Erythropoietin Receptor Expression and Correlation to Tamoxifen Response and Prognosis in Breast Cancer

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Abstract Purpose: The main function of erythropoietin (EPO) is to stimulate erythropoiesis. EPO receptors (EPOR) are expressed in other cell types, including tumor cells, suggesting that the EPO/EPOR pathway governs additional cellular processes besides erythropoiesis. Recombinant EPO (rhEPO) is frequently given to anemic cancer patients, although data on clinical outcome are conflicting. In an attempt to understand these clinical data, we analyzed EPO and EPOR expression in breast cancer and evaluated EPOR as a putative prognostic and predictive marker in breast cancer patients treated with tamoxifen.

Experimental Design: EPO mRNA/protein and EPOR mRNA were quantified by PCR and ELISA, respectively. Tissue microarrays containing 500 breast tumors from premenopausal women randomized to tamoxifen or no adjuvant treatment were evaluated for EPOR expression by immunohistochemistry. Predictive and prognostic information was evaluated using Kaplan-Meier curves and log-rank tests to estimate recurrence-free survival (RFS).

Results: EPO and EPOR were expressed in cultured cells, and breast tumor specimens expressed EPOR at varying levels. Tamoxifen treatment significantly increased RFS in patients with estrogen receptor–positive/progesterone receptor–positive (ER+/PR+) tumors with low EPOR expression (P = 0.001) but had no effect on RFS in patients with tumors with high EPOR expression (P = 0.98). In the untreated cohort, RFS was significantly improved for patients with ER+ tumors with high EPOR expression.

Conclusion: EPOR is abundantly expressed in breast cancer specimens. The fact that high expression of EPOR is related to an impaired tamoxifen response in ER+/PR+ tumors and to improved survival in untreated patients suggests that EPOR expression in breast cancer affects tumor behavior. (Clin Cancer Res 2009;15(17):5552–9)

Erythropoietin (EPO) is a 30.4-kDa glycoprotein hormone produced mainly in the adult kidney and in fetal liver primarily in response to hypoxia (1, 2). Its main function is to stimulate erythropoiesis. The biological effects are mediated through the binding to the erythropoietin receptor (EPOR). In erythroid precursors, receptor binding of EPO induces the activation of intracellular signaling cascades that lead to enhanced proliferation, differentiation, and survival (3–5). EPO production has been found in erythroid progenitors that co-express EPOR, and an autocrine-paracrine mechanism of erythropoiesis regulation has been implicated (6). EPO expression has also been shown in nonhematopoietic cells and tissues like endothelial cells (7), brain (8), female genital tract (9), placenta (10), myoblasts (11), kidney (12), and intestine (13). The function(s) of EPO/EPOR in these tissues is not known in any detail. In recent years, EPO and EPOR expression has also been detected in various cancer forms, including breast cancer (14–18). Based on these findings, autocrine or paracrine growth-stimulatory EPO-EPOR loops in malignant tumors have been suggested (19), although
Erythropoietin Receptors in Breast Carcinoma

Translational Relevance

Erythropoietin receptor (EPOR) mRNA is detected in many normal and malignant nonhematopoietic cells, including breast cancer, but EPOR protein expression in tumor cells has been questioned. We show EPOR expression at the protein level in breast cancer cells. Thus, treatment of anemic patients with breast cancer with recombinant EPO might result in direct effects on tumor cell behavior. Using a unique breast tumor material from patients receiving either tamoxifen or no treatment at all, we find that EPOR levels correlate to tamoxifen response in patients with estrogen receptor-positive (ER+) tumors, suggesting that EPOR is functional in these tumors and that a cross-talk between the EPO/EPOR and ER pathways may exist. Our data imply that direct tumor effects of recombinant EPO in women with ER+ breast cancer have to be considered and further evaluated. The correlation between EPOR expression and tamoxifen response also suggests that EPOR could be used as a predictive factor for tamoxifen response.

Materials and Methods

Patient material, tissue microarray, and immunohistochemistry. During 1984 to 1991, 564 premenopausal patients or patients <50 y with stage II (pT2N0M0, pT2N1M0) invasive breast cancer were enrolled in a randomized trial of 2 y of tamoxifen treatment with a daily dosage of either 40 mg (study center 1) or 20 mg (study center 2) or no adjuvant treatment. All node-positive patients received locoregional postoperative radiotherapy, but <2% of the patients received adjuvant polychemotherapy or goserelin. The median follow-up time for patients without breast cancer event was 13.9 y. The study design is described in detail elsewhere (25).

Paraffin-embedded specimens from 500 cases included in the study described above could be retrieved from the archives (the study was approved by the Ethics Committees at Lund and Linköping University). Representative areas with invasive cancer were marked on H&E-stained slides and two 0.6-mm tissue cores were taken from each donor block and 5-µm-thick in triplicate recipient blocks using an automated arrayer (ATA-27, Beecher, Inc.) as described previously (26). Four-micrometer sections were dried, deparaffinized, rehydrated, and microwavetreated for 2 × 5 min in TR-buffer high pH (DAKO), before being stained in a Techmate 500 machine (DAKO) using the C-20 anti-EPOR antibody (sc-695, Santa Cruz Biotechnology; dilution 1:300). Staining intensity was evaluated semiquantitatively using a classification from 1 to 3, representing low staining intensity (grade 1), intermediate staining intensity (grade 2), or high staining intensity (grade 3). The tissue microarray was evaluated separately by two independent observers blinded to clinical outcome data. Conflicting results (<10%) were revised and a consensus was reached. Estrogen receptor (ER) status, progesterone receptor status (PR), vascular endothelial growth factor (VEGF)-A, VEGF receptor 2 (VEGFR2), and HIF-1 were evaluated by immunohistochemistry and scored as previously described (27, 28). Cultured cells were harvested, pelleted, fixed and treated as surgical specimens, and paraffin-embedded. Immunohistochemistry was done as described above.

Cell cultures. The human breast cancer cell lines MCF7, T-47D, and MDA-MB-468 were maintained under standard conditions as described (29). Hepatoma HepG2 cells were grown in RPMI 1640 and the erythroleukemic EPO-dependent cell line UT7 (30) in αMEM with 2 units rhEPO/mL, supplemented with 10% FCS and antibiotics. Hypoxia experiments were done in a hypoxia chamber at 1% O2 as described (29). For small interfering RNA (siRNA) knockdown experiments, MCF7 and UT7 cells were grown in six-well plates and transfected with siRNA against either EPOR or HSPA5 (coding for HSP70 protein 5), using Lipofectamine in OptiMEM for 4 to 6 h as described (31). Scrambled nonspecific or no siRNA was used as controls. Sequences are given in Supplementary Table S1. After transfection, cells were grown under standard conditions and harvested for RNA or protein, or fixed and embedded for immunohistochemistry.

Quantitative real-time PCR. RNA extraction, cDNA synthesis, and quantitative PCR were done as previously described (32). For relative quantification of expression levels, the comparative Ct method was used (33). Expression levels of the investigated genes were normalized to the expression of three housekeeping genes (SDHA, YWHAZ, and UBC for MCF7, T-47D, and UT7 cells; SDHA, YWHAZ, and GAPDH for MDA-MB-468 cells). Primers were designed using Primer express (Applied Biosystems) and primer sequences are specified in Supplementary Table S1.

Western blot and EPO ELISA. Breast cancer and UT7 cells were harvested and lysed in radioimmunoprecipitation assay buffer, and equal amounts of protein were processed by SDS-PAGE for Western blotting as described (34). EPOR protein was detected using the polyclonal C-20 anti-EPOR antibody (27). Expression of EPOR in the investigated cell lines was evaluated semiquantitatively using a classification from 1 to 3, representing low intensity (grade 1), intermediate intensity (grade 2), or high intensity (grade 3). For small interfering RNA (siRNA) knockdown experiments, MCF7 and UT7 cells were grown in serum-free medium for 24 h at 21% or 1% O2. EPO in culture supernatants was determined using a commercial ELISA (R&D Systems), according to the manufacturer’s protocol. To determine intracellular EPO content, cells were lysed in NP40 buffer [1% NP40, 10% glycerol, 20 mmol/L Tris-HCl (pH 8.0), 137 mmol/L NaCl] containing complete protease inhibitor (Roche), and equal amounts of protein were analyzed using ELISA. HepG2 cells were used as positive control for EPO production.
Statistics. The $\chi^2$ test was used for comparison of categorized tumor variables and the Mann-Whitney $U$ test for continuous variables. Survival analyses were done according to the intention-to-treat rule, and recurrence-free survival (RFS) included local, regional, and distant metastasis and breast cancer-related death as primary events. RFS was illustrated using Kaplan-Meier plots and the log-rank test was used to test for equality of survivorship curves. Corresponding hazard ratios (HR) with 95% confidence intervals (95% CI) for RFS were calculated using the Cox proportional hazards model in univariate and multivariate analysis. The null hypothesis of tamoxifen treatment predictive effect by EPOR status in hormone receptor-positive patients was evaluated using a Cox model with a term for the interaction between tamoxifen treatment and EPOR status. All statistical analyses were two sided, and $P \leq 0.05$ was considered statistically significant. The statistical calculations were done using SPSS version 12 (SPSS, Inc.).

Results

EPO and EPOR are expressed in cultured breast cancer cells and breast tumor specimens. EPO mRNA was detected in normoxic ER+ (MCF7, T-47D) and ER- (MDA-MB-468) breast cancer cells, although the expression levels differed substantially in the three tested cell lines (Supplementary Fig. S1A-C). At hypoxic conditions (1% $O_2$), the EPO mRNA levels increased in all cell lines. Surprisingly, no detectable EPO protein was found in breast cancer cell-conditioned medium (Supplementary Fig. S1D) even after 20-fold concentration or in cell homogenates from these cells (data not shown). As a positive control, conditioned medium from the EPO-expressing HepG2 cells was used. In these cells, EPO expression is induced by hypoxia and, as expected, the concentration of EPO protein increased in conditioned medium at hypoxic growth conditions (Supplementary Fig. S1D). Taken together, our results suggest that EPO is expressed and up-regulated by hypoxia in breast cancer cells, but the EPO protein does not seem to be produced in detectable amounts at normoxia or hypoxia in these cells under the growth conditions investigated.

Quantitative PCR further showed constitutive EPOR expression in all cell lines tested with robust mRNA levels at normoxia that slightly increased at prolonged hypoxia (Fig. 1A-C). EPOR was also expressed at the mRNA level in breast tumor homogenates and in normal breast tissue (Fig. 1D).

Immunodetection of EPOR and antibody specificity. We next wanted to verify EPOR expression in breast cancer cells and tissues at the protein level. Although EPOR protein expression has been reported in tumor tissues, including breast cancer, by using the C-20 antibody (14, 18), the specificity of this anti-EPOR antibody has recently been questioned (20, 21). We therefore tested the specificity of the C-20 antibody with focus on its use in immunohistochemistry, using MCF7 and the EPO-dependent UT7 erythroleukemia cells with EPOR.
expression knocked down by siRNA. The knockdown effect in MCF7 cells at mRNA level was efficient with an 80% reduction in EPOR mRNA compared with controls transfected with scramble siRNA (Fig. 2A). At the protein level, a reduction in EPOR in MCF7 cells was also seen, using UT7 erythroleukemia cells as positive controls to identify the EPOR specific band (≈66 kDa; Fig. 2B). As described by several laboratories, the C-20 antibody detects more than one protein (20, 21), of which some have been referred to as molecular weight variants of EPOR (35). Without further optimizing our Western blot conditions, we saw three additional bands in MCF7 cell lysates, including a protein migrating in the 70- to 80-kDa region claimed to be a HSP70 protein, where HSP70-5 shares a 6-amino-acid-long peptide sequence with the C-20 epitope (20). We consider these bands unspecific as they were not reduced by EPOR siRNA treatment (Fig. 2B).

Immunostaining of fixed, embedded, and sectioned siRNA-transfected MCF7 cells using the C-20 anti-EPOR antibody revealed a marked decrease in immunoreactivity in the EPOR knockdown cells compared with controls (Fig. 2C-D). Likewise, EPOR protein, mRNA in, and immunostaining of siRNA-treated UT7 erythroleukemia cells, expressing functional EPOR, were also drastically reduced (Fig. 2E-H). In both MCF7 and UT7 cells, immunohistochemistry staining was cytoplasmic and plasma membrane associated. Furthermore, knockdown of HSPA5 (encoding HSP70-5) by siRNA in MCF7 cells did not affect EPOR immunostaining intensities or Western blot EPOR signal in these cells (Supplementary Fig. S2B-C).

To optimize our EPOR immunohistochemistry protocol, sections with ductal carcinoma in situ were stained, revealing a strong, predominantly cytoplasmic EPOR expression (Supplementary Fig. S2A) with no discernable difference between normoxic and the hypoxic cells adjacent to necrotic zones, as defined previously (36). Importantly, the anti-EPOR antibody stained only tumor cells proper and not tumor stroma, and antibody block with the immunization peptide impaired the immunostaining (Supplementary Fig. S2A). In conclusion, our C-20 antibody immunohistochemistry protocol primarily detects EPOR epitope(s) and does so with high sensitivity, although inherent in the technique, an element of nonspecific staining can never be completely ruled out.

**EPOR expression in breast cancer specimens from patients in a randomized tamoxifen treatment trial.** Using a tissue microarray with tumors from the tamoxifen trial described in Materials and Methods, EPOR expression was investigated by immunohistochemistry. Evaluation of EPOR staining was possible...
in 382 cases; 118 cases were missing due to loss of tissue cores in the staining process or lack of invasive tumor cells in the cores. The clinicopathologic characteristics in the group of tumors with EPOR expression information did not differ from the total material (Supplementary Table S2). EPOR expression was present in tumors with varying staining intensity. Weak expression was found in 25, intermediate expression in 187, and high expression in 170 tumors. To obtain statistical power, tumors with low (grade 1) and intermediate expression (grade 2) were further analyzed together and referred to as low expression as opposed to tumors with high expression (grade 3).

Table 1 summarizes the correlations between EPOR expression and important patient and tumor characteristics. EPOR expression was significantly associated with age (P = 0.001) and inversely associated with tumor size (P = 0.011). There was also a difference in EPOR expression with respect to tumor types (Table 1). There was no association between EPOR expression and hormone receptor status or Nottingham histologic grade (NHG). To investigate a putative effect of hypoxia on EPOR expression, we evaluated HIF-1α expression in relation to EPOR expression but found no significant correlation. There was a positive correlation between EPOR and VEGFR2 (P = 0.005), but not VEGF-A expression (Table 1).

EPOR expression in relation to tamoxifen response and prognosis. In patients with ER+ and/or PR+ tumors and low expression of EPOR, tamoxifen significantly increased RFS (P = 0.001), whereas in tumors with a high expression of EPOR, no statistically significant difference was detected in the tamoxifen-treated patient cohort compared with controls (P = 0.98; Fig. 3). Similar results were obtained when subgroups of ER+ or PR+ tumors, respectively, were analyzed separately (data not shown). Using a Cox proportional hazards model including a treatment interaction variable [EPOR (low/high) × TAM (±)], there was a significant association between tamoxifen treatment response and EPOR expression (HR, 2.3; 95% CI, 1.1-5.0; P = 0.03). When adjusting this model for age, tumor size, nodal status, and NHG, this association was still significant (HR, 2.2; 95% CI, 1.0-4.8; P = 0.05; Table 2).

Table 1. EPOR expression in relation to different clinicopathologic variables

<table>
<thead>
<tr>
<th>Category</th>
<th>EPOR, low expression</th>
<th>EPOR, high expression</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>n = 212 (%)</td>
<td>n = 170 (%)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>43.2 (25-56)</td>
<td>45.2 (26-57)</td>
<td>0.001</td>
</tr>
<tr>
<td>&lt;40</td>
<td>40 (18.9)</td>
<td>25 (14.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>40-49</td>
<td>156 (73.6)</td>
<td>109 (64.1)</td>
<td></td>
</tr>
<tr>
<td>≥50</td>
<td>16 (7.5)</td>
<td>36 (21.2)</td>
<td></td>
</tr>
<tr>
<td>Tumor size (mm)</td>
<td>n = 212 (%)</td>
<td>n = 170 (%)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>26.4 (2-50)</td>
<td>24.3 (8-75)</td>
<td>0.012</td>
</tr>
<tr>
<td>&lt;20</td>
<td>59 (27.8)</td>
<td>68 (40.2)</td>
<td>0.011</td>
</tr>
<tr>
<td>≥20</td>
<td>153 (72.2)</td>
<td>101 (59.8)</td>
<td></td>
</tr>
<tr>
<td>Tumor type</td>
<td>n = 212 (%)</td>
<td>n = 170 (%)</td>
<td></td>
</tr>
<tr>
<td>IDC/ductal</td>
<td>175 (85.0)</td>
<td>154 (92.2)</td>
<td>0.004</td>
</tr>
<tr>
<td>ILC/lobular</td>
<td>19 (9.2)</td>
<td>2 (1.2)</td>
<td></td>
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<tr>
<td>IMC/medullary</td>
<td>12 (5.8)</td>
<td>11 (6.6)</td>
<td></td>
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<tr>
<td>Node status</td>
<td>n = 212 (%)</td>
<td>n = 170 (%)</td>
<td></td>
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<tr>
<td>0</td>
<td>64 (30.2)</td>
<td>44 (25.9)</td>
<td>0.63</td>
</tr>
<tr>
<td>1-3</td>
<td>100 (47.2)</td>
<td>85 (50.0)</td>
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</tr>
<tr>
<td>&gt;4</td>
<td>47 (22.2)</td>
<td>41 (24.1)</td>
<td></td>
</tr>
<tr>
<td>NHG</td>
<td>n = 212 (%)</td>
<td>n = 170 (%)</td>
<td></td>
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<tr>
<td>1</td>
<td>18 (8.8)</td>
<td>24 (14.4)</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>86 (42.0)</td>
<td>61 (36.5)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>101 (49.3)</td>
<td>82 (49.1)</td>
<td></td>
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<tr>
<td>ER</td>
<td>n = 212 (%)</td>
<td>n = 170 (%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>69 (33.0)</td>
<td>52 (31.3)</td>
<td>0.73</td>
</tr>
<tr>
<td>Positive</td>
<td>140 (67.0)</td>
<td>114 (68.7)</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>n = 212 (%)</td>
<td>n = 170 (%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>75 (36.4)</td>
<td>54 (32.9)</td>
<td>0.49</td>
</tr>
<tr>
<td>Positive</td>
<td>131 (63.6)</td>
<td>110 (67.1)</td>
<td></td>
</tr>
<tr>
<td>Ki-67 index (%)</td>
<td>n = 212 (%)</td>
<td>n = 170 (%)</td>
<td></td>
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<tr>
<td>0-1</td>
<td>24 (12.4)</td>
<td>21 (14.0)</td>
<td>0.57</td>
</tr>
<tr>
<td>2-10</td>
<td>57 (29.5)</td>
<td>44 (29.3)</td>
<td></td>
</tr>
<tr>
<td>10-25</td>
<td>51 (26.4)</td>
<td>43 (28.7)</td>
<td></td>
</tr>
<tr>
<td>25-50</td>
<td>27 (14)</td>
<td>25 (16.7)</td>
<td></td>
</tr>
<tr>
<td>&gt;50</td>
<td>34 (17.6)</td>
<td>17 (11.3)</td>
<td></td>
</tr>
<tr>
<td>VEGFR2</td>
<td>n = 212 (%)</td>
<td>n = 170 (%)</td>
<td></td>
</tr>
<tr>
<td>0, absent staining</td>
<td>51 (24.4)</td>
<td>23 (14.1)</td>
<td>0.005</td>
</tr>
<tr>
<td>1, weak staining intensity</td>
<td>79 (37.8)</td>
<td>54 (33.1)</td>
<td></td>
</tr>
<tr>
<td>2, intermediate staining intensity</td>
<td>58 (27.8)</td>
<td>53 (32.5)</td>
<td></td>
</tr>
<tr>
<td>3, intense staining intensity</td>
<td>21 (10.0)</td>
<td>33 (20.2)</td>
<td></td>
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</table>

Abbreviations: IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; IMC, invasive medullary carcinoma.
In the untreated cohort, RFS was significantly improved for patients with ER+ tumors with high compared with low EPOR expression (Fig. 4). In ER- tumors, there was no difference in RFS in relation to EPOR expression. The same pattern was seen when analyzing subgroups of PR+ versus PR- tumors (data not shown). By multivariate analysis, EPOR expression in ER+ tumors was still significantly associated with RFS (HR, 0.54; 95% CI, 0.33-0.90; \( P = 0.018 \)) after adjusting for age, tumor size, node, and NHG status (Table 2). To further corroborate these findings, we analyzed the data from a clinical breast cancer microarray study (37) with regard to EPOR expression in ER+ tumors and disease-specific survival. As shown in Fig. 4C and in support of our protein tissue microarray data, high EPOR expression correlated to favorable outcome, whereas patients with tumors with low EPOR expression had a poorer outcome.

**Discussion**

The recent observations of adverse effects of rhEPO on patient outcome (23, 24) raise the question of whether functional EPORs are expressed in tumor cells (20, 38) and whether this expression could affect tumor behavior and clinical outcome (39). Here we show that EPOR is expressed in breast cancer cell lines and breast tumor tissues, but as one would anticipate, EPOR expression levels differ among individual tumors. Regarding prognosis, high EPOR protein expression was significantly associated with an improved survival in untreated patients with hormone receptor-positive tumors. This association was significant also in multivariate analysis and seems to be an independent prognostic factor. Importantly, our immunohistochemistry-based EPOR protein data were corroborated by independent microarray analyses, showing that high EPOR mRNA in ER+ breast cancer associates with favorable outcome. Because a minority of patients in the Miller data set (37) had received adjuvant treatment with chemotherapy or endocrine treatment, the materials are not completely comparable but nevertheless suggest that EPOR expression is related to prognosis. The gene signature presented by Sörlie et al. (40) has given important information regarding breast cancer classification where ER has shown to be an important discriminator for the activity of other important genes. The finding that EPOR gives prognostic information in ER+ but not in ER- has to be further elucidated but can support that EPOR has a specific functional role associated with ERα.

We further show that high EPOR expression associates with an impaired tamoxifen response in ER+/PR+ tumors. The strong correlation between EPOR expression and tamoxifen response indicates that high EPOR expression could be involved in tamoxifen resistance and suggests that the EPORs in breast cancer tissues are functional, with capacity to transduce EPO-dependent responses. It has previously been shown that high VEGFR2 expression in the same tumor material correlates to...
impaired tamoxifen response (27), and we show here that EPOR and VEGFR2 protein levels also correlate positively. Furthermore, high expression of either receptor associates with poor tamoxifen response in hormone receptor–positive tumors. One potential mechanism behind these observations could be that activated growth factor receptors lead to activation of ER by tyrosine phosphorylation–induced pathways in an estrogen-independent way, thereby counteracting the effects of tamoxifen (41, 42). In addition, increased growth factor signaling has been suggested to cause a down-regulation of ER and thereby a more endocrine-independent phenotype (42). The observation that high EPOR expression correlates to improved prognosis in ER+ but not in ER- tumors from untreated patients is a novel finding, which together with the finding that tamoxifen effects correlate to EPOR expression levels could suggest a cross-talk between the ER and EPOR signaling pathways.

A randomized controlled trial with an untreated cohort is a unique opportunity to distinguish between prognostic and predictive information yielded by a biomarker or gene signature, whereas information from cohorts where all patients have been allocated to a specific treatment gives both prognostic and treatment predictive information. In this randomized study, we found that high EPOR expression correlated to better prognosis among untreated patients with ER+ tumors and tamoxifen resistance when analyzing the treatment effect. Similar data have been presented from our group regarding cyclin D1, which has been shown to be an indicator of treatment resistance as well as a biomarker of good prognosis (43). We then hypothesized that cyclin D1 might partially block the effect of tamoxifen on ERα, at the same time causing an estrogen-independent low activation (43). In line with this hypothesis, EPOR could theoretically also be involved in modulating ERα-dependent signaling.

With established EPOR expression in breast and other tumor cells follows the question of whether the adverse rhEPO effects on patient outcome involves rhEPO activation of its receptor expressed in tumor cells. EPOR protein has been proposed to be nonfunctional in tumor cells due to a non–cell surface location, and therefore, presumably, is not available for activation by rhEPO (38). Our immunohistochemistry data show both cytoplasmic and plasma membrane location of EPOR in breast cancer cells, and the correlation between EPOR expression and outcome and tamoxifen response suggests active EPOR in breast cancer, although we have not experimentally proved active plasma membrane–located EPOR in these cells. Furthermore, receptors can be activated by ligand-independent mechanisms, and we suggest that quantification of EPOR activity in cancer cells and tissues should be the next step in understanding the role(s) of EPOR in cancer disease. Thus, whether EPORs in breast cancer are active or can be activated by EPO or EPO independent mechanisms, or if EPOR expression merely is a marker of tamoxifen response and breast cancer prognosis, are open questions and need further attention.

The question of EPOR expression and the potential EPOR functionality in tumor tissue is clinically very important as rhEPO is a major treatment regimen for cancer patients with anemia. The research field has suffered from lack of a generally accepted methodology to assess EPOR protein and activity. Needless to say, with an increasing number of clinical EPO trials reporting adverse effects in the EPO arm (23, 24), the question of EPOR expression and direct effects of rhEPO on tumor cells has to be considered seriously. Our contribution unequivocally shows
EPOR expression in breast cancer at the mRNA and protein levels. We also show that EPOR expression is associated with prognosis and tamoxifen response, which might suggest that EPOR-activated pathways contribute to the overall clinical behavior of breast cancers. Our data highlight EPOR as a potential predictive marker of tamoxifen response in hormone receptor-positive tumors.

References
Clinical Cancer Research

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