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Review

Defective insulin signaling and mitochondrial dynamics in diabetic cardiomyopathy

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ABSTRACT

Diabetic cardiomyopathy (DCM) is a common consequence of longstanding type 2 diabetes mellitus (T2DM) and encompasses structural, morphological, functional, and metabolic abnormalities in the heart. Myocardial energy metabolism depends on mitochondria, which must generate sufficient ATP to meet the high energy demands of the myocardium. Dysfunctional mitochondria are involved in the pathophysiology of diabetic heart disease. A large body of evidence implicates myocardial insulin resistance in the pathogenesis of DCM. Recent studies show that insulin signaling influences myocardial energy metabolism by impacting cardiomyocyte mitochondrial dynamics and function under physiological conditions. However, comprehensive understanding of molecular mechanisms linking insulin signaling and changes in the architecture of the mitochondrial network in diabetic cardiomyopathy is lacking. This review summarizes our current understanding of how defective insulin signaling impacts cardiac function in diabetic cardiomyopathy and discusses the potential role of mitochondrial dynamics.

1. Introduction

Diabetic cardiomyopathy (DCM) is defined as the presence of left ventricular (LV) dysfunction beyond that which can be accounted for by arterial hypertension, coronary artery disease (CAD) or evidence of any other structural cardiac disease in individuals with diabetes [1,2]. From a cellular standpoint, changes in the energetics of the heart have been proposed to contribute to the development of DCM [3]. Because mitochondria are the major source of ATP to meet the energy demands of the heart, it has been proposed that mitochondrial dysfunction is an underlying cause of metabolic disorders and insulin resistance-associated heart disease [4]. Extensive experimental and clinical evidence indicates that mitochondrial dynamics (fusion, fission and mitophagy) are essential for mitochondrial quality control and sustained function in several tissues [5] including the cardiovascular system [6,7]. In addition, prolonged hyperglycemia and insulin resistance in individuals with T2DM can lead to dramatic changes in cardiac mitochondrial dynamics and function [8–10]. Studies by Battiprolu *et al.* showed that mice developed myocardial insulin resistance in response to high fat-diet (HFD) characterized by a down-regulation of IRS1 activity, decreased AKT signaling, and a shift from glucose to fatty acid (FA) utilization [11]. Mice with cardiomyocyte-selective ablation of the insulin receptor (CIRKO) showed defects in FA and pyruvate metabolism and reduced tricarboxylic acid flux associated with mitochondrial uncoupling. Thus, altered insulin signaling in the heart may contribute directly to mitochondrial dysfunction in the setting of obesity and T2DM [4]. Our group recently demonstrated a link between insulin and the regulation of mitochondrial dynamics, particularly mitochondrial fusion, in neonatal rat cardiomyocytes [12], raising new questions regarding the interplay among mechanisms affecting the architecture of

Abbreviations: DCM, Diabetic cardiomyopathy; T2DM, Type 2 diabetes mellitus; LV, Left ventricular; CAD, Coronary artery disease; HFD, High fat-diet; FA, Fatty acid; IRS1, Insulin receptor substrate 1; CIRKO, Cardiomyocyte-specific knockout of the insulin receptor; Mnf1, Dynamin-related GTPase mitofusin 1; Mnf2, Dynamin-related GTPase mitofusin 2; Opa-1, Optic atrophy protein 1; IMM, Inner mitochondrial membrane; OMM, Outer mitochondrial membrane; Fis1, Mitochondrial fission 1 protein; Drp1, Dynamin related protein 1; ROS, Reactive oxygen species; SOD, Superoxide dismutase; O-Glc-NAcylation, O-linked-β-N-acetylglucosamine modification; HF, Heart failure; mTOR, Mammalian target of rapamycin; NFκB, Nuclear factor kappa-light-chain-enhancer of activated B cells; Pink1, Serine/threonine kinase PTEN-induced putative kinase 1; Parkin, E3 ubiquitin ligase; AMPK, 5′ AMP-activated protein kinase; Drp1-CKO, Cardiomyocyte-specific Drp1 knockout; T1DM, Type 1 diabetes mellitus; LAMP1, Lysosomal-associated membrane protein 1; IRS2, Insulin receptor substrate 2; CIRS12KO, Cardiomyocyte-specific deletion of both IRS1 and IRS2

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the mitochondrial network in DCM. In this review, we will summarize the available evidence that links insulin signaling to DCM and discuss the potential functional roles of mitochondrial dynamics.

2. Diabetic cardiomyopathy and mitochondrial dynamics in the heart

Myocardial energy deficiency is closely related to the initiation and progression of various cardiac pathologies, such as those observed during insulin resistance and T2DM [13,14]. Under normal physiological conditions, the heart utilizes energy from substrates (FA and carbohydrates) based on metabolic demand and availability [15]; however, in the setting of insulin resistance, the myocardium's ability to use glucose as an energy source is reduced [16,17]. This change in substrate preference plays a critical role in the pathophysiology of DCM [11,18]. Mitochondria are highly abundant and represent 30% of cardiac cell volume, generating more than 90% of the intracellular ATP consumed by the heart [15,19]. Thus, many studies are currently focused on mitochondrial dysfunction as a causative factor in metabolic disorders and insulin resistance-associated heart diseases [20,21].

In mammalian cells the mitochondria are dynamic organelles that continuously change their morphology through fusion and fission events in response to intracellular circumstances, and changes in the balance of these processes have been implicated in different biological events such as cell division, apoptosis, autophagy, and metabolism [6,22]. Nonetheless, it is important to consider that evidence available in the literature about cardiac mitochondrial dynamics differs according to the type of cardiac cells analyzed. For example, mitochondria are distributed throughout the cytoplasm in a reticular network and are unrestricted in their movements in neonatal cardiomyocytes or immortal cardiac cell lines (H9c2, HL-1 cells). Conversely, in adult cardiomyocytes the mitochondria are located under the sarcolemma, around nuclei and between myofibrils, providing enough ATP for muscle contraction; however their ability to move is limited due to these cellular constraints [6,19]. Thus, mitochondrial division is estimated to only occur at an extremely low frequency in adult cardiomyocytes under physiological conditions [23]. However, despite the evidence mentioned above the proteins implicated in mitochondrial dynamics are present in the adult heart, suggesting that adult cardiac mitochondria have retained their ability to undergo fusion, fission and mitophagy (mitochondrial degradation), which are key processes associated with quality control of mitochondria, mitochondrial turnover and mitochondrial homeostasis in adult cardiomyocytes [19]. However, this is not the only task carried out by mitochondrial dynamics [5]. Recent studies have linked mitochondrial dynamics to the balance between energy demands and nutrient supply, suggesting that changes in mitochondrial morphology can act as a mechanism for energetic adaptation to changing cardiac metabolic necessities [24]. Indeed, alterations in mitochondrial structure and function have been linked to cardiovascular diseases [6] including DCM [25].

Despite the apparent correlation between the dysregulation of mitochondrial dynamics and myocardial energy deficiency, the precise mechanism of how this dysregulation contributes to the pathogenesis of DCM is still unclear. Moreover, current understanding of the importance of mitochondrial dynamics in the heart seems to contradict the long-standing paradigm in the mitochondrial field that cardiac mitochondria are relatively static [26]. Although recent *in vivo* observations support the presence of morphological changes, the question that still remains is whether these changes are cause or consequence of disease progression.

2.1. Diabetic cardiomyopathy and mitochondrial fission/fusion in the heart

In mammalian cells, the main regulators of mitochondrial fusion are the dynamin-related GTPases mitofusins (Mnf1 and Mfn2) specialized proteins localized on the outer mitochondrial membrane, and optic atrophy protein 1 (Opa-1), a protein localized on the inner mitochondrial membrane (IMM). On the other hand, the mitochondrial fission 1 protein (Fis1), localized on the outer mitochondrial membrane (OMM), and the cytoplasmic GTPase dynamin related protein 1 (Drp1) are involved in mitochondrial fission [24,27] (Fig. 1A).

Several studies have indicated that mitochondrial quality control plays a pivotal role in protecting the heart against stress, although the presence of fission and fusion has not been well documented in adult cardiomyocytes [26], extremely well organized cells in which mitochondrial movements are greatly restricted [19]. In this sense, initial studies were done with neonatal cardiomyocytes or with immortalized cardiac cell lines (H9c2, HL-1) [28,29] in which the mitochondrial network does not directly reflect that of an adult cardiomyocyte [19]. Chen *et al.* reported that the fusion/fission cycle would last 14–16 days in adult cardiomyocytes, being slower than that seen in neonatal cardiomyocytes [30], suggesting that mitochondrial dynamics is a process that depends on the cardiomyocyte cell architecture. Studies in non-cardiac cells [31–35] have shown that low expression of fusion proteins is associated with mitochondrial network fragmentation. However, Papanicolau *et al.* showed that mice harboring a cardiomyocyte-specific knockout of Mfn2 exhibited larger mitochondria [36]. This was also observed in Opa1 (+/−) mice, which accumulated large clusters of fused mitochondria with altered cristae [37]. Cardiomyocyte-specific Mfn1-null mice showed fragmentation of their mitochondrial network [38] and mice with an adult cardiomyocyte-specific conditional ablation of Mfn1/Mfn2 together developed a fragmented mitochondrial network and progressed to dilated cardiomyopathy [30]. Thus, mitochondrial dynamics is a complex process in the adult heart and may depend upon the specific cell architecture. Moreover, in terms of cardiac contractile function, increases in mitochondrial volume may directly impact the force developed by myofibrils [39], suggesting a direct link between mitochondrial morphology and cardiac contractile function. On the one hand, enhanced mitochondrial fragmentation may lead to cardiac disorders [24]; on the other hand, Ishihara *et al.* have shown that mitochondrial fission is required for neonatal cardiomyocyte development, participating in the formation of highly organized myofibrils and maintenance of uniformly active mitochondria with mitochondrial DNA (mtDNA) nucleoids in cardiomyocytes [40], suggesting that mitochondrial fission is a key process during heart development.

Studies specifically addressing the direct relationship between fission and fusion of cardiac mitochondrial and insulin resistance and T2DM are limited. However, we know that a major contributory factor linked to the onset of DCM is hyperglycemia-induced oxidative stress (Fig. 1B). In this context, *in vitro* data from Yoon's group suggest that hyperglycemia induces mitochondrial fragmentation in neonatal rat ventricular myocytes (H9c2 cells) [41]. Furthermore, in the same model Yu *et al.* also demonstrated that sustained hyperglycemia induces mitochondrial fission together with mitochondrial reactive oxygen species (ROS) production, which in turn promotes activation of a proapoptotic pathway [42]. These pathological changes could be prevented by transfecting the cells with a dominant-negative form of Drp1, DrpK38A, suggesting that mitochondrial fragmentation and dysfunction in the setting of hyperglycemia is Drp1-dependent [42]. Similarly, in a recent article, Watanabe *et al.* showed that Drp1 and ROS act synergistically to promote mitochondrial dysfunction and inhibit insulin signal transduction in H9c2 cells [43]. This effect could be partially reversed when these cells were treated with the superoxide dismutase (SOD) mimetic TMPyP [43]. In a different, but related model, Makino *et al.* showed mitochondrial fragmentation in coronary endothelial cells from murine diabetic hearts that was associated with reduced levels of Opa-1 and increased levels of Drp1 [44]. Studies in models of T2DM suggest that insulin resistance might contribute to reduced myocardial recovery after ischemia [45]. Interestingly, pretreatment of adult rat cardiomyocytes with Mdivi-1 (pharmacological inhibitor of Drp1) reduced cell death and protected the heart from ischemia/reperfusion injury [46]. Transfection of neonatal rat cardiomyocyte with Drp1K38A to inhibit fission was likewise protective [47].