

REVIEW

Fibroblast growth factor receptors, developmental corruption and malignant disease

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Fibroblast growth factors (FGF) are a family of ligands that bind to four different types of cell surface receptor entitled, FGFR1, FGFR2, FGFR3 and FGFR4. These receptors differ in their ligand binding affinity and tissue distribution. The prototypical receptor structure is that of an extracellular region comprising three immunoglobulin (Ig)-like domains, a hydrophobic trans-membrane segment and a split intracellular tyrosine kinase domain. Alternative gene splicing affecting the extracellular third Ig loop also creates different receptor isoforms entitled FGFRIIIb and FGFRIIIc. Somatic fibroblast growth factor receptor (FGFR) mutations are implicated in different types of cancer and germline FGFR mutations occur in developmental syndromes particularly those in which craniosynostosis is a feature. The mutations found in both conditions are often identical. Many somatic FGFR mutations in cancer are gain-of-function mutations of established pre-clinical oncogenic potential. Gene amplification can also occur with 19–22% of squamous cell lung cancers for example having amplification of FGFR1. Ontologic comparators can be informative such as aberrant spermatogenesis being implicated in both spermatocytic seminomas and Apert syndrome. The former arises from somatic FGFR3 mutations and Apert syndrome arises from germline FGFR2 mutations. Finally, therapeutics directed at inhibiting the FGF/FGFR interaction are a promising subject for clinical trials.

Introduction

FGF ligands and receptors

Fibroblast growth factors (FGFs) are a family of homologous polypeptide ligands that bind to their cognate fibroblast growth factor receptors (FGFRs). Their effects are exerted in a context-dependent manner with different roles in different tumor types. For example, FGFs have tumor suppressive activity in medulloblastomas and prostate cancer but are tumor drivers in other malignancies. Signaling mediated by FGFs can cause mitogenesis, proliferation, differentiation, cellular migration, angiogenesis and repair of tissue injury.

There are 22 FGF ligand genes in humans comprising six sub-families based on differing phylogeny and sequence homology. The encoded ligands were previously considered to be paracrine factors; however, the ligands FGF19, FGF21 and FGF23 have more recently been recognized as endocrine factors, which are dependent

on the presence of Klotho proteins in the target tissue. These FGFs participate in the regulation of bile acid, cholesterol, glucose, vitamin D and phosphate homeostasis (1–4). There are also four FGF homologous factors (previously designated FGF11–FGF14) that have high sequence identity with FGF family members. They do not activate FGFRs and therefore are no-longer considered to be FGF family members. Furthermore, FGF15 is the mouse ortholog of human FGF19 thereby giving a total of 18 recognized biologically active FGF ligands (5).

There are four cell surface FGFR isoforms that are differentially activated by FGF ligands in conjunction with the scaffold protein heparan sulfate proteoglycan (6). FGF ligand binding to the FGFR causes receptor dimerization, transphosphorylation and activation of an intracellular tyrosine kinase domain that is separated into two contiguous active regions (7,8). Activation also includes FGFR substrate 2 (FRS2) that activates the growth factor receptor bound 2 (Grb2)/son of sevenless 1 complex and thereby the mitogen-activated protein kinase (MAPK) pathway when phosphorylated (Figure 1c) (9–12). There is also a non-tyrosine kinase receptor called FGFR1 (7). Oncogenic mutations of the FGFRs are detailed in Table I. These can sometimes cause receptor activation by creating a cysteine residue that forms an intermolecular disulfide bond with consequent ligand-independent receptor dimerization, for example, R248C. In another circumstance, a conformational change of the activation loop of the receptors tyrosine kinase domain can arise, for example, K650/652E (6,13,14).

The ligand specificity of FGFR1, FGFR2 and FGFR3 is partially determined by alternative splicing within the C-terminal half of the third immunoglobulin (Ig) loop of the extracellular FGF binding domain. This is illustrated in Figure 1b. This alternative splicing creates a IIIb isoform (in which exon 8 is used) that is preferentially expressed in epithelial cells and a IIIc isoform (in which exon 9 is used) that is preferential expression in mesenchymal cells. The IIIb isoform preferentially binds secreted FGF ligands from adjacent mesenchyme, and the IIIc isoform usually binds ligands secreted from the adjacent epithelium. The configuration of this paracrine arrangement has the benefit of obviating inadvertent autocrine stimulation. This safeguard fails in disorders such as Apert's syndrome in which there is incorrect FGF ligand binding with inappropriate FGFR autocrine activation. Normally, the fibroblast ligands FGF3, 7, 10 and 22 exclusively bind to the IIIb isoform, FGF1 binds to both the IIIb and IIIc isoforms, whereas the other 13 FGF ligands with established FGFR stimulatory effects have preferential binding to the IIIc isoforms (16). Interestingly, murine studies have established an important role for the IIIb isoform of FGFR2 in mesenchymal–epithelial signaling during organogenesis (17). Finally, in stem cells, FGF signaling cascades can interact with the Hedgehog, Wnt, Notch and bone morphogenetic protein signaling pathways (18,19).

FGFR and developmental biology

(i) *Craniosynostosis.* Craniosynostosis is the premature closure of sutures, which are the lines where two bones formed by intramembranous ossification meet. The incidence of craniosynostosis is 1 in 2000 live births with 80–90% being isolated and 10–20% occurring as a phenotypic feature of a syndrome. In isolated cases, the sagittal suture is most frequently affected (55%), followed by the coronal (20%), lambdoid (5%) and metopic sutures (5%). The majority of cases of craniosynostosis occurring as part of a recognized syndrome arise from dominant gain-of-function mutations within the *FGFR1*, *FGFR2* or *FGFR3* genes, leading to constitutive ligand-independent receptor activation. These include Crouzon, Pfeiffer, Apert, Jackson-Weiss, Beare-Stevenson and Muenke syndromes. In

Abbreviations: EGFR, epidermal growth factor receptor; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; FRS2, FGFR substrate 2; GRB2, growth factor receptor bound 2; HCC, hepatocellular carcinoma; Ig, immunoglobulin; MAPK, mitogen-activated protein kinase; NSCLC, non-small cell lung carcinoma; TACC, transforming acidic coiled-coil; VEGFR, vascular endothelial growth factor receptor.

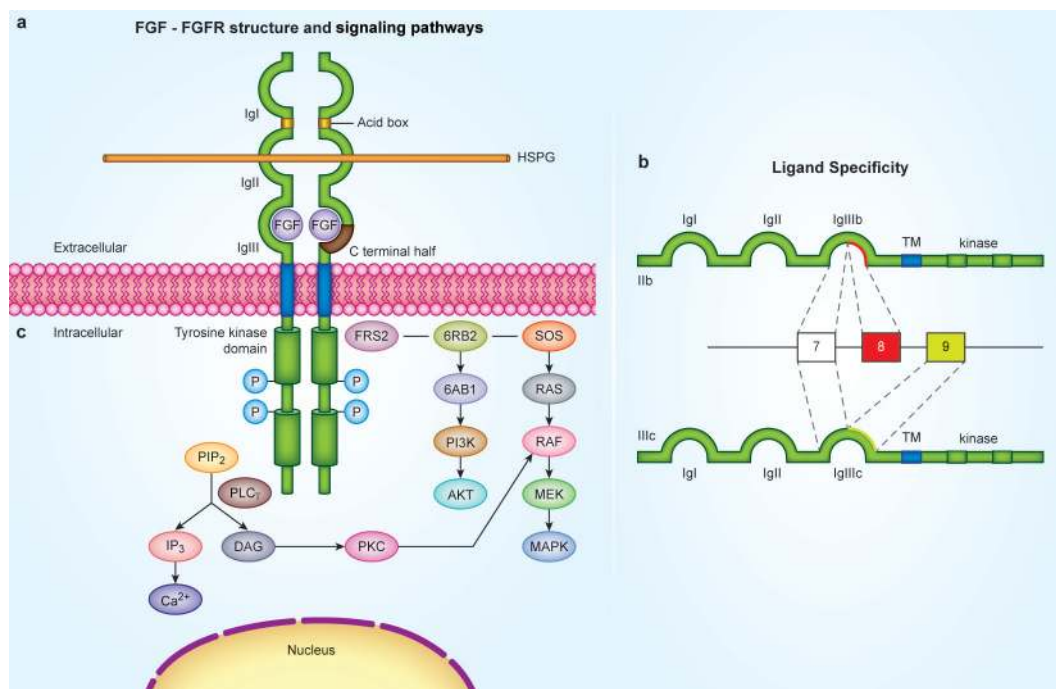


Fig. 1. FGFRs. (a) FGF–FGFR structure. The FGF–FGFR complex consists of two receptor molecules, two FGFs and a heparan sulfate proteoglycan (HSPG). The HSPG stabilizes and sequesters FGFs. The FGFR comprises three extracellular Igs, a transmembrane helix and an intracellular split tyrosine kinase domain. IgI and IgII are separated by an acidic box. (b) Splicing: ligand specificity of FGFR is controlled by alternative splicing of the third Ig loop (IgIII) in the ligand binding domain (C-terminal half), resulting in a IIIb isoform and a IIIc isoform. Exon 8 produces the IIIb isoform and exon 9 produces the IIIc isoform. Note: FGF 3, FGF 7, FGF 10 and FGF 22 exclusively bind IIIb, whereas FGF 1 binds both and the remaining 13 bind IIIc. (c) Signaling: after ligand binding and FGFR dimerization, the kinase domains transphosphorylate each other, leading to the docking of adapter proteins and the activation of downstream pathways. Ligand-stimulated FGFRs phosphorylate the FGFR-associated cytosolic docking protein FRS2. Once phosphorylated, FRS2 recruits son of sevenless adapter protein (SOS) and GRB2 to activate RAS and the downstream RAF-MAPK pathway. A different complex involves GRB2-associated binding protein 1 (GAB1), and it recruits phosphoinositide 3-kinase (PI3K) and this activates AKT-dependent antiapoptotic pathway. Phospholipase C γ (PLC γ) hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP₂) to phosphatidylinositol (3,4,5)-triphosphate (PIP₃) and diacylglycerol (DAG). PIP₃ releases calcium, whereas DAG activates protein kinase C, which helps reinforce the activation of the MAPK pathway by phosphorylating RAF in a RAS-independent manner.

addition to mutations in the *FGFR* genes leading to syndromic cases of craniosynostosis, abnormalities involving the transcription factors *MSX2* (Boston-type craniosynostosis), *TWIST* (Saethre-Chotzen and Baller-Gerold) and the ligand *EPHRIN-B1* can also cause craniosynostosis.

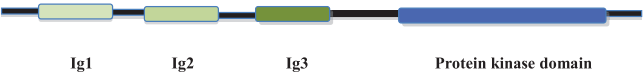
Physiologically, FGFs are expressed in the osteoblastic front of the membranous bones and at low levels mediate cellular proliferation through *FGFR2*. At high levels, they cause osteoblastic differentiation mediated by *FGFR1*. The concentration of FGF ligand diminishes with increasing distance from the suture. Interestingly, a recent study found evidence that *FGFR1* amplification predicts sensitivity to the pan-FGFR kinase inhibitor *NVP-BGJ398* in osteosarcoma (20). The relationship between osteoblast differentiation and FGF–FGFR-mediated signaling in osteosarcoma is a subject for further research.

Crouzon syndrome has an autosomal dominant pattern of inheritance. It is the most common syndrome in which craniosynostosis is a feature with an incidence of 1 in 25 000 children. It is caused by mutations of *FGFR2* exon 9(B) or exon 7(U) that affect the Ig-like domain III of the encoded *FGFR2*. Symptoms and signs of Crouzon syndrome are restricted to the head and neck. Pfeiffer syndrome is usually caused by *FGFR2* exon 9(B) mutations that also affect Ig-like domain III of *FGFR2*, but 5% of cases arise from a *FGFR1* exon 5, P252R mutation. Craniofacial abnormalities in Pfeiffer syndrome resemble those of Crouzon syndrome, but in addition, there is broadening of the great toes and thumb with variable cutaneous syndactyly. Coronal craniosynostosis and midface hypoplasia are also characteristic. Experimental introduction of the gain-of-function mutation *Fgfr2^{c342Y/+}* to create *Fgfr2c* Cys342Tyr heterozygous mice demonstrated that the arising mutant phenotype including craniosynostosis is related to *FGFR2c* regulation of the osteoblast lineage (21). *FGFR2* missense gene mutations that cause Crouzon syndrome in humans

include the *FGFR2*, Cys342Tyr amino acid substitution. An effect on the proliferation of chondrocytes but not gene expression suggested that *FGFR2c* co-operates with *FGFR3* to create the cartilaginous model for endochondral ossification.

Apert syndrome usually arises from *FGFR2* exon 7(u), Ser252Trp or Pro253Arg mutations of the highly conserved region linking *FGFR2* Ig-like domains II and III. The disorder is less common but phenotypically more severe than other syndromes in which craniosynostosis is a feature. Experimentally, it has been found that by a dominant negative effect, soluble *FGFR2* inhibits the enhanced osteoblastic differentiation seen in this condition (22). In general, the majority of *FGFR* mutations are ligand independent; however, some mutations such as Ser252Trp and Pro253Arg in the translated *FGFR2* ectodomain cause phenotypic alterations by altered ligand binding affinity. Apert syndrome arises because of enhanced ligand binding affinity and concurrent promotion of inappropriate ligand binding (23,24). The Ser252Trp substitution allows *FGFR2c* to bind to and be activated by the mesenchymally expressed ligands, FGF7 or FGF10. Epithelially expressed *FGFR2b* is activated by FGF2, FGF6 and FGF9 ligands, again violating the cardinal governing rules of *FGFR2* ligand specificity (25). Another disorder, Muenke syndrome has comparatively mild phenotypic manifestations and accounts for 8% of cases of craniosynostosis. Affected patients may have deafness or brachydactyly. These patients usually have a *FGFR3*, Pro250Arg mutation that affects the region between Ig-like domains II and III of *FGFR3*. The described gain-of-function mutations all increase the affinity of FGF ligand binding to their respective FGFRs.

Investigators at Children’s Hospital of Philadelphia noted that there is an identical amino acid substitution in *FGFR1*, *FGFR2* and *FGFR3* in different craniosynostosis syndromes. They performed a PCR restriction enzyme assay on 113 patients with differing

Table I. Established oncogenic mutations of the FGFR. Schematic diagram of FGFR regions


FGF receptor	Mutation	Mutation
FGFR1	P252S	N546K
	P252T	K656E
FGFR2	D101Y	N550K
	S252W	K660E
	P253R	K660M
	S267P	K660N
	W290C	L764fs*4
	A(-2)G	
	A314D	
	A315T	
	S373C	
	Y376C	
	C383R	
FGFR3	R248C	N540S
	S249C	K650E
	G370C	K650M
	S371C	K650N
	Y373C	K650Q
	G380R	K650T
	A391E	795fs*139
FGFR4	Y367C	N535D
		N535K
		V550E
		V550L

Adapted from Greulich *et al.* (15) and COSMIC database (Catalogue of Somatic Mutations in Cancer, Sanger, UK). Documents in descending order oncogenic mutations from the extracellular encoding region to the intracellular encoded domain for each individual FGFR (15). Described mutations are not solely restricted to the Ig or protein kinase domains, but colors green/blue are used to assist.

craniosynostosis syndromes to identify the analogous Pro246Arg mutation in FGFR4 (26). The anticipated amino acid substitution or other mutations within the conserved linker region were not identified with the inference that *FGFR4* mutations are not likely to be an important factor in craniosynostosis syndromes. Of potential clinical use Table II documents individual FGFR genetic alterations in genetic syndromes and specific malignancies.

(ii) *A selfish path.* Activating point mutations of *FGFR3* usually causes autosomal dominant skeletal dysplasias with defective growth of long tubular bones. Achondroplasia is the most common cause of dwarfism and nearly always arises from a Gly380Arg transmembrane *FGFR3* mutation. Functionally, this mutation promotes an interaction between transmembrane helices (27). *FGFR3* mutations are usually inherited as spontaneous new mutations through the paternal lineage and the incidence of achondroplasia increases with increasing paternal age. In an apparent epidemiologic–pathogenic discordance in achondroplasia, sperm derived from cohorts of normal men of differing ages only exhibit a slight increase in mutant sperm with increased paternal age (28). A vicarious clarifying insight was provided by investigators from the University of Oxford. In a separate investigation on Apert syndrome, they looked for mutation of cytosine 755 within *FGFR2*, which has the highest inferred mutation within this gene, from sperm of men with different ages. Sixty-six percent of cases of Apert syndrome arise from *FGFR2* 755 C-to-G transversions. Paradoxically, though these mutations are detrimental to embryonic development, they are enriched because they lead to a selective advantage to the spermatogonia in which they arise. The observed birth prevalence of Apert's syndrome was 200- to 800-fold greater than that which would be expected from the background 755 C-to-G transversions rate. This disparity was because mutant spermatogonia were positively selected

for before the commencement of meiosis (the two cellular divisions that create sperm) (29). This evidence of a selective advantage of pathogenic *FGFR2* mutations in the male germ line was commented on by experts as evidence of a new category of gene mutation. Descriptively, this category consists of 'hot-spot' gene mutations clustered at just one or two nucleotide sites leading to developmental disorders, which occur almost exclusively in males and rise markedly with age (30). It is now established that exemplary examples of this hot-spot class are *FGFR3* and achondroplasia, and *FGFR2* and Apert's syndrome. *RET* gene mutations that are found in multiple endocrine neoplasia are also included within this descriptive category. Interestingly, *RET* is functionally important in spermatogonia and all three genes encode receptor tyrosine kinases.

Importantly, *FGFR3* mutations are also linked with spermatocytic seminomas, which occur more frequently in older men (31). In a screen of 30 spermatocytic seminomas tested for oncogenic mutations in 17 genes, 2 mutations in *FGFR3* (both 1948A>G encoding K650E that incidentally causes thanatophoric dysplasia in the germline) and 5 mutations in *HRAS* were identified (32–34). The investigators inferred that a common 'selfish' pathway is activated by paternal age effect mutations that contribute to proliferation within the testis with consequential diverse next generation phenotypes of malignancies, fetal lethality and congenital syndromes (29). This was supported by an immunohistochemical study that found localized cellular aggregates with enhanced *FGFR3* immunohistochemical detection and pAKT a marker of downstream signal activation. It appears that populations of spermatogonia in individual seminiferous tubules are clonally mosaics in older men (35). As afore-detailed *FGFR2* mutations in Apert syndrome increase the clonal expansion of male germ cells. Therefore, selective advantages are conferred on spermatogonia that possess the *FGFR* mutations found in spermatocytic seminoma and Apert syndrome.

The *FGFR3* gene and cancer

The *FGFR3* gene is located on chromosome 4p16.3. *FGFR3* mutations occur in urothelial bladder tumors. These tumors are classified as either superficial (stage pTa or pT1) or muscle invasive (stage pT2 or greater) and are graded from grade 1 to grade 3 (G1–G3). A prospective Spanish study of *FGFR3* mutation status in 772 superficial urothelial tumors evaluated the prevalence and prognostic significance of *FGFR3* gene mutations. These mutations were more frequent in TaG1 (61%) and TaG2 (58%) tumors compared with TaG3 (34%) or T1G3 (17%) tumors (36). Overall, *FGFR3* gene mutations were associated with lower tumor grade and stage with the prevalence of *FGFR3* mutations decreasing as the depth of invasion and grade increased. The study supported the preceding concept that *FGFR3* mutant tumors are associated with a good prognosis. Ninety-one percent of *FGFR3* sequence changes were accounted for by S249C, Y375C, S248C or G372C. F386L polymorphisms occurred more often in low-grade tumors (odds ratio, 6.97) and the A393E amino acid substitution was significantly more common in tumors of low malignant potential. Multivariate analysis of all the superficial tumors did establish that *FGFR3* mutations are associated with an increased risk of recurrence. Stratified subset analysis, however, found that this was restricted to the TaG1 *FGFR3* mutant cohort (hazard ratio 2.12, 95% confidence interval 1.28–3.53; $P = 0.004$).

In another European study of ~4700 cases of urothelial bladder cancer and 45 000 controls, the T allele of rs798766 on chromosome 4p16.3 was linked with urothelial bladder cancer (odds ratio 1.24) (37). The locus of this allele which is within one intron of the transforming acidic coiled-coil (*TACC3*) gene, 70 kb from *FGFR3*, was associated with a greater risk of recurrence in low-grade Ta bladder tumors. Furthermore, rs798766 is more common in Ta tumors with *FGFR3* activating gene mutations than wild-type *FGFR3*. In a final reflection of practical significance, *FGFR3* mutation status was found to be superior to grade in predicting clinical outcome in a series of 286 well-differentiated urothelial cell carcinomas in which *FGFR3* mutations were detected in 60% of cases (36,38).

Glioblastomas multiforme is the most common malignant brain tumor in adults and fusion genes involving *FGFR* members are present

Table II. Germline and somatic genetic alterations of FGFR gene receptors

Gene	Genetic alteration	Syndrome	Genetic alteration	Malignancy
<i>FGFR1</i>	P252R mutation G48S and L245P mutations	Pfeiffer syndrome (46) Idiopathic hypo-gonadotropic hypo-gonadism (48)	Amplicon on 8p11.2 8p11 translocation	Breast cancer (47) Myeloproliferative syndrome (49)
	Heterozygous loss of function mutations Y372C mutation C342R mutation	Kallmann syndrome type 2 (10%) (50) Osteoglophonic dysplasia (52) Jackson-Weiss syndrome (53)	Amplification	Lung cancer (51)
<i>FGFR2</i>	W290G and C342W mutations S252W and P253R mutations W290C, Y340C mutations Y375C	Crouzon syndrome (53) Apert syndrome(56) Pfeiffer syndrome (58) Beare-Stevenson cutis gyrata syndrome (60)	FGFR-TACC fusion genes rs2981582C/T, rs1219648A/G, rs2420946C/T, polymorphisms A314D, A315T, S373C, Y376C, C383R, N550K mutations K660M mutations W290C mutation S267P mutation	Glioblastoma (38) Breast cancer (54) Endometrial cancer (55) Cervical cancer (57) Lung cancer (59) Gastric cancer (61)
	K526E mutation	Familial scaphocephaly syndrome (62)	Gene amplification	Gastric cancer(63)
	I156R LADD mutation	Lacrimo-auriculo-dento- digital syndrome (64)	Allelic loss at 10q26	Osteosarcoma (65)
	<i>FGFR3</i>	G1138A	Achondroplasia (66)	R248C, S249C, G370C, S371C, Y373C S249C
Ala391Glu substitution		Crouzon syndrome with acan- thosis nigricans (68)	795FS*139	Multiple myeloma (70)
C742T mutation		Thanatophoric dysplasia type I and type II (33)	S249C Y367C N535K, V550E G388R polymorphism	Low-grade prostate cancer (72) Breast cancer (73) Rhabdomyosarcoma (74) High risk of breast, lung and prostate cancer (75,76)
<i>FGFR4</i>	Asn540Lys substitution —	Hypochondroplasia (71) —	—	—

This list does not include all known *FGFR* mutations implicated in cancer because of space constraints.

in a small subgroup. These fusion genes were identified by investigators using short-term cultures of stem-like cells derived from nine glioblastomas multiformes. Subsequent massively parallel, paired-end sequencing of expressed transcripts established a *FGFR-TACC* fusion gene frequency of 3%. The fusion gene is an in-frame fusion of the tyrosine kinase coding domain of *FGFR1* or *FGFR3* with the *TACC* encoding regions of the *TACCC1* or *TACCC3* genes, respectively (39). Functionally, the *FGFR-TACC* fusion protein product disrupts the mitotic spindle leading to cellular aneuploidy. Experimentally, viral transfection of *Ink4A*; *Arf*^{-/-} astrocytes with either *FGFR3-TACCC3* or *FGFR1-TACCC1* followed by subcutaneous injection into immunodeficient mice leads to glial fibrillary acidic-protein positive, gliomas. Cells containing the *FGFR-TACC* fusion proteins were also stereotactically transduced into normal mice. It was found that the *FGFR3-TACCC3* fusion possesses constitutive phosphorylation of the tyrosine kinase domain and the adaptor protein *FRS2*. This was ablated by treatment with PD173074, which specifically inhibits *FGFR*-associated tyrosine kinase activity. A K508M mutation had a similar effect. When mice with xenografted intracranial *FGFR3-TACCC3* initiated glioma were treated with PD173074, tumor growth was inhibited. Furthermore, treatment with another *FGFR* inhibitor, *AZD4547* doubled the survival time compared with control. This was inferred to be evidence of the fusion gene's oncogenic effect and the potential therapeutic efficacy that may arise from targeting this gene fusion.

FGFR3 mutations are also important in the pathogenesis of seborrheic keratosis. Transgenic mice with an activating S249C *Fgfr3* mutation targeted to the epidermal basal cell layer have ligand-independent activation of *Fgfr3*. This is the most frequent *FGFR3* mutation in carcinomas and the identical mutation also causes thanatophoric dysplasia, the most common lethal skeletal dysplasia (40–42). These transgenic mice develop benign epidermal tumors with phenotypic similarity to seborrheic keratosis and acanthosis nigricans. In one publication, 62 cases of seborrheic keratosis were screened for *FGFR3* mutations and 39% were found to have somatic activating *FGFR3* mutations. There

are three main histologic variants of seborrheic keratosis: hyperkeratosis, acanthotic and seborrheic (43). In a further study evaluating 65 acanthotic seborrheic keratoses, 57% had somatic activating *FGFR3* mutations (44). These mutations were present in both flat (initial) and thick keratosis and were associated with increased patient age, localization to the head and neck ($P < 0.01$) as well as increased expression of *bcl-2*. *FGFR3* mutations have also been found in cervical cancer. Lastly, gain-of-function mutations and overexpression of *FGFR3* are the most common mutations seen in multiple myeloma (45). Also of interest to hematologists, *FGFR1* translocations have been identified in patients with chromosome 8p11 myeloproliferative syndrome (46).

FGFR2 abnormalities in cancer

The *FGFR2* gene is located on chromosome 10q26. *FGFR2* tyrosine kinase domain mutations have been identified in squamous cell lung cancer, endometrial and cervical cancer. These mutations are often identical to those found in selected craniofacial syndromes. *FGFR2* missense mutations can also occur in ovarian and gastric cancer (47–49). In progression of the genitourinary malignancies prostate and bladder cancer, spliceosome dysregulation that switches expression of *FGFR2b* to the *FGFR2c* isoform has also been found (50,51). Pathophysiologically, mutations around the third Ig-like domain of *FGFR2* lead to autocrine FGF signaling, in contrast, mutations of the *FGFR2* kinase domain causes signal activation by releasing *FGFR2* from autoinhibition (19).

In breast cancer, germline polymorphisms of the second intron of *FGFR2* occur and single nucleotide polymorphisms involving *FGFR2c* are associated with *BRCA2* mutated disease (52,53). A proposed estrogen binding site is formed by the rs10736303 breast cancer susceptibility single nucleotide polymorphism, and in estrogen receptor-positive breast cancer, there is preferential upregulation of *FGFR2* (52,54). A C-terminal truncated *FGFR2* protein also causes constitutive ligand-independent activation of *FGFR2* signaling in breast malignancy (53,55). *FGFR* aberrations in breast cancer are not just restricted to *FGFR2*. *FGFR1* amplification also occurs in 10% of cases, particularly luminal subtype B tumors and are correlated with

resistance to endocrine therapy and an unfavorable prognosis. A further 15–30% of breast cancers have amplification of *FGFR3*, which is correlated with an aggressive clinical phenotype.

Oncogenic activating mutations of the *FGFR2* tyrosine kinase domain occur in 12% of cases of endometrial cancer (56). Cell line studies of malignant endometrial cells with activating *FGFR2* mutations have shown that inhibition of *FGFR2* kinase activity using PD173074 inhibited transformation as well as decreased anchorage-independent cell growth and survival. This infers an oncogenic importance and a potential therapeutic benefit in targeting this mutant receptor. An investigation not restricted to *FGFR* evaluation was performed in 233 gastric cancers (193 primary tumors and 40 cell lines) and 98 primary non-malignant-matched gastric samples (57). The intent was to determine the most prevalent molecular targets and to find systemic patterns of exclusivity and co-occurrence within the molecular targets identified. Five gastric cancer subgroups were identified, which were defined by signature genomic alterations: *FGFR2* (9%), *KRAS* (9%), epidermal growth factor receptor (*EGFR*; 8%), *ERBB2* (8%) and *MET* (4%). Evidently, these subgroups are related to receptor tyrosine kinase/*RAS* signaling and were amplified in a mutually exclusive fashion. Receptor tyrosine kinase/*RAS* amplification occurred in ~37% of the gastric cancer cases. Of potential therapeutic importance, the *FGFR2*-amplified tumors were sensitive to the FGFR/vascular endothelial growth factor receptor (VEGFR) inhibitor Dovitinib. In diffuse-type gastric cancer, *FGFR2* overexpression is correlated with an unfavorable prognosis (58). In these cases, *FGFR2* amplification can be identified by FISH (59). A FISH image of an *FGFR2*-amplified gastric cancer is shown in Figure 2. In this case, many of the cells are triploid/tetraploid/polysomic, but it is a case of true *FGFR2* amplification. In another study, direct sequencing of exons IIIa and IIIc on 30 matched primary gastric tumors and normal tissue identified two heterozygous *FGFR2* mutations: (i) a missense mutation (Ser267Pro) in exon IIIa and (ii) a splice site mutation (940-2A G) in exon IIIc (49). These mutations are identical to the activating mutations found in Crouzon, Apert and Pfeiffer syndrome (60).

FGFR and lung cancer

Frequent and focal *FGFR1* amplification occurs in ~9–22% of cases of squamous cell lung cancer (61). In a high-resolution genomic analysis of 232 primary lung cancers, 155 of which were squamous cell lung cancer, this abnormality was not identified in subtypes other than squamous cell carcinoma. Twenty-five significant amplification peaks were identified, which included *SOX2* on chromosome 3q26.33, and a peak on chromosome 8p12, which included *FGFR1* and *FLJ43582*. Of the

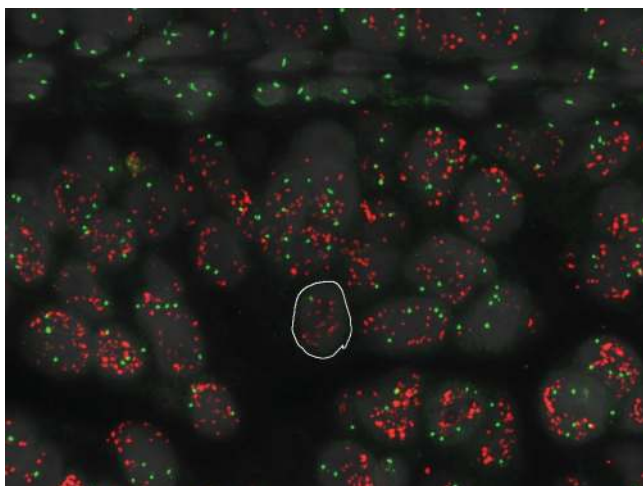


Fig. 2. *FGFR2*-amplified gastric cancer FISH analysis. Green signals within the circle are CEP10 (centromere probe); red signals within the circle represent *FGFR2* genes. In addition to *FGFR2* gene amplification, there is evidence of triploidy/quadruploidy in some cells.

cases with *FGFR1* amplification ($n = 15$), 73% were from smokers with none of the remaining cases arising from patients who had never smoked. In an independent validation series of squamous cell lung cancers ($n = 153$), a 22% frequency of *FGFR1* amplification (four or more copies) was established by FISH. A separate evaluation of a publicly available single nucleotide polymorphism array data set of 581 lung cancers found *FGFR1* amplification in ~1% of non-squamous cell lung cancers. Cell-based screening using the non-isoform-specific FGFR inhibitor PD173074 found that it inhibited growth and induces apoptosis in cell lines with the *FGFR1* amplification. *FGFR1* was validated as the critical target of PD173074-mediated antitumor efficacy in *FGFR1*-amplified cell lines using knockdown and ectopic expression of the *FGFR1*-resistant allele *FGFR1*^{V561M}. Mice engrafted with *FGFR1*-amplified cells treated with PD173074 had tumor regression. MAPK signaling is the key pathway engaged by *FGFR1* amplification.

FGFR1 amplification is not the only important recent molecular discovery in squamous cell lung cancer. In a separate investigation, investigators sequenced the tyrosine kinome in squamous cell lung cancers and cell lines (62). They found gain-of-function mutations in the discoidin domain receptor (*DDR2*) tyrosine kinase gene, a putative oncogenic event, in 3.8% of cases. These mutations were associated with sensitivity to Dasatinib preclinically and in a single demonstrative clinical case. An independent series of 524 patients were enrolled in a study searching for driver gene mutations in non-small cell lung carcinoma (NSCLC). A *DDR2* mutation frequency of 4.4% was found, with a *FGFR2* mutation frequency of 2.2%. The *FGFR2* mutations only occurred in cases of squamous cell lung cancer arising in smokers (63).

Finally, ~10% of cases of NSCLC (squamous cell carcinoma, large cell carcinoma and adenocarcinoma) have mutations in exons 18, 19 or 21 of the tyrosine kinase domain of the *EGFR*. Somatic mutations of the *EGFR* gene are small mutations, which affect amino acid residues from 747 to 750 or point mutations, most frequently replacement of leucine by arginine at codon 858 (64,65). These alterations confer sensitivity to treatment with the adenosine triphosphate-competitive anilinoquinazoline inhibitors, gefitinib and erlotinib (66). Unfortunately, secondary acquired resistance with progressive disease arises in all treated cases, and molecularly, this acquired resistance can occur in different ways. The first such resistance mechanism described was the gatekeeper *EGFR* mutation T790M (67). Acquired resistance to gefitinib can also arise from amplification of chromosome region 7q31.1–q33.3, which contains the *c-Met* gene, as well as by phosphorylation of the insulin-like growth factor type 1 receptor and constitutive activation of phosphatidylinositol 3-kinase (68,69). More recently, it has been discovered that de-repression of *FGFR2* and *FGFR3* can cause secondary acquired resistance (70). Gene expression analysis of NSCLC cell lines with *EGFR* signaling that had been treated with gefitinib demonstrate increased expression of *FGFR2* and *FGFR3* (70). In a luciferase reporter construct, inhibitors of MEK (MAP/ERK kinase) and c-Src stimulated *fgfr2-luc* activity at similar levels to gefitinib. It was inferred that *FGFR2* and *FGFR3* signaling is a mechanism of acquired resistance to tyrosine kinase inhibition and combination treatment with both *EGFR* and *FGFR* tyrosine kinase inhibitors is a possible therapeutic potential.

FGFR4 and cancer

The *FGFR4* gene is located at chromosome 5q35–qter. An increased incidence of prostate cancer in Caucasian males is associated with homozygosity of the *FGFR4* Arg388 allele, and this mutation is associated with clinically aggressive disease (71). This mutant allele is also significantly associated with advanced tumor stage and reduced overall survival in squamous cell carcinoma of the oral cavity and oropharynx (72).

Prognostic importance of FGF's in pancreatic cancer

Correlation of gene expression with prognosis is of oncologic interest because if a gene's expression is associated with an adverse prognosis, this may vicariously infer it having a possible driver oncogenic role. FGFs have been found to be of prognostic importance in pancreatic cancer. An immunohistochemical analysis of 78 pancreatic carcinomas

found a significant proportion of pancreatic cancer cells overexpress acid FGF (60%) and basic FGF (56%) (73). High expression was also found in adjoining atrophic exocrine pancreatic cells. The presence of either type of FGF was significantly correlated with advanced tumor stage. Basic FGF overexpression was also associated with a shorter survival. The mean postoperative survival time was 15.9 ± 1.2 months for cases of pancreatic cancer with absent basic FGF, compared with 9.1 ± 0.8 months for pancreatic cancers that were positive for basic FGF.

Anti-FGFR drugs

Targeting the FGF ligand–FGFR interaction is promising for supportive care and the treatment of cancer and has been the subject of other reviews (74–76). In supportive care, Palifermin is an N-truncated form of FGF7 (keratinocyte growth factor). A double-blind trial has been performed on patients receiving treatment for hematologic cancers, of Palifermin compared with placebo. Palifermin was administered for three consecutive days prior to high-dose chemotherapy, as well as 3 days subsequent to hematopoietic stem cell transplantation. The median duration and incidence of grade 4 mucositis were reduced from 9 to 6 days and from 62 to 20%, respectively, in the treatment arm (77).

FGFR-directed therapeutics are subcategorized as either selective FGFR inhibitors or multitargeted tyrosine kinase inhibitors. Selective inhibitors include NVP-BGJ398, AZD4547 and LY287445. Non-selective inhibitors include those that target FGFR and VEGFRs (e.g. LY2874455 or Brivanib), triple angiokinase inhibitor (e.g. BIBF 1120) or inhibitors with a wide target spectrum (e.g. Regorafenib or Pazopanib). Most compounds of therapeutic interest have an $IC_{50} < 200$ nmol against one of the FGFR subtypes. The literature is dominated by preclinical and phase I trials. A phase II trial of TKI258 (Dovitinib), a tyrosine kinase inhibitor of FGFR1, FGFR2, FGFR3, VEGFR and PDGFR (platelet-derived growth factor receptor), was however conducted in breast cancer. A preceding study of 880 breast tumors detected amplification of *FGFR1* in 8.7% of cases. *FGFR1* amplification was more common in Her-2 negative disease and was the strongest independent predictor of a poor prognosis (78). The phase II clinical trial was performed on heavily pretreated patients with HER2-negative, visceral metastatic disease (79). Patients were stratified into four groups based on *FGFR1* amplification and hormone receptor status. Dovitinib was efficacious in the *FGFR1*-amplified hormone receptor positive subset (25% had a non-confirmed partial response rate and/or stable disease rate of ≥ 4 weeks).

Toxicity data of the consequences of FGFR inhibition are often confounded in studies of non-selective tyrosine kinase inhibitors, but the FGFR specificity of BGJ398, AZD4547 and LY287445 have been suggested as probably more informative (74). Hyperphosphatemia and calcification of tissue arising from blockade of FGFR23 signaling are clinically important unanticipated toxicities. Hyperphosphatemia may be managed with phosphate binders and diuretics.

Specific therapeutics

NVP-BGJ398 is a pan-FGFR kinase inhibitor (20). A preclinical study of 500 osteosarcoma cell lines found that amplification of *FGFR1* may be a predictive biomarker of sensitivity to treatment with NVP-BGJ398. Furthermore, data derived from the Cancer Cell Line Encyclopedia (a collection of 1000 cell lines derived from multiple differing malignancies) demonstrated that cancer cell lines with FGF19 copy number gain at the 11q13 amplicon are sensitive to NVP-BGJ398. This sensitivity was restricted to a cohort that had concomitant expression of the glycosidase I, β -Klotho in the target tissue. A proportion of hepatocellular carcinomas (HCCs) with this molecular description are sensitive to NVP-BGJ398. In three liver cancer cell lines sensitive to NVP-BGJ398, constitutive FRS2 Tyrosine phosphorylation was ablated. These cell lines also expressed FGFR4 with short hairpin RNA studies providing evidence that although the majority of chromosome 11q13 amplified tumor types may not respond to FGF19/FGFR4 inhibition, a subgroup of *FGF19*-amplified liver cancers with concomitant β -Klotho may benefit. Additionally, an anti-FGF19 monoclonal antibody has been found to prevent hepatocellular tumors in transgenic mice (80,81).

AZD4547 inhibits the tyrosine kinases FGFR1, FGFR2 and FGFR3. AZD4547 caused tumor regression in a xenografted mouse model of a human cancer. Antitumor activity was associated pharmacodynamics modulation of phospho-FGFR3. A phase II of AZD4547 versus paclitaxel in advanced gastric carcinoma or gastro-esophageal junction cancers with FGFR2 amplification or polysomy is accruing (clinicaltrials.gov: NCT01457846).

LY2874455 is a FGFR dominant inhibitor with lower VEGFR2 activity. Antitumor effects have been observed in several cancer cell lines treated with LY2874455, including those derived from gastric cancer, NSCLC, multiple myeloma and bladder cancer. It also had antitumor activity in different tumor xenograft models of these cancer types (82).

Non-specific therapeutics

Brivanib is an inhibitor of FGF tyrosine kinase signaling and a potent inhibitor of VEGFR2 (83). In a mouse model, it was shown to inhibit growth of human HCC (84). A phase II study of Brivanib as first-line treatment involving 55 patients with advanced HCC demonstrated a response rate of 7.3% and a disease control rate of 51%. Median progression-free survival was 2.7 months and overall survival was 10 months (85). Most frequent adverse effects included hypertension, fatigue and diarrhea. A subsequent phase II trial involving Brivanib in the second line was conducted on 46 patients with advanced HCC who had failed prior antiangiogenic therapy (86). The oral antiangiogenic multikinase inhibitor exclusive of FGFR, Sorafenib is already Food and Drug Administration approved for the systemic treatment of advanced HCC based on the results of a phase III trial (87,88). In that trial, the partial response rate was 4.6%, with a disease control rate of 45.7%. Median overall survival was 9.79 months with a median time to disease progression of 2.7 months.

BIBF 1120 is a triple angiokinase inhibitor that targets VEGF, PDGFR and FGFR (R18). A phase I dose-escalation study of BIBF 1120 with pemetrexed was conducted on patients with NSCLC previously treated with one first-line platinum-based chemotherapy regimen (89). The stable disease rate was 50% with a mean progression-free survival of 5.4 months. In a comparative study of interest, a phase III trial of pemetrexed versus docetaxel in patients with NSCLC who had previously been treated with chemotherapy demonstrated a mean progression-free survival of 2.9 months in each arm (90).

Finally, therapeutics that target FGF ligand–FGFR-mediated signaling can possess a target profile that far exceeds the FGF–FGFR axis alone. This makes it difficult to establish the true benefit accruing from selective FGFR inhibition. An especially permissive example is Regorafenib, which is an oral multikinase inhibitor of angiogenesis (VEGFR1, VEGFR2, VEGFR3 and TIE2), of oncogenic kinases (KIT, RAF and RET) as well as stromal PDGFR- β and FGFR1 (91). Evidently, much of its therapeutic efficacy is probably attributable to its wide spectrum of therapeutic targets in addition to FGFR1. Its efficacy in selected malignancies is established.

Conclusion

Therapeutic targeting of the FGF–FGFR interaction offers a new molecular avenue to treat cancer. The treatment possibilities are dominated by malignancies with FGFR abnormalities such as mutations or amplification. This review covers the implications of FGFR abnormalities in both development and cancer. Lacunae of information remain to be filled by further research in this branch of ontology, but the potential of what remains to be found is tremendously exciting.

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