Chapter 15
Mitochondria in Structural and Functional Cardiac Remodeling

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The heart is the organ responsible for pumping blood to the whole body, allowing thus the distribution of oxygen and nutrients to peripheral tissues and the elimination of toxic products through excretory organs. Cardiac tissue is composed of multiple cell types, including cardiomyocytes, fibroblasts, endothelial cells, vascular smooth muscle cells, macrophages and mast cells. Cardiomyocytes are the specialized cells responsible for myocardial contraction. Although they correspond to only 30–40% of the total number of cells in the heart, cardiomyocytes represent almost 75% of the heart volume [1]. Contractile function is manifest from the fetal stage forward, however, mitotic capacity of cardiomyocytes diminishes in the later stages of embryogenesis and stops almost completely after birth [2]. Therefore, the ability of the heart to respond to physiological or pathological conditions involves cellular remodeling processes rather than cellular proliferation. Cardiac remodeling is classically defined as changes in the structure of cardiac tissue, evidenced by changes in

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ventricle mass, volume or shape, fibrotic content, vascularization, cellular hypertrophy, and cell death. For instance, when the heart faces sustained work overload, compensatory mechanisms are activated in order to increase the capacity to pump blood. Initial mechanisms involve an increase in the frequency of contraction. When this is not sufficient, cardiac remodeling pathways are triggered. Cardiac hypertrophy can be caused by an increase in the size of individual cardiomyocytes [3] as well as by an increase in fibroblast proliferation (hyperplasia) [4]. It can be classified as adaptive or pathological, depending on the mechanisms activated. In adaptive hypertrophy muscular, vascular, and interstitial compartments maintain normal proportionality and collagen content, thus preserving tissue structure and function. This type of hypertrophy occurs in response to exercise training or during pregnancy, and changes to the heart can revert when the workload normalizes [5]. In contrast, during pathological hypertrophy, intercompartmental proportionality is ultimately lost, leading to structural heterogeneity of the myocardium. Importantly, relative collagen content of the heart increases, leading to myocardial fibrosis and detrimental structural remodeling [6].

Cardiac remodeling also occurs after a myocardial infarction (MI), where a large number of cardiac cells undergo cell death by necrosis, necroptosis, and apoptosis initiated by nutrient and oxygen deprivation. In an attempt to maintain cardiac output surviving cardiomyocytes increase in size, mass and volume. The left ventricle generally undergoes the most pronounced remodeling following MI, because it is the primary chamber responsible for pumping blood to the rest of the body [7]. Similar changes occur in the spared myocardium following damage from ischemia reperfusion (I/R).

Other stimuli leading to cardiac remodeling, and especially to hypertrophy, are elevated blood pressure and volume overload, both regulated primarily through the sympathetic and renin-angiotensin-aldosterone (RAA) systems. The sympathetic system is mediated by the action of norepinephrine and epinephrine on adrenergic receptors, regulating the rate and strength of cardiac contraction, as well as blood pressure in blood vessels [8]. Adrenergic receptors are G protein-coupled receptors that are desensitized upon sustained exposure to agonists, leading to attenuation of receptor responsiveness. Depressed receptor function is strongly associated with ventricular dysfunction, hypertrophy and heart failure [8].

Similarly, the function of the RAA system is to regulate blood pressure. When cardiac function becomes less efficient, secretion of renin by the kidney is increased, leading to an increase in renin- dependent conversion of angiotensinogen into angiotensin I (Ang I). In turn, angiotensin-converting enzyme (ACE) converts Ang I into angiotensin II (Ang II), which binds to angiotensin receptor (AT1) mediating changes in cells of both the vascular system and the heart, primarily involving vasoconstriction of vessels that increases blood pressure. Activation of the RAA is closely associated with the severity of heart failure, dilated cardiomyopathy and left ventricular (LV) hypertrophy [9].

Cardiac fibroblasts are responsible for secreting components of the extracellular matrix (ECM). During cardiac remodeling, fibroblasts can differentiate to myofibroblasts, that express contractile proteins including alpha-smooth muscle actin

(α-SMA), and exhibit increased migratory, proliferative and secretory properties [4]. Cardiac myofibroblasts are activated by proinflammatory cytokines (e.g. tumor necrosis factor alpha (TNFα), interleukin-1 (IL-1), interleukin-6 (IL-6), transforming growth factor beta (TGF-β)), vasoactive peptides (e.g. Ang II, endothelin-1, natriuretic peptides) and hormones (e.g. noradrenaline). This leads to increased cell proliferation and migration, enhanced secretion of the previously mentioned cytokines and vasoactive peptides, and secretion of additional growth factors such as VEGF. This can further increase fibrosis, and remodeling of the heart in a feed-forward mechanism. Myofibroblasts are also be activated directly by mechanical stretch and in response to I/R [4].

In addition to changes in organ structure, cardiac remodeling includes changes in structure or function on the subcellular level. Although many organelles in cardiomyocytes undergo remodeling, this chapter will focus on those changes directly related to mitochondria. In particular, we will discuss the ability of mitochondria to undergo dynamic changes that affect both their outer membrane structure (fusion/fission) and their inner membrane structure (cristae remodeling), and how these processes contribute to the development or progression of cardiac pathologies. In addition we will address the cardiovascular consequences of changes to mitochondrial function, including loss of mitochondrial respiration, increase in ROS production, diminished ATP synthesis and the release of pro-death factors into the cytosol.

In the human heart, ATP production is carried out primarily by mitochondria, and is estimated to be as much as 6 kg ATP per day [10]. Although, during the fetal stage, lactate and glucose are the major sources for energy production, the heart undergoes a metabolic remodeling at birth, switching substrate oxidation from glucose to fatty acids. Thus, β-oxidation of free fatty acids and oxidative phosphorylation become the primary mechanisms for ATP production, producing approximately 70% of the ATP consumed by the heart [11].

In addition to ATP production, mitochondria participate in processes involving cell proliferation, differentiation, cellular immunity, calcium regulation, iron homeostasis, lipid metabolism, cellular aging, cell death, production of reactive oxygen species (ROS) and ROS scavenging [12].

In neonatal cardiomyocytes, mitochondria are distributed throughout the cytoplasm and around the nucleus. In contrast, in the adult heart they are packaged and aligned [13], located in parallel, longitudinal rows interleaved with the contractile machinery (interfibrillary mitochondria, IFM) or in monolayers immediately underneath sarcolemma (subsarcolemmal mitochondria, SSM) [14]. These differentially distributed mitochondria, also differ in the morphology of their cristae, being mostly tubular in the IFM and mainly lamelliform in SSM [15]. The contrasting morphologies of the two populations imply metabolic differences, as IFM display a higher rate of oxidative phosphorylation and enzymatic activity than SSM [16]. SSM also exhibit a lower capacity for calcium uptake than IFM [17], potentially leading to increased susceptibility to cytochrome c release and initiation of death signals in this population. Thus these two functionally distinct pools of mitochondria may play different roles in the pathologies leading to cardiac remodeling.

Mitochondria have their own genome (mtDNA), which in mammals is a densely packed double- stranded DNA molecule of 16.6 kb, containing 37 genes encoding 11 messenger RNAs (mRNAs; translated to 13 proteins), 2 ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs) [18]. The proteins encoded in the mitochondrial genome are core constituents of the mitochondrial respiratory complexes I, III, IV and V that are embedded in the inner mitochondrial membrane (IMM) [19]. Similarly, the tRNAs and rRNAs encoded in mtDNA are essential for mitochondrial ribosome assembly the translation of mitochondrial mRNAs [20].

Although mitochondria are the primary organelles responsible for energy production, the nuclear genome is the primary site for encoding the bulk of mitochondrial proteins. Therefore, the two organelles need to communicate closely. This allows nuclear control of mitochondrial ATP production [21] and conversely, changes in mtDNA or mitochondrial function are capable of modifying nuclear gene expression.

Mitochondria are highly dynamic organelles, continuously undergoing repeated cycles of fusion and fission in response to environmental changes and to the metabolic status of the cell (Fig. 15.1). These processes are strictly controlled by the activity of a group of guanosine triphosphatases (GTPases) related to the dynamin family [13]. Mitochondrial fission creates smaller and more circular mitochondria, through the activity of Dynamin-1-like protein (DRP1) and its adapter proteins mitochondrial fission 1 protein (FIS1) and mitochondrial fission factor (MFF) [22]. This process is required for redistribution of mitochondria in mitosis, release of cytochrome c during cell death by apoptosis and for selective mitochondrial degradation (mitophagy) [22]. On the other hand, mitochondrial fusion generates fewer, larger and more elongated mitochondria, in a process regulated by mitofusin (MFN) 1 and 2, both situated in the outer mitochondrial membrane (OMM), and by optical atrophy protein 1 (OPA1), located in the IMM. This process allows the exchange of material (matrix components, damaged mtDNA) and promotes balance in bioenergetics properties (e.g. mitochondrial membrane potential) [11].

Increased oxidative stress, altered calcium load or cellular pH are each capable of impacting mitochondrial dynamics, disrupting mitochondrial membrane potential, and inducing opening of the mitochondrial permeability transition pore (MPTP). This latter process has been widely associated with remodeling of the mitochondrial cristae, especially during I/R [23].

Existing therapies to prevent the progression of cardiac remodeling primarily act at the cardiac or vascular level. These include drugs for reducing hypertension, such as inhibitors of ACE (enalapril), angiotensin receptor blockers (losartan) and beta-blockers (atenolol); and drugs that reduce cholesterol levels like statins and fibrates [4]. However, no drugs currently in use are targeted directly at the causes of subcellular remodeling of mitochondria, warranting further research aimed at understanding the possible relation between the prevention of functional or metabolic remodeling and the prevention or amelioration of cardiac diseases.

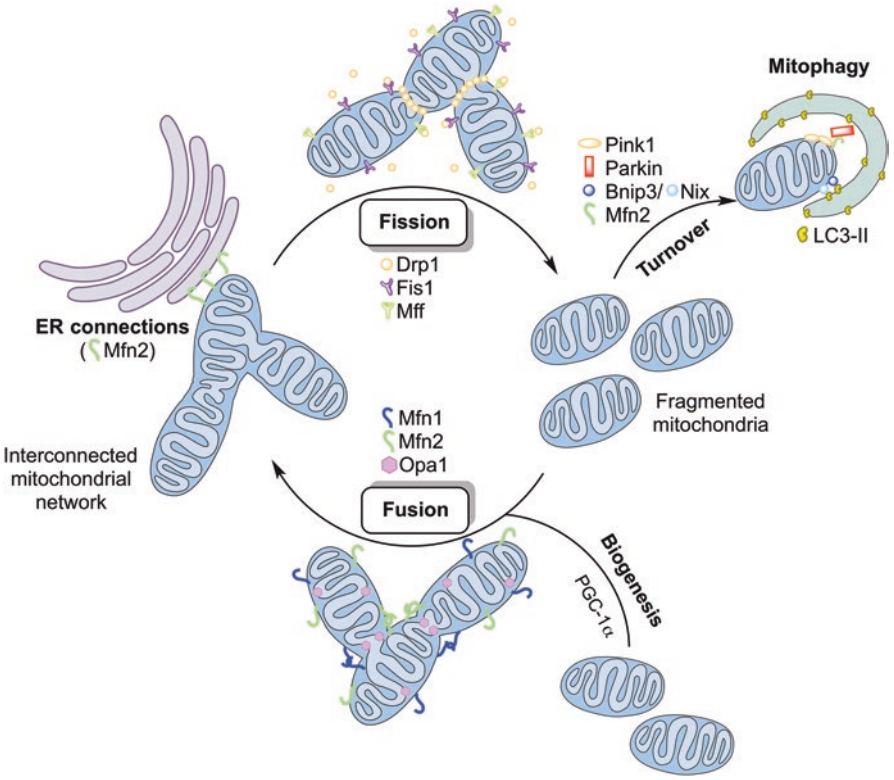

Fig. 15.1 Mitochondrial life cycle. Mitochondrial dynamics enable mitochondria to respond to external stimuli or metabolic signals in a way that maintains proper function. Mitochondrial fusion is regulated by mitofusin (*Mfn*) 1 and 2 and optical atrophy protein 1 (*Opa1*), at the outer and inner mitochondrial membranes, respectively. This process results in a more interconnected mitochondrial network that enhances communication with the endoplasmic reticulum (*ER*) through mitochondria-associated endoplasmic reticulum membranes (*MAM*) and a complex mechanism involving the presence of Mfn2 amongst other proteins, thus enhancing calcium movement from ER to mitochondria and regulating its function. Conversely, Drp1 and its adapter proteins Fis1 and Mff control mitochondrial fission. This process is required for selective mitochondrial turnover (mitophagy), which allows isolation of damaged mitochondria to ensure proper mitochondrial quality control. Here, the mitochondrial kinase Pink1 phosphorylates Mfn2 which allows Parkin E3 ligase bind phosphorylated Mfn2 to catalyze the ubiquitination of several proteins on the OMM that promotes interaction with LC3 at nascent phagophores, initiating mitochondrial autophagy. Nix and Bnip3 are mitophagy regulators that sense myocardial damage and facilitate the elimination of damaged cardiac mitochondria. Peroxisome proliferator activated receptor gamma coactivator 1α (PGC-1α) is a critical factor that coordinates mitochondrial biogenesis and the expression of metabolic genes and therefore critical to the synthesis aspect of mitochondrial dynamics

Mitochondrial Dynamics

The term mitochondrial dynamics comprises at least three processes. One is the capability of mitochondria to undergo fission and fusion processes, changing its network between punctate and fragmented, and elongated and interconnected mitochondria, respectively [6]. Secondly remodeling of the mitochondrial matrix, which influences metabolite diffusion, and thus, mitochondrial metabolism [24]. And finally, mitochondrial motility within the cell, which is mediated by the interplay of mitochondrial fission and fusion proteins with kinesin 1 and 3 motors and the adaptors Milton and Miro (Miro/Milton/kinesin complex), thus allowing interaction of mitochondria with cytoskeletal components to facilitate their movement along microtubules [25].

In cardiac cells, the most prominent events related to mitochondrial dynamics are fission and fusion and remodeling of the cristae. These dynamic processes occur in response to external stimuli and metabolic signals. Changes in mitochondrial morphology have been implicated in cell division, embryonic development, apoptosis, autophagy, metabolism, development, and differentiation [26]. In the heart, disruption of these mechanisms has been identified in multiple cardiac diseases, including cardiac hypertrophy, heart failure, dilated cardiomyopathy, and ischemic heart disease. In addition, modification of these processes has been shown to protect the heart against injury in specific settings [27].

Mitofusins 1 and 2

Mitofusins are present in all tissues, but in the heart the levels of mRNA encoding these proteins are particularly high, with MFN2 in greater abundance than MFN1 [28]. Both are members of the family of transmembrane GTPase and reside in the OMM. They mediate OMM fusion through GTP hydrolysis. MFN1 has a higher GTPase activity than MFN2, making it more efficient in the fusion process [29]. The C- terminal end of the proteins is oriented towards the cytosol and contains both a coiled-coil domain 2 (also called hepta-repeat domain or HR2) and a transmembrane domain. The N-terminal end contains the GTP binding domain and another coiled-coil domain (HR1) [30]. To facilitate fusion of the OMM, the HR2 domains of MFN1 or MFN2 form dimeric complexes with MFN1 or MFN2 on adjacent mitochondrion, allowing tethering of the two mitochondria.

OPA1

OPA1 is expressed throughout the body, but is present in greater quantity in the retina, brain, testis, heart and skeletal muscle [31]. This protein is a large GTPase located in the IMM, whose function is to promote mitochondrial fusion of the IMM and is also involved in remodeling of mitochondrial cristae [32]. The protein is

comprised of a N-terminal domain containing a mitochondrial import sequence (MIS, which is removed by a mitochondrial matrix protease when the protein is imported to the mitochondria), a transmembrane domain (TM), a region of alternative splicing, a coiled-coil region, a GTPase domain, a middle domain, and a GTPase effector domain (GED or assembly domain) at the C-terminus [26]. In addition to the GTPase domain, OPA1 requires the presence of MFN1 to induce mitochondrial fusion [33]. OPA1 undergoes post-translational modifications by alternative splicing at exons 4, 4b, and 5b, generating eight human isoforms of OPA1. Four of these isoforms (3, 5, 6, and 8) can be cleaved by YME1L, an intermembrane AAA (ATPase associated with diverse cellular activities) protease, generating shorter forms of OPA1 (S-OPA1), which are soluble and reside in the mitochondrial intermembrane space. The other isoforms of OPA1 (1, 2, 4, and 7) do not normally undergo cleavage and constitute the long forms of OPA1 (L-OPA1), which contain the TM domain, and are anchored into the IMM. Both, long and short isoforms are required for mitochondrial fusion [26]. Under conditions of mitochondrial membrane depolarization, ATP deficiency and apoptosis induction, the L-OPA1 isoforms undergo inducible cleavage by OMA1 generating short forms of OPA1. This prevents the pro-fusion activity of OPA1, resulting in mitochondrial fragmentation. Through this process, depolarized fragmented mitochondria can be removed by mitophagy [34]. In addition, short and long forms of OPA1 form oligomers, keeping cristae junctions tightly closed, thereby opposing cytosolic release of cytochrome c that can initiate apoptosis [35].

DRP1

DRP1 is abundant in brain, heart and muscle [36]. It is located in the cytosol and translocates to the OMM to induce fission or division of mitochondria [26]. DRP1 contains a GTPase domain, a central domain and a GTPase effector domain or assembly domain [36], which regulates the GTPase activity of the protein and directs it to the mitochondria [37]. To initiate fission, DRP1 translocates to the OMM where it binds to the receptor, FIS1 or MFF, undergoes oligomerization, and then begins constriction of the mitochondrial scission site using GTP to power the process [37]. DRP1 is regulated by a diversity of post- translational modifications such as phosphorylation and nitrosylation [38]. Particularly important in cardiac remodeling, dephosphorylation of DRP1 by calcineurin initiates fission, whereas phosphorylation of the same site by PKA opposes it.

FIS1

FIS1 is uniformly distributed in the OMM and is comprised of an N-terminal domain, required for protein–protein interactions, FIS1 oligomerization, and mitochondrial fission; a transmembrane domain and a C-terminal domain exposed to the intermembrane space, that are essential for mitochondrial localization and the

induction of apoptosis [39]. It has been shown that, although FIS1 is required for mitochondrial fission, it is not a limiting factor in the process and mitochondrial recruitment of DRP1 can be regulated by other factors [45].

MFF

Mitochondrial fission factor (MFF) mediates both mitochondrial and peroxisome fission and is highly expressed in heart, kidney, liver, brain, muscle, and stomach [40]. It has a C-terminal transmembrane domain essential for its localization to mitochondria, anchoring to the OMM, and the ability to multimerize, exhibiting similar properties to FIS1 [40]. MFF (and specifically its N-terminal cytosolic region) is required for DRP1 recruitment to mitochondria and together MFF and DRP1 are capable of mediating mitochondrial fission independent of FIS1 [41]. This indicates that FIS1 and MFF are independent of one another and may activate fission in response to different stressors. Interestingly, both proteins also mediate fission of peroxisomes [40].

Mitochondrial Elongation Factors

The mitochondrial elongation factor proteins, MIEF1 and MEIF2 are also known as mitochondrial dynamics proteins MiD49 and MiD51 respectively [49]. The levels of MIEF1 mRNA are high in adult human heart, skeletal muscle, pancreas and kidney [42]. The activity of these proteins has not been fully characterized, however, studies indicate that although overexpression of MiD49/MiD51 recruits DRP1 to mitochondria, it actually reduces DRP1's GTP-binding activity, thereby promoting mitochondrial fusion and increasing mitochondrial elongation. Furthermore, knockdown of MiD49/MiD51 increases fragmentation [42] and suggests that these proteins may prevent DRP1's interaction with FIS1, and thus, its fission-related function. In contrast, studies from other investigators [43] indicate that, rather than blocking fission, MiDs might recruit DRP1 to mitochondria and maintain it in an inactive state until a cellular signal triggers fission. Palmer et al. also demonstrated that siRNA knockdown of both MiD49 and MiD51 reduced translocation of DRP1 to mitochondria and resulted in mitochondrial fusion and elongation [44]. Yet, overexpression of MiD49/51 blocked fission by sequestering DRP1 specifically at mitochondria, and promoted MFN1 and MFN2-dependent mitochondrial fusion [45]. Thus, the exact function and effect of these proteins remains to be elucidated.

Pathological Mitochondrial Remodeling

As will be expanded upon later in the chapter, pathological remodeling of mitochondria can be particularly onerous in the heart as it can lead to depletion of ATP and increases in intracellular calcium and ROS, thereby triggering mechanisms

such as mitophagy or apoptosis, outcomes often observed in the failing heart or following cardiac I/R. In this sense, proteins involved in mitochondrial fission and fusion are particularly important, as they can be critical for maintaining or restoring mitochondrial integrity and function. Cellular imbalances such as oxidative stress, damage to mtDNA or loss of mitochondrial membrane potential, among others, affect the integrity of mitochondria, leading to energy insufficiency, and thus, an inability to support cell function that may eventually lead to cell death (Fig. 15.2).

Remodeling of mitochondrial cristae is a necessary prerequisite for apoptosis. Loss of cristae structure allows redistribution of cytochrome c in the intermembrane space and its release into the cytoplasm through pores in the OMM generated by Bax and Bak, initiating activation of caspase cascades leading to cell death [46]. In the IMM, OPA1 oligomerizes and stabilizes the morphology of the cristae, preventing remodeling of such structures and thus opposing the release of cytochrome c and subsequent apoptosis [35]. For cytochrome c release to occur, L-OPA1 isoforms are processed to S-OPA1, allowing the disassembly of oligomers [47]. Therefore, OPA1 acts as an anti-apoptotic protein, preventing remodeling, whereas the cleaved forms are pro-apoptotic.

The combined actions of DRP1 and MFF are also important in this process. Otera and colleagues demonstrated that knockdown of DRP1 and MFF compromised mitochondrial fission and apoptosis induced by exogenous stimuli. In turn, MFF overexpression induced extensive mitochondrial fragmentation concomitant with an increased sensitivity to apoptosis, suggesting that mitochondrial fission facilitates apoptotic cristae remodeling [41]. hFIS1 likewise displays dual functionality, participating in mitochondrial fission as a component of normal mitochondrial dynamics, as well as being capable of trigger caspase-dependent cell death causing the release of cytochrome c from mitochondria, in the setting of calcium release from the endoplasmic reticulum (ER) and opening the MPTP [44].

MFN2 is another key player that carries out multiple functions. In addition to its role in mitochondrial fusion, it also modulates mitochondrial apoptotic pathways. Recent studies have shown that MFN2 is also present on ER where it assists in the tethering of ER to mitochondria. This contact between ER and mitochondria, enables calcium signaling, inositol trisphosphate (IP3) signaling, and calcium-dependent induction of apoptotic signaling [48]. MFN2 protein levels and apoptotic cell death increased in rat cardiomyocytes exposed to hydrogen peroxide. Whereas, siRNA depletion of MFN2 suppressed apoptosis [49]. Activation of apoptosis by oxidative stress is likewise reduced in the hearts of mice with a genetic disruption of MFN2 [50]. Therefore, MFN2 appears to play an active role in cardiomyocyte death mediated by oxidative stress [49]. In contrast, in other contexts, MFN2 may exert an anti-apoptotic effect. When cardiomyocytes are treated with ceramide, DRP1/FIS1-dependent mitochondrial fragmentation and apoptosis occurs [51]. When MFN2 is depleted, ceramide's effects are exacerbated (mitochondrial fragmentation, DRP1 co-localization with FIS1, mitochondrial membrane depolarization, cytochrome c release, and cell death), suggesting that MFN2 may exert an anti-apoptotic effect against ceramide [51]. Therefore, the role of MFN2 in the apoptotic process appears to be mediated by the nature of the apoptotic stimulus.

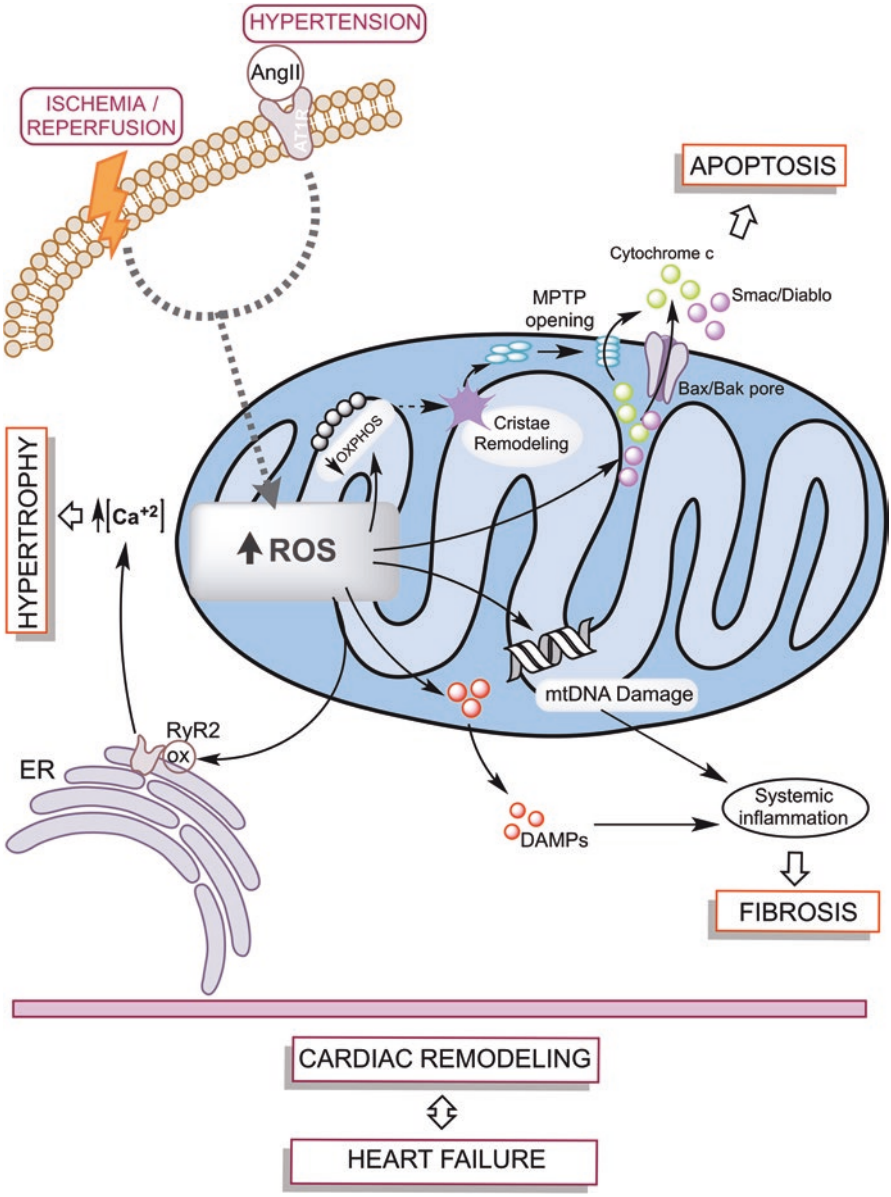

Fig. 15.2 Mitochondrial ROS in cardiac remodeling. Cardiac remodeling is induced by several mitochondrial ROS-related mechanisms. Cardiac pathological conditions like hypertension and ischemia/reperfusion (I/R) both induce an increase in mitochondrial ROS production. This leads to cristae remodeling, which in turn triggers the opening of the mitochondrial permeability transition pore (*MPTP*) and the assembly of the Bax/Bak pore. Both pores allow the release of pro-apoptotic proteins (like cytochrome c and Smac/Diablo), inducing the activation of the intrinsic cell death pathways in cardiac cells. Mitochondrial ROS can also cause the release of intra-mitochondrial damage-associated molecular patterns (*DAMPs*) and damage mitochondrial DNA, leading to systemic inflammation and triggering cardiac fibrosis. ROS generated by mitochondria can increase cytosolic calcium levels through oxidative damage of the ryanodine receptor (*RyR2*), leading to the activation of pro- hypertrophic pathways. Together, the cumulative effects of mitochondrial ROS triggers cardiac remodeling that ultimately leads to chronic heart failure

MARCH5, an E3 ubiquitin ligase associated with the OMM can act as a negative regulator of mitochondrial fission by mediating proteasomal degradation of MiD49 [52]. When cells were subjected to pro-apoptotic stimuli or to stimuli that affects mitochondrial activity, protein levels of MiD49 were decreased in a MARCH5-dependent manner. This data suggests that MARCH5, by increasing MiD49 degradation, inhibits fission and may protect cell from apoptosis induced by stress.

Proteins involved in mitochondrial dynamics are also affected during I/R, a prominent initiator of myocardial remodeling. In this context, Pei and colleagues [53] reported a reduction in MFN2 after I/R. The authors also observed a significant decrease in ATP production and an increase in ROS generation in MFN2 deficient mice subjected to I/R compared to the response of wild type mice. Subsequent suppression of cardiac function and the extent of myocardial fibrosis were likewise greater in the MFN2-deficient mice. They also showed that melatonin can increase MFN2 expression in ischemic myocardium by activating Notch1, thereby preserving mitochondrial integrity, increasing ATP production, reducing ROS production and preventing cardiomyocyte apoptosis after I/R, leading to an improved cardiac function and reduced fibrosis in myocardium, and attenuating cardiac remodeling.

Changes to mitochondrial form and function are a prominent feature of cardiac remodeling is observed in heart failure. Chen et al. [54] documented decreases in OPA1 protein levels in failing hearts, that occurred in conjunction with an increase in smaller and fragmented mitochondria. Mitochondrial fragmentation and reduction in OPA1 protein levels likewise occurs in vitro in H9c2 myoblasts subjected to simulated ischemia [34]. In addition, reduction of OPA1 levels with shRNA increased mitochondrial fragmentation, cytochrome c release, and apoptosis in response to simulated ischemia [54]. All these findings suggest that OPA1 helps limit fission of mitochondria during ischemia, and reduction of OPA1 levels following ischemia may contribute to heart failure progression.

DRP1 plays a central role promoting mitochondrial fragmentation and death during simulated ischemia, which can be prevented by expressing a dominant negative mutant of DRP1 [55, 56]. Treatment with a pharmacological inhibitor of DRP1, mdivi-1, increases the proportion of elongated mitochondria in adult cardiomyocytes, protecting them against simulated I/R in vitro and decreasing the myocardial infarct size in vivo [41]. This suggests that inhibition of DRP1 may be a good therapeutic target to mitigate the damage caused by I/R injury, thus preventing the subsequent pathological remodeling of the heart. Consistent with this, the prohyperthrophic-activation of calcineurin has been shown to recruit DRP1 to mitochondria, thereby stimulating mitochondrial fission, and the depletion of MFN2 to stimulate cardiac hypertrophy [57]. Either calcineruin inhibition or expression of a dominant-negative of Drp1 (K38A) was sufficient to prevent norepinephrine-induced hypertrophy, demonstrating that fission is a requisite step for calcineurin-mediated hypertrophic remodeling [57].

Disatnik and colleagues [58] demonstrated that treatment with p110 (a peptide that selectively inhibits the FIS1/DRP1 interaction, thereby preventing fission) restores mitochondrial functions in different models of cardiac I/R injury, including isolated primary cardiomyocytes, ex vivo heart, and in vivo myocardial infarction

models. The authors showed that inhibition of fission during reperfusion preserves cardiac tissue integrity and cardiac function. This highlights the importance of mitochondrial dynamics in the progression of heart disease and supports the notion that intervening in such processes can help stop its progression.

Mitochondrial ROS, Cell Death in Cardiac Remodeling

Mitochondria are broadly recognized as one of the main sources of oxidative stress and ROS production [59]. This is particularly true in cardiomyocytes, in which several studies have shown that approximately 2% of the oxygen reduced during the normal functioning of the electron transport chain is lost as superoxide anion (O2·-) due to leak of the electron transport chain, mainly at the level of complexes I and III [60]. Thus, it is not surprising that mitochondria are involved in many oxidative stress-related cardiac pathologies, including cardiac hypertrophy [61], fibrosis [62], diabetic [63] or dilated [64] cardiomyopathies, I/R damage [53], and heart failure [65]. ROS can impact many signaling pathways. It is therefore capable of triggering a diversity of signaling cascades relevant to cardiomyocyte hypertrophy [66]. Moreover, ROS generation plays an important role in both Ang II and α-adrenergic-receptor induced hypertrophy [67] as well as in the pro-hypertrophic activation of NFκB [68]. ROS can likewise stimulate proliferation of cardiac fibroblast [69] as well as enhancing expression of matrix metalloproteinases (MMPs) [70] and their activation [71]. Based on the pro-hypertrophic action of ROS, antioxidant compounds such as omega-3 fatty acids have been proposed as protective against pressure overload-induced myocardial hypertrophy [72].

I/R damage to the heart is broadly linked to mitochondrial ROS production [53]. During ischemic events, mitochondrial respiration is lowered to near-zero, and energy production through oxidative phosphorylation is almost stopped. Later, when reperfusion is achieved and oxygen flow through the electron transport chain restored abruptly, the initial burst in ROS production is thought to overwhelm cellular antioxidant mechanisms [73]. This increase in mitochondrial generation ROS is thought to underlie most of the cellular damage associated with I/R. The antioxidant compounds picroside II [74], lycopene [75], all-trans retinoic acid [76] and vitamin C [77] have been shown to diminish ROS production during I/R in cardiomyocytes, thereby reducing mitochondrial damage and cellular death. These studies support the contention that increased ROS production activate cellular death pathways to effect cardiac damage during I/R.

Over the course of time, each of the above-discussed cardiac pathologies can lead to heart failure [78]. Thus, heart failure is considered and end-phase condition, triggered by accumulative damage in both the structure and function of the heart [79]. Given the cumulative, progressive nature of the disease, it is perhaps not surprising that ROS is capable of damaging a range of macromolecules relevant to cardiac excitation-contraction coupling and mitochondrial energy production rather than targeting a single substrate [65]. Additional support to idea that ROS plays an

fundamental role in the development of heart failure comes from the evidence of changes in uncoupling proteins (UCPs) [65]. For instance, mRNA and protein levels of UCP2 and UCP3 are reported to decrease in the heart during the early stages of heart failure in both animal and human models [80, 81]. UCPs are endogenous proteins capable of dissipating the mitochondrial membrane potential and increasing efficient electron flow through the electron transport chain by transporting of protons across the IMM [82]. UCPs increase oxygen consumption but diminish ROS production, by reducing electron leak responsible for O2·– production [83]. Thus, reduced UCP expression may represent an attempt to preserve oxygen and ATP in the early stages of heart failure that could come at the cost of increasing the capacity for generating ROS. In the later stages of heart failure, however, UCP2 and UCP3 protein levels are reported to increase [84, 85] following the activation of PPARα by free fatty acids in plasma. It is unclear whether this late stage increase in UCP ultimately plays a protective or detrimental role in chronic heart failure [65].

Mitochondrial induction of apoptosis has been broadly associated with cardiac remodeling. Indeed, the frequency of apoptotic cardiomyocytes has been shown to be 10–100-fold higher after myocardial infarction than those observed in control hearts [86]. Mitochondria contain several intrinsic apoptosis- inducing factors, such as cytochrome c, the SMAC/Diablo protein and some of the calpain proteases [87]. Upon internal damage signals, such as DNA fragmentation, accumulation of intracellular toxic compounds, or uncontrolled oxidative stress, mitochondrial inner membrane is permeabilized, thereby releasing these pro-apoptotic factors into the cytosol and triggering the activation of the intrinsic cell death pathway [88]. Oxidative stress in particular, when not controlled by the antioxidant systems, is able to rapidly destabilize the structure of the IMM [89]. Therefore, it is likely that many of the cardiac pathologies linked to increased ROS production involve mitochondrial activation of cell death signals.

One of the main avenues by which mitochondrial pro-apoptotic molecules are released into the cytosol is via a pore formed by the pro-apoptotic Bcl-2 family proteins [90]. Upon apoptosis-inducing stimuli, Bcl-2 proteins (such as Bak and Bax) oligomerize on the IMM, opening a pore that allows the release of pro- apoptotic factors. Another important channel in mitochondria-activated apoptosis is the MPTP [90]. Such channel is thought to be formed by several mitochondrial membrane proteins and is activated upon IMM damage, thereby contributing to cell death [90]. The importance of MPTP opening in cardiac damage is supported by several articles reporting on the potential of MPTP inhibition to diminish apoptotic death, offering cardioprotection in diverse cardiac pathological states [91–93].

Mitophagy in Cardiac Remodeling

Autophagy is a catabolic recycling pathway triggered by various intra or extracellular stimuli that is conserved from yeast to mammals. During autophagy diverse cytosolic constituents are enveloped by double-membrane vesicles named

autophagosomes, which later fuse with lysosomes in order to degrade their cargo. Dysregulation in autophagy is associated with a diverse range of pathologies including cardiovascular diseases [94]. Mitochondrial autophagy or mitophagy, which literally means "eating mitochondria," is the term applied to the cellular mechanism for identifying and selectively eliminating dysfunctional mitochondria as part of the overall mitochondrial quality control process. Increasing lines of evidence indicate that autophagy is intimately involved in the survival and death of cardiomyocytes under stress, however it is likely also involved in maintaining normal cardiac function and metabolism (Fig. 15.3). The beneficial effects of autophagy are partly mediated by the elimination of damaged mitochondria and the consequent prevention of mitochondrial dysfunction, oxidative stress increase, and cell death [95].

The most well studied mechanism of mitophagy in cardiomyocytes is the one mediated by the cytosolic E3 ubiquitin ligase Parkin [96] and the mitochondrial membrane kinase PTEN-induced putative kinase-1 (Pink1) [97]. Pink1-Parkin-mediated autophagy participates in mitochondrial quality control and in the maintenance of cardiac function in the heart at baseline. Cardiomyocytes in which Bnip3 is overexpressed are able to eliminate damaged mitochondria through autophagy via a Parkin-dependent mechanism, indicating that Parkin-mediated mitochondrial autophagy exists in cardiomyocytes [98]. The lack of Parkin-mediated mitophagy in heart-specific MFN2 KO mice is compensated by activation of non-selective autophagy in cardiomyocytes [95], thereby allowing cardiomyocytes to maintain mitochondrial quality.

PINK1−/− mice develop LV dysfunction and pathological cardiac hypertrophy [99], which is mediated by increased oxidative stress and dysfunctional mitochondria in cardiomyocytes. In the same line, PINK1 protein levels are significantly decreased in heart failure [99]. Consistently, mouse hearts lacking MFN2 develop cardiac dysfunction with age [100], suggesting that mitophagy regulated by the PINK1-MFN2 pathway plays an important role in maintaining cardiac function at baseline.

Translocation of Parkin to mitochondria and ubiquitination of mitochondrial proteins occurs during the acute phase of I/R, suggesting that Parkin-mediated mitophagy is stimulated [101]. Parkin-deficient mice exhibit a decrease in mitochondrial autophagy, accumulation of dysfunctional mitochondria and a reduced survival rate after I/R [101]. Taken together, these results suggest that mitophagy is stimulated in the heart during the chronic phase of cardiac remodeling and that it would be protective for the heart.

In cardiomyocytes, mitochondrial dynamics play an important role in mitophagy quality control through the process of DRP1-mediated asymmetric fission [102]. Accordingly, mitochondria in the early stages of senescence or those that have sustained moderate damage, will segregate dysfunctional components into one of the two daughter organelles generated by a fission event. The damaged daughter mitochondrion will be promptly identified as such and removed via Pink1-Parkin mediated mitophagy, whereas the healthy daughter will re-join the cellular mitochondrial pool, likely by fusing with other similarly fit mitochondria [103].

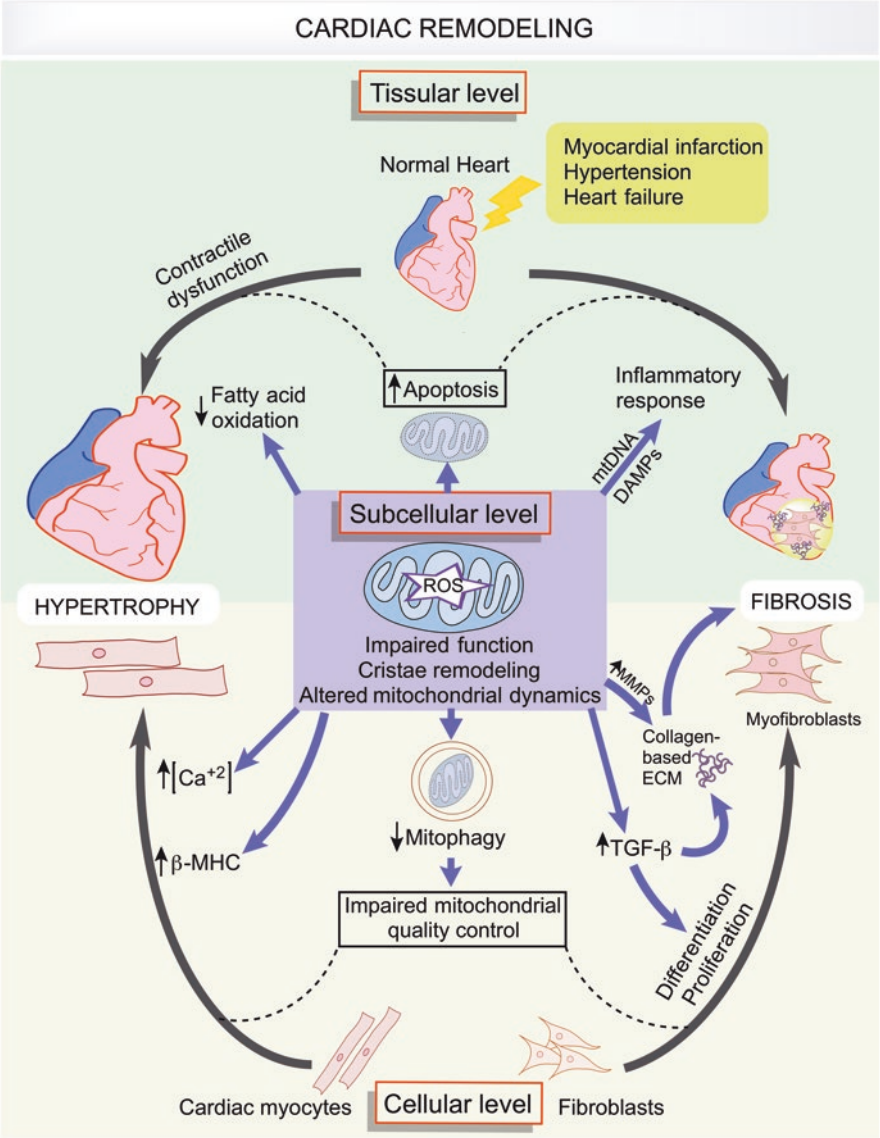

Fig. 15.3 Cardiac remodeling. Cardiac remodeling may occur at tissue, cellular or subcellular levels. At the tissue level, pathological conditions such as myocardial infarction, hypertension or heart failure lead to contractile myocardial dysfunction, cardiac hypertrophy and cardiac fibrosis. At the subcellular level, impaired mitochondrial function, cristae remodeling, altered mitochondrial dynamics and decreased mitophagy, together with increased ROS production, trigger a variety of downstream effects, including increased β-MHC expression, elevated cytosolic calcium, and decreased fatty acid oxidation. Mitochondrial function also impacts collagen-based extracellular matrix homeostasis by increasing the activity and synthesis of matrix metalloproteinases (MMPs) and the levels of TGF-β. Increased TGF-β production leads to differentiation and proliferation of cardiac fibroblasts into myofibroblasts, thus increasing cardiac fibrosis. Similarly, increased mitochondrial ROS production damages mtDNA and leads to the release of DAMPs, triggering an inflammatory response that further enhances cardiac fibrotic remodeling. Finally, damage to mitochondrial function can leads to the activation of cell death pathways, causing further contractile dysfunction, hypertrophy and cardiac fibrosis

The protective effect of mitophagy in the heart generally appears most prominently during stress. During ischemia, autophagy is triggered as an adaptive mechanism providing nutrients and eliminating damaged mitochondria, which could otherwise release damaging ROS and initiate apoptosis [104]. In fact, pharmacological inhibition of autophagy increases cardiomyocyte death by I/R, indicating that autophagy functions as a pro-survival mechanism in such conditions [105]. After reperfusion, triggered cardiac autophagy can be either adaptive or detrimental [94], and the impact of these processes on cardiac remodeling remains to be fully understood. Moreover, in response to myocardial infarction, activation of autophagy in the border zone is increased at an early stage, returning to normal during the chronic phase [106]. The autophagic vacuoles in the border zone are over-sized and contain organelles, including degraded mitochondria, in contrast with normalized autophagosomes which are upregulated in the remote zone during the chronic phase [106]. Pharmacological enhancing of autophagy with rapamycin 2 weeks after coronary ligation, ameliorates cardiac dysfunction and maladaptive remodeling, whereas inhibition of this process with bafilomycin A1 worsens them [106].

In mice, myocardial ischemia induced by permanent coronary artery ligation upregulates p53 and TP53-induced glycolysis and apoptosis regulator (TIGAR). Genetic deletion of p53 or TIGAR in mice stimulates mitophagy and inhibits accumulation of damaged mitochondria and apoptosis, an effect abolished by chloroquine. The authors suggest that the beneficial effects of the p53 or TIGAR downregulation may be mediated by mitophagy [107].

During myocardial reperfusion after a period of myocardial ischemia, mitochondria generate ROS, initiating a feedforward mechanism of oxidative stress, mitochondrial injury and cell death [108]. Selective elimination of damaged mitochondria by mitophagy is predicted to protect cardiomyocytes during reperfusion.

Despite the discussed reports, further investigations are needed in order to introduce modification of autophagy and mitophagy as useful therapeutic targets in the treatment of cardiovascular diseases, and to better understand where and when autophagy is activated or inhibited and how it affects the function of mitochondria in response to a wide variety of cardiovascular stress conditions that leads to heart remodeling.

Mitochondria in Cardiac Pathology

As previously discussed in this chapter, the main causes of cardiac remodeling that lead to heart failure are hypertension and I/R. At a cellular level, the processes that contribute to cardiac remodeling include cardiomyocyte hypertrophy, cell death and increased interstitial fibrosis. In this section, we will discuss the role of mitochondria in this processes and its potential as a therapeutic target.

Mitochondria and Cardiac Hypertrophy

It is generally accepted that mitochondrial dysfunction develops in the failing heart, and in many of the studies the model used was one of pathological cardiac hypertrophy. Cardiac hypertrophy can be divided into pathological or physiological hypertrophy. Thus, if after an initial phase of compensation, the growth response leads to contractile dysfunction, ventricular dilation, and heart failure, hypertrophy is considered pathological [45]. Pathological cardiac hypertrophy is a chronic complex disease that occurs in response to hemodynamic overload and is accompanied by oxidative stress and mitochondrial dysfunction [109]. In most cases of pathological hypertrophy, mitochondrial changes in structure and function have been described. In contrast, physiological cardiac hypertrophy is associated with enhanced mitochondrial function [45].

Myocardial oxidative stress has been implicated in the transition from compensated cardiac hypertrophy to heart failure, and evidence exists to support a role for mitochondrial and non-mitochondrial sources of ROS [45]. Oxidative stress can further impair mitochondrial function by leading to oxidative modifications of mitochondrial proteins, mutations of mtDNA and activation of the MPTP [77].

Pathological cardiac hypertrophy is associated with activation of many signaling pathways, exemplified by the calcineurin/nuclear factor of activated T-cell pathway, histone deacetylases, phosphatidyl inositol 3 kinase (PI3K)/Akt/Forkhead box protein O1 (FoxO1)/ mammalian target of rapamycin (mTOR) signaling networks, the ERK signaling pathway, G-protein-coupled receptor signaling pathways, among others [45, 110]. Change in calcium homeostasis is a critical factor in the deterioration of cardiac hypertrophy. Calcium affects the integrity of cardiomyocyte function in two ways: one is through calcium/calcineurin signaling pathway that directly activates/inactivates calcium/calcineurin dependent kinase [111, 112]. Another is through calcium channels that control the calcium flow in and out of organelles like mitochondria and ER [110]. Under normal conditions, mitochondria play a key role in cytosolic calcium clearance [113].

In vivo studies using pigs with hypertrophic cardiomyopathy revealed swollen cardiac mitochondria with disrupted cristae and substantial mtDNA depletion. Complex I and complex IV activity were also reduced in this model of hypertrophy [54].

Modulation of mitochondrial energy supply may provide means for therapy against cardiac hypertrophy. In cardiomyocytes, mitochondria produce ATP mainly through fatty acid oxidation (FAO) and oxidative phosphorylation. Many groups have described reduced myocardial FAO in pressure overload cardiac hypertrophy and heart failure. However, during the early stages of hypertrophy, which precede heart failure, changes in, or potential mechanisms for reduced FAO are less clear [45].

Currently, there are no effective methods to treat cardiac hypertrophy. It is reasonable to conclude that compensated cardiac hypertrophy is associated with relatively preserved mitochondrial function and that the development of mitochondrial dysfunction occurs in parallel with the development of heart failure. It remains to be

definitively proved if adapting mitochondria to the hypertrophic state and maintaining mitochondrial function can be a promising therapeutic method in preventing the deterioration of cardiac hypertrophy and remodeling.

Mitochondrial Metabolism in Cardiac Fibrosis

Cardiac fibrosis is characterized by accumulation of ECM in the myocardium and it is present in most cardiac pathological conditions [114]. Because the adult mammalian myocardium has negligible regenerative capacity, the most extensive fibrotic remodeling of the ventricle is found in diseases associated with acute cardiomyocyte death. Following acute I/R, the sudden loss of a large number of cardiomyocytes triggers an inflammatory reaction, ultimately leading to replacement of dead myocardium with a collagen- based scar [115]. Similarly, pressure overload induced by hypertension, results in extensive cardiac fibrosis that is initially associated with increased stiffness and diastolic dysfunction. A persistent pressure load may lead to ventricular dilation and further diastolic and systolic heart failure [114]. In addition, hypertrophic cardiomyopathy has been associated with the development of significant cardiac fibrosis [116].

Several cell types are implicated in fibrotic remodeling of the heart, either directly by producing matrix proteins (fibroblasts) or indirectly by secreting fibrogenic mediators (macrophages, mast cells, lymphocytes, cardiomyocytes, and vascular cells). In all conditions associated with cardiac fibrosis, fibroblast transdifferentiation into secretory and contractile cells, termed myofibroblasts, is the key cellular event that drives the fibrotic response [117]. Regardless of the aetiology of cardiac injury, myofibroblasts are prominently involved in both reparative and fibrotic processes. Indeed, myofibroblast accumulation in the cardiac interstitium has been reported, not only in myocardial infarction [118] but also in the pressure and volume overloaded myocardium [119].

Oxidative stress has also been implicated in the pathogenesis of cardiac fibrosis, both through direct actions and through its involvement in cytokine and growth factor signaling. ROS are able to directly regulate the quantity and quality of interstitial ECM by modulating both matrix protein expression and metabolism. Inappropriate production of ROS in mitochondria, overwhelming the antioxidant defense systems, and the resulting oxidative damage to mtDNA and mitochondrial proteins have long been recognized as playing a causative role in the development and progression of cardiac remodeling and dysfunction [120]. Recent data support an essential role for the mitochondrial antioxidant enzyme thioredoxin 2 in preserving cardiac function by suppressing mitochondrial ROS production [64]. Mice with cardiac specific deletion of thioredoxin 2 develop progressive dilated cardiomyopathy with impairment of contractile function, heart chamber dilation, and marked interstitial fibrosis [121].

TGF-β1 is required for fibroblast differentiation into a pro-fibrotic myofibroblast phenotype, and it has been established that NADPH oxidase 4 (NOX)-dependent

O2• − production by TGF-β1 is required for this differentiation [122, 123]. Moreover, ROS generated from mitochondrial electron transport chain complex III are required for TGF-β1–mediated transcription of NOX4 [124] and the initial activation of NOX4 amplifies and sustains TGF-β1–induced oxidative stress. Therefore, prevention of early mitochondrial O2• − formation and ensuing NOX4 up-regulation might be a key mechanism involved in the anti-fibrotic effect of PETN [125]. In conclusion, long-term PETN treatment targeting superoxide generation and NO• bioavailability most likely prevented the changes of mitochondrial scavenging pathways and progressive fibrotic remodeling, leading to improved cardiac functional performance in congestive heart failure.

Alterations in the balance of MMPs and tissue inhibitors of metalloproteinases (TIMPs) are also involved in myocardial matrix remodeling. MMPs are expressed at very low levels in normal myocardium but markedly increased expression and activity of MMPs have been shown in human and animal hearts during the remodeling process after I/R [126, 127]. Increased oxidative stress activates MMPs and decreases fibrillar collagen synthesis in cardiac fibroblasts [62]. On the other hand, the TGF-β activating effects of ROS may enhance ECM deposition in the cardiac interstitium [128]. In adult rat cardiac fibroblasts, Ang II-stimulated collagen production is mediated through ROS generation [129]. When the mitochondrial bioenergetics and ROS production is pharmacologically restored, up-regulation of MMP9 is completely prevented, while maintaining TIMP1 gene expression, suggesting a reduction of MMP activity and less pronounced ventricular remodeling in this condition [130].

Injured mitochondria possess inflammatory properties, including damage-associated molecular patterns (DAMPs), which might produce cardiomyocyte injury [58]. Cellular injury releases mitochondrial DAMPs into the circulation eliciting a sepsis-like neutrophil mediated organ injury [131]. Furthermore, mitochondrial DAMPs activate the inflammasome, a group of intracellular multiprotein complexes that activates caspase-1 and proinflammatory cytokines [132]. Alternatively, mtDNA that escapes from autophagy leads to inflammatory responses in cardiomyocytes, inducing myocarditis and dilated cardiomyopathy [43]. Hypertension-induced inflammatory mediators may also compromise mitochondrial integrity and function. Progressive increase in interleukin (IL)-1 levels in diabetic rats is associated with decreased activity of the cardiac mitochondrial aldehyde dehydrogenase-2 [76]. Similarly, TNF-α treatment in vitro magnifies morphological changes in mitochondria and decreases membrane potential and ATP production in adipocytes [133], implicating mitochondria as targets of systemic inflammation that can lead to immunologic and fibrotic response. Taken together, these observations implicate mitochondria in the genesis of chronic inflammation in failing hearts.

Mitochondrial dysfunction also alters intracellular calcium homeostasis, which in smooth muscle cells regulates peripheral vascular resistance, influencing the risk of hypertension. Mitochondrial calcium additionally promotes biogenesis by upregulating PGC-1α expression [134]. In addition, increased myocardial expression of mitophagy markers is associated with changes in calcium cycling proteins,

contributing to LV interstitial fibrosis and diastolic dysfunction [135]. Hence, mitochondrial abnormalities might also provoke myocardial fibrotic remodeling and dysfunction through alterations in intracellular calcium signaling.

Mitochondria During Hypertension

Myocardial mitochondria are vulnerable to the effects of hypertension, which most commonly impairs mitochondrial structure, bioenergetics, or homeostasis. Although antihypertensive drugs have the capacity to attenuate mitochondrial injury secondary to hypertension, drugs that specifically target the mitochondria may prove more efficacious in ameliorating hypertensive mitochondrial dysfunction or end-organ damage. Structural mitochondrial alterations secondary to hypertension may include decreased mass and density, swelling, as well as cristae remodeling, fragmentation, or loss. Decreased mitochondrial mass and density have ramifications for mitochondrial function, as fewer and smaller-sized mitochondria compromise their oxidative capacity and energy production. Hypertension has been prominently associated with damage and loss of cardiolipin, a phospholipid uniquely found in the IMM and necessary for proper cristae formation [136]. Cardiolipin regulates mitochondrial dynamics and prevents the formation and opening of the MPTP, and release of cytochrome c from into the cytosol where it triggers apoptosis [64].

Several studies provide evidence of hypertension-induced mitochondrial structural and functional abnormalities, including alterations of biogenesis and dynamics, RAAS-induced mitochondrial damage (ATP synthase, and creatine kinase activity are reduced in hypertensive heart mitochondria, while cytochrome c release and caspase-3 expression are upregulated, implying stimulated apoptosis), ROS overproduction, apoptosis, and mtDNA mutations. For example, despite similar volume, cardiac mitochondria from hypertensive rats are disorganized and show reduced number of cristae [74]. Cardiac mitochondria are normally in continuous interaction with the sarcoplasmic reticulum and sarcomeric structures forming ‘intracellular energetic units’ to allow optimal energy transfer. Thus, alterations in mitochondrial arrangement might impair muscle power and contractile function [137]. Mitochondrial structural changes are also accompanied by reduced mitochondrial respiration, triggered by decreased complex-I activity. The notion that mitochondrial damage compromises cardiomyocyte contractility suggests a possible role of mitochondria in the development of hypertension induced cardiac dysfunction. Conversely, ECM expansion and fibrosis secondary to hypertension may directly contribute to structural mitochondrial abnormalities and contractile dysfunction [75]. Therefore, despite the link between damaged mitochondria and hypertensive heart disease, a cause effect relationship remains to be established.

In hypertensive rats, mRNA levels of the fusion proteins MFN1 and MFN2, and OPA1 were decreased, suggesting a shift towards increased mitochondrial fragmentation. Furthermore, hypertensive rats exhibited reduced enzyme activities and

expression of ETC subunits, indicating that mitochondrial function and remodeling are compromised in hypertension-induced cardiac hypertrophy. However, whether mitochondrial alterations are the primary cause or downstream consequence of hypertensive myocardial injury remains obscure.

Mitochondria-targeted antioxidants are novel cell-permeable compounds that may attenuate hypertensive injury by protecting mitochondria from oxidative damage. MitoQ is a ubiquinone derivative that prevents lipid peroxidation and mitochondrial damage in several experimental models. MitoQ also blunts hypertension, improves endothelial function, and reduces cardiac hypertrophy in spontaneously hypertensive rats [69]. Furthermore, its combination with low-dose losartan provides additive therapeutic benefit, by attenuating hypertension and reducing LV hypertrophy in spontaneously hypertensive rats, and by a direct anti-hypertrophic effect on rat cardiomyocytes in vitro [71]. Hence, MitoQ provides both hemodynamic and mitochondria-specific beneficial effects.

Mitochondria During Cardiac I/R Injury

I/R injury occurs when the blood supply to a tissue is blocked for minutes to hours (ischemia) and then restored (reperfusion). This important pathological mechanism underlies a range of disorders, including heart attack and stroke, where the prevention of blood flow to the tissue, for example by a blood clot, is followed by reperfusion when the blockage is removed mechanically or pharmacologically [138].

The current consensus is that a period of ischemia primes the tissue for subsequent damage upon reperfusion. Ischemic cells will eventually die if blood flow is not restored, but it is during reperfusion itself that most I/R damage is initiated. Thus, paradoxically, the essential therapeutic intervention to treat ischemia drives tissue pathophysiology [139]. The first damaging event upon reperfusion is a burst of ROS production from mitochondria [136]. Mitochondrial ROS not only drive acute damage but also initiate the pathology that develops over the minutes, days, and weeks following reperfusion [140]. The initial burst of ROS production upon reperfusion directly causes oxidative damage to mitochondria, thereby disrupting ATP production [141]. The mitochondria are recognized as a key player in cardiomyocyte death after myocardial infarction and cardiomyopathies. The events occurred during cardiac I/R significantly alter mitochondrial structure and function: reduce membrane potential, impair oxidative phosphorylation and decrease high energy phosphate synthesis, dysregulate calcium homeostasis, increase ROS production, matrix swelling and membrane permeability, and release of cytochrome c and other apoptotic factors leading to cell death. These events are initiated during ischemia and extend throughout reperfusion to severely compromise cardiac post-ischemic functional recovery and cell viability [142, 143].

During reperfusion, the reoxygenation of ischemic tissue results in mitochondrial calcium overload and renormalization of intracellular and matrix pH which are

accompanied by the prodigious generation of ROS that synergistically induce the opening of the MPTP [144–146]. Together, this mitochondrial disruptions are the principal mechanism of apoptotic/necrotic cardiomyocyte death accounting for the majority of I/R injury [136, 139, 147, 148].

ROS-mediated mitochondrial damage also releases DAMPs, such as mtDNA, which can initiate the sterile inflammatory response [149, 150]. This activation of the innate immune system causes inflammation that contributes to I/R injury and can continue for days after the initial damage [149]. Therefore, during the weeks and months following the initial I/R injury, fibrotic scar tissue will form replacing dead cells along with extensive tissue remodeling [115]. The end result is often an organ that is structurally altered and functions poorly, frequently leading to persistent long-term pathology such as chronic heart disease [117].

Improvement in the clinical management of ischemic heart disease remains elusive despite the discovery of many molecular and cellular mechanisms that may be valuable targets against I/R injury. The importance of mitochondrial bioenergetics and function in contributing not only to cardiac injury but also to reducing cardiac injury is now well recognized. There are several cardioprotective strategies or treatments against I/R injury directed to mitochondria. For instance, cyclosporine administered at the time of reperfusion seems to have a sustained beneficial effect on infarct size reduction, which might improve the post-infarction remodeling [151]. Pharmacological modulation with the mitochondria-targeting peptide bendavia leads to sustainment of $\Delta\Psi m$ in cardiomyocytes during I/R, suggesting that bendavia might act as a direct MPTP blocker [152]. Moreover, mitochondria can be delivered to the ischemic heart through the vasculature and transplantation of these organelles affords an important functional benefit [153].

Conclusions

- Cardiac remodeling not only occurs at the organ or tissue levels, but can be also evidenced at the level of organelles like mitochondria.
- Mitochondrial dynamics remodeling has shown to be a cause or consequence of the development of several cardiac pathologies.
- Increased ROS production derived from mitochondrial metabolism has been associated with the development of cardiac pathologies.
- Mitophagy is stimulated in the heart during the chronic phase of cardiac remodeling and would be protective for the heart.
- The mitochondria is a therapeutic target in cardiac remodeling that leads to heart failure, hypertension and I/R.

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