New Molecular Insights of Insulin in Diabetic Cardiomyopathy

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Type 2 diabetes mellitus (T2DM) is a highly prevalent disease worldwide. Cardiovascular disorders generated as a consequence of T2DM are a major cause of death related to this disease. Diabetic cardiomyopathy (DCM) is characterized by the morphological, functional and metabolic changes in the heart produced as a complication of T2DM. This cardiac disorder is characterized by constant high blood glucose and lipids levels which eventually generate oxidative stress, defective calcium handling, altered mitochondrial function, inflammation and fibrosis. In this context, insulin is of paramount importance for cardiac contractility, growth and metabolism and therefore, an impaired insulin signaling plays a critical role in the DCM development. However, the exact pathophysiological mechanisms leading to DCM are still a matter of study. Despite the numerous questions raised in the study of DCM, there have also been important findings, such as the role of micro-RNAs (miRNAs), which can not only have the potential of being important biomarkers, but also therapeutic targets. Furthermore, exosomes also arise as an interesting variable to consider, since they represent an important inter-cellular communication mechanism and therefore, they may explain many aspects of the pathophysiology of DCM and their study may lead to the development of therapeutic agents capable of improving insulin signaling. In addition, adenosine and adenosine receptors (ARs) may also play an important role in DCM. Moreover, the possible cross-talk between insulin and ARs may provide new strategies to reverse its defective signaling in the diabetic heart. This review focuses on DCM, the role of insulin in this pathology and the discussion of new molecular insights which may help to understand its underlying mechanisms and generate possible new therapeutic strategies.

Keywords: insulin, diabetic cardiomyopathy, miRNAs, exosomes, adenosine, adenosine receptors

DIABETIC CARDIOMYOPATHY: PATHOPHYSIOLOGY AND CLINICAL FEATURES

Over the past two decades, the prevalence of type 2 diabetes mellitus (T2DM) has steadily increased as a consequence of higher rates of obesity, an increase in sedentary lifestyle, and changes in the environment. If unaddressed, nearly half a billion people will be burdened by this chronic disease in 2030 (Shaw et al., 2010). At one time, whether T2DM contributed to cardiac

dysfunction in diabetic patients was a matter of debate (Rubler et al., 1972). However, diabetic cardiomyopathy (DCM)—with a prevalence of 12% in T2DM patients (Bertoni et al., 2004)—is now recognized as a serious complication of T2DM resulting from sustained hyperglycaemia and hyperlipidemia that leads to increases in cardiac oxidative stress, inflammation, and myocardial fibrosis, as well as detrimental changes in Ca^{2+} handling and mitochondrial function (Bugger and Abel, 2014; Jia et al., 2016). Several signaling pathways converge in the development of DCM, and the goal of finding suitable therapeutic targets remains a challenge. From a clinical standpoint, DCM is defined as the presence of left ventricular (LV) dysfunction in patients with T2DM in the absence of arterial hypertension, coronary artery disease (CAD) or evidence of any other structural cardiac disease (Battiprolu et al., 2010, 2013; Maisch et al., 2011; Adeghate and Singh, 2014; Isfort et al., 2014). Its clinical presentation ranges from early-stage DCM, which is characterized by abnormal myocardial energy metabolism, diastolic dysfunction and reduced LV strain to overt heart failure (HF), associated with cardiomyocyte hypertrophy, myocardial fibrosis and cardiomyocyte death (Voulgari et al., 2010; Falcao-Pires and Leite-Moreira, 2012; Battiprolu et al., 2013; Dhalla et al., 2014; Go et al., 2014; Isfort et al., 2014).

The first stage of DCM is clinically asymptomatic, but is evidenced by a characteristic diastolic dysfunction (increased ventricular stiffness, left atrial enlargement, and elevated LV end-diastolic pressure) (Battiprolu et al., 2010). The prevalence of diastolic dysfunction is several times higher in T2DM patients than in sex- and age-matched populations and can impact up to 40% of T2DM patients (Poulsen et al., 2010). Several mechanisms contribute to ventricular stiffness in T2DM: for instance, sustained hyperglycemia promotes formation of advanced glycation end products (AGEs), which are particularly prone to cross-linking with extracellular proteins, such as collagen and elastin, altering their structural properties and impairing cardiac relaxation (Murarka and Movahed, 2010; Jia et al., 2016).

The second stage of DCM is characterized by cardiac remodeling, LV hypertrophy (LVH), and the emergence of clinical indications of HF (Ozasa et al., 2008); however, whether LV systolic dysfunction ultimately develops is a matter of debate. Although some authors have suggested that there is an association between systolic dysfunction and long-standing diabetes (Mbanya et al., 2001), most publications indicate that resting systolic function is normal in the majority of T2DM patients, as assessed by LV ejection fraction (LVEF), although some subjects may exhibit systolic impairment during exercise (Vered et al., 1984). However, LVEF can be a poor approximation of cardiac contractility and may not detect minor changes in myocardial function (Sonoyama et al., 2007). These abnormalities in myocardial function correlate closely with changes in Ca^{2+} handling, caused in part by AGEs that decrease mechanical efficiency of the sarcomere and impair electromechanical coupling, ultimately leading to reduced contractility (Bugger and Abel, 2014). In addition, the activity of the sarco(endo)plasmic reticulum Ca^{2+}-ATPase isoform 2 (SERCA2), the ryanodine receptor (RyR) and Na^+/Ca^{2+}

exchanger (NCX1) are markedly reduced in DCM, leading to diminished release of Ca^{2+} from endoplasmic reticulum stores and slowed replenishment of these stores during relaxation. These changes, in turn, lead to elevated end-diastolic Ca^{2+} levels and a progressive decrease in the frequency of Ca^{2+} transients (Zhao et al., 2014). There is evidence that hyperglycemia may activate caspase-3 directly (Cai et al., 2002) causing apoptosis of cardiomyocytes and increased interstitial fibrosis (Fiordaliso et al., 2000). Thus, increased cardiomyocyte apoptosis may be a critical step in the transition from compensated to decompensated HF (Frustaci et al., 2000). It has also been proposed that insulin-resistance in DCM may be related to increased risk of cardiac LVH or HF (Abel et al., 2012). Altered myocardial insulin signaling is a hallmark of DCM (Battiprolu et al., 2012; Bugger and Abel, 2014; Jia et al., 2016); however, the molecular mechanisms that have been proposed to contribute to the development of insulin resistance in DCM are not fully understood. In this review, we summarize the current knowledge about cardiac insulin signaling, with emphasis on new molecular insights regarding its role in DCM.

INSULIN SIGNALING IN THE NORMAL HEART

An appreciation for the role of insulin in glucose homeostasis began with its discovery in 1922 (Banting et al., 1922). The ability of insulin to induce glucose uptake (Levine et al., 1949) by activating specific cell surface receptors on target tissues (Kahn et al., 1974; Ebina et al., 1985; Ullrich et al., 1985), thus, triggering the translocation of the glucose transporter 4 (GLUT4) to the plasma membrane (Cushman and Wardzala, 1980; Suzuki and Kono, 1980), is well documented. However, much remains to be learned regarding both the physiological and pathophysiological impact of insulin signaling in the diversity of tissues, such as adipose tissue and skeletal muscle, upon which it acts (Leto and Saltiel, 2012). Although a comprehensive understanding of the mechanisms underlying insulin action in the heart is only just emerging, there is abundant evidence that insulin impacts cardiac metabolism by influencing a wide range of cellular processes, such as glucose transport, glycolysis, glycogen synthesis (Abel, 2004), cardiac hypertrophy, protein synthesis, lipid metabolism (Belke et al., 2002). Moreover, insulin also participates in myocardial contractility (Fu et al., 2014), protection against ischemia-induced necrotic death (Diaz et al., 2015), autophagy (Riehle et al., 2013), and cell survival, either directly or through the closely associated insulin-like growth factor-1 (IGF-1) (Troncoso et al., 2014).

Cardiac Metabolism

Insulin-stimulated glucose uptake in cardiomyocytes is mediated primarily through mobilization of glucose transporter 4 (GLUT4). Basal cardiac glucose uptake is mediated by glucose transporter 1 (GLUT1), however, contraction-mediated activation of GLUT4 translocation may also contribute significantly to myocardial glucose uptake (Abel, 2004). We have reported that GLUT4-mediated glucose uptake
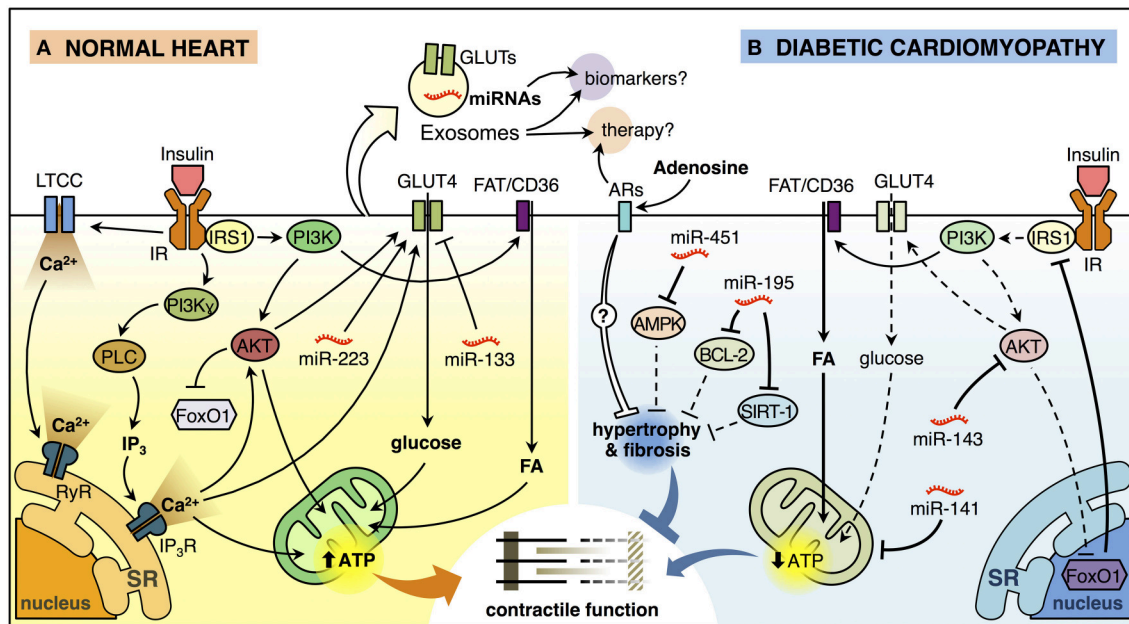
FIGURE 1 | Insulin signaling in the normal heart and diabetic cardiomyopathy. (A) Insulin binding to its receptor (insulin receptor, IR) promotes a biphasic $[Ca^{2+}]_i$ response in cardiomyocytes. The first phase of insulin-dependent $[Ca^{2+}]_i$ increase involves extracellular Ca^{2+} influx through L-type calcium channel (LTCC) and activation of ryanodine receptor (RyR) Ca^{2+} channel which in turn releases Ca^{2+} from the sarcoendoplasmic reticulum (SR). The second phase involves a non-inhibitory G protein coupled receptor ($G\beta\gamma$ subunits) that activates downstream effectors, including phosphatidylinositol 3-kinase (PI3K)γ and phospholipase C (PLC). PLC generates inositol-1,4,5-trisphosphate (IP_3) that opens the IP_3-ligated Ca^{2+}channels in the SR. These mechanisms trigger the translocation of glucose transporter 4 (GLUT4) from an intracellular store to the plasma membrane (PM) and increase glucose uptake. Insulin also induces the translocation of the fatty acid (FA) transporter FAT/CD36 to the PM trough PI3K activation promoting the FA uptake. Increased lipid and glucose uptake increase mitochondrial oxidative metabolism generating adenosine triphosphate (ATP) that supports myocardial contractile function. Insulin activation of AKT, downstream of PI3K, inactivates the transcription factor FoxO1. In addition, miR-223 stimulates and miR-133 reduces GLUT4-dependent glucose uptake. **(B)** Insulin signaling is deficient in diabetic cardiomyopathy (DCM). FoxO1-dependent downregulation of insulin receptor substrate 1 (IRS1) and AKT are associated with reduced insulin-induced GLUT4 translocation to the PM and lower glucose uptake. At the same time, cardiomyocytes accumulate lipids, reducing mitochondrial oxidative metabolism and promoting mitochondrial uncoupling, which in turn, affects cardiomyocyte function. Also, miR-143 inhibits insulin signaling, whereas miR-141 may impact mitochondrial function and the production of ATP. On the other hand, miR-451 is associated with inhibition of AMPK, while miR-195 inhibits the expression of BCL-2 and SIRT-1. Both of these miRNAs are associated with hypertrophy, which will consequently generate contractile dysfunction. Adenosine may have a paracrine effect on cardiomyocytes, which could reduce hypertrophy and myocardial fibrosis through the activation of adenosine receptors (ARs). Exosomes can ferry miRNAs and proteins such as GLUT4 from one cell to another and therefore, they can be used as potential biomarkers or therapeutic agents/targets for DCM.

in cardiomyocytes can be activated through a G protein, phosphatidylinositol 3-kinase (PI3K)γ and 1,4,5-inositol-triphosphate (IP_3) receptor (IP_3R) signaling axis, leading to generation of a cytoplasmic Ca^{+2} signal that mobilizes GLUT4 (Contreras-Ferrat et al., 2010; **Figure 1A**). Although the mechanisms of insulin regulation of cardiac GLUT4 are not yet fully defined, recent data show that constitutive activation of PI3K and AKT can reduce glucose uptake in mouse cardiomyocytes through a mechanism that does not involve GLUT4 translocation, suggesting that insulin impacts GLUT4 activity beyond the ability to regulate translocation to the plasma membrane (Zhu et al., 2013a). Moreover, in the heart of mice with a cardiomyocyte-selective deletion of GLUT4 (G4H$^{-/-}$), there was a reduction in the levels of insulin-responsive aminopeptidase (IRAP) which was related to impaired GLUT4 endosomal trafficking (Abel et al., 2004), further suggesting that GLUT4 may play a role in regulating glycolysis beyond its ability to transport glucose (Tian and Abel, 2001), evidence that highlight the critical role of GLUT4 and

GLUT1 in the insulin-mediated glucose transport on cardiac metabolism.

Cardiac Contractility

Several animal studies have shown the cardioprotective effects of insulin (Das, 2003). In addition, clinical studies have suggested an association between insulin resistance and HF (Arnlov et al., 2001; Ingelsson et al., 2005). However, excessive cardiac insulin signaling can exacerbate systolic dysfunction induced by pressure overload in mice (Shimizu et al., 2010). Cardiac specific insulin receptor knockout (CIRKO) hearts show a mild reduction in cardiac function (Belke et al., 2002) and developed more severe contractile dysfunction compared with control hearts in response to isoproterenol exposure, a combined β_1- and β_2-adrenergic receptor (βAR) agonist (McQueen et al., 2005). Fu et al. proposed an interesting model for crosstalk between the insulin receptor (IR) and β_2AR signaling in the heart (complex IR/β_2AR) that could impact cardiac contractility (Fu et al., 2014). In their model, increased IR signaling promotes dissociation of the IR/β_2AR

complex, leading to an impaired contractile response to β_2AR subsequent activation. This effect was seen in isolated neonatal and adult cardiomyocytes, as well as Langendorff-perfused hearts (Fu et al., 2014). Moreover, CIRKO mice, which are deficient for IR signaling, display defective electrical impulse propagation, elevated K^+ extracellular concentrations (Punske et al., 2004), and diminished levels of several key K^+ channel components involved in ventricular repolarization (Lopez-Izquierdo et al., 2014). Moreover, insulin can diminish markers of cytosolic oxidative stress (Li et al., 2012) and improve impaired cardiac electrical activity during hypoxia in G4H$^{-/-}$ hearts (Sohn et al., 2013). Altogether, this evidence suggests a link between insulin signaling and the electrical function in the heart independent of its activation of GLUT4-dependent glucose uptake.

Cardiac Growth

In the adult heart, myocardial growth is achieved primarily through hypertrophic expansion of individual cardiomyocytes (Pasumarthi and Field, 2002). Heart size increases during post-natal development in response to several stimuli (Olson and Schneider, 2003), and these responses can be classified either as "pathological" or "physiological" (Richey and Brown, 1998). Insulin is considered an anabolic hormone that promotes protein synthesis, cell growth and hypertrophy (Shiojima et al., 2002). CIRKO mice are characterized by a 30% reduction in heart size (Belke et al., 2002), consistent with the effects observed in cardiomyocytes with reduced PI3K/AKT signaling, where insulin-stimulated hypertrophy is blunted (Shioi et al., 2000; Shiojima et al., 2002). G4H$^{-/-}$ mice developed modest cardiac hypertrophy, with preserved contractile function, that is associated with an increase in cardiomyocyte size (Abel et al., 1999). Another study conducted by O'Neill et al. suggests that PI3K activates AKT-independent signaling pathways that mediate metabolic and mitochondrial adaptations during physiological cardiac hypertrophy (O'Neill et al., 2007). Cardiomyocyte-specific overexpression of constitutively activated mechanistic target of rapamycin (mTOR), an important target and modulator of the insulin-stimulated AKT pathway, did not elicit a significant increase in adult heart weight (Shen et al., 2008), even though mTOR is essential for cardiac development and growth during embryogenesis (Zhu et al., 2013b). Cardiomyocytes treated with the peroxisome proliferator-activated receptor (PPAR)γ agonist troglitazone also respond by increasing protein content (Bell and Mcdermott, 2000); however, the hypertrophic response of cardiomyocytes to PPARγ agonists does not appear to be mediated by changes in myocardial insulin signaling (Sena et al., 2007).

Studies of mice with cardiomyocyte-specific deletion of either insulin receptor substrate 1 (IRS1) (CIRS1KO mice) or IRS2 (CIRS2KO mice) reported that at baseline, adult CIRS1KO hearts were reduced in size, whereas CIRS2KO hearts exhibited cardiac hypertrophy. However, following a swim training exercise regimen, mitochondrial capacity and substrate oxidation rates increased in wild-type controls, but not in IRS-deficient hearts, suggesting that although different IRS isoforms may perform different roles during resting conditions, both are required for the hypertrophic and metabolic adaptation to exercise (Riehle

et al., 2014). In summary, these studies suggest an important role for insulin signaling in physiological cardiac growth, however studies by Ikeda et al. indicated that in response to exercise IGF-1 may actually activate both the IGF-1 receptor (IGF-1R) and IR in the heart (Ikeda et al., 2009). Thus, deciphering the crosstalk between the IGF-1 and insulin signaling pathways will be crucial to understanding the role of IR in exercise-induced cardiac hypertrophy.

INSULIN SIGNALING IN DIABETIC CARDIOMYOPATHY

Defective insulin signaling in DCM has been described in both animal models (Wright et al., 2009) and humans (Cook et al., 2010), primarily observed as reduced GLUT4 translocation to the plasma membrane. Cardiac dysfunction induced by pressure overload in mice is associated with insulin resistance, hyperinsulinemia and increased AKT activation (Shimizu et al., 2010). Hearts from obese and insulin-resistant rodent models (*ob/ob* and *db/db* mice) exhibit higher rates of fatty acid (FA) oxidation and MVO$_2$, but lower rates of glucose oxidation and cardiac efficiency, which precede the development of hyperglycemia (Mazumder et al., 2004; Buchanan et al., 2005). Moreover, CIRKO mice exhibit decreased LV function associated with mitochondrial dysfunction and reduced mitochondrial FA oxidative capacity following a myocardial infarct (Sena et al., 2009), as well as an enhanced decline in cardiac function when treated with streptozotocin (STZ) compared to wild-type mice (Bugger et al., 2012). In addition, these animals have 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 (Boudina et al., 2009). In contrast, the type 1 diabetic Akita mouse model (Akita) is characterized by normal cardiac function and preserved myocardial insulin sensitivity, and does not exhibit FA-induced mitochondrial uncoupling (Bugger et al., 2008). These results suggest that the activity of pathways controlling cardiac mitochondrial function may be different in insulin-responsive type 1 and insulin-resistant T2DM hearts.

Interestingly, FoxO (forkhead box-containing protein, O subfamily) proteins have been described as important regulators of several cellular processes in the myocardium such as cardiac growth (Skurk et al., 2005; Ni et al., 2006), and autophagy (Sengupta et al., 2009; Schips et al., 2011). In addition, Ni et al. (2007) showed that FoxO activation leads to altered insulin signaling and decreased glucose uptake in cardiac myocytes (Ni et al., 2007). In this sense, activation of FoxO1 in response to the HFD was directly related to down-regulation of IRS1 activity, decreased AKT signaling, and insulin resistance, suggesting that FoxO1 might be a key step in the development of DCM preventing cardiac dysfunction by restoring insulin responsiveness (Battiprolu et al., 2012; **Figure 1B**). Recently, it has been shown that impaired cardiac insulin signaling FoxO1-mediated stimulates the β-myosin heavy chain (β-MHC) expression promoting cardiac dysfunction (Qi et al., 2015). Thus,

FoxO1 plays a key role in the control of cardiac insulin signaling both in physiological and pathophysiological conditions, and it might prove a novel therapeutic or preventive strategy for treating individuals with DCM.

On the other hand, FAs are the major substrate for ATP synthesis in the heart; nevertheless, excess lipid accumulation is associated with decreased cardiac function in obesity and diabetes (Finck et al., 2002, 2003). In this context, insulin is also able to induce FA uptake in cardiomyocytes, promoting the translocation of the FA transporter FAT/CD36 (Glatz et al., 2006; Coort et al., 2007) to the plasma membrane mediated by PI3K activation (Luiken et al., 2002; **Figure 1A**). Overexpression of PPARγ led to cardiac lipid accumulation and cardiomyopathy associated with increased FAT/CD36 mRNA, but this effect was antagonized by WY-14,643, a potent PPARα agonist (Son et al., 2010). Nevertheless, comprehensive understanding of the relationship between PPARs and insulin in DCM are not yet fully understood (Jia et al., 2016). The heart can metabolize either non-esterified FA (bound to albumin) or FA obtained from lipolysis of lipoprotein-bound triglycerides (TG). Hearts from mice with cardiomyocyte-specific knockout of lipoprotein lipase (LpL) showed reduced FA oxidation and increased glucose oxidation, associated with increased expression of GLUT4, and reduced expression of pyruvate dehydrogenase kinase 4 (PDK4), suggesting that heart is able to adapt by balancing between the use of glucose and FA (Augustus et al., 2006). However, recent studies indicate that deletion of mTOR impairs myocardial palmitate oxidation and reduces expression of several genes associated with FA metabolism while also reducing insulin-stimulated AKT Ser^{473} phosphorylation (Zhu et al., 2013c). Thus, intact mTOR signaling may be essential for maintaining appropriate control over substrate usage. In addition, cardiomyocytes from HFD-fed mice exhibit increased FA oxidation rates and myocardial oxygen consumption (MVO$_2$), correlating with reduced GLUT4 content and impaired GLUT4 translocation in response to insulin (Wright et al., 2009; **Figure 1B**) and consistent with earlier studies involving the response of cardiomyocytes to the adipocytokine resistin (Graveleau et al., 2005). In summary, cardiac lipotoxicity involves detrimental effects that may adversely impact cardiomyocyte function and participate in progression of DCM.

New Insights
miRNAs

miRNAs are small, single-strand non-coding RNA, that are transcribed by RNA polymerase II (Pol II), generating a primary miRNA (pri-miRNA). In the nucleus, the RNase III endonuclease Drosha and the double-stranded RNA-binding domain protein DGCR8/Pasha cleave the pri-miRNA to produce the precursor miRNA (pre-miRNA) of ~70-nt. Exportin-5 transports the pre-miRNA into the cytoplasm, where it is cleaved by another RNase III endonuclease, Dicer, together with the protein TRBP/Loquacious, releasing miRNA containing a 21- to 23-nt (Bushati and Cohen, 2007; Pasquinelli, 2012). They serve as important post-transcriptional regulators that down-regulate the expression of their target genes (Pritchard et al., 2012) The exact and detailed role of miRNAs in the heart is far from

being completely understood, but there is mounting evidence showing that alterations in many miRNAs correlate with the development and progression of human diseases, including cardiovascular diseases, such as DCM (Asrih and Steffens, 2013). In this context, Zheng et al. (2015) found that miR-195 is up-regulated in the hearts of C57BL/6 mice treated with STZ and this was associated with a decrease expression of its target proteins B cell leukemia/lymphoma 2 (BCL-2) and sirtuin 1 (SIRT-1) (Zheng et al., 2015). Moreover, inhibition of miR-195 inhibited apoptosis, reduced oxidative stress and hypertrophy in diabetic hearts. Additionally, cardiac function and coronary blood flow was improved and the expression of BCL-2 and SIRT-1 was increased, suggesting that targeting miR-195 may attenuate DCM (Zheng et al., 2015). In order to further elaborate on the subject, we briefly discuss about a few miRNAs and their potential role in DCM. The models used, beneficial or detrimental effect on cardiac function and possible mechanisms suggested so far are summarized in **Table 1**.

miR-133a,b expression have been shown to participate in metabolic control by reducing the expression of GLUT4 and insulin-mediated glucose uptake in neonatal rat cardiomyocytes (NRCs) (Horie et al., 2009). In contrast, Chen et al. (2014) found that miR-133a expression was reduced in the hearts of diabetic mice and this was associated with an increase in the expression of fibrosis markers. Moreover, they observed that overexpression of miR-133a in the heart attenuated cardiac fibrosis, which is one of the central causes for the HF generated by DCM (Chen et al., 2014). Considering the apparent contradiction between these two studies performed in different models, the results should be analyzed with precaution and further *in vivo* studies are required to fully clarify the role of miR-133a in DCM.

On the other hand, overexpression of miR-223 in NRCs significantly increased glucose uptake, in similar magnitude to that observed in cells stimulated with insulin. *In vitro*, overexpression of miR-223 in cardiomyocytes increased total GLUT4 level and induced GLUT4 translocation from the cytoplasmic compartment to the cell membrane. *In vivo*, inhibition of miR-223 in the heart resulted in a significant decrease in GLUT4 expression (Lu et al., 2010). miR-141 may be another miRNA of importance in DCM, since it was found to be significantly increased in the diabetic heart and its overexpression decreased the content of the inner mitochondrial membrane phosphate transporter, solute carrier family 25 member 3 (Slc25a3) in the mouse atrial cardiomyocyte cell line HL-1 (Baseler et al., 2012), suggesting that altered miR-141 expression may influence cardiac mitochondrial function and ATP generation (Baseler et al., 2012).

Further extending the evidence for the role of miRNA in the pathophysiology DCM, miR-451 levels were found to be markedly increased in palmitate-stimulated NRCs and DIO mouse heart (model of pre-T2DM and obesity with elevated blood glucose and impaired glucose tolerance). It was found that Cab39 (Calcium-binding protein 39, a scaffold protein of LKB1, a kinase upstream of AMPK) was a direct target to miR-451 in the heart. Furthermore, miR-451 knockdown partly rescued lipotoxicity *in vitro*, and HFD-induced cardiac hypertrophy was ameliorated in miR-451 KO mice through the LKB1/AMPK

TABLE 1 | Effects of microRNAs (miRs) on diabetic cardiomyopathy (DCM) models: beneficial or detrimental effect on cardiac function and possible mechanisms.

miR	Model	Cardioprotective effect	Proposed mechanism	References
195	STZ mice	↓	↑ Apoptosis ↑ Oxidative stress ↑ Hypertrophy ↓ Coronary blood flow	Zheng et al., 2015
133a,b	NRCs	↓	↓ Glucose uptake	Horie et al., 2009
133a	STZ mice	↑	↓ TGF-β1 ↓ SMAD2 ↓ p-ERK1/2	Chen et al., 2014
223	NRCs	↑	↑ Glucose uptake	Lu et al., 2010
141	HL-1, STZ mice	↓	↓ ATP production	Baseler et al., 2012
451	HFD-fed mice, NRCs	↓	↓ LKB1/AMPK	Kuwabara et al., 2015
143	HL-1, ARCs	↓	↓ p-AKT ↓ ORP8 ↓ Glucose uptake	Blumensatt et al., 2013
29a,b,c	HL-1, ZDF rat	↓	↓ mTORC1/MCL-1	Arnold et al., 2014
322	*ob/ob* mice	↑	↓ p-AKT	Marchand et al., 2016

Abbreviations: AKT, protein kinase B; AMPK, AMP-activated protein kinase; ARCs, adult rat cardiomyocytes; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; LKB1, liver kinase B1; MCL-1, myeloid leukemia cell differentiation protein; mTORC1, mammalian target of rapamycin complex 1; NRCs, neonatal rat cardiomyocytes; ORP8, Oxysterol-binding protein-related protein 8; SMAD2, SMAD family member 1; STZ, streptozotocin; TGF-b1, transforming growth factor beta 1; ZDF, Zucker Diabetic Fatty rat. ↓, Decreased; ↑, Increased.

pathway (Kuwabara et al., 2015). Moreover, activin A released from epicardial adipose tissue in T2DM, inhibits insulin action via the induction of miR-143 in cardiomyocytes. This miRNA inhibits the AKT pathway through down-regulation of the novel regulator of insulin action, ORP8 (Blumensatt et al., 2013). ORP8 binds 25-OH-cholesterol, and this regulates AKT signaling in apoptosis through the induction of AKT degradation via proteasome and/or inhibition of PI3K in macrophages (Jordan et al., 2011). Moreover, activation of AKT in response to insulin and the consequent phosphorylation of glycogen synthase kinase 3β (GSK3β) were diminished in HepG2 cells depleted of ORP8 (Jordan et al., 2011). Future studies should confirm these results in the heart to further clarify the role of miR-143 and ORP8 in DCM.

Regarding insulin, it has been shown that miR-29, which downregulates the pro-survival protein myeloid cell leukemia 1 (MCL-1) (Roggli et al., 2012), may be involved in the development of DCM. Arnold et al., reported that insulin inhibited the expression of miR-29a, b and c and increased the mRNA levels of MCL-1 in HL-1 cells. Additionally, they observed that dysregulation of the miR-29-MCL-1 axis as a consequence of low levels of insulin or inhibition of mTORC1 in Zucker Diabetic Fatty (ZDF) rats was associated with structural damage of the diabetic myocardium (Arnold et al., 2014). In addition, Marchand et al. have recently reported that miR-322 modulates insulin pathway, protecting the heart against the cardiac dysfunction observed in C57Bl/6N mice fed with HFD, showing that miR-322 overexpression reduced AKT phosphorylation induced by insulin. Thus, miR-322 regulates insulin signaling and might protect the heart against consequences of hyperinsulinemia (Marchand et al., 2016). Moreover, Costantino et al. showed that altered miRs

profile induced by hyperglycaemia in the diabetic heart is not reverted by the restoration of normal glycaemia levels with insulin (Costantino et al., 2016). This study also showed through ingenuity pathway analysis that the miRs increased in the diabetic heart were involved in myocardial cell signaling pathways associated with apoptosis, fibrosis, hypertrophic growth, autophagy, oxidative stress, and HF and this may explain why the detrimental effects of DCM persist even after the restoration of normal glucose levels (Costantino et al., 2016). Considering all the evidence shown above, miRNAs clearly participate in the pathophysiological mechanisms involved in DCM and more studies are required to fully understand and control their diagnostic and therapeutic potential.

Exosomes

Exosomes are nanosized extracellular vesicles ranging from 50 to 90 nm in diameter. These nanovesicles are formed in multivesicular bodies, which after fusing with the plasma membrane; release their content to the extracellular medium (Thery et al., 2006). Exosomes can carry proteins, RNA or miRNA from one cell to another through the bloodstream, protecting its content from enzymatic degradation and thus, these extracellular vesicles may be mediating intercellular communications (Yellon and Davidson, 2014). Moreover, de Jong et al reported that cellular stress may induce variations in the protein and mRNA content of endothelial cell-derived exosomes (De Jong et al., 2012), which suggests that exosomes may represent the physiological state of the cell (Yellon and Davidson, 2014). Therefore, exosomes are a very attractive target to develop possible biomarkers for different diseases.

The potential role of exosomes in cardiovascular diseases is starting to be explored. In this context, it has been described

that exosomes exert a cardioprotective effect in the myocardium against ischemia-reperfusion (Giricz et al., 2014; Vicencio et al., 2015). It has also been reported that exosomes may play a role in septic cardiomyopathy, cardiac hypertrophy, and peripartum cardiomyopathy (Ailawadi et al., 2015).

Regarding DCM and the importance of miRNAs in this disease, exosomes arise as potential biomarkers for diagnosis. However, much remains to be elucidated about these nanovesicles and their role in DCM. In this context, it has been suggested that most of the plasma miRNAs are contained in exosomes, however, it has also been reported that plasma miRNAs can be bound to complexes of proteins like argonaute or high density lipoproteins (Yellon and Davidson, 2014) and therefore, further studies are required to clarify this point.

In the context of understanding the mechanisms involved in diabetes-induced ischemic cardiac disease (Ailawadi et al., 2015), Wang et al showed that cardiomyocyte-derived exosomes isolated from Goto-Kakizaki (GK) diabetic rats inhibited the proliferation and migration of endothelial cells. Interestingly, exosomes isolated from Wistar rat's cardiomyocytes generated the opposite effect in the endothelial cells, promoting migration and proliferation. In addition, the effects of both GK and Wistar rat's exosomes on endothelial cells were reversed by the exosome inhibitor GW4869. Moreover, GK-exosomes contained higher levels of miR-320 and lower levels of miR-126 compared to Wistar rat's cardiomyocyte-derived exosomes (Wang et al., 2014). Therefore, these results suggest that reduced angiogenesis in the diabetic myocardium may be mediated by exosomes with anti-angiogenic effects released from cardiomyocytes to the endothelium. These findings also suggest a possible role for exosomes for the treatment of DCM ischemic complications.

It has also been recently reported that cardiomyocyte-derived exosomes can regulate glucose transport in endothelial cells (Garcia et al., 2016). This study showed that under glucose deprivation conditions, the production of exosomes from cardiomyocytes was increased and these nanovesicles contained glucose transporters and enzymes involved in glycolysis, which in turn lead to an increase in glucose uptake, glycolysis and the production of pyruvate in the endothelial cells (Garcia et al., 2016). Taken the current evidence together, exosomes have massive potential to give new insights that could help to understand the pathophysiology of DCM and they can not only serve as potential biomarkers, but also act as therapeutic targets or agents that could reverse the impaired insulin signaling observed in DCM.

Adenosine

The purine nucleoside adenosine is an important paracrine/autocrine signaling molecule that modulates a wide range of biological processes activating four subtypes of G protein-coupled adenosine receptors (ARs), A_1, A_{2A}, A_{2B}, and A_3 (Fredholm, 2014). The ARs play a key role in tissue homeostasis both in physiological and pathophysiological conditions, such as inflammation, ischemia, hypoxia and cancer, regulating tissue homeostasis (Antonioli et al., 2013).

Interestingly, new evidence has emerged suggesting a link between adenosine signaling and T2DM (Antonioli et al., 2015).

In this context, it has been described that adenosine modulates insulin secretion (Johansson et al., 2007; Yang et al., 2012; Ohtani et al., 2013) and also controls the proliferation and regeneration of β pancreatic cells (Ohtani et al., 2013; Andersson, 2014). Moreover, several studies have proposed that adenosine regulates the insulin signaling in muscle (Faulhaber-Walter et al., 2011; Figler et al., 2011; Johnston-Cox et al., 2012), liver (Figler et al., 2011; Johnston-Cox et al., 2012), and adipose tissue (Faulhaber-Walter et al., 2011; Figler et al., 2011; Johnston-Cox et al., 2012; Csoka et al., 2014).

In the cardiovascular system, the expression of ARs have been reported in vascular smooth muscle cells (Zhao et al., 1997), human umbilical vein endothelial cells (Wyatt et al., 2002), coronary artery endothelial cells (Olanrewaju et al., 2000), cardiac fibroblasts and cardiomyocytes (Sassi et al., 2014). In STZ-treated diabetic rats, cardiomyocytes exhibited increased levels of A_1 and A_3, reduced levels of A_{2A} and unaltered levels of A_{2B} (Grden et al., 2005); however, cardiac fibroblasts showed increased levels of A_1 and A_{2A}, reduced levels of A_3 but did not change the levels of A_{2B} (Grden et al., 2006). Thus, the evidence described above highlights the need to assess the functional role of ARs in DCM.

As we mentioned before, early-stage DCM is characterized by cardiomyocyte hypertrophy and myocardial fibrosis (Battiprolu et al., 2013; Go et al., 2014). In this context, Puhl et al. (2016) recently showed that A_1 attenuates phenylephrine-mediated (α-1 adrenoceptor) cardiomyocyte hypertrophic and cardiac fibrosis, suggesting that A_1 activation might be a novel therapeutic target to prevent cardiac remodeling (Puhl et al., 2016). In addition, cell-to-cell communication via cAMP/adenosine activating A_1 and A_{2A}/A_{2B} in cardiomyocytes and fibroblast, respectively, may be also relevant in preventing isoproterenol-mediated (βAR agonist) cardiomyocyte hypertrophy and cardiac fibrosis, suggesting an interesting paracrine cardiac action of adenosine (Sassi et al., 2014). In summary, whether ARs participates in the molecular mechanisms of DCM, or whether they might be promoting the DCM-related defective insulin signaling are important issues that need to be addressed.

CONCLUDING REMARKS AND OPEN QUESTIONS

The complete pathophysiology of DCM and the role of insulin in it are still being elucidated. Further studies are certainly required to clarify many aspects of this cardiovascular disease. However, there has also been emerging information on different mechanisms that may help answer the variety of questions raised in the study of DCM. Despite increasing amount of evidence that highlights the role of ARs in the regulation of insulin signaling in several tissues such as muscle, liver and adipose tissue, there are many open questions to deeply understand their roles both in the healthy heart and DCM. Thus, could insulin signaling in DCM be improved by activation of ARs? In addition, miRNAs may play a key role for early diagnosis of DCM and evidence such as the altered miRNA landscape in the diabetic heart suggests that new strategies are required to reverse the detrimental effects

of DCM that persists despite normalization of blood glucose levels. In this context, is it possible to reverse the cardiovascular complications of DCM by targeting the miRNAs associated with these deleterious effects? Also, exosomes may also serve as potential biomarkers of DCM. These nanovesicles are a mechanism of inter-cellular communication and therefore, there is much excitement about the possibility of harnessing their power as therapeutic agents. Could normal exosomes isolated from healthy patients reverse the phenotype of patients with DCM? Is it possible to exogenously control the release of beneficial exosomes? Answers to these questions will help to elucidate the complex mechanisms underlying the defective insulin signaling in DCM, opening new fields for basic and clinical studies.

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