Fibroblast growth factor receptors in breast cancer: expression, downstream effects, and possible drug targets

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Abstract

Cancer treatments are increasingly focusing on the molecular mechanisms underlying the oncogenic processes present in tumors of individual patients. Fibroblast growth factor receptors (FGFRs) are among the many molecules that are involved in oncogenesis and are currently under investigation for their potential as drug targets in breast cancer patients. These receptor tyrosine kinases play a role in several processes including proliferation, angiogenesis, and migration. Alterations in these basal processes can contribute to the development and progression of tumors. Among breast cancer patients, several subgroups have been shown to harbor genetic aberrations in FGFRs, including amplifications of *FGFR1*, *FGFR2*, and *FGFR4* and mutations in *FGFR2* and *FGFR4*. Here, we review *in vitro* and *in vivo* models that have partly elucidated the molecular implications of these different genetic aberrations, the resulting tumor characteristics, and the potential of FGFRs as therapeutic targets for breast cancer treatment.

Endocrine-Related Cancer (2012) 19 R115–R129

Introduction

Breast cancer is the most commonly occurring cancer in women with 1.4 million new cases diagnosed worldwide annually (Jemal *et al.* 2010). Currently, breast surgery and irradiation are the local therapies of choice, and chemohormonal and antihuman epidermal growth factor receptor 2 (HER2 (ERBB2)) therapies are commonly used as a systemic treatment to prevent outgrowth of distant metastases (Prosnitz *et al.* 2001). Progress in local and systemic treatment has clearly improved the prognosis of breast cancer patients, but still 458 503 women died from the disease in 2008 (Ferlay *et al.* 2008).

Therefore, the development of new therapies is focused on the specific genetic abnormalities in individual cancers, resulting in more personalized treatment (Bild *et al.* 2006, Alvarez *et al.* 2010). In order to develop these personalized therapies, it is important to fully understand the molecular basis of the oncogenic pathways that can be targeted in breast cancer.

Receptor tyrosine kinases (RTKs) regulate cell proliferation, differentiation, and apoptosis: processes

that are often deregulated in cancer. Several RTKs, including epidermal growth factor receptor (EGFR), HER2, and platelet-derived growth factor receptor (PDGFR (PDGFRB)), have been extensively studied leading to the development of targeted inhibitors, such as trastuzumab and imatinib, that have been successfully used to treat cancer patients overexpressing *HER2* and *PDGFR* respectively (Arteaga 2003, Mukai 2010, Peterson 2011).

This review focuses on the fibroblast growth factor receptor (FGFR) family of RTKs. FGFR signaling cascades, the genetic aberrations in FGFRs, and their correlations with other genotypic and phenotypic features found in breast cancer will be summarized. Finally, therapies currently under development as well as new possibilities to target the FGFR pathway will be discussed. Articles were retrieved through PubMed using the search items ‘breast’ or ‘mammary’ and ‘FGFR’. In addition, the references of the retrieved papers were screened for additional papers. Only papers in English were further considered.

FGFRs: expression and signaling

The FGFR family comprises four members, *FGFR1*, -2, -3, and -4 (Turner & Grose 2010), located on chromosomes 8p12, 10q26, 4p16.3, and 5q35.1-qter respectively. All FGFRs contain an extracellular ligand-binding domain, a transmembrane domain, and an intracellular tyrosine kinase (TK) domain (Eswarakumar et al. 2005). The extracellular domain has three immunoglobulin (Ig)-like domains (IgI–III, Fig. 1). The second and third Ig domains are responsible for binding the FGF ligand (Beenken & Mohammadi 2009). The FGF family of secreted glycoproteins consists of 18 members. The different FGFs and their corresponding receptors are expressed in a tissue-specific way. This tissue-specific expression pattern and the differences in binding affinity

contribute to the specificity of the ligand–receptor interaction (Eswarakumar et al. 2005, Turner & Grose 2010). Furthermore, this specificity is also regulated by splicing. *FGFR1*, -2, and -3 have two splice variants of the IgIII domain, resulting in IIIb and IIIc isoforms. The IIIb isoform is present on the epithelial cells and IIIc is expressed by mesenchymal cells (Beenken & Mohammadi 2009).

FGFs are released from the extracellular matrix (ECM) by heparins, proteases, or specific FGF-binding proteins. FGFs bind FGFRs and form a stable structure together with heparin sulfate proteoglycans on the cell surface (Harmer et al. 2004, Mohammadi et al. 2005, Turner & Grose 2010). Klotho family proteins also facilitate the FGF–FGFR interaction (Wu et al. 1991, Kurosu et al. 2006) by binding to FGFRs and increasing the affinity of FGF for its receptor, thus

Figure 1 FGF–FGFR structure and downstream signaling. The extracellular domain of FGFRs consists of three ligand-binding Ig domains. Binding of the ligand is stabilized by HSPG and Klotho. Intracellular tyrosine kinase domains (TK) are present. Upon ligand binding, the receptors dimerize, resulting in cross-phosphorylation of the tyrosine kinase domains. This leads to binding of several docking proteins, which can also be phosphorylated. Downstream signaling occurs through four main pathways: PLC γ , STATs, AKT, and MAPK. FGF, fibroblast growth factor; HSPG, heparin sulfate proteoglycans; TK, tyrosine kinase domain; STAT, signal transducer and activator of transcription; FRS2 α , fibroblast growth factor receptor substrate 2 α ; GRB2, growth factor receptor-bound protein 2; GAB1, GRB2-associated binding protein 1; PI3K, phosphoinositide-3 kinase; AKT, v-akt murine thymoma viral oncogene; SOS, son of sevenless; RAS, rat sarcoma; MAPK, mitogen-activated protein kinase; PLC γ , phospholipase C γ ; PIP2, phosphatidylinositol 4,5-bisphosphate; IP3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C.

resulting in increased receptor activation (Kurosu *et al.* 2006). Each FGFR isoform can bind multiple FGF ligands and some ligands are common to several receptors (Eswarakumar *et al.* 2005).

Ligand binding results in FGFR dimerization and subsequent activation of the intracellular kinase domain, leading to cross-phosphorylation of tyrosine residues present on the intracellular tail of the receptor (Turner & Grose 2010). Several docking proteins are able to bind to these phosphorylated residues, resulting in their phosphorylation and subsequent activation. Major pathways downstream of activated FGFRs include the rat sarcoma mitogen-activated protein kinase (RAS–MAPK) pathway and the phosphoinositide-3 kinase ν -akt murine thymoma viral oncogene (PI3K (PIK3CA)–AKT (AKT1)) pathway whose activation is mediated via FGFR substrate 2 α (FRS2 α (FRS2)) and several other adaptor molecules. Furthermore, protein kinase C is activated via phospholipase C γ (PLC γ (PLCG)), and finally activation of signal transducer and activator of transcription (STAT) signaling can take place (Acevedo *et al.* 2009, Turner & Grose 2010; Fig. 1).

All these pathways play an important role in cell proliferation, differentiation, inhibition of apoptosis, and migration. In addition, FGFR signaling is involved in angiogenesis and wound repair (Eswarakumar *et al.* 2005, Turner & Grose 2010). Downstream effector activation is cell type specific and may be dependent on cross talk with other signaling pathways (Dailey *et al.* 2005). Although different FGFRs signal via similar pathways, differences in downstream effector activation have been described. Activation of downstream targets by FGFR4 is less strong than FGFR1 (Vainikka *et al.* 1994), and FGFR1 signaling sustains longer than FGFR2 because of faster degradation of FGFR2 after activation (Xian *et al.* 2007). Finally, even though FGFR isoforms have some specificity for

different FGF ligands (Eswarakumar *et al.* 2005), no conclusive evidence has been found that specific FGFs activate specific downstream pathways (Dailey *et al.* 2005).

Aberrations in FGFRs found in breast cancer

Deregulation of FGFR activity could contribute to cancer development by increasing cell proliferation, angiogenesis, and inhibiting apoptosis. Genetic aberrations in FGFRs have indeed been found to be associated with breast cancer and are described below.

FGFR1

No germ-line mutations in *FGFR1* have been identified to date. However, a somatic mutation (*K566R*) was found in a sample from a patient with basal-like, triple-negative breast cancer (Kan *et al.* 2010). Whether this mutation is present in other breast cancer patients remains to be investigated. Furthermore, a *S125L* mutation was described in a ductal breast carcinoma cell line (HCC1395; Stephens *et al.* 2005, Greenman *et al.* 2007). However, whether these mutations have consequences for FGFR1 function and its role in breast cancer development and/or progression have not been investigated.

Several studies have identified amplifications of *FGFR1* in breast cancer (Chin *et al.* 2006, Letessier *et al.* 2006, Reis-Filho *et al.* 2006, Elbauomy Elsheikh *et al.* 2007, Marchio *et al.* 2008, Andre *et al.* 2009, Kadota *et al.* 2009, Moelans *et al.* 2010; Table 1). The percentage of *FGFR1* amplifications found using different techniques ranges from 7.5 to 17%. This variability could be caused by differences between tumor samples, which were not selected for histopathological type or clinical parameters. Furthermore, each study used a different technique to assess the copy number alterations, which can also account for the

Table 1 Copy number alterations in *FGFR1* identified in breast cancer patients

Breast cancer cases (n)	Technique, whole genome/gene specific	Results: <i>FGFR1</i> amplification/loss	Reference
106	aCGH: 70 kb interval, whole genome	Highly amplified in 10%	Andre <i>et al.</i> (2009)
161	SNP DNA microarray, whole genome	Amplified in 7.5%	Kadota <i>et al.</i> (2009)
1319	FISH, gene specific	Amplified in 9.4%	Letessier <i>et al.</i> (2006)
496	CISH, gene specific	Amplified in 8.7%	Elbauomy Elsheikh <i>et al.</i> (2007)
104	MLPA, gene specific	Amplified in 17% (7%: highly amplified) Lost in 10%	Moelans <i>et al.</i> (2010)
24	CISH, gene specific	Amplified in 16.6%	Marchio <i>et al.</i> (2008)

aCGH, array comparative genome hybridization; FISH, fluorescent *in situ* hybridization; CISH, chromogenic *in situ* hybridization; MLPA, multiplex ligation-dependent probe amplification.

discrepancies in the results. Microarray expression analyses showed that *FGFR1* amplifications were associated with increased *FGFR1* RNA expression (Chin et al. 2006, Andre et al. 2009).

Several studies have described that *FGFR1* amplifications were not associated with some pathological characteristics (Table 2). Associations with proliferation and HER2 status are inconsistent between studies, possibly due to differences in techniques. Proliferation was estimated by assessing the percentage of Ki-67-positive cells (Letessier et al. 2006) or the mitotic activity index (Moelans et al. 2010), and *HER2* expression was also evaluated in different ways (Letessier et al. 2006, Elbauomy Elsheikh et al. 2007, Kadota et al. 2009, Moelans et al. 2010). Even though

estrogen receptor (ER) and progesterone receptor (PR) statuses were assessed in the same way in the different studies, the correlations with *FGFR1* amplification were inconsistent. Confounder effects by clinical or pathological parameters could provide an explanation for the differences in association between expression of these hormone receptors and *FGFR1* amplification.

In addition to pathological features, association analysis of *FGFR1* amplification with clinical outcome has also been performed (Table 2). Patients with an *FGFR1* amplification were more likely to develop distant metastases (Elbauomy Elsheikh et al. 2007). In addition, a trend for *FGFR1* amplification and a shorter disease-free survival (DFS) was observed. Finally, amplification of *FGFR1* was significantly

Table 2 Association of *FGFR1* amplification in unselected cohorts with clinical and pathological parameters

Parameter	Association: yes/no	Reference
Development of distant metastasis	Yes: positive association	Elbauomy Elsheikh et al. (2007)
Grade	No	Letessier et al. (2006), Elbauomy Elsheikh et al. (2007), and Moelans et al. (2010)
Tumor size	No	Letessier et al. (2006), Elbauomy Elsheikh et al. (2007), Kadota et al. (2009), and Moelans et al. (2010)
Histopathological type	No	Letessier et al. (2006), Kadota et al. (2009), and Moelans et al. (2010)
Lymph node invasion	No	Letessier et al. (2006), Elbauomy Elsheikh et al. (2007), and Kadota et al. (2009)
Vascular invasion	No	Letessier et al. (2006) and Moelans et al. (2010)
Tumor stage	No	Elbauomy Elsheikh et al. (2007) and Kadota et al. (2009)
Molecular subtypes (basal-like, luminal A/B, ERBB2-positive, normal-like)	No	Letessier et al. (2006) and Moelans et al. (2010)
P53 status	No	Letessier et al. (2006)
EGFR status	No	Elbauomy Elsheikh et al. (2007)
Expression of low- and high-molecular weight cytokeratins	No	Elbauomy Elsheikh et al. (2007)
Androgen receptor status	No	Elbauomy Elsheikh et al. (2007)
Proliferation	No	Moelans et al. (2010)
ER status: positive or negative	Yes: positive association No	Letessier et al. (2006) Letessier et al. (2006), Elbauomy Elsheikh et al. (2007), and Kadota et al. (2009)
PR status: positive or negative	Yes: associated with a positive status No	Moelans et al. (2010) Letessier et al. (2006), Kadota et al. (2009), and Moelans et al. (2010)
Age (<50/≥50)	Yes: trend with a negative status No	Elbauomy Elsheikh et al. (2007) Letessier et al. (2006) and Moelans et al. (2010)
HER2 status	Yes: if amplified, more likely to be older than 50 No	Elbauomy Elsheikh et al. (2007)
	Yes: inverse correlation with Her2 overexpression	Letessier et al. (2006) and Moelans et al. (2010) Elbauomy Elsheikh et al. (2007)
	Yes: amplification was positively associated with Her2+ status	Kadota et al. (2009)

EGFR, epidermal growth factor receptor; ER, estrogen receptor; PR, progesterone receptor.

associated with a shorter overall survival (OS), independent of other prognosticators such as grade, tumor size, lymph node invasion, and ER status. Interestingly, *FGFR1* amplification remained a significant independent risk factor for poor DFS and OS in ER-positive but not in ER-negative cases (Elbauomy Elsheikh *et al.* 2007). This suggests that there may be an interaction between *FGFR1* and ER signaling resulting in a poor prognosis. The observation that *FGFR1* amplification is associated with a poor prognosis in ER-positive breast cancer was confirmed in a group of ER-positive tumor samples from patients treated with adjuvant endocrine therapy (tamoxifen). In this group, *FGFR1* amplification was significantly associated with decreased metastasis-free survival compared with samples with normal *FGFR1* levels (Turner *et al.* 2010b). Finally, two studies showed an association between amplification of 8p11–12 where *FGFR1* is located and poor disease outcome. Although these analyses do not specifically show *FGFR1* amplification in the breast cancer samples, they support the findings in previous studies (Chin *et al.* 2006, Letessier *et al.* 2006).

The function of *FGFR1* as an oncogene has been disputed due to the results of functional studies in cell lines carrying 8p11–12 amplifications, which showed that FGFR inhibition did not affect the proliferation (Ray *et al.* 2004). Several studies have identified other potential oncogenes located on 8p11–12, including *PPAPDC1B*, *RAB11FIP1*, *LSM1*, *BAG4*, *C8ORF4*, and *WHSCIL1* (Garcia *et al.* 2005, Gelsi-Boyer *et al.* 2005, Yang *et al.* 2006, Bernard-Pierrot *et al.* 2008). These results do not exclude the possibility of multiple genes in the 8p11–12 region acting as oncogenes, which might even cooperate.

In addition to its suggested role as an oncogene, there is some evidence that FGFR1 may have a tumor suppressor function in breast cancer. Loss of *FGFR1* was found in 10% of invasive breast cancer samples (Moelans *et al.* 2010). Another study identified a deletion of 8p11–12 in breast carcinomas, which was shown to be associated with a poor outcome (Chin *et al.* 2006). However, as this region contains many genes, it is not certain whether poor prognosis can be attributed to the specific loss of *FGFR1*.

FGFR2

So far, only one somatic mutation (*R203C*) in *FGFR2* has been identified in the breast cancer cell line HCC1143 (Stephens *et al.* 2005, Sjöblom *et al.* 2006, Greenman *et al.* 2007). However, several single nucleotide polymorphisms (SNPs) in *FGFR2* were

found to be highly associated with breast cancer risk and identified *FGFR2* as a breast cancer susceptibility gene (Hunter *et al.* 2007). Four of these SNPs are located in intron 2 of *FGFR2*. An independent study, using a different panel of SNPs, found an association of another SNP in intron 2 of *FGFR2* with breast cancer (Easton *et al.* 2007). The risk allele of this SNP was significantly associated with a positive ER and PR status and a lower grade (Garcia-Closas *et al.* 2008). Further analysis of the SNPs located in the second intron of *FGFR2* identified a haplotype block of eight SNPs as the minor disease-predisposing allele (Easton *et al.* 2007, Meyer *et al.* 2008). This haplotype block is not in linkage disequilibrium with any coding region of *FGFR2*, excluding a change in protein sequence. Also, when comparing the expression levels of the two most common splicing variations (including or excluding exon 3), no significant difference was observed between the minor and common homozygotes (Meyer *et al.* 2008). These results indicate that the change in the protein sequence due to the SNPs does not result in a functional difference.

However, when correlating the expression levels of *FGFR2* in invasive breast cancer samples with the haplotype of the eight SNPs, mRNA levels of *FGFR2* were significantly increased in tumor samples that were homozygous for the minor alleles when compared with samples homozygous for the common alleles (Meyer *et al.* 2008). This indicates that the risk genotype results in increased expression of *FGFR2*, which could contribute to the development of breast cancer.

In a genome-wide screen identifying copy number alterations, *FGFR2* was found to be amplified in only two (1.2%) out of 161 primary breast cancer samples (Kadota *et al.* 2009). Other genome-wide screens analyzing large groups of unselected breast cancer samples also showed that *FGFR2* is rarely amplified (Adelaide *et al.* 2007), and others did not find any amplifications or losses of *FGFR2* (Andre *et al.* 2009). However, *FGFR2* was amplified in 4% of triple-negative breast cancer samples, whereas no amplification was found in other molecular subtypes. In addition, the mRNA expression levels of *FGFR2* were significantly increased in amplified vs nonamplified tumor samples (Turner *et al.* 2010a), suggesting a potential role for *FGFR2* in triple-negative breast cancer. *FGFR2* is also suggested to play a role in a subgroup of familial breast cancer patients. When comparing the RNA expression profiles of breast cancer samples of patients with a germ-line mutation in breast cancer 1 and 2 (*BRCA1* and -2), *FGFR2*

expression was significantly higher in breast cancers of *BRCA2* mutation carriers (Bane et al. 2009). Finally, increased FGFR2 protein levels in samples from invasive ductal carcinoma (IDC) patients were found to be associated with lower (disease-free) survival rates (Sun et al. 2011). How FGFR2 is regulated in these and *BRCA2*-mutated patients and whether it is involved in the development or progression of breast cancer are not clear.

FGFR3

Even though activating mutations in *FGFR3* have been identified in several cancer types (Cappellen et al. 1999, Sibley et al. 2001, Zhang et al. 2005, Hafner et al. 2006), no *FGFR3* mutations have been found in breast cancer (Sibley et al. 2001, Koziczak et al. 2004, Greenman et al. 2007). *FGFR3* mutations cause Saethre–Chotzen syndrome, consisting of premature fusion of one or more skull sutures often accompanied by facial asymmetry and fusion of digits (Sahlin et al. 2009). There is one documented case of a Saethre–Chotzen patient with a heterozygous missense mutation in exon 7 of *FGFR3* (*P250R*) who also developed breast cancer. This mutation is located in the ligand-binding domain of *FGFR3*, which leads to activation of the kinase domain (Sahlin et al. 2009). Moreover, this mutation is located near mutational hot spots found in the bladder and cervical carcinomas (*R248C* and *S249C*; Sibley et al. 2001). It is unclear whether the mutation found to cause Saethre–Chotzen syndrome also increases the risk of developing breast cancer; therefore, more studies are required to further elucidate this.

A possible role for FGFR3 in therapy resistance was identified in a group of 429 ER-positive breast cancer patients who received tamoxifen as primary therapy. FGFR3 protein levels were significantly increased in patients who did not respond to the treatment (Tomlinson et al. 2012).

FGFR4

A Gly388Arg (*Arg*³⁸⁸) missense mutation, now known as SNP rs351855, was identified in the transmembrane domain of *FGFR4* (Bange et al. 2002). Immunohistochemical analysis of breast cancer samples did not show any correlation of the *Arg*³⁸⁸ SNP genotype with *FGFR4* expression levels (Thussbas et al. 2006). Sequence analysis of breast cancer samples revealed that the *Arg*³⁸⁸ genotype, either heterozygous or homozygous, was present in 37–43% and 8–11% respectively (Table 3; Bange et al. 2002, Jezequel et al. 2004, Thussbas et al. 2006, Marme et al. 2010). No significant difference could be identified when comparing the distribution of the *Arg*³⁸⁸ allele in healthy controls and breast cancer patients. This indicates that this SNP is not associated with the initiation of breast cancer (Bange et al. 2002).

*Arg*³⁸⁸ did not correlate with age, HER2, ER and PR status, and grade (Bange et al. 2002, Jezequel et al. 2004, Thussbas et al. 2006, Marme et al. 2010). Correlations were found for tumor stage (Thussbas et al. 2006, Marme et al. 2010), tumor size (Thussbas et al. 2006), and axillary lymph node involvement (Bange et al. 2002, Jezequel et al. 2004, Thussbas et al. 2006, Marme et al. 2010), but not all of these associations were confirmed by other studies (Bange et al. 2002, Jezequel et al. 2004, Thussbas et al. 2006). Finally, when analyzing the DFS period, no correlation was found with the *Arg*³⁸⁸ allele (Bange et al. 2002). However, in primary lymph node-positive patients (N+), DFS was significantly shorter in *Arg*³⁸⁸ than *Gly*³⁸⁸ carriers (Bange et al. 2002, Thussbas et al. 2006). This suggests that *Arg*³⁸⁸ could be a marker for increased tumor aggressiveness in advanced breast cancer. However, another study did not find a significant association of *Arg*³⁸⁸ with DFS in unselected N+ breast cancer cases (Jezequel et al. 2004). *Arg*³⁸⁸ was not associated with DFS in patients who did not receive adjuvant systemic therapy.

Table 3 *FGFR4 Arg*³⁸⁸ allele distribution in breast cancer patients and healthy controls and associations with clinical or pathological parameters

Cases (n)	Gly/Gly (%)	Gly/Arg (%)	Arg/Arg (%)	Correlations with Arg allele	Reference
Healthy controls (n= 123)	45	49	6		Bange et al. (2002)
145	46	43	11	Axillary lymph node involvement	Bange et al. (2002)
234	52	37	11	Axillary lymph node involvement	Jezequel et al. (2004)
372	49	43	8	Tumor stage, tumor size, axillary lymph node involvement	Thussbas et al. (2006)
352	46	43	11	Tumor stage, axillary lymph node involvement	Marme et al. (2010)

When adjuvant systemic therapy was used, DFS and OS were shorter in patients carrying one or two *Arg*³⁸⁸ alleles, making it a possible marker for therapy resistance in this patient group (Thussbas *et al.* 2006).

Interestingly, in patients who received neoadjuvant chemotherapy, the *Arg*³⁸⁸ allele was significantly and independently associated with a better clinical and pathological response (Marme *et al.* 2010).

In addition to the *Arg*³⁸⁸ germ-line mutation, a somatic mutation (*V510M*) in *FGFR4* was identified in a tumor sample from a lobular carcinoma patient (Stephens *et al.* 2005, Greenman *et al.* 2007). This mutation has not been found in other (breast) cancer patients to date, making its significance unclear.

Amplification of *FGFR4* has been reported in breast cancer samples. In a small study, 10% of breast tumors had *FGFR4* amplifications, which were associated with ER and PR positivity and lymph node metastases (Jaakkola *et al.* 1993). Furthermore, *FGFR4* mRNA levels were elevated in 32% of breast cancer samples (Penault-Llorca *et al.* 1995). In a retrospective study analyzing ER-positive breast cancers from patients treated with neoadjuvant tamoxifen, high levels of *FGFR4* were independently associated with tumor response and survival after treatment (Meijer *et al.* 2008).

Molecular implications of genetic aberrations in *FGFRs* on tumor characteristics

As described earlier, genetic aberrations in *FGFRs* have been shown to contribute to breast cancer risk, tumor progression, and response to therapy. These different outcomes can be due to the fact that *FGFRs* signal through downstream signaling pathways whose activation depends on several factors. The *in vitro* and *in vivo* studies described below give more insight into the functional effects of abnormal *FGFR* signaling.

FGFR1

Inducible activation of *Fgfr1* (*iFgfr1*) in transgenic mice resulted in increased cell proliferation in the lateral buds and ductal branching of the mammary epithelium (Welm *et al.* 2002). Furthermore, there was recruitment of macrophages, which are required for lateral bud formation and also play a role in the formation of small blood vessels (Schwertfeger *et al.* 2006) through the production of interleukin 1 β (Reed *et al.* 2009). After sustained induction of *FGFR1*, epithelial cells further proliferated, forming multicellular lesions that eventually became invasive. These invasive lesions were characterized by loss of cell

polarity, cell detachment from the basement membrane, and anoikis resistance. Finally, the invasive lesions in *iFgfr1* transgenic mice were surrounded by an increased number of highly branched small blood vessels (Welm *et al.* 2002).

The observed changes in the breast epithelium of *iFgfr1* mice were recapitulated in 3D cultures of HC11 and MCF10A cells transfected with inducible *FGFR1* (Welm *et al.* 2002, Xian *et al.* 2005, 2009). These models have shown that the induction of *Fgfr1* expression results in epithelial to mesenchymal transition.

The molecular downstream targets that are activated upon induction of *iFGFR1* include ribosomal protein S6 kinase (RSK (RPS6KA1)), the PI3K–Akt pathway, and the MAPK–ERK pathway (Welm *et al.* 2002, Xian *et al.* 2005, 2009). Target genes activated by *FGFR1* include several clusters that are linked to tumor formation through angiogenesis, cell cycle regulation, chemotaxis, and the response to inflammation (Schwertfeger *et al.* 2006).

Activation of *FGFR1* does not only affect the characteristics of epithelial cells but also influences the myoepithelium and microenvironment. Long-term activation of *FGFR1* in transgenic mice resulted in a disruption of the myoepithelial cell barrier and ECM disorganization (Welm *et al.* 2002).

Activation of *FGFR1* signaling using a small amount of FGF2 in the MDA-MB-134, CAL120, JIMT-1, MFM223, S68, SUM44 (Turner *et al.* 2010b), CMA, MDA-MB-361, and HCC38 (Shiang *et al.* 2010) cell lines, which have endogenous *FGFR1* amplifications, resulted in downstream activation of multiple pathways, including FRS2, ERK1/2 (MAPK3/1), and RSK phosphorylation. A different study showed that these pathways were already basally activated without ligand (Turner *et al.* 2010b). Also, cells harboring *FGFR1* amplifications seem to have an oncogenic addiction to *FGFR1* signaling as they are highly sensitive to inhibition of *FGFR1* by siRNA or SU5402, an *FGFR1* TK inhibitor (TKI; Reis-Filho *et al.* 2006). Furthermore, *FGFR1*-amplified cell lines were resistant to treatment with 4-hydroxytamoxifen (4-OHT), suggesting that *FGFR1* amplification is involved in resistance to endocrine therapy (Turner *et al.* 2010b). This notion supports the observation that amplification of *FGFR1* is associated with a poor prognosis in ER-positive patients that were treated with tamoxifen as an adjuvant endocrine therapy. Interestingly, inhibition of *FGFR1* by siRNA increased the sensitivity of *FGFR1*-amplified cell lines to 4-OHT, making this a possible drug therapy (Turner *et al.* 2010b).

Another indication for interacting pathways comes from the mouse mammary tumor virus–wingless type 1 (MMTV–*Wnt1*) mouse model. Crossing these mice with *iFgfr1* transgenic mice and subsequent activation of FGFR1 dramatically accelerated tumor formation, suggesting an interaction between FGFR1 and WNT (WNT) signaling (Pond et al. 2010). Further experiments are needed to explore whether genetic aberrations in *FGFRs* affect tumor formation in other mouse mammary tumor models harboring mutations in oncogenes that frequently occur in breast cancer patients.

FGFR2

The *FGFR2*-amplified SUM52PE and MFM223 cell lines depend on FGFR2 signaling for survival. This addiction to FGFR2 is due to activation of the PI3K–AKT signaling pathway that results in inhibition of apoptosis. Furthermore, downstream targets of FGFR signaling like FRS2, AKT, and ERK were phosphorylated in the absence of serum and inactivated by an FGFR inhibitor. This indicates that the phosphorylation of these targets is independent of ligand binding and is dependent on FGFR kinase activity. In contrast, the HCC1143 cell line, in which a mutation in *FGFR2* has been described, showed no evidence of FGFR-dependent signaling and is not dependent on FGFR for proliferation (Turner et al. 2010a).

As described earlier, SNPs in intron 2 of *FGFR2* correlate with higher *FGFR2* mRNA expression levels (Meyer et al. 2008). As the sequence of intron 2 includes a regulatory region, SNPs could alter one or more transcription factor binding sites, thereby regulating the expression levels of *FGFR2* (Easton et al. 2007). The SNPs rs35054928 and rs2981578 are located next to an organic cation transporter (OCT)-binding site. The risk allele of rs2981578 also creates a putative binding site for runt-related transcription factor (RUNX) and the risk allele of rs10736303 one for ER (Katoh & Katoh 2009). The risk allele of rs7895676 has reduced binding capacity to CCAAT/enhancer binding protein β (C/EBP β (CEBPB)). Whereas the rs2981578 variants have equal affinity for OCT1 (POU2F1), the high-risk allele has much higher affinity for RUNX2 (Meyer et al. 2008), thought to be due to differences in H3/H4 acetylation of the SNP sites (Zhu et al. 2009). Overall, the differences in binding affinity result in a higher expression of *FGFR2* in cell lines carrying the high-risk alleles. This is in accordance with the increased expression levels of *FGFR2* in breast cancer samples that were homozygous for the high-risk alleles (Meyer et al.

2008). Remarkably, ER binding was not altered for any of the SNPs, whereas the increased risk for breast cancer due to the high-risk alleles of *FGFR2* are highly associated with ER-positive tumors (Garcia-Closas et al. 2008). A possible explanation for this association is that ER and OCT1/RUNX may cooperate to increase the expression of *FGFR2* (Meyer et al. 2008), which is supported by the finding that OCT and ER sites often cluster together (Carroll et al. 2006). The exact role of ER in the *FGFR2* allele resulting in increased breast cancer risk remains to be investigated.

FGFR3

MCF7 cells were used to study the molecular mechanisms behind the involvement of FGFR3 in tamoxifen resistance in ER-positive breast cancer. Inducible activation of FGFR3 decreased the sensitivity of MCF7 cells to 4-OHT treatment independent of ER activity. Activation of PLC γ 1 (PLCG1) was essential for this resistance phenotype and downstream activation of PI3K signaling was an important component of this reduced sensitivity to endocrine therapy (Tomlinson et al. 2012).

FGFR4

The poor response to chemotherapy associated with overexpression of *FGFR4* (Meijer et al. 2008) has been recapitulated in several *FGFR4* overexpressing breast cancer cell lines, which are resistant to doxorubicin and cyclophosphamide (Roidl et al. 2009). *FGFR4* contributes to this resistance by activating antiapoptotic signaling via activation of MAPK and subsequent increase in the B-cell lymphoma-extra large (BCL-XL) levels. In accordance with this, inhibition of FGFR4 reduced ERK phosphorylation and decreased levels of BCL-XL, ultimately leading to increased chemosensitivity to drugs (Roidl et al. 2009).

The Arg³⁸⁸ SNP in *FGFR4* affects its structure, but TK activity was not increased in breast cancer cells expressing this FGFR variant (Bange et al. 2002). This suggests that FGFR4-mediated resistance to chemotherapy may not be due to increased kinase activity of the receptor. Interestingly, Gly³⁸⁸-expressing cells are less invasive compared with Arg³⁸⁸-expressing cells or cells that have no endogenous *FGFR4* expression (Stadler et al. 2006). This indicates that the Gly³⁸⁸ allele protects patients from tumor invasion, which is consistent with a better prognosis (Bange et al. 2002). The kinase domain was proven to be essential for this protective effect (Stadler et al. 2006), suggesting that downstream signaling plays an important role. Gene expression analysis revealed

upregulation of the lysophosphatidic acid (LPA) receptor gene (*EDG2* (*LPAR1*)), matrix metalloproteinase 1 (*MMP1*), and downregulation of plasminogen activator inhibitor (*PAI1* (*SERPINE1*)) in *Arg*³⁸⁸-compared with *Gly*³⁸⁸-expressing cells, possibly contributing to increased cell motility and invasion. *EDG2* induces cell migration by activation of PI3 and AKT upon binding of the LPA ligand, which is consistent with increased cell motility in cells expressing *Arg*³⁸⁸. The observed upregulation of *MMP1* in *Arg*³⁸⁸-expressing cells may contribute to increased invasion due to increased degradation of the ECM (Stadler *et al.* 2006). *PAI1* has been shown to inhibit urokinase plasminogen activator (uPA) resulting in a decrease in migration and invasion. In addition, upregulation of uPA sensitizes the *Arg*³⁸⁸-expressing cells to chemotherapy-induced apoptosis (Whitley *et al.* 2004). All these (indirect) downstream targets of the *Arg*³⁸⁸*FGFR4* variant seem to increase tumor invasion and decrease sensitivity to chemotherapy, explaining the worse clinical prognosis of *Arg*³⁸⁸ carriers (Bange *et al.* 2002, Thussbas *et al.* 2006).

Decreased invasion and no change in proliferative capacity of *Gly*³⁸⁸-expressing breast cancer cells was also observed in the whey acidic protein–transforming growth factor α (WAP–TGF α) breast cancer mouse model in which either the *Arg*³⁸⁵ or *Gly*³⁸⁵ allele (the mouse equivalents of the human 388 alleles) was knocked in. The tumors of the mice carrying the *Arg*³⁸⁵ allele were more invasive than in their *Gly*³⁸⁵ littermates due to increased migration (Seitzer *et al.* 2010).

In addition to increased invasion, WAP–TGF α mice carrying the *Arg*³⁸⁵ allele also developed highly proliferative lung metastases. Genes suggested to contribute to this effect are cyclin-dependent kinase (CDK) inhibitor 1A (p21), which is downregulated in *Arg*³⁸⁵ tumor samples and CDK1, FLK1 (KDR; fetal liver kinase-1), CD44, MMP13, and MMP14, which are upregulated in the *Arg*³⁸⁵ tumors and are involved in cell invasion (Seitzer *et al.* 2010). Interestingly, the involvement of MMPs in *Arg*³⁸⁸-mediated tumor progression has also been suggested in breast cancer patients (Sugiyama *et al.* 2010a,b).

Therapeutic targets and drugs in development

Based on the genetic aberrations in FGFRs identified in breast cancer patients and their consequences on a molecular level, several approaches can be used to target FGFR signaling.

Upstream intervention

As described earlier, cell lines with *FGFR1* amplifications and overexpression have low levels of downstream signaling in the absence of ligand. However, ligand binding strongly induces downstream activation, making inhibition of ligand–receptor interaction a useful approach to inhibit FGFR1 signaling. In *FGFR2*-amplified cell lines, downstream signaling is ligand independent. Whether the presence of a ligand increases signaling, making ligand interference a useful target, is yet unclear. For patients overexpressing *FGFR4*, the dependence on ligand binding has yet to be investigated in more detail to determine whether it is useful to target ligand binding.

A possible method to inhibit ligand binding is by designing FGF ligand traps such as FP-1039 (Turner & Grose 2010): a fusion protein comprising the extracellular domain of FGFR1 and the Fc region of IgG. FP-1039 has been shown to have antiangiogenic effects *in vivo*. Moreover, FP-1039 was able to block tumor formation of breast cancer cell line xenografts, depending on their expression of FGFs and FGFRs (Zhang *et al.* 2007). FP-1039 is currently being tested in a phase I clinical trial (Keer 2010). Furthermore, as the FGFRs have several ligands in common, not only FGFR1 activation but also activation of FGFR3 and FGFR4 is blocked by binding of FP-1039 to FGFs (Zhang *et al.* 2007), making FP-1039 a rather universal blocker of FGFR signaling.

A second method to interfere with ligand binding is the use of antagonistic peptide mimics. These could be effective drugs for patients harboring *FGFR* amplifications that depend on the presence of a ligand to induce signaling. However, at present, only agonistic peptide mimics have been designed for FGFR1-IIIc and FGFR2-IIIb. These were proven to be functional FGFR agonists with apparent therapeutic potential (Li *et al.* 2008).

Intervention on the FGFR level

Several methods can be used to target the FGFRs at the receptor level. First of all, numerous TKIs targeting FGFRs have been developed. These small molecules compete with ATP to bind to the receptor resulting in reduced activity of the kinase domain (Katoh & Katoh 2009, Turner & Grose 2010). However, as the kinase domains of RTKs are very similar, most TKIs are not specific for one FGFR and also inhibit the activity of VEGFRs and/or PDGFRs (Turner & Grose 2010). This may in fact increase their activity in cancer patients but perhaps cause more side effects. Brivanib alaninate is an example of a TKI targeting both FGFRs and VEGFRs. Its effect in breast cancer has so far only been

tested *in vitro*: cell lines with FGFR1 amplification/overexpression are more sensitive than nonamplified ones (Marme et al. 2010). The effect of this drug on other FGFRs and in patients remains to be investigated.

Another multitarget drug is dovitinib, an inhibitor of FGFRs, PDGFRs, and VEGFRs. This TKI was tested in a phase II clinical trial (NCT00958971) including HER2-negative breast cancer patients and only showed antitumor activity in patients with an FGFR1 amplification and an ER- and/or PR-positive status, whereas other subgroups only showed stabilization of tumor growth (Andre et al. 2011). Another phase II trial (NCT01262027) with dovitinib has been started to control inflammatory breast cancer and also evaluate the safety of this drug. E-3810 is a multitarget small-molecule inhibitor targeting VEGFRs and FGFR1, which is currently being tested in a phase I clinical trial. After initial testing in patients with solid tumors, breast cancer patients with *FGFR1* amplifications are included in the dose expansion phase to obtain preliminary data on the antitumor activity of this drug (clinical trial: NCT01283945). Finally, BIBF1120 was developed as a kinase inhibitor targeting FGFRs, PDGFRs, and VEGFRs. Testing of this drug in multiple cancer mouse models and clinical trials including different cancer types has already been shown to be successful (Hilberg et al. 2008, Antoniu & Kolb 2010). A phase I/II clinical trial (NCT01484080) is currently investigating its efficacy in combination with paclitaxel (a mitotic inhibitor used to treat several types of cancer) in patients with early HER2-negative breast cancer.

PD173074 and SU5402 are the first compounds that have been designed as FGFR-specific TKIs. PD173074 was effective in ER-, PR-, and HER2-negative cell lines with *FGFR2* amplification/overexpression (Turner et al. 2010a). SU5402 decreased proliferation exclusively in cell lines with *FGFR1* amplification and overexpression (Reis-Filho et al. 2006). It must be noted, however, that not every cell line with *FGFR1* amplifications is sensitive to FGFR1 inhibition, which was found to depend on the oncogenic background (Reis-Filho et al. 2006). In this regard, further *in vitro* and *in vivo* studies will have to be performed in order to identify the right subgroup among patients with *FGFR1* amplification and overexpression who will respond to FGFR-specific drugs.

The efficacy and safety of AZD4547, a TKI that targets FGFRs, are currently being tested in a phase IIa clinical trial (NCT01202591). The patients included are ER positive and have *FGFR1* polysomy or gene amplification and are also treated with exemestane, an aromatase inhibitor used to treat ER-positive breast

cancer (Lintermans et al. 2011). Another pan-FGFR inhibitor (BGJ398) is also currently being tested in a phase 1 clinical trial to determine the maximum tolerated dose (clinical trial NCT01004224). This trial includes patients with solid tumors with *FGFR1* or -2 amplifications or *FGFR3* mutations.

Monoclonal antibodies may be a good solution to circumvent the side effects of unspecific TKIs. There are FGFR1-IIIc- and FGFR3-specific antibodies that have not yet been tested in any breast cancer model (Turner & Grose 2010). An anti-FGFR4 antibody (10F10) strongly increased sensitivity to doxorubicin in cell lines naturally overexpressing *FGFR4* (Roidl et al. 2009).

Another potentially highly specific way to target *FGFRs* is by RNA aptamers. These short RNA oligonucleotides are selected for their high affinity and specificity for their target protein, thereby acting as synthetic antibodies (Ireson & Kelland 2006). Currently, RNA aptamers that target the *FGFR2* kinase domain, the *FRS2* interacting domain, and the extracellular domain of *FGFR2* are being developed, but their effectiveness remains to be tested (Katoh & Katoh 2009). Therapeutic strategies using siRNA or miRNA are still in a very early stage of development focusing on off-target effects and delivery (Katoh & Katoh 2009).

Downstream intervention

Many of the proteins involved in FGFR signaling also play a role in other signaling pathways and in healthy cells. This makes it very difficult to target these proteins without side effects. No extensive research has been performed on targeting the downstream effectors of FGFR signaling. The targets suggested below are therefore mostly hypothetical, whereas for some, preliminary data are present.

In FGFR1-transfected MCF10A cells, treatment with chloromethylketone (CMK), an irreversible small molecule inhibitor specific for RSK (Cohen et al. 2005), reduced proliferation and restored sensitivity to anoikis (Xian et al. 2009). Interestingly, the inhibitory effect of CMK was only observed in breast cancer cell lines overexpressing *FGFR1* (Xian et al. 2009). The effect of CMK *in vivo* remains to be investigated.

MMPs could also be possible drug targets downstream of FGFR1. Inhibition of MMPs in HC11 cells overexpressing *FGFR1* with a pan-MMP inhibitor (GM6001) resulted in decreased invasion and epithelial mesenchymal transition (EMT), although proliferation, apoptosis, and cell polarity were not changed (Xian et al. 2005). This indicates that inhibition of MMP activity may not suffice as mono

treatment but could be useful when used in combination with other drugs.

PI3 kinase is a possible drug target for patients with *FGFR2* amplifications, as *FGFR2*-amplified cell lines are dependent on PI3K-mediated inhibition apoptosis for their survival. This idea is supported by a study in *FGFR2*-amplified cell lines, which found that dual targeting of *FGFR2* and PI3 kinase by PD173074 and BEZ235 (a PI3 kinase/mTOR inhibitor), respectively, was very effective (Adelaide *et al.* 2007). As PI3K signaling is also suggested to be important in *FGFR3*-mediated resistance to tamoxifen, the inhibition of this pathway could also be a potential treatment for this group of patients.

In patients with *FGFR4* overexpression, targeting the antiapoptotic pathway that activates BCL-XL via MAPK might be a possibility. As these proteins have been shown to mediate chemotherapy resistance in this group of patients, drugs targeting them could increase the response rate. No clinical studies in this field have been performed so far, but administration of UO126 (a specific MEK inhibitor) to *FGFR4*-expressing breast cancer cells has been shown to decrease BCL-XL expression (Roidl *et al.* 2009). In addition to a broad panel of MEK inhibitors that have been developed to treat different types of cancer (Fremin & Meloche 2010), BCL-XL inhibitors are also available for clinical research (Kang & Reynolds 2009).

As the *FGFR4*-*Arg*³⁸⁸ polymorphism does not result in increased TK activity (Bange *et al.* 2002), drugs interfering with ligand binding or the TK activity of the receptor are unsuitable for treating *FGFR4*-*Arg*³⁸⁸ breast cancer patients. However, as described earlier, numerous downstream effectors of *FGFR4* play a role in the increased migration capacity of *Arg*³⁸⁸-expressing cells. Interference with any of these molecules could be a potential therapeutic strategy for breast cancer patients harboring the *Arg*³⁸⁸ allele. This is supported by the observation that siRNA targeting *EDG2* significantly reduces the migration of cells carrying the *Arg*³⁸⁸*FGFR4* allele (Stadler *et al.* 2006).

In general, when targeting *FGFR* signaling, its role in proliferation, differentiation, migration, and angiogenesis should be kept in mind. Interfering in these important basic cell processes could have many side effects. Further investigation of the context-dependent activation of downstream pathways is necessary to anticipate these side effects.

Conclusion

In the past decade, our understanding of the important role of *FGFRs* in breast cancer has been growing.

Genetic aberrationsTcharrsid10362901 in different *FGFRs* have been identified in subgroups of breast cancer patients, and their (molecular) implications on tumor characteristics have been partly elucidated. Overall, it is very clear that *FGFRs* are promising drug targets. *FGFR2* amplifications could be a very interesting drug target in triple-negative breast cancer patients, as other targeted therapies are not effective. Although several mutations in *FGFR3* have been found in other types of cancer and its overexpression has been associated with endocrine resistance, genetic aberrations affecting *FGFR3* have not been identified in breast cancer patients (Sibley *et al.* 2001). Future experiments should be focused on the molecular mechanism behind the increased breast cancer risk associated with the SNPs in intron 2 of *FGFR2*, especially addressing the role of ER. Some contradicting results regarding the involvement of the *Arg*³⁸⁸*FGFR4* allele in the prognosis and therapy resistance of breast cancer patients should be clarified by large-scale studies in which the clinical and pathological parameters are monitored closely. Furthermore, the *FGFR1* gene amplification observed that several different types of breast cancer make it a very relevant drug target. As the inducible *FGFR1* models have greatly increased the understanding about its molecular mechanism, the next step should be to screen for possible drugs. Interestingly, in contrast to *FGFR1*, expression of *FGFR2* did not induce neoplasia in the prostate (Freeman *et al.* 2003), suggesting that *FGFR1* is more oncogenic than *FGFR2*. However, whether this is also the case in mammary tissue or this oncogenic potency is an issue of context remains to be investigated.

Several *FGFR*-targeting strategies are in different phases of development. In addition to the drugs described earlier, several other compounds targeting *FGFRs* are being tested on other types of cancer, including urothelial carcinoma, non-small-cell lung cancer, and ovarian cancer (Katoh & Katoh 2009, Antoniu & Kolb 2010, Turner & Grose 2010, Bello *et al.* 2011, Lamont *et al.* 2011). Even though most of these drugs are not specific for *FGFRs*, but also target *PDGFRs* and *VEGFRs*, this might actually be an advantage as these RTKs are known for their roles in tumor angiogenesis and proliferation (Petrelli & Giordano 2008). Likely, further refinements in *FGFR*-targeting drugs will be necessary to increase effectiveness and limit side effects. At the same time, translational studies need to identify the subgroup of breast cancer patients that will best respond to the various *FGFR*-targeted therapies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

Funding

Supported by unrestricted educational grants from Aegon, Inc. and Pink Ribbon, The Netherlands.

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Received in final form 11 April 2012

Accepted 16 April 2012

Made available online as an Accepted Preprint
16 April 2012