Resistance to Chemotherapy Is Associated with Fibroblast Growth Factor Receptor 4 Up-Regulation

Andreas Roidl, Hans-Jürgen Berger, Sushil Kumar, Johannes Bange, Pjotr Knyazev, and Axel Ullrich

Abstract

Purpose: Establishment of antiapoptotic signaling pathways in tumor cells is a major cause for the failure of chemotherapy against cancer. To investigate the underlying mechanisms, we developed an experimental approach that is based on the genetic plasticity of cancer cells and the selection for cell survival on treatment with chemotherapeutic agents.

Experimental Design: Gene expression changes of surviving cell clones were analyzed by macroarrays. Involvement of fibroblast growth factor receptor 4 (FGFR4) in antiapoptotic pathways was elucidated by apoptosis assays, small interfering RNA experiments, and an antagonistic antibody.

Results: We show that FGFR4 gene expression is up-regulated in doxorubicin-treated, apoptosis-resistant cancer cell clones. Ectopic expression of FGFR4 in cancer cells led to reduced apoptosis sensitivity on treatment with doxorubicin or cyclophosphamide, whereas knockdown of endogenous FGFR4 expression in breast cancer cell lines had the opposite effect. FGFR4 overexpression resulted in Bcl-xl up-regulation at both mRNA and protein levels. Knockdown of FGFR4 expression by small interfering RNA caused a decrease in phospho-extracellular signal-regulated kinase 1/2 levels and reduced Bcl-xl expression. Moreover, an antagonistic FGFR4 antibody suppressed the resistance of cancer cells with endogenous FGFR4 expression against apoptosis-inducing chemotherapeutic agents.

Conclusion: Based on these findings, we propose an antiapoptotic signaling pathway that is initiated by FGFR4 and regulating the expression of Bcl-xl through the mitogen-activated protein kinase cascade. Our findings are exemplary for a novel strategy toward the elucidation of diverse signaling pathways that define antiapoptotic potential in cancer cells. These observations open new avenues toward the diagnosis of chemoresistant tumors and therapies targeting FGFR4-overexpressing cancers.

Breast cancer is the most frequent malignancy among women in the western world. Although much effort has been invested into designing new therapies, classic chemotherapeutic agents such as doxorubicin (Adriamycin) are still widely employed in the clinic. Chemotherapeutic drugs are used as primary or adjuvant therapy with response rates from 60% to 100% (2, 3). A major problem of most of the current therapies are the sometimes severe side effects and the intrinsic or acquired drug resistance of cancer cells to these drugs, which lead to relapse and metastatic progression of the tumor. This is reflected by the decline in the response rates with second-line chemotherapy to 20% to 30% (4).

Motivated by these facts, a comprehensive characterization of genes contributing to a drug resistance phenotype has been done in this study. Based on an approach of Hudziak et al. (5) and Abraham et al. (6), where tumor necrosis factor-α-resistant and Fas ligand-resistant cell clones were established, we respectively took advantage of the genomic instability of breast cancer cell lines to generate doxorubicin-resistant cell clones. When treated with chemotherapeutic drugs, cell clones survived the treatment because of their ability to adapt to the selection pressure by using antiapoptotic mechanisms. Subsequent comparison of gene expression profiles of apoptosis-sensitive breast cancer cells and corresponding apoptosis-resistant cell clones led to the identification of fibroblast growth factor receptor 4 (FGFR4) as a gene with increased gene expression in response to treatment with doxorubicin or cyclophosphamide.

The four closely related members of the human FGFRs (FGFR1-FGFR4) belong to the receptor tyrosine kinase family and consist of three IG domains, an acidic box, except for

FGFR2, a HAV motive, and a split tyrosine kinase domain (7). Currently, 23 ligands are known to activate FGFRs and stimulate a multitude of signaling pathways (8). On ligand binding, FGFRs become transphosphorylated, which results in the recruitment of the central adapter protein FGFR substrate 2. FGFR substrate 2 is a binding platform for various adaptor proteins (e.g., antagonistic antibodies) and chemotherapeutic drugs in FGFR4-overexpressing cancers. These results present new options toward the early diagnosis of chemoresistance and suggest novel combinations of chemotherapy and anti-FGFR4 agents to prevent or significantly delay the onset of therapy resistance.

Translational Relevance

Treatment of patients suffering from breast cancer is still largely based on classic chemotherapy. Beside serious side effects, formation of resistance to these drugs is hampering successful cancer therapy. Here, we describe a novel strategy toward the elucidation of signaling pathways and targets that define chemoresistance in cancer cells. Using our cell genetics-based strategy, we identified the fibroblast growth factor receptor 4 (FGFR4) to contribute to the cancer cell resistance phenotype. We show that doxorubicin-resistant cancer cells become sensitive after being treated with small interfering RNA or a blocking antibody against FGFR4. In a combined application of FGFR4-blocking antibody and the chemotherapeutic drug doxorubicin, we could further increase the apoptosis rate of cancer cell lines, suggesting a combinatorial strategy of FGFR4 inhibitors (e.g., antagonistic antibodies) and chemotherapeutic drugs in FGFR4-overexpressing cancers. These results present new options toward the early diagnosis of chemoresistance and suggest novel combinations of chemotherapy and anti-FGFR4 agents to prevent or significantly delay the onset of therapy resistance.

Materials and Methods

Reagents and antibodies. FGF ligands were purchased from TEBU. The antibodies used were polyclonal anti-FGFR4, anti-extracellular signal-regulated kinase (ERK) 2, and anti-Akt1/2 (Santa Cruz Biotechnology), rabbit polyclonal anti-phospho-Akt (Ser473; New England Biolabs), mouse monoclonal anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology), mouse monoclonal anti-tubulin (Sigma), rabbit polyclonal anti-phospho-ERK (Cell Signalling), mouse monoclonal anti-Bcl-xl (BD Biosciences), mouse monoclonal anti-VSV antibody (Sigma), and mouse monoclonal anti-FGFR4 antibody 10F10 (I3 Pharma).

Secondary horseradish peroxidase-conjugated antibodies were goat anti-mouse (Sigma) and goat anti-rabbit (Bio-Rad). If required, cells were starved by total serum withdrawal for 48 h and treated with growth factors as indicated in the figure legends.

Cell culture, plasmids, and retroviral infections. Cell lines 769-P, TE671, MDA-MB-453, BT-474, MDA-MB-361, MDA-MB-231, ZR75-1, and MCF7 were obtained from the American Type Culture Collection and cultivated following the supplier’s instruction, except MDA-MB-453 cells, which were maintained in RPMI supplemented with 10% FCS and glutamine (Life Technologies/Invitrogen).

pLXSN vectors containing human FGFR4-encoding cDNA have been described before (13). The polyclonal MCF7 cell lines expressing FGFR4 were generated by retroviral gene transfer. Amphotropic retroviral supernatants were produced by transfection of Phoenix packaging cells using the appropriate vector constructs by the calcium phosphate/chloroquine method as described previously. At 48 h post-transfection, the tissue culture medium was filtered through a 0.45 µm filter, mixed with polybrene (4 µg/mL final), and used for infection of the cells. Cells were infected three times for at least 4 h and allowed to recover for 24 h with fresh medium. Polyclonal cells stably expressing FGFR4 were selected with G418 (2 mg/mL) for 2 weeks.

To target FGFR4 protein for down-regulation by siRNA, we generated pRetroSuper-FGFR4 constructs. The target sequences of siRNAs used in this article were GAGCAGAGGCTGACAGAGT (si66), CTACCGTCTAGATGGCTGTCG (siCtrl), and GTGCCCGACCTTGAAGCA (si74). Polyclonal cells carrying pRetroSuper-FGFR4 were selected with puromycin (2 µg/mL) for 2 days.

Reverse transcription-PCR and semiquantitative PCR. RNA was isolated using the RNeasy Mini Kit (Qiagen) and reversely transcribed into cDNA using the AMV reverse transcriptase (Roche) and oligo(dT) primer (Life Technologies). The RNA and cDNA concentration was measured using the NanoDrop ND1000 (PegLab) system. For the semiquantitative PCR amplification, the following primers (MWG) have been used: 5'-ACCACAGCTCATGCGCAAC3' (GAPDH forward) and 5'-TCCACCACCATGTTGCGTA3' (GAPDH reverse) and 5'-AGAATTTCTGCACCATGCTTCAGGCACACC3' (Bcl-xl forward) and 5'-GGGTGATGTGGAGCTGGGATGTC-3' (Bcl-xl reverse). PCR products were subjected to electrophoresis on a 2% agarose gel and DNA was visualized by ethidium bromide staining.

Cell lysis, immunoprecipitation, and immunoblotting. Cell cultures were washed with PBS and incubated at 4°C with lysis buffer [50 mmol/L Hepes/NaOH (pH 7.5), 150 mmol/L NaCl, 1 mmol/L EDTA, 10% glycerol, and 1% Triton X-100] supplemented with
phosphatase and protease inhibitors (10 mmol/L sodium pyrophosphate, 1 mmol/L phenylmethylsulfonyl fluoride, 2 mmol/L sodium orthovanadate, and 10 μg/mL aprotinin). Cellular debris was removed by centrifugation. Protein concentration measurements were done using the Micro-BCA Protein Assay Kit (Pierce). For immunoprecipitations, whole-cell lysates were combined with antibody and 30 μL protein A/G-Sepharose slurry (GE Healthcare). The samples were incubated for 3 h on a rotation wheel at 4°C. The precipitates were washed three times with HNTG buffer [20 mmol/L HEPES/NaOH (pH 7.5), 150 mmol/L NaCl, 10% glycerol, 0.1% Triton X-100, and 10 mmol/L sodium pyrophosphate] suspended in 3× SDS sample buffer, boiled for 3 min, and subjected to SDS-PAGE. For Western blot analysis, proteins were transferred to nitrocellulose membranes and incubated with the appropriate antibodies. Signals were developed via an enhanced chemiluminescence detection system (Perkin-Elmer). Before reprobing, membranes were stripped with 65 mmol/L Tris-HCl (pH 6.8) containing 2% SDS at 50°C for 1 h. For the quantification of immunoblots, scanned images were analyzed with Aida Image Analyzer software (Raytest).

RNA interference. Transfection of 21-nucleotide siRNA duplexes (Ambion) for targeting endogenous genes was carried out using Lipofectamine 2000 (Invitrogen) and 0.84 μg siRNA duplexes per 6-well plate as described. Transfected cells were assayed on indicated time points. Sequences of siRNA used were FGFR4 5′-GCGUCUACGCGGAAUACUUC-3′ and GL2-luciferase 5′-CGUACCGCGAAUACGUIC-3′. Specific silencing of FGFR4 was confirmed by immunoblot analysis as described before.

Cell death assays. Cell death was measured by counting the percentage of hypodiploid cells using flow cytometry as described previously. Propidium iodide fluorescence was measured in the FL2 channel on a FACS Calibur (BD Biosciences). Cell culture supernatant and trypsinized cells were pooled.

cDNA array preparation. Gene expression analysis was performed by hybridization of nylon filter arrays with radioactive targets (cDNA). The homemade arrays comprised 1,100 gene inserts (all protein kinases, protein phosphatases, and other signaling proteins). The vector with different gene inserts was spotted with a biogrid roboter in triplicates.

cDNA array hybridization. Radioactive labeling of the cDNAs was achieved using the Megaprime DNA labeling kit (Amersham Biosciences) and 50 μCi [α-33P]ATP per reaction. The labeled cDNA was purified via the Nucleotide Removal Kit (Qiagen) and incubated with 0.5 mg/mL COT-DNA (Invitrogen) in hybridization buffer (5× SSC, 0.1% SDS) for 5 min at 95°C and 30 min at 68°C to block repetitive sequences in the cDNA. The cDNA was added to prehybridized (68°C) hybridization buffer containing 100 μg/mL tRNA (baker’s yeast; Roche Applied Science). The cDNA arrays were incubated in prehybridization buffer (5× Denhardt’s, 5× SSC, 100 mmol/L NaPO4, 2 mmol/L Na2P2O7, and 100 μg/mL tRNA) for 4 h and subsequently with the labeled cDNA in hybridization buffer for 16 h.

Statistical analysis. The raw DNA microarray intensity data were pre-processed using total intensity normalization. Mean gene expression of 10 independent arrays of MDA-MB-453 cell line and at least 3 independent arrays of doxorubicin-treated clones were compared.

Results

Resistance of MDA-MB-453 breast cancer cells to doxorubicin correlates with FGFR4 up-regulation. We performed an assay to identify antiapoptotic mechanisms in the breast cancer cell line MDA-MB-453. Cells were treated with 0.5 μM doxorubicin for 48 h and surviving clones were isolated after 8 weeks.

On average, a 2-fold up-regulation of FGFR4 mRNA was found in clones surviving the doxorubicin treatment when the gene expression profiles generated by array analysis were compared (Fig. 1A). About half of the clones showed an up to 4-fold increase in FGFR4 mRNA levels, whereas others showed weak or no increase (Fig. 1B). The FGFR4 expression levels were confirmed by semiquantitative PCR analysis (Supplementary Fig. S1).

FGFR4 expression affects resistance of cancer cells to doxorubicin. To study the role of FGFR4 in chemoresistance, we used two of the resistant MDA-MB-453 clones (453R1 and 453R9) for stable knockdown of FGFR4 by FGFR4 siRNAs (si66 and si74). Transfection of siRNAs 66 and 74 efficiently suppressed receptor expression in 453R1 (Fig. 2A) and 453R9 (data not shown) cells and exhibited a significant increase in the sensitivity to apoptosis induction by doxorubicin treatment when compared with control siRNA-infected cells (Fig. 2A).

Furthermore, we stably overexpressed the FGFR4 in MCF7 breast cancer cells, which do not express FGFR4 endogenously (Fig. 2B). As expected, MCF7-FGFR4-overexpressing cells treated with doxorubicin showed less apoptosis than MCF7-parental control cells (Fig. 2B).

In addition, we analyzed the apoptosis rate of MCF7 cells ectopically overexpressing FGFR4 and 453R1 cells after knockdown of FGFR4 expression at different doxorubicin concentrations. In MCF7-FGFR4 cells, apoptosis was reduced at different doxorubicin concentrations. Consequently, the 453R1 FGFR4 knockdown cells showed higher apoptosis rates compared with control 453R1 cells (Fig. 2C).

Finally, we analyzed if ectopic overexpression of FGFR4 in MCF7 or a FGFR4 knockdown in 453R1 cells affects sensitivity against other chemotherapeutic drugs such as cyclophosphamide, cisplatin, or Taxotere. Interestingly, only with cyclophosphamide reduced apoptosis sensitivity was
observed in MCF7-FGFR4-overexpressing cells. Accordingly, 453R1 siRNA 66 and 74 cells exhibit a higher apoptosis rate than the control cells when treated with cyclophosphamide (Fig. 2D). However, no effect could be observed when cells were treated with Taxotere and cisplatin. These experiments show that overexpression of FGFR4 increases apoptosis resistance, whereas a stable knockdown of FGFR4 sensitizes cancer cells toward specific chemotherapeutic drugs.

Gene expression analysis of ectopic FGFR4-expressing cells reveals increased Bcl-xl levels. To gain further insight into the molecular mechanism mediating the chemoresistance by FGFR4, we performed a gene expression array analysis of MCF7 cells ectopically expressing FGFR4. Here, we could detect the Bcl-xl expression to be 2-fold up-regulated (Fig. 3A). We validated the differences in expression levels of Bcl-xl by semiquantitative PCR (Fig. 3A). Furthermore, an increased amount of Bcl-xl protein was detected in the FGFR4-expressing MCF7 cell line (data not shown). One of the FGFR4 downstream signaling pathways is the MAPK cascade. As MAPK cascade is also an important regulator of Bcl-xl expression,
MCF7-FGFR4 cells were treated with UO126, a specific MEK inhibitor. The semiquantitative PCR showed that UO126 treatment abolished Bcl-xL expression (Fig. 3B). Therefore, we conclude that Bcl-xL expression is dependent on FGFR4 expression and MAPK activity.

Knockdown of FGFR4 affects MAPK signaling pathway and Bcl-xL expression. To prove the concept of FGFR4 mediating drug resistance by activating MAPK cascade and thereby increasing Bcl-xL levels, we used different breast cancer cell lines, which are endogenously expressing high amounts of FGFR4. As expected, the transient knockdown with synthetic siRNA directed against FGFR4 (siFGFR4) in BT-474 breast cancer cells showed a reduced apoptosis rate on several time points (Fig. 4A).

On FGFR4 knockdown, we could observe lower phospho-ERK levels at 96 h after transfection than in control experiments (Fig. 4B). However, Akt phosphorylation was unchanged by the knockdown (data not shown).

As expected, the transient knockdown of FGFR4 also affected Bcl-xL expression. One hundred forty-four hours after transfection, we could detect reduced Bcl-xL protein levels (Fig. 4C). These results showed that Bcl-xL expression is partially dependent on FGFR4 expression and could be suppressed by FGFR4 knockdown.

A FGFR4-blocking antibody inhibits FGF19-induced MAPK activation and reduces the chemoresistance of doxorubicin-treated cancer cells. Next, we wanted to address the question if the enhanced chemoresistance is linked with the FGFR4 activity in the different cancer cell lines. For this purpose, we generated a FGFR4-blocking antibody (10F10). We stimulated various breast cancer cell lines, which endogenously express FGFR4, with its specific ligand FGF19. When FGFR9 treated, phospho-ERK levels were increased compared to not stimulated control cells. After preincubation of the cell lines with the FGFR4-blocking antibody and subsequent FGF19 stimulation, reduced phospho-ERK levels compared with sole FGF19-treated cancer cell lines could be detected (Fig. 5A). In these experiments, we clearly could show that the FGFR4-blocking antibody (10F10) is inhibiting the FGFR4-mediated downstream signaling.

To verify if the blocking of the FGFR4 activity results in increased sensitivity to chemotherapeutic drugs, we incubated the breast cancer cell lines MDA-MB-361, BT-474, and ZR75-1 with doxorubicin, VSV/10F10 antibody, or in different combinations and measured the apoptosis rate after 48 h of doxorubicin treatment. Here, we could show that the blocking of FGFR4 by 10F10 antibody resulted in increased apoptosis rate after doxorubicin induction, whereas the VSV antibody incubation had no effect on apoptosis (Fig. 5B).

In cDNA macroarray experiments, we could observe that FGFR4 is highly expressed in ~20% of the available breast cancer cell lines, which is consistent with other published data in human cancers (refs. 19, 20; Fig. 5C). High expression of the receptor was observed in the cell lines BT-474, ZR75-1, MDA-MB-361, and MDA-MB-453. Therefore, these cell lines were used to inhibit FGFR4 signaling and to sensitize the cancer cells for chemotherapy. We compared the apoptosis rates of FGFR4/doxorubicin-treated cancer cell lines with cell lines additionally treated with FGFR4-blockading antibody. Doxorubicin/FGFR4 treatment of the different cell lines induced apoptosis in a low to medium extent, whereas the additional treatment with FGFR4-blockading antibody significantly further increased the apoptosis rates (Fig. 5D): ~40% in MDA-MB-361, ~29% in ZR75-1, and ~10% in BT-474. In MDA-MB-453 cells, we could not detect increased apoptosis with 10F10 antibody treatment. This might be due to the constitutive activation of the FGFR4 in this cell line, which could not be blocked by the 10F10 antibody (data not shown). However, in these experiments, we could show that FGFR4 activity supports resistance of cancer cells to doxorubicin, whereas inversely the inhibition of FGFR4 activation by a FGFR4-blocking antibody (10F10) leads to increased chemosensitivity of cancer cells.

The Oncomine database (21) allows searching for genes, which are up-regulated in tumors, in many published array experiments. For FGFR4, we observed a significant increase in expression in oncogenic transformed tissues compared with normal tissues in six different studies (Supplementary Fig. S2). This shows the frequency of FGFR4 overexpression and the relevance of FGFR4 signaling in human cancers. Therefore, we analyzed the efficacy of the blocking antibody 10F10 in cancer cell lines of other origin than breast tissue. As an example, we used the 769-P kidney carcinoma and the TE671 glioblastoma cell lines. Likewise, additional treatment with the blocking antibody increased the apoptosis rate in these cells (Fig. 5D).

Discussion

Mitotic instability is a hallmark of cancer and allows tumor cells to adapt to the changing microenvironment when drugs are applied. Chemotherapy imposes such a selective process and the tumor cells harboring genetic changes benefit under such conditions and survive (22). We tried to simulate the selective process in normal cell culture conditions by treating MDA-MB-453, a breast cancer cell line, with the commonly

![Fig. 3. FGFR4 governs Bcl-xL expression. A, array analysis of Bcl-xL expression in MCF7-Mock and ectopically expressing FGFR4 cells. Validation by semiquantitative reverse transcription-PCR of Bcl-xL expression in MCF7 cells. B, MCF7-FGFR4 cells were treated with 10 μmol/L U0126 for 6 and 12 h. The cDNA of U0126 treated cells was used to perform reverse transcription-PCR with Bcl-xL primers. The integrity and amount of cDNA used in each reverse transcription-PCR were measured by GAPDH amplification.](image-url)
used chemotherapeutic drug doxorubicin. The doxorubicin-resistant cancer cells were used to analyze the gene expression changes, which could be advantageous for the cells to withstand doxorubicin treatment. FGFR4 expression was significantly higher in the resistant clones, and as FGFRs are known to provide apoptotic resistance (23), we predicted it to be one of the events that take place during the selection process. This change in expression has presumably occurred at transcriptional level as there was found no gene amplification for FGFR4 in resistant clones (data not shown).

The FGFR4 gene is known to be regulated by two transcription factors Ikaros and HNF1 (24–27) where deregulation could result in increased expression. Therefore, it would be interesting to study if such a regulatory mechanism exists in chemoresistant tumors. A comparison of published gene expression studies revealed the increased expression of FGFR4 in several breast cancer cell lines (Fig. 5C) and oncogenically transformed tissues (Supplementary Fig. S2). Various studies found an overexpression of FGFR4 in breast and other cancer types (19, 20, 25, 27, 28). This, for example, leads to an unfavorable disease progression and reduced survival of the patients with prostate cancer and astrocytomas. We could show an antiapoptotic effect of FGFR4 by overexpression as well as repression of the mRNA by siRNA experiments. This effect could only be observed when cells were treated with doxorubicin or cyclophosphamide, both DNA-damaging agents, suggesting common mechanisms of these drugs. Several polymorphisms of FGFR family members have been reported and linked to different diseases (29–31). Up to now, antiapoptotic effects have only been reported for FGFR1 and FGFR3 (23, 32). Translocation or constitutive active mutations of FGFR1 or FGFR3 result in hyperactivity and can lead to
activation or up-regulation of antiapoptotic proteins such as STATs, McI1, Bcl2, or Bcl-xl (33, 34), which contribute to cancer cell survival. Inhibition of FGFR1 or FGFR3 by inhibitors, antisense approaches, or short hairpin RNA experiments induced apoptosis in cells of different origin (17, 35). Apart from that, we show that FGFR4 also provides apoptotic resistance.

Although FGFR activation is associated with multiple physiologic effects, the induction or repression of apoptosis is increasingly intriguing considering recent reports. FGFR activation leads to MAPK or phosphatidylinositol 3-kinase signaling depending on the cellular context (12). Recently, it has been reported that FGFR signaling converges the HER-2 signaling and modulates translation (10). Although all these observations have been instrumental in our understanding of FGFR signaling, the majority of them struggles with the intrinsic redundancy of FGFRs and cannot associate specific FGFRs with these effects and therefore warrants a candidate-based approach to study their functions. Our studies overcome these problems as we have used overexpression, siRNA knockdown, specific blocking antibody approaches, and the specific FGFR4 ligand FGF19 (36) to implicate the FGFR4 as an important component of antiapoptotic signaling in cancers during the chemotherapeutic treatment. The Bcl-xl and FGFR4 coexpression in endogenous and overexpressing systems and the direct dependence of Bcl-xl expression on FGFR4 expression is of immense relevance. Bcl-xl as a prominent downstream effector molecule in antiapoptosis is also up-regulated in various tumors such as breast, squamous cell, and liver carcinoma (37–39). It is well known that Bcl-xl is responsible for the accomplishment of chemoresistance of cancer cells against, for example, doxorubicin, leading to decreased apoptosis and increased survival (40, 41).

It is shown that MAPK activation via various signaling pathways leads to the expression of antiapoptotic molecules

**Fig. 5.** Usage of a FGFR4-blocking antibody mediates inhibition of FGF19-induced MAPK activation and decreases the chemoresistance of doxorubicin-treated cancer cells. A, different breast cancer cell lines (BT-474, MDA-MB-361, and ZR75-1) were starved 48 h with 0% FCS/medium. Cells were treated with either FGF19 alone or 10F10 plus FGF19 and compared with the untreated control cells. Stimulation and inhibition of the cells is shown by a TL immunoblot with phospho-ERK antibody. B, apoptosis rate of BT-474, MDA-MB-361, and ZR75-1 breast cancer cell line treated with 2 μmol/L doxorubicin for 48 h and/or different stimuli. C, cDNA macroarray analysis shows the relative FGFR4 expression in various breast cancer cell lines. D, induction of apoptosis with doxorubicin in FGF19-treated or FGF19/10F10-treated breast cancer cell lines showing high FGFR4 expression. Quantification of the apoptosis rates by propidium iodide staining and subsequent fluorescence-activated cell sorting analysis. Apoptosis rate of two cancer cell lines with other origin than mammary gland assessed as described above.
and the rescue of cells from apoptosis. On the other hand, the MAPK has also been implicated in apoptosis induction actively via up-regulation of caspases, which could be reversed by blocking MEK (42). Therefore, it is attractive to hypothesize that certain growth signals converge the MAPK pathway and, in addition to activating it, lead to up-regulation of both apoptotic and antiapoptotic molecules depending on the cellular context. Furthermore, MAPK signaling is indispensable for up-regulation, as Bcl-xl expression has been reduced to basal levels on UO126, a specific MEK inhibitor, treatment (Fig. 4C).

In principle, STAT3 or STAT5, among other transcription factors, is known to be mainly responsible for activating Bcl-xl transcription in epithelial cancers (43). We have not been able to see any difference in phospho-STAT expression levels in our experiments, which let us predict the existence of another alternative mechanism how FGFR4 signaling controls Bcl-xl expression. In another study, it was shown that Bcl-xl expression is dependent on prolactin stimulation (44). Interestingly, FGFR1 stimulation of FGFR4 is increasing the prolactin promoter activity (45). These observations might be the basis to study the link between FGFR4 and Bcl-xl expression. As Bcl-xl expression is very often counteracting, the chemotherapy of cancer patients, small peptide antagonists, or antisense oligonucleotides against Bcl-xl are already used in the clinical therapy to extend the disease-free survival time of cancer patients (46–49).

Our findings open up new possibilities in cancer therapy. Patients can be treated with alternative therapeutic drugs or antibodies against FGFR4 to decrease the receptor tyrosine kinase expression or activity, which leads to the expression of antiapoptotic molecules (e.g., Bcl-xl) during the applied chemotherapy. Consequently, FGFR4 could serve as a prognostic marker and target for novel cotherapies in cancer patients.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**

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