Primary Tumor Levels of Tissue Inhibitor of Metalloproteinases-1 Are Predictive of Resistance to Chemotherapy in Patients with Metastatic Breast Cancer

Anne-Sofie Schrohl, Marion E. Meijer-van Gelder, Mads N. Holten-Andersen, Ib Jarle Christensen, Maxime P. Look, Henning T. Mouridsen, Nils Brünner, and John A. Foekens

Abstract

Purpose: Only about 50% of metastatic breast cancer patients benefit from cytotoxic chemotherapy. Today, no validated markers exist for prediction of chemotherapy sensitivity/resistance in this patient group. Tissue inhibitor of metalloproteinases-1 (TIMP-1) has been shown to protect against apoptosis, and the purpose of the present study was to test the hypothesis that tumors expressing high levels of TIMP-1 are protected against apoptosis-inducing agents and thus less sensitive to apoptosis-inducing chemotherapeutic drugs.

Experimental Design: We investigated the association between primary tumor expression levels of TIMP-1 protein and objective response to first-line chemotherapy in 173 patients with metastatic breast cancer.

Results: When analyzed as a continuous log-transformed variable, increasing TIMP-1 levels were significantly associated with lack of response to cyclophosphamide/methotrexate/5-fluorouracil and anthracycline-based chemotherapy (P = 0.01; odds ratio, 2.0; 95% confidence interval, 1.1-3.3). In a multivariate model, including lymph node status, steroid hormone receptor status, menopausal status, dominant metastases site, type of chemotherapy, and disease-free interval, TIMP-1 was significantly associated with resistance to treatment (P = 0.03; odds ratio, 1.7; 95% confidence interval, 1.1-3.3).

Conclusions: In the present exploratory study, we showed that elevated tumor tissue TIMP-1 levels were significantly associated with a poor response to chemotherapy. By using TIMP-1, we identified a group of patients with metastatic breast cancer, which hardly respond to the most frequently used chemotherapy regimes (i.e., cyclophosphamide/methotrexate/5-fluorouracil and anthracyclines).

The use of chemotherapy for treatment of patients with metastatic breast cancer is routine. A large proportion of the patients, however, does not benefit from the treatment and may unnecessarily suffer from substantial side effects. The only biomarkers for use as predictors of response to specific therapy regimens are the estrogen and progesterone receptors (ER and PR) for predicting response to hormonal treatment and HER-2/neu expression for predicting response to trastuzumab (1). The need for markers used to individualize treatment will continue to increase as new therapy regimens are introduced.

Tissue inhibitor of metalloproteinases-1 (TIMP-1) is one of four endogenous protease inhibitors belonging to the matrix metalloproteinase proteolytic system (reviewed in ref. 2). In recent years, an increasingly complex role of TIMPs in cancer disease has emerged, and, today, TIMPs are recognized as multifunctional molecules with complicated effect on tumor development and growth (for a review, see ref. 3). TIMP-1 inhibits matrix metalloproteinase–mediated proteolytic degradation of extracellular matrix, and, besides this, TIMP-1 is capable of stimulating cell growth (4, 5) and of inhibiting apoptosis (6–11). Clinically, in primary breast cancer tissue, an association between high levels of TIMP-1 mRNA or protein and a poor patient prognosis has been established (12–16). Furthermore, a recent study including 251 patients indicated that high plasma levels of TIMP-1 are associated with a poor response to hormone therapy in patients with metastatic breast cancer (17).

The purpose of the present study was to test the hypothesis that tumors expressing high levels of TIMP-1 are protected against apoptosis-inducing agents and thus less sensitive to chemotherapeutic drugs that work through induction of apoptosis. To test...
this hypothesis, we investigated the association between primary tumor expression levels of TIMP-1 protein and objective response to chemotherapy in patients with metastatic breast cancer. In 173 patients with recurrent breast cancer, who were all treated with cyclophosphamide/methotrexate/5-fluorouracil (CMF) or an anthracycline-containing regimen (cyclophosphamide/epirubicin/5-fluorouracil or cyclophosphamide/Adriamycin/5-fluorouracil or Adriamycin only) as first-line systemic treatment, we evaluated objective responses to chemotherapy and then analyzed whether these were related to primary tumor tissue levels of TIMP-1, as determined by ELISA. In addition, we analyzed whether the response to chemotherapy was associated with other clinicopathologic variables.

Patients and Methods

Patients. The present study, in which coded tumor tissues collected between 1979 and 1993 were used, was done in accordance to the Code of Conduct of the Federation of Medical Scientific Societies in the Netherlands. The study design was approved by the institutional Medical Ethical Committee (MEC no. 02.953). Originally, samples were selected according to the criteria described previously (18, 19) and included in prognostic and predictive studies based on the availability of stored cytosol extracts (in liquid nitrogen), which remained after routine ER and PR analysis. For the present retrospective study, samples were selected from those patients who received first-line chemotherapy (CMF in 94 patients and an anthracycline-containing regimen, cyclophosphamide/epirubicin/5-fluorouracil, cyclophosphamide/Adriamycin/5-fluorouracil or single agent Adriamycin, in 79 patients) for metastatic disease. At the primary diagnosis, 16 patients presented with distant metastasis (M1 patients), and 157 patients were M0. At the start of chemotherapy, 79 patients (visceral or soft tissue versus bone, 27-79 years). Ninety-two were premenopausal, and 81 were postmenopausal. The median age of the patients at the time of surgery was 47 years (range, 24-79 years) and at the start of chemotherapy was 50 years (range, 27-79 years). Ninety-two were premenopausal, and 81 were postmenopausal. None of the patients had received neoadjuvant systemic therapy. One hundred thirteen of the patients received no adjuvant therapy; 24 patients received adjuvant hormonal treatment; 13 received only adjuvant anthracycline containing chemotherapy; 22 received only adjuvant non-anthracycline chemotherapy; and one patient received adjuvant hormonal treatment and non-anthracyline chemotherapy.

Tissue samples. Tissue samples were stored in liquid nitrogen and pulverized with a Microdismembrator II (Braun, Melsungen, Germany) as recommended by the European Organization for Research and Treatment of Cancer for determination of levels of ER and PR (21). Tissue powder was suspended in European Organization for Research and Treatment of Cancer receptor buffer [10 mmol/L K2HPO4 buffer, containing 1.5 mmol/L diphosphat de EDTA, 3 mmol/L sodium azide, 10 mmol/L monothioglycerol, 10% (v/v) glycerol (pH 7.4)] and centrifuged for 30 minutes (100,000 × g), and the cytosolic fraction was collected as the supernatant. Cytosolic samples were stored in liquid nitrogen, or at −80°C for short-term storage, until analysis.

Patients were selected from those patients who received first-line chemotherapy (CMF in 94 patients and an anthracycline-containing regimen, cyclophosphamide/epirubicin/5-fluorouracil, cyclophosphamide/Adriamycin/5-fluorouracil or single agent Adriamycin, in 79 patients) for metastatic disease. At the primary diagnosis, 16 patients presented with distant metastasis (M1 patients), and 157 patients were M0. At the start of chemotherapy, 79 patients (visceral or soft tissue versus bone, 27-79 years). Ninety-two were premenopausal, and 81 were postmenopausal. None of the patients had received neoadjuvant systemic therapy. One hundred thirteen of the patients received no adjuvant therapy; 24 patients received adjuvant hormonal treatment; 13 received only adjuvant anthracycline containing chemotherapy; 22 received only adjuvant non-anthracycline chemotherapy; and one patient received adjuvant hormonal treatment and non-anthracyline chemotherapy.

Statistical analysis. The SAS software package (version 8.2; SAS Institute, Cary, NC) was used for statistical calculations. The association of TIMP-1 and the clinical baseline covariates to the objective response was analyzed using logistic regression analysis. The probability of nonresponse is modeled. TIMP-1 was scored as a continuous variable (natural log transformed to assure a reasonable fit to the data, 1 added if TIMP-1 level was 0). All Ps < 0.05 were considered significant.

Association of TIMP-1 with response to chemotherapy. When analyzed as a continuous log-transformed variable, increasing tumor tissue TIMP-1 levels were significantly associated with decreasing probability of response to CMF or anthracycline-based chemotherapy (P = 0.01; odds ratio, 2.0; 95% CI, 1.1-3.3). Note that the odds ratio shows the difference in 1 unit on the log scale that is a ratio of TIMP-1 levels differing by ~2.7 times. The area under the receiver operating characteristic curve was 0.62 (Fig. 1). In addition to TIMP-1, site of metastasis (visceral or soft tissue versus bone, P = 0.03) and type of chemotherapy (CMF versus anthracycline-containing regimens, P = 0.03) were significantly associated with a poor rate of response. M stage at the time of primary diagnosis (M1, versus M0) was associated with a poor response rate with a P of 0.05 (odds ratio, 0.2; 95% CI, 0.05-1.0). None of the following variables were significant in univariate logistic regression analysis: age at the start of chemotherapy (<40 versus 40-55 versus 56-70 versus >70 years), menopausal status at the start of chemotherapy (postmenopausal versus premenopausal), lymph node status (N1, versus N0), hormone receptor status (ER or PR positive versus negative), and disease-free interval (>12 versus ≤12 months). The results of the univariate logistic regression analysis are shown in Table 1.

5 See http://www.fmwv.nl.

© 2006 American Association for Cancer Research.

www.aacrjournals.org

Clin Cancer Res 2006;12(23) December 1, 2006

7055

Downloaded from clincancerres.aacrjournals.org on April 20, 2017.
TIMP-1 levels in nonresponders (i.e., patients with stable disease >6 months, stable disease <6 months, and patients with progressive disease) were not significantly different ($P = 0.22$, Kruskal-Wallis test). Eighteen nonresponding patients (~10%, 12 CMF treated and 6 anthracycline treated) had the highest tumor tissue TIMP-1 levels, all above 33.4 ng TIMP-1/mg of total protein. This means that TIMP-1 analyses could identify 18 of 109 (17%) of the nonresponders.

**Multivariate analysis.** The association between tumor tissue levels of TIMP-1 and response to treatment was studied by multivariate logistic regression analysis. In addition to tumor tissue TIMP-1 levels, the multivariate model included menopausal status at the start of chemotherapy, lymph node status, ER and PR status, dominant site of relapse, disease-free interval, and type of chemotherapy. All covariates were scored as described above. In the multivariate model, a high TIMP-1 level was significantly associated with a poor response to treatment ($P = 0.03$; odds ratio, 1.7; 95% CI, 1.1-3.3).

**Fig. 1.** Receiver operating characteristic curve modeling the probability of nonresponse to chemotherapy. Specificity, proportion of responders classified correctly; sensitivity, proportion of nonresponders classified correctly. The area under the curve was 0.62.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>$P$</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\leq 40$</td>
<td>1</td>
<td>0.77</td>
</tr>
<tr>
<td>40-55</td>
<td>1.2 (0.5-2.9)</td>
<td>0.06</td>
</tr>
<tr>
<td>55-70</td>
<td>1.1 (0.4-2.9)</td>
<td>0.23</td>
</tr>
<tr>
<td>$&gt; 70$</td>
<td>0.5 (0.1-3.1)</td>
<td>0.28</td>
</tr>
<tr>
<td>Menopausal status*</td>
<td>0.5 (0.3-1.0)</td>
<td>0.23</td>
</tr>
<tr>
<td>Lymph node status$^1$</td>
<td>0.7 (0.3-1.3)</td>
<td>0.23</td>
</tr>
<tr>
<td>Steroid hormone receptor status$^1$</td>
<td>0.7 (0.4-1.3)</td>
<td>0.28</td>
</tr>
<tr>
<td>Dominant metastasis site$^1$</td>
<td>2.9 (1.1-7.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Disease-free interval$^1$</td>
<td>1.5 (0.8-2.9)</td>
<td>0.25</td>
</tr>
<tr>
<td>Type of chemotherapy$^1$</td>
<td>0.5 (0.3-0.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>TIMP-1**</td>
<td>2.0 (1.1-3.3)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Postmenopausal versus premenopausal.
$^1$N = 171 patients, N$_1+$ versus N$_0$.
$^2$N = 171 patients, ER or PR positive versus ER or PR negative.
$^3$Visceral or soft tissue versus bone.
$^4$Disease-free interval: >12 versus $\leq$12 months.
$^5$CMF versus anthracycline-containing regimens.
$^6$$\log_8$ TIMP-1 (ng/mg of total protein).
better response to treatment. Age was not included in the multivariate analysis due to its strong association to menopausal status. The results of the multivariate analysis are summarized in Table 1.

Discussion

Today, chemotherapy is routinely used in the management of metastatic breast cancer. We know that some tumors are resistant to treatment, but, at present, it is not possible to predict which patients will benefit from chemotherapy. Unfortunately, treatment of metastatic breast cancer in general does not cure the patient; moreover, the treatment is associated with substantial side effects, making tailoring of treatment highly important in this setting. Hypothetically, by employing predictive markers, this current problem of administering drugs to which the patients are already resistant may be overcome.

In the present study, we found that primary tumor tissue levels of TIMP-1 were associated with the probability to respond to chemotherapy (anthracyclines, CMF), higher tumor tissue levels of TIMP-1 being predictive of a smaller chance of responding to chemotherapy. As shown at the receiver operating characteristic curve, at a specificity of 100%, the sensitivity was ~17%. This suggest that without misclassifying any responders, we could identify a group of nonresponding patients with metastatic breast cancer, in this study about 17%, having only a minimal chance of responding to chemotherapy with some of the most frequently used regimens (i.e., CMF and anthracyclines). Ideally, these patients should be offered an alternative treatment. When performing a multivariate analysis, tumor tissue TIMP-1 together with bone metastasis were significantly associated with a poor response to chemotherapy.

To our knowledge, this report is the first one to indicate an association between high levels of TIMP-1 protein in primary breast tumor tissue and a poor response to chemotherapy when the tumor has metastasized. In support of this finding is a recent report describing an association between high plasma levels of TIMP-1 and a poorer response to endocrine therapy when patients with metastatic breast cancer (17). However, another recent study on TIMP-1 suggested the association between tumor tissue levels of TIMP-1 mRNA and efficacy of adjuvant chemotherapy, and, here, no association was found (24). The benefit from adjuvant chemotherapy and from therapy for metastatic disease could indeed be different as has been suggested when related to another proteinase inhibitor (plasminogen activator inhibitor type-1): In the adjuvant setting, high levels of tumor tissue plasminogen activator inhibitor-1 protein have been found predictive of benefit from chemotherapy (25, 26), whereas in patients with metastatic breast cancer, high levels of plasminogen activator inhibitor-1 have been associated with a poor response to endocrine therapy (27, 28). Although comparing different treatment types (chemotherapy versus endocrine therapy), for plasminogen activator inhibitor-1, it has been suggested that the reason for this apparent discrepancy relates to tumor phenotype (26). Tumors with high levels of plasminogen activator inhibitor-1 are assumed to be rather aggressive and thus susceptible to treatment in the adjuvant setting. Later, when these tumors have metastasized, they apparently become resistant to therapy.

We have, at present, no explanation to this difference. Whether this difference is true for TIMP-1 as well remains to be analyzed.

We, and others, have previously shown that high levels of TIMP-1 tumor tissue protein are associated with a poor prognosis in patients with primary breast cancer (12–16); this indicates that TIMP-1 may correlate with an aggressive phenotype. Future studies should analyze in more detail the benefit from adjuvant systemic therapy according to TIMP-1 levels.

The possible explanations for a correlation of TIMP-1 with an aggressive, poor-prognosis, treatment-resistant tumor phenotype are numerous. The increased level of TIMP-1 in these aggressive tumors could be merely a response to increased proteolysis. In support of this view are recent findings that indicate the free fraction of TIMP-1 in tumor tissue to be associated with good prognosis and only TIMP-1 in complex with matrix metalloproteinases to be associated with poor prognosis in primary breast cancer (29). However, as discussed below, TIMP-1 has been shown to inhibit apoptosis, and as some chemotherapeutic drugs, including CMF and anthracyclines, induce apoptosis, protection from apoptotic cell death by TIMP-1 could prevent these drugs from working properly.

The ability of TIMP-1 to influence apoptotic cell death has been shown in several cell types, including human breast epithelial cells (6, 7) and human breast carcinoma cells (8). In vitro, high levels of endogenous (6, 7) and exogenously added TIMP-1 (6–8) significantly improves cell survival after various apoptotic stimuli. The mechanism whereby TIMP-1 acts to inhibit apoptosis is not yet entirely explained; however, some studies have revealed parts of possible pathways. In breast epithelial cells, overexpression of TIMP-1 leads to constitutive activation of focal adhesion kinase (6), and in malignant and in nonmalignant human breast epithelial cells, the activation of phosphatidylinositol 3-kinase and extracellular signal-regulated kinases seems to be a central part of TIMP-1-mediated anti-apoptotic signaling (7, 8). Accordingly, a well-known cell survival pathway involving focal adhesion kinase, phosphatidylinositol 3-kinase, and Akt is pointed to, which mediates the activation of antiapoptotic Bcl-2 family members Bcl-2 and Bcl-XL (7). In a study by Lee et al., involvement of Akt after stimulation of cells with TIMP-1 was shown (8), and in this study, the survival pathway further seemed to involve pertussis toxin–sensitive G-protein and an src family kinase, besides phosphatidylinositol 3-kinase and extracellular signal-regulated kinases. Thus, by initiating survival pathways involving the abovementioned molecules, TIMP-1, potentially, is capable of inhibiting cells from undergoing apoptosis induced by chemotherapeutic drugs. In human breast epithelial cells in culture, the antiapoptotic effect of TIMP-1 does not seem to depend on its ability to inhibit matrix metalloproteinases (6–8). It should be mentioned, however, that in certain other cell types, this ability of TIMP-1 to inhibit apoptosis does seem to depend on its matrix metalloproteinase inhibitory function (11).

The association of TIMPs with apoptosis is not unfamiliar; within the TIMP family, TIMP-3 mRNA in tumor tissue was recently linked with predicting response to therapy in patients with primary breast cancer (24). In a study of 273 patients, it was found that high levels of TIMP-3 mRNA were predictive of a good response to adjuvant tamoxifen therapy. Interestingly, it was suggested that this could be explained by the involvement of TIMP-3 in pathways of tamoxifen-induced apoptosis, as TIMP-3 seems capable of inducing apoptosis via Fas (30). Furthermore, a recent study including 251 patients indicated that high plasma levels of TIMP-1 protein are associated with a
poor response to hormone therapy in patients with metastatic breast cancer (17). Similar to these findings by Span et al. and Lipton et al., our results further contribute to the emerging picture of TIMPs as complex proteins that, although related, have highly distinct functions.

Based on the abovementioned antiapoptotic functions of TIMP-1, one could hypothesize that by neutralizing TIMP-1 by means of small-molecule inhibitors or antibodies, a response to apoptosis-inducing drugs would be possible. In vitro, addition of anti-TIMP-1 antibodies can reverse the antiapoptotic effect of TIMP-1 as shown by Guedez et al. (9). In vivo studies are still lacking in this area, but one could imagine combining TIMP-1 neutralizing treatment with conventional chemotherapy.

In conclusion, the present exploratory study supports the hypothesis that tumors containing large amounts of the protease inhibitor TIMP-1 are less sensitive to treatment with chemotherapeutic drugs. However, more clinical studies including large and independent patient groups are needed to investigate further the strength of TIMP-1 as a possible predictive marker of chemotherapy efficacy and to study whether looking at TIMP-1 fractions or combining TIMP-1 with other markers can improve the predictive sensitivity.

References