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SPOTLIGHT REVIEW

New roles for mitochondria in cell death in the reperfused myocardium

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Abstract Mitochondria play an important role in regulating the life and death of cells. They provide the cell with energy via oxidative phosphorylation but can quickly turn into death-promoting organelles in response to stress by disrupting adenosine triphosphate synthesis, releasing pro-death proteins, and producing reactive oxygen species. Due to their high-energy requirement, cardiac myocytes are abundant in mitochondria and as a result, particularly vulnerable to mitochondrial defects. Myocardial ischaemia and reperfusion are associated with mitochondrial dysfunction and cell death. Therefore, future therapies will focus on preserving mitochondrial integrity and function in hopes of minimizing the impact of ischaemia/reperfusion (I/R) injury. It is well established that myocardial I/R activates both necrosis and apoptosis, and that blocking either process reduces the levels of injury. However, recent studies have demonstrated that alterations in mitochondrial dynamics or clearance of mitochondria via autophagy also can contribute to cell death in the myocardium. In this review, we will discuss these new developments and their impact on the role of cardiac mitochondria in cell death following reperfusion in the heart.

Keywords Mitochondria • Fission • Fusion • Autophagy • Myocytes

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1. Introduction

Mitochondria are important gate-keepers of life and death in the cell. In cardiac myocytes, the mitochondria occupy up to 30% of the total volume and provide energy to the contracting cell in the form of adenosine triphosphate (ATP) via oxidative phosphorylation. The mitochondria are also very sensitive to alterations in the cellular environment and can quickly switch from being a supporter of life to a promoter of cell death. Therefore, it is not surprising that mitochondrial dysfunction is associated with the loss of myocytes and subsequent development of heart failure. It is well known that myocardial ischaemia and reperfusion results in mitochondrial dysfunction and cell death via both necrosis and apoptosis. Necrosis is induced via opening of the mitochondrial permeability transition pore (mPTP) which results in swelling and subsequent rupture of mitochondria.[1] Apoptosis is activated by permeabilization of the outer mitochondrial membrane and release of pro-death proteins, such as cytochrome *c* by the pro-apoptotic Bcl-2 members Bax and Bak.[2,3] Recently, it has become clear that alterations in mitochondrial dynamics and autophagy also contribute to loss of myocytes during ischaemia/reperfusion (I/R).

Studies have shown that mitochondria are highly dynamic organelles that can undergo fission or fusion in response to changes in the cellular environment. In fact, mitochondrial fission has been linked to increased mitochondrial production of reactive oxygen species (ROS),[4,5] impaired function,[6] and activation of cell death.[7–9] In addition, mitochondria are cleared in the cell by autophagy (also called mitophagy) and both defective and excessive mitophagy have been linked to cell death.[10–14] Mitochondrial fission and mitophagy are activated in response to ischaemia and reperfusion in the myocardium. In this review, we discuss the roles of mitochondrial dynamics and mitophagy in myocardial cell death following the reperfusion phase.

2. Ischaemia–reperfusion injury and opening of the mPTP

Myocardial infarction occurs when the blood flow to the myocardium is disrupted due to a sudden and sustained thrombotic occlusion of a coronary artery. As a consequence, anaerobic glycolysis is initiated, lactate is produced, and the physiological pH is reduced in the myocytes.[15] The influx of hydrogen ions into the mitochondria dissipates the mitochondrial membrane potential ($\Delta\psi_m$) and activates the Na^+–H^+ exchanger (NHE) causing an elevation in sodium levels. To reduce the levels of Na^+ in the mitochondria, the sodium–calcium exchanger (Na^+–Ca^{2+} exchanger) is activated, thus introducing Ca^{2+} into the mitochondria. Upon reperfusion, the Na^+–Ca^{2+} exchanger is reversed and starts extruding Ca^{2+} from mitochondria.[16] The

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endoplasmic reticulum (ER) is now known to be tethered to mitochondria which facilitates rapid uptake of Ca^{2+} into mitochondria released from the ER.[17,18] It was recently reported that Ca^{2+} release from the ER was initiated upon reperfusion with subsequent uptake by the mitochondria and loss of $\Delta\psi_m$.[19] During reperfusion, Ca^{2+} uptake into mitochondria occurs via the mitochondrial calcium uniporter.[20,21]

The re-introduction of blood flow to the heart in a timely manner is of paramount importance to salvage the tissue. Paradoxically, this reperfusion phase is also the stage where most of the cells in the heart die. During ischaemia, the mPTP in the inner mitochondrial membrane, which is responsible for causing primarily necrotic cell death, remains closed as the high levels of hydrogen ions inhibits the opening.[15] Instead, excess uptake of Ca^{2+}, increased pH, and elevated production of ROS at the onset of reperfusion promote opening of the mPTP. ROS are normal by-products of oxidative phosphorylation. However, the sudden return of oxygen during reperfusion and restoration of mitochondrial respiration will increase mitochondrial ROS formation at levels that exceed the cells antioxidant capacity.[22–24] Opening of the mPTP leads to an influx of osmolytes, depolarization of the $\Delta\psi_m$, and hydrolysis of ATP. Further insult will cause the inner membrane in the mitochondria to swell with subsequent rupture of the outer membrane, which disrupts its function and releases pro-death proteins into the cytosol. Necrotic cells undergo extensive organelle and cell swelling with subsequent plasma membrane rupture. Also, the release of cellular components into the extracellular space induces inflammation in the tissue. Studies have demonstrated that either inhibiting the mPTP directly[25–27] or targeting the upstream regulators of mPTP, e.g. the NHE, lead to cardioprotection.[28] Also, mice lacking cyclophilin D (CypD), an essential component of the mPTP, are resistant to I/R injury.[29,30] Nevertheless, transient opening of the mPTP has also been demonstrated to play a physiological role in regulating mitochondrial calcium levels for proper metabolic function.[31]

3. Activation of pro-apoptotic Bax/Bak in the reperfused myocardium

I/R also activates apoptosis in the heart.[2,3,32] Apoptosis is an energy-dependent process that triggers cell death through programmed self-destruction. Cells undergoing apoptosis are characterized by cell shrinkage, plasma membrane blebbing, condensation of the nucleus, fragmentation of the DNA, and nucleus. In contrast to necrosis, apoptosis does not trigger an inflammatory response as cellular contents are not released into the extracellular space but are instead engulfed by macrophages. The Bcl-2 proteins are important regulators of mitochondrial integrity in cells. These proteins are categorized into three groups based on their domain architecture: (1) anti-apoptotic Bcl-2, Bcl-X_L and Mcl-1, which contain all four Bcl-2 homology (BH) regions and maintain mitochondrial integrity, (2) pro-apoptotic proteins containing only the BH1–3 regions and function to permeabilize the outer mitochondrial membrane (Bax and Bak), and (3) BH3-only proteins, such as Bad, Bnip3, Nix, Bid, and Puma. The BH3-only proteins are initially activated by various cellular stressors, such as oxidative stress, DNA damage, and hypoxia, and they activate downstream effectors Bax and/or Bak. The resulting permeabilization of the outer mitochondrial membrane causes the release of pro-apoptotic proteins, such as cytochrome *c*, apoptosis-inducing factor, and Smac/DIABLO (Second Mitochondria-derived Activator of Caspases/Direct IAP Binding Protein with Low PI).[33] The Bcl-2 proteins have been found to play major roles in I/R. For instance, transgenic mice overexpressing Bcl-2 in the heart had reduced levels of apoptosis, smaller infarcts, and improved cardiac function after I/R compared with wild-type mice.[34–36] Similarly, Bax-deficient mice had reduced mitochondrial damage and infarct size after I/R.[37]

4. Cell death by necrosis, apoptosis, and autophagy in the infarcted myocardium

Necrosis, apoptosis, and autophagy all contribute to cell death in the reperfused heart. During ischaemia, most myocytes are lost via necrosis due to extended oxygen deprivation and ATP depletion. Upon reperfusion, both apoptosis and necrosis are rapidly activated in cells that are still viable in the risk zone. As discussed earlier, mitochondrial Ca^{2+} overload upon reperfusion and increased oxidative stress results in opening of the mPTP pore, a major contributor of necrotic cell death.[16,22,24,29,38] Similarly, increased production of ROS activates the mitochondrial cell death pathway in myocytes.[39] Although both necrosis and apoptosis are activated upon reperfusion, the acute loss of cells during reperfusion is primarily due to necrosis. Instead, apoptotic cell death occurs at a lower incidence than necrosis but takes places for an extended period of time after I/R. Studies have demonstrated the presence of apoptotic myocytes as early as 30 min[40–42] and as late as 3 days after initiation of reperfusion.[43,44] This continuous activation of apoptosis in the myocardium after I/R can result in substantial loss of myocytes overtime which contributes to development of heart failure.

The autophagic–lysosomal pathway is responsible for the degradation and recycling of cytoplasmic components and organelles.[45] Autophagy is rapidly enhanced in response to nutrient deprivation to provide the cells with amino acids and fatty acids. It is also important in cellular quality control by degrading cytotoxic protein aggregates and damaged organelles.[45] Autophagy is also rapidly enhanced during the ischaemic phase and upon reperfusion.[11,14,46] Most studies suggest that a moderate level of autophagy in response to stress is beneficial,[11,47,48] whereas excessive autophagy is detrimental to the cell.[11,12] A recent study found that autophagy was enhanced as early as 30 min after coronary ligation in both the ischaemic and non-ischaemic regions and that inhibition of autophagy resulted in increased injury.[49] This study also found that prolonged ischaemia resulted in impaired autophagy. Other studies have found that inhibition of autophagy genetically or pharmacologically results in increased susceptibility to I/R injury.[10,11,49] Similarly, enhancing autophagy in the heart can reduce acute I/R injury.[11,47,49] In contrast, some studies have found that enhanced autophagy can be detrimental during reperfusion.[11,12] It is very possible that the duration and levels of autophagy plays an important role in determining whether autophagy will be protective or detrimental to myocytes during reperfusion. Excessive autophagy can result in removal of too many essential organelles, such as mitochondria, and therefore contribute to the development of heart failure.

5. Mitochondrial dynamics

It has become clear that mitochondrial fission and fusion play important roles in regulating life and death of cells (*Figure 1*). Mitochondria are highly dynamic organelles that are constantly undergoing fission and fusion to adapt to changes in the cellular environment. These processes are regulated by several different GTPases. Fusion of the outer mitochondrial membrane is regulated by mitofusins 1 and 2 (Mfn1 and Mfn2), whereas fusion of the inner membrane is regulated by optic atrophy protein 1 (Opa1).[50–53] During mitochondrial fission, dynamin-related protein 1 (Drp1) translocates to the outer mitochondrial membrane, where it interacts with fission protein 1 (Fis1) to promote division of mitochondria.[54,55] All of these proteins are expressed in the mammalian heart[56–60] and recent studies suggest that they play important roles in regulating cell survival and death in the myocardium.

6. Functional role of mitochondrial dynamics in the myocardium

Studies have been initiated to investigate the functional role of mitochondrial dynamics in the myocardium and it is clear that mitochondrial fission and fusion are important for normal heart function. For instance, Wakabayashi *et al.*[61] discovered that deletion of Drp1 was embryonic lethal and that Drp1-deficient embryonic myocytes had reduced contractility. Dorn and colleges found that knockdown of fusion proteins mitochondrial assembly regulatory factor or Opa1 led to development of cardiomyopathy in *Drosophila*.[57] Another study found that a mutation in Drp1 resulted in cardiomyopathy in mice.[62] Although these studies suggest that proper fission is important for normal function of mitochondria, alterations in mitochondrial dynamics have also been implicated in I/R injury. Drp1-dependent mitochondrial fission occurs following myocardial ischaemia and pharmacological inhibition of fission reduced I/R injury.[56] This study discovered that inhibition of fission during I/R reduced mPTP opening and cell death in cardiac myocytes.[56] In addition, Chen *et al.*[63] demonstrated that coronary ligation in adult rat caused a reduction in Opa1 levels which correlated with increased mitochondrial fragmentation. Furthermore, ischaemia induced loss of Opa1 and subsequent mitochondrial fission in H9c2 cells, and overexpression of Opa1 preserved mitochondrial morphology during ischaemia.[63]

Many studies have reported that excessive Drp1-mediated mitochondrial fission contributes to apoptotic cell death.[7–9,64,65] Mitochondrial fission occurs within the same time frame as activation of the proapoptotic Bcl-2 family member Bax and permeabilization of the mitochondrial outer membrane. Drp-1 also co-localizes with Bax at defined foci on the mitochondrial membrane at the onset of apoptosis.[8,66] However, fission is not required for Bax/Bak-dependent apoptosis and inhibiting Drp1 only delays cell death.[64,65] Parone *et al.*[64] discovered that inhibiting the fission machinery partially prevented cytochrome *c* release from mitochondria but had no effect on the release of Smac/DIABLO. Similarly, Sugioka *et al.*[65] found that cells with fragmented mitochondria were more sensitive to apoptotic stimuli. Thus, these studies suggest that Drp1-mediated fission is not a prerequisite for apoptosis but rather increases sensitivity to apoptotic stimuli perhaps by enhancing cytochrome *c* release.

There is evidence that aberrant mitochondrial fission impairs mitochondrial bioenergetic function. Excessive fission results in abnormally small mitochondria with fragmented cristae[67] and smaller mitochondria are less efficient in ATP production.[6] Other studies have found that fragmented mitochondria have enhanced mitochondrial ROS production.[4,5] Since ROS are a major by-product of oxidative phosphorylation, it is possible that fission alters the spatial orientation of the enzymes in the electron transport chain. This in turn may lead to uncoupling of respiration and increased ROS. Alternatively, it is possible that fission of mitochondria results in uneven distribution of mitochondrial components, such as antioxidant enzymes.

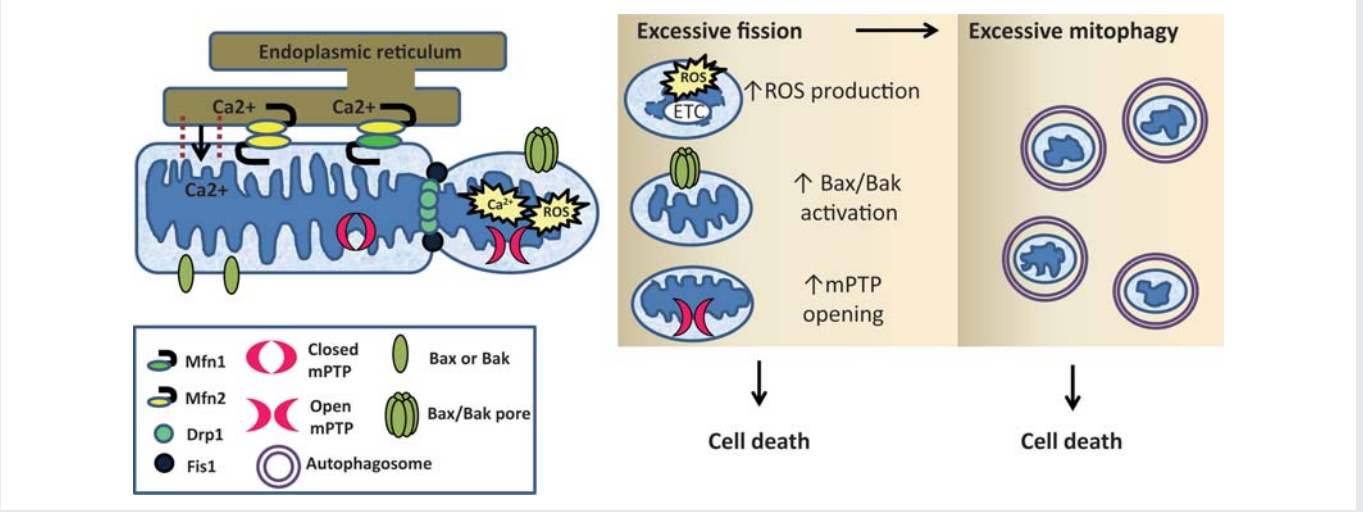

Figure 1 Recently elucidated pathways leading to mitochondrial-dependent cell death include excessive mitochondrial fission and mitophagy. Mfn2 on the mitochondria will form homo- or heterodimeric complexes with Mfn2 or Mfn1 on the ER for calcium transfer into the mitochondria. Overloading of mitochondrial calcium leads to opening of the mPTP at the onset of reperfusion. Excessive mitochondrial fission by Drp1 and Fis1 produces dysfunctional mitochondrial fragments that have increased production of ROS. These abnormally small mitochondrial fragments are also more sensitive to mPTP opening and Bax/Bak activation. Moreover, smaller dysfunctional mitochondria are more likely to be removed by autophagy and excessive mitophagy following mitochondrial fission will lead to energetic failure and cell death.

The role of mitochondrial fusion proteins in the myocardium is also under investigation. Papanicolaou *et al.*[58] discovered that conditional deletion of Mfn2 in cardiac myocytes resulted in development of mild cardiac hypertrophy and systolic dysfunction in mouse hearts. Surprisingly, the same study found that $Mfn2^{-/-}$ hearts were more resistant to I/R injury and exhibited less cell death after I/R.[58] Mfn2-deficient myocytes were more resistant to oxidative stress-induced cell death and Ca^{2+}-mediated mPTP opening,[58] suggesting that Mfn2 plays a role in promoting mPTP opening. Apart from mediating mitochondrial fusion, Mfn2 on the mitochondria also functions to tether the mitochondria to the ER. The resulting close proximity of these sub-cellular organelles allow the mitochondria to absorb excess cytotoxic Ca^{2+} that is released from the ER.[17] It is therefore possible that Mfn2-mediated tethering of ER to mitochondria may affect mPTP opening at reperfusion via uptake of excessive levels of Ca^{2+}. Nevertheless, it should be noted that Mfn2 has pleiotropic effects and there are conflicting data with respect to whether Mfn2 promotes cellular survival or death. Several studies have found that Mfn2 can also inhibit apoptosis in cells. For instance, overexpression of a dominant-active form of Mfn2 protected against Bax-mediated cytochrome *c* release,[68] down-regulation of Mfn2 exacerbated ceramide-induced mitochondrial dysfunction and cytochrome *c* release,[58] and Mfn2 overexpression protected against IR injury in HL-1 cells.[56] In contrast, elevated Mfn2 levels were detected in myocytes undergoing apoptosis following H_2O_2 treatment, myocardial infarction, or I/R injury.[69] Clearly, further studies are needed to understand the functional role of Mfn2 in cardiac cells.

7. Mitochondrial autophagy

Autophagy is a process involved in the elimination of unnecessary or dysfunctional organelles in the cell. The removal of dysfunctional mitochondria by autophagy is also referred to as mitophagy. The importance of regular turnover of mitochondria by autophagy in the heart was demonstrated in the study by Nakai *et al.*[10] who found that genetic disruption of autophagy led to rapid accumulation of dysfunctional mitochondria in myocytes and development of cardiac dysfunction. Autophagy and mitophagy are also induced in the heart in response to I/R.[14,47] Properly targeted mitophagy leads to decreased cell death in the ischaemic myocardium.[47] Conversely, a disrupted or over-activated autophagic mechanism is implicated in pathological cell death during heart failure,[70] hypertrophy,[71] and during reperfusion.[11,47]

It is clear that removal of dysfunctional mitochondria is important for cellular survival. Damaged mitochondria can serve as a source of ROS which can cause further damage to adjacent mitochondria. It was demonstrated that in the rigid and well-defined spatial arrangement of mitochondria in the adult cardiac myocyte, damage to a few mitochondria via laser quickly propagated the deleterious signals to the neighbouring mitochondria via a ROS-dependent process.[72] Damaged mitochondria can also leak pro-apoptotic proteins such as cytochrome *c* which results in activation of caspases and cell death.[33,73] Mitophagy has also been shown to play an important role in ischaemic preconditioning presumably by removing weak mitochondria that might be more prone to mPTP opening during reperfusion.[74] Thus, mitophagy protects by preventing activation of unnecessary cell death which is important in a post-mitotic cell such as the myocyte. However, it is not surprising that excessive mitophagy is detrimental to myocytes. The contracting myocyte is a high energy requiring cell and removal of too many mitochondria by autophagosomes will create an energy deficiency and result in cell death. In summary, further studies are required to determine exactly what the threshold for mitophagy is in myocytes and why only certain conditions induces excess mitophagy.

8. Regulation of mitophagy by Bnip3 and Nix

Although the Bcl-2 proteins are important regulators of the mitochondrial cell death pathway, they are also important regulators of autophagy. It has been demonstrated that binding of Bcl-2 and Bcl-X_L to Beclin-1 at the BH3 region prevents formation of the omegasome, an ER-associated platform for the initial formation of pre-autophagosomal vesicles.[75] The omegasome is created when Beclin-1 associates with vesicles containing Vps34, a class III phosphatidylinositol 3-kinases (PI3K).[76] The Bcl-2/Beclin-1 complex is also regulated by BH3-only proteins. A BH3-like domain was detected in Beclin-1 and disruption of this domain interfered with the ability to bind to Bcl-X_L.[75,77] The usage of the mimetic drug ABT-737 for Bad as well as the Bad protein itself disrupted the Beclin-1-Bcl-2/Bcl-X_L complex and restored autophagy.[75] Expression of Bad also impaired the interaction of Beclin-1 and Bcl-X_L at the ER interface.[75] Similarly, phosphorylation of Bcl-2 by c-Jun N-terminal kinase 1 (JNK1) inhibited this binding.[78] In contrast, binding of Bcl-2 to Beclin-1 is enhanced by the presence of the recently characterized nutrient-deprivation autophagy factor-1.[79]

Bcl-2/adenovirus E1B nineteen-kilodalton interacting protein (Bnip3) and its homologue Nix (also called Bnip3L) are pro-apoptotic BH3-only proteins which play key roles in the pathogenesis of heart failure.[80,81] Nix has been implicated in cardiac hypertrophy and development of cardiomyopathy,[81] whereas Bnip3 plays a role in I/R injury and post-infarct remodelling.[82–84] Both Bnip3 and Nix have been reported to mediate mitochondrial dysfunction via opening of the mPTP[80,85,86] and via activation of Bax/Bak.[87,88] Recent studies have demonstrated that Bnip3 and Nix are potent inducers of mitophagy. We found that mitophagy was specifically induced in myocytes over-expressing Bnip3 and that inhibiting this process resulted in increased cell death.[13,14,89] Interestingly, Bnip3 induced mitochondrial autophagy in cells lacking a functional mPTP[90] and in Bax/Bak-deficient fibroblasts,[89] suggesting that Bnip3 activates this process even in the absence of mitochondrial permeabilization and apoptosis. Moreover, Nix has been found to be essential for mitochondrial clearance via autophagy during reticulocyte maturation.[91] Similar to our findings with Bnip3, Nix-mediated mitochondrial clearance was normal in Bax/Bak-null reticulocytes and unaffected by inhibitors of the mPTP in wild-type reticulocytes.[91] Thus, these data suggest that the induction of Bnip3- and Nix-dependent mitophagy is a separately activated process that is independent of Bax/Bak and the mPTP.

It is still unclear exactly how Bnip3 and Nix mark mitochondria for removal by autophagosomes. It has been suggested that the Nix-dependent loss of $\Delta\psi m$ plays an important role in targeting the mitochondria to autophagosomes for clearance during erythroid maturation.[92] Sandoval *et al.*[92] found that treatment with compounds that caused mitochondrial depolarization restored mitophagy in $Nix^{-/-}$ cells. We found that Bnip3 selectively induced degradation of proteins involved in oxidative phosphorylation.[89] Thus, it is possible that the impairment in the electron transport chain and reduced ATP

synthesis serve to target the mitochondria for autophagy. We also recently found that Bnip3-mediated mitophagy involves recruitment of Parkin to mitochondria prior to their autophagy.[93] Parkin is an E3 ubiquitin ligase that is selectively recruited to dysfunctional mitochondria to promote their removal by autophagy.[94] In addition, Nix and Bnip3 can directly interact with autophagy proteins microtubule-associated protein 1 light chain 3 (LC3) and gamma-aminobutyric acid receptor-associated protein (GABARAP) on the autophagosome,[89,95] which might also serve to tether the mitochondrion to the autophagosome.

9. Role of the mPTP in mitophagy

The opening of the mPTP at the onset of reperfusion is synonymous with cardiac cell death. However, opening of the mPTP has also been implicated in the selective removal of damaged mitochondria.[96–98] In the heart, starvation-induced mitophagy was found to be reduced in CypD-deficient hearts compared with wild-type mice.[99] Cyclosporine A, a known inhibitor of the mPTP, has also been demonstrated to inhibit upregulation of autophagy following reperfusion.[29] Although Bnip3 induced opening of the mPTP,[86] we found that the presence of CsA had no effect on Bnip3-mediated mitophagy in cardiac myocytes.[90] Also, Bnip3 was still a potent inducer of autophagy in mouse embryonic fibroblasts (MEFs) isolated from the CypD$^{-/-}$ mice. CypD$^{-/-}$ MEFs lack a functional mPTP and are resistant to opening induced by hydrogen peroxide. These studies suggest that the role of mPTP in mitophagy is context dependent.

10. Mitochondrial dynamics and mitophagy

It has become clear that mitochondrial dynamics play an important role in regulating mitophagy. The link between mitochondrial morphology and mitophagy was initially established when Twig *et al.*[100] demonstrated that mitochondrial fission produced fragments with different membrane potential. The mitochondrial fragment with higher membrane potential had a higher chance of re-fusion, while the fragment with lower membrane potential was subjected to autophagy.[100] Gomes *et al.*[6] subsequently demonstrated that fused mitochondria were spared from starvation-induced autophagy. Since then, many studies have reported that inhibition of mitochondrial fission or induction of fusion abolished mitophagy in cells.[67,93] We recently reported that Drp1-mediated mitochondrial fission is required for mitophagy by Bnip3 in adult cardiac myocytes and inhibition of fission or induction of fusion inhibited mitophagy.[93] It is still unknown why mitochondria must undergo fission before removal by autophagosomes. However, it is possible that mitochondria are too large to be engulfed by autophagosomes and fission will produce smaller fragments that can more easily be engulfed by the autophagosomes or fission is required to segregate dysfunctional mitochondria prior to removal by autophagy. Interestingly, since fission is a prerequisite for mitophagy, it is possible that too much fission will also lead to excessive mitophagy and subsequent cell death.

11. Conclusion

It is clear that mitochondrial dysfunction is a major contributor to loss of myocytes during myocardial ischaemia and subsequent reperfusion. With a reduced number of myocytes, the heart is no longer able to sustain contractility and heart failure develops. This is a major clinical problem and development of novel therapeutic interventions to reduce myocardial cell death following infarction is ongoing. Since mitochondria play key roles in regulating life and death of the cardiac myocytes, future therapies should be aimed at preserving or restoring mitochondrial function in the myocytes. Mitochondrial activations of necrosis and apoptosis are both known contributors to cell death in the reperfused myocardium. Mitochondrial dynamics and mitophagy are also important processes involved in regulating both cell death and survival in the myocardium. Thus, these pathways might represent novel therapeutic targets to preserve mitochondrial function in the reperfused myocardium. However, many important questions regarding the effect of modulating these processes in the heart remain to be answered. A better understanding of the complex mechanisms associated with mitochondrial dysfunction is necessary to identify potential novel targets and therapeutic strategies to preserve mitochondrial function and cell viability in the reperfused myocardium.

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