Mitochondrial dysfunction in cardiac aging

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ARTICLE INFO

Article history:
Received 16 March 2015
Received in revised form 6 July 2015
Accepted 9 July 2015
Available online 17 July 2015

Keywords:
Mitochondrial dysfunction
Heart
Aging
Mitostasis

ABSTRACT

Cardiovascular diseases are the leading cause of death in most developed nations. While it has received the least public attention, aging is the dominant risk factor for developing cardiovascular diseases, as the prevalence of cardiovascular diseases increases dramatically with increasing age. Cardiac aging is an intrinsic process that results in impaired cardiac function, along with cellular and molecular changes. Mitochondria play a great role in these processes, as cardiac function is an energetically demanding process. In this review, we examine mitochondrial dysfunction in cardiac aging. Recent research has demonstrated that mitochondrial dysfunction can disrupt morphology, signaling pathways, and protein interactions; conversely, mitochondrial homeostasis is maintained by mechanisms that include fission/fusion, autophagy, and unfolded protein responses. Finally, we describe some of the recent findings in mitochondrial targeted treatments to help meet the challenges of mitochondrial dysfunction in aging.

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1. Introduction

The heart is a highly metabolic organ that is reliant on the maintenance of cellular-energetic homeostasis, precisely regulated mitochondrial dynamics, and optimal mitochondrial function. Mitochondria are important determinants of cellular homeostasis and longevity since they are the main producers of cellular ATP and play a vital role in regulation of apoptotic death pathways in many tissues. Due to its high energetic demand and high density of mitochondria, the heart is especially vulnerable to mitochondrial dysfunction via structural disruption, energetic fluctuations, and mitochondrial signaling. Cardiac senescence is accompanied by a general decline in mitochondrial function, clonal expansion of dysfunctional mitochondria, increased production of reactive oxygen species (ROS), suppressed mitophagy, and dysregulation of mitochondrial quality control processes such as fusion and fission [1–4]. These detrimental alterations in mitochondrial function have been widely correlated with several age-related cardiac diseases, as will be described below. The mechanisms responsible for age-related mitochondrial dysfunctions in cardiac tissue are only partially defined and it is not yet clear the extent to which mitochondrial dysfunction is directly linked to aging [5]. Nevertheless, the information below will illustrate that there is abundant evidence that mitochondrial function is intimately tied to cardiac health and, likely, largely related to cardiac aging.

2. Mitochondrial energetics in cardiac aging

Given the high energetic demand of the heart, it is not surprising that age-related defects in mitochondrial bioenergetics have been related to normal cardiac aging [5–7]. Many factors contribute to the reduced energetic capacity of the cardiac mitochondria including increased ROS, mutation and deletions in the mitochondrial genome, and dysregulation of proteostasis and mitochondrial biogenesis [5–9]. In rodent models, the total mitochondrial content does not change in liver and brain with age, however, the activity levels of components of the electron transport chain decrease [10].

It has been documented that the activity of mitochondrial respiratory chain complexes declines with age in skeletal muscle [11], brain [12], and heart [6], particularly in complex I and IV [6,13]. Complexes II, III, and V remain less affected by age in cardiomyocytes [6,9]. Differences in the reported activity levels of complex III in aging heart may be due to the inclusion or exclusion of two separate populations of cardiac mitochondria, interfibrillar (IFM) and subsarcolemmal (SSM), a unique aspect of cardiac structure. Of these two populations, complex III activity may only decrease in the IFM with aging [14]. The decreased activity of complexes I and V (and possibly III and IV) may be partially compensated for by increased expression of the mitochondrial genes within those complexes in adult mice, but this overexpression was reversed in aged mice [15].

Levels of mitochondrial respiratory proteins and other key proteins involved in mitochondrial metabolism decline in the old heart, including those in fatty acid metabolism. Conversely, glycolytic metabolic pathways as well as extracellular structural proteins increase significantly with age [16]. Increased expression of glycolytic proteins, together with a decline in fatty acid oxidation, TCA cycle, and amino acid

http://dx.doi.org/10.1016/j.bbabio.2015.07.009
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metabolism, indicates a metabolic remodeling with age that bears some similarity to heart failure in younger individuals [17,18]. As described in a subsequent section, treatment of old mice with CR or rapamycin reverses this metabolic substrate shift in the heart, restoring a greater dependence on fatty acid oxidation and mitochondrial function [16]. As the heart has uniquely high and continuous energetic requirements, many of the energetic and metabolic changes seen in cardiac aging and failure may be more apparent in the heart than in other organs.

3. ROS, DNA damage and the aging heart

Mitochondria are the main source and target of ROS produced as by-products of cellular respiration [19,20]. ROS production increases with age and higher ROS content limits proper functioning of macromolecules and signaling pathways [21]. The mitochondria free radical theory of aging (MFRTA) hypothesized that age-related increases in mitochondrial ROS resulted in accumulation of mtDNA mutations and oxidized proteins and lipid that impaired mitochondrial respiratory (RC) efficiency, leading to further ROS production in a viscous cycle [22–24]. Many studies showed data consistent with this theory, including increased ROS production, increases in mitochondrial DNA (mtDNA) mutations, and respiratory chain dysfunction in aging tissues [25–31,6,32–35]. Decreased mtDNA quantity and deteriorating replication fidelity with age contribute to an accumulation of dysfunctional mitochondria often resulting in pathological outcome [36–40]. However, there is increasing recognition that ROS and ROS signaling have beneficial roles, and many studies of mouse models in which cytoplasmic antioxidant enzymes are reduced or increased have failed to support a causal connection to aging [41]. The study that is most supportive of the mitochondrial variant of the free radical theory of aging is of transgenic mice that express catalase that is targeted to mitochondria (mCAT); these mice has increased lifespan [42] and numerous reports of resistance to diseases of aging [43]. Most relevant to this review, mCAT mice have a phenotype of delayed cardiac aging that includes both functional and molecular parameters [44]. Furthermore, mice with mutation in the mitochondrial Polg exonuclease proofreading domain (PolgD181A) have elevated mitochondrial DNA mutations and deletions, exhibit a progeria phenotype and can develop cardiomyopathy leading to congestive heart failure [45]. This cardiomyopathy is attenuated in mCAT mice, indicating that this phenotype is partly mediated by mitochondrial oxidative stress [46].

4. Mitochondrial structural changes with aging

In several model systems, and in humans, evidence suggests that the structure of mitochondria in the heart is disrupted by the aging process. Studies in human and hamster hearts show that mitochondria may increase in size with age [47,48]. Electron microscopy has been used to demonstrate a disrupted morphology of mitochondria with age in mice [49]. It has been shown that the mouse cardiac inner mitochondrial membrane displays a loss of cristae with aging [50], although crista morphology does not appear to change with age in Fischer 344 rats both in situ and in isolated subsarcomal and interbrillar mitochondria [51].

Mitochondrial structure is intimately associated with functional integrity and the cristae provide essential scaffolding for RC complexes; thus it would be no surprise that alterations in mitochondrial structure might be integrally related to the age-related decline in mitochondrial activity. Indeed, reversing the loss of youthful mitochondrial structure with age may result in improved electron transport activity [52].

4.1. Cardiolipin in the aging heart

The inner mitochondrial membrane contains cardiolipin (1′- [1,2-diacyl-sn-glycero-3-phosphoryl]-3′- [1′,2′-diacyl-sn-glycero-3′-phosphoryl]-sn-glycerol, 1,3- dihydroxytriglycerolic acid), which is almost entirely absent from the rest of the mammalian cell [53–55]. Cardiolipin (CL) was first purified from beef heart in 1942 and has since been classified as a tetra–acyl phospholipid essential for the structural integrity of the mitochondrial membrane [56] (reviewed in [53]). The acyl chains on CL can vary depending on which kingdom, species and tissue is investigated [57–60] and what diet is being consumed [61,62].

CL has an essential role in maintaining optimal mitochondrial structure and function through its ability to maintain curvature of cristae, supporting the assembly and interaction of mitochondrial respiratory chain complexes and supercomplexes, modulating and maintaining the proton gradient, and preventing apoptosis [reviewed in [63,64]]. Recent studies have begun to help clarify some of these complex interactions between cristae structure, CL, respiratory complexes and the proteins that facilitate their assembly [65,66]. A considerable body of evidence [54,67–70], with rare exception [71] suggests that CL is selectively lost and/or remodeled in aging mitochondria.

Modification or restoration of CL content has been proposed as a method of reducing age-associated declines in mitochondrial function. Age-dependent loss of CL may be due to oxidative stress [72], which can be due to extrinsic ROS or by peroxidase activity of cytochrome C that is closely associated with CL [73]. Prevention of peroxidation of CL may attenuate or abrogate mitochondrial dysfunction [73]. In the brain, melatonin might help to preserve the structural integrity of cardiolipin by preventing age-related peroxidation of the cardiolipin [74]. This was observed alongside other improved parameters of mitochondrial aging in the brain and suggests that preservation of intact cardiolipin is an avenue of abrogating age-related declines in mitochondrial function. Similarly, Paradies and colleagues have reported that acyl-carnitine supplementation in aged rats restored CL levels to that of young controls, and that some CL-dependent processes were improved [68,75].

Barth syndrome is an example of CL disease with cardiomyopathies being the most deadly symptom. In Barth syndrome, tafazzin, another protein located in the mitochondria, is mutated or lost. CL is modified into its final 18:2 form from monolysocerlipin (MLCL) by adding and removing acyl chains in two different tafazzin-dependent mechanisms [76]. The exact mechanism is still unknown for how tafazzin and CL interact, but the disease state suggests an important relationship between the two.

More recently, it has been suggested that the protective effects of mitochondrial targeted SS-31 peptide is due to its affinity for CL and the prevention of cytochrome C peroxidation (see the section on Cardiolipin-Targeted Therapies, below).

5. Dietary intervention and the aging heart

CR, the reduction of total calories without nutritional deficits, is the longest studied and most reproducibly successful method of extending lifespan and improving healthspan in model organisms. CR exerts some of its effects through modulating TOR signaling (mTORC1 in particular in mammals), and seems to have a wide range of effects, including modulation of tissue maintenance (reviewed extensively in [77]). In multiple organisms, including humans, rodents, and monkeys, chronic CR delays the onset of cardiac aging. This can be seen as a reduction of aging-associated cardiac functional decline, cardiac hypertrophy, and cardiomyopathy [16,78–82].

CR protects against cardiomyopathy, at least in part, by reducing age-associated apoptosis. This is partially accomplished by a reduced susceptibility to DNA damage, improved DNA repair, and apoptosis-related gene expression alterations [80,83]. Expression of genes involved in numerous other processes important for mitochondrial function in aging are also modulated by CR, including extracellular matrix maintenance, inflammation, oxidative phosphorylation, and glucose and fatty acid metabolism [16,83]. Other protective effects of CR in the myocardium include the reduction of fibrosis and perivascular collagen deposition, reduced vascular inflammation and left ventricular
cardiac hypertrophy, along with protective effects against ischemia [83–85].

While it is unknown exactly how CR modulates cardiac aging, an attractive hypothesis is that limited nutrient and energy availability allows tissues to switch to a somatic maintenance state that may include optimization of existing cellular resources. For example, Drake and colleagues (2013) found that proliferative rates in heart, while low in controls, were further reduced by life-long CR (measured by DNA synthesis) while measures of mitochondrial biogenesis were maintained [86,87]. Short term (10 weeks) CR has been shown to result in improved cardiac function accompanied by a 30% reduction in protein turnover rates, and remodeling of the cellular and mitochondrial cardiac proteome and metabolome toward an abundance profile more similar to that of young mice, as well as with lower oxidative damage [16].

Oxidative stress increases with age, concurrent with a decreasing ability to prevent or recover from oxidative stress in the heart [88]. While some evidence suggests that mitochondrial dysfunction may not be due to damage from age-associated ROS alone [25], modulation of this stress either by direct targeting of catalase to the mitochondria (mCAT) [42,89], or by CR [16,79,81,90] results in improvements in cardiac function and in molecular changes indicative of an improved response to oxidative stress. For example, long-term, but not short-term, CR has been shown to dramatically reduce mitochondrial H₂O₂ production while lowering oxidative damage to mtDNA [91]. A clear understanding of the mechanisms of CR enhancement of cardiac mitochondrial function should provide greater insight into future protective intervention strategies.

6. Signaling pathways

Modulation of cardiac health and aging, including the effects of CR, is mediated through several signaling pathways, the best characterized of which include mTOR and Insulin-like Growth Factor signaling and downstream of these, regulation of histone acetylation by sirtuins.

6.1. mTOR pathway

Rapamycin inhibits mTOR (mechanistic target of rapamycin) and is the best studied CR mimetic. mTOR modulates several important growth and cellular quality control mechanisms including ribosomal biogenesis, autophagy, lipid synthesis, and protein translation (reviewed in [92]). Following the National Institute on Aging Intervention Testing Program’s [93] demonstration of enhanced longevity after chronic rapamycin treatment of mice [94], several other publications have demonstrated that long-term rapamycin treatment of mice improves healthspan measures and/or extends lifespan [95,96]. Inhibitors of TOR (both genetic and pharmacological) also extend lifespan and healthspan in other model organisms including flies [97], nematodes [98,99], and yeast [100,101]. The liver, adrenal glands, tendons, bone marrow, and heart have all been observed to be affected by rapamycin during aging [102,103].

Rapamycin confers functional benefits to the aging heart. Wilkinson and colleagues found that many measures of healthspan were positively affected by life-long rapamycin treatment in 20–22-month-old genetically heterogeneous mice. In the heart, they found that the incidence of nuclear atypia was reduced in rapamycin treated animals [102]. Pressure-overload-induced cardiac hypertrophy in young mice is reduced by rapamycin [104]. Recently, it has been shown that short-term (10–12 weeks) rapamycin treatment in late-life reversed age-related cardiac functional declines in mice, including improvement in systolic and diastolic dysfunction, and a reversal of cardiac hypertrophy [16,105]. Investigators at the Buck Institute reported that this was accompanied by a reduction in age-related sterile inflammation [105], while our laboratory showed that rapamycin recapitulated the CR effect of remodeling the old heart proteome to a more youthful abundance of proteins associated with young mitochondrial function (ETC, TCA cycle, fatty acid metabolism) and decreased abundance of glycolytic pathway proteins [16]. These results may point to proteomic and metabolic remodeling as a mechanism behind the cardiac functional benefits granted by rapamycin.

6.2. Insulin-like growth factor

The insulin/IGF-1 signaling pathway helps regulate cellular proliferation, survival, and autophagy [106,107]. This pathway is one of the best characterized determinants of lifespan, as deficiency in insulin/IGF-1 signaling is associated with increased lifespan in both invertebrate and vertebrate models of aging and IGF-1 activity is also down regulated in CR [108,109]. In general, IGF-1 has been shown to be cardio protective, allowing for suppression of ROS and autophagy in the cardiovascular system [107,110–112]. Notably, reduction in insulin/IGF-1 signaling improved cardiac performance at advanced age in Drosophila [113]. In contrast, an age-dependent decline in serum IGF-1 correlates with an increased risk of heart failure in humans [114]. It has therefore been proposed that treatments to increase IGF-1 signaling, including growth hormone therapy, may actually be beneficial in some patients with heart failure [115]. Thus, much remains to be learned before we understand the full role the IGF-1 pathway plays in cardiac aging.

6.3. Sirtuins

Sirtuins (Sirt) are a family of proteins deacetylases. There are seven members of the family, with Sirt3, Sirt4, and Sirt5 being targeted to the mitochondria [116,117]. Sirt3 in particular has been studied in the cardiovascular system and has been shown to prevent apoptosis, interact with nutrient sensing, and post-translationalily modify proteins, while also being the only Sirt to be linked to an increase in human lifespan [118,119]. Sirt3 overexpression leads to a decrease in cardiac hypertrophy via activation of Foxo3a-dependent defenses, while the loss of Sirt3 in cell lines and mice, leads to an increase [119,120]. Sirt3 helps prevent apoptosis by inhibiting upstream effectors of Bax, including Ku70 [121]. Other studies have shown that Sirt3 reduces levels of ROS by regulating antioxidant enzymes such as SOD2, MnSOD, and catalase [116,118,119]. By responding to mitochondrial NAD status, Sirt3 has a key metabolic regulatory role; this is shown mice lacking Sirt3 by reductions in complex I and III of the ETC, decreased in fatty acid oxidation, and a glycolygetic state that leads to accelerated cardiac aging [117,122,123]. Sirt3 is thought to be the main deacetylase in the mitochondria, which is supported by the fact that when Sirt3 knockout mice are investigated, there is an increase in acetylation in the ETC, especially complex I [120]. Sirt3 is able to maintain mitochondrial integrity by deacetylating cyclophilin D, a protein that helps open the mitochondrial permeability transition pore [124]. Calcium induced mitochondrial swelling was increased in Sirt3 deficient cells [123]. Resveratrol has been shown to activate Sirt3 by a variety of labs [125,126]. This interaction has been linked to both the NF-ΚΒ signaling and TGF-β/Smad3. With the induction of NF-ΚΒ pathway, it is suggested that apoptosis is inhibited by increasing the expression of SOD2 and Bcl2, while decreasing the Bax [125]. In models of mouse TAC surgery, when resveratrol was given, the mice had less fibrosis, which was linked to the TGF-β/Smad3 pathway preventing the transition of myoblasts to fibroblasts [126].

Sirtuins that are not targeted to the mitochondria have also been linked to the heart and aging. Overexpression of Sirt1 caused early heart failure with a decrease in oxidative respiration and an increase in degenerated mitochondria [127]. Some have suggested that this interaction might be signaled through ALD2, a mitochondrial encoded gene whose overexpression accentuates myocardial remodeling and contractile dysfunction in aging [128]. Sirt7 deficient mice have shortened lifespans demonstrating cardiac hypertrophy and inflammatory cardiomyopathy [119]. Sirt7 deacetylates a protein involved in
mitochondrial homeostasis [129]. When Sirt7 is lacking, apoptosis was shown to increase in primary cultured cardiomyocytes [130].

7. Proteostasis and cardiac aging

Protein homeostasis (proteostasis) is the equilibrium between protein synthesis, maintenance, and degradation. Maintaining the proteome is integral to maintaining cellular functions and organismal health, and many studies have demonstrated that inability to remove unwanted proteins and/or replace them with functional proteins can be detrimental [131]. Age-related conditions are generally accompanied by a decline in protein quality control mechanisms, thereby causing changes in the global proteome. A few well studied examples include cardiac dysfunction [132,133], neurodegenerative disease [134], cataracts [135], and sarcopenia [136–138]. While dysfusion of protein quality control mechanisms is a hallmark of aging, interventions that improve protein quality can enhance organismal health and longevity [131,134,139,140]. For example, the characteristic accumulation of damaged proteins and declines in mitochondrial respiratory capacity with age have been alleviated in models with over-expression of mitochondrial-targeted catalase [42], CR [141,16], reduced IGF-1 signaling [142,143], and rapamycin treatment [92,16]. That this mechanism has been implicated in interventions that inhibit mTOR may not be surprising, given its known effects on protein translation and degradation (see above). Collectively, these studies suggest that dysfunctional proteostasis has a causative role in aging and that restoration of protein homeostasis machinery is protective against aging and age-related disease. However, many mechanistic question of how these processes extend lifespan and healthspan remain unanswered. Fortunately, these processes are receiving increased attention as their roles are becoming more recognized [131,140].

The aging cardiac proteome recapitulates most hallmarks of the aged cellular proteome including the appearance of protein aggregates and lipofuscin, increased protein oxidation and damage, increased ubiquitination, and declines in autophagy and the ubiquitin proteinosome system [16,144–146]. All of these changes are consistent with altered proteostasis during cardiac aging. Consistent with this, we have observed increased protein ubiquitination and carboxylation in old hearts [16]. However, this is not accompanied by increased protein turnover in old hearts; in fact, slower turnover is observed in aging rodents [16,147]. Together, observation of increased protein ubiquitination and carbonylation, but decreased protein turnover is suggestive of a defect in cardiac proteostasis. These changes may owe to an underlying decline in major protein quality control systems with age, which in turn leads to low quality and damaged proteins which become increasingly unable to perform their roles efficiently. Given the importance of mitochondrial energetics in the heart, mechanisms of mitochondrial quality control are particularly relevant to cardiac aging. The next sections focus on mitochondrial fission, fusion, unfolded protein response and autophagy as critical components of protein quality control.

8. The role of fusion/fission dysregulation in Age-related cardiac bioenergetics deficiencies

As noted above, mitochondrial dysfunction, and in particular, bioenergetic deficiencies are an important hallmark of cardiac aging. Age-related decline in mitochondrial activity and impaired mitochondrial dynamics offer a potent explanation for deteriorating cardiac performance with age. Dysregulation of mitochondria quality control processes are widely reported in aging and although few studies have focused on the role of dysfunctional mitochondrial dynamics in cardiac senescence, there is extensive evidence to indicate that healthy cardiac performance is highly reliant on precise balance of mitochondrial fission and fusion. Mitochondria are highly motile organelles that constantly change morphology, fuse, divide, and move depending on energy demands and integrity of individual mitochondria. Following fission, segments of mitochondrial that are dysfunctional, sensed as reduced membrane potential, are targeted for mitophagy [148]. This homeostatic process helps to ensure optimal mitochondrial quality and supply of ATP to meet energy demand [149]. The key regulators of mitochondrial dynamics, Mfn1, Mfn2, Opal1, hFis1, Drp1, Mff, MiD49, and MiD51 show high expression in normal cardiac tissue, consistent with their pivotal role in mitochondrial dynamics and bioenergetics [150,151]. Genetic defects of proteins regulating fusion/fission are correlated with severe alterations in mitochondria morphology, decreased mtDNA integrity, increased oxidative stress, susceptibility to apoptosis, and metabolic dysregulation [152]. Mitofusion 1 and mitofusion 2 null mice are embryonic lethal, while knock-downs have fragmented mitochondria, characteristic of declining mitochondrial fusion; mfn1 deficiency is observed in giant cells similar to those present in age-related cardiac hypertrophy [153]. Genetic aberrations of mfn1 and 2 are consistent with increased respiratory dysfunction and higher frequencies of mtDNA mutations [154]. Dysregulation of fission may cause permeabilization of the mitochondrial membrane and release of caspase-3, a key modulator of myopathic apoptosis observed in senescent heart [155–157] that can trigger several other cytosolic death pathways [158,159] likely similar to those observed in heart failure [155] and possibly other cardiac pathologies.

Mitochondrial fusion is regulated by Mfn1, Mfn2, and Opa1. Opa1 mice missing one allele, develop cardiomyopathy late in life, and the acetylation of Opal1 has been linked to the development of heart disease when mice are pharmacologically, dietary, or surgically stressed [160]. The general loss of Opal1 in MEFs has been shown to give fragmented mitochondrial populations. Recently, in a fly model, suppression of Opa1 led to worsened contractility and increased dilation. These challenges were traced back to increased ROS production, and could be reversed by increasing ROS scavenging proteins [161]. Cardiac specific Mfn1/Mfn2 KOs have been shown to develop early onset heart disease [162]. Mfn1/2 are found on the outer mitochondrial membrane, where they can make hetero- or homo-dimeric interactions with neighboring mitochondria. Mfn1/2, unlike Opa1, are increased in some forms of heart failure [163]. Mfn1/Mfn2 plays a large role in autophagy that is often difficult to separate from their roles in fusion, which is an area of intense research. Mfn2 plays a key role in mitochondria–sarcoplasmic reticulum tethering for calcium signaling. In fact, loss of outer membrane mitofusins (MARP) led to fragmented mitochondria with higher ROS, which was repaired by increasing XBPI expression, a protein involved in ER stress [161].

Mitochondrial fission is managed by Drp1, Fis1, and Mff. Drp1 is localized to the cytosol until it is attracted to the mitochondrial surface for a fission event [164]. Drp1 has recently been suggested to help protect cardiac cells from IR injury by allowing them to be less reliant on oxidative phosphorylation and delaying or suppressing apoptosis [164,165]. The depletion of Drp1 in cardiomyocytes or in mouse hearts leads to mitochondrial dysfunction and heart disease, respectively [166]. A study by Ikeda et al. demonstrated that unchecked mitochondrial fusion, by Drp1 knock out was just as detrimental as is unchecked fission [167]. MiD49/Mid51 has been shown to recruit to the peroxisome as well [151]. MiF1/Mid51 plays a large role in autophagy that is often difficult to separate from their roles in fusion, which is an area of intense research. Mitofusins play a key role in mitochondria–sarcoplasmic reticulum tethering for calcium signaling. In fact, loss of outer membrane mitofusins (MARP) led to fragmented mitochondria with higher ROS, which was repaired by increasing XBPI expression, a protein involved in ER stress [161].
9. Autophagy and mitophagy

Autophagy is a major quality control pathway essential for the removal of unwanted proteins, macromolecules and organelles to maintain mitochondrial function. Cellular degradation involving lysosomes, a single membrane vesicle containing enzymes for the digestion of macromolecules, is generally categorized under the umbrella term "autophagy" [140]. There are three major ways by which proteins can be delivered to a lysosome for degradation, defining the primary categories of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy. Many details of these processes are outside the scope of this review, and readers are referred to detailed reviews on each topic [131,140,144]. This section will focus on macroautophagy and a mitochondrial-specific form of macroautophagy termed "mitophagy", as these are well characterized processes which may be important in both mitochondrial function and aging. Knocking down components of macroautophagy strongly diminishes mitochondrial function [16,144,145,169], demonstrating that it plays a key role in mitochondrial maintenance and homeostasis.

Two well characterized regulators of mitophagy are PINK1 and Parkin [170,171]. PINK1, aka phosphatase and tensin (PTEN) homologue-induced kinase 1, is a mitochondria-targeted serine/threonine kinase which serves to protect the cell from mitochondrial dysfunction and apoptosis [172]. Mutations in this protein are the most common cause of recessive familial Parkinsonism in humans [173]. In addition, PINK1 KO mice have severe deficiencies in mitochondrial homeostasis accompanied by morphological changes in the mitochondrial network, increased ROS, and susceptibility to heat shock [172]. Together this evidence suggests PINK1 has an important role in Parkinson’s disease as well as mitochondrial quality in normal cells, and possibly plays an important role in aging.

Under healthy conditions, PINK1 is imported into mitochondria via the TOM complex and is actively degraded by mitochondrial processing peptidase (MPP) and presenilin-associated rhomboid-like protease (PARL) [172,174,175]. Upon loss of mitochondrial membrane potential, PINK1 accumulates on the mitochondrial outer membrane and recruits Parkin, an E3 ubiquitin ligase, leading to the poly-ubiquitination of many mitochondrial outer membrane proteins such as Hexokinase I, VCAC1, MFN1/2, and Miro [176]. These ubiquitinated proteins are recognized by autophagy proteins P62, LC3 II, and BNIP3 to promote fusion with the lysosome and clearance of the dysfunctional organelle via mitophagy [176,177]. Many of the details surrounding this pathway and the interactions starting at PINK1 and leading up to mitophagy have been studied in detail and reviewed elsewhere [170,172,176,177].

A few studies have shown that PINK1/Parkin mediated mitophagy is important for heart function, particularly in the context of adaptation and recovery from stress. Parkin KO rats, in contrast to wild type, lack cardioprotection following ischemic preconditioning [178]. Parkin deficient mice exhibit impaired recovery of cardiac function after sepsis and mediating cardiac aging. Like normally aging mice, cardiac specific PINK1 knockdown of ATG5 in mice has conversely been shown to accelerate aspects of aging in the heart, suggesting that autophagy plays an important role in maintaining normal heart function and mediating cardiac aging. Like normally aging mice, cardiac specific ATG5 mutants develop left ventricular hypertrophy, but they also develop accelerated heart failure with decreased fractional shortening, abnormal mitochondrial morphology, decreased respiratory capacity, and die prematurely [175,189,190]. While the mechanism by which autophagy maintains cardiac function is not fully understood, fragmentation of mitochondria and accumulation of ubiquitinated proteins and p62 in mice lacking ATG5 suggests that this is an essential protective mechanism [189,191]. In agreement with this, a study performed on cardiomyocyte cell lines found that induction of autophagy was protective against oxidative stress-induced protein aggregation, reduced levels of protein ubiquitination, improved mitochondrial function, and reduced cell death [175,191].

Inhibition of the mTOR pathway (see above) is well known to increase autophagy and extend lifespan. In fact, the mTOR inhibitor rapamycin is one of the few drugs available which can be used to increase autophagy. Longevity studies with rapamycin and other forms of mTOR inhibition have reported increased autophagy in animals across many studies [92,139,140,143], and offer further evidence that autophagy may play a central role in aging. Even so, due to the difficulty of specifically over-expressing autophagy components without more detailed review details the role of mitophagy, including the less known role of BNIP3, in the heart [182].

Even though there were obvious morphological differences in the mitochondria of Parkin deficient animals, one common observation of these studies was that under normal conditions there was no apparent difference in cardiac function compared to wild type mice until advanced age or animals were first subjected to stress. PINK1 KO mice also show increased vulnerability to ischemic injury [183], but unlike Parkin deficiency, loss of PINK1 has been reported to show signs of cardiac dysfunction in mice as young as 2 months [184]. By six months of age, PINK1 KO and heterozygous mutants show increased heart weight, cardiomyocyte hypertrophy, decreased fractional shortening, and increases in hypertrophic gene expression [184]. Again, in contrast to Parkin deficient mice under normal conditions, this study also reported reductions in mitochondrial biogenesis and bioenergetics starting at 2 months of age. Collectively, studies in PINK1/Parkin have shown that these mediators are important for cardiac function, particularly in response to stressors, and compensatory increases in other degradation pathways may alleviate the dysfunction resulting in reduced mitophagy. However, considerable uncertainty remains in understanding relative roles of mitophagy vs. other proteostatic processes in maintaining mitochondrial protein quality control. A key observation is that half-lives of different respiratory chain complexes and even different proteins within a complex are highly variable, including in the heart [185,186]. This would not be expected on the basis of the common perception of mitophagy was a bulk recycling process. It has been suggested, however, that damaged proteins can be preferentially segregated to the mitochondrial components that are degraded by mitophagy [187], but there is also evidence that proteasomal activity correlates with respiratory chain protein half-lives [16,186]. Further studies will be needed to more clearly determine the relative roles of mitophagy and other proteostatic mechanisms in mitochondria, including their importance in age-related declines in the heart.

Modulation of macroautophagy has shown a mix of positive and negative results in various heart disease models; however, numerous lines of evidence have shown that macroautophagy has an important role in organismal and cardiac aging. A recent report found that genetic over-expression of ATG5, a vital autophagy protein involved in autophagosome formation, improved mitochondrial morphology, respiratory rates, and extended lifespan in mice [188]. ATG5 has been shown to have a pro-apoptotic function, and this activity in reducing cancer deaths C57BL/6 mice may be a longevity-promoting component. Cardiac-specific knockdown of ATG5 in mice has conversely been shown to accelerate aspects of aging in the heart, suggesting that autophagy plays an important role in maintaining normal heart function and mediating cardiac aging. Like normally aging mice, cardiac specific ATG5 mutants develop left ventricular hypertrophy, but they also develop accelerated heart failure with decreased fractional shortening, abnormal mitochondrial morphology, decreased respiratory capacity, and die prematurely [175,189,190]. While the mechanism by which autophagy maintains cardiac function is not fully understood, fragmentation of mitochondria and accumulation of ubiquitinated proteins and p62 in mice lacking ATG5 suggests that this is an essential protective mechanism [189,191]. In agreement with this, a study performed on cardiomyocyte cell lines found that induction of autophagy was protective against oxidative stress-induced protein aggregation, reduced levels of protein ubiquitination, improved mitochondrial function, and reduced cell death [175,191].

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targeting non-specific processes, direct evidence that activating autophagy can extend lifespan is not yet available.

10. Mitochondrial unfolded protein response

The mitochondrial unfolded protein response (UPRMT) is another aspect of protein quality control implicated in cardiac aging via its effects on mitochondrial function. UPRMT was first proposed in 1996, and described as a stress response involving mitochondrial chaperones and heat shock proteins [192]. Various models have been investigated to help reduce the amount of stress that occurs in the mitochondria, helping to decrease the UPRMT [17,193]. Dietary supplementation with taurine, a key nutrient for cardiac health, was shown to decrease oxidative stress and inhibit mitochondria-dependent cell apoptosis [193]. Prohibins (Phbs), highly conserved proteins in the mitochondria, have pivotal roles in the UPRMT. Phb make hetero-multimeric ring complexes that help with proper mitochondrial protein folding, ETC, assembly, and the regulation of proteases [194,195]. Phb2 helps ensure that OPA1 is functional for mitochondrial fusion. In a complex with DNAJC19, Phb2 is responsible for maintaining cardiolipin and mitochondrial cristae structure for healthy mitochondrial function [195]. Other proteins involved in this process are mShp70, Hsp60, and Hsp10. Mitochondrial Unfolded Response Elements 1 and 2 (MURE1 and MURE2) help upregulate Hsp60 and ClpP during mitochondrial homeostasis [196].

Recently, the UPRMT was investigated in an in vivo model of electron transport deficiency mice. Surf1−/− and litter mate control hearts were investigated for their induction of UPRMT proteins under the constant stress of COX assembly deficiency. The authors found significant increases in Lon and Trx2, with a trend of increased CHOP, all implicated in the UPRMT, demonstrating the role that the mitochondrial UPRMT can play to help relieve mitochondrial dysfunction in hearts in a stressed environment [196]. This study was performed in young mice, leaving the exact role of the UPRMT in aging still open; however, this remains an area of active investigation [197,198]. CHOP, Lon, and Trx2 are key proteins in the stress response pathways. CHOP is activated by the marking of unfolded proteins by Bip/GRP78, helping to prevent aggregation of the misfolded proteins [199]. Lon and Trx2 have recently been shown to play a key role in decreasing ROS in the mitochondria and preventing apoptosis within the cardiomyocytes. Recent work suggests that Lon helps prevent ROS induced apoptosis in a hypoxia model, and one can hypothesize that Lon would perform this role in any stressed environmental situation, not only in hypoxia [200]. Trx2 has been known to be important in preventing apoptosis, since Trx2 knock-out mice are embryonically lethal. Work from the Min lab shows that there is a decrease in Trx2 expression in human dilated cardiomypathy patients, and that mice with Trx2 deleted from their heart also develop this disease [201]. The loss of Trx2 increased oxidative stress, apoptosis, fibrosis, and contractile dysfunction, due to Trx2 not being around for decreasing ROS production or binding ASK1 to prevent apoptosis [201]. This encourages the notion that an increased expression of Trx2 helps maintain a stable and healthy environment in stressed hearts by decreasing ROS production and blocking mass apoptotic cell death [201]. Another study using BXM mice demonstrated that the transcriptional regulation and protein regulation of the same protein can vary in the UPRMT in opposite directions [202]. Thus, while there is considerable interest in UPRMT as a new and potentially underappreciated mechanism of proteostasis, it is too early to know its significance in normative cardiac aging.

11. Mitochondrial targeted therapies

Due to the critical importance of mitochondria in insuring adequate cellular energetics and function, there has been great interest in discovering mitochondrial targeted therapies for various diseases and conditions, including cardiac dysfunction and aging. Some of these therapies attempt to decrease the oxidative stress in the mitochondrial environment [203], while others focus on structural components of mitochondria [204–208]. It can be argued that CR and inhibition of mTOR signaling can do both, and thus, these two interventions, described above, and may also be considered to be mitochondrial therapies.

11.1. Mitochondrial antioxidants

The triphenylallylphosphonium ion (TPP+) has been conjugated to coenzyme Q (MitoQ) and plastoquinone (SKQ1) to deliver these redox-active compounds into the mitochondrial matrix, utilizing the negative potential gradient across the inner mitochondrial membrane. MitoQ has been shown to help maintain eNOS availability and reduce hypertension. MitoQ has been shown together with losartan, an angiotensin receptor blocker, that did not decrease ROS production in the mitochondria, but the combined therapy did lead to an improvement in cardiovascular function [207]. SKQ1 is another mitochondrial targeted antioxidant that has been reported to extend lifespan in male BALB/c mice and dwarf hamsters [209]. In the BALB/c mice there was also a reduction in age-related cardiac hypertrophy [209,210]. Pretreatment with MitoQ and SKQ1 have both been shown to have beneficial effects in animal models of ischemia-induced cardiac dysfunction [211,212]. The role that mitochondrial-targeted antioxidants might be able to play in protecting or repairing cardiac mitochondrial dysfunction in aging is thus a promising area of study. CoQ10, a mimic of a naturally occurring antioxidant of the electron transport chain has also showed promise improving mitochondrial function in the heart. Mouse studies involving ApoA1−/− mice demonstrate that addition of CoQ10 improves infarct size to that of a wild-type mouse [213]. Current human studies using CoQ10 in dietary supplements in adults have hint at improved health with an optimal diet [214,215], and clinical study in children with primary mitochondrial diseases is underway [214,215].

11.2. Cardiolipin-targeted therapies

Two CL targeted drugs have been studied, TPP-n-ISA and SS-31. TPP-n-ISA, studied primarily in brain injury and radiation, helped maintain CL in a structural arrangement that makes peroxidation more difficult [208]. The Szeto–Schiller (SS) compounds are tetrapeptides that preferentially concentrate in the mitochondrial inner membrane independent of the mitochondrial potential gradient. SS-31 (or as an acetate salt MTP-131, aka Bendavia™), the best studied of these, has been shown to reduce ROS levels and prevent ischemia–reperfusion injury in a variety of infarct models [206,216]. In our laboratory, we have found SS-31 to be protective of angiotensin II induced cardiac hypertrophy, as well as G alpha q-induced cardiac failure [217]. The protective effect of SS31 in the TAC model of heart failure was as great as that of mCAT, and conferred an even more complete protection of failure-related proteomic alterations than did mCAT [218]. It has recently been shown that SS-31 targets CL, altering the CL/cytochrome c interaction to optimize electron transfer, inhibit ROS generation and cytochrome c peroxidase activity. In a number of disease models SS-31 appears to help maintain mitochondrial cristae density, presumably by preserving the tetralinoleoyl isoform of CL which is vital to maintaining cristae curvature [204,205,219]. By stabilizing the CL–cytochrome c interaction, SS-31 may also prevent the pro-apoptotic activity of cytochrome c, although this has not been proven. In its clinical formulation, Bendavia, SS-31 may also prevent the pro-apoptotic activity of cytochrome c, although this has not been proven. In its clinical formulation, Bendavia, SS-31 is currently in multiple phase II studies, including a study to examine its impact to improve outcome in patients with acute myocardial infarction [220], as well as for treatment of patients with mitochondrial myopathy in primary mitochondrial disease, including Barth syndrome (NCT02367014).

The application of mitochondrial targeted therapies appears to be an exciting area of growth, as additional druggable targets to protect or improve the function of this important organelle are discovered.
11.3. Signaling pathway therapies

As previously mentioned above, the regulation of NAD is key to allowing the sirtuins pathways to maintain their function, which helps with healthspan and lifespan. With this idea, more NAD+/NADH therapies are being created. In a study of gas-1 mutant worms, nicotinic acid (NA) and resveratrol both improved survival of the worms [221], NA helped decrease the amount of ROS in the worms system and it fib.

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