MINI REVIEW

Hormone-like (endocrine) Fgfs: their evolutionary history and roles in development, metabolism, and disease

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Abstract Fibroblast growth factors (Fgfs) are proteins with diverse functions in development, repair, and metabolism. The human *Fgf* gene family with 22 members can be classified into three groups, canonical, intracellular, and hormone-like *Fgf* genes. In contrast to canonical and intracellular *Fgf*s identified in invertebrates and vertebrates, hormone-like *Fgf*s, *Fgf15/19*, *Fgf21*, and *Fgf23*, are vertebrate-specific. The ancestral gene of hormone-like *Fgf*s was generated from the ancestral gene of canonical *Fgf*s by gene duplication early in vertebrate evolution. Later, *Fgf15/19*, *Fgf21*, and *Fgf23* were generated from the ancestral gene by genome duplication events. Canonical Fgfs act as autocrine/paracrine factors in an Fgf receptor (Fgfr)-dependent manner. In contrast, hormone-like Fgfs act as endocrine factors in an Fgfr-dependent manner. Canonical Fgfs have a heparin-binding site necessary for the stable binding of Fgfrs and local signaling. In contrast, hormone-like *Fgf*s acquired endocrine functions by reducing their heparin-binding affinity during their evolution. Fgf15/19 and Fgf23 require βKlotho and αKlotho as cofactors, respectively. However, Fgf21 might physiologically require neither. Hormone-like Fgfs play roles in metabolism at postnatal stages, although they also play roles in development at embryonic stages. Fgf15/19 regulates bile acid metabolism in the liver. Fgf21 regulates lipid metabolism in the white adipose tissue. Fgf23 regulates serum phosphate and active vitamin D levels. Fgf23 signaling disorders caused by hereditary diseases or tumors result in metabolic disorders. In addition, serum Fgf19 or Fgf21 levels are significantly increased by metabolic disorders. Hormone-like Fgfs are newly emerging and quite unique in their evolution and function.

Keywords Fgf · Endocrine · Evolution · Metabolism · Disease

Introduction

Fibroblast growth factors (Fgfs) are proteins with diverse functions in development, repair, and metabolism. The prototypic Fgfs, Fgf1 (acidic Fgf) and Fgf2 (basic Fgf), which were originally isolated as mitogens for fibroblasts from the brain and pituitary, are widely expressed in developing and adult tissues. Fgfs have since been isolated as growth factors for cultured cells or identified by homology-based polymerase chain reaction (PCR) and/or homology-based searches in DNA databases. A few Fgfs have also been identified in human hereditary diseases or human/mouse tumors (Itoh 2007; Itoh and Ornitz 2008; Krejci et al. 2009; Beenken and Mohammadi 2009).

The human *Fgf* gene family comprises 22 members including *Fgf1–Fgf23*. *Fgf15* has not been identified in humans. Although the human genome has been completely elucidated, no other member of the human *Fgf* family has been identified. Human Fgfs, which comprise ~150–300 amino acids, have a conserved ~120-residue core with ~30–60% identity. Human *Fgf* genes were generated partly by gene duplications early in metazoan evolution but mainly by two large-scale genome duplication events early in vertebrate evolution (Itoh and Ornitz 2004).

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The mouse is a widely used mammal model for studying gene functions. The mouse *Fgf* family also comprises 22 members including *Fgf1–Fgf23*. *Fgf19* has not been identified in mice. *Fgf15* and *Fgf19* are orthologs in vertebrates (Itoh and Ornitz 2004). The zebrafish is also a widely used vertebrate model for studying gene functions. The zebrafish *Fgf* family comprises 28 members with several paralogs generated by an additional genome duplication event shortly after the teleost radiation (Itoh and Konishi 2007; The Zebrafish Model Organism Databases, http://zfin.org).

The *Fgf* family can be divided into seven subfamilies by phylogenetic and gene locus analyses. These subfamilies can also be classified into three groups, the intracellular *Fgf11/12/13/14* subfamily, the hormone-like (endocrine) *Fgf19/21/23* subfamily, and the canonical *Fgf* subfamily comprising the *Fgf1/2/5*, *Fgf3/4/6*, *Fgf7/10/22*, *Fgf8/17/18*, and *Fgf9/16/20* subfamilies, by their action mechanisms (Itoh and Ornitz 2004, 2008). Canonical Fgfs mediate their biological responses as extracellular proteins by binding to and activating cell surface tyrosine kinase Fgf receptors (Fgfrs) with heparin/heparan sulfate as a cofactor. They act as local signaling molecules in an autocrine/paracrine manner. In the development of multicellular organisms, various signaling pathways are activated in a highly coordinated manner to ensure proper morphogenesis. Secreted signaling molecules such as canonical Fgfs, bone morphogenic proteins (Bmps), Wnts, and Hedgehogs play crucial roles in development by influencing the intracellular signaling events of their neighbors from a distance (Itoh and Ornitz 2004, 2008; Thisse and Thisse 2005; Mikels and Nusse 2006). In contrast, intracellular Fgfs, Fgf11–Fgf14, act as intracellular signaling molecules in an Fgfr-independent manner. They interact with intracellular domains of voltage-gate sodium channels and with a neuronal mitogen-activated protein kinase scaffold protein, islet-brain-2, and mainly play roles in neuronal functions at postnatal stages (Goldfarb 2005; Goldfarb et al. 2007; Xiao et al. 2007).

Canonical and intracellular *Fgf*s have been identified in invertebrates and vertebrates. However, hormone-like *Fgf*s, *Fgf15/19*, *Fgf21*, and *Fgf23*, have been identified in vertebrates but not invertebrates (Itoh and Ornitz 2004; Itoh and Konishi 2007). Hormone-like Fgfs also mainly mediate their biological responses in an Fgfr-dependent manner. However, they bind to Fgfrs with quite low affinity even in the presence of heparin/heparan sulfate (Zhang et al. 2006). Hormone-like Fgfs are unique in their evolution and function. Several excellent reviews on the roles of hormone-like Fgfs in metabolism have been published (Fukumoto 2009; Kurosu and Kuro-o 2009; Kharitonenkov 2009; Razzaque 2009). This article provides a review of hormone-like Fgfs, from their evolutionary history to their roles in development, metabolism, and disease.

Identification of hormone-like Fgf genes

Fgf15/19

Pbx1 is a homeodomain transcription factor that has the ability to form heterodimers with homeodomain proteins encoded by the homeotic selector (*Hox*) gene complexes. In pre B cell leukemias, Pbx1 is converted into a strong transactivator by fusion to the activation domain of the bHLH transcription factor E2A. The E2A-Pbx1 fusion protein should therefore activate the transcription of genes normally regulated by Pbx1. *Fgf15* was originally identified as a downstream target of the chimeric homeodomain oncoprotein E2A-Pbx1 in mice (McWhirter et al. 1997).

A new human *Fgf* was identified by a DNA database search with the conserved amino acid core of known Fgfs. As the human Fgf is not highly similar (less than 55% amino acid identity) to any other known Fgfs, it was named Fgf19 (Nishimura et al. 1999; Xie et al. 1999). However, the *Fgf19* gene was later found to be the human ortholog of mouse *Fgf15* based on conserved synteny around their loci (Itoh and Ornitz 2004). Only the mouse and rat orthologs were named *Fgf15*. The orthologs in other vertebrates were named *Fgf19*.

Fgf21

Fgf21 was originally identified in mice and humans by homology-based PCR with the conserved amino acid core of human Fgf19 (Nishimura et al. 2000). Later, human Fgf21 was identified as a stimulator of glucose uptake in mouse 3 T3-L1 adipocytes by a glucose uptake assay to search for novel proteins with therapeutic potential to treat diabetes mellitus (Kharitonenkov et al. 2005). *Fgf21* was also identified as a gene inducible by fasting or a high-fat, low-carbohydrate ketogenic diet in mice (Inagaki et al. 2007; Badman et al. 2007)

Fgf23

Fgf23 was originally identified in mice and humans by a DNA database search with the conserved amino acid core of mouse Fgf15 (Yamashita et al. 2000). Human *Fgf23* was simultaneously identified as a gene responsible for autosomal dominant hypophosphatemic rickets (ADHR) (ADHR consortium 2000). ADHR is characterized by low serum phosphate levels, rickets, osteomalacia, lower extremity deformities, short stature, bone pain and dental abscesses. Later, human *Fgf23* was identified as a causative humoral protein for human tumor-induced osteomalacia (TIO) (Shimada et al. 2001). TIO is a paraneoplasitc disease characterized by hypophosphatemia caused by renal phosphate wasting.

Identification of the Fgf19/21/23 gene subfamily

Although Fgf15/19, Fgf21, and Fgf23 are not very similar (~22–35% amino acid identity), phylogenetic and gene locus analyses indicate that they are all members of the *Fgf19/21/23* subfamily (Itoh and Ornitz 2004, 2008).

Evolutionary history of hormone-like Fgf genes in vertebrates

The Fgf signaling system has been conserved throughout metazoan evolution. Most ancestral genes of the human *Fgf* subfamilies have been identified in the ascidian, *Ciona intestinalis*. Ascidians belong to the subphylum Urochordata, the earliest branch in the phylum Chordata (Satou et al. 2002). These results indicate that most ancestral genes of the *Fgf* subfamilies were generated by gene duplication events early in metazoan evolution. The evolutionary history of the *Fgf* family has been proposed (Fig. 1) (Itoh and Ornitz 2004, 2008). An ancestral gene of the intracellular *Fgf* subfamily, *Fgf13-like*, is an ancestral gene of the *Fgf* family. An ancestral gene of the canonical *Fgf* subfamily, *Fgf4-like*, was generated from *Fgf13-like* by gene duplication during the early stages of metazoan evolution. In contrast, no ancestral gene of the hormone-like *Fgf19/21/23* subfamily has been identified in *Ciona intestinalis*. The ancestral gene, *Fgf19-like*, was generated from *Fgf4-like* by local gene duplication early in vertebrate evolution. Later, *Fgf19*, *Fgf21*, and *Fgf23* were generated from *Fgf19-like* by two large-scale genome duplication events (Itoh and Ornitz 2004; Itoh and Ornitz 2008). The evolutionary history suggests that hormone-like *Fgf*s are vertebrate-specific.

Fgf15/19 and *Fgf23* have been identified in all vertebrates examined including teleosts, amphibians, reptiles, birds, and mammals. *Fgf21* has also been identified in most vertebrates though not birds. *Fgf21* might have been lost in the bird lineage (Itoh 2007; Itoh, unpublished observation). Intracellular and canonical *Fgf* subfamilies have several teleost-specific paralogs, which were generated by an additional teleost-specific genome duplication event, in zebrafish. However, the hormone-like *Fgf* subfamily has no teleost-specific paralog in zebrafish (Itoh and Konishi 2007). Canonical Fgfs have a heparin-binding site. The site is necessary for the stable binding of Fgfrs and local signaling in an autocrine/paracrine manner. In contrast, hormone-like Fgfs have endocrine functions with reduced heparin-binding affinity (Goetz et al. 2007). Hormone-like *Fgf*s acquired systemic signaling in an endocrine manner during their evolution (Itoh and Ornitz 2008).

Roles of hormone-like Fgfs in development and metabolism

Fgf15/19

In mice, the expression of *Fgf15* exhibits a regionally restricted pattern in the developing nervous system, suggesting that Fgf15 has roles in regulating cell division and patterning within specific regions of the embryonic brain, spinal cord, and sensory organs (McWhirter et al. 1997). Transgenic mice over-expressing human *Fgf19* showed a significant and specific reduction in fat mass and an increase in energy expenditure, indicating potential roles for Fgf19 in energy metabolism (Tomlinson et al. 2002).

To examine the physiological roles of Fgf15 in vivo, *Fgf15* knockout mice were generated. The mice survived at a normal Mendelian ratio until embryonic day (E) 10.5, but, then gradually died. Most of them had died by postnatal day (P) 7. Heart defects were observed in the embryonic stages, indicating that Fgf15 is required for proper

Fig. 1 Evolutionary history of the *Fgf* gene family. *Fgf13-like* is an ancestral gene of the *Fgf* family. *Fgf4-like* is an ancestral gene of the canonical *Fgf* family. *Fgf4-like* was generated from *Fgf13-like* by gene duplication. *Fgf15/19-like*, an ancestral gene of the hormone-like *Fgf* subfamily, was generated from *Fgf4-like* by local gene duplication early in vertebrate evolution. Later, *Fgf15/19*, *Fgf21*, and *Fgf23* were generated by two large-scale genome duplication events during the evolution of early vertebrates
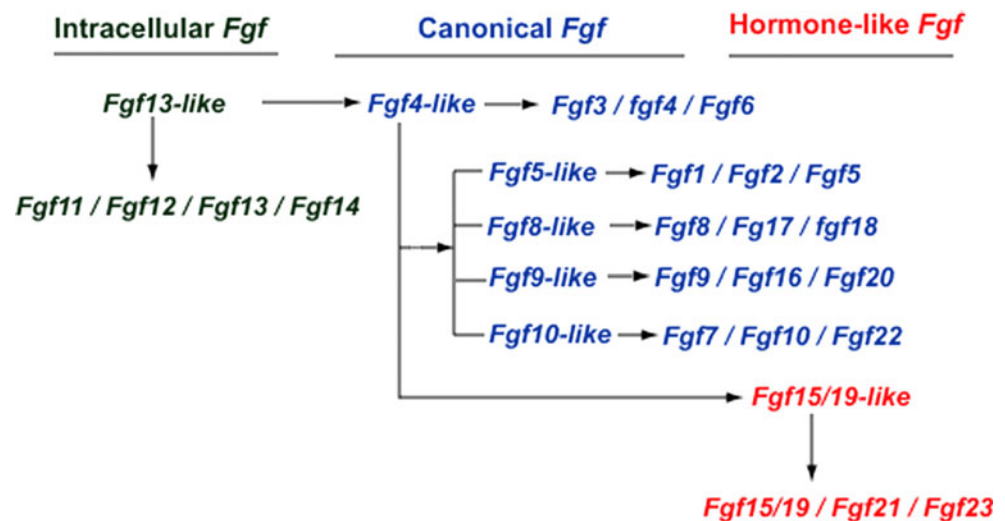
morphogenesis of the cardiac outflow tract during embryonic development (Table 1) (Vincentz et al. 2005). *Fgf19* knockdown zebrafish embryos were also generated by the injection of *Fgf19* Morpholino antisense oligonucleotides. Their phenotypes show essential roles of Fgf19 in the forebrain, lens, and retina during embryonic development (Table 1) (Miyake et al. 2005; Nakayama et al. 2008). These results indicate that Fgf15/19 acts as a growth/ differentiation factor in the heart and brain at embryonic stages.

Although most *Fgf15* knockout mice die by P7, a few survive with a normal appearance. Hepatic *cholesterol 7α-hydroxylase* (*Cyp7a1*) gene expression is increased in the surviving mice. Cyp7a1 catalyzes the first and rate-limiting step in the classical pathway of bile acid synthesis. Fecal bile acid excretion is increased in *Fgf15* knockout mice (Inagaki et al. 2005). A similar phenotype is observed in *Fgfr4* knockout mice (Yu et al. 2000). The liver and intestine play crucial roles in maintaining bile acid homeostasis. *Fgf15* and *Fgfr4* are expressed in the intestine and liver, respectively. These results indicate that intestinal Fgf15 plays a crucial role in regulating hepatic bile acid synthesis at postnatal stages by activating hepatic Fgfr4 in an endocrine manner (Table 1 and Fig. 2) (Inagaki et al. 2005).

Fgf21

In mice, *Fgf21* is most abundantly expressed in the liver. *Fgf21* is also expressed in the pancreas, white adipose tissue, and muscle (Nishimura et al 2000; Kharitonenkov and Shanafelt 2009). Potential roles of Fgf21 in energy metabolism were first shown by experiments in vitro. Fgf21 stimulated glucose uptake in cultured mouse and human adipocytes (Kharitonenkov et al. 2005). The phenotypes of *Fgf21* transgenic mice with over-expression in the liver also indicated potential roles in energy metabolism including improved insulin sensitivity and reduced serum triglycerides levels (Kharitonenkov et al. 2005).

Mammals have evolved complex metabolic responses to fasting. During fasting, nonesterified fatty acid (NEFA) is released from the white adipose tissue into the liver where it is converted to acetyl-CoA by oxidation. Hepatocytes synthesize ketone bodies from acetyl-CoA. Ketone bodies become the predominant energy source for the brain during fasting. Peroxisome proliferator-activated receptor α (PPARα), a nuclear receptor activated by NEFA, is crucial to the normal adaptive response to fasting. Ketogenesis in the liver during fasting is greatly impaired in *PPARα* knockout mice (Kersten et al. 1999; Leone et al. 1999). Hepatic *Fgf21* expression is induced directly by PPARα in response to fasting. The phenotypes of *Fgf21* transgenic mice indicate that Fgf21 stimulates lipolysis in the white adipose tissue and ketogenesis in the liver (Inagaki et al. 2007). The expression of *Fgf21* was also induced in the liver by a high-fat, low-carbohydrate ketogenic diet. In addition, adenoviral knockdown of hepatic *Fgf21* in mice fed the ketogenic diet caused reduced serum ketone body levels, fatty liver, and lipemia (Badman et al. 2007). Hepatic triglyceride levels were also significantly decreased in *Fgf21* transgenic mice (Inagaki et al. 2007). These results suggest that Fgf21 acts as a metabolic regulator of lipolysis in the white adipose tissue and is required for ketogenesis and triglyceride clearance in the liver.

To elucidate the physiological roles of Fgf21, *Fgf21* knockout mice were generated. The mice were viable, fertile, and seemingly normal. They showed hypertrophy of adipocytes and decreased lipolysis in adipocytes. Serum NEFA levels were significantly decreased, although serum glucose and triglyceride levels were essentially unchanged. In contrast, fasting *Fgf21* knockout mice showed increased lipolysis in adipocytes and increased serum NEFA levels. These results indicate that Fgf21 stimulates lipolysis in the white adipose tissue during feeding but inhibits it during fasting. Unexpectedly, serum ketone levels were increased by fasting in *Fgf21* knockout mice, indicating that ketogenesis was not impaired (Hotta et al. 2009). These results indicate that Fgf21 regulates lipolysis in adipocytes in response to the metabolic state but is not required for ketogenesis or triglyceride clearance in the liver (Table 1). However, *Fgf21* knockout mice fed a ketogenic diet showed partial impairments in ketogenesis and glucose control (Badman et al. 2009).

Peroxisome proliferation-activated receptor γ coactivator-1α (Pgc-1α), a transcriptional coactivator, interacts with several DNA-binding proteins to regulate metabolism in response to changes in nutritional status. Fgf21 induces *Pgc-1α* expression in the liver. Fgf21 cannot induce gluconeogenic gene expression in *Pgc-1α* knockout mice. In addition, *Fgf21* knockout mice did not fully express *Pgc-1α* in response to prolonged fasting and exhibited impaired glucogenesis and ketogenesis (Potthoff et al. 2009). These results indicate that the metabolic actions of Fgf21 are mediated in part through Pgc-1α. However, as described above, it

Table 1 Roles of hormone-like Fgfs in mice and zebrafish

	Mice	Zebrafish
At embryonic stages		
Fgf15/19	Cardiac outflow development	Brain development
Fgf21	-	Hematopoiesis
Fgf23	-	-
At postnatal stages		
Fgf15/19	Bile acid metabolism	-
Fgf21	Lipid metabolism	-
Fgf23	Phosphate/vitamin D metabolism	-
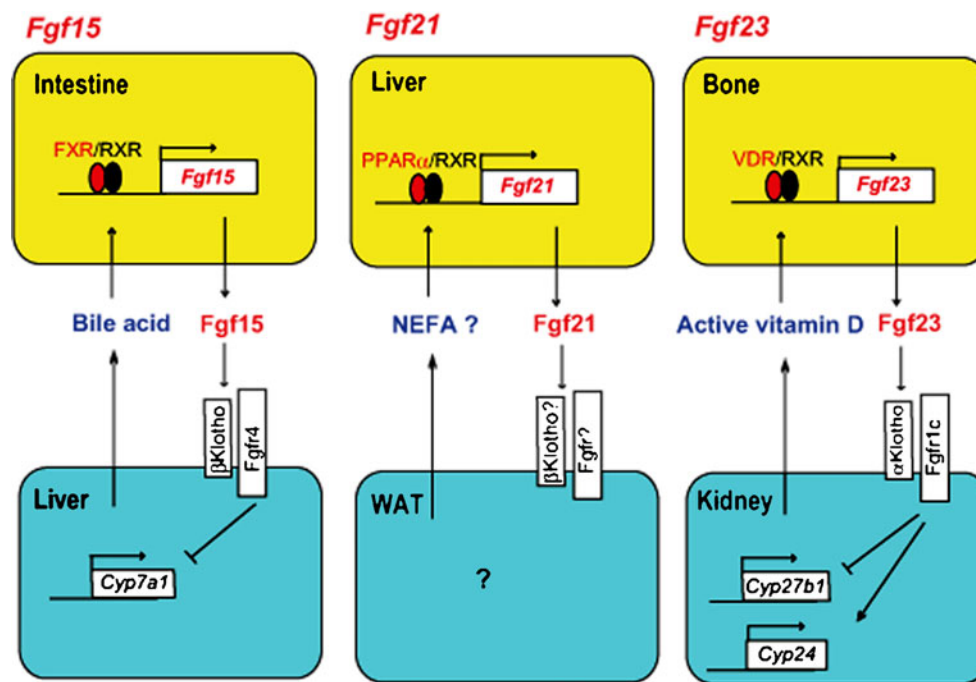
Fig. 2 Action mechanisms of hormone-like Fgfs and regulatory mechanisms of their gene expression. (*Fgf15*) Intestinal *Fgf15* expression is regulated by bile acid produced in the liver. The ligand-bound FXR forms a heterodimer with RXRs and induces the expression of *Fgf15*. The Fgf15 suppresses the expression of *Cyp7a1* in the liver by activating the βKlotho-Fgfr4 complex. The regulatory process forms a negative feedback loop in the regulation of bile acid homeostasis by Fgf15. (*Fgf21*) Hepatic *Fgf21* expression is induced by the activation of PPARα. NEFA binds to and activates PPARα. The ligand-bound PPARα forms a heterodimer with RXRs and induces the expression of *Fgf21*. However, the regulatory mechanism of *Fgf21* expression remains unclear. (*Fgf23*) Active vitamin D binds the vitamin D receptor (VDR). The ligand-bound VDR forms a heterodimer with retinoid X receptors (RXRs) and induces the expression of *Fgf23*. The increased Fgf23 suppresses the expression of *Cyp27b1* and induces the expression of *Cyp24* by activating the αKlotho-Fgfr1c complex. The regulatory process forms a negative feedback loop in the regulation of vitamin D homeostasis

has also been reported that Fgf21 is not required for glucogenesis and ketogenesis in the liver (Hotta et al. 2009).

Fgf21 regulates the insulin-independent transport of glucose in cultured 3 T3-L1 adipocytes. Functional interplay between the Fgf21 and peroxisome proliferation-activated receptor γ (PPARγ) pathways leads to a marked stimulation of glucose transport (Moyers et al. 2007). These results suggest a novel synergy between Fgf21 and PPARγ homeostasis.

Fgf21 stimulates *insulin* gene expression but not glucose-induced insulin secretion in isolated rat pancreatic islets. Although Fgf21 has no effect on islet cell proliferation, it preserves β-cell function and survival (Wente et al. 2006). *Fgf21* expression was markedly increased in mouse pancreatic acinar cells during cerulein-induced pancreatitis and following injury in vitro. *Fgf21* transgenic mice exhibited decreased serum amylase levels and decreased pancreatic stellate cell activation. In addition, *Fgf21* knockout mice showed increased serum amylase levels and tissue damage (Johnson et al. 2009). These results indicate a function of *Fgf21* as an immediate response gene protecting pancreatic acini from overt damage.

Starvation inhibits growth by blocking the growth hormone/insulin-like growth factor I (Igf-I) signaling pathway (Thissen et al. 1999). *Fgf21* transgenic mice are 40–50% smaller than their wild-type counterparts (Inagaki et al. 2008). Fgf21 causes resistance to growth hormone in the liver. Fgf21 reduces concentrations of the active form of signal transducer and activator of transcription 5, a major mediator of growth hormone actions, and causes corresponding decreases in the expression of its target genes including *Igf-1*. Fgf21 also induces the hepatic expression of genes encoding IGF-1-binding protein 1 and suppressor of cytokine signaling 2, which blunt growth hormone signaling (Inagaki et al. 2008). These results indicate a central role for Fgf21 in inhibiting growth as part of its broader role in inducing the adaptive response to starvation. However, body size and tibia length are essentially unchanged in *Fgf21* knockout mice (Hotta et al 2009). In addition, growth hormone signaling in the liver was essentially unaffected in *Fgf21* knockout mice by normal feeding or fasting for 24 h (Itoh et al. unpublished observation). These results indicate that Fgf21 is not a major physiological regulator for growth hormone signaling in the liver.

Torpor, the controlled lowering of metabolic rates, body temperature, and physical activity, is an adaptation that allows various mammals to cope with periods of low food availability (Melvin and Andrews 2009). *Fgf21* transgenic mice show torpor-like phenomena such as hypoglycemia, ketosis, and hypothermia (Inagaki et al. 2007). However, fasting-induced hypothermia and locomotor activity were essentially unchanged in *Fgf21* knockout mice (Oishi et al. 2010). These results indicate that Fgf21 is not a major physiological regulator for the hypothermia that is associated with the early stages of fasting.

Fgf21 knockdown zebrafish embryos were also generated by injection of *Fgf21* Morpholino antisense oligonucleotides. The embryos lacked erythroid and myeloid cells, indicating that Fgf21 is essential for hematopoiesis in zebrafish. The potential action mechanism of Fgf21 indicates an essential role in hematopoiesis as a differentiation factor (Table 1) (Yamauchi et al. 2006).

Fgf23

Fgf23 is mainly expressed in the bone. Fgf23 decreases serum phosphate levels (Shimada et al. 2004). Type 2a and 2c sodium-phosphate cotransporters (NaPi-2a and NaPi-2c) mediate proximal tubular phosphate reabsorption in the kidney. Fgf23 suppresses the expression of *NaPi-2a* and *NaPi-2c* in proximal tubular cells. Serum phosphate levels are decreased by their suppression. In addition, Fgf23 also suppresses the expression of *Cyp27b1* (1α-hydroxylase) and stimulates the expression of *Cyp24* (24-hydroxylase). 1α-hydrooxylase and 24-hydroxylase mediate the synthesis of active vitamin D and degrade active vitamin D, respectively. By altering these gene expression levels, Fgf23 decreases levels of active vitamin D in serum. As active vitamin D stimulates intestinal phosphate reabsorption, decreased levels result in decreased serum phosphate levels (Table 1) (Shimada et al. 2004).

To examine the physiological roles of Fgf23 in mice, *Fgf23* knockout mice were generated (Shimada et al. 2004). The phenotypes of these mice mirror ADHR and TIO phenotypes. The mice showed hyperphosphatemia and increased active vitamin D levels, indicating that Fgf23 is a physiological regulator for phosphate and active vitamin D levels in serum.

Action mechanism of hormone-like Fgfs with Klothos

αKlotho

αKlotho was originally identified as a gene with a loss-of-function mutation in a mouse strain that developed multiple aging-like phenotypes (Kuro-o et al. 1997). The

phenotypes are essentially similar to those of *Fgf23* knockout mice (Shimada et al. 2004). These results indicate that Fgf23 and Klotho may function in a common signal transduction pathway. αKlotho is a 130-kDa single-pass transmembrane protein with a short cytoplasmic domain (10 amino acids). The extracellular domain has two homologous domains with sequence similarity to β-glucosidase. However, β-glucosidase-like activity is not detected in αKlotho (Kuro-o et al. 1997). In cultured cells, αKlotho efficiently bound to Fgfrs, Fgfr1, and Fgfr3 but not to Fgfr2. αKlotho significantly enhanced the ability of Fgf23 to induce phosphorylation of a substrate of the Fgf receptor and Erk in cultured cells (Kurosu et al. 2006). Alternative splicing in the third immunoglobulin-like domain occurs in Fgfr1, Fgfr2, and Fgfr3 but not in Fgfr4. αKlotho most efficiently binds to and activates Fgfr1c among several isoforms of Fgfrs in cultured cells (Urakawa et al. 2006). These results indicate that Fgf23 activates Fgfr1c, which forms a complex with αKlotho (Fig. 2).

βKlotho

βKlotho is a protein that shares structural identity (41% amino acid identity) and characteristics with αKlotho. *βKlotho* is expressed predominantly in the liver, pancreas, and adipose tissue (Ito et al 2000). The synthesis and excretion of bile acids are dramatically increased in *βKlotho* knockout mice. In addition, *βKlotho* knockout mice exhibit resistance to gallstone formation (Ito et al. 2005). Their phenotypes overlap those of *Fgfr4* knockout mice (Yu et al. 2000) and viable *Fgf15* knockout mice (Inagaki et al. 2005), indicating that Fgf15/19, Fgfr4, and βKlotho are essential components in the regulation of bile acid synthesis (Fig. 2). Fgf19 can bind to the βKlotho-Fgfr4 complex in cultured cells. Fgf19 also activates Fgf signaling in hepatocytes that primarily express *Fgfr4* and reduces the expression of *Cyp7a1* encoding the rate-limiting enzyme for bile acid synthesis (Kurosu et al. 2007).

As described above, Fgf15/19 plays roles in development as a growth/differentiation factor (Vincentz et al. 2005; Miyake et al. 2005; Nakayama et al. 2008). However, it remains unclear whether βKlotho is required for Fgf15/19 signaling at embryonic stages.

βKlotho is also essential for Fgf21 signaling in cultured cells (Ogawa et al. 2007; Kharitonenkov et al. 2008; Suzuki et al. 2008). However, *Fgf21* knockout mouse phenotypes (Hotta et al. 2009) are distinct from *βKlotho* knockout mouse phenotypes (Ito et al. 2005). In addition, administration of recombinant human Fgf21 to *βKlotho* knockout mice indicates that Fgf21 signals are transduced in the absence of βKlotho (Tomiyama et al. 2010). These results indicate the existence of a βKlotho-independent Fgf21 signaling pathway in which undefined

cofactors might be involved (Fig. 2). One-to-one functional interactions such as αKlotho/Fgf23, βKlotho/Fgf15/19, and undefined cofactor/Fgf21 would result in tissue-specific signal transduction of the hormone-like Fgf subfamily (Tomiyama et al. 2010). The beneficial pharmacological effects of Fgf21 such as decrease of blood glucose and lipid levels and suppression of weight gain have been observed in mice after its administration or its over-expression by transgene (Kharitonenkov 2009). However, as the pharmacological effects have not been examined in βKlotho knockout mice, the involvement of βKlotho in the pharmacological effects remains unclear.

Regulatory mechanism of hormone-like Fgf gene expression

Fgf15

Intestinal *Fgf15* expression is regulated by bile acid produced in the mouse liver. This bile acid is released into the intestinal lumen and binds to its nuclear receptor, the farnesoid X receptor (FXR), in intestinal epithelial cells. The ligand-bound FXR forms a heterodimer with retinoid X receptors (RXRs) and induces the expression of *Fgf15*. The Fgf15 suppresses the expression of *Cyp7a1* in the liver by activating Fgfr4. Cyp7a1 catalyzes the rate-limiting step in bile acid synthesis in the liver. The regulatory process forms a negative feedback loop in the regulation of bile acid homeostasis by Fgf15 (Fig. 2) (Inagaki et al. 2005; Kuro-o 2008).

Fgf21

Hepatic *Fgf21* expression is greatly induced in wild-type mice but not *PPARα* knockout mice by fasting for 24 h. In addition, hepatic *Fgf21* expression is also greatly induced by GW7647, a PPARα—selective agonist (Inagaki et al. 2007). These results indicate that hepatic *Fgf21* expression is induced by the activation of PPARα. NEFA also binds to and activates PPARα. The ligand-bound PPARα forms a heterodimer with RXRs and induces the expression of *Fgf21*. Fasting increases the amounts of NEFA released from adipocytes. Hepatic *Fgf21* expression during fasting might be induced through the activation of PPARα by the NEFA (Moore 2007; Kuro-o 2008). However, although hepatic *Fgf21* expression is greatly induced in wild-type mice by fasting for 24 h, serum NEFA levels are essentially unchanged (Badman et al. 2007; Hotta et al. 2009). These results indicate that PPARα is not activated by NEFA during fasting for 24 h. The regulatory mechanism of *Fgf21* expression remains unclear (Fig. 2).

Fgf23

Active vitamin D produced in the kidney increases the expression of *Fgf23* in the bone and serum Fgf23 levels in mice. Active vitamin D binds the vitamin D receptor (VDR). The ligand-bound VDR forms a heterodimer with retinoid X receptors (RXRs) and induces the expression of *Fgf23* in the bone. The Fgf23 suppresses the expression of *Cyp27b1* and induces the expression of *Cyp24* in the kidney (Shimada et al 2004). *Cyp27b1* and *Cyp24* encode enzymes, 1a-hydroxylase and 24-hydroxylase, which synthesize and inactivate active vitamin D, respectively. The suppression of *Cyp27b1* expression and induction of *Cyp24* expression by Fgf23 result in decreased levels of active vitamin D in serum. The regulatory process forms a negative feedback loop in the regulation of vitamin D homeostasis (Fig. 2) (Tsujikawa et al. 2003; Moore 2007; Kuro-o 2008).

Roles of hormone-like Fgfs in disease

Fgf19

Serum Fgf19 levels are markedly elevated in patients with extrahepatic cholestasis caused by a pancreatic tumor. Although not expressed in the normal liver, *Fgf19* is abundantly expressed in the liver of cholestatic patients. This is accompanied by a number of adaptations aimed at protecting the liver against bile salt toxicity. Fgf19 signaling may be involved in some of these adaptations (Table 2) (Schaap et al. 2009). Serum Fgf19 levels are also significantly increased in patients on chronic hemodialysis (Table 2) (Reiche et al. 2010). Hepatic lipid metabolism is disturbed in patients with nonalcoholic fatty liver disease (NAFLD). The hepatic response to Fgf19 is impaired in NAFLD patients with insulin resistance. This impaired hepatic response to Fgf19 may contribute to the disturbance of lipid homeostasis in NAFLD (Table 2) (Schreuder et al. 2010).

Fgf21

Serum Fgf21 levels are increased in patients with type 2 diabetes and obesity (Table 2) (Zhang et al 2008; Chen et al 2008; Mraz et al 2009). They are also increased in patients with Cushing's syndrome (Table 2) (Durovcová et al. 2010). In contrast, serum Fgf21 levels are decreased in patients with anorexia nervosa (Table 2) (Dostálová et al. 2008).

Fgf23

Hereditary Fgf23 signaling disorders result in metabolic disorders (Razzaque 2009). AHDR is caused by missense

Table 2 Human diseases caused by hormone-like Fgf signaling disorder

Gene	Disease	Gene mutation	Fgf signaling
Hereditary disease			
Fgf23	ADHR	Gain-of-function mutations of *Fgf23*	Increase
	XLH	Loss-of-function mutations of *Phex*	Increase
	ARHR	Loss-of-function mutations of *Dmp1*	Increase
	FTC	Loss-of-function mutations of *Fgf23* or *Galnt3*	Decrease
Paraneoplastic disease			
Fgf19	Extrahepatic cholestasis		Increase
Fgf23	TIO		Increase
Metabolic disease			
Fgf19	Chronic hemodialysis		Increase
	NAFLD		Decrease
Fgf21	Type 2 diabetes		Increase
	Obesity		Increase
	Cushing's syndrome		Increase
	Anorexia nervosa		Decrease
Fgf23	Renal failure		Increase

mutations of *Fgf23* with gain-of-function (ADHR consortium 2000). A part of Fgf23 is cleaved by intracellular proteolytic processing. The cleaved Fgf23 forms lose their biological activity. *Fgf23* mutations in ADHR result in impaired proteolytic processing of Fgf23 and increased active Fgf23 levels in serum (Table 2) (White et al. 2001). *Phex* encodes endopeptidase. X-linked hypophosphatemia (XLH) is caused by loss-of-function *Phex* mutations that increase serum Fgf23 levels (Table 2) (Johnson et al. 2009). *Dmp1* encodes dentin matrix acidic phosphoprotein, an extracellular matrix protein. Autosomal recessive hypophosphatemic rickets/ osteomalacia (ARHR) is caused by loss-of-function *Dmp1* mutations that increase serum Fgf23 levels (Table 2) (Lorenz-Depiereux et al. 2006). *Dmp1* knockout mouse phenotypes are similar to ARHR phenotypes. *Dmp1/Fgf23* double knockout mouse phenotypes are similar to *Fgf23* single knockout mouse phenotypes. These results indicate that the hypophosphatemia in patients with loss-of-function *Dmp1* mutations is induced by increased serum Fgf23 levels (Liu et al. 2008). However, the mechanism regulating serum Fgf23 levels has been unclear.

Reduced Fgf23 signaling also causes human metabolic disorders. Familial tumoral calcinosis (FTC) is characterized by ectopic calcification and hyperphosphatemia. Missense mutations of *Fgf23* with loss-of-function result in FTC. These mutations destabilize the tertiary structure of Fgf23 and increase its susceptibility to degradation (Table 2) (Benet-Pagès et al. 2005). *Galnt3* encodes uridine diphosphate-N-acetly-α-D-galactosamine: polypeptide N-acetylgalactosaminytransferase. FTC is also caused by loss-of-function *Galnt3* mutations (Table 2) (Topaz et al. 2004; Frishberg et al. 2005). The enzyme is a Golgi-associated enzyme that initiates mucin-type O-glycosylation at proteins. Fgf23 is glycosylated by Galnt3. The O-glycosylation prevents proteolytic processing of Fgf23 and allows the secretion of an intact Fgf23 (Kato et al. 2006; Frishberg et al. 2007). *Galnt3* knockout mice develop hyperphosphatemia without apparent calcification. The loss-of-function mutation of *Galnt3* impairs secretion of intact Fgf23, leading to decreased serum Fgf23 levels and hyperphosphatemia (Ichikawa et al. 2009). These results provide in vivo evidence that Galnt3 plays an essential role in the proper secretion of Fgf23.

In addition to hereditary Fgf23 signaling disorders, tumors that over-produce Fgf23 also cause metabolic disorders. TIO is a paraneoplasitc disease characterized by hypophosphatemia caused by renal phosphate wasting (Table 2) (Shimada et al. 2001). Serum Fgf23 levels are also greatly increased in patients with renal failure, partly owing to decreased renal clearance. These results suggest that Fgf23 to have a compensatory role in the disease (Table 2) (Larsson et al. 2003; Imanishi et al. 2004).

Conclusions

Hormone-like Fgfs, Fgf15/19, Fgf21, and Fgf23, are newly emerging and unique in evolution and function. Although hormone-like *Fgf* genes have been identified in most vertebrates examined, they have not been identified in invertebrates, indicating that hormone-like Fgfs are vertebrate-specific. Hormone-like Fgfs mainly act as endocrine factors in an Fgfr-dependent manner. Fgf15/19 and Fgf23 physiologically require βKlotho and αKlotho and as a

cofactor, respectively. However, Fgf21 might physiologically require neither. Recent studies have revealed important roles of hormone-like Fgfs in humans, mice, and zebrafish. Hormone-like Fgfs participate in metabolism at postnatal stages, although they also have roles in development at embryonic stages. Fgf15/19 regulates bile acid metabolism in the liver. Fgf21 regulates lipid metabolism in the white adipose tissue. Fgf23 regulates serum phosphate and active vitamin D levels. Hormone-like Fgf signaling disorders caused by hereditary diseases or tumors result in metabolic diseases. In addition, serum hormone-like Fgf levels are significantly affected by some metabolic diseases. Further understanding of the roles of hormone-like Fgfs will provide clues to their roles in metabolism and clinical treatments for metabolic diseases.

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