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REVIEW

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The role of nonmyocardial cells in the development of diabetic cardiomyopathy and the protective effects of FGF21: a current understanding

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Abstract

Diabetic cardiomyopathy (DCM) represents a unique myocardial disease originating from diabetic metabolic disturbances that is characterized by myocardial fibrosis and diastolic dysfunction. While recent research regarding the pathogenesis and treatment of DCM has focused primarily on myocardial cells, nonmyocardial cells—including fibroblasts, vascular smooth muscle cells (VSMCs), endothelial cells (ECs), and immune cells—also contribute significantly to the pathogenesis of DCM. Among various therapeutic targets, fibroblast growth factor 21 (FGF21) has been identified as a promising agent because of its cardioprotective effects that extend to nonmyocardial cells. In this review, we aim to elucidate the role of nonmyocardial cells in DCM and underscore the potential of FGF21 as a therapeutic strategy for these cells.

Keywords Diabetic cardiomyopathy, Nonmyocardial cells, Fibroblast growth factor 21

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Introduction

Diabetes is a chronic condition characterized by hyperglycemia resulting from impaired insulin secretion and/or action. It represents a heterogeneous metabolic disorder primarily characterized by elevated blood sugar levels [1]. According to the International Diabetes Federation (IDF), there are over 400 million people living with diabetes worldwide, 91% of whom have type 2 diabetes. IDF data indicate that diabetes affects 8.8% of the global population, and this number is projected to increase to 642 million by 2040 [2]. Over the past few decades, the incidence of diabetes has steadily increased globally across nearly all regions, making it a pressing global health concern [3]. Cardiovascular disease (CVD) is the leading cause of mortality among diabetic patients. Diabetic cardiomyopathy (DCM), a severe cardiovascular complication associated with diabetes, represents a pathophysiological state induced by diabetes mellitus

(DM). Importantly, DCM can lead to heart failure (HF) independent of coronary artery disease, hypertension, and heart valve disorders [4]. Multiple factors contribute to the onset and progression of diabetic cardiomyopathy. These include insulin resistance, disorders in myocardial cell glucose and lipid metabolism, oxidative stress, myocardial cell apoptosis, myocardial inflammation, and fibrosis. Both individually and in combination, these factors collectively promote the development of DCM [5–8].

Recently, most research on the pathogenesis and treatment of DCM has focused primarily on myocardial cells. However, the pathophysiology of DCM cannot be attributed solely to pathological changes in myocardial cells. The heart is a composite structure comprising both myocardial cells and nonmyocardial cells. Myocardial cells account for approximately 40% of cardiac cells; fibroblasts constitute the second largest proportion of approximately 20%; vascular cells contribute to approximately 15% of cardiac cells; immune cells represent approximately 10% of cardiac cells; and adipocytes constitute approximately 0.5% of cardiac cells [9]. The nonmyocardial cell population plays a critical role in maintaining healthy cardiac function and, under diabetic conditions, contributes to myocardial disease through fibrosis, inflammation, and vascular dysfunction[10] (Fig. 1). Furthermore, cardiomyocytes are impacted by fibroblast activation and transformation into myofibroblasts, leading to excessive extracellular matrix (ECM) deposition and fibrosis. This process increases myocardial stiffness and disrupts normal cardiomyocyte contractility. VSMCs contribute to diabetic cardiomyopathy by promoting vascular remodeling and stiffness in response to hyperglycemia and other metabolic stressors. This vascular dysfunction leads to impaired blood flow and increased afterload on the heart, which negatively impacts cardiomyocyte function and promotes hypertrophy. Endothelial dysfunction and endothelial-to-mesenchymal transition (EndMT) in diabetes reduce angiogenesis and impair nutrient delivery, contributing to cardiomyocyte ischemia, apoptosis, and dysfunction. Activated macrophages and other immune cells release pro-inflammatory cytokines that exacerbate inflammation, leading to cardiomyocyte hypertrophy, apoptosis, and further deterioration of cardiac function. These processes collectively lead to the deterioration of cardiac function and structure in DCM [10].

Fibroblast growth factor 21 (FGF21), a peptide hormone that regulates energy homeostasis, it is expressed in various mouse tissues, including the liver, brown adipose tissue, white adipose tissue (WAT), and pancreas. The liver is the primary source of circulating FGF21 in the bloodstream [11]. FGF signaling is regulated in a tissue-specific manner through the interaction of FGF, fibroblast growth factor receptor (FGFR), heparan sulfate proteoglycan (HSPG), and Klotho-type co-receptors [12]. The FGFR family comprises FGFR1b, FGFR1c, FGFR2b, FGFR2c, FGFR3b, FGFR3c, and FGFR4 [13]. FGF21 was reported to requires βklotho (KLB) to assist in binding FGF21 to FGFR1c, FGFR2c, and FGFR3c, thereby initiating its biological effects [14–16]. βklotho expression is tissue-specific, with high levels found predominantly in

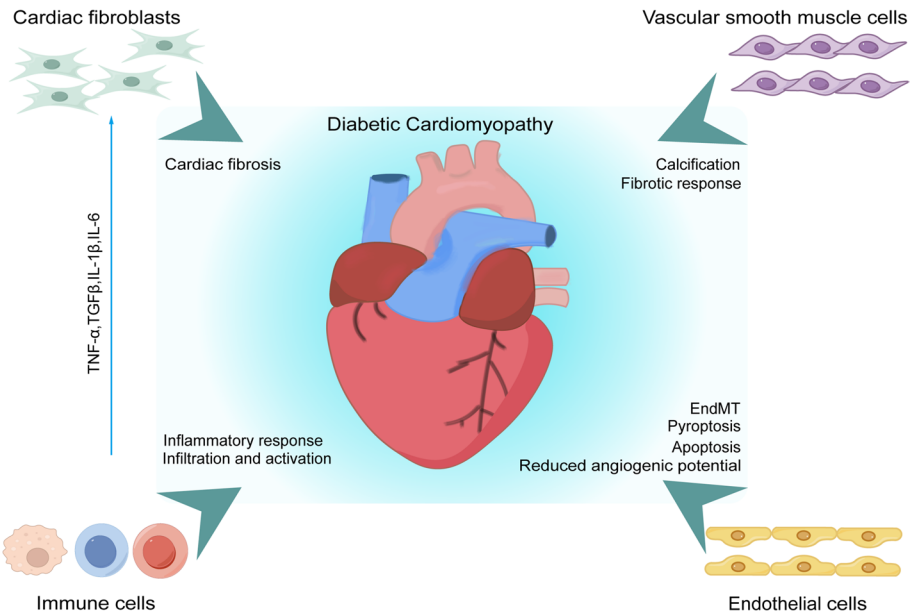


Fig. 1 Contribution of nonmyocardial cells to the development of DCM

adipose tissue, liver, and pancreas [17]. Studies have also shown that βklotho is expressed in smooth muscle cells [18–20], endothelial cells [21–23], and macrophages [21, 24]. While βklotho expression is very low in fibroblasts, it may be activated under pathological conditions [25]. Receptor activation can initiate various intracellular signaling cascades, including direct interaction with the FRS2 docking protein, and downstream activation of the Ras/MAPK, PKC, Jak/STAT, and PI3K/mTOR pathways, affecting metabolism, proliferation, and survival [26] Studies have shown that FGF21 can promote the uptake of glucose by adipocytes independent of insulin and can upregulate the expression of Glucose transporter 1 (GLUT1). Additionally, FGF21 can lower blood glucose and triglyceride levels in OB/OB, DB/DB, and ZDF rats and improve insulin sensitivity and glucose tolerance without causing hypoglycemia or weight gain [27]. FGF21 plays a protective role in the hearts of mice with DCM.

A previous study detailed the relationship between FGF21 and cardiomyocytes in DCM. The authors reported that FGF21 has various physiological functions, such as antiapoptotic, anti-inflammatory, antioxidative, and fatty acid oxidation effects, and that it protects cardiomyocytes during DCM pathogenesis [28]. However, there is a lack of comprehensive reviews on the role of FGF21 in nonmyocardial pathology in DCM to date; this review aims to shed light on its impacts beyond myocardial cells.

Diabetic cardiomyopathy (DCM) involves complex interactions between various cell types in the heart, not just cardiomyocytes. Nonmyocardial cells, including fibroblasts, vascular smooth muscle cells, endothelial cells, and immune cells, play critical roles in the pathogenesis of DCM. Cardiac fibroblasts contribute to fibrosis, which is a hallmark of DCM. They proliferate and produce ECM proteins, leading to stiffening of heart tissue and impaired cardiac function. Smooth muscle cells undergo phenotypic shifts, and DCM-related metabolic adaptations correlate with the proliferation, migration, calcification, and inflammation of these cells. Endothelial dysfunction is common in patients with DCM, resulting in impaired angiogenesis and reduced capillary density. This compromises myocardial perfusion and contributes to ischemia and cardiomyocyte death. Inflammatory cells, including macrophages and T-cells, infiltrate the myocardium in response to hyperglycemia and other metabolic stressors. These cells release proinflammatory cytokines, exacerbating myocardial inflammation and fibrosis.

Cardiac fibroblasts

Role of cardiac fibroblasts in DCM

Cardiac fibroblasts (CFs) represent the predominant nonmuscle cell type within the heart. They play a pivotal role in maintaining normal cardiac morphology and function and actively participate in cardiac remodeling processes under pathological conditions. These fibroblasts engage in intricate interactions with other cardiac cells that are mediated by chemical, mechanical, and electrical signals, thereby influencing diverse cellular signaling pathways and gene expression [29, 30]. Notably, CFs receive profibrotic cues from immune and vascular cells, allowing them to dynamically regulate the ECM. Conversely, they contribute to vascular neogenesis and immune cell infiltration by secreting vascular growth factors and chemokines, thereby eliciting an inflammatory response. In diabetic contexts, these intercellular interactions may intensify, contributing to the development of DCM. Furthermore, under high glucose (HG) conditions, CFs increase collagen synthesis while concurrently suppressing the overall activity of matrix metalloproteinases (MMPs), ultimately promoting cardiac fibrosis and heart failure [31].

Transition of CFs into myofibroblasts

The differentiation of CFs into myofibroblasts is a key cellular event in DCM-induced myocardial fibrosis. Under high-glucose conditions, CFs tend to differentiate into myofibroblasts while promoting CF proliferation. CFs isolated from adult rats showed a significant increase in alpha smooth muscle actin (α-SMA) expression after 24 h of high glucose treatment compared with that in CFs cultured in low glucose medium, promoting spontaneous differentiation into myofibroblasts [32]. CFs isolated from diabetic animals presented increased α-SMA expression, indicating a phenotypic transition of diabetic CFs into myofibroblasts [33]. The balance between ECM synthesis and degradation plays a crucial role in myocardial fibrosis. The ECM is primarily composed of type I and type III collagen. CFs, as the primary effector cells in cardiac fibrosis, are responsible for producing the ECM and maintaining its dynamic balance. CFs isolated from Zucker diabetic rats synthesize angiotensin II (Ang II), activating the renin–angiotensin–aldosterone system and ACE signaling pathways and promoting collagen matrix contraction and deposition [33]. Moreover, culturing CFs isolated from db/db mice under 5 mM and 25 mM glucose conditions led to a significant increase in type I collagen expression. These findings suggest that under high-glucose conditions, myocardial fibroblasts exhibit elevated collagen expression, leading to ECM deposition, increased cardiac stiffness, and diastolic dysfunction.

Myofibroblasts secrete a large amount of ECM, as well as MMPs and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), which participate in the fibrotic remodeling process.

Mechanisms underlying CF transition and fibrosis in DCM

High glucose/hyperglycemia

To identify potential targets for treating DCM, numerous studies on the phenotype and function of CFs have been conducted. TGF-β1 (transforming growth factor-β1), an inducer, facilitates the differentiation of CFs into myofibroblasts [34]. In isolated rat CFs, HG stimulation promoted TGF-β1 expression. HG conditions stimulate increases in ERK1/2 activity in CFs in vitro, and the ERK1/2 inhibitors PD98059 and U0126 inhibit HG-induced fibroblast proliferation and collagen expression while also suppressing the HG-induced upregulation of TGF-β1 expression [35]. The calcium-sensing receptor (CaSR), a member of the G protein-coupled receptor superfamily, regulates the intracellular calcium concentration. HG treatment of CFs promotes CaSR expression through changes in cellular activity. Activated CaSR subsequently upregulates the expression of Smad ubiquitin regulatory factor 2 (Smurf2), resulting in increased ubiquitination levels of proto-oncogenes and Smad7. Additionally, autophagy is activated, leading to excessive CF proliferation and extensive collagen deposition via the TGF-β pathway [36, 36]. Furthermore, CFs have an intracellular renin–angiotensin system (RAS). Upon HG stimulation, intracellularly synthesized Ang II, a potent vasoconstrictor and fibrotic factor, upregulates thrombospondin-1 (TSP1) expression [37]. This activation of TSP1 promotes TGF-β1 bioactivation, thereby enhancing the synthesis of TGF-β1 and collagen type I in CFs [38]. TSP-1, a multidomain protein synthesized and secreted by various cells, has been shown to have upregulated expression in CFs under HG conditions. TSP-1 activates the latent complex of TGF-β1 and participates in the upregulation of TGF-β1 and type III collagen expression [39]. HG increases the activity of the transcription coactivator p300, thereby increasing TGF-β1 activity through Smad2 acetylation [40]. The expression of the transcription factor FoxO1 is upregulated by HG, resulting in increased TGF-β1 expression and FoxO1 activity. FoxO1 promotes connective tissue growth factor (CTGF) expression, facilitating CF phenotypic transformation and augmenting the synthesis and secretion of ECM proteins, leading to cardiac fibrosis and cardiac dysfunction [41].

Hyperglycemia induces an increase in DNA methyltransferase 1 (DNMT1) expression in CFs. DNMT1-mediated hypermethylation of the suppressor of cytokine signaling 3 (SOCS3) promoter inhibits the downregulation of SOCS3 expression in diabetic cardiac fibrosis, thereby promoting the activation of STAT3, leading to cardiac fibroblast activation and collagen deposition [42]. HG suppresses RASSF1A expression, accompanied by an increase in MeCP2 expression, which activates the RASSF1A/ERK1/2 signaling pathway to promote cardiomyocyte fibroblast proliferation. Abnormal cardiomyocyte fibroblast proliferation can lead to heart failure and ECM deposition, thereby exacerbating the development of DCM [43].

Cytokines

Cytokines are also involved in cardiac fibrosis. In STZ-induced diabetic mice with IL-6 gene expression knockout, cardiac interstitial fibrosis was significantly reduced compared with that in STZ-induced wild-type (WT) diabetic mice. IL-6 gene knockout mitigates the upregulation of TGFβ1 expression in the hearts of diabetic mice treated with HG or cultured CFs treated with IL-6 [44]. IL-17 plays a similar role in diabetic mice. It binds to the receptors IL-17RA and IL-17RC on the surface of CFs to activate the PKCβ/Erk1/2/NF-κB signaling pathway. This activation leads to increased expression levels of collagen I and III in CFs, ultimately promoting collagen synthesis and deposition in cardiac tissue [45]. HG treatment significantly promotes the production of proinflammatory cytokines (IL-1β) and activates NF-κB in CFs, increasing the expression of fibrotic markers (CTGF, FN, and α-SMA) and extracellular matrix proteins (Col-I and Col-III) [46]. In one STZ-induced model of diabetic mice, IL-33 exhibited antifibrotic cytokine function. Diabetes-related hyperglycemia causes stress in cardiomyocytes, leading to the release of DAMPs and HMGB1. Interstitial HMGB1 interacts with Toll-like receptor 4 (TLR4) receptors on adjacent fibroblasts, leading to reduced expression of IL-33 and increased collagen production [47].

Energy metabolism

The heart adapts its energy metabolism by utilizing various substrates. During development, the heart predominantly relies on aerobic glycolysis and lactate oxidation. In contrast, the adult heart primarily generates ATP through oxidative metabolism, with fatty acids serving as the principal energy source [48]. CFs mirror the substrate utilization characteristics of the heart. CFs isolated from db/db mice under normal blood glucose conditions exhibit a profibrotic phenotype characterized by increased collagen synthesis and reduced TGF-β sensitivity [49]. Additionally, under high-fat diet (HFD) conditions, CFs can differentiate into adipocytes [50]. Adipocytes synthesize and secrete circulating regulators, including resistin and leptin, which promote cardiac fibrosis development. In hearts overexpressing resistin,

the mRNA expression levels of collagen subtypes I and III, as well as those of CTGF and fibronectin, are elevated [51]. Furthermore, resistin promotes CF proliferation in adult mice by activating Janus kinase 2 (JAK2) through TLR4 binding. This activation leads to STAT3 phosphorylation, translocation to the nucleus, and subsequent promotion of fibroblast-to-myofibroblast differentiation and fibrosis via the JNK/c-Jun signaling pathway, independent of TGFβ1 signaling [52]. The profibrotic effects of leptin in the heart are realized mainly by enhancing collagen synthesis. Cardiomyocytes cultured in vitro and treated with leptin for 24 h presented significantly increased procollagen Iα (1) expression and decreased expression of MMPs 8, 9, and 13 [53]. Additionally, leptin expression increases MT1-MMP transport to the cell surface in primary adult rat CFs, resulting in increased extracellular MMP-2 precursor activation and increased fibroblast migration, ultimately leading to increased cardiac collagen deposition [54]. Elevated expression of cell death-inducing DFFA-like effector C (CIDEC), a lipid droplet-associated protein that can prevent lipid mobilization and promote intracellular lipid storage, is associated with DCM in rats [55]. Insulin resistance significantly increases CIDEC expression in CFs, promoting CIDEC nuclear translocation. This inhibits AMP-activated protein kinase α (AMPK) phosphorylation and amplifies collagen synthesis [56].

Advanced glycation end products (AGEs)

Advanced glycation end products (AGEs) constitute a heterogeneous group of molecules formed through non-enzymatic glycation and the oxidation of proteins, lipids, and nucleic acids. AGE formation and accumulation are increased in individuals with DM [57]. Research has indicated that AGEs stimulate type I collagen expression via ERK 1/2- and p38-MAPK-dependent pathways [58]. Furthermore, diabetes upregulates CTGF expression, promoting the binding of AGEs to their receptors (receptors for advanced glycation end products, RAGEs). This interaction contributes to regulating extracellular matrix (ECM) synthesis, accumulation, and fibroblast differentiation [59, 60]. These findings underscore the direct role of AGEs in promoting cardiac fibrosis.

Protective effect of FGF21 against CF transition and fibrosis

The heart serves as both a target and a source of FGF21. CF is known to express FGFR1c [61] and FGFR2 [62]. Recent research has underscored that CFs exhibit minimal FGF21 synthesis but are direct targets of its effects. FGF21 expression is significantly increased in hypertensive humans and mice, which coincides with pronounced cardiac hypertrophy and fibrosis. Upon the onset of hypertension, both locally produced and systemically circulating FGF21 are induced to exert protective effects on the heart [63]. Compared with their wild-type counterparts, mice lacking FGF21 expression display increased susceptibility to cardiac hypertrophy and impaired pump function following isoproterenol treatment [64]. In the context of DCM, FGF21 expression deficiency contributes to myocardial lipid accumulation via Nrf2 (nuclear transcription factor E2-related factor 2)-driven upregulation of CD36 expression, thereby promoting DCM development [65].

Mechanistically, FGF21 inhibits the TGF-β1-Smad 2/3-MMP 2/9 signaling pathway and collagen synthesis, mitigating fibrosis [66]. Furthermore, it suppresses tachycardia-induced TGF-β1 expression, impedes collagen expression upregulation in fibroblasts, and attenuates rapid pacing-induced oxidative stress in atrial myocytes through ROS, TGF-β, and ox-CaMKII signaling. These actions collectively improve rapid pacing-induced myofibrillar degradation, counteract L-type calcium channel expression downregulation, and increase p-RyR2 expression, effectively mitigating atrial remodeling [67]. Additionally, FGF21 activates cell surface FGFR, promoting the expression of the transcription factor early growth response protein 1 (EGR1) while inhibiting NLRP 3, IL-1, IL-6, IL-18, COL 1, COL 3, Acta 2, and TGF-β1 activity [68].

Furthermore, FGF21 has been unequivocally established as an effective therapeutic agent for type II diabetes and obesity. The administration of recombinant FGF21 protein to ob/ob, db/db, or HFD-fed, in addition to obese Zucker diabetic fatty (ZDF) rats, robustly reduced obesity, lowered blood glucose and triglyceride levels, and improved insulin sensitivity [69]. In mice with streptozotocin (STZ)-induced type I diabetes, inhibiting FGF21 expression exacerbated cardiac hypertrophy and fibrosis [70]. This was accompanied by elevated expression levels of atrial natriuretic factor, α-SMA, type I and III collagen, and TGF-β. Additionally, lipid droplet numbers increased, cardiac triglyceride concentrations rose, plasma triglyceride and cholesterol levels increased, and peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) expression was downregulated. CD36 expression was upregulated, contributing to lipid accumulation and further compromising cardiac function. Adiponectin, a hormone secreted by adipocytes, plays a pivotal role in regulating energy metabolism, body weight, and blood glucose balance. Treatment with FGF21 induced adiponectin expression, which, in turn, reduced resistin-induced inflammation and probably inhibited the profibrotic effects of resistin in the heart [71].

In summary, under high glucose/hyperglycemia conditions, CFs undergo a transition into myofibroblasts. This process, along with the influence of AGEs and other pathological insults, leads to an increase in collagen

synthesis and extracellular matrix deposition, culminating in cardiac stiffness and fibrosis. FGF21 exerts a protective effect by inhibiting CF differentiation through multiple pathways while mitigating damage caused by HG, disruption of lipid metabolism, and other insults mediated by CFs during myocardial fibrosis development (Fig. 2).

In DCM, CFs play a crucial role in myocardial fibrosis. This condition involves the accumulation of ECM proteins, particularly collagen types I and III, leading to ventricular wall stiffness and impaired heart function.

As the most important signaling pathway in CF transition, TGF-β1 expression is induced by many factors during DCM, such as high glucose, HG, AGEs and adipocyte-derived resistin and leptin. Cytokines are also involved in cardiac fibrosis, causing the deposition of collagens in CFs. FGF21 forms a complex with FGFR and βklotho, which inhibits the TGF-β1-Smad 2/3-MMP 2/9 signaling pathway and collagen synthesis. This inhibition prevents CF differentiation into myofibroblasts, thereby reducing fibrosis and offering cardioprotection against the detrimental effects of HG and dysregulated lipid metabolism.

Endothelial cells

Role of endothelial dysfunction in DCM

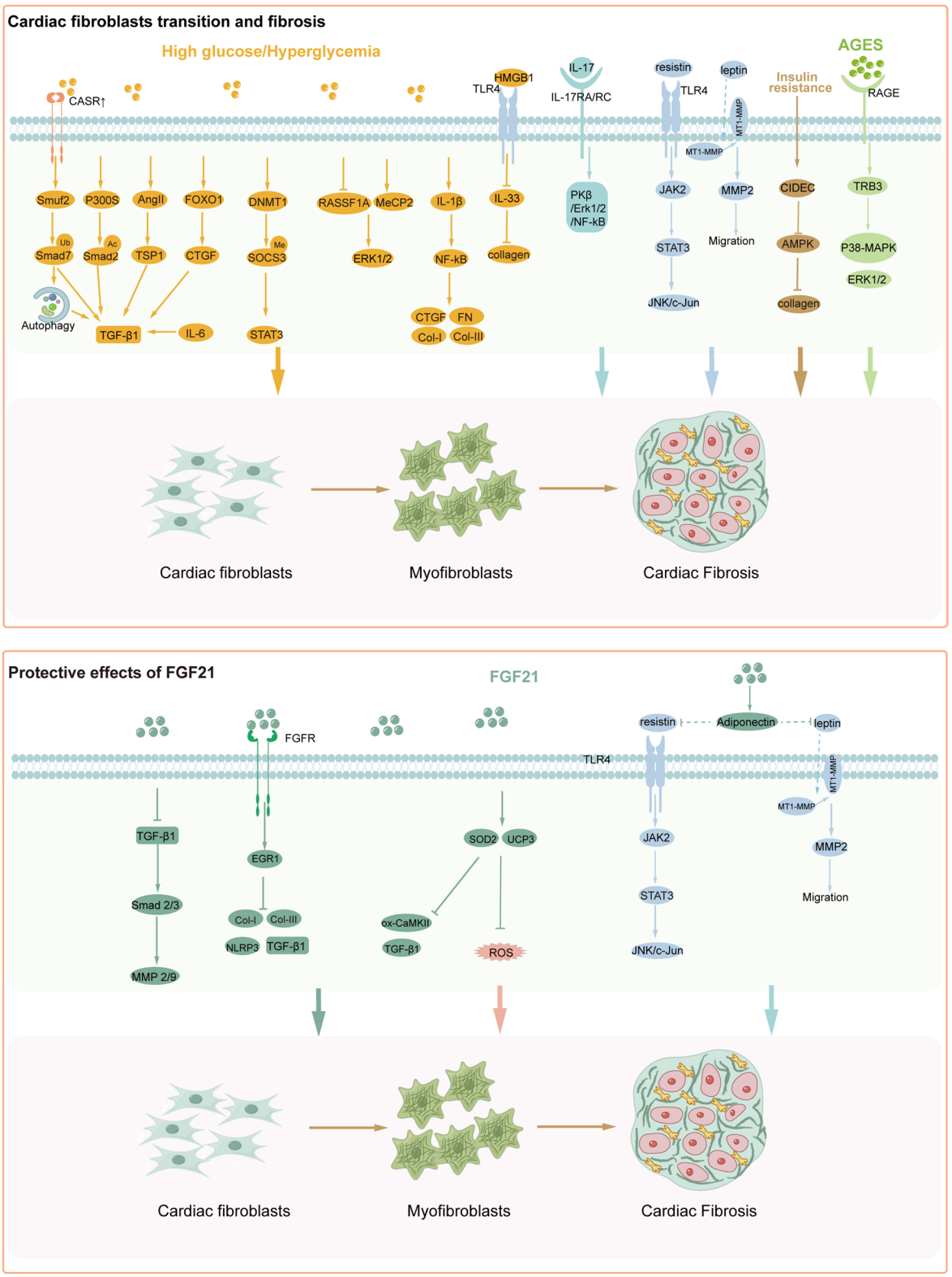
An early hallmark of CVD is endothelial dysfunction, which is characterized by disruptions in the normal physiological functions of endothelial cells. Endothelial cells, a highly heterogeneous cell type, exhibit different phenotypes and functions across various tissues and microenvironments. Their primary role is to maintain and regulate normal vascular function. By secreting various bioactive substances, such as nitric oxide (NO), endothelin-1 (ET-1), nerve growth factor-1 (NGF-1), and prostacyclin I2, endothelial cells modulate the contraction, metabolism, survival, and proliferation of myocardial cells in the heart [72]. Additionally, endothelial cells influence myocardial remodeling through signaling molecules, including those distributed via extracellular vesicles [73]. Elevated blood sugar levels, hyperinsulinemia, and insulin resistance can inflict damage upon endothelial cells, subsequently disrupting myocardial metabolism. These cellular perturbations manifest as abnormal calcium ion balance [74], endoplasmic reticulum stress [75], mitochondrial impairments [76], the accumulation of AGEs [77], and ECM deposition [78]. These processes ultimately culminate in myocardial rigidity, fibrosis, and structural remodeling, leading to compromised cardiac diastolic and systolic function and resulting in HF. Additionally, diabetes exacerbates these effects by promoting endothelial-to-mesenchymal transition (EndMT), which may contribute to myocardial fibrosis and remodeling [79, 80].

Some studies suggest that cardiac fibrosis could be associated with the emergence of fibroblasts derived from ECs via a process known as EndMT that can be induced by TGF-β1 activity [81]. However, it's worth noting that some research challenges this perspective because the specific markers used in these studies may not definitively confirm endothelial origin. Studies utilizing lineage tracing and potentially more specific genetic markers have shown that in disease models such as pressure overload, AngII/PE infusion, TAC, and myocardial infarction (MI) injury, resident cardiac fibroblasts primarily contribute to the pool of myofibroblasts in fibrotic tissue [82, 83]. Nonetheless, in certain contexts of diabetes, endothelial cells may undergo a transition into mesenchymal-like cells that produce extracellular matrix components. Consequently, EndMT offers valuable insights into the potential plasticity of endothelial cells in pathological conditions like diabetic cardiomyopathy. While more research is required to definitively establish this connection, EndMT could be a potential mechanism contributing to myocardial fibrosis, especially in these particular pathological contexts.

Under normal physiological conditions, ECs maintain vascular function by releasing vasodilators (such as NO, prostacyclin, and bradykinin) and vasoconstrictors (such as prostaglandins, endothelin, and angiotensin-II). In the context of DCM, an imbalance exists in the release of vasoconstrictors and vasodilators by ECs, contributing to endothelial dysfunction. As a vasodilator released by ECs, nitric oxide (NO) plays a crucial role in endothelial function. NO is synthesized by endothelial nitric oxide synthase (eNOS) and has a very short half-life. Its action is limited to the site of production, making eNOS predominantly expressed in ECs to supply the vasculature [84]. In diabetic patients, the NO-dependent vasodilatory response may be compromised, possibly due to eNOS uncoupling caused by vascular oxidative stress, resulting in reduced NO production and diminished bioavailability [85]. Additionally, endothelial-myocyte uncoupling plays a significant role in DCM. Functionally, the ECM connects ECs with myocardial cells, and under diabetic conditions, the activation of MMPs leads to oxidative matrix accumulation, inducing endothelial-myocyte uncoupling and contributing to impaired diastolic function in diabetic patients [86].

Mechanisms underlying cardiac endothelial dysfunction in DCM-related endothelial-to-mesenchymal transition (EndMT)

ECs serve as the primary targets of hyperglycemic damage. Prolonged blood glucose levels exceeding 7 mmol/L can result in metabolic disruptions within ECs. The injury induced by HG in ECs plays a crucial role in the



Me: methylation; Ub: ubiquitination; Ac:acetylation

Fig. 2 Role of CFs in DCM and the corresponding protective effects of FGF21

pathogenesis of DCM. In wild-type diabetic mice, ECs within the heart undergo a process known as EndMT [87].

In primary human aortic endothelial cells (HAECs), exposure to high glucose (HG) results in reduced expression of the endothelial markers CD31 and VE-cadherin, whereas the expression of mesenchymal markers such as α-SMA, FSP-1, and FN increases. These findings indicate that HAECs acquire a mesenchymal phenotype through the EndMT process [88]. Additionally, HG stimulation induces the synthesis of Ang II in HAECs, leading to the loss of CD31 expression. However, these changes can be reversed via the administration of angiotensin receptor antagonists [89]. Under HG conditions, the TGF-β signaling pathway is activated, promoting the phosphorylation and nuclear translocation of Smad proteins. Simultaneously, intracellular ROS levels increase, triggering oxidative stress reactions and disrupting the balance of intracellular redox reactions, thereby exacerbating the transformation of ECs into mesenchymal cells [90]. In heart microvascular endothelial cells (CMECs) treated with HG + PA (palmitic acid), the expression of Sirtuin 6 (Sirt 6) is significantly downregulated. Endothelial-specific Sirt 6 expression knockout exacerbates DCM in mice, whereas in vitro knockdown of Sirt 6 expression promotes the proliferation and migration of HG + PA-induced CMECs. The results of this study suggest that Sirt 6 inhibits EndMT in CMECs stimulated with HG + PA by downregulating Notch 1 expression [91]. In diabetic mouse hearts, excessive activation of FoxO1 promotes EndMT in heart microvascular endothelial cells by regulating DDAH1 expression [80].

Advanced glycation end products (AGEs)

Recent studies have indicated that endothelial dysfunction is closely related to AGEs. AGEs activate RAGE, leading to the expression upregulation of NF-κB and its target genes. This, in turn, reduces the bioavailability and activity of endothelial-derived NO and promotes the generation of reactive oxygen species, ultimately contributing to endothelial dysfunction [92].

Autophagy, apoptosis, and pyroptosis

Autophagy, which is a step in programmed cell death, is dysregulated in DCM, leading to severe coronary microvascular dysfunction (CMD) [93]. Long-term exposure of fetal mouse hearts to nonmetabolic sugars induces severe lysosomal disruption and autophagic dysregulation [94]. Extracellular vesicles released from diabetic CMECs carry deleterious Mst1 proteins, initiating a cascade that amplifies myocardial cell damage. The disease-related signals received by myocardial cells lead to a further reduction in autophagy and exacerbate apoptosis, ultimately impairing heart function. Additionally, Mst1 inhibits GLUT4 membrane translocation in myocardial cells cultured under HG conditions, potentially contributing to insulin resistance and DCM [95]. Furthermore, Mst1 contributed to CMD in DM by directly inhibiting autophagy and inducing apoptosis in CMECs [96]. Research has indicated that HG stress reduces autophagy in CMECs, promoting cell apoptosis [97]. Additionally, exposing human microvascular ECs to HG and high-fat environments leads to mitochondrial dysfunction, triggering caspase-3 and PARP cleavage and inducing EC apoptosis and senescence [98]. Under elevated blood glucose and hyperlipidemia conditions, miR-125a-5p expression is upregulated. It contributes to cellular apoptosis and simultaneously participates in oxLDL-induced pyroptosis of vascular endothelial cells (VECs) [99]. Extensive research has underscored the pivotal role of endothelial dysfunction in DCM, which impacts vascular function, inflammatory status, and cardiac fibrosis, among other pathological processes.

Protective effect of FGF21 against cardiac endothelial dysfunction

ECs express FGFR1c [100–102], FGFR2 [103, 104], and FGFR3[105]. The FGFR1-βklotho complex modulates EndMT, AMPKα activity and nuclear factor erythroid 2-related factor 2 (NRF-2) signaling, thereby inhibiting endothelial dysfunction.

Anti-EndMT response

FGF21 plays a protective role in DCM by modulating glucose and lipid metabolism, endothelial cell function, and the metabolic state, thereby mitigating cardiac damage. Systemic administration of an adenovirus vector expressing FGF21 (Ad-FGF21) in wild-type mice enhances blood flow restoration, capillary density, and eNOS phosphorylation in ischemic limbs [106]. In human umbilical vein endothelial cells (HUVECs), FGF21 suppresses H2O2-induced cell apoptosis by inhibiting the activation of the mitogen-activated protein kinase (MAPK) signaling pathway [107]. Notably, βklotho expression deficiency induces EndMT through the MEK and ERK pathways. Conversely, AcSDKP increases βklotho expression in an FGFR1-dependent manner, forming an FGFR1-βklotho complex that modulates EndMT by inhibiting MEK and ERK pathway induction. This highlights the synergistic effect of FGF21 and AcSDKP in enhancing the anti-EndMT response [108].

Anti-eNOS dysfunction

Nitric oxide (NO), which is released by ECs, serves as a vasodilator and plays a critical role in endothelial function. eNOS is responsible for NO synthesis and supports

the vasculature. In DCM, the vasodilatory response may be compromised due to eNOS dysfunction, resulting in reduced NO production and cardiac damage. FGF21 binding to FGFRs, specifically FGFR1, enhances AMPKα activity and increases the expression of catalase (CAT), nuclear factor erythroid 2-related factor 2 (NRF-2), and heme oxygenase-1 (HO-1), thereby inhibiting oxidative stress. Furthermore, FGF21 directly enhances eNOS activity, promoting NO production and restoring endothelium-dependent vascular dilation function [109]. Moreover, FGF21 attenuates HG-induced oxidative stress in HUVECs and increases eNOS phosphorylation in an AMPK-dependent manner, thereby alleviating eNOS dysfunction [22]. FGF21 inhibitors impede HUVEC growth, migration, and invasion, significantly reducing eNOS, phosphoinositide 3-kinase (PI3K), and AKT mRNA and protein expression in HUVECs. These findings suggest that FGF21 regulates eNOS expression by activating the PI3K/AKT pathway [110]. In brain microvascular endothelial cells (BMECs), rhFGF21 promotes the vascular generation and migration of HBMECs by activating PPARγ and upregulating eNOS to form the FGF21/FGFR1/βklotho complex [23].

Antioxidative stress and apoptosis

In studies related to the blood–brain barrier following cerebral hemorrhage, FGF21 upregulated the expression of SIRT6 by promoting the AMPK–Foxo3a pathway. This regulatory mechanism mitigates mitochondrial morphological damage in ECs, reduces ROS accumulation, restores ATP synthesis, and inhibits apoptosis [111]. Similarly, in HG-treated HUVECs, FGF21 administration increases the phosphorylation of Akt and Foxo3a, which is reduced by HG, thereby protecting HUVECs from HG-induced oxidative stress and apoptosis [112]. In vitro experiments involving HUVECs treated with hydrogen peroxide (H2O2), recombinant human FGF21 (rhFGF21), an FGFR1 inhibitor (PD166866), and a PI3K inhibitor (LY294002) revealed that FGF21 significantly promotes angiogenesis by activating the FGFR1/PI3K/AKT/VEGF pathway [113]. Additionally, FGF21 counteracts the decrease in cell viability, increase in apoptosis, and increase in ROS levels induced by HG in HUVECs through activation of the PI3K/AKT/mTOR pathway [114]. FGF21 delays EC aging by upregulating SIRT1 expression while also protecting HUVECs from H2O2-induced accumulation of intracellular ROS and DNA damage [115]

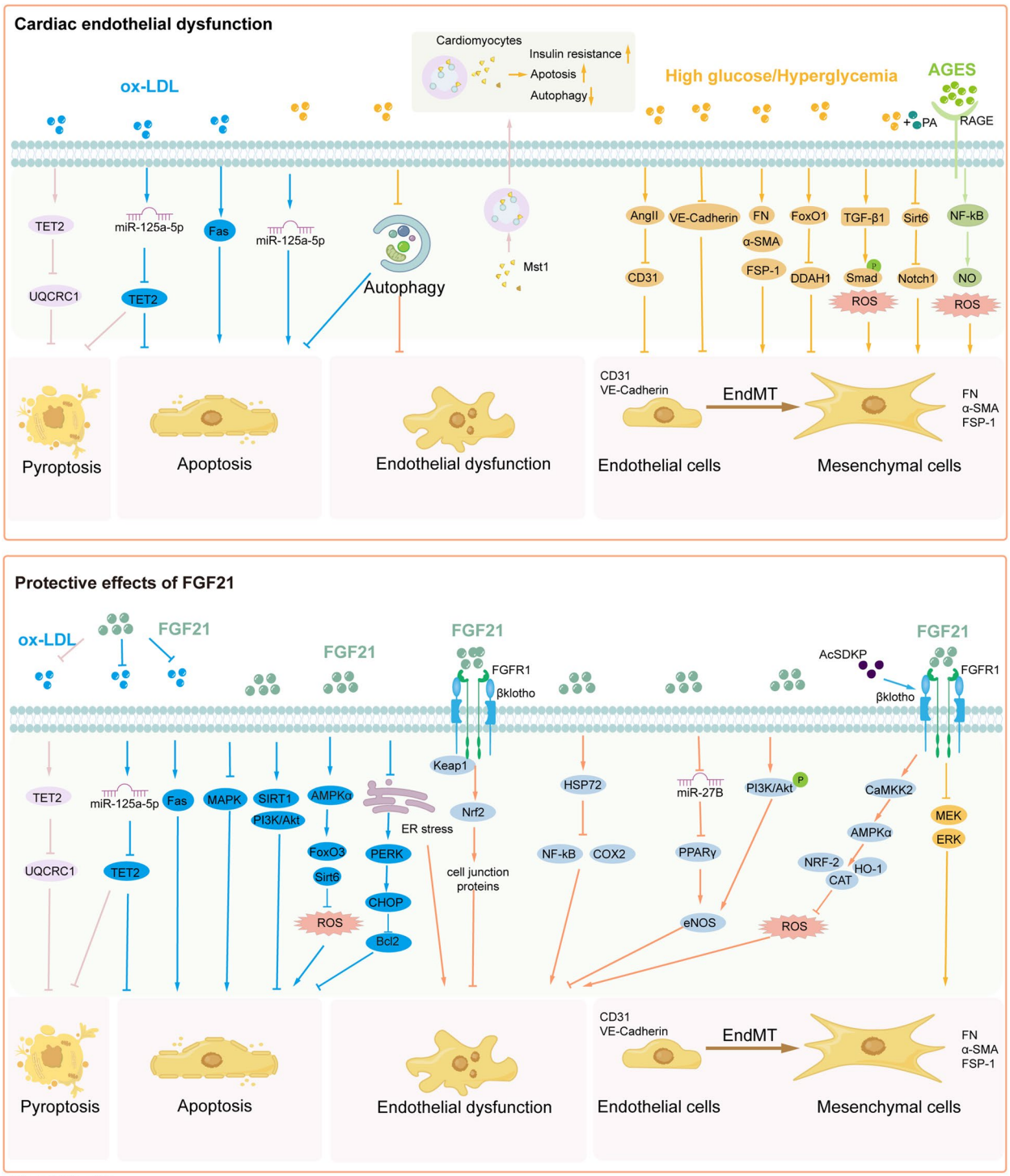
In ox-LDL-treated HUVECs, FGF21 mRNA and protein expression increase. The application of PPARα ligands can significantly induce the expression of FGF21, indicating that ECs can secrete FGF21 in response to stress. Elevated expression of FGF21 can inhibit ox-LDL-induced cell apoptosis and endothelial dysfunction [116]. Additionally, FGF21 reverses the downregulation of UQCRC1 expression induced by ox-LDL, inhibits ox-LDL-induced apoptosis and related molecular expression in HUVECs, and reverses mitochondrial dysfunction and ROS production [117]. FGF21 also acts independently of the ERK1/2 pathway and exerts antiapoptotic effects by inhibiting Fas expression in ox-LDL-induced apoptosis in HUVECs and apoE$^{-/-}$ mice [118].

In addition, FGF21 inhibits miR-27b expression, targeting the PPARγ-inhibitory NF-κB signaling pathway and the expression of inflammatory factors, thereby alleviating hypoxia-induced HPAEC dysfunction and inflammation [119]. In cerebral microvascular endothelial cells (CMECs), FGF21 promotes the mRNA and protein expression of HSP72 while simultaneously inhibiting the activity of the proinflammatory factors cyclooxygenase-2 and NF-κB (p65), providing protective effects against CMEC damage caused by hypoxic stress [120]. In vascular ECs of atherosclerotic rats, FGF21 activates the NF-κB signaling pathway while inhibiting the nuclear translocation of activated NF-κB (p65) and the expression levels of inflammatory factors in vascular ECs, thereby inhibiting oxidative stress and improving and maintaining the morphology of the vascular endothelium in atherosclerotic rats [121]. Under diabetic conditions, Nrf2 is negatively regulated by Keap1 binding, leading to proteasomal ubiquitination and Nrf2 degradation. rFGF21 activates FGFR1 to increase its binding with Keap1 (an inhibitor of Nrf2), reducing the interaction between Keap1 and Nrf2, releasing Nrf2, and promoting its translocation to the cell nucleus, ultimately promoting the expression of cell junction proteins [122]. Reconstruction of gap junction proteins helps prevent significant changes in cardiac structure and electrophysiological characteristics under diabetic conditions, thereby preserving ventricular function [123]. In HPAECs subjected to hypoxia, FGF21 can alleviate hypoxia-induced EC apoptosis by inhibiting the PERK/CHOP signaling pathway, downregulating caspase-4 expression, upregulating Bcl2 expression, and improving endothelial dysfunction by inhibiting ERS [124].

In summary, HG targets ECs, triggering EndMT and leading EC dysfunction, which may contribute to diabetic fibrosis. EC dysfunction may also result from oxidative stress, apoptosis, and pyroptosis. These factors compromise myocardial perfusion and contribute to ischemia and cardiomyocyte death. FGF21 prevents EndMT in ECs via the FGFR1-βklotho complex, thereby mitigating fibrosis. FGF21 also modulates glucose and lipid metabolism and EC function, thereby mitigating cardiac damage. It enhances blood flow, capillary density, and eNOS

phosphorylation and suppresses apoptosis, oxidative stress, and ER stress in ECs (Fig. 3).

In DCM, endothelial dysfunction is common, resulting in impaired angiogenesis and reduced capillary density. HG affects ECs, causing endothelial-to-mesenchymal transition (EndMT), which contributes to diabetic fibrosis. HG also induces oxidative stress, apoptosis, and pyroptosis in ECs. These factors compromise myocardial



PA: palmitic acid

Fig. 3 Role of endothelial dysfunction in DCM and the corresponding protective effects of FGF21

perfusion and contribute to ischemia and cardiomyocyte death. FGF21 modulates glucose and lipid metabolism and EC function, thereby mitigating cardiac damage. It enhances blood flow, capillary density, and eNOS phosphorylation and suppresses apoptosis, oxidative stress, and ER stress in ECs. FGF21 prevents EndMT in ECs via the FGFR1-βklotho complex, thereby mitigating fibrosis.

Vascular smooth muscle cells (VSMCs)

Role of VSMC dysfunction in DCM

VSMCs constitute a critical component of arterial physiology and pathology. They actively participate in regulating vascular constriction and dilation, thereby modulating cardiac perfusion and function [125]. Abnormal VSMC function significantly contributes to cardiovascular complications in individuals with diabetes. VSMCs experience phenotypic shifts and metabolic adaptations in the context of vascular diseases. These alterations primarily manifest as increased glycolysis, compromised mitochondrial respiration, perturbed fatty acid oxidation, and modified amino acid metabolism. Importantly, these metabolic changes are correlated with critical processes, including vascular remodeling, proliferation, migration, apoptosis, calcification, and inflammation [126].

Mechanisms underlying cardiac VSMC dysfunction in DCM

Phenotypic transitions of VSMCs

VSMCs are the fundamental components of the medial layer of blood vessels and primarily regulate vascular tension to control blood pressure and flow, which are essential for preserving vascular physiological integrity. VSMCs predominantly display a "contractile" phenotype characterized by relative quiescence and a "synthetic" phenotype marked by robust proliferation and migration, with metabolic alterations principally driving phenotypic transitions [127]. Upon vascular injury, differentiated "contractile" VSMCs transition to a "synthetic" phenotype, facilitating cell proliferation and migration to the site of damage [128].

HG conditions promote this phenotypic shift and augment VSMC proliferation and migration. Compared with those cultured under normal glucose (NG) conditions, porcine VSMCs (PVSMCs) cultured under HG conditions exhibit markedly elevated basal NF-κB activity, with the protein kinase C inhibitor Calphostin C attenuating HG-induced NF-κB activation [129]. HG further stimulates NF-κB-dependent VSMC proliferation and reinforces the "synthetic" phenotype via the lactate/GPR81 pathway, significantly increasing collagen I production and cellular proliferation and migration [130, 131]. Additionally, HG transiently enhances ERK1/2 phosphorylation and upregulates Ang II synthesis in rats via vascular proteases; a concurrent decrease in angiotensin-converting enzyme 2 expression under HG conditions leads to increased Ang II accumulation in VSMCs [132]. HG also promotes adiponectin synthesis and leptin expression in VSMCs, resulting in notable increases in ROS production and oxidative stress [133].

Calcification

The calcification of heart valves (e.g., aortic or mitral valves) can impair valve function, leading to valvular stenosis or regurgitation. Exosomes secreted by HUVECs treated with HG (HG-HUVEC-Exos) are abundant in expression of the multifunctional proteoglycan versican (VCAN), which predominantly localizes to the mitochondria of VSMCs, precipitating mitochondrial dysfunction and promoting VSMC calcification and senescence [134]. Moreover, recent research revealed enrichment of Notch3 protein expression in HG-HUVEC-Exos, which facilitates VSMC calcification and aging via the Notch3-mTOR signaling pathway [135]. In Wistar rats, type II diabetes induced by a HFD supplemented with a low dose of STZ revealed that telomerase activity and VSMC proliferation in diabetic and high glucose-insulin (HGI) treatment groups were significantly elevated compared with those in the control group, and inhibition of telomerase activity was found to mitigate VSMC proliferation [136]. Elevated insulin levels may also contribute to vascular stiffness by increasing the expression of receptor activator of NF-κB ligand (RANKL), increasing alkaline phosphatase activity, osteocalcin expression, and the formation of calcification nodules in VSMCs [137, 138].

Advanced glycation end products (AGEs)

Chronic hyperglycemia promotes the synthesis and accrual of AGEs [139]. In primary rat VSMCs, AGEs promote autophagy via the ERK and Akt signaling pathways, thereby increasing VSMC proliferation and migration in response to AGEs [140, 141]. Furthermore, AGEs increase ROS production by increasing Bcl-2-associated athanogene 3 (BAG3) expression, which in turn promotes VSMC proliferation and migration [142]. In vitro studies have demonstrated that AGEs significantly increase cell proliferation and migration in a concentration-dependent manner, which is mediated in part by the RAGE/PI3K/AKT pathway [143]. Additionally, AGEs can temporally induce the expression of the fibrogenic mediator CTGF through the ERK1/2, JNK, and Egr-1 pathways, contributing to VSMC proliferation, migration, and ECM deposition [144]. AGE incubation activates the apoptosis signal-regulating kinase 1 (ASK1)/mitogen-activated protein kinase kinase (MKK)/p38MAPK pathway, leading to an enhanced fibrotic response in human coronary smooth muscle cells (HCSMCs) [145].

Protective effects of FGF21 against VSMC dysfunction in DCM

Anti-VSMC calcification

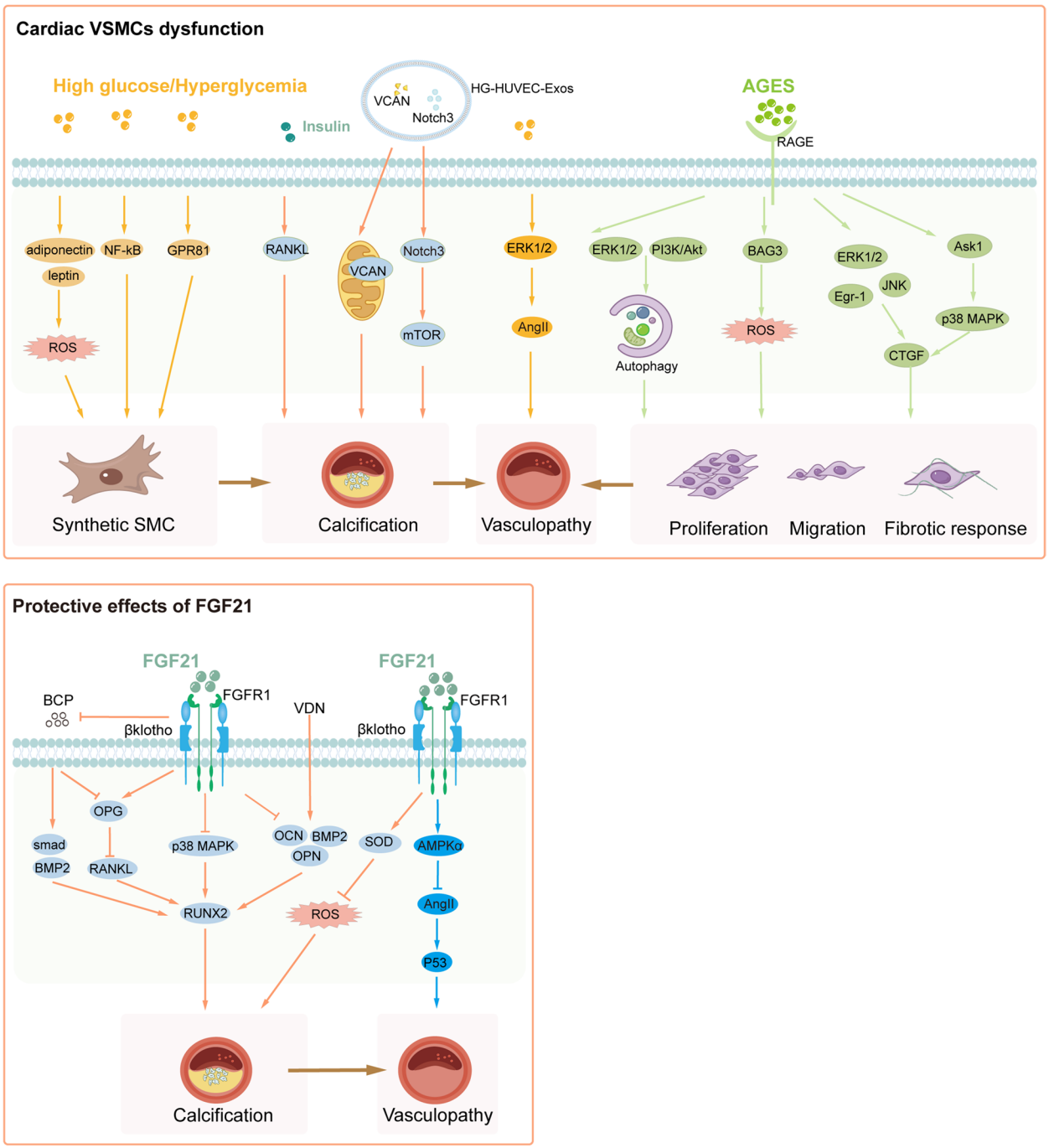
In diabetic mice, FGF21 significantly inhibits neointimal proliferation in the ligated carotid artery. In vitro, VSMCs cultured in 30 mM glucose for 48 h presented upregulated expression of PCNA, a hallmark of proliferation; concurrently, cell migration was increased, and FGF21 treatment reversed these aberrations. Moreover, FGF21 markedly suppresses the release of active caspase-1 (p20) and IL-1β in VSMCs exposed to HG. Studies have demonstrated that FGF21 inhibits Syk phosphorylation via FGFR1, which modulates the NLRP3 inflammasome through ASC phosphorylation and oligomerization, thereby exerting anti-inflammatory effects [146].

DCM does not directly lead to vascular calcification; however, diabetes-associated arteriosclerosis may increase the risk of cardiovascular complications, including vascular calcification. When vessels incur damage or inflammatory stimulation, VSMCs may contribute to the repair process by differentiating into osteoblast-like cells, a transformation that can result in calcification and fibrosis of the vessel wall, increasing stiffness and the risk of CVD. Additionaly, VSMC express FGFR1c [61] and FGFR2 [147]. Research has indicated that FGF21/FGFR1/3/βklotho signaling plays a pivotal role in vascular calcification [18–20]. In a CKD model established by 5/6 nephrectomy and a high-phosphate diet, which led to vascular calcification, increased expression of the FGF21, FGFR1, and βklotho receptors was observed in the aorta; these receptors are predominantly located in the arterial media, with FGFR1 and βklotho being expressed primarily in VSMCs. The transcription levels of genes associated with vascular calcification in FGF21-KO mice were significantly greater than those in control mice. Mechanistic studies have suggested that FGF21 inhibits vascular calcification by restoring antioxidant SOD levels and reducing vascular oxidative stress [20].

In vitro, FGF21 administration inhibits BGP-induced BMP2/Smad signaling pathway expression and the expression of osteoblast differentiation markers in VSMCs, preventing vascular calcification [148]. In human cerebral VSMCs, Ang II induces cellular senescence and promotes ROS/superoxide anion production, whereas FGF21 inhibits Ang II-induced p53 activation, partially preventing Ang II-induced senescence-related changes [18]. Additionally, FGF21 suppresses the osteogenic transformation of VSMCs by downregulating the expression of bone-related proteins such as osteopontin (OPN) [149]. In cultured rat VSMCs, FGF21 significantly attenuates mineral deposition and cell apoptosis, inhibits VSMC calcification through the OPG/RANKL system, and modulates the calcification process via the P38 and PI3K/AKT pathways [150]. FGF21 enhances βklotho expression and increases FGFR1 and FGFR3 mRNA expression. The FGFR-1 inhibitor SU5402 partially blocks the inhibitory effect of FGF21 on BMP-2 and RUNX-2 expression, whereas the P38 inhibitor SB203580 weakens the downregulation of RUNX-2 expression. These findings suggest that FGF21 inhibits VSMC calcification through the FGF21/FGFR1/3/βklotho/P38MAPK/RUNX-2 signaling pathway [19]. FGF21 regulates VSMC function and gene expression through various pathways, thereby inhibiting the occurrence of vascular calcification.

In summary, VSMCs undergo phenotypic changes under HG conditions, thereby shifting from a "contractile" to a "synthetic" phenotype. HG conditions stimulate various molecular pathways in VSMCs, including NF-κB activation, ERK1/2 phosphorylation, mitochondrial dysfunction, and increased synthesis of Ang II, contributing to the dysfunctional state of these cells. AGEs can induce the expression of CTGF in VSMCs. AGES can also induce autophagy and oxidative stress. These factors contribute to VSMC proliferation, migration, and ECM deposition in VSMCs. These phenotypic shifts lead to VSMC calcification and inflammation, contributing to the dysfunctional state of the cells and ultimately DCM. FGF21 inhibits VSMC calcification by inhibiting calcification-related gene expression, restoring antioxidant SOD levels and reducing vascular oxidative stress. In addition, FGF21 inhibits Ang II-induced p53 activation, partially preventing Ang II-induced senescence-related changes and thereby inhibiting the occurrence of vasculopathy (Fig. 4).

In DCM, VSMCs undergo phenotypic changes under HG conditions, thereby shifting from a "contractile" to a "synthetic" phenotype. HG conditions stimulate various molecular pathways in VSMCs, including NF-κB activation, ERK1/2 phosphorylation, mitochondrial dysfunction, and increased synthesis of Ang II, contributing to the dysfunctional state of these cells. AGEs can temporally induce the expression of CTGF through the ERK1/2, JNK, and Egr-1 pathways. AGES can also induce autophagy and oxidative stress. These factors contribute to VSMC proliferation, migration, and ECM deposition. These phenotypic shifts lead to VSMC calcification and inflammation, contributing to the dysfunctional state of the cells and ultimately DCM. FGF21 inhibits VSMC calcification by inhibiting calcification-related gene expression, restoring antioxidant SOD levels and reducing vascular oxidative stress. In addition, FGF21 inhibits Ang II-induced p53 activation, partially preventing Ang II-induced senescence-related changes and thereby inhibiting the occurrence of vasculopathy.



BCP: beta-glycerophosphate

VDN: vitamin D3 plus nicotine

Fig. 4 Role of cardiac VSMC dysfunction in DCM and the corresponding protective effects of FGF21

Immune cells

Role of immune cell activation in DCM

The heart harbors a variety of immune cells, including macrophages, mast cells (MCs), and dendritic cells [151, 152]. Immune cells play a key role in DCM, and their hyperactivation leads to a sustained inflammatory response that promotes myocardial injury and increased cardiovascular risk. Abnormal activity of the immune system may become an important driver of DCM development. The infiltration and activation of immune cells

are key pathogenic mechanisms in DCM, ultimately leading to myocardial fibrosis and cardiac insufficiency.

Mechanisms underlying immune cell activation in DCM

Many macrophages are present in the heart. In diabetic patients, M1-type macrophages dominate and promote persistent low levels of inflammation and insulin resistance, and M2-type macrophages help reduce cardiac inflammation. The number of macrophages was significantly greater in the hearts of STZ-induced diabetic mice than in those of nondiabetic mice, and M1-type macrophage infiltration was observed [153, 154]. An imbalance in the M1/M2 macrophage ratio promotes DCM. The onset of oxidative stress and alterations in fatty acid metabolism during DCM can cause macrophage polarization, in which the M1 type predominates [155, 156]. In vitro, macrophages cultured under HG conditions exhibit an M1 proinflammatory phenotype [157, 158], and miR-32/Mef2d/cAMP signaling promotes M1 macrophage polarization by inhibiting autophagy [159]. Furthermore, AGE treatment of RAW264.7 cells exerted proinflammatory effects by promoting the production of NO, TNF-α, PGE2, iNOS, and COX-2 [160]. Moreover, miR-471-3p expression was significantly upregulated in AGE-treated RAW264.7 cells, which increased the proportion of M1-type macrophages by negatively regulating SIRT1 expression [154]. Cardiac immune cells also affect cardiac fibrosis and remodeling through interactions with other cardiac nonmyocytes. TNFα promotes CF proliferation and collagen production through WISP1 signaling [161]. M1-type macrophages secrete IL-1β, which interacts with receptors on the surface of CFs to promote myocardial fibrosis [162]. Macrophages can also transdifferentiate into myofibroblasts and deposit collagen and ECM after cardiac damage, promoting the development of fibrosis [163]. MCs promote cardiomyocyte death, CF proliferation, TGF-β signaling, and collagen synthesis and deposition by releasing IL-6 and TNF-α in mice with DCM [161, 164].

Protective effects of FGF21 against immune cell activation in DCM

Macrophages are reported to express FGFR like FGFR1c [61] and FGFR3 [165]. Macrophages are the target cells through which FGF21 exerts its anti-inflammatory effects, mainly by enhancing Nrf2-mediated antioxidant capacity and inhibiting the NF-κB signaling pathway through FGFR1-βklotho complex. FGF21 also promotes M2 macrophage polarization by regulating adiponectin [166] and modulates the immune response by affecting glucose uptake in monocytes [167].

In vitro, FGF21 administration decreased the expression of TNF-α, IL-1β, IL-6, and IFN-γ while increasing the level of IL-10 in LPS-treated RAW264.7 macrophages [24]. In addition, FGF21 inhibited ROS production, increased oxidative stress, and modulated the inflammatory response by inhibiting the activation of the NF-κB signaling pathway. FGF21 induced HO-1 expression in LPS-treated RAW264.7 macrophages and exerted an anti-inflammatory effect by increasing Nrf2 expression [24]. In mice with T2DM, the combined action of FGF21 and insulin promotes the shift of macrophages from the M1 type to the M2 type to further reduce the expression of IL-1β, IL-6, and TNF-α [168]. Upregulation of circulating FGF21 expression in obesity promotes healthy expansion of subcutaneous adipose tissue (SAT) to improve systemic insulin sensitivity, which is achieved by upregulation of lipocalin expression in SAT, a process that is accompanied by an increase in M2-type macrophage polarization [166]. In addition to its role in DCM, FGF21 plays a role in other CVDs through its actions on immune cells. During the development of atherosclerosis, FGF21 inhibits cholesterol accumulation in macrophages, thereby inhibiting foam cell formation, and significantly reducing the expression of the inflammatory factors IL-1α, IL-6, and tumor necrosis factor-α, effects that can be blocked by FGFR inhibitors [169]. It has been hypothesized that FGF21 may prevent atherosclerosis by increasing the proportion of M2-type macrophages through inhibition of miR-33 expression [170]. Furthermore, FGF21 regulates the coupling of metabolism and the immune system, modulating the dynamic balance of peripheral T-cells by regulating thymic development and age-related degeneration [171, 172] Monocytes exhibit metabolic changes during inflammation, during which increased glucose utilization is critical for immune and inflammatory responses, whereas FGF21 expression is regulated by the PI3K/Akt signaling pathway, which indirectly regulates the immune response by affecting glucose uptake in monocytes [167].

In summary, in DCM including HG conditions and exposure to AGEs, macrophages adopt a proinflammatory M1 phenotype, resulting in increased production of inflammatory markers. This inflammatory environment contributes to fibrosis and cardiac remodeling. Specifically, TNFα stimulates CFs to proliferate and produce collagen, whereas M1 macrophages secrete IL-1β, further promoting myocardial fibrosis. FGF21 reduces inflammatory responses and protects the myocardium from oxidative stress and injury by inhibiting the expression of inflammatory cytokines, enhancing antioxidant capacity and promoting macrophage polarization. These effects contribute to a reduction in diabetes-induced myocardial inflammation and cardiac injury, providing a potential new strategy for the treatment of DCM (Fig. 5).

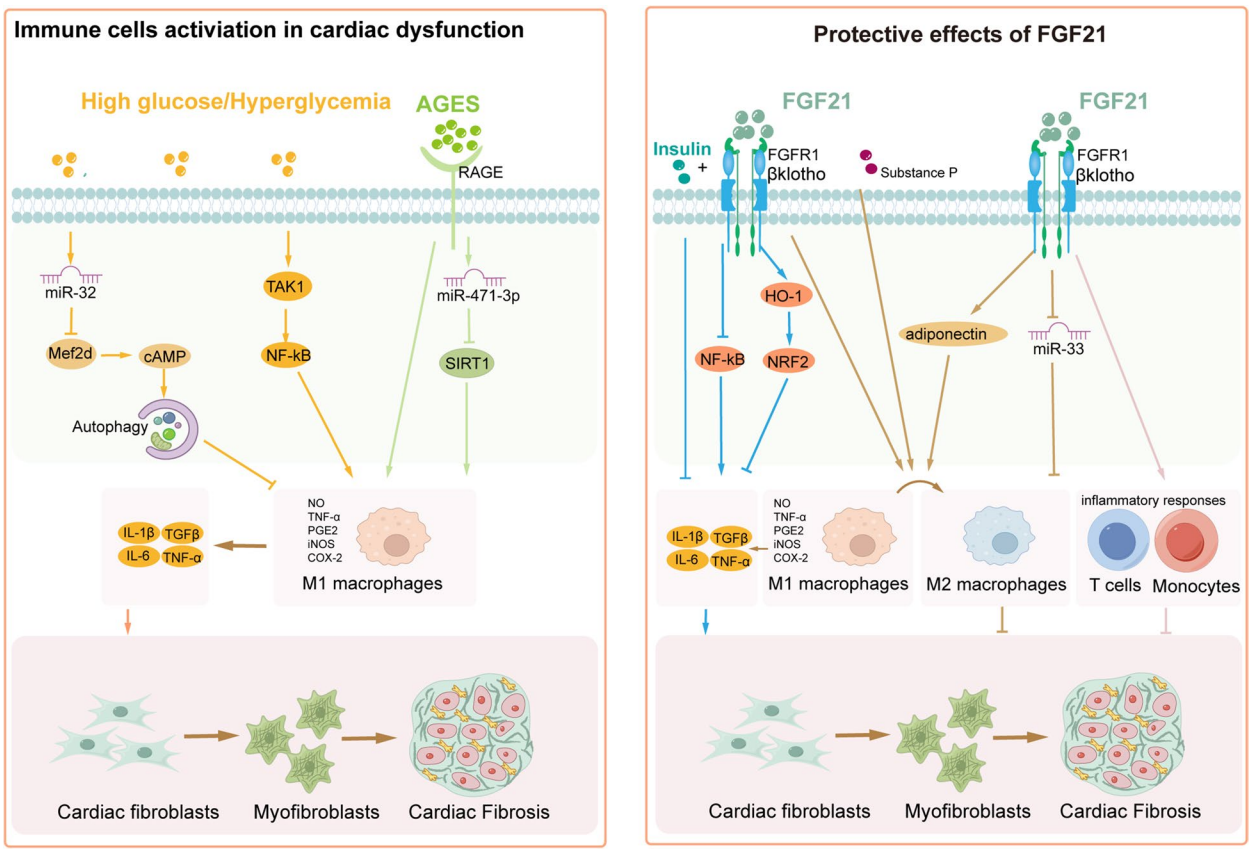


Fig. 5 Role of immune cell activation in DCM and the corresponding protective effects of FGF21

Immune cell infiltration and activation play pivotal roles in DCM, ultimately leading to myocardial fibrosis and cardiac dysfunction. Under HG conditions and exposure to AGEs, macrophages adopt a proinflammatory M1 phenotype, resulting in increased production of inflammatory markers. This inflammatory environment contributes to fibrosis and cardiac remodeling. Specifically, TNFα stimulates CFs to proliferate and produce collagen, whereas M1 macrophages secrete IL-1β, further promoting myocardial fibrosis. FGF21 inhibits the expression of inflammatory cytokines, enhances antioxidant capacity, and orchestrates a shift in macrophage

Table 1 Preclinical research on FGF21 targeting nonmyocardial cells in CVDs

Conditions	Animal models	Recombinant human FGF21	Observed effects	Cell types	References
MI	Exercise Training-MI	100 ng/ml, 15 h	Cell apoptosis and collagen production ↓	CFs	[66]
Cardiac hypertrophy	SIRT1-iKO mice	2.5 mg/kg/d, i.p., 4wk;	Ang II-induced cardiac hypertrophy ↓	CFs	[175]
Neointima hyperplasia	Wire-mediated vascular injured diabetic mice	5 mg/kg/d, i.v., 4wk;	neointima hyperplasia ↓	VSMCs	[146]
Vascular calcification	Vascular calcification rat models	70 μg/kg/d, osmotic pump,4wk;	osteogenic transition↓	VSMCs	[149]
Atherosclerosis	Atherosclerotic rats	6 mg/kg/d, i.v., 40d;	Inflammation and oxidative stress↓	ECs	[121]
Diabetes	HFD-STZ-induced T2D mice, db/db mice and T1D mice	0.5 mg/kg/d, i.v., 33d;	Blood glucose in T2DM and oxidative stress↓	ECs	[109]
Atherosclerosis	High-fat diet-fed ApoE-/- mice	1.0 mg/kg/d, i.p., 8wk;	Atherosclerotic plaques↓	ECs	[118]
Diabetes	Db/db mice	5 μg/kg/d, s.c.,10d;	inflammation↓; Angiogenesis↑	ECs	[176]
Atherosclerosis	High-fat diet-fed ApoE-/- mice	10 mg/kg/d, p.o., 12wk;	atherogenesis↓	Macrophage	[177]

i.p. intraperitoneal injection, *i.m.*, intramuscular injection, *i.v.*, intravenous injection, *s.c.*, subcutaneous injection, *p.o.* orally administered, MI Myocardial infarction

polarization from proinflammatory (M1-type) to anti-inflammatory (M2-type) phenotypes. Additionally, FGF21 modulates the inflammatory response of immune cells, including T-cells. These combined effects contribute to mitigating diabetes-induced myocardial inflammation and protecting against cardiac injury in DCM.

Therapeutic potential of FGF21 administration for DCM

Several preclinical studies have demonstrated the cardioprotective effects of FGF21 administration in animal models of CVD (Table 1). These findings have led to the development of FGF21 analogs and mimetics that are currently being evaluated in clinical trials. In fact, several FGF21 analogs and mimetics have advanced to the early stages of clinical trials, specifically for patients diagnosed with obesity, T2DM, and nonalcoholic steatohepatitis (NASH) [173, 174] (Table 2).

One clinical trial performed in patients with obesity and T2DM highlighted the potential of LY2405319 to improve lipid profiles and insulin sensitivity, despite the lack of a significant impact on glycemic control. Early improvements in lipid parameters were observed as soon as two days after the first injection. This study underscores the complexity of treating metabolic diseases and the need for comprehensive approaches beyond glycemic control [181]. Another clinical study

Table 2 Clinical trials involving FGF21 analogues

FGF21 analogues	Clinical trial ID	Phase	Status	Condition/disease	Heart effects observed	References
Pegozafermin	NCT04048135	Phase 2	Completed	NASH	N/A	[178, 179]
	NCT06318169	Phase 3	Recruiting	MASH / NASH With Fibrosis	/	/
	NCT05852431	Phase 3	Recruiting	Severe Hypertriglyceridemia	/	/
	NCT06419374	Phase 3	Recruiting	MASH / NASH With Compensated Cirrhosis	/	/
	NCT04541186	Phase 2	Completed	Severe Hypertriglyceridemia	N/A	[180]
LY2405319	NCT01869959	Phase 1	Completed	T2DM	N/A	[181]
BMS-986036	NCT02097277	Phase 2	Completed	T2DM	N/A	[182]
	NCT02413372	Phase 2	Completed	NASH	N/A	[183, 184]
	NCT03198182	Phase 1	Completed	Overweight/ Obesity	N/A	[183]
	NCT03674476	Phase 1	Completed	NAFLD、NAFLD、NASH	N/A	N/A
	NCT03400163	Phase 2	Completed	Non-Alcoholic Steatohepatitis	N/A	N/A
	NCT04493567	Phase 1	Completed	Healthy Participants	N/A	N/A
	NCT03445208	Phase 1	Completed	Hepatic Cirrhosis、Liver Fibrosis、NAFLD	N/A	N/A
	NCT04634149	Phase 1	Completed	Moderate Liver Impairment、Severe Liver Impairment	N/A	N/A
	NCT03611101	/	Completed	NASH	N/A	N/A
	NCT03486912	Phase 2	Completed	Hepatic Cirrhosis、Liver Fibrosis、NAFLD	N/A	[185, 186]
	NCT03486899	Phase 2	Completed	Liver Fibrosis、NAFLD、NASH	N/A	[185, 187, 188]
	NCT04649710	Phase 1	Withdrawn	Healthy Participants	N/A	N/A
BIO89-100	NCT05022693	Phase 1	Completed	NASH	NA	N/A
	NCT04929483	Phase 2	Active, not recruiting	NASH	N/A	[189, 190]
BMS-986171	NCT02538874	Phase 1	Completed	Liver Fibrosis/NASH	N/A	N/A
Efruxifermin	NCT06528314	Phase 3	Recruiting	NASH、MASH	/	/
	NCT06215716	Phase 3	Recruiting	NASH With Fibrosis	/	/
	NCT06161571	Phase 3	Recruiting	NASH/MASH、NAFLD/MASLD	/	/
	NCT05039450	Phase 2	Active, not recruiting	NASH	/	[191]
	NCT03976401	Phase 2	Completed	NASH	N/A	[192, 193]
	NCT04767529	Phase 2	Active, not recruiting	NASH	N/A	[194]
PF-05231023	NCT01285518	Phase 1	Completed	T2DM	N/A	N/A
	NCT01396187	Phase 1	Completed	T2DM	N/A	N/A
	NCT01673178	Phase 1	Completed	T2DM	Heart rate↑	[195]
	NCT01923389	Phase 1	Terminated	T2DM	N/A	N/A
LLF580	NCT03466203	Phase 1	Completed	Obesity		[196]

MASH Metabolic Dysfunction-Associated Steatotic Liver Disease, *N/A* Not Available, *Not NASH* Nonalcoholic steatohepatitis, *NAFLD* Nonalcoholic Fatty Liver Disease, *T2DM* Diabetes Melliuts, Type 2

involving PF-05231023 showed promise in improving lipid profiles and adiponectin levels. However, its impact on glycemic control remains unclear, emphasizing the need for further investigation [197]. Regrettably, cardiac outcomes were not thoroughly investigated in these clinical studies (Table 2). Considering the potential cardioprotective role of FGF21 in DCM, future clinical data are necessary to refine the therapeutic efficacy of FGF21, including the development of treatments that specifically target cardiac tissue.

Conclusions and outlook

This review highlights the critical role of nonmyocardial cells, specifically CFs, ECs, VSMCs, and immune cells, in the progression of DCM. These cells significantly contribute to myocardial injury and fibrosis, which are characteristic features of DCM. They interact with myocardial cells and the ECM, influencing cardiac function through their roles in inflammation, fibrosis, and angiogenesis. Further investigation into the specific mechanisms of these interactions could lead to the identification of novel therapeutic targets for DCM.

Fibroblast growth factor 21 (FGF21), a metabolic regulator, has demonstrated antiapoptotic and antifibrotic effects in cardiac cells. The protective role of FGF21 against DCM underscores its potential as a therapeutic target for DCM. However, the clinical application of FGF21 in CVD patients has not been fully validated and warrants further research. Current limitations, such as poor drug bioavailability and biophysical properties, restrict the therapeutic potential of FGF21, necessitating the development of new FGF21-based drugs to overcome these challenges. Moreover, clinical trials have shown inconsistent pathological manifestations in different tissues and cells in obesity and T2DM, with obesity even inducing FGF21 resistance [173].

In addition, cardiac outcomes have not been thoroughly investigated in current clinical studies. Therefore, considering the complexity of the physiological and pharmacological effects of FGF21, future efforts should focus on confirming the specific target organs and cellular pathways involved in DCM. These findings will facilitate the development of FGF21 receptor agonists, sensitizers, or analogs with greater selectivity and safety. Thus, investigating the role of noncardiomyocytes in DCM and the corresponding protective effect of FGF21 on these cells is highly important. This is crucial to determine the therapeutic specificity of FGF21.

In conclusion, our study highlights the importance of nonmyocardial cells in the development of DCM and the corresponding protective effect of FGF21. Future research in this area is expected to contribute to the development of more effective treatments for DCM.

Abbreviations

α-SMA	Alpha-Smooth Muscle Actin
AGEs	Advanced glycation end products
AMPK	AMP-activated protein kinase
Ang II	Angiotensin II
ASK1	Apoptosis signal-regulating kinase 1
BMECs	Brain microvascular endothelial cells
CaSR	Calcium-sensing receptor
CAT	Catalase
CFs	Cardiac fibroblasts
CIDEC	Cell death-inducing DFFA-like effector C
CMD	Coronary microvascular dysfunction
CTGF	Connective tissue growth factor
CVD	Cardiovascular disease
DCM	Diabetic cardiomyopathy
DM	Diabetes mellitus
ECM	Extracellular matrix
ECs	Endothelial cells
EGR1	Early growth response protein 1
EndMT	Endothelial–mesenchymal transition
eNOS	Endothelial nitric oxide synthase
FGF21	Fibroblast growth factor 21
FGFR	Fibroblast growth factor receptor
FoxO1	Forkhead box transcription factor O1
GLUT1	Glucose transporter 1
HCSMCs	Human coronary smooth muscle cells
HAECs	Human aortic endothelial cells
HF	Heart failure
HFD	High-fat diet
HG	High glucose
HO-1	Heme oxygenase-1
HUVECs	Human umbilical vein endothelial cells
IDF	International Diabetes Federation
JAK2	Janus kinase 2
MC	Mast cells
MECP2	Methyl CpG binding protein 2
MKK	Mitogen-activated protein kinase kinase
MMPs	Matrix metalloproteinases
NASH	Nonalcoholic steatohepatitis
NO	Nitric oxide
NRF-2	Nuclear factor erythroid 2-related factor 2
ox-LDL	Low-density lipoprotein oxidation
PA	Palmitic acid
PGC-1α	Peroxisome proliferator-activated receptor γ coactivator 1α
PI3K	Phosphoinositide 3-kinase
RAGEs	Receptors for advanced glycation end products
RANKL	Receptor activator of nuclear factor κB ligand
RASSF1A	Ras association domain family 1 isoform A
rhFGF21	Recombinant human FGF21
ROS	Reactive oxygen species
SAT	Subcutaneous adipose tissue
Sirt 6	Sirtuin 6
Smurf2	Smad ubiquitin regulatory factor 2
SOCS3	Suppressor of cytokine signaling 3
T2DM	Type 2 diabetes mellitus
TGF-β1	Transforming growth factor-beta 1
TIMPs	Tissue inhibitors of metalloproteinases
TLR4	Toll-like receptor 4
TSP1	Thrombospondin-1
VSMCs	Vascular smooth muscle cells
WAT	White adipose tissue
WT	Wild-type
ZDF	Zucker diabetic fatty

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Authors' contributions

Tianyi Zhang and Donghui Jiang contributed equally to preparing the original draft of the manuscript. Tianyi Zhang, Donghui Jiang and Shengbiao Li prepared the figures. Xiao Zhang, Ligang Chen and Jun Jiang revised the

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Data availability
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Declarations

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The authors declare no competing interests.

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