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Cardiac mitochondrial dynamics: miR-mediated regulation during cardiac injury

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ABSTRACT

Mitochondrial integrity is indispensable for cardiac health. With the advent of modern imaging technologies, mitochondrial motility and dynamics within the cell are extensively studied. Terminally differentiated and well-structured cardiomyocytes depict little mitochondrial division and fusion, questioning the contribution of mitochondrial fusion proteins (Mitofusin 1/2 and Optic Atrophy 1 protein) and fission factors (Dynamin-like protein 1 and mitochondrial fission 1 protein) in cardiomyocyte homeostasis. Emerging evidences suggest that alterations in mitochondrial morphology from globular, elongated network to punctate fragmented disconnected structures are a pathological response to ensuing cardiac stress and cardiomyocyte cell death, bringing forth the following question, "what maintains this balance between fusion and fission?" The answer hinges upon the classical "junk" DNA: microRNAs, the endogenous non-coding RNAs. Because of their essential role in numerous signaling pathways, microRNAs are considered to play major roles in the pathogenesis of various diseases. Mitochondria are not exempted from microRNA-mediated regulation. This review defines the importance of mitochondrial structural integrity and the microRNA-mitochondrial dynamics tandem, an imminent dimension of the cardiac homeostasis network. Elucidating their coordinated interaction could spur RNA-based therapeutics for resuscitating functional mitochondrial population during cardiovascular disorders.

1. Introduction

With the escalating prevalence of Cardiovascular Disorders (CVDs) worldwide, burgeoning efforts are put forth to unravel the molecular and pathophysiological characteristics of disease progression. Statistics of The American Heart Association in 2016, report that more males died of CVDs in 2013 than females [1] and their 2015 statistics postulate that CVD-associated global mortality could escalate from 17.3 million of 2012 to 23.6 million by 2030 [2].

Heart failure (HF) is a cumulative result of pressure or volume overload, ischemia, infarction, or other cardiomyopathies. It is associated with reduced cardiac output and capacity to pump blood to other organs [3]. Left ventricular hypertrophy is major predictor of developing heart failure, shifting from initial adaptive to decompensation in the presence of progressive pathological signals [4,5]. It is executed by a number of interlinked and dysregulated signaling cascades triggered during neurohormonal changes, ischemic environment, inflammation, ionic imbalance and several other factors. At the molecular level, it is associated with the development of cardiac fibrosis [6], expression of fetal genes and the down-regulation of adult isoforms of these fetal genes essential for cardiac functions [7]. Increasing evidence points to cardiac mitochondrial damage and dysfunction as a potential source for metabolic defects and bioenergetics crisis in the failing heart [8] [9].

Mitochondria are gregarious subcellular organelles orchestrating cell fate. Originating as a proteobacterium invasion during eukaryotic evolution [10], mitochondria have established efficient and mutual alliance in eukaryotes. Like the brain [11], the heart has a high demand for ATP (Adenosine triphosphate) for contractility and 20–40% of the myocardial mass is composed of mitochondria [12]. "Mitochondrial dynamics" is a collective term for mitochondrial movement, the counterbalanced fusion-fission events, and mitophagy. This phenomenon is highly cell-type specific [13]. While mitochondria of fibroblasts, neurons and other cells have actively moving mitochondria, it was believed, until now, that those of cardiomyocytes are restricted by the myofilaments and cytoskeleton [14]. A recent study has shown that fusion activity is frequent in cultured adult cardiomyocytes, enabling exchange of matrix contents between mitochondria. However, the kinetics are slower compared to neonatal cardiomyocytes and other cell types [15]. The fusion and fission events are regulated by specific GTP-dependent proteins that allow a tubule reticular networking morphology *via* fusion of the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) fusion with redistribution of matrix components [16]. Another check

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point in this dynamic mitochondrial motion within the cell is structured by endogenous, small noncoding RNA, the microRNAs (miRNA/miRs). These are ~21 nucleotides long RNAs involved in post-transcriptional gene regulation through translational repression or mRNA degradation by binding 3′-UTR (Untranslated Region) of target messenger RNAs (mRNAs) [17]. Numerous comprehensive reviews have summarized, in detail, the mechanism of miRNA biogenesis and its mode of translational repression [18–21]. Recent researchers have identified a discrete pool of nuclear and mitochondrial genome-encoded microRNAs within the organelle (mitomiR/mt-miR) [22,23] pertinent to its health and disease. In this review, we address the implications of differential mitochondrial dynamics and their regulatory miRNAs during cardiac pathogenesis. Understanding this interlink between mitochondrial structure and function may help identify novel therapeutic targets for cardiac disorders.

2. Core machinery of mitochondrial fusion and fission

Mitochondria are mobile, semi-autonomous subcellular organelles existing in interconnected network through opposing fusion and fission episodes. The structural integrity of mitochondria helps to maintain of their function in persistently changing physiological conditions, contributing to mitochondrial genome integrity [24] and turnover number [25]. The precise mechanism that triggers and executes mitochondrial membrane fusion and motility within a cell remains ambiguous. One proposed model for mitochondrial fusion event is the "kiss and run" interplay by Liu et al., whereby transient fusion allows partial exchange of integral membrane proteins, stimulating mitochondrial motility. However, a complete fusion allows redistribution of both soluble and membrane components after OMM fusion, kissing of IMM, and complete IMM fusion [26].

Mitofusins 1 and 2 (MFN1/2) are integral OMM proteins involved in fusion of outer membrane by acting *in trans* and forming homo and/or hetero dimers through their coiled-coil domain, tethering juxtaposed mitochondria together [27]. The Optic Atrophy Protein 1 (OPA1) is critical for the fusion of IMM and cristae remodelling [28] while the GTP-dependent Dynamin Related Protein-1 (DRP1) directs fission by forming helical loop structures around the mitochondria [29,30] and pinching them apart. Mitochondrial fission protein 1 (Fis1) [31], mitochondrial fission factor (Mff) [32], and mitochondrial dynamics protein 49 and 51 kDa (MiD49 and MiD51, respectively) [33] are speculated OMM receptors for DRP1.

Table 1 enlists other accessory proteins involved in regulation of mitochondrial dynamics. The shift in equilibrium towards fusion generates a network of long, tubular mitochondria that are beneficial to metabolically active cells while a shift in the direction of fission generates small, globular, fragmented mitochondria that are generally a precursor for apoptosis. Although cardiomyocytes are highly metabolically active, adult cardiomyocytes display fragmented mitochondria [13,34], raising the question of the role of these mitochondrial fusion/fission proteins.

3. Mitochondrial remodelling in cardiac disorders

Adequate contraction of normal adult heart requires a supply of ~30 kg of ATP, *i.e.* 70 times more than the actual weight of the organ itself [35] and a dynamic Ca^{2+} environment. The myocardium utilizes variety of substrates like fatty acids (FA), glucose, lactate, ketones and amino acids for ATP generation. 60–90% of the total ATP is produced from B-oxidation of fatty acids during resting state of adult heart [36] and the efficient phosphotransfer enzyme system ensures its availability for utilization. Mitochondrial creatine kinase allows for energy transfer by functionally coupling with ANT (Adenine nucleotide translocase) [37], ensuring local supply of ADP and bridging mitochondrial inner and outer membranes [38,39].

Early stages of pathological hypertrophy or myocardial dysfunction are associated with shift in substrate utilization from fatty acid to glucose oxidation. It appears protective as the ratio of ATP generation to oxygen consumption is increased. This compensation, however, is lost with the progression of heart failure [40,41]. Some clinical studies report that abnormalities in FAO does not occur during early stages of hear failure but dramatic reduction in FAO enzymes is observed during advanced or end-stage heart failure [42–44]. Contrastingly, one study reported reduced glucose uptake and increased fatty acid uptake in human heart failure condition [45].

The cardiac contractile unit, sarcomere, is dependent on Ca^{2+} fluxes across sarcoplasmic reticulum (SR), mitochondria and cytoplasm. SR is the primary calcium storage-release organelles that permeate myofibrils and associate with mitochondria, ensuring that calcium release and reuptake are closely monitored. Mitochondrial membrane consists of ion exchange complexes sensitive to ionic homeostatic imbalances. Ca^{2+} enters into the mitochondria *via* the mitochondrial calcium uniporter (MCU), a multimeric Ca^{2+} selective complex activated by mitochondrial membrane potential and finite cytoplasmic Ca^{2+} ($[Ca^{2+}]_c$) [46]. The complex is regulated by MICU1 [47,48], MICU2 (Mitochondrial Ca^{2+} uptake] [49], MCUR1 (mitochondrial regulator 1) [50,51], EMRE (essential MCU regulator) [52] and MCUb [53]. Research by Luongo et al. [54] using MCU-null cardiomyocytes show that ablation of MCU promotes resistance to Ca^{2+} overload and swelling while deletion in other cells of the heart, like fibroblasts and endothelial cells, worsened the injury [54].

Conventional transmission electron microscopy typifies cardiac mitochondria into subsarcolemmal (SSM) and interfibrillar mitochondria (IFM). The repeating network cluster of IFM interweaves myofibrils in parallel to cardiomyocytes, while SSM are tucked beneath the sarcolemma membrane [55]. Because of the highly structured architecture of the myocardium, cardiac mitochondria display restricted movement, unlike those of other cell types [14]. Majority of the cardiac mitochondrial fusion/fission events have been studied in immortalized cell types or neonatal cardiomyocytes [56–60] rather than adult cardiomyocytes. An interesting research by Huang et al. [61] report that adjacent mitochondria communicate with each other by "kissing" and remote mitochondria by "nanotunneling", accounting for local communication in adult cardiomyocytes. Fusion was more frequent in mitochondria of perinuclear region not restricted by the sarcomeres than interfibrillar mitochondria [61].

However, ironically, this organization of mitochondria in cardiomyocytes also warrants lethality with accumulating ROS (Reactive Oxygen Species) and damaged mitochondria. Electron microscopic images of end stage post-ischemia conditioned mice hearts portray reduced SSM and atypical heaps of IFM beneath the membrane [62] while

Table 1
Mitochondrial dynamics regulatory/accessory proteins.

Mammalian protein	Function	Reference
Stomatin-like protein 2 (SLP-2)	Hyperfusion	[155]
Mitochondrial fission process 1 (MTFP1)	Fission	[156]
BCL-2 associated X-protein (Bax)	MFN2 complex assembly	[157]
PTEN-induced putative kinase (PINK1)	Mitophagy	[158]
Membrane associated ring finger (C3HC4) (MARCH5)	Ubiquitin ligase	[159]
Mitochondrial-anchored protein ligase (MAPL)	SUMO Ligase	[160]
Presenilin-associated rhomboid-like protein (PARL)	OPA1 processing	[161]
SUMO1/Sentrin specific eptidase 5 (SENP5)	SUMO protease	[162]