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MINIREVIEW

Fibroblast Growth Factor 21: A Novel Metabolic Regulator With Potential Therapeutic Properties in Obesity/Type 2 Diabetes Mellitus

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Summary

Fibroblast growth factor 21 (FGF21) is a novel metabolic regulator produced primarily by the liver that exerts potent antidiabetic and lipid-lowering effects in animal models of obesity and type 2 diabetes mellitus. This hormone contributes to body weight regulation and is strongly involved in the response to nutritional deprivation and ketogenic state in mice. The principal sites of metabolic actions of FGF21 are adipose tissue, liver and pancreas. Experimental studies have shown marked improvements in diabetes compensation and dyslipidemia after FGF21 administration in diabetic mice and primates. Positive metabolic actions of FGF21 without the presence of apparent side effects make this factor a hot candidate to treat type 2 diabetes and accompanying metabolic diseases. The aim of this review is to summarize the current knowledge about the metabolic effects of FGF21 including some preliminary data on changes of its levels in humans with a special emphasis on its therapeutic potential in type 2 diabetes mellitus.

Key words

Fibroblast growth factor 21 • Fasting • Obesity • Type 2 diabetes • Peroxisome proliferator-activated receptor • Glucose uptake

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Introduction

Rapidly increasing prevalence of obesity and its comorbidities commonly referred to as metabolic or insulin resistance syndrome in virtually all developed countries worldwide has stimulated an intensive research exploring the etiopathogenesis of these diseases and the possibilities of its prevention and/or treatment. Adipose tissue is now recognized as an important endocrine organ producing numerous factors involved in energy homeostasis and metabolic regulations (Haluzík *et al.* 2004, Havel 2002, Housa *et al.* 2006). In addition to the adipose tissue-derived hormones, also the muscle- and the liver-produced factors are intensively studied as potential regulators of energy homeostasis. Fibroblast growth factor-21 (FGF21) produced predominantly by the liver and, to a lesser extent, also by adipose tissue, represents an example of hepato-adipokine with recently discovered effects on glucose and lipid homeostasis. Positive metabolic effects observed after FGF21 administration in diabetic rodents and monkeys make it an interesting candidate for novel therapeutic agent to treat patients with obesity, type 2 diabetes mellitus and/or other features of metabolic syndrome.

Family of fibroblast growth factors

Fibroblast growth factors (FGFs) are hormonal factors with diverse biological functions. Human FGF family includes 22 members that are divided into seven subfamilies based on phylogeny and sequence identity (Ornitz and Itoh 2001, Itoh and Ornitz 2004). While most of FGFs act as local regulators of cell growth and differentiation, recent studies indicated that FGF19 subfamily members including FGF15/19, FGF21 and FGF23 exert important metabolic effects by an endocrine fashion.

The members of FGF19 subfamily regulate diverse physiological processes that are not affected by classical FGFs. The wide variety of metabolic activities of these endocrine factors include the regulation of the bile acid, carbohydrate and lipid metabolism as well as phosphate, calcium and vitamin D homeostasis (Tomlinson *et al.* 2002, Holt *et al.* 2003, Shimada *et al.* 2004, Kharitonenkov *et al.* 2005, Inagaki *et al.* 2005, Lundasen *et al.* 2006).

FGF21 was originally isolated from mouse embryos. FGF21 mRNA was most abundantly expressed in the liver, and to lesser extent in the thymus (Nishimura *et al.* 2000). Human FGF21 is highly identical (approximately 75 % amino acid identity) to mouse FGF21. Among human FGF family members, FGF21 is the most similar (approximately 35 % amino acid identity) to FGF19 (Nishimura *et al.* 2000). FGF21 is free of the proliferative and tumorigenic effects (Kharitonenkov *et al.* 2005, Huang *et al.* 2006, Wente *et al.* 2006) that are typical for majority of the members of FGF family (Ornitz and Itoh 2001, Nicholes *et al.* 2002, Eswarakumar *et al.* 2005).

Special features of FGF21 signaling

FGFs exert their biological actions by at least five distinct subfamilies of high-affinity FGF receptors (FGFRs) that belong to a family of tyrosine kinase receptors (Mohammadi *et al.* 2005). Activation of the FGFRs induces stimulation of divergent downstream pathways mediated by FRS-2, MAPK, SHP-2, PI3K and $p70^{S6K}$, Raf, Stat and other signaling molecules (Pelech *et al.* 1986, Carballada *et al.* 2001, Deo *et al.* 2002, Kontaridis *et al.* 2002).

FGF21 is a typical FGF molecule with respect to its ability to stimulate FGFR substrate 2α (FRS2α) phosphorylation and activation of ERK1/2 and Akt signaling pathways (Ibrahimi *et al.* 2004, Kharitonenkov *et al.* 2005, Mohammadi *et al.* 2005, Zhang *et al.* 2006). However, efforts to demonstrate a direct interaction between FGFRs and FGF21 have failed so far (Kharitonenkov *et al.* 2005). This observation suggested that a cofactor(s) might be necessary for FGF21 to activate FGF signaling in the tissue. βKlotho, a homologous single-pass transmembrane protein that binds to specific FGFRs, was identified as a cofactor essential for FGF21 activity (Ogawa *et al.* 2007, Suzuki *et al.* 2008). βKlotho brings about binding affinity that is just sufficient to produce a metabolic but not mitogenic response. The expression of βKlotho, in combination with particular FGFR isoforms, determines the tissue-specific metabolic activities of FGF21 (Kurosu *et al.* 2007). βKlotho is expressed in adipose tissue, liver, and pancreas (Ito *et al.* 2000) and thus the major actions of FGF21 are located to these tissues. Cells lacking βKlotho do not respond to FGF21 and the introduction of βKlotho to these cells confers FGF21-responsiveness and recapitulates the entire scope of FGF21 signaling observed in naturally responsive cells (Kharitonenkov *et al.* 2007).

Another important regulatory factor of FGF21-FGFR binding and dimerization is heparan sulfate (Mohammadi *et al.* 2005). In contrast to other FGFs, which require heparan sulfate for high affinity receptor binding and activation, FGF21 binds heparan sulfate with low affinity (Goetz *et al.* 2007). The weak heparan sulfate binding affinity keeps FGF21 from being captured in extracellular matrices and thus to function as an endocrine factor (Mohammadi *et al.* 2005). In addition, FGF21 contains intramolecular disulfide bonds, which may increase its stability in plasma and allow it to function as a hormone (Harmer *et al.* 2004).

Hepatic action of FGF21

Hepatic expression and circulating levels of FGF21 in mice were strongly induced by fasting (12-24 hours) and were rapidly suppressed by refeeding (Badman *et al.* 2007, Inagaki *et al.* 2007, Lundasen *et al.* 2007). In fact, the expression of FGF21 in the liver was very low in the fed state. FGF21 secreted upon fasting from the liver then acted on adipose tissue to induce metabolic adaptation to fasting. Specifically, FGF21 stimulated lipolysis in adipocytes with subsequent conversion of released fatty acids to ketones in the liver (Badman *et al.* 2007, Inagaki *et al.* 2007). In the study comparing FGF21 overexpressing mice with wild-type animals FGF21 overexpression reduced physical activity and promoted torpor, a short-term hibernation-like state of regulated hypothermia that conserves energy in small mammals (Inagaki *et al.* 2007).

FGF21 was markedly induced in the liver of ketogenic diet-fed mice (78.9 % fat, 9.5 % protein, 0.76 % carbohydrate) compared with animals fed F6 rodent diet (6.5 % fat, 24.8 % protein, 39.7 % carbohydrate). The *in vivo* suppression of hepatic FGF21 expression in ketogenic diet-fed mice with shRNA-expressing adenovirus caused fatty liver, lipemia, and reduced serum ketones. These effects were at least in part mediated by altered expression of the key genes

governing lipid and ketone metabolism. Hence, the induction of FGF21 was required for the normal activation of hepatic lipid oxidation, triglyceride clearance, and ketogenesis in response to nutritional challenges (Badman *et al.* 2007).

Multiple findings have indicated that the expression of FGF21 is at least in part downstream of peroxisome proliferator-activated receptor-α (PPARα) (Badman *et al.* 2007, Inagaki *et al.* 2007, Lundasen *et al.* 2007). Treatment of mice and human primary hepatocytes with the PPARα agonist Wy-14,643 markedly induced hepatic mRNA expression of FGF21 (Lundasen *et al.* 2007). In contrast, FGF21 mRNA was low in PPARα-deficient mice, and 24-h fasting or treatment with Wy-14,643 in these mice did not change its expression. Obese leptin-deficient *ob/ob* mice with markedly increased hepatic PPARα levels displayed a 12-fold increased hepatic FGF21 mRNA levels (Memon *et al.* 2000). Tentative PPARα responsive elements were present in the promoter regions of both mouse and human FGF21 genes (Lundasen *et al.* 2007).

Stimulation of PPARα receptors by its exogenous ligands fibrates and FGF21 administration exert several similar metabolic effects. For example, both fibrates and FGF21 administration lowered LDL-cholesterol, raised HDL-cholesterol, and improved insulin sensitivity in dyslipidemic rhesus monkeys (Winegar *et al.* 2001, Kharitonenkov *et al.* 2007). Furthermore, both PPARα agonists and FGF21 prevented diet-induced obesity and enhanced insulin sensitivity in rodents (Guerre-Millo *et al.* 2000, Chou *et al.* 2002, Kharitonenkov *et al.* 2005). These overlapping effects suggest that FGF21 contributes to many of the actions of PPARα agonists. The finding that FGF21 is induced by PPARα in human hepatocytes raises the intriguing possibility that FGF21 mediates some of the therapeutic actions of the PPARα agonists – fibrates – that are used as hypolipidemic drugs in humans.

Actions of FGF21 in adipose tissue

Although liver is a candidate for the paracrine and autocrine actions of FGF21, the most dramatic effects of FGF21 have been found in adipose tissue where neither FGF21 nor FGFR4 are significantly expressed compared to high expression in the liver (Kharitonenkov *et al.* 2005, Moyers *et al.* 2007).

FGF21 is a potent stimulator of glucose uptake in mouse 3T3-L1 adipocytes as well as in differentiated human adipocytes (Kharitonenkov *et al.* 2005). The 72-h combined treatment of 3T3-L1 adipocytes with FGF21 and the PPAR-γ agonist rosiglitazone in markedly increased the expression of the GLUT1 glucose transporter and synergistically stimulated glucose transport (Moyers *et al.* 2007). Recent studies have shown that FGF21 gene is in addition to its stimulation by PPARα a direct target gene of PPARγ (Wang *et al.* 2008).

FGF21 effects are insulin-independent and additive to insulin-induced metabolic effects. While insulin works in a rapid manner increasing glucose uptake within minutes, FGF21 activity is very likely mediated through the changes in gene expression. FGF21 stimulatory effect on glucose uptake in adipocytes thus occurs as late as within several hours. In contrast to insulin, which functions through GLUT4 translocation from the intracellular pool to plasma membrane (Shepherd and Kahn 1999), FGF21 appears to upregulate the cellular GLUT1 expression (Kharitonenkov *et al.* 2005, Ogawa *et al.* 2007).

FGF21-overexpressing transgenic mice were resistant to diet-induced obesity. The administration of FGF21 to obese leptin-deficient *ob/ob* and leptin receptor-deficient *db/db* mice and obese ZDF rats significantly lowered blood glucose and triglycerides, decreased fasting insulin levels and improved glucose clearance during an oral glucose tolerance test. FGF21 did not affect food intake or body weight/composition of diabetic or lean mice and rats over the course of 2 weeks of administration. Importantly, FGF21 did not induce mitogenicity, hypoglycemia, or weight gain at any dose tested in diabetic or healthy animals or when overexpressed in transgenic mice (Kharitonenkov *et al.* 2005).

The administration of FGF21 to diabetic rhesus monkeys for 6 weeks dramatically reduced fasting plasma glucose, fructosamine, triglyceride, insulin and glucagone levels. Importantly, hypoglycemia was not observed during the study despite of significant glucose-lowering effects. FGF21 administration also significantly lowered LDL-cholesterol and increased HDL-cholesterol and, in contrast to mice (Kharitonenkov *et al.* 2005), slightly but significantly decreased body weight (Kharitonenkov *et al.* 2007).

Action of FGF21 in pancreas

FGF21 was detected in human, rat and mouse pancreatic islets as well as in purified rat β-cells and INS-1E cells (Wente *et al.* 2006). In pancreatic islets

isolated from healthy rats, FGF21 increased insulin mRNA and protein levels but did not potentiate glucose-induced insulin secretion. In islets isolated from diabetic rodents, FGF21 treatment increased islet insulin content and glucose-induced insulin secretion. Short-term treatment of normal or diabetic *db/db* mice with FGF21 lowered plasma insulin levels and improved glucose clearance during oral glucose tolerance test. The ability of FGF21 to increase insulin biosynthesis and promote β-cell survival without inducing mitogenicity resulted in a dramatic reduction in glucose levels and increased number of islets and β-cells in *db/db* mice after long-term (constant infusion for 8 weeks) administration of FGF21 (Wente *et al.* 2006).

FGF21 production and secretion might be related to the metabolic status of the β-cells. Because FGF21 has a short half-life (Kharitonenkov *et al.* 2005), local release of FGF21 during high metabolic demand could represent a physiologically important mechanism to maintain β-cell performance and enhance insulin effects *via* inhibition of glucagon release from pancreatic α-cells and stimulation of glucose uptake in adipocytes (Kharitonenkov *et al.* 2005).

Circulating FGF21 levels in humans

At present, very little is known about changes of serum FGF21 levels in humans. Two cross-sectional studies described increased FGF21 levels in patients with obesity and type 2 diabetes mellitus (Zhang *et al.* 2008, Chen *et al*. 2008). Furthermore, FGF21 levels correlated with several features of metabolic syndrome. Interestingly, FGF21 mRNA expression was also detected in subcutaneous adipose tissue and it positively correlated with circulating FGF21 levels (Zhang *et al.* 2008). Our preliminary data have shown that chronic malnutrition in patients with anorexia nervosa significantly decreases serum FGF21 levels relative to healthy normal-weight women (Dostálová *et al*. 2008).

Conclusions and Perspectives

FGF21 is a novel metabolic factor produced predominatly by the liver that exerts the unique role in the regulation of carbohydrate and lipid metabolism in the liver, adipose tissue and pancreas. FGF21 is tightly nutritionally-regulated in animal models and might represent a missing link in the adaptive response to long-term nutritional deprivation. FGF21 exhibits the therapeutic characteristics essential for an effective treatment of several components of metabolic syndrome including diabetes, dyslipidemia and obesity. Its administration in diabetic primates led to a sustainable improvement in glucose control without occurrence of hypoglycemia, a significant improvement of diabetic dyslipidemia and a mild weight loss without any significant side effects (Kharitonenkov *et al.* 2007). All of these characteristics make FGF21 a hot candidate for the treatment of patients with obesity and type 2 diabetes mellitus. Future studies are highly required to unravel whether the unique metabolic effects of FGF21 shown in rodents and primates are also present in humans.

Conflict of Interest

There is no conflict of interest.

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