Review Article

FGF21 as an Endocrine Regulator in Lipid Metabolism: From Molecular Evolution to Physiology and Pathophysiology

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The FGF family comprises twenty-two structurally related proteins with functions in development and metabolism. The Fgf21 gene was generated early in vertebrate evolution. FGF21 acts as an endocrine regulator in lipid metabolism. Hepatic Fgf21 expression is markedly induced in mice by fasting or a ketogenic diet. Experiments with Fgf21 transgenic mice and cultured cells indicate that FGF21 exerts pharmacological effects on glucose and lipid metabolism in hepatocytes and adipocytes via cell surface FGF receptors. However, experiments with Fgf21 knockout mice indicate that FGF21 inhibits lipolysis in adipocytes during fasting and attenuates torpor induced by a ketogenic diet but maybe not a physiological regulator for these hepatic functions. These findings suggest the pharmacological effects to be distinct from the physiological roles. Serum FGF21 levels are increased in patients with metabolic diseases having insulin resistance, indicating that FGF21 is a metabolic regulator and a biomarker for these diseases.

1. Background

Prototypes of fibroblast growth factors (FGFs), FGF1 and FGF2, were originally isolated as mitogens for cultured fibroblasts from the brain and pituitary [1, 2]. The human/mouse Fgf gene family comprises twenty-two members, including Fgf1–Fgf23, all of which are evolutionarily related. However, as mouse Fgf15 and human Fgf19 are orthologous, we refer to these genes as Fgf15/19 in this paper. Human/mouse FGFs are proteins of ~150–300 amino acids with 13–71% identity. All FGFs with a ~120 amino acid conserved core region (~30–60% identity) are signaling molecules with diverse functions in development and metabolism. Fgf genes are widely expressed in developing and adult tissues [3–6].

FGFs can be classified into three types, paracrine, intracrine, and endocrine FGFs, by their mechanisms of action [6]. Paracrine FGFs (FGF1~FGF10, FGF16~FGF18, FGF20, and FGF22) function as secreted local paracrine signaling molecules in multiple developmental processes, including differentiation, cell proliferation, and migration. They mediate biological responses by binding to cell surface tyrosine kinase FGF receptors (FGFRs) [4, 6, 7]. Intracrine FGFs (FGF11~FGF14) function as nonsecreted signaling molecules. They mainly play roles in neuronal functions at postnatal stages in an FGFR-independent manner [8–10]. Endocrine FGFs (FGF15/19, FGF21, and FGF23) mediate biological responses as secreted proteins in an FGFR-dependent manner. Endocrine FGFs function over long distances in an endocrine manner and mainly play roles in metabolism at postnatal stages [11–13].

Paracrine and intracrine FGFs have been identified in both invertebrates and vertebrates. However, endocrine FGFs have only been identified in vertebrates [6]. Endocrine FGFs are emerging in evolution. FGF15/19 and FGF23 play roles as metabolic regulators in bile acid metabolism and phosphate and active vitamin D metabolism, respectively [11, 13–15]. FGF21 exerts diverse pharmacological effects on glucose and lipid metabolism, ketogenesis, and growth hormone signaling in hepatocytes in mice. However, FGF21 may not be a physiological regulator for these hepatic functions. FGF21 physiologically regulates lipid metabolism in adipocytes and torpor. Serum FGF21 levels are significantly increased in patients with metabolic diseases having insulin resistance. These results indicate the physiological and pathophysiological roles of FGF21. Several excellent review articles on FGF21,
focusing on its pharmacological effects on metabolism and therapeutic uses for metabolic diseases have been published [16–20]. This paper concentrates on the molecular evolution and physiological and pathophysiological roles of FGF21.

2. Identification of Fgf21

The Fgf21 gene was originally identified in mice by the polymerase chain reaction with the amino acid sequence of human FGFI5/19. Human Fgf21 was also identified by homology-based searching in the human DNA database [21]. Later, human FGFI5/19 was found to act as a stimulator of glucose uptake in mouse 3T3-L1 adipocytes in an assay used to search for novel proteins with therapeutic potential to treat diabetes [22]. Mouse Fgf21 was also identified as a hepatic gene inducible by fasting or a ketogenic diet [23, 24].

Human FGFI5/19 is a secreted protein of 209 amino acids with a 29-amino-acid amino-terminal secreted signal sequence and a ~120-amino-acid conserved core region. Human FGFI5/19 is highly homologous to mouse FGFI5/19 (~75% identity). However, its low homology with other human FGFs (less than 35% identity) indicates that FGFI5/19 is structurally unique [21]. Paracrine FGFs have a heparin-binding site, which is necessary for stable interaction with FGFRs and heparin-like molecules [25]. However, FGFI5/19 also as well as FGFI5/19 and FGFI5/23 lack a typical heparin-binding site [25, 26].

3. Molecular Evolution of Fgf21

The FGF signaling system has been conserved throughout metazoan evolution. Potential evolutionary relationships in the Fgf family have been proposed based on results of gene location and phylogenetic analyses. These analyses have identified seven subfamilies: Fgf1/2/5, Fgf3/4/6, Fgf7/10/22, Fgf8/17/18, Fgf9/16/20, Fgf11/12/13/14, and Fgf15/19/21/23 [4, 6]. Ascidians belong to the subphylum Urochordata, the earliest branch in the phylum Chordata. Ancestral genes of paracrine and intracrine Fgfs have been identified in the ascidian, Ciona intestinalis [27]. However, no ancestral gene of endocrine Fgfs has been identified in Ciona intestinalis. The sea lampreys, Petromyzon marinus, are cyclostomes, the most basal extant group of vertebrates [28]. An ancestral gene of endocrine Fgfs has been identified in the lamprey genome, and tentatively named Fgf15/19-like (Itoh et al., unpublished observation) (Figure 1(a)). Lamprey Fgf15/19-like also lacks a typical heparin-binding site. These results suggest that lamprey Fgf15/19-like is an ancestral endocrine Fgf, which was generated from the ancestral paracrine Fgf gene, Fgf4-like, by local gene duplication early in vertebrate evolution (Figure 1(b)). Later, Fgf19, Fgf21, and Fgf23 were generated from the ancestral endocrine Fgf gene by two genome duplication events early in vertebrate evolution [4, 6]. The evolutionary history suggests that endocrine FGFs are vertebrate specific. As described above, paracrine FGFs have a heparin-binding site. The site is necessary for the stable binding of FGFRs/heparin-like molecules and local signaling in a paracrine manner. Endocrine FGPs potentially acquired systemic signaling in an endocrine manner by reducing heparin-binding affinity early in vertebrate evolution [6, 13, 25].

Endocrine Fgf genes have been identified in all vertebrates examined, including teleosts, amphibians, reptiles, birds, and mammals. Fgf21 has also been identified in most vertebrates. However, Fgf21 has not been identified in the chicken and zebra finch genomes (Ensemble Genome Browser; Itoh et al., unpublished observation) (Figure 1(a)). Genome sizes and gene numbers are smaller in birds than in mammalian species. Although the evolutionary implications of these changes remain to be understood, the reduced genome sizes and gene numbers may have evolved in response to the physiological demands of flight [29]. Fgf21 might therefore have been lost in the bird lineage.

4. Roles of FGF21 in Glucose Metabolism

FGF21 is expressed abundantly in the liver, and also in the pancreas, white adipose tissue, muscle, and testis [17, 21, 30]. Potential roles of FGF21 in glucose metabolism were first shown by experiments with cultured cells [22]. FGF21 stimulated glucose uptake in cultured mouse and human adipocytes. Functional interplay between the FGF21 and peroxisome proliferation-activated receptor γ (PPARγ) pathways led to a marked stimulation of glucose transport, suggesting a novel synergy between FGF21 and PPARγ homeostasis [31]. In addition, Fgf21 transgenic mice were resistant to diet-induced obesity. Serum glucose levels were also reduced to near normal levels in both ob/ob and db/db mice by the administration of FGF21. These findings indicate that FGF21 plays a role in glucose metabolism and has potential therapeutic effects on metabolic diseases [16–19].

To elucidate the physiological roles of FGF21, Fgf21 knockout mice have been generated. These mice had normal food intake and energy expenditure levels, serum glucose and insulin levels, and hepatic glycogen levels, indicating that FGF21 is not to be a physiological regulator for glucose metabolism [32].

5. Roles of FGF21 in Lipolysis in Adipocytes

Mammals have evolved complex metabolic responses to fasting. During fasting, nonesterified fatty acid (NEFA) is released from adipocytes into the blood and taken up by hepatocytes. Peroxisome proliferator-activated receptor α (PPARα) is a nuclear receptor. Hepatic Fgf21 expression was greatly induced by fasting for 24 h in wild-type mice but not PPARα knockout mice. It was also markedly induced by a PPARα-selective agonist [23]. These results indicate that hepatic Fgf21 expression is induced by the activation of PPARα. NEFA binds to and activates PPARα. The ligand-bound PPARα forms a heterodimer with RXRs and induces the expression of Fgf21 [33]. Fasting increases the amount of NEFA released from adipocytes. Hepatic Fgf21 expression during fasting is probably induced through the activation of PPARα by NEFA (Figure 2(a)) [34, 35]. Fgf21 knockout mice fasted for 24 h showed increased lipolysis in adipocytes,
which resulted in decreased adipocyte size and increased serum NEFA levels [32]. These results indicate that FGF21 inhibits lipolysis in adipocytes during fasting. The regulatory process forms a negative feedback loop in the control of lipolysis by FGF21 (Figure 2(a)). FGF21 also regulates mitochondrial activity and enhances oxidative capacity through an AMP-activated protein kinase-AMPK- sirtuin 1-SIRT1- peroxisome proliferator-activated receptor-γ coactivator-1α-PGC-1α- dependent mechanism in adipocytes [36].

6. Roles of FGF21 in Ketogenesis and Triglyceride Clearance in Hepatocytes

In hepatocytes, NEFA is converted to acetyl-CoA by oxidation, and ketone bodies are produced from acetyl-CoA. Ketone bodies become the predominant energy source for the brain during fasting. Hepatic ketogenesis during fasting was greatly impaired in PPARα knockout mice, indicating that PPARα is crucial to the normal adaptive response to fasting [37, 38]. As described above, hepatic Fgf21 expression is induced in response to fasting and PPARα agonists. In addition, the phenotypes of Fgf21 transgenic mice demonstrate that FGF21 stimulates hepatic ketogenesis, indicating that FGF21 plays a role in hepatic ketogenesis [23]. Feeding with a ketogenic diet (KD) mimics the metabolic conditions of chronic starvation. Adenoviral knockdown of hepatic Fgf21 in mice fed KD caused reduced blood ketone levels, fatty liver, and lipemia, suggesting that FGF21 is required for hepatic ketogenesis and triglyceride clearance in mice fed KD [24]. In addition, serum triglyceride levels were reduced to near normal levels in both ob/ob and db/db mice by the administration of FGF21 [22]. These findings also indicate functions of FGF21 in ketogenesis and triglyceride metabolism and potential therapeutic effects on metabolic diseases [16–19]. However, hepatic ketogenesis and triglyceride levels were essentially normal in Fgf21 knockout mice fasted or fed KD, indicating FGF21 not to be a physiological regulator for hepatic ketogenesis and triglyceride clearance in mice [32]. These results suggest the physiological roles of FGF21 to be distinct from the pharmacological effects of FGF21 indicated by experiments with Fgf21 transgenic mice. In humans, serum FGF21 levels are also increased by fasting for 7 days or PPARα activation. In contrast, ketogenesis is independent of serum FGF21 levels [39, 40]. However, it also has been reported that Fgf21 knockout mice fed KD developed hepatosteatosis and showed partial impairment in ketogenesis [41].

Peroxisome proliferation-activated receptor γ coactivator-1α-PGC-1α- regulates metabolism in response to changes in nutritional status. PGC-1α negatively regulated hepatic Fgf21 expression [42]. In contrast, FGF21 induced hepatic Pgc-1α expression. Fgf21 knockout mice did not fully express Pgc-1α in response to prolonged fasting and exhibited
impaired gluconeogenesis and ketogenesis. In addition, FGF21 could not induce gluconeogenic gene expression in Fgf1a knockout mice [43]. These results indicate that gluconeogenesis and ketogenesis by FGF21 are mediated in part through PGC-1α. However, as described above, other experiments with Fgf21 knockout mice suggest that FGF21 may not be required for gluconeogenesis and ketogenesis [32].

7. Roles of FGF21 in Growth Hormone Signaling in Hepatocytes

Starvation inhibits growth by blocking the growth hormone (GH)/insulin-like growth factor 1 (IGF1) signaling pathway [44]. Fgf21 transgenic mice are 40–50% smaller than their wild-type counterparts. Tibia length is also significantly reduced in Fgf21 transgenic mice. FGF21 causes resistance to GH in the liver [45]. Actions of GH are mostly mediated by IGF1. IGF1 signaling is induced by the GH/STAT5 (signal transducer and activator of transcription) signaling pathway. The phosphorylation of STAT5 and the expression of IGF1 are significantly decreased in livers of Fgf21 transgenic mice. IGF-binding protein 1 (IGFBP1), which is involved in sequestering IGF1, inhibits IGF1 signaling. A suppressor of cytokine signaling 2 (SOCS2) also inhibits GH signaling by binding to the tyrosine-phosphorylated GH receptor. The expression of Igfbp1 and Socs2 was greatly enhanced in Fgf21 transgenic livers. These results indicate the important role of FGF21 in the inhibition of GH/IGF1 signaling [45]. However, Fgf21 knockout mice are apparently healthy, and their body and tibia lengths are essentially normal [32]. In addition, hepatic Igf1, Igfbp1, and Socs2 expression was essentially normal in Fgf21 knockout mice (Murata et al., unpublished observation). The expression of Igf1 was slightly decreased by fasting for 24 h in wild-type mice. In contrast, the expression of Igfbp1 and Socs2 was greatly and slightly increased in fasted wild-type mice, respectively. In addition, the expression of Igf1, Igfbp1, and Socs2 in fasted Fgf21 knockout mice was similar to that in fasted wild-type mice (Murata et al., unpublished observation). The Fgf21 knockout phenotypes indicate that FGF21 is not a physiological regulator essential for GH/IGF1 signaling.

8. Roles of FGF21 in Torpor

Torpor, the controlled lowering of metabolic rates, body temperature, and physical activity, is an adaptation that various mammals use to cope with periods of low food availability [46]. The basal core body temperature of Fgf21 transgenic mice is consistently lower than that of wild-type mice. Moreover, Fgf21 transgenic mice enter torpor on fasting for 24 h, whereas wild-type mice do not [23]. In addition, a PPAR pan-agonist reduced body temperature late at night in concert with the induction of hepatic FGF21 expression [47]. However, body temperature and physical activity were essentially normal in Fgf21 knockout mice fasted for 24 h, indicating that FGF21 is not physiologically required for torpor induced by fasting for 24 h [48]. Hepatic Fgf21 expression and torpor were also induced in mice fed KD for 5 days (Figure 2(b)). However, torpor was attenuated in Fgf21 knockout mice fed KD for 5 days. These results indicate that FGF21 is potentially involved in the torpor induced by KD [48] (Figure 2(b)).

9. Mechanism of FGF21 Action

FGF signaling is mostly mediated by the activation of FGFRs. Four Fgfr genes, Fgfr1–Fgfr4, have been identified in humans and mice [3, 7, 49]. These genes encode proteins (~800 amino acids) that contain an extracellular ligand-binding domain with three immunoglobulin-like domains (I, II, and III), a transmembrane domain, and intracellular tyrosine kinase domains. Fgfr1–Fgfr3 encode two major variants of immunoglobulin-like domain III (IIIb and IIIc) generated by alternative splicing. The immunoglobulin-like domain III is an essential determinant of ligand-binding specificity [26]. Thus, seven FGFR proteins (FGFR 1b, 1c, 2b, 2c, 3b, 3c, and 4) with differing ligand-binding specificity are generated from Fgfr1–Fgfr4. The binding of FGFs to FGFRs induces receptor dimerization and transphosphorylation and the
activation of downstream signaling pathways: RAS-RAF-MAPK, PI3K-AKT, STAT, and PLCγ [7, 49].

Endocrine FGFs also mediate biological responses in an FGFR-dependent manner. However, they activate FGFRs with very low activity even in the presence of heparin/heparan sulfate, as they bind to heparin/heparan sulfate with very low affinity [25, 26]. Aklotho is a transmembrane protein of ~1,000 amino acids with a short cytoplasmic domain [50]. βKlotho shares structural identity (41% amino acid identity) and characteristics with αKlotho [51]. Fgf23 and αKlotho knockout mouse phenotypes indicate that FGF23 and αKlotho function in a common signal transduction pathway. FGF23 can bind to the αKlotho-FGFR1c complex in cultured cells [52], suggesting that αKlotho is a cofactor essential for FGF23 signaling. βKlotho, Fgf15/19, and Fgfr4 knockout mouse phenotypes also indicate that FGF15/19, Fgfr4, and βKlotho function in a common signal transduction pathway [53–55]. FGF15/19 can bind to the βKlotho-FGFR4 complex in cultured cells. FGF15/19 also activates FGF signaling in hepatocytes that primarily express Fgfr4 [56].

In the presence of βKlotho, FGF21 can bind to and activate FGFR1c, FGFR2c, FGFR3c, or FGFR4, which activates the MAP kinase pathway, in cultured cells, indicating that βKlotho is also essential for FGF21 signaling in cultured cells [57–59]. However, Fgf21 knockout mouse phenotypes [32] are distinct from βKlotho knockout mouse phenotypes [51]. In addition, the administration of a recombinant human FGF21 to βKlotho knockout mice showed that FGF21 signaling is transduced in the absence of βKlotho [60]. These results indicate the existence of a βKlotho-independent FGF21 signaling pathway in which undefined cofactors might be involved [60].

10. FGF21 Signaling in Metabolic Diseases

Nonalcoholic fatty liver disease (NAFLD) is a hepatic manifestation of metabolic syndrome and ranges from simple fatty liver to nonalcoholic steatohepatitis. Its prevalence has increased dramatically over recent years in developed countries [61]. The pathophysiological hallmark of NAFLD is insulin resistance. NAFLD may increase the risk of type 2 diabetes and atherosclerosis [62]. Serum FGF21 levels are significantly increased in NAFLD (Table 1) [63–65]. Serum FGF21 levels are positively correlated with intrahepatic triglyceride levels [65]. As NAFLD is now recognized as a major public health problem, reliable biomarkers for NEFLD are needed. Serum FGF21 levels might be useful as a biomarker for NEFLD [61].

Type 2 diabetes connected with visceral obesity and insulin resistance has become a global health concern. Serum FGF21 levels are increased in patients with type 2 diabetes, gestational diabetes, and obesity, indicating FGF21 to be a potential new marker in patients with type 2 diabetes (Table 1) [66–71]. Serum FGF21 levels are independently associated with markers of insulin resistance and an adverse lipid profile [66, 70]. The upregulation of serum FGF21 levels might be a compensatory mechanism to improve glucose metabolism when insulin resistance is present. Diet-induced obese mice also have increased serum (endogenous) FGF21 levels and respond poorly to exogenous FGF21, indicating that obesity is an FGF21-resistant state [72]. Impaired glucose tolerance (IGT) is an important category of prediabetes. Serum FGF21 levels are also increased in Chinese subjects with IGT. However, serum FGF21 levels do not correlate with insulin resistance in the subjects [73].

Cushing’s syndrome is a hormone disorder caused by high levels of cortisol (hypercortisolism) in the blood. Patients with Cushing’s syndrome frequently suffer from visceral obesity, insulin resistance/diabetes, and other abnormalities similarly to patients with metabolic syndrome. Serum FGF21 levels are also increased in patients with Cushing’s syndrome. The increased FGF21 levels are due to excessive fat accumulation and related metabolic abnormalities rather than a direct effect of cortical on FGF21 production (Table 1) [74].

Lipodystrophy is a common alteration in HIV-1-infected patients under antiretroviral treatment. This syndrome is usually associated with peripheral lipoatrophy, central adiposity, and, in some cases, lipomatosis, as well as systemic insulin resistance and hyperlipidemia [75]. Serum FGF21 levels are increased in HIV-1-infected patients with lipodystrophy. This increase is closely associated with insulin resistance, metabolic syndrome, and markers of liver damage. FGF21 might be a biomarker of altered metabolism in HIV-1-infected, antiretroviral-treated patients (Table 1) [76].

Serum FGF21 levels correlate with renal function and are markedly increased in chronic kidney disease patients receiving hemodialysis, suggesting a possible link between their FGF21 levels and renal function [77]. Patients with end-stage renal disease (ESRD) show insulin resistance. Serum FGF21 levels are also markedly increased in patients with ESRD, suggesting FGF21 to play a role in insulin resistance in these patients (Table 1) [78].

11. Conclusion

Endocrine FGFs, FGF15/19, FGF21, and FGF23, are emerging in evolution and unique in function. The Fgf21 gene, which was generated early in vertebrate evolution, is specific to vertebrates. Fgf21 has been identified in most vertebrate genomes, but not in bird genomes, indicating that it might be lost in the bird lineage. Genome sizes and gene numbers

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<td>Type 2 diabetes</td>
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are smaller in birds than in mammalian species. As the differences might have evolved in response to the physiological demands of flight, Fgf21 also might have been lost. FGF21 mainly acts as an endocrine factor in an FGFR-dependent manner. FGF21 requires βKlotho as a cofactor in cultured cells. However, it may not require βKlotho in mice. FGF21 exerts pharmacological effects on hepatic glucose and lipid metabolism and growth hormone signaling. These effects might be useful for treating metabolic diseases. However, experiments with Fgf21 knockout mice indicated FGF21 not to be physiologically essential for hepatic glucose and lipid metabolism, ketogenesis, and growth hormone signaling. In contrast, FGF21 inhibited lipolysis in adipocytes of fasted mice and attenuated torpor induced by KD, indicating that Fgf21 may be a “thrifty gene.” Serum FGF21 levels are increased in patients with metabolic diseases having insulin resistance including NAFLD, type 2 diabetes, Cushing’s syndrome, and HIV-1-induced lipodystrophy. Although it remains unclear whether serum FGF21 levels are increased by FGF21 resistance or an adaptive response to metabolic disorders, these findings indicate that FGF21 potentially functions as a metabolic regulator in relation with insulin resistance and is a biomarker for metabolic diseases. Further study of FGF21 may provide clues as to its roles in lipid metabolism and clinical treatments for metabolic diseases.

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