in the game of ROS production and/or proton-motive cascade. Loss of its activity has been attributed to the mutations in mitochondrial DNA (mtDNA) in aging animals and recently was linked to the apoptotic cell death pathway [32].

Reduced complex I activity was seen in the rat heart [33], brain synaptic mitochondria [34] as well as continual decrease in the frontal cortex of Parkinson disease patients [35]. It is important to note that the activity and stability of this respiratory complex are determined by its abundance, PTMs and/or specific protein–protein interactions. In contrary, other studies have reported no age-related decrease in complex I activity [36]. Most of the inconsistencies are related to the complexes II, III and IV, where activities of respiratory complexes have been also shown to decline [37], remain unchanged or increased during aging [38]. Our data show age-dependent decrease in all ETC enzyme activities, although the extent of inhibition is different (**Figure 1**). Among them, complex IV was most affected throughout aging and reached 63.4% of adult respiring mitochondria. While complexes I, II and III maintained activities in 14 months old rat mitochondria, cytochrome c oxidase showed deprivation by

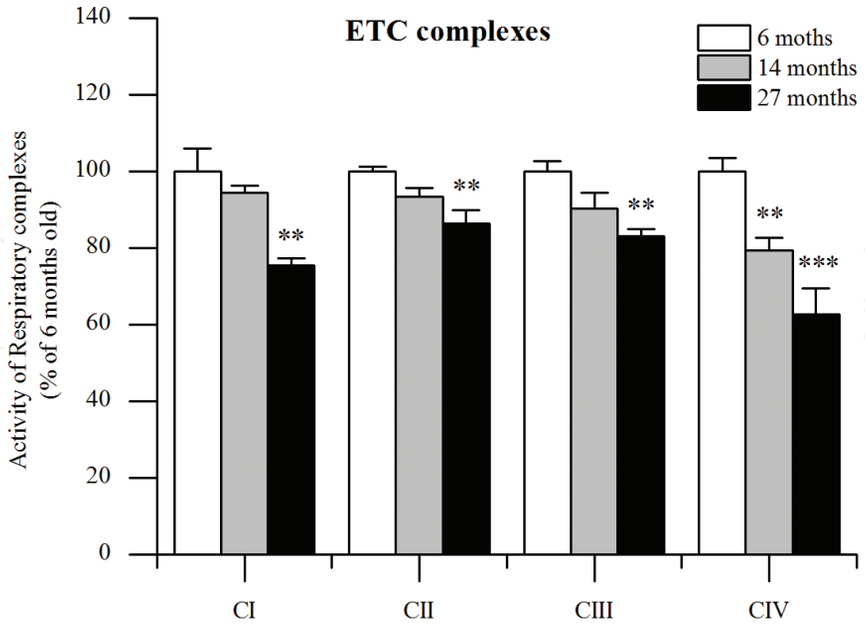

Figure 1. Activities of ETC complexes in heart mitochondria during aging (yet unpublished data). Values are expressed as Mean ± SEM of 5 individual experiments, $^{**}p < 0.01$, $^{***}p < 0.001$; significantly different in comparison to 6 months old rats.

21.6% ($p < 0.01$) when compared to the adults. Unique role in both, Krebs cycle and ETC, has in inner mitochondrial membrane (IMM) embedded complex II. Its function is cardiolipin-independent and is not taking part in respiratory supercomplexes but has been identified as an isolated entity in mildly solubilized mitochondrial membranes [39]. This confirms the least damage by 13.6% in senescent mitochondria when compared to the other respiratory complexes. Interestingly, recent works described the direct connection of the $O_2^{\bullet-}$ formation and respiratory chain complex II [40] when lack of succinate was present. At saturated succinate concentration and high membrane potential complex II is tightly bound with reverse transfer of electrons to $O_2^{\bullet-}$ -producing complex I [41].

Several factors have impact on ETC with specific role of mtDNA. ETC complexes are composed of both nuclear DNA-encoded (more than 80 proteins subunits) and 13 mtDNA-encoded subunits proteins. Respiratory chain and F_1F_0-ATPase deficits are adverse effects on a variety of cellular and tissue functions, causing a wide range of complex clinical phenotypes. The incidence of inherited mitochondrial diseases is estimated to be about 1 in 5000 but a much larger population may be affected when somatic genetic defects, such as mtDNA mutation and deletions accompanying normal aging, are considered. The frequency of the common 4977-bp mtDNA deletion, a typical consequence of oxidative stress [42], increases with age in the human heart and is estimated to be 5- to 15-fold higher in people over 40 years of age relative to younger individuals [43]. This deletion affects genes encoding 7 polypeptide components of the mitochondrial ETC. Bioenergetic consequences of 4977-bp deletions will be reflected when the proportion of deleted mtDNA exceeds 50–55% of total mtDNA. The involvement of mtDNA mutations in cardiac aging is supported by findings in mice that express a proof reading deficient version of mtDNA polymerase (PolG) [44]. A high load of mtDNA mutations and deletions accumulate in the heart of these mice, in conjunction with the early onset of several age-associated changes including cardiac enlargement, fibrosis, impairment of systolic and diastolic function [30], and reduced activity of ETC complexes [44].

To maintain proton-motive force and electron transfer through ETC, reduced equivalents are required. In mitochondria, the Krebs cycle generates NADH, which delivers electrons to the ETC inducing translocation of protons across the inner mitochondrial membrane. This establishes a membrane potential that fuels the F_1F_0-ATPase to generate ATP. At the ETC, electrons can leak to produce $O_2^{\bullet}$ which is dismutated to H_2O_2 by Mn-dependent superoxide dismutase (Mn-SOD) and, in turn, is detoxified by enzymes that require NADPH. Accordingly, equilibrium exists between reduced and oxidized forms of equivalents NADH/NAD^+ and NADPH/$NADP^+$ [45].

In experiments, citrate synthase is frequently used as basal mitochondrial activity marker to avoid possible effects of different yield of mitochondria. Increase in its activity shown in **Figure 2** led to more pronounced activity changes in ETC complexes with age. Starting from citrate synthase, some of the Krebs cycle enzymes play inevitable role in reduced equivalents machinery, such as NAD(P)$^+$-isocitrate dehydrogenase (ICDH) and α-ketoglutarate dehydrogenase (KGDH). Both of the enzymes share some common features as NADH production and they are regulated by calcium (Ca^{2+}). Calcium uptake dynamically controls the redox state of NAD(P)H in working cardiac myocytes [28] and availability of NADPH together with reduced glutathione is required for H_2O_2 removal by glutathione peroxidase/glutathione reductase

http://dx.doi.org/10.5772/intechopen.71789

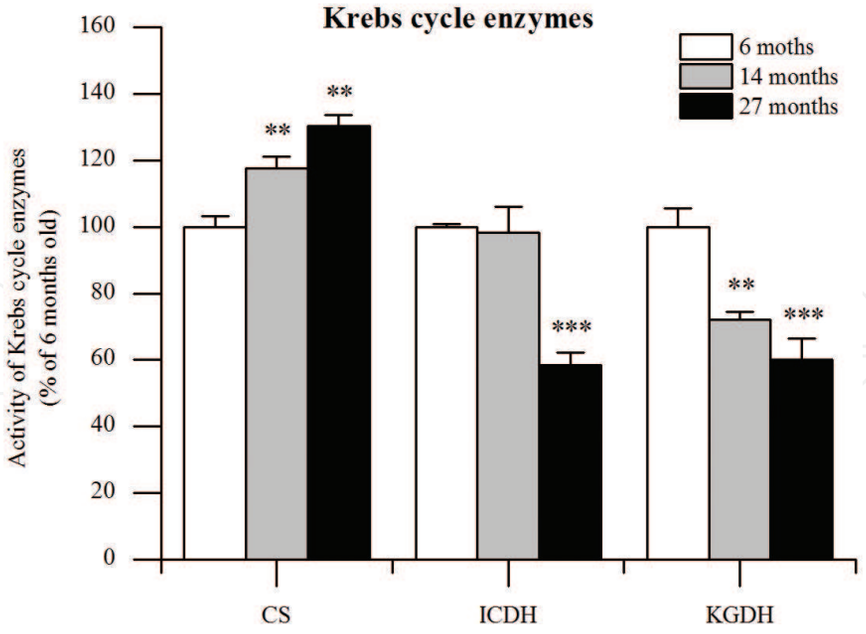

Figure 2. Activities of Krebs cycle enzymes in heart mitochondria during aging (yet unpublished data). Values are expressed as Mean ± SEM of 5 individual experiments, $^{**}p < 0.01$, $^{***}p < 0.001$; significantly different in comparison to 6 months old rats.

cycling. Therefore regeneration of NADPH is very important and depends on Krebs cycle enzymes, especially ICDH [46]. Interestingly, the activity of ICDH was affected only in senescent mitochondria but by a significant 42.5% decrease (from 246.52 ± 1.90 to 144.04 ± 5.58) μmol/min/mg protein. Almost equivalent to ICDH activity in senescent hearts was drop in KGDH activity but this enzyme has shown gradual decline during whole aging process (**Figure 2**). In heart failure, elevated intracellular Na^+ promotes ROS-formation by reducing mitochondrial Ca^{2+} uptake. Therefore, mitochondrial Ca^{2+} uptake critically regulates mitochondrial ROS production/removal [28] in cooperation with Krebs cycle enzymes and other ions to maintain mitochondrial homeostasis. Activation of ion channels in the IMM such as mitochondrial permeability transition pore (MPTP), the inner membrane anion channel (IMAC) and ATP-dependent K^+-channel causes depolarization of IMM. This is accompanied by an increase in electron flux required to maintain ATP production. Opening of these channels might promote ROS production, but interestingly they can be activated by ROS themselves. Moreover, $O_2^{\bullet-}$ can be released from mitochondria via MPTP and IMAC. ROS in such a situation trigger oscillations of membrane potential leading to higher incidence of arrhythmias during reperfusion in the heart [47]. It seems that oxidative stress is one of the key events in myocardial senescence progression and development; however, during evolution cells were equipped with antioxidant defense mechanisms that can prevent/recover cells from an oxidative to a reductive state.

3. Redox homeostasis in mitochondria

Mitochondria play important role in generation of ROS and RNS but they are themselves players in different signaling pathways, in which the mitochondrial oxidative defense system

contributes to maintain redox homeostasis. Cellular redox state is determined by the reduction potentials and reducing capacities of the redox couples, such as GSH/GSSG, NAD(P)H/NAD(P)$^+$, thioredoxin (reduced/oxidized), glutaredoxin (reduced/oxidized) and cysteine/cystine, From these the GSH/GSSG system is considered to be the most abundant among endogenous antioxidants with 2 to 4-fold higher abundance than other redox systems.

3.1. Thiol–disulfide redox state of mitochondria

Reduced form of GSH (γ-L-glutamyl-L-cysteinyl glycine) is two electron donating molecule and in humans is almost uniquely present in a quite high concentration (1–10 mmol/L). This allows GSH to scavenge ROS either directly or indirectly. As an antioxidant, it reacts with reactive forms and radicals produced in association with electron transport, xenobiotic metabolism and inflammatory responses [48]. GSH homeostasis is not only regulated by its *de novo* synthesis, but also by other factors such as utilization, recycling and cellular export. Cooperation with other antioxidant, redox-related enzymes is important for recycling and maintenance of the optimal redox environment. It was reported that GSH level decreases over time in heart mitochondria [49] and several brain regions [50]. This phenomenon was confirmed by GSH measurement in three age groups (**Table 2**). Rapid decline of GSH level was accompanied by increase in oxidized GSSG form in senescent rat hearts. Interestingly, 14 months old hearts were able to maintain basal concentrations of that present in adult ones. The relative GSH/GSSG ratio indicates a decrease in GSH levels leading to more oxidized environment in senescent cardiomyocytes and experimentally dilated cardiomyopathy in mice [51]. The total content of thiol-containing compounds (R-SH), in contrary to GSH, decreases very slowly (by 19.2% in senescent) during aging process.

(μmol/g of tissue)	**Age (months)**		
	6	**14**	**27**
R-SH content	8.66 ± 0.46	7.12 ± 0.26	7.00 ± 0.09*
GSH content	3.00 ± 0.25	3.74 ± 0.39	1.68 ± 0.19**
GSSG content	1.77 ± 0.21	1.61 ± 0.25	3.81 ± 0.31**
GSH/GSSG ratio	1.64	2.10	0.51
Enzyme activity (μmol/min/mg protein)			
GPx activity	9.033 ± 0.860	8.239 ± 0.081***	6.489 ± 0.112***
GR activity	4.790 ± 0.093	4.194 ± 0.306**	3.960 ± 0.124***
TrxR activity	0.085 ± 0.018	0.050 ± 0.008**	0.052 ± 0.013**

Values are expressed as means ± SEM of 5 individual experiments. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; significantly different in comparison to 6 months old rats.

Table 2. The content and activities of GSH cycle-related molecules in heart during aging (yet unpublished data).

Aerobic respiration may result in an increase of H_2O_2, which is metabolized by glutathione peroxidase (GPx), while GSH is recycled by the action of glutathione reductase (GR). Since both of the enzymes were affected in old as well as senescent rat hearts, overproduction of H_2O_2 or lack of reduced NADPH was present. Moreover, peroxidase-mediated elimination of H_2O_2 will also augment the level of GSSG. This in turn, may not only lower the glutathione redox potential, but also increase in amount of protein mixed disulfides. Addition of GSH to protein cysteine residues results to post-translational modification known as S-glutathionylation. It is a reversible process with potential to activate or inactivate protein function by modulating different cellular pathways. It is able to influence gene expression by affecting different transcription factors such as Nrf2 (nuclear factor erythroid 2-related factor 2) or NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) [52]. The Nrf2-Keap1 (Kelch-like ECH-associated protein 1) pathway is the major regulator of cytoprotective responses to oxidative and electrophilic stress. In the presence of ROS, critical cysteine residues in Keap1 become oxidized leading to a conformational change, which prevents its binding to Nrf2. As a consequence, Nrf2 degradation is stopped and its nuclear translocation promoted [53]. Recently was shown that S-glutathionylation of endothelial NO synthase (eNOS) at Cys^{689} and Cys^{908} leads to eNOS uncoupling, diminished NO production and enhanced oxidative stress linked with superoxide overproduction [54]. S-glutathionylation as well as GSH alone interacts with earlier mentioned nuclear factor Nrf2. This factor is key transcription factor of 4-hydroxy-2-nonenal (HNE). HNE is highly reactive aldehyde product of lipid peroxidation with potential to modulate function of proteins and lipids. The Nrf2 under stress conditions activates HNE-mediated antioxidant protection, when at a sub-lethal concentration 5 μmol/l HNE stimulates biosynthesis of GSH in cardiomyocytes. In contrary, glutathione after oxidation to the glutathione radical ($GS^{\bullet}$) has deleterious effects. It is able to take H^+ from lipid side chains and polyunsaturated fatty acids or to induce lipid peroxidation [55]. Altogether, S-glutathionylation may be a double-edged sword in the sense that it promotes antioxidant or pro-oxidant responses.

Peroxidase activity is found in GPx and in the thiol-specific proteins called peroxiredoxins (Prx). They react with H_2O_2 at a very high rate and their activity depends on cysteine residue in the active site. Peroxiredoxins can also reduce and detoxify peroxynitrite anion and a wide range of organic hydroperoxides. The highest reaction rate and abundance has Prx2 which traps almost all H_2O_2 *in vivo* [56]. The level of this powerful thiol-specific protein was maintained until the age of 14 months, but senescent mitochondria lost 35.1% of Prx2 amount (**Figure 3**). Oxidized cysteine residues of Prx are specifically reduced by Trx. Oxidized Trx as well as other oxidized cellular proteins can be reversibly reduced by TrxR in a NADPH-dependent manner. The Trx/TrxR system appears to have a protective function against oxidative stress, e.g. supports the activity of ribonucleotide reductase and inhibits apoptosis signal-regulated kinase-1 [57]. Thioredoxin reductase has been severely affected during aging process by 41.9% in group of 14 months old hearts with an extension until the age of 27 months (**Table 2**). The same scenario was observed in Trx protein level where the amount of protein has decreased to 62.4% in 14 months old as well as 27 months

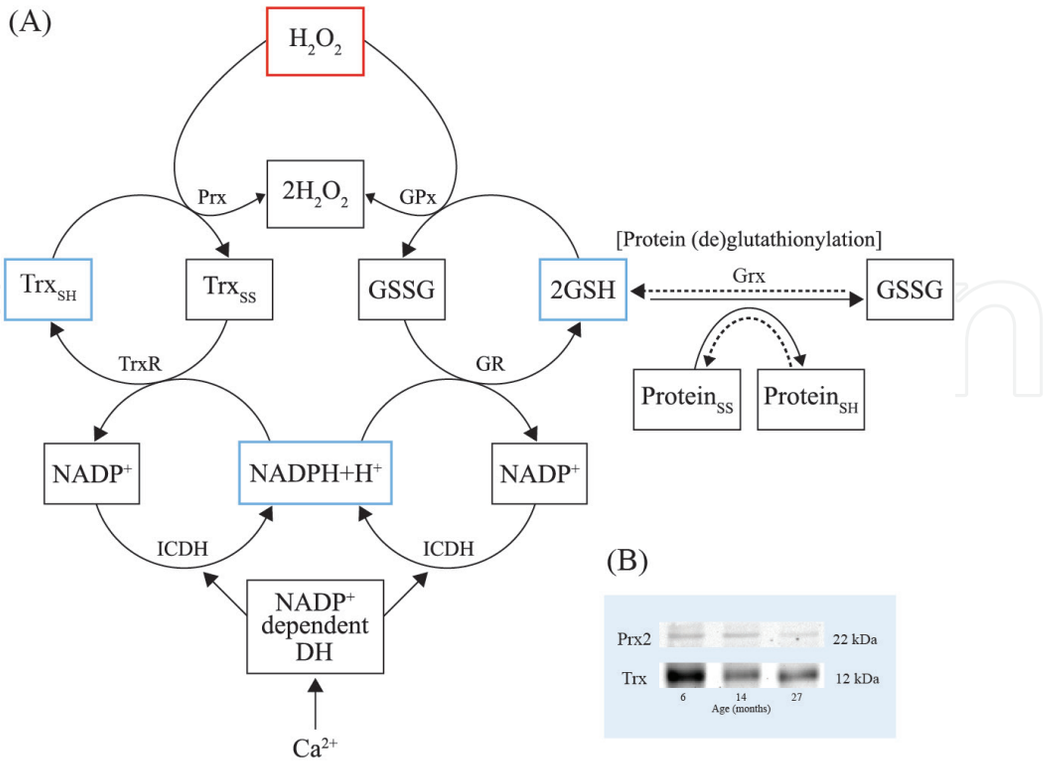

Figure 3. Scheme of (A) Thiol-disulfide network and (B) protein level for Prx2, Trx (yet unpublished data). GSH-reduced glutathione, GSSG-oxidized glutathione, GPx-Glutathione peroxidase, GR-Glutathione reductase, Grx-Glutaredoxin, Prx-Peroxiredoxin, Trx-Thioredoxin, TrxR-Thioredoxin reductase, SH-reduced and SS-oxidized form of Protein, ICDH-isocitrate dehydrogenase.

old rat hearts (**Figure 3**). Low Trx level is probably result of TrxR malfunctioning due to lack of NADPH, overproduction of ROS and/or RNS. Thioredoxin also plays a role in the reversible S-nitrosylation of cysteine residues in target proteins, and thereby contributes to the response of intracellular nitric oxide (NO). In addition, Trx is able to block caspase-3 activity through nitrosylation of the active cysteine site in response to NO. Therefore, Trx protein deficiency may contribute to the stimulation of caspase-dependent apoptosis. The most studied enzyme in the process of protein S-glutathionylation is glutaredoxin (Grx). The high specificity of Grx to S-glutathionylated proteins is used as a tool for studying and identifying them. In general, it is stated that the main task of Grx is to remove GSH from S-glutathionylated proteins. Thus, reduced thiol-containing protein is restored, which was confirmed in experiments with siRNA (small interfering RNA). The suppression of Grx genes with siRNA is an approach to study not only the "antioxidant" properties of Grx but also the role of protein S-glutathionylation [58]. Grx2 catalyzes S-glutathionylation of IMM proteins in a relatively reduced GSH/GSSG ratio equal to 6. The fact that thiol-disulfide oxidoreductases can catalyze both oxidizing and reducing reactions is not exclusive to Grx. Thioredoxin can act as an oxidant in oxidizing environment. Currently, the biggest challenge of researchers is to identify which enzymes are responsible for (de)glutathionylation, and if it is spontaneous process or catalyzed by enzymes. Existing information suggests that the

http://dx.doi.org/10.5772/intechopen.71789

mechanisms involved in resistance to various types of stress together with maintenance of bioenergetic capacity and redox homeostasis may be critical in the evolution of longevity.

3.2. Mitochondrial protein-protein network

One of the major goals of gerontology is to understand the comprehensive mechanisms involved in aging at different levels and hopefully to help understand age-related diseases. The complexity of the proteome supersedes that of the genome, due to alternative splicing events and PTMs of proteins. Proteomes are expected to be two to three orders of magnitude more complex than would be predicted from numbers of protein-encoding genes present in the respective genomes [59]. It is widely recognized that cellular aging causes changes in the proteome. However, the nature and targets of these changes and their consequences have not yet been completely identified. In recent years, mass spectrometry (MS) has been recognized as a golden standard tool for the identification and analysis of individual proteins. For further understanding of the molecular changes during heart aging, we have identified several proteins and compared the differences in the mitochondrial protein expression profiles among two age groups.

Precipitated proteins from mitochondria of 6 and 27 months old rat hearts were separated with two-dimensional electrophoresis (2-DE) to provide a protein profile (**Figure 4**). Interestingly, the change in protein level was statistically significant (1.5-fold change, 95% confidence interval) in only 12 proteins (marked with red circles) from the total protein pool of mitochondria. All the proteins were down-regulated in senescent mitochondria in comparison to the adult ones. Despite the small number of quantitatively modified proteins, these create an interesting protein-protein network. The strength of data support represents line thickness of protein-protein interaction network generated by String

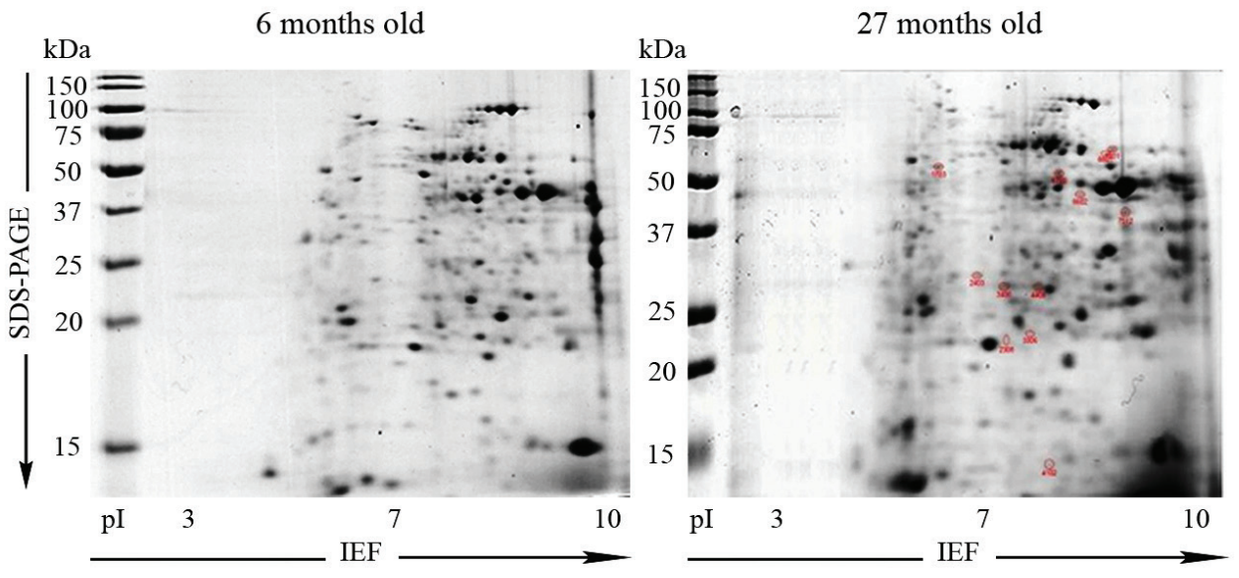

Figure 4. Representative 2-DE analysis of mitochondrial proteins in aging rat heart (yet unpublished data).

software and using *gene IDs* (**Figure 5**). Two proteins, methylmalonate-semialdehyde dehydrogenase [acylating] (Gene ID: *Aldh6a1*) and carnitine O-palmitoyltransferase 2 (*Cpt2*) did not fit to the protein-protein interaction map. However, their role in fatty acid metabolism is very important in connection to the heart muscle work and energy production. The rest of the proteins participate in various metabolic pathways of mitochondria. Three take part in Krebs cycle – malate dehydrogenase (*Mdh1*), dihydrolipoyl dehydrogenase (*Dld*) and aconitate hydratase (*Aco2*). Post-translational cysteine-related modification of aconitase was reported to be a key in linkage between Krebs cycle, redox signaling and metabolism of ROS [60]. Next five down-regulated proteins were directly connected with cardiac muscle contraction and the risk of developing age-related neuronal disorders - Huntington, Parkinson and Alzheimer disease. All of them are subunits of ETC, thus regulate production of energy in mitochondria: NADH dehydrogenase 1 alpha subcomplex subunit 5 (*Ndufa5*), cytochrome bc1 complex subunit 1 (*Uqcrc1*) and cytochrome bc1 complex subunit Rieske (*Uqcrsf2*), cytochrome c oxidase subunit 5B (*Cox5b*), electron transfer flavoprotein subunit beta (*Etfb*). Combined defects in oxidative phosphorylation and fatty acid beta oxidation were detected in mitochondrial diseases [61]. Creatine kinase M-type (*Ckm*) was only one cytoplasmic enzyme interacting with mitochondria through transfer of phosphate and the last one is voltage-dependent anion-selective channel protein 1 (*Vdac1*),

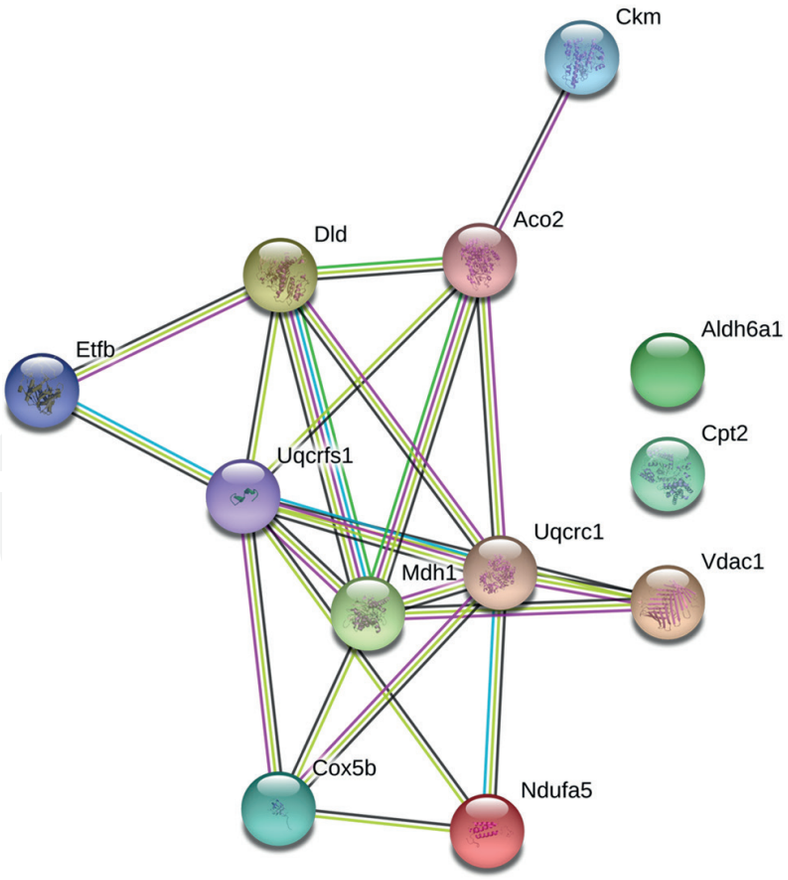

Figure 5. Functional protein-protein interactions during aging in rat mitochondria (yet unpublished data).

which plays a role in outer mitochondrial membrane permeabilization and cellular death. This outer mitochondrial membrane protein is tightly bound with Alzheimer disease, where mediates amyloid β toxicity and represents a potential target for Alzheimer disease therapy [62].

Currently available evidence indicates that the steady-state amounts of structural damage to proteins accumulated during life are relatively small, and are often present only in trace amounts. On the other hand, the pro-oxidizing changes in the redox state reflected by the decline in redox potential, increases in production of H_2O_2 and level of protein modifications are significant and ubiquitous. However, the oxidative PTMs are relevant only if these are connected to functional consequences. It is important to consider that a slight modification in low abundance proteins may be of physiological importance. Distinguishing between inconsequential modifications and functionally significant ones requires careful biochemical/biophysical analysis of target proteins [63]. Thus, proteomic approaches represent powerful tools to address these questions by identifying the targeted proteins and the extent of their modifications.

3.3. Post-translational modifications of proteins during aging

The detailed examination of enzyme molecules by mass spectrometry and other techniques continues to identify hundreds of distinct PTMs. Global analyses of enzymes using proteomics revealed widespread distribution of PTMs on many key enzymes located in all cellular compartments. Multiple PTMs within a single enzyme molecule and their mutual interplays are critical for the regulation of catalytic activity. Enzymatic PTMs can be detected in ever increasing amounts and they appear to be critical for folding and assembly (e.g. glycosylation), function as key regulators of catalytic activity of enzymes (e.g. binding of prosthetic groups, phosphorylation), or mark enzyme molecules for targeted destruction (e.g. ubiquitylation). In parallel with these processes there are non-enzymatic PTMs caused by ROS and RNS continuously interacting with individual enzyme molecules. These PTMs contribute to molecular aging and may also be involved in regulation of enzymes' catalytic activity [64]. There are two groups of PTMs observed during oxidative stress mediated aging, reversible and irreversible. The major types of *irreversible PTMs* are carbonylation and 3-nitrotyrosilation.

- *Carbonylation* is covalent adduction of lipid aldehyde to the side chains of lysine, histidine or cysteine residues. Extensive amount of information about this modification can be found in the recently published book [65].
- *3-nitrotyrosilation*, frequently called tyrosine nitration is formed between RNS (peroxynitrite anion) and a tyrosine residue of target protein. Extensive research was done during last years in failing human [66] and rat hearts [67].

Second group of *reversible PTMs* which are related to the aging process are sulfur-mediated S-Sulfenylation, S-nitrosylation, S-glutathionylation and lipid peroxidation pathway-related HNE modification.

- Protein *S-Sulfenylation* leads to the production of sulfur-hydroxylation product (P-SOH), disulfide bond and sulfenyl-amide bond formation. It may be a precursor to the process of S-sulfinylation and S-sulfonylation. This type of modification is believed to be a fleeting molecular switch that regulates non-enzymatic oxidative folding [68].
- *S-nitrosylation* occurs when NO is covalently incorporated into the Cysteine thiol group forming S-nitrosothiol (SNO). It plays important role in redox metabolism through GSH interaction in failing heart in rats [67] and human heart disease [69]. This PTM has been progressively implicated in virtually every NO-regulated process within the cardiovascular system. The current, widely-held paradigm is that S-nitrosylation plays an equivalent role as phosphorylation, providing a stable and controllable PTM [70].
- *S-glutathionylation* is covalent attachment of GSH to protein thiol groups. The function of protein S-glutathionylation reactions in metabolism is a rapid and reversible redox signaling mechanism that involves the conjugation and removal of glutathione from cysteine switches. Several observations have shown that unlike other redox modifications S-glutathionylation reactions fulfill the requisite criteria to serve as an effective PTM that controls protein function, links energy metabolism to redox signaling in mitochondria. Because of its role in modulation of ROS production in myocardial mitochondria, currently the usage of mitochondria penetrating antioxidants is discussed in context of the heart disease treatment [71].
- During last decade *HNE modification* was under extensive research because of its dual role as pro- and anti-oxidant. This most abundant reactive aldehyde attacks predominantly nitrogen of histidine, lysine (less commonly arginine) or cysteine, and it is related to wide range of metabolic diseases [72].

Above mentioned PTMs occur the most frequently during oxidative stress-related aging process. However, there are virtually hundreds of additional PTMs that may occur in enzymes. To support results from protein profiling of mitochondria is important to focus on deep protein analyzes of individual selected proteins, their interactions within the individual compartments, between different organelles or the intercellular communication. For this purpose, proteomics is now integrated with molecular genetics, transcriptomics, and other areas leading to systems biology strategies.

3.4. Turnover of mitochondrial proteins - role of mitophagy in cardiomyocytes

Proper functioning of mitochondria is crucial for cardiac function. Damaged mitochondria produce less ATP, release greater amounts of ROS, and have a lower threshold for cytochrome c release resulting in apoptosis, undergo mitochondrial permeability transition pore opening resulting in necrosis or may release mitochondrial components into cytosol where are recognized by receptors for removal. Mitochondrial turnover is therefore an integral aspect of quality control in which dysfunctional mitochondria are selectively eliminated through autophagy or mitochondrial autophagy (mitophagy) and replaced through expansion of preexisting mitochondria (biogenesis). In the heart mitochondria turnover is with a half life of 14 days. Rat cardiomyocytes have roughly 1000 mitochondria per cell,

suggesting that under basal resting conditions, one mitochondrion per cell is replaced every 40 minutes [73]. In order to facilitate and initiate mitophagy, mitochondrial fusion and fission play a critical role in mitochondrial turnover. Fission of mitochondria into smaller fragments is a crucial requirement for mitophagy to occur. The key regulator of this process is dynamin-related protein 1 (Drp1), which in concert with proteins fission 1 (Fis1), mitochondrial fission factor (Mff), mitochondrial dynamics proteins of 49 kDa (MiD49) and 51 kDa (MiD51) is responsible for mitochondrial fragmentation. The role of last three proteins appears essential. Mff assists in the assembly of Drp1. MiD49 and MiD51 may play a regulatory role by recruiting Drp1 and maintaining it in inactive state until fission is required [74]. Proteins that promote outer mitochondrial membrane (OMM) fusion such as Mitofusin 1 and 2 (Mfn1 and 2) are ubiquitinated and eliminated by the ubiquitin proteasome system. E3 ubiquitin ligase Parkin (also known as Park2) and PTEN-induced putative kinase 1 (PINK1) have been shown to play an important role in mitophagy. PINK1 targets to the mitochondria but is normally degraded by presenilin associated rhomboid-like protease (PARL). In response to loss of mitochondrial membrane potential, PARL is inactivated; PINK1 is stabilized and recruits Parkin. Parkin ubiquitinates several mitochondrial associated proteins and they are then recognized by p62 and bring mitochondria to the autophagosomes. Thus mitochondria with membrane potential loss can be selectively degraded. Parkin substrates include e.g. voltage-dependent anion-selective channel protein (VDAC), translocase of the outer membrane (TOM), mitochondrial fission 1 (FIS1), hexokinase, mitochondrial Rho-GTPase (MIRO) 1 and 2, although whether ubiquitination of these proteins is required or sufficient for mitophagy is unclear and highly dependent on the specific cellular context [75].

The importance of mitophagy for the preservation of cardiovascular homeostasis, the cardiomyocyte-specific deletion of Parkin and the expression of a mutant Mfn2 (mitofusin 2) at birth prevented the switch from fetal to adult mitochondria in the mice heart [76]. Another example, mice bearing a heart-specific deletion of Mfn2 prematurely succumbed to a progressive cardiomyopathy characterized by impaired contractile function [77]. Such a detrimental phenotype could be reversed, at least partially by Mfn2 to prevent the targeted mitochondria from rejoining the mitochondrial network through fusion. Under basal conditions Mfn2 functions in mitochondrial fusion events and links endoplasmic reticulum to mitochondria. Also acts as Parkin receptor during mitophagy following phosphorylation by PINK1 and recruiting Parkin to the mitochondria. So even in case of Parkin-dependent mitophagy, some outer mitochondrial membrane proteins are recycled through transfer to the endoplasmic reticulum. Most studies have relied on the systemic modulation of autophagy with nutritional or pharmacological interventions or the homozygous/heterozygous deletion of a relevant gene. Nutritional and pharmacological interventions commonly used to modulate autophagy in the cardiovascular system *in vivo*, including caloric restriction or caloric restriction mimetics, rapamycin, 3-methyladenine, and lysosomal inhibitors, are rather nonspecific. Genetic interventions offer increased specificity, but are not devoid of potential problems that should be kept under attentive consideration. Linking mitophagy with cardiomyocytes is the field of interest in many publications [78]. While mitophagy is responsible for bulk degradation of mitochondria, turnover of individual components may proceed at asynchronous rates

through redistribution of components via fusion events, selective degradation of proteins by mitochondrial proteases, and proteasomal elimination of some outer mitochondrial membrane proteins. Some studies suggest that inner mitochondrial membrane proteins, especially oxidative phosphorylation constituents, may be primarily cleared via mitophagy. There is more to discuss, especially communication between intracellular organelles (mitochondria and endoplasmic reticulum) and oxidative phosphorylation events in mitochondria in relation to redox network, apoptosis/necrosis or mitophagy. These appear to be key players in cardiomyocytes survival during aging.

4. Conclusions

Maintenance of mitochondrial function and energy/redox homeostasis requires both generation of newly synthesized and elimination of dysfunctional mitochondria. Taken together, age-dependent decline of mitophagy inhibits removal of dysfunctional or redundant mitochondria as well as impairs mitochondrial biogenesis. It results to progressive mitochondrial accretion and consequently, deterioration of cardiomyocytes function. At present it is pointed out that the steady-state amounts of structural damage accumulated during life are relatively small. However, changes in the redox state reflected by the decline in redox potential, increases in production of H_2O_2 and level of protein modifications are significant and ubiquitous. Activities of ETC complexes revealed that the most affected throughout aging was complex IV, in contrast to relatively small age-related changes in complex II. These non-uniform changes in ETC enzyme complexes may lead to altered electron transfer through the chain, leading to impairment of ATP synthesis and overall functionality of cardiomyocytes during aging. The ability of the main regulatory Krebs cycle enzymes to supplement NADH to ETC was altered in senescent mitochondria. ICDH and KGDH had only half activity in senescent mitochondria; however ICDH was not affected in group of 14 months old. This might support promotion of H_2O_2 in senescent cardiac myocytes where peroxiredoxin 2 ability to trap H_2O_2 was also significantly affected. Decrease in GSH levels indicates low GSH/GSSG ratio leading to more oxidized environment in senescent cardiomyocytes but 14-month old hearts were able to maintain basal levels of adult mitochondria. Despite the variations in enzyme activities, the overall proteomic analyses revealed only 12 significantly affected proteins during aging. All these were more or less deprived but question is what is responsible for such a changes. Are these proteins really relevant? Is change in protein amount important? These and other questions are waiting for us and other researchers to be answered.

Specific marker of senescence was yet not identified, but evaluating a series of markers can help to define the senescent state. Senescent cells have greater proportion of protein content, which might be modulated by several PTMs. If these protein modifications are connected to functional consequences and protein-protein interactions are revealed, link may lead to the solution. Unfortunately, the proteome is much more complicated than the genome. To fit all the proteins together with their different characteristics in various pathways and cellular compartments is almost infeasible. At present, various nutritional, pharmacological and genetic interventions have higher or lower specificity, but are not devoid of potential problems that

should be kept under attentive consideration. Collectively, these studies suggest that dysfunctional proteostasis has a causative role in aging and that restoration of protein homeostasis is protective against age-related diseases. New window is opened and hopefully with help of bioinformatics collecting huge amounts of data from proteomics, genomics and other scientific approaches will lead to personalized therapeutic procedures for individual patient and age-related disorder(s).

Acknowledgements

This work was supported by the project APVV-15-0107 and the project "Competence Center for Research and Development in the field of Diagnostics and Therapy of Oncological diseases", ITMS: 26220220153, co-financed from EU sources. We thank Mrs. Zdena Cetlova for technical assistance.

Author details

Zuzana Tatarkova[1]*, Martin Kolisek[2], Ivana Pilchova[2], Peter Racay[1,2] and Peter Kaplan[1,2]

*Address all correspondence to: tatarkova@jfmed.uniba.sk

1 Department of Medical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia

2 Biomedical Center Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia

References

[1] Steenman M, Lande G. Cardiac aging and heart disease in humans. Biophysical Reviews. 2017;**9**(2):131-137. DOI: 10.1007/s12551-017-0255-9

[2] Lakatta EG, Sollott SJ. Perspectives on mammalian cardiovascular aging: Humans to molecules. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology. 2002;**132**(4):699-721. DOI: 10.1016/S1095-6433(02)00124-1

[3] Höhn A, Weber D, Jung T, Ott C, Hugo M, Kochlik B, et al. Happily (n)ever after: Aging in the context of oxidative stress, proteostasis loss and cellular senescence. Redox Biology. 2017;**11**:482-501. DOI: 10.1016/j.redox.2016.12.001

[4] Lopez-Lluch G, Hunt N, Jones B, Zhu M, Jamieson H, Hilmer S, et al. Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**:1768-1773. DOI: 10.1073/pnas.0510452103

[5] Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Experimental Cell Research. 1961;**25**:585-621

[6] Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. Genes & Development. 2010;**24**(22):2463-2479. DOI: 10.1101/gad.1971610

[7] Marzetti E, Csiszar A, Dutta D, Balagopal G, Calvani R, Leeuwenburgh C. Role of mitochondrial dysfunction and altered autophagy in cardiovascular aging and disease: From mechanisms to therapeutics. American Journal of Physiology. Heart and Circulatory Physiology. 2013;**305**:H459-H476. DOI: 10.1152/ajpheart.00936.2012

[8] Ago T, Matsushima S, Kuroda J, Zablocki D, Kitazono T, Sadoshima J. The NADPH oxidase Nox4 and aging in the heart. Aging (Albany NY). 2010;**2**:1012-1016. DOI: 10.18632/aging.100261

[9] Barger JL, Kayo T, Vann JM, Arias EB, Wang J, Hacker TA, et al. A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. PLoS One. 2008;**3**(6):e264. DOI: 10.1371/journal.pone.0002264

[10] Shirakabe A, Ikeda Y, Sciarretta S, Zablocki DK, Sadoshima J. Aging and autophagy in the heart. Circulation Research. 2016;**118**:1563-1576. DOI: 10.1161/CIRCRESAHA.116.307474

[11] Flatt T. A new definition of aging? Frontiers in Genetics. 2012;**23**(3):148. DOI: 10.3389/fgene.2012.00148

[12] Scorrano L. Keeping mitochondria in shape: A matter of life and death. European Journal of Clinical Investigation. 2013;**43**(8):886-893. DOI: 10.1111/eci.12135

[13] Hu J, Dong L, Outten CE. The redox environment in the mitochondrial intermembrane space is maintained separately from the cytosol and matrix. The Journal of Biological Chemistry. 2008;**283**(43):29126-29134. DOI: 10.1074/jbc.M803028200

[14] Herrmann JM, Riemer J. The intermembrane space of mitochondria. Antioxidants & Redox Signaling. 2010;**13**(9):1323-1326. DOI: 10.1089/ars.2010.3270

[15] Nuebel E, Manganas P, Tokatlidis K. Orphan proteins of unknown function in the mitochondrial intermembrane space proteome: New pathways and metabolic cross-talk. Biochimica et Biophysica Acta. 2016;**1863**(11):2613-2623. DOI: 10.1016/j.bbamcr.2016.07.004

[16] Young AR, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JF, et al. Autophagy mediates the mitotic senescence transition. Genes & Development. 2009;**23**(7):798-803. DOI: 10.1101/gad.519709

[17] Lee BY, Han JA, Im JS, Morrone A, Johung K, Goodwin EC, et al. Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. Aging Cell. 2006;**5**(2):187-195. DOI: 10.1111/j.1474-9726.2006.00199.x

[18] Beauséjour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, et al. Reversal of human cellular senescence: Roles of the p53 and p16 pathways. The EMBO Journal. 2003;**22**(16):4212-4222. DOI: 10.1093/emboj/cdg417

[19] Helman A, Klochendler A, Azazmeh N, Gabai Y, Horwitz E, Anzi S, et al. p16(Ink4a)-induced senescence of pancreatic beta cells enhances insulin secretion. Nature Medicine. 2016;**22**(4):412-420. DOI: 10.1038/nm.4054

[20] Muñoz-Espín D, Cañamero M, Maraver A, Gómez-López G, Contreras J, Murillo-Cuesta S, et al. Programmed cell senescence during mammalian embryonic development. Cell. 2013;**55**(5):1104-1118. DOI: 10.1016/j.cell.2013.10.019

[21] Barja G. The mitochondrial free radical theory of aging. Progress in Molecular Biology and Translational Science. 2014;**127**:1-27. DOI: 10.1016/B978-0-12-394625-6.00001-5

[22] Cedikova M, Pitule P, Kripnerova M, Markova M, Kuncova J. Multiple roles of mitochondria in aging processes. Physiological Research. 2016;**65**(5):5519-5531

[23] Gnaiger E. Capacity of oxidative phosphorylation in human skeletal muscle: New perspectives of mitochondrial physiology. The International Journal of Biochemistry & Cell Biology. 2009;**41**(10):1837-1845. DOI: 10.1016/j.biocel.2009.03.013

[24] Milane L, Trivedi M, Singh A, Talekar M, Amiji M. Mitochondrial biology, targets, and drug delivery. Journal of Controlled Release. 2015;**207**:40-58. DOI: 10.1016/j.jconrel.2015.03.036

[25] Boudina S, Graham TE. Mitochondrial function/dysfunction in white adipose tissue. Experimental Physiology. 2014;**99**(9):1168-1178. DOI: 10.1113/expphysiol.2014.081414

[26] Bonomini F, Rodella LF, Rezzani R. Metabolic syndrome, aging and involvement of oxidative stress. Aging and Disease. 2015;**6**(2):109-120. DOI: 10.14336/AD.2014.0305

[27] Maurel A, Hernandez C, Kunduzova O, Bompart G, Cambon C, Parini A, et al. Age-dependent increase in hydrogen peroxide production by cardiac monoamine oxidase A in rats. American Journal of Physiology. Heart and Circulatory Physiology. 2003;**284**(4):H1460-H1467. DOI: 10.1152/ajpheart.00700.2002

[28] Kohlhaas M, Liu T, Knopp A, Zeller T, Ong MF, Böhm M, et al. Elevated cytosolic Na^+ increases mitochondrial formation of reactive oxygen species in failing cardiac myocytes. Circulation. 2010;**121**(14):1606-1613. DOI: 10.1161/CIRCULATIONAHA.109.914911

[29] Dai DF, Chen T, Wanagat J, Laflamme M, Marcinek DJ, Emond MJ, et al. Age-dependent cardiomyopathy in mitochondrial mutator mice is attenuated by overexpression of catalase targeted to mitochondria. Aging Cell. 2010;**9**(4):536-544. DOI: 10.1111/j.1474-9726.2010.00581.x

[30] Dai DF, Rabinovitch PS, Ungvari Z. Mitochondria and cardiovascular aging. Circulation Research. 2012;**110**(8):1109-1124. DOI: 10.1161/CIRCRESAHA.111.246140

[31] Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. Cell. 2005;**120**(4):483-495. DOI: 10.1016/j.cell.2005.02.001

[32] Shulga N, Pastorino JG. GRIM-19-mediated translocation of STAT3 to mitochondria is necessary for TNF-induced necroptosis. Journal of Cell Science. 2012;**125**:2995-3003

[33] Preston CC, Oberlin AS, Holmuhamedov EL, Gupta A, Sagar S, Syed RH, et al. Aging-induced alterations in gene transcripts and functional activity of mitochondrial oxidative phosphorylation complexes in the heart. Mechanisms of Ageing and Development. 2008;**129**(6):304-312. DOI: 10.1016/j.mad.2008.02.010

[34] Petrosillo G, Matera M, Casanova G, Ruggiero FM, Paradies G. Mitochondrial dysfunction in rat brain with aging Involvement of complex I, reactive oxygen species and cardiolipin. Neurochemistry International. 2008;**53**(5):126-131. DOI: 10.1016/j.neuint.2008.07.001

[35] Navarro A, Boveris A. Brain mitochondrial dysfunction in aging, neurodegeneration, and Parkinson's disease. Frontiers in Aging Neuroscience. 2010;**2**:34. DOI: 10.3389/fnagi.2010.00034

[36] Cocco T, Sgobbo P, Clemente M, Lopriore B, Grattagliano I, Di Paola M, et al. Tissue-specific changes of mitochondrial functions in aged rats: Effect of a long-term dietary treatment with N-acetylcysteine. Free Radical Biology & Medicine. 2005;**38**(6):796-805. DOI: 10.1016/j.freeradbiomed.2004.11.034

[37] Kumaran S, Subathra M, Balu M, Panneerselvam C. Age-associated decreased activities of mitochondrial electron transport chain complexes in heart and skeletal muscle: Role of L-carnitine. Chemico-Biological Interactions. 2004;**30**(148):11-18. DOI: 10.1016/j.cbi.2003.10.010

[38] Choksi KB, Papaconstantinou J. Age-related alterations in oxidatively damaged proteins of mouse heart mitochondrial electron transport chain complexes. Free Radical Biology & Medicine. 2008;**44**(10):1795-1805. DOI: 10.1016/j.freeradbiomed.2008.01.032

[39] Lenaz G, Genova ML. Supramolecular organisation of the mitochondrial respiratory chain: A new challenge for the mechanism and control of oxidative phosphorylation. Advances in Experimental Medicine and Biology. 2012;**748**:107-144. DOI: 10.1007/978-1-4614-3573-0_5

[40] Dröse S. Differential effects of complex II on mitochondrial ROS production and their relation to cardioprotective pre- and postconditioning. Biochimica et Biophysica Acta. 2013;**1827**(5):578-587. DOI: 10.1016/j.bbabio.2013.01.004

[41] Quinlan CL, Orr AL, Perevoshchikova IV, Treberg JR, Ackrell BA, Brand MD. Mitochondrial complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions. The Journal of Biological Chemistry. 2012;**287**(32):27255-27264. DOI: 10.1074/jbc.M112.374629

[42] Puche JE, García-Fernández M, Muntané J, Rioja J, González-Barón S, Castilla Cortazar I. Low doses of insulin-like growth factor-I induce mitochondrial protection in aging rats. Endocrinology. 2008;**149**(5):2620-2627. DOI: 10.1210/en.2007-1563

[43] Mohamed SA, Hanke T, Erasmi AW, Bechtel MJ, Scharfschwerdt M, Meissner C, et al. Mitochondrial DNA deletions and the aging heart. Experimental Gerontology. 2006;**41**(5):508-517. DOI: 10.1016/j.exger.2006.03.014

[44] Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature. 2004;**429**(6990):417-423. DOI: 10.1038/nature02517

[45] Aon MA, Cortassa S, O'Rourke B. Redox-optimized ROS balance: A unifying hypothesis. Biochimica et Biophysica Acta. 2010;**1797**(6-7):865-877. DOI: 10.1016/j.bbabio.2010.02.016

[46] Moreno-Sánchez R, Gallardo-Pérez JC, Rodríguez-Enríquez S, Saavedra E, Marín-Hernández Á. Control of the NADPH supply for oxidative stress handling in cancer cells. Free Radical Biology & Medicine. 2017;**112**:149-161. DOI: 10.1016/j.freeradbiomed.2017.07.018

[47] Akar FG, Aon MA, Tomaselli GF, O'Rourke B. The mitochondrial origin of postischemic arrhythmias. The Journal of Clinical Investigation. 2005;**115**:3527-3535. DOI: 10.1172/JCI25371

[48] Ray PD, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cellular Signalling. 2012;**24**(5):981-990. DOI: 10.1016/j.cellsig.2012.01.008

[49] van der Vlies D, Woudenberg J, Post JA. Protein oxidation in aging: Endoplasmic reticulum as a target. Amino Acids. 2003;**25**(3-4):397-407. DOI: 10.1007/s00726-003-0025-9

[50] Tong J, Fitzmaurice PS, Moszczynska A, Mattina K, Ang LC, Boileau I, et al. Do glutathione levels decline in aging human brain? Free Radical Biology & Medicine. 2016;**93**:110-117. DOI: 10.1016/j.freeradbiomed.2016.01.029

[51] Rajasekaran NS, Connell P, Christians ES, Yan LJ, Taylor RP, Orosz A, et al. Human alpha B-crystallin mutation causes oxido-reductive stress and protein aggregation cardiomyopathy in mice. Cell. 2007;**130**(3):427-439. DOI: 10.1016/j.cell.2007.06.044

[52] Pastore A, Piemonte F. S-S-glutathionyation signaling in cell biology: Progress and prospects. European Journal of Pharmaceutical Sciences. 2012;**46**(5):279-292. DOI: 10.1016/j.ejps.2012.03.010

[53] Ungvari Z, Bailey-Downs L, Sosnowska D, Gautam T, Koncz P, Losonczy G, et al. Vascular oxidative stress in aging: A homeostatic failure due to dysregulation of nrf2-mediated antioxidant response. The American Journal of Physiology. 2011;**301**:H363-H372. DOI: 10.1152/ajpheart.01134.2010

[54] De Pascali F, Hemann C, Samons K, Chen CA, Zweier JL. Hypoxia and reoxygenation induce endothelial nitric oxide synthase uncoupling in endothelial cells through tetrahydrobiopterin depletion and S-glutathionyation. Biochemistry. 2014;**53**(22):3679-3688. DOI: 10.1021/bi500076r

[55] Zhang Y, Sano M, Shinmura K, Tamaki K, Katsumata Y, Matsuhashi T, et al. 4-hydroxy-2-nonenal protects against cardiac ischemia-reperfusion injury via the Nrf2-dependent pathway. Journal of Molecular and Cellular Cardiology. 2010;**49**(4):576-586. DOI: 10.1016/j.yjmcc.2010.05.011

[56] Sies H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress. Redox Biology. 2017;**11**(6):613-619. DOI: 10.1016/j.redox.2016.12.035

[57] Go YM, Jones DP. Redox control systems in the nucleus: Mechanisms and functions. Antioxidants & Redox Signaling. 2010;**13**(4):489-509. DOI: 10.1089/ars.2009.3021

[58] Qanungo S, Starke DW, Pai HV, Mieyal JJ, Nieminen AL. Glutathione supplementation potentiates hypoxic apoptosis by S-glutathionylation of p65-NFkappaB. The Journal of Biological Chemistry. 2007;**282**(25):18427-18436

[59] Černý M, Skalák J, Cerna H, Brzobohatý B. Advances in purification and separation of posttranslationally modified proteins. Journal of Proteomics. 2013;**9**:2-27. DOI: 10.1016/j.jprot.2013.05.040

[60] Lushchak OV, Piroddi M, Galli F, Lushchak VI. Aconitase post-translational modification as a key in linkage between Krebs cycle, iron homeostasis, redox signaling, and metabolism of reactive oxygen species. Redox Report. 2014;**19**(1):8-15. DOI: 10.1179/1351000213Y.0000000073

[61] Nsiah-Sefaa A, McKenzie M. Combined defects in oxidative phosphorylation and fatty acid β-oxidation in mitochondrial disease. Bioscience Reports. 2016;**36**(2):e00313. DOI: 10.1042/BSR20150295

[62] Smilansky A, Dangoor L, Nakdimon I, Ben-Hail D, Mizrachi D, Shoshan-Barmatz V. The voltage-dependent anion channel 1 mediates amyloid β toxicity and represents a potential target for Alzheimer disease therapy. The Journal of Biological Chemistry. 2015;**290**(52):30670-30683. DOI: 10.1074/jbc.M115.691493

[63] Ortuño-Sahagún D, Pallàs M, Rojas-Mayorquín AE. Oxidative stress in aging: Advances in proteomic approaches. Oxidative Medicine and Cellular Longevity. 2014;**2014**:573208. DOI: 10.1155/2014/573208

[64] Ryšlavá H, Doubnerová V, Kavan D, Vaněk O. Effect of posttranslational modifications on enzyme function and assembly. Journal of Proteomics. 2013;**92**:80-109. DOI: 10.1016/j.jprot.2013.03.0252

[65] Ros J, editor. Protein Carbonylation: Principles, Analysis, and Biological Implications. 1st ed. Hoboken, New Jersey, USA: John Wiley & Sons; 2017. 416 p

[66] Sheeran FL, Pepe S. Posttranslational modifications and dysfunction of mitochondrial enzymes in human heart failure. American Journal of Physiology. Endocrinology and Metabolism. 2016;**311**(2):E449-E460. DOI: 10.1152/ajpendo.00127.2016

[67] Wang X, Shults NV, Suzuki YJ. Oxidative profiling of the failing right heart in rats with pulmonary hypertension. PLoS One. 2017;**12**(5):e0176887. DOI: 10.1371/journal.pone.0176887

[68] Beedle AE, Lynham S, Garcia-Manyes S. Protein S-sulfenylation is a fleeting molecular switch that regulates non-enzymatic oxidative folding. Nature Communications. 2016;**7**:12490. DOI: 10.1038/ncomms12490

[69] Zhang YH. Nitric oxide signalling and neuronal nitric oxide synthase in the heart under stress. F1000Res. 2017;**6**:742. DOI: 10.12688/f1000research.10128.1

[70] Wolhuter K, Eaton P. How widespread is stable protein S-nitrosylation as an end-effector of protein regulation? Free Radical Biology & Medicine. 2017;**109**:156-166. DOI: 10.1016/j.freeradbiomed.2017.02.013

[71] Mailloux RJ, Treberg JR. Protein S-glutathionlyation links energy metabolism to redox signaling in mitochondria. Redox Biology. 2016;**8**:110-118. DOI: 10.1016/j.redox.2015.12.010

[72] Castro JP, Jung T, Grune T, Siems W. 4-Hydroxynonenal (HNE) modified proteins in metabolic diseases. Free Radical Biology & Medicine. 2017;**111**:309-315. DOI: 10.1016/j.freeradbiomed.2016.10.497

[73] Carreira RS, Lee Y, Ghochani M, Gustafsson ÅB, Gottlieb RA. Cyclophilin D is required for mitochondrial removal by autophagy in cardiac cells. Autophagy. 2010;**6**(5):462-472. DOI: 10.4161/auto.6.4.11553

[74] Losón OC, Song Z, Chen H, Chan DC. Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fission. Molecular Biology of the Cell. 2013;**24**(5):659-667. DOI: 10.1091/mbc.E12-10-0721

[75] Zhang J. Teaching the basics of autophagy and mitophagy to redox biologists—Mechanisms and experimental approaches. Redox Biology. 2015;**4**:242-259. DOI: 10.1016/j.redox.2015.01.003

[76] Gong G, Song M, Csordas G, Kelly DP, Matkovich SJ, Dorn GW II. Parkin-mediated mitophagy directs perinatal cardiac metabolic maturation in mice. Science. 2015;**350**: aad2459. DOI: 10.1126/science.aad2459

[77] Chen Y, Dorn GW II. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. Science. 2013;**340**:471-475. DOI: 10.1126/science.1231031

[78] Bravo-San Pedro JM, Kroemer G, Galluzzi L. Autophagy and mitophagy in cardiovascular disease. Circulation Research. 2017;**120**(11):1812-1824. DOI: 10.1161/CIRCRESAHA.117.311082

IntechOpen