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FGF21

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Version 1.0, December 15, 2011 [DOI: 10.3998/panc.2011.35]

Gene symbols: Fgf21

1. General Information

FGF21 belongs to the FGF sub family of endocrine factors (21) that also includes FGF15 (FGF19 in rodents) and FGF23. These factors, unlike other FGFs, are characterized by the absence of the conventional FGF heparin-binding domain (6, 11) making them capable of diffusing away from the tissue of origin, and thus acting as hormones. Each of these factors plays an important role in cell metabolism. Recent studies in humans, rodents and chimpanzees correlated increased circulating FGF21 to decreased obesity and increased sensitivity to insulin, thereby positively affecting cell metabolism (5, 10, 16). In addition, FGF21 levels are elevated during nutrient deprivation and promote ketogenesis as an alternative energy source (2). The initial link connecting FGF21 to metabolism was provided by Kharitonenkov et al (15) who demonstrated that FGF21 stimulated glucose uptake both in mouse and human adipocytes. Subsequent studies showed that FGF21 administration not only led to reduced plasma glucose, insulin, and lipid concentrations, but also improved hepatic and prepheral insulin sensitivity (4, 15, 31).

FGF21 is highly expressed in liver, pancreas, and white adipose tissue (WAT) (13, 20, 21). FGF21 executes its biological effects through cell-surface receptors composed of the classic FGF receptors, which are tyrosine kinases, in complex with β-klotho (14, 17, 22, 24). Moreover, the tissues expressing FGF21 also abundantly express β-klotho, further strengthening the possibility that FGF21 mediates its effects through β-klotho (12, 22). However, some recent evidence suggests that FGF21 signals can be transduced in the absence of β-klotho in vivo, suggesting that an alternative receptor may exist for FGF21 (25).

Fgf21 is upregulated by starvation and increased fat consumption, suggesting a complex role in metabolism. In these situations, *Fgf21* expression appears to be mediated mostly by nuclear receptors. In WAT, peroxisome proliferator-activated receptor (PPAR) γ robustly activates *Fgf21* expression (20, 29). PPARα, on the other hand, regulates *Fgf21* expression in liver tissue (10). This effect is direct as analysis of the *Fgf21* promoter reveals the presence of a PPAR binding site that, when mutated, causes the loss of *Fgf21* gene activation. Various reports linked FGF21 expression in liver to diverse regulatory factors, such as carbohydrate responsive element-binding protein (ChREBP) (9), the retinoic acid receptor-related orphan receptor (ROR) α (30) and PPARα (2, 10, 19) and negative regulation by liver X

receptor (LXR) (23), circadian output protein, E4BP4 (26), and NFE2-related factor 2 (Nrf2) (3). The regulation of *Fgf21* by various transcriptional regulators and the presence of regulatory elements in *Fgf21* promoter imparts molecular flexibility to FGF21 expression not only during fed but also during fasting conditions (27). However, there is still limited knowledge on the factors that regulate FGF21 expression under normal conditions or how it is regulated in tissues other than liver and WAT.

2. FGF21 in the Pancreas

Fgf21 expression is robustly activated upon injury in the exocrine pancreas. FGF21 appears to be an immediate response gene to pancreatic injury as expression is elevated within 15 minute of initiating injury in cerulein-induced pancreatitis, and increases 100-fold within 1 hour of initial cerulein injection. Similar responses were observed in L-arginine models of injury (13).

A recent report also observed an increased expression of *Fgf21* within 1 hour along with other stress related molecules when mice are fed with protease inhibitor, which leads to increased endogenous CCK release and results in pancreatic growth (7). This suggests a potential role of FGF21 in adaptive growth of pancreas. Therefore, it may be that increases in Fgf21 expression during pancreatitis are related to an adaptive role for acinar cells. Interestingly, the target of FGF21 in these situations appears to be acinar cells. Elevated levels of active ERK1/2 were observed within minutes of stimulating primary acinar cells or AR42J acinar cells with purified FGF21 protein (13).

The role of FGF21 in pancreatitis was further explored in genetically modified mouse lines that harbor a targeted deletion of the *Fgf21* gene (*Fgf21*$^{-/-}$) or that maintain high levels of circulating FGF21 (*ApoE-FGF21Tg*). The severity of cerulein-induced pancreatitis was inversely correlated to the amount of FGF21 expressed in the pancreas of these animals, based on the activation of pancreatic stellate cells and tissue fibrosis. In addition, the expression of *Early growth response 1* (*Egr1*), an immediate response gene that is enhanced by cell stress is also inversely correlated to the presence of *Fgf21.* Targeted ablation of *Egr1* reduces the severity of cerulein-induced pancreatitis. Combined, these results suggest that FGF21 may act as defense molecule to protect pancreas against the pancreatic injury (13).

3. Future questions

While studies on the regulation of Fgf21 in liver and WAT have identified several important regulatory factors, our knowledge on how Fgf21 is regulated in pancreas remains elusive. PPARγ, which is activated during pancreatitis (23) and regulates *Fgf21* expression in adipocytes, lacks the ability to activate Fgf21 in primary acinar cells (13). Therefore future research is required to delineate factors and mechanisms that regulate *Fgf21* expression in pancreas. Moreover, while it has been shown that FGF21 signals through β-klotho and activates ERK1/2, the details of the underlying signaling pathways that limit pancreatic injury are yet to be unraveled. A recent report linked an SNP in 3'UTR of *Fgf21* to obesity (32). Owing to its important protective role during pancreatitis, it is imperative to scrutinize if there is any SNP in *Fgf21* that might be associated with pancreatitis or other pancreatic anomalies.

4. Tools to study FGF21

a. cDNA clones

Mammalian expression vector for human Fgf21 is available in which *Fgf21* cDNA was cloned in pcDNA3.1 vector (33).

b. Antibodies

Antibodies raised against FGF21 are commercially available from various suppliers. We

have used anti-FGF21 (R & D Systems, goat polyclonal) in our lab for western blotting.

c. Recombinant FGF21

In order to see the impact on cultured cells and in vivo administration, recombinant human FGF21 has been purified in the *Escherichia coli* using a pET30a vector (15).

d. Mouse lines

here are at least 4 *Fgf21* knockout and transgenic mouse lines available. Both Fgf21 knockout and transgenic mice are viable and fertile. Mouse line with targeted deletion of *Fgf21* using pGTN29 vector has been generated which replaced part of exon 1, all of exon 2 and 5' region of exon 3 of *Fgf21* with neomycin resistance gene (1) and is available from Eleftheria Maratos-Flier. Another Fgf21 knockout line has been described in which part of exon1 and all of exons 2 and 3 have been replaced with IRES-LacZ-polyA/PGK-neo cassette (8). This line is available from Nobuyuki Itoh. To specifically knockdown *Fgf21* in mice, adenovirus vectors encoding shRNA against *Fgf21* has also been described (2).

Transgenic mouse line expressing human *Fgf21* cDNA under the control of apolipoprotein E (ApoE) promoter has also been described (15) and is available from Alexei Kharitonenkov. Another transgenic mouse line expressing mouse *Fgf21* coding sequence under ApoE promoter has also been independently generated (10) and is available from Steven Kliewer.

e. siRNA

To specifically knockdown Fgf21 in cultured cells, siRNA sequences have been described (18). siRNAs are also available from various commercial suppliers.

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Acknowledgement: I am thankful to Dr. Christopher Pin for the inspiration and discussion.

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