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Mitochondrial Quality Control in the Diabetic Heart

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Abstract

Diabetes is a well known risk factor for heart failure. Diabetic heart damage is closely related to mitochondrial dysfunction and increased ROS generation. However, clinical trials have shown no effects of antioxidant therapies on heart failure in diabetic patients, suggesting that simply antagonizing existing ROS by antioxidants is not sufficient to reduce diabetic cardiac injury. A potentially more effective treatment strategy may be to enhance the overall capacity of mitochondrial quality control to maintain a pool of healthy mitochondria that are needed for supporting cardiac contractile function in diabetic patients. Mitochondrial quality is controlled by a number of coordinated mechanisms including mitochondrial fission and fusion, mitophagy and biogenesis. The mitochondrial damage consistently observed in the diabetic hearts indicates a failure of the mitochondrial quality control mechanisms. Recent studies have demonstrated a crucial role for each of these mechanisms in cardiac homeostasis and have begun to interrogate the relative contribution of insufficient mitochondrial quality control to diabetic cardiac injury. In this review, we will present currently available literature that links diabetic heart disease to the dysregulation of major mitochondrial quality control mechanisms. We will discuss the functional roles of these mechanisms in the pathogenesis of diabetic heart disease and their potentials for targeted therapeutical manipulation.

Keywords

Diabetic Cardiomyopathy; Mitochondria Quality Control; Mitochondrial Dynamics; Mitophagy; Mitochondrial Biogenesis

1. Overview

Diabetic heart damage is closely related to mitochondrial dysfunction and increased generation of reactive oxygen species (ROS) [1–6]. Clinical trials have shown that simply antagonizing existing ROS by antioxidants is not sufficient to reduce diabetic heart failure [7–12]. A presumably more effective treatment strategy may be to enhance the overall capacity of mitochondrial quality control to maintain a pool of healthy mitochondria that are

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needed for supporting cardiac contractile function. Mitochondrial quality control can be performed at multiple points during the mitochondrial life cycle through a group of interrelated inducible processes, ranging from protein folding and ATP production to mitochondrial dynamics and motility as well as mitochondrial degradation and biogenesis.

Mitochondria undergo constant fusion and fission (collectively termed mitochondrial dynamics) to change their shape and size to meet the metabolic demand in a cell. Fusion is controlled by mitofusion 1 (Mfn1), Mfn2 and optic atrophy 1 (Opa1), while fission is controlled by dynamin-related protein 1 (Drp1), mitochondrial fission protein 1 (Fis1), mitochondrial fission factor (MFF), and mitochondrial dynamics proteins 49 (MiD49) and MiD51[13]. Superfluous or injured mitochondria are removed through autophagy that specifically targets mitochondria for lysosomal elimination, a process termed mitophagy. Mitophagy achieves its selectivity and specificity through a well established pathway composed of a serine/threonine kinase PINK1(phosphatase and tensin homolog–induced putative kinase 1) and an E3 ubiquitin ligase Parkin. The specificity is also mediated by a number of adaptors or receptors that are found in cytosol or on mitochondrial membranes, including sequestosome 1 (p62), histone deacetylase 6 (HDAC6), BCL2/adenovirus E1B interacting protein 3 (BNIP3), BNIP3-like (BNIP3L or NIX) [14, 15], FUN14 domain containing 1 (FUNDCl) [16], Bcl-2-like protein 13 (Bcl2L13) [17, 18], and Optineurin and Nuclear dot protein 52 (NDP52)[19]. Normally tightly coupled to mitophagy, the mitochondrial biogenesis is a process that generates new mitochondria to replenish the mitochondrial pool. The mitochondrial proteins are encoded by both nuclear genome and mitochondrial genome which are synchronized by PGC-1 α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha). PGC-1 α is a master regulator that activates and coordinates mitochondrial biogenesis through its effects on multiple transcription factors including PPAR γ and PPAR α , estrogen receptor–related α (ERR α), nuclear respiratory factor 1 and 2 (NRF1/2), and mitochondrial transcription factor A (mtTFA)[20].

The mitochondrial damage consistently observed in the diabetic heart indicates a failure of the mitochondrial quality control. In this review, we will present currently available literature that links diabetic cardiac injury to the dysregulation of major mitochondrial quality control mechanisms including mitochondrial dynamics, mitophagy and mitochondrial biogenesis. We will discuss the functional roles of these processes in the pathogenesis of diabetic cardiomyopathy and explore their potentials for targeted therapeutical manipulation.

2. Diabetic Heart Disease and Mitochondrial Dysfunction

Diabetes is a risk factor for the development of various cardiovascular complications, which constitute the leading causes of death in both type 1 and type 2 diabetic populations. Diabetic patients are prone to heart damage due to multiple diabetes-associated risk factors or processes such as obesity, hypercholesterolemia, atherosclerosis, impaired microcirculation and hypertension. Nevertheless, diabetic cardiomyopathy, a heart muscle-specific disease independent of vascular pathology, also significantly contributes to the increased risk of heart failure and mortality in diabetic patients[1, 2]. Thus, cardiac dysfunction in diabetic patients are caused by multiple pathologic mechanisms.

Interestingly, all these mechanisms are associated with mitochondrial injury which has been proposed to underlie the pathophysiology of diabetic heart disease[5, 6]. Indeed, numerous animal and human studies demonstrate the frequent occurrence of damaged mitochondria in the diabetic hearts[21–27]. Dysfunctional mitochondria can cause more ROS production and release pro-death factors such as cytochrome C, apoptosis-inducing factor, and Smac/DIABLO[26, 28–31]. Various ROS scavengers or antioxidants are able to reduce cardiomyocyte death and attenuate diabetic cardiac injury in experimental animal models [4, 31–35]. Unfortunately, the antioxidant-based therapies have been generally disappointing in diabetic patients [7–12], suggesting that simply antagonizing existing ROS by antioxidants is not sufficient to reduce diabetic cardiac injury. A potentially more effective treatment strategy may be to enhance the overall capacity of mitochondrial quality control to maintain a pool of healthy mitochondria that are needed for supporting cardiac contractile function in diabetic patients. Presumably, this could be achieved by manipulating and coordinating the major quality control mechanisms to maintain a delicate balance between mitochondrial fusion and fission and a tight coupling between mitochondrial dynamics, mitophagy and biogenesis (Figure 1).

3. Mitochondrial Dynamics in the Diabetic Heart

Mitochondria in most mammalian cells are highly dynamic organelles that undergo constant fusion and fission to change their shape, size and number to meet the metabolic demand of the cell. Mitochondrial morphology at any given time is determined by the net balance of fission and fusion, which are controlled by a group of dynamin-related large GTPases that include fusion proteins Mfn1, Mfn2 and Opa1 as well as fission protein Drp1 and its receptors Fis1, MFF, MiD49 and MiD51[13]. Mitochondrial dynamics is vital for mitochondrial function and cell survival, but it is also implicated in apoptosis, necrosis and necroptosis[36, 37]. Mitochondrial fusion is mediated by Mfn1/2 in the outer mitochondrial membrane (OMM) and by Opa1 in the inner mitochondrial membrane. Opa1 is processed into several isoforms including the short and long forms (S-Opa1 and L-Opa1). L-Opa1 is located in the inner membrane and is important for stabilizing mitochondrial cristae for efficient mitochondrial respiration[38]. Mitochondrial fusion events allow the mixing of the contents of different mitochondria thereby diluting injured mitochondrial proteins and DNA. Fusion also promotes the formation of elongated tubular mitochondria which may have increased ability to produce ATP and reduced likelihood to be degraded by mitophagy[39]. In contrast, fission occurs upon the recruitment Drp1 to the OMM where it binds to Fis1, MFF, MiD 49 or MiD51[13]. Drp1 function is tightly regulated by reversible phosphorylation by Cdk1/Cyclin B and cyclic AMP-dependent protein kinase (PKA) and its dephosphorylation by phosphatase calcineurin and the mitochondrial phosphatase phosphoglycerate mutase family member 5 (PGAM5) [39]. Fission events produce smaller fragmented mitochondrial subunits which permit even distribution of mitochondria in a cell. Importantly, fission can segregate damaged segments of mitochondria and facilitate their removal by mitophagy[40]. However, enhanced mitochondrial fragmentation is also linked to the initiation of apoptosis[36]. A reduction in mitochondrial fragmentation has been shown to attenuate apoptosis in several cell types [41–43].

Mitochondrial fission and fusion are readily observable in H9c2 myoblast cells[44–46] and neonatal cardiomyocytes[47, 48] since mitochondrial movements are not severely restricted. In contrast, mitochondria of adult cardiomyocytes are relatively static and organelle fission and fusion occur only at very low frequency because the densely packed mitochondria lack motility between myofibrils and under the sarcolemma [49]. In adult heart, the mitochondrial fusion and fission cycle is estimated to require 16 days [50]. Nevertheless, interruption of mitochondrial fusion or fission pathways has dramatic effects on cardiomyocyte functionality [51–54]. Opa1 heterozygous knockout mice develop late-onset cardiomyopathy characterized by mitochondrial dysfunction, increased ROS and reduced cardiac function[55]. Deletion of both Mfn 1 and Mfn 2 leads to excessive mitochondrial fragmentation and fatal cardiomyopathy due to unopposed fission [50]. Conversely, moderate overexpression of Opa1 leads to mild cardiac hypertrophy that is resistant to ischemic injury. The beneficial cardiac effects of Opa1 are associated with increased L-Opa1 levels, stabilized mitochondrial cristae, enhanced mitochondrial respiratory efficiency, and blunted cytochrome c release and ROS production[38]. These studies demonstrate a cardioprotective role of mitochondrial fusion proteins. However, the role of mitochondrial fission appears to be context-dependent. A Drp1 mutation (C452F) in mice leads to autosomal dominant dilated cardiomyopathy and heart failure with impaired mitochondrial dynamics and mitophagy[56, 57]. Cardiac specific deletion of Drp1 uniformly produces large and elongated dysfunctional mitochondria, leading to heart failure and premature death in several different studies[58–62], which underscores the importance of mitochondrial fission in maintaining cardiac homeostasis at baseline. Nonetheless, suppressing mitochondrial fission by chemical inhibitors attenuates cardiac damage induced by ischemia/reperfusion[54, 63, 64], pressure overload[65] and doxorubicin[66]. Collectively, these results demonstrate that mitochondrial fragmentation is a double-edged sword that can be either protective or detrimental depending on the specific context and the extent of fragmentation induced. Thus, the functional role of mitochondrial fragmentation in the heart under different conditions has to be individually determined. One potential determinant for the ultimate cardiac effect of mitochondrial fragmentation is its coupling status with mitophagy. If mitophagy functions normally, it may quickly remove the injured daughter mitochondria thereby protecting the heart. By contrast, if mitophagy is defective or fission is excessive, the fragmented dysfunctional mitochondria may accumulate leading to cardiac injury. Therefore, a delicate balance between mitochondrial fusion and fission and a tight coupling between mitochondrial fragmentation and mitophagy may be the key for maintaining a healthy mitochondrial network and cardiac homeostasis.

The research of mitochondrial dynamics in diabetes is limited, but there is strong evidence to indicate that mitochondrial dynamics are altered in high glucose treated cardiomyocytes and in the diabetic hearts. For example, high glucose induces the formation of short and small mitochondria in H9c2 cardiac myoblast cells [44–46], which is dependent on the phosphorylation of Drp1 at serine 616 by ERK1/2 [46]. Inhibition of mitochondrial fission by dominant negative Drp1 mutant (K38A) attenuates high glucose-induced ROS production and cell death [45], suggesting that the fragmented mitochondria contribute to high glucose toxicity. Similarly, high glucose triggers mitochondrial fragmentation in neonatal rat cardiomyocytes[47, 48]. This is related to increased O-linked-N-acetyl-glucosamine

glycosylation (O-GlcNAcylation) of Drp1, decreased phosphorylation of Drp1 at serine 637, and increased mitochondrial translocation of Drp1 [47]. Also, high glucose reduces the protein levels of Opa1 and increases its O-GlcNAcylation [48]. Increasing Opa1 protein levels or reducing Opa1 O-GlcNAcylation blocks high glucose-induced mitochondrial fragmentation and dysfunction [48], further supporting a detrimental role of mitochondrial fragmentation in high glucose toxicity. In the diabetic mouse heart, STZ type 1 diabetes induces the fragmentation of interfibrillar mitochondria as assessed by light scattering [67] and electron microscopy [68], which is associated with increased proteolytic cleavage of Opa1 [69]. Coronary endothelial cells isolated from STZ diabetic mice also show increased Drp1, reduced Opa1 and enhanced mitochondrial fission [70]. Increased O-GlcNAcylation of Drp1 is observed in the heart of type 2 diabetic mice [47]. Significantly, decreased expression of Mfn1 and Atg5 as well as enhanced mitochondrial fragmentation are seen in the right atria of type 2 diabetic patients [71]. Functionally, the increased mitochondrial fission has been proposed to be responsible for enhanced ROS production in diabetic cardiomyopathy [69]. This is supported by the ability of Drp1-K38A mutant to attenuate oxidative stress in the liver and the kidney of STZ diabetic mice [72]. Unfortunately, the effect of Drp1-K38A on diabetic heart is not determined in this study. Given its ability to impair the ROS-stimulated mitophagy [73], Drp1-K38A overexpression may not necessarily protect against diabetic cardiac injury. More importantly, knocking out Drp1 invariably results in heart failure in mice [58–61], casting further doubt on the hypothesis that disrupting mitochondrial fission could be beneficial to the diabetic heart. Thus, it remains an open question how manipulating mitochondrial fission process would affect the development of diabetic cardiomyopathy.

4. Autophagy, Mitophagy and their Regulatory Pathways

The macro-autophagy-lysosomal degradation pathway is a cytoplasmic quality control system through which long-lived proteins and organelles are delivered to and degraded in the lysosomes. In response to starvation or stress, autophagy is activated to degrade and recycle cellular components that may indiscriminately include mitochondria, which is thought to be a non-selective bulk degradation pathway. However, when mitochondria are injured, they need to be specifically targeted and escorted into the lysosomes for elimination, a process termed mitophagy (14). The general autophagy and the selective mitophagy do not necessarily change together in the same direction. For example, mitophagy can be induced under nutrient-rich conditions to remove superfluous or injured mitochondria when general autophagy is inhibited [74]. Conversely, starvation activates autophagy in several cell types but it actually inhibits mitochondrial degradation [39]. Thus, mitophagy must be mechanistically separable from general autophagy and be regulated by different signaling pathways [75, 76] to ensure the selective nature of mitophagy that eliminates only dysfunctional or superfluous mitochondria.

4.1. The Autophagy Pathway

Autophagy involves several sequential steps including the engulfment of cellular constituents, the formation of double-membrane autophagosome, its fusion with a lysosome to form autolysosome, and the degradation and recycling of the cargoes (Figure 2). Induction

of autophagy is coordinately controlled by many positive and negative regulators including the mechanistic target of rapamycin complex1 (mTORC1), a growth factor-regulated and nutrient-sensing kinase, and the AMP-activated protein kinase (AMPK), an energy-sensing kinase [77]. Indeed, mTORC1 is well known to inhibit autophagy under nutrient-rich conditions, while AMPK stimulates autophagy under nutrient-restricted conditions (Figure 2). The opposing effects of mTORC1 and AMPK on autophagy are mediated by autophagy-related protein kinase 1 (Atg1), known as Unc-51-like kinase 1 (ULK1) in mammalian cells. ULK1 forms a stable complex with Atg13, FIP200, and Atg101[78], and its activity is inhibited by mTORC1 and activated by AMPK through reversible and competitive phosphorylation[79]. ULK1 induces autophagy by phosphorylating and activating the class III phosphatidylinositol 3-kinase (PI3K)/vacuolar protein sorting 34 (Vps34) complex[80]. The Vps34 complex is essential for the generation of autophagosomes and is formed by Beclin 1, Vps34 and Vps15 through interaction with several cofactors including Atg14L, UVRAG, Ambra1, Bif-1, Rubicon, HMGB1, nPIST, VMP1 and SLAM [81]. During autophagosome formation, the microtubule-associated protein 1 light chain 3 (LC3-I/Atg8) is lipidated by Atg7 and Atg3 to form LC3-II that is incorporated into the double membranes of the autophagosomes. The Atg12–Atg5–Atg16 complex is also required for the formation of LC3-II and autophagosomes [82]. The protein levels of LC3-II are commonly used to estimate the total number of autophagosomes and autolysosomes, collectively referred to as autophagic vacuoles (AVs) [83]. However, a single snapshot of LC3-II at a given time point can not directly inform the functional status of autophagy due to the dynamic nature of this multi-step process. The autophagic activity can be assessed more accurately by so-called autophagic flux that reflects the number of AVs that are delivered to and degraded in the lysosome. Accordingly, autophagic flux is calculated as the difference in the protein levels of the LC3-II or the numbers of AVs in the absence and presence of lysosomal inhibitors such as Pepstatin A, E64d, chloroquine and Bafilomycin A1[84]. The importance of using autophagic flux instead of static levels of LC3-II as a marker for autophagic activity has been demonstrated in many different studies[85].

4.2. Mitophagy and its Regulatory Pathways

Mitophagy may recruit the same core machinery as general autophagy. It may also use other alternative pathways which may or may not be dependent on Atg5/7. Mitophagy achieves its selectivity and specificity through PINK1/Parkin pathway and a number of adaptors or receptors including p62, HDAC6, BNIP3/NIX, FUNDC1, Bcl2L13, and Optineurin and NDP52 (Figure 3). Some atypical forms of mitophagy have been reported but their regulation and significance in mitochondrial quality control need further elucidation.

4.2.1. PINK1/Parkin-dependent mitophagic pathway—PINK1 and Parkin comprise a well characterized pathway that regulates the initiation of mitophagy[86]. A common trigger for mitophagy is the mitochondrial depolarization. PINK1 is stabilized and activated on the surface of depolarized mitochondria [87] to phosphorylate Mfn2 that help recruit Parkin [88], an E3 ubiquitin (Ub) ligase that normally resides in the cytosol. PINK1 also phosphorylates both Parkin[89, 90] and ubiquitin[91] at Ser65, which is required for efficient recruitment and full activation of Parkin. Recent studies have elucidated the mechanism by which phosphorylated ubiquitin associates with and activates phosphorylated

Parkin[92, 93]. The activated Parkin then adds Ub-chain to mitochondrial proteins on the outer membrane including Mfn1/2, translocase of outer mitochondrial membrane 20 (TOM20), and voltage-dependent anion channel (VDAC). Ubiquitination and proteasomal degradation of Mfn1/2 promote mitochondrial fission and mitophagy initiation. This sets in motion a feed-forward mitochondrial ubiquitination pathway in which PINK1 phosphorylates the Ub chain causing further recruitment and enzymatic amplification of Parkin that in turn ubiquitinates more mitochondrial proteins [94]. These ubiquitinated mitochondria bind p62 or Optineurin and HDAC6 through the ubiquitin-binding domain and are transported along microtubules to cluster in the perinuclear region [95–97]. P62 or optineurin then recruits autophagosomal membranes via LC3 through its LC3-interacting domain, leading to the formation of mitophagosome which then fuses with the lysosome where mitochondria are degraded [96–98].

Ambra1 (activating molecule in Beclin1-regulated autophagy) is another player involved in Parkin- dependent and independent mitophagy. Ambra1 interacts with Parkin and is recruited to perinuclear clusters of depolarized mitochondria[99]. By binding to LC3, Ambra1 anchors damaged mitochondria to the autophagosomal membrane where Ambra1 activates class III PI3K to induce the formation of mitophagosome[100]. However, this process is not dependent on Parkin and p62 [100] despite the facts that Parkin can enhance the mitochondrial recruitment of Ambra1[99] and p62 is required for mitochondrial perinuclear clustering [95]. Parkin-mediated mitophagy has been reported to require another E3 ligase SMAD-specific E3 ubiquitin protein ligase 1 (Smurf1). However, Smurf1 does not need its ubiquitin ligase activity to promote the selective degradation of damaged mitochondria [101]. Instead, Smurf1 facilitates the delivery of the Ub-decorated mitochondria to the nascent autophagosomal membrane through its C2 membrane-targeting domain[101]. Nevertheless, it remains unknown whether the C2 domain directly binds to autophagosomal membrane or it cooperates with other partners such as p62, HDAC6 and Ambra1 to accomplish this task. Interestingly, PINK1/Parkin needs to arrest mitochondrial movement before mitophagy by phosphorylating, ubiquitinating and degrading the mitochondrial Rho-GTPase (Miro), an outer membrane protein that is required for mitochondria trafficking to various cellular locations [102, 103]. Miro-mediated mitochondrial movement normally prevents mitophagy. Taken together, it appears that PINK1 and Parkin regulate mitophagy at multiple levels including mitochondrial fragmentation (via Mfn1/2), mitochondrial motility (via Miro), autophagy receptor protein (via p62 or optineurin), and autophagy machinery (via Ambra1).

Parkin-mediated mitophagy is affected by the loss-of-function of Clec16a, a membrane-associated endosomal protein that has been identified as a disease susceptible gene for type1 diabetes. Clec16a interacts with and stabilizes neuregulin receptor degradation protein 1(Nrdp1), an E3 ubiquitin ligase that mediates the degradation of Parkin. Clec16a deficiency reduces Nrdp1 protein levels and correspondingly increases Parkin expression, triggering the initiation of mitophagy[104]. However, loss of Clec16a also impairs the fusion between autophagosome and lysosome thus blocking mitophagic flux. This results in the accumulation of unhealthy mitochondria thereby reducing β -cell function[104]. It remains unknown if Clec16a overexpression can affect mitophagy initiation and progression.

Parkin-mediated mitophagy is antagonized by USP30, a deubiquitinase localized to mitochondria. Overexpression of USP30 counteracts Parkin-mediated ubiquitin chain formation in damaged mitochondria and blocks Parkin's ability to drive mitophagy. Conversely, reducing USP30 activity enhances mitochondrial degradation in neurons and protects flies against paraquat toxicity in vivo [105], suggesting that USP30 inhibition promotes mitophagy and cell survival under this condition. However, another study shows that depletion of USP30 enhances depolarization-induced cell death in Parkin-overexpressing cells, indicating a pro-death function for USP30 inhibition [106]. These results suggest the importance of keeping a delicate balance between ubiquitination and deubiquitination in maintaining mitochondrial homeostasis and cell survival.

Pink1 and Parkin can induce mitophagy independently of each other. PINK1 does so by recruiting two autophagy receptors Optineurin and NDP52 to the surface of mitochondria, which in turn recruit other protein molecules including ULK1, DFCP1 and WIPI1 that mark the mitochondria for degradation. This process is not dependent on Parkin, suggesting a previously unrecognized ability of PINK1 to directly induce mitophagy [19]. Similarly, Parkin can translocate to damaged mitochondria to induce mitophagy in PINK1 deficient cardiomyocytes or neuronal cells, suggesting the existence of as yet unidentified PINK1-independent mechanisms that can activate Parkin and induce mitophagy [107, 108].

4.2.2. NIX or BNIP3—NIX or BNIP3 may serve as a mitophagy receptor that bridges mitochondria and LC3 to selectively induce mitochondrial degradation [14–16]. NIX/Bnip3 is inserted into the outer mitochondrial membrane through its C-terminal transmembrane domains, and recruits autophagy machinery by directly binding to LC3/GABARAP proteins on the autophagosome via the N-terminal LC3-interacting motifs [109, 110]. Also, NIX/BNIP3 may directly trigger mitochondrial depolarization to induce mitophagy [40, 111]. Of note, NIX/BNIP3 can induce cell death, but it is currently unclear how the pro-death and pro-mitophagy functions of NIX/BNIP3 are coordinated or differentially regulated [15]. In addition, NIX/BNIP3 may interact with Parkin pathway to regulate mitophagy. For example, BNIP3-induced mitophagy is associated with increased Parkin translocation to mitochondria and is attenuated by Parkin deficiency [112]. Conversely, mitochondrial uncoupler Carbonyl cyanide m-chlorophenylhydrazone (CCCP)-induced Parkin translocation was inhibited in cells deficient in NIX [113]. These results suggest a mutual requirement of NIX/BNIP3 and Parkin in their ability to induce mitophagy. Nevertheless, the functional significance and the regulatory mechanism of their interaction remain to be defined.

4.2.3. FUNDC1—FUNDC1 is a mitochondrial outer-membrane protein that serves as a mitophagy receptor to recruit the autophagosomal membrane through its typical LC3-binding motif. [16]. FUNDC1-induced mitophagy is Atg5 dependent but Beclin 1 independent. FUNDC1 can induce mitophagy in HeLa cells that do not express Parkin, suggesting that FUNDC1-mediated mitophagy is Parkin-independent. Nevertheless, recent studies have shown that FUNDC1-mediated mitophagy can be modulated by multiple signaling pathways. The reversible phosphorylation of FUNDC1 appears to be an essential step that is exquisitely regulated by several different kinases and phosphatase. FUNDC1 activity is inhibited by its phosphorylation at tyrosine 18 by Src kinase that prevents its

interaction with LC3[16], but increased by its phosphorylation at serine 17 by ULK1 which enhances FUNDC1 binding to LC3[114]. FUNDC1 is also phosphorylated at Ser13 by Casein kinase 2 (CK2) under basal conditions, but it is dephosphorylated by the mitochondrially localized PGAM5 phosphatase in response to hypoxia. Dephosphorylation of FUNDC1 by PGAM5 enhances its interaction with LC3, leading to the activation of mitophagy [115]. To add another layer of complexity, the anti-apoptotic protein Bcl-xL suppresses FUNDC1-mediated mitophagy due to its ability to interact with PGAM5 and inhibit the dephosphorylation of FUNDC1 at Ser13 [116]. Lastly, FUNDC1 expression is repressed post-transcriptionally by miR-137, a hypoxia-responsive microRNA that specifically inhibits mitophagy with no effect on general autophagy[117]. Collectively, these results demonstrate that diverse signaling pathways converge on FUNDC1 to control mitophagy within the physiological range, suggesting FUNDC1 as a potentially important regulator of mitophagy that may be readily targeted therapeutically. Given that FUNDC1 does not like NIX/BNIP3 to induce cell death, FUNDC1-targeted therapies may be relatively safe with little adverse effects.

4.2.4. Bcl2L13—Bcl-2-like protein 13 (Bcl2L13) is a newly identified mitophagy receptor that is a functional homolog of Atg32, the yeast mitophagy receptor. BCL2L13 is localized to outer mitochondrial membrane and interacts with LC3 through its WXXI motif, the LC3-interacting region required for mitophagy [17, 18]. Bcl2L13 induces mitophagy in a Parkin-independent manner without mitochondrial ubiquitination. It is also sufficient to induce mitochondrial fragmentation, which needs the BH domains but is independent of Drp1. BCL2L13 is necessary for mitochondrial damage-induced fragmentation and mitophagy. Bcl2L13 may undergo phosphorylation but the responsible kinase and regulatory mechanism are unclear. It remains controversial whether or not Bcl2L13 can induce cell death[17, 118].

4.2.5. AMPK—AMPK has been recognized as a positive regulator of general autophagy[77], but its role in selective mitophagy remains unclear. One study shows that inhibition or knockdown of AMPK prevents ULK1 mitochondrial translocation and inhibits hypoxia-induced mitophagy in MEF cells, suggesting a requirement of AMPK for hypoxia-induced mitophagy[119]. In contrast, another study demonstrates that the percentage of CCCP-induced colocalization of mitochondria and RFP-LC3 puncta is similar in WT and AMPK α 1/ α 2 double knockout MEFs, indicating that AMPK is not essential for CCCP-induced mitophagy[120]. Thus, further studies are needed to clarify if AMPK is either sufficient or necessary for mitophagy induction.

4.2.6. Atypical Forms of Mitophagy—Dr. Shimizu's group has described a non-canonical alternative autophagy that is regulated by ULK1 and beclin 1. The formation of the autophagosome depends on the small GTPase Rab9, but not Atg5, which facilitates the fusion of the phagophore with vesicles derived from the trans-Golgi and late endosomes. Interestingly, the Rab9 dependent alternative autophagic pathway is responsible for mitochondrial degradation during erythrocyte differentiation [121], raising the possibility that the Rab9-dependent non-canonical autophagy may also contribute to mitophagy in other cell types. Indeed, starvation or hypoxia induces mitophagy in Atg5 knockout mouse embryonic fibroblasts and in HeLa cells deficient in Atg7, Atg12 and LC3. However, this

mitophagy is severely suppressed by the knockdown of Rab9A and Rab9B, suggesting that mitophagy occurs predominantly through the Rab9-dependent alternative autophagy pathway[122]. Whether this is true in other cell types remains to be determined.

Another novel atypical mitophagy pathway has been described that regulates mitochondrial protein turnover and quality through the formation of mitochondria-derived vesicles (MDVs) in a PINK1 and Parkin dependent manner[123, 124]. This pathway distinguishes itself from canonical mitophagy in that it is triggered by ROS but not mitochondrial depolarization. These MDVs contain oxidized proteins and other components, bud off mitochondria and deliver the cargos to lysosome for degradation, which is independent of ATG5 and LC3. This pathway appears to selectively degrade damaged mitochondrial contents to repair the mitochondria rather than completely eliminate them as does canonical mitophagy.

5. Autophagy and Mitophagy in the Diabetic Heart

5.1. Autophagy in the Diabetic Heart

Numerous studies have demonstrated autophagy as a double-edged sword that could be either protective or detrimental to the heart depending on the specific context. As a result, either activation or inhibition of autophagy could be utilized as a viable strategy to treat heart disease and heart failure of different origins[125, 126].

Several studies has shown that autophagy is inhibited in the hearts of OVE26 and streptozotocin (STZ)-induced type 1 diabetic mice as determined by several methods including autophagy flux assays[127, 128]. Another recent study claimed an increased autophagy in STZ diabetic mouse hearts as shown by increased LC3-II levels and GFP-LC3 puncta. However, chloroquine treatment reduced rather than further increased the LC3-II levels[129], suggesting that cardiac autophagic flux was actually inhibited. Cardiac autophagy is also reduced in several forms of metabolic syndrome and type 2 diabetic animal models[127–129], but it is not as consistent since other studies reported either increased[130] or unchanged autophagy in the type 2 diabetic hearts[127, 128]. These different conclusions could be attributed partly to the fact that not all studies have followed the suggested guidelines to determine autophagic flux [84, 85]. Interestingly, the functional states of the mTORC1 and AMPK signaling pathways appear to be correlated with autophagic activities in the diabetic hearts [127, 128]. Thus, the activities of mTORC1 and AMPK may be used as markers to predict the functional status of cardiac autophagy (Figure 2).

The functional role of autophagy in the diabetic heart was determined by using both gain- and loss-of-function mouse models. Somewhat surprisingly, the type 1 diabetes-induced cardiac damage was attenuated in beclin 1-deficient or Atg16L-deficient mice and exacerbated in beclin 1 overexpressing mice [131], suggesting that the diminished autophagy is an adaptive response that limits diabetic cardiac injury. This effect may be related to an up-regulation of a non-canonical alternative autophagy and a restoration of the selective mitophagy[131]. In contrast, high fat diet-induced cardiac injury was substantially reduced by either mTOR deficiency or rapamycin treatment that restored cardiac autophagy [132], suggesting that autophagy inhibition in type 2 diabetes contributes to cardiac damage.

However, it remains unknown why inhibition of cardiac autophagy appears adaptive in type 1 diabetic models but maladaptive in most type 2 diabetic models. This discrepancy may be explained by the difference in etiologies and coexisting conditions between type 1 and type 2 diabetes[127].

5.2. Mitophagy in the Diabetic Heart

Recent studies suggest an indispensable role for mitophagy in maintaining cardiac homeostasis. For example, mice deficient in Parkin [133], SMURF1 [101] or BNIP3 and NIX [134] uniformly exhibit reduced mitophagy and increased accumulation of abnormal mitochondria in cardiomyocytes. Also, Parkin overexpression is sufficient to attenuate functional decline of aged hearts [135], while Parkin inactivation abolishes the cardioprotective effect of ischemic preconditioning [136] and renders mice more sensitive to doxorubicin cardiotoxicity[135] and myocardial infarction [137]. On the other hand, too much mitophagy may cause heart damage due to excessive destruction of mitochondria. For instance, increased Parkin may be responsible for the exaggerated mitophagy and impaired cardiac function in Drp1 knockout mice since Parkin ablation partially rescued the cardiac phenotype of Drp1 knockout mice [62]. In support of this possibility, HeLa cells overexpressing Parkin die in galactose after CCCP-treatment due to mitochondrial loss, while HeLa cells lacking Parkin are able to survive[86].

The functional status of mitophagy and the specific regulatory mechanisms of mitophagy in the diabetic heart remain largely unknown. The protein levels of PINK1 and Parkin are consistently decreased in many diabetic animal tissues including the heart [131, 138], suggesting that mitophagy may be inhibited in the diabetic hearts. Interestingly, mice deficient in Beclin 1 or Atg16L1 had partially restored expression and mitochondrial localization of PINK1, Parkin, and LAMP1 under overt diabetic conditions[131]. There was also increased mitochondrial expression of Rab9, a small GTP binding protein required for mitochondria degradation in erythrocytes [121]. It is thus possible that when canonical autophagy is inhibited, alternative autophagy is up-regulated, which may trigger mitophagy to protect the diabetic heart. Future studies need to fully characterize mitophagy in the diabetic hearts using multiple methods including mitophagy flux determination.

6. Mitochondrial Biogenesis in the Diabetic Heart

Mitochondrial biogenesis is stimulated by various forms of stress such as exercise, caloric restriction, cold temperature, oxidative stress, cell proliferation and differentiation. Mitochondria are not made *de novo*. Rather, mitochondrial biogenesis is achieved by the growth and division of pre-existing organelles, and is coupled with mtDNA replication as well as fusion and fission to generate new functional mitochondria with their own genome, conferring cells stress resistance [139]. The nuclear genome encodes more than 1000 mitochondrial proteins that are imported into mitochondria, while the mitochondrial genome encodes 13 subunits of the electron transport chain complexes along with mitochondrial rRNA and tRNA that are necessary for translation of the respiratory subunit mRNAs within the mitochondrial matrix. The two genomes are synchronized by PGC-1 α , a master regulator that activates and coordinates mitochondrial biogenesis. PGC-1 α binds to and

activates various transcription factors including PPAR γ , PPAR α , ERR α , and NRF1/2. NRF1/2 activate mitochondrial mtTFA that in turn drives transcription and replication of mtDNA[20]. PGC-1 α expression is enhanced in response to a plethora of stimuli by multiple transcription factors including myocyte enhancer factor 2 (MEF2), forkhead box class-O (FoxO1), activating transcription factor 2 (ATF2), and cAMP response element-binding protein (CREB). These factors, in turn, are modulated by different signaling pathways such as Akt, p38 mitogen-activated protein kinase, calmodulin-dependent protein kinase IV, calcineurin A, and protein kinase A. PGC-1 α levels and activity are also regulated by numerous posttranslational modifications including phosphorylation, acetylation, methylation, ubiquitination, and O-linked N-acetylglucosylation[20].

The role of PGC-1 α in the heart has been tested by the gain- and loss-of-function studies. Overexpression of PGC-1 α is sufficient to induce the expression of nuclear and mitochondrial genes and mitochondrial proliferation in the mouse hearts [140]. However, higher expressing lines also show attenuated sarcomeric structure and dilated cardiomyopathy[140], suggesting that uncontrolled mitochondrial biogenesis from birth is not desirable. Conversely, whole body ablation of the PGC-1 α gene suppresses cardiac expression of mitochondrial genes, which causes a marked deficiency in energy reserve, leading to reduced cardiac function in response to aging, exercise and pressure overload[141–143]. Concurrent knockout of both PGC-1 α and PGC-1 β genes during postnatal cardiac growth leads to altered expression of the genes involved in mitochondrial dynamics and marked mitochondrial structural derangements, which culminates in a lethal cardiomyopathy [144], suggesting that PGC-1 coactivators are required for normal postnatal cardiac development. Surprisingly, inducible knockout of PGC-1 α/β in the adult heart does not disturb mitochondrial dynamics or cardiac function despite that it inhibits the expression of nuclear- and mitochondrial-encoded genes involved in mitochondrial dynamics and energy transduction in the adult heart[144]. These results suggest that the general activity of mitochondrial biogenesis, dynamics and mitochondrial turnover is substantially lower in the adult heart than in the developing heart. Although PGC-1 coactivators are required for maintaining high-capacity mitochondrial respiratory function by driving expression of related genes, they are dispensable for maintenance of mitochondrial density and cardiac function under basal conditions in the adult.

There may be other mechanisms that regulate mitochondrial biogenesis independently of PGC-1 α expression. For example, PGC-1 α is not increased in ob/ob mouse heart, but mitochondrial DNA and mitochondrial number are increased[145]. Mitochondrial biogenesis is severely impaired as evidenced by reduced mtDNA replication and depletion of mtDNA in the human failing heart, but there is no corresponding decrease in PGC-1 α expression [146]. More directly, cardiac specific overexpression of the transcription factor c-Myc simultaneously reduces PGC-1 α levels and promotes mitochondrial biogenesis. Conversely, inactivation of Myc in the adult myocardium decreases the expression of mitochondrial biogenesis genes in response to hemodynamic load. These results suggest that Myc directly regulates mitochondrial biogenesis without the involvement of PGC-1 α [147].

In patients with insulin resistance and type 2 diabetes (T2D), mitochondrial biogenesis appears to be reduced, at least in later stage, in several organs including skeletal muscle and

the heart, as shown by decreased mtDNA content and mitochondrial volume[146, 148–150]. In fact, increasing mitochondrial biogenesis by pharmacological agents has been proposed as a potential therapeutic strategy for the treatment of insulin resistance and T2D [151, 152]. Nevertheless, a number of type 1 and type 2 diabetic mouse models show significantly increased mitochondrial area and number in the heart, which is accompanied by increased PGC-1 α expression and mtDNA content, suggesting an enhanced mitochondrial biogenesis in the diabetic hearts[22, 24, 25, 145, 153]. However, the increased mitochondria are morphologically and functionally defective as indicated by mitochondrial ultrastructural abnormalities as well as reduced mitochondrial respiratory function and ATP production[22, 153], suggesting a uncoupling between mitochondrial biogenesis and mitochondrial function. It is possible that diabetes may trigger mitochondrial biogenesis, but impairs mitochondrial degradation by mitophagy, the net result is the accumulation of defective mitochondria. It is also possible that the change in mitochondrial number in diabetic hearts may reflect alterations in rates of mitochondrial fission and/or fusion. Apparently, further studies are needed to differentiate these possibilities.

7. Interactions between Mitochondrial Quality Control Mechanisms

7.1. The Interplay between Mitochondrial Dynamics and Mitophagy

Mitochondrial dynamics serves as a quality control mechanism to preserve healthy mitochondria via fusion and eliminate dysfunctional mitochondria via fission and subsequent mitophagy. Thus, mitochondrial fission segregates damaged segments of mitochondria and facilitate their removal by mitophagy [40]. Inhibition of fission by inactivating Drp1 often prevents mitophagy in various cell types, suggesting a necessary role for fission in mitophagy [16, 51, 74, 154, 155]. However, it remains debatable how mitochondrial dynamics affects mitophagy in adult cardiomyocytes. Increased mitochondrial fragmentation by knocking out both Mfn1 and Mfn2 in mice is not accompanied by increased mitophagy in cardiomyocytes [61], suggesting that mitochondrial fission alone is not sufficient to induce mitophagy. Mfn2 per se may also be required to recruit Parkin to the depolarized mitochondria to ensure a successful induction of mitophagy in the heart[88].

It is even more uncertain whether or not fission is required for mitophagy in adult cardiomyocytes. The controversy is highlighted in 4 recent publications that report either decreased [58, 59] or increased cardiac mitophagy[61, 62] in mice with cardiac specific deletion of fission protein Drp1 that invariably produces enlarged mitochondria, cardiac dysfunction and/or heart failure. Of note, despite large and elongated mitochondria in Drp1 knockout heart, Dorn's group reported enhanced mitophagy as indicated by elevated Parkin protein expression as well as increased levels of p62, LC3-II and ubiquitinated proteins in mitochondria. More strikingly, ablation of Parkin decreased mitochondrial ubiquitination and partially rescued the cardiac phenotype of Drp1 knockout mice, suggesting that Parkin-mediated mitophagy likely contributed to cardiac damage[62]. In sharp contrast, Sesaki's group showed that Parkin/Drp1 double knockout mice had the same levels of mitochondrial ubiquitination, further reduced mitophagy and worse cardiac function when compared with Drp1 single KO mice [58], suggesting that Parkin was not responsible for the increased mitochondrial ubiquitination and was cardioprotective in Drp1 knockout heart. Dorn's group

induced cardiac specific deletion of Parkin and Drp1 at adult stage, while Sesaki's group used Parkin whole body knockout mice and induced Drp1 deletion around perinatal period, which may partially explain the discrepancy in cardiac phenotype between the two studies. It is possible that knockout of Parkin in non-cardiac tissues may have contributed to the worse heart function in Sesaki's study. Alternatively, an unidentified mechanism rather than Parkin-mediated mitophagy might have been activated in the adult heart to cause the cardiac effects observed in Dorn's study. It is equally possible that mitophagy was initiated but not completed in Drp1 KO hearts. The increased accumulation of LC3-II and p62 in mitochondria was not necessarily an indication of enhanced mitophagy. It could have been caused by a reduction in lysosomal degradation due to a defective maturation of mitophagosome or impaired lysosomal function. Measuring mitophagic flux using a lysosomal inhibitor may help resolve this issue. In support of this possibility, Sesaki's group showed that ubiquitin-decorated mitochondria were not colocalized with the lysosomal marker Lamp1 in Drp1 KO hearts[58], suggesting a defect in the formation of the mitolysosome. Similarly, Parkin expression levels are markedly increased in Clec16a deficient β -cells, which triggers the initiation of mitophagy[104]. However, the fusion between mitophagosome and lysosome is impaired, which results in the overall inhibition of mitophagic flux leading to the accumulation of dysfunctional mitochondria.

With regard to the effects of mitophagy on mitochondrial dynamics, studies have produced contradictory results probably due to different cell types used in these studies. One study showed that the PINK1/Parkin pathway promoted Drp1-dependent mitochondrial fragmentation in COS7 cells [156], suggesting a feed-back activation of the fission process by mitophagy which may generate more fragmented mitochondria further stimulating mitophagy. In contrast, other studies showed completely the opposite results in SH-SY5Y cells, i.e., PINK1/Parkin inhibited mitochondrial fragmentation [155, 157, 158], demonstrating a feed-back inhibition of fission by mitophagy that may serve to limit mitophagy within certain range once it is started. Mechanistically, Parkin was able to ubiquitinate Drp1 leading to its degradation by the ubiquitin proteasome system [158]. However, it remains unknown whether and how mitophagy affects mitochondrial dynamics in cardiomyocytes. In this respect, Parkin knockout hearts had increased Drp1 levels [58]. This may suggest increased mitochondrial fragmentation in Parkin knockout hearts, which remains to be determined.

7.2. The interplay between mitochondrial dynamics and mitochondrial biogenesis

Studies have assessed the effects of mitochondrial dynamics on mitochondrial biogenesis. Dorn's group showed that interrupting either mitochondrial fusion or fission by ablating Mfn1/Mfn2 or Drp1 impaired mitochondrial biogenesis in mouse hearts as indicated by the reduced expression levels of mtTFA, PGC-1 α , and PGC1- β genes [61], suggesting that an intact fusion or fission machinery is necessary for mitochondrial biogenesis to occur. This was supported by Sesaki's study showing reduced levels of several components of the electron transport chain in Drp1 knockout hearts[58]. On the contrary, Sadoshima's group did not observe a reduction in the protein expression levels of PGC1 α and mtTFA in Drp1 knockout heart, suggesting that Drp1 is not required for mitochondrial biogenesis[59]. Regardless of the discrepancy between these studies, even if mitochondrial dynamics does

affect mitochondrial biogenesis, the underlying molecular mechanism remains to be determined.

On the other hand, ablation of both PGC-1 α/β genes right after birth reduced the expression levels of Mfn1, Mfn2, Opa1, and Fis1, leading to marked mitochondrial morphological derangements [144], suggesting that PGC-1 coactivators are required for normal mitochondrial dynamics and postnatal cardiac development. However, this does not hold true at adult stage since ablation of PGC-1 α/β in the adult heart only inhibited the expression of related genes, but it did not disturb mitochondrial dynamics or cardiac function[144]. This may be related to the relatively moderate inhibition of Mfn1/Mfn2 expression by PGC-1 α/β deletion and very rare fission/fusion events in adult heart.

7.3. The interplay between mitophagy and mitochondrial biogenesis

It is increasingly clear that mitophagy and mitochondrial biogenesis are two opposing but tightly coupled processes that determine mitochondrial content, structure, and function. These two processes must be coordinated to ensure the proper regulation of mitochondrial quantity and quality. Recent studies have identified several signaling pathways that may regulate the coordination between mitophagy and mitochondrial biogenesis[159]. For example, mitochondrial stress induces cell death in SH-SY5Y cells, which is associated with the activation of extracellular signal-regulated protein kinases 1/2 (ERK1/2)[160]. Inhibiting ERK1/2 not only blunts mitophagy, but also increases mitochondrial biogenesis, resulting in restoration of normal mitochondrial structure and function, suggesting ERK1/2 as a dual regulator for both mitophagy and biogenesis [160]. Moreover, PINK1 not only positively regulates mitophagy but also is required for mitochondrial biogenesis because inactivation of PINK1 results in decreased mitochondrial DNA levels, electron transport activities and ATP production [161, 162]. Similarly, Parkin has been shown to stimulate mitochondrial biogenesis through multiple mechanisms including enhancing the activities of mtTFA and mitochondrial DNA[163, 164] as well as promoting the degradation of PARIS (ZNF746), a zinc finger protein that can bind to PGC-1 α promoter and repress the expression of PGC-1 α and its target gene NRF-1[165]. Thus, Parkin is able to simultaneously control both mitophagy and mitochondrial biogenesis [166]. Finally, AMP-activated protein kinase (AMPK) can coordinately regulate both mitophagy and mitochondrial biogenesis[167, 168]. AMPK up-regulates autophagy/mitophagy through its effects on mTOR and ULK1, and promotes mitochondrial biogenesis via SIRT1-dependent deacetylation of PGC-1 α or upregulation of PGC-1 α expression.

Conversely, except for regulating mitochondrial biogenesis, PGC-1 α can enhance autophagy and mitophagy via upregulation of transcription factor EB (TFEB), the master regulator of autophagy-lysosome system. This may account for the ability of PGC-1 α to enhance the elimination of HTT aggregates in a Huntington disease model [169] and to coordinately upregulate mitochondrial biogenesis and mitophagy in skeletal muscle [170, 171]. However, how PGC-1 α regulates mitophagy in the heart remains unknown.

7.4. The effects of diabetes on the Interactions between Mitochondrial Quality Control Mechanisms

Diabetes has dramatic effects on each of the mitochondrial quality control mechanisms. Mitochondrial fragmentation is increased in the diabetic hearts [67–71], but this may not necessarily lead to increased cardiac mitophagy given the reduced levels of PINK1 and Parkin [131, 138]. However, this possibility can not be completely ruled out since the functional status of Parkin-independent mitophagy is unknown and there is increased Rab9 expression in the diabetic hearts [131] which may activate mitophagy through an alternative autophagic pathway. Apparently, one has to directly determine the mitophagic activity in the diabetic hearts to resolve this issue. Mitochondrial biogenesis is likely reduced in the diabetic hearts [146, 148–150], consistent with the reduced expression of Parkin and PINK1 which positively regulate biogenesis [161–166]. However, some studies suggest an increased mitochondrial biogenesis in the diabetic hearts [22, 24, 25, 145, 153], in line with elevated mitochondrial fission which may promote mitochondrial biogenesis [58, 61]. Thus, although diabetes does appear to affect the interactions between mitochondrial quality control mechanisms, the effects are rather complicated. Further studies are needed to more carefully characterize each of the mitochondrial quality control mechanisms in the diabetic hearts and to determine the exact nature of their interplay by modulating each quality control process in the diabetic hearts.

8. Conclusions and Directions

The mitochondrial damage consistently observed in the diabetic hearts indicates a failure of the mitochondrial quality control mechanisms including mitochondrial dynamics, mitophagy and biogenesis. Indeed, dysregulation of each of these processes has been observed in the diabetic hearts. However, the relative contribution of each dysregulated mechanism to diabetic cardiac injury remains largely unknown. In order to harness the potential of targeting mitochondrial quality control mechanisms for the treatment of diabetic cardiomyopathy, it is necessary to determine whether the observed changes in each mechanism are adaptive or maladaptive using appropriate gain- and loss-of-function approaches. Given the interplay between different mechanisms, it is also important to identify the molecular underpinnings that regulate and coordinate these processes to achieve optimal mitochondrial quality control thereby reducing diabetic cardiac injury. It is expected that the next few years will see an explosion of studies aiming to understand the molecular mechanisms and develop therapeutic strategies that target the mitochondrial quality control processes individually or in combination.

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Abbreviations

ROS	reactive oxygen species
Mfn1/2	mitofusin 1/2
Opt1	optic atrophy 1
Drp1	dynammin-related protein 1
Fis1	mitochondrial fission protein 1
MFF	mitochondrial fission factor
MiD49/51	mitochondrial dynamics proteins 49 and 51
PINK1	phosphatase and tensin homolog (PTEN)–induced putative kinase 1
p62	sequestosome 1
HDAC6	histone deacetylase 6
BNIP3	BCL2/adenovirus E1B interacting protein 3
NIX/BNIP3L	BNIP3-like
FUNDC1	FUN14 domain containing 1
Bcl2L13	Bcl-2-like protein 13
NDP52	nuclear dot protein 52
PPARγ	peroxisome-proliferator-activated receptor γ
PGC-1α	PPAR γ co-activator-1 α
ERRα	estrogen receptor–related α
NRF1/2	nuclear respiratory factor 1 and 2
mtTFA	mitochondrial transcription factor A
PKA	cyclic AMP-dependent protein kinase
PGAM5	mitochondrial phosphatase phosphoglycerate mutase family member 5
mTOR	mammalian or mechanistic target of rapamycin
ULK	unc-51 like autophagy activating kinase
AMPK	AMP-activated protein kinase
Atg	autophagy-related

AVs	autophagic vacuoles
Vps34	vacuolar protein sorting 34
PI3K	phosphatidylinositol 3-kinase
LC3	microtubule-associated protein 1 light chain 3
STZ	streptozotocin
TOM20	translocase of outer mitochondrial membrane 20
VDAC1	voltage-dependent anion channel 1
Ub	ubiquitin
Ambra1	activating molecule in Beclin1-regulated autophagy
Miro	mitochondrial Rho-GTPase
GFP	green fluorescent protein
RFP	red fluorescent protein
Smurf1	SMAD-specific E3 ubiquitin protein ligase 1
LAMP1	lysosomal-associated membrane protein 1.
GABARAP	gamma-aminobutyric acid receptor-associated protein.
CK2	Casein kinase 2
Usp30	ubiquitin-specific peptidase 30
CCCP	Carbonyl cyanide m-chlorophenylhydrazone
Nrdp1	neuregulin receptor degradation protein 1

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Highlights

- Diabetic cardiac injury is closely related to mitochondrial dysfunction
- Diabetes induces mitochondrial fragmentation in the heart
- Diabetes inhibits autophagy and may alter mitophagy in the heart
- Mitochondrial biogenesis is dysregulated in the diabetic heart
- Coordination of mitochondrial quality control mechanisms is essential for limiting diabetic cardiac injury.

Figure 1. Mitochondrial quality control mechanisms and their potential interplay
Mitochondria undergo fusion and fission to change their shape and size. Mitophagy sequesters injured mitochondria within the autophagosome and deliver them to the lysosome for degradation. Mitochondrial biogenesis generates new mitochondria to replenish the mitochondrial pool. These processes are coordinately regulated by several different pathways and interact with each other to meet the metabolic demand of the cell under various conditions (see section 7 for details).

Figure 2. The autophagy pathway in the diabetic heart

Insulin deficiency/hyperglycemia (type 1 diabetes), insulin resistance/hyperinsulinemia (type 2 diabetes) and other metabolic signals in the diabetic heart simultaneously activate mTOR and inhibit AMPK, which inactivates ULK1 complex, a kinase essential for the activation of Vps34-Vps15-Bec1 complex. Activated Vps34 induces the formation of double-membrane vesicles termed autophagosomes that sequester unwanted cellular components and deliver them to the lysosomes for degradation. LC3 and Atg12-Atg5-Atg16 complex also participate in the formation of autophagosomes. LC3-II is a commonly used marker for autophagosomes and autolysosomes.

Figure 3. PINK1/Parkin dependent and independent mitophagy pathways

Mitochondrial depolarization activates PINK1 which phosphorylates Mfn2, Parkin and ubiquitin, promoting Parkin mitochondrial translocation and activation. PINK1 and Parkin regulate mitophagy at multiple levels including mitochondrial fragmentation (via Mfn1/2), mitochondrial motility (via Miro), autophagy receptor protein (via p62 or optineurin), and autophagy machinery (via Ambra1). Parkin-mediated mitophagy requires Smurf1 and is antagonized by the deubiquitinase USP30. PINK1 can also induce mitophagy by recruiting Optineurin and NDP52 independently of Parkin. Alternatively, Nix or BNIP3, FUNDC1 and Bcl2l13 serve as mitophagy receptors that bridge mitochondria and LC3 to induce mitophagy independently of Parkin. PGAM5 dephosphorylates FUNDC1 to promote mitophagy.