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Review

Hormone-like fibroblast growth factors and metabolic regulation

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ABSTRACT

The family of fibroblast growth factors (FGFs) consisting now of 22 members is generally considered to control a wide range of biological functions such as development, differentiation and survival. However, research during the past decade provided substantial evidence that a so called "hormone-like" subgroup of FGFs, comprised of FGF19, FGF21 and FGF23, is involved in the regulation of diverse metabolic pathways to control glucose, lipid, bile acid, phosphate and vitamin D metabolism. The unique properties of these FGFs include predominant production of the factors in selective tissues, their abundance in the blood due to the lack of extracellular heparin-mediated sequestration, and highly specific tissue-targeted action via engagement of their respective co-receptors. The important metabolic context of FGF19, FGF21, and FGF23 actions has revealed important novel roles for FGFs and provided significant means to explore an opportunity for therapeutic targeting of these factors and their corresponding pathways.

1. Introduction

Energy homeostasis is critical for life; therefore diverse hormonal mechanisms have evolved to regulate cellular energy utilization as well as inter-tissue communication to coordinate metabolic pathways. Although regulation of cellular metabolism by major controllers such as insulin and glucagon is well-established, a new distinct subgroup of fibroblast growth factors consisting of FGF19, FGF21 and FGF23, has been recently identified to play critical roles in the metabolic network. Within FGF superfamily, these three factors share the highest level of homology with one another and propagate their effects via FGFRs, although each of them displays a diverse mechanism of receptor activation. In contrast to classical FGFs that require heparin for efficient FGFR engagement, FGF19, FGF21 and FGF23 lack conventional heparin-binding domains. This property allows these factors to elude body's vast depot of extracellular heparan sulphate proteoglycans and be readily present in the circulation, thus putatively function in an endocrine-like manner [1,2].

Despite the absence of heparin-binding domains, FGF19, FGF21 and FGF23 still require cofactors, type 1 transmembrane proteins Klotho [3] or βKlotho [4], for efficient FGFR activation [5–7]. In contrast to ubiquitously expressed FGFRs, patterns of Klotho and βKlotho expression in the body are fairly restricted. Thus, Klotho and βKlotho, not FGFRs, define tissue selectivity of action for the hormone-like FGFs and determine the distinct physiological roles of these factors: FGF19 regulates cholesterol/bile acid (BA) synthesis, FGF21 controls glucose and lipid metabolism, and FGF23 modulates phosphate/vitamin D metabolism [8–10].

2. FGF19

Transcripts of FGF19 or its mouse ortholog FGF15 are detected in brain, cartilage, skin, retina and gall bladder, but are primarily present in the gut [9,11]. The mechanism of FGFR activation by FGF19 is more complex than other endocrine FGFs. FGF21 and FGF23 do not bind to FGFR directly or function in the absence of their corresponding Klotho cofactors, and their actions are diversely specific toward βKlotho and Klotho, respectively [5,6]. In contrast, FGF19 can directly interact with FGFR4 [12–14], or it may recruit either βKlotho or Klotho as a coreceptor [7,12]. Indeed, in 293 and 3T3-L1 cells that are devoid of endogenous FGFR4, forced expression of βKlotho or Klotho is sufficient to institute FGF19 action [5,12]. Therefore, FGF19 can specifically function in a Klotho independent manner but only via FGFR4, or through multiple FGFRs in the context of Klotho coreceptors expression. FGF19 binds both βKlotho Klotho or via its C-terminus, while N-terminal part of FGF19 is involved in FGFR interaction and activation [13].

The initial evidence that FGF19 regulates body metabolism came from FGF19 transgenic mice. These animals were lean and protected against diet-induced obesity as a result of elevated energy expenditure, enhanced lipid oxidation and increased brown tissue mass [15]. The FGF19 transgenic mice also displayed lower plasma glucose, triglycerides and cholesterol, as well as reductions in the levels of the major metabolic hormones such as insulin, glucagon, leptin and IGF1. The striking metabolic phenotype in FGF19 overexpressing animals can be partly explained by changes in liver gene expression. In the

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FGF19 transgenic mice, there was a lower liver expression of enzymes that are involved in lipogenesis including acetyl CoA carboxylase 2 (ACC2) and stearoyl CoA desaturase 1 (SCD1), as well as decreased expression of CYP7A1 and CYP8B1 that is involved in cholesterol/BA synthesis. Comparable pharmacology and molecular events were recapitulated in naive animals dosed with FGF19 protein [16]. Consistent with FGF19 effect on CYP7A1 and CYP8B1 it is not surprising that this factor plays an important role in cholesterol/BA metabolism [9,17,18]. FGF19 production occurs primarily in the ileum upon bile acid-induced activation of farnesoid X receptor (FXR). When released into circulation, FGF19 reaches liver and activates FGFR4 to suppress CYP7A1/8B1 expression leading to a consequent reduction in BA production [9,17,18]. The existence of a negative FGF19 (FGF15)/FGFR4 enterohepatic feedback loop that effectively controls bile acid synthesis is additionally evidenced by elevated levels of CYP7A1 and BA in livers of both, FGF15 and FGFR4 knockout animals [9]. The existence of gut-to-liver axis of communication is further evidenced by the findings that only intestinal, but not portal nor intravenous administration of BAs inhibited CYP7A1 expression and BA production [9]. In man, blood FGF19 levels display well pronounced diurnal rhythm, with 2.5-fold increase in serum level after feeding when increase in blood bile acids occurs [19].

Consistent with the phenotypes observed in FGF19 transgenic and FGF19-treated animals, FGFR4 knockout mice on a normal diet exhibited features of metabolic syndrome that included increased adiposity, dyslipidemia, glucose intolerance, insulin resistance as well as hypercholesterolemia [20]. However, the mechanism by which FGFR4 is involved in glycemic regulation remains unknown as reconstitution of this receptor in liver, the main tissue of its expression, surprisingly did not correct the impaired glucose homeostasis in FGFR4 knockout mice. It is also unclear why FGFR4 knockout mice are protected from diet-induced fatty liver while FGF19 is potent in ameliorating hepatosteatosis [20]. Furthermore, it is intriguing that FGF19 C-terminal deletion mutant that lacks the ability to activate FGFR1, 2, and 3 due to its inability to bind βKlotho but still able to act via FGFR4, retained its full hepatic action but failed to improve glucose control in these mice [21]. Thus, the regulation of glucose homeostasis by FGF19 may require other FGFRs and/or target organs beyond liver.

It is rather remarkable that the in vivo pharmacological effects of FGF19 [15,16] and FGF21 [10,22] in rodents on glucose and lipid metabolism are nearly identical. This is likely due to the fact that both factors in part utilize a common βKlotho-dependent mechanism of FGFR activation, and suggest that FGF19 and FGF21 may share overlapping functions in metabolic regulation. However, a critical pharmacological feature that differentiates both factors is that FGF21 is nonmitogenic [5] and may even delay tumor formation [20], whereas FGF19 transgenic mice develop hepatocellular carcinomas [23]. The latter has been recently suggested to be mediated via FGFR4 activation in the liver [24].

3. FGF21

FGF21 is produced and possibly secreted in active form by peripheral tissues such as liver, pancreas, adipose, and skeletal muscle [25–28]. The expression of FGF21 in these tissues is regulated by critical pathways involved in metabolic regulation including PPARα, PPARγ [27,29,30] and Akt-dependent signaling [25], consistent with the role of FGF21 as an energy homeostasis controller.

Similar to the biology of many other metabolic hormones, FGF21 level in general circulation is responsive to the energy state of the body. Prolonged fasting and activation of PPARα, a transcriptional regulator of fasting adaptations, induce hepatic FGF21 expression and plasma FGF21 levels [29,30], supporting involvement of FGF21 in fasting-induced response. The role of PPARα in the FGF21 expression is suggested by the PPARα recognition sites on the FGF21 gene promoter [30], and the findings that PPARα knockout mice failed to respond to fasting- or PPARα agonist-induced hepatic FGF21 expression [29,30]. The PPARα-mediated FGF21 expression during fasting is presumably physiologically induced via activation of PPARα by free fatty acid, an energy substrate that is elevated when plasma glucose and insulin decline. Insulin and glucagon, two primary hormonal switches between feeding and fasting conditions have also been shown to regulate FGF21 expression and its levels in blood of rodents. In isolated primary hepatocytes, glucagon induces FGF21 expression whereas insulin has an inhibitory role [31]. In muscle and fat cells, however, insulin stimulates FGF21 expression [25]. In glucagon receptor knockout mice, fasting or exercise is unable to induce a normal level of hepatic FGF21 expression [32], supporting the notion that glucagon elevates hepatic FGF21 expression in vivo. Total lack of glucagon action in glucagon receptor knockout mice, however, also leads to the elevated FGF21 expression in liver, suggesting rather an inhibitory role of glucagon signaling in FGF21 production [33].

FGF21 mediates metabolic changes such as elevation of hepatic lipid oxidation, ketogenesis and gluconeogenesis [29,30,34] that are reminiscent of the body's adaptation to fasting state. Liver-specific FGF21 transgenic mice exhibited increased hepatic ketone production and decreased triglyceride storage, which is associated with enhanced expression of key genes involved in ketogenesis and lipid metabolism [30]. Under ketogenic diet, adenovirus-mediated knockdown of hepatic FGF21 in mice results in liver steatosis and elevated plasma triglyceride, concomitant with a reduction in the expression of genes critical for lipid metabolism [29]. However, the physiological consequences of whole-body FGF21 ablation differ significantly, based on the findings of three independently generated knockout models [34–36]. When fed a ketogenic diet for 28–30 weeks, mice lacking FGF21 developed fatty liver, hypertriglyceridemia, increased serum free fatty acids and cholesterol, impaired ketogenesis, glucose intolerance and insulin resistance [35]. However, another cohort of FGF21 null mice on 8 weeks of normal chow displayed reduced fasting plasma free fatty acids level, with no other metabolic derangement [36]. Finally, a third line of FGF21 null animals of undisclosed age and diet regimen had elevated fasting free fatty acids and mild hypoglycemia [34]. Although the discrepancies in the mice phenotypes require reconciliation through further research, it may be the results of differences in the animal age, diet, genetic background and experimental procedures. Indeed, the most striking phenotype of FGF21 knockout mice was observed in older animals fed a ketogenic diet [35], as compared to younger mice without a dietary challenge [34,36].

The tissue-specific metabolic action of FGF21 on liver, adipose tissue and pancreas is achieved by restrictive expression of βKlotho, a single-pass transmembrane protein which is essential for FGF21 signaling [5,37,38]. However, it was reported that FGF21 induced Egr-1 transcriptional activation in white and brown adipose tissues of βKlotho knockout mice [17]. The report proposed the possibility of a βKlotho-independent pathway for FGF21 signaling [17]. Nonetheless, it remains to be studied whether the finding is valid for more integrated metabolic actions of FGF21 beyond signaling, or the observation [38] is simply a reflection of secondary systemic effects. Indeed, the recent report on adipose-specific deletion of βKlotho indicates that the anti-obesity effect of FGF21 treatment is completely abolished in mice [39], supporting the critical role of βKlotho to propagate FGF21 signal. Several potential downstream mediators of the FGF21 have been reported recently. In the liver, FGF21 induces the expression of peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α), a critical transcriptional regulator of fatty acid oxidation, mitochondrial function and gluconeogenesis [34]. In cultured adipocytes, FGF21 is shown to activate AMP-activated protein kinase (AMPK), and sirtuin 1 (SIRT1) which deacetylate PGC1α to increase mitochondrial gene expression and oxidative capacity [40]. This is consistent with the elevation of whole body energy expenditure, and reduction of adiposity and hepatic steatosis in FGF21-treated mice

[22,41]. In the pancreas, FGF21 has been shown to preserve β-cell function and survival [28], and to protect pancreatic acini cells against injury [42].

While FGF21 appears to be therapeutically beneficial in rodent studies, it is however unclear how much of these findings will translate to human background as some fundamental differences in FGF21 function in rodents and humans have already been noted. For example, in mouse models FGF21 has been shown to induce lipolysis in adipocytes, hepatic ketogenesis and state of torpor [29,30]. All of the above does not seem to be the case in human background. Indeed, FGF21 has been shown to inhibit lipolysis in human adipocytes [43]. Further, in humans the induction of ketogenesis appears to be independent of FGF21, FGF21 does not reveal a pronounced diurnal rhythm, and FGF21 protein in plasma is increased only after extreme fasting condition for 7 days [44]. Finally, given that torpor is an adaptive metabolic response in small mammals, the direct extrapolation of FGF21 rodent biology to man may be largely misleading.

A critical feature of FGF21 metabolic action is its capacity to sensitize insulin action in vivo. Systemic administration of FGF21 in mice reduces plasma glucose and triglyceride, enhances insulin sensitivity, and lowers obesity and hepatosteatosis [22,29,41]. In diabetic rhesus monkeys, FGF21 treatment caused a marked amelioration in fasting plasma glucose, fructosamine, triglycerides, insulin, and glucagon [45]. Chronic FGF21 treatment of ob/ob mice improved fasting hyperglycemia via improved hepatic insulin sensitivity and decreased liver glucose production [46]. Interestingly, neither chronic nor acute FGF21 treatment altered skeletal muscle glucose uptake in the ob/ob mouse model, consistent with the lack of βKlotho in this tissue [46]. The insulin-sensitizing effects of FGF21 are apparent in animals that display the metabolic syndrome or insulin resistance, but otherwise FGF21 does not affect metabolism in normal animals. Therefore, the attractive preclinical safety features of FGF21 include absence of hypoglycemia, lipodystrophy, edema and excessive weight loss [47]. Despite the wealth of data on the therapeutic effects of this peptide, the molecular mechanism for FGF21 insulin-sensitizing effects is not fully understood but may be related to the observed elevation of insulin receptor expression in peripheral tissues [22].

Elevation of circulating FGF21 to a modest 1.5-fold to 2-fold is observed in diabetic, obese, and dyslipidemic individuals, and appeared to correlate with reduced insulin sensitivity and HDL-cholesterol as well as clinical signs of metabolic syndrome including increased adiposity, blood glucose, insulin and triglycerides [44,48,49]. These findings suggest that elevated FGF21 may manifest a possible physiological response to compensate for cellular resistance to FGF21 action in a metabolically compromised state. Consistent with the notion of FGF21 resistance, the acute effect of FGF21 on signaling and metabolic responses is indeed impaired in diet-induced obese and diabetic ob/ob mice [46,50]. It remains therefore unclear why these animals showed apparent metabolic improvement upon chronic treatment at therapeutic doses of FGF21 [22,41,46], or whether prolonged FGF21 therapy would eventually lead to desensitization of this therapeutic in man.

4. FGF23

Since FGF23 gene was discovered mutated in patients with autosomal dominant hypophosphatemic rickets, FGF23 has been identified as a critical hormonal regulator of phosphate homeostasis [8]. In this genetic disorder, the mutation in a subtilisin-like proprotein convertase cleavage site in FGF23 renders the protein resistant to proteolysis, leading to increased half-life of active FGF23 in blood and eventual hypophosphatemia [8]. Conversely, attenuation of FGF23 signaling caused by missense mutations in the gene leads to familial tumoral calcinosis with hyperphosphatemia calcinosis in humans [51]. Elevated FGF23 levels are also found in patients with X-linked hypophosphatemic rickets osteomalacia [52]. In patients with chronic renal disease, FGF23 is increased by 100- to 1000-fold, and its circulating levels appeared to inversely correlate with renal function [53].

While FGF23 expression is detected in multiple tissues [54], the protein is primarily produced in the bone [55] from which it circulates to regulate phosphate and vitamin D metabolism in the kidney. In FGF23-transgenic mice, renal phosphate reabsorption is decreased [56,57], caused by lower levels of sodium/phosphate cotransporter type IIa and IIc in the kidney [58,59]. FGF23 also reduces plasma phosphate concentration by suppressing the CYP27B1/CYP24 pathway-mediated production of 1, 25-dihyroxyvitamin D level, the active form of vitamin D which facilitates intestinal phosphate absorption [58,60]. In corroboration with these findings, animals with genetic ablation of FGF23 in mice display high plasma phosphate, calcium and 1, 25-dihyroxyvitamin D levels, impaired bone mineralization, calcification of soft tissues including kidney and vasculature, and shortened life-span [61]. Taken together, the human and mouse genetic and pathophysiologic data highlight the primary function of FGF23 as a hormone of phosphate and vitamin D metabolism.

The ability of FGF23 to target kidney via bone-kidney axis of cross-tissue communication is determined by high renal expression of its coreceptor Klotho that is enriched in distal convoluted tubules where the early response to FGF23 takes place. Nonetheless, FGF23-regulated phosphate homeostasis and vitamin D metabolism are localized in the far distant proximal tubules, suggesting the presence of a distal-to-proximal communication in FGF23 renal action that mechanistically may involve additional molecular partners such as soluble Klotho. FGF23 binds to Klotho via its C-terminus and this interaction is required for activation of FGFR [6,62]. In a mutant mouse model of hypophosphatemia (Hyp mice), genetic inactivation of Klotho is able to induce hyperphosphatemia even in the presence of high circulating FGF23 levels [63]. The requirement of Klotho in FGF23-mediated phosphate homeostasis is further supported by the findings that FGF23 treatment failed to lower serum phosphate in Klotho knockout mice and FGF23/Klotho double knockout mice [63]. Although FGFR1c/Klotho complex has been proposed to constitute a canonical renal FGF23 receptor [59], FGF23 may mediate its signal via several other FGFRs [6]. Therefore, FGF23 receptor specificity is determined by Klotho, consistent with the absence of renal phenotype in animals with ablation of specific FGFR isoforms [64].

FGF23 also acts directly on the Klotho-expressing parathyroid gland to inhibit the secretion parathyroid hormone (PTH) which increases vitamin D activation and elevates intestinal phosphate absorption. Interestingly, elevated levels of FGF23 and Klotho are associated with hyperparathyroidism [65,66], which may be due to altered metabolism of vitamin D or direct regulation of FGF23 expression by PTH. Knockout mouse models provided evidence that FGF23 is required for skeletal mineralization and normal bone development [61,67], and the action is independent of its serum phosphate functions [67]. Given the expression of Klotho in the reproductive organs, brain, choroid plexus, and pituitary, there may be physiological role of FGF23 in these tissues which remains to be demonstrated [68].

5. Conclusions

FGF19, FGF21 and FGF23 are present systemically in the blood and may act on respective target tissues through tissue-specific expression of their Klotho coreceptors. These hormone-like FGFs hold the major promise for therapeutic development among other FGF family members. FGF21 is leading in clinical development owing to its disease-modifying pharmacology and remarkable safety profile in animal studies that is yet to be confirmed in humans [69]. Although FGF19 is also an attractive drug candidate, the development of hepatocellular carcinoma in aged FGF19 transgenic mice [23] positions this factor as a drug target for cancer [70,71] rather than a viable therapeutic for the treatment of chronic metabolic disorders. Targeting FGF23 may have a therapeutic benefit for the treatment of

hypophosphatemic diseases as its role in the pathogenesis of human is established [8,51,52], and neutralizing antibodies against FGF23 ameliorate phosphate and vitamin D metabolism in mice [72]. The elevation of FGF23 in chronic renal disease points to the possibility of this protein to be involved in the pathophysiology of this disease in man [53]. However, it still remains uncertain whether FGF23 action needs to be pharmacologically inhibited or activated given the positive correlations between serum FGF23 levels with poor prognosis in patients with end-stage renal disease [73]. The role of FGF23 in renal diseases therefore requires further clarification. Given the broad spectrum effects endocrine-like FGFs have on metabolic regulation, the pharmacological significance of FGF19, FGF21, and FGF23-based therapeutics encompassing recombinant proteins and/or neutralizing antibodies need to be ultimately explored in the future.

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