Serum Levels of Transforming Growth Factor β1 in Patients With Breast Cancer

Shyr-Ming Sheen-Chen, MD; Han-Shiang Chen, MD; Chih-Wei Sheen; Hock-Liew Eng, MD; Wei-Jen Chen, MD, MS

Hypothesis: Transforming growth factor β1 (TGF-β1) may be related to breast cancer progression.

Design: Prospective study.

Setting: University hospital.

Patients: Sixty consecutive patients with invasive breast cancer undergoing surgery were prospectively included and evaluated. The control group consisted of 14 patients with benign breast tumors (7 with fibrocystic disease and 7 with fibroadenoma).

Intervention: Venous blood samples were collected before the surgery. Sera were obtained by centrifugation and stored at –70°C until assayed. Serum concentrations of TGF-β1 were measured by quantitative sandwich enzyme immunoassay. Data on primary tumor stage, age, estrogen receptor status, lymph node status, distant metastases, and TNM staging (according to the Union Internationale Contre le Cancer) were reviewed and recorded.

Main Outcome Measures: Measurements of preoperative serum TGF-β1 levels in patients with breast cancer.

Results: The mean±SD value of serum TGF-β1 in patients with invasive breast cancer was 498.7±249.7 pg/mL and in the control group was 495.2±225.5 pg/mL (P = .96). However, there were significantly higher serum levels of TGF-β1 in patients with more advanced lymph node status (P = .04), more advanced TNM stage (P = .005), and poorer histological grade (P = .02). In multivariate analysis, TNM staging (P = .02) was demonstrated to be the independent factor related to significantly higher serum levels of TGF-β1.

Conclusions: Patients with more advanced TNM stages were shown to have higher serum TGF-β1 levels. Thus, serum TGF-β1 levels may reflect the severity of invasive breast cancer.

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Many growth factors and their receptors have been reported to play an important role in the growth regulation of breast cancer.1,2 One of these is transforming growth factor β (TGF-β).2,3 The TGF-β superfamily contains proteins that serve a wide variety of biological activities, including growth control, cellular differentiation, embryologic morphology, and immunity.4 Transforming growth factor β1 is the predominant form found in humans and is widely expressed in a variety of normal cells and organs. It is a multifunctional polypeptide, promoting angiogenesis, accumulation of extracellular matrix glycoproteins, and cell adhesion proteins, while inhibiting growth of both epithelial and immune cells.4 Transforming growth factor β1 has been reported to be overexpressed in many tumors including those of the breast and is thought to be related to tumor transformation and progression.5-8 Nevertheless, there are conflicting reports about the role of the expression of TGF-β1 in breast cancer.9-12 Thus, this study was designed with the aim to elucidate the possible relationship between TGF-β1 and breast cancer.

See Invited Critique at end of article

RESULTS

The mean±SD value of serum TGF-β1 was 498.7±249.7 pg/mL in patients with invasive breast cancer and 495.2±225.5 pg/mL in the control group (P = .96). However, with the stratification of the above-mentioned clinicopathologic variables, patients with more advanced lymph node status (P = .04), more advanced TNM stage (P = .005), and poorer histological grade (P = .02) were
PATIENTS, MATERIALS, AND METHODS

From November 1998 to February 2000, 60 consecutive patients with invasive breast cancer were included in the study. Venous blood samples were collected before surgery. Serum samples were obtained by centrifugation and stored at -70°C until assayed. All 60 patients were women aged between 32 and 79 years (mean, 50.3 years). Except for those with distant metastases, all patients underwent modified radical mastectomy, and the diagnosis of breast cancer was confirmed by histological examination. Invasive breast cancer was defined as carcinoma, regardless of origin (duct or lobule), with invasion to or beyond the basement membrane. Tumors were graded according to the criteria described by Bloom and Richardson. Primary tumor stage, age, estrogen receptor status, lymph node status, distant metastases, and TNM stage were recorded for all patients. Each patient was assessed preoperatively by a thorough physical examination, chest radiography, level of serum alkaline phosphatase, and mammogram. Bone scan and abdominal ultrasonography were performed for all patients with provisional clinical stage III disease to rule out the presence of distant metastases. Regardless of the provisional clinical stage, all patients with elevated serum alkaline phosphatase, special complaints such as bone pain, or any specific findings indicating the possibility of distant metastases such as hepatomegaly also underwent bone scan and abdominal ultrasonography to detect possible distant metastases. Estrogen receptor status was determined by immunohistochemical staining.

Fourteen patients with benign breast tumor (7 with fibrocystic disease and 7 with fibroadenoma) were used as the control group.

MEASUREMENT OF TGF-β1

An enzyme-linked immunosorbent assay kit was used for the quantitative determinations of serum TGF-β1 concentration. In brief, each serum sample was diluted and added to the microtiter wells pre-coated by TGF-β soluble receptor type II, followed by an additional incubation with an enzyme-linked polyclonal antibody specific for TGF-β1. Color development was performed using a tetramethyl benzidine–hydrogen dioxide mixture and terminated with sulfuric acid. The absorbance of each well was determined using a spectrophotometer.

STATISTICAL ANALYSIS

An independent sample t test and analysis of variance were used to compare the serum TGF-β1 levels between subgroups of the clinicopathologic variables as the univariate analysis. Multivariate analysis was performed using the linear regression stepwise method to compare the serum TGF-β1 levels in patients with breast cancer. A P value of less than .05 was considered statistically significant.

<table>
<thead>
<tr>
<th>Table 1. Serum Levels of TGF-β1 in Relation to Clinicopathologic Variables*</th>
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<tbody>
<tr>
<td>Age, y</td>
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<tr>
<td>No. (%)</td>
</tr>
<tr>
<td>&lt;50 (53)</td>
</tr>
<tr>
<td>≥50 (47)</td>
</tr>
<tr>
<td>Estrogen receptor status</td>
</tr>
<tr>
<td>Negative (42)</td>
</tr>
<tr>
<td>Positive (58)</td>
</tr>
<tr>
<td>Primary tumor staging</td>
</tr>
<tr>
<td>T1 (10)</td>
</tr>
<tr>
<td>T2 (55)</td>
</tr>
<tr>
<td>T3 (7)</td>
</tr>
<tr>
<td>T4 (28)</td>
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<tr>
<td>Lymph node status</td>
</tr>
<tr>
<td>N0 (45)</td>
</tr>
<tr>
<td>N1 (25)</td>
</tr>
<tr>
<td>N2 (30)</td>
</tr>
<tr>
<td>Distant metastases</td>
</tr>
<tr>
<td>Absent (88)</td>
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<tr>
<td>Present (12)</td>
</tr>
<tr>
<td>TNM staging</td>
</tr>
<tr>
<td>Early stage (I and II)</td>
</tr>
<tr>
<td>Late stage (III and IV)</td>
</tr>
<tr>
<td>Histologic grade</td>
</tr>
<tr>
<td>Well differentiated (43)</td>
</tr>
<tr>
<td>Moderate and poorly</td>
</tr>
<tr>
<td>differentiated (57)</td>
</tr>
</tbody>
</table>

*TGF-β1 indicates transforming growth factor β1. †Independent sample t test and analysis of variance.

shown to have significantly higher serum levels of TGF-β1 (Table 1). In multivariate analysis, TNM staging (P = .02) was demonstrated to be the independent factor related to significantly higher serum levels of TGF-β1 (Table 2).

COMMENT

The evaluation of the possible outcome of patients with breast cancer is important for planning treatment. Because no single prognostic factor can determine the status of a patient with breast cancer, physicians must consider all available prognostic data. Angiogenesis is essential for tumor growth and metastasis and is regulated by numerous angiogenic factors. The recent study of Gunningham et al showed that the short form of the alternatively spliced flt-4—not its ligand, vascular endothelial growth factor C—is related to lymph node metastasis in human breast cancer. The recent study of Pawlowski et al confirmed that the 4 members of the type 1 growth factor receptor gene family such as epidermal growth factor, c-erb-b2, c-erb-b3, and c-erb-b4 are prognosis markers of tumor aggressiveness in breast cancer. We conducted this study to investigate any correlation between serum TGF-β1 and the clinicopathologic variables and to elucidate the possible relationship between the TGF-β1 and breast cancer. We included patients with invasive breast cancer only since the clinical course of intraductal, noninvasive breast cancer is usually quite different.
Transforming growth factor β1 has been reported to be overexpressed in many tumors, including those of the breast, and is thought to be related to tumor transformation and progression. The following mechanisms have been suggested to be related to tumor transformation and progression: (1) TGF-β1 production by tumor cells can enhance tumor growth through promoting angiogenesis and evading immune surveillance; (2) TGF-β1 can promote accumulation of extracellular matrix glycoproteins and cell adhesion proteins, which may enhance the metastatic potential of the tumor; (3) in vitro studies have demonstrated that the induction of TGF-β1 secretion may increase the cellular motility and the production of protease, thus enhancing the invasive potential of fibrosarcoma; and (4) in addition to elevated TGF-β1, some tumor cells with mutations in the type II TGF-β receptor gene leading to the absence of the cell surface type II TGF-β receptor may avoid the TGF-β-mediated growth inhibitory effect.

There are conflicting reports about the role of the expression of TGF-β1 in breast cancer. Some authors have claimed that the immunohistochemical staining intensity for TGF-β1 is positively associated with the rate of disease progression. To the contrary, Auvinen et al. found that immunohistochemical staining for the expression of TGF-β1 is related to favorable prognostic factors. Also, Murray et al. found that patients with high levels of TGF-β1 messenger RNA (mRNA) expression have a longer disease-free interval. The diversity of results among different series may be owing to different antibodies used to detect the expression of TGF-β1. Moreover, TGF-β protein and TGF-β mRNA expressions may not always be comparable. Peton et al. demonstrated that TGF-β proteins are frequently found in the same cells as TGF-β mRNA but occasionally differences exist in the location of cells where TGF-β protein or TGF-β mRNA expressions are found. Finally, although semiquantitative evaluation is sufficient to differentiate negative from positive reactions, it may not be accurate enough to evaluate the intermediate patterns of staining.

We used the quantitative sandwich enzyme immunnoassay to measure serum concentrations of TGF-β1, and our preliminary results showed that patients with more advanced lymph node status (P = .04), more advanced TNM stage (P = .005), and poorer histologic grade (P = .02) have significantly higher serum levels of TGF-β1 (Table 1). In multivariate analysis, TNM staging (P = .02) was demonstrated to be the independent factor related to significantly higher serum levels of TGF-β1 (Table 2). Such findings are more similar to reports by Gorsch et al. and Walker and Dearing and suggest that TGF-β1 is related to tumor transformation and progression. The choice of serum for a quantitative analysis in our study could possibly avoid the above-mentioned disadvantages of a semiquantitative analysis by immuno histochemical staining.

Based on our preliminary results, the preoperative level of serum TGF-β1 is closely related to a more advanced TNM stage. Thus, serum TGF-β1 levels may reflect the severity of invasive breast cancer. Although the preliminary results seem promising, the follow-up in this series is still short; longer follow-up is needed before a substantial conclusion can be achieved.

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### REFERENCES


### Table 2. Multivariate Analysis of Serum TGF-β1 Level in Patients With Breast Cancer

<table>
<thead>
<tr>
<th>TNM staging</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early stage (I and II)</td>
<td>Constant</td>
<td>. . . . . .</td>
<td>. .</td>
</tr>
<tr>
<td>Late stage (III and IV)</td>
<td>192.7</td>
<td>70.8-314.6</td>
<td>.02</td>
</tr>
</tbody>
</table>

* TGF-β1 indicates transforming growth factor β1; CI, confidence interval; and ellipses, not applicable.
† Linear regression stepwise method.

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Transferring growth factor β is a member of a superfamily of polypeptides with diverse and complex biological functions. The report by Sheen-Chen et al suggests an association between serum expression of TGF-β and breast cancer stage and grade of disease. However, as noted by the authors, analyses of the relationship between TGF-β and breast cancer have previously resulted in a number of conflicting reports.1-4 In fact, this study demonstrated no difference in TGF-β expression in patients with benign breast disease compared with patients with breast cancer. Some reports have demonstrated results completely contrary to the present report, finding decreased TGF-β serum expression, while others have reported results similar to that of Sheen-Chen et al, that decreased TGF-β receptor expression is associated with more advanced breast cancer. These latter studies seem more consistent with the well-recognized growth inhibitory effect of TGF-β on normal mammary epithelial cells. Such data are certainly consistent with a more general phenomenon of malignant disease; that is, regulation of tumor progression is multifactorial. While this report provides a reasonable addition to the already controversial literature on the subject of TGF-β and breast cancer, it aptly demonstrates the broader conclusion that determining reproducible relationships between tumor cell products and clinical prognosis is often difficult.

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