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Review

The role of mitochondrial fusion and fission in the process of cardiac oxidative stress

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Summary. Mitochondria are the energy suppliers in the cell and undergo constant fusion and fission to meet metabolic demand during the cell life cycle. Well-balanced mitochondrial dynamics are extremely important and necessary for cell survival as well as for tissue homeostasis. Cardiomyocytes contain large numbers of mitochondria to satisfy the high energy demand. It has been established that deregulated processes of mitochondrial dynamics play a major role in myocardial cell death. Currently, cardiac mitochondrial cell death pathways attract great attention in the cell biology and regenerative medicine fields. Importantly, mitochondrial dynamics are tightly linked to oxidative stress-induced cardiac damage. This review summarizes molecular mechanisms of mitochondrial fusion and fission processes and their potential roles in myocardial cell death triggered by oxidative stress. Advances in understanding the effect of both normal and abnormal mitochondrial dynamics on heart protection will lead to significant improvement of therapeutic discoveries.

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Introduction

Mitochondria are two-layer membrane structure organelles that continuously fuse, divide, generate ATP and provide energy for most cells (de Brito and Scorrano, 2008, 2009). Each human heart contracts approximately 38 million times per year (Li and Liu, 2018). Due to the high energy demand of the heart, mitochondria occupy 30-35% of the cardiomyocyte volume and play vital roles in regulating cellular biological processes (Kubli and Gustafsson, 2012; Chandhok et al., 2018; Li and Liu, 2018). A shift toward fission may lead to mitochondrial fragmentation, observed in quiescent cells, while a shift toward fusion will result in the formation of large mitochondrial networks, found in metabolically active cardiomyocytes (Youle and van der Bliek, 2012). Many studies have indicated that mitochondria can respond to metabolic or environmental stresses and mediate cell metabolism (Bach et al., 2003, Shaughnessy et al., 2014; Wan et al., 2018). However, mitochondrial dysfunction would activate mechanisms leading to different types of cell death, including autophagy and apoptosis (Lemasters et al., 1998; Luongo et al., 2017). Furthermore, defects in mitochondrial dynamics are associated with various human cardiovascular disorders such as ischemia reperfusion injury, heart failure and abnormal heart

aging (Archer, 2013; Vasquez-Trincado et al., 2016; Li and Liu, 2018). A series of mitochondrial fusion and fission regulatory proteins have been found to play important roles in deciding cell survival or death (Bach et al., 2003; Westermann, 2010; Ikeda et al., 2014). Importantly, the myocardial tissue is greatly susceptible to oxidative stress-induced heart pathologies. Abnormalities in mitochondrial fusion and fission can cause oxidative stress leading to impairment of cardiac function. In this review, we discuss the role of mitochondrial fusion and fission in the process of cardiac oxidative stress under physiological and pathological conditions.

Mitochondrial fusion and fission proteins

Mitochondrial fusion and fission processes are mediated by the dynamin-family guanosine triphosphatases (GTPases) (Lackner et al., 2009). It has been well established that dynamin-related protein 1 (Drp1), mitochondrial fission 1 protein (Fis1), mitofusins (Mfn1 and Mfn2) and optic atrophy 1 (Opa1) are the important regulators of mitochondrial dynamics (Fig. 1) (Bach et al., 2003; Ikeda et al., 2014). These proteins are normally conserved from yeast to human (Abdelwahid et al., 2007, 2011). The fusion and fission of mitochondria is mainly regulated by dynamics of a double-layered membrane. Modulation of mitochondrial morphology requires various proteins related to the dynamin motor GTPases that control fusion and fission of inner and outer membranes of the mitochondria. During the fission process, Drp1 translocates from the cytoplasm to the outer mitochondrial membrane. The mitofusion proteins contain trans-membrane domains

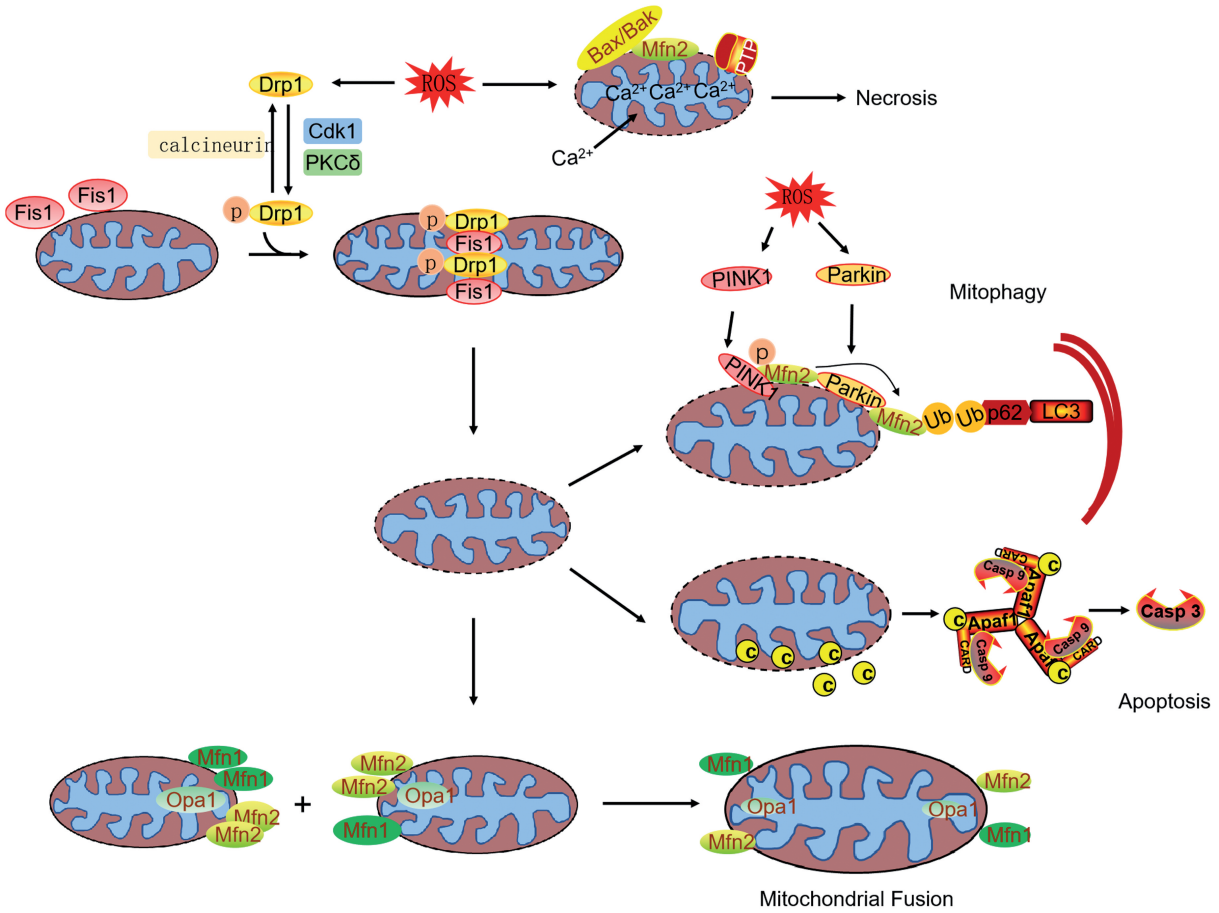

Fig. 1. Mitochondrial dynamics under physiological conditions, involving the processes of mitochondrial fission, mitochondrial fusion, mitophagy and programmed cell death. Both Fis1 and Drp1 are key players for mitochondrial fission. Fis1 is anchored on the outer mitochondrial membrane. Drp1 is recruited to the mitochondria upon phosphorylation by Cdk1 or PKCδ to promote the fission process. Calcineurin could dephosphorylate Drp1. Intracellular ROS promote the Drp1 phosphorylation process. The damaged mitochondria could be separated through mitochondrial fission process and cleared by mitophagy. Parkin and Pink1 are major molecules for mitophagy initiation. Phosphoubiquitination of Mfn2 mediates autophagosome formation by interacting with p62/LC3. Moreover, excessive mitochondrial fission promotes the release of cytochrome C and apoptosis. Mfn2 could also co-operate with Bax/Bak and promote necrosis induced by ROS and calcium overload. Mfn1 and Mfn2 are two major mitofusin proteins that mediate the fusion of the outer mitochondrial membrane. Opa1 is mainly localised in the inner mitochondrial membrane and promotes fusion of the inner mitochondrial membrane. The functional role of Opa1 in mitochondrial fusion can be dependent on Mfn1.

and promote membrane fusion by forming the oligomers through hydrolysis of GTP domains (Zorzano et al., 2010; Chan, 2012; Picard et al., 2015).

Mitochondrial fission is necessary and is important for providing the cells with enough mitochondria (Mitra, 2013). After translocation to the mitochondrial membrane, Drp1 forms spirals, which constrict to sever both the inner and outer mitochondrial membranes (Fig. 1). Phosphorylation of Drp1 is crucial for mediating mitochondria fission (Chang and Blackstone, 2007). In the cardiomyocytes, a series of protein kinases are involved in the regulation of Drp1 phosphorylation, such as the Cdk1 and PKCδ (Zaja et al., 2014). It has been shown that calcineurin can dephosphorylate Drp1 thus reducing its accumulation in mitochondria, as well as inhibiting Drp1-mediated activation of the mitochondrial fission (Wang et al., 2011). In addition, it has been demonstrated that excessive mitochondrial fission is the leading cause of myocardial cell death under oxidative stress conditions (Estaquier and Arnoult, 2007). Drp1 phosphorylation and its mitochondrial translocation were shown to be critical determinants of apoptosis induction (Frank et al., 2001). Many studies showed that mitochondrial fission in cardiomyocytes is mediated by Drp1 which promotes the release of cytochrome c from the mitochondria leading to subsequent apoptosis (Estaquier and Arnoult, 2007; Anzell et al., 2018). Inhibition of Drp1 by a dominant-negative form of Drp1, Drp_{K38A}, blocks the release of cytochrome c and inhibits cell death (Frank et al., 2001). It has also been shown that the fission of mitochondria could promote the mitophagy process, which degrades the damaged mitochondria of cardiomyocytes under normal conditions (Frank et al., 2012). However, excessive autophagy induced by mitochondrial fission accelerates myocardial cell death during ischemia/reperfusion (I/R) injury (Zepeda et al., 2014; Dorn, 2016).

Fis1 is another type of mitochondrial fission protein and is located in the outer mitochondrial membrane. Fis1 participates in mitochondrial fission through an interaction that recruits Drp1 from the cytosol to the mitochondria. Fis1 is a limiting factor in mitochondrial fission and the number of Fis1 molecules on the mitochondrial surface determines the fission frequency (Yoon et al., 2003). Fis1 is also involved in the apoptosis process that is associated with various diseases (Lee et al., 2004). Triggering mitochondrial fragmentation by Fis1 overexpression could induce potent cytochrome c release and apoptosis (Lee et al., 2004). The apoptotic protein Bcl-x_L has been shown to inhibit cytochrome c release and cell death but it did not appear to prevent mitochondrial fragmentation (Finucane et al., 1999; Sheridan et al., 2008). These results indicate that mitochondrial fragmentation could alter mitochondrial outer-membrane permeability leading to subsequent cytochrome c release (James et al., 2003). Moreover, inhibition of Fis1 significantly decreased the extent of apoptotic cell death, which is greater than that induced by Drp1 downregulation (Lee et al., 2004; Loson et al., 2013). In addition, knockdown of human Fis1 does not have an influence on the distribution of Drp1 in mitochondria, and deletion of two Fis1 homologous genes in *Caenorhabditis elegans* does not produce a strong mitochondrial fission phenotype, which suggests that additional pathways of Drp1 recruitment exist in metazoans (Westermann, 2010). In the mammalian cell, there are three structurally distinct classes of recruitment factors on the mitochondrial outer membranes: Fis1, Mff, and the two related proteins MiD49 and MiD51 (Gandre-Babbe and van der Bliek, 2008; Palmer et al., 2011). Fis1-mediated mitochondrial fission was also demonstrated to participate in myocardial cell death (Wang et al., 2012). Interestingly, miR-484 played a crucial role in cardioprotection through targeting Fis1 (Wang et al., 2012).

Mitochondrial fusion is a particularly complex process. This is because mitochondria are two-layer membrane structure organelles and mitochondrial fusion involves the coordination of various membranous activities (Chen et al., 2003). It is generally accepted that both mammalian Mfn1 and Mfn2 mediate mitochondrial outer membrane fusion while Opa1 regulates the mitochondrial inner membrane fusion (Fig. 1). However, the molecular mechanisms of mitochondrial fusion are still under debate, mainly due to the controversy about the structure of mitofusin. For example, Mfn2 consists of four parts: a conserved GTP domain, two coiled-coil domains (first heptad repeat (HR1), second heptad repeat (HR2)) and transmembrane domain(s). In a commonly accepted model, Mfn2 contains two neighboring lipophilic transmembrane domains, leading to conserved GTP domain, HR1 domain and HR2 domain facing the cytoplasm (Yu et al., 2018). However, the novel research by Mattie et al. (2018) underlined only a single transmembrane domain present in Mfn2 structure. Therefore, conserved GTP domain and HR1 domain are exposed to the cytosol while HR2 domain protrudes into the mitochondrial intermembrane space (IMS). The IMS environment is sensitive to changes in oxidative stress, e.g. excessive reactive oxygen species (ROS) production (Giacomello and Scorrano, 2018), which may have an effect on the state of HR2 domain, further influencing the mitochondrial fusion.

The role of Mfn2 in myocardial cell death is still elusive. As a mitofusion protein, Mfn2 functions to antagonize the mitochondrial fission process. Mfn2 mediates the outer mitochondrial membrane fusion according to various models of Mfn2 structures (Yu et al., 2018). In addition to its role in mitochondrial fusion, Mfn2 plays other signaling roles in the cell (Yu et al., 2018). In particular, Mfn2 serves as a receptor of Parkin during Parkin-mediated mitophagy (Chen and Dorn, 2013). Knockout of Mfn2 in mouse cardiomyocytes is capable of inhibiting mitophagy (Chen and Dorn, 2013). In addition, it has been demonstrated that Mfn2 could regulate the mitochondria and endoplasmic reticulum/sarcoplasmic reticulum (ER/SR) tethering in addition to mediating Ca^{2+} cross-talk, which in turn are

regulated by Ca^{2+} uptake (de Brito and Scorrano, 2009). It should be noted that Mfn2 cannot be deemed to a tether since it is unclear whether Mfn2 serves a negative or positive regulator in ER/SR-mitochondria tethering (Cosson et al., 2012; Filadi et al., 2015, 2017; Li et al., 2015; Wang et al., 2015b; Leal et al., 2016; Naon et al., 2016; Harmon et al., 2017; Naon et al., 2017; Cieri et al., 2018).

It has been reported that Mfn2 knockout can protect adult mice from cardiac I/R injury, hypoxia or H_2O_2 treatment (de Brito and Scorrano, 2008; Papanicolaou et al., 2011). Other investigators indicated that that Mfn2 promotes cardiomycyte apoptosis under oxidative stress through activation of the Ras-PI3K-Akt signaling pathway (Shen et al., 2007). However, there is evidence suggesting that knockout of Mfn2 resulted in cardiomyopathy in mice, which was mainly due to impairment of the mitophagy (Gong et al., 2015). The ongoing controversies about Mfn2 functions could be explained as follows. On one hand, mitophagy helps clear the damaged mitochondria and relieve cellular stress under normal conditions. However, when cells experience acute damage, such as myocardial infarction or I/R injury, excessive mitophagy and various forms of cell death may occur (Gong et al., 2015). On the other hand, Mfn2 promotes mitochondrial Ca^{2+} overload and subsequent myocardial cell death through mediating the SR-mitochondria tethering. It has been found that Mfn2 could promote cell necrosis through mediating mitochondrial fusion in combination with Bax (Whelan et al., 2012). Overall, Mfn2 appears to play a complex role in myocardial cell death.

It has been proposed that Mfn1 plays a more dominant role in fusing the mitochondria compared to Mfn2 (Santel et al., 2003), mainly due to its higher activity and greater tethering efficiency (Ishihara et al., 2004). Interestingly, the pro-fusion protein Opa1 promotes the inner mitochondrial membrane fusion through cooperation with Mfn1 (Cipolat et al., 2004). It has been reported that Opa1 is the only mitochondria-shaping protein associated with the inner mitochondrial membrane (Frezza et al., 2006). The bulk of cytochrome c is known to localize in the cristae of the inner mitochondrial membrane. Opa1 has been found to protect the cardiomyocyte from apoptosis by controlling cristae remodeling and restricting cytochrome c release (Frezza et al., 2006). In addition, Opa1 was thought to play an important role in modulating cell necrosis under oxidative stress through interacting with Bnip3, an important myocardial necrosis regulator (Landes et al., 2010).

Pink1 and Parkin were shown to be involved in mitochondrial dynamics (Fig. 1), however, their role in different models is not consistent (Poole et al., 2008; Yu et al., 2011). Loss of Pink1 and Parkin has opposite effects on mitochondrial morphology in organisms (Yu et al., 2011). For instance, Pink1 and Parkin can promote mitochondrial fission in Drosophila, whereas they act as pro-fusion factors in human cells (Poole et al., 2008). Thus, it needs to be further explored how Pink1, Parkin and mitochondrial dynamics can be integrated in mitochondrial quality control pathways in human cells.

Mitochondrial dynamic and programmed cell death

The major role of cardiac mitochondria is to provide the contracting cardiomyocytes with a continuous energy supply in the form of ATP. In response to the different metabolic or environmental stress signals, mitochondria may become producers of ROS and release pro-death proteins, resulting in disrupted ATP synthesis and activation of cell death pathways (Fig. 1) (Quinlan et al., 2012). As mentioned above, the mitochondrial fission machinery is associated with ROS generated during cardiomyocyte apoptotic and other types of cell death. Moreover, Drp1 mutation can inhibit the occurrence of mitophagy (Kageyama et al., 2014). Thus efficient therapeutic strategies could target molecular and biological mitochondrial dynamics and ROS mechanisms at different levels.

While mitochondrial outer membrane permeabilization (MOMP) plays a dominant role in apoptosis, early opening of inner membrane mitochondrial permeability transition pore (MPTP) is considered to be a key event in primary necrosis (Whelan et al., 2012). Upon cellular stress, the outer mitochondrial membrane is unable to expand and mitochondrial swelling results in rupture and release of pro-apoptotic proteins into the cytosol (Elmore, 2007). The MPTP is an inner mitochondrial membrane channel that allows passage of molecules up to 1.5 kDa (Xu et al., 2019). Opening of the pore causes a collapse of the proton gradient and electrical potential across the inner mitochondrial membrane, leading to disruption of oxidative phosphorylation and cessation of ATP synthesis. In addition, opening of the MPTP causes an influx of solutes and water followed by swelling of the inner membrane, precipitating mitochondrial dysfunction (Xu et al., 2019).

MPTP opening is the major cause of myocardial cell death during the cardiac I/R injury. Interestingly, modulation of mitochondrial dynamics is closely related to the opening of MPTP (Halestrap, 2010). However, the composition of the MPTP remains elusive. To date, the only protein identified to be an essential component in the latter process is cyclophilin D (Fayaz et al., 2015). In addition, the MPTP is a major contributor of myocardial I/R injury, and inhibitors of the MPTP can reduce infarct size in *ex vivo* I/R. Moreover, cyclophilin D deficient mice are resistant to I/R injury (Di Lisa et al., 2011). Interestingly, cells lacking cyclophilin D are still sensitive to apoptotic stimuli, suggesting that MPTP opening is not required for induction of apoptosis via the mitochondrial pathway (Nakagawa et al., 2005).

It is generally believed that Bax (Bcl-2 associated X) and Bak (Bcl-2 antagonist killer 1) could induce activation of MOMP and apoptosis (Fig. 1) (Pena-Blanco and Garcia-Saez, 2018). In response to apoptotic

stimuli, Bax can be recruited on mitochondria from cytosol followed by Drp1 translocation, further inducing release of cytochrome c and activation of caspases (Huang et al., 2007; Chandhok et al., 2018). It has been recently reported that absence of Bax and Bak can reduce infarct size during I/R injury, suggesting that deletion of Bax and Bak significantly inhibits MPTP opening and necrosis (Whelan et al., 2012). Moreover, the mechanisms of Bax-regulated necrosis or apoptosis are different (Korytowski et al., 2011; Whelan et al., 2012). Mitochondrial fragmentation has been reported in Bax/Bak knockout cells, exhibiting similar fusion defects seen in cells lacking Mfn2 where Mfn2 was found to localize at site of Bax punctate foci on the mitochondrial outer membrane (Karbowski et al., 2002). It has been suggested that Bax-driven mitochondrial fusion is critical for MPTP opening and necrosis (Whelan et al., 2012). Thus, mitochondrial morphology is tightly related to mechanisms controlling the intrinsic cascades of cell death.

Mitochondrial dynamics and cardiac oxidative stern

The fusion and fission processes significantly contribute to mitochondrial quality control and function and ROS production. Mitochondrial activity is the major intracellular source of ROS generated at complex I and III of the respiratory chain. In addition, the mitochondria are important targets for the damaging effects of ROS. Indeed, the accumulation of dysfunctional and abnormal mitochondria in the diseased heart is tightly associated with excessive production of ROS (Newsholme et al., 2007).

Mitochondrial fission and ROS production

Mitochondrial fission pathways contribute to ROS pathways in various pathological conditions. Considerable attention is given to research focusing on high glucose-induced myocardial cell death where mitochondrial fission is the major cause of mitochondrial ROS production (Yu et al., 2008). Mitochondria undergo rapid fission with an increase in ROS formation when exposed to a high concentration of glucose. However, some experiments showed that suppression of ROS increase did not prevent mitochondrial fission under high glucose conditions. In hyperglycemia, researchers found that the mitochondrial fission process is necessary for high glucose-induced respiration and ROS overproduction, and that inhibition of mitochondrial fission decreased the ROS level (Yu et al., 2006). In cardiomyopathy induced by obesity and cardiac insulin resistance, Drp1 mediated mitochondria fission promotes insulin resistance, which is related to ROS production (Chang et al., 2019). In the doxycycline (DOX) toxicity-induced myocardial cell death, excessive mitochondrial fission was observed and a series of proteins involved in the regulation of mitochondrial dynamics played important roles in cardiomyocyte damage (Govender et al., 2014).

Apoptosis repressor with caspase recruitment domain (ARC) is a cardio-protective protein and is significantly decreased during oxidative stress-induced myocardial damage (Xu et al., 2019). It has been shown that ARC is engaged in the mitochondrial fission process and reduces DOX-induced myocardial apoptosis through inhibition of mitochondrial fission (Wang et al., 2015a). Recently, ARC has been shown to be involved in oxidative stress-induced myocardial necrosis through inhibition of MPTP opening (Xu et al., 2019). P21 is an important cell circle regulator that can be transcriptionally regulated by P53 (Wan et al., 2018). P21 is an important target of miR-499 and plays an emerging role in mitochondrial fission process, although the detailed mechanism of P21 in regulating mitochondrial fission is still elusive (Wan et al., 2018). During myocardial ischemia/reperfusion injury, excessive mitochondrial fission was observed and was found to be involved in the burst of mitochondrial ROS that leads to the opening of MPTP. It should be emphasized that excessive mitochondrial fission is responsible for the ROS production during the myocardial ischemia/reperfusion injury (Ong et al., 2010; Webster, 2012). Moreover, inhibition of mitochondrial fission and ROS production after myocardial infarction can improve the long-term cardiac function (Disatnik et al., 2013). This evidence indicates that mitochondrial fission is closely associated with ROS production and may thus represent an effective therapeutic target for treatment of cardiac diseases.

Mitochondrial fusion and ROS

Mitochondrial fusion proteins could inhibit excessive mitochondrial fission under stress conditions. Indeed mitochondrial fusion proteins could agonize the mitochondrial fission process and prevent ROS production. However, certain mitochondrial fusion proteins could also promote the production of ROS. Several studies showed that Mfn2 may promote myocardial cell death under ROS stress conditions (Shen et al., 2007; Papanicolaou et al., 2011; Hall et al., 2016). Mfn2 knockout mice showed a protective effect against myocardial ischemia reperfusion injury. Further studies showed that Mfn2 could modulate mitochondrial calcium signaling and promote the MPTP opening (Papanicolaou et al., 2011). Interestingly, Mfn2 was found to promote the MPTP opening through regulating mitochondrial fusion (Whelan et al., 2012). These results indicate complex roles of mitochondrial dynamics in myocardial cell death and survival. Therefore, future studies are needed to illustrate the clear relationship between mitochondrial dynamics and ROS production during progression of cardiac pathology.

Mitochondrial dynamics and ROS in cell therapy

Mitochondrial dynamics are tightly linked to ROS in

modulating stem cell homeostasis, differentiation and heart development. ROS are known to be important for the stem cell response to changes in oxygen level (Brunelle et al., 2005). Physiological generation of ROS may trigger the perinuclear mitochondrial network that controls cell fate (Facucho-Oliveira et al., 2007; Prigione et al., 2010; Mandal et al., 2011). While genetic ablation of Drp1 is lethal on embryonic day 12.5 (Manczak et al., 2012), the deletion of Mfn1 and Mfn2 in the hearts of mouse embryos was found to lead to abnormal cardiac development and lethality on embryonic days E9.5–10.5 (Chen et al., 2011). Various mitochondrial dynamics genes including Dnm1l, Mtp18, Opa1, Dap3, Mfn2, mitofilin have been shown to change expression levels to promote cardiogenesis (Smirnova et al., 2001; Mukamel and Kimchi, 2004; Chen and Chan, 2005; John et al., 2005; Tondera et al., 2005; Misaka et al., 2006). Indeed, in mouse embryonic cell models and cell cultures, fusion of the mitochondria contributes to cascades regulating differentiation of mesodermal cells into cardiac cells (Kasahara et al., 2013). Moreover, ablation of Mfn2 or Opa1 in mouse ESCs disrupts differentiation of ESCs into cardiac cells (Kasahara et al., 2013). Impairment of mitochondrial fusion and fission genes in stem cell-derived cardiomyocytes can modulate expansion of the cardiogenic phenotype. In particular, cell-to-cell communications facilitate passage of mitochondria and promote acquiring cardiomyogenic phenotype by progenitor cells (Koyanagi et al., 2005). Mitochondrial dynamics genes have been shown to interact with calcineurin to modulate the Notch1 pathway that directs differentiating cardiac cells (Kasahara et al., 2013). Cardiomyocytes may depend on mitochondrial fusion regulators including mitofusins and Opa1 via OXPHOS metabolism (Kasahara et al., 2013; Fang et al., 2016). Previous investigators have used iPSCs and demonstrated a role for mitochondrial dynamics in regulating pluripotency induction (Vazquez-Martin et al., 2012; Son et al., 2015). Loss of Mfn1 and Mfn2 promotes somatic cell reprogramming to pluripotent stem cells (Son et al., 2015). On the other hand, mitochondrial fission machinery was shown to regulate pluripotency and pluripotent cell reprogramming via a Drp1 dependent mechanism (Cho et al., 2015; Choi et al., 2015; Kim et al., 2015). Mesenchymal stem cells (MSCs) are known to differentiate into a multitude of cell lineages (Colter et al., 2000). MSCs promote repair of damaged tissue via donor cell engraftment, stimulation of molecular networks as well as providing healthy mitochondria (Shi et al., 2010; Bernardo and Fibbe, 2012; Islam et al., 2012). MSC therapy has been shown to rescue the abnormalities of mitochondrial dynamics and augments host metabolism (Newell et al., 2018). Although various studies have revolutionized the understanding of the link between mitochondrial dynamics, ROS and stem biology, the molecular characterization of these aspects is only in its infancy. Future cardiac studies are required to clarify regulatory mechanisms implicating mitochondrial fusion and fission in ROS generation by stem cells.

Abnormal mitochondrial fusion/fission in heart diseases

Mitochondrial dynamics are essential for healthy cardiac tissue. While a shift toward mitochondrial fusion promotes formation of interconnected mitochondria, a shift toward mitochondrial fission could mediate fragmentation and dysfunctions of mitochondria in various human heart diseases (Youle and van der Bliek, 2012; Archer, 2013). Thus, identifying the regulatory networks of mitochondrial fusion and fission may provide promising information for preventing cardiac disease pathologies. It has been reported that abnormalities in mitochondrial dynamics are primarily responsible for the progression in myocardial I/R injury, cardiac hypertrophy, heart failure, drug-induced cardiac toxicity and heart aging (Fig. 2, Table 1) (Yu et al., 2008; Chen et al., 2009; Ikeda et al., 2014, 2015; Reddy, 2014; Vasquez-Trincado et al., 2016; Lesnefsky et al., 2017; Anzell et al., 2018; Wan et al., 2018). It is generally accepted that when the cell experiences oxidative stress, mitochondrial fusion helps repair small amounts of mitochondrial damage, facilitating complementation between damaged mitochondria. Mitochondrial fusion is beneficial to mitigate the effects of environmental stress by mixing the contents of partially damaged mitochondria with other mitochondria (Youle and van der Bliek, 2012). On the other hand, mitochondrial fission could produce new mitochondria and separate the seriously damaged mitochondria. Moreover, when cells experience high levels of oxidative stress, mitochondrial fission could be increased, e.g. during removal of seriously damaged mitochondria or occurrence of cell apoptosis (Youle and van der Bliek, 2012). In the cardiac system, it has been reported that promoting mitochondrial fusion or inhibiting mitochondrial fission play a significant role in protecting the heart against I/R injury (Table 1) (Ong et al., 2010). The decreased expression of Opa1 has been found in failing (rat and human) hearts (Ikeda et al., 2015). Moreover, Opa1 deficiency was also related to increased sensitivity to I/R injury (Le Page et al., 2016). It has been demonstrated that overexpression of Opa1 protected cardiomyocytes from injury by enhancing mitochondrial fusion, suggesting that fused mitochondria are more tolerant to I/R injury (Table 1) (Varanita et al., 2015). Besides, mitochondrial fission mediates both cell apoptosis and cell necrosis (Vasquez-Trincado et al., 2016). Based on the relationships between mitochondrial fission and cardiomyocyte death, numerous studies focused on the inhibiting mitochondrial fission as the potential regulator of cardiac diseases (Ong et al., 2010; Ikeda et al., 2015; Maneechote et al., 2018). Ong et al. (2010) have reported that the mitochondrial fission inhibitor-1 (Mdivi-1), antagonist of Drp1, could decrease infarction size during I/R injury. It was found that inhibition of Drp1 by Mdivi-1 can increase mitochondrial length and

inhibit mitochondrial fission, decreasing cardiomyocyte apoptosis and programmed necrosis (Maneechote et al., 2018) (Table 1). In addition, Bax and Bak exerted broad effects on cell death and were clearly related to changes of normal and abnormal mitochondrial fusion and fission diseases (Whelan et al., 2012). Recent reports indicated that cardiac microRNAs may be therapeutically useful for I/R injury conditions (Wang et al., 2011). For example, miR-499 downregulates Drp1 and prevents mitochondrial fission (Table 1). Moreover, the overexpression of miR-499 had few influences on normal hearts but protected the heart from myocardial infarction (Wang et al., 2011). The above mentioned data strongly support the relationship between mitochondrial dynamics and cardiovascular diseases and highlight the possibility of modulating the mitochondrial fusion and fission in generating potential therapies (Table 1).

Conclusion

Over the past years, researchers have documented that maintenance of cardiomyocyte viability and function are largely dependent on mitochondrial fusion and fission processes. It has been established that Drp1,

Table 1. Potential application of mitochondrial dynamic proteins in the treatment of cardiac diseases.

Pathological Condition	ROS Levels	Mode of Cell Death	Experimental Observation	Therapeutic Application	Reference
Cardiac ischemia/ reperfusion injury	Acute ROS production	Apoptosis, Necrosis and autophagic cell death	Drp1 phosphorylation; Drp1 mitochondrial trans-localization	Inhibition of mitochondrial fission using Mdiv-1, dynasore, P110	Vasquez-Trincado et al., 2016; Anzell et al., 2018
Left ventricular hypertrophy	Mild ROS production and maladaptive hypertrophy	Apoptosis and Autophagic cell death	Down-regulation of Mfn2 or Opa1	Activation of Mfn2 or Opa1 to prevent left	Vasquez-Trincado et al., 2016
Drug induced cardiac toxicity	Acute ROS production	Apoptosis and Necrosis	Down-regulation of Mfn1 and Mfn2; Drp1 mitochondrial trans-localization	Inhibition of mitochondrial fission using Mdiv-1, dynasore, P110; Or Activation of Mfn2 and Mfn1	Govender et al., 2014; Reddy, 2014
Heart failure	Acute ROS production or mild ROS production	Apoptosis and Necrosis	Down-regulation of Opa1 and Mfn2; Up-regulation of Drp1	Inhibition of mitochondrial fission using Mdiv-1, dynasore, P110; Or Activation of Mfn2 and Mfn1	Chen et al., 2009, Reddy, 2014
Aging heart	Mild ROS production	Apoptosis, Necrosis and Autophagic cell death	Down-regulation Mfn1 in age-related cardiac hypertrophy; Opa1-deficinecy and loss of cristae with aging	Manipulation of mitochondrial dynamic proteins to improve the impaired mitochondrial function with aging	Ikeda et al., 2014

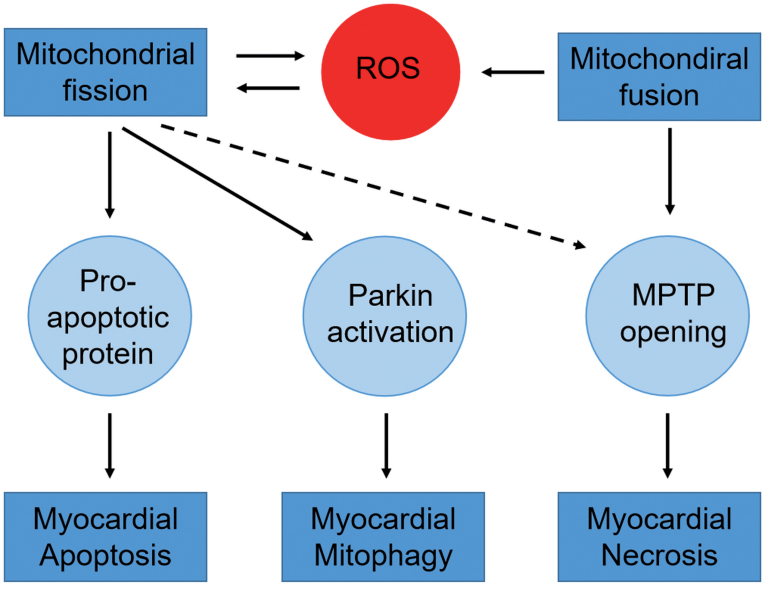

Fig. 2. The relationship of mitochondrial dynamics with different modes of myocardial cell death. Mitochondrial fission is associated with cardiomyocyte apoptosis and mitophagy. Mitochondrial fission could also promote the opening of MPTP and myocardial necrosis under some pathological conditions. ROS could promote mitochondrial fission; and excessive mitochondrial fission could also produce intracellular ROS reciprocally.

Fis1, Mfn1/2 and Opa1 are the key mitochondrial fission/fusion proteins within cardiac cells. Other associated proteins like Pink1 and Parkin are also involved in mitochondrial dynamics roles in cell survival and death. Abnormalities of mitochondrial dynamics are the main causes of cellular death under oxidative stress in the heart. Although, mitochondrial dynamics correlates with ROS level and cardiomyocyte fate, the direct role of mitochondrial dynamics in ROS production awaits future research. Further experimental studies are required to dissect the precise molecular mechanisms involving mitochondrial dynamics in various deleterious effects during cardiac oxidative stress. To conclude, advancement of research exploring the regulatory networks connecting mitochondrial fusion and fission, ROS and cell death in the heart should improve therapeutic approaches for cardiovascular diseases.

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