Cell. Mol. Life Sci. (2009) 66:2067–2073
DOI 10.1007/s00018-009-0003-9

Cellular and Molecular Life Sciences

REVIEW

Fibroblast growth factor 21: an overview from a clinical perspective

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Received: 30 January 2009 / Revised: 11 February 2009 / Accepted: 13 February 2009 / Published online: 11 March 2009

Abstract Fibroblast growth factor 21 (FGF21) has been proposed as a novel putative therapeutic agent in type 2 diabetes. A large amount of data, predominantly obtained from murine models but also from non-human primates, suggest that FGF21 ameliorates obesity-associated hyperglycemia and hyperlipidemia primarily via effects on adipose tissue and the pancreas. In addition, FGF21 has been reported to play a pivotal regulatory role in starvation and ketosis. However, while it is clear that FGF21 has potent effects in vivo in several animal models, the exact mechanisms remain elusive. Moreover, very recent results from different human cohort studies have shown a paradoxical regulation of plasma FGF21 in obesity and type 2 diabetes as well as other important qualitative differences in the effects and regulation of FGF21 between rodents and humans. This review focuses on the most recently published data on FGF21 with emphasis on results obtained in humans.

Keywords Adipocyte · Diabetes · Insulin · Metabolism · Obesity

Fibroblast growth factor 21, a member of the FGF-family superfamily

The mammalian fibroblast growth factor (FGF)-family currently consists of 22 members subdivided into seven subfamilies based on their structural similarities and modes of action [1]. Most FGFs play an important role as paracrine factors regulating cell growth and differentiation, including angiogenesis and transformation [2]. However, members of the FGF-19 subfamily, which also includes FGF21 [3] and FGF23 [4–6], differ in two important aspects from all other FGF proteins. First, they have no or very small mitogenic effects and, second, they exert their action via systemic, hormone-like effects. Thus, FGF19 (the human orthologue of murine FGF15) [7] is primarily expressed in the intestine but regulates bile acid synthesis in the liver in both rodents [8] and humans [9]. FGF23 is produced in bone tissue and regulates phosphate and vitamin D metabolism via effects on the kidney [10], while FGF21 is predominantly expressed in the liver and has beneficial effects on several metabolic parameters in different animal models of obesity [11]. These three members of the FGF19 subfamily share approximately 30% amino acid sequence homology. The human *FGF21* gene is located on chromosome 19 and encodes a 209-amino acid-long protein with an amino-terminal signaling peptide which after cleavage results in a mature protein of 181 amino acids. Human and mouse FGF21 share 75% sequence identity at the amino acid level. A recent review has presented a comprehensive overview of FGF21 as a metabolic regulator based on data in animal models [12]. However, very recent results published only in the last few months show that FGF21 expression and regulation in human subjects appear to differ considerably from those in animal models. As such, these results have generated discussions on the possible clinical implications of FGF21. This overview provides a brief background on FGF21 and focuses on the species differences between rodents and humans, a crucial aspect when considering FGF21 as a possible therapeutic agent for obesity and type 2 diabetes patients.

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Fibroblast growth factor 21 signaling mechanisms

FGFs mediate their action via a set of membrane-bound FGF receptors (FGFRs) that in turn are expressed in multiple splice variants. FGFR1-R4 contains an intracellular tyrosine kinase domain that is activated upon ligand binding. leading to the activation of a number of downstream signals, including MAPKs, RAF1, AKT1 and STATs [12]. However, FGFs cannot interact with FGFRs directly since they require a co-factor to bind and activate FGFR signaling efficiently. For most FGFs, this co-factor has been shown to be heparin/heparan sulphate, and binding is mediated via conserved heparin-binding regions. In contrast, crystallographic analysis of members of the FGF19 subfamily has shown that their heparin-binding regions diverge significantly from those of other FGFs [13, 14]. The weak binding affinity of the former for heparin/heparan sulphate enables them to avoid being trapped in the extracellular matrix, thereby allowing them to function as endocrine factors (although auto- or paracrine effects may still be exerted). Instead, as first shown for FGF23, members of the FGF19 subfamily require the presence of specific transmembrane proteins from the klotho-family for FGFR binding and activation [15, 16]. β-Klotho, a protein that shares 41% amino acid identity with klotho, has been shown to be required for the bioactivity of FGF21 and FGF19 [17, 18]. A study in murine 3T3-L1 cells, which predominantly express FGFR1 and, to a lesser extent, FGFR2, suggests that β-klotho forms a preformed complex with either of these two receptors, which is then activated upon FGF21 binding [19]. Several reports suggest that the "c"-receptor splice isoforms of FGFR1-3 exhibit a particular affinity to β-klotho and could thus act as endogenous receptors for FGF21 [17–19]. These results fit well with a recent study in BaF3 cells (a murine pro-B cell line), which do not express any endogenous FGFRs [20]. In these cells, FGF21 was shown to be bioactive in cells stably expressing β-klotho and FGFR1c or -R3c. In 3T3-L1 cells, transient overexpression of FGFR4 (endogenously mainly expressed in the liver) also resulted in an interaction with β-klotho [19]. However, although both FGF19 and FGF21 can bind to the β-klotho–FGFR4 complex, only incubations with FGF19 result in efficient receptor activation [17]. Taken together, these results suggest that FGF21 may interact with different FGFRs in specific cellular environments and that β-klotho expression is required for receptor activation. β-Klotho is almost exclusively expressed in adipose tissue, pancreas and liver [21], which may explain why the main effects of FGF21 in mice have been observed in the former two tissues. To date, no direct effects of FGF21 on hepatocytes have been reported. There is currently no definitive evidence for why FGF19 (and not FGF21) displays a specific action in liver cells [17], but the fact that liver predominantly expresses FGFR4 may be an important contributing factor. In 3T3-L1 cells and white adipose tissue, FGFR1 is by far the most abundant receptor, and it is therefore most likely that FGF21's main functional receptors in this tissue are preformed complexes of β-klotho with FGFR1c [17, 19]. Although FGFR2 is also expressed in adipose tissue, the levels are much lower and, consequently, this receptor probably plays a minor role [17]. An insight into how FGF21 interacts with the FGFR-β-klotho complex was recently presented by two separate groups using similar approaches [22, 23]. By assessing the binding affinity of consecutively amino- or carboxy-terminally truncated FGF21 proteins, both groups concluded that the carboxy-terminus region is essential for β-klotho binding while the amino-terminus is important for FGFR activation. This information is important for a possible future development of more selective or potent receptor agonists and could also be helpful in the development of non-peptide ligands. It should be stressed, however, that although the initial steps in FGF21 signaling are rather well characterized, the downstream target genes affected by FGF21 signaling remain to be defined.

Fibroblast growth factor 21 effects in animals

The beneficial metabolic effects of FGF21 were first identified in a high-throughput screen for secreted factors that promote glucose uptake in murine 3T3-L1 adipocytes [11]. A detailed examination showed that FGF21 stimulated insulin-independent glucose uptake by increasing GLUT-1 expression in both 3T3-L1 and human preadipocytes. Furthermore, FGF21 administration in genetic models of murine obesity lowered blood glucose and triglyceride levels; consequently, transgenic mice overexpressing FGF21 in the liver were protected against diet-induced obesity (DIO) and insulin resistance [11]. Subsequent studies in murine DIO-models confirmed that FGF21 can reverse hepatic steatosis and insulin resistance but also reduce body weight by up to approximately 20% [24, 25]. These effects are obviously not limited to rodents since beneficial effects of FGF21 on hyperglycemia and hyperlipidemia have also been observed in diabetic rhesus monkeys [26]. Despite the glucose-lowering effects of FGF21, there have been no reports on an increased rate of hypoglycemic events in any of the studies published to date. Moreover, in contrast to many other agents with glucose-lowering effects, FGF21 does not induce weight gain, which is a big caveat with many currently used therapies in type 2 diabetes. Given the tissue distribution of β-klotho–FGFR discussed above, FGF21 is believed to exert its activity mainly via effects on adipose tissue and the pancreas. However, the cellular mechanisms are not entirely clear. In murine adipocytes in vitro, murine FGF21 increases glucose uptake via increased GLUT1 and

stimulates the enzymatic breakdown of stored triglycerides via lipolysis [11]. The latter effect may promote a reduction in fat depots although, as discussed below, increased lipolysis is generally associated with insulin resistance. Even though it is tempting to speculate that FGF21 mediates its insulin sensitizing effects via increased GLUT1, this is not likely to be the main mechanism for a number of reasons. First, the increase in GLUT1 following FGF21 administration in mice is very modest and limited to adipose tissue [11]. Second, increases in GLUT1 could hardly explain the effects on hyperlipidemia or weight reduction. In the pancreas, FGF21 inhibits glucagon secretion and preserves insulin content and release by increasing islet survival via a reduction of glucolipotoxicity and cytokine-mediated apoptosis [27]. The effects on islet function are probably not a main mechanism either since they are unable to explain the weight reduction observed in DIO-models or the reduction in hepatosteatosis. A number of alternative mechanisms through which FGF21 may act have been recently proposed [24] based on a study in which mice treated with FGF21 displayed increased energy expenditure and a reduced respiratory quotient (RQ) indicative of a preferential utilization of fat as an energy source. A detailed expression analysis showed that in the liver, genes involved in fatty acid oxidation were increased, while genes involved in de novo lipogenesis were decreased. In adipose tissue, genes involved in uncoupling, lipolysis and lipogenesis were all concomitantly increased, and the authors hypothesized that the coordinated changes in liver and adipose tissue expression could result in a state of futile cycling and increased energy expenditure. Interestingly, out of a panel of 67 studied hormones or cytokines, FGF21 treatment led to a significant reduction of only two proteins, leptin and insulin. Since the reduction of these two hormones was most probably secondary to weight reduction, these results suggest that the anti-obesity effect of FGF21 is probably not mediated via changes in circulating factors. Another recent study performed a similar analysis in a DIO-model using the same mouse strain (C57BL/6) [25]. In this work, FGF21 treatment reduced body weight to a similar degree (approx. 20% at the maximum dose), mainly via decreased adiposity and increased energy expenditure, without affecting total caloric intake. A reduction in the expression of hepatic lipogenic genes was also observed. However, in somewhat contrast with the data presented by Coskun et al. [24], no changes in RQ were observed, suggesting that there were no alterations in fuel selection between fatty acids and carbohydrates. Instead, a significant increase in physical activity was observed in animals treated with FGF21, and this effect could be observed as early as a few days after starting the administration of the FGF21 (before any notable weight changes). This result was in contrast to that obtained by Coskun et al. [24] where no changes in physical activity were observed. It is therefore still unclear whether FGF21 increases locomotor activity or not, and this should be addressed in future studies.

In addition to its insulin sensitizing effect, FGF21 has also been proposed to play an important role in the physiological response to food deprivation/starvation and was recently hypothesized to be the primary factor initiating the production of ketone bodies [28–30]. In these elegant murine studies, FGF21 expression in the liver was shown to be under the control of the transcription factor peroxisome proliferator-activated receptor α (PPARα), which is activated during starvation [28, 29]. The authors [29] proposed that this activation results in an increase in circulating FGF21 levels, which in turn promotes lipolysis in adipose tissue and the release of fatty acids into the circulation. Fatty acids are then taken up by the liver and converted into ketone bodies. These studies, as well as other reports [31], not only suggest a novel role for FGF21 but also demonstrate that FGF21 expression can be regulated. In the original work describing the cloning of FGF21, an mRNA expression analysis showed that expression was predominantly found in the adult liver and, to a lesser extent, in the thymus [3]. However, recent studies have shown that FGF21 expression is rather plastic and can be induced in a number of cell types, including adipocytes [32, 33] and muscle cells [34, 35]. In the former, FGF21 expression can be induced by PPARγ activation, and simultaneous treatment with FGF21 and PPARγ agonists leads to a synergistic effect on glucose uptake in 3T3-L1 cells [36]. There appears to be a crosstalk between PPARγ and FGF21 that has led some authors to propose the hypothesis that the insulin sensitizing effects of thiazolidinediones may at least in part be mediated via changes in FGF21 expression [32]. Moreover, FGF21 expression in liver, and to a lesser extent in adipose tissue, is increased not only by fasting but also by a high fat diet [32]. Recent studies have shown that FGF21 mRNA levels in liver and adipose tissue are increased in mice with genetic obesity [31, 37]. This could indicate that increased FGF21 expression may constitute a protective pathway in the metabolic alterations induced by obesity.

Human FGF21, in vitro effects and in vivo expression

While there is little question that FGF21 has potent metabolic effects in different animal models of obesity and may also play a part in the physiological response to fasting, data presented in humans are less convincing. To date, there have been no published studies on the effects of FGF21 in vivo in humans, and conclusions can therefore only be drawn from in vitro studies in human cells and cross-sectional analysis of FGF21 levels in different

cohorts. Important differences have emerged from these studies in comparison to data obtained in animal models. For example, while murine FGF21 appears to promote lipolysis in 3T3-L1 cells [11, 29], experiments using human FGF21 in primary cultures of human adipocytes have failed to demonstrate any significant effects on non-stimulated (basal) lipolysis [38]. Quite the contrary, FGF21 attenuates hormone-stimulated lipolysis in these cultures—although only at very high (supraphysiological) concentrations. Human adipocytes express FGFR1 and -R2 as well as β-klotho and are clearly responsive to FGF21, as evidenced by the phosphorylation of downstream targets, such as MAPKs [38]. This demonstrates that FGF21 may have qualitatively different effects in human adipocytes than in murine ones. It should be stressed, however, that these data do not exclude the possibility that FGF21 may have positive effects on insulin sensitivity in man. The increased release of fatty acids into the circulation is a well-established factor underlying the development of insulin resistance [39], and FGF21-mediated attenuation of stimulated lipolysis could arguably result in reduced circulating FA levels in vivo, although this remains to be shown. An interesting study assessing the plasma levels of FGF21 in different human subjects was recently published [40]. FGF21 levels were assessed by an enzyme-linked immunosorbent assay (ELISA) in samples from 76 healthy normal-weight subjects ranging in age from 20 to 80 years. The interindividual variation in plasma levels was considerable—from 21 to 5300 pg/mL with a mean and median level of 450 and 156 pg/mL, respectively. In this cohort, there was no difference in FGF21 levels between genders and no correlation to the body mass index (BMI), age, blood glucose, serum-lipids or markers for bile acid and cholesterol synthesis. The authors also analyzed plasma FGF21 levels in a separate set of five healthy subjects from whom blood samples were drawn every 90 min overnight. In these five individuals, FGF21 levels were remarkably stable, there were no circadian changes nor any changes in relation to feeding or fasting. This result was in contrast to the highly fluctuating values of FGF19 assayed in the same subjects [9]. Moreover, in two separate cohorts, short-term fasting (12–20 and 48 h, respectively) did not alter FGF21 levels, although the longer fasting period resulted in a significant ketogenic response, as evidenced by a 40-fold increase in serum 3-hydroxybutyrate. The authors were able to retrieve serum samples from a small cohort ($n = 5$) of females with rheumatoid arthritis that underwent a 7-day strict water fast. In these subjects, a 74% increase in FGF21 levels could be observed at the end of the fasting period, although the levels were still in the normal range previously observed in the healthy normal-weight cohort described above. The authors also assessed whether fibrates, a triglyceride (TG)-lowering class of pharmaceutic agents that activate PPARα, had any effect on circulating FGF21 levels. They found that FGF21 levels at baseline were twofold higher in 19 subjects with hypertriglyceridemia (HTG) than in the healthy controls. Fibrate treatment for 3 weeks resulted in a significant reduction of TG (60%) and a small (28%) but significant increase in FGF21. It should be stressed that this study only evaluated the circulating levels of FGF21 and that no tissue expression was assessed in any of the cohorts. Nevertheless, these results demonstrate that (1) FGF21 levels are highly variable and do not strongly correlate with age, BMI or gender in normal-weight subjects, (2) ketogenesis in humans is clearly induced independently of plasma FGF21 levels and (3) plasma FGF21 is elevated in hypertriglyceridemia (HTG), and its expression may be somewhat enhanced via PPARα activation. A recent study from the Czech Republic reported that plasma FGF21 levels in 17 anorectic women (BMI approx. 16 kg/m^2) were significantly lower than those in the age-matched controls [41]. In addition, realimentation for 2 months in ten of the subjects (reaching a mean BMI approx. 17.5 kg/m^2) resulted in a further reduction of FGF21 levels. In contrast to these results, those from a recent cross-sectional report from Hong Kong involving 232 Chinese subjects with or without obesity [37] showed that the circulating levels of FGF21 in this cohort were significantly higher in obese subjects and correlated positively with adiposity, serum insulin and TG but negatively with high-density lipoprotein (HDL)-cholesterol. An independent association between serum FGF21 and the metabolic syndrome was also found. Furthermore, FGF21 mRNA expression in subcutaneous fat correlated significantly with its circulating levels, suggesting that adipose tissue may contribute to plasma FGF21 levels. In another cross-sectional study of 162 Chinese subjects, FGF21 plasma levels were found to be significantly increased in type 2 diabetic patients with or without ketosis compared to age- and BMI-matched controls [42]. A caveat pertaining to both these studies is that plasma FGF21 levels were only assessed at one time-point. Interestingly, a very recent study in 120 patients with or without chronic renal insufficiency reported that circulating FGF21 levels were significantly and inversely related to markers of renal function [43]. The FGF21 levels were >15-fold higher in subjects with chronic hemodialysis. Kidney function is therefore an important aspect to consider when circulating FGF21 levels are being assessed. A word of caution is appropriate with respect to these human studies: the pronounced variations in plasma FGF21 levels imply that large study populations are required in order to detect significant differences between groups with sufficient statistical power. Moreover, although FGF21 is primarily believed to act via endocrine mechanisms, a caveat to all of these human studies is that a detailed assessment of tissue

expression is lacking; consequently, local auto-or paracrine effects of FGF21 cannot be excluded. This issue should be assessed in future studies.

Taken together, the results from these studies display both similarities and differences with previously published data in animals and suggest that FGF21 biology may be more complex than previously thought. First, plasma FGF21 levels are highly variable and do not correlate with BMI in normal-weight subjects [40]. A significant positive correlation is observed with obesity, the metabolic syndrome [37] and type 2 diabetes [42], which is in accordance with tissue expression of FGF21 in obese mice, as discussed above. This association parallels the elevated levels of insulin and leptin observed in type 2 diabetes and obesity, respectively. The physiological role of FGF21 in man, however, remains elusive. Speculatively, it could play a role in modulating adipose tissue lipolysis and possibly the endocrine function of the pancreas. Clearly, more studies in humans are warranted. Thus, the question remains, are pathophysiological conditions associated with the metabolic syndrome characterized by FGF21 resistance or are the elevated circulating levels part of a protective mechanism? Alternatively, are there other causal relationships, such as reduced plasma clearance or increased FGF21 secretion from tissues other than liver? Another putative mechanism that should be taken into consideration is that FGF21 in the circulation may undergo truncations in either the amino- or carboxy-terminal region, thereby losing part or all of its bioactivity. Given the importance of these peptide sequences for receptor binding/activation, this possibility is of great interest but has not yet been assessed. Thus, the elevated plasma levels observed in different clinical conditions in man could be characterized by the presence of non-functional FGF21. Could the administration of FGF21 in man alleviate some of the negative cardiometabolic consequences of obesity? One should bear in mind the disappointing fate of leptin as a therapeutic agent in human obesity following the finding that obese subjects display hyperleptinemia due to leptin resistance [44]. Consequently, leptin administration fails to evoke any significant weight reduction in man [45] (except for extremely rare cases with null mutations in the leptin gene [46]). Moreover, it appears clear that although FGF21 levels are slightly increased by prolonged (7 days)—but not short-term (up to 2 days)—fasting, ketosis can occur independently of plasma FGF21 level, which is in sharp contrast to what has been shown for mice.

Conclusion

While FGF21 certainly has potent effects in different animal models of type 2 diabetes, without promoting weight gain or inducing hypoglycemia, data in humans are so far lacking. The effects on human adipocytes are qualitatively different from those observed in murine fat cells and only detected at very high concentrations. No data have so far been presented on the effects of FGF21 in human islets. Ketosis in man occurs independently of plasma FGF21. Moreover, the levels of circulating FGF21 are significantly higher in subjects with obesity and type 2 diabetes. Since the physiological role of FGF21 in man is unclear, one can only speculate whether the increase in FGF21 is a protective mechanism, an indirect sign of FGF21 resistance or a phenomenon secondary to reduced plasma clearance, increased ectopic expression or the presence of truncated, inactive forms of the protein in the circulation. Nevertheless, in view of the lack of studies assessing the effect of FGF21 administration in humans, caution should be taken in extrapolating data in rodents to humans. Hopefully, even small prospective clinical studies will be able to answer whether FGF21 has effects in man and whether it will indeed constitute a putative therapeutic agent in obesity and type 2 diabetes.

Acknowledgments I would like to thank Prof Mats Rudling, Prof Bo Angelin and Niklas Mejhert for valuable discussions during the preparation of this manuscript.

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