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Fibroblast Growth Factor-21 as a Therapeutic Agent for Metabolic Diseases

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Contents

Abstract

Fibroblast growth factor (FGF)-21 is a unique member of the FGF family, with several molecular characteristics that differ from classical FGFs and exhibiting a pharmacologic profile that includes a variety of metabolic responses *in vitro* and when tested *in vivo* in animal models. FGF21 represents a novel and attractive therapeutic agent for type 2 diabetes mellitus, because of its ability to modulate disease phenotype in preclinical settings without inducing any apparent adverse effects. Although FGF21 was discovered relatively recently, the understanding of its biology and therapeutic utility is rapidly evolving. A number of key metabolically linked molecules and pathways have been suggested to be involved in the mechanism of action of FGF21, depending on the specific target tissue/organ. Further research into these mechanisms should lead to important advances in the understanding of FGF21 biology and pave the way for novel therapeutic strategies. The specifics of FGF21 activities both in cell culture and *in vivo*, its potential as a target for diabetes, and insights into the molecular mechanisms of FGF21 metabolic actions will be discussed in this review.

1. The Role of Fibroblast Growth Factors (FGFs) in the Regulation of Metabolic Processes

Fibroblast growth factors (FGFs) and their corresponding receptors (FGFRs) have been primarily associated with the processes of development, transformation, and angiogenesis.[1-4] However, over the last decade, new data have emerged showing that FGF/FGFR-mediated pathways may play important roles in defining and regulating functions of endocrine-relevant tissues and organs, as well as modulating various metabolic processes. For example, FGF10 and FGF16 play important roles in adipocyte and pancreatic biology,[5-7] and FGF19 and its mouse ortholog FGF15, regulate cholesterol and bile-acid metabolism in humans and rodents, respectively.[8,9] When tested in mice, FGF19 provides resistance to obesity and insulin desensitization.[10,11] Another member of the FGF family, FGF23, is a key player in the regulation of phosphate and calcium metabolism.[12,13] Likewise, the overexpression of a dominant-negative form of FGFR1 in pancreatic β cells leads to diabetes mellitus in mice.[14] FGFR2 appears to be a key molecule in the process of pancreatic development.[15-17] FGFR3 is a critical player in bone,[18] whereas FGFR4 has been implicated in cholesterol metabolism and bile-acid synthesis.[19] The metabolic roles of FGF/FGFR are still not precisely defined and are currently under intense investigation.

2. Overview of FGF21 and its *In Vitro* Effects

FGF21, a relatively novel member of the FGF family, has been recently shown to control various endocrine functions, which has led to its identification as a novel metabolic regulator.[20] The human *FGF21* gene was initially cloned in 2000 and mapped to chromosome 19.[21] Full-length FGF21 is 209 amino acids long, with a typical signal peptide at its N terminus, resulting in a mature FGF21 polypeptide of 181 amino acids. FGF21 is a secreted molecule[21] and can be expressed and purified from *Escherichia coli* paste in a bioactive form.[20] Human and mouse FGF21 orthologs share 75% sequence identity at the amino acid level.[21]

Within the FGF family, human FGF21 is most similar to FGF19 and FGF23 (31% and 26% amino acid sequence identity, respectively). Based on their structural relatedness and the ability to regulate metabolic processes, FGF19, FGF21, and FGF23 together form a unique subfamily of 'hormone-like' FGFs,[22] as, unlike classical FGFs, they can be detected in plasma.[9,12,13,23] Nevertheless, it remains to be determined whether FGF21 functions primarily as an autocrine, paracrine, or endocrine factor.

Initial characterization of the tissue/cell-type distribution of FGF21 expression and its regulation has been completed, but primarily at the mRNA level. According to published reports, endogenous FGF21 mRNA is detected in just a few types of tissue, such as adult thymus and liver,[21] whereas FGF21 expression is limited to mature hepatocytes[24] and the pancreas.[25,26] However, the actual profile of FGF21 expression may in fact be broader than reported, because of its exceptionally dynamic regulation. FGF21 mRNA levels can be profoundly modulated in different types of tissue and cells by a variety of conditions; for example, in hepatocytes on high glucose and fatty acid challenge,[23,26,27] in C_2C_{12} myotubes under 'starvation' conditions[28] or in the liver of rodents on sucrose- and fat-rich diets,[23,29,30] on fasting/fed transition,[23,26,29] and after partial hepatectomy or CCl_4 insult.[24] Peroxisome-proliferator activated receptor (PPAR)-α agonists also induce a substantial elevation of FGF21 in primary hepatocytes and in the liver of treated rodents.[23,26,29] Importantly, PPARα appears to directly regulate FGF21 expression.[26]

Although contributing to a better understanding of FGF21 biology in a variety of ways, most of the currently available FGF21 expression/expression regulation data come from mRNA analyses in rodents, which limits their utility in elucidating FGF21 functions in humans and the potential of FGF21 as a therapeutic agent. Because various analytical reagents for this emerging molecule are now becoming available from several commercial sources, it is expected that the actual profile of FGF21 expression and parameters of its regulation will soon be clarified at the protein level and studied in humans.

The bioactivity of FGF21 was first discovered through a cell-based functional screen aimed at identifying novel secreted molecules with therapeutic potential to treat diabetes. FGF21 stimulates glucose uptake in differentiated mouse 3T3-L1 cells and human primary adipocytes.[20] In contrast to the rapid action of insulin, the predominant effect of FGF21 on glucose uptake requires several hours of cell treatment, implying that transcriptional activation is part of the FGF21 mechanism of action. Importantly, the FGF21 effect on glucose uptake is insulin independent, additive to the activity of insulin on co-treatment, and not modulated by the addition of exogenous heparin.[20] The latter is probably explained by the observation that FGF21 does not appreciably interact with heparin,[20,31,32] which is unusual for FGFs, as they generally bind to, and require, heparin as a co-factor for full activity.[33]

In addition, when tested on isolated pancreatic islets, FGF21 suppressed glucose-mediated glucagon release and stimulated insulin accumulation and secretion, suggesting that the FGF21 has a direct effect on pancreatic α and β cells, as well as protecting islets from glucolipotoxicity and cytokine-induced cell apoptosis.[25] Overall, the *in vitro* data available to date indicate that FGF21 is functionally active only on cells of fat and pancreatic origins.[20,25,26,31,34]

Growth-promoting activity is one of the best-documented functions for most FGFs; however, FGF21 has not been shown to induce proliferation when tested on several primary and immortalized cells that are typically sensitive to treatment with classical FGFs.[20,25] Moreover, in co-stimulation experiments, FGF21 does not block the proliferative activity induced in these cells by other FGFs.[20] Thus, FGF21 does not appear to be mitogenic and is not a natural antagonist of typical FGF actions. This conclusion is further reinforced in a recent report, which describes the receptor specificity of FGF family members, including FGF21.[22] Indeed, evoking even marginal effects on mitogenicity in BaF3 cells overexpressing individual FGFRs requires exposing the cells to physiologically irrelevant concentrations of FGF21 (>200 nM, which is well above the reported EC_{50} (50% of the effective concentration) of ≈0.5 nM for FGF21 in glucose uptake and MAP kinase assays)[20,25] and the addition of massive doses of heparin at concentrations of ≥10 μg/mL.

Strikingly, treatment of 3T3-L1 adipocytes with the combination of FGF21 and a PPARγ agonist, rosiglitazone, leads to a remarkable increase in glucose uptake over the agents independently.[31] Moreover, based on FGFR activation data, rosiglitazone appears to sensitize cells to FGF21 stimulation, and FGF21 in turn

induces upregulation of PPARγ protein. Although it is currently unclear what particular impact each of these effects has on glucose transport, these data reveal potential synergy in the actions between these two regulators of glucose homeostasis, FGF21 and PPARγ.[31]

3. FGF21 *In Vivo* Pharmacology

The *in vivo* bioactivity of FGF21 has been observed using numerous biomarkers that illustrate the effects of FGF21 actions in a broad range of animal studies covering a variety of disease models.[20,23-26,35]

The consequences of enforced expression of human[20] and mouse FGF21[23,26] have been evaluated in transgenic mice.[20,24,26] FGF21 transgenic animals appear viable, normal at birth, and essentially indistinguishable from their wild-type littermates. Noticeably, they do not possess signs of neoplasia, tumors, or any other overt abnormalities throughout their lifespan, as demonstrated by extensive morphologic and histologic analyses.[20,23] Thus, prolonged exposure of mice to FGF21 does not lead to carcinogenic events. Nevertheless, a specific phenotype of FGF21 transgenic mice was revealed upon a variety of challenges.

C57Bl/6 mice overexpressing human FGF21 from the liver (circulating levels of 70–150 ng/mL) were evaluated for changes in metabolic parameters.[20] When fed a high fat, high carbohydrate (HFHC) diet for 15 weeks, FGF21 transgenic mice were resistant to weight gain and fat accumulation, despite an increased caloric intake. Consistent with low adiposity, the levels of leptin were noticeably reduced, as well as those of glucagon. Importantly, these effects were not associated only with the HFHC diet, as they were also observed in aged FGF21 transgenic mice on a normal chow diet. At 9 months of age, they weighed significantly less, had lower fasting glucose levels, less total and liver fat, more brown adipocytes, and showed improved glucose clearance and insulin sensitivity compared with control animals.[20]

Analysis of FVB mice with liver-specific overexpression of mouse FGF21 confirmed the absence of neoplasias or tumors.[24] Moreover, when compared with control animals, no change was observed in the incorporation of bromodeoxyuridine (BrdU) into the livers of FGF21 transgenic mice after partial hepatectomy or CCl_4 insult, suggesting that FGF21 is unlikely to play a role in a compensatory liver restoration process. Unexpectedly, forced expression of FGF21 markedly reduces tumor incidence, measured by the frequency of diethylnitrosamine-induced adenomas and the timing of their first appearance.[24] However, although FGF21 appears to delay the process of tumor initiation in the liver, the incidence of hepatocellular carcinomas in FGF21 transgenic mice at 12 months of age was similar to that in control animals. Thus, if FGF21 negatively regulates chemically induced hepatocarcinogenesis, it is likely to interfere with disease progression at its early stages.[24]

Recently, another line of FGF21 transgenic mice on a C57BL/6J background has been described.[26] These animals had 50–150 times higher levels of FGF21 mRNA in the liver than control animals, and had lowered levels of serum cholesterol, triglycerides, glucose and insulin (indicative of the improved lipid and glycemic metabolism), and ameliorated insulin resistance, consistent with previously published data.[20] FGF21 transgenic mice were also found to have elevated serum levels of β-hydroxybutyrate, suggesting the induction of ketogenesis, as well as having adipocytes of smaller size, increased lipase expression in white adipose tissue and elevated serum levels of free fatty acids, all evocative of the induction of lypolysis.[26] Importantly, as urine concentrations of adrenaline and noradrenaline were reduced in FGF21 transgenic animals, FGF21 does not appear to stimulate lipolysis through a systemic increase in catecholamines.[26] Surprisingly, these FGF21 transgenic mice were also reported to have 1–2ºC lower core body temperatures, reduced locomotor activity, and enhanced torpor during 24-hour fasting compared with their control littermates.[26] Although intriguing, the latter observations have not been reported by others[20,24] and, therefore, need to be explored further.

Adenovirus-mediated knockdown of FGF21 with short hairpin RNA (shFGF21) in mice led to a substantial decrease in circulating FGF21 levels and resulted in serious metabolic abnormalities, such as fatty liver, severe hypertriglyceridemia (predominantly as a result of the increase in chylomicron/very low-density lipoprotein fraction), a significant elevation in serum free fatty acids and total cholesterol, and a reduction in serum ketones.[23] Importantly, these changes were apparent only in animals fed a high-fat diet and not on a normal chow diet. This correlates with a dramatic elevation in the endogenous expression of FGF21 in animals on a ketogenic diet, and provides further evidence that the outcomes of shFGF21 experiments are indeed a result of a direct attenuation of endogenous FGF21 expression/function.[23]

Systemic administration of FGF21 in rodents via subcutaneous injections or through constant infusion leads to a variety of metabolic consequences. FGF21 effectively lowers fed and fasted plasma glucose levels, stimulates glucose disposal, and increases insulin sensitivity in oral glucose tolerance tests.[20] Importantly, the FGF21 glucose-lowering effects are long lasting, as they persist for at least 24 hours following the cessation of FGF21

administration. Moreover, despite near normalization of glucose levels in diabetic animals, no incidence of hypoglycemia induced by the administration of FGF21 has been observed in either healthy or diseased rodents in a fasting or fed state, even at supra-pharmacological doses of FGF21.[20,26] The reason for that is unknown; as normoglycemic animals still respond to FGF21 treatment in a variety of ways,[23,24,26] the absence of hypoglycemia clearly distinguishes the glucose-lowering effects of FGF21 from those of insulin. In addition, FGF21 efficiently reduces serum and liver triglycerides, circulating insulin and glucagon levels, and elevates serum β-hydroxybutyrate.[20,23,26] Because no changes in the levels of fed and fasted glucose, lipids, insulin, and glucose disposal during oral glucose tolerance tests were observed with a single subcutaneous injection of FGF21, it appears that beneficial FGF21-dependent effects require repeated exposure of animals to the protein.[20,26]

When administered to diabetic *db/db* mice through continuous infusion for 8 weeks, FGF21 corrected hyperglycemia and preserved β-cell function and mass.[25] There was an elevation of circulating plasma insulin levels in the FGF21-treated group compared with control animals. Moreover, histologic examination revealed a 340% increase in the number of islets per section, 280% more β cells per section, and 130% more β cells per islet. This correlates with the FGF21-mediated attenuation in cytokine-induced caspase 3/7 activation and DNA fragmentation observed in INS-1E cells, and evokes the Akt-dependent phosphorylation of BAD (BCL2-antagonist of cell death), a suppressor of apoptosis and promoter of cell survival,[36] as a potential basis for the *in vivo* effect.[25] Finally, a small reduction in proliferating cell nuclear antigen (PCNA) staining has been observed in islets, corroborating the nonmitogenic character of FGF21 action.[25]

The therapeutic potential of FGF21 has also been evaluated in nonhuman primates.[35] Daily administration of FGF21 in a dose-escalating fashion for 6 weeks to diabetic rhesus monkeys (*Macaca mulatta*) caused a dramatic decline in levels of fasting plasma glucose, fructosamine, triglycerides, insulin, and glucagon. Significant improvements in lipoprotein profiles, including lowering of low-density lipoprotein (LDL)-cholesterol and raising of high-density lipoprotein (HDL)-cholesterol, beneficial changes in the circulating levels of several cardiovascular risk markers/factors, and the induction of a moderate weight loss are also seen in FGF21-treated animals. As in rodents, no incidence of hypoglycemia was demonstrated in monkeys at any point during the study. Overall, the outcomes of this study strongly suggest that FGF21 is an effective metabolic regulator in the context of type 2 diabetes and other metabolic diseases.[35]

A zebrafish otholog of human FGF21 has been identified and detected in developing embryos.[37] Studies using antisense FGF21 morpholino oligonucleotides (MOs), as well as the FGFR inhibitor SU5402, show a loss of erythroid and myeloid cells and their progenitors in FGF21 knockdown embryos, but not blood vessels and lymphoid cells, and the injection of FGF21 RNA rescues this phenotype. Importantly, FGF21 MOs have no effect on cell proliferation and apoptosis in the developing embryos. This report concludes that FGF21 is essential for hematopoietic development in zebrafish.[37] However, as no link between FGF21 and hematopoiesis in mammals has so far been reported, these intriguing observations need to be evaluated in higher species.

4. Insights into FGF21 Mechanism of Action

Despite exhibiting atypical FGF-family activity, FGF21 appears to induce its signal via classical FGFRs. As an initial event, FGF21 stimulates tyrosine phosphorylation of FGFRs,[20,25,31,34] which appears to be critical for FGF21 function, since no FGF21 activity is detected in the presence of the specific FGFR inhibitor SU5402.[25] Moreover, FGF21 induces a typical 'FGF-like' signaling pattern in responsive cells, such as the phosphorylation of proteins FRS2, MAPK, MAP2K1, RAF1, AKT1, GSK3, RPS6KB2, STAT3, and PTPN11, and Ca^{2+} flux.[20,25,31,34] The fact that many of these *in vitro* effects can also be caused by archetypal FGFs such as FGF1,[20,25,31,34] is additional evidence that FGF21 signals through activation of a conventional FGFR-mediated pathway.

Nevertheless, FGF21 is a unique member of the FGF-ligand family. In contrast to many other FGFs, it does not bind or require heparin for its bioactivity,[20,31,32,34] fails to directly interact with soluble FGFRs,[20] shows, to date, an activity profile restricted to cells of adipose and pancreatic origins,[20,25,26,31,34] is not mitogenic *in vitro*, and does not induce mitogenicity in FGF21 transgenic mice or following systemic administration in rodents or primates.[20,22,24-26,35]

The nature of some of these unusual characteristics has been clarified by the demonstration that βKlotho is a critical component of the FGF21 receptor complex.[34] βKlotho[38] is a type I transmembrane protein, structurally similar to Klotho,[13,39] and its function is not well understood. A recent report links this molecule to cholesterol and bile-acid metabolism;[40] however, the exclusive expression of βKlotho in pancreas, adipose, and liver[38] is suggestive of a broader biological role.

Based on its structural similarity to Klotho, which has been shown to support activity of FGF23,[41,42] βKlotho has been tested

as a co-factor for FGF21 signaling. First, in transient expression experiments in HEK293 cells and using a pull-down approach, βKlotho was found to physically interact with multiple 'c' isoforms of FGFRs, but more efficiently with FGFR1, FGFR4, and FGF21. Moreover, although HEK293 cells transfected with mock plasmid did not respond to FGF21 stimulation, expression of βKlotho recapitulated FGF21 activity in a MAPK activation assay.[34] Although βKlotho has not been detected in 3T3-L1 preadipocytes, it was found to be abundantly expressed in adipocytes,[34] which correlates with the ability of FGF21 to act on differentiated 3T3-L1 cells but not on preadipocytes.[20] Furthermore, siRNA-mediated knockdown of βKlotho in 3T3-L1 adipocytes impedes FGF21-induced GLUT1 upregulation and glucose uptake.[34] Thus, functional studies in cells show that cultures lacking this molecule do not respond to FGF21, that introduction of βKlotho confers FGF21 responsiveness and recapitulates FGF21 signaling observed in naturally responsive cells, and that disruption of βKlotho function impedes FGF21 activity.[34]

As βKlotho appears to be a necessary component of the FGF21 receptor, several unique features of FGF21 biology can now be rationally explained based on this finding. The fact that βKlotho is critically required for FGF21/FGFR/βKlotho complex formation clarifies the inability of FGF21 to directly interact with FGFRs.[20] βKlotho may, thus, serve a parallel role to FGF21, as heparin does for conventional FGFs.[33] The selective expression pattern of βKlotho in the liver, pancreas, and adipose explains the specificity of FGF21 for cells of adipose and pancreatic origins.[20,25,26,31,34] Although a direct effect of FGF21 on liver-derived cells has not yet been reported, it is likely, given the profound impact of FGF21 on lipid metabolism in animals. The absence of mitogenicity with FGF21 may be a direct consequence of βKlotho in the FGF21 receptor complex, paralleling that of the FGF23/Klotho system[32,42] in which signaling was shown to be nonproliferative.

Despite the identification of βKlotho as a critical component of FGF21 activity, several outstanding questions about the molecular specifics of the FGF21 mechanism of action need to be further clarified. FGF21 functions through a typical FGFR-mediated pathway, activating various FGFRs in the context of βKlotho co-overexpression in HEK293 cells.[34] However, in native conditions, FGF21 functions through FGFR1 and FGFR2 on adipocytes and pancreatic cells. As multiple splice isoforms of these receptors were detected in 3T3-L1 and INS-1E cells,[20,25,34] it is currently unclear which particular variants serve as the specific FGF21 receptors within the FGF21/FGFR/βKlotho complex, or if any of them can suffice. Moreover, as no FGFR4 and only marginal levels of FGFR3 were detected in 3T3-L1 adipocytes,[20,31] it remains to be seen whether FGF21 can signal through these FGFRs when they are present at appreciable levels. Moreover, no information currently exists on specific FGFR target(s) to propagate FGF21 actions *in vivo*. Their identification, however, would be of significant value while assessing the future effectiveness of FGF21-based clinical interventions, tailoring disease indications and patient populations for this agent, and identifying novel molecular candidates for further drug discovery around the FGF21 mode of action. As this line of research is of critical importance, it is worth mentioning the potential technical challenges it poses, given the structural complexity within the FGFR family.[2] Likewise, the ability of βKlotho to mediate the activities of other FGF family members has not been fully evaluated. Whereas the effects of FGF1 are not βKlotho dependent, and FGF23 utilizes Klotho instead of βKlotho to mediate its action via FGFRs,[41,42] other FGFs need to be explored, FGF19 in particular.[8-11] Moreover, βKlotho is structurally similar to Klotho, which has been shown to possess a β-glucuronidase activity.[43] Whether βKlotho is also an enzyme, and what role, if any, its putative enzymatic activity plays in the propagation of FGF21 action, needs to be clarified. Finally, the biggest remaining questions regard the mechanism(s) underlying the multiple metabolically beneficial effects that are consistently observed in animals following FGF21 administration.

To date, several molecules and their corresponding pathways have been suggested to contribute to the FGF21 mode of action *in vivo*. As FGF21 is a potent modulator of GLUT1-dependent glucose uptake in 3T3-L1 adipocytes,[20,31] it is tempting to speculate that GLUT1 may mediate FGF21 effects *in vivo*. Indeed, GLUT1 levels were augmented in mice that were administered FGF21.[20] However, since this effect was only modest and limited to adipose tissue, GLUT1 does not seem to be a plausible candidate to have a major impact on the glucose-lowering function of FGF21. Moreover, the role of GLUT1 in the regulation of lipid metabolism and β-cell function has not been defined. Thus, GLUT1 is very unlikely to be an underlying cause of any FGF21 *in vivo* effects beyond glucose control.

Although several lines of evidence suggest an insulin-independent FGF21 mechanism of action, FGF21 induces an increase in insulin content and glucose secretion in cultures of isolated islets.[25] Moreover, insulin levels were found to be elevated in *db/db* mice that were administered FGF21 for 8 weeks.[25] Nevertheless, FGF21-dependent lowering of circulating insulin has been consistently observed in other rodent animal models and in diabetic monkeys, suggesting improvements in insulin sensitivity.[20,35] It is conceivable that elevated insulin levels in FGF21-treated *db/db*

mice that are prone to early pancreatic failure simply arose as a result of significant improvements in β-cell function and mass.[25]

Glucagon secretion from isolated islets is impaired by FGF21,[20,25] and its levels are lowered after FGF21 administration in diabetic rodents and monkeys.[20,35] As glucagon is a known contributor to the pathophysiology of diabetes,[44] this suggests an alternative glucagon-mediated mechanism of FGF21 action *in vivo*. In addition, the levels of several other circulating cytokines, including adiponectin,[45] were beneficially modulated in FGF21-treated diabetic monkeys and, along with others, could also be candidates mediating FGF21 activity.[35] Alternatively, alterations in cardiovascular risk factor profiles may be indirect effects reflecting FGF21-induced improvements in glucose and lipid control, as many of these molecules are considered biomarkers for various metabolic dysfunctions.[46]

Although FGF21 induces moderate weight loss in diabetic monkeys,[35] no bodyweight effect has been demonstrated with FGF21 in rodents.[20,24-26] It is possible, however, that human FGF21 is more biologically active in the primate, resulting in a more pronounced pharmacologic response than that observed in mice, or that bodyweight lowering in rodents requires higher doses of the protein than those that can establish effective glucose control. Alternatively, most of the experimentation in rodents was carried out in genetically compromised leptin- or leptin-receptor-deficient models of type 2 diabetes, which differ in their disease pathology from diabetic monkeys with naturally occurring disease. If true, it would be suggestive of leptin as a potential mediator of FGF21 *in vivo* effects. In fact, FGF21 transgenic mice with normal leptin signaling are protected from weight gain and fat accumulation.[20] Furthermore, since βKlotho has been linked to bile-acid metabolism,[40] and the appreciation of bile acids as fine regulators of metabolism is growing,[47,48] their role as downstream mediators of FGF21 actions needs to be ultimately studied.

Recent reports suggest interplay between FGF21 and PPAR pathways and shed additional and critical light on the mechanism of action of FGF21. A cross-talk and synergy between PPARγ and FGF21 pathways has been documented in 3T3-L1 adipocytes.[31] An increase in the ability of FGF21 to activate FGFRs has been shown in rosiglitazone-treated adipocytes, suggesting that it is possible that PPARγ ligands may in fact function as FGF21 sensitizers. Although our understanding of this effect at the mechanistic level is still lacking, the demonstration of FGF21/PPARγ synergy *in vivo* is becoming critical and may add value to the therapeutic potential of FGF21. Moreover, PPARα, a key mediator of starvation response,[49] is directly involved in the regulation of FGF21 expression and appears to be upstream of FGF21 actions.[23,26,29] These elegant studies employing various techniques showed that fasting, ketogenic diets and free fatty acids, and stimulation with synthetic PPARα agonists, led to a profound increase in FGF21 levels in general circulation and in the liver, and these effects were significantly attenuated in PPARα$^{-/-}$ mice. Although these animals are known to be deficient in β-oxidation and ketogenesis,[50] the administration of recombinant FGF21 rescues the normal fasting response in PPARα$^{-/-}$ mice. This correlated with the ability of FGF21 to regulate sets of specific genes controlling triglyceride clearance, production of ketone bodies and fatty acid oxidation in the liver, and lipolysis in adipose tissue. Importantly, most of the physiological effects attributed to the actions of endogenous FGF21 were observed in fasted animals, a physiological state in which FGF21 is readily expressed, instituting this protein as an important endocrine factor in response to starvation.

This metabolic condition, however, may not be the only one in which FGF21 is destined to play an important regulatory role. Indeed, FGF21 is also remarkably upregulated in the livers of genetic *ob/ob* and diet-induced obese (DIO) mice[29,30] that are hyperphagic. As suggested, this effect may be related to the activation of PPARα function in response to the abundance of free fatty acids in these animals.[29] Alternatively, because hepatocytes that were cultured in the presence of high concentrations of glucose show induction of FGF21 expression,[27] it could also be a consequence of elevated blood glucose in *ob/ob* and DIO mice as part of a natural compensatory mechanism to correct for hyperglycemia. It is worth noting that even though endogenous FGF21 levels are significantly elevated in diabetic *ob/ob* mice, these animals respond to FGF21 administration in a variety of beneficial ways.[20] Furthermore, FGF21 has been found to be efficacious in diabetic Rhesus monkeys.[35] Collectively, these recent observations add significantly to the therapeutic potential of FGF21 to efficiently function in a disease state, and further establish FGF21 as a major metabolic regulator.[20,23,26,29,30]

Thus, several key metabolically linked molecules and therapeutically relevant pathways have been suggested to participate in the mechanism of action of FGF21. This makes good sense, as it is highly unlikely that one specific molecule/pathway could underlie the activities of FGF21 *in vivo*, given the magnitude and extent of metabolic changes and the complex regulation of FGF21 expression observed in a variety of animal models. Rather, FGF21 acts *in vivo* in a pleiotropic fashion, utilizing diverse downstream molecules/mechanisms in different target tissues/organs that in turn determine the broad positive metabolic outcome. Determining their specific roles and impact on the propagation of the FGF21

signal, and defining pathway interactions, should lead to important advances in the elucidation of FGF21 biology and pave the way for novel strategies in optimizing FGF21 therapeutic properties.

5. Conclusion

Despite recent progress, the overall mechanism of FGF21 action, and particularly its primary determinants *in vivo*, are still poorly understood. Nevertheless, FGF21 shows promise as a revolutionary disease-modifying therapy for type 2 diabetes and associated metabolic disorders. In preclinical testing, FGF21 provides sustained glucose and lipid control, amelioration of insulin resistance, improvements in β-cell function and mass, beneficial changes in lipoprotein and cardiovascular risk factor profiles, and bodyweight reduction in nonhuman primates. Of equal importance, these striking metabolic outcomes do not arise at the expense of adverse effects such as hypoglycemia, edema, or increased adiposity, which are commonly associated with therapies currently available for diabetic patients.

As the incidence of type 2 diabetes continues to be a growing global health concern, the need for more effective and safer therapies is increasing.[51,52] Future studies in humans should explore the therapeutic potential of FGF21 and provide a better understanding of its mechanism of action.

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