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Xavier Palomer, Javier Pizarro-Delgado, Manuel Vázquez-Carrera

Trends

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The current criteria to diagnose DCM include left ventricular (LV) diastolic dysfunction, reduced LV ejection fraction, pathological LV hypertrophy and interstitial fibrosis. However, it is difficult to identify DCM in the early stages because of its heterogeneity.

There are some validated biomarkers for the diagnosis and risk assessment of numerous cardiac diseases, but none of them is able to discriminate those patients with DCM.

The diabetic heart is characterized by metabolic disturbances that are often accompanied by local inflammation, oxidative stress, myocardial fibrosis and cardiomyocyte apoptosis. The discovery of selected biomarkers that integrate these processes is of great interest to detect or prevent DCM in the early stages, or even to treat it once established.

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Emerging actors in diabetic cardiomyopathy: heartbreaker biomarkers or therapeutic targets?

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Abstract

The diabetic heart is characterized by metabolic disturbances that are often accompanied by local inflammation, oxidative stress, myocardial fibrosis and cardiomyocyte apoptosis. Overall changes result in contractile dysfunction, concentric left ventricular hypertrophy and dilated cardiomyopathy, altogether affecting cardiac output and eventually leading to heart failure, the foremost cause of death in diabetic patients. There are currently a number of validated biomarkers for the diagnosis and risk assessment of several cardiac diseases, but none of them is capable of discriminating patients with diabetic cardiomyopathy. In this review we point to several novel candidate biomarkers from new activated molecular pathways (including microRNAs) with the potential to detect or prevent diabetic cardiomyopathy in the early stages, or even to treat it once established. The prospective use of selected biomarkers that integrate inflammation, oxidative stress, fibrosis and metabolic dysregulation is widely discussed.

Nonstandard abbreviations and acronyms: AGE, advanced glycation end-products; AMPK, AMP-activated protein kinase; ANP, natriuretic peptide A; AP-1, activator protein-1; BNP, natriuretic peptide B; CHI3L1, chitinase-3-like protein 1 (YKL-40); CPT-1, carnitine palmitoyl-transferase 1; DCM, diabetic cardiomyopathy; DM1, type 1 diabetes; DM2, type 2 diabetes; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FA, fatty acid; FABP3, fatty acid binding protein 3; FAT/CD36, fatty acid translocase; FGF, fibroblast growth factor; GDF15, growth differentiation factor 15; GLUT4, glucose transporter 4; HMGB1, high-mobility group box1; IGFBP-7, insulin-like growth factor binding protein-7; IL, interleukin; JAK, janus kinase; JNK, c-Jun N-terminal kinase; LVEF, left ventricular ejection fraction; MAPK, mitogen-activated protein kinase; MCP1, monocyte chemoattractant protein 1; MHC, myosin heavy chain; MICU1, mitochondrial calcium uptake 1; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; NFAT, nuclear factor in activated T cells; PARP, poly ADP ribose polymerase; PDK4, pyruvate dehydrogenase kinase 4; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; PI3K, phosphoinositide 3 kinase; PPAR, peroxisome proliferator-activated receptor; RAGE, receptor for AGE; ROS, reactive oxygen species; Sp1, specificity protein 1; ST2, suppression of tumorigenicity 2; STAT, signal transducer and activator of transcription; TGFβ, transforming growth factor β: Tn, troponin; TNF-α, tumour necrosis factor-α; TP53INP2, tumour protein p53 inducible nuclear protein 2; TRIM72, tripartite motif containing 72; TZD, thiazoledinediones

Diabetic cardiomyopathy: where we are

Diabetic cardiomyopathy (DCM) is currently defined as myocardial dysfunction that develops in diabetic patients, and which is not directly attributable to hypertension, valve disease or coronary artery disease. Its prevalence ranges from 20% to 60% in the diabetic population[1, 2], and affects both people with type 1 (DM1) and type 2 (DM2) diabetes[3]. The diabetic heart is characterized by metabolic disturbances, which, together with subcellular component abnormalities and immunological alterations, locally prompt inflammation, oxidative stress, mitochondrial dysfunction and **apoptosis** (see Glossary). Overall changes result in extracellular **cardiac remodeling** and fibrosis, contractile dysfunction, concentric left ventricular hypertrophy and **dilated cardiomyopathy**, which affect **cardiac output** and eventually lead to heart failure, the foremost cause of death in diabetic patients[4].

Current criteria to diagnose DCM (Box 1) include left ventricular **diastolic dysfunction**, reduced left ventricular ejection fraction (LVEF), pathological **cardiac hypertrophy** and interstitial fibrosis[5]. However, it is often difficult to identify DCM in the early stages because of its heterogeneity. For this reason, it is essential that early molecular events are identified that would allow early diagnosis. This would enable a reliable assessment of the disease, and would boost the use of novel therapeutic strategies to delay or prevent its progression to heart failure. Nowadays, there is an increased trend towards the identification of new activated molecular pathways and mediators involved in the pathogenesis of DCM. Quantification of molecular biomarkers could provide an interesting tool to both improve DCM detection, and to discover new therapeutic targets (Box 2). In spite of the considerable panel of candidates, however, no molecule has proven to be useful in clinical practice to date. Thus, the identification of potential cardiac biomarkers that are released during early and reversible alterations is of great interest. Numerous molecules with autocrine and paracrine effects are excreted by the heart in response to the structural and functional alterations associated with diabetes. These molecules are involved in processes as diverse as

inflammation, metabolism, contractility, fibrosis, hypertrophy and apoptosis. Interestingly, increasing evidence points to a potential link between chronic low-grade inflammation, fibrosis and metabolic dysregulation in heart diseases[4]. For that reason, we focus on those molecules of cardiac origin that could mediate the crosstalk between inflammation, cardiac remodelling and metabolic dysregulation in the failing heart during diabetes.

Molecular basis of diabetic cardiomyopathy

Metabolic remodeling plays a key role in diabetic cardiomyopathy

The pathophysiological mechanisms of DCM are multifactorial (Figure 1), although it is widely accepted that metabolic dysregulation plays a pivotal role in its development. Free fatty acids (FFA) are the preferred energy substrate in the adult heart, although other substrates such as glucose, lactate or ketone bodies may provide additional fuel sources. At the transcriptional level, cardiac metabolism is mostly regulated by the **PPAR** (peroxisome proliferator-activated receptor)/PGC-1α (PPARγ coactivator-1α) axis, which along with its transcriptional targets, plays a central role in DCM[6]. One of these target genes, pyruvate dehydrogenase kinase 4 (PDK4), which catalyzes the rate-limiting step of glucose oxidation, is chronically elevated in the diabetic heart[7]. In contrast, the uptake of FA and glucose in the heart is mediated, respectively, by fatty acid translocase (FAT/CD36) and the insulin-induced glucose transporter 4 (GLUT4), which are both transcriptionally regulated by PPAR/PGC-1α. Under **insulin resistance**, GLUT4 is internalized while FAT/CD36 becomes preferentially localized to the sarcolemma, thus promoting a substrate shift toward increased mitochondrial FA β-oxidation as the sole fuel source[8-10]. Despite the higher FA oxidation rate, myocardial lipid accumulation is a hallmark of the diabetic heart, and **cardiac steatosis** is currently regarded as a major cause of DCM[4, 11]. The ensuing accumulation of toxic lipid intermediates (lipotoxicity) is characterized by the activation of the proinflammatory transcription factor nuclear factor-κB (NF-κB), and the appearance of

endoplasmic reticulum (ER) stress and mitochondrial dysfunction, all linked to myocyte apoptosis, myocardial fibrosis and contractile dysfunction[3, 12].

The role of inflammation and fibrosis

Elevated FFA plasma levels and hyperglycemia activate NF-κB in cardiomyocytes[4]. The resultant increased expression and secretion of **cytokines** and **chemokines** exerts several autocrine effects via downstream activation of activator protein-1 (AP-1) and NF-κB itself, which are actively involved in the development of heart failure during DCM[4, 13].

Hyperglycemia also induces the formation of **advanced glycation end-products** (AGE) within the cardiomyocytes[14], which, through the interaction with their receptor (RAGE, receptor for AGE), trigger NF-κB activation, hence modifying the overall gene expression in cardiomyocytes and reducing cardiac contractility[13]. Hyperglycemia-derived AGE induce the formation of structural proteins, type I and III collagens, promote the covalent crosslinking between various extra- and intracellular proteins, and elicit a switch in the expression from the α myosin heavy chain (MHC) isoform to the β-MHC isoform[3, 15]. These changes favor interstitial fibrosis, myocardial stiffness and subsequent left ventricular diastolic dysfunction.

Oxidative stress and apoptosis

Hyperglycemia and undue FA oxidation in mitochondria during DCM promote reactive oxygen species (ROS) accumulation in cardiomyocytes, which lead to **oxidative stress** and are involved in all stages of DCM, including cardiac hypertrophy, fibrosis, contractile dysfunction and heart failure[15]. An excess of ROS brings on the uncoupling of oxidative metabolism, induces DNA, protein and lipid oxidative damage, triggers NF-κB activation, and gives rise to endoplasmic reticulum (ER) stress[3]. As a result, myocardial energy generation is impaired, calcium handling disturbed, and cardiac contractility and efficiency are reduced[3, 12]. In murine cardiomyocytes,

oxidative stress directly induces insulin resistance via up-regulation of the extracellular signal-regulated protein kinase (ERK)1/2 activity, which inhibits the NF-E2-related factor 2 (Nrf2), a transcription factor that protects against sustained oxidative stress[16].

ROS accumulation also hastens cardiomyocyte apoptosis, which is often observed in the myocardium of animal models and patients with diabetes[15]. Apoptosis is caused by several mechanisms, including the activation of DNA reparative enzymes such as poly ADP ribose polymerase (PARP), or through interference with nitric oxide[15]. PARP also activates NF-κB and diverts glucose metabolism from its usual glycolytic pathway, thus favoring hyperglycemia-induced cell injury. The ensuing accumulation of glycolytic intermediates will, in turn, harm cardiac tissue via AGE formation and protein kinase C activation[15, 17].

Other pathophysiological mechanisms

ER stress is partly responsible for cardiomyocyte cell death in the heart of diabetic rats[18]. When ER stress is limited, activation of the unfolded protein response potentiates **autophagy** as a short-term strategy to protect cardiac cells during diabetes[19]. In contrast, persistent ER stress will activate NF-κB, p38 MAPK (mitogen-activated protein kinase) and JNK (c-Jun N-terminal kinase) pathways, which in turn are responsible for the induction of ER stress-mediated cardiomyocyte apoptosis[20]. ER stress also alters cardiomyocyte calcium uptake and handling, thus aggravating diastolic relaxation dysfunction[3]. In fact, calcium ions are pivotal regulators of the process of excitation–contraction coupling in the heart (Box 3), and dysfunctional intracellular calcium signaling and transport have been observed in various murine models of diabetes[21-23], and also in diabetic patients with diastolic dysfunction[24].

Where are we going: Emerging actors in DCM

Metabolism-related molecular biomarkers

The metabolic inflexibility that distinguishes the diabetic myocardium is characterized by increased rates of fatty acid uptake and mitochondrial oxidation as the predominant energy source. This affects the cardiac muscle by diverse mechanisms and acting on different cardiomyocyte compartments[25]. The inability of the diabetic heart to switch between substrates in the presence of different stressors may offer novel biomarkers representing the "struggle" of the heart to deal with these conditions. For instance, the phosphocreatine (PCr)/ATP ratio (Figure 2) is significantly reduced in the diabetic heart. Magnetic resonance spectroscopic imaging is suitable for the determination of phosphocreatine, ATP and triglyceride accumulation in the myocardium[3]. A recent study demonstrates that the PCr/ATP ratio predicts diastolic dysfunction in obese patients without cardiovascular risk factors[26]. However it is not able to discriminate those patients with DCM from other pathologies[3]. For instance, the PCr/ATP ratio is also reduced in patients with hypertensive heart disease without diabetes or overt systolic dysfunction[27].

The striated muscle-specific E3 ligase TRIM72 (tripartite motif containing 72, or mitsugumin 53, MG53) is another potential therapeutic target because it induces systemic insulin resistance, dyslipidemia and hyperglycemia. Its expression is also increased in the myocardium during diabetes[28]. Cardiac-specific overexpression of TRIM72 in mice results in insulin resistance, because it drives ubiquitin-dependent degradation of the insulin receptor and insulin receptor substrate 1[28]. This is followed by steatosis and, finally, severe DCM characterized by myocardial hypertrophy, fibrosis and cardiac dysfunction. Further metabolic effects of TRIM72 arise from the transcriptional upregulation of PPARα-target genes involved in FA uptake and metabolism, together with the PDK-mediated repression of glycolysis[28]. Remarkably, PDKs and, in particular PDK4, have emerged as interesting candidates for diabetes therapy as they might link inflammation, apoptosis and insulin resistance[29, 30]. TRIM72 also plays an important role in cardiac fibrosis, probably through the modulation of transforming growth factor (TGF)β[31].

Conversely, several evidences have identified TRIM72 as an important cardioprotective factor. Cardiac ischemia/reperfusion insults downregulate myocardial TRIM72 protein levels, whereas elevation of TRIM72 protects cardiomyocytes from oxidative injury and alleviates cardiac ischemia/reperfusion injury[32]. Overall data suggest that, in acute myocardial infarction and cardiac ischemia/reperfusion injury, a transient and short-term increase of TRIM72 could protect against acute damage, whereas specific inhibition of its E3 ligase activity might prevent insulin resistance and lipid dysregulation in the heart.

Similarly, TP53INP2 (tumor protein p53 inducible nuclear protein 2), the expression of which is induced by PPARα, regulates the expression of important glycolytic enzymes involved in glucose uptake and glycogen storage in cardiomyocytes[33]. Its protein levels are reduced in muscle from streptozotocin-induced diabetic mice and in obese diabetic db/db mice[28] and in muscle from DM2 patients[34]. TP53INP2 is a bifunctional protein that acts as a nuclear coactivator and key regulator of basal autophagy and protein degradation; as a consequence, it hinders the expression of hypertrophic genes, particularly in the context of hyperglycemia[33, 34]. Therefore, its activation in the heart might be therapeutically useful to delay cardiac hypertrophy and to prevent the metabolic dysregulation characteristic of DCM.

Since cardiac steatosis is a hallmark of DCM, the identification of factors released during this process could be useful to detect or prevent the disease. The fatty acid binding protein 3 (FABP3), a cardiac-specific PPAR-induced protein that transports FA from the plasma membrane to the mitochondria for their subsequent oxidation after conjugation with carnitine by CPT-1 (carnitine palmitoyl-transferase 1), is released to the plasma after the onset of cell damage in patients with systolic dysfunction or heart failure[35]. FABP3 is detected in the serum of DM2 patients with early cardiac injury[36] and is correlated with cardiac insulin resistance in mice[37]. FABP3 is also increased in patients with hypertrophic and dilated cardiomyopathy, or heart failure[36]. In contrast, patients with reduced LVEF due to doxorubicin-induced cardiotoxicity display lower

plasma levels of FABP3[38]. FABP3 also protects against oxidative stress and mitochondrial dysfunction, and exerts an antiapoptotic role in cardiac cells[39]. Overall data suggest that FABP3 could be a suitable diagnostic tool, but also a promising therapeutic target for treating DCM.

Some autocrine and paracrine molecules secreted by the heart or the epicardial adipose tissue could also be useful as metabolic-related DCM biomarkers. Activin A is released in DM2 patients and is capable of inhibiting insulin secretion[40]. Moreover, plasma activin A levels are inversely correlated with myocardial glucose metabolism[41]. The activin A-mediated blockade of insulin-mediated phosphorylation of phosphoinositide 3-kinase (PI3K)/Akt[40], a key regulator of myocardial glucose uptake, could account for these metabolic changes, and could reflect early DCM development. An even more promising biomarker is insulin-like growth factor binding protein-7 (IGFBP-7), a modulator of insulin receptor activity and signaling that displays a positive correlation with increased collagen deposition, fibrosis and cardiac hypertrophy in diabetes[37, 42]. In fact, serum IGFBP-7 and TGFβ levels are elevated in diabetic patients, particularly in those displaying diastolic dysfunction[42]. In support of this, IGFBP-7 has been identified as a biomarker associated with cardiac hypertrophy and heart failure through systematic proteomic studies[43] and in patients with diastolic dysfunction and heart failure[44]. Of note, IGFBP-7 displayed a similar performance and discrimination capacity than the natriuretic peptide B.

Molecular biomarkers involved in inflammation and fibrosis

Several proinflammatory cytokines (interleukins [IL]1 and IL6; monocyte chemoattractant protein 1, MCP1; and tumor necrosis factor-α, TNF-α) involved in the progression of heart failure are secreted by both cardiomyocytes and cardiac fibroblasts of diabetic patients, particularly in those with diastolic dysfunction[15, 42]. These classical inflammatory biomarkers exert various autocrine and paracrine effects, including insulin resistance, mitochondrial injury, oxidative stress, fibrosis and apoptosis (Figure 3)[42]. Proinflammatory cytokines have previously been designated

as potential biomarkers in the early detection of DCM, although their plasma levels do not discriminate between DCM and other cardiac pathologies nor are they useful for stratifying patients according to their risk[45]. Furthermore, therapies aimed at inhibiting these cytokines (TNF-α, IL1β) have failed to ameliorate inflammation-induced insulin resistance and DCM in clinical studies[46].

Cardiotrophin-1 (CT-1), which belongs to the IL6 family of cytokines, is released by cardiac fibroblasts and cardiomyocytes in response to mechanical, metabolic and hypoxic stress[47]. Through the activation of gp130, CT-1 promotes cell survival by PI3K/Akt phosphorylation-induced inactivation of the proapoptotic Bcl-2-associated death promoter (BAD) protein[47]. If the stressor persists, CT-1 promotes deleterious cardiac fibrosis and remodeling, cardiac hypertrophy and dysfunction and, eventually, heart failure[47]. CT-1 promotes cardiomyocyte hypertrophy through the JAK/STAT3 (janus kinase/signal transducer and activator of transcription 3) and ERK5 pathways, whereas by activating p42/44 MAPK and PI3K/Akt it promotes cardiomyocyte survival[48]. CT-1 has also been proposed to activate NF-κB and to induce insulin-stimulated glucose uptake by cardiac cells, thus playing a crucial role in regulating glucose metabolism[37]. Plasma CT-1 levels are increased in patients with DM2 or impaired glucose tolerance, and these levels positively correlate with glycemia and left ventricular hypertrophy[49, 50]. However, its high expression in other tissues besides the heart, and its increased expression in other non-diabetic cardiomyopathies hinder its usefulness as a biomarker in DCM [47].

Soluble ST2 (suppression of tumorigenicity 2) is a cardiac biomarker cleared by the Food and Drug Administration (FDA) to aid in assessing the prognosis of patients diagnosed with chronic heart failure[47]. ST2 is regarded as a decoy receptor of IL33, thus preventing the protective effects of this cytokine in obesity and cardiac remodeling[51]. Interestingly, DM2 patients exhibit higher plasma levels of soluble ST2, especially those people presenting diastolic dysfunction and inappropriate glycemic control[52]. However, it remains to be elucidated whether this is a

consequence rather than a cause of the inflammatory activation observed in the heart. In support of ST2 as a suitable biomarker for DCM, plasma levels of this receptor were independently associated with cardiovascular mortality in patients with heart failure and diabetes and the combination of ST2 with troponin T significantly increased its discrimination potential[53]. These biomarkers could be useful to predict the clinical course of heart failure in both the general population and in diabetic patients.

Chitinase-3-like protein 1 (CHI3L1 or YKL-40) is a glycoprotein whose plasma levels are elevated in patients with diabetes or obesity and in people suffering myocardial infarction, coronary artery disease or ischemic heart disease[54]. Its plasma levels positively correlate with insulin resistance and the lipid profile (free FFA and triglycerides) in DM2 patients[55]. YKL-40 is involved in cell proliferation and differentiation, and could potentially protect the heart by attenuating inflammation, apoptosis, tissue remodeling and fibrosis[54, 56]. YKL-40 inhibits p38 and JNK MAPKs, a fact that counteracts the inflammatory responses induced by TNF-α and IL1, and reduces the expression of matrix metalloproteinases (MMP)[55]. Secretion of YKL-40 is regulated by NF-κB[56] and Sp1 (specificity protein 1)[57]. The latter is a transcription factor that, together with PPARα and NR2F2 (nuclear receptor subfamily 2 group F member 2) is responsible for decreasing FA utilization in the hypertrophied heart[58]. Thus, YKL-40 has the potential to become a biomarker for DCM, but also to prevent or even to treat it once established. However, the fact that insulin treatment does not correct YKL-40 serum levels in persons with type 2 diabetes mellitus[59] casts a doubt on its feasibility as a suitable biomarker for DCM.

Another interesting mediator of the inflammatory response is HMGB1 (high-mobility group box 1), the expression of which is increased in the heart during diabetes[60]. During DCM, HMGB1 translocates from the nucleus to the cytoplasm and even to the myocardial interstitium, where it boosts fibrosis and inflammation[60]. These effects rely on the activation of MAPK and NF-κB pathways, and subsequent stimulation of collagen deposition, increases in MMP2 and MMP9

activity, and production of TNF-α and IL6[60, 61]. Inhibition of HMGB1 decreases myocardial inflammation and fibrosis in diabetic mice, thus ameliorating left ventricular dysfunction and cardiac remodeling[60]. It also protects the heart against hyperglycemia-induced apoptosis by downregulating ERK1/2 and caspase-3 activities[62], thus pointing to HMGB1 inhibition as a novel therapeutic target in the treatment of DCM, even once established[63]. In this regard, downregulation of HMGB1 has been reported to attenuate diabetes-induced cardiac dysfunction by the inhibition of inflammation, oxidative stress, fibrosis and apoptosis[63, 64].

During DCM the deposition of extracellular matrix protein impairs contractility and ultimately leads to cardiac stiffness and progression towards heart failure. Therefore, extracellular matrix synthesis and turnover could be a useful biomarker for the recognition, diagnosis and even prevention of fibrosis in DCM. In this regard, plasma levels of procollagen type-1 propeptide and MMP7 are positively correlated with diastolic dysfunction in diabetic patients, while MMP2 levels are reduced in experimental models of DM1 and DM2 displaying cardiac fibrosis and diastolic dysfunction [37]. MMP9 levels are increased in myocardial fibrosis and heart failure, and, interestingly, pharmacological or genetic inhibition of MMPs ameliorates cardiac remodeling in mice[65]. Likewise, procollagen type III aminopeptide in serum is an indicator of extracellular matrix turnover that has been proposed as a marker of early left ventricular dysfunction in subjects with insulin resistance[66].

The proinflammatory transcription factors NF-κB and AP-1 contribute in the course of DCM to the overexpression of collagens, fibronectin and TGFβ, in this way enhancing extracellular matrix protein accumulation[13]. Cardiac and plasma TGFβ levels are correlated with the degree of fibrosis in DCM, particularly in those subjects presenting concomitant diastolic dysfunction, and, notably, inhibition of TGFβ in animal models of DM2 prevents diastolic dysfunction[37, 42]. AP-1, which is mostly composed of JUN and FOS heterodimers, regulates extracellular matrix deposition and decreases contractility and cell permeability, inducing cardiomyocyte hypertrophy

and fibrosis[67]. It is worth mentioning that downregulation of FOS transcription by miR-146a has the capacity to inhibit MMP9 activity[68], thus suggesting that this **microRNA** (miRNA) could be a promising therapeutic tool for preventing cardiac disorders associated with enhanced inflammation and fibrosis in the heart. Furthermore, miR-146a might prevent myocardial lipid accumulation and subsequent lipotoxic cardiomyopathy, because FOS is also an activator of lipid biosynthesis via a transcriptional-independent mechanism[69].

Growth differentiation factor 15 (GDF15), which belongs to the TGFβ superfamily and is highly expressed in the heart, suppresses the synthesis and secretion of proinflammatory cytokines (TNF-α and IL6) and modulates cell growth and differentiation[70]. Its plasma levels are increased in diabetic patients, and positively correlate with obesity, fasting glucose levels, insulin resistance, plasma triglycerides and the proinflammatory marker C-reactive protein (CRP)[45], thus representing another potential biomarker. However, its usefulness in predicting disease progression, prognosis or protection of the heart is hindered by the fact that GDF15 is non-specific for metabolic diseases and also due to it is associated with increased cancer incidence in patients with type 2 diabetes[70].

Finally, galectin-3 (or Mac-2) is a beta-galactoside-binding lectin that is regarded as one of the key links between fibrosis, inflammation and adverse cardiac remodeling in heart failure[71]. In rodent models of heart failure, galectin-3 is locally secreted by activated macrophages and fibroblasts, where it exerts its profibrotic action by enhancing myofibroblast proliferation, accumulation of extracellular matrix, macrophage infiltration and cardiac hypertrophy, via stimulation of the TGFβ signaling pathway[71, 72]. Measurement of galectin-3 plasma levels has been proposed as a good predictor and prognostic biomarker of left ventricular systolic dysfunction and heart failure in diabetic subjects[73]. Galectin-3 might also be of therapeutic interest in DCM, since its inhibition prevents the increase of profibrotic and proinflammatory markers in some organs[74]. Similarly, its administration to mice causes insulin resistance and glucose intolerance,

whereas its pharmacological or genetic inhibition improves insulin sensitivity in obese mice[46]. The same authors demonstrated that galectin-3 interferes with insulin signaling by directly binding to the insulin receptor.

Myocardial contractile and prohypertrophic biomarkers

Troponins are multiprotein complexes constituted by Troponin I (TnI), C (TnC) and T (TnT) that regulate calcium-mediated interaction between actin and myosin, thus being directly related to myocardial contractility. They are released into the circulation from the myocardium during inflammatory processes and in experimental models of diabetes, particularly in those animals also presenting heart failure[3, 75], suggesting that they could be useful biomarkers for DCM. TnI and TnT isoforms are currently used as necrosis markers in clinical practice, since their serum levels may be predictive for cardiovascular death in patients with myocardial infarction and heart failure[76].

In addition, mitochondrial calcium uptake 1 (MICU1), a key regulator of mitochondrial Ca^{2+} uptake, is downregulated in the heart during diabetes[77]. This contributes to myocardial mitochondria-dependent intrinsic apoptosis and the progression of DCM. Of note, MICU1 downregulation caused by hyperglycemia and hyperlipidemia in cardiomyocytes is due to the inhibition of Sp1, which is diminished in several tissues from diabetic patients[77]. Importantly, its overexpression in the heart partially prevents the development of DCM by activating the antioxidant system and reducing myocardial fibrosis and subsequent cardiac hypertrophy[77], thus pointing to MICU1 activation as a novel therapeutic target in DCM.

Non-physiological hypertrophy is characterized by cardiomyocyte enlargement, increased protein synthesis and reactivation of fetal gene expression, including β-MHC and the natriuretic peptides A and B (ANP and BNP). These neurohormones are produced and secreted by cardiac cells during heart failure to counteract the onset of volume and pressure overload via their vasodilator and

natriuretic effects[78]. Not surprisingly, plasma ANP and BNP, as well as the biologically inactive precursor N-terminal fragment of BNP (NTproBNP), are currently utilized as biomarkers for heart failure and myocardial infarction[37, 45], and could also be valuable in the diagnosis or prevention of DCM. In fact, raised plasma BNP levels are positively correlated with insulin resistance in prediabetic subjects[79], and display a good predictive capacity for left ventricular dysfunction and heart failure in DCM[37, 75]. However, the specific role, if any, of natriuretic peptides in DCM onset and development remains controversial. Some studies report reduced plasma levels and deficient ANP and BNP signaling in obese, insulin-resistant and diabetic subjects[80], but the opposite has also been described[81]. This apparent contradiction could be explained by the proposed biphasic association of BNP with DM2, in which the diabetes risk would decrease when BNP concentrations lie within the so-called "physiological range", but would rise when BNP levels increase due to pathophysiological conditions (i.e. myocardial infarction and heart failure).

Miscellaneous biomarkers: miRNAs and fibroblast growth factors (FGFs)

Numerous miRNAs are differentially expressed in cardiac tissue of a streptozotocin-induced mouse model of DM1; some of them exert their deleterious effects through targeting genes associated with cardiac hypertrophy and fibrosis[82]. Among those regulating cardiac fibrosis miR-21, miR-29b, miR-142-3p and miR-700[83], which act through pathways requiring p38 MAPK activation, modulation of TGFβ activity and synthesis of type I collagen, are of particular interest. Other interesting miRNAs include miR-133a, miR-150, and miR-373, which regulate cardiac hypertrophy and fibrosis by modulating the activity of the myocyte enhancer factor 2C transcription factor[82-85]. In a similar way, miR-208a is a prohypertrophic cardiac-specific miRNA that acts through the repression of myostatin and GATA4[83].

Hyperglycemia induces the expression of miR-1 and miR-34a in cardiomyoblasts, which promote apoptosis[82]. miR-1 also negatively regulates calcium signalling during cardiac contractility by

targeting Junctin, a component of the ryanodine receptor Ca^{2+} release channel complex[86]. In a DM2 rat model, miR-320 promotes apoptosis by targeting genes such as vascular endothelial growth factor and **fibroblast growth factors** (FGF)[82], while key regulators of apoptosis, including p53 and p21, are induced by miR-30c and miR-181a during DCM[87]. Also, overexpression of miR-141 in streptozotocin-induced diabetic mice disrupts mitochondrial energy production by inhibiting the expression of an inner mitochondrial phosphate transporter, which eventually results in cell death[82]. On the other hand, miR-30d, which is enhanced in a DM1 animal model, promotes cardiomyocyte proinflammatory-induced apoptosis through the activation of caspase-1 and secretion of proinflammatory cytokines[88]. The same research group suggested an opposite role for miR-9[89]. More recently, Deng et al. reported a significant reduction in the expression levels of circulating miR-24 in blood of DM2 patients with coronary heart disease[90]. Since miR-24 directly targets YKL40, this results in an interesting and potentially beneficial appealing increase in YKL40 expression. Finally, studies investigating the role of miRNAs in the regulation of autophagy and mitophagy during DCM have revealed an important role for miR30a, miR-133a, miR-212 and miR-221[83].

MicroRNAs also stand out due to their extensive effects on the control of glucose uptake and cardiac metabolism in the diabetic heart. For instance, miR-133a reduces the levels of GLUT4 expression[91], whereas miR-223 induces GLUT4 protein expression[92]. Other examples include the miR-199a/miR-214 cluster, which inhibits PPARβ/δ to impair mitochondrial fatty acid oxidation[93], and miR-451, which exacerbates lipotoxicity and cardiac hypertrophy in a mouse model of DM2 through the suppression of AMP-activated protein kinase (AMPK) activity, a master regulator of cardiac glucose and FA metabolism[94].

In summary, the expression of numerous miRNAs is dysregulated in the heart and even modified in the plasma of diabetic individuals. For this reason, miRNAs have been proposed as potential serum biomarkers for the prognosis and diagnosis of patients with DCM. As an example, a recently

published study indicated that plasma miR-19b-3p and miR-181b-5p levels could be suitable biomarkers of DCM in asymptomatic diabetic patients[95]. Moreover, since several of these miRNAs are causatively associated with the disease, the development of anti-miRNAs (antagomiRs) and miRNA mimics to target them could be a potential approach for future therapeutic intervention. For instance, intravenous administration of antagomiRs targeting miR-132 and miR-133 protects the heart against cardiac fibrosis, hypertrophy and heart failure[96, 97], and cardiomyocyte-specific miR-451 knockout mice are partially resistant to diabetes-induced cardiac hypertrophy and contractile dysfunction[94].

With regard to fibroblast growth factors (FGF), the most notable in the heart are FGF1, FGF2 and, above all, FGF21 and FGF23. FGF1 displays high expression in the heart and is transcriptionally induced by PPARα, PPARβ/δ and PPARγ agonists[98]. FGF2, which is expressed in both cardiomyocytes and fibroblasts, causes extracellular matrix deposition and apoptosis, thus leading to pathological cardiac hypertrophy[98]. FGF21 and FGF23 function as local signaling molecules in metabolism through activation of FGF receptors, using α-Klotho or β-Klotho as a cofactor.

The liver is the main site of expression and release of FGF21 into the blood, although it is also produced by cardiomyocytes, where it acts in an autocrine manner[99]. In the heart, FGF21 is under the transcriptional control of the sirtuin 1-PPARα pathway[99]. FGF21 displays powerful antidiabetic effects due to its broad systemic metabolic actions, which include the improvement of glucose homeostasis and insulin sensitivity[100]. However, a paradoxically abnormal elevation of serum FGF21 levels is observed during obesity and, especially, insulin resistance[100]. In response to prohypertrophic stimuli, FGF21 knockout mice exhibit enhanced cardiac hypertrophy and inflammation compared to wild-type controls, in addition to greater repression of FA oxidation[99]. Treatment with exogenous FGF21 reverses all these changes. Likewise, a more recent study highlighted the antioxidative role of FGF21 in the heart[101]. As a result, FGF21 reduces ROS production and protects the heart against inflammatory or prohypertrophic

stimuli[101]. Interestingly, ablation of FGF21 in a mouse model of DM1 exacerbates DCM through a FAT/CD36-mediated increase in cardiac lipid uptake and accumulation, which in turn impairs cardiac lipid and glucose utilization and mitochondrial function[102]. This exacerbates cardiac oxidative stress and provokes inflammation and cardiomyocyte apoptosis. Conversely, FGF21 overexpression improves hyperglycemia-induced DCM via attenuation of cardiac hypertrophy, fibrosis, oxidative stress and inflammation, in a process requiring both β-klotho and AMPK[102].

Less is known about FGF23. It regulates phosphorus homeostasis in the kidney and parathyroid glands, and some studies have unveiled a causal role for FGF23 in the pathogenesis of left ventricular hypertrophy via activation of phospholipase Cγ and calcineurin-NFAT (nuclear factor in activated T cells) signaling pathways[103]. In the diabetic population, plasma levels of FGF23 are increased[104], and some studies point to its suitability as an atherosclerosis biomarker in these patients[105].

Concluding remarks

The relatively slow evolution of DCM and its associated high morbidity and mortality make the validation of new, reliable and specific biomarkers that would allow more efficient and early diagnosis of utmost importance. Several standardized biomarkers have been validated by the FDA for the diagnosis and risk assessment of various cardiac diseases to date, but they are not able to distinguish patients with DCM, particularly in the early stages, i.e. when still asymptomatic but after dysregulation in cardiac metabolism, inflammation and fibrosis has already begun. Moreover, assessment of DCM is puzzling in clinical practice owing to the atypical and diverse presentation of signs and symptoms.

The pathophysiology of DCM has several interconnected backgrounds, including metabolic dysregulation, low-grade inflammation, oxidative stress, myocardial fibrosis and cardiomyocyte

apoptosis, which, overall, contribute to the progression and fatal outcomes of the disease. Therefore, the best approach to detect DCM prior to the appearance of clinical symptoms and irreversible complications will likely involve combining echocardiographic imaging techniques to evaluate changes in cardiac structure and function, and the quantification of a panel of minimally-invasive biomarkers covering the main diabetes-induced alterations in the heart (see Outstanding Questions). This multi-biomarker approach would be quite time-consuming and may incur a higher cost than traditional biomarkers, but would improve the diagnosis and prognosis of the disease, and would bypass the problems observed when relying on single biomarkers.

In this review we highlight several candidates that fulfil the requirements for becoming biomarkers with the potential to detect or prevent DCM, or even to treat it once established. Among them, FABP3, activin A, CT-1, YKL40, galectin-3, IGFBP-7, FGF21 and several miRNAs stand out because they can be measured by minimally-invasive methods, they integrate several pathophysiological pathways and, last but not least, they are potentially capable of distinguishing patients with DCM also in the early stages. Of course, further preclinical studies are required to unequivocally elucidate the role of these biomarkers in the development and progression of DCM and to fine-tune their detection methods. Afterwards, additional clinical data will be necessary to explain some of the conflicting results observed in clinical trials and to establish key values or ranges of these biomarkers according to the type and severity of diabetes, and the characteristics of the patients.

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Glossary

Advanced glycation end-products: proteins or lipids that are glycated and oxidized as a consequence of persistent exposure to high concentrations of reducing sugars (e.g. glucose), and that are causatively associated with complications of diabetes.

Apoptosis: genetically programmed cell death that is characterized by the fragmentation of nuclear DNA, and which aims to eliminate DNA-damaged, superfluous or unwanted cells.

Autophagy: homeostatic process that involves cell degradation of unnecessary or dysfunctional cytoplasmic components ranging from protein aggregates to whole organelles through the action of lysosomes.

Cardiac hypertrophy: abnormal thickening of the heart muscle that results from cardiomyocyte enlargement and changes in the extracellular matrix.

Cardiac output: volume of blood being pumped by the heart per unit time.

Cardiac remodeling: is the result of an imbalance between pro- and antifibrotic factors that promotes extracellular matrix protein deposition. The resultant cardiac fibrosis impairs cardiomyocyte contractility and, ultimately, leads to cardiac stiffness and heart failure.

Cardiac steatosis: excessive accumulation of triglycerides in cardiomyocytes.

Cytokines and chemokines: extracellular mediators that participate in the regulation of acute and chronic inflammation.

Dilated cardiomyopathy: condition in which the heart becomes enlarged and cannot pump blood efficiently.

Diastolic dysfunction: impaired ventricular relaxation and filling resulting in a higher end-diastolic pressure for a given end-diastolic volume.

Endoplasmic reticulum (ER) stress: ER is the subcellular organelle responsible for protein folding and maturation. Any perturbation that hinders these processes will give rise to the accumulation of unfolded or misfolded proteins, which leads to the activation of the unfolded

protein response (UPR) by the ER. The purpose of the UPR is to promote cell survival by mitigating the adverse effects of ER stress, and this is achieved by arresting general mRNA translation, facilitating protein degradation and enhancing the production of molecular chaperones involved in protein folding.

Fibroblast growth factors (FGF): small signaling proteins (~150–300 amino acids) with diverse biological functions, mainly in development and metabolism. The family comprises up to 23 members (FGF1-FGF23), which display paracrine, intracrine, and endocrine actions.

Insulin resistance: condition in which the cells of the body do not respond properly to the hormone insulin.

microRNA: endogenous non-coding small RNA that modulates gene expression by targeting mRNAs for post-transcriptional repression.

Oxidative stress: refers to elevated intracellular levels of reactive oxygen species (ROS) that cause damage to lipids, proteins and DNA. It is caused by an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects by antioxidants.

Peroxisome proliferator-activated receptors (PPAR): subfamily of the nuclear receptor superfamily that regulates transcriptional gene expression and plays essential roles in the regulation of cellular differentiation, development and metabolism. It comprises three isotypes: PPARα, PPARβ/δ and PPARγ.

Box 1

Clinical features and diagnostic methods of diabetic cardiomyopathy

The diabetic heart is mostly characterized by concentric left ventricular hypertrophy, dilated cardiomyopathy and extracellular fibrosis. Cardiac remodeling during diabetic cardiomyopathy (DCM) occurs in different phases. The first area in which obvious pathological changes usually occur is the myocardial interstitium. This is often followed by perivascular fibrosis and left ventricular hypertrophy, which result in impaired relaxation and passive filling of the left ventricle (diastolic dysfunction)[3]. Diastolic dysfunction is habitually the earliest functional abnormality detected in DCM, and may even be found in asymptomatic diabetic patients and patients with good glycemic control. Conversely, systolic dysfunction, defined as the inability of the myocardium to eject an adequate blood volume, is less frequent and typically develops in the later stages of the disease.

Despite the different techniques available nowadays, there is no gold-standard diagnostic test for DCM. Indeed, DCM is usually detected through identification of systolic dysfunction at late stages of the disease, when heart failure is already established. Endomyocardial biopsy sampling and cardiac catheterization are accurate methods for diagnosing diastolic dysfunction in the early stages, but because of the high risk they pose, their use has been confined to research settings[37]. Two-dimensional, and more recently three-dimensional, echocardiography are valuable inexpensive and non-invasive tools to detect functional and structural cardiac disorders in real time, including early fibrosis and cardiac hypertrophy[37]. Magnetic resonance imaging displays greater accuracy and reproducibility than echocardiography, and is valid for measuring left ventricular mass, myocardial steatosis and diastolic dysfunction, besides providing data about myocardial fibrosis. Nevertheless, it is currently only used for research purposes due to its demanding time, cost and expertise requirements. Finally, positron emission tomography scanning

has been successfully used to detect myocardial metabolic abnormalities in murine models of DCM and asymptomatic DM2 patients.

Box 2

Treatment approaches for diabetic cardiomyopathy

There are no formal guidelines regarding the management of diabetic cardiomyopathy (DCM) but, as a general rule, it includes lifestyle modifications (weight loss, restriction of total energy intake and regular physical activity), improvement of glycemic control, lipid-lowering drugs, and the management of heart failure itself.

The PPARγ agonists thiazoledinediones (TZD) are hypoglycemic insulin-sensitizing drugs that improve myocardial glucose metabolism in addition to displaying some beneficial antiinflammatory and profibrinolytic effects[106]. However, chronic TZD treatment may favor the occurrence of symptoms that resemble heart failure, and for that reason they are not recommended in patients suffering this pathology[3]. Likewise, metformin is a first-line medication for the treatment of DM2 that prevents DCM in experimental models and reduces morbidity and mortality in overweight patients with heart failure and diabetes, but that was till recently contraindicated in patients with heart failure because it causes lactic acidosis[3]. Similar conflicting results have been reported with other classic antidiabetic medicines (sulfonylureas and insulin)[15]. With regard to newer antidiabetic drugs (glucagon-like peptide-1 mimetic agents and dipeptidyl peptidase 4 inhibitors)[3, 15], or lipid-lowering drugs (statins)[107], there are some encouraging data pointing to their cardioprotective effects, but additional clinical trials are required to determine their efficacy and safety in DCM.

The antihypertensive drugs angiotensin converting enzyme inhibitors, renin inhibitors and angiotensin II receptor blockers also protect the heart in both human and animal models of DCM[3]. Similarly, β-adrenoreceptor antagonists[108] and calcium channel blockers[109] have

both shown protective effects against DCM, suggesting their putative suitability in hypertensive patients with DCM. Finally, antianginal metabolic modulators (trimetazidine, ranolazine) are also promising drugs for treating DCM in the near future, since they may correct the metabolic dysregulation occurring during this pathology, at the same time they display beneficial pleiotropic effects on oxidative stress, lipotoxicity, calcium handling and apoptosis[3].

Box 3

Regulation of heart contraction by calcium

Calcium ions are pivotal regulators in the process of excitation-contraction coupling in the heart. Once the action potential reaches the cardiomyocyte, the cell membrane is depolarized and calcium enters the cell through voltage-dependent L-type calcium channels in the sarcolemma. This increased intracellular calcium activates the ryanodine receptors located in the sarcoplasmic reticulum to trigger the massive release of further calcium ions from this store. Then, cytosolic calcium will bind to myofilaments in order to begin cardiomyocyte contraction. Removal of calcium from the cytosol results in the opposite process of cardiomyocyte relaxation, and this is mostly carried out by pumping calcium back into the sarcoplasmic reticulum by SERCA (sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase), although other processes may intervene (mitochondrial calcium uniport, PMCA or plasma-membrane Ca^{2+}-ATPase and Na^{+}/Ca^{2+} exchange pumps).

Figure legends

Figure 1. **Major pathophysiological mechanisms of diabetic cardiomyopathy**. Diabetes induces an increase in plasma free fatty acids (FA) and glucose levels, which are internalized into cardiomyocytes by fatty acid translocase (FAT/CD36) and the insulin-induced glucose transporter 4 (GLUT4), respectively. Excess FA stimulates the peroxisome proliferator-activated receptor (PPAR)/PGC-1α (PPARγ coactivator-1α) pathway, leading to increased transcription of genes involved in FA uptake and oxidation (CPT1, carnitine palmitoyl-transferase 1, and FAT/CD36). Another PPAR-induced gene, the pyruvate dehydrogenase kinase 4 (PDK4), decreases glucose oxidation by inactivating the pyruvate dehydrogenase complex (PDC). This causes an increase in fatty acid oxidation, which will lead to mitochondrial dysfunction, the loss of metabolic flexibility and energy production efficiency (ATP) by the heart, and the generation of reactive oxygen species (ROS) by the mitochondria. On the other hand, downregulated insulin signaling as a consequence of insulin resistance prevents GLUT4 translocation from intracellular vesicles towards the sarcolemma, thus reducing glucose uptake and utilization, and promoting a substrate shift toward increased mitochondrial FA β-oxidation. Despite the higher FA oxidation rate, myocardial lipid accumulation (cardiac steatosis) occurs, and the subsequent formation of toxic lipid intermediates (ceramides and diacylglycerol, DAG) contributes to the development of heart failure. These intermediates activate the proinflammatory NF-κB and activator protein-1 (AP-1) transcription factors and favor the onset of endoplasmic reticulum (ER) stress and mitochondrial dysfunction. On the other hand, hyperglycemia also induces the formation of advanced glycation end-products (AGE) and ROS within the cardiomyocytes, which trigger NF-κB activation, hence inducing inflammation (cytokines and chemokines) and interstitial fibrosis (MMP, matrix metalloproteinase; TGFβ, transforming growth factor β). ROS accumulation also hastens apoptosis and brings on ER stress in cardiomyocytes. As a consequence, calcium handling is disturbed and cardiac contractility is reduced, overall leading to cardiac dysfunction.

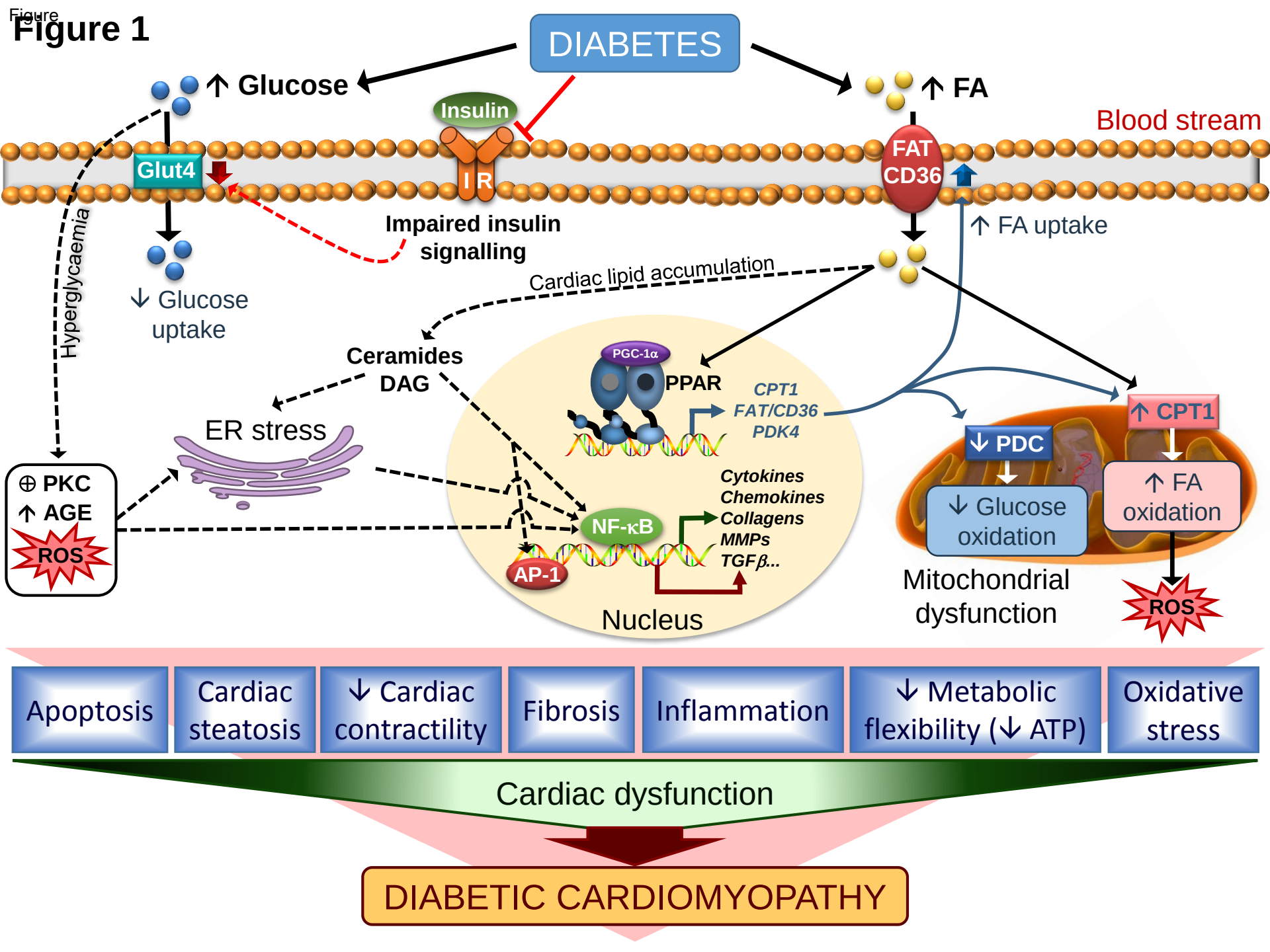
Figure 2. **Emerging metabolism-related components in diabetic cardiomyopathy**. In the phosphocreatine (PCr)/creatine (Cr) "shuttle" system, phosphate is transferred from the ATP formed in the mitochondria to Cr via mitochondrial creatine kinase (mtCK), generating PCr. Then, PCr diffuses into the cytoplasm, where the muscle-type creatine kinase (mmCK) forms Cr and ATP, the latter participating in myofibril (cardiomyocyte) contraction. The fatty acid binding protein 3 (FABP3) is a cardiac-specific PPAR-target protein induced during diabetes that transports long-chain fatty acids (FA) to the mitochondria for their subsequent oxidation, and also to the nucleus to activate PPAR-dependent gene expression. FABP3 is involved in cardiac steatosis and mitochondrial dysfunction by excess FA delivery, and also has an antiapoptotic role. The formation of toxic lipid intermediates (ceramides and diacylglycerol, DAG) induces endoplasmic reticulum (ER) stress, thus altering membrane synthesis and reducing calcium release (i.e. cardiomyocyte contraction). TRIM72 (tripartite motif containing 72) impairs insulin signaling because it drives ubiquitin-dependent degradation of insulin receptor (IR) and insulin receptor substrate 1 (IRS1), thus increasing the reliance of the heart on FA as an energy source and PPAR activity. TRIM72 also plays an important role in cardiac fibrosis through the modulation of the transforming growth factor (TGF)β. In a similar way, TP53INP2 (tumour protein p53 inducible nuclear protein 2), the expression of which is induced by PPARα, regulates the expression of important glycolytic enzymes involved in glucose uptake and glycogen storage in cardiomyocytes. TP53INP2 is also a key regulator of autophagy and protein degradation, thus hindering the expression of hypertrophic genes, particularly in the context of hyperglycaemia.

Figure 3. **Emerging molecules involved in inflammation and fibrosis**. Cardiotrophin-1 (CT-1) promotes cardiac fibrosis and remodeling and inhibits apoptosis by activating the JAK/STAT3 (janus kinase/signal transducer and activator of transcription 3) pathway. Galectin-3 (Gal-3),

which is locally secreted by macrophages and fibroblasts, promotes inflammation and fibrosis by enhancing myofibroblast proliferation, accumulation of extracellular matrix and macrophage infiltration, via stimulation of the TGFβ (transforming growth factor β) signaling pathway. Gal-3 also interferes with insulin signaling by binding to the insulin receptor (IR). YKL-40 attenuates NF-κB-dependent inflammation (cytokines and chemokines), apoptosis, tissue remodeling and fibrosis (MMPs, collagens, TGFβ). Likewise, through the inhibition of NF-κB, GDF15 (growth differentiation factor 15) suppresses the synthesis and secretion of proinflammatory cytokines (TNF-α, IL6). HMGB1 (high-mobility group box 1), the expression of which is induced in cardiomyocytes and cardiac fibroblasts by hyperglycemia, and may be released to the myocardial interstitium, boosts fibrosis and inflammation through the activation of MAPKs (ERK1/2 and JNK) and NF-κB. Its effects rely on binding to the receptor for advanced glycation end products (RAGE) and toll-like receptors (not shown). HMGB1 also displays negative inotropic effects. Downregulation of MICU1 (mitochondrial calcium uptake 1) in the heart during diabetes contributes to myocardial mitochondria-dependent intrinsic apoptosis and fibrosis, and also favors oxidative stress. Finally, insulin-like growth factor binding protein-7 (IGFBP-7) increases collagen deposition, fibrosis and cardiac hypertrophy and interferes with insulin signaling.

Figure

Figure 1



Figure

Figure 2

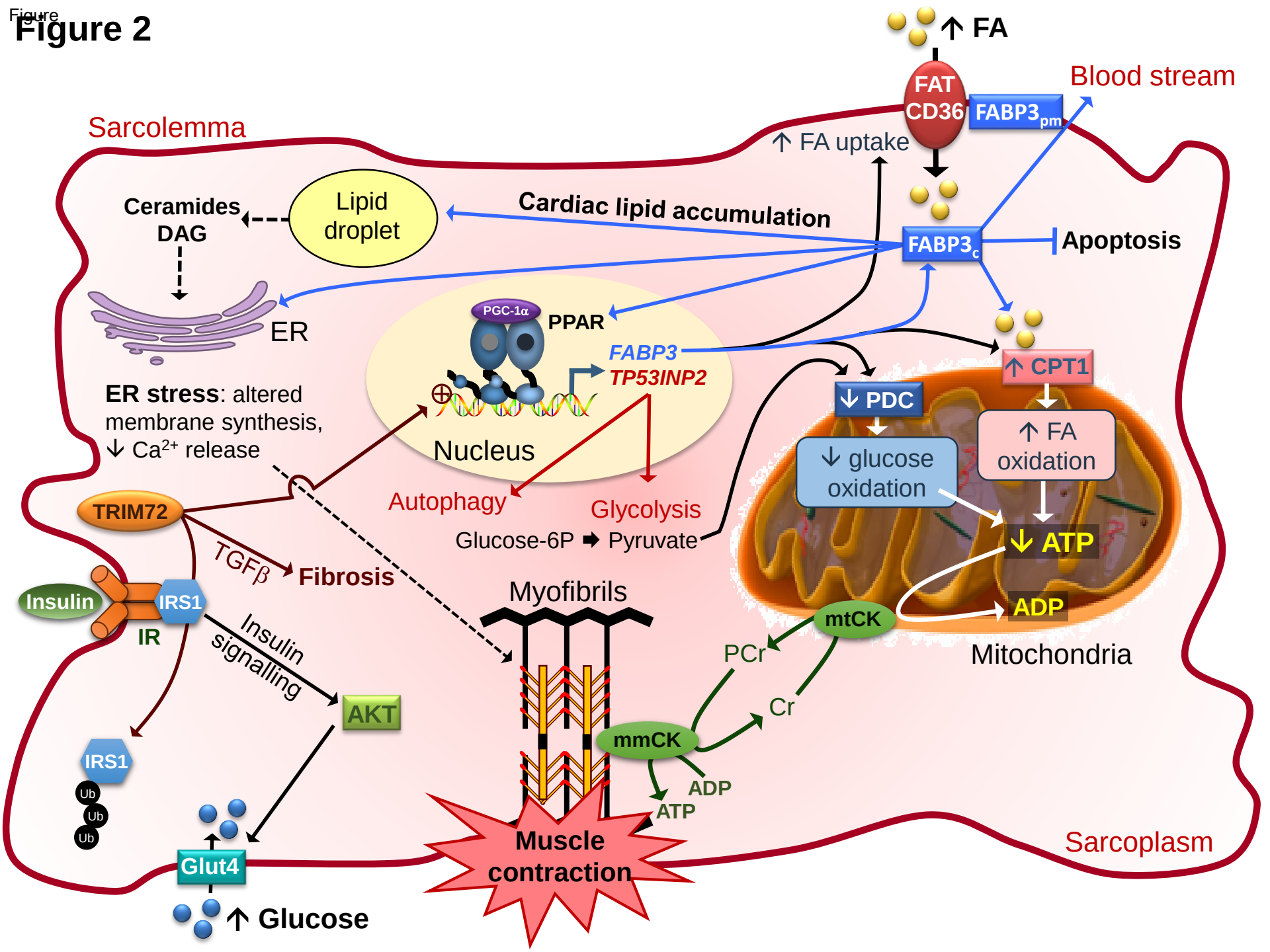


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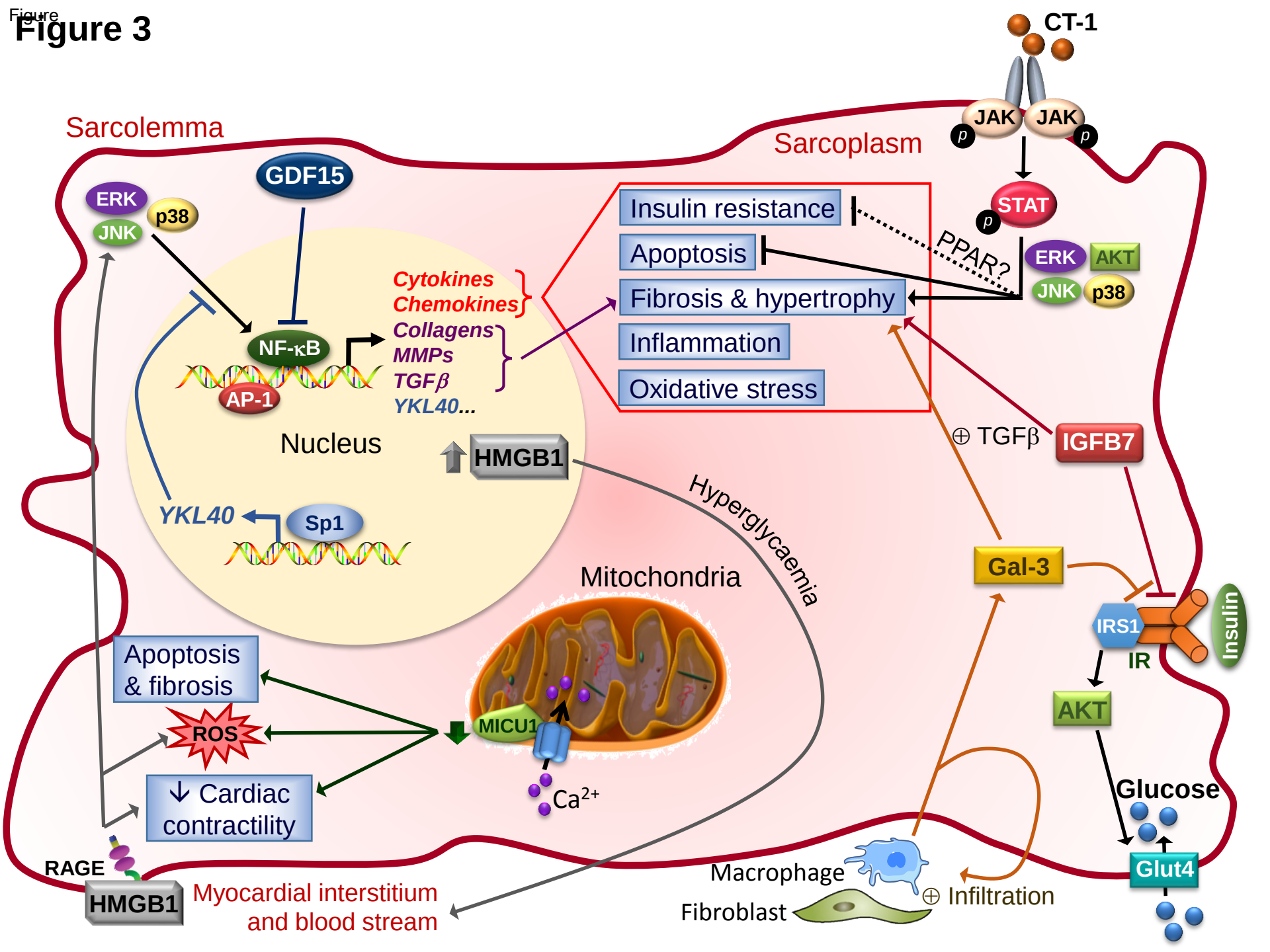
Figure 3



Emerging actors in diabetic cardiomyopathy: heartbreaker biomarkers or therapeutic targets?

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Outstanding Questions

Will it be possible in the near future to detect or prevent diabetic cardiomyopathy in the early stages, or even to treat it once established?

Is the combination of imaging techniques with the quantification of a panel of biomarkers the best approach to detect diabetic cardiomyopathy?

Will it be possible to develop novel biomarkers with potential to discriminate diabetic cardiomyopathy from other cardiac diseases? Will it be the combination of biomarkers that integrate inflammation, oxidative stress, fibrosis and metabolic dysregulation useful to selectively discriminate diabetic cardiomyopathy?

Will candidate biomarkers belonging to the newly discovered molecular pathways provide new tools to improve the diagnosis of diabetic cardiomyopathy? Will these biomarkers also be useful as therapeutic strategies to treat diabetic cardiomyopathy? Are non-coding RNAs appropriate as novel biomarkers with potential to detect or prevent diabetic cardiomyopathy, or even to treat it once established?

Is the quantification of a panel of biomarkers (the multi-biomarker approach) covering the main diabetes-induced alterations in the heart (i.e. inflammation, oxidative stress, fibrosis and metabolic

dysregulation) a suitable, reliable and feasible method to improve diagnosis and prognosis of the disease? Which is the best approach to develop, optimize and validate this multi-biomarker panel?