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REVIEW

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Hepatic hormone FGF21 and its analogues in clinical trials

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Abstract

Fibroblast growth factor 21 (FGF21) is a fasting or stress inducible metabolic hormone produced mainly in the liver. It plays important roles in regulating both glucose and lipid homeostasis via interacting with a heterodimeric receptor complex comprising FGF receptor 1 (FGFR1) and β-klotho (KLB). For the past decade, great effort has been made on developing FGF21 derivatives or specific FGF21 receptor agonists into therapeutic agents for various metabolic disorders including type 2 diabetes (T2D), obesity, and more importantly, nonalcoholic fatty liver disease (NAFLD). Here we have reviewed FGF21 gene and protein structures, its expression pattern, cellular signaling cascades that mediate FGF21 production and function. We have then summarized the six clinical trials utilizing four FGF21 analogues. Finally, two recent literatures on the development of GLP-1 and FGF21 dual agonists were presented briefly.

KEYWORDS

dual agonists, fibroblast growth factor 21, lipid metabolism, metabolic diseases

1 | BRIEF SUMMARY ON FGF21 STRUCTURE AND SIGNALING PATHWAYS THAT MEDIATES ITS FUNCTIONS

The fibroblast growth factor (FGF) family is comprised of secreted proteins that are encoded by 22 genes in both humans and rodent species.[1] Among the FGF family members, FGF21, FGF19 (the murine orthologue is FGF15) and FGF23, form a special subfamily for possessing low heparin-/heparan sulfate-binding ability.[2] Thus, they can be released into the circulation, serving as endocrine hormones,[2–4] while other members exert their functions mainly in paracrine, intracrine, or autocrine manners.[1]

Human and mouse *FGF21* cDNAs were initially cloned in 2000 by Nishimura and colleagues, and were found to be predominantly expressed in the liver, in contrast to other members of the FGF family.[5] The murine *Fgf21* gene is located on chromosome 7 while the human *FGF21* gene is located on chromosome 19. The pre-protein of FGF21 consists of 210 amino acid residues for mice and 209 amino acid residues for humans (Figure 1), sharing 79% amino acid sequence identity. The pre-protein in both humans and mice contains a 30 amino acid hydrophobic domain. This domain has the characteristics of a signal sequence, allowing FGF21 secretion.[5,6] Thus, mature human FGF21 hormone contains 179 amino acid residues, while mature mouse FGF21 hormone contains 180 amino acid residues.

Although mouse *Fgf21* mRNA can be detected in organs including pancreas, thymus, testes, gastrointestinal tract (GI), brain, skeletal muscle, as well as brown and white adipose tissues (BAT and WAT), circulating FGF21 is considered as liver derived.[7] In humans, FGF21 is expressed in fewer tissues or organs, including the liver, skeletal muscles, and brain; but not in adipose tissues.[8,9]

FGF21 binds to and activates its receptors, known as FGF receptors (FGFRs); a family of receptor tyrosine kinases (RTKs). There are seven primary FGFR isoforms identified in mammals, known as 1b, 1c, 2b, 2c, 3b, 3c, and 4.[10]

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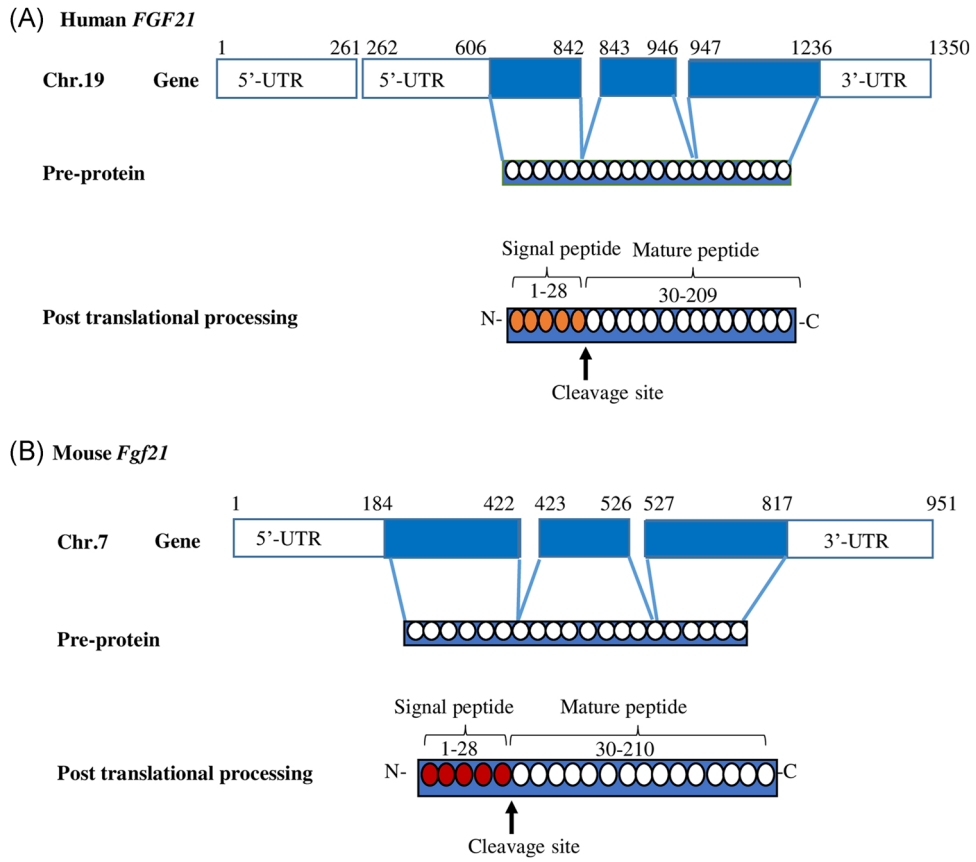

FIGURE 1 Gene and protein structures of human and mouse FGF21. Human *FGF21* gene is located on chromosome 19 (A), while mouse *Fgf21* gene is located on chromosome 7 (B). Both mouse and human FGF21 pre-hormones possess a signal peptide with 30 amino acid residues. Native mature human FGF21 contains 179 amino acid residues while mature mouse FGF21 contains 180 amino acid residues. Human FGF21 shares 79% amino acid sequence identify with mouse FGF21. National Center for Biotechnology Information (NCBI) designations: NC_000019.10 for human *FGF21* and NC_000073.6 for mouse *Fgf21*

Importantly, FGFR activation by FGF21, FGF19 and FGF23 is dependent on the transmembrane protein β-klothos (KLB), which is known to be expressed in the liver, adipose tissues, pancreas, gut, hypothalamus and gallbladder.[11] Based on tissue distribution of FGFRs and KLB, as well as cell-based receptor activation assays and *in vivo* genetic models,[12,13] it is generally accepted that functions of FGF21 is mainly mediated by FGFR1c/KLB and FGFR3c/KLB.[12–14] Via interacting with FGFR1c/KLB or FGFR3c/KLB, FGF21 up-regulates fatty acid β-oxidation, ketogenesis, and gluconeogenesis in the liver. It also stimulates insulin synthesis in pancreatic islets and white adipose tissue (WAT) browning, as well as glucose uptake. Together, these effects lead to the attenuation of obesity, dyslipidemia and insulin intolerance. *In vitro* FGF21 treatment in mouse primary hepatocytes leads to the activation of extracellular signal-regulated kinases (ERKs) and the activation of *c-fos* and early growth response protein 1 *(Egr-1)* gene expression. [15]

Plasma FGF21 hormone, hepatic *Fgf21* mRNA and FGF21 protein levels can be increased by high-fat and low-carbohydrate ketogenic diet (KD) consumption, fasting, fructose or alcohol consumption.[4,16] The nuclear receptor peroxisome proliferator-activated receptor α (PPARα) has been recognized as a major transcriptional activator of *FGF21/Fgf21*.[16–20] PPARα knockout mice were shown to have 50% reduction in plasma FGF21 hormone level.[16] Indeed, PPARα is usually activated during energy deprivation. PPARα agonists, such as fibrates, can be utilized in treating hyperlipidemia. PPARα knockout mice, however, also displayed hepatic *Fgf21* mRNA and plasma FGF21 hormone level elevation in response to ketogenic diet challenge,[16] indicating the existence of PPARα-independent mechanisms, that are implicated in regulating *Fgf21* expression and FGF21 hormone production. Other documented stimuli of *Fgf21* gene transcription include endoplasmic reticulum (ER) stress and the hepatic lipogenic transcription factor, carbohydrate response element binding protein (ChREBP)[4]. We and others have shown that *Fgf21* expression and FGF21 sensitivity can be regulated by dietary polyphenols including resveratrol, curcumin, and anthocyanin.[15,21,22] We found that in mice on low fat diet feeding, curcumin administration stimulates FGF21 production, while in obese mouse model induced by high fat diet (HFD) feeding, curcumin intervention attenuates HFD induced FGF21 overexpression and improves FGF21 sensitivity. [15]

During the past a few years, four researcher teams have independently reported that hepatic *Fgf21* expression in various rodent models can be stimulated by exenatide or liraglutide treatment.[23–27] Exenatide and liraglutide are type 2 diabetes (T2D) drugs, known as glucagon-like peptide 1 receptor (GLP-1R) agonists or GLP-1 analogues.[28] We have demonstrated very recently by RNA-seq and other tools that GLP-1R is not expressed in mouse liver. Although *in vivo* liraglutide treatment increased hepatic *Fgf21* expression, the stimulation was not observed in mouse primary hepatocytes with direct liraglutide treatment. Furthermore, the stimulation was not observed in GLP-1R knockout mice. Liver specific FGF21 knockout mice on high fat high fructose diet challenge show comparable metabolic impairment with that in wild type mice.[29] However, body weight lowering and lipid profile homeostatic effects of liraglutide were severely impaired in liver specific FGF21 knockout mice.[29] Thus, liraglutide may stimulate hepatic *Fgf21* expression via GLP-1R expressed in extra-pancreatic organs, such as the brain; and FGF21 is required for liraglutide to exert its certain therapeutic functions.[29] For additional information on mechanisms underlying hepatic FGF21 expression, please see articles or studies elsewhere.[4,17,30]

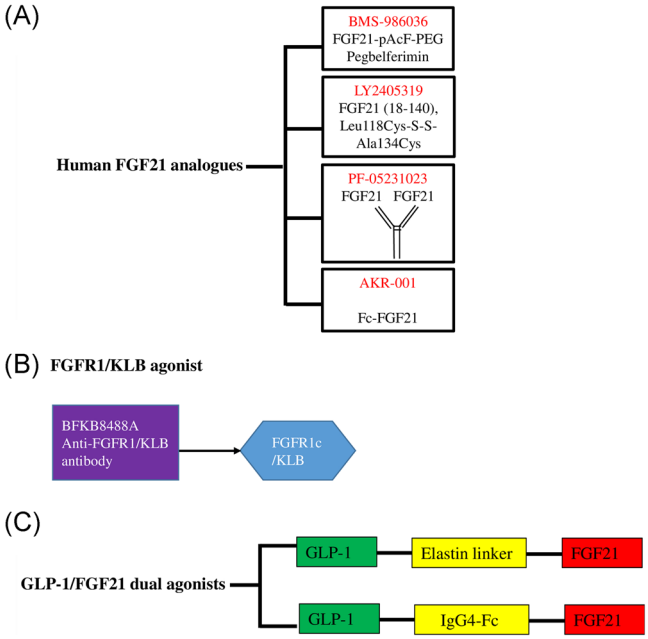

FIGURE 2 List of three categories of potential FGF21 based therapeutic agents. FGF21 analogues (A). FGFR1/KLB agonist. (C) FGF21 and GLP-1 dual agonists (B)

2 | RECENT FGF21-BASED CLINICAL TRIALS

To explore patho-physiological functions of FGF21, two transgenic mouse models have been generated in which FGF21 is over-expressed in the liver, utilizing the liver specific apolipoprotein E (*ApoE*) promoter or the *pLiv7* promoter.[17,31] These mice showed reduced body weight, associated with improved glucose and lipid homeostasis.[17,31] FGF21 knockout mice were then generated.[32] FGF21 knockout mice showed no difference, when compared with age and sex matched wild type littermates, on body weight and plasma insulin levels.[33] These observations indicate the existence of yet to be identified compensatory mechanisms on body mass homeostasis in FGF21 knockout mice. Nevertheless, FGF21 knockout mice showed reduced hepatic fatty acid activation and β-oxidation, associated with reduced expression of genes that are involved in gluconeogenesis and lipolysis. Importantly, these defects can be reversed by exogenous FGF21 administration.[33] Exogenous FGF21 administration on improving energy homeostasis were also observed in animal models including that in non-human primates.[31,34–37] These promising outcomes in intensive pre-clinical investigations then triggered further investigations in clinical trials.

To date, six randomized clinical trials have been conducted utilizing four human FGF21 analogues in testing their therapeutic potential in T2D or obesity, with or without another defined metabolic disorder such as fatty liver disease.[37–42] Native FGF21 possesses a short half-life of 30 min to 2 hours. For conducting these clinical trials, four different human FGF21 analogues have been created for increasing their stability by various means (Figure 2A). Among them, BMS-986036 (also known as pegbelfermin) is a PEGylated human FGF21 analogue.[40,41,43] LY2405319 is an engineered human FGF21 variant produced in the fungus *Pichia pastoris* with improved formulation stability and protein expression.[38,44,45] PF-05231023 is a long-acting FGF21 analogue, which contains two modified human FGF21 molecules that are linked to a humanized immunoglobulin 1 antibody backbone.[37,39,46,47] Finally, AKR-001 [formally known as Fc-FGF21 (RGE), AMG 876] is an Fc-FGF21 fusion protein, with a half-life of 3.0–3.5 days.[42,48]

Table 1 provides a summary of these human FGF21 analogues and their utilization in clinical trials. Briefly, between 2013 and 2020, six clinical trials were conducted in subjects with obese and T2D (by Gaich *et al* in 2013 with LY2405319, by Talukdar *et al* in 2016 with PF-05231023, and by Sanyal *et al* in 2018 with BMS-986036), or in subjects with obese only (by Kim *et al* in 2017 with PF-05231023), or in subjects with obese and fatty liver disease (by Charles *et al* in 2019 with BMS-986036), or in subjects with T2D (by Kaufman et al with AKR-001). Overall, these six clinical trials cannot provide a clear conclusion on reducing body weight in obese subjects they have been tested yet, although such effect could be observed in rodent model studies,[43] and body weight reduction was observed in one trial with PF-05231023.[37]

TABLE 1 A brief summary on FGF21 clinical trials

Name	Chemical features	Clinical trial by	Year	Main beneficial effects observed
LY2405319	Modified human FGF21 expressed in yeast	Gaich et al	2013	Reduced plasma lipid and lipoproteins, increased HDL-C, and reduced fasting insulin level.
PF-05231023	Two FGF21 joint with an IgG backbone	Talukda et al	2016	Increased HDL-C, reduced total cholesterol, LDL-C, and fasting TG, fasting glucose and insulin.
PF-05231023	Two FGF21 joint with an IgG backbone	Kim et al	2017	Increased HDL-C, adiponectin, and whole-body insulin sensitivity, reduced LDL-C, fasting glucose and insulin level.
BMS-986036	Peglylated human FGF21	Sanyal et al	2018	Increased HDL-C and adiponectin; reduced LDL-C, fasting TG and hepatic fat fraction.
BMS-986036	Peglylated human FGF21	Charles et al	2019	Increased HDL-C, adiponectin, and whole body insulin sensitivity, reduced LDL-C, fasting TG, and fasting glucose and insulin levels.
AKR-001	Fc-FGF21 engineered fusion protein	Kaufman et al	2020	Increased HDL-C and adiponectin, decreased TG, and improved glycemic control and markers of insulin sensitivity under both fasting and fed conditions.

Importantly, the effects of these four human FGF21 analogues on improving lipid homeostasis, including the reduction on low-density lipoprotein cholesterol (LDL-C), fasting plasma triglyceride (TG), the increase on high-density lipoprotein cholesterol (HDL-C) and adiponectin, are consistent for all the five clinical trials. The four human FGF21 analogues were also shown to attenuate hyperinsulinemia or improve insulin sensitivity in five clinical trials (Table 1). For the BMS-986036 trial conducted by Sanyal *et al*, assessment on fasting insulin level was not made.

As FGF21 exerts its metabolic functions via FGFR1/KLB, another approach is to generate specific FGFR1/KLB agonist. Very recently, Baruch and colleagues[49] reported their intensive observations on antibody-mediated activation of the FGFR1/KLB complex in rodents, nonhuman primates and in human subjects. The humanized bispecific antibody utilized in this study is known as BFKB8488A (Figure 2B), which was shown to enhance the dimerization of FGFR1c only when KLB is present on the cell surface by a previous investigation.[50] Baruch *et al*[49] observed that BFKB8488A can induce weight loss in obese cynomolgus monkeys. The treatment in monkeys also increased serum adiponectin levels and FGFR1 target gene expression in their adipose tissues. In obese human subjects, a single dose BFKB8488A injection resulted in a transient body weight reduction and sustained improvement in cardiometabolic parameters. The treatment also led to a trend towards reduction in preference for sweet taste and carbohydrate intake. The authors have suggested that specific FGFR1/KLB complex activation with a bispecific antibody is a potential therapy for obesity-related metabolic disorders.[49]

It is worth mentioning that NGM282 (Aldafermin), an engineered FGF19 analogue, has also been utilized in clinical trials for patients with Non-alcoholic steatohepatitis (NASH), with promising outcomes including the reduction in absolute liver fat content.[51–54] In a phase 2 trial with NASH patients, Aldafermin was shown to reduce liver fat and generated a trend towards the improvement of hepatic fibrosis.[52]

3 | DEVELOPMENT OF GLP-1 AND FGF21 DUAL AGONISTS

Peptide-based multi-agonists is a new paradigm in the field of metabolic pharmacology.[55] Effort has been made in the development of a monomeric peptide, targeting GLP-1R, receptors for glucose-dependent insulinotropic polypeptide (GIP) and glucagon.[56] It has been postulated that the GLP-1/GIP/glucagon triagonist may exert its metabolic beneficial function partially via increasing hepatic FGF21 production.[57] Two very recent investigations described the work in the development of the GLP-1 and FGF21 dual agonist.[57,58] Figure 2C shows the overall structure of these two dual agonists.

Gilroy et al[57] took the approach in fusing GLP-1 to FGF21 with an elastin-linker polypeptide (ELP). In such a fusion protein, the ELP linker serves as a sustained release module. Specifically, modified GLP-1 (with enhanced stability) is located at the N terminus while modified FGF21 (with enhanced stability) is located at the C terminus, while the intervening ELP is in the middle. GLP-1-ELP-FGF21 fusion protein was expressed in *Escherichia coli*. Followed by the fusion protein purification, it was tested in the *db/db* diabetic mouse model. Gilroy *et al*[57] reported that once-weekly treatment with GLP-1-ELP-FGF21 fusion protein resulted in much potent body weight lowering effect and enhanced glycemic control, which cannot be reached with the utilization of either one of the agonist alone. They have also claimed that the dual-agonist has superior efficacy when compared to a GLP-1/FGF21 mixture, indicating the advantage for combining structurally distinct peptides into one multi-functional molecule.

Pan *et al*[58] took a different approach. They employed the phage display high-throughput screening approach in identifying FGF21 mutations that showed improved KLB binding property. They then utilized IgG4 Fc to fuse the identified FGF21 variants to extend their half-life in the circulation. Furthermore, they explored the potential synergistic effect of FGF21 with GLP-1 by generating the dual agonist. They reported that one of the dual agonists, namely GLP-1-Fc-FGF21 D1, showed stronger glucose lowering effect in a diabetes mouse model. This dual agonist also showed better anti-NASH effect, when compared with the use of either FGF21 or GLP-1 alone.

4 | SUMMARY

Following the discovery of FGF21 in 2000, our understanding on the biology and pathophysiology of this liver derived peptide hormone have been advanced rapidly. Native mature human FGF21 hormone contains 179 amino acid residues, much bigger than GLP-1 (30 or 31 amino acid residues), GIP (42 amino acid residues), glucagon (29 amino acid residues) and glucagon-like peptide 2 (GLP-2, 33 amino acid residues), making the journey of developing it into a therapeutic agent much longer than those gut or pancreatic hormones. In addition, as recently commented by Geng and colleagues,[59] the existence of endogenous FGF21 inactivation enzymes and obesity-mediated FGF21 resistance represent the major obstacles to the clinical implementation of FGF21-based pharmacotherapies.

The six clinical trials conducted to date provide us not only the hope but also further challenges. Consistent and profound effects of the four FGF21 analogues in the six clinical trials in obese subjects include insulin signaling sensitization and lipid profile improvement. We, however, are disappointed at current stage on the lack of clear conclusion on the body weight lowering effect and the potential systematic side effect, especially those on bone loss and the cardiac system. Whether the development of bispecific antibodies against the FGFR1/KLB complex will lead to a novel FGF21 based therapy is yet to be further determined. As FGFR1 and KLB are also involved in other physiological events or activities, whether their activation leads to potential side effect needs to be carefully assessed in both preclinical and clinical investigations. Further investigations in combining multiple disciplinary efforts may lead to the generation of better FGF21 analogues for both hyperlipidemia and obesity treatment. Alternatively, better clinical outcomes may be achieved with improved formulation and administration duration with the current human FGF21 analogues.

As mentioned above, four research laboratories have reported independently that GLP-1 based diabetes drugs, or GLP-1R agonists, can stimulate hepatic FGF21 production in various rodent models.[23–27] We reported very recently that in mice, certain functions of GLP-1R agonists, including body weight lowering and lipid profile improvement, are mediated by hepatic FGF21.[29] Physiologically, GLP-1 is the gut hormone released postprandially while FGF21 is considered as a "fasting" hormone with profound release during starvation. These two hormones, however, do possess both overlapping and unique metabolic homeostatic beneficial functions. The development of GLP-1 and FGF21 dual agonist represents a novel strategy in making FGF21 into therapeutic agents for metabolic disorders including T2D, obesity and fatty liver diseases. Future clinical trials may also answer the question whether GLP-1/FGF21 double agonists bring better treatment for atherosclerosis and diabetic nephropathy, and the concerns whether the double agonists generate additional side effect.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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