Effects of fibroblast growth factor 21 on the heart

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Abstract

Fibroblast growth factor 21 (FGF21) is a novel polypeptide ligand that has been shown to be involved in several physiological and pathological processes including regulation of glucose and lipids as well as reduction of arteriosclerotic plaque formation in the great vessels. It has also been shown to exert cardioprotective effects in myocardial infarction, cardiac ischemia-reperfusion injury, cardiac hypertrophy and diabetic cardiomyopathy. Moreover, FGF21 protects the myocardium and great arteries by attenuating remodeling, inflammation, oxidative stress and also promoting the energy supply to the heart through fatty acid $\beta$-oxidation. This growing evidence emphasizes the important roles of FGF21 in cardioprotection. This review comprehensively summarizes and discusses the consistent and inconsistent findings regarding the beneficial effects of FGF21 on the heart available from both basic research and clinical reports. The details of the signaling, biological and pharmacological effects of FGF21 with regard to its protection of the heart are also presented and discussed in this review.

Key Words
- fibroblast growth factor 21
- myocardial injury
- cardiac metabolism
- oxidative stress

Introduction

Fibroblast growth factors (FGFs) are polypeptide chains that have paracrine, autocrine or endocrine functions. The paracrine FGFs are further divided into five subfamilies, whereas the autocrine and endocrine FGFs are composed of one subfamily each (Itoh & Ornitz 2011, Itoh & Ohta 2013) (Fig. 1). FGFs act through cell surface FGF receptors (FGFRs), which are regulated by four types of genes including FGFR1, FGFR2, FGFR3 and FGFR4 (Mohammadi et al. 2005, Beenken & Mohammadi 2009, Goetz & Mohammadi 2013). Although FGFRs are essential for FGF action on the target cells, they cannot activate intracellular signaling without co-receptors (Kharitonenkov 2008). Previous studies show that heparan sulphate proteoglycans are essential co-receptors for paracrine and autocrine FGFs (Beenken & Mohammadi 2009, Goetz & Mohammadi 2013), whereas Klothos are essential co-receptors for endocrine FGFs to mediate their attachment to and activation of target FGFRs (Suzuki et al. 2008, Beenken & Mohammadi 2009, Goetz & Mohammadi 2013).

FGF21 is an endocrine FGF that consists of 209 amino acids. The FGF21 ligand is produced from several organs such as the liver and adipose tissue (Ito et al. 2000), skeletal muscle (Joki et al. 2015), and the heart (Nishimura et al. 2000, Kharitonenkov 2009, Planavila et al. 2013, Patel et al. 2014). To activate FGF21 signaling, FGF21 binds to FGFR1c with its C-terminus, and also with $\beta$-Klotho as its co-receptor with its N-terminus, to form the FGFR/$\beta$-Klotho complex (Kharitonenkov 2008, Suzuki et al. 2008, Yie et al. 2009, Ding et al. 2012, Hale et al. 2012).
The FGFR/β-Klotho complex then stimulates the autophosphorylation of the fibroblast receptor substrate 2 alpha (FRS2α), which is the first step in the downstream signaling of FGF21 (Kharitonkov 2008, Suzuki et al. 2008). However, FGF21 is believed to have no action in physiological conditions since FGF21 knockout (FGF21-KO) mice were found to have normal development (Badman et al. 2009), and did not develop any pathological conditions such as insulin resistance (Hotta et al. 2009, Potthoff et al. 2009). Nevertheless, future studies are needed to evaluate this hypothesis.

FGF21 has been shown to play an important role in pathological processes, such as the regulation of plasma glucose level (Nishimura et al. 2000) and fatty acid β oxidation (FAO) which is the primary energy source for the myocardium (Vega et al. 2000, Planavila et al. 2013).

Under stress conditions, FGF21 has been shown to reduce the apoptosis of Islet β cells (Wente et al. 2006), hepatocytes (Yu et al. 2015), vascular cells (Wu et al. 2014), cardiac endothelial cells (Lu et al. 2010) and cardiomyocytes (Cong et al. 2013, Liu et al. 2013). Interestingly, FGF21 also protects the heart from apoptosis and remodeling through the activation of adiponectin release to activate the adiponectin signaling pathways (Joki et al. 2015). Currently, the biological and pharmacological mechanism of FGF21 in cardioprotection is still to be elucidated. This review will focus on the effects of FGF21 and its roles in the heart. The consistent and inconsistent findings regarding the beneficial effects of FGF21 in the heart available from both basic research and clinical reports are comprehensively summarized and discussed. The details of the signaling, biological and pharmacological effects of FGF21 with regards to its protection of the heart are also presented and discussed in this review.

**Effects of FGF21 on the heart**

FGF21 is synthesized and expressed in the heart by cardiomyocytes (Planavila et al. 2013) and cardiac microvascular endothelial cells (CMECs) (Lu et al. 2010). A previous study demonstrated that cardiomyocytes secrete FGF21 into the media culture in basal conditions at a rate of ~0.05 ng/ml per 24 h (Planavila et al. 2013). In the heart, FGF21 ligands act via the FGFR1c (Suzuki et al. 2008, Liu et al. 2013, Planavila et al. 2013, Wu et al. 2014), and FGFR3 (Suzuki et al. 2008, Liu et al. 2013), utilizing β-Klotho as a co-receptor (Suzuki et al. 2008, Liu et al. 2013, Planavila et al. 2013). Endogenous and exogenous FGF21 plays an anti-apoptotic role in both in vitro and in vivo models, partially through the adiponectin signaling cascade (Joki et al. 2015). Recent studies found that FGF21 protects against isoproterenol (ISO) induced cardiac hypertrophy by activating anti-oxidative pathways (Planavila et al. 2013, 2014) and promoting FAO (Planavila et al. 2013). FGF21 also protects the heart from ischemic reperfusion (I/R) injury and myocardial infarction (MI) by activating several survival pathways (Cong et al. 2013, Liu et al. 2013, Patel et al. 2014). Moreover, FGF21 deficiency accelerated the development of diabetic cardiomyopathy (DCM) (Yan et al. 2015). In contrast, FGF21 administration also prevents lipotoxicity and diabetes induced cardiac apoptosis in DCM (Zhang et al. 2015a).

Interestingly, Liu and colleague demonstrated that the endogenous FGF21 which acted as endocrine protection in the ischemic myocardium was not from the heart but from the liver and adipose tissue (Liu et al. 2013),...
indicating that the major endogenous FGF21 proteins which preserve cardiac function are from the liver and adipose tissues. Although FGF21 from cardiomyocytes is not a major source, previous studies demonstrated that the autocrine action of FGF21 from cardiomyocytes is essential and could protect the heart from pathological conditions such as cardiomyocyte hypertrophy and I/R injury (Planavila et al. 2013, 2014).

**Effects of FGF21 on myocyte apoptosis and myocardial infarction**

Myocardial ischemia and I/R injury induce cell apoptosis and MI, leading to an impairment in cardiac function. Growing evidence from both *in vitro* and *in vivo* studies demonstrate that exogenous FGF21 protected the cardiomyocytes from apoptosis and MI, and improved cardiac function through activating the PI3K-Akt1-BAD pathway in FGF21-KO mice (Liu et al. 2013), and Akt-GSK3β-caspase 3 dependent pathways in H9c2 cell lines (Cong et al. 2013), resulting in the suppression of caspase 3 induced apoptosis. It was proposed that the activation of these pathways would lead to a decrease in the myocardial infarct area and increase cardiac function (Liu et al. 2013, Patel et al. 2014).

Evidence regarding the effects of FGF21 on inhibiting cardiovascular cell apoptosis in *in vitro* models is summarized in Table 1. FGF21 protects H9c2 cells from I/R injury in a dose dependent manner by promoting the energy supply, and reducing inflammation and apoptosis through the Akt-GSK3β pathway (Cong et al. 2013). On other hand, a previous study found peroxisome proliferator activated receptor alpha (PPARα) activation led to the synthesis and release of FGF21. FGF21 was released into the culture media, and protected the CMECs from lipotoxicity induced by Ox-LDL by decreasing DNA fragmentation in an autocrine manner (Lu et al. 2010). In an *ex vivo* model of global cardiac ischemia, it has been shown that recombinant rat FGF21 infusion 10 min prior to ischemia can protect the heart from I/R injury by decreasing MI and increasing the cardiac function through activation of the MAPK-PI3k-Akt signaling pathway (Patel et al. 2014). Moreover, FGF21 prevented oxidative stress (Cong et al. 2013, Planavila et al. 2014), and also increased the energy supply for cardiomyocytes in H9c2 cell lines under I/R injury conditions (Cong et al. 2013).

In addition to *in vitro* reports, evidence regarding the effects of FGF21 on cell apoptosis and myocardial infarction in *in vivo* models is summarized in Table 2. In FGF21-KO mice, FGF21 given intravenously at 50 ng/g per day for 3 days with the first dose being given immediately after I/R injury (I=30 min, R=1–30 days), had been shown to protect the heart from apoptosis, MI, and also increase cardiac function through activation of the FGFRI/β-Klotho-PI3K-Akt1-BAD signaling cascade (Liu et al. 2013). The acute MI in C57BL/6 mice showed that an i.v. injection of Recombinant mouse FGF21 10 ng/g in a single dose immediately post MI, which was caused by a left anterior descending coronary artery ligation, decreased the infarction area. It was also shown that these protective effects could be reversed by SiRNA-FGF21 intravenously injected 1 day prior to MI (Liu et al. 2012). Moreover, in chronic MI (2 weeks) C57BL6 and adiponectin-KO mice models it was demonstrated that FGF21 protein derived from skeletal muscles protected the heart from apoptosis through adiponectin signaling (Joki et al. 2015). In addition, FGF21 100 μg/kg per day s.c. injections for 4 weeks could protect the abdominal aorta from arteriosclerotic lesions through lipid regulation and ER stress induced vascular cell apoptosis in the ApoE-KO model (Wu et al. 2014).

All of these findings indicate that exogenous and endogenous FGF21 play an important role in protecting the heart from apoptosis via several pathways including PI3K-Akt1-BAD and Akt-GSK3β-caspase 3 dependent mechanisms, leading to decreased infarction and increased left ventricular function under I/R injury, lipotoxic and MI conditions.

**Molecular basis of anti-apoptosis signaling cascades of FGF21**

The anti-apoptotic signaling cascade of FGF21 from in *in vitro* and in *in vivo* models previously mentioned are summarized in Fig. 2. After FGF21 binding to FGFRI and β-klotho via its N-terminus and C-terminus, respectively, the FGF21 ligand induces dimerization of receptors, and the autophosphorylation of tyrosine kinase recruits and phosphorylates FRS2α. In later steps, the anti-apoptotic signaling pathways in cardiomyocytes could be activated through 4 major survival pathways, including Erk1/2, RORα, PI3K-Akt and AMPK signaling pathways. Currently, the downstream signaling proteins involved in these processes are still unclear (Patel et al. 2014).

Previous studies demonstrated that the downstream signaling cascades of FGF21 begin with the autophosphorylation of the receptor after the binding of FGF21. This leads to the phosphorylation of FRS2α, and subsequent activation of PI3K (Liu et al. 2013, Patel et al. 2014, Yu et al. 2015) following its phosphorylation at Serin458
### Table 1  Effects of FGF21 on apoptosis in cardiomyocytes and endothelial cells

<table>
<thead>
<tr>
<th>Model</th>
<th>Methods</th>
<th>Dose</th>
<th>Results</th>
<th>Interpretation</th>
<th>References</th>
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| H9c2 cells (rat cardiomyocytes) | I/R injury (I=3 h, R=1, 3, 6 h) | FGF21 0.25, 0.5, 1, 1.5, 2, 4, 5, 6, 7, 8 µg/ml applied immediately at reperfusion | ↑ Cell viability (dose-dependent)  
↑ pAkt, GSK3β  
↑ ATP synthase-α  
↓ Apoptosis  
↓ Cleaved caspase-3/pro-caspase 3 ratio  
↓ TNFα, PAI1  
↓ H2O2 damaged cells | FGF21 protects H9c2 cells from I/R injury by promoting the energy supply, and reducing inflammation and apoptosis in cardiomyocytes through Akt-GSK3β pathway in a dose-dependent manner. | Cong et al. (2013) |
| CMECs                       | Lipotoxicity induced by Ox-LDL50 or 100 µg/ml 37 °C for 12 h | Bezafibrate (PPAR-α ligand) 50, 100, 200 µmol/l for 12 h, Transfected with shRNA-FGF21 vector | ↑ Culture media FGF21 concentration  
↓ DNA fragmentation  
↓ PPAR-α ligand 200 µmol/l  
↓ shRNA-FGF21 transfection  
↓ DNA fragmentation  
↓ Culture media FGF21 concentration | PPAR-α activation increases FGF21 synthesis and release into culture media and protects the CMECs from lipotoxicity. | Lu et al. (2010) |
| Male wistar rats            | I/R injury; ex vivo (I: 30 min, R: 120 min) | Rr FGF21 100 nM (in normal tyrodes solution) infusion 10 min prior to ischemia | ↑ Rate pressure production  
↑ MAPK-Pi3k-Akt signaling  
↓ Total infarct size, LVDP | FGF21 increases cardiac function and decreases infarct size due to I/R injury through the activation of MAPK-Pi3k-Akt signaling. | Patel et al. (2014) |

IR, ischemic reperfusion; GSK3β, glycogen synthase kinase 3β; TNFα, tumor necrosis factors alpha; PAI1, plasminogen activator inhibitor 1; CMECs, Cultured cardiac micro vascular endothelial cells; Ox-LDL, oxidized low density lipoprotein; PPARα, Peroxisome proliferator-activated receptor alpha; PGC1α, PPARα coactivated 1 alpha; Rr, recombinant rats; LVDP, left ventricular diastolic pressure; MAPK, AMP-activated protein kinase; PI3K, phosphatidylinositol 3 kinase.
Table 2  Effects of FGF21 on myocardial infarction and apoptosis in in vivo models

<table>
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<th>Model</th>
<th>Methods</th>
<th>Dose</th>
<th>Results</th>
<th>Interpretation</th>
<th>References</th>
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| FGF21-KO mice             | I/R injury (I = 30 min, R = 1, 3, 5, 10, 20, 30 days) | FGF21 50 ng/g per day, i.v. immediately after myocardial injury, and for the next 3 days | ↑ ± dp/dt, %FS  
↑ FGF21 sensitivity  
↓ Apoptosis, MI  
↑ FGFR1/β-Klotho–PI3K–Akt1–BAD signaling | FGF21 decreases cellular apoptosis and increases cardiac function due to I/R injury through an activation of FGFR1/β-Klotho–PI3K–Akt1–BAD signaling. | Liu et al. (2013) |
| ApoE⁻/⁻ mice              | Abdominal aortic (AA) arteriosclerosis for 4 weeks | FGF21 100 μg/kg per day, s.c. injection for 4 weeks | ↑ AA luminal diameter  
↓ Arteriosclerotic lesion area  
↓ TC, TG  
↓ Cleaved caspase 12, CHOP, GRP94  
↓ Apoptotic rate  
↓ Infarct size by siRNA-FGF21  
↓ Infarct size by FGF21 | FGF21 protects the AA from arteriosclerotic lesion through lipid regulation and ER stress induced by vascular cell apoptosis. | Wu et al. (2014) |
| C57BL/6 mice              | MI for 24 h                                  | At MI, RmFGF21 10 ng/g, i.v. injection     | ↓ Capillary density (CD31)  
↑ Cardiac function  
↑ TNFα, IL6 mRNA  
Reversed by adiponectin-KO | FGF21 protects heart from acute MI by decreasing myocardial infarct area | Liu et al. (2012) |
| C57BL6, adiponectin-KO mice | Chronic MI (2 weeks) | Ad-FGF21 1 × 10⁻⁹ pfu/mouse, i.m. injection 3 days prior to MI | | FGF21 protein was derived from skeletal muscle and protects the heart from apoptosis through adiponectin signaling. | Joki et al. (2015) |

FGF21-KO, FGF21 knockout; ApoE⁻/⁻, apolioprotein E knockout; I/R, ischemic reperfusion; MI, myocardial ischemia; Rm, Recombinant mouse; i.v., intravenous; PI3K, phosphatidylinositol 3 kinase; BAD, BCL2 antagonist of cell death; AA, Abdominal aortic; TC, total cholesterol; TG, triglyceride; CHOP, C/EBP homologous protein; GRP94, glucose-regulated proteins 94; ER, endoplasmic reticulum; LVDP, left ventricular diastolic pressure; MAPK, AMP-activated protein kinase.
This leads to the recruitment and phosphorylation of a secondary messenger Akt1 by phosphorylation at Serine473 (pS473). Akt1 in turn activates the BCL2 antagonist of cell death (BAD) by inducing the phosphorylation of BAD at Serine136 (pS136). This causes BCL2 and BCL-XL to inhibit BAX and BAK induced caspase3/7 activity, which leads to decreased apoptosis in cardiomyocytes (Liu et al. 2013). In addition, FGF21 has...
been shown to inhibit apoptosis through another alternative pathway by activating Akt, thereby inhibiting GSK3β, thus leading to decreased caspase 3 activity (Akt-GSK3β-caspase 3 dependent pathways) (Cong et al. 2013) (Fig. 2). Moreover, FGF21 can protect the heart from apoptosis by activation of the Erk1/2-p38 MAPK-AMPK survival pathway (Zhang et al. 2015a). Evidence from these reports confirmed that FGF21 plays a critical role in myocardial protection and anti-apoptosis following myocardial injury (Patel et al. 2014, Joki et al. 2015, Zhang et al. 2015a).

Due to the potential cardioprotective benefits of FGF21, it is possible that FGF21 could be used to prevent and/or treat the myocardial apoptosis due to I/R injury or MI. However, evidence related to the roles of the time course of FGF21 administration and its beneficial effects to the pathological heart are still lacking.

Effects of FGF21 on cardiac hypertrophy and adverse cardiac remodeling

Myocardial ischemia resulting from coronary artery disease (CAD) is the primary cause of MI which could impair cardiac function by reducing the ejection fraction (EF), leading to insufficient oxygen supply to body tissues (Gheorghiade & Bonow 1998, Joki et al. 2015). This contributes to progression to cardiac hypertrophy and heart failure due to the compensatory mechanisms of the circulatory system to maintain the EF and carry oxygen to peripheral metabolic tissues, known as cardiac remodeling. This long-term maladaptive remodeling can cause increased ventricular hypertrophy, ventricular dilatation, interstitial growth and cardiac fibrosis (Neely et al. 1972).

Evidence regarding the effects of FGF21 on protection against adverse cardiac remodeling and hypertrophy in in vitro and in vivo models is summarized in Table 3. In a single in vitro study, pre-treatment with FGF21 protects neonatal cardiomyocytes (NCMs) from phenylephrine induced hypertrophy by promoting FAO gene expression, attenuating inflammation and oxidative stress through the activation of Sirt1 and Erk1/2-CREB signaling pathways (Planavila et al. 2013). This study also demonstrated that the Sirt1-PPARα pathway plays an important role in the control of FGF21 expression in the heart.

Evidence from in vivo studies demonstrate that continuous administration of ISO via s.c. infusion for 7 days in FGF21-KO mice induced cardiomyopathy and led to MI, impaired cardiac metabolism and loss of cardiac function in the rat heart (Heather et al. 2009, Planavila et al. 2013). Interestingly, the endocrine function of FGF21 derived from skeletal muscles attenuated cardiac hypertrophy, and reversed the adverse cardiac remodeling process, leading to improved left ventricular function in this chronic MI mice model (Joki et al. 2015). In FGF21-KO mice, it has been shown that FGF21 attenuated cardiac hypertrophy by decreasing hypertrophic markers including atrial natriuretic factor (ANF) and α-skeletal actin (αSKA) (Planavila et al. 2013). Moreover, FGF21 decreased the heart weight/body weight ratio and cardiomyocytes area, and also improved cardiac function (Planavila et al. 2013, 2014).

In summary, the protective effects of FGF21 against cardiac hypertrophic damage have been evidenced. Conversely, FGF21 deficiency was found to enhance the induction of cardiac hypertrophy by promoting pro-inflammatory pathways, oxidative stress, cardiac fibrosis and impairing cardiac metabolism (Planavila et al. 2013, 2014). Results confirmed that cardiac FGF21 has an impact on activation of the autocrine loop and plays a protective role against cardiac hypertrophy and remodeling. However, further investigation and clinical studies are needed to warrant the usefulness of FGF21 against cardiac hypertrophy.

Molecular basis of anti-hypertrophic signaling cascades of FGF21

The FGF21 activates cells to autocrine function by binding to FGR1 on the cell membrane, using β-Klotho as a co receptor. This event activates the dimerization of the receptor and causes autophosphorylation of tyrosine kinase. Tyrosine kinase then recruits and phosphorylates FRS2α. The FRS2α in turn affects four primary pathways, which in turn leads to the attenuation of cardiac hypertrophy. An illustrated diagram of the anti-hypertrophic effects of FGF21 in both the autocrine and endocrine manner by the loop autocrine function of FGF21 through the Sirt1/PPARα pathway is shown in Fig. 3.

The first of these four pathways is the activation of the Erk1/2-CREB-Sirt1-PGC1α signaling pathway as an autocrine function and autocrine loop regulation in FGF21-KO cardiomyocytes. This pathway leads to increased mitochondrial FAO enzyme genes expression including MCAD and mcpt1α, indicating increased cardiac mitochondrial FAO (Planavila et al. 2013). The second pathway involves the inhibition of the translocation of pro-inflammatory cytokines NFκβ into the nucleus to activate inflammatory cytokine expression including TNFα, IL6, and MCP1, resulting in a decrease in the
### Table 3  Effects of FGF21 on cardiac hypertrophy and remodeling

<table>
<thead>
<tr>
<th>Model</th>
<th>Methods</th>
<th>Dose</th>
<th>Results</th>
<th>Interpretation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGF21-KO mice</td>
<td>Cardiac hypertrophy induced by ISO 15 mg/kg per day for 7 days via s.c. Osmotic pump</td>
<td>FGF21 2.5 μg/kg per day, s.c. for 7 days</td>
<td>↓ HW/BW, cardiomyocyte area</td>
<td>FGF21 protects heart from ISO induced cardiac hypertrophy via promoting FAO gene expression, and reducing inflammation.</td>
<td>Planavila et al. (2013)</td>
</tr>
<tr>
<td>FGF21-KO mice</td>
<td>Cardiac hypertrophy induced by ISO</td>
<td>ISO 15 mg/kg per day, s.c. for 7 days</td>
<td>In FGF21-KO mice</td>
<td>FGF21 protects heart from ISO induced cardiac hypertrophy through an anti-oxidative stress mechanism.</td>
<td>Planavila et al. (2014)</td>
</tr>
<tr>
<td>C57BL6 and adiponectin-KO mice</td>
<td>Chronic M1 (2 weeks)</td>
<td>Ad-FGF21 1×10⁻⁹ pfu/mouse i.m. injection 3 days prior MI</td>
<td>↑ Capillary density (CD31)</td>
<td>FGF21 protein was derived from skeletal muscle, it protects the heart by attenuating cardiac remodeling in chronic MI.</td>
<td>Joki et al. (2015)</td>
</tr>
<tr>
<td>NCMs</td>
<td>Cardiomyocyte hypertrophy induced by PE</td>
<td>FGF21 pre-treatment (5 nM) for 24 h</td>
<td>↑ PGC1α, mcpt1α genes</td>
<td>FGF21 protects NCMs from PE induced hypertrophy by promoting FAO gene expression, attenuating inflammation and oxidative stress through the activation of Sirt1 and Erk1/2-CREB signaling pathways.</td>
<td>Planavila et al. (2013)</td>
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FGF21-KO, FGF21 knockout; ANF, atrial natriuretic factor; αSKA, alpha skeletal actin; IL6, interleukin 6; PGC1α, coactivated 1 alpha; MCAD, medium chain acetyl CoA dehydrogenase; mcpt1α, mitochondrial carnitine palmitoyltransferase 1; ISO, isoproterenol; LPS, lipopolysaccharide; ROS, reactive oxygen species; pfu, plaque-forming units; i.m., intramuscular; TNFα, TNFα tumor necrosis factor alpha; NCMs, neonatal cardiomyocytes; ROS, reactive oxygen species; NFκB, nuclear factor kappa beta; IL6, interleukin 6; PE, phenylephrine; PPARα, Peroxisome proliferator-activated receptor alpha; FAO, fatty acid oxidation; Sirt1, Sirtuin 1; Erk1/2, Extracellular signal-regulated kinases 1/2; CREB, CAMP response element-binding protein.
inflammatory processes (Planavila et al. 2013). The third pathway involves the inhibition of cardiac MMP9, which indicates a decrease in cardiac fibrotic formation following cardiac remodeling (Planavila et al. 2013). Finally, FGF21 activates the anti-oxidative pathway, resulting in the reduction of oxidative stress in the cells (Planavila et al. 2013, 2014).

**Figure 3**

FGF21 signaling cascade in anti-hypertrophic effects of FGF21. FGF21 exerts anti-hypertrophic effects in autocrine and endocrine manners by loop autocrine function through the Sirt1/PPARα pathway. FAO, fatty acid β oxidation; PGC1α, peroxisome proliferator-activated receptor 1γ coactivator 1α; NFkB, nuclear factor Keppa B; SIRT1, sirtuin1; FGF21, fibroblast growth factors 21; FGFR1, fibroblast growth factors receptors 1; PPARα, peroxisome proliferator activated receptor α; MCAD, medium-chain acyl-CoA dehydrogenase; mcpt 1α, carnitine palmitoyltransferase Iα; Erk1/2, extracellular-signal-regulated kinases 1/2; CREB, cAMP response element-binding protein; RXR, retinoid X receptor; TNFα, tumour necrosis factors α; IL6, interleukin 6; MCP1, monocyte chemoattractant protein 1; MMP9, matrix metallopeptidase 9.

**FGF21 protects the heart from diabetes induced cardiomyopathy**

Evidence regarding the protection of the heart from diabetes induced cardiomyopathy by FGF21 is summarized in **Table 4**. In FGF21 deficient mice, it has been shown that FGF21 is essential in the prevention of the
Table 4  Protective effects of FGF21 on the heart from diabetes induced cardiomyopathy

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<th>Model</th>
<th>Methods</th>
<th>Dose</th>
<th>Results</th>
<th>Interpretation</th>
<th>References</th>
</tr>
</thead>
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<tr>
<td>FGF21-KO mice</td>
<td>T1DM was induced by STZ 60 mg/kg once a day via i.p. injection for 6 days</td>
<td>–</td>
<td>↑ Blood glucose, TG, ↑ Cardiac Nrf2 and CD36, ↑ Cardiac 3NT and 4HNE, ↑ Cardiac TG, ↑ Cardiac lipid accumulation, ↑ Cardiac collagen accumulation, ↑ Cardiac CTGF, ↑ Cardiac PGC1α, ↑ %EF, ↑ %FS</td>
<td>FGF21 deficiency up-regulation of Nrf2 driven CD36 expression exacerbates cardiac lipid uptake and accumulation, oxidative stress, impairs cardiac lipid and glucose utilization, leading to acceleration of the development of DCM.</td>
<td>Yan et al. (2015)</td>
</tr>
<tr>
<td>FGF21-KO and WT mice</td>
<td>NEFA 0.1 g/10 g, i.p. injection T1DM was induced by STZ</td>
<td>FGF21 100 µg/kg per day for 10 days, i.p. injection</td>
<td>↑ Cardiac function, ↑ Blood glucose, plasma TAG, ↑ Cleaved caspase 3, ↑ Apoptosis, ↑ Collagen contents, ↑ Erk1/2-p38MAPK-AMPK signaling pathway, ↑ tPTEN, ↑ pPTEN, ↑ Apoptosis</td>
<td>FGF21 protects the heart from lipotoxicity and diabetes induced apoptosis through activation of the Erk1/2-p38MAPK-AMPK signaling pathway.</td>
<td>Zhang et al. (2015a,b)</td>
</tr>
<tr>
<td>H9C2 cells and cardiomyocytes</td>
<td>Pre-treated with pharmaceutical inhibitors or specific small interfering (si) RNAs against Erk1/2, p38MAPK, AMPK, PTEN and pPTEN</td>
<td>FGF21 50 ng/ml, followed palmitate treatment for 15 min</td>
<td>↑ Apoptosis</td>
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FGF21-KO, FGF21 knockout; T1DM, type 1 diabetes mellitus; STZ, streptozotocin; i.p., intraperitoneal; TG, triglyceride; Nrf2, nuclear factor (erythroid-derived 2)-like 2; 3NT, 3 nitrotyrosine; 4HNE, 4 hydroxyynonenal; CTGF, connective tissue growth factor; PGC1α, peroxisome proliferator-activated receptor gamma co-activator 1α; %EF, percent ejection fraction; %FS, percent fractional shortening; DCM, diabetic cardiomyopathy; NEFA, non esterified fatty acid.
progression of T1DM induced cardiomyopathy (Yan et al. 2015). It has been proposed that four potential mechanisms are responsible for this adverse effect due to FGF21 deficiency. First, increased cardiac oxidative stress was observed, as shown by increased 3 nitrotyrosine (3NT) and 4 hydroxynonenal (4HNE). Second, increased nuclear factor (erythroid derived 2) like 2 (Nrf2) activated CD36 expression was seen, which led to increased plasma lipid uptake and accumulation into the cells. Third, decreased PGC1α protein expression was observed, which led to decreased FAO thus promoting lipid uptake and accumulation in cardiomyocytes via the CD36 receptor on the cell membranes. Fourth, increased myocardial collagen accumulation was observed as shown by increased connective tissue growth factor (CTGF). In contrast, FGF21 administration can protect the heart from lipotoxicity and diabetes induced cardiac apoptosis by the activating of the Erk1/2-p38 MAPK-AMPK survival pathway leading to decreased cardiac apoptosis and improved cardiac function (Zhang et al. 2015a). An illustrated diagram of these mechanisms is shown in Fig. 4. It has been proposed that these four potential mechanisms cause left ventricular dysfunction and accelerate the development and progression of DCM (Yan et al. 2015) and the effects can be reversed by FGF21 treatment (Zhang et al. 2015a).

Fgf21 regulates energy supply in the heart

FAO is the major source of energy for cardiomyocytes, generating 50–70% of ATP in a normal adult heart, while only 20–30% of energy is released by glycolysis, and <5% from other sources (Neely et al. 1972, Neely & Morgan 1974). It has been shown that the transition from fetal glycolysis (fetal pattern) to FAO in the neonatal stage (Lockwood & Bailey 1970, Kelly et al. 1989) is brought about by increased PGC1α, PPARα, and FGF21 mRNA expression (Planavila et al. 2013). In contrast, the chicken ovalbumin upstream promoter transcription factor (COUP-TF) that regulates glycolysis is down regulated (Sack et al. 1997). PPARα is expressed at a high rate in mitochondrial FAO tissue, and is situated on the nuclear membrane with the retinoid X receptor (RXR). PGC1α binding to the PPARα/RXR on the nuclear membrane leads to the increased expression of FAO genes including MCAD and mcpt1α, hence promoting increased FAO and synthesis of the ATP supply for the heart in physiological conditions (Vega et al. 2000) (Fig. 5A).

Under pathological conditions such as cardiac hypertrophy, myocardium FAO enzyme genes are down regulated (Sack et al. 1997, Razeghi et al. 2001), while the COUP-TF is up regulated (Sack et al. 1997). This caused the switch of the energy source back to the fetal glycolysis pattern again (Fig. 5B). A recent study demonstrated that myocardium PGC1α, MCAD, and mcpt1α mRNA expression is regulated by FGF21 to promote FAO for the energy supply to the heart (Planavila et al. 2013). The deletion of FGF21 has been shown to increase CD36 and decrease PGC1α, leading to acceleration of DCM through aggravating cardiac lipid accumulation (Yan et al. 2015). Therefore, FGF21 might be beneficial as a pharmacological intervention under these conditions. Further studies are needed to give more evidence for the substantiation to this hypothesis.

Molecular basis of the antioxidant signaling cascade of FGF21 in cardiomyocytes

Previous studies demonstrated that following ISO induced cardiac hypertrophy by causing cardiac oxidative stress and inflammation, and that FGF21 was secreted from cardiomyocytes via Sirt1 activation. Sirt1 was found to stimulate FGF21mRNA and protein expression and secretion into the circulation, where FGF21 proceeded to act in a paracrine, autocrine and endocrine manner. In the autocrine loop function, FGF21 induces anti-oxidant gene expression through the Erk/Sirt1 pathway, including uncoupling protein 3 (UCP3), superoxide dismutase 2 (Sod 2), peroxiredoxin 5 (Prdx5), glutathione peroxidase 1 (GPX1), Catalase (CAT) and Sequestosome 1 (Sqstm1), resulting in a reduction in cardiac tissue injury (Planavila et al. 2014). Furthermore, FGF21 has been shown to activate the Nrf2 pathway in hepatocytes, which was found to lead to increased anti-oxidant gene expression, resulting in the reduction of liver tissue injury (Yu et al. 2015) (Fig. 6).

The stimulation of antioxidative pathways by FGF21 led to an increase in antioxidative gene and enzyme expression, and prevented oxidative stress by decreasing ROS production in cardiomyocytes. Therefore, this protected the myocardium (Planavila et al. 2014) from oxidative stress and subsequent injury. Interestingly, a clinical study demonstrated that FGF21, UCP3, and Sod2 levels were increased in dilated cardiomyopathy patients in the final stages of heart failure (Planavila et al. 2014). This indicated that FGF21 could protect the cardiomyocytes or slow down the degree of damage following oxidative stress in a failing human heart.
It has been shown that these signaling pathways of FGF21 have the crosstalk at the secondary messenger levels such as ERK1/2 for anti-apoptosis (Zhang et al. 2015a), anti-hypertrophic (Planavila et al. 2013, 2014), and anti-oxidative stress (Yu et al. 2015) in the heart. Activation of FGFR/β-Klotho complex therefore could activate these effects of FGF21 simultaneously at this crosstalk. However, further studies are needed to investigate this issue.

Clinical evidence of the association between FGF21 and cardiovascular alteration

Evidence regarding the correlation of plasma FGF21 with cardiovascular alteration in clinical reports are summarized in Table 5. Serum FGF21 levels have been shown to have a strong correlation with waist circumference, systolic blood pressure, lower extremity arteriosclerotic disease (Zhang et al. 2015b) and carotid intima.
media thickness (Chow et al. 2013) in T2DM (type 2 diabetes mellitus) patients. FGF21 level was also increased in atrial fibrillation (AF) patients and was shown to be an independent risk factor for AF (Han et al. 2015). In the cases of non-alcoholic fatty liver disease (NAFLD) and CAD, the serum FGF21 was associated with an adverse lipid profile and also showed a positive correlation with total cholesterol (TC) and triglycerides (TG) (Shen et al. 2013). Moreover, the CAD patient’s serum FGF21 levels were also positively correlated with TG, fasting blood glucose, ApoB100, insulin, and HOMA-IR, and also have a negative correlation with HDL, and ApoA1 (Lin et al. 2010). Recent studies demonstrated that serum FGF21 levels correlate with metabolic status in patients. High serum FGF21 levels in several pathological conditions of the heart under metabolic dysregulation may be explained by FGF21 resistance conditions, which have been observed in ex vivo experiments with obese rat hearts (Patel et al. 2014) and in vivo experiments with DIO mice liver and white adipose tissue (Fisher et al. 2010). Therefore, serum FGF21 levels may be indicators of adverse metabolic dysregulation and prognosis for CVD.

The term ‘FGF21 resistance’ in the heart was first mentioned in chronic DIO rats by Patel and colleagues (Patel et al. 2014). They found that obese rat hearts had increased FGF21 mRNA, and FGF21 protein expression and secretion levels. Despite the high level of FGF21, disrupted FGF21-FGFR1-β-Klotho signaling and decreased ERK1/2, Akt and AMPK phosphorylation were observed under this condition (Patel et al. 2014). These findings indicate that obese condition caused the impairment of the FGF21 signaling cascades, and that the feedback mechanism allowed the increased production of FGF21 to overcome the FGF21 receptor signaling dysfunction. Unfortunately, the increased endogenous FGF21 level was not sufficient when the exogenous FGF21 administration comes into play a role for therapeutic strategy. The FGF21 resistance was also observed in clinical reports where serum FGF21 level was significantly increased in non-NAFLD (Shen et al. 2013), coronary heart disease (Lin et al. 2010, Shen et al. 2013), metabolic syndrome (Lee et al. 2014), and T2DM (Lenart-Lipinska et al. 2013). This condition is similar to what has been observed in subjects under ‘insulin resistance’ condition in which the impairment of insulin receptor and signaling cascades was found with increased plasma insulin level (Pratchayasakul et al. 2011, Pipatpiboon et al. 2012).

The cross sectional study in 15 male patients who underwent aorto-coronary bypass surgery showed that

Figure 5
Relationship of FGF21 mRNA expression and FAO control in cardiomyocytes in different periods of age, and its alteration under physiological and pathological stages. (A) Relative transcriptional level of FGF21 mRNA and cardiac FAO enzyme gene regulator expression in different age brackets. (B) Relative transcriptional level of FGF21 mRNA and cardiac FAO enzyme gene regulator expression in physiological and pathological stages. PGC1α, peroxisome proliferator-activated receptor 1α; coactivator 1α; RXR, retinoid X receptor; PPAR, peroxisome proliferator activated receptor-α; COUP-TF, chicken ovalbumin upstream promoter transcription factor; NRRE, nuclear receptor response element; FAO, fatty acid β oxidation; ATP, Adenosine triphosphate.
serum FGF21 levels increased to a peak at 6 h into surgery, and were associated with increased serum glucose, insulin, pro-inflammatory cytokines (TNFα, MCP1) and inflammatory cytokines (IL6, 8), but returned to baseline at 96 h after surgery (Kotulak et al. 2011). Moreover, epicardial fat and muscular FGF21 mRNA expression increased after surgery has reacted positively with blood glucose levels at the end of surgery (Kotulak et al. 2011) indicating that FGF21 mRNA expression and serum FGF21 levels regulated glucose homeostasis, increased the insulin sensitivity and attenuated the inflammatory process.

In a cross sectional study of 189 patients who underwent cardiac multidetector coronary computed tomography, it was found that serum FGF21 levels were associated with an adverse lipid profile and pericardial fat volume only in metabolic syndrome patients (Lee et al. 2014). Interestingly, cardiac tissue peroxidase 1; Sptsm1, sequestosome 1; ROS, reactive oxygen species; Nrf2, Nuclear factor erythroid 2-related factor 2; PI3K, phosphatidylinositol 3-kinase. Data from Planavila et al. (2014) and Yu et al. (2015).

FGF21 mRNA and antioxidant genes (UCP3 and Sod2) are upregulated in six failing human hearts which may be mechanisms to preserve myocardial function in cases of heart failure (Planavila et al. 2014). All of these findings indicate that FGF21 plays an important role in metabolic regulation and attenuates cardiac oxidative stress in heart failure patients. The increased FGF21 level observed in heart failure patients was due to the FGF21 resistance as shown by a previous report (Planavila et al. 2014). Despite the increased endogenous FGF21 under this pathological condition, its level was still not sufficient to overcome the FGF21 resistance. Therefore, the role of exogenous FGF21 is considered as a potential therapeutic strategy to provide cardioprotective effects (Lu et al. 2010, Cong et al. 2013, Planavila et al. 2013, 2014, Zhang et al. 2015a). Previous reports at least from basic studies using exogenous FGF21 demonstrating the
<table>
<thead>
<tr>
<th>Models</th>
<th>Type of study</th>
<th>N</th>
<th>Major findings</th>
<th>Interpretation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2DM and CV risk factor prevalence study</td>
<td>Cohort study</td>
<td>670 (158: T2DM, 502: CV risk factor prevalence study)</td>
<td>– FGF21 shows a positive correlation with CIMT in women</td>
<td>Serum FGF21 levels are associated and independent established risk factor with carotid atherosclerosis.</td>
<td>Chow et al. (2013)</td>
</tr>
<tr>
<td>NAFLD, CAD, non NAFLD and CAD</td>
<td>Cohort study</td>
<td>253</td>
<td>↑ FGF21 in NAFLD vs non NAFLD</td>
<td>Serum FGF21 levels increased and were associated with adverse lipid profiles in NAFLD and CAD.</td>
<td>Shen et al. (2013)</td>
</tr>
<tr>
<td>DCMP and final stage HF</td>
<td>Cross-sectional study</td>
<td>6 HF 10 donor</td>
<td>↑ Cardiac tissue FGF21 mRNA</td>
<td>Serum FGF21 expression is up-regulated in the failing human heart</td>
<td>Planavila et al. (2014)</td>
</tr>
<tr>
<td>CAD</td>
<td>Cross-sectional study</td>
<td>135 patients</td>
<td>– Positively correlated with TG, FBG, Apo B100, insulin, and HOMA-IR</td>
<td>Serum FGF-21 level is associated with adverse lipid profiles in CAD patients</td>
<td>Lin et al. (2010)</td>
</tr>
<tr>
<td>IHD (Aorto-coronary bypass surgery)</td>
<td>Prospective</td>
<td>15 male patients</td>
<td>↑ Serum FGF21 levels during surgery; peak at 5 h</td>
<td>FGF21 regulated glucose homeostasis, insulin sensitivity and attenuated inflammatory process.</td>
<td>Kotulak et al. (2011)</td>
</tr>
<tr>
<td>Patients had undergone 64 slice cardiac MDCT</td>
<td>Cross-sectional study</td>
<td>189 patients</td>
<td>↑ Serum FGF21 in MS, but not in diabetes or CAD</td>
<td>Serum FGF21 is associated with lipid profiles, insulin resistance, PFV and MS, but not altered in DM or CAD.</td>
<td>Lee et al. (2014)</td>
</tr>
<tr>
<td>T2DM</td>
<td>Prospective</td>
<td>9697</td>
<td>↑ Higher baseline plasma FGF21 levels had ↑ Total CV events</td>
<td>High baseline plasma FGF21 levels in T2DM patients is associated with CV events.</td>
<td>Ong et al. (2015)</td>
</tr>
<tr>
<td>AF</td>
<td>Cross-sectional study</td>
<td>113 AF patients, 60 healthy volunteers</td>
<td>↑ FGF21 in permanent AF &gt; persistent and paroxysmal AF</td>
<td>Serum FGF21 levels are increased in AF patients and are an independent risk factor for AF.</td>
<td>Han et al. (2015)</td>
</tr>
</tbody>
</table>

T2DM, type 2 diabetes mellitus; CV, cardiovascular; CIMT, carotid intima media thickening; hsCRP, high sensitivity C-reactive protein; FGF21, fibroblast growth factor 21; NAFLD, nonalcoholic fatty liver disease; CAD, coronary artery disease; TG, total cholesterol; TG, triglyceride; LDL, low density lipoprotein; DCMP, dilate cardiomyopathy; HF, heart failure; UCPs, uncoupling proteins; Sod, superoxide dismutase; FBG, fasting blood glucose; Apo, apolipoprotein; HOMA-IR, homeostatic model assessment insulin resistance; HDL, high density lipoprotein; IHD, ischemic heart disease; IL, interleukin; MCP1, monocyte chemo attractant protein 1; TNFα, tumor necrosis factor alpha; cardiac MDCT, cardiac multidetector coronary computed tomography; MS, metabolic syndrome; PFV, pericardial fat volume; DM, diabetes mellitus; CVD, cardiovascular diseases; AF, atrial fibrillation; LA, left atrium.
improved cardiac function in cardiac I/R injury (Cong et al. 2013, Liu et al. 2013, Patel et al. 2014), cardiac hypertrophy (Planavila et al. 2013), and DCM (Zhang et al. 2015a) supported this hypothesis.

Although previous studies indicated that PPARα is the essential downstream signaling protein in regulating the expression of FGF21 mRNA in cardiomyocytes, and preserving the myocardial metabolism through regulating the FAO (Lu et al. 2010, Planavila et al), PPARα agonist (Finofibrate) has been shown to be unable to reduce cardiovascular events in T2DM patients after a 5-year follow-up (Ong et al. 2015). Moreover, the risk cardiovascular events in T2DM patients showed a correlation with baseline plasma FGF21 levels. Higher baseline plasma FGF21 levels correlated with an increased risk of cardiovascular events (Ong et al. 2015). This suggests that plasma FGF21 levels could be used as a biomarker of metabolic dysregulation, and increased plasma FGF21 levels may be indicative of a higher risk of cardiovascular events.

Despite the fact that FGF21 can preserve the heart in several pathological conditions, FGF21 resistance may be a limitation for the potential role of exogenous FGF21 administration. However, previous studies demonstrated that exogenous FGF21 exerted its effects in a dose-dependent manner (Cong et al. 2013, Planavila et al. 2014, Yu et al. 2015). Moreover, it is possible that FGF21 replacement during an early stage of FGF21 resistance may be more effective than late replacement. Future studies are needed to prove this hypothesis. Furthermore, long-term FGF21 treatment (Zhang et al. 2015a) may provide better outcome than the acute intervention (Patel et al. 2014). Lastly, combined therapy of FGF21 with specific drugs may provide better efficacy than FGF21 monotherapy. All of these hypothetical strategies still need to be verified in future studies.

Conclusion

Experimental studies of FGF21 in the heart have consistently demonstrated its beneficial effects in in vitro, ex vivo and in vivo models. Evidence has shown that FGF21 is crucial for cardioprotection in myocardial hypertrophy, ischemia, DCM and I/R injury. FGF21 provides its therapeutic benefits by attenuating apoptosis, oxidative stress and inflammation, and improving energy supply, and therefore could be used as an indicator of metabolic dysregulation. Moreover, FGF21 could be a potential therapeutic target for metabolic disorders and CVD in the future.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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