Fibroblast growth factors in cardiovascular disease: The emerging role of FGF21

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FGFs constitute a vast family of polypeptides with important biological effects. FGF family in humans includes 22 members (FGF1 to FGF14 and FGF16 to FGF23; FGF15 is the mouse ortholog of FGF19) located in different tissues and having specific functions. Phylogenetic analysis of the human FGF gene family has identified seven subfamilies of FGFs that are also functionally classified according to their intracrine/intracellular, paracrine, and endocrine actions (46). The seven FGF subfamilies are classified as follows: subfamily 1 comprises FGF1 and FGF2; subfamily 4 comprises FGF4, FGF5, and FGF6; subfamily 7 comprises FGF3, FGF7, FGF10, and FGF22; subfamily 8 comprises FGF8, FGF17, and FGF18; subfamily 9 comprises FGF9, FGF16, and FGF20; subfamily...
11 comprises FGF11, FGF12, FG13, and FGF14; and subfamily 19 comprises FGF19, FGF21, and FGF23. Subfamily 11 consists of the intracrine factors; subfamily 19 consists of the endocrine factors, and the rest of the subfamilies consist of the paracrine FGFs (24). Intracrine FGFs act as intracellular molecules by regulating electrical excitability (100). The paracrine and endocrine factors are secreted or released from the cell and function by binding to specific FGF receptors (FGFRs). The endocrine FGFs have very low heparin-binding affinity, which enables them to be released in the circulation (46).

Various FGFs along with other cytokines, such as vascular endothelial growth factors (VEGFs), have been examined as therapeutic agents in cardiovascular disease (93–94, 97). Most of them have common effects in biological processes, including angiogenesis, wound healing, neurogenesis, and neuroprotection (19, 48, 86). Recently, the endocrine member FGF21 has been shown to be a potent metabolic regulator and to have multiple beneficial effects in conditions that are considered to be major cardiovascular risk factors, such as hyperlipidemia, obesity, and diabetes (61, 74, 85).

In the present review, we describe the role of FGFs in cardiovascular disease. The potential use of the angiogenic capacity of FGFs for treatment has been the main focus of research, and many studies evaluating this potential have already progressed to advanced-phase clinical trials. We then specifically review the background knowledge and the mechanisms of action of FGF21, the newest member of the FGF family, and the evidence regarding its role and potential protective value in cardiovascular disease and especially atherosclerosis.

Metabolic Effects of FGFs and Association with Cardiovascular Disease

Members of the endocrine family have been shown to exert significant metabolic actions (Table 1). FGF19, a member of the endocrine FGF15/19 family, is characteristically expressed in ileal enterocytes regulating bile acid synthesis (38, 44). In transgenic mouse models and mouse models of dietary- and leptin-induced diabetes, FGF19 increases metabolic rate, decreases adiposity, and restores glucose homeostasis, suggesting a multiple metabolic role (25, 109). FGF19 is also associated with an intense liver-proliferating response and hepatocellular carcinoma in mice overexpressing FGF19 in skeletal muscle although it could also be used as an effective biomarker for patients most likely to respond to anti-FGF19 treatment for hepatocellular carcinoma (79, 95). In patients with type 2 diabetes and metabolic syndrome as well as those with suspected or established CAD, FGF19 is negatively correlated with metabolic indices and known cardiovascular risk factors [e.g., 2-h postprandial glucose, serum fasting insulin, body mass index (BMI), triglycerides, and glycosylated hemoglobin] (4, 34). Patients with CAD were found to have lower levels of FGF19 than those without CAD, adjusting for other factors, whereas FGF19 was also an independent predictor of the extent of CAD (34).

FGF23, also a member of the endocrine FGFs, is secreted by osteocytes in the serum, and it is essential in phosphate and vitamin D metabolism (65, 102). FGF23 serum levels have been found to be associated with cardiovascular risk factors such as apolipoprotein A1 and high-density lipoprotein (HDL) in subjects with and without chronic kidney disease (76–77). Elevated serum levels of FGF23 are also associated with the progression and development of left ventricular hypertrophy in patients with chronic kidney disease but are also independently associated with heart disease as well as the severity and extent of CAD in patients undergoing coronary angiography (Table 1) (32, 42, 47, 73, 112). This is supported by evidence that FGF23, except for binding to FGFR and the coreceptor klotho in the kidney and parathyroid gland, acts directly to the heart via a klotho-independent signaling pathway (20). In a recent large study, increased levels of FGF23 were associated with cardiovascular death and incident heart failure in 3,627 patients with stable ischemic heart disease; FGF23 levels also indicated a better response to therapy with angiotensin-converting enzyme inhibitor therapy (110). The link of FGF23 with direct cardiac effects and the potential association of serum levels with cardiovascular risk factors warrant further research.

Table 1. FGF family members and cardiovascular effects

<table>
<thead>
<tr>
<th>FGF Subfamily</th>
<th>FGF Involved</th>
<th>Cardiovascular Effect</th>
<th>Studied Cardiovascular End Point in Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGF1</td>
<td>FGF1</td>
<td>Angiogenesis</td>
<td>Peripheral artery disease (7, 80, 107) (pharmacological effect)</td>
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<tr>
<td></td>
<td>FGF2</td>
<td>Angiogenesis</td>
<td>Coronary artery disease (94, 103)</td>
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<tr>
<td>FGF4</td>
<td>FGF4</td>
<td>Angiogenesis</td>
<td>Peripheral artery disease (56, 117) (pharmacological effect)</td>
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<tr>
<td></td>
<td>FGF5</td>
<td>Angiogenesis</td>
<td>Coronary artery disease (30–31, 37) (pharmacological effect)</td>
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<tr>
<td></td>
<td>FGF19</td>
<td>Not established</td>
<td>Coronary artery disease (34) (correlation with serum levels)</td>
</tr>
<tr>
<td></td>
<td>FGF23</td>
<td>Cardiomyocyte hypertrophy</td>
<td>Left ventricular hypertrophy in chronic kidney disease (32, 42)</td>
</tr>
<tr>
<td></td>
<td>FGF21</td>
<td>Lipid lowering</td>
<td>Coronary artery disease (112)</td>
</tr>
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</table>

The cardiovascular-related end point as a result of fibroblast growth factor (FGF) pharmacologic action or observed correlation to FGF serum levels in humans is shown.
FGFs and Therapeutic Angiogenesis in Ischemic Vascular Disease

FGF1 was first described as having angiogenic potential in animal studies by Schumacher and associates (97–98). Available data on this molecule are not clear despite encouraging results from phase 1 and 2 clinical trials for therapeutic angiogenesis in ischemic vascular disease (6, 80); a phase 3 trial showed no positive effects from the treatment with this factor (7). FGF2, namely basic FGF, along with VEGFs, is known to act in multiple stages of angiogenesis (Table 1) (92, 107). In animal studies, FGF2 leads to improved myocardial perfusion, can provide cardioprotection during ischemia-perfusion injury (5, 35, 39, 41, 43, 55), and has a strong link with cardiac hypertrophy (40, 49, 96). In humans, FGF2 protein delivery resulted in significant improvement in angina (Table 1) (94, 103). Recombinant FGF2 has also been tested in peripheral artery disease with improvements of limb function (Table 1) (56, 117).

FGF4 (a member of the FGF4 subfamily that includes FGF4, FGF5, and FGF6) promotes the growth of human embryonic stem cells and their pluripotency (75). In a rabbit hind-limb ischemia model, FGF4 administered by adenoviral gene transfer induced vascular permeability, therapeutic angiogenesis, and arteriogenesis (91). Subsequently, a series of clinical trials, Angiogenic GENe Therapy (AGENT), AGENT2, AGENT3, and AGENT4, were carried out to test the safety and efficacy of FGF4 as gene therapy. During these studies FGF4 was found to have no difference between the treated and the placebo group in myocardial perfusion in patients with stable angina and reversible ischemia and to have no significant effect on exercise tolerance at 12 wk (Table 1) (30 –31, 37).

FGF5, also a member of the FGF4 subfamily, is known to be highly expressed in the central nervous system and in the skin, where it regulates hair growth (36, 105). FGF5 is reported to increase myocardial blood flow and function, decrease myocyte apoptosis, and increase myocyte number after gene transfer of the growth factor in swine (28, 70, 104). There are no available results from extensive studies on FGF5 and its effects in human cardiovascular disease, which would provide more definitive insight into the role of this molecule.

FGF21: Expression and Mode of Action

FGF21 is emerging as the newest candidate from the FGF family with a potentially critical role in the cardiovascular system. Many of the above-mentioned members of the FGF family are different from FGF21, as they are paracrine factors acting as secreted or released extracellular proteins instead of being released in the blood stream as endocrine FGF21 (46). The initially purified from bovine brain FGF1 and FGF2 were identified to be mitogenic for a wide variety of mesoderm-derived cells, whereas FGF21 is mostly known for its metabolic effects rather than the mitogenic ones, with so far only one study in the literature documenting a potential angiogenic effect of this molecule (29, 115).

FGF21 was initially identified in mouse embryos, and it is a member of the subfamily 15/19 of the FGFs, together with FGF19 and FGF23 in humans (24, 81). Gene expression in C57/BL6 mice has shown that FGF21 is expressed predominantly in pancreas, testis, liver, and brown adipose tissue and in lower amounts in other tissues, including the aorta (24). FGF21 acts through the known FGFRs as a complex with α-klotho protein (50, 82), and, following the formation of α-klotho protein (50, 82), and, following the formation of α-klotho protein (50, 82), and, following the formation...
of the complex on the cell surface, a signaling cascade is activated (45, 50, 82). The β-klotho gene was identified in mice to encode for a type I membrane protein β-klotho, member of the klotho family, and it is predominantly expressed in liver, pancreas, and adipose tissue and in lower amounts in mouse aorta (24, 45). The expression of mRNA-encoding FGFRs in cultured human endothelial cells (ECs) and smooth muscle cells showed that both cell types express FGFR1, but ECs also express low levels of FGFR4, whereas smooth muscle cells also express low levels of FGFR2 (8). Importantly, mouse cardiomyocytes express FGFR1 and FGFR3, as well as β-klotho (66).

Recent in vivo findings demonstrate that FGF21 has several metabolic effects, and its serum levels have also been correlated with many cardiovascular risk factors, summarized in Fig. 1.

**Metabolic Effects of FGF21**

Many animal and, recently, clinical studies have highlighted the actions and beneficial role of FGF21 in metabolic diseases (Fig. 2). It was initially found to increase glucose uptake in mouse 3T3-L1 and primary human adipocytes (51). Reduced plasma glucose and triglyceride levels were observed when FGF21 was administered in genetic mouse models of obesity and type 2 diabetes, leptin-deficient ob/ob mice, and leptin receptor-deficient db/db mice (51). Furthermore, FGF21 administration in diet-induced obese mouse models lowered their mean body weight and fat mass, increased their energy expenditure, ameliorated their lipid and glucose metabolism, improved hepatic and peripheral insulin sensitivity, and reduced hepatic steatosis (12, 113). When FGF21 was administered daily for 6 wk in diabetic obese rhesus monkeys, a dramatic decrease in fasting blood glucose and insulin was observed along with significant improvements in the lipid and lipoprotein profile, including a decrease in triglycerides and low-density lipoprotein (LDL) and an increase in HDL (52). FGF21 has also been shown to be critical in promoting adaptation in ketotic states and fasting according to animal studies (15). When mice consume a ketogenic diet and when they are on a fasting regime, serum FGF21 levels increase, whereas they rapidly decrease after animals are refed from the fasted condition (3). When mice lacking FGF21 are put on a ketogenic diet, multiple metabolic impairments including hyperlipidemia, impaired glucose tolerance, and steatosis are observed (2–3). FGF21 has a potent role in various tissues such as in white adipose tissue by regulating browning in adaptive thermogenesis and in liver, which constitutes a target tissue for FGF21 through which FGF21 effects on lipid and glucose metabolism are mediated (22–23).

**FGF21: A biomarker for metabolic diseases**

Studies in humans with nonalcoholic fatty liver disease, obesity, and diabetes document increased serum levels of FGF21 (Fig. 1). Baseline FGF21 serum levels are significantly higher in obese people and patients with type 2 diabetes compared with controls, and treatment with fenofibrate further increases these levels (78, 85). Greater FGF21 concentrations were observed in a group of patients with type 2 diabetes, and these were significantly correlated to adiponectin, weight, glucose, HDL cholesterol, and triglycerides (74). In two other studies of patients with type 2 diabetes, fasting serum FGF21 was increased and correlated with multiple metabolic parameters such as blood glucose and lipids, blood pressure, and HbA1c (Fig. 1). Thus a role of FGF21 in the pathogenesis of insulin resistance and type 2 diabetes was suggested (9, 61).

The metabolic syndrome has also been associated with increased serum FGF21 levels, whereas an increase in FGF21 serum levels has been suggested as a new biomarker for nonalcoholic fatty liver disease or steatohepatitis (17, 54, 60, 62, 116, 118). A study on obese children confirmed that increased serum FGF21 is correlated to BMI and free fatty acids (90). When serum FGF21 levels were tested after an oral load of fructose, it was interestingly shown that FGF21 values acutely spike, presenting a similar curve as serum glucose and insulin after a glucose load. This finding shows that FGF21 presents a typical hormonal response possibly mediated by carbohydrate-responsive element-binding protein that is activated by fructose (18).

FGF21 is involved in glucose and lipid metabolism, which indicates that elevated serum concentrations of this molecule in conditions such as obesity, diabetes, hyperlipidemia, and fatty liver disease could be used as a biomarker, although one could also speculate that FGF21 plays a protective role in the course of pathophysiological processes involved in atherosclerosis in humans. The prognostic value of serum FGF21 was assessed in...
patients with type 2 diabetes, and it was shown that FGF21 levels are predictive of combined cardiovascular morbidity and mortality (Fig. 3) (59). Increased baseline serum levels of this molecule were found to be associated with a higher risk for cardiovascular events in patients with type 2 diabetes in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, and interestingly this association tended to be stronger in the patient group that presented higher total cholesterol levels (84). The authors speculate that the increased basal levels of FGF21 in this group of patients may be an indication of the potential role of FGF21 as a biomarker for the early detection of cardiometabolic risk and furthermore that it may reflect a compensatory response or the need of supraphysiological doses of FGF21 as a result of FGF21 resistance, a hypothesis proven in obese mouse models (21). In the clinical setting, when an FGF21 analog was administered to patients with obesity and type 2 diabetes, there was improvement in dyslipidemia with modest effects on glucose homeostasis (26).

A strong link between FGF21 and mitochondrial diseases was recently uncovered when serum levels of this protein were shown to be significantly elevated and when FGF21 was found to be the most sensitive predictor compared with other known predictors in detecting the presence of the disease (13). This aspect of FGF21 properties may be considered similar to the known increase of FGF21 in states of fasting, as mitochondrial aspects of FGF21 properties may be considered similar to the basal levels of FGF21 in this group of patients may be an indication of the potential role of FGF21 as a biomarker for cardiovascular risk (72). Obesity and pericardial fat accumulation (Table 1), indicating that FGF21 may be related to fat deposition and dyslipidemia independent of obesity (58). Pericardial fat has been previously shown to be related to cardio-metabolic risk factors and contribute to CAD; this could further extend the role of FGF21 as a biomarker for cardiovascular risk (72). Obesity and pericardial fat are also associated with the presence and the severity of atrial fibrillation (106), and an association between elevated FGF21 serum levels and atrial fibrillation has been shown independently of risk factors, such as BMI, presence of hypertension, and levels of high-sensitivity C-reactive protein (33).

Markers of subclinical atherosclerosis have been evaluated in relation to serum FGF21 levels, which were found to be positively correlated to carotid intima-media thickness in female patients (10). When 744 community-dwelling adults participating in the Baltimore Longitudinal Study of Aging were assessed for FGF21 serum levels, an independent association with hypertension was discovered (99). Moreover, measurements of brachial-ankle pulse-wave velocity, indicative of arterial stiffness, in obese nondiabetic women were found to be correlated with serum FGF21 levels. When the same women were followed up with after a 3-mo exercise program, FGF21 levels decreased along with improvement of brachial-ankle pulse-wave velocity measurements (114).

**Protective Role of FGF21 in Cardiovascular Disease**

The protective role of FGF21 for the cardiovascular system is supported by in vitro studies, which provide evidence that one of the pharmacological effects of FGF21 is to increase cholesterol uptake in human hepatocytes and mouse macrophages, by increasing LDL receptor (LDL-R) through reduction of the myosin regulatory light chain-interacting protein/
inducible degrader of the LDL-R, an effect additive to that of statins (14, 16, 53, 71) (Fig. 1).

FGF21 is upregulated by peroxisome proliferator-activated receptor-α (PPAR-α) in the animal and human liver and by PPAR-γ in adipose tissue (27, 69, 111). PPAR-α and PPAR-γ are both known to play an important role in lipid and glucose regulation and in cardiovascular disease (57). A recent in vitro study has provided evidence that both FGF21 and PPAR-α mRNA are expressed in rat cardiovascular ECs (Fig. 1) (68). In this study, a mechanistic insight into the dual role for FGF21 was provided; taking into account the lipid-lowering effects of both FGF21 and PPAR-α, the two molecules could cooperate to create an autocrine feedback loop in conditions of increased presence of lipids. Moreover, the finding in the same study that, by inducing apoptosis of cardiovascular ECs with oxidized LDL (ox-LDL), a dose-dependent induction of FGF21 was observed (Fig. 1) and the apoptotic effect of ox-LDL was influenced by the FGF21 response leads to the speculation that FGF21 could be a biological marker of EC stress and injury and, more importantly, that it may play a physiological role in improving endothelial function at an early stage of atherosclerosis, as the authors conclude. Use of bezafibrate, a PPAR-α ligand, has been shown to induce FGF21 expression, further supporting a protective role of FGF21 (Fig. 1) (68).

FGF21, both endogenous and exogenously administered, successfully interacts with cardiomyocytes to protect them after myocardial ischemia/reperfusion injury (Fig. 4) (66). FGF21 was shown to be the main mediator (via β-klotho-dependent signaling pathways) of a cardioprotective endocrine response in the liver and adipose tissue activated by experimental myocardial ischemia (66). These findings indicate β-klotho-dependent pathways of action of FGF21 on cardiac...
tissue and support a potential therapeutic role of this molecule. The protective action of FGF21 on ischemic/injured cardiac cells is further supported by more studies in vitro and in vivo (11, 67, 87). By regulating genes in antioxidant pathways, such as uncoupling protein 3 and peroxiredoxin 5, FGF21 prevents oxidative stress mediated by reactive oxygen species in cardiomyocytes (88). Protection of the heart from hypertrophy is another targeted action of FGF21 that has been discovered in mice, as FGF21 knockout mice seem to be unprotected from hypertrophic insults, whereas protection is restored as soon as FGF21 is replaced (89).

There is also evidence that, in adipose tissue, β-klotho-independent signaling pathways of FGF21 exist, highlighting the necessity to assess signaling alternatives in other tissues as well, such as the coronary artery wall (108). In a double knockout mouse model of atherosclerosis, with both FGF21 and apolipoprotein E deficiency, an accelerated plaque-formation process was observed compared with the single apolipoprotein E deficiency control group (63). The protective effects of FGF21 presence were found to be connected to metabolic effects, such as cholesterol lowering by suppressing sterol-responsive element protein 2 expression in the liver as well as induction of the antiatherosclerotic molecule adiponectin in adipose tissue (63, 83).

Conclusions

FGF family members have a potent intracellular, paracrine, and endocrine activity with multiple effects on metabolism and potential actions on the cardiovascular system. Members of the endothelial family have been shown to be associated with metabolic markers and the presence/extent of CAD predicting cardiovascular morbidity. The therapeutic value of FGFs related to angiogenesis has been investigated in clinical trials, but current evidence remains inconclusive; thus further research is required.

FGF21 is emerging as a new promising member of the FGF family with a potentially important role in cardiovascular disease and especially atherosclerosis. FGF21 levels have been shown to be strongly related to traditional cardiovascular risk factors and conditions such as hyperlipidemia, hypertension, diabetes, and obesity in humans. However, multiple beneficial metabolic effects of FGF21 have been previously demonstrated in experimental and animal models, suggesting that FGF21 is not a simple marker of cardiovascular risk but may have a protective effect on the cardiovascular system, contributing to a reduction in risk. Emerging evidence demonstrating the protective role of FGF21 against endothelial damage, atherosclerotic plaque formation, and ischemic injury of cardiomyocytes related to oxidative stress has provided a greater insight toward that direction. The role of FGF21 in the development of atherosclerosis and whether FGF21 could serve as a novel therapeutic agent during the early stages of atherosclerotic disease need to be studied further.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


REFERENCES

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