Fibroblast growth factor receptor 4 predicts failure on tamoxifen therapy in patients with recurrent breast cancer

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Abstract

Tamoxifen treatment of estrogen-dependent breast cancer ultimately loses its effectiveness due to the development of resistance. From a functional screen for identifying genes responsible for tamoxifen resistance in human ZR-75-1 breast cancer cells, fibroblast growth factor (FGF) 17 was recovered. The aim of this exploratory study was to assess the predictive value of FGF17 and the receptors FGFR1–4 for the type of response to tamoxifen treatment (clinical benefit) and the duration of progression-free survival (PFS) in patients with recurrent breast cancer. mRNA levels of FGF17 and FGFR1–4 were quantified by real-time reverse transcriptase PCR in 285 estrogen receptor-positive breast carcinomas with clinical follow-up. All patients had recurrent disease and were treated with tamoxifen as first-line systemic therapy for local or distant relapse. FGF17 and FGFR1–3 mRNA levels had no significant predictive value for this group of patients. However, high FGFR4 mRNA levels analyzed as a continuous log-transformed variable predicted poor clinical benefit (odds ratio = 1.22; P = 0.009) and shorter PFS (hazard ratio = 1.18; P < 0.001). In addition, in multivariable analysis, the predictive value of FGFR4 was independent from the traditional predictive factors. Our analyses show that FGFR4 may play a role in the biological response of the tumor to tamoxifen treatment. In addition, as altered expression of FGF17 causes tamoxifen resistance in vitro, the FGF signaling pathway could be a valuable target in the treatment of breast cancer patients resistant to endocrine treatment.

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Introduction

The fibroblast growth factors (FGFs) make up a large family of ligands that signal through cell surface tyrosine kinase FGF receptors (FGFRs). In humans, 22 distinct FGFs and 4 FGFRs have been identified. All FGFs share important similarities, including significant sequence homologies at both the DNA and the protein levels (reviewed by Ornitz & Itoh 2001). During embryonic development, FGF signaling has been associated with proliferation, migration, and differentiation. In adults, FGFs are homeostatic factors that function in control of the nervous system, tissue repair, response to injury, and tumor angiogenesis (reviewed by Ornitz & Itoh 2001, Eswarakumar et al. 2005).

The four FGFRs have an extracellular ligand-binding domain, a transmembrane domain and a split intracellular tyrosine kinase domain. The ligand-binding domain of the receptors is composed of three immunoglobulin-like domains I, II, and III. FGFR1–3 all undergo alternative splicing using two alternative exons (IIIb and IIIc) to encode the carboxy terminal half of immunoglobulin domain III. These splice variants are expressed in a tissue-specific manner with exon IIIb variant expression restricted to epithelial lineages, and exon IIIc variant expression preferentially in mesenchymal lineages. Alternative splicing also contributes to the receptor-binding specificity. Unlike FGFR1–3, FGFR4 is not alternatively spliced in this region (Ornitz et al. 1996,
Eswarakumar et al. 2005), but other variants have been reported (Ezzat et al. 2002).

FGF17 was identified in our functional screen as a gene causing tamoxifen resistance in ZR-75-1 human breast cancer cells (Meijer et al. 2006). Among known FGF family members, the FGF17 protein shows the highest similarity with FGF8 (Hoshikawa et al. 1998). Together with FGF8 and FGF18, it forms a subfamily, having similar gene structures, overlapping patterns of expression and receptor-binding specificities (Ornitz & Itoh 2001). Overexpression of FGF8 and FGF17 in NIH-3T3 cells results in a transforming and tumorigenic phenotype (Kouhara et al. 1994, Xu et al. 1999). Furthermore, FGF8 expression has been demonstrated to be significantly higher in breast cancer than in non-malignant breast tissues (Marsh et al. 1999). No data were available for FGF17 expression in breast cancer yet.

In order to investigate the association of FGF17 expression and expression of the receptors FGFR1–4 with tamoxifen treatment in breast cancer patients, a retrospective study was performed. Gene mRNA levels were measured by real-time quantitative reverse transcriptase PCR (RT-PCR) in 285 estrogen receptor (ER)-positive-frozen primary breast tumors from patients who developed recurrent disease that was treated with tamoxifen as first-line therapy. This report describes the association of FGF17 and the FGF receptors with first-line tamoxifen treatment in patients with recurrent breast cancer.

Patients and methods

Patients

The institutional Medical Ethical Committee approved our study design (MEC 02.953), which was carried out according to the Code of Conduct of the Federation of Medical Scientific Societies in The Netherlands (http://www.fmwv.nl/). Frozen tumor samples were originally submitted to our reference laboratory from regional hospitals for measurements of steroid hormone receptors and have been stored in our tumor bank at the Erasmus Medical Center (Rotterdam, The Netherlands). Guidelines for primary treatment were similar for all hospitals. All available frozen tumor specimens from female patients with breast cancer who entered the clinic during 1979–1995 and from whom detailed clinical follow-up was available (Foekens et al. 1999, 2001) were processed for mRNA analysis. Further inclusion criteria were: > 100 mg frozen tissue available, invasive breast cancer, no previous other cancer (except basal cell skin cancer or early stage cervical cancer stage Ia/Ib), no second primary breast tumor at first relapse, no adjuvant systemic hormonal treatment, > 30% invasive tumor cell nuclei, and good RNA quality. Samples were rejected because of insufficient frozen tumor material (~50%), too low percentage tumor cells (~15%), and poor RNA quality (~9%). A total of 285 primary, ER-positive breast tumor samples were included in this retrospective study. These patients were treated either with breast-conserving surgery (36%) or with modified mastectomy (64%). An axillary node dissection was performed in 93% of the patients (n = 268, 148 patients node positive). In contrast to current clinical practice, not all node-positive patients received adjuvant systemic therapy. This is due to the fact that tumors that were surgically removed from 1979 onwards were included in this study, and up to around 1990 it was not common clinical practice in The Netherlands to offer adjuvant systemic therapy to all node-positive patients. In total, 211 patients were postmenopausal (64% at primary surgery and 74% at start of first-line therapy) and all had ER-positive tumors. ER status was determined by routine ligand-binding assays or enzyme immunoassays, and pathological examination was not performed centrally and reflects daily clinical practice in the various participating regional hospitals as described previously (Foekens et al. 1989). None of the patients had received neoadjuvant therapy or were exposed to hormonal adjuvant treatment. Half of the patients received adjuvant radiotherapy and 52 patients were treated with adjuvant chemotherapy (19 patients anthracyclin-based (FAC/FEC) and 33 patients non-anthracyclin-based (CMF)). Thirty patients had metastasis at diagnosis or developed distant metastasis (including supraclavicular lymph node metastasis) within 1 month after primary surgery.

All patients, including 32 patients with a local recurrence, were treated for recurrent disease with first-line tamoxifen therapy (40 mg daily). Median time to treatment was 28 months. The median age of the patients at the time of primary surgery was 57 years (ranges 26–89 years) and at the start of tamoxifen therapy for recurrent disease 61 years (ranges 29–90 years). Patients were routinely followed at the outpatient clinic, generally once every 3 weeks during the first 6 months, and in case of objective response approximately every 6 weeks later on (Martens et al. 2005). Skin metastases were assessed clinically by palpation and documented by photography; lymph node metastases were assessed by palpation and sometimes if necessary by ultrasound; lung metastases were routinely followed by X-thorax (once every 6–12 weeks), and by CT-thorax where applicable; liver metastases were
always followed by CT of the liver, in general once every 12 weeks; brain metastases were assessed by magnetic resonance imaging if indicated; and bone metastases were followed by X-rays (every 6–12 weeks) and bone scan (every 6–12 months) as a standard, and by magnetic resonance imaging if indicated. Furthermore, plasma tumor marker levels (CA15.3 and/or CA125) were regularly measured. The type of response to tamoxifen therapy was recorded as defined by standard Union Internationale Contra Cancrum criteria (Hayward et al. 1977). In total 179 patients, with complete remission (i.e., complete disappearance of all metastases, \( n = 13 \)), partial remission (i.e., at least 50% reduction, \( n = 39 \)), or with stable disease longer than 6 months \( (n = 127) \), were classified as patients having clinical benefit (responders) as defined in the manual for clinical research and treatment in breast cancer of the European Organization for Research and Treatment of Cancer (EORTC Breast Cancer Cooperative Group 2000). Fifteen patients with stable disease shorter than or equal to 6 months and 91 patients of disease (25% or more increase) were classified as non-responders. Median follow-up time for treatment of recurrent disease was 42.7 months.

**Tissue processing**

Primary tumor tissue processing was done as described previously (Sieuwerts et al. 2005). In summary, 20–60 cryostat sections of 30 \( \mu m \), corresponding to 30–100 mg, were cut from frozen tissues for RNA isolation. To assess the amount of tumor cells relative to the amount of surrounding stromal cells, 5 \( \mu m \) sections were cut for hematoxylin and eosin staining, before, in between, and after cutting the sections for RNA isolation. For this study, only specimens with at least 30% tumor nuclei, distributed uniformly over at least 70% of the section area, were included.

**RNA isolation, cDNA synthesis, and quantification of mRNA**

RNA isolation, cDNA synthesis, and quantification of mRNA and quality control checks were done as described in detail before (Sieuwerts et al. 2005). RNA samples without distinct rRNA peaks or failing to amplify efficiently with the housekeeper primer sets were excluded from this study. Real-time quantitative RT-PCR was performed using an ABI Prism 7700 Sequence detection system (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) and Assay-on-Demand kits from Applied Biosystems. The following assays were used: Hs00182599_m1 (FGF17), Hs00241111_m1 (FGFR1), Hs00240796_m1 (FGFR2), Hs00179829_m1 (FGFR3), and Hs002425 58_m1 (FGFR4). We used a protocol as recommended by the manufacturer. Quantitative RT-PCR assays for EGFR and ERBB2 have previously been described (Dorssers et al. 2005). To enable comparison of the mRNA levels in different samples, values were normalized using the average expression levels of a set of housekeeping genes containing \( PBGD \), \( HPRT \), and \( B2M \). Levels of the target genes expressed relative to this housekeeping set were quantified as follows: mRNA target = \( 2^{(\text{mean } C_t \text{ housekeeping} - \text{mean } C_t \text{ target})} \) (Sieuwerts et al. 2005). \( C_t \) is defined as the detection threshold cycle number.

**Statistical analysis**

Computations were done with the use of STATA statistical package, release 9.2 (STATA Corp., College Station, TX, USA). Differences in levels were assessed with the Mann–Whitney \( U \) test or Kruskal–Wallis test, including a Wilcoxon-type test for trend, when appropriate. In these tests, patient and tumor characteristics were used as grouping variables. The strengths of the associations between continuous variables were tested with the Spearman rank correlation \( (r_s) \). To reduce the skewness, most variables were log-transformed while ER-\( \alpha \) and FGFR3 data were Box–Cox transformed. Logistic regression analysis was used to examine the relationship between the mRNA levels measured and clinical benefit of tamoxifen therapy. Odds ratios (ORs) were calculated and presented with their 95% confidence interval. The likelihood ratio test in logistic regression models was used to test for differences. The Cox proportional hazard model was used to calculate the hazard ratio (HR) and 95% confidence interval in the analyses of progression-free survival (PFS) and post-relapse overall survival. The proportional hazards assumption was tested using Schoenfeld residuals. In most cases, tamoxifen treatment is started at the end of the disease-free interval, except for those patients that were first treated with surgery and/or radiotherapy for local recurrence \( (n = 16) \). For all cases, the starting point of PFS is defined as the start of the first line of systemic treatment with tamoxifen for recurrence. The endpoint is the first detection of progression of the disease. For visualization, levels of FGFR4 were divided into four equal parts. Survival curves were generated using the Kaplan & Meier’s (1958) method, and the log-rank test was used to test for differences. A two-sided \( P \) value of <0.05 was considered statistically significant.
Table 1: Associations of biological factors with clinicopathological factors

<table>
<thead>
<tr>
<th>Clinicopathological factors</th>
<th>No. of patients</th>
<th>ER-α (×10⁻¹)</th>
<th>PGR (×10⁵)</th>
<th>FGFR1 (×10⁻¹)</th>
<th>FGFR2 (×10⁻²)</th>
<th>FGFR3 (×10⁻²)</th>
<th>FGFR4 (×10⁻¹)</th>
<th>FGF17 (×10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Menopausal status</strong></td>
<td></td>
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<tr>
<td>Prenomenopausal</td>
<td>74</td>
<td>0.37 (0.18–0.72)</td>
<td>1.04 (0.33–2.06)</td>
<td>1.61 (0.80–3.47)</td>
<td>2.88 (1.21–6.77)</td>
<td>2.09 (0.57–5.65)</td>
<td>0.24 (0.12–0.61)</td>
<td>1.58 (0.61–3.31)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>211</td>
<td>0.88 (0.39–1.81)</td>
<td>0.62 (0.07–3.05)</td>
<td>1.94 (0.95–3.48)</td>
<td>3.95 (1.76–8.80)</td>
<td>2.57 (0.87–5.77)</td>
<td>0.35 (0.11–1.17)</td>
<td>1.36 (0.51–3.03)</td>
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<td><strong>Age (years)</strong></td>
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<td>≤ 40</td>
<td>18</td>
<td>0.50 (0.17–1.02)</td>
<td>0.99 (0.27–2.42)</td>
<td>1.40 (0.90–2.31)</td>
<td>4.61 (1.21–13.26)</td>
<td>2.92 (0.74–7.26)</td>
<td>0.26 (0.14–1.11)</td>
<td>1.29 (0.61–2.98)</td>
</tr>
<tr>
<td>41–55</td>
<td>93</td>
<td>0.40 (0.18–0.79)</td>
<td>1.01 (0.19–1.94)</td>
<td>2.27 (0.97–3.51)</td>
<td>3.14 (1.35–6.14)</td>
<td>2.04 (0.63–5.28)</td>
<td>0.25 (0.13–0.76)</td>
<td>1.69 (0.61–3.81)</td>
</tr>
<tr>
<td>56–70</td>
<td>100</td>
<td>1.08 (0.38–1.88)</td>
<td>0.56 (0.06–3.31)</td>
<td>1.99 (1.00–3.93)</td>
<td>4.40 (1.57–6.69)</td>
<td>2.32 (0.77–6.69)</td>
<td>0.41 (0.11–1.31)</td>
<td>1.43 (0.51–2.81)</td>
</tr>
<tr>
<td>&gt; 70</td>
<td>74</td>
<td>0.99 (0.66–1.97)</td>
<td>0.63 (0.07–3.05)</td>
<td>1.69 (0.75–3.00)</td>
<td>3.90 (2.11–10.76)</td>
<td>3.78 (1.21–10.18)</td>
<td>0.28 (0.10–0.77)</td>
<td>1.37 (0.39–2.99)</td>
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<tr>
<td><strong>Tumor size</strong></td>
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<tr>
<td>≤ 2 cm</td>
<td>78</td>
<td>0.85 (0.31–1.49)</td>
<td>0.56 (0.08–2.06)</td>
<td>2.06 (0.77–3.51)</td>
<td>4.46 (1.71–10.19)</td>
<td>2.10 (0.82–7.39)</td>
<td>0.27 (0.14–0.62)</td>
<td>1.44 (0.61–3.06)</td>
</tr>
<tr>
<td>&gt; 2–≤5 cm</td>
<td>169</td>
<td>0.70 (0.31–1.53)</td>
<td>1.01 (0.13–3.27)</td>
<td>1.84 (1.00–3.48)</td>
<td>3.64 (1.71–7.11)</td>
<td>2.86 (0.76–3.23)</td>
<td>0.35 (0.12–0.88)</td>
<td>1.52 (0.67–3.14)</td>
</tr>
<tr>
<td>&gt; 5 cm+pT4</td>
<td>38</td>
<td>0.54 (0.23–1.78)</td>
<td>0.42 (0.06–1.80)</td>
<td>1.29 (0.57–3.42)</td>
<td>2.51 (0.93–8.61)</td>
<td>1.94 (0.79–5.15)</td>
<td>0.36 (0.10–1.96)</td>
<td>0.61 (0.32–3.53)</td>
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<tr>
<td><strong>Tumor grade</strong></td>
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<tr>
<td>Good/moderate</td>
<td>35</td>
<td>1.06 (0.49–1.81)</td>
<td>0.79 (0.04–2.60)</td>
<td>2.09 (1.00–3.57)</td>
<td>5.16 (2.36–10.92)</td>
<td>4.88 (2.36–10.41)</td>
<td>0.61 (0.16–2.32)</td>
<td>2.43 (1.01–6.60)</td>
</tr>
<tr>
<td>Poor</td>
<td>158</td>
<td>0.58 (0.25–1.14)</td>
<td>0.66 (0.08–2.55)</td>
<td>1.67 (0.77–3.32)</td>
<td>3.42 (1.33–7.11)</td>
<td>2.02 (0.63–5.34)</td>
<td>0.39 (0.12–1.08)</td>
<td>1.28 (0.46–2.25)</td>
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<tr>
<td><strong>Histological type</strong></td>
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<tr>
<td>IDC</td>
<td>163</td>
<td>0.78 (0.30–1.53)</td>
<td>0.47 (0.06–2.33)</td>
<td>1.84 (0.90–3.80)</td>
<td>4.13 (1.71–8.85)</td>
<td>2.78 (0.89–7.39)</td>
<td>0.35 (0.13–1.27)</td>
<td>1.46 (0.53–3.16)</td>
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<tr>
<td>ILC</td>
<td>28</td>
<td>0.72 (0.31–1.71)</td>
<td>1.46 (0.42–4.36)</td>
<td>2.10 (1.06–3.99)</td>
<td>3.80 (1.92–6.84)</td>
<td>2.37 (0.67–7.31)</td>
<td>0.16 (0.09–0.34)</td>
<td>2.01 (0.48–4.23)</td>
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<tr>
<td>DCIS+IDC</td>
<td>24</td>
<td>0.51 (0.17–0.97)</td>
<td>0.98 (0.33–1.22)</td>
<td>2.46 (0.97–3.61)</td>
<td>2.84 (1.12–8.24)</td>
<td>1.93 (0.69–6.57)</td>
<td>0.47 (0.16–0.64)</td>
<td>1.22 (0.45–2.36)</td>
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<tr>
<td><strong>Nodal status</strong></td>
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<tr>
<td>N0</td>
<td>120</td>
<td>0.86 (0.33–1.63)</td>
<td>0.40 (0.07–2.21)</td>
<td>2.16 (1.00–4.02)</td>
<td>4.41 (1.73–9.00)</td>
<td>2.78 (0.90–8.80)</td>
<td>0.32 (0.13–0.68)</td>
<td>1.85 (0.79–4.39)</td>
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<tr>
<td>N1–3</td>
<td>66</td>
<td>0.61 (0.26–1.43)</td>
<td>1.05 (0.13–3.10)</td>
<td>1.56 (0.96–2.60)</td>
<td>3.41 (1.52–7.11)</td>
<td>2.45 (0.60–4.91)</td>
<td>0.45 (0.16–1.17)</td>
<td>1.27 (0.54–2.27)</td>
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<tr>
<td>N&gt;3</td>
<td>82</td>
<td>0.57 (0.27–1.26)</td>
<td>1.04 (0.23–2.64)</td>
<td>1.69 (0.75–3.03)</td>
<td>3.40 (1.30–8.65)</td>
<td>2.08 (0.75–6.81)</td>
<td>0.24 (0.08–1.40)</td>
<td>0.92 (0.32–2.68)</td>
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<td><strong>DSR</strong></td>
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<tr>
<td>LRR</td>
<td>32</td>
<td>0.47 (0.20–1.48)</td>
<td>0.47 (0.11–2.73)</td>
<td>1.18 (0.53–2.06)</td>
<td>2.36 (1.27–4.93)</td>
<td>2.06 (0.57–8.64)</td>
<td>0.51 (0.23–2.03)</td>
<td>1.29 (0.46–4.47)</td>
</tr>
<tr>
<td>Bone</td>
<td>146</td>
<td>0.70 (0.30–1.29)</td>
<td>0.75 (0.09–2.52)</td>
<td>1.93 (0.99–3.30)</td>
<td>3.79 (1.75–8.47)</td>
<td>2.54 (0.83–7.04)</td>
<td>0.26 (0.12–1.01)</td>
<td>1.32 (0.59–2.74)</td>
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<tr>
<td>Viscera</td>
<td>107</td>
<td>0.77 (0.34–1.79)</td>
<td>0.95 (0.07–3.28)</td>
<td>2.16 (0.81–4.54)</td>
<td>4.37 (1.41–8.85)</td>
<td>2.46 (0.70–7.16)</td>
<td>0.35 (0.11–0.75)</td>
<td>1.74 (0.51–3.35)</td>
</tr>
</tbody>
</table>
Results

Correlations between biological factors

All tissue samples were analyzed for ER-α and PGR mRNA expression and none tested negative for ER-α mRNA in concordance with the selection of ER protein-positive samples (Sieuwerts et al. 2005). Spearman’s rank correlation revealed associations (P<0.001) between mRNA levels of FGFR4 and FGFR1 ($r_s = 0.23$), FGFR3 ($r_s = 0.29$), and PGR ($r_s = -0.31$). In addition, FGFR1 mRNA levels correlated with those of FGF17 ($r_s = 0.32$). ER-α mRNA levels correlated with those of PGR ($r_s = 0.22$), FGFR2 ($r_s = 0.34$), and FGFR3 ($r_s = 0.38$).

Association of biological factors with clinicopathological factors

In Table 1, the associations of the median mRNA levels of the factors measured with clinicopathological factors are shown. ER-α, FGFR2, FGFR3, and FGF17 mRNA levels were inversely related with tumor grade. Furthermore, ER-α mRNA expression levels were higher in tumors of postmenopausal patients, and mRNA levels of FGF17 were inversely related with nodal status. PGR mRNA levels varied between histological subtypes, and mRNA levels of FGFR1 varied between the different categories of dominant site of relapse. None of the mRNA levels determined correlated with tumor size, disease-free interval (Table 1), or adjuvant systemic treatment (data not shown).

Uni- and multivariable analysis for clinical benefit

The main clinical endpoints of this study were the measurable effect of tamoxifen therapy on tumor size (clinical benefit) and the length of PFS of the patients after start of first-line therapy for recurrent disease. In univariate logistic regression analysis using log-transformed continuous variables, high expression levels of ER-α and PGR predicted a favorable clinical benefit (Table 2). In contrast, high levels of FGFR4 predicted a worse outcome for the patient (OR = 1.22, $P = 0.009$). For FGF17, FGFR1–3, no significant associations with clinical benefit were observed (Table 2). The estimate for FGFR4 was similar when patients with a local recurrence were excluded. To further explore the predictive power of FGFR4, the patients were divided into four equal quarters, ranging from low to high FGFR4 mRNA levels. Compared with patients with low tumor mRNA levels of FGFR4 (OR set at 1.0), those with high FGFR4 mRNA levels showed a worse outcome.
Patients with intermediate levels of FGFR4 (Q2 and Q3) showed intermediate ORs (Table 2). The proportion of patients that experienced clinical benefit was 75% (54/72) for those with the lowest, and 51% (36/71) for those with the highest FGFR4 mRNA levels.

The predictive value of FGF17 and FGFR1–4 was further studied with multivariable logistic regression analysis (Table 2). For this analysis, we used the base multivariable model including traditional predictive factors (menopausal status, dominant site of relapse, disease-free interval, and ER), as described previously for a larger group of 691 patients treated with first-line tamoxifen for recurrent disease (Meijer-van Gelder et al. 2004), with age and PGR added. The traditional prognostic factors ‘nodal status’, ‘tumor size’, and ‘grade’ were not included, because they did not have any predictive value as expected in an analysis for response to first-line therapy for recurrent disease. ‘Adjuvant chemotherapy’ was also omitted, since the estimates of our base model were not different with adjuvant therapy included or excluded.

(OR = 2.92, CI = 1.44–5.92, \( P = 0.003 \)). Patients with intermediate levels of FGFR4 (Q2 and Q3) showed intermediate ORs (Table 2). The proportion of patients that experienced clinical benefit was 75% (54/72) for those with the lowest, and 51% (36/71) for those with the highest FGFR4 mRNA levels.

The predictive value of FGF17 and FGFR1–4 was further studied with multivariable logistic regression analysis (Table 2). For this analysis, we used the base multivariable model including traditional predictive factors (menopausal status, dominant site of relapse, disease-free interval, and ER), as described previously for a larger group of 691 patients treated with first-line tamoxifen for recurrent disease (Meijer-van Gelder et al. 2004), with age and PGR added. The traditional prognostic factors ‘nodal status’, ‘tumor size’, and ‘grade’ were not included, because they did not have any predictive value as expected in an analysis for response to first-line therapy for recurrent disease. ‘Adjuvant chemotherapy’ was also omitted, since the estimates of our base model were not different with adjuvant therapy included or excluded. FGF17 and FGFR1–4 were separately added as transformed continuous variables to the base model. These analyses showed that the predictive value of FGFR4 (OR = 1.21, \( P = 0.031 \)) for clinical benefit was independent from the traditional predictive factors of the base model (Table 2).
In univariate analysis using log-transformed continuous variables, high tumor levels of ER-α and PGR mRNA were correlated with longer PFS and thus with better outcome for the patient (Table 3). On the other hand, high mRNA levels of FGFR4 were significantly associated with shorter PFS (log-transformed continuous, HR = 1.18, P < 0.001). To allow visualization by Kaplan–Meier plots, patients were divided into four equal quartiles according to their FGFR4 mRNA levels. Patients having low FGFR4 mRNA levels (quarter 1) had a median PFS of more than twice the PFS of patients having high FGFR4 mRNA levels (quarter 4; 14 vs 6 months Fig. 1A).

### Post-relapse overall survival

Post-relapse overall survival (Fig. 1B) was also significantly better in patients with low FGFR4 mRNA levels. In multivariable analysis, the predictive value of FGFR4 was independent of the traditional predictive factors (Table 3). When divided into quartiles, the hazard ratios compared with the lowest quartile (Q1) were 1.15 (Q2), 1.14 (Q3), and 2.11 (Q4). The proportional hazards assumption was not violated for FGFR4.

### Discussion

Recently, we have identified FGF17 in a functional screen for genes responsible for tamoxifen resistance in a human breast cancer cell line (Meijer et al. 2006).
FGF17 is predominantly expressed in the brain and the nervous system, and no data were available for its expression in breast cancer until now. In contrast, other FGFs and the FGFRs have been reported to be expressed and to have a potential role in breast cancer (reviewed by Dickson et al. 2000, Zammit et al. 2001). Furthermore, FGF2 has been linked to tumor aggressiveness (Sieuwerts et al. 2002, Van der Auwera et al. 2004) and FGFR3 and FGF5 to metastatic preferences of breast cancer (Kang et al. 2003, Smid et al. 2006).

In the current exploratory study, quantitative RT-PCR was used to evaluate whether mRNA expression levels of FGF17 and the receptors FGFR1–4 in primary tumors can predict the outcome of first-line tamoxifen treatment. The endpoints of this study were clinical benefit of the therapy and PFS in patients with recurrent breast cancer. Because of the retrospective nature of this study, we have defined the type of response strictly beforehand. The size of the metastases or the occurrence of new lesions was used as objective measures of treatment effect. We included only hormone-naive patients to exclude acquired therapy resistance to tamoxifen.

Our study shows that FGF17 mRNA levels did not associate with clinical benefit and PFS of patients treated with tamoxifen. In contrast, increasing levels of its receptor FGFR4 were related with a higher probability of tamoxifen failure. In multivariable analysis, high tumor levels of FGFR4 provided additional predictive information, independent of the traditional predictive factors menopausal status, dominant site of relapse, disease-free interval, ER, and PGR status. Moreover, addition of the mRNA levels of EGFR and ERBB2 (HER2) as variables to the base model did not alter the estimates for FGFR4 for PFS or clinical benefit (Table 4). No significant correlation was observed with disease-free survival in this patient series (Table 1), nor in an independent set of 604 node-negative breast cancer patients.
FGFR4 (Zhang et al. 2006) that FGF17 in epithelial lineages mainly activates directly remains to be solved. The observation that FGFR4 mRNA levels have been found in breast cancer (Jaakkola et al. 1993, Penault-Llorca et al. 1995). In addition, a germ line polymorphism in this gene, resulting in expression of FGFR4 variants containing either glycine (Gly388) or arginine (Arg388) at codon 388, has been identified (Bange et al. 2002). However, there has been some debate as to whether the Arg388 allele is relevant for breast cancer prognosis (Bange et al. 2002, Becker et al. 2003, Jezequel et al. 2004, Spinola et al. 2005, Thussbas et al. 2006). Furthermore, Thussbas et al. (2006) reported a survival difference favoring FGFR4 wild type in patients treated with adjuvant chemotherapy. In addition, no significant survival difference between the variants was seen in patients treated with adjuvant endocrine therapy. Future analysis of this polymorphism in our patient series may establish its predictive value.

FGFR4 adds to an expanding list of biological factors which provide predictive information for tamoxifen treatment of breast cancer (Milano et al. 2006). Prominent examples are uPA/PAI-1 (Foekens et al. 1995, Meijer-van Gelder et al. 2004), cyclin E (Span et al. 2003), HOXB13/IL17BR (Ma et al. 2004, Jansen et al. 2007), the 81-gene signature (Jansen et al. 2005), and BCAR1, a gene identified in another functional screen (Van der Flier et al. 2000).

The question whether FGFR4 association with development of tamoxifen resistance is caused in a direct manner remains to be solved. The observation that FGF17 in epithelial lineages mainly activates FGFR4 (Zhang et al. 2006) supports the theory that the biological effects seen in our functional screen were caused by activation of this particular receptor. In concordance with this hypothesis, we have confirmed expression of FGFR4 (at levels comparable with the housekeepers) and the other FGFR family members in our cell model using quantitative RT-PCR analysis (unpublished results). The absence of association between FGF17 and FGFR4 levels in the breast tumors may be explained by the balance between the receptor concentration and the amount and affinity of all ligands. Activation of FGFRs may lead to activation of the MAPK and the PI3K/AKT pathways (Katoh 2002). Increasing evidence indicates that changes occurring in growth factor signaling pathways, as currently well documented for EGFR and ERBB2, may dramatically influence steroid hormone action and may be critical to anti-hormone-resistant breast cancer cell growth (Newby et al. 1997, De Placido et al. 1998, Kurokawa & Arteaga 2003, Nicholson et al. 2003, Hayes 2004, Schiff et al. 2004, Shou et al. 2004).

In conclusion, the established association between FGFR4 mRNA levels and clinical benefit of therapy suggests that FGFR4 plays a role in the biological response of the tumor to tamoxifen treatment. As altered FGF signaling causes tamoxifen resistance in vitro, this pathway could be a valuable target in the treatment of breast cancer patients resistant to endocrine treatment.

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Table 4 Fibroblast growth factor receptor (FGFR4) is independent of EGFR and ERBB2

<table>
<thead>
<tr>
<th>Factor</th>
<th>Clinical benefit</th>
<th>Progression-free survival</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>EGFR</td>
<td>0.186</td>
<td>0.84</td>
</tr>
<tr>
<td>ERBB2</td>
<td>0.545</td>
<td>1.28</td>
</tr>
<tr>
<td>FGFR4</td>
<td>0.045</td>
<td>1.20</td>
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</tbody>
</table>

* Multivariable logistic regression analysis for clinical benefit of patients receiving first-line tamoxifen therapy with simultaneous addition of the factors and corrected for the base model as detailed in Table 2.
* Multivariable Cox regression analysis for progression-free survival of patients receiving first-line tamoxifen therapy with simultaneous addition of the factors and corrected for the base model as detailed in Table 3.
* Factor added as dichotomized variable representing high (14% of the patients) and low levels of mRNA.
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