The FGF family: biology, pathophysiology and therapy

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Abstract | The family of fibroblast growth factors (FGFs) regulates a plethora of developmental processes, including brain patterning, branching morphogenesis and limb development. Several mitogenic, cytoprotective and angiogenic therapeutic applications of FGFs are already being explored, and the recent discovery of the crucial roles of the endocrine-acting FGF19 subfamily in bile acid, glucose and phosphate homeostasis has sparked renewed interest in the pharmacological potential of this family. This Review discusses traditional applications of recombinant FGFs and small-molecule FGF receptor kinase inhibitors in the treatment of cancer and cardiovascular disease and their emerging potential in the treatment of metabolic syndrome and hypophosphataemic diseases.

Autosomal dominant hypophosphataemic rickets
A hereditary disorder of phosphate wasting characterized by rickets, lower extremity deformities and osteomalacia.

Lacrimo-auriculo-dento-digital syndrome
(LADD). A syndrome characterized by abnormalities of the digits and teeth, low-set ears and aplasia of the lacrimal and salivary glands. Mutations in FGFR2 and FGF10 are known to cause LADD.

Kallmann syndrome
This syndrome results from a deficiency of gonadotropin-releasing hormone, which leads to hypogonadism. Mutations in FGFR1c and FGF8 are known to cause Kallmann syndrome.

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There are 18 mammalian fibroblast growth factors (FGF1–FGF10 and FGF16–FGF23) which are grouped into 6 subfamilies based on differences in sequence homology and phylogeny: FGF1 and FGF2; FGF3, FGF7, FGF10, FGF22; FGF4, FGF5 and FGF6; FGF8, FGF17 and FGF18; FGF9, FGF16 and FGF20; and FGF19, FGF21 and FGF23 (REF. 1). The numbered 'FGFs' that are unassigned to subfamilies — the FGF homologous factors (previously known as FGF11–FGF14) — have high sequence identity with the FGF family but do not activate FGF receptors (FGFRs) and are therefore not generally considered members of the FGF family[2] (BOX 1); FGF15 is the mouse orthologue of human FGF19. FGFs are classically considered to be paracrine factors and are known for their roles in tissue patterning and organogenesis during embryogenesis: the first five subfamilies fall into this category. By contrast, the FGF19, FGF21 and FGF23 subfamily has recently been shown to function in an endocrine manner, dependent on the presence of klotho proteins in their target tissues, to regulate bile acid, cholesterol, glucose, vitamin D and phosphate homeostasis[3–6].

The involvement of FGF signalling in human disease is well documented. Deregulated FGF signalling can contribute to pathological conditions either through gain- or loss-of-function mutations in the ligands themselves — for example, FGF23 gain of function in autosomal dominant hypophosphataemic rickets[7], FGF10 loss of function in lacrimo-auriculo-dento-digital syndrome (LADD syndrome)[8], FGF3 loss of function in deafness[9] and FGF8 loss of function in Kallmann syndrome[10] — or through gain- or loss-of-function mutations in FGFRs, which contribute to many skeletal syndromes[41], Kallmann syndrome[36], LADD syndrome[54] and cancer. Therapeutic approaches using exogenous FGFs, antibodies or small molecules are still relatively new, and many avenues of investigation remain open. Recombinant FGF7 is already in use for the treatment of chemoradiation-induced oral mucositis. Future application of the FGFs in renal disease, glucose and phosphate homeostasis, stem cell research, tissue repair and bioengineering, and angiogenesis is expected. Continued efforts to understand the structural biology of FGF–FGFR interactions will play a key part in driving the discovery of new therapies.

In this article, we briefly review current knowledge regarding FGF–FGFR signalling and then focus on the biology, pathology and recent developments regarding the pharmacological applications of each ligand.

The FGF–FGFR signalling system

FGFs. All FGFs, except those in subfamilies FGF1 and FGF2, and FGF9, FGF16 and FGF20, have signal peptides. The FGF9, FGF16 and FGF20 subfamily is nonetheless secreted through the traditional endoplasmic reticulum (ER)–Golgi secretory pathway[11], whereas the FGF1 and FGF2 subfamily is secreted independently[12]. FGFs have a homologous core region that consists of 120–130 amino acids ordered into 12 antiparallel β-strands (β1–β12) flanked by divergent amino and carboxyl termini (FIG. 1a). In general, primary sequence variation of the N- and C-terminal tails of FGFs accounts for the different biology of the ligands[13] (FIG. 1b). The heparan sulphate glycosaminoglycan (HSGAG) binding site (HBS) within the FGF core is composed of the β1–β2 loop and parts of the region spanning β10 and β12. For paracrine FGFs,

Box 1 | **Fibroblast homologous factors**

Although fibroblast homologous factors (FHFs) have high sequence and structural homology with fibroblast growth factors (FGFs) and bind heparin with high affinity, they do not activate FGF receptors (FGFRs). The FHF core structure is similar to that of FGFs: they exhibit the same β-trefoil core that consists of 12 antiparallel β-strands. However, several key receptor-binding residues are divergent or occluded in FHFs. Val157, unique to FHFs, reduces binding to FGFRs by eliminating important hydrogen bonds with the D2–D3 linker of FGFR that are formed by asparagine, threonine or aspartate in FGFs[2]. Furthermore, the carboxyl terminus of FHF packs against the rest of the ligand in such a way as to preclude many FGFR binding residues from interacting[278]. Owing to the inability of FHFs to bind FGFRs, the inclusion of FHFs in the FGF family should be reconsidered. The principal targets of FHFs are the intracellular domains of voltage-gated sodium channels. FHF mutations in mouse models cause a range of neurological abnormalities and FHF mutations in humans are implicated in cerebellar ataxia[263]. Accordingly, FHFs are an intriguing area of research in their own right.

the elements of the HBS form a contiguous, postively charged surface. By contrast, the HBS of the FGF19, FGF21 and FGF23 subfamily contains ridges formed by the β1–β2 loop and the β10–β12 region that sterically reduce HSGAG binding to the core backbone of the FGFs and lead to the endocrine nature of this subfamily[14].

FGFRs. The FGF ligands carry out their diverse functions by binding and activating the FGFR family of tyrosine kinase receptors in an HSGAG-dependent manner. There are four FGFR genes (*FGFR1–FGFR4*) that encode receptors consisting of three extracellular immunoglobulin domains (D1–D3), a single-pass transmembrane domain and a cytoplasmic tyrosine kinase domain[13]. A hallmark of FGFRs is the presence of an acidic, serine-rich sequence in the linker between D1 and D2, termed the acid box. The D2–D3 fragment of the FGFR ectodomain is necessary and sufficient for ligand binding and specificity, whereas the D1 domain and the acid box are proposed to have a role in receptor autoinhibition[15] (FIG. 2a). Several FGFR isoforms exist, as exon skipping removes the D1 domain and/or acid box in FGFR1–FGFR3. Alternative splicing in the second half of the D3 domain of FGFR1–3 yields b (FGFR1b–3b) and c (FGFR1c–3c) isoforms that have distinct FGF binding specificities[16] and are predominantly epithelial and mesenchymal, respectively. Each FGF binds to either epithelial or mesenchymal FGFRs, with the exception of FGF1, which activates both splice isoforms.

After the binding of ligand and HSGAGs, FGFRs dimerize[17–19], enabling the cytoplasmic kinase domains to transphosphorylate on A loop tyrosines to become activated (FIG. 3). A loop phosphorylation is followed by phosphorylation of tyrosines in the C tail, kinase insert and juxtamembrane regions[20]. The two main intracellular substrates of FGFR are phospholipase C (PLC) γ1 (also known as FRS1) and FGFR substrate 2 (also known as FRS2). Phosphorylation of an FGFR-invariant tyrosine (Y766 in FGFR1) at the C tail of FGFR creates a binding site for the SH2 domain of PLCγ and is required for PLCγ phosphorylation and activation. By contrast, FRS2 associates constitutively with the juxtamembrane region of the FGFR. Phosphorylation of FRS2 is essential for activation of the Ras–mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase–Akt signalling pathways[21]. FGFs are also known to function in the cytosol and nucleus of cells, both through endocytosis of activated FGF–FGFR complexes and through endogenous sources of ligand[22].

FGF–FGFR specificity. FGF–FGFR binding specificity is regulated both by primary sequence differences between the 18 FGFs and the 7 main FGFRs (FGFR1b, FGFR1c, FGFR2b, FGFR2c, FGFR3b, FGFR3c and FGFR4) and by temporal and spatial expression patterns of FGFs, FGFRs and HSGAGs. The alternative splice isoforms of FGFRs are generally tissue specific: the b isoform is usually expressed in epithelial tissue, whereas the c isoform is usually expressed in mesenchymal tissue[23]. Ligands are produced in either epithelial or mesenchymal tissue and generally activate receptors of the opposite tissue specificity: in normal physiology, a ligand produced in the epithelium will activate a mesenchymal receptor and vice versa. Several ligands, including FGF1 in particular, pose an exception to this general understanding by promiscuously binding to both b and c isoforms of certain FGFRs. Pathological states can result from a breakdown in binding specificity, as is common in cancers in which FGFs are overexpressed[24]. Structural studies of FGF1, FGF2, FGF8 and FGF10 with their cognate FGFRs show that sequence diversity at FGF N termini, variation in β1 strand length (FIG. 1b) and the alternatively spliced regions in D3 dictate their binding specificities (FIG. 2b,c).

The FGF–FGFR dimer. A functional FGF–FGFR unit consists of two 1:1:1 FGF–FGFR–HSGAG complexes juxtaposed in a symmetrical dimer[18]. Each ligand in the dimer binds both receptors, and the two receptors contact each other directly through a patch at the base of D2. Each ligand interacts with the D2 domain of a second receptor through a secondary receptor binding site, and mutation of ligand residues within this site reduces receptor dimerization and signalling without affecting ligand–receptor binding[25].

HSGAG binding. HSGAG binds to a basic canyon formed on the membrane-distal end of the symmetric dimer to strengthen protein–protein contacts. HSGAG facilitates FGF–FGFR dimerization by simultaneously binding both FGF and FGFR, thereby promoting and stabilizing protein–protein contacts between ligand and receptor both within the 1:1 FGF–FGFR complex and between the two complexes in the 2:2 FGF–FGFR dimer. In addition to facilitating FGF–FGFR binding, HSGAGs stabilize FGFs against degradation, act as a storage reservoir for ligand and determine the radius of ligand diffusion[26]. Interestingly, the divergence in the morphogenetic activities of FGF7 and FGF10 on branching organs appears to correlate with the differences in their HSGAG affinity and the HSGAG-dependent diffusion of these two ligands through the extracellular matrix (H. Makarenkova *et al.*, unpublished observations).

Oral mucositis
This condition results from injury to the epithelium of the oral cavity and can vary widely in severity. In the worst cases, oral mucositis can lead to ulceration, infection and the need for assisted feeding.

Heparan sulphate glycosaminoglycan
(HSGAG). HSGAGs are long chains of repeating disaccharide units that can be variably sulphated or acetylated, allowing for considerable structural diversity. HSGAGs are located in the extracellular matrix at the surface of every cell, where they modulate the activity of a wide range of growth factors and morphogens.

Exon skipping
A specific type of alternative splicing in which an exon is entirely skipped.

Alternative splicing
This process increases protein diversity by dividing up the primary RNA gene transcript, excluding certain exons, and then reconnecting the transcript. These alternative ribonucleotide sequences are then translated, giving a variety of protein isoforms.

Modulators of FGF signalling. FGF-binding protein (FGFBP) is a carrier protein[27] that activates FGFs by releasing them from the extracellular matrix, where they are bound by HSGAGs[28]. FGFBP has been shown to increase FGF2-dependent proliferation of fibroblast cells[29] and may have an important role in the development of some cancers[30]. Other activators of FGF signalling include fibronectin leucine-rich transmembrane protein 3 (FLRT3), which facilitates FGF8 activity through the MAPK pathway[31].

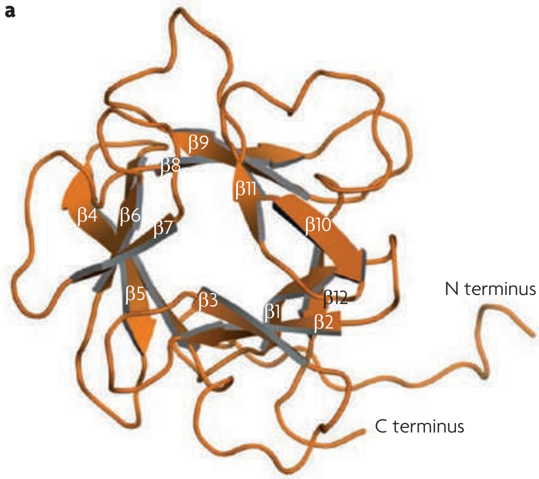
The sprouty family of proteins play an important part in inhibiting receptor tyrosine kinase (RTK) signalling and were first discovered as inhibitors of FGFs in *Drosophila melanogaster*[32]. FGF signalling activates sprouty proteins, which can then in turn inhibit FGF stimulation of the MAPK pathway by interacting with GRB2 (growth factor receptor bound protein 2), SOS1 or RAF1 (REF. 33). MKP3 (MAPK phosphatase 3) is another general inhibitor of RTK signalling that also impinges on FGF activity by dephosphorylating extracellular signal-regulated kinase (ERK)[34]. SEF is a specific inhibitor of FGFs that can function at multiple points along the signalling pathway to attenuate signalling[34].

FGFR pathophysiology and therapy

Germline gain-of-function mutations in FGFRs are responsible for various diseases, such as craniosynostosis, dwarfing syndromes and cancer. Most of the FGFR mutations are ligand independent, but a few — such as Ser252Trp and Pro253Arg in the ectodomain of FGFR2 — manifest only during ligand binding. These mutations cause Apert's syndrome by enhancing ligand binding affinity and promoting the binding of inappropriate ligands[35,278–280]. Remarkably, many of the germline mutations that cause skeletal syndromes also contribute, through somatic mutations, to the development of cancer. Furthermore, mutations in FGFR1–FGFR3 often occur in homologous residues and account for multiple pathologies.

FGFR1. At least three genetic disorders can be attributed to mutations in FGFR1: Kallman's syndrome[36], osteoglophonic dyplasia and Pfeiffer's syndrome[37]. Pathological FGFR1 signalling also occurs in various malignancies. Glioblastoma brain tumours exhibit FGFR1 kinase domain gain-of-function mutations[38], and FGFR1 is abnormally activated

a



b

																		β1			
FGF1	14	E	K	F	N	L	P	P	G	N	Y	K	K	P	K	L	L	Y	C	31
FGF2	37	G	S	G	A	F	P	P	G	H	F	K	D	P	K	R	L	Y	C	34
FGF3	34	G	G	V	Y	E	H	L	G	G	A	P	R	R	R	K	L	Y	C	51
FGF7	55	R	S	Y	D	Y	M	E	G	G	D	I	R	V	R	R	L	F	C	72
FGF10	68	V	R	S	Y	N	H	L	Q	G	D	V	R	W	R	K	L	F	S	85
FGF22	31	P	R	S	Y	P	H	L	E	G	D	V	R	W	R	R	L	F	S	48
FGF4	72	S	G	A	G	D	Y	L	L	G	I	K	R	L	R	R	L	Y	C	89
FGF5	67	Q	S	S	F	Q	W	S	P	S	G	R	R	T	G	S	L	Y	C	84
FGF6	74	N	W	E	S	G	Y	L	V	G	I	K	R	Q	R	R	L	Y	C	91
FGF8	42	L	V	T	D	Q	L	S	R	R	L	I	R	T	Y	Q	L	Y	S	59
FGF17	43	A	M	T	D	Q	L	S	R	R	Q	I	R	E	Y	Q	L	Y	S	60
FGF18	43	R	A	R	D	D	V	S	R	K	Q	L	R	L	Y	Q	L	Y	S	60
FGF9	49	V	T	D	L	D	H	L	K	G	I	L	R	R	R	Q	L	Y	C	66
FGF16	48	P	T	D	F	A	H	L	K	G	I	L	R	R	R	Q	L	Y	C	65
FGF20	52	A	A	Q	L	A	H	L	H	G	I	L	R	R	R	Q	L	Y	C	69
FGF19	33	P	H	V	H	Y	G	W	G	D	P	I	R	L	R	H	L	Y	T	50
FGF21	35	S	S	P	L	L	Q	F	G	G	Q	V	R	Q	R	Y	L	Y	T	52
FGF23	27	N	A	S	P	L	L	G	S	S	W	G	G	L	I	H	L	Y	T	44

c

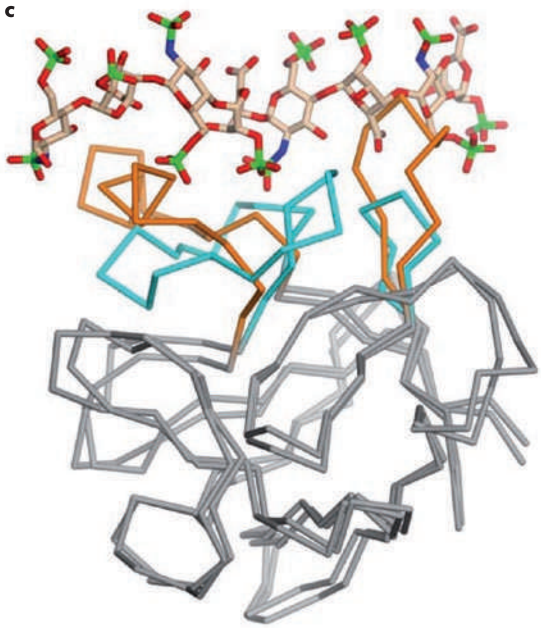


Figure 1 | **Structural features of fibroblast growth factors (FGFs). a** | FGF1, showing its 12 antiparallel β-sheets and amino and carboxyl termini. **b** | The 18 FGFs, grouped according to subfamily. The sequence alignment in the region of the divergent N terminus proximal to the β-trefoil core is given. The β1 strand of FGF1 is provided to indicate the limit of the N terminus. **c** | FGF19 superimposed onto FGF2 from the FGF2–FGF receptor 1–heparin ternary structure (Protein Data Bank). FGF2 and FGF19 are rendered as ribbons and heparin is shown as sticks: oxygen (red), nitrogen (blue), carbon (beige), and sulphur (green) atoms are shown. The core regions of both ligands are coloured grey, and the heparin binding regions of FGF2 and FGF19 are coloured cyan and orange, respectively. Heparin from 1FQ9 clashes with the ridges in the heparin binding region of FGF19. To eliminate these clashes, heparin must translocate away from FGF19 but, in doing so, crucial contacts between heparin and the FGF19 backbone cannot be made. The weakened heparin binding observed in the FGF19 subfamily members is responsible for their endocrine behaviour.

Craniosynostosis
This condition results from the premature closure of sutures of a developing skull before the completion of brain growth. The brain continues to grow in areas of the skull where sutures have not closed, leading to a malformed cranium.

Apert's syndrome
One of the most common craniosynostosis syndromes that exhibits severe syndactyly (digit fusion) of the hands and feet. Apert's syndrome is often associated with visceral abnormalities of the cardiovascular, respiratory and urogenital systems.

Osteoglophonic dysplasia
A bone disorder presenting with dwarfism, vertebral fragility, craniosynostosis and failure to thrive. The term osteoglophonic refers to the 'hollowed out' appearance of the metaphyses in X-rays, which are the growth zones of long bones.

Pfeiffer's syndrome
A craniosynostosis disorder that can also present with polydactyly.

Glioblastoma
An aggressive tumour derived from glial cells that exhibits high levels of neovascularization.

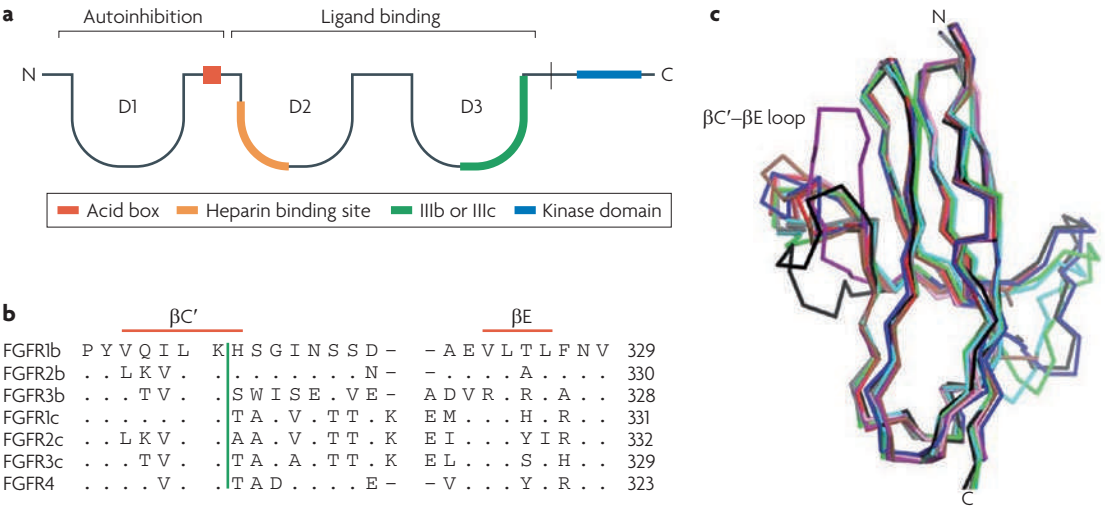


Figure 2 | **Structural features of fibroblast growth factor receptors (FGFRs). a** | A schematic of the FGFR structure. **b** | The sequence alignment of the seven main FGFRs in the region of the βC′–βE loop, including the βC′ and βE strands. The vertical green bar divides the unspliced portion of the receptor at the left from the spliced portion that follows. **c** | A superimposition of the D3 domains of solved FGF–FGFR complex structures. FGF2–FGFR2c is shown in red, FGF8–FGFR2c is shown in purple, FGF1–FGFR1c is shown in blue, FGF1–FGFR2b is shown in green, FGF1–FGFR3c is shown in black, FGF1–FGFR2c is shown in brown, FGF2–FGFR1c is shown in pink, FGF3–FGFR2b is shown in grey and FGF10–FGFR2b is shown in cyan. The variation in the conformation of the βC′–βE loop between the structures as it interacts with divergent amino termini is evident. The plasticity of this loop is a major determinant of FGF–FGFR binding specificity.

Myeloproliferative syndrome
A progressive disease that can transform into acute leukaemia. Also known as stem cell leukaemia or lymphoma syndrome, it often presents with a T-cell lymphoblastic lymphoma and eosinophilia.

Crouzon's syndrome
A craniosynostosis syndrome presenting with a beaked nose and bulging, excessively separated eyes (exopthalmos and hypertelorism, respectively).

Callosal agenesis
An absence of the corpus callosum, the tissue that connects the two hemispheres of the brain.

Ventriculomegaly
A condition associated with enlarged lateral ventricles in the brain. Ventriculomegaly can have many causes, one of which is callosal agenesis.

Hypochrondroplasia
A mild dwarfism syndrome generally presenting with nearly normal cranial and facial characteristics.

Thanatophoric dysplasia type II
A lethal neonatal skeletal dysplasia associated with a severe cloverleaf-shaped skull deformity.

Severe achondroplasia with developmental delay and acanthosis nigricans syndrome
This dwarfism syndrome is accompanied by substantial neurological disorders and acanthosis nigricans, which involves a hyperpigmentation of the skin.

in malignant prostate cells[39]. In 8p11 myeloproliferative syndrome (EMS), translocations fuse different proteins in frame with the FGFR1 kinase domain, causing constitutive dimerization of the kinase[40].

FGFR2. Mutations in the kinase domain of FGFR2 have been identified in patients with various craniosynostosis syndromes, including Crouzon's syndrome and Pfeiffer's syndrome[41]. These mutations constitutively activate FGFRs by disengaging an autoinhibitory molecular brake at the hinge region of the kinase domain[42]. Many of these mutations that lead to skeletal deformity are also commonly observed in endometrial cancers[43,44]. Ectodomain FGFR2 mutations cause ligand-independent disulphide-mediated covalent receptor dimerization and activation in pathologies such as Crouzon's syndrome[45]. Ligand-dependent gain-of-function ectodomain mutations in FGFR2c allow binding to FGFR2b-binding ligands[46,278,280], which contributes to the development of Pfeiffer's syndrome and Apert's syndrome. The mutations involved in these syndromes also cause white matter pathologies, including callosal agenesis and ventriculomegaly[47]. Interestingly, through a dominant-negative effect, soluble FGFR2 can inhibit the osteoblastic differentiation typically observed in Apert's syndrome[48]. Notably, single nucleotide polymorphisms (SNPs) in FGFR2c are associated with BRCA2 mutation-carrying breast cancers[49,286].

FGFR3. Transmembrane mutations, such as Gly380Arg in FGFR3, promote non-covalent interactions between transmembrane helices and occur in nearly all cases of achrondroplasia, which is the most common genetic form of dwarfism[50]. Kinase-domain FGFR3 mutations increase catalytic activity independently of receptor dimerization[51] by disengaging the molecular brake at the kinase hinge region[4]. A range of germline mutations affect three codons (Ile538, Asn540 and Lys650) in the FGFR3 kinase domain, yielding three dwarfing syndromes of varying clinical severity: hypochondroplasia, thanatophoric dysplasia type II and severe achondroplasia with developmental delay and acanthosis nigricans syndrome (SADDAN syndrome)[52–53]. Furthermore, overexpression and gain-of-function mutations in FGFR3 occur in multiple myeloma, an incurable B-cell malignancy[55]. Gain-of-function FGFR3 mutations are the most commonly observed mutations in bladder cancer[56], and activating FGFR3 mutations are also observed in benign skin tumours[57,58]. Most of the FGFR3 mutations found in cancer are identical to the FGFR2 mutations involved in skeletal disorders. Kinase domain loss-of-function mutations also occur in FGFR2 and FGFR3 in LADD syndrome[54] and in FGFR2 in melanoma[281].

FGFR4. FGFR4 has potential value as a prognostic marker in cancer. Arg388 in FGFR4 is associated with increased aggressiveness of prostate cancer, and promotes metastasis by increasing cellular motility and invasiveness[59]. This same allele in FGFR4 is a predictor of poor clinical prognosis in head and neck squamous cell carcinoma[60]. In recurrent breast cancer, high FGFR4 expression correlates with low efficacy of tamoxifen treatment[61].

Therapeutic potential of FGFRs. Direct inhibition of FGFRs may prove to be of clinical value. Sunitinib is a receptor tyrosine inhibitor that has received Food and Drug Administration (FDA) approval for indications in renal cell carcinoma and gastrointestinal stromal tumours,

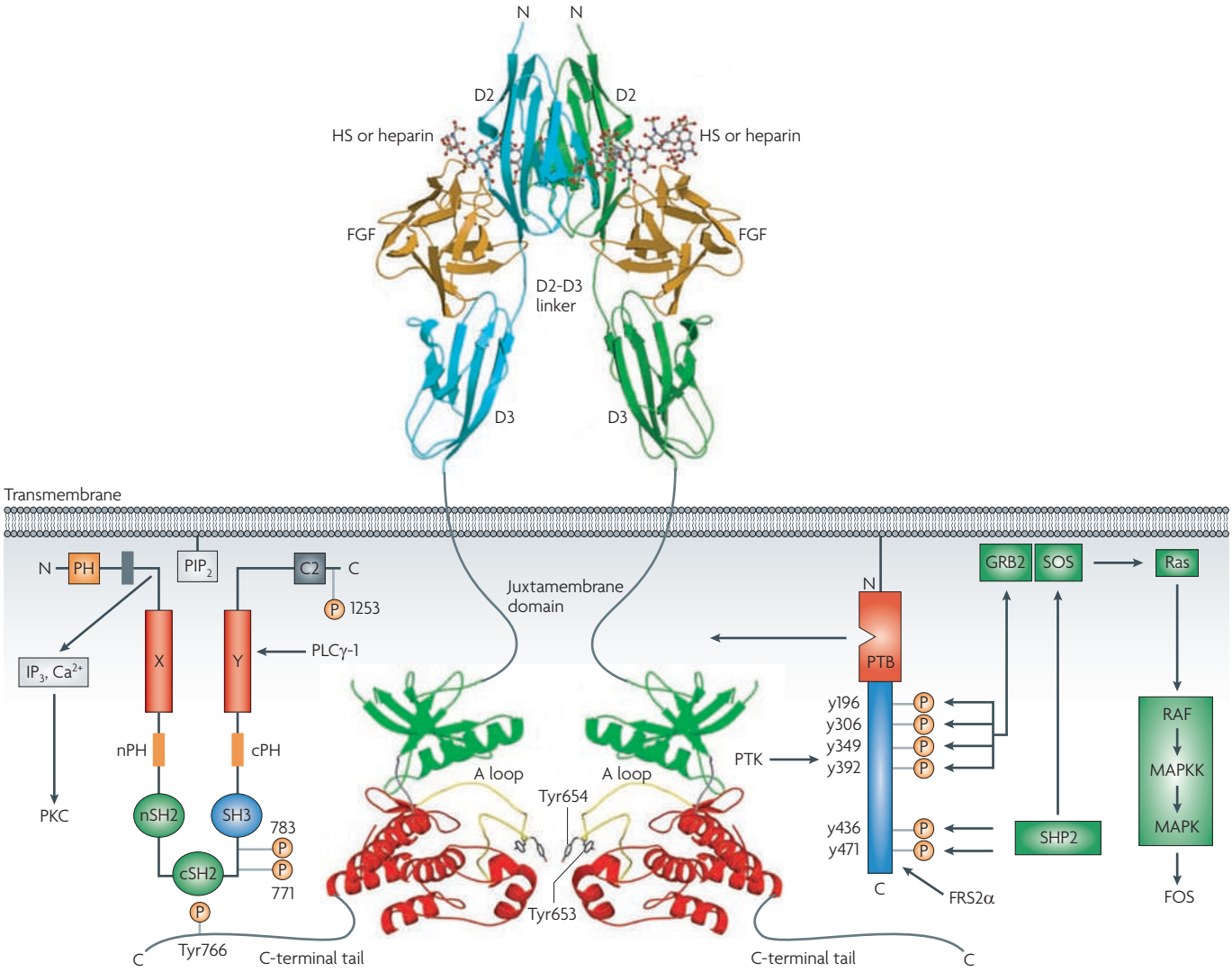


Figure 3 | **Fibroblast growth factor receptor (FGFR) signalling.** Structurally unresolved regions are shown as grey lines. Amino-terminal and carboxy-terminal lobes of the kinase domain are coloured green and red, respectively. The two major intracellular targets, phospholipase (PLC)γ1 and FGFR substrate 2α (FRS2α), are shown. A loop, activation loop; GRB2, growth factor receptor bound 2; HS, heparan sulphate; IP_3, inositol-1,4,5-trisphosphate; MAPK, mitogen-activated protein kinase; MAPKK, mitogen-activated protein kinase kinase; PH, pleckstrin homology domain; PIP_2, phosphatidylinositol-4,5-bisphosphate; PKC, protein kinase C; PTB, phosphotyrosine binding domain; PTK, protein tyrosine kinase; SH, Src homology domain. Figure is modified, with permission, from REF. 13 © (2005) Elsevier Science.

and, unlike imatinib mesylate (Gleevec; Novartis), it does have some activity against FGFRs[62]. SU5402, PD173074 and nordihydroguaiaretic acid are small-molecule FGFR inhibitors that have efficacy in multiple myeloma cell lines with deregulated FGFR3 expression[63,64]. Furthermore, PD173074 has the ability to induce cell cycle arrest in endometrial cancer cells with mutated FGFR2 (REF. 65). In addition to small-molecule inhibition, antibodies against FGFR3 have been shown to effectively cause apoptosis in mouse models of multiple myeloma and bladder cancer[66,67]. These instances are proof of principle that FGFR inhibition could be efficacious in the treatment of malignancy (TABLE 1). Mutation of Tyr766 in the PLCγ1 binding site of FGFR1 attenuates EMS[68]; therefore, interference with the FGFR–PLCγ1 interaction could prove to be a promising therapeutic strategy in the treatment of EMS. The use of PLCγ inhibitors alongside tyrosine kinase inhibitors could also slow the development of drug resistance to these tyrosine kinase inhibitors[24].

Paracrine FGF ligands

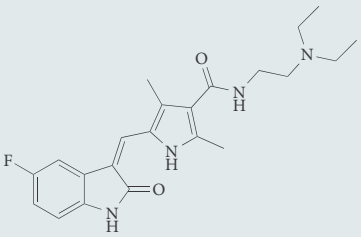
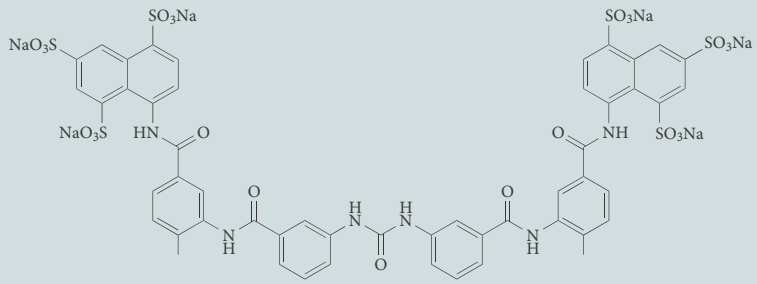
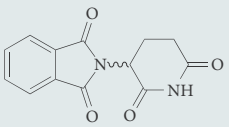
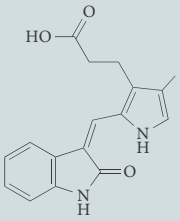
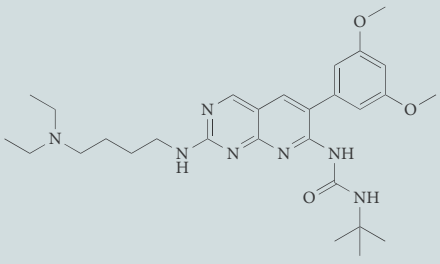
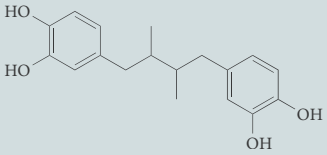
The paracrine FGF families are FGF1 and FGF2; FGF3, FGF7, FGF10 and FGF22; FGF4, FGF5 and FGF6; FGF8, FGF17 and FGF18; and FGF9, FGF16 and FGF20. Their high affinity for HSGAG causes them to act in a localized manner near the source of their expression (TABLE 2). Paracrine FGFs are being explored for their therapeutic potential in angiogenesis, cytoprotection and tissue repair (TABLE 3). For example, recombinant FGF7 is already used in the clinic to treat chemoradiation-induced

mucositis; applications of recombinant FGF1, FGF2 and of FGF4 gene therapy to cardiovascular pathologies are being explored; and recombinant FGF18 is in the early stages of development for osteoarthritis treatment. Many paracrine FGFs are deregulated in cancers, and their overexpression stimulates proliferation and angiogenesis, which can contribute to cancer growth[24].

The FGF1 subfamily

Biology. As both *Fgf1*$^{-/-}$ and *Fgf2*$^{-/-}$ mice are viable and fertile and *Fgf1*$^{-/-}$ mice are apparently completely normal[69], the physiological roles of FGF1 and FGF2 are still unclear. However, it is likely that FGF1 and FGF2 play some physiological part in the maintenance of vascular tone, as administration of FGF1 and FGF2 lowers blood pressure

Table 1 | **Selected inhibitors of FGF signalling**

Drug	Structure	Function	Refs
Sunitinib		• A tyrosine kinase inhibitor that acts on FGFR in addition to many other RTKs • Inhibition occurs at concentrations in the low nanomolar range *in vitro* • 800 ng per ml plasma concentration inhibits FGFR, but only 50–100 ng per ml is needed for efficacy	62
Suramin		• Mimics heparin and interferes with FGF–FGFR binding • Efficacious in a range of cancers but also has the dangerous side effect of coagulopathy	103–107
Thalidomide		• Interferes with FGF2-induced angiogenesis • Phase II trials demonstrated its efficacy in treating cancer, including prostate and renal malignancies	100–102
SU5402		• SU5402, PD173074 and NDGA have shown efficacy against multiple myeloma cell lines through inhibition of the FGFR3 kinase domain • PD173074 has also shown *in vitro* efficacy against FGFR2-deregulated endometrial cancers	63–65
PD173074			
NDGA			

FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; NDGA, norhydroguaiaretic acid; RTK, receptor tyrosine kinase.

in rats[70] and can restore nitric oxide synthase activity in spontaneously hypertensive rats[71]. In addition, isolated vessels from *Fgf2*$^{-/-}$ mice have a reduced response to vasoconstrictors[72]. Although *Fgf2*$^{-/-}$ mice experience some hypotension owing to decreased smooth muscle contractility[72], they are still able to regulate their blood pressure[73].

The angiogenic properties of FGF2 are well known. Exogenous FGF2 stimulates migration and proliferation of endothelial cells *in vivo*[74], has anti-apoptotic activity[75] and encourages mitogenesis of smooth muscle cells and fibroblasts, which induces the development of large collateral vessels with adventitia[76]. However, as overexpression of *Fgf2* does not lead to spontaneous vascular defects[77], and normal vascularization is retained in double knockout *Fgf2*$^{-/-}$;*Fgf1*$^{-/-}$ mice[69], the physiological relevance of these effects is uncertain. Evidently, there is a high level of compensation among the growth factors mediating angiogenesis[78].

Other possible physiological roles for FGF2 include inflammation, in which stress-induced activation of caspase 1 leads to release of FGF2 (REF. 79); and asthma, as FGF2 enables airway smooth muscle cells to proliferate in response to asthma triggers[80].

Interestingly, FGF1 is a proliferative factor for human preadipocytes and may be important to the overall regulation of human adipogenesis[81].

Pathophysiology. A possible role for FGF1 in humans is suggested by its increased levels in the pericardial fluid of patients with cardiac ischaemia[82]. Incubation of endothelial cells with FGF1 leads to microvascular branching[83], and the ligand also has anti-apoptotic activity[84], suggesting mechanisms through which it might function in vascular injury.

Therapeutic potential of FGF1. FGF1 has some therapeutic potential for cardiovascular disorders. Phase I trials have shown that intramyocardial injection of FGF1 during coronary artery bypass graft surgery improves collateral artery growth and capillary proliferation[85]. Beneficial effects of FGF1 on the peripheral circulation have also been shown. Injection of a plasmid that encodes FGF1 (NV1FGF) into the leg improved perfusion of end-stage lower-extremity ischaemia in a Phase I trial[86] and led to a twofold reduction in the need for amputation in patients with critical limb ischaemia in a recent Phase II study[87]. Interestingly, distal blood and oxygen pressure were similar after injection of either NV1FGF or placebo[88] and the mode of action of FGF1 might not have been primarily angiogenic. The TAMARIS (Therapeutic Angiogenesis for the Management of Arteriopathy in a Randomized International Study) Phase III trial is underway to evaluate NV1FGF and will further address the possibility of a systemic mechanism of FGF1 action.

FGF1 can repair nerve injuries. It enabled functional regeneration of transected spinal cords in rats[89] and restored some motor function to paralyzed limbs in a 6-month-old boy with brachial plexus avulsion[90]. FGF1 administration has benefited patients with chronic transverse myelitis[91], and the combination of sural nerve grafts with FGF1 treatment partly restored ambulation to a paraplegic[92].

Therapeutic potential of FGF2 in cardiovascular disease. In an unblinded trial, a single bolus of FGF2 reduced the size of ischaemic regions in the myocardium, improved treadmill performance and reduced the frequency of angina[93,94]. However, in the FGF Initiating RevaScularization Trial (FIRST), FGF2 treatment conferred some benefit in the first few months, but these improvements were not sustained, whereas continued improvement was seen in the placebo group[95,96]. Using a different protocol, implanting heparin beads containing adsorbed FGF2 over ischaemic myocardium reduced the size of the ischaemic region and ameliorated the associated symptoms, with beneficial effects being retained for 3 years of follow-up[97]. This treatment method does require open-chest delivery, but it is one of the few examples of a sustained positive response among Phase I trials with FGF2.

FGF2 has also been examined for its efficacy in the peripheral circulation. Patients suffering from claudication who received intra-arterial FGF2 showed improved calf blood-flow compared with patients who received placebo[98]. However, in the TRAFFIC (Therapeutic Angiogenesis with Recombinant Fibroblast Growth Factor-2 for Intermittent Claudication) study, none of the immediate improvements, such as peak walking time, was ultimately statistically significant[99] (BOX 2).

Therapeutic potential of FGF2 in cancer. Thalidomide is an inhibitor of FGF2-induced angiogenesis[100], and Phase II trials have demonstrated its benefit in patients suffering from androgen-independent metastatic prostate cancer[101] or renal cancer[102]. Suramin, a polysulphated naphylurea, interferes with FGF signalling by mimicking heparin, and is efficacious in bladder, kidney and prostate cancers[103–107]. Treatment of prostate cancer with suramin also enhances the activity of other chemotherapeutics, such as doxorubicin[108] (TABLE 1), possibly by reducing the ability of FGF1 and FGF2 to enable broad-based resistance to anticancer drugs[109]. The high doses of suramin required for clinical efficacy produce substantial side effects, however, including coagulopathy[110]. Suramin is only one example of many heparinoids[111]. One of the most promising heparinoids is PI-88, a heparanase inhibitor that has been studied widely in recent clinical trials[112].

Interferon-α (IFNα) and IFNβ can downregulate FGF2 in kidney, bladder and prostate human cell lines[113]; accordingly, administration of these interferons inhibits FGF2 expression and the growth of bladder carcinoma cells[114]. Some evidence suggests that inhibition of FGF2 signalling slows the growth of tumours by inhibiting vascularization[115]. However, FGF2 levels do not generally correlate with microvessel density in tumours[116], indicating that the mechanism underlying the anti-tumour effects of interferons mediated through FGF2 may not be solely angiogenesis based. In 1995, the success of the ECOG (Eastern Cooperative Oncology Group) Trial 1684 led to the approval of IFNα for the treatment of patients with melanoma[117]. However, because of high toxicity, the use of IFNs in biochemotherapy regimens for metastatic melanoma is no longer recommended[118].

Nitric oxide
Among its many functions, this small molecule relaxes the smooth muscle surrounding blood vessels.

Brachial plexus
The bundle of nerves located in the axilla (armpit) that descends into the upper limb to provide sensation and motor control.

Chronic transverse myelitis
Inflammation across the width of one segment of the spinal cord that can lead to destruction of myelin and neurological impairment.

Heparin
A highly sulphated heparan sulphate glycosaminoglycan (HSGAG). Although it does not act physiologically on FGF–FGFR signalling, it can substitute for other HSGAGs in experimental studies.

Table 2 | **The physiology of FGFs**

Fibroblast growth factor (FGF)	Phenotype of knockout mouse	Physiological role
FGF1	Normal[69]	Not established
FGF2	Loss of vascular tone Slight loss of cortex neurons[72–73]	Not established
FGF3	Inner ear agenesis in humans[9]	Inner ear development[9]
FGF4	Embryonic lethal[128]	Cardiac valve leaflet formation Limb development[126–128]
FGF5	Abnormally long hair[129]	Hair growth cycle regulation[129–131]
FGF6	Defective muscle regeneration[133]	Myogenesis[132,133]
FGF7	Matted hair Reduced nephron branching in kidney[137,138]	Branching morphogenesis[138]
FGF8	Embryonic lethal[162]	Brain, eye, ear and limb development[160,161]
FGF9	Postnatal death Gender reversal Lung hypoplasia[170]	Gonadal development Organogenesis[170,171]
FGF10	Failed limb and lung development[142]	Branching morphogenesis[142]
FGF16	Embryonic lethal[172]	Heart development[172]
FGF17	Abnormal brain development[163]	Cerebral and cerebellar development[163]
FGF18	Delayed long-bone ossification[164,165]	Bone development[164,165]
FGF19	Increased bile acid pool[189]	Bile acid homeostasis Lipolysis Gall bladder filling[3,6,197–201]
FGF20	No knockout model	Neurotrophic factor[175]
FGF21	No knockout model	Fasting response Glucose homeostasis Lipolysis and lipogenesis[4,208–225]
FGF22	No knockout model	Presynaptic neural organizer[143]
FGF23	Hyperphosphataemia Hypoglycaemia Immature sexual organs[185,235]	Phosphate homeostasis Vitamin D homeostasis[226–261]

Gene silencing by antisense targeting of FGF2 and FGFR1 in models of human melanoma caused a dramatic reduction in the size of tumours[115], but many challenges remain for the application of antisense technology in general[119].

Therapeutic potential of FGF2 in other disorders. Interestingly, patients suffering from major depressive disorder have deregulated FGF transcript levels that are restored by serotonin reuptake inhibitors[120]. In rats, *Fgf2* and *Fgfr1* mRNA levels were downregulated in the hippocampi following social defeat[121], and intracerebroventricular administration of FGF2 produced antidepressant-like effects[122]. These data suggest that manipulation of FGF signalling could yield benefits in the treatment of mood disorders.

FGF2 has also been studied in cartilage homeostasis[123], recombinant FGF2 has been shown to have some efficacy in wound healing in patients suffering from ulcers[124], and a recent Phase II study has suggested that recombinant FGF2 (trafermin) could aid in regenerating alveolar bone in patients with periodontitis[125].

FGF4 subfamily

Biology. FGF4 has wide-ranging functions in development, including cardiac valve leaflet formation[126] and limb development[127]. *Fgf4* knockout mouse embryos experience post-implantation lethality owing to the necessity of FGF4 for trophoblast proliferation[128]. FGF5 negatively regulates a step of the hair follicle growth cycle. *Fgf5* knockout mice exhibit abnormally long hair in the absence of any other defect[129], and loss-of-function mutations in the *Fgf5* gene account for hereditary variations in hair length in canines and felines[130,131]. FGF6 plays a part in myogenesis[132], and *Fgf6* knockout mice have defective muscle regeneration, with significantly increased fibrosis following a freeze–crush injury[133].

Therapeutic potential. Whereas FGF2 has primarily been studied as preparations of recombinant protein, FGF4 has been administered by means of gene therapy. Alferminogene tadenovec (Ad5FGF4) is *FGF4* encoded within replication-deficient human adenovirus serotype 5. Phase I and II clinical trials revealed improvements in treadmill exercise capacity, but Phase III trials were discontinued when a high placebo response was revealed[134]. The Phase III Angiogenic Gene Therapy trial (AGENT) demonstrated the safety of the therapeutic method, as flu-like symptoms or hepatic toxicity were rarely observed[135]. A review of all patients showed no significant benefit from the treatment, but a reanalysis revealed a gender-specific response that was traced in part to a reduced placebo response in women[136]. Cardium Therapeutics has initiated the AWARE (Angiogenesis in Women with Angina pectoris who are not candidates for Revascularization) Phase III trial to study the gender-specific response of Ad5FGF4 in women.

FGF7 subfamily

Biology. FGF7, also known as keratinocyte growth factor, is expressed specifically in mesenchyme. *Fgf7*$^{-/-}$ mice are viable and fertile, exhibiting only minor abnormalities, such as matted hair[137], and about 30% fewer nephrons compared with controls[138]. FGF7 levels are increased by up to 150-fold in skin after cutaneous injury[139], also being increased after bladder and kidney injury[140,141].

Homozygous deletions in FGF3 were shown to cause hereditary deafness, leading to total inner ear agenesis in humans[9]. The specificity of FGF3 for this effect is impressive: this *FGF3*$^{-/-}$ human knockout showed no other symptoms apart from a few dental defects. *Fgf10* (also known as *Kgf2*) knockout mice lack limbs and pulmonary structures[282,283], in addition to exhibiting defects in all other branching organs. FGF22, along with FGF7 and FGF10, is a presynaptic organizer with roles in vesicle clustering and neurite branching[143].

Trophoblast
These cells form the outer layer of the developing embryo and are responsible for its implantation into the endometrium.

Table 3 | **Applications of FGFs and FGFRs**

Ligand or receptor	Current/potential therapeutic application	Refs
FGF1	Recombinant FGF1 used with nerve grafts Treatment of peripheral ischaemia with FGF1 plasmids	87,88, 90–92
FGF2	Use of thalidomide in prostate and renal cancer Implantation of FGF2-coated heparin beads post-MI Recombinant FGF2 modulates mood in mice	97, 101–102, 122
FGF4	Potential gene therapy for stable angina in women	136
FGF5	Potential of FGF5 inhibitors to aid hair growth	129
FGF7	Treatment of mucositis (known as the drug palifermin) Recombinant FGF7 improves wound healing	149, 157
FGF18	Recombinant FGF18 has an anabolic effect on cartilage	167
FGF19	Potential of recombinant FGF19 in diabetes	3,6,199,200
FGF20	Potential in Parkinson's disease	176
FGF21	Potential of recombinant FGF21 in diabetes	4,208–225
FGF23	Use of anti-FGF23 antibodies in hypophosphataemia	260,261
FGFR1	PLCγ inhibitors in the treatment of EMS and as an adjunct to TKIs	24,68
FGFR2	Small-molecule inhibitors and anti-FGFR2 antibodies in endometrial cancer	65
FGFR3	Small-molecule inhibitors and anti-FGFR3 antibodies in multiple myeloma	63,64,66,67
FGFR4	Prognostic marker in prostate cancer and squamous cell carcinoma	59,60

EMS, 8p11 myeloproliferative syndrome; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; MI, myocardial infarction; PLCγ, phospholipase Cγ; TKI, tyrosine kinase inhibitor.

Pathophysiology. LADD syndrome, an autosomal-dominant disease characterized by hearing loss, dental anomalies, and lacrimal and salivary gland hypoplasia, is caused by FGF10 loss-of-function mutations[8].

A correlation between disease and FGF7 subfamily expression is observed for several disorders. Overexpression of FGF7 correlates with inflammation in patients suffering from inflammatory bowel disease, suggesting that FGF7 may have a compensatory role[144]. FGF7 and FGF10 are also overexpressed in psoriatic skin[145,146]. FGF10 and FGF7 are thought to act as andromedins (mediators of androgen action)[147,148] and as such could have a role in the pathogenesis of prostate cancer by facilitating epithelial cell proliferation.

Therapeutic potential. Palifermin, an N-terminally truncated form of FGF7 with increased stability, is FDA approved for the treatment of chemoradiation-induced oral mucositis in patients undergoing bone marrow transplantation. When administered on 3 consecutive days before high-dose chemotherapy, as well as for 3 days following haematopoietic stem cell transplantation, palifermin reduced the median duration of mucositis from 9 to 6 days, and reduced the incidence of grade 4 mucositis from 62% to 20%. This corresponds with a significant improvement in the patients' quality of life, as grade 4 mucositis is of such severity that oral feeding is impossible. Importantly, palifermin also reduced the patients' use of opioid analgesics, which indicates that there was reduced pain. The adverse events associated with palifermin were mild and transient, and most were attributable to the underlying cancer or concurrent chemotherapeutic regimen[149].

Palifermin acts mainly by increasing cell proliferation. Studies indicate that the increased epithelium thickness produced by a dose of palifermin can be maintained for up to 1 week[150,151].

Other mechanisms of FGF7 action could include upregulation of *NRF2*, which activates genes that encode antioxidant enzymes[152]. Inflammatory cytokines are important to the pathogenesis of mucositis, and FGF7 may affect this aetiology both by reducing the ratio of T-helper type 1 to T helper type 2 cytokines[153] and by reducing tumour necrosis factor-α and IFNγ levels through its induction of interleukin 13 (REF. 154).

New applications for palifermin are being investigated. Palifermin reduces the incidence of graft-versus-host disease and also improves immune function in animal models[155]. However, these findings have not yet been corroborated in clinical trials, perhaps because of the inclusion of methotrexate in the stem cell transplantation regimen, a compound that is cytotoxic to epithelial cells and could counteract the beneficial proliferative effects of palifermin[156]. Treatment of injured epithelia with FGF7 results in an improved wound-healing response[157], suggesting the potential use of FGF7 for tissue engineering.

Human Genome Science explored recombinant FGF10 (repifermin) as a treatment option for ulcerative colitis and mucositis, but its development was terminated in 2004 after it failed in several clinical trials[158,159].

FGF8 subfamily

Biology. FGF8 is involved in brain, limb, ear and eye development[160] and, along with FGF17, is crucial for forebrain patterning[161]. *Fgf8*$^{-/-}$ mice do not undergo gastrulation[162], *Fgf17*$^{-/-}$ mice exhibit abnormalities in the development of cerebral and cerebellar structures[163], and *Fgf18*$^{-/-}$ mice have decreased expression of osteogenic markers and delayed long-bone ossification[164,165].

Pathophysiology. Loss-of-function mutations in FGF8 that affect its binding to FGFR1c or cause degradation of FGF8 lead to Kallmann's syndrome, a developmental disorder characterized by anosmia and hypogonadism[10].

Therapeutic potential. FGF18 is currently under investigation by Merck Serono for the treatment of osteoarthritis, which is a disease involving degeneration of cartilaginous tissue. FGF18 has an anabolic effect on cartilage: a single intravenous injection of FGF18 leads to increased deposition of cartilage in the ribs, trachea, spine and joints[166]. In a rat model of osteoarthritis, intra-articular injection of FGF18 increased cartilage formation[167]. Merck Serono is now following up this preclinical data with Phase I clinical trials to study the effects of FGF18 on osteoarthritis progression in humans.

Monoclonal antibodies against FGF8 have shown some efficacy in mouse models of breast cancer and prostate cancer[168,169].

Box 2 | **Challenges to the treatment of cardiovascular disease with fibroblast growth factors**

The best method for administering growth factors for the purpose of angiogenic stimulation has been a matter of some discussion. The long-term presence and slow release of growth factors in the tissue is important for maintenance of new vasculature[264], but the mean half-life of fibroblast growth factor 2 (FGF2) in the body is only about 7.6 hours. This half-life is extended when heparin is co-administered[265,266]. Protein engineering may prove useful, as the half-life of FGF1 in the presence of heparin can be increased by a single amino acid mutation[267], and recent developments have shown that stabilizing mutations within the β-barrel can dramatically decrease the likelihood of protein unfolding[268].

Only 3–5% of the dose is typically retained in the myocardium 150 minutes after intracoronary injection[269,270]. At 24 hours after intracoronary infusion, the myocardium no longer retains any portion of the dose[94]. The intravenous route is even less effective because of first-pass pulmonary metabolism of FGFs. Intramyocardial delivery of FGFs delivers the best dose of growth factor, as it allows targeting of ischaemic areas of the heart and has prolonged tissue retention — up to tenfold higher than that achieved by intracoronary injection[271]. However, intramyocardial injection may not be the most appropriate therapy if the goal is to cause growth of epicardial vessels. Adenoviral vectors may also not produce expression of FGFs for a sufficient length of time to achieve beneficial effects[272].

It seems that a single intracoronary or intra-arterial injection of FGF, although helpful in animals, will be unlikely to affect clinical progress in patients[78]. Intramuscular or intramyocardial administration might yet be feasible, owing to a high retention of protein and its slow removal[273]. The potential for haemangioma formation[274] or neovascularization of atherosclerotic plaques[275] is a concern, however, for long-term safety. The advantages of protein therapy include precision in dosing, the ability to combine multiple proteins in a treatment and a well-characterized safety profile[276]. In summary, therapy with exogenous FGFs has not yet altered the course of cardiovascular disease in humans. Heparin derivatives are perhaps one alternative route of angiogenesis therapy[277] and vascular endothelial growth factor A also holds promise, given its greater specificity for angiogenesis.

FGF9 subfamily

Biology. *Fgf9* knockout mice demonstrate male-to-female sex reversal and lung hypoplasia that quickly leads to postnatal death[170]. Importantly, the FGF9 subfamily, which signals from epithelium to mesenchyme, functions in a reciprocal way to the FGF7 subfamily, which signals from mesenchyme to epithelium. FGF9 stimulates mesenchymal proliferation, and mesenchyme produces ligands of the FGF3, FGF7, FGF10 and FGF22 subfamily. Accordingly, knocking out *FGF9* disrupts the mesenchymal–epithelial signalling loop that helps regulate these FGFRb-binding ligands. Reduced mesenchymal proliferation in turn leads to a reduced production of FGF3, FGF7, FGF10 and FGF22 subfamily ligands, which is the proximate cause of lung hypoplasia[171]. *Fgf16* knockout mice exhibit significant cardiac defects[172].

Pathophysiology. SNPs in *FGF20* have recently been associated with Parkinson's disease[173], in which they have been shown to increase FGF20 translation *in vivo*, leading to increased expression of α-synuclein, one of the causative agents of this disease [174].

Therapeutic potential. The potential therapeutic application of FGF20 in Parkinson's disease is beginning to be explored. FGF20 is a neurotrophic factor for rat midbrain dopaminergic neurons[175], and monkey stem cells differentiated *in vitro* into dopaminergic neurons after treatment with exogenous FGF20 and FGF2 have been transplanted into a primate Parkinson's disease model, which alleviated some symptoms[176]. Thus, despite the negative role of FGF20 in Parkinson's disease aetiology *in vivo*, the ligand shows some promise in stem cell biology *in vitro*.

Under the name velafermin, FGF20 was investigated by Curagen for the purpose of alleviating oral mucositis. Although Phase I clinical trials were promising[177], the project was terminated in October 2007 when Phase II trials failed to meet therapeutic targets.

Endocrine FGF ligands

The endocrine ligands, FGF19, FGF21 and FGF23, currently have the greatest promise for pharmacological development among the FGFs (FIG. 4; TABLE 3). The decreased HSGAG binding of the endocrine FGFs leads to increased diffusion of these FGFs from their source, but it also reduces the ability of HSGAGs to promote the binding of these FGFs to their receptors. In order to signal, the endocrine FGFs depend on the presence of α-klotho or β-klotho (encoded by *Kl* and *Klb*, respectively) in their respective target tissues. The klotho proteins bind both the endocrine FGFs and their cognate FGFRs to increase ligand–receptor affinity[186,187,191–196].

α-Klotho was first discovered when mice that lacked the gene aged prematurely[178]. Overexpression of *Kl* can extend the lifespan of mice[179]. The extracellular domain of the α-klotho protein is secreted into the blood and cerebrospinal fluid, where it acts as a humoral factor[180,181]. In particular, α-klotho regulates Ca^{2+} metabolism by binding the Na^+–K^+-ATPase[182] and by acting as a β-glucuronidase to hydrolyse the extracellular sugar residues of the TRPV5 ion channel, thereby trapping the channel on the cell membrane[183].

Abnormalities of phosphate metabolism and bone mineral density in $Kl^{-/-}$ mice were similar to those observed in *Fgf23* knockout mice[184,185]. This phenotypical similarity led to the discovery that FGF23 requires α-klotho to activate FGFRs[186,187]. Similar reasoning identified the necessity of β-klotho for FGF19 signalling: both $Klb^{-/-}$ and $Fgf15^{-/-}$ (the orthologue of human *FGF19*) mice have increased expression of the liver-specific gene *CYP7A1* (cytochrome P450 7A1) and increased bile acid pools[188,189]. A similar phenotype is also seen in $Fgfr4^{-/-}$ mice[190], which lack a principal receptor for FGF19 and the principal liver FGFR. *In vitro* studies have confirmed that FGF19 requires β-klotho for signalling[191–193]. Some overexpression studies also

suggested that FGF19 might bind α-klotho, but this may only occur in non-physiological conditions[193]. FGF21 is also a β-klotho-dependent ligand[191,193–196].

FGF19

Biology. FGF19 transcripts are found in brain, cartilage, skin, retina, kidney, gall bladder and small intestine[197,198]. Expression occurs primarily in the ileum from which the ligand circulates to the liver and carries out its main functions[189]. Interest in FGF19 was stimulated after decreased adiposity, increased energy expenditure, reduced liver triglycerides, increased fatty acid oxidation, reduced glucose levels and improved insulin sensitivity were observed in *Fgf19* transgenic mice[6]. Moreover, these mice did not become obese or diabetic on a high-fat diet. These metabolic effects were not mediated through insulin-like growth factor 1, growth hormone, the thyroid hormone triiodothyronine or leptin, none of which was increased in the transgenic mice[6]. Metabolic rate was similarly increased in mice given recombinant FGF19, thereby confirming the genetic data. FGF19 treatment was also able to prevent or reverse diabetes in mice that were made obese by ablation of brown adipose tissue or genetic knockdown of leptin[3].

FGF19 mediates its physiological effects in the liver through the regulation of transcription[3]. FGF19 gene expression is directly induced by the farnesoid X receptor, a nuclear receptor that recognizes bile acids. In turn, FGF19 inhibits CYP7A1, the enzyme that catalyses the rate-limiting step in bile acid synthesis[199]. Studies in humans have shown that serum FGF19 levels vary diurnally, with rises in serum FGF19 of up to 250% occurring 1–2 hours after a post-prandial increase in bile acids[200]. FGF19 also downregulates acetyl CoA carboxylase 2 (ACC2), which converts acetyl CoA to malonyl CoA, a repressor of carnitine palmitoyl transferase 1 (CPT1)-initiated fatty acid oxidation. By reducing ACC2 activity, FGF19 increases fatty acid oxidation. FGF19 also downregulates the lipogenic enzyme stearoyl CoA desaturase 1 (SCD1)[3,199]. FGF19 additionally regulates gall bladder filling in part by a cAMP-dependent relaxation of gall bladder smooth muscle[201].

FGFR4 is the predominant receptor by which FGF19 mediates its liver-specific effects. Experiments in *Fgfr4*$^{-/-}$ mice showed that FGF15 (the mouse orthologue of FGF19) was unable to repress CYP7A1 activity[189], and *Fgfr4*$^{-/-}$ mice have a phenotype that is indicative of reduced FGF19 activity, such as increased bile acid pools[190]. FGF19 has been believed to be specific for FGFR4 since 3T3 fibroblast cell lines, which lack FGFR4, were found to be unresponsive to FGF19 (REF. 198). This is supported by more recent *in silico* modelling of the interaction of FGF19 with FGFRs[202] and pull-down experiments in the presence of β-klotho[192,193]. However, it is unlikely that FGF19 is entirely specific for FGFR4. Overexpression studies in HEK293 and 3T3-L1 cells show that FGF19 can bind and activate other FGFRs in the presence of β-klotho[191,194]. Most importantly, FGF19 can also cause an increase in gall bladder volume in *Fgfr4*$^{-/-}$ mice, indicating that FGFRs other than FGFR4 can mediate the effects of FGF19 in gall bladder[201].

Although the overlapping phenotypes of FGF19- and FGF21-overexpressing mice suggest that FGF19, like FGF21, might act on adipose tissue that predominantly expresses FGFR1, FGF19 only weakly activates cells in excised white adipose tissue[191].

Pathophysiology. Deregulated FGF19 signalling or FGF19 mutant proteins have not yet been associated with human metabolic disease, and plasma FGF19 levels in patients with anorexia nervosa are the same as in controls[203].

Therapeutic potential. One major concern for the potential translation of FGF19 to the clinic is the evidence that *Fgf19* transgenic mice develop hepatocellular carcinomas with age[204]. Nonetheless, it might be possible to find a therapeutic window in which FGF19 is efficacious but not tumorigenic.

Interestingly, Genentech has shown that anti-FGF19 monoclonal antibodies inhibit growth of colon tumour xenografts *in vivo* and prevent hepatocellular tumours in FGF19 transgenic mice[205]. Some of this tumour growth inhibition is mediated by downregulating β-catenin signalling[206]. This further confirms a role for FGF19 in oncogenesis and suggests that its mitogenicity could be controlled pharmacologically.

The need for experimental data on FGF19 action in primates has been noted in another review[270], and target identification through FGF19 administration to mice lacking different metabolic enzymes should be useful[207].

If FGF19 does eventually prove to be safe for use in humans, it might represent an important therapeutic option in the treatment of type 2 diabetes and its associated disorders.

FGF21

Biology. FGF21 is expressed in liver and thymus[208], adipose tissue[209] and islet β-cells in the pancreas[210]. Its expression can be induced in skeletal muscle in response to Akt activation[211]. The role of FGF21 in metabolic regulation was first discovered in association with its adipocyte-specific ability to cause glucose uptake, which is accomplished in part by upregulating transcription of the glucose transporter GLUT1 (REF. 4). Daily injections of FGF21 for 7 days in various murine models of diabetes (*ob–ob* mice, *db–db* mice, and Zucker diabetic fatty rats) reduced the levels of plasma glucose, triglycerides, glucagon and insulin[4]. Administration of FGF21 to *ob–ob* mice for 2 weeks reduced body weight by 20% and ameliorated hyperglycaemia[212], and similar results were found in mice with diet-induced obesity[213]. Likewise, *Fgf21* transgenic mice exhibited improved insulin sensitivity and glucose clearance, lower fasting glucose levels, lower glucagon levels, reduced weight, leaner hepatic tissue, increased retention of brown adipose tissue and smaller adipocytes, relative to controls[4]. The *Fgf21* transgenic mice consumed twice as much food as did control mice, but were nonetheless resistant to diet-induced obesity. In fact, *Fgf21* transgenic mice are markedly smaller than their control littermates, which may be a consequence

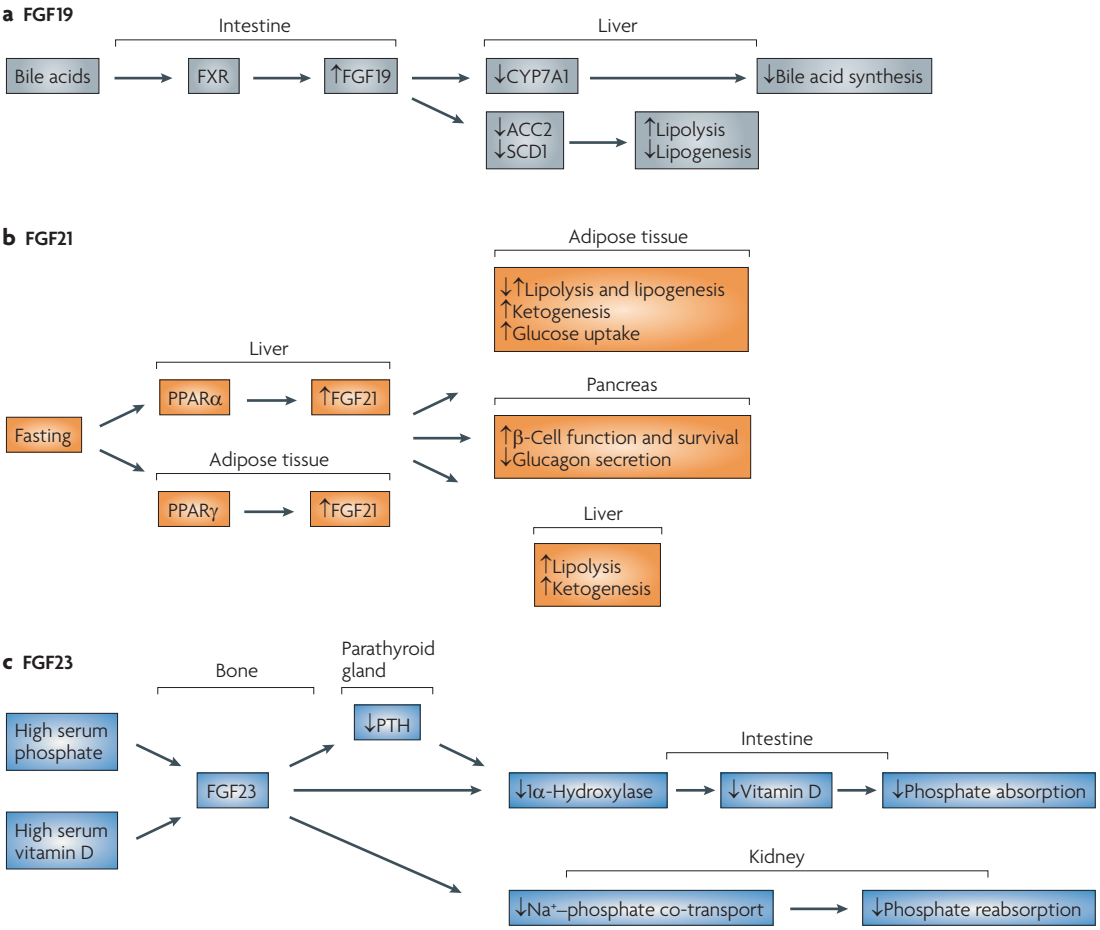


Figure 4 | **The physiology of fibroblast growth factor 19 (FGF19), FGF21 and FGF23. a** | Bile acids activate the FXR receptor in the intestine, leading to expression of FGF19 in the ileum. FGF19 circulates to the liver, where it acts through FGF receptor 4 (FGFR4) to inhibit bile acid synthesis and lipogenesis. **b** | FGF21 mediates the fasting response and is regulated by peroxisome proliferator-activated receptor-α (PPARα) and PPARγ in liver and adipose tissue, respectively. The biology of FGF21 in model systems and humans is still being elucidated, but among its many functions are increasing glucose uptake in adipose tissue, improving β-cell function, inhibiting glucagon secretion, increasing ketogenesis and regulating lipolysis and lipogenesis in a complex manner. FGF21 is expressed in liver, adipose and pancreatic tissue. It acts primarily on adipose tissue. The effects of FGF21 on liver function are probably accomplished through indirect mechanisms as it does not signal through FGFR4. **c** | FGF23 production is upregulated in bone in response to high serum phosphate and vitamin D levels. FGF23 then circulates to the parathyroid gland, intestine and kidney. In the intestine, FGF23 downregulates 1α-hydroxylase so as to reduce the levels of activated vitamin D, thereby inhibiting absorption of phosphate from the diet. The repression of parathyroid hormone (PTH) by FGF23 also helps to downregulate 1α-hydroxylase. In the kidney, FGF23 inhibits Na^+–phosphate ion co-transport and thus increases excretion of phosphate. CYP7A1, cytochrome P450 7A1; SCD1, stearoyl CoA desaturase 1.

of the suppression of STAT5 (signal transducer and activator of transcription 5) — a mediator of growth hormone signalling — by FGF21 (REF. 214) Adenoviral knockdown of FGF21 in mice leads to fatty liver, lipaemia, reduced levels of serum ketones and increased cholesterol levels[215]. These results are consistent with data from experiments with rhesus monkeys, in which levels of fasting glucose, triglycerides, glucagon and insulin were reduced after FGF21 administration[216]. A small reduction in weight and improved lipoprotein profiles were also observed. In particular, high density lipoprotein c levels were 80% higher than control levels after 6 weeks of FGF21 administration.

Another action of FGF21 is the preservation of β-cell function. Although FGF21 has no effect on normal rat pancreatic islets, it does increase insulin secretion from diabetic islets and protects β-cells from apoptosis by activating the ERK1–ERK2 and Akt pathways, respectively. Under conditions of glucolipotoxicity or inflammation, FGF21 reduces caspase 3 and caspase 7 activity, probably by Akt-induced phosphorylation of BCL2-antagonist of cell death (BAD), a suppressor of apoptosis[210]. The anti-apoptotic effects of FGF21 are probably also exerted by reducing glucose and triglyceride levels, creating a less toxic environment for β-cells[210]. β-Cell mass was preserved in *db–db* mice after 8 weeks of FGF21

administration, and treated animals had 280% more β-cells per histological section than untreated animals[210]. Under diabetic conditions, insulin biosynthesis becomes important for the insulin response, and FGF21 supports this function by preserving β-cells.

A role for FGF21 in the fasting response became apparent when it was observed that FGF21 expression was induced in mice by starvation or a ketogenic diet[215,217] and that FGF21 is also induced by fasting and suppressed by refeeding in rats[218]. In fact, microarray data show that FGF21 is the most markedly upregulated gene in ketotic mice[215].

The molecular mechanisms by which FGF21 mediates the fasting response is still being elucidated. It is known that peroxisome proliferator-activated receptor-α (PPARα) regulates FGF21 activity[215,217]. PPARα is a nuclear receptor that responds to fatty acid metabolites, mediates the starvation response and upregulates genes that are involved in fatty acid transport and oxidation[219]. PPARα directly induces *FGF21* mRNA transcription in mouse liver and human hepatocytes, and chromatin immunoprecipitation experiments show that PPARα binds to the *Fgf21* promoter in mouse liver tissue[217]. PPARα is not solely responsible for inducing *FGF21* expression, however, as FGF21 expression can be induced by a ketogenic diet even in *Ppara*$^{-/-}$ mice[215]. FGF21 also reduces physical activity and induces torpor in fasting mice, indicating that FGF21 may also be involved in a neurological response to fasting[217].

Several lines of evidence suggest that FGF21 is involved in lipolytic processes. Adenovirus-mediated short hairpin RNA knockdown of FGF21 in mice downregulated genes that are involved in β-oxidation as well as triglyceride accumulation[215]. Although the PPARα target genes were not upregulated in *Fgf21* transgenic mice, increased numbers of mRNA transcripts of lipases were observed in the liver. Two mediators of ketogenesis — CPT1a (carnitine palmitoyltransferase 1a) and HMGCS2 (hydroxymethylglutaryl-CoA synthetase 2) — were also post-transcriptionally upregulated[217]. Interestingly, as there was less adrenaline in the urine of transgenic mice compared with controls[217], the lipolytic activities of FGF21 are not mediated through catecholamines.

In tension with these results is the observation that FGF21 is also a target of PPARγ, which is a key regulator of adipogenesis[220]. Moreover, PPARγ agonists act in synergy with FGF21 to promote glucose transport and triglyceride formation[221]. Some experiments have shown that FGF21 does not upregulate lipolytic genes in adipocytes[222], which contradicts data from earlier experiments[217]. Furthermore, FGF21 significantly attenuated noradrenaline-stimulated lipolysis in human adipocytes *in vitro*[222]. These observations are consistent with the fact that *Fgf21* knockdown in mice leads to lipaemia[215], and perhaps that some of the pro-lipolytic changes seen in transgenic mice are adaptative[217]. It has also been proposed that the anti-lipolytic effect of FGF21 contributes to its role in insulin sensitization[222].

Some coherence may be brought to the conflicting data by the fact that PPARγ agonists upregulate FGF21 expression in adipose tissue but not in liver, whereas PPARα agonists upregulate FGF21 expression in liver, but not in adipose, tissue[223]. Furthermore, administration of FGF21 to *ob–ob* mice for 2 weeks dramatically suppressed liver lipogenic genes, such as stearoyl CoA desaturase 1 (*Scd1)*, and at the same time upregulated the expression of *Scd1* and other lipogenic genes such as acetyl CoA carboxylase 2 (*Acc2*) in white adipose tissue[212]. Several lipases and PPARγ co-activator 1α (PGC1α), a regulator of oxidative metabolism, were also upregulated in white adipose tissue. This led to the hypothesis that FGF21 leads to a futile cycling of lipogenesis and lipolysis in white adipose tissue[212].

The potential for tissue-specific activity of FGF21 raises the question of its receptor specificity. FGFR1 and FGFR4 are the principal FGFRs in white adipose tissue and liver, respectively[191]. FGF21 can bind FGFR4 in *in vitro* overexpression experiments[195], but cannot activate H4IIE hepatocytes that express β-klotho[191]. This suggests that the action of FGF21 on liver may be indirect, as already suggested by its repression of STAT5 levels[214]. FGFR1 from adipose tissue is therefore probably the main receptor for FGF21.

Studies in humans have further clarified the profile of FGF21 biology. Although a ketotic diet induces FGF21 expression in mice[215], it does not do so in humans, in whom ketogenesis is independent of FGF21 and FGF21 levels only become increased after prolonged fasting for 7 days[224]. Earlier induction of FGF21 expression may occur in liver and adipose tissue during fasting, but these tissues were not specifically examined. In humans, FGF21 levels varied 250-fold among 76 healthy individuals and did not correlate with serum triglycerides, glucose, body mass index, age or gender. There was no diurnal variation and FGF21 was unrelated to bile acid synthesis. FGF21 expression is induced by PPARα agonists in humans[224]. Interestingly, although acute fasting increases FGF21 levels, FGF21 levels are significantly reduced in individuals suffering from chronic malnourishment as a result of anorexia nervosa[203].

Pathophysiology. As for FGF19, deregulated FGF21 has not been shown to be a causative factor in human metabolic disorders. In human cross-sectional studies, a positive association of serum FGF21 with adiposity, insulin resistance, and adverse lipid profiles has been observed[209], although the correlation with insulin resistance is abolished when controlling for body mass index. Fasting FGF21 levels are also increased in individuals with type 2 diabetes[225], which may indicate that resistance develops to FGF21 or may represent a compensatory increase in FGF21.

Therapeutic potential. FGF21 is currently of great therapeutic promise as, unlike FGF19, it has an excellent safety profile. FGF21 did not show significant mitogenic potential in cell lines and *Fgf21* transgenic mice did not demonstrate any tissue hyperplasia until they were 10 months of age[4]. Furthermore, FGF21 administration does not lead to either hypoglycaemia or oedema, which are two common side effects of current diabetes therapies[4,212,213,216].

The biological profile of FGF21 gives this ligand the potential to address the causative factors of type 2 diabetes. The progressive loss of β-cells through β-cell apoptosis and hyperglycaemia caused by inappropriately increased glucagon levels that are unrepressed following feeding are among the aetiologies of type 2 diabetes. FGF21 has been shown to increase β-cell survival[210] and inhibit glucagon secretion[4,216]. The ability of FGF21 to normalize glucose levels and facilitate insulin sensitization is well attested and reproducible[4,212,216].

The pharmacology of FGF21 remains to be fully elucidated but it seems that, by initiating a wide range of cellular responses, FGF21 has a pharmacodynamic action that long outlasts the presence of the ligand[4]. Interestingly, the ability of FGF21 to ameliorate hyperglycaemia was apparent at doses of 0.1 mg per kg per day that achieved steady-state levels of about 7.4 ng per ml in mice, whereas the effect of FGF21 on weight loss was increased by higher doses[212].

FGF23

Biology. FGF23 was initially shown to be preferentially expressed in the ventrolateral thalamic nucleus[226]. FGF23 was also identified as a gene that is mutated in patients with hypophosphataemic rickets[7]. Since then, studies have revealed that FGF23 is a key humoral regulator of phosphate homeostasis.

FGF23 is most highly expressed in bone[227,228], from which it circulates through the blood to regulate vitamin D and phosphate metabolism in kidney. Renal phosphate reabsorption is suppressed in *Fgf23*-overexpressing mice[229,230], owing to downregulation of type IIa and IIc sodium–phosphate co-transport on the apical surface of renal proximal tubular epithelial cells[231–233]. FGF23 also downregulates enzymes that metabolize vitamin D, leading to reduced levels of available active 1,25-dihydroxyvitamin D. Because 1,25-dihydroxyvitamin D enhances intestinal phosphate absorption, this effect of FGF23 also leads to reduced phosphate levels[231].

FGF23 also acts on the parathyroid gland to inhibit parathyroid hormone (PTH) secretion[234]. PTH increases the uptake of phosphate from bone and upregulates 1α-hydroxylase, leading to increased vitamin D activation and enhanced phosphate reabsorption in the intestine. Notably, FGF23 can still normalize serum phosphorous levels in thyroparathyroidectomized rats[232].

Fgf23$^{-/-}$ mice suffer from hyperphosphataemia, increased 1,25-dihydroxyvitamin D levels, hypoglycaemia, atrophy of the thymus, immature reproductive organs and increased serum triglycerides[185,235]. Hyperphosphataemia and soft tissue calcification in *Fgf23*$^{-/-}$ mice are ameliorated by additionally knocking down the genes encoding 1α-hydroxylase or the vitamin D receptor[236–238]. This indicates that an increase in 1,25-dihydroxyvitamin D levels is responsible for the hyperphosphataemia and calcification seen in FGF23-deficient mice[239]. Indeed, FGF23 has been shown to lower 1α-hydroxylase levels by a vitamin D receptor-independent mechanism[240]. High vitamin D levels lead to tissue atrophy through apoptosis, and so FGF23 can prevent vitamin D-induced apoptosis by suppressing 1α-hydroxylase[241].

Pathophysiology. Mutations in FGF23 are implicated in a wide range of disorders. Autosomal dominant hypophosphataemic rickets is caused by mutations in a subtilisin-like proprotein convertase cleavage site in FGF23 that render the protein less susceptible to degradation, thereby increasing the biological activity of FGF23 and leading to hypophosphataemia[7]. X-linked hypophosphataemic rickets is caused by inactivating mutations of *PHEX*, a gene that encodes a metalloprotease of the M13 family[242]. By an unknown mechanism, this leads to increased FGF23 levels in many patients with this disease[243,244].

FGF23 levels are increased tenfold above controls in patients with tumour-induced osteomalacia, a tumour-associated syndrome of renal phosphate wasting[243,244]. These data, combined with the observation that FGF23 serum concentrations decrease after tumour removal[245,246], show that FGF23 is important to the pathology of phosphate wasting in TIO. Circulating FGF23 levels are also increased and correlate with disease burden in patients with fibrous dysplasia, a disorder in which normal bone is replaced by fibro-osseous tissue[227].

Reduced FGF23 signalling also causes pathology. Familial tumoural calcinosis (FTC) is a disorder marked by hyperphosphataemia in which individuals develop calcified masses, often within the joints[247]. Even though *Fgf23*$^{-/-}$ mice do not develop calcified masses[185], several mutations in the *Fgf23* gene have been shown to contribute to hyperphosphataemic tumoral calcinosis[248–250]. These missense mutations destabilize the tertiary structure of the FGF23 protein and increase its susceptibility to degradation, such that full-length species of FGF23 occur at low concentrations in affected patients[284]. Mutations in α-klotho have also been implicated in FTC[251,285]; in such cases, insensitivity to circulating FGF23 causes FTC, rather than the increased processing of FGF23 that results from *FGF23* mutations. Further defects that cause FTC include loss-of-function mutations in the glycosyltransferase GALNT3. Although FGF23 is *O*-glycosylated[252] and GALNT3 selectively directs *O*-glycosylation at Thr178 of FGF23 (REF. 253), FGF23 probably does not contribute to FTC caused by GALNT3 mutations, as FGF23 is increased in these patients, probably in compensation for their hyperphosphataemia[254].

FGF23 is increased in patients with renal failure by 100–1,000-fold, partly owing to decreased renal clearance but also suggesting that it might have a compensatory role in this disease[255,256]. However, whether the increased FGF23 levels in chronic kidney disease are beneficial or harmful is still a matter of debate[257]. In any case, FGF23 levels do correlate strongly with disease outcome. Increased levels of serum FGF23 at the beginning of dialysis treatment predict a significant increase in 1 year mortality in patients with chronic kidney disease[258]. FGF23 serum levels are also predictive of the development of secondary hyperparathyroidism[259].

Therapeutic potential. Given its involvement in the pathogenesis of human disease, FGF23 holds promise as a therapeutic target, and a range of studies have confirmed its potential. Administration of neutralizing antibodies that target FGF23 normalized phosphate

Osteomalacia
Demineralization of the bones often associated with a lack of vitamin D.

Secondary hyperparathyroidism
This condition is marked by excessive secretion of parathyroid hormone as a result of low serum calcium levels. It is often seen in patients suffering from kidney disease.

and vitamin D concentrations in the serum of mice with hypophosphataemia[260], which points to the possible application of FGF23 neutralizing antibodies to the treatment of hypophosphataemic disorders. Neutralizing antibodies against N- and C-terminal regions of FGF23 have also proved successful at increasing serum phosphate and activated vitamin D levels in mice[261]. Another potential avenue of therapy could be the use of C-terminal peptides of FGF23. FGF23 binds to klotho through its C-terminal region, and these peptides could therefore abrogate binding and eliminate FGF–FGFR klotho-dependent signalling[14]. This possibility is currently being investigated in our laboratory (R. Goetz *et al.,* unpublished observations). The contribution of FGF23 to chronic kidney disease is unclear, but this ligand may also have pharmacological significance in this context.

Concluding remarks

The mitogenic and cytoprotective properties of FGF7 are already being put to advantageous use in the clinic. Other FGFs, including FGF1, FGF2 and FGF4, have been tested in clinical trials and may eventually be used to treat cardiovascular disease. FGF18 is in the beginning stages of development for the treatment of osteoarthritis, and FGF5 inhibitors may find a niche in the treatment of some forms of non-autoimmune alopecia. The precise role of FGFs in mood disorders requires considerably more investigation[262], but it is possible that some therapeutic application will arise in this field, especially as so many of the FGFs are involved in brain patterning and neurological development.

Therapies that target RTKs are already common. It remains to be seen whether FGFR-specific inhibitors will have an impact on the treatment of cancer. The recent work that revealed the ability of small-molecule FGFR inhibitors to cause cell death in cancer cells is at least a proof of principle[65]. Currently, the development of inhibitors of the FGFR–PLCγ interaction looks promising because their concomitant use with RTK inhibitors may slow the onset of drug resistance.

Of the endocrine ligands, FGF21 currently holds the most potential as a drug target, owing to its beneficial impact in animal models of diabetes and its lack of toxicity. Although recombinant FGF19 also improves aspects of the metabolic syndrome in mouse models, its ability to initiate tumour growth in transgenic mice as well as its expression in human tumours is a significant cause for concern. Further investigation of its side-effect profile is vital.

The involvement of FGF23 in disease makes it a particularly attractive therapeutic target. Antibodies against FGF23 or peptide analogues of the FGF23 C terminus should ultimately prove useful in combating human hypophosphataemic diseases. Increased levels of FGF23 in chronic kidney disease is a subject of intense study; further applications of the ligand to the treatment of renal disorders may yet be found.

FGF-based therapies are still relatively new to the clinic and the broad biology of this family of growth factors has yet to be fully exploited in the treatment of human disease. Many new developments, both in further elucidation of FGF biology and in their pharmacological application, are expected in the future.

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DATABASES
UniProtKB: http://www.uniprot.org
FGF1 | FGF2 | FGF4 | FGF7 | FGF8 | FGF9 | FGF19 | FGF21 | FGF23 | FGFR1 | FGFR2 | FGFR3 | FGFR4

FURTHER INFORMATION
Moosa Mohammdi's homepage:
http://saturn.med.nyu.edu/~mohammad/
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