Journal of Molecular and Cellular Cardiology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Review article

Molecular mechanisms mediating mitochondrial dynamics and mitophagy and their functional roles in the cardiovascular system

Yoshiyuki Ikeda [a,b], Akihiro Shirakabe [a], Christopher Brady [a], Daniela Zablocki [a], Mitsuru Ohishi [b], Junichi Sadoshima [a,*]

[a] Department of Cell Biology & Molecular Medicine, NJ Medical School, Rutgers University, USA
[b] Department of Cardiovascular Medicine and Hypertension, Graduate School of Medical and Dental Science, Kagoshima University, Japan

ARTICLE INFO

Article history:
Received 5 August 2014
Received in revised form 12 September 2014
Accepted 16 September 2014
Available online xxxx

Keywords:
Mitochondria
Fusion
Fission
Mitophagy
Drp1

ABSTRACT

Mitochondria are essential organelles that produce the cellular energy source, ATP. Dysfunctional mitochondria are involved in the pathophysiology of heart disease, which is associated with reduced levels of ATP and excessive production of reactive oxygen species. Mitochondria are dynamic organelles that change their morphology through fission and fusion in order to maintain their function. Fusion connects neighboring depolarized mitochondria and mixes their contents to maintain membrane potential. In contrast, fission segregates damaged mitochondria from intact ones, where the damaged part of mitochondria is subjected to mitophagy whereas the intact part to fusion. It is generally believed that mitochondrial fusion is beneficial for the heart, especially under stress conditions, because it consolidates the mitochondria's ability to supply energy. However, both excessive fusion and insufficient fission disrupt the mitochondrial quality control mechanism and potentiate cell death. In this review, we discuss the role of mitochondrial dynamics and mitophagy in the heart and the cardiomyocytes therein, with a focus on their roles in cardiovascular disease. This article is part of a Special Issue entitled 'Mitochondria'.

Contents

1. Introduction . . . 0
2. Mitochondrial dynamics: fission and fusion . . . 0
3. Physiological role of mitochondrial dynamics . . . 0
4. Elimination of damaged mitochondria by autophagy . . . 0
5. Mitochondrial dynamics, mitophagy and cardiovascular disease . . . 0
6. Perspectives . . . 0
Sources of funding . . . 0
Disclosures . . . 0
References . . . 0

1. Introduction

Mitochondria are essential sources of energy in cells and, thus, are particularly important intracellular organelles in ventricular cardiomyocytes, which require regular frequent contraction. However, mitochondria are also major intracellular sources of reactive oxygen species (ROS), which are produced as byproducts of ATP synthesis through the electron transport chain or through upregulation of ROS producing enzymes, such as Nox4, or downregulation of anti-oxidants. Although ROS produced in mitochondria can act as second messengers to trigger adaptive processes [1–3], mitochondrial damage caused by pathological stress often leads to production of excessive ROS, which develops into a vicious cycle of oxidative stress and mitochondrial damage and spreads rapidly into the intact mitochondria within the same cell through a mechanism known as ROS-induced ROS release [4]. Eventually, the mitochondria release cytochrome c into the cytosol by increasing outer mitochondrial membrane (OMM)

* Corresponding author at: Department of Cell Biology and Molecular Medicine, Cardiovascular Research Institute, New Jersey Medical School, Rutgers, The State University of New Jersey, 185 South Orange Ave., MSB G609, Newark, NJ 07103, USA. Tel.: +1 973 972 8619; fax: +1 973 972 8919.
E-mail address: sadoshju@njms.rutgers.edu (J. Sadoshima).

http://dx.doi.org/10.1016/j.yjmcc.2014.09.019
0022-2828/

permeability and activate apoptosis, a cellular suicide mechanism, in order to avoid a series of catastrophic events. In the presence of severe stress, such as prolonged cardiac ischemia, mitochondrial permeability transition pore (mPTP) opening abrogates the H^+ gradient, which is essential for ATP synthesis, and cells undergo necrosis [5]. Histological analysis shows that the heart contains a large volume of mitochondria, indicating that cardiomyocytes rely heavily upon mitochondrial oxidative metabolism as a source of energy supply [5]. In order to prevent the vicious cycle of mitochondrial damage and ROS production, myocardial cells appears to have intrinsic quality control mechanisms by which they protect themselves from minor injury and maintain their function, such as mitochondrial autophagy [6]. In this review, we discuss the role of mitochondrial dynamics in the cardiovascular system, with a special emphasis on the function of Drp1, a molecule involved in fission and mitophagy, in cardiomyocytes.

2. Mitochondrial dynamics: fission and fusion

Mitochondria are dynamic organelles that constantly undergo fusion and fission, collectively termed "mitochondrial dynamics", to adapt to changes in the cellular environment and to maintain their function [6]. Fission produces small spherical mitochondria, whereas fusion produces tubular or elongated-shaped mitochondria [6]. Disruption of mitochondrial fission leads to formation of fused mitochondria, whereas that of fusion leads to formation of small and divided mitochondria, suggesting that the morphological changes in mitochondria are balanced by opposing events. It should be noted that the continuous occurrence of mitochondrial fusion and fission has not been tracked in normal adult ventricular cardiomyocytes and, thus, their roles have been inferred based on pharmacological or genetic manipulation. Although we discuss molecular mechanisms controlling fission and fusion of mitochondria in the following section, almost all works have been conducted using non-cardiac cell types. Thus, caution should be exercised regarding whether the findings from other cell types can be applicable to adult ventricular cardiomyocytes.

Mitochondrial dynamics are regulated by several different guanosine triphosphatases (GTPases), which are well-conserved among yeast, flies, and mammals [7–9]. Mitofusin 1 and 2 (Mfn1 and Mfn2) and optic atrophy 1 (Opa1) are involved in regulating mitochondrial fusion in the outer and inner mitochondrial membranes, respectively. On the other hand, mitochondrial fission is regulated by mitochondrial fission 1 (Fis1) and mitochondrial fission factor (Mff), localized on the outer mitochondrial membrane, and by recruitment of dynamin-related protein 1 (Drp1) from the cytosol to mitochondrial fission sites, where it interacts with Mff to promote fission [7–9].

Mitochondrial fission is initiated by constriction of mitochondria at points where endoplasmic reticulum (ER) tubules surround mitochondria and mark mitochondrial division sites, which is followed by recruitment of Drp1 to the mitochondria [10]. Although the mechanism by which initial constriction of mitochondria occurs remains unknown, subsequent constriction and scission processes are mediated by Drp1 [11,12]. Drp1 is an ~80 kDa dynamin GTPase superfamily protein with an N-terminal GTPase domain thought to provide mechanical force, a dynamin-like middle domain, a variable domain, and a C-terminal GTPase effector domain (GED) [13,14]. Drp1 is abundantly expressed in the skeletal muscle, heart, kidney, and brain of adult humans. Drp1 primarily exists in the cytosol as a dimer/tetramer. During mitochondrial fission, Drp1 translocates from the cytosol to mitochondria, where it oligomerizes around and constricts the mitochondria, thereby leading to severing of the mitochondrial membrane by GTP hydrolysis [15–19]. Drp1 is regulated by a variety of post-translational modifications, including phosphorylation, S-nitrosylation, small ubiquitin-like modifier (SUMO)-ylation, and ubiquitination, in response to diverse cellular stimuli [20–29]. Mitochondrial localization of Drp1 is positively regulated by protein kinase A, calcineurin, PUMA, Bax/Bak, ceramide, and O-linked-β-N-acetylglucosamine (O-GlcNAcylation) modification, and is negatively regulated by miR-499 and Pim1 [28,30–34]. Drp1 lacks a mitochondrial targeting sequence. Therefore, Drp1-mediated mitochondrial fission requires a receptor to promote Drp1 recruitment to the outer mitochondrial membrane [35,36]. Fis1 is a 17-kDa protein that anchors to the outer mitochondrial membrane with its N-terminal multiple tetratricopeptide repeat motif exposed to the cytoplasm and was thought to serve as a receptor for Drp1 [37]. Indeed, overexpression of Fis1 in cells promotes mitochondrial fission. However, Fis1 knockdown affects neither recruitment of Drp1 to mitochondria nor fission in HeLa cells and HCT 116 cells [38]. Mff is a C tail-anchored protein [39]. In cells with Mff knockdown, mitochondrial localization of Drp1 is decreased and Drp1 is dispersed in the cytoplasm. In contrast, Mff overexpression induces mitochondrial fission with increased Drp1 recruitment to mitochondria, suggesting that Mff acts as a Drp1 receptor to promote mitochondrial fission [38].

Mitochondrial fusion is regulated by GTPase dynamin-family proteins, including Mfn1, Mfn2, and Opa1 [40,41]. Mfn1 and Mfn2 localize to the outer mitochondrial membrane and share approximately 77% sequence similarity. Mfn1 and Mfn2 have both redundant and distinct functions to promote mitochondrial fusion by forming either homotypic or heterotypic complexes [42]. Downregulation of Mfn1 or Mfn2 shows fragmentation of mitochondria in MEF cells, suggesting that Mfn1 and Mfn2 have non-redundant functions to promote mitochondrial fusion [42]. It should be noted that although cardiac-specific Mfn1 KO mice exhibited fragmented mitochondria [43], cardiac-specific Mfn2 KO mice exhibited enlarged mitochondria in the heart [44,45], suggesting that Mfn1 and Mfn2 have distinct roles in regulating mitochondrial fusion in the mouse heart. Mfn2 also regulates shape and function of the endoplasmic/sarcoplasmic reticulum [46,47]. Mfn2 plays an important role in mediating autophagosome-lysosome fusion in cardiomyocytes [45]. Furthermore, Mfn2 is phosphorylated by PTEN-induced kinase 1 (PINK1) and serves as a receptor for Parkin during mitophagy [48].

Opa1 is a dynamin-related protein that localizes to and tethers the inner mitochondrial membrane to maintain the integrity of the cristae. Overexpression of Opa1 promotes the formation of a branched network of elongated mitochondria, whereas downregulation of Opa1 induces fragmentation of mitochondria and disorganization of the cristae structures [49,50]. Opa1 cannot tubulate and fuse mitochondria lacking the outer membrane protein Mfn1, but that is not the case in those lacking Mfn2 [41]. Decreases in the electrochemical potential across the inner mitochondrial membrane or proapoptotic stimuli induce paraplegin-dependent proteolytic cleavage of Opa1, thereby stimulating mitochondrial fragmentation [51].

3. Physiological role of mitochondrial dynamics

Mitochondrial dynamics are crucial for compensating for mitochondrial damage and for eliminating mitochondria with unrecoverable damage through fusion and fission, respectively. The physiological role of fission is believed to be segregation of unrecoverable damaged mitochondria in order to maintain overall mitochondrial quality and to preserve the health of the mitochondrial network [6,52]. Mitochondrial fission divides the mitochondrion into functionally uneven daughter mitochondria. In order to achieve asymmetric separation of mitochondria, there may be a sorting event preceding fission, but molecular mechanisms mediating the sorting are currently unknown. The daughter mitochondrion with a normal membrane potential can undergo fusion with other mitochondria. However, the daughter mitochondrion with decreased membrane potential is unable to fuse with other mitochondria, resulting in elimination by mitophagy (Fig. 1) [52,53]. Mitochondrial fission is also necessary to redistribute mitochondrial DNA and transport mitochondria to daughter cells during mitosis [54]. Drp1-mediated fragmentation of mitochondria protects HeLa cells from ceramide-induced, Ca^{2+}-mediated apoptosis, suggesting that fission may segregate intact mitochondria from dysfunctional ones [55].

Please cite this article as: Ikeda Y, et al, Molecular mechanisms mediating mitochondrial dynamics and mitophagy and their functional roles in the cardiovascular system, J Mol Cell Cardiol (2014), http://dx.doi.org/10.1016/j.yjmcc.2014.09.019