De-regulated FGF receptors as therapeutic targets in cancer

Victoria Knights, Simon J. Cook *

Laboratory of Molecular Signalling, The Babraham Institute, Babraham Research Campus, Cambridge, CB22 3AT, England, UK

ARTICLE INFO

ABSTRACT

Fibroblast growth factors (FGFs) acting through their cognate receptors (FGFRs) play vital roles in development and de-regulation of FGF/FGFR signalling is associated with many developmental syndromes. In addition there is much interest in inhibiting FGF/FGFR signalling as a therapeutic approach to cancer. FGF/FGFR signalling is certainly important in tumour angiogenesis but studies in the last few years have uncovered increasing evidence that FGFRs are driving oncogenes in certain cancers and act in a cell autonomous fashion to maintain the malignant properties of tumour cells. These observations make FGFRs increasingly attractive as targets for therapeutic intervention in cancer. In this article, we review FGFR signalling and describe recent advances in cancer genomics and cancer cell biology that demonstrate that specific tumour types are dependent upon or addicted to de-regulated FGFR. We also describe the range of therapeutic strategies currently employed or in development to antagonise de-regulated FGFRs including antibodies and small molecule tyrosine kinase inhibitors.

© 2009 Elsevier Inc. All rights reserved.

Contents

1. Introduction

To date, 22 structurally related signalling ligands have been classified as fibroblast growth factors (FGFs) in mammals. These factors all share a homologous core region of 120 amino acids that is arranged into 12 anti-parallel β-strands, and is flanked by divergent amino- and carboxy-termini. FGFs can elicit numerous cellular and physiological responses during embryonic development as well as in the adult organism, and have been implicated in processes that include proliferation, differen-tiation and angiogenesis. FGF1–FGF10 and FGF16–FGF23 mediate these
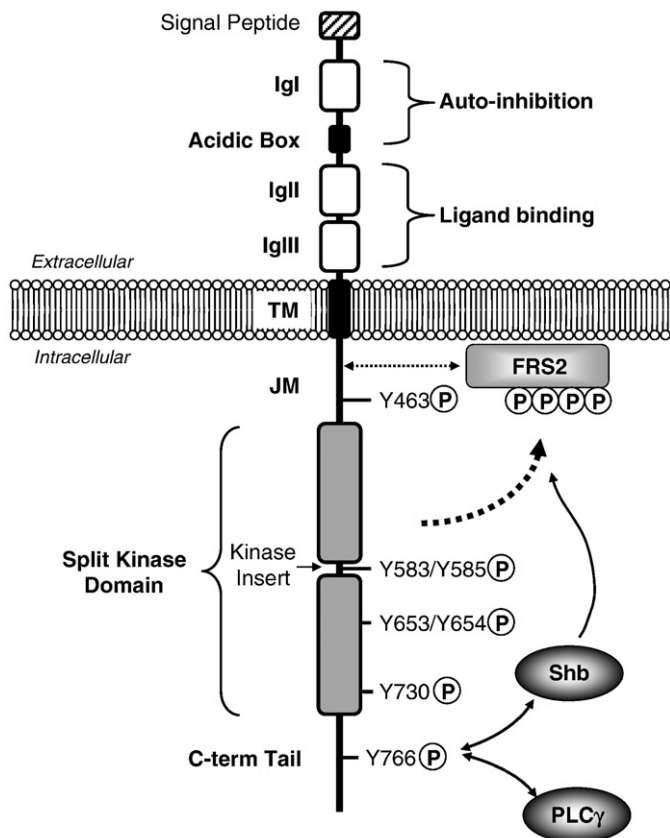
---

effects by binding to a family of five structurally related receptor tyrosine kinases (RTKs), designated FGF receptors (FGFR1–5) (Beenken & Mohammadi, 2009). Although FGF11–FGF14 are structurally related to the other FGF molecules, they do not bind FGFRs and instead interact with voltage-gated sodium channels (Olsen et al., 2003). Like other RTKs, FGFR1–4 are comprised of an extracellular domain, a single-pass trans-membrane domain, and a carboxy-terminal cytoplasmic domain (Fig. 1). The extracellular portion contains three immunoglobulin-like (Ig) folds, IgI, IgII and IgIII, with a stretch of eight consecutive acidic residues between IgI and IgII (the acidic box). Whilst the IgII and IgIII domains are necessary and sufficient for ligand binding (Plotnikov et al., 1999), the amino-terminal portion of the receptor containing IgI and the acidic box is thought to possess an auto-inhibitory function (Olsen et al., 2004). The intracellular region contains a juxta-membrane domain (JM), a split kinase domain that contains the classical tyrosine kinase motifs, and a carboxy-terminal tail (Eswarakumar et al., 2005). Like FGFR1–4, FGFR5 contains an extracellular ligand binding domain and a trans-membrane domain, but lacks an intracellular kinase domain. The function of this receptor is unclear but since it is expressed at the plasma membrane it could potentially form heterodimers with other FGFR proteins and influence signalling (Sleeman et al., 2001).

In addition to multiple FGFR genes, further diversity is achieved through alternative splicing of FGFR1–3. A plethora of splice variants are possible and produce receptors that are prematurely truncated and secreted, lack Ig domains or utilise different coding regions for the

carboxy-termini (Powers et al., 2000). Of particular significance is the differential use of exons 8 or 9 in the second half of the IgIII domain (Fig. 1), which results in expression of either the IIIb or IIIc receptor isoform. These isoforms have different ligand binding affinities and are expressed in a tissue specific manner so that the IIIb isoform is predominantly expressed in epithelial lineages, whilst the IIIc isoform is expressed in mesenchymal tissues (Ornitz & Itoh, 2001).

An important aspect of FGF biology is the interaction between FGFs and heparin or heparin sulphate glycosaminoglycans (HSGAGs) within the extracellular matrix. HSGAG binding serves to protect FGFs from protease-mediated degradation, and generates a local reservoir of FGF that can be spatially controlled by HSGAG expression patterns (Häcker et al., 2005). In addition, activation of the FGFR is modulated by HSGAGs which directly bind to the receptor via the IgII domain and are believed to increase FGF:FGFR affinity and half life (Ornitz & Itoh, 2001; Beenken & Mohammadi, 2009).

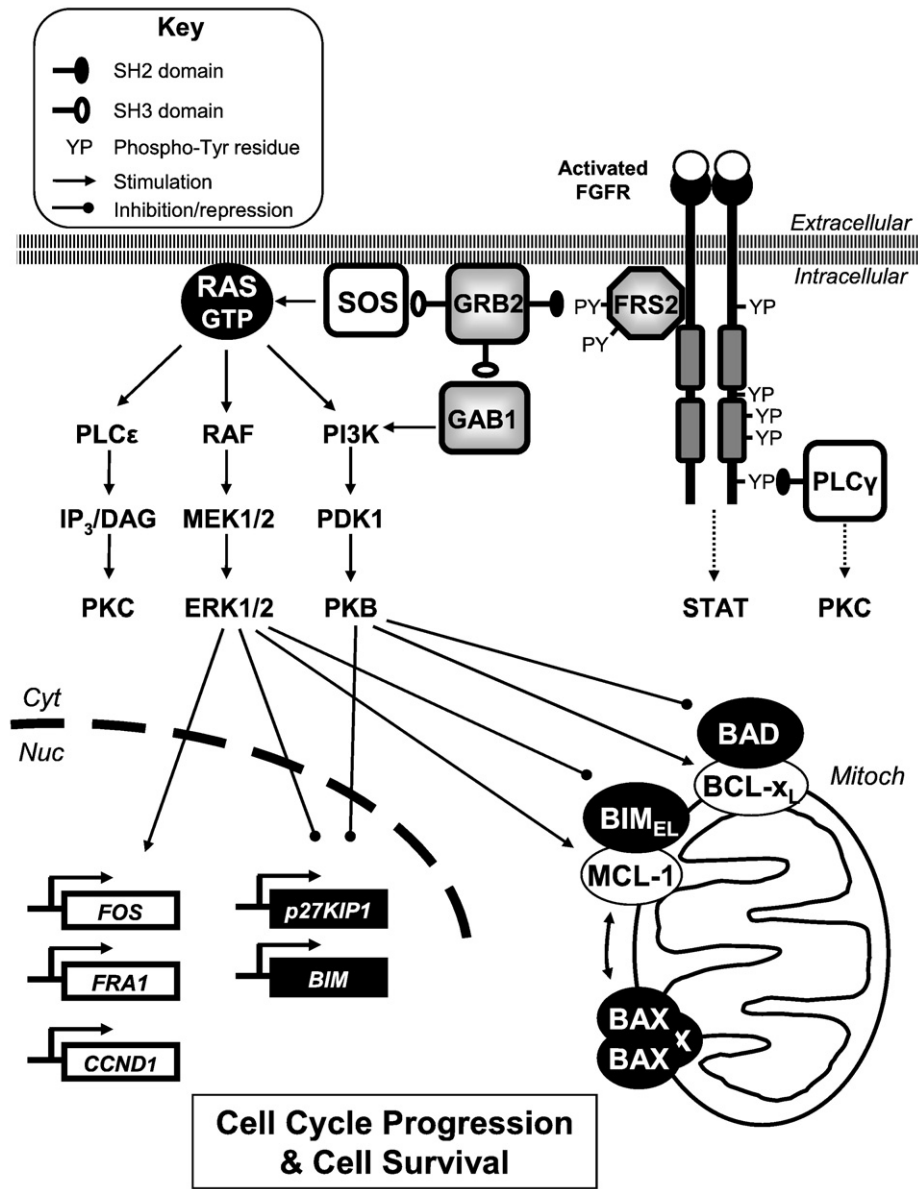## 2. Fibroblast growth factor receptor signalling

2.1. Receptor activation

Formation of the HSGAG:FGF:FGFR ternary complex stabilises receptor dimerisation and promotes FGFR trans-phosphorylation (Plotnikov et al., 1999; Furdui et al., 2006), which in the case of FGFR1, occurs in three discrete stages. The first phosphorylation event takes place at Y653, a residue that lies in the activation loop of the kinase domain of FGFR1 (Fig. 1), resulting in a 50- to 100-fold increase in its kinase activity (Furdui et al., 2006). Residues Y583 (kinase insert), Y463 (JM), Y766 (carboxy-terminal tail), and Y585 (kinase insert) are then phosphorylated, and this allows recruitment of SH2 and PTB domain-containing proteins (Furdui et al., 2006; Lew et al., 2009). Finally, another activation loop residue, Y654, is phosphorylated, resulting in a further 500–1000-fold increase in tyrosine kinase activity and the subsequent phosphorylation of FGFR substrates (Furdui et al., 2006).

2.2. Downstream signalling

Ligand-activated FGFRs can couple to the activation of several intracellular signalling pathways, often in a highly cell type-specific manner (Fig. 2). The two main substrates of FGFR1 are FRS2 and phospholipase-Cγ (PLCγ). FRS2 associates with the JM region of the inactive receptor in a constitutive, phosphotyrosine-independent manner (Ong et al., 2000); however, receptor-mediated phosphorylation of FRS2 requires phosphorylation of FGFR1 at Y766 and subsequent recruitment of the Shb adaptor protein (Cross et al., 2002). Phosphorylation of FRS2 permits recruitment of GRB2 (both directly and indirectly via SHP2), which in turn recruits the RAS guanine nucleotide exchange factor (GEF) SOS1, as well as the scaffolding molecule GAB1. SOS1 activates the RAS GTPases by catalysing GDP–GTP exchange, whilst GAB1 binds the regulatory subunit of phosphoinositide-3 kinase (PI3K), and so leads to RAS-independent PI3K activation (Ong et al., 2001). RAS itself can of course activate a number of effector proteins such as RAF, PI3K, RAL-GEFs and PLCε (Rhee, 2001; Downward, 2003), and so can promote signalling down several pathways including the RAF–MEK1/2–ERK1/2 (RAF–MAPK and ERK kinase—extracellular signal-regulated kinase) and PI3K–PDK1–PKB/AKT (PI3K–3-phosphoinositide-dependent kinase–protein kinase B).

Like the Shb adaptor, which is required for FGFR-mediated FRS2 phosphorylation, PLCγ binds FGFR1 directly though a conserved motif (YLDL) containing phosphorylated Y766 (Mohammadi et al., 1991); indeed, the YLDL motif is invariant in FGFRs1–4. Since the Shb and PLCγ proteins both bind to the same region of FGFR it is conceivable that they may compete with one another for FGFR binding (Fig. 1) so different pools of activated receptor may engage Shb or PLCγ, presumably with

Fig. 1. Structure of the fibroblast growth factor receptor (FGFR) family of receptor tyrosine kinases. The major sites of tyrosine phosphorylation in FGFR1 are shown. FGFR2, -3 and -4 all contain tyrosine residues corresponding to Y653, Y654, Y730 and T766 in FGFR1. The positions of the Immunoglobulin-like domain (Ig), acidic box, trans-membrane domain (TM), juxta-membrane domain (JM) and C-terminal cytoplasmic tail are indicated. FRS2 associates with the receptor in a phosphotyrosine-independent manner at the JM domain, and is then phosphorylated at multiple sites by the activated FGFR tyrosine kinase to initiate downstream signalling; FRS2 phosphorylation requires the recruitment of the Shb adaptor protein to phosphorylated Y766. PLCγ binding to the C-terminal cytoplasmic tail also requires phosphorylation of FGFR1 Y766.

Fig. 2. Signalling pathways activated by the fibroblast growth factor receptors (FGFRs). Ligand activation of the FGFR results in trans-phosphorylation, followed by recruitment and phosphorylation of intracellular substrates. FGFR substrate 2 (FRS2) associates with FGFR in a constitutive manner and is phosphorylated following receptor activation, allowing FRS2 to bind SH2 domain-containing proteins, such as GRB2. Through its SH3 domains, GRB2 associates with the RAS guanine nucleotide exchange factor, SOS1/2, and the scaffold molecule, GAB1, which in turn results in activation of RAS and phoshoinositide-3 kinase (PI3K). Activation of the RAF–MEK1/2–ERK1/2 and PI3K–PKB pathways can occur downstream of RAS, as can activation of PLCε. FGFR signalling also couples to phospholipase Cγ (PLCγ) in an FRS2-independent manner, along with several STAT molecules. These effector signalling pathways are responsible for regulating downstream biological responses either by controlling gene expression in the nucleus or by directly regulating (phosphorylating) key effector molecules outside the nucleus. For example, both the ERK1/2 and PKB pathways are implicated in promoting cell cycle progression by increasing expression of the D-type cyclins (e.g., CCND1) and repressing expression of CDK inhibitors such as p27^{KIP1}. Similarly both pathways can promote cell survival by repressing pro-apoptotic proteins or increasing the abundance of pro-survival proteins of the BCL-2 family.

different consequences for downstream signalling, or there may be temporal coordination to allow each in turn to be recruited to activated receptor complexes. FGFR-mediated phosphorylation of PLCγ results in hydrolysis of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P$_2$) within the inner leaflet of the plasma membrane, generating two second messengers, diacylglycerol (DAG) and inositol (1,4,5)-trisphosphate (Ins(1,4,5)P$_3$). Ins(1,4,5)P$_3$ acts as a ligand for the Ins(1,4,5)P$_3$ receptor, a large calcium channel present in the membranes of intracellular calcium stores, most notably the endoplasmic reticulum. Binding of Ins(1,4,5)P$_3$ to its receptor causes channel opening and diffusion of Ca^{2+} down its electrochemical gradient into the cytosol. In this way PLCγ activity promotes activation of Ca^{2+}-dependent protein kinases such as the calmodulin-dependent protein kinases and Ca^{2+}- and DAG-dependent proteins such as classical and novel isoforms of the

protein kinase C (PKC) family of kinases (Rhee, 2001). In addition, studies with constitutively active FGFR3 mutants have shown that various signal transduction and activation of transcription (STAT) factors, including STAT1, STAT3, STAT5a and STAT5b, can be activated downstream of this receptor (Li et al., 1999; Hart et al., 2000). However, the precise mechanism by which FGFR promotes the tyrosine phosphorylation and activation of the STATs is currently unclear.

The consequences of activating these intracellular signalling pathways are of course varied and far-reaching. In the context of cancer, the RAF–MEK–ERK1/2 and PI3K–PKB pathways are of great interest as they are important in mediating the effects of a variety of other oncoproteins including other RTKs, such as the epidermal growth factor receptor (EGFR), RAS proteins and BRAF. Both the ERK1/2 and PKB pathways promote cell cycle progression and cell survival and

frequently act in a partially redundant fashion (Fig. 2). For example, ERK1/2 can drive the *de novo* expression of *CCND1* whilst PKB promotes the stabilisation of its gene product cyclin D1 and both pathways can repress expression of the cyclin dependent kinase inhibitor p27[KIP1] (Meloche & Pouysségur, 2007). Both pathways can promote cell survival by antagonising pro-death proteins such as BIM and BAD. For example, ERK1/2 can phosphorylate BIM[EL] directly, thereby targeting it for proteasomal degradation (Ley et al., 2003) whilst PKB can phosphorylate BAD directly, promoting it's sequestration by 14-3-3 proteins (Datta et al., 1997), and can phosphorylate FOXO3a, which also promotes 14-3-3 binding and nuclear exclusion (Brunet et al., 1999) thereby preventing expression of BIM. Both the ERK1/2 and PKB pathways can also promote the expression of pro-survival proteins such as BCL-x[L] and MCL-1 (Balmanno & Cook, 2009; Gillings et al., 2009). Since these pathways are frequently activated upon engagement of FGFRs they are likely to play a major role in mediating the effects of FGFR oncoproteins on cell cycle and cell survival.
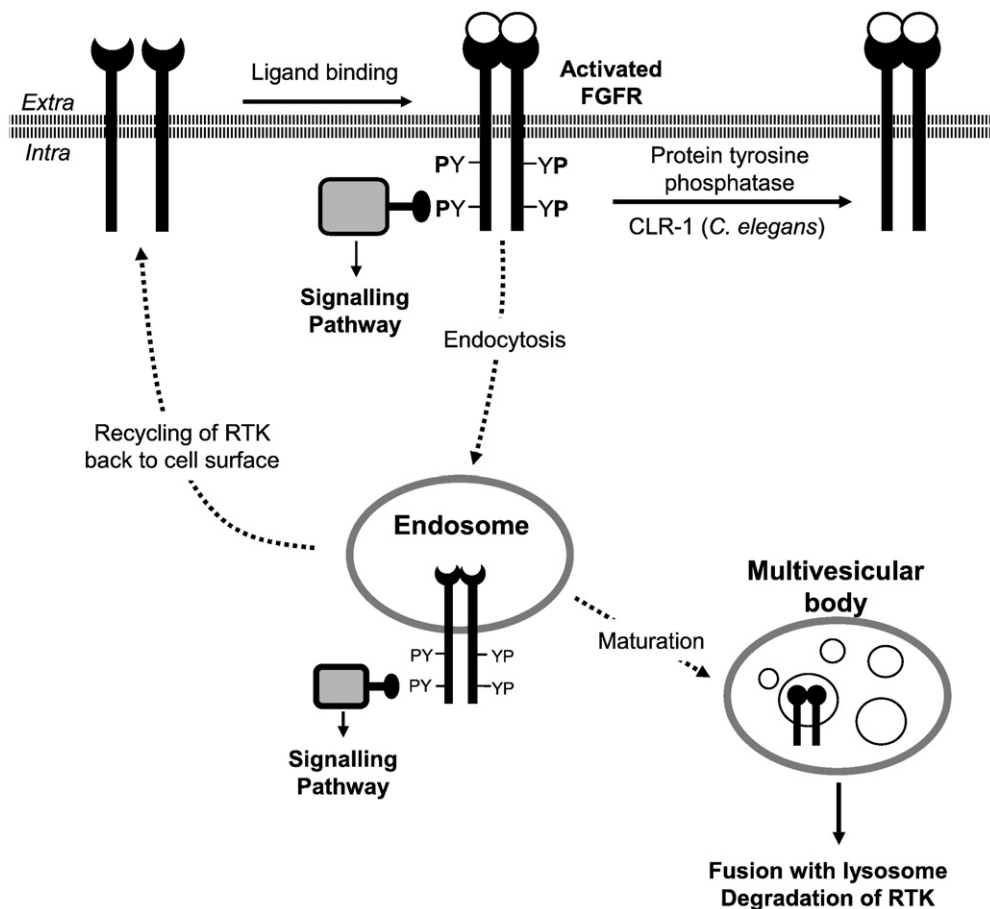
2.3. Signal regulation and termination

Various methods are employed by the cell to regulate FGFR signalling, both at the level of the receptor (Fig. 3) as well as further downstream in the signalling cascade (Fig. 4). Auto-inhibition of receptor activation represents one mechanism of regulation, and is achieved by association of the IgI and acidic box with the ligand binding region of the receptor comprising the IgII and IgIII domains (Fig. 1) (Olsen et al., 2004). In addition the carboxy portion of FGFR2

may possess an inhibitory function because truncation mutants derived from this receptor possess increased transforming capacity in NIH3T3 cells compared to the full length receptor (Lorenzi et al., 1997).

The phosphorylation status of an RTK reflects a balance between the intrinsic kinase activity of the receptor and the activity of opposing protein tyrosine phosphatases (PTPs). CLR-1 is a PTP that antagonises the activity of EGL-15 FGFR, in *C. elegans* (Kokel et al., 1998). The identity of the mammalian PTP(s) that de-phosphorylate FGFR is not yet known; however, their discovery is anticipated with much interest since their ability to antagonise FGFR signalling makes them potential tumour suppressor genes. Ligand-mediated receptor endocytosis represents yet another mechanism that allows regulation of FGFR signal intensity, and is believed to be initiated by c-CBL-mediated FGFR ubiquitination (Wong et al., 2002) (Fig. 3).

Feedback inhibition is a cellular control mechanism where an enzyme promotes the activity or expression of its own negative regulators. In many cases, feedback mechanisms that control signalling downstream of FGFR exert their effects on the RAS–RAF–MEK1/2–ERK1/2 pathway (Fig. 4). Indeed, fine tuning of the ERK1/2 pathway can occur though the direct binding and phosphorylation of ERK1/2 regulators by the ERK1/2 kinases directly. For example, ERK1/2 have several substrates that act upstream in the pathway, including MEK1, BRAF and CRAF, SOS1 and FRS2; in each case, ERK1/2-mediated phosphorylation can result in reduced signal flux down this cascade (Langlois et al., 1995; Brummer et al., 2003; Shaul & Seger, 2007). Moreover, certain dual specificity phosphatases (DUSPs, also known as

Fig. 3. Mechanisms for termination of active FGFR. Ligand binding results in FGFR dimerisation, trans-phosphorylation and transmission of the signal to intracellular signalling pathways. Termination of active receptor signalling can occur through RTK dephosphorylation by protein tyrosine phosphatases. In *C. elegans* this is performed by the CLR-1 phosphatase; the identity of the PTP responsible for FGFR dephosphorylation in humans is not known but could be a potential tumour suppressor. Alternatively, active receptor complexes may be internalised by endocytosis to endosomes and then either recycled back to the plasma membrane, or targeted via multivesicular bodies to the lysosome for degradation.
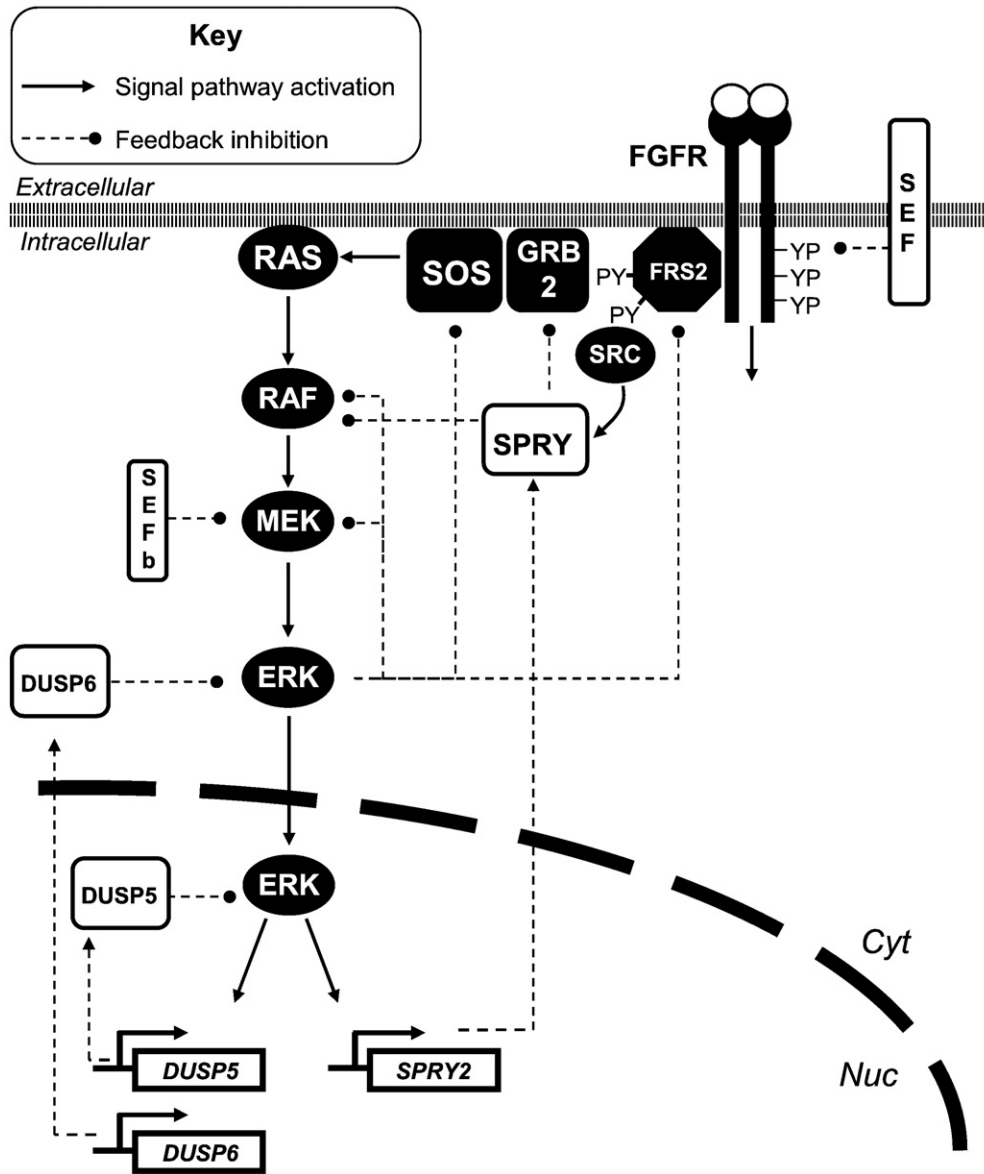
Fig. 4. Mechanisms of negative feedback inhibition acting on fibroblast growth factor receptor (FGFR) signalling. The dual-specificity phosphatases (DUSPs) inactivate ERK1/2 by dephosphorylation. DUSP5 and DUSP6 are expressed as a consequence of ERK1/2 activation and serve to inactivate ERK1/2 in the nucleus or cytoplasm respectively. The SPROUTY (SPRY) family are regulated transcriptionally and post-translationally downstream of FGFR. SPRY2 expression is induced by ERK1/2 activity, and its activity is enhanced following phosphorylation by SRC-family kinases that are recruited to FRS2. SPRY negatively regulates RAF–MEK1/2–ERK1/2 signalling, but it is unclear as to whether it acts upstream or downstream of RAS. The trans-membrane protein, SEF, inhibits FGFR signalling at the level of the receptor, whilst an alternative splice variant, SEF-b, is localised to the cytosol and appears to negatively regulate RAF/MEK1/2–ERK1/2 signalling at the level of MEK1/2. ERK1/2 can also phosphorylate several components including MEK, RAF and FRS2 to inhibit signalling down this pathway.

MAPK phosphatases or MKPs) that catalyse the dephosphorylation and inactivation of ERK1/2 are also expressed as a consequence of ERK1/2 activation. DUSP1 (also known as MKP1), can be phosphorylated by ERK1/2 resulting in increased stability (Brondello et al., 1999), whilst expression of DUSP6 (also known as MKP3) is induced by FGF signalling in an ERK1/2-dependent fashion in the chick embryonic limb (Smith et al., 2006) and acts to de-phosphorylate ERK1/2 in the cytoplasm. DUSP5 is also an ERK1/2 inducible gene and can de-phosphorylate ERK1/2 in the nucleus (Mandl et al., 2005).

SPROUTY (SPRY) was first identified as a negative regulator of FGF signalling in drosophila, which expresses a single isoform, dSPRY (Hacohen et al., 1998), but since then has been shown to regulate signalling downstream of many growth factors in mammals, including FGF, EGF and VEGF (Cabrita & Christofori, 2008). The SPRY2 promoter contains ETS-1 and CREB binding elements (Ding et al., 2003), placing its transcription downstream of the ERK1/2 cascade. The exact mode of action of the SPRY proteins has not been fully elucidated and is likely to vary depending on the cell type, the identity of the RTK being activated and the particular SPRY isoform in question. FGF stimulation results in phosphorylation of several tyrosines including a highly conserved residue near the amino-terminus of SPRY2 (Y55) by a SRC-family kinase, and this is required for the inhibitory effect of SPRY2 (Mason et al., 2004; Rubin et al., 2005). SRC kinases are recruited to phosphorylate SPRY proteins by their binding to phosphorylated FRS2 (Li et al., 2004) and play a key role in controlling FGFR signalling dynamics (Sandilands et al., 2007). In addition, dephosphorylation of S112 and S115 by PP2A results in a conformational change that allows SPRY2 to bind GRB2 (Lao et al., 2006). Whilst some reports show that GRB2-binding is sufficient for SPRY-mediated inhibition of the RAS–RAF–MEK1/2–ERK pathway, this finding is by no means universal (Cabrita & Christofori, 2008; Mason et al., 2006), and it has also been suggested that SPRY may inhibit the ERK1/2 cascade through binding

to CRAF and/or BRAF (Mason et al., 2006; Yusoff et al., 2002). Moreover, this system is further complicated by the finding that SPRY proteins can both homo- and hetero-oligomerise and that hetero-oligomers display differential potencies for inhibition of the ERK1/2 pathway downstream of the FGFR (Ozaki et al., 2005).

The SEF gene encodes a single-pass trans-membrane protein that is expressed in response to FGF stimulation and acts to specifically inhibit FGFR signalling (Tsang et al., 2002; Kovalenko et al., 2003). The exact mechanism of FGFR inhibition is still unclear, but there is evidence to suggest that it may act at the level of the receptor. SEF co-immunoprecipitates with both FGFR1 and FGFR2, and overexpression of this protein leads to a decrease in receptor phosphorylation, along with decreased ERK1/2 and PKB signalling (Tsang et al., 2002; Kovalenko et al., 2003). An alternative splice form, SEF-b, lacks the signal peptide present in SEF and is localised to the cytosol. Unlike the trans-membrane isoform, SEF-b inhibits PDGF-induced proliferation, does not affect FGF-induced PKB signalling, and is likely to inhibit the ERK1/2 pathway at the level of, or downstream of, MEK1/2 (Preger et al., 2004).

3. Fibroblast growth factor receptors in cancer

Cancer encompasses an array of diseases that are characterised by uncontrolled proliferation/survival and the eventual invasion of resultant cells into adjacent or distant tissues within the body. To achieve this all cancer cells possess specific properties, or 'hallmarks', that allow this; namely self sufficiency in growth signals and insensitivity to anti-growth signals, evasion of apoptosis, immortality, the ability to initiate angiogenesis and the acquisition of invasiveness and metastatic ability (Hanahan & Weinberg, 2000). In addition, a cancer cell must also evade elimination by the immune system.

Generation of fully transformed cells in vitro requires the de-regulation of several oncogenes and tumour suppressor genes and tumour cells typically accrue multiple genetic alterations in their lifetime. The challenge is to identify which of these mutations are 'drivers' that underpin the tumour phenotype, and which are simply 'passengers' that are picked up during tumour progression (perhaps arising as a result of genomic instability) but confer no selective advantage. It is becoming increasingly apparent that maintenance of the malignant phenotype requires the continued activity of one or a few specific driving oncogenes; a phenomenon termed 'oncogene addiction' (Weinstein & Joe, 2006). Tumours evolve to be dependent on their driving oncogenes, and the pathways they control, for the maintenance of tumour hallmarks. Whilst normal cells typically use several redundant pathways to control vital processes such as cell cycle progression and cell survival, this redundancy may be reduced in cancer. Indeed it is thought that the tumour cell evolves a greater dependency upon pathways controlled by its driving oncogene than surrounding normal cells so that inactivation of these pathways becomes more catastrophic for the cancer cell than for other cells in the body (Weinstein & Joe, 2006). This effectively creates a 'therapeutic window' and pharmacological inhibitors of oncogenes that are required for maintenance of a specific tumour therefore represent an attractive and viable therapeutic strategy.

FGF signalling can produce mitogenic, anti-apoptotic and angio-genic responses in cells, each of which are considered to be hallmarks of cancer when de-regulated. Moreover, evidence from in vitro and in vivo tumour models have shown that FGFs and FGFRs can act as oncogenes (Jeffers et al., 2002), and consequently there has been considerable recent interest in FGFR inhibitors as anti-cancer agents.

FGFR family members are found to be de-regulated in a variety of cancers including solid tumours and haematological malignancies. Gene amplification or aberrant transcriptional regulation can result in receptor overexpression, whilst a number of point mutations have been identified that produce receptors that are either constitutively active or exhibit a reduced dependence on ligand binding for

activation. In addition translocations can result in expression of FGFR-fusion proteins with constitutive FGFR kinase activity. Finally, isoform switching alters the ligand binding specificity of resulting receptors and hence sensitizes cells to FGFs that they would not normally be responsive to (Jeffers et al., 2002; Eswarakumar et al., 2005).

Aberrant expression of FGF family members represents another mechanism through which FGF:FGFR signalling can become de-regulated in cancer. Indeed, mouse mammary tumour virus (MMTV) insertion can induce murine mammary gland cancer by increasing the expression of FGF3 and FGF4 (Basilico & Moscatelli, 1992). Moreover, amplification and/or overexpression of FGF proteins has also been observed in various cancers (Basilico & Moscatelli, 1992; Powers et al, 2000; Jeffers et al., 2002), suggesting a possible role for FGF autocrine signalling in tumour development.

This review will focus on examples of FGFRs as cell autonomous oncoproteins in tumour cells, focusing on breast cancer, urothelial carcinoma, gastric cancer and the haematological malignancies multiple myeloma and 8p11 myeloproliferative syndrome. These exemplify the various strategies employed to de-regulate FGFR signalling in tumours. However, it should be noted that FGFR de-regulation occurs in numerous other tumour types, including prostate cancer (Kwabi-Addo et al., 2001), astrocytoma (Yamaguchi et al., 1994), thyroid carcinoma (Onose et al., 1999), cervical carcinoma (Cappellen et al., 1999), head and neck squamous cell carcinoma (Streit et al., 2004), colorectal cancer (Jang et al., 2001) and peripheral T cell lymphoma (Yagasaki et al., 2001). Furthermore, the angiogenic activities of FGF/FGFR may be important in the progression to malignancy and readers are directed to recent reviews on this topic (Suhardja & Hoffman, 2003; Chen & Forough, 2006).

4. Breast cancer

Worldwide, breast cancer is the second most common cancer after lung cancer, representing about 10% of all new cancer cases, and 23% of all cancer cases in women. The majority of breast tumours are ductal adenocarcinomas (~85%), whilst the remainder are derived from lobular tissue. Due to improvements in screening, most tumours are detected at an early stage with ~90% of all new cases being non-invasive at diagnosis (ductal carcinoma in situ). Invasive tumours are staged according to the TNM staging system (Sellers, 1960) and are examined for the expression status of the oestrogen receptor (ER), progesterone receptor and HER2, in order to plan efficient treatment regimes. HER2 is amplified and/or over expressed in about 30% of cases (Slamon et al., 1987), and antagonism of this receptor has prolonged disease-free survival; however, the molecular mechanism for tumour progression in the remaining 70% is poorly understood.

In normal breast tissue, expression of FGFs and FGFRs is highest during ductal morphogenesis, which represents a highly proliferative stage of development when epithelial tissue invades the stroma (Welm et al., 2002). This expression profile suggests that FGFs act as potent mitogens in the mammary gland and could therefore potentially play a role in mammary tumorigenesis. Indeed, FGFR family members are frequently over expressed in breast cancer, and this is often accompanied by increased, or altered, expression of FGF ligands. The 8p11-p12 amplicon, which contains FGFR1, is observed in about 10–15% breast cancer patients, and a subset of these over express FGFR1 protein (Ray et al., 2004). Interestingly, FGFR1 appears to be significantly more highly expressed in lobular carcinomas compared to ductal tumours, with one study reporting FGFR1 amplification and overexpression in 7 out of 13 tumours derived from lobular tissue (Reis-Filho et al., 2006; Xian et al., 2009). In addition, conditional activation of FGFR1 in non-transformed mouse or human mammary cells resulted in morphological transformation (Xian et al., 2005; Xian et al., 2009), whilst transgenic mice expressing AP20187-activated FGFR1 under the control of the mammary-specific

MMTV promoter developed alveolar hyperplasia and invasive lesions following sustained treatment with AP20187 (Welm et al., 2002). Moreover, inhibition of FGFR1 kinase activity causes death of breast cancer-derived cell lines that over express FGFR1 (Reis-Filho et al., 2006) indicating that these cells are addicted to continued FGFR1 signalling for viability.

Amplification and overexpression of FGFR2 is observed in about 4–12% breast tumours (Ray et al., 2004), whilst FGFR4 protein is over expressed in around 30% patients (Koziczak et al., 2004). Inhibition of FGFR signalling causes a proliferative block in breast cancer-derived cell lines that over express FGFR2 or FGFR4 (Ray et al., 2004; Koziczak et al., 2004), and thus both receptors are likely to act as oncogenes in this disease. In addition to this, genome-wide association studies have shown that single nucleotide polymorphisms (SNPs) that lie within intron 2 of *FGFR2* are associated with an increased risk of breast cancer (Easton et al., 2007). Since several of these SNPs lie within close proximity to transcription factor binding sites, it has been postulated that their association with breast cancer risk may be due to changes in *FGFR2* gene expression. In particular, one SNP identified generates a putative ER binding site, and this may be relevant to the pathology of ER + breast cancer (Easton et al., 2007).

Alternative splicing may represent another mechanism by which FGF:FGFR signalling can become de-regulated and contribute to breast tumorigenesis. Differential splicing of FGFR2 can produce a variety of receptors that differ in their extracellular and carboxy-terminal domains; at least three different carboxy-terminal variants have been described for FGFR2, designated C1, C2 and C3. The C1 and C2 variants are produced from the same exon with different splice acceptor sites, whilst C3 utilises a separate exon, is shorter than the other two variants and has different signalling properties (Tannheimer et al., 2000; Moffa et al., 2004). This FGFR2-C3 variant lacks the PLCγ binding site and appears to phosphorylate the FRS2 adaptor protein more efficiently than its C1 counterpart (Moffa et al., 2004). FGFR2-C3 is also more transforming than the other two carboxy-terminal variants, and since this isoform is expressed in breast cancer-derived cells, but not normal human mammary epithelial cells (Moffa et al., 2004), isoform switching from FGFR2-C1 to FGFR2-C3 may play a role in neoplastic progression. This theory is further supported by the finding that FGFR2-C3 is more highly expressed in stomach cancer cells when compared to normal stomach epithelium (Moffa et al., 2004).

Anti-oestrogen therapy is routinely used as a first line treatment in ER + patients, but acquired resistance has become a problem in the clinic. Resistance to endocrine therapy is thought to occur through the tumour cell's use of alternative signalling pathways to bypass the ER and FGF:FGFR signalling has been implicated as one mechanism by which oestrogen-independence arises (McLeskey et al., 1998). Since a significant proportion of breast cancer patients display de-regulated FGFR signalling in some form or another, it is anticipated that inhibition of these receptors may be useful in the treatment of this disease.

5. Urothelial carcinoma (UC)

Urothelial carcinoma (UC) is the most common type of bladder cancer, representing about 90% of all cases. The disease is broadly divided into two subtypes: papillary and muscle invasive UC. Over 70% of UC tumours are papillary (stage Ta on the TNM staging system), and well differentiated (low grade) at diagnosis, and this form of the disease has a good prognosis with relatively infrequent progression to invasive UC (10–20%). Those cases that are invasive at diagnosis, on the other hand, have a very poor prognosis with metastasis being a major clinical problem. Invasive disease is staged on a scale of T1–T4 depending on the extent of invasion, and the majority of cases are thought to evolve from a superficial, but poorly differentiated (high grade), lesion designated carcinoma in situ (Knowles, 2006).

Whilst many genetic alterations have been documented for invasive UC, only a small number are found in low grade Ta tumours;

the most common being deletions involving chromosome 9 and activating mutations affecting FGFR3. Over 60% of low grade and low stage tumours express mutant FGFR3, but this abnormality is rarely found in invasive bladder cancer (Kimura et al., 2001; Billerey et al., 2001; Bakkar et al., 2003; Tomlinson et al., 2007a). However, FGFR3 overexpression does occur in both forms of the disease (Tomlinson et al., 2007a), and changes to FGFR3 splicing may represent another mechanism by which FGF signalling contributes to pathology. In normal urothelial cells two FGFR3 isoforms are expressed: the epithelial splice form, FGFR3IIIb, and a truncated form (FGFR3Δ8–10) that is secreted, binds FGF1 and FGF2, and acts as a dominant negative regulator of FGF1-induced proliferation (Tomlinson et al., 2005). Expression of FGFR3Δ8–10 is decreased in several bladder cancer cell lines (Tomlinson et al., 2005) and this could potentially increase FGF signalling through decreased ligand sequestration. In addition, isoform switching from FGFR3IIIb to the mesenchymal isoform, FGFR3IIIc, has been documented for cell lines derived from invasive UC (Tomlinson et al., 2005), and since FGFR3IIIc binds a larger repertoire of FGF ligands than its epithelial counterpart, these tumour cells may acquire responsiveness to FGFs that they were not previously sensitive to. As epithelial-derived tumour cells acquire increased migratory ability they often lose differentiation and begin to express mesenchymal markers, a process known as the epithelial-to-mesenchymal transition. Whether the FGFR3IIIb to FGFR3IIIc isoform switch actively drives this increased migratory capacity, or whether the switch is a result of the epithelial-to-mesenchymal transition process, remains to be seen. Since FGFR3IIIb remains the dominant isoform expressed in cell lines derived from low grade and stage tumours (Tomlinson et al., 2005), signalling by this receptor could potentially limit the invasive capacity of tumour cells. Indeed, mutant FGFR3IIIb is frequently expressed in benign urothelial papilomas, along with benign skin tumours such as epidermal nevi and sebhorreic keratoses (Bernard-Pierrot et al., 2006).

Although papillary UC has a good prognosis, multiple recurrences are common and patients therefore require long term monitoring and repeated surgery. The high frequency of FGFR3 mutations in low grade and stage tumours, along with the fact that relatively few other genetic abnormalities are found for papillary UC, has pinpointed it as a potential target for therapeutic inhibition. This concept is supported by two studies where RNA interference or FGFR kinase inhibition caused reduced proliferation and colony-forming ability of UC-derived cell lines that express activated FGFR3 (Bernard-Pierrot et al., 2006; Tomlinson et al., 2007b). However, whilst FGFR inhibitors may prevent the need for repeated surgery in order to remove benign bladder polyps, papillary UC is already relatively well served in terms of therapies: BCG therapy promotes a local immune reaction against tumour cells and provides cheap and effective treatment for about 2/3 papillary UC patients (http://www.cancerhelp.org.uk), whilst intravesical valrubicin (Valstar) treatment can be used to treat BCG-refractory UC (http://www.valstarsolution.com). Thus, although FGFR inhibition is well validated as an approach to low grade papillary UC, the effectiveness of current therapies and the costs of new inhibitors mean that FGFR inhibitors may not be an economically viable strategy for treatment of these patients. It remains to be seen whether FGFR inhibitors would be beneficial for those patients with invasive forms of the disease that exhibit de-regulated FGFR signalling.

6. Gastric cancer

Gastric cancer is a particularly lethal cancer and although the overall incidence is declining worldwide, the incidence in parts of Asia remains high. Gastric cancer is broadly divided into the well-differentiated intestinal subtype and a poorly differentiated diffuse subtype with a less favourable prognosis and these subtypes are characterised by different underlying oncogenic lesions. *HER2* amplification (Yokota et al., 1988) and *KRAS* mutations (Yashiro et al., 2005) are found in the well-differentiated subtype whereas *MET* and *FGFR2* amplification is found in

the more aggressive diffuse subtype (Kuniyasu et al., 1992; Tsujimoto et al., 1997). *FGFR2* amplification was first detected in gastric cancer cell lines (Hattori et al., 1990) and has since be found in up to 10% of primary gastric cancers (Yoshida et al., 1993; Mor et al., 1993; Hara et al., 1998). In addition, activating mutations have also been found in FGFR2 in primary gastric cancers (Jang et al., 2001).

Whilst these reports serve as a 'smoking gun' implicating FGFR2 as a driving oncogene more recent interventional studies have provided evidence that gastric cancer cell lines with amplified *FGFR2* may respond well to anti-FGFR therapeutics. AZD2171, a mixed vascular endothelial growth factor receptor (VEGFR)/FGFR inhibitor, and Ki23057 both show good anti-tumour activity against FGFR2-positive gastric cancer cell lines (Nakamura et al., 2006; Takeda et al., 2007). A more recent study of a panel of 12 gastric cancer cell lines showed that only those that exhibited amplification of *FGFR2* were sensitive to the growth inhibitory effects of the FGFR1–3 selective tyrosine kinase inhibitor PD173074 (Kunii et al., 2008). More specifically gastric cancer cell lines with *FGFR2* amplification were 200-fold more sensitive to PD173074 than those without, revealing a substantial therapeutic window for selective intervention. These studies provide strong evidence that gastric cancer cells with FGFR2 amplification are indeed addicted to FGFR2 activity for proliferation and/or viability, providing a rationale for the use of FGFR inhibitors in such instances. This last study was also notable for demonstrating that gastric cancer lines with amplified *FGFR2* also exhibit elevated phosphorylation of at least three EGFR family members: EGFR1, HER2 (also known as EGFR2) and Erbb3 (EGFR3). This phosphorylation was a consequence of FGFR2 activity since it was abolished by PD173074 and could be further enhanced by FGF7, a dedicated ligand for FGFR2. Furthermore, shRNA-mediated inhibition of Erbb3 inhibited the growth of FGFR2 amplified gastric cancer cell lines suggesting that activation of EGFR family members contributes to their FGFR2-dependent proliferation. Finally, FGFR2-dependent phosphorylation of EGFRs in these cells was not inhibited by EGFR inhibitors such as erlotinib or gefitinib suggesting that the amplified FGFR2 may phosphorylate these EGFRs directly (Kunii et al., 2008). This apparent coupling of FGFR2 to EGFRs has only been demonstrated in gastric cancer cell lines to date so it will be important to confirm these observations in primary tumour tissue; nonetheless, it raises the possibility that amplification of FGFR2, like MET (Engelman et al., 2007), may be another mechanism for gefitinib resistance (Thomson et al., 2008; Kono et al., 2009). In summary, these data provide strong evidence that FGFR inhibitors may be particularly effective in cases of gastric cancer that exhibit FGFR2 amplification.

7. Haematological malignancies

Haematological malignancies derive from cells of haematopoietic origin and affect the bone marrow, blood and lymph nodes. They are classified according to lineage: lymphoid neoplasms, myeloid neoplasms, mast cell disorders and histiocytic neoplasms. Within each category, individual diseases are defined according to immunophonotype, genetic features, morphology and clinical symptoms (Harris et al., 2000). Although rare in solid tumours, chromosomal translocations represent a common cause of these malignancies and translocations affecting *FGFR1* or *FGFR3* in particular have been observed in certain disease types.

7.1. Multiple myeloma (MM)

Multiple myeloma (MM) is a cancer of bone marrow plasma cells (and therefore is classified as a lymphoid neoplasm) and accounts for ~10% of all haematological malignancies. The disease is characterised by the uncontrolled proliferation of transformed plasma cells within the bone marrow and is thought to evolve from a pre-malignant and asymptomatic stage termed monoclonal gammopathy of undetermined significance (MGUS) (Kyle & Rajkumar, 2008). MM is

designated symptomatic when tissue impairment becomes apparent. Although myeloma cells have a lower rate of antibody secretion than normal plasma cells, clonal growth results in a high concentration of monoclonal Ig (also known as paraprotein, or M protein) within the serum and urine. Tumour cells produce cytokines that stimulate osteoclast-mediated bone resorption, and this ultimately results in bone lesions and the release of calcium into the blood, causing hypercalcaemia. Normal bone marrow cells are replaced by infiltrating tumour cells, haematopoiesis is disrupted, and patients become both anaemic and immunocompromised. In addition, renal failure may occur as a result of hypercalcaemia, recurrent infections, and accumulation of paraprotein and/or tumour cells within the kidney (Jagannath, 2008).

Lymphoid neoplasms are often characterised by translocations affecting the immunoglobulin heavy chain locus (IgH) on chromosome 14, and these are thought to occur through aberrant class switch recombination. Class switch recombination is the process by which one antibody isotype is converted to another, and involves the excision of a segment of DNA and the joining of discontinuous sequences within the IgH locus. Translocations that place proto-oncogenes under the transcriptional control of the strong IgH enhancer regions are frequently observed in MM (~60% MM), and are thought to represent an initiating event in disease pathogenesis (Fenton et al., 2002). The t(4;14)(p16;q32) reciprocal translocation is the second most common IgH translocation and is observed in about 15% of MM (Keats et al., 2006). This genetic abnormality brings FGFR3 on derivative chromosome 14 (der(14)), and an IgH-MMSET fusion product on der(4), under the influence of the IgH enhancer regions Eα and Eμ respectively (Dring et al., 2004), and in the majority of cases, results in high levels of FGFR3 expression. A subset of t(4;14)(p16;q32) patients also carry point mutations within the translocated FGFR3 allele that are thought to arise later in the disease process and confer ligand-independent activation of the mutant FGFR3 (Chesi et al., 2001).

De-regulated FGFR3 probably acts as a driving oncogene in t(4;14) (p16;q32) positive MM, but it is currently unclear whether over-expression of the wild type receptor alone is sufficient for transformation. Whilst activated forms of FGFR3 induced transformation of NIH3T3 cells, wild type FGFR3 was found to be non-transforming in this system even in the presence of its ligand (Chesi et al., 2001). Conversely, ectopic expression of wild type FGFR3 in interleukin 6 (IL6)-dependent murine B9 MM cells resulted in IL6-independent proliferation when exogenous FGF1 and heparin was added (Plowright et al., 2000), suggesting that wild type FGFR3 may be transforming only in a limited number of cell types. Importantly, when murine bone marrow cells were transduced with retroviral vector containing either wild type or activated FGFR3 and then transplanted into mice, wild type FGFR3-expressing bone marrow cells developed pro-B cell leukaemia/lymphoma ~1 year after transplantation (Li et al., 2001), demonstrating that wild type FGFR3 can indeed contribute to the pathogenesis of lymphoid malignancy. Consistent with activated FGFR3 being more strongly transforming than its wild type counterpart, mice transplanted with activated FGFR3-expressing bone marrow cells exhibited leukocytosis with circulating pre-B cell tumours within six weeks of transplantation (Li et al., 2001). Furthermore, transgenic mice expressing activated FGFR3 under the control of the lymphoid specific enhancer Eμ developed lethal pro-B-cell lymphoma within six weeks after birth (Chen et al., 2005).

Multiple myeloma is still considered an incurable disease, with treatment focusing on containment and suppression. In recent years, treatment with thalidomide/lenalidomide or bortezomib in combination with steroids, alkylating agents and haematopoietic stem cell transplantation has been the treatment of choice for treatment-naïve MM. Those patients carrying the t(4;14)(p16;q32) translocation are classified into the 'high risk' category of MM with a poor prognosis of just 2–3 years median survival (Fenton et al., 2002; Keats et al., 2006). These patients do not respond well to current treatments (Keats et al.,

2006), and as such new regimes are needed for second and third line treatment of these t(4;14)(p16;q32) positive cases. Whilst bortezomib has offered some patients prolonged survival a recent study has shown that FGFR3 expression does not influence response rates to this drug (Dawson et al., 2009). Thus, there may be utility for FGFR inhibitors in the treatment of these t(4;14)(p16;q32) patients with FGFR3 expression. Indeed, many studies have shown that FGFR3 inhibition by antagonistic antibodies or small molecule inhibitors causes reduced proliferation and increased apoptosis of FGFR3-positive MM cell lines (Trudel, et al., 2004; Grand et al., 2004a, 2004b, Trudel, et al., 2005, Trudel, et al., 2006) and thus inhibition of FGFR3 signal transduction may represent a viable therapeutic strategy for this group of MM patients.

7.2. 8p11 myeloproliferative syndrome (EMS)

Myeloproliferative disorders are a heterogeneous group of diseases that are caused by uncontrolled proliferation of cells of the myeloid lineage. 8p11 myeloproliferative syndrome (EMS) is a rare atypical myeloproliferative disorder that is characterised by myeloid hyperplasia, eosinophilia and is sometimes associated with T cell lymphoblastic lymphoma (Grand et al., 2004a, 2004b). Clinically, EMS is an aggressive disease with a short chronic phase rapidly followed by transformation to acute myeloid leukaemia, which can result in death within weeks or months if left untreated.

A characteristic feature of EMS is the presence of chimeric proteins that contain the kinase domain of FGFR1 fused to the oligomerisation domain of one of a number of unrelated partners, and this occurs following reciprocal chromosomal translocations involving FGFR1 at 8p11. Ten partner genes have been identified to date (ZNF198, FOP, CEP1, BCR, FGFR1OP2, TIF1, MYO18A, CPSF6, HERV-K and LRRFIP1) (Soler et al., 2009), and in each case the partner gene drives ligand-independent dimerisation of the fusion protein, resulting in trans-phosphorylation and activation of the FGFR1 kinase. In cases where EMS is associated with T cell lymphoblastic lymphoma, these translocations are observed in both the myeloid leukaemia and lymphoma cells, suggesting that both cell types are derived from a single transformed haematopoetic stem cell that carries this genetic abnormality (Xiao et al., 1998). ZNF198–FGFR1 and BCR–FGFR1 fusion proteins have been shown to exhibit constitutive FGFR1 kinase activity that is localised to the cytoplasm, and are capable of transforming IL3-dependent Ba/F3 cells to growth factor independence in a PI3K- and p38 MAPK-dependent fashion (Demiroglu et al., 2001). Moreover retroviral transduction of ZNF198–FGFR1 into bone marrow of immunocompromised mice results in EMS-like disease with myeloproliferation as well as T cell lymphoma, demonstrating that the translocation event is sufficient for EMS pathogenesis (Roumiantsev et al., 2004).

Currently the only effective treatment option available for EMS patients is allogenic stem cell transplantation, but studies with FGFR inhibitors suggest that such drugs may be useful in managing this disease. Growth of ZNF198–FGFR1- or BCR–FGFR1-transformed Ba/F3 cells is inhibited by FGFR inhibition (Demiroglu et al., 2001; Chase et al., 2007), and treatment of FGFR1OP2–FGFR1 positive cell lines (KG1 and KG1A) with the multi-targeted tyrosine kinase inhibitor, TK1258, results in significant apoptosis (Chase et al., 2007). Furthermore, this compound also inhibits proliferation and survival of primary cells from EMS patients, demonstrating the potential effectiveness of FGFR inhibitors in a more clinically relevant system (Chase et al., 2007).

8. Therapeutic strategies

Since FGFR inhibition can reduce proliferation and induce cell death in a variety of in vitro and in vivo tumour models, inhibitors of FGFR or FGFR-dependent downstream signalling pathways may represent useful anti-cancer agents in the clinic. Since tumour cells with de-regulated

FGFR frequently exhibit hyper-activation of RAF–MEK1/2–ERK1/2 and PI3K–PKB there could be merit in using therapeutics targeted against these pathways. For example, expression of FGFR1 in normal human urothelial cells confers FGF-dependent cell proliferation and cell survival that can be blocked by ERK1/2 pathway inhibitors (Tomlinson et al., 2009). It follows then that tumour cells with high ERK1/2 activity resulting from FGFR1 de-regulation are likely to evolve some degree of addiction to ERK1/2 signalling and consequent sensitivity to ERK1/2 pathway inhibitors. However, the situation in tumour cells is frequently more complex; the degree of downstream pathway activation can vary substantially between tumour cell lines and will determine the sensitivity to MEK1/2 and PI3K inhibitors. For example, the degree of redundancy between the RAF–MEK1/2–ERK1/2 and PI3K–PKB pathways in cell proliferation and cell survival signalling (see Section 2.2, Fig. 2) means that concomitant activation of these pathways may reduce the efficacy of targeting either of them individually; certainly this is the case in colorectal cancer cell lines where coincident activation of the PI3K pathway confers intrinsic resistance to the MEK1/2 inhibitor AZD6244 (Balmanno et al., 2009). For these reasons, it is far more likely that efficacy (tumour cell cycle arrest or death) will be achieved by targeting the upstream driving oncoprotein, FGFR, rather than one of several pathways downstream. FGFR inhibition can be achieved by several approaches and both small molecule tyrosine kinase inhibitors directed against FGFR activity, and FGFR-antagonistic antibodies have been described.

8.1. Small molecule fibroblast growth factor receptor tyrosine kinase inhibitors

Protein kinases catalyse the transfer of the terminal phosphate group of ATP onto serine, threonine or tyrosine residues of their protein substrates. Approximately 518 kinases are encoded by the human genome (Zhang et al., 2009), and all share a common structure characterised by two lobes connected by a hinge region. The ATP-binding site is situated at the interface of the two lobes, whilst the protein substrate binding site is located mostly in the carboxy-terminal lobe (Johnson et al., 1998). Many small molecule kinase inhibitors discovered to date associate with the ATP-binding pocket of the enzyme, and mimic hydrogen bonding patterns normally produced by ATP binding. These drugs are classed as ATP-competitive, and are subdivided into Type I and Type II inhibitors that recognise the 'active' or 'inactive' conformation of the active site, respectively. All kinases possess an activation loop, and it is the conformation that this loop adopts that determines whether the active site is 'active' or 'inactive'. In many cases, an open conformation is produced by phosphorylation of activation loop residues, whilst a closed, 'inactive' conformation of the loop sterically hinders substrate binding and therefore produces a catalytically incompetent enzyme (Zhang et al., 2009). Other classes of small molecule kinase inhibitors include allosteric inhibitors that bind to a site other than the ATP-binding site and are usually non-competitive with respect to ATP, and irreversible inhibitors that form covalent bonds with the ATP-binding site, usually through cysteine residues (Zhang et al., 2009).

The first tyrosine kinase inhibitor to be approved for clinical use was the ABL, KIT and platelet-derived growth factor receptor (PDGFR) Type II ATP-completive inhibitor, imatinib (Glivec), which induces remission of virtually all chronic myeloid leukaemia (CML) patients when administered during the chronic phase of the disease (Azam et al., 2003). The effectiveness of imatinib treatment in such a large group of CML patients lies in the fact that this disease is clearly linked to a very specific genetic abnormality, the Philadelphia chromosome translocation; as such, all CML patients express BCR–ABL, a constitutively active kinase chimera, which is the target of kinase inhibition. Imatinib is now also licensed for treatment of gastrointestinal stromal tumour (GIST), and several other kinase inhibitors have subsequently been approved for anti-cancer therapy, including gefitinib (Iressa) and erlotinib (Tarceva) (EGFR inhibitors for treatment of a variety of cancers including advanced or metastatic non-small cell lung cancer (NSCLC)),

sunitinib (Sutent) (VEGFR, PDGFR, KIT and FLT3 inhibitor for treatment of GIST and renal cancer), lapatinib (Tyverb) (EGFR and HER2 inhibitor for treatment of trastuzumab (Herceptin)-refractory breast cancer) and sorafenib (Nexavar) (BRAF, VEGFR2, EGFR and PDGFR inhibitor for treatment of renal cancer) (Imai & Takaoka, 2006).

Many small molecule inhibitors of FGFR tyrosine kinase activity have been described in the literature and some are currently in clinical trials. For example, PD173074 is a potent ATP-competitive and reversible inhibitor of FGFR1–3, with in vitro IC_{50} values of 3.6 nM for FGFR1, 3.3 nM for FGFR2 and 5.3 nM for FGFR3 (IC_{50}~5–10 μM for FGFR4 in cells) (Ezzat et al., 2005; Kunii et al., 2008). This compound inhibits FGFR-dependent growth and/or survival in several cancer cells, including t(4;14)(p16;q32) positive MM cell lines (Trudel et al., 2004), mutant FGFR2-expressing endometrial cancer cell lines (Byron et al., 2008) and FGFR2-amplified gastric cancer cell lines (Kunii et al., 2008), demonstrating the potential use of FGFR-targeted kinase inhibitors as anti-cancer therapy. PD173074 has now been dropped from clinical development owing to toxicity issues, and as a result is now primarily used by the academic community as a research tool. Likewise, SU5402, which was a precursor compound in development of SU6668 (Table 1), is used as a tool for studying FGFR signalling (Laird et al., 2000), whilst other compounds such as Ki23057 (Kyowa Hakko Kirin Pharmaceuticals) are currently at the development stage (Nakamura et al., 2006). Other FGFR inhibitors remain in the pipelines of several pharmaceutical companies (see Table 1 for a list of FGFR-targeted inhibitors currently in clinical development) and their entry into the clinic is anticipated. Since the catalytic domains of various kinases exhibit significant structural homology, small molecule inhibitors generally demonstrate activity against more than one kinase. Indeed, a striking feature of these FGFR inhibitors is that they almost invariably have activity against VEGFR and/or PDGFR, two structurally related RTKs (Table 1); the converse is also true, as some compounds isolated as VEGFR inhibitors have activity against FGFR (Table 1). Although this means that the patient may be subject to toxicities associated with inhibition of each kinase, these side effects are often mild compared to those seen with conventional cytotoxic chemotherapies. For example, in Phase I clinical trials the main adverse effects associated with the Novartis RTK inhibitor, CHIR-258 (TKI-258) (which has activity against VEGFR, PDGFR, FGFR1–3, FLT-3 and c-KIT), were fatigue, hypertension and gastrointestinal complications (including nausea, vomiting, anorexia and diarrhoea). Importantly gastrointestinal effects were easily controlled with antiemetic treatment, whilst hypertension was resolved when dosing was modified (Sarker et al., 2008). This is in stark contrast with cytotoxic drugs that affect all areas of the body where cells rapidly divide, including the lining of the gut, the skin, hair and bone marrow (it should be noted here that RTK inhibitors are usually administered in combination with conventional chemotherapeutics, and so their use will not preclude such adverse effects). It is now recognised that inhibition of more than one kinase by a drug may increase effectiveness in the treatment of a particular tumour type by disrupting redundant pathways (which might otherwise drive resistance). In addition, multi-kinase inhibition may also allow the use of a particular drug for other tumour types or may inhibit tumorigenesis at multiple stages. For example, multi-kinase inhibitors that target FGFR and VEGFR may have superior efficacy by inhibiting both the cell autonomous growth of FGFR-dependent tumour cells and by preventing tumour angiogenesis; whether this is associated with greater toxicity remains to be seen.

8.2. Antagonistic antibodies to fibroblast growth factor receptors

Monoclonal antibodies (MAbs) that bind and antagonise RTKs represent another weapon in the fight against cancer. Such drugs can directly inhibit cancer progression by blocking ligand binding and RTK dimerisation (and hence exploit the tumours cell's addiction to a specific oncogene), but can also act indirectly to promote tumour cell removal by the immune system. In addition, antibodies can be conjugated to a

Table 1
Small molecule inhibitors of the FGFR tyrosine kinase currently in clinical development.

Drug	Company	Inhibits	Comments	Refs
BMS-540215 (Brivanib)	Bristol-Myers Squibb	FGFR, VEGFR	Prodrug hydrolysed to BMS-540215 in vivo.	Huynh et al., 2008
CHIR-258 (TKI-258)	Novartis	VEGFR, PDGFR, FGFR, FLT-3, c-KIT	Effective in t(4;14) (p16;q32) positive MM cells that express either wild type or activated FGFR3.	Sarker et al., 2008; Trudel et al., 2005
BIBF 1120 (Vargatef)	Boehringer Ingleheim	FGFR, VEGFR and PDGFR		Hilberg et al., 2008
AB1010 (Masitinib)	AB-Science	c-KIT, PDGFR and FGFR3		Hahn et al., 2008
SU6668	Sugen	FGFR, PDGFR, VEGFR2		Fabbro and Manley, 2001
GW786034 (Pazopanib)	Glaxo-Smith-Kline	VEGFR, PDGFR, c-KIT, (FGFR1/3)	Primarily developed as a VEGFR inhibitor but also inhibits FGFR1 and FGFR3 with IC_{50} values of 140 nM and 130 nM respectively in vitro. Inhibits FGF2-driven angiogenesis in vivo.	Kumar et al., 2007
RO438596	Roche	VEGFR, PDGFR, FGFR	Inhibits proliferation and anchorage-independent growth of NSCLC cell lines that coexpress FGF2 or FGF9 and FGFRs.	Marek et al., 2009; McDermott et al., 2005

The table lists small molecule FGFR inhibitors that are currently under development and/or undergoing clinical evaluation and notes other RTKs that are also targeted by these drugs.

variety of molecules, including toxins or radioisotopes, and this may provide a mechanism by which chemotherapy or radiotherapy can be targeted primarily at tumour cells. Although the production of MAbs is more costly than the production of small molecules, clinical approval of chimeric and humanised antibodies has been superior over that of kinase inhibitors (Reichert et al., 2005), and indeed the pharmaceutical industry has shown interest in both drug types. Antibodies are relatively large proteins, and as such must be administered intravenously, in contrast to small molecules that are much smaller and often orally available. However, the less convenient administration of antibodies may be reconciled with a longer half life compared to small molecules (the EGFR-targeted MAb, cetuximab (Erbitux), has a half life of 3.1–7.8 days whilst the EGFR kinase inhibitors gefitinib and erlotinib have half lives of 48 h and 36 h respectively) (Dancey & Sausville, 2003), meaning that antibodies can be administered on a weekly basis whilst small molecules must be taken daily. Nonetheless, in the case of brain cancers, antibodies need to be administered directly into the tumour itself owing to inefficient delivery across the blood–brain barrier, and therefore administration of antibody therapy to such patients requires a relatively invasive procedure.

Binding of an antibody to its target is incredibly specific and therefore therapeutic MAbs can be produced that exhibit high specificity for a particular molecule expressed on the cancer cell surface. Indeed, antibodies can be raised that are specific for particular FGFR isoforms, including defined splice variants, and these may be expected to target fewer normal cells in the body and hence produce fewer side effects. What is more, since different FGFR isoforms often have opposing effects in target cells, this technique would also allow specific inhibition of oncogenic FGFR molecules, including splice variants that are selectively up-regulated in tumour cells.

Ligand-competitive antibodies have been shown to inhibit proliferation of wild type FGFR3-expressing MM and UC cell lines

(Martínez-Torrecuadrada et al., 2005; Trudel et al., 2006), and more recently an FGFR3-specific antagonistic antibody (R3Mab) has been described that inhibits proliferation driven by wild type FGFR3 or by two of the common ligand-independent FGFR3 mutants expressed in UC (FGFR3^{R248C} and FGFR3^{S249C}) (Qing et al., 2009). This suggests that antibody therapy may be viable for treatment of wild type and mutant FGFR3-driven tumours. The FGFR3^{R248C} and FGFR3^{S249C} mutations both generate unpaired cysteine residues between the IgII and IgIII domains, resulting in inter-molecular disulphide bond formation and constitutive FGF-independent dimerisation. Crystallographic studies showed that R3Mab binds to an epitope that includes the IgII and IgIII region of the receptor (Qing et al., 2009) and so is likely to disrupt dimerisation, and hence activation, of the FGFR3^{R248C} and FGFR3^{S249C} mutants. However, it did not inhibit in vitro proliferation of the MM-derived KMS-11 cell line that expresses ligand-independent FGFR3^{Y373C} at high levels (Qing et al., 2009), probably reflecting the fact that these mutants form dimers through inter-molecular disulphide bonds that are localised outside of the IgII and IgIII regions. Interestingly, despite being ineffective against KMS-11 cells in vitro, growth of KMS-11 cells subcutaneously transplanted in mice was inhibited by R3Mab, likely reflecting an immune response generated by R3Mab-coating of these cells (Qing et al., 2009). Thus, although these antibodies may not inhibit proliferation and/or survival driven by all activated FGFR mutants in human tumours, they may still be used to eliminate tumour cells through complement-dependent or antibody-dependent cellular cytotoxicity. It should be noted however that such antibody therapies would only be useful for treating tumour cells that express FGFR at the plasma membrane, and so are unlikely to be effective against diseases where de-regulated FGFR activity is localised to the cytoplasm (such as EMS, which is driven by chimeric FGFR1 fusion proteins that do not reside at the plasma membrane). Small molecule inhibitors of FGFR, on the other hand, do not rely on plasma membrane localisation of FGFR and so could potentially be useful for treating a larger cohort of diseases in which FGFR activity is de-regulated.

9. Future directions

De-regulated FGFR signalling is clearly important for growth and/or viability of several tumours, including a subset of breast cancer, UC, gastric and haematological malignancies. By all commonly used criteria the de-regulated FGFRs observed in these diseases are acting as driving oncoproteins and as such they represent attractive and increasingly well validated therapeutic targets. Several pharmaceutical companies currently have FGFR-directed therapies in their pipelines and these drugs are likely to be available for use in the clinic in the near future. What remains to be seen is the longevity of responses to this class of compounds. Other kinase-directed inhibitors have produced promising responses in specific tumour types, but this has been thwarted by the emergence of drug-resistant tumour cells and relapse. Experience therefore suggests that FGFR-directed therapies may be subject to the same problem. A better understanding of possible mechanisms of resistance, both intrinsic and acquired, may be useful for guiding discovery of second generation inhibitors and drug combinations, and this will hopefully prolong the use of this promising class of drugs in the fight against cancer.

Acknowledgments

References

Azam, M., Latek, R. R., & Daley, G. Q. (2003). Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of BCR–ABL. Cell 112(6), 831−843.

Bakkar, A. A., Wallerand, H., Radvanyi, F., Lahaye, J. B., Pissard, S., Lecerf, L., et al. (2003). FGFR3 and TP53 gene mutations define two distinct pathways in urothelial cell carcinoma of the bladder. Cancer Res 63(23), 8108−8112.

Balmanno, K., & Cook, S. J. (2009). Tumour cell survival signalling by the ERK1/2 pathway. Cell Death Differ 16(3), 368−377.

Balmanno, K., Chell, S. D., Gillings, A. S., Hayat, S., & Cook, S. J. (2009). Intrinsic resistance to the MEK1/2 inhibitor AZD6244 (ARRY-142886) is associated with weak ERK1/2 signalling and/or strong PI3K signalling in colorectal cancer cell lines. Int J Cancer 125(10), 2332−2341.

Basilico, C., & Moscatelli, D. (1992). The FGF family of growth factors and oncogenes. Adv Cancer Res 59, 115−165.

Beenken, A., & Mohammadi, M. (2009). The FGF family: biology, pathophysiology and therapy. Nat Rev Drug Discov 8(3), 235−253.

Bernard-Pierrot, I., Brams, A., Dunois-Lardé, C., Caillault, A., Diez de Medina, S. G., Cappellen, D., et al. (2006). Oncogenic properties of the mutated forms of fibroblast growth factor receptor 3b. Carcinogenesis 27(4), 740−747.

Billerey, C., Chopin, D., Aubriot-Lorton, M. H., Ricol, D., Gil Diez de Medina, S., Van Rhijn, B., et al. (2001). Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. Am J Pathol 158(6), 1955−1959.

Brondello, J. M., Pouysségur, J., & McKenzie, F. R. (1999). Reduced MAP kinase phosphatase-1 degradation after p42/p44MAPK-dependent phosphorylation. Science 286(5449), 2514−2517.

Brummer, T., Naegele, H., Reth, M., & Misawa, Y. (2003). Identification of novel ERK-mediated feedback phosphorylation sites at the C-terminus of B-Raf. Oncogene 22(55), 8823−8834.

Brunet, A., Bonni, A., Zigmond, M. J., Lin, M. Z., Juo, P., Hu, L. S., Anderson, M. J., Arden, K. C., Blenis, J., & Greenberg, M. E. (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96(6), 857−868.

Byron, S. A., Gartside, M. G., Wellens, C. L., Mallon, M. A., Keenan, J. B., Powell, M. A., et al. (2008). Inhibition of activated fibroblast growth factor receptor 2 in endometrial cancer cells induces cell death despite PTEN abrogation. Cancer Res 68(17), 6902−6907.

Cabrita, M. A., & Christofori, G. (2008). Sprouty proteins, masterminds of receptor tyrosine kinase signaling. Angiogenesis 11(1), 53−62.

Cappellen, D., De Oliveira, C., Ricol, D., de Medina, S., Bourdin, J., Sastre-Garau, X., et al. (1999). Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. Nat Genet 23(1), 18−20.

Chase, A., Grand, F. H., & Cross, N. C. (2007). Activity of TKI258 against primary cells and cell lines with FGFR1 fusion genes associated with the 8p11 myeloproliferative syndrome. Blood 110(10), 3729−3734.

Chen, G. J., & Forough, R. (2006). Fibroblast growth factors, fibroblast growth factor receptors, diseases, and drugs. Recent Pat Cardiovasc Drug Discov 1(2), 211−224.

Chen, J., Williams, I. R., Lee, B. H., Duclos, N., Huntly, B. J., Donoghue, D. J., et al. (2005). Constitutively activated FGFR3 mutants signal through PLCgamma-dependent and -independent pathways for hematopoietic transformation. Blood 106(1), 328−337.

Chesi, M., Brents, L. A., Ely, S. A., Bais, C., Robbiani, D. F., Mesri, E. A., et al. (2001). Activated fibroblast growth factor receptor 3 is an oncogene that contributes to tumor progression in multiple myeloma. Blood 97(3), 729−736.

Cross, M. J., Lu, L., Magnusson, P., Nyqvist, D., Holmqvist, K., Welsh, M., et al. (2002). The Shb adaptor protein binds to tyrosine 766 in the FGFR-1 and regulates the Ras/MEK/MAPK pathway via FRS2 phosphorylation in endothelial cells. Mol Biol Cell 13(8), 2881−2893.

Dancey, J., & Sausville, E. A. (2003). Issues and progress with protein kinase inhibitors for cancer treatment. Nat Rev Drug Discov 2(4), 296−313.

Datta, S. R., Dudek, H., Tao, X., Masters, S., Fu, H., Gotoh, Y., & Greenberg, M. E. (1997). Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91(2), 231−241.

Dawson, M. A., Opat, S. S., Taouk, Y., Donovan, M., Zammit, M., Monaghan, K., et al. (2009). Clinical and immunohistochemical features associated with a response to bortezomib in patients with multiple myeloma. Clin Cancer Res 15(2), 714−722.

Demiroglu, A., Steer, E. J., Heath, C., Taylor, K., Bentley, M., Allen, S. L., et al. (2001). The t(8;22) in chronic myeloid leukemia fuses BCR to FGFR1: transforming activity and specific inhibition of FGFR1 fusion proteins. Blood 98(13), 3778−3783.

Ding, W., Bellusci, S., Shi, W., & Warburton, D. (2003). Functional analysis of the human Sprouty2 gene promoter. Gene 322, 175−185.

Downward. (2003). Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer 3(1), 11−22.

Dring, A. M., Davies, F. E., Fenton, J. A., Roddam, P. L., Scott, K., Gonzalez, D., et al. (2004). A global expression-based analysis of the consequences of the t(4;14) translocation in myeloma. Clin Cancer Res 10(17), 5692−5701.

Easton, D. F., Pooley, K. A., Dunning, A. M., Pharoah, P. D., Thompson, D., Ballinger, D. G., et al. (2007). Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 447(7148), 1087−1093.

Engelman, J. A., Zejnullahu, K., Mitsudomi, T., Song, Y., Hyland, C., Park, J. O., et al. (2007). MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 316(5827), 1039−1043.

Eswarakumar, V. P., Lax, I., & Schlessinger, J. (2005). Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev 16(2), 139−149.

Ezzat, S., Huang, P., Dackiw, A., & Asa, S. (2005). Dual inhibition of RET and FGFR4 restrains medullary thyroid cancer cell growth. Clin Cancer Res 11(3), 1336−1341.

Fabbro, D., & Manley, P. W. (2001). Su-6668. SUGEN. Curr Opin Investig Drugs 2(8), 1142−1148.

Fenton, J. A. L., Pratt, G., Rawstron, A. C., & Morgan, G. J. (2002). Isotype class switching and the pathogenesis of multiple myeloma. *Hematol Oncol 20*, 75–85.

Furdui, C. M., Lew, E. D., Schlessinger, J., & Anderson, K. S. (2006). Autophosphorylation of FGFR1 kinase is mediated by a sequential and precisely ordered reaction. *Mol Cell 21*(5), 711–717.

Gillings, A. S., Balmanno, K., Wiggins, C. M., Johnson, M., & Cook, S. J. (2009). BIM as a mediator of tumour cell death in response to oncogene-targeted therapeutics. *FEBS J 276*(21), 6050–6062.

Grand, E. K., Chase, A. J., Heath, C., Rahemtulla, A., & Cross, N. C. (2004). Targeting FGFR3 in multiple myeloma: inhibition of t(4;14)-positive cells by SU5402 and PD173074. *Leukemia 18*(5), 962–966.

Grand, E. K., Grand, F. H., Chase, A. J., Ross, F. M., Corcoran, M. M., Oscier, D. G., et al. (2004). Identification of a novel gene, FGFR1OP2, fused to FGFR1 in 8p11 myeloproliferative syndrome. *Genes Chromosomes Cancer 40*(1), 78–83.

Häcker, U., Nybakken, K., & Perrimon, N. (2005). Heparan sulphate proteoglycans: the sweet side of development. *Nat Rev Mol Cell Biol 6*(7), 530–541.

Hacohen, N., Kramer, S., Sutherland, D., Hiromi, Y., & Krasnow, M. A. (1998). Sprouty encodes a novel antagonist of FGF signaling that patterns apical branching of the Drosophila airways. *Cell 92*(2), 253–263.

Hahn, K. A., Ogilvie, G., Rusk, T., Devauchelle, P., Leblanc, A., Legendre, A., et al. (2008). Masitinib is safe and effective for the treatment of canine mast cell tumors. *J Vet Intern Med 22*(6), 1301–1309.

Hara, T., Ooi, A., Kobayashi, M., Mai, M., Yanagihara, K., & Nakanishi, I. (1998). Amplification of c-myc, K-sam, and c-met in gastric cancers: detection by fluorescence in situ hybridization. *Lab Invest 78*(9), 1143–1153.

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *Cell 100*(1), 57–70.

Harris, N. L., Jaffe, E. S., Diebold, J., Flandrin, G., Muller-Hermelink, H. K., Vardiman, J., et al. (2000). The World Health Organization classification of hematological malignancies report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997. *Mod Pathol 13*(2), 193–207.

Hart, K. C., Robertson, S. C., Kanemitsu, M. Y., Meyer, A. N., Tynan, J. A., & Donoghue, D. J. (2000). Transformation and Stat activation by derivatives of FGFR1, FGFR3, and FGFR4. *Oncogene 19*(29), 3309–3320.

Hattori, Y., Odagiri, H., Nakatani, H., Miyagawa, K., Naito, K., Sakamoto, H., et al. (1990). K-sam, an amplified gene in stomach cancer, is a member of the heparin-binding growth factor receptor genes. *Proc Natl Acad Sci U S A 87*(15), 5983–5987.

Hilberg, F., Roth, G. J., Krssak, M., Kautschitsch, S., Sommergruber, W., Tontsch-Grunt, U., et al. (2008). BIBF 1120: triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. *Cancer Res 68*(12), 4774–4782.

Huynh, H., Ngo, V. C., Fargnoli, J., Ayers, M., Soo, K. C., Koong, H. N., et al. (2008). Brivanib alaninate, a dual inhibitor of vascular endothelial growth factor receptor and fibroblast growth factor receptor tyrosine kinases, induces growth inhibition in mouse models of human hepatocellular carcinoma. *Clin Cancer Res 14*(19), 6146–6153.

Imai, K., & Takaoka, A. (2006). Comparing antibody and small-molecule therapies for cancer. *Nat Rev Cancer 6*(9), 714–727.

Jagannath, S. (2008). Pathophysiological underpinnings of multiple myeloma progression. *JMCP 14*(7), S7–S11.

Jang, J. H., Shin, K. H., & Park, J. G. (2001). Mutations in fibroblast growth factor receptor 2 and fibroblast growth factor receptor 3 genes associated with human gastric and colorectal cancers. *Cancer Res 61*(9), 3541–3543.

Jeffers, M., LaRochelle, W. J., & Lichenstein, H. S. (2002). Fibroblast growth factors in cancer: therapeutic possibilities. *Expert Opin Ther Targets 6*(4), 469–482.

Johnson, L. N., Lowe, E. D., Noble, M. E., & Owen, D. J. (1998). The Eleventh Datta Lecture. The structural basis for substrate recognition and control by protein kinases. *FEBS Lett 430*(1–2), 1–11.

Keats, J. J., Reiman, T., Belch, A. R., & Pilarski, L. M. (2006). Ten years and counting: so what do we know about t(4;14)(p16;q32) multiple myeloma. *Leuk Lymphoma 47*(11), 2289–2300.

Kimura, T., Suzuki, H., Ohashi, T., Asano, K., Kiyota, H., & Eto, Y. (2001). The incidence of thanatophoric dysplasia mutations in FGFR3 gene is higher in low-grade or superficial bladder carcinomas. *Cancer 92*(10), 2555–2561.

Knowles, M. A. (2006). Molecular subtypes of bladder cancer: Jekyll and Hyde or chalk and cheese? *Carcinogenesis 27*(3), 361–373.

Kokel, M., Borland, C. Z., DeLong, L., Horvitz, H. R., & Stern, M. J. (1998). clr-1 encodes a receptor tyrosine phosphatase that negatively regulates an FGF receptor signaling pathway in Caenorhabditis elegans. *Genes Dev 12*(10), 1425–1437.

Kono, S. A., Marshall, M. E., Ware, K. E., & Heasley, L. E. (2009). The fibroblast growth factor receptor signaling pathway as a mediator of intrinsic resistance to EGFR-specific tyrosine kinase inhibitors in non-small cell lung cancer. *Drug Resist Updat 278*(4–5), 14087–14091.

Kovalenko, D., Yang, X., Nadeau, R. J., Harkins, L. K., & Friesel, R. (2003). Sef inhibits fibroblast growth factor signaling by inhibiting FGFR1 tyrosine phosphorylation and subsequent ERK activation. *J Biol Chem 278*(16), 14087–14091.

Koziczak, M., Holbro, T., & Hynes, N. E. (2004). Blocking of FGFR signaling inhibits breast cancer cell proliferation through downregulation of D-type cyclins. *Oncogene 23*(20), 3501–3508.

Kumar, R., Knick, V. B., Rudolph, S. K., Johnson, J. H., Crosby, R. M., Crouthamel, M. C., et al. (2007). Pharmacokinetic-pharmacodynamic correlation from mouse to human with pazopanib, a multikinase angiogenesis inhibitor with potent antitumor and antiangiogenic activity. *Mol Cancer Ther 6*(7), 2012–2021.

Kunii, K., Davis, L., Gorenstein, J., Hatch, H., Yashiro, M., Di Bacco, A., et al. (2008). FGFR2-amplified gastric cancer cell lines require FGFR2 and Erbb3 signaling for growth and survival. *Cancer Res 68*(7), 2340–2348.

Kuniyasu, H., Yasui, W., Kitadai, Y., Yokozaki, H., Ito, H., & Tahara, E. (1992). Frequent amplification of the c-met gene in scirrhous type stomach cancer. *Biochem Biophys Res Commun 189*(1), 227–232.

Kwabi-Addo, B., Ropiquet, F., Giri, D., & Ittmann, M. (2001). Alternative splicing of fibroblast growth factor receptors in human prostate cancer. *Prostate 46*(2), 163–172.

Kyle, R. A., & Rajkumar, S. V. (2008). Multiple myeloma. *Blood 111*(6), 2962–2972.

Laird, A. D., Vajkoczy, P., Shawver, L. K., Thurnher, A., Liang, C., Mohammadi, M., et al. (2000). SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. *Cancer Res 60*(15), 4152–4160.

Langlois, W. J., Sasaoka, T., Saltiel, A. R., & Olefsky, J. M. (1995). Negative feedback regulation and desensitization of insulin- and epidermal growth factor-stimulated p21ras activation. *J Biol Chem 270*(43), 25320–25323.

Lao, D. H., Chandramouli, S., Yusoff, P., Fong, C. W., Saw, T. Y., Tai, L. P., et al. (2006). A Src homology 3-binding sequence on the C terminus of Sprouty2 is necessary for inhibition of the Ras/ERK pathway downstream of fibroblast growth factor receptor stimulation. *J Biol Chem 281*(40), 29993–30000.

Lew, E. D., Furdui, C. M., Anderson, K. S., & Schlessinger, J. (2009). The precise sequence of FGF receptor autophosphorylation is kinetically driven and is disrupted by oncogenic mutations.*Sci Signal 2*(58) ra6.

Ley, R., Balmanno, K., Hadfield, K., Weston, C., & Cook, S. J. (2003). Activation of the ERK1/2 signaling pathway promotes phosphorylation and proteasome-dependent degradation of the BH3-only protein, Bim. *J Biol Chem 278*(21), 18811–18816.

Li, X., Brunton, V. G., Burgar, H. R., Wheldon, L. M., & Heath, J. K. (2004). FRS2-dependent SRC activation is required for fibroblast growth factor receptor-induced phosphorylation of Sprouty and suppression of ERK activity. *J Cell Sci 117*(25), 6007–6017.

Li, C., Chen, L., Iwata, T., Kitagawa, M., Fu, X. Y., & Deng, C. X. (1999). A Lys644Glu substitution in fibroblast growth factor receptor 3 (FGFR3) causes dwarfism in mice by activation of STATs and ink4 cell cycle inhibitors. *Hum Mol Genet 8*(1), 35–44.

Li, Z., Zhu, Y. X., Plowright, E. E., Bergsagel, P. L., Chesi, M., Patterson, B., et al. (2001). The myeloma-associated oncogene fibroblast growth factor receptor 3 is transforming in hematopoietic cells. *Blood 97*(8), 2413–2419.

Lorenzi, M. V., Castagnino, P., Chen, Q., Chedid, M., & Miki, T. (1997). Ligand-independent activation of fibroblast growth factor receptor-2 by carboxyl terminal alterations. *Oncogene 15*(7), 817–826.

Mandl, M., Slack, D. N., & Keyse, S. M. (2005). Specific inactivation and nuclear anchoring of extracellular signal-regulated kinase 2 by the inducible dual-specificity protein phosphatase DUSP5. *Mol Cell Biol 25*(5), 1830–1845.

Marek, L., Ware, K. E., Fritzsche, A., Hercule, P., Helton, W. R., Smith, J. E., et al. (2009). Fibroblast growth factor (FGF) and FGF receptor-mediated autocrine signaling in non-small-cell lung cancer cells. *Mol Pharmacol 75*(1), 196–207.

Martínez-Torrecuadrada, J., Cifuentes, G., López-Serra, P., Saenz, P., Martínez, A., & Casal, J. I. (2005). Targeting the extracellular domain of fibroblast growth factor receptor 3 with human single-chain Fv antibodies inhibits bladder carcinoma cell line proliferation. *Clin Cancer Res 11*(17), 6280–6290.

Mason, J. M., Morrison, D. J., Bassit, B., Dimri, M., Band, H., Licht, J. D., et al. (2004). Tyrosine phosphorylation of Sprouty proteins regulates their ability to inhibit growth factor signaling: a dual feedback loop. *Mol Biol Cell 15*(5), 2176–2188.

Mason, J. M., Morrison, D. J., Basson, M. A., & Licht, J. D. (2006). Sprouty proteins: multifaceted negative-feedback regulators of receptor tyrosine kinase signaling. *Trends Cell Biol 16*(1), 45–54.

McDermott, L. A., Simcox, M., Higgins, B., Nevins, T., Kolinsky, K., Smith, M., et al. (2005). RO4383596, an orally active KDR, FGFR, and PDGFR inhibitor: synthesis and biological evaluation. *Bioorg Med Chem 13*(16), 4835–4841.

McLeskey, S. W., Zhang, L., El-Ashry, D., Trock, B. J., Lopez, C. A., Kharbanda, S., et al. (1998). Tamoxifen-resistant fibroblast growth factor-transfected MCF-7 cells are cross-resistant in vivo to the antiestrogen ICI 182, 780 and two aromatase inhibitors. *Clin Cancer Res 4*(3), 697–711.

Meloche, S., & Pouysségur, J. (2007). The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. *Oncogene 26*(22), 3227–3239.

Moffa, A. B., Tannheimer, S. L., & Ethier, S. P. (2004). Transforming potential of alternatively spliced variants of fibroblast growth factor receptor 2 in human mammary epithelial cells. *Mol Cancer Res 2*(11), 643–652.

Mohammadi, M., Honegger, A. M., Rotin, D., Fischer, R., Bellot, F., Li, W., et al. (1991). A tyrosine-phosphorylated carboxy-terminal peptide of the fibroblast growth factor receptor (Flg) is a binding site for the SH2 domain of phospholipase C-gamma 1. *Mol Cell Biol 11*(10), 5068–5078.

Mor, O., Ranzani, G. N., Ravia, Y., Rotman, G., Gutman, M., Manor, A., et al. (1993). DNA amplification in human gastric carcinomas. *Cancer Genet Cytogenet 65*(2), 111–114.

Nakamura, K., Yashiro, M., Matsuoka, T., Tendo, M., Shimizu, T., Miwa, A., et al. (2006). A novel molecular targeting compound as K-samII/FGF-R2 phosphorylation inhibitor, Ki23057, for Scirrhous gastric cancer. *Gastroenterology 131*(5), 1530–1541.

Olsen, S. K., Garbi, M., Zampieri, N., Eliseenkova, A. V., Ornitz, D. M., Goldfarb, M., et al. (2003). Fibroblast growth factor (FGF) homologous factors share structural but not functional homology with FGFs. *J Biol Chem 278*(36), 34226–34236.

Olsen, S. K., Ibrahimi, O. A., Raucci, A., Zhang, F., Eliseenkova, A. V., Yayon, A., et al. (2004). Insights into the molecular basis for fibroblast growth factor receptor autoinhibition and ligand-binding promiscuity. *Proc Natl Acad Sci U S A 101*(4), 935–940.

Ong, S. H., Guy, G. R., Hadari, Y. R., Laks, S., Gotoh, N., Schlessinger, J., et al. (2000). FRS2 proteins recruit intracellular signaling pathways by binding to diverse targets on fibroblast growth factor and nerve growth factor receptors. *Mol Cell Biol 20*(3), 979–989.

Ong, S. H., Hadari, Y. R., Gotoh, N., Guy, G. R., Schlessinger, J., & Lax, I. (2001). Stimulation of phosphatidylinositol 3-kinase by fibroblast growth factor receptors is mediated by coordinated recruitment of multiple docking proteins. *Proc Natl Acad Sci U S A 98*(11), 6074–6079.

Onose, H., Emoto, N., Sugihara, H., Shimizu, K., & Wakabayashi, I. (1999). Overexpression of fibroblast growth factor receptor 3 in a human thyroid carcinoma cell line results in overgrowth of the confluent cultures. *Eur J Endocrinol 140*(2), 169–173.

Ornitz, D. M., & Itoh, N. (2001). Fibroblast growth factors. *Genome Biol 2*(3) REVIEWS3005.

Ozaki, K., Miyazaki, S., Tanimura, S., & Kohno, M. (2005). Efficient suppression of FGF-2-induced ERK activation by the cooperative interaction among mammalian Sprouty isoforms. *J Cell Sci 118*(24), 5861–5871.

Powers, C. J., McLeskey, S. W., & Wellstein, A. (2000). Fibroblast growth factors, their receptors and signaling. *Endocr Relat Cancer 7*(3), 165–197.

Plotnikov, A. N., Schlessinger, J., Hubbard, S. R., & Mohammadi, M. (1999). Structural basis for FGF receptor dimerization and activation. *Cell 98*(5), 641–650.

Plowright, E. E., Li, Z., Bergsagel, P. L., Chesi, M., Barber, D. L., Branch, D. R., et al. (2000). Ectopic expression of fibroblast growth factor receptor 3 promotes myeloma cell proliferation and prevents apoptosis. *Blood 95*(3), 992–998.

Preger, E., Ziv, I., Shabtay, A., Sher, I., Tsang, M., Dawid, I. B., et al. (2004). Alternative splicing generates an isoform of the human Sef gene with altered subcellular localization and specificity. *Proc Natl Acad Sci U S A 101*(5), 1229–1234.

Qing, J., Du, X., Chen, Y., Chan, P., Li, H., Wu, P., et al. (2009). Antibody-based targeting of FGFR3 in bladder carcinoma and t(4;14)-positive multiple myeloma in mice. *J Clin Invest 119*(5), 1216–1229.

Ray, M. E., Yang, Z. Q., Albertson, D., Kleer, C. G., Washburn, J. G., Macoska, J. A., et al. (2004). Genomic and expression analysis of the 8p11–12 amplicon in human breast cancer cell lines. *Cancer Res 64*(1), 40–47.

Reichert, J. M., Rosensweig, C. J., Faden, L. B., & Dewitz, M. C. (2005). Monoclonal antibody successes in the clinic. *Nat Biotechnol 23*(9), 1073–1078.

Reis-Filho, J. S., Simpson, P. T., Turner, N., Lambros, M. B., Jones, C., Mackay, A., et al. (2006). FGFR1 emerges as a potential therapeutic target for lobular breast carcinomas. *Clin Cancer Res 12*(22), 6652–6662.

Rhee, S. G. (2001). Regulation of phosphoinositide-specific phospholipase C. *Annu Rev Biochem 70*, 281–312.

Roumiantsev, S., Krause, D. S., Neumann, C. A., Dimitri, C. A., Asiedu, F., Cross, N. C., et al. (2004). Distinct stem cell myeloproliferative/T lymphoma syndromes induced by ZNF198–FGFR1 and BCR–FGFR1 fusion genes from 8p11 translocations. *Cancer Cell 5*(3), 287–298.

Rubin, C., Zwang, Y., Vaisman, N., Ron, D., & Yarden, Y. (2005). Phosphorylation of carboxyl-terminal tyrosines modulates the specificity of Sprouty-2 inhibition of different signaling pathways. *J Biol Chem 280*(10), 9735–9744.

Sandilands, E., Akbarzadeh, S., Vecchione, A., McEwan, D. G., Frame, M. C., & Heath, J. K. (2007). Src kinase modulates the activation, transport and signalling dynamics of fibroblast growth factor receptors. *EMBO Rep 8*(12), 1162–1169.

Sarker, D., Molife, R., Evans, T. R., Hardie, M., Marriott, C., Butzberger-Zimmerli, P., et al. (2008). A phase I pharmacokinetic and pharmacodynamic study of TKI258, an oral, multitargeted receptor tyrosine kinase inhibitor in patients with advanced solid tumors. *Clin Cancer Res 14*(7), 2075–2081.

Sellers, A. H. (1960). The staging of breast cancer, trial of the TNM method. *Can J Surg 4*, 116–117.

Shaul, Y. D., & Seger, R. (2007). The MEK/ERK cascade: from signaling specificity to diverse functions. *Biochim Biophys Acta 1773*(8), 1213–1226.

Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., & McGuire, W. L. (1987). Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science 235*(4785), 177–182.

Sleeman, M., Fraser, J., McDonald, M, Yuan, S, White, D., Grandison, P., et al. (2001). Identification of a new fibroblast growth factor receptor, FGFR5. *Gene 271*(2), 171–182.

Smith, T. G., Karlsson, M., Lunn, J. S., Eblaghie, M. C., Keenan, I. D., Farrell, E. R., et al. (2006). Negative feedback predominates over cross-regulation to control ERK MAPK activity in response to FGF signalling in embryos. *FEBS Lett 580*(17), 4242–4245.

Soler, G., Nusbaum, S., Varet, B., Macintyre, E. A., Vekemans, M., Romana, S. P., et al. (2009). LRRFIP1, a new FGFR1 partner gene associated with 8p11 myeloproliferative syndrome. *Leukemia 23*(7), 1359–1361.

Streit, S., Bange, J., Fichtner, A., Ihrler, S., Issing, W., & Ullrich, A. (2004). Involvement of the FGFR4 Arg388 allele in head and neck squamous cell carcinoma. *Int J Cancer 111*(2), 213–217.

Suhardja, A., & Hoffman, H. (2003). Role of growth factors and their receptors in proliferation of microvascular endothelial cells. *Microsc Res Tech 60*(1), 70–75.

Takeda, M., Arao, T., Yokote, H., Komatsu, T., Yanagihara, K., Sasaki, H., et al. (2007). AZD2171 shows potent antitumor activity against gastric cancer over-expressing fibroblast growth factor receptor 2/keratinocyte growth factor receptor. *Clin Cancer Res 13*(10), 3051–3057.

Tannheimer, S. L., Rehemtulla, A., & Ethier, S. P. (2000). Characterization of fibroblast growth factor receptor 2 overexpression in the human breast cancer cell line SUM-52PE. *Breast Cancer Res 2*(4), 311–320.

Thomson, S., Petti, F., Sujka-Kwok, I., Epstein, D., & Haley, J. D. (2008). Kinase switching in mesenchymal-like non-small lung cancer lines contributes to EGFR inhibitor resistance through pathway redundancy. *Clin Exp Metastasis 25*(8), 843–854.

Tomlinson, D. C., Baldo, O., Harnden, P., & Knowles, M. A. (2007). FGFR3 protein expression and its relationship to mutation status and prognostic variables in bladder cancer. *J Pathol 213*(1), 91–98.

Tomlinson, D. C., Hurst, C. D., & Knowles, M. A. (2007). Knockdown by shRNA identifies S249C mutant FGFR3 as a potential therapeutic target in bladder cancer. *Oncogene 26*(40), 5889–5899.

Tomlinson, D. C., L'Hôte, C. G., Kennedy, W., Pitt, E., & Knowles, M. A. (2005). Alternative splicing of fibroblast growth factor receptor 3 produces a secreted isoform that inhibits fibroblast growth factor-induced proliferation and is repressed in urothelial carcinoma cell lines. *Cancer Res 65*(22), 10441–10449.

Tomlinson, D. C., Lamont, F. R., Shnyder, S. D., & Knowles, M. A. (2009). Fibroblast growth factor receptor 1 promotes proliferation and survival via activation of the mitogen-activated protein kinase pathway in bladder cancer. *Cancer Res 69*(11), 4613–4620.

Trudel, S., Ely, S., Farooqi, Y., Affer, M., Robbiani, D. F., Chesi, M., et al. (2004). Inhibition of fibroblast growth factor receptor 3 induces differentiation and apoptosis in t(4;14) myeloma. *Blood 103*(9), 3521–3528.

Trudel, S., Li, Z. H., Wei, E., Wiesmann, M., Chang, H., Chen, C., et al. (2005). CHIR-258, a novel, multitargeted tyrosine kinase inhibitor for the potential treatment of t(4;14) multiple myeloma. *Blood 105*(7), 2941–2948.

Trudel, S., Stewart, A. K., Rom, E., Wei, E., Li, Z. H., Kotzer, S., et al. (2006). The inhibitory anti-FGFR3 antibody, PRO-001, is cytotoxic to t(4;14) multiple myeloma cells. *Blood 107*(10), 4039–4046.

Tsang, M., Friesel, R., Kudoh, T., & Dawid, I. B. (2002). Identification of Sef, a novel modulator of FGF signalling. *Nat Cell Biol 4*(2), 165–169.

Tsujimoto, H., Sugihara, H., Hagiwara, A., & Hattori, T. (1997). Amplification of growth factor receptor genes and DNA ploidy pattern in the progression of gastric cancer. *Virchows Arch 431*(6), 383–389.

Weinstein, I. B., & Joe, A. K. (2006). Mechanisms of disease: oncogene addiction—a rationale for molecular targeting in cancer therapy. *Nat Clin Pract Oncol 3*(8), 448–457.

Welm, B. E., Freeman, K. W., Chen, M., Contreras, A., Spencer, D. M., & Rosen, J. M. (2002). Inducible dimerization of FGFR1: development of a mouse model to analyze progressive transformation of the mammary gland. *J Cell Biol 157*(4), 703–714.

Wong, A., Lamothe, B., Lee, A., Schlessinger, J., & Lax, I. (2002). FRS2 alpha attenuates FGF receptor signaling by Grb2-mediated recruitment of the ubiquitin ligase Cbl. *Proc Natl Acad Sci U S A 99*(10), 6684–6689.

Xian, W., Schwertfeger, K. L., Vargo-Gogola, T., & Rosen, J. M. (2005). Pleiotropic effects of FGFR1 on cell proliferation, survival, and migration in a 3D mammary epithelial cell model. *J Cell Biol 171*(4), 663–673.

Xian, W., Pappas, L., Pandya, D., Selfors, L. M., Derksen, P. W., de Bruin, M., et al. (2009). Fibroblast growth factor receptor 1–transformed mammary epithelial cells are dependent on RSK activity for growth and survival. *Cancer Res 69*, 2244–2251.

Xiao, S., Nalabolu, S. R., Aster, J. C., Ma, J., Abruzzo, L., Jaffe, E. S., et al. (1998). FGFR1 is fused with a novel zinc-finger gene, ZNF198, in the t(8;13) leukaemia/lymphoma syndrome. *Nat Genet 18*(1), 84–87.

Yagasaki, F., Wakao, D., Yokoyama, Y., Uchida, Y., Murohashi, I., Kayano, H., et al. (2001). Fusion of ETV6 to fibroblast growth factor receptor 3 in peripheral T-cell lymphoma with a t(4;12)(p16;p13) chromosomal translocation. *Cancer Res 61*(23), 8371–8374.

Yamaguchi, F., Saya, H., Bruner, J. M., & Morrison, R. S. (1994). Differential expression of two fibroblast growth factor-receptor genes is associated with malignant progression in human astrocytomas. *Proc Natl Acad Sci U S A 91*(2), 484–488.

Yashiro, M., Nishioka, N., & Hirakawa, K. (2005). K-ras mutation influences macroscopic features of gastric carcinoma. *J Surg Res 124*(1), 74–78.

Yokota, J., Yamamoto, T., Miyajima, N., Toyoshima, K., Nomura, N., Sakamoto, H., et al. (1988). Genetic alterations of the c-erbB-2 oncogene occur frequently in tubular adenocarcinoma of the stomach and are often accompanied by amplification of the v-erbA homologue. *Oncogene 2*(3), 283–287.

Yoshida, T., Sakamoto, H., & Terada, M. (1993). Amplified genes in cancer in upper digestive tract. *Semin Cancer Biol 4*(1), 33–40.

Yusoff, P., Lao, D. H., Ong, S. H., Wong, E. S., Lim, J., Lo, T. L., et al. (2002). Sprouty2 inhibits the Ras/MAP kinase pathway by inhibiting the activation of Raf. *J Biol Chem 277*(5), 3195–3201.

Zhang, J., Yang, P. L., & Gray, N. S. (2009). Targeting cancer with small molecule kinase inhibitors. *Nat Rev Cancer 9*(1), 28–39.

http://www.cancerhelp.org.uk.

http://www.valstarsolution.com.