Roles of FGFR in oral carcinogenesis

Xiaoyan Xie¹, Zhiyong Wang¹, Fangman Chen, Yao Yuan, Jiayi Wang, Rui Liu and Qianming Chen

State Key Laboratory of Oral Diseases, West China College of Stomatology, Sichuan University, Chengdu 610041, China,

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Abstract

Fibroblast growth factor receptors (FGFRs) play essential roles in organ development during the embryonic period, and regulate tissue repair in adults. Accumulating evidence suggests that alterations in FGFR signalling are involved in diverse types of cancer. In this review, we focus on aberrant regulation of FGFRs in pathogenesis of oral squamous cell carcinoma (OSCC), including altered expression and subcellular location, aberrant isoform splicing and mutations. We also provide an overview of oncogenic roles of each FGFR and its downstream signalling pathways in regulating OSCC cell proliferation and metastasis. Finally, we discuss potential application of FGFRs as anti-cancer targets in the preclinical environment and in clinical practice.

Introduction

Oral squamous cell carcinoma (OSCC) ranks among the most frequent cancer worldwide and is associated with severe morbidity and high mortality. Despite therapeutic improvements, the clinical outcome of this disease remains stagnant, and the 5-year survival rate is around 60% during the last decades (1–3). Development of OSCC is a multiple and complex process; however, the key oncogenic factors involved in this process is not fully illustrated.

FGFRs belong to a family of receptor tyrosine kinases (RTKs), and each family member possesses an extracellular ligand-binding region, an intracellular tyrosine kinase domain and a single-pass transmembrane domain. FGFRs are activated after extracellular part binding to cognate ligands (FGFs), and in turn trigger

intracellular downstream signalling cascades by phosphorylating the tyrosine residue in their substrates (4). Aberrant regulations in FGFRs, including altered expression and subcellular location, aberrant isoform splicing and mutations, are frequently observed in various tumours.

Structure of Fibroblast Growth Factor Receptor

The overall structure of FGFRs is similar to other receptor tyrosine kinases (RTKs). A total of five FGFRs (FGFR1–FGFR5) are identified so far, of which four FGFRs (FGFR1–FGFR4) are composed of an extracellular ligand-binding domain, a single transmembrane domain and a cytoplasmic domain containing the catalytic protein tyrosine kinase core as well as a carboxy-terminal tail (Fig. 1.). In contrast, FGFR5, which is usually referred as FGFR1, lacks the intracellular tyrosine kinase domain (5). The extracellular ligand-binding part of FGFR has three extracellular immunoglobulin (Ig)-like domains (D1–D3). The second and the third Ig-like domains of FGFRs are decisive and adequate for ligand binding and specificity, while the first Ig-like domain with the presence of an acid box is proposed to play a role in receptor auto-inhibition (6).

FGFR signal transduction

Initiation of FGFR signalling cascade

After binding to FGFs (Table 1), activated FGFR phosphorylates intracellular proteins, such as phospholipase C γ (PLC γ) and FGFR substrate 2 (FRS2), triggering the downstream signalling cascades (Fig. 1). The Src homology 2 (SH2) domain of PLC γ binds to a phosphotyrosine residue within FGFR carboxyl terminus, which is the critical step of PLC γ activation. Activated PLC γ catalyses phosphatidylinositol diphosphate (PIP₂) to inositol triphosphate (IP₃) and diacylglycerol (DAG), which results in the Ca²⁺-release and protein kinase C (PKC) activation. PKC in turn phosphorylates RAF and

Correspondence: Rui Liu and Jiayi Wang, State Key Laboratory of Oral Diseases, West China College of Stomatology, Sichuan University, Chengdu, China. Tel.: +86 28 85503484; Fax: +86 28 85501484; Emails: liurui_scu@hotmail.com and jiaiyimail@163.com

¹These authors contributed equally to this work.

Figure 1. The FGFR structure and signalling cascades. FGFRs are single-pass transmembrane receptors with an extracellular ligand-binding domain containing 3 Ig-like domains (Ig I-III) and an intracellular tyrosine kinase domain. The ligand-receptor binding is stabilized by the interaction with HSPG, thus inducing receptor dimerization and transphosphorylation. After ligand-induced FGFR activation, two signalling branches, RAS/MAPK and PI3K/AKT pathways, are initiated via FRS2. FGFR signals are also transduced to the DAG-PKC and IP3-Ca²⁺-releasing signalling branches via PLCγ. Several negative regulators are also associated with FGFR signalling (in violet).

Table 1. Summary of fibroblast growth factor (FGF) ligand subfamilies

FGF1 subfamily	FGF4 subfamily	FGF7 subfamily	FGF8 subfamily	FGF9 subfamily	FGF19 subfamily
FGF1	FGF4	FGF3	FGF8	FGF9	FGF19
FGF2	FGF5	FGF7	FGF17	FG16	FGF21
	FGF6	FGF10	FGF18	FGF20	FGF23

further enhances the mitogen-activated protein kinase (MAPK) pathway (5). FRS2 binds to the juxtamembrane region of FGFRs and is then phosphorylated by activated FGFR. FRS2 serves as a key docking protein that allows the recruitment of growth factor receptor bound protein 2 (GRB2) and the adaptor proteins son of sevenless (SOS), which can subsequently activates RAS, RAF and MAPK pathways (7). In addition, GRB2 can also activate Akt by recruiting phosphoinositide 3-kinase (PI3K) (8).

Termination of FGFR signalling cascade

It is widely accepted that FGFR signalling is terminated by FGFR endocytosis and subsequent lysosomal degradation (Fig. 2). FGFRs are recruited to pre-formed clathrin-coated pits (CCPs), forming FGFRs and clathrin clusters. FRS2 recruits the E3 ubiquitin ligase Cbl to activated FGFR through Grb2 and leads to ubiquitination of both FRS2 and FGFR. Src and its downstream effector Eps8 can recruit new clathrin at the plasma membrane, facilitating clathrin-mediated FGFR endocytosis (9). After internalization, endocytosed FGFRs are delivered to early/sorting endosomes, where

ubiquitinated FGFRs are recognized by Hrs and the ESCRT complexes, then sorted into intraluminal vesicles (ILVs) of multivesicular endosomes (MVEs), leading to lysosomal degradation (10,11).

Aberrant regulation of FGFRs in OSCC

Altered expression

Abnormal expression of FGFRs has been extensively studied in OSCC. It is reported that up-regulation of FGFR1 and FGFR2 was more frequent in early clinical stages than in advanced stages (12,13). Amplification of both FGFR1 and FGFR2 is likely to be an important reason for their overexpression. It is shown that increased FGFR1 gene copies were found in approximately 17% of OSCC and 10% of oral tongue squamous cell carcinomas (OTSCC) (12,14). In addition, the FGFR1 amplification is more frequent in men and is significantly associated with a habit of smoking (13,14), but it is barely observed in HPV-positive OSCC (15). Amplification of FGFR2 is reported to be accompanied with loss of its C-terminal sequence, which results in the expression of a C-terminally truncated FGFR2. This

Figure 2. Termination of FGFR signalling.

FGFR signalling is terminated by clathrin-mediated endocytosis. The receptors are routed to early endosomes, from where they can be sorted into ILVs of the MVE and subsequently targeted for lysosomal degradation (ubiquitylated receptors) or recycled to the plasma membrane (non-ubiquitylated receptors). The CBL family of ubiquitin ligases plays a key role in mediating RTK ubiquitylation. Ubiquitin is an essential signal for endosomal sorting of FGFRs into the ILVs of MVEs. Components of the endocytic machinery, including EPS8, Scr, the ESCRT and Hrs. DUBs catalyse the removal of ubiquitin from receptors before their translocation into the ILV, without allowing cargo to escape.

C-terminal truncation decreases FGFR2 internalization and prolongs FGFR2 half-life, therefore leading to the activation of FGFR2 (16).

The expression pattern of FGFR3 in OSCC is relatively unclear yet. By a hamster cheek pouch oral cancer model, high level of FGFR3 expression persisted in epithelium, fibroblasts and vascular endothelia at all stages of malignant transformation (17,18). However, the expression of FGFR3 was found to be more pronounced at the early dysplasia stages, by analysing clinical samples from normal epithelium, mild dysplasia and moderate/severe dysplasia.

FGFR fusion proteins

Enhanced FGFR activity can also result from chromosomal rearrangements, by which FGFR genes are fused with other genes and generate a fused protein. The fused protein might acquire functional properties from each of the original proteins, inducing permanent dimerization and activation of the tyrosine kinase (19). As these fusion proteins are usually located in the cytosol, rather than cell surface, they can escape the routing degradation mechanisms for wild-type FGFRs. Therefore, FGFR fusion proteins are probably potent oncogenes that can drive proliferation of cancer cells.

Most FGFR fusion proteins are identified in haematologic malignancies, such as SCLL (stem cell leukaemia lymphoma syndrome) and chronic myelogenous leukaemia-like diseases (20–24). 5' gene fusion of FGFR3 with TACC3 was the first reported FGFR fusion protein in OSCC and head and neck squamous cell carcinoma (HNSCC). FGFR3 fusion partner, TACC3,

mediates oligomerization of FGFR3 and augments FGFR3 kinase activity. Loss of FGFR 3' UTR and miR-99a was evidenced to be responsible for FGFR3-TACC3 fusion in glioblastoma multiforme (GBM) (25,26). However, it is recently reported that FGFR3-TACC3 fusion is more frequent in HPV-positive HNSCC cases, suggesting that HPV infection may facilitate this process (15).

Aberrant isoform splicing

Alternative splicing of FGFR transcripts results in distinct FGFR isoforms, including FGFRs with truncated C-terminal domain, soluble secreted FGFRs, FGFRs with either two or three Ig-like domains and alternative splicing in the third Ig-like domain of the receptor. It has been shown that exon 7 of all FGFR genes encodes for the N-terminal half of D3 (designated 'a form'), whereas exons 8 and 9 alternatively encode for the C-terminal half of D3, termed as 'b' and 'c' forms of FGFR, respectively. Alternative splicing in the second half of the third Ig-like domain (D3) creates IIIb and IIIc isoforms, which markedly differs from each other in ligand-binding specificity (27,28). Thus, switch between different FGFR isoforms allows tumour cells to respond to and utilize a broader range of FGFs in the microenvironment (29,30). Several reports have compared the oncogenic potential of different isoforms of the FGFRs (16,31,32). The isoform splicing pattern of FGFR2 was shown to be associated with the pathological classification of OSCC. Well-differentiated squamous cell carcinoma cell lines (H357 and H413) expressed more FGFR2IIIb than IIIc, whereas the poorly differentiated

cells (H314) expressed more FGFR2IIIc than IIIb. Interestingly, an additional transcription product was detected in normal and tumour-derived human oral keratinocytes, which harbour both the IIIb and IIIc splice variants of FGFR2 joined by an additional base pair and with the intronic sequence between the exons removed (33). Expression of similar transcript was previously reported in normal and hyperplastic prostate epithelium and normal and malignant breast epithelium (34,35); however, the exact function of this transcript remains unclear yet.

Mutations

Mutations of FGFRs have been identified in diverse types of tumours, including human lung cancer, bladder cancer, urothelial papilloma, breast cancer, gastric and colorectal cancers and prostate cancer (36–44). Mutations within the extracellular domain may alter ligand-binding affinity and specificity. Certain mutations are capable to promote dimerization of the FGFR or activate the tyrosine kinase domain even in the absence of ligands. Further, those mutations located in the kinase domain of FGFR may result in conformational change, leading to permanent kinase activation. It should be noted that loss-of-function mutations in FGFRs were also identified in human cancers (45), suggesting that FGFRs may play an anti-proliferative role in such context.

A heterozygous missense mutation (G2128T) in exon 17 of FGFR3b, causing a glycine-to-cysteine substitution at position 697 (G697C) in the cytoplasmic kinase domain, was observed in 62% (44/71) of the OSCC cases. This mutation increased FGFR3b expression on the plasma membrane, promoted FGFR3 dimerization and, more importantly, enhanced the FGFR3b auto-phosphorylation, suggesting that this cysteine/glycine substitution might cause constitutive ligand-independent FGFR3 activation. (46).

Genome-wide analysis of clinical tumour samples has demonstrated that a single-nucleotide polymorphism (SNP) in FGFR4 exon 9 resulted in substitution of a glycine residue for an arginine-Gly388Arg within transmembrane domain. Although the precise mechanism is unclear yet, this mutation increases FGFR4 stability and prolonged its half-life. Incidence of this mutation is associated with prognostic parameters in several human cancers (47–49). Particularly in OSCC, FGFR4 Gly388Arg polymorphism is found to be strongly associated with poor prognosis, such as lymphatic embolization and premature disease-related death (50,51). The allele type of FGFR4 amino acid 388 in OSCC was Arg/Arg (8.3%), Arg/Gly (54.2%) and Gly/Gly (37.5%) (47–49,52). Furthermore, during 60 months of

observation after radical surgery, a majority of 150 OSCC patients bearing homozygous Arg388 FGFR4 plus mutated TP53 died from cancer, while more than 90% patients with homozygous Gly388 FGFR4 plus wild-type TP53 survived. Therefore, FGFR4 Arg388 allele and mutations in TP53 probably synergistically affected the survival of OSCC patients, and combination of these two factors may serve as a potential prognostic predictor of OSCC (53).

Implication of aberrant FGFR signalling in OSCC

FGFR signalling pathway is considered as important oncogenic pathway in various cancers and plays a significant role in regulating cancer cell proliferation and metastasis (Fig. 3). The involvement of FGFRs in the development of OSCC is summarized in the following section.

Tumour cell proliferation

A recent study showed that FGFR3 expression was considerably higher in all except in one of the 14 OSCC

Figure 3. Aberrant FGFR signalling in tumorigenesis. The effects of deregulated FGFR signalling are wide ranging, involving both tumour cells and the surrounding stroma, including the vasculature. (a) FGFRs enhance tumour cell proliferation and survival under radiotherapy and chemotherapy. (b) Multiple FGFRs exert angiogenic functions on endothelial cells by interplay with other angiogenic pathways. (c) FGFRs are critical factors in tumour–stroma interactions to promote tumour metastasis. (d) FGFRs also play important roles in glycolytic metabolism by regulating PKM2.

cell lines than that in the normal oral epithelial cells. Knockdown of FGFR3 resulted in a 35% decrease in cell proliferation of OSCC cell lines (54). Regulation of FGFR3 expression was shown to be at translational level rather than transcriptional level. It is reported that FGFR3 is the target of miR-100, whose expression is substantially lower in OSCC cell lines compared with that in normal human oral keratinocyte controls. Overexpression of miR-100 in OSCC cells significantly decreased the expression of FGFR3 and the proliferation of tumour cells (55). FGFR4 is believed to be the predominant receptor for FGF19, and anti-FGF19 monoclonal antibodies can inhibit tumour cell survival and proliferation by down-regulating β -catenin signalling (56). High expression of FGFR4 was found to be associated with the reduced overall survival in head and neck squamous cell carcinoma (HNSCC) (51).

Tumour cell survival under stress

A major survival advantage of cancer cells is natural or acquired resistance to anti-cancer therapy, including radiotherapy and chemotherapy. This occurs mainly through activating survival pathways or inhibiting apoptotic pathways.

By a high-throughput gene expression microarray containing 54675 oligonucleotide-based probe sets, expression levels of 167 genes (220 probes) were found to be elevated at least 5-fold or more by X-ray irradiation at all doses (0, 2, 4 and 8 Gy) in the radio-resistant OSCC cell lines, HSC2, compared with radio-sensitive OSCC cell lines, HSC3. Among them, FGFR3 expression was significantly elevated in radio-resistant cells (57). Accordingly, another study reported that FGFR3 expression level was apparently associated with the sensitivity of OSCC cells to ionizing radiation. It is shown that knockdown of FGFR3 caused 50%, 60% or 96% reduction in cell viability when companied with 1, 2.5 or 5 Gy ionizing radiation, respectively (54).

FGFR4 Arg388 mutation was implicated in radio-resistance and chemo-resistance in breast cancer (58,59) and oesophageal cancer (60). Ansell *et al.* analysed FGFR4 Arg388 polymorphism in around 40 HNSCC cell lines; however, no apparent correlation was observed between Arg388 mutation and radio/chemo-sensitivity, suggesting that the role of FGFR4 in regulating radio/chemo-sensitivity might be context dependent (61).

Tumour metastasis

Accumulating evidences suggest that FGFR1 is probably the major effector for FGF2-induced OSCC metastasis

(62–69). It is reported that the immunoreactivity of bFGF and FGFR1 expressions in OSCC cells was relatively low at non-invasive areas; however, strong expression of both bFGF and FGFR1 was observed in metastatic tumours. Importantly, expression patterns of FGF2 and FGFR1 were positively correlated with the mode of tumour invasion. Interestingly, concurrent bFGF and FGFR1 expressions in tumour-associated fibroblasts were detected in majority (67%) of OSCC patients, and 83% specimens with positive bFGF fibroblasts were also found to be FGFR1 positive (70). More importantly, markedly enhanced expression of FGF2 and FGFR1 was observed in tumour-associated fibroblast from OSCC tumours with lymph node metastasis compared with those cases without metastasis. It has been demonstrated that FGF2 promotes the production of cancer cell proteinases and enhances their invasive ability (71). Furthermore, FGF2 was reported to induce the production of hepatocyte growth factor, transforming growth factor- β from fibroblasts and matrix metalloproteinase-2, which are relevant to tumour invasion (72). Therefore, FGFR1 is proposed to play an important role in the invasion and metastasis of OSCC by means of interaction with bFGF.

Recently, FGFR4 Arg allele carrier SNP is involved in OSCC metastasis. It is reported that a total of 24 OSCC patients were enrolled to analyse the pattern of SNPs in FGFR4 gene and to evaluate the association between SNPs and prognostic parameters (51). The patients carrying FGFR4 allele Arg/Arg or Arg/Gly at amino acid 388 were associated with advanced nodal stage (pathologic N2 + N3), compared with those carrying Gly/Gly allele. However, no apparent association of FGFR4 genotype and other clinicopathologic variables, such as age, gender, tumour size, depth of invasion and extracapsular spread, was noted.

FGFRs as therapeutic targets in cancer

The pivotal roles of FGFRs in carcinogenesis highlight FGFRs as potential anti-cancer drug targets. Inhibition of FGFRs can be achieved by several approaches, including small-molecule tyrosine kinase inhibitors and neutralizing monoclonal antibodies (Table 2).

Small-molecule FGFR inhibitors

The first generation of FGFR small molecules is mainly tyrosine kinase inhibitors (TKI) with cross-interaction with VEGFRs and PDGFRs, and several of them are currently in preclinical or clinical development (73). TKI-258 (Novartis) is an inhibitor of FGFRs, VEGFRs, PDGFRs, KIT and FLT3 (74), which is now being

Table 2. Small-molecule inhibitors of the FGFR tyrosine kinase currently in clinical development

Chemical name (drug name)	Pharmaceutical company	Multikinase activity	Clinical development (indication)
Mixed pharmacology			
TKI-258 Dovitinib	Novartis	FGFRs, VEGFRs, FLT3, PDGFRs, KIT	Phase II (breast, bladder, myeloma)
AZ2171 (Cediranib)	Astra Zeneca	VEGFRs, FGFRs, KIT	Phase I (gastric, breast), Phase II (endometrial)
BMS582664 (Brivanib)	Bristol Myers Squibb	VEGFRs, FGFRs	Phase II (endometrial, gastric, bladder)
E7080	Eisai	VEGFRs, FGFRs, PDGFRs, KIT	Phase II (endometrial cancer)
FGFR selective			
AZD4547	AstraZeneca	FGFR1-3	Phase II (breast, gastric)
BGJ398	Novartis	FGFR1-3	Phase I (solid tumours)
LY2874455	Eli Lilly	FGFR1-4	Phase I (solid tumours)

tested in bladder cancer patients with FGFR3 mutation (NCT00790426) and in advanced breast cancer patients with and without FGFR1 amplification (NCT00958971), and relapsed multiple myeloma cases with and without the t (4,14) translocation that often associates with FGFR3 amplification (NCT01058434). AZD2171 (Astra Zeneca) is a pan-VEGF receptor TKI, but it also showed inhibitory effects against FGFR1 and FGFR2 (75). Treatment with AZD2171 counteracts cell proliferation in KATO-III and OCUM-2M gastric cancer cell lines, both of which harbour FGFR2 amplifications (76). Brivanib (Bristol Myers Squibb) is a dual tyrosine kinase inhibitor against VEGFRs and FGFRs, which was found to be effective in metastatic solid malignancies resistant to standard therapy and is currently being developed as an anti-angiogenic agent in Phase II clinical trials (77). E7080 (Eisai) shows inhibitory activity against VEGFRs, FGFRs and PDGFRs and is being tested in Phase II clinical trials, examining the efficacy in patients with metastatic endometrial cancer (NCT01111461) (78).

The second-generation compounds are more potent FGFR inhibitors with increased specificity for the FGFR family, including AZD4547 (AstraZeneca) (79), BGJ398 (Novartis) (80) and LY2874455 (Eli Lilly) (81). AZD4547 (AstraZeneca) is a more specific pan-FGFR inhibitor that is being tested in Phase I clinical trials for patients with FGFR1 and FGFR2 gene amplification (NCT00979134) and in Phase IIa trials for FGFR1 gene-amplified ER-positive breast cancer and FGFR2 gene-amplified gastric cancer (NCT01202591 and NCT01457846, respectively). BGJ398 (Novartis) is being evaluated in a Phase I study for advanced solid malignancies with either FGFR3 mutation or FGFR1 and FGFR2 amplification for those patients failed in other treatment (NCT01004224). LY2874455 inhibits FGFR activity by occupying the ATP-binding pocket of the enzyme. It is currently in Phase I trials for an unselected cancer patient population (NCT01212107). Unlike other FGFR inhibitor mainly targeting intracellular

kinase domain, SSR128129E (SSR) binds to FGFR extracellular domain. SSR128129E does not prevent FGFR binding to FGF ligand, but inhibits FGF-induced subsequent conformational changes in FGFR through allosteric mechanisms, which finally block FGF signal transduction (82).

Monoclonal antibodies

Monoclonal antibodies (MAbs) highly specific for particular FGFR isoform can reduce the side effects mediated by pan-FGFR inhibitors, representing another strategy in developing FGFR inhibitors. Although antibodies may not directly inhibit cell proliferation in human tumours, they can recruit the immune system to eliminate tumour cell *via* antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity. In addition, anti-FGFR monoclonal antibodies can be conjugated to radioisotopes or toxins, providing a mechanism by which radiotherapy or chemotherapy can be targeted primarily at tumour cells. As the first FGFR antibody with potential clinical utility, MFGR1877S (Genentech) was shown to be effective in treating multiple myeloma cell lines harbouring oncogenic FGFR3 mutations and is currently in Phase I trials (83). An FGFR3-specific antagonistic antibody, R3Mab, which disrupts receptor dimerization and activates FGFR3^{R248C} and FGFR3^{S249C} mutants, exerts a potent anti-tumour effect in KMS 11 (human myeloma cell line) subcutaneous xenografts, through induction of antibody-dependent cell-mediated cytotoxicity (84). Several anti-FGFR2 monoclonal antibodies are being developed, including HuGAL-FR21 (Galaxy) and GP369 (Aveo), which show efficacy in mouse xenograft models of FGFR2-amplified gastric cancer (SNU16) and breast cancer (MFM-223) (85,86). Moreover, administration of a humanized anti-FGFR4 monoclonal antibody, LD1 (chLD1), showed promising anti-tumour effects in the HUH7 HCC xenograft model (87). It should be noted that monoclonal antibody works only when the majority of FGFR is

expressed on tumour cell surface, while small-molecule inhibitors of FGFR, on the contrary, can target both surface and intracellular FGFR.

Summary

In the past decade, a large body of studies markedly increase our knowledge on the clinical relevance of FGFRs in cancer biology. Enhanced FGFR signalling transduction, due to increased expression, activating mutations, abnormal isoform splicing or impaired termination of signalling, is connected with proliferative and invasive phenotype of OSCC cells. Apparently, aberrant regulation of FGFRs can contribute to the development of OSCC and could thus be potential therapeutic targets. Nevertheless, FGFR-based anti-cancer drug discovery is still challenging, since each FGFR is intimately involved in biological processes in normal cells, and patient response to these FGFR inhibitors is relatively uncertain. Thus, more efforts both in further elucidation of FGFR biology and in pharmacological innovation are expected in the future.

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Conflicts of interest

The authors declare no conflicts of interest.

References

- Messadi DV (2013) Diagnostic aids for detection of oral precancerous conditions. *Int. J. Oral Sci.* **5**, 59–65.
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. *CA Cancer J. Clin.* **61**, 69–90.
- Chinn SB, Myers JN (2015) Oral cavity carcinoma: current management, controversies, and future directions. *J. Clin. Oncol.* **33**, 3269–3276.
- Katoh M, Nakagama H (2014) FGF receptors: cancer biology and therapeutics. *Med. Res. Rev.* **34**, 280–300.
- Eswarakumar VP, Lax I, Schlessinger J (2005) Cellular signaling by fibroblast growth factor receptors. *Cytokine Growth Factor Rev.* **16**, 139–149.
- Beenken A, Mohammadi M (2009) The FGF family: biology, pathophysiology and therapy. *Nat. Rev. Drug Discov.* **8**, 235–253.
- Gotoh N (2008) Regulation of growth factor signaling by FRS2 family docking/scaffold adaptor proteins. *Cancer Sci.* **99**, 1319–1325.
- Altomare DA, Testa JR (2005) Perturbations of the AKT signaling pathway in human cancer. *Oncogene* **24**, 7455–7464.
- Auciello G, Cunningham DL, Tatar T, Heath JK, Rappoport JZ (2013) Regulation of fibroblast growth factor receptor signalling and trafficking by Src and Eps8. *J. Cell Sci.* **126**, 613–624.
- Belleudi F, Leone L, Maggio M, Torrisi MR (2009) Hrs regulates the endocytic sorting of the fibroblast growth factor receptor 2b. *Exp. Cell Res.* **315**, 2181–2191.
- Chanut-Delalande H, Jung AC, Baer MM, Lin L, Payre F, Affolter M (2010) The Hrs/Stam complex acts as a positive and negative regulator of RTK signaling during Drosophila development. *PLoS ONE* **5**, e10245.
- Freier K, Schwaenen C, Sticht C, Flechtenmacher C, Muhling J, Hofe C *et al.* (2007) Recurrent FGFR1 amplification and high FGFR1 protein expression in oral squamous cell carcinoma (OSCC). *Oral Oncol.* **43**, 60–66.
- Ozretic L, Wagner S, Huebbers CU, Gattenlohner S, Klussmann JP, Beutner D *et al.* (2016) FGFR1 amplification and co-overexpression of c-MYC in oropharyngeal squamous cell carcinoma. *Oral Oncol.* **54**, e7–9.
- Young RJ, Lim AM, Angel C, Collins M, Deb S, Corry J *et al.* (2013) Frequency of fibroblast growth factor receptor 1 gene amplification in oral tongue squamous cell carcinomas and associations with clinical features and patient outcome. *Oral Oncol.* **49**, 576–581.
- Cancer Genome Atlas Network (2015) Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* **517**, 576–582.
- Cha JY, Maddileti S, Mitin N, Harden TK, Der CJ (2009) Aberrant receptor internalization and enhanced FRS2-dependent signaling contribute to the transforming activity of the fibroblast growth factor receptor 2 IIIB C3 isoform. *J. Biol. Chem.* **284**, 6227–6240.
- Raimondi AR, Molinolo AA, Itoiz ME (2006) Fibroblast growth factor-2 expression during experimental oral carcinogenesis. Its possible role in the induction of pre-malignant fibrosis. *J. Oral Pathol. Med.* **35**, 212–217.
- Nayak S, Goel MM, Makker A, Bhatia V, Chandra S, Kumar S *et al.* (2015) Fibroblast Growth Factor (FGF-2) and Its Receptors FGFR-2 and FGFR-3 May Be Putative Biomarkers of Malignant Transformation of Potentially Malignant Oral Lesions into Oral Squamous Cell Carcinoma. *PLoS ONE* **10**, e0138801.
- Jackson CC, Medeiros LJ, Miranda RN (2010) 8p11 myeloproliferative syndrome: a review. *Hum. Pathol.* **41**, 461–476.
- Roumiantsev S, Krause DS, Neumann CA, Dimitri CA, Asiedu F, Cross NC *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**, 287–298.
- Guasch G, Delaval B, Arnoulet C, Xie MJ, Xerri L, Sainty D *et al.* (2004) FOP-FGFR1 tyrosine kinase, the product of a t(6;8) translocation, induces a fatal myeloproliferative disease in mice. *Blood* **103**, 309–312.
- Chen J, Deangelo DJ, Kutok JL, Williams IR, Lee BH, Wadleigh M *et al.* (2004) PKC412 inhibits the zinc finger 198-fibroblast growth factor receptor 1 fusion tyrosine kinase and is active in treatment of stem cell myeloproliferative disorder. *Proc. Natl Acad. Sci. USA* **101**, 14479–14484.
- Ren M, Li X, Cowell JK (2009) Genetic fingerprinting of the development and progression of T-cell lymphoma in a murine model of atypical myeloproliferative disorder initiated by the ZNF198-fibroblast growth factor receptor-1 chimeric tyrosine kinase. *Blood* **114**, 1576–1584.
- Demiroglu A, Steer EJ, Heath C, Taylor K, Bentley M, Allen SL *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**, 3778–3783.

- 25 Singh D, Chan JM, Zoppoli P, Niola F, Sullivan R, Castano A *et al.* (2012) Transforming fusions of FGFR and TACC genes in human glioblastoma. *Science (New York, N.Y.)* **337**, 1231–1235.
- 26 Parker BC, Annala MJ, Cogdell DE, Granberg KJ, Sun Y, Ji P *et al.* (2013) The tumorigenic FGFR3-TACC3 gene fusion escapes miR-99a regulation in glioblastoma. *J. Clin. Invest.* **123**, 855–865.
- 27 Miki T, Bottaro DP, Fleming TP (1992) Determination of ligand-binding specificity by alternative splicing: two distinct growth factor receptors encoded by a single gene. *Proc. Natl Acad. Sci. USA* **89**, 246–250.
- 28 Yayon A, Zimmer Y, Shen GH, Avivi A, Yarden Y (1992) A confined variable region confers ligand specificity on fibroblast growth factor receptors: implications for the origin of the immunoglobulin fold. *EMBO J.* **11**, 1885–1890.
- 29 Zhang X, Ibrahimi OA, Olsen SK (2006) Receptor specificity of the fibroblast growth factor family The complete mammalian fgf family. *J. Biol. Chem.* **281**, 15694–15700.
- 30 Wesche J, Haglund K, Haugsten EM (2011) Fibroblast growth factors and their receptors in cancer. *Biochem. J.* **437**, 199–213.
- 31 Cha JY, Lambert QT, Reuther GW, Der CJ (2008) Involvement of fibroblast growth factor receptor 2 isoform switching in mammary oncogenesis. *Mol. Cancer Res.* **6**, 435–445.
- 32 Itoh H, Hattori Y, Sakamoto H, Ishii H, Kishi T, Sasaki H *et al.* (1994) Preferential alternative splicing in cancer generates a K-sam messenger RNA with higher transforming activity. *Cancer Res.* **54**, 3237–3241.
- 33 Drugan CS, Paterson IC, Prime SS (1998) Fibroblast growth factor receptor expression reflects cellular differentiation in human oral squamous carcinoma cell lines. *Carcinogenesis* **19**, 1153–1156.
- 34 Story MT, Hopp KA, Molter M, Meier DA (1994) Characteristics of FGF-receptors expressed by stromal and epithelial cells cultured from normal and hyperplastic prostates. *Growth Factors* **10**, 269–280.
- 35 Wilson SE, Weng J, Chwang EL, Gollahon L, Leitch AM, Shay JW (1994) Hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), and their receptors in human breast cells and tissues: alternative receptors. *Cell. Mol. Biol. Res.* **40**, 337–350.
- 36 Greenman C, Stephens P, Smith R, Dalgleish GL (2007) Patterns of somatic mutation in human cancer genomes. *Nature* **446**, 153–158.
- 37 Davies H, Hunter C, Smith R, Stephens P, Greenman C (2005) Somatic mutations of the protein kinase gene family in human lung cancer. *Cancer Res.* **65**, 7591–7595.
- 38 Ruhe JE, Streit S, Hart S, Wong CH, Specht K (2007) Genetic alterations in the tyrosine kinase transcriptome of human cancer cell lines. *Cancer Res.* **67**, 11368–11376.
- 39 Tomlinson DC, Hurst CD, Knowles MA (2007) Knockdown by shRNA identifies S249C mutant FGFR3 as a potential therapeutic target in bladder cancer. *Oncogene* **26**, 5889–5899.
- 40 van Rhijn BW, van Tilborg AA (2002) Novel fibroblast growth factor receptor 3 (FGFR3) mutations in bladder cancer previously identified in non-lethal skeletal disorders. *Eur. J. Hum. Genet.* **10**, 819–824.
- 41 van Rhijn BW, Montironi R, Zwarthoff EC (2002) Frequent FGFR3 mutations in urothelial papilloma. *J. Pathol.* **198**, 245–251.
- 42 Stephens P, Edkins S, Davies H, Greenman C, Cox C (2005) A screen of the complete protein kinase gene family identifies diverse patterns of somatic mutations in human breast cancer. *Nat. Genet.* **37**, 590–592.
- 43 Jang JH, Shin KH, Park JG (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**, 3541–3543.
- 44 Hernández S, de Muga S, Agell L, Juanpere N (2009) FGFR3 mutations in prostate cancer: association with low-grade tumors. *Mod. Pathol.* **22**, 848–856.
- 45 Gartside MG, Chen H, Ibrahimi OA, Byron SA (2009) Loss-of-function fibroblast growth factor receptor-2 mutations in melanoma. *Mol. Cancer Res.* **7**, 41–54.
- 46 Zhang Y, Hiraishi Y, Wang H, Sumi KS, Hayashido Y, Toratani S *et al.* (2005) Constitutive activating mutation of the FGFR3b in oral squamous cell carcinomas. *Int. J. Cancer* **117**, 166–168.
- 47 Stadler CR, Knyazev P, Bange J, Ullrich A (2006) FGFR4 GLY388 isotype suppresses motility of MDA-MB-231 breast cancer cells by EDG-2 gene repression. *Cell. Signal.* **18**, 783–794.
- 48 Sugiyama N, Varjosalo M, Meller P (2010) FGF receptor-4 (FGFR4) polymorphism acts as an activity switch of a membrane type 1 matrix metalloproteinase–FGFR4 complex. *Proc. Natl Acad. Sci. USA* **107**, 15786–15791.
- 49 Heinzle C, Gsur A, Hunjadi M, Erdem Z, Gauglhofer C (2012) Differential effects of polymorphic alleles of FGF receptor 4 on colon cancer growth and metastasis. *Cancer Res.* **72**, 5667–5777.
- 50 Dutra RL, de Carvalho MB, Dos SM, Mercante AM, Gazito D, de Cicco R *et al.* (2012) FGFR4 profile as a prognostic marker in squamous cell carcinoma of the mouth and oropharynx. *PLoS ONE* **7**, e50747.
- 51 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**, 213–217.
- 52 Choi KY, Rho YS, Kwon KH, Chung EJ, Kim JH, Park IS *et al.* (2012) ECRG1 and FGFR4 single nucleotide polymorphism as predictive factors for nodal metastasis in oral squamous cell carcinoma. *Cancer Biomark* **12**, 115–124.
- 53 Tanuma J, Izumo T, Hirano M, Oyazato Y, Hori F, Umemura E *et al.* (2010) FGFR4 polymorphism, TP53 mutation, and their combinations are prognostic factors for oral squamous cell carcinoma. *Oncol. Rep.* **23**, 739–744.
- 54 Henson BJ, Gollin SM (2010) Overexpression of KLF13 and FGFR3 in oral cancer cells. *Cytogenet. Genome Res.* **128**, 192–198.
- 55 Henson BJ, Bhattacharjee S, O'Dee DM, Feingold E, Gollin SM (2009) Decreased expression of miR-125b and miR-100 in oral cancer cells contributes to malignancy. *Genes Chromosom. Cancer* **48**, 569–582.
- 56 Pai R, Dunlap D, Qing J, Mohtashemi I, Hotzel K (2008) Inhibition of fibroblast growth factor 19 reduces tumor growth by modulating β -catenin signaling. *Cancer Res.* **68**, 5086–5095.
- 57 Ishigami T, Uzawa K, Higo M, Nomura H, Saito K, Kato Y *et al.* (2007) Genes and molecular pathways related to radioresistance of oral squamous cell carcinoma cells. *Int. J. Cancer* **120**, 2262–2270.
- 58 Marne F, Werft W, Benner A, Burwinkel B, Sinn P, Sohn C *et al.* (2010) FGFR4 Arg388 genotype is associated with pathological complete response to neoadjuvant chemotherapy for primary breast cancer. *Ann. Oncol.* **21**, 1636–1642.
- 59 Thussbas C, Nahrig J, Streit S, Bange J, Kriner M, Kates R *et al.* (2006) FGFR4 Arg388 allele is associated with resistance to adjuvant therapy in primary breast cancer. *J. Clin. Oncol.* **24**, 3747–3755.
- 60 Shim HJ, Shin MH, Kim HN, Kim JH, Hwang JE, Bae WK *et al.* (2016) The Prognostic Significance of FGFR4 Gly388 Polymorphism in Esophageal Squamous Cell Carcinoma after Concurrent Chemoradiotherapy. *Cancer Res. Treat* **48**, 71–79.
- 61 Ansell A, Farnebo L, Grenman R, Roberg K, Thunell LK (2009) Polymorphism of FGFR4 in cancer development and sensitivity to cisplatin and radiation in head and neck cancer. *Oral Oncol.* **45**, 23–29.

- 62 Sato T, Oshima T, Yoshihara K (2009) Overexpression of the fibroblast growth factor receptor-1 gene correlates with liver metastasis in colorectal cancer. *Oncol. Rep.* **21**, 211–216.
- 63 Wang J, Li J, Wang X, Zheng C, Ma W (2013) Downregulation of microRNA-214 and overexpression of FGFR-1 contribute to hepatocellular carcinoma metastasis. *Biochem. Biophys. Res. Commun.* **439**, 47–53.
- 64 Göke F, Franzen A, Menon R, Goltz D (2012) Rationale for Treatment of Metastatic Squamous Cell Carcinoma of the Lung Using Fibroblast Growth Factor Receptor Inhibitors. *Chest* **142**, 1020–1026.
- 65 Brunello E, Brunelli M, Bogina G, Calìo A (2012) FGFR-1 amplification in metastatic lymph-nodal and haematogenous lobular breast carcinoma. *J. Exp. Clin. Cancer Res.* **31**, 103.
- 66 Preusser M, Berghoff AS, Berger W, Ilhan-Mutlu A (2014) High rate of FGFR1 amplifications in brain metastases of squamous and non-squamous lung cancer. *Lung Cancer* **83**, 83–89.
- 67 Kamura S, Matsumoto Y, Fukushi JJ, Fujiwara T (2010) Basic fibroblast growth factor in the bone microenvironment enhances cell motility and invasion of Ewing's sarcoma family of tumours by activating the FGFR1-PI3K-Rac1 pathway. *Br. J. Cancer* **103**, 370–381.
- 68 Rachagani S, Macha MA, Ponnusamy MP (2012) MUC4 potentiates invasion and metastasis of pancreatic cancer cells through stabilization of fibroblast growth factor receptor 1. *Carcinogenesis* **33**, 1953–1964.
- 69 Yang F, Zhang Y, Ressler SJ, Ittmann MM, Ayala GE (2013) FGFR1 is essential for prostate cancer progression and metastasis. *Cancer Res.* **73**, 3716–3724.
- 70 Hase T, Kawashiri S, Tanaka A, Nozaki S, Noguchi N, Kato K *et al.* (2006) Correlation of basic fibroblast growth factor expression with the invasion and the prognosis of oral squamous cell carcinoma. *J. Oral Pathol. Med.* **35**, 136–139.
- 71 Kramer RH, Vogel KG, Nicolson GL (1982) Solubilization and degradation of subendothelial matrix glycoproteins and proteoglycans by metastatic tumor cells. *J. Biol. Chem.* **257**, 2678–2686.
- 72 Uchida D, Kawamata H, Omotehara F, Ki N, Kimura-Yanagawa T, Hino S *et al.* (2001) Role of HGF/c-met system in invasion and metastasis of oral squamous cell carcinoma cells in vitro and its clinical significance. *Int. J. Cancer* **93**, 489–496.
- 73 Lim SM, Kim HR, Shim HS, Soo RA, Cho BC (2013) Role of FGF receptors as an emerging therapeutic target in lung squamous cell carcinoma. *Future Oncol.* **9**, 377–386.
- 74 Lee SH, de Menezes DL, Vora J, Harris A, Ye H, Nordahl L *et al.* (2005) In vivo target modulation and biological activity of CHIR-258, a multitargeted growth factor receptor kinase inhibitor, in colon cancer models. *Clin. Cancer Res.* **11**, 3633–3641.
- 75 Wedge SR, Kendrew J, Hennequin LF, Valentine PJ (2005) AZD2171: a highly potent, orally bioavailable, vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for the treatment of cancer. *Cancer Res.* **65**, 4389–4400.
- 76 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**, 3051–3057.
- 77 Jonker DJ, Rosen LS, Sawyer MB, de Braud F, Wilding G, Sweeney CJ *et al.* (2011) A phase I study to determine the safety, pharmacokinetics and pharmacodynamics of a dual VEGFR and FGFR inhibitor, brivanib, in patients with advanced or metastatic solid tumors. *Ann. Oncol.* **22**, 1413–1419.
- 78 Matsui J, Yamamoto Y, Funahashi Y, Tsuruoka A, Watanabe T, Wakabayashi T *et al.* (2008) E7080, a novel inhibitor that targets multiple kinases, has potent antitumor activities against stem cell factor producing human small cell lung cancer H146, based on angiogenesis inhibition. *Int. J. Cancer* **122**, 664–671.
- 79 Gavine PR, Mooney L, Kilgour E, Thomas AP (2012) AZD4547: an orally bioavailable, potent, and selective inhibitor of the fibroblast growth factor receptor tyrosine kinase family. *Cancer Res.* **72**, 2044–2056.
- 80 Guagnano V, Furet P, Spanka C, Bordas V, Le DM, Stamm C *et al.* (2011) Discovery of 3-(2,6-dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea (NVP-BGJ398), a potent and selective inhibitor of the fibroblast growth factor receptor family of receptor tyrosine kinase. *J. Med. Chem.* **54**, 7066–7083.
- 81 Brooks AN, Kilgour E, Smith PD (2012) Molecular pathways: fibroblast growth factor signaling: a new therapeutic opportunity in cancer. *Clin. Cancer Res.* **18**, 1854–1862.
- 82 Bono F, De Smet F, Herbert C, De Bock K, Georgiadou M, Fons P *et al.* (2013) Inhibition of tumor angiogenesis and growth by a small-molecule multi-FGF receptor blocker with allosteric properties. *Cancer Cell* **23**, 477–488.
- 83 Lonial S, Niesvizky R, McCulloch L, Rajangam K, Vij R. (2012) Cardiac and pulmonary safety profile of single-agent carfilzomib from four phase 2 studies in patients with relapsed and/or refractory multiple myeloma. *Blood* **120**, 4037.
- 84 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**, 1216–1229.
- 85 Zhao WM, Wang L, Park H, Chhim S, Tanphanich M, Yashiro M *et al.* (2010) Monoclonal antibodies to fibroblast growth factor receptor 2 effectively inhibit growth of gastric tumor xenografts. *Clin. Cancer Res.* **16**, 5750–5758.
- 86 Bai A, Meetze K, Vo NY, Kollipara S, Mazsa EK, Winston WM *et al.* (2010) GP369, an FGFR2-IIIb-specific antibody, exhibits potent antitumor activity against human cancers driven by activated FGFR2 signaling. *Cancer Res.* **70**, 7630–7639.
- 87 Bumbaca D, Wong A, Drake E, Reyes AE 2nd, Lin BC, Stephan JP *et al.* (2011) Highly specific off-target binding identified and eliminated during the humanization of an antibody against FGF receptor 4. *MAbs* **3**, 376–386.