Review

Cancer Research

# Potential for Targeting the Fibroblast Growth Factor Receptors in Breast Cancer

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## Abstract

Breast cancer is the most common cancer of women, accounting yearly for approximately 30% of newly diagnosed cases and ranking second as a cause of death. Despite improvements in breast cancer detection and development of new therapeutic approaches, there are still tumors for which no targeted therapies are available. This review summarizes recent findings on the fibroblast growth factor receptors (FGFR) and the data supporting their role in breast cancer. We will describe the approaches being made to develop therapeutics targeting these receptors. Finally, to improve the chances for success with FGFR signal transduction inhibitors, strategies to choose appropriate breast cancer patients for treatment will be discussed. *Cancer Res; 70(13); 5199–202. ©2010 AACR.*

Breast cancer is a heterogeneous disease, from the cellular morphology to the array of expressed genes in individual tumors. Despite this heterogeneity, there are specific genetic alterations that are found in a relatively high percentage of breast cancers, such as the *ERBB2* amplicon on chromosome 17q21 present in approximately 25% of primary tumors. As a transmembrane receptor with kinase activity, ErbB2 has proved to be an excellent target for cancer therapy. Various types of signal transduction inhibitors, including monoclonal antibodies and tyrosine kinase inhibitors (TKI), are used to treat patients whose tumors possess the *ERBB2* amplicon and overexpress the receptor (reviewed in ref. 1). Currently much effort is going into targeting additional genetic alterations driving breast cancer. Here, we will discuss the fibroblast growth factor/fibroblast growth factor receptor (FGF/FGFR) network, whose members have multiple, essential developmental roles and have also been implicated in different types of human tumors, including breast cancer.

In mammals, there are four *FGFRs* that encode transmembrane receptors with tyrosine kinase activity. Furthermore, alternative splicing of FGFR 1, 2, and 3 in the third Ig-like loop domain gives rise to the IIIb and IIIc isoforms, which are expressed in epithelial or mesenchymal compartments, respectively. There are 22 FGF ligands, 18 of which that bind specific FGFR isoforms, inducing receptor dimerization, kinase activation, and autophosphorylation of intracellular tyrosine residues (reviewed in ref. 2). In addition to phosphorylation of tyrosine residues on the receptors and on PLCγ, the adaptor protein FRS2 that links FGFRs to the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways, is heavily phosphorylated in response to receptor activation. Other effector proteins including STAT transcription factors and Src are also activated by FGFRs (Fig. 1, center panel; reviewed in ref. 3).

Different types of FGF/FGFR alterations, including abnormal expression levels, single nucleotide polymorphisms (SNP), mutations, and amplifications, have been described in cancer (reviewed in ref. 4). The association between aberrant FGF/FGFR expression and mammary cancer was established more than 20 years ago in mouse mammary tumor virus (MMTV)–induced tumors, in which proviral insertional mutagenesis induced FGF3 transcriptional activation. Indeed, in >65% of mammary tumors, MMTV proviruses transcriptionally activate different members of the FGF/FGFR network (e.g., Fgf4, Fgf6, Fgf8, Fgf10, and Fgfr2; refs. 5–7). In this review we will concentrate on breast cancer, presenting the evidence for FGF/FGFR involvement in human disease and discussing current approaches that might be used for targeting oncogenic FGFRs in breast cancer.

Large-scale analyses of human cancer genomes have revealed that FGFRs are often amplified or mutated (8). In breast cancer, different types of genetic alterations in FGF/FGFR have been described, with amplification the most common and activating mutations being rarer. The 8p11-12 amplicon harboring *FGFR1* has been detected in 8 to 10% of breast cancers (9). This is a complex amplicon with at least two cores, which had led to variation in reports of FGFR1 overexpression (10). In two large studies, 8p11-12 amplification was shown to correlate with poor outcome (9, 10), and, in a recent study, high FGFR1 levels in the estrogen receptor (ER)–positive, highly proliferative luminal B subtype, correlated with tamoxifen resistance (11). *FGFR2* amplification (10q26) and overexpression have also been described in a small subgroup (6 of 165 = 4%) of triple-negative breast tumors [negative for ER, for progesterone receptor (PR), and for high ErbB2]. Interestingly, no case of *FGFR2* amplification was found in >200 tumors that were not triple negative (12).

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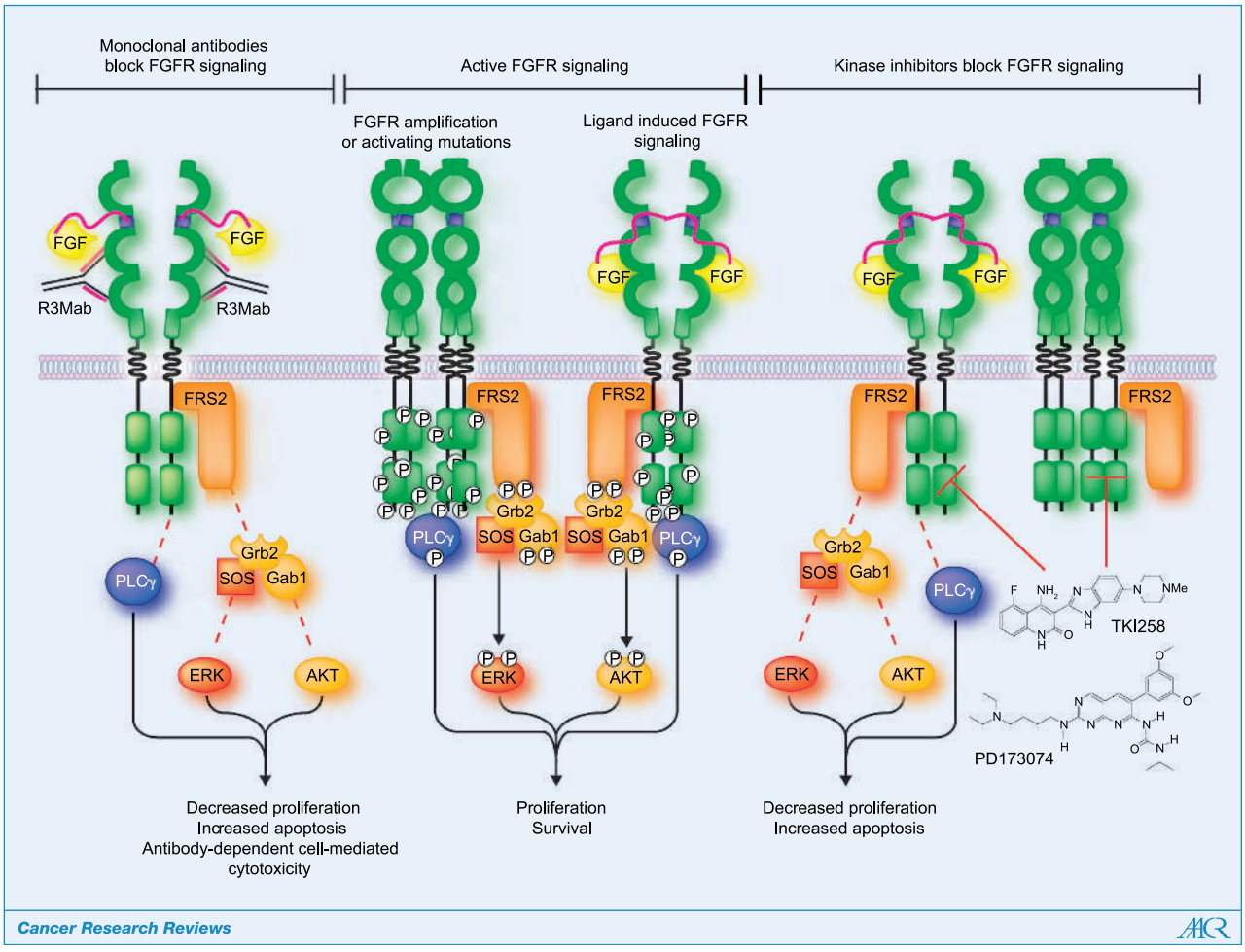


**Figure 1.** Center panel, under normal conditions, heparin (pink)-bound FGF mediates FGFR dimerization, leading to kinase activation and phosphorylation of Tyr residues in the kinase domain and the C-terminal tail; PLCγ associates with the latter, leading to its phosphorylation and activation. The major FGFR effector, the adaptor protein FRS2, is constitutively associated with the receptor and following activation becomes phosphorylated on Tyr and Ser/Thr residues. Phosphorylated FRS2 acts as a docking site for Grb2 that then activates extracellular signal-regulated kinase (ERK) and AKT via SOS and Gab1, respectively. These pathways in turn regulate cell proliferation and cell survival. In cancer, activating mutations or gene amplification, common causes of deregulated FGFR signaling, lead to constitutive activation of downstream signaling pathways and aberrant cell proliferation and increased cell survival. Left panel, FGFR-specific monoclonal antibodies bind the extracellular domain of the receptor and inhibit FGFR signaling, causing changes in tumor cell proliferation and survival. In the example shown, the R3MAb binds FGFR3 and decreases the signaling potential of autocrine-activated as well as mutant receptors (33). Right panel, treatment of tumor cells with TKIs such as PD173074 or TKI258 blocks ligand-induced FGFR activity and constitutive FGFR signaling from mutated or amplified receptors. FRS2 Tyr phosphorylation decreases, causing an uncoupling of Grb2 from the adaptor protein and a decrease in ERK and AKT activity. Both antibodies and TKIs have effects on tumor cells, e.g., decreasing proliferation and stimulating apoptosis. Unlike TKIs, antibodies also have the potential to recruit immune effector cells to the tumor via their ability to bind of the Fcγ receptors, which can lead to antibody-dependent cellular cytotoxicity.

Activating mutations in *FGFR3* have been found in many types of human tumors; however, in breast cancer these seem to be very rare. There is one report on a Saethre-Chotzen syndrome patient with breast cancer whose tumor carries a gain-of-function *FGFR3* mutation (P250R; ref. 10). Whether additional breast cancer patients with activating mutations in FGFR3 will be uncovered, or if this mutation had a causal role in the disease, remains to be studied. Regarding FGFR4, an activating mutation (Y367C) that causes constitutive receptor dimerization was discovered in the MDA-MB453 breast tumor cell line (13). The mutation is in a homologous region to mutations in FGFR2 and FGFR3, which are associated with human diseases. Currently, activating FGFR4 mutations do not seem to be common in primary breast tumors.

SNPs associated with breast cancer risk and with breast cancer severity have also been described. Genome-wide screens aimed at uncovering breast cancer-associated genes identified SNPs in intron 2 of *FGFR2* (14, 15). These SNPs were shown to predispose selectively for ER-positive cancer (15) and, *in vitro*, to alter Oct-1/Runx2 binding, leading to an increase in FGFR2 transcription (16). FGFR2 was also shown to be transcriptionally activated in some MMTV-induced mammary tumors (7), and in human tumors there is evidence

that FGFR2 ligands are abnormally expressed. Indeed, amplification of *FGF3* (11q13) occurs in 15% of breast tumors and was shown to correlate with increased aggressiveness in node-negative breast carcinoma (17). Taken together, these data suggest that inappropriate expression or activation of FGFR2, which has an important role in normal development of the gland (18), might predispose to breast cancer.

A SNP in *FGFR4*, which converts Gly to Arg at codon 388 in the transmembrane region of the receptor, has been implicated in poor prognosis of various types of human tumors (mentioned in ref. 19). In some breast cancer cohorts, the *FGFR4Arg388* allele has been correlated with poor response to chemotherapy (20) and endocrine therapy (21). Interestingly, MDA-MB-453 breast tumor cell clones resistant to the DNA-damaging agents doxorubicin or cyclophosphamide showed upregulation of FGFR4 (22). The significance of this SNP has been tested by introducing the *FGFR4Arg388* allele into a transgenic mammary tumor model in which it was shown to cause a more rapid tumor development and tumor progression compared with the *FGFR4Gly388* allele (19). Although the molecular changes underlying the ability of the *FGFR4Arg388* allele to promote tumor progression have not been molecularly clarified, evidence for enhanced receptor kinase activity as well as increased FGFR4 protein stability have been presented (19). The latter activation mechanism has also been reported for other FGFRs. Using a mammary epithelial 3-D cell-culture model, it was shown that active FGFR1 is more stable and promotes prolonged signaling in comparison to FGFR2 (23).

From this brief discussion it should be clear that there is good evidence supporting distinct roles for specific FGF/FGFR family members in breast cancer. The next step will be to use *in vitro* and *in vivo* preclinical tumor models to examine the potential of blocking FGF/FGFR activity. The most advanced approaches for targeting the network are the small molecule TKIs and blocking antibodies for specific receptors or ligands (Fig. 1, left and right panels; reviewed in 4). Because of space constraints, we will concentrate on TKIs that have been tested in breast cancer models. The important points to be considered are: FGFR inhibitor activity, how to choose appropriate breast cancer patients for treatment, and potential side-effects of blocking FGFRs.

There are a number of small molecule TKIs that block FGFR activity and are either used for *in vitro* experimental work or are in various stages of clinical development (for a complete list see ref. 4). Most of the published data on FGFR inhibitors have been done with inhibitors referred to as multitargeted because they block not only the FGFRs (class V family), but also class III [platelet-derived growth factor receptor (PDGFR) family] and IV [vascular endothelial growth factor receptor (VEGFR) family] receptor tyrosine kinases (RTK) at similar low nmol/L concentrations (Fig. 1, right panel; ref. 24), e.g., PD173074, TKI168, or BMS-582664 (Brivanib; ref. 25). For TKI168, a phase I, first-in-human study in patients with advanced solid cancers, including breast, showed that 2 of 35 treated patients had a partial response (26). There was some dose-limiting toxicity reported, although overall the inhibitor was well tolerated (26). The activity as well as the side effects of the multitargeted inhibitors might reflect blockade of FGFR as well as the inhibition of the other classes of RTKs that they affect. Newer, more selective FGFR inhibitors are being developed; however, no data on efficacy or toxicity are available. Although it is only possible to speculate on possible side-effects, the importance of the metabolic FGFs (FGF19, 21, and 23) in many physiological functions (reviewed in ref. 27) must be considered when blocking FGFR activity.

What is known on the activity of the FGFR TKIs in preclinical models? Data generated with breast tumor cell lines displaying *FGFR1* (11) or *FGFR2* (12, 28) amplification showed that they were very sensitive to treatment with the multitargeted inhibitor PD173074. MDA-MB453 breast tumor cells with the activating FGFR4 Y367C mutation (13) are also very sensitive to PD173074, showing a strong proliferative block following treatment with the inhibitor (28). Moreover, a breast cancer model showing autocrine ligand-mediated FGFR activity was very responsive to TKI168 both *in vitro* and *in vivo* (29). Despite these good responses, it has become increasingly clear that clinical management of breast cancer will require rational combinations of signal transduction inhibitors (30). Regarding this, it was shown that FGFR1-driven breast cancer models were very sensitive to a small-molecule ribosomal S6 kinase (RSK) inhibitor (31), suggesting that this kinase could be targeted together with FGFR. Overexpression and activation of FGFR and ErbB2 has also been observed in a breast cancer model and combined inhibition of both RTKs had stronger antitumor activity than individual treatments (32). With increasing knowledge of essential effectors downstream of FGFRs, it is likely that other appropriate combinations will emerge and potentially prolong the appearance of FGFR inhibitor resistance.

In considering choice of patients to be treated with FGFR inhibitors, tumor-specific gene amplification and protein overexpression are generally good indicators for sensitivity to a targeted inhibitor. ErbB2 is a good example because patients whose tumors do not show the 17q21 amplicon generally do not respond to an ErbB2-targeted therapy. Thus, screening for amplification of *FGFR1* and *FGFR2*, which is rarer at 0.5 to 1%, should help in choosing patients for FGFR inhibitor treatment. The *FGFR* amplicons can be screened for via standard FISH procedures; however, it would be very important to develop diagnostic antibodies that can be used to choose those patients that do overexpress these receptors, in particular because the *FGFR1* amplicon is so complex (10). It has recently been shown that ER-positive tumors with the *FGFR1* amplicon tend to be PR negative, which reflects FGFR-mediated transcriptional repression of PR expression (11). Thus, high FGFR1 levels and ER positivity accompanied by PR negativity could be a characteristic of breast tumors with active FGFR1 signaling and might be useful in choosing patients for treatment. In breast cancer cell lines, it has been reported that increased levels of FGFR4 are found in cells resistant to chemotherapeutics (22). When treated with doxorubicin plus an FGFR4-blocking antibody, the cells responded showing increased cell death when the receptor was blocked (22). Whether elevated FGFR4 levels can be used to predict

response to TKIs in combination with chemotherapeutics remains to be tested in the clinical setting. Finally, FRS2 tyrosine phosphorylation is another biomarker that might also be considered as a reporter for activity of any of the FGFRs. It might be useful to test for an association between FGFR amplification or activation together with FRS2 phosphorylation. In conclusion, although there are still some hurdles to be overcome, there is enough good evidence suggesting that targeting FGFRs in certain subtypes of breast cancer would be a valuable approach in the future.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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