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FGF21 Prospects for Applications in Clinical Practice

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AS, EK and GL gathered the literature and author AN wrote the manuscript. Authors DVC and IH corrected the manuscript. All authors read and approved the final manuscript.

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Mini-review Article

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ABSTRACT

History of FGF21: FGF21, firstly identified in 2000, was reported as a novel endocrine metabolic regulator in 2005. FGF21 has attracted great attention for its therapeutic potentials, because it has strong influence on metabolism, without displaying the proliferative activities of other members of the FGF family.
Physiological Roles of FGF21: It has been shown that various kinds of stress can raise the circulated levels of FGF21, including malnutrition, cold exposure and exercise. FGF21 promotes lipid catabolism, ketogenesis, gluconeogenesis and glucose uptake, and also protects tissues from stress-induced cytotoxicity.
Aim of the Article: This mini review is a summary of the potential uses of FGF21 for diagnostic and therapeutic purposes. High levels of circulating FGF21 have been associated with various pathologic situations, whereas different forms of the FGF21 molecule have been developed and evaluated on animal models and humans for therapeutic use.

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Keywords: FGF21; biomarker; clinical trials; therapeutic potential; analogs.

ABBREVIATIONS

AF: Atrial Fibrillation; AN: Anorexia Nervosa; AMI: Acute Myocardial Infarction; BMI: Body Mass Index; BP: Diastolic Blood Pressure; CAD: Coronary Artery Disease; FGFs: Fibroblast Growth Factors; FGFRs: FGF Receptors; GH: Growth Hormone; IMT: Intima-media Thickness; NAFLD: Non-alcoholic Fatty Liver Disease; NWC: Normal-weight Controls; LAD: Left Atrial Diameter; LEAD: Lower Extremity Atherosclerotic Disease.

1. INTRODUCTION

Fibroblast growth factors (FGFs) superfamily is currently comprised of 22 autocrine, paracrine and endocrine signaling proteins in mammals, which regulate a broad spectrum of biological processes like proliferation, differentiation and metabolism. Endocrine and paracrine FGFs act through FGF receptors (FGFRs) 1b, 1c, 2b, 2c, 3b, 3c and 4. Endocrine FGFs also require α or β-Klotho cofactor for activation [1,2]. FGF21 is the 21st discovered member of the superfamily of FGFs, firstly identified in 2000 [3] and reported as a novel metabolic regulator in 2005 [4]. Human FGF21 is an endocrine protein hormone, 208 amino acids long, which acts through FGFR1c with β-Klotho as a cofactor [5]. FGF21 has attracted great attention for its therapeutic potentials, because it has strong influence on metabolism, without displaying the proliferative activities of other FGFs [6,7]. Circulating FGF21 levels under standard conditions are maintained basal, by liver secretion [8]. However, under stress, the FGF21 levels rise, and besides liver, other tissue types including white and brown adipose, muscle and pancreas also secrete FGF21 [9]. It has been shown that many different kinds of stress can increase the circulating levels of FGF21, including malnutrition [10], cold exposure [11] and exercise [12]. Generally, FGF21 contributes to stress adaptation and restoration of metabolic balance, by stimulating lipid catabolism, ketogenesis [13], gluconeogenesis and glucose uptake [14]. FGF21 as a fasting-induced hormone in humans, appears to contribute to the late stages of adaptive starvation, when it may regulate the utilization of fuel derived from tissue breakdown [15]. Furthermore, FGF21 was identified as a promoter of endothelial cell angiogenesis, acting via FGFR1 and β-Klotho coreceptor, through a dynamin-2 and Rab5 dependent pathway [16]. This mini review aims to summarize the FGF21 prospects for applications in clinical practice.

2. THE POTENTIALS OF FGF21 AS A BIOMARKER

A number of recent studies render FGF21 promising as a biomarker for several types of human diseases. Elevated serum levels of FGF21 were found to be an independent biomarker of non-alcoholic fatty liver disease (NAFLD). Patients with NAFLD showed significantly higher baseline FGF21 concentration than the control group [17,18]. Another study determined that maternal FGF21 serum levels were significantly higher in preeclampsia patients than in healthy, age-matched pregnant women [19]. FGF21 serum levels were associated with specific cardiovascular diseases. Patients with coronary artery disease CAD not only showed significantly higher serum FGF21 levels over non CAD subjects but also after adjustment of sex, age, and body mass index (BMI), FGF21 was identified as an independent factor of CAD [18]. Also, circulating FGF21 levels were significantly higher in atrial fibrillation (AF) patients than in the control group. After the adjustment of the gender, age and body mass index, circulating FGF21 levels were positively associated with left atrial diameter (LAD). Multivariate logistic regression analysis revealed that both FGF21 and LAD were correlated with AF [20]. Additionally, serum FGF21 levels were correlated with carotid atherosclerosis. Serum FGF21 levels were found positively correlated with carotid intima-media thickness (IMT). Elevated serum FGF21 levels were associated with carotid atherosclerosis in women, but not in men, independent of established risk factors, including C-reactive protein and adverse lipid profiles [21]. Furthermore, the levels of serum FGF21 were independently associated with the presence of Acute Myocardial Infarction (AMI) in Chinese patients. Also high FGF21 levels were found to be possibly related to the incidence of re-infarction within 30 days after onset [22].

FGF21 serum levels in diabetic patients are associated with several lesions resulting from the high glucose levels. Serum FGF21 levels were found independently correlated with the extent and severity of CAD. FGF21 was elevated in type 2 diabetes mellitus patients with CAD [23]. Moreover, circulating FGF21 levels were correlated with lower extremity atherosclerotic disease (LEAD) in Chinese female diabetic patients. Serum FGF21 levels were significantly higher in LEAD group than in non-LEAD group in females, but not in male patients [24]. Furthermore, circulating FGF21 levels were associated with the severity of diabetic retinopathy model. FGF21 levels were found higher in patients with type 2 diabetes than in age-, body-mass index-, and sex-matched controls and even higher in diabetic patients with diabetic retinopathy than those without retinopathy [25]. In addition, serum FGF21 levels predicted progressive kidney disease in patients with type 2 diabetes and normoalbuminuria. The baseline of circulating FGF21 levels were correlated with the estimated glomerular filtration rate category [26]. Furthermore, higher baseline FGF21 levels are seen in patients with type 2 diabetes and established microvascular disease and predict the future development of new microvascular disease. Of 6465 patients without baseline total microvascular disease, 1517 developed new on-study total microvascular disease over 5 years of follow-up. Higher baseline FGF21 levels were associated with a higher risk of new on-study total microvascular disease after adjusting for potential confounding factors [27].

Of course, as a metabolic and stress hormone, FGF21 is associated with metabolic disorders. FGF21 is deemed as an independent predictor of the metabolic syndrome and type 2 diabetes. Caucasian patients with metabolic syndrome had higher FGF21 baseline levels. Logistic regression analysis revealed that FGF21 independently predicted the risk of metabolic syndrome development, after adjustment for age, sex, body mass index, and time [28]. Moreover, circulating FGF21 levels were determined significantly elevated with an increasing number of metabolic disorders. Univariate correlation analysis revealed that in Japanese subjects serum FGF21 levels were significantly correlated with systolic and diastolic blood pressure (BP), pulse pressure, age, BMI, fasting triglyceride levels, plasma glucose levels, and total cholesterol levels. Multiple regression analysis (adjusted for age, BMI, and gender) showed that serum FGF21 levels were independently and significantly associated with triglyceride levels and systolic BP. Serum FGF21 levels were significantly higher in subjects with high systolic BP and triglyceride levels compared with those who had normal systolic BP and triglyceride levels [18,29]. FGF21 serum levels were also associated with aspects of the metabolic syndrome, hepatocellular function and growth hormone (GH) status in human subjects. Multiple regression analyses indicated that circulating FGF21 concentrations remained independently and positively correlated with systolic blood pressure, triglycerides, and gamma-glutamyl transferase [30].

Serum FGF21 levels were also associated with disorders of musculoskeletal system and they were proposed as an indicator of mitochondrial disease. Circulating FGF21 concentrations were determined significantly elevated in patients with mitochondrial disease [31]. This observation, together with the fact that none of the classical biomarkers such as lactate, pyruvate, their ratio, creatine kinase and amino acids are not as sensitive and specific as FGF21 is, render FGF21 levels the best predictor of this disorder [32]. Moreover, circulating FGF21 levels were found associated with worsened radial trabecular bone microarchitecture and decreased radial bone strength in women with anorexia nervosa (AN). There is a statistically significant inverse correlation between log FGF21 and trabecular number in the radius in both AN and normal-weight controls (NWC) and a statistically significant positive correlation between log FGF21 and trabecular separation in the radius in both AN and NWC. Estimates of radial bone strength were inversely correlated with log FGF21 in AN for both stiffness and failure load [33]. Furthermore GDF-15 and FGF-21 are elevated in mitochondrial diseases. It was found that elevated levels of GDF-15 and or FGF-21 correctly identified a larger proportion of patients than elevated levels of GDF-15 or FGF-21 alone [34].

3. THERAPEUTIC POTENTIAL OF FGF21

The beneficial effects of FGF21 administration were originally demonstrated in obese mice in the study of Coskun and coworkers [35]. Diet-induced and *ob/ob* obese mice lost 20% of their body weight, mainly from the adipose tissue upon FGF21 administration. No reduction in food consumption or increase in physical activity was observed, but an upregulation in energy

expenditure was described. Furthermore, a reduction of hepatosteatosis and glycemia was observed. In the study of Huang and coworkers [36], FGF21 also reversed hepatic steatosis and increased insulin sensitivity in diet-induced obese mice. Moreover, exogenous FGF21 ameliorated collagen-induced arthritis by decreasing oxidative stress and also by inactivation of nuclear factor κB pathway [37].

In order to solve the problem of short circulating half-life and improve the pharmacological properties of the native FGF21 molecule, genetic modulations and conjugation with molecules that prolong the stability of proteins in vivo have been utilized. In the study of Xu and coworkers [38] site-directed polyethylene glycol (PEG) conjugation was used not only in order to elongate the short circulating half-life of FGF21, but also to minimize its side effects. In this study, the site of PEGylation was found to be significant for the bioactivity of FGF21, while the configuration and number of PEG molecules conjugated on FGF21 were found to be associated with the intensity of side effects. In the study of Camacho and coworkers [39] low doses of FGF21 PEGylated at selected amino acid residues restored the insulin sensitivity in diet-induced insulin resistant mice. The long-lasting anti-diabetic effects of PEGylated FGF-21 were also confirmed in type 1 and 2 diabetic mice [40,41]. FGF21 selectively conjugated to a scaffold antibody (CovX-Body) showed up to 70-fold increased half-life in mice, compared with wild-type FGF21, while retaining its bioactivity. In ob/ob mice, a single injection of the conjugated FGF21 (CVX-343) improved glucose tolerance for 6 days [31].

LY2405319 is a genetically modified human FGF21, rationally designed for improved biopharmaceutical properties. LY2405319 presented the same biological characteristics with native FGF21 in *in vitro* and *in vivo* assays while its pharmacological properties make it a good candidate for clinical trials [42]. Subcutaneous administration of LY2405319 once daily in diabetic rhesus monkeys, led to improvement of significant metabolic parameters, including body weight, glucose, cholesterol, insulin and triglyceride levels [43]. The daily administration of LY2405319 to human subjects with obesity and type 2 diabetes for 28 days improved dyslipidemia. Low-density lipoprotein cholesterol and triglycerides decreased, whereas high-density lipoprotein cholesterol increased. Furthermore, body weight, fasting insulin, and adiponectin were also ameliorated, but unfortunately the reduction of glucose levels was not satisfactory [44]. PF-05231023 is another long-acting FGF21 construct, consisting of two molecules of modified FGF21 conjugated to an antibody scaffold (CovX-2000). Similar to the LY2405319 molecule, PF-05231023 caused body weight loss and decreased glucose levels in diet-induced and *ob/ob* obese mice [45]. Upon administration in human subjects with type 2 diabetes mellitus, PF-05231023 reduced triglyceride levels, total cholesterol and low-density lipoprotein cholesterol, and raised high-density lipoprotein cholesterol, but like LY2405319, no significant decrease in plasma glucose levels was recorded [46]. Recently, the *in vivo* effects of the long-acting FGF21 molecule PF-05231023 was tested in overweight/obese human subjects with type 2 diabetes and obese cynomolgus monkeys. PF-05231023 did not affect the glycemic control, but improved plasma lipoprotein profile and displayed a significant decrease in body weight [47].

Instead of using native or modified FGF21, for improving the outcome in metabolic diseases, other molecules that mimic its effects have been engineered. Wu and coworkers reported the usage of an agonistic anti-FGFR1 monoclonal antibody, which ameliorate metabolic parameters by binding to the FGF21 receptor. A single administration of this antibody into obese diabetic mice decreased hyperglycemia, hyperlipidemia, and hepatosteatosis [48]. Improved biological effects for an anti-FGFR1c/β-Klotho bispecific protein compared to native FGF21 have also been reported in the study of Smith and coworkers [49]; the bispecific protein was administrated to monkeys and led to ameliorated metabolic parameters and body weight loss than in animals treated with FGF21. Last but not least, FGF21 analogs were reported to have potential beneficial effects on lipids and lipoproteins for familial hypercholesterolemia (FH) patients [50].

4. CONCLUSIONS- FUTURE PERSPECTIVES

Although FGF21 was initially described as a hormone that can ameliorate some metabolic parameters in obesity and type 2 diabetes, it is evident that its role and function is more extensive. High levels of FGF21 have been associated with several pathologies, indicating that FGF21 could be a good candidate for use in clinical diagnosis. However, further and larger clinical studies need to be performed so as to

establish FGF21 as a biomarker useful for clinical diagnosis. Furthermore the continuous efforts to improve the pharmacological properties of FGF21 and the results from the first clinical trials in human subjects are an auspicious start for the development of a FGF21 form with clinical applications, at least in patients with obesity and metabolic syndrome. Thus, FGF21 could be useful in clinical practice.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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