Serum Soluble Epidermal Growth Factor Receptor Concentrations Decrease in Postmenopausal Metastatic Breast Cancer Patients Treated with Letrozole

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Abstract

Previous studies have implicated estrogen as a regulator of epidermal growth factor receptor (EGFR) expression in breast tumors. We therefore speculated that estrogen might modulate serologic soluble EGFR (sEGFR) concentrations in breast cancer patients. Accordingly, we measured serum sEGFR concentrations in postmenopausal women with metastatic breast cancer (MBC) treated with letrozole, an aromatase inhibitor that blocks estrogen synthesis. Serum specimens were obtained prior to and following 1 and 3 months of letrozole therapy. We report that sEGFR concentrations do not differ between MBC patients prior to letrozole treatment and age- and postmenopause-matched healthy women (P = 0.468). In contrast, however, sEGFR concentrations decreased significantly in 76% of MBC patients after both 1 month (P = 0.006) and 3 months (P = 0.003) of letrozole therapy versus pretreatment concentrations. Within the limitations of this study, we found no evidence for an association between pretreatment sEGFR concentrations or decreased treatment sEGFR concentrations and either progression-free or overall survival. Nonetheless, we conclude that future prospective studies are warranted to determine if baseline and/or longitudinal serum sEGFR concentrations may be useful for predicting disease progression and survival, and/or for monitoring responsiveness to aromatase inhibitors or other endocrine therapies in breast cancer patients. (Cancer Res 2005; 65(8): 3059-62)

Introduction

Among postmenopausal women, the majority of all breast cancers are hormone-dependent; in these patients, tumor growth is regulated primarily by estrogen. As a result, selection of chemotherapy for breast cancer patients is based on estrogen receptor status. Clinically, patients with estrogen receptor–positive tumors respond well to antihormonal therapies that target the estrogen-estrogen receptor signaling pathway; these patients, consequently, have a better outcome than patients with estrogen receptor–negative tumors (1, 2). For more than two decades, the antiestrogen tamoxifen has been the standard of choice for first-line therapy in postmenopausal women with advanced, estrogen receptor–positive breast cancer. Unfortunately, 25% to 35% of patients with estrogen receptor–positive tumors do not respond to tamoxifen treatment and virtually all patients later develop tamoxifen-resistant tumors (1, 3). Third-generation aromatase inhibitors (e.g., anastrozole, letrozole, and exemestane), which block the synthesis of estrogens from androgens, have been developed to treat tamoxifen resistant tumors (3). All three aromatase inhibitors have been found to be effective in the adjuvant disease setting (4). Moreover, recent randomized trials have provided sufficient evidence to justify choosing either anastrozole or letrozole over tamoxifen to treat advanced breast cancer in patients (5). Thus, third-generation aromatase inhibitors are assuming an increasingly important role in the management of women with breast cancer.

Although the estrogen-dependent growth of estrogen receptor–positive breast cancer is well established, the proliferative pathways stimulating estrogen receptor–negative breast cancer cell growth have not been completely defined. In this regard, signal transduction pathways involving the epidermal growth factor receptor (EGFR/ErbB1/HER1) family, which includes ErbB2 (HER2/Neu), have been implicated in the etiology and progression of estrogen receptor–negative breast cancer. Interestingly, estrogens and estrogen receptor have been shown to regulate expression of EGFR. Furthermore, numerous studies have shown that expression of estrogen receptor and EGFR are inversely correlated in breast tumors and breast cancer–derived cell lines (1, 3). Thus, EGFR and EGF-related growth factors are often overexpressed in estrogen receptor–negative breast tumors, where these growth factors are thought to stimulate estrogen-independent breast tumor growth via autocrine and/or paracrine mechanisms. Clinically, EGFR–positive breast cancer patients are more likely to fail hormonal therapy, and to relapse and die regardless of estrogen receptor status. Interestingly, Ellis et al. (6) showed that responsiveness to neoadjuvant letrozole therapy (88%) was more effective than tamoxifen therapy (21%) in patients with estrogen receptor–positive plus EGFR and/or ErbB2-positive breast tumors. Thus, tumor expression of EGFR may be an important predictive factor to consider when selecting a specific endocrine therapy for the treatment of breast cancer patients.

Elevated pretreatment serum concentrations of soluble ErbB2 have been associated with decreased progression-free and overall survival for metastatic breast cancer (MBC) patients treated with various hormonal regimens (7). Accordingly, soluble isoforms of EGFR

Note: Jacqueline M. LaFky and Andre T. Baron are the principal co-authors.

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(sEGFR) might also be clinically useful serologic biomarkers in breast cancer patients (8). Recently, we have identified a 110 kDa soluble EGFR isoform (p110 sEGFR) in human sera (9) and have developed an acridinium-linked immunosorbent assay to quantify this biomarker (10). Using this assay, we have shown that sEGFR concentrations are significantly lower in ovarian cancer patients than in healthy women, and that sEGFR may be useful in the risk assessment, screening, and/or diagnosis of ovarian cancer (9, 11). Although sEGFR isoforms have been shown to exhibit growth-inhibitory properties in vitro (12–14), their functions in vivo remain to be elucidated. Regardless of the potential functional role of sEGFR in regulating normal and neoplastic cell proliferation, serum sEGFR concentrations may be useful in cancer risk assessment, screening, and diagnosis, as well as for predicting and/or monitoring therapeutic responsiveness, disease recurrence, tumor progression, and survival.

Letrozole treatment reduces circulating and local tissue estrogen concentrations (4). Considering that estrogens and estrogen receptor can regulate EGFR expression, we hypothesized that letrozole treatment would affect circulating sEGFR levels and that altered serum concentrations of sEGFR might have potential diagnostic, prognostic, and theranostic utility in breast cancer patients treated with letrozole.

Materials and Methods

Serum specimens were collected prior to treatment, and following 1 and 3 months of letrozole therapy from postmenopausal women with MBC who had failed two prior hormonal regimens, and who were subsequently entered in a North Central Cancer Treatment Group randomized phase II trial of 0.5 or 2.5 mg letrozole daily, as described previously (15). Written informed consent was obtained from each participant in this Mayo Foundation Institutional Review Board–approved study. Sixty-four of the 93 eligible patients enrolled in this clinical trial provided a pretreatment serum specimen. Among 89 patients who completed 1 month of treatment, 51 patients provided a 1-month serum specimen; 44 of these 51 patients provided both a pretreatment and 1-month serum specimen. Three of these 44 patients had progressive disease after 1 month of treatment. Of 66 patients who completed 3 months of treatment, 41 patients provided a 3-month serum specimen;

<table>
<thead>
<tr>
<th>Study group</th>
<th>sEGFR concentration (fmol/mL)</th>
<th>Mean (± SD)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matched healthy women (n = 43)</td>
<td></td>
<td>4,962 (± 6,417)</td>
<td>2,525</td>
<td>114-31,465</td>
</tr>
<tr>
<td>Matched MBC patients (n = 43)</td>
<td></td>
<td>4,957 (± 4,574)</td>
<td>3,857</td>
<td>ND-19,447</td>
</tr>
<tr>
<td>Pretreatment (n = 64)</td>
<td></td>
<td>4,833 (± 4,498)</td>
<td>3,768</td>
<td>ND-19,447</td>
</tr>
<tr>
<td>Letrozole for 1 month (n = 44)</td>
<td></td>
<td>2,013 (± 2,04)</td>
<td>1,280</td>
<td>ND-10,617</td>
</tr>
<tr>
<td>Letrozole for 3 months (n = 32)</td>
<td></td>
<td>1,886 (± 2,113)</td>
<td>1,438</td>
<td>ND-8,553</td>
</tr>
</tbody>
</table>

Table 1. Descriptive statistics of serum sEGFR concentrations in age-matched postmenopausal healthy women compared with MBC patients prior to letrozole treatment, and in postmenopausal MBC patients prior to letrozole treatment compared with 1 and 3 months of letrozole treatment

Figure 1. A, scattergram comparing serum sEGFR concentrations between age-matched postmenopausal healthy women and postmenopausal MBC patients before letrozole treatment. Each point represents the median value for one serum sample assayed thrice in duplicate. Serum specimens with sEGFR concentrations below the inter-assay biological detection limit (BDL) of 23.2 fmol/mL were arbitrarily assigned a value of 15 fmol/mL. Horizontal lines indicate median serum sEGFR concentration for each group of women. Horizontal lines in the box plot represent the first, second (the median), and third quartiles; whiskers extend from the box to a distance of 1.5 interquartile ranges. B, scattergram comparing the percent change in sEGFR concentrations between MBC patients prior to letrozole treatment and following either 1 or 3 months of letrozole treatment. C, longitudinal serum sEGFR concentrations in MBC patients at 1 month of letrozole treatment versus pretreatment were compared to determine if sEGFR concentrations changed over time. Points to the left, on, or to the right of the bisecting line indicate an increase, no change, or a decrease, respectively, in sEGFR concentrations after 1 month of letrozole therapy compared with pretreatment sEGFR values.

Abbreviation: ND, not detectable.
32 of these 41 patients gave both a pretreatment and 3-month serum specimen, and 31 of these 41 patients gave both a 1-month and 3-month serum specimen. Nine of the 32 patients who provided both a pretreatment and a 3-month specimen had progressive disease after 3 months of treatment. Serum specimens from healthy women were collected under a Mayo Foundation Institutional Review Board–approved “Normal Values Study” as described previously (10, 16) and were compared with pretreatment breast cancer serum samples. We were able to age-match (± 2 years) 43 of the 64 pretreatment postmenopausal MBC patients to 43 healthy postmenopausal women from this Normal Values Study cohort. Serum sEGFR concentrations were determined by acridinium-linked immunosorbent assay as outlined previously (10, 16).

Results and Discussion

Descriptive statistics of serum sEGFR concentrations are provided in Table 1 for age- and postmenopause-matched MBC patients and healthy women, as well as for MBC patients at each collection time point. Consistent with a prior report from our group (17), and in contrast to what we have observed in epithelial ovarian cancer patients (9, 11), we observed no difference between pretreatment serum sEGFR concentrations in MBC patients and age- and postmenopause-matched healthy women (Wilcoxon signed-rank test, \( P = 0.468 \); Fig. 1A). We did, however, observe a significant decrease in the percent change in log sEGFR concentrations after both 1 month (Wilcoxon signed-rank test, \( P = 0.006 \)) and 3 months (Wilcoxon signed-rank test, \( P = 0.003 \)) of letrozole therapy compared with pretreatment values (Fig. 1B).

Further analysis revealed that after 1 and 3 months of letrozole treatment, 73% (32 of 44, Fig. 1C) and 75% (24 of 32, data not shown) of MBC patients had lower sEGFR concentrations compared with pretreatment values, respectively. Collectively, 76% (39 of 51, data not shown) of the MBC patients who provided at least one longitudinal serum specimen showed a decrease in sEGFR concentrations compared with pretreatment values. For the 31 letrozole-treated MBC patients who provided both 1- and 3-month serum specimens, we observed no difference in sEGFR concentrations between these time points (Wilcoxon signed-rank test, \( P = 0.25 \); data not shown). In addition, we observed no evidence that the percent change in log sEGFR concentrations differ with respect to treatment dose 1 month after initiation of letrozole treatment (0.5 mg per dose, 24 patients versus 2.5 mg per dose, 27 patients; Wilcoxon rank-sum test, \( P = 0.67 \); data not shown) or 3 months after initiation of letrozole therapy (0.5 mg dose, 19 patients versus 2.5 mg dose, 22 patients; Wilcoxon rank-sum test, \( P = 0.46 \); data not shown).

To assess whether progression-free survival or overall survival are associated with pretreatment sEGFR concentrations, Cox proportional hazards models were fit to these data. Among the 64 patients with pretreatment sEGFR concentrations, 58 had progressed and 48 had died. In this small study population, we found no significant association between pretreatment sEGFR concentrations and either progression-free survival or overall survival (data not shown), and we were unable to determine if changes in sEGFR concentrations could predict or monitor disease progression. However, the small population, incomplete sets of pretreatment versus longitudinal serum samples, and/or a lack of serum samples at the time of progression may have negated our ability to discern any statistically significant associations.

Incidentally, the obvious biomarker of choice to assess responsiveness to letrozole therapy is estrogen. However, serum estrogens (i.e., estradiol) in postmenopausal women normally circulate at concentrations well below the detection limit of most standard laboratory assays (reference range, not detectable to 31 pg/mL). Circulating estradiol concentrations for patient sera collected in this study were determined by the Mayo Foundation Immunoochemical Core Laboratory using the Estradiol-6 Assay on the ACS:180 Immunoassay Analyzer (Bayer Corporation, Diagnostics Division, Tarrytown, NY). The detection limit of this assay was 35 pg/mL, which is slightly higher than the upper reference limit for estradiol in postmenopausal women. As expected, we observed that 52% (29 of 56) of pretreatment, 81% (39 of 48) of 1-month, and 84% (31 of 37) of 3-month serum specimens collected from letrozole-treated postmenopausal MBC patients had nondetectable estradiol concentrations (data not shown). Using a more sensitive assay with a lower limit of detection for estradiol (0.572 pg/mL), Geisler et al. (18), were unable to detect estradiol in any of the 12 women treated with letrozole at the standard dose of 2.5 mg per day. Thus, limitations associated with measuring estradiol concentrations in postmenopausal women currently preclude the clinical use of this steroid hormone as a serum biomarker, and further indicate the need for a surrogate marker as a measure of responsiveness to letrozole therapy in patients with breast cancer.

In summary, we report that treatment with letrozole, an aromatase inhibitor that blocks estrogen production, decreases circulating serum sEGFR concentrations in the majority (76%) of postmenopausal women with MBC. These observations further implicate estrogen as an important regulator of EGFR expression in vivo; however, the mechanism by which estrogen regulates sEGFR concentrations requires additional study. Nonetheless, we believe that these results are particularly important in light of recent studies suggesting that tumor EGFR expression, in combination with estrogen receptor expression, may be a better indicator of responsiveness to letrozole treatment compared with estrogen receptor expression alone (6). Recognizing the limitations of this study, we propose that prospective clinical studies are warranted to test further the potential utility of sEGFR as a serum biomarker for predicting disease progression and survival, and/or for monitoring responsiveness to letrozole therapy or other endocrine therapies in MBC patients.

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References


2. Rochefort H, Glondu M, Sahla ME, Platet N, Garcia M. sEGFR Concentrations in Letrozole-treated MBC Patients


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