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Molecular pathology of the fibroblast growth factor family

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Abstract

Human fibroblast growth factor (FGF) family contains 22 proteins that regulate a plethora of physiological processes in both developing and adult organism. The mutations in the FGF genes were not known to play role in human disease until the year 2000, when mutations in FGF23 were found to cause hypophosphataemic rickets. Nine years later, seven FGFs have been associated with human disorders. These include FGF3 in Michel aplasia; FGF8 in cleft lip/palate and in hypogonadotropic hypogonadism; FGF9 in carcinoma; FGF10 in the lacrimal/salivary glands aplasia, and lacrimo-auriculo-dento-digital syndrome; FGF14 in spinocerebellar ataxia; FGF20 in Parkinson disease; and FGF23 in tumoral calcinosis and hypophosphataemic rickets. The heterogeneity in the functional consequences of FGF mutations, the modes of inheritance, pattern of involved tissues/organs, and effects in different developmental stages provide fascinating insights into the physiology of the FGF signaling system. This article reviews the current knowledge about the molecular pathology of the FGF family.

Keywords

Fibroblast growth factor; FGF; disease; mutation; genetics; human

BRIEF OVERVIEW OF THE FGF SIGNALING SYSTEM

The majority of the 22 known FGFs are secreted proteins that signal, as true growth factors, via activation of their cognate receptors, consisting of four transmembrane FGF-receptor tyrosine kinases (FGFR1-4). Only FGF11-14 represent exceptions from this paradigm, as they are not capable of FGFR activation and function instead as mediators of intracellular signaling (Goldfarb, 2005). The following section outlines the major features of the FGF signaling systems.

FGF signaling via FGFR activation

After their release from cells, FGFs bind and activate their cognate FGFRs located at the surface of the recipient cell. Encoded by four genes (FGFR1-4), FGFRs exist in seven

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principal variants generated by alternative splicing in the extracellular Ig-like domain adjacent to the membrane. As the variable region involves the ligand-binding site, the FGFR isoforms differ in their binding affinity towards the FGF ligands. Experimental studies analyzing receptor specificity of the entire FGF family demonstrate a significant redundancy in FGF-FGFR interactions, with all the major FGFR variants being activated by at least five FGF ligands. For instance, the prevalent isoform of FGFR3 (FGFR3c) appears to be strongly activated by FGF1, 2, 4, 8, 9, 17–20 *in vitro* (Ornitz et al., 1996; Zhang et al., 2006). This redundancy appears, in contrast, limited *in vivo*. One of the most striking examples is the FGF-mediated activation of FGFR3 on developing cartilage. While human cartilage itself expresses at least four FGFR3-cognate ligands (FGF1, 2, 17, 19) (Krejci et al., 2007a) their removal produces no skeletal phenotype in mice suggesting that cartilage-borne (autocrine) FGFs are not the physiological ligands of FGFR3 (Miller et al., 2000; Searce-Levie et al., 2008). This is instead (paracrine) FGF18, which is expressed in perichondrial tissues adjacent to cartilage and its removal results in skeletal overgrowth from apparent lack of FGFR3 activation (Liu et al., 2002). These data suggest that there exist another important regulators of the specificity of the FGF-FGFR interaction *in vivo*, such as cell surface heparan sulphate polysaccharides.

Regulation of FGF signaling by heparan sulphate

Heparan sulphate (HS) originates from heparan – a linear polymer of glucuronate (GlcA) and *N*-acetylglucosamine monosaccharides (GlcNAc), which is modified by partial *N*-sulfation of GlcNAc (to create glucosamine *N*-sulfate, GlcNS) and epimerization of GlcA (to create iduronic acid, IdoA) during its transit through the ER-Golgi apparatus (Kusche-Gullberg and Kjellen, 2003). The resulting extracellular HS is a heterogeneous polysaccharide with different degrees of GlcA and IdoA content and 2-*O*-, 3-*O*- and 6-*O*-sulfation of GlcA/IdoA and GlcNS, respectively.

HS is a critical component of the FGF signaling system both *in vitro* and *in vivo* (Lin et al., 1999). In classical experiments performed by Rapraeger et al. and Yayon et al., FGFR activation by FGF2 was abolished in CHO or Swiss 3T3 cells genetically, chemically or enzymatically engineered to lack endogenous HS, thus demonstrating the absolute requirement of HS for proper FGFR activation (Rapraeger et al., 1991; Yayon et al., 1991). Later studies showed that the optimal biologically active HS length is an octasaccharide and that the 6-*O*-Sulfation is essential for the proper FGFR activation (Ornitz et al., 1992; Guimond et al., 1993). According to the current model of FGF-FGFR-HS interactions, the FGF, FGFR and HS form a complex of 2:2:2 stoichiometry with both FGF and FGFR HS binding sites contributing equally to formation of the HS binding site. Many different HS variants are expressed in a tissue- and developmentally-specific manner, resulting in a spectrum of binding specificities for different FGF/FGFR/Hs combinations (Mohammadi et al., 2005). As demonstrated for FGF4 signaling in embryonal mouse development, HS constitute a powerful, tissue-specific regulatory mechanism that allows for a precise tuning of the cellular response to FGFs (Allen et al., 2001).

FGF/FGFR-mediated intracellular signaling

Recruitment of two FGFR molecules into the FGF-HS complex results in FGFR dimerization, activation of intrinsic tyrosine kinase activity and autophosphorylation at several tyrosine residues within the cytoplasmic domain of the receptor (Mohammadi et al., 1996). Activated FGFRs employ several signaling pathways in their downstream intracellular signaling, including ERK and p38 mitogen activated kinases, phospholipase gamma (PLC γ), protein kinase C (PKC), phosphatidylinositol 3-kinase (PI3K) and src (reviewed by Klint and Claesson-Welsh, 1999). With exception of PLC γ that binds directly on the phosphorylated Y766 of the activated FGFR (Peters et al., 1992), FGFRs recruit their

downstream signaling via phosphorylation of several signaling adaptors such as Gab1, SHB, SHC and FRS2 (Klint et al., 1995; Hadari et al., 2001; Ong et al., 2001; Krejci et al., 2007b). Among those, FRS2 plays a dominant role, being critical for FGFR-mediated activation of at least the ERK and PI3K pathways (Hadari et al., 2001).

The individual FGFRs have unique expression patterns *in vivo*, and affect cell behavior differently in both *in vitro* and *in vivo* experimental models (Wang et al., 1994; Shaoul et al., 1995; Umbhauer et al., 2000). Studies comparing the signaling capacity among the individual FGFRs however show that they activate their downstream signaling in a similar fashion, with the major differences being quantitative (Raffioni et al., 1996; Hart et al., 2000). The signaling specificity of individual FGFR type is further complicated by the fact that most cells express more than one FGFR variant and FGFRs can form active heterodimers (Bellot et al., 1991).

Intracellular signaling of FGF11–FGF14

FGF11–FGF14 (also known as fibroblast growth factor-homologous factors or FHF1–FHF4) were identified via a combination of genetic approaches aimed at identification of genes similar to known FGFs (Smallwood et al., 1996). FHF proteins share significant sequence and structural homology with FGFs and bind HS in a manner similar to the FGFs. In contrast, analysis of FHF-mediated FGFR activation showed that FHFs are unable to activate any of the four FGFRs, likely due to the structural incompatibility of the FGFR-interacting region (Olsen et al., 2003; Mohammadi et al., 2005). According to the present stage of knowledge (reviewed by Goldfarb, 2005), FHFs act as intracellular signaling molecules that function independently of FGFRs, via interaction with islet brain-2 scaffold protein and voltage-gated sodium channels, as discussed in detail later.

MOLECULAR PATHOLOGY OF FGF FAMILY

Over the past thirty years, multiple members of FGFR family were found to contribute to human disorders. These include conditions associated mostly with mutations in FGFRs, such as skeletal and craniofacial dysplasias (FGFR2 and FGFR3), myeloproliferative syndromes (FGFR1) and multiple myeloma (FGFR3) (reviewed by Grose and Dickson, 2005; Wilkie, 2005). This review focuses specifically on mutations in members of FGF family, newly discovered as candidates for human diseases.

FGF3 in Michel aplasia with microtia and microdontia

Michel aplasia is a rare form of congenital sensorineural hearing loss characterized by complete absence of the inner ear structures, including the cochlea, vestibule and semicircular canals (Michel, 1863). In 2007, Tekin et al. described a novel, autosomal recessively inherited form of Michel aplasia in three unrelated Turkish families accompanied by type I microtia with anteverted ears, and microdontia with widely spaced teeth (referred here as MMM - Michel aplasia with microtia and microdontia; MIM# 610706). Other anatomical abnormalities were mild when present, and patients had normal cognitive abilities and anatomically normal middle ear, cerebral and cerebellar structures, demonstrating that MMM affects mostly the inner and outer ear (Tekin et al., 2007). Positional cloning followed by sequencing analysis identified one missense and two nonsense mutations in FGF3 (MIM# 164950), resulting in S156P, R104X and V206SfsX117 alterations of FGF3 amino acid sequence (Tekin et al., 2007). Later studies identified additional FGF3 mutations associated with MMM, resulting in I85MfsX15, L6P and G66C alterations in the FGF3 protein sequence (Table 1) (Tekin et al., 2008; Alsmadi et al., 2009).

In embryonal development, the inner ear forms from the otic vesicle, an invagination of the ectodermal otic placode induced by the adjacent rhombencephalic neural tube (hindbrain

rhombomeres 5 and 6), which expresses FGF3 (Wilkinson et al., 1989; Mansour et al., 1993). The elucidation of FGF3's role in inner ear development began in 1991, when Represa et al. demonstrated that inhibition of FGF3 expression repressed the induction of the otic vesicle in chick embryo explant cultures (Represa et al., 1991). Ectopic expression of FGF3 was capable of inducing otic placodes and subsequent otic vesicles, thus further confirming the role of FGF3 in early development of the chicken inner ear (Vendrell et al., 2000).

In contrast, mice homozygous for *Fgf3^{neo}* (*neo^r* cassette targeted into the first protein coding exon) show a significantly milder phenotype compared to humans suffering with MMM or the aforementioned chick model. Although grossly abnormal in their labyrinth section with reduced or missing endolymphatic ducts, the inner ears of *Fgf3^{neo}* mice contain all the major structures, apparently due to normal induction of the otic vesicle (Mansour et al., 1993). This phenotype is in further discrepancy with the *Fgf3^{-/-}* mice (lacking the entire coding region of FGF3), that show apparently normal inner ear development and function (Alvarez et al., 2003). Interestingly, mice lacking both FGF3 and FGF10 (MIM# 602115) developed no or severely impaired otic vesicles with the lack of cochleovestibular ganglion and dramatic reduction or absence of expression of otic marker genes *Pax2*, *Dlx5* and *Sox9* (Alvarez et al., 2003; Wright et al., 2003). FGFR2b appears to be the FGF3/FGF10-cognate receptor as evidenced by inner ear defects in *FGFR2b null* mice, similar to mice lacking both FGF3 and FGF10 (Pirvola et al., 2000). Neither proliferation nor survival of the ectoderm cells were affected by *Fgf3/Fgf10* deletion, suggesting that the major role of FGF signaling in otic induction is to establish the appropriate patterns of gene expression in both the otic ectoderm (*Pax2*, *Dlx5* and *Sox9*) and dorsal hindbrain (*Wnt3a*) (Wright and Mansour, 2003; Hatch et al., 2007).

Taken together, FGF3 and FGF10 act as redundant factors during early inner ear initiation in mice (Alvarez et al., 2003; Pauley et al., 2003), which appears to be at variance with the human data, as patients with FGF3 mutations suffer complete loss of the inner ear without carrying concomitant FGF10 mutations (Tekin et al., 2007). Whether this discrepancy is simply a result of incomplete penetrance and variable expressivity of the inner ear phenotype in both MMM and *Fgf3* or *Fgf3/Fgf10 null* mice (Mansour et al., 1993; Wright and Mansour, 2003; Hatch et al., 2007), remains to be answered. Another important question is whether the MMM-associated mutations lead to simple loss of FGF3 function (Table 1). FGF3 has a dual action within the cells. In addition to the mitogenic signaling of secreted FGF3 that acts in a paracrine manner via cell-surface FGFR2b, FGF3 is known to inhibit cell growth via a direct, intracrine signaling in nucleus of its producing cell (Kiefer et al., 1995; Antoine et al., 1997). Although paracrine FGF3 signaling is very likely impaired in MMM, the opposite might be the truth in the case of nuclear signaling. For instance, L6P mutation lies within the signal peptide and may disturb FGF3 secretion, altering the balance between secreted and nuclear FGF3 (Tekin et al., 2007). Future functional studies should determine the effects of intracellular FGF3 signaling in context of inner ear development.

FGF8 and FGF10 in nonsyndromic cleft lip or palate

Cleft lip and/or palate (CLP) appears when the two halves of the craniofacies that form the hard palate and/or lip fail to fuse completely. Non-syndromic (without other associated abnormalities) cleft lip and/or palate (NS-CLP) is a common congenital anomaly affecting 1:700 births worldwide (Jugessur and Murray, 2005). Both environmental and genetic factors play roles in NS-CLP development with as many as 14 loci involved in clefting (Schliekelman and Slatkin, 2002). When screening the multiple members of FGF/FGFR family for mutations, Riley et al. found a D73H missense mutation in FGF8 (MIM# 600483) in a patient with CLP. This mutation is predicted to cause loss-of-function through

destabilizing FGF8's N-terminal conformation important for FGFR binding affinity and specificity (Table 1) (Riley et al., 2007).

The mammalian secondary palate develops from the maxillary processes that first outgrow from the sides of the embryonal oronasal cavity vertically by the sides of the tongue to form the palatal shelves. Later, the shelves elevate above the tongue, grow horizontally to meet in the midline and adhere to each other (Ferguson 1988; Dudas et al., 2007). Failure of any of these processes results in cleft palate. Multiple members of FGF/FGFR signaling system are involved in palatogenesis as evidenced by the phenotype of *Fgf10*^{-/-}, *Fgfr2b*^{-/-} mice as well as humans carrying apparent loss-of-function mutations in FGFR1, FGFR2, FGFR3, FGF8 and FGF10, that all develop cleft palate (De Moerloose et al., 2000; Rice et al., 2004; Riley et al., 2007).

It is likely that different FGFs and FGFRs regulate different stages of palatogenesis, and thus particular FGF or FGFR mutations may produce the NS-CLP phenotype by different mechanisms. For instance, FGF10 and its receptor FGFR2b are important for initial stages of palatogenesis prior to shelf elevation. FGFR2b is expressed in developing palatal epithelium and receives its activating ligand FGF10 from the adjacent mesenchyme. FGFR2b signaling, in turn, upregulates sonic hedgehog production in epithelia, which signals back to the mesenchyme where it upregulates cell proliferation. Thus cell proliferation of both palatal epithelia and mesenchyme are impaired in *Fgf10*^{-/-} and *Fgfr2b*^{-/-} mice, underlying the cleft palate phenotype (Rice et al., 2004). Such data suggest that at least the loss-of-function mutations in FGF10 and FGFR2 may lead to NS-CLP by disrupting epithelial-mesenchymal communication in initial stages of palatogenesis (Riley et al., 2007).

Interestingly, hypomorphic *Fgf8* mice show abnormal palate with reduced or absent palatine bones, however this phenotype is a part of much broader craniofacial abnormalities caused by impaired survival of neural crest cells, which populate the pharyngeal arches of developing embryo and contribute to development of many facial structures (Abu-Issa et al., 2002). It is unlikely that the same mechanism underlies the NS-CLP phenotype in human with FGF8-D73H mutation, where the defect appears to be limited only to the lip and palate (Riley et al., 2007). FGF8 may therefore regulate later stages of palatogenesis in humans such as growth or fusion of the palatal shelves.

FGF8 in idiopathic hypogonadotropic hypogonadism

Idiopathic hypogonadotropic hypogonadism (IHH; MIM# 146110) is characterized by absent or delayed puberty, hypogonadism and low serum levels of gonadotropins (Fechner et al., 2008). When IHH is associated with anosmia, the resulting condition is termed Kallmann syndrome (KS; MIM# 147950). Several loci have been implicated in IHH development such as *KAL1*, GnRH receptor, nasal embryonic LHRH factor, G protein-coupled receptor 54 and others, altogether accounting for 30% of IHH cases. When screening 461 unrelated IHH probands, Falardeau et al. found 6 mutations in FGF8 in 3 familial and 3 sporadic cases of both IHH and KS (Falardeau et al., 2008). The mutations included heterozygous H14N, P26L, K100E, R127G, T229M as well as homozygous F40L substitutions in FGF8 amino acid sequence (Table 1). Interestingly, the F40L and K100E cases carried also mutations in FGFR1 (double heterozygous Q764H/D768Y and R250Q, respectively) (Falardeau et al., 2008), in addition to the loss-of-function mutations in FGFR1 reported to associate with both KS and IHH previously (Dode et al., 2003; Pitteloud et al., 2006). *In silico* structural analysis followed by biochemical studies show that mutations have a loss-of-function effect on FGF8, affecting FGF8-mediated FGFR activation by impairing its interaction with HS (K100E) or FGFR (R127G), or via an unknown mechanism (T229M).

Gonadotropin-releasing hormone (GnRH) is produced by GnRH neurons in preoptic brain area and regulates reproduction by stimulation of production of gonadotropins which, in turn, stimulate gonadal gametogenesis. FGF8 appears essential for development and/or maintenance of GnRH neurons, as the hypomorphic *Fgf8* mice lack GnRH neurons in brain regions ranging from preoptic areas to hypothalamus. Correspondingly, GnRH is absent from hypothalamic extracts of mice homozygous for a hypomorphic allele (Falardeau et al., 2008). Although the IHH and KS patients carrying the FGF8 mutations displayed mostly reproductive and olfactory phenotypes, the cleft lip/palate, scoliosis and low bone density were also found in some patients (Falardeau et al., 2008). Despite not having complete penetrance, these phenotypes point to much broader function of FGF8 in development as discussed above.

FGF9 in colorectal, endometrial and ovarian carcinoma

When searching for gene targets of the Wnt/ β -catenin pathway in familial colorectal tumors, Abdel-Rahman et al. found a six distinct mutations in FGF9 (MIM# 600921) associated with as much as 5% of colorectal, endometrial and ovarian cancer samples tested (Abdel-Rahman et al., 2008). The mutations caused one frameshift (L188YfsX18), four missense (G84E, R173K, V192M, D203G), and one nonsense (E142X) change in FGF9 protein sequence that were all predicted to result in a loss of FGF9 function, albeit through different mechanisms. The V192M, D203G and L188YfsX18 mutations are likely to impact FGF9 interaction with FGFR. In contrast, G84E and E142X substitutions may interfere with protein folding. Finally, R173K is predicted to impair FGF9's interaction with HS, necessary for FGFR activation. When compared with wild-type (wt) FGF9, mutants exhibited reduced ability to activate a major intracellular effector of FGFR signaling, ERK MAP kinase, thus confirming the predicted negative functional effect (Abdel-Rahman et al., 2008).

What is the role of FGF9 in carcinoma? FGFs are known oncogenes, with several members of the family being implicated in a different cancers such as leukemia, Kaposi sarcoma and breast and stomach carcinomas (Dickson et al., 1984; Sakamoto et al., 1986; Delli-Bovi et al., 1988; Zhan et al., 1988; Krejci et al., 2001; Krejci et al., 2007c). In contrast, various FGFs including FGF9 are known to induce differentiation and/or inhibit growth, invasion and *in vivo* tumor formation of several cancer cell lines, such as MDA-MB-231 breast cancer cells, RCS chondrosarcoma cells and pancreatic adenocarcinoma cells BxPc3, T3M4 and HPAF (McLeskey et al., 1994; Korah et al., 2000; El-Hariry et al., 2001; Krejci et al., 2004). Such data demonstrate that FGFs can function as inhibitors of tumor formation, depending on the cellular environment. The loss-of-function nature of the FGF9 mutations is in agreement with the mutations being either homozygous or correlated with a partial loss of heterozygosity at the FGF9 region (Abdel-Rahman et al., 2008). Although these data identify FGF9 as a tumor suppressor in carcinoma, they are in variance with experiments identifying FGF9 as a critical downstream oncogene target of Wnt/ β -catenin signaling (Hendrix et al., 2006). Future experiments are therefore necessary to unravel the exact nature of FGF9 signaling in carcinoma.

FGF10 in aplasia of lacrimal and salivary glands and in lacrimo-auriculo-dento-digital syndrome

Autosomal dominant aplasia of lacrimal and salivary glands (ALSG; MIM# 180920) is a rare condition caused by aplasia or hypoplasia of various glands including those of lacrimal, parotid, submandibular and sublingual origin (Wiedemann, 1997). ALSG is characterized by irritable eyes, epiphora (constant tearing), and xerostomia (dryness of the mouth), increased risk of dental erosion, caries and periodontal disease (Entesarian et al., 2005). ALSG overlaps clinically with the lacrimo-auriculo-dento-digital (LADD; MIM# 149730) syndrome. In LADD, aplasia of lacrimal and salivary glands is present together with

multiple congenital malformations of variable expressivity affecting the teeth (microdontia), ears (small, cup-shaped, and low-set ears; hearing loss), distal limb (multiple thumb malformations), respiratory system (complex pulmonary malformations) and genitalia (Hollister et al., 1973; Francannet et al., 1994; Rohmann et al., 2006).

Several studies have reported mutations in FGF10 associated with ALSG and LADD. These include nucleotide substitutions leading to R80S, G138E, W169X, R193X amino acid changes as well as deletion of exons 2 and 3 (Δ exons 2/3) reported in ALSG; and C106F, I156R and K137X amino acid changes reported in LADD (Table 1) (Entesarian et al., 2005; Rohmann et al., 2006; Milunsky et al., 2006). ALSG and LADD are allelic disorders as demonstrated by appearance of ALSG and LADD in a mother and daughter pair, both carrying a FGF10-K137X mutation (Milunsky et al., 2005).

The importance of FGF10 for embryogenesis is highlighted by the phenotype of *Fgf10*^{-/-} mice that die shortly after birth and show virtual absence of lungs and fore- and hindlimbs, due to the impaired induction of primary lung and limb buds, respectively (Sekine et al., 1999; Min et al., 1998). Although *Fgf10*^{+/-} heterozygotes originally appeared normal, detailed analysis revealed aplasia/hypoplasia of the lacrimal and salivary glands, phenocopying ALSG (Min et al., 1998; Sekine et al., 1999; Entesarian et al., 2005). Ectopic expression of FGF10 results in induction of several types of glands *in vitro* and *in vivo*, including those of lacrimal, Harderian (nasal) and ocular origin, confirming the essential role of FGF10 in induction of glandular morphogenesis (Govindarajan et al., 2000; Makarenkova et al., 2000). In this process, FGFR2b appears to be the receptor for FGF10, as evidenced by their high binding affinity and similarities between the *Fgf10* and *Fgfr2b* knock-out mice (Igarashi et al., 1998; Sekine et al., 1999; De Moerloose et al., 2000; Ohuchi et al., 2000; Jaskoll et al., 2005). Correspondingly, mutations in FGFR2b (A628T, A648T and R649S Δ Asp650) have also been associated with LADD, again confirming the importance of the FGF10-FGFR2b interaction for this disorder (Rohmann et al., 2006). Experimental studies have shown impaired FGF10-FGFR2b signaling with LADD mutants when compared to wt FGF10-FGFR2b, due to rapid degradation of the ligand (FGF10-K137X and C106F), decreased receptor affinity (FGF10-I156R), or weak tyrosine kinase activity by the receptor (FGFR2b-A628T, A648T and R649S) (Shams et al., 2007; Lew et al., 2007).

Lacrimal gland development involves complex branching morphogenesis, which begins when epithelial-mesenchymal interactions initiate epithelial budding. FGF10-mediated FGFR2b signaling initiates glandular morphogenesis by stimulating cell division and matrix remodelling in cellular precursors to the gland epithelia (Makarenkova et al., 2000; Steinberg et al., 2005). This process is dependent on SHP2, ERK and PI3K signaling and induction of matrix metalloproteinase 2 (Steinberg et al., 2005). It is important to note that loss of both copies of *Fgf10* results in major developmental disturbances in mice, i.e. complete loss of lungs and limbs (Min et al., 1998; Sekine et al., 1999), whereas *Fgf10* haploinsufficiency causes human and murine ALSG (Entesarian et al., 2005). Glandular induction thus appears to be much more sensitive to the signaling strength of FGF10/FGFR2b than are limb or lung bud induction.

FGF14 in autosomal-dominant spinocerebellar ataxia 27

The spinocerebellar ataxias (SCA) are a group of hereditary neurodegenerative diseases for which more than 14 different genetic loci have been identified. In 2003, van Swieten et al. described a large family of Dutch origin with progressive autosomal dominant cerebellar ataxia accompanied by tremor, low cognitive performance, memory impairment and various behavioral problems (van Swieten et al., 2003; Brusse et al., 2006), that was later classified as SCA27 (MIM# 609307). Genetic analysis revealed a nucleotide transition at the position

434 of the FGF14 gene (MIM# 601515), resulting in F145S substitution in the amino acid sequence (van Swieten et al., 2003). Later, Dalski et al. described a patient with clinical features similar to SCA27 that carried a truncating mutation in FGF14 (D163fsX12), as a result of a single nucleotide deletion at position 487, causing a frameshift and premature stop codon 12 amino acids later (Table 1) (Dalski et al., 2005).

According to the *in silico* modeling of the FGF14 structure, the F145S substitution decreases FGF14 protein stability and thus has an apparent loss-of-function effect (van Swieten et al., 2003). This is confirmed by the phenotype of the *Fgf14* null or combined *Fgf12/Fgf14* null mice, which phenocopies human SCA27. Such animals are viable and anatomically normal, but develop profound ataxia, a paroxysmal movement disorder and cognitive defects. This suggests an involvement of several brain structures such as cerebellar granular neurons, Purkinje cells, and hippocampal neurons, which all represent the prominent sites of FGF14 expression in the brain (Munoz-Sanjuan et al., 1999; Wang et al., 2002; Goldfarb et al., 2007; Wozniak et al., 2007; Xiao et al., 2007; Shakkotai et al., 2009).

Fgf14^{-/-} mice show normal cerebellar architecture and granular neuron morphology, demonstrating that the cause of ataxia and paroxysmal movement disorder stems from impaired cellular function rather than a gross developmental or degenerative defect (Goldfarb et al., 2007). Indeed, various experiments demonstrate that neurons lacking FGF14 (or those expressing FGF14-F145S) have marked deficits in their excitability and basal synaptic function, due to the altered inactivation and recovery of their voltage-gated Na channels, presynaptic vesicular trafficking and docking and expression of synaptic proteins (Goldfarb et al., 2007; Laezza et al., 2007; Xiao et al., 2007). FGF14 colocalizes with voltage-gated Na_v channels in the soma and initial segment of the neurons, via binding on the cytoplasmic C-terminal tail of the channel's α -subunit (Na_v α) (Liu et al., 2001; Wittmack et al., 2004; Goldfarb et al., 2007). It appears that this interaction is necessary for proper functioning of the channel, explaining the neuronal excitability defects in *Fgf14*^{-/-} mice (Goldfarb et al., 2007). In contrast, the SCA27-associated FGF14-F145S mutant seems to exert a dominant-negative effect on wt FGF14 function. FGF14-F145S does not interact with Na_v α , but rather binds wt FGF14 preventing its interaction with Na_v α (Laezza et al., 2007).

FGF20 in Parkinson disease

Parkinson disease (PD; MIM# 168600) is the second most common neurodegenerative disease affecting approximately 1% of population over age 50. PD is manifested by resting tremor, bradykinesia and rigidity caused by the loss of dopaminergic neurons within substantia nigra. The cause of dopaminergic cell death is not clear, with multiple genetic and environmental factors contributing to the disease development in most cases (de Lau et al., 2006).

By linkage analysis performed in 174 families with multiple members affected, Scott et al. found five distinct loci to account for an increased risk of PD (Scott et al., 2001). Later, FGF20 (MIM# 605558) was identified as a candidate gene that influences PD, lying within the area of strongest linkage result identified in original genomic screen (Scott et al., 2001; van der Walt et al., 2004). By pedigree disequilibrium test performed on 644 PD families, one intronic SNP and two SNPs located within the FGF20's 3' untranslated region (UTR) showed significant association with PD risk (van der Walt et al., 2004). As one of the 3' UTR SNPs (rs12720208) disrupts the predicted binding site for the brain-expressed microRNA miR-433, it may affect the biogenesis of FGF20 by causing its upregulation. This prediction was confirmed in experiments where SNP rs12720208 rendered expression of a luciferase construct (containing the full-length 3' UTR of FGF20) insensitive to miR-433-mediated downregulation (Wang et al., 2008).

How can FGF20 upregulation cause PD? Within the brain, FGF20 is preferentially expressed in substantia nigra and has neurotrophic and pro-survival actions on dopaminergic neurons (Ohmachi et al., 2000; Murase et al., 2006). However, as shown in dopaminergic neuroblastoma cell line SH-SY5Y, FGF20 can also upregulate α -synuclein, a principal protein component of filamentous Lewy bodies, which represent the pathological hallmark of PD (Spillantini et al., 1997; Wang et al., 2008). α -synuclein mutations, overexpression or gene duplication causes PD in various experimental systems (Polymeropoulos et al., 1997; Kirik et al., 2002; Chartier-Harlin et al., 2004). Antagonistic pleiotropy may thus apply to the FGF20 role in PD, where it positively regulates proliferation, differentiation and stress-resistance of dopaminergic neurons during early stages of life. In elders, however, chronically elevated FGF20 may contribute to deterioration of dopaminergic neurons by upregulation of α -synuclein (Wang et al., 2008).

FGF23 in autosomal-dominant hypophosphataemic rickets

Autosomal dominant hypophosphataemic rickets (ADHR; MIM# 193100) is a disorder of renal phosphate wasting, characterized by hypophosphataemia, rickets (defective cartilage mineralization), osteomalacia (defective bone mineralization), short stature, bone pain, dental abscesses and low or inappropriately normal circulating levels of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) (Econs and McEnery, 1997). As ADHR-associated osteomalacia resembles tumor-induced osteomalacia (TIO), and TIO is usually resolved by removal of the responsible tumor, it was speculated that a humoral phosphaturic factor, so called "phosphatonin", is responsible for both conditions (Cai et al., 1994; Strewler, 2001). In 2000, positional cloning and subsequent sequencing identified missense mutations in FGF23 (MIM# 605380) in all four ADHR families tested, specifically causing R176Q, R179Q or R179W amino acid substitutions in FGF23 (Table 1) (ADHR Consortium, 2000). Shortly afterwards, overexpression of FGF23 was shown to be the causative factor of TIO (Shimada et al., 2001).

Several *in vivo* models confirm that FGF23 is indeed a "phosphatonin" hormone, essential for the control over phosphate homeostasis. Animals implanted with FGF23-R176Q-expressing CHO cells, injected with naked FGF23-R176Q DNA or overexpressing FGF23-R176Q in a liver-specific manner all suffer from hypophosphatemia, osteomalacia and low 1,25(OH)₂D₃ levels (Saito et al., 2003; Bai et al., 2003; 2004).

As ADHR has autosomal dominant inheritance, the clustering of FGF23 missense mutations suggested they are of a gain-of-function nature. This was also suggested by a phenotype of transgenic mice overexpressing FGF23 under the $\alpha 1(I)$ collagen promoter, which resemble ADHR in their growth retardation, osteomalacia and disturbed phosphate homeostasis (Larsson et al., 2004). In fact, all three ADHR mutations stabilize full-length FGF23 by disrupting the ¹⁷⁶RHTR¹⁷⁹ motif that serves as a cleavage site for subtilisin-like proprotein convertase or PHEX endopeptidase (The HYP Consortium 1995; White et al., 2001). Correspondingly, mutated FGF23 is resistant to intracellular proteolytic processing and circulates as a full-length 32kDa variant only, in contrast to wt FGF23 that is released from cells as a mixture of full-length and truncated 12 kDa variant (White et al., 2001).

How is FGF23 action mediated? Although the target organ of endocrine FGF23 action is clearly the kidney proximal tubules, the source of circulating FGF23 is less obvious. Several studies addressing this question report both wide-spread and restricted FGF23 expression, with mineralized tissues being the prominent source (ADHR Consortium, 2000; Cormier et al., 2005; Yoshiko et al., 2007). Thus FGF23, produced by bone, downregulates phosphate via inhibiting its reabsorption in the renal proximal tubules, possibly through the inhibition of Na/P_i co-transporters (Npt). Correspondingly, the kidney expression of Npt1, Npt2a and Npt2c is inhibited by FGF23 (Saito et al., 2003; Bai et al., 2004; Larsson et al., 2004; Inoue

et al., 2005). In addition to Npt, FGF23 might influence other regulators to exert its systemic effects such as vitamin D, which is decreased in ADHR due to FGF23 effects on the expression of its key metabolizing enzymes CYP27B1 and CYP24A1 (Bai et al., 2003; Saito et al., 2003; Bai et al., 2004; Shimada et al., 2004a).

Detailed analyses of the FGF23 signaling mechanisms lead to the question of its cognate FGFR. The activation of intracellular mediators of FGF23 signaling such as ERK MAP kinase pathway was detected in kidney but not in other 10 organs tested suggesting the existence of renal receptor unique for FGF23. Analyzing the murine renal homogenates for proteins that bind FGF23, Urukawa et al. identified Klotho as the major FGF23-binding protein in kidney, experimentally capable of restoring the cell's responsiveness to FGF23 both *in vitro* and *in vivo* (Urukawa et al., 2006; Kurosu et al., 2006).

Klotho is a type I membrane protein that has large 980 amino acid extracellular domain followed by short 11 amino acid cytoplasmic domain, originally described as an anti-ageing molecule (Kuro-o et al., 1997). According to the present stage of knowledge (reviewed by Drücke et al., 2007), Klotho functions as co-receptor for FGF23. FGF23 binds to HS poorly, resulting in its restricted ability to activate its cognate FGFR1c, FGFR3c and FGFR4. This phenotype is rescued by Klotho, which interacts simultaneously with both FGF23 and FGFR, to increase FGF23's binding affinities to the level sufficient for FGFR activation (Kurosu et al., 2006; Urukawa et al., 2006; Goetz et al., 2007).

FGF23 in familial tumoral calcinosis

Familial tumoral calcinosis (FTC; MIM# 211900) is a rare metabolic disorder characterized by the progressive deposition of basic calcium phosphate crystals in periarticular spaces and often found in the hip, elbow or shoulder (Palmer 1966). Calcifications lead to painful skin ulcerations, secondary skin and bone infections, contractures, which often necessitate surgical removal and result in incapacitating damage (Topaz et al., 2005). Recently, several missense mutations leading to H41Q, Q54K, S71G, M96T, S129F substitutions in the FGF23 amino acid sequence were identified in patients suffering from FTC (Table 1) (Araya et al., 2005; Chefetz et al., 2005; Benet-Pagès et al., 2005; Larsson et al., 2005; Garringer et al., 2008).

FTC patients present with hyperphosphataemia and inappropriately normal or elevated $1,25(\text{OH})_2\text{D}_3$, a phenotype that mirrors ADHR and resembles *Fgf23*^{-/-} mice or patients with loss-of-function mutations in Klotho (Shimada et al., 2004b; Ichikawa et al., 2007), all suggesting an FTC mutations lead to the loss of FGF23 function. Molecular structure modelling suggests that FTC mutations disrupt FGF23 structure, thus resulting in putative loss-of-function (Chefetz et al., 2005; Larsson et al., 2005). This is supported by experimental evidence demonstrating poor maturation and secretion of FGF23 carrying FTC mutations, due to the retention of mutated FGF23 within the Golgi complex of producing cells (Benet-Pagès et al., 2005; Garringer et al., 2008).

Apart from FGF23, FTC can also be caused by inactivating mutations in UDP-GalNAc: polypeptide *N*-acetylgalactosaminyltransferase 3 (GALNT3) (Topaz et al., 2004). GALNT3 is a Golgi-associated enzyme that mediates *O*-linked glycosylation of proteins by attaching GalNAc residues to serine or threonine (Ten Hagen et al., 2003). Intriguingly, the FTC patients carrying GALNT3 have low secreted levels of bioactive full-length FGF23 but high levels of its inactive C-terminal fragment, suggesting enhanced proteolytic processing of FGF23 (Topaz et al., 2004; Frishberg et al., 2007). Recently, Kato et al. showed that GALNT3 *O*-glycosylates the threonine 178 of FGF23, localized within the proprotein convertase cleavage site ¹⁷⁶RHTR¹⁷⁹. This glycosylation seems to prevent FGF23

degradation either by modifying FGF23 structure or by prohibiting the proprotein convertases from accessing the cleavage motif (Kato et al., 2006; Fukumoto et al., 2007).

FUTURE PERSPECTIVES

At the present time, the FGF9 mutations in carcinoma represent the only known somatic FGF mutations, in contrast to other FGF mutations which appear to be of a germline origin. The disorders caused by mutations in FGF3, FGF8, FGF10 and, to some extent, FGF14 are developmental defects where anatomical or physiological impairment of the target tissue occurs early in the embryonal development. In contrast, the conditions caused by disturbed signaling of FGF23 offer several treatment possibilities. In ADHR, the Klotho/FGFR complex may be targeted, for example by a neutralizing antibody, to suppress the FGF23 signaling in order to negate the effect of stabilizing FGF23 mutations. In FTC on the other hand, the stable FGF23 ADHR mutants may prove useful to restore the FGF23 signaling impaired by the loss-of-function mutations.

Although FGFs play critical roles in development and life, the molecular pathology of the FGF family was only appreciated recently, with the discovery of FGF23 mutations associated with ADHR in a year of 2000 (ADHR Consortium, 2000). Nine years later, we know at least eight different human conditions caused by mutations in FGF3, FGF8, FGF9, FGF10, FGF14 and FGF23 (Table 1). This list is very likely to expand in near future. The FGF2, FGF15, FGF16, FGF17 and FGF18 represent another possible candidates for human diseases as suggested by the phenotypes of their knockout mice (reviewed by Itoh, 2007), that are briefly discussed below.

Fgf2 knockout mice are morphologically normal but show decreased vascular smooth muscle contractility, low blood pressure and thrombocytosis suggesting the essential role of FGF2 in vascular tone (Zhou et al., 1998). In addition, *Fgf^{-/-}* mice develop decreased bone mass and bone formation rates with age demonstrating the role of FGF2 in maintaining the proper bone function (Montero et al, 2000).

Mice lacking both *Fgf15* alleles die in late embryonic or early postnatal stages due to heart malfunction caused by misaligned aorta and pulmonary trunk. These defects correlate with early morphological defects of the outflow tract due to aberrant behavior of the cardiac neural crest cells (Vincentz et al., 2005).

Fgf16 knockout mice die at E11.5, showing hemorrhage in the heart and ventral body region as well as multiple facial abnormalities. Morphological analysis of developing hearts demonstrates poor trabeculation, thinning of the myocardial wall and dilation of cardiac chambers in mid gestation *Fgf16 null* embryos, possibly due to the impaired proliferation of embryonic myocardial cells (Yan Lu et al., 2008).

Mouse *Fgf17^{-/-}* mutants are viable and anatomically normal but show abnormalities in frontal cortex (FC), mainly reduction of the dorsal FC size and complementary rostral shift of caudal cortical areas (Cholfin and Rubenstein, 2007). *Fgf17^{-/-}* mice present with striking abnormalities during several social behaviours such as offspring-to-mother vocalization, male-to-female interaction, and exploration of novel environment, all suggesting that aberrant FGF17 signaling may contribute to the neurological disorders that affect such behaviour (Searce-Levie et al., 2008).

The phenotype of *Fgf18* knockout mice demonstrates an important role of FGF18 in bone and cartilage physiology. *Fgf18^{-/-}* mice present with expanded zones of proliferating and hypertrophic chondrocytes and increased chondrocyte proliferation and differentiation. This phenotype is similar to that observed in mice lacking functional FGFR3, implying that

FGF18 acts as a physiologic ligand for FGFR3 in mice (Liu et al., 2002; Deng et al., 1996). In addition, *Fgf18*^{-/-} mice display delayed calvaria ossification and decreased expression of bone markers *osteopontin* and *osteocalcin*, possibly due to the impaired proliferation and terminal differentiation of osteogenic mesenchymal cells (Ohbayashi et al., 2002).

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TABLE 1

FGF mutations in human diseases and the corresponding mouse models.

FGF mutation coding DNA protein		Human condition	Corresponding mouse model/phenotype	References
<i>FGF3</i> (NM_005247)				
c.17 T>C	p.Leu6Pro (loss?)	Deafness associated with complete absence of inner ear structures (Michel aplasia), microtia and microdontia	Impaired inner ear development in <i>Fgf3</i> knock-out mice and in <i>Fgf3/Fgf10</i> knockout mice	Tekin et al., 2007, 2008; Alsmadi et al., 2009; Mansour et al., 2003; Alvarez et al., 2003; Hatch et al., 2007
c.196 G>T	p.Gly66Cys (loss?)			
c.254 delT	p.Ile85MetfsX15 (loss?)			
c.310 C>T	p.Arg104X (loss?)			
c.466 T>C	p.Ser156Pro (loss?)			
c.616 delG	p.Val206SerfsX117 (loss?)			
<i>FGF8</i> (NM_033165)				
	p.Asp73His (loss)	Non-syndromic cleft lip and palate	Cleft palate in <i>Fgf8</i> hypomorphic mice	Riley et al., 2007; Meyers et al., 1998
c.40 C>A	p.His14Asn (loss)	Hypogonadotropic hypogonadism and Kallmann syndrome	Lack of GnRH neurons in <i>Fgf8</i> hypomorphic mice	Falardeau et al., 2008
c.77 C>T	p.Pro26Leu (loss)			
c.118 T>C	p.Phe40Leu (loss)			
c.298 A>G	p.Lys100Glu (loss) *			
c.379 C>G	p.Arg127Gly (loss) *			
c.686 C>T	p.Thr229Met (loss) *			
<i>FGF9</i> (NM_002010)				
c.563 delT	p.Leu188TyrfsX18 (loss) *	Colorectal, endometrial and ovarian carcinoma	No	Abdel-Rahman et al., 2008
c.251 G>A	p.Gly84Glu (loss)			
c.424 G>T	p.Glu142X (loss)			
c.518 G>A	p.Arg173Lys (loss)			
c.574 G>A	p.Val192Met (loss) *			
c.608 A>G	p.Asp203Gly (loss) *			
<i>FGF10</i> (NM_004465)				
c.240 A>C	p.Arg80Ser (loss)	Autosomal-dominant aplasia of lacrimal and salivary glands	Aplasia/hypoplasia of lacrimal and salivary glands in <i>Fgf10</i> ^{+/-} mice	Entesarian et al., 2005, 2007; Milunsky et al., 2005
c.413 G>A	p.Gly138Glu (loss)			
	p.Trp169X (loss)			
c.577 C>T	p.Arg193X (loss)	Autosomal-dominant lacrimo-auriculo-dento-digital syndrome	No	Rohmann et al., 2006; Milunsky et al., 2006; Shams et al., 2007
c.317 G>T	p.Cys106Phe (loss) *			
c.409 A>T	p.Lys137X (loss) *			
c.467 T>G	p.Ile156Arg (loss) *	Non-syndromic cleft lip and palate	Cleft palate in <i>Fgf10</i> ^{-/-} mice	Riley et al., 2007; Rice et al., 2004
	p.Ser59Phe (loss)			
<i>FGF14</i> (NM_175929)				
c.434 T>C	p.Phe145Ser (loss) *	Autosomal-dominant spinocerebellar ataxia 27	Impaired cognitive abilities, ataxia and paroxysmal dyskinesia in <i>Fgf14</i> ^{-/-} and	van Swieten et al., 2003; Wang et al., 2002; Dalski et al., 2005;
c.487 delA	p.Asp163fsX12 (loss)			

FGF mutation coding DNA protein		Human condition	Corresponding mouse model/phenotype <i>Fgf11^{-/-}/Fgf14^{-/-}</i> mice	References Goldfarb et al., 2007; Wozniak et al., 2007; Laezza et al., 2007
<i>FGF23</i> (NM_020638)				
c.527 G>A	p.Arg176Gln (gain) *	Autosomal-dominant hypophosphataemic rickets	Hypophosphataemia, rickets and osteomalacia in <i>Fgf23-Arg176Gln</i> transgenic mice	ADHR Consortium, 2000; White et al. 2001; Bai et al., 2004
c.535 C>T	p.Arg179Trp (gain) *			
c.536 G>A	p.Arg179Gln (gain) *			
c.123 C>A	p.His41Gln (loss) *	Familial tumoral calcinosis	Hypercalcemia and vascular calcifications in <i>Fgf23^{-/-}</i> mice	Chefetz et al., 2005; Benet-Pages et al., 2005; Larsson et al., 2005; Shimada et al., 2004; Araya et al., 2005; Garringer et al., 2008; Masi et al., 2009
c.160 C>A	p.Gln54Lys (loss) *			
c.211 A>G	p.Ser71Gly (loss) *			
c.287 T>C	p.Met96Thr (loss) *			
	p.Ser129Phe (loss) *			

A putative effect of mutation on FGF function is shown, with asterisk (*) indicating whenever the effect of mutation was confirmed experimentally. GenBank reference sequence numbers (mRNA or cDNA) for FGF genes are indicated. The DNA sequence data for FGF8-Asp73His, FGF10-Trp169X, FGF10-S59F, and for FGF23-Ser129Phe mutations were not available in the original references.

Mutation numbering follows the HGVS guidelines (www.hgvs.org/mutnomen) with +1 of the cDNA as the A of the ATG initiation codon (codon 1).