Serum sErbB1 and Epidermal Growth Factor Levels As Tumor Biomarkers in Women with Stage III or IV Epithelial Ovarian Cancer

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Abstract

Epithelial ovarian cancer (EOC) has a high mortality rate, which is due primarily to the fact that early clinical symptoms are vague and nonspecific; hence, this disease often goes undetected and untreated until in its advanced stages. Sensitive and reliable methods for detecting earlier stages of EOC are, therefore, urgently needed.

Epidermal growth factor (EGF) is a ligand for EGF receptor (ErbB1); this receptor is the product of the c-erbB1 proto-oncogene. ErbB1 overexpression is common in human ovarian carcinoma-derived cell lines and tumors, in which overexpression is thought to play a critical role in tumor etiology and progression. Furthermore, ErbB1 overexpression is associated with disease recurrence and decreased patient survival.

Recently, we have developed an acridinium-linked immunosorbent assay that detects a ~110-kDa soluble analogue of ErbB1, i.e., sErbB1, in serum samples from healthy men and women (A. T. Baron, et al., J. Immunol. Methods, 219: 23–43, 1998). Here, we demonstrate that serum p110 sErbB1 levels are significantly lower in EOC patients with stage III or IV disease prior to (P < 0.0001) and shortly after (P < 0.0001) cytoreductive staging laparotomy than in healthy women of similar ages, whereas EGF levels are significantly higher than those of age-matched healthy women only in serum samples collected shortly after tumor debulking surgery (P < 0.0001). We observe that the preoperative serum sErbB1 concentration range of advanced stage EOC patients barely overlaps with the serum sErbB1 concentration range of healthy women. In addition, we show that serum sErbB1 and EGF levels changed temporally for some EOC patients who were surgically debulked of tumor and who provided a second serum sample during the course of combination chemotherapy. Finally, we observe a significant positive association between sErbB1 and EGF levels only in serum samples of EOC patients collected prior to cytoreductive surgery (correlation coefficient = 0.61968; P = 0.0027). These data suggest that epithelial ovarian tumors concomitantly affect serum sErbB1 and EGF levels. In conclusion, these data indicate that serum sErbB1 and EGF (postoperative only) levels are significantly different between EOC patients and healthy women and that altered and/or changing serum sErbB1 and EGF levels may provide important diagnostic and/or prognostic information useful for the management of patients with EOC.

Introduction

In women over the age of 35, the vast majority of ovarian tumors (90–95%) are of epithelial origin (1, 2). Globally, EOC represents the seventh most common type of women’s cancer after breast, cervix, colon and rectum, stomach, corpus uteri, and lung cancer (3); in the United States, EOC is the fifth most common women’s cancer (4). Although the incidence of EOC varies worldwide, the highest incidence rates are found in industrialized countries, with the exception of Japan. In the United States, approximately 12–13 new cases of EOC per 100,000 people are diagnosed each year (1, 2). It is estimated that 1 woman in 70 will be afflicted with EOC in her lifetime (5, 6) and that approximately 14,500 women will die from this disease in the United States in 1998 (7). In general, EOC is not curable and is the leading cause of death from gynecological malignancy in the United States. In addition, the risk of developing EOC is increased up to 4-fold for women with a prior history of breast carcinoma, and the relative risk of subsequently being diagnosed with breast cancer is increased 10–40% for women who first develop ovarian cancer (1). EOC, therefore, represents a major women’s health problem.

The high mortality rate of EOC is due primarily to the fact that clinical symptoms of this disease are often vague and nonspecific during its early stages. EOC, therefore, typically goes undetected and untreated until in its advanced stages; approximately 70% of all EOC patients are diagnosed with stage III or IV disease (8, 9). Although stage I EOC has a 90% 5-year survival rate, patients with stage III and IV disease have 5-year survival rates of just 15–20% and disease-free survival rates of less than 10%. Physicians rely on the patient’s history

1 The abbreviations used are: EOC, epithelial ovarian cancer; EGF, epidermal growth factor; MAb, monoclonal antibody; TGF, transforming growth factor; ECD, extracellular domain; ALISA, acridinium-linked immunosorbent assay.
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TGF-\(\beta\)-ploid carcinomas have been shown to release more EGF and TGF-\(\beta\). Meden therapy for patients with metastatic breast cancer (50–52). Levels are positive predictors of responsiveness to hormonal therapy for patients with metastatic breast cancer (50–52). Meden et al. (56) have recently observed a positive association between levels of a p80 sErbB1 protein and full-length ErbB1 in tissue samples of serous cystadenocarcinomas of the ovary. In contrast, patients with bladder cancer appear to have decreased sErbB1 levels in their urine (57). Together, these studies suggest that levels of sErbB1 proteins in serum, urine, and tumor tissues can change in response to disease state and therefore may provide useful prognostic and perhaps diagnostic information to physicians and their patients.

We have recently developed a sensitive ErbB1 ECD-specific ALISA to detect sErbB1 molecules in patient body fluids and have shown that serum samples of healthy men and women contain a ~110-kDa sErbB1 protein (58). Here, we report that (a) serum samples from patients with advanced stage EOC contain p110 ErbB1; (b) serum sErbB1 and EGF (post-operative only) levels differ significantly between healthy women and patients with stage III or IV EOC; (c) serum levels of sErbB1 and EGF change temporally in some surgically debulked EOC patients during the course of chemotherapy; and (d) serum sErbB1 and EGF levels are positively associated only in EOC patients prior to cytoreductive surgery. This study is the first report of serum sErbB1 and EGF levels in patients with EOC.

Materials and Methods

Cell Culture and Antibody Reagents. Hybridoma cells synthesizing MAbs 15E11 were grown as described previously (59). MAbs 15E11 was used in the form of high titer conditioned culture medium. MAbs R.1 and 528 were obtained as purified IgGs from Amersham Pharmacia Biotech (Arlington Heights, IL) and Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), respectively. Each of these MAbs is specific for epitopes of the ECD of ErbB1.

Serum Samples. Blood from healthy women was collected by the Department of Laboratory Medicine & Pathology, Mayo Medical Laboratories, in accordance with an ongoing institutional review board-approved Normal Values Study program and processed into serum. All serum samples from healthy women used in this study were collected between 1981 and 1984. Each healthy donor was required to provide a recent medical history that included a physical exam and the results of the following tests: hematology group, chemistry group, lipids, thyroid function, and urinalysis. Chest X-ray and ECG also were performed on age-appropriate subjects. These healthy women were not recovering from a surgical procedure. Detailed clinical records from these women are available.

Between 1985 and 1994, serum samples from women presenting to the Mayo Clinic for gynecological surgery were collected and stored to study the reproducibility of CA-125 measurements in women with EOC (60–63). Patients with ovarian cancer were classified as having International Federation of Gynecology and Obstetrics stage I, II, III, or IV disease at the time of staging laparotomy and tumor reductive surgery. Pathological diagnosis of EOC was made by histological examination following surgery by the Department of Laboratory Medicine & Pathology, Mayo Medical Laboratories. Serum samples were considered preoperative if they were collected within 30 days prior to surgery. Patients with a prior diagnosis of EOC that had received previous cytoreductive surgery, ra-
diation, or chemotherapy were eliminated from our study. We identified serum samples from 21 patients, ranging in age from 15 to 83 years, that fit these criteria. Although many preoperative serum samples were collected to study the reproducibility of CA-125 measurements in women with EOC (60–63), only these 21 samples remained from these studies. Of these 21 patients, 18 had stage III disease, and 3 had stage IV disease at the time of diagnosis. Pathological examination revealed that all 21 tumors were adenocarcinomas with the following histological tumor subtypes: 11 papillary serous, 5 serous, 1 endometrioid, 1 adenosquamous, and 3 not otherwise specified.

Postoperative serum samples from patients with stage III or IV EOC were collected in accordance with North Central Cancer Treatment Group and Mayo Clinic Protocol 90-61-54, entitled “Cyclophosphamide plus carboplatin: comparison of conventional dose and double-dose carboplatin in patients with stage III or IV ovarian carcinoma—a Phase III study.” All serum samples were collected between 1992 and 1994. Seventy-nine eligible patients were randomized to treatment on this study within 1 month after staging laparotomy and cytoreductive surgery. Chemotherapy was initiated within 3 days of randomization. Initial serum samples were collected within 34 days after staging laparotomy and cytoreductive surgery for all but six EOC patients; these six patients provided a serum sample 35–287 days after cytoreductive surgery and were eliminated from further study. The cohort of 73 EOC patients who provided serum samples within 34 days of randomization ranged in age from 24 to 74 years; 65 of these patients had stage III disease, and 8 had stage IV disease at the time of diagnosis. Pathological examination revealed that their tumors were of the following histological subtypes: 50 serous adenocarcinomas, 1 serous/endometrioid adenocarcinoma, and 22 tumors of other subtypes not specified in the database. Although most of the initial serum samples were collected prior to the start of chemotherapy, 15 patients had begun chemotherapy just prior (1–9 days) to their initial venipuncture. Follow-up serum samples were collected at various time points between 35 and 287 days following debulking surgery from 33 of the 73 patients who provided initial serum samples; these 33 patients were randomized to both treatment arms of this clinical trial.

Following collection, all blood samples were allowed to clot at room temperature for 30 min. The serum was separated from the clot and cells by centrifugation at 2000 g for 10 min, divided into 1-ml aliquots, and stored at −70°C. Each serum sample was thawed after transfer to our laboratory, aliquoted into smaller volumes, and refrozen at −70°C to prevent sErbB1 and EGF degradation. Each serum sample was, therefore, frozen and thawed only twice.

**Immunoprecipitation of sErbB1 Analogues from Human Sera.** To identify sErbB1 proteins, sera from healthy women and patients with advanced stage EOC were immunoprecipitated with protein G MAb-minus and protein G MAb R.1-coupled resins, separated by SDS-PAGE, and Western blotted according to Baron et al. (58). MAb 15E11 (IgG1 isotype), in the form of undiluted high titer-conditioned culture medium, was used as the primary antibody for Western immunoblot analyses. Rabbit antimouse IgG1 specific peroxidase secondary antibody, diluted 1:4000 into RPMI 1640 supplemented with 20% fetal bovine serum, 20 mM Hepes, pH 7.3, 2 mM l-glutamine, 1 mM sodium pyruvate, and 0.01% thimerosal (mercury-[o-carboxyphenyl]thio)-ethyl sodium) as a preservative was used as the secondary antibody for Western immunoblot analyses. Antibody binding was visualized with the enhanced chemiluminescent substrate luminol (Amersham Pharmacia Biotech).

**ErbB1 ECD-specific ALISA.** Serum sErbB1 levels were determined with an ALISA specific for epitopes of the ECD of ErbB1 according to Baron et al. (58) with the following ALISA blocking buffer (2.0% BSA, 10 mM Trizma, pH 7.4, 150 mM NaCl, 0.01% normal rabbit serum, 0.01% normal mouse serum, 0.02% Na3). Human sera were assayed undiluted or at dilutions of either 1:25 or 1:10 in ALISA blocking buffer. Initially, each serum sample was tested in duplicate at a 1:25 dilution in three separate experiments. Each serum sample was then tested in duplicate at a 1:10 dilution in three separate experiments. Finally, those serum samples that yielded undetectable sErbB1 levels at dilutions of 1:25 and 1:10 were tested undiluted in duplicate in three separate experiments. Undiluted serum samples that yielded values in relative light units below the interassay biological detection limit of 24 fmol/ml for this ALISA were considered undetectable. The sErbB1 concentration reported in the scattergrams for each serum sample represents the median of the mean sErbB1 level determined in three separate assays.

**ELISA.** Serum EGF levels were determined with the QuantiQine human EGF ELISA (R & D Systems, Minneapolis, MN) according to the manufacturer’s instructions, but with the following modifications. All serum samples were tested in duplicate at a 1:5 dilution in calibrator diluent (RD6H). In addition, assay diluent (RD1) was added to each well of EGF standards, as well as unknown serum samples. Serum samples that yielded absorbance values below the interassay biological detection limit of 0.44 fmol/ml for this ELISA were considered undetectable. The mean serum EGF level for each serum sample used in this study is shown in the scattergrams. One serum sample was depleted during the course of this study; therefore, we report EGF concentrations for only 72 serum samples from EOC patients who provided serum samples 0–34 days after cytoreductive surgery.

**Results**

**Serum Samples from EOC Patients Contain p110 sErbB1.** Recently, we have developed a sandwich type ALISA specific for the ECD of the EGF receptor that is useful for quantifying ErbB1 and sErbB1 molecules in human cell lines, tissues, and body fluids (58). Furthermore, we have shown by immunoprecipitation and Western immunoblot analysis that our ALISA specifically measures a ~110-kDa sErbB1 protein in serum samples of healthy men (n = 2) and women (n = 13) and that our ALISA does not detect ErbB2, ErbB3, or ErbB4. To identify the specific sErbB1 analogue(s) present in serum samples of patients with EOC, we immunoprecipitated five serum samples (postoperative) from patients with stage III EOC with protein G affinity resin alone (control) or protein G resin coupled covalently to the capture MAb used in our ALISA (MAb R.1; Ref. 64). We previously had determined that these serum samples contained high levels of MAb used in our ALISA (MAb R.1; Ref. 64). We previously had determined that these serum samples contained high levels of sErbB1 by ALISA. Immunoprecipitates were subsequently Western blotted with anti-ErbB1 ECD-specific MAb 15E11 (59). Fig. 1 shows a Western immunoblot of immunoprecipitates from a healthy woman and a patient with stage III EOC. A ~110-kDa protein eluted from the MAb R.1 affinity resin, but not from the MAb-minus resin of both serum samples. We have observed this ~110-kDa protein in all five of the stage III EOC serum samples, and in all of the serum samples from healthy women analyzed to date (data not shown). This ~110-kDa protein did not react with peroxidase-conjugated secondary antibody alone.
These results demonstrate that sera from healthy women and patients with advanced stage EOC contain a p110 sErbB1 protein.

**EOC Patients Have Significantly Lower Serum sErbB1 Levels.** Age is a known prognostic factor for patients with EOC (1, 2). However, there is presently no evidence that serum sErbB1 levels differ with respect to age for either healthy men or women (58). We have identified serum samples collected within a period of 30 days prior to staging laparotomy and cytoreductive surgery from 21 stage III or IV EOC patients; none of these patients had received prior chemotherapy, radiation, or debulking surgery. We compared the serum sErbB1 levels in these EOC patients with the serum sErbB1 levels in a group of 21 healthy women of similar ages (Fig. 2; Table 1).

The median (range) serum sErbB1 concentration of the 21 age-matched healthy women is 6,395 fmol/ml (1,846–23,708 fmol/ml). In contrast, the median (range) preoperative serum sErbB1 concentration of the 21 patients with stage III or IV EOC is 284 fmol/ml (30–1,350 fmol/ml). These data indicate that preoperative serum sErbB1 levels in patients with stage III or IV EOC are significantly lower than serum sErbB1 levels in healthy women of similar ages (Wilcoxon rank sum test, \( P < 0.0001 \)).

**Serum sErbB1 Levels Change Temporally in EOC Patients.** We also have examined serum samples collected after staging laparotomy and cytoreductive surgery from 73 patients with stage III or IV EOC, who presented for treatment between 1992 and 1994. These patients had not received prior debulking surgery, radiation, or chemotherapy for EOC, and were enrolled (data not shown). These results demonstrate that sera from healthy women and patients with advanced stage EOC contain a p110 sErbB1 protein.

**Fig. 1.** To identify endogenous sErbB1 molecules, serum samples from healthy women and patients with advanced stage EOC were immunoprecipitated with protein G MAb minus (−; Lanes 1 and 3) or protein G MAb R.1-coupled (+; Lanes 2 and 4) resins and Western blotted with anti-ErbB1 ECD-specific MAb 15E11. This Western immunoblot of immunoprecipitates from a healthy woman (NHS, normal human serum; Lanes 1 and 2) and a patient with stage III EOC (EOCS, EOC serum; Lanes 3 and 4) shows that a single comigrating band of \( \sim 110\)-kDa labels specifically with MAb 15E11. This \( \sim 110\)-kDa band is only present in the MAb R.1-coupled immunoprecipitation samples. Shown from top to bottom is the relative mobility of prestained molecular mass markers (Bio-Rad Laboratories) corresponding to myosin (197 kDa), β-galactosidase (117 kDa), BSA (89 kDa), and ovalbumin (52 kDa).

**Fig. 2.** A. sera from healthy women and patients with stage III or IV EOC were assayed by ALISA to quantify their sErbB1 levels; scattergrams of five patient groups are plotted. Patient groups include healthy women (\( n = 21 \)) age-matched to a cohort of EOC patients (\( n = 21 \)) who provided preoperative serum samples prior to cytoreductive surgery, healthy women (\( n = 73 \)) age-matched to a cohort of EOC patients (\( n = 73 \)) who provided initial postoperative serum samples 0–34 days after cytoreductive surgery, and EOC patients who provided follow-up postoperative serum samples 35–287 days after cytoreductive surgery (\( n = 33 \)). Serum samples with sErbB1 levels below the interassay biological detection limit (horizontal line with arrow) of 24 fmol/ml were arbitrarily assigned values of 20 fmol/ml for graphing purposes. Each data point represents the median of the mean sErbB1 concentration for one serum sample tested in duplicate from a minimum of three separate assays. The median sErbB1 concentration for each group of patients is indicated by the horizontal line and is given in Table 1. B. serum sErbB1 levels from EOC patients (\( n = 33 \)) who provided an initial postoperative serum sample at 0–34 days and a second follow-up postoperative serum sample at 35–287 days are compared to determine whether sErbB1 levels changed over time. Data points to the left of the bisecting line indicate an increase in sErbB1 concentration relative to the initial serum sample, whereas data points on or to the right of this line indicate either no change or a decrease in sErbB1 concentration relative to the initial serum sample.
in a phase III randomized clinical trial to study the efficacy of cyclophosphamide plus conventional dose carboplatin versus cyclophosphamide plus an intensive dose of carboplatin in patients with stage III and IV EOC following surgery. Chemotherapy was initiated within approximately 1 month after surgery. Although most of the initial serum samples were collected prior to the start of chemotherapy, 15 patients had begun chemotherapy just prior to (1–9 days) their initial venipuncture. We compared the initial postoperative (0–34 days) serum sErB1 levels in these 73 EOC patients to serum sErB1 levels in a group of 73 healthy age-matched women (Fig. 2A, Table 1). The median (range) serum sErB1 concentration of the 73 healthy women was 6,113 fmol/ml (1,292–51,358 fmol/ml). In contrast, the median (range) initial postoperative serum sErB1 concentration of the 73 EOC patients was 1,799 fmol/ml (nondetectable to 11,035 fmol/ml). These data indicate that the initial postoperative sErB1 levels in patients with stage III or IV EOC differ significantly from sErB1 levels in an age-matched group of healthy women (Wilcoxon rank sum test, \( P < 0.0001 \); the one patient with a nondetectable serum sErB1 level was excluded from this analysis).

Thirty-three patients enrolled in the aforementioned phase III study provided a second serum sample 35–287 days after cytoreductive surgery. The median (range) serum sErB1 concentration of these 33 serum samples was 6,434 fmol/ml (nondetectable to 29,666 fmol/ml). The median (range) serum sErB1 concentration of these 33 serum samples appeared higher than those seen in healthy women (Wilcoxon rank sum test, \( P < 0.0001 \); the one patient with a nondetectable serum sErB1 level was excluded from this analysis).

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>Median age (range), yrs</th>
<th>Mean sErB1 (fmol/ml)</th>
<th>Mean sErB1 (fmol/ml)</th>
<th>Range of sErB1 (fmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy women age-matched to EOC patients who gave preoperative serum samples (( n = 21 ))</td>
<td>68 (25–76)</td>
<td>8,166</td>
<td>6,395</td>
<td>1,846–23,708</td>
</tr>
<tr>
<td>Stage III or IV EOC patients who gave preoperative serum samples (( n = 21 ))</td>
<td>68 (15–83)</td>
<td>429</td>
<td>284</td>
<td>30–1,350</td>
</tr>
<tr>
<td>Healthy women age-matched to EOC patients who gave initial postoperative serum samples between days 0 and 34 (( n = 73 ))</td>
<td>54 (25–74)</td>
<td>8,298</td>
<td>6,113</td>
<td>1,292–51,358</td>
</tr>
<tr>
<td>Stage III or IV EOC patients who gave initial postoperative serum samples between days 0 and 34 (( n = 73 ))</td>
<td>56 (24–74)</td>
<td>2,695</td>
<td>1,799</td>
<td>ND–11,035</td>
</tr>
<tr>
<td>Stage III or IV EOC patients who gave second postoperative serum samples between days 35 and 287 (( n = 33 ))</td>
<td>55 (24–71)</td>
<td>9,256</td>
<td>6,434</td>
<td>ND–29,666</td>
</tr>
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</table>

\( \text{ND} \) indicates nondetectable values below the interassay biological detection limit of 24 fmol/ml.

EOC Patients Have Significantly Higher Postoperative Serum EGF Levels. EGF and sErB1 molecules have been shown to bind each other in vitro (65–69). Because EGF and sErB1 may interact in serum, we compared the EGF levels between the groups of EOC patients and healthy age-matched women (Fig. 3, Table 2). The median (range) preoperative serum EGF concentration of the 21 stage III or IV EOC patients was 8.1 fmol/ml (1.1–84.9 fmol/ml). The median (range) serum EGF concentration of the 21 healthy women age-matched to these EOC patients was 8.6 fmol/ml (nondetectable to 38.8 fmol/ml). No significant difference in EGF levels was found between the 21 preoperative stage III or IV EOC serum samples and those of the 21 healthy age-matched women (Wilcoxon rank sum test, \( P = 0.8449 \); the one patient with a nondetectable serum sErB1 level was excluded from this analysis). The median (range) postoperative serum EGF concentration of the 72 EOC patients who provided initial serum samples 0–34 days after cytoreductive surgery was 26.3 fmol/ml (1.0–133.1 fmol/ml); only 72 serum samples were available to quantify EGF levels. In contrast, the median (range) serum EGF concentration of the 72 healthy women age-matched to these EOC patients was 11.2 fmol/ml (nondetectable to 43.5 fmol/ml). These data indicate that postoperative serum EGF levels in patients with stage III or IV EOC are significantly higher than those in an age-matched group of healthy women (Wilcoxon rank sum test, \( P < 0.0001 \); the one patient with a nondetectable serum sErB1 level was excluded from this analysis).

Serum EGF Levels Change Temporally in EOC Patients. The median (range) EGF concentration in the 33 serum samples collected from stage III or IV EOC patients between postoperative days 35–287 was 22.1 fmol/ml (nondetectable to 195.4 fmol/ml). These follow-up EGF levels were similar to those seen in the 72 initial postoperative serum samples (median, 26.3 fmol/ml; range, 1.0–133.1 fmol/ml; Table 2), with the exception of one patient who had an undetectable level of serum EGF (Fig. 3A). Statistical comparison between the EGF levels observed in the serum samples from postoperative days 0–34 versus days 35–287 was not performed, because the latter serum samples were collected from a subset of the 72 patients enrolled in this phase III study, as well as over a prolonged period of time, \( i.e., 253 \) days. We also did not perform a statistical comparison with a group of healthy women for the same reason. Examination of the EGF concentration in the initial versus the second serum sample from each of the 33 patients who underwent cytoreductive surgery and received...
combination chemotherapy shows that EGF levels changed temporally for several, but not for all, of these 33 patients during the course of combination chemotherapy; in fact, some patient’s serum EGF levels increased, whereas others’ decreased (Fig. 3B).

**sErbB1 and EGF Levels Are Positively Associated in Preoperative Serum Samples of EOC Patients.** To determine whether an association between serum sErbB1 and EGF levels exists, we graphed the concentrations of these molecules against each other for the following groups of patients: all of the healthy women used in this study, all of the EOC patients from whom preoperative serum samples were collected, all of the EOC patients from whom postoperative serum samples were collected 0–34 days after cytoreductive surgery, and all of the EOC patients from whom postoperative serum samples were collected 35–287 days after cytoreductive surgery. These graphs demonstrate no obvious visual relationship between sErbB1 and EGF levels was seen in serum samples collected from EOC patients prior to cytoreductive surgery, i.e., higher sErbB1 levels are associated with higher EGF levels (Fig. 4). We found this relationship in the preoperative EOC serum samples to be statistically significant (Spearman’s rank order correlation coefficient = 0.61968; \( P = 0.0027 \)), whereas no evidence for a significant association between sErbB1 and EGF levels was found to exist in the 21 healthy women age-matched to this cohort of EOC patients (Spearman’s rank order correlation coefficient = 0.10075; \( P = 0.6726 \)). In addition, we found no evidence for an association between sErbB1 and EGF levels in the 72 EOC patients who provided postoperative serum samples 0–34 days after cytoreductive surgery (Spearman’s rank order correlation coefficient = 0.07923; \( P = 0.5113 \)), or in the 72 healthy women age-matched to this cohort of EOC patients (Spearman’s rank order correlation coefficient = 0.17033; \( P = 0.1526 \)).

**Discussion**

Several previous studies have described sErbB1 proteins in human tissues and body fluids, with apparent molecular masses ranging from 41 to 95 kDa (40–42, 55, 70–72). Ilekis et al. (41) used MAb LA22- and EGF-affinity chromatography to purify a 80-kDa protein from term human placenta that is detected by Western immunoblot analysis with anti-ErbB1 ECD-specific MAb LA22. Witters et al. (55) identified a 95-kDa sErbB1 analogue in human urine by immunoprecipitation and Western immunoblot analysis. We previously reported that human placenta expresses a 1.8-kb alternative mRNA transcript that encodes a truncated, glycosylated 60-kDa form of sErbB1 in transfected tissue culture cells (42). Recently, we isolated a second 3.0-kb alternative mRNA transcript from human placenta, which encodes a glycosylated \(~\sim 110-\)kDa form of sErbB1 containing extracellular subdomains I–IV.\(^4\) In addition, we have shown that sera from both healthy men and women contain a \(~\sim 110-\)kDa sErbB1 analogue (58). Here, we show that serum samples from patients with advanced stage EOC also contain a \(~\sim 110-\)kDa sErbB1 protein. However, we have not yet clarified the relationship between this serum sErbB1 protein and the p110 sErbB1 product encoded by the 3.0-kb mRNA

\(^4\) J. L. Reiter and N. J. Mailhe, manuscript in preparation.
Data suggest that epithelial ovarian tumors affect circulating the concentration range seen in all of the healthy women EOC patients (30 –1,350 fmol/ml; Table 1) barely overlaps with sErbB1 in preoperative serum samples of these advanced stage of similar ages. Furthermore, the concentration range of p110 preoperative serum levels of p110 sErbB1 than healthy women sErbB1 molecules into the circulatory system. Here, we report indicate that normal male and female tissues deliver p110 fmol/ml (467–51,583 fmol/ml), respectively (58). These data men and women have median (range) serum p110 sErbB1 protein is generated either by tran-
mRNA, or by proteolytic cleavage of the full-length ErbB1 holoreceptor in various tu-
mRNA, or by proteolytic cleavage of the full-length sErbB1 molecules have been shown to bind EGF in vitro, or during wound repair.

Ovarian tumors have been shown to release more EGF and TGF-α than benign tissues, and aneuploid ovarian carcinomas have been shown to release more EGF and TGF-α than diploid ovarian carcinomas (23). We did not find a statistically significant difference between preoperative serum EGF levels in patients with stage III or IV EOC and serum EGF levels in healthy women of similar ages. However, we report that EGF levels are significantly higher in 72 stage III or IV EOC patients who provided serum samples 0–34 days after cytoreductive surgery than in healthy women of similar ages. However, we report that EGF levels return to normal values following tumor debulking surgery.

We show here that serum samples collected 0–34 days after cytoreductive surgery from 73 stage III or IV EOC patients also have significantly lower levels of p110 sErbB1 than healthy women of similar ages. Serum samples collected 35–287 days after cytoreductive surgery from 33 of these 73 EOC patients contain sErbB1 levels that appear similar to those seen in healthy women. Interestingly, both the initial and second postoperative serum sErbB1 levels appear higher than those seen in preoperative serum samples of patients with stage III or IV EOC. Examination of the sErbB1 concentration in the initial versus the second serum sample from each of the 33 patients who underwent cytoreductive surgery revealed that sErbB1 levels increased temporally for many, but not for all, of these 33 patients during the course of combination chemotherapy (Fig. 2B). Because these 33 patients may represent a biased subset of the 79 women enrolled in this clinical trial, we were not able to determine whether changing postoperative serum sErbB1 levels are useful biomarkers of responsiveness to chemotherapy. Although the data presented here were generated from preoperative and postoperative serum samples from two cohorts of EOC patients, these data lead us to hypothesize that serum concentrations of p110 sErbB1 are significantly lower in advanced stage EOC patients than in healthy women and that sErbB1 levels return to normal values following tumor debulking surgery.

Ovarian tumors have been shown to release more EGF and TGF-α than benign tissues, and aneuploid ovarian carcinomas have been shown to release more EGF and TGF-α than diploid ovarian carcinomas (23). We did not find a statistically significant difference between preoperative serum EGF levels in patients with stage III or IV EOC and serum EGF levels in healthy women of similar ages. However, we report that EGF levels are significantly higher in 72 stage III or IV EOC patients who provided serum samples 0–34 days after cytoreductive surgery than in healthy women of similar ages. It is likely that small patient sample size (n = 21) diminished our ability to detect anything but large differences in serum EGF levels between the EOC patients who provided preoperative serum samples and the group of healthy age-matched women. These data lead us to hypothesize that serum EGF levels may either be higher in stage III or IV EOC patients before and after cytoreductive surgery than in healthy women or, alternatively, that serum EGF levels may rise in response to surgical wounding and/or during wound repair.

sErbB1 molecules have been shown to bind EGF in vitro (65–69). Here, we report a positive association between

**Table 2** Comparison of serum EGF concentrations between healthy women and patients with stage III or IV EOC

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>Median age (range), yrs</th>
<th>Mean EGF (fmol/ml)</th>
<th>Median EGF (fmol/ml)</th>
<th>Range of EGF (fmol/ml)</th>
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</thead>
</table>
| Healthy women age-matched to EOC patients who gave preoperative serum samples (n = 21) | 68 (25–76) | 11.9 | 8.6 | ND–38.8 
| Stage III or IV EOC patients who gave preoperative serum samples (n = 21) | 68 (15–83) | 23.0 | 8.1 | 1.1–84.9 |
| Healthy women age-matched to EOC patients who gave initial postoperative serum samples between days 0 and 34 (n = 72) | 54 (25–74) | 15.1 | 11.2 | ND–43.5 |
| Stage III or IV EOC patients who gave initial postoperative serum samples between days 0 and 34 (n = 72) | 56 (24–74) | 37.3 | 26.3 | 1.0–133.1 |
| Stage III or IV EOC patients who gave second postoperative serum samples between days 35 and 287 (n = 33) | 55 (24–71) | 34.1 | 22.1 | ND–195.4 |

* ND, nondetectable values below the interassay biological detection limit of 0.44 fmol/ml.
* Wilcoxon rank sum test; patients with nondetectable EGF levels were not used in these analyses.

**Fig. 4.** Serum sErbB1 and EGF concentrations are graphed against each other to determine whether an association between these two molecules exists in EOC patients who provided serum samples prior to cytoreductive surgery. The biological detection limits for sErbB1 (horizontal line) and EGF (vertical line) are shown.

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transcript identified from human placenta. It is possible that this serum p110 sErbB1 protein is generated either by transcription from this 3.0-kb mRNA, by transcription from another c-erbB1 mRNA, or by proteolytic cleavage of the full-length ErbB1 receptor from the cell surface.

Overexpression of the ErbB1 holoreceptor in various tumor cell lines and neoplasms (26, 28) has led us, as well as others, to hypothesize that sErbB1 levels in human body fluids may be altered during oncogenesis, tumor progression, and metastasis. We previously demonstrated by ALISA that healthy men and women have median (range) serum p110 sErbB1 levels of 24,512 fmol/ml (6,751–48,826 fmol/ml) and 3,716 fmol/ml (467–51,583 fmol/ml), respectively (58). These data indicate that normal male and female tissues deliver p110 sErbB1 molecules into the circulatory system. Here, we report that patients with stage III or IV EOC have significantly lower preoperative serum levels of p110 sErbB1 than healthy women of similar ages. Furthermore, the concentration range of p110 sErbB1 in preoperative serum samples of these advanced stage EOC patients (30–1,350 fmol/ml; Table 1) barely overlaps with the concentration range seen in all of the healthy women studied to date (467–51,583 fmol/ml; Ref. 58). Together, these data suggest that epithelial ovarian tumors affect circulating sErbB1 levels and that low serum sErbB1 levels may be diagn-

nostic indicators of EOC. In addition, these data lead us to hypothesize that a low serum sErbB1 level may be a risk factor for EOC.

We show here that serum samples collected 0–34 days after cytoreductive surgery from 73 stage III or IV EOC patients also have significantly lower levels of p110 sErbB1 than healthy women of similar ages. Serum samples collected 35–287 days after cytoreductive surgery from 33 of these 73 EOC patients contain sErbB1 levels that appear similar to those seen in healthy women. Interestingly, both the initial and second postoperative serum sErbB1 levels appear higher than those seen in preoperative serum samples of patients with stage III or IV EOC. Examination of the sErbB1 concentration in the initial versus the second serum sample from each of the 33 patients who underwent cytoreductive surgery revealed that sErbB1 levels increased temporally for many, but not for all, of these 33 patients during the course of combination chemotherapy (Fig. 2B). Because these 33 patients may represent a biased subset of the 79 women enrolled in this clinical trial, we were not able to determine whether changing postoperative serum sErbB1 levels are useful biomarkers of responsiveness to chemotherapy. Although the data presented here were generated from preoperative and postoperative serum samples from two cohorts of EOC patients, these data lead us to hypothesize that serum concentrations of p110 sErbB1 are significantly lower in advanced stage EOC patients than in healthy women and that sErbB1 levels return to normal values following tumor debulking surgery.

Ovarian tumors have been shown to release more EGF and TGF-α than benign tissues, and aneuploid ovarian carcinomas have been shown to release more EGF and TGF-α than diploid ovarian carcinomas (23). We did not find a statistically significant difference between preoperative serum EGF levels in patients with stage III or IV EOC and serum EGF levels in healthy women of similar ages. However, we report that EGF levels are significantly higher in 72 stage III or IV EOC patients who provided serum samples 0–34 days after cytoreductive surgery than in healthy women of similar ages. It is likely that small patient sample size (n = 21) diminished our ability to detect anything but large differences in serum EGF levels between the EOC patients who provided preoperative serum samples and the group of healthy age-matched women. These data lead us to hypothesize that serum EGF levels may either be higher in stage III or IV EOC patients before and after cytoreductive surgery than in healthy women or, alternatively, that serum EGF levels may rise in response to surgical wounding and/or during wound repair.

sErbB1 molecules have been shown to bind EGF in vitro (65–69). Here, we report a positive association between
sErbB1 and EGF levels only in EOC patients prior to tumor debulking surgery. This pattern of association was not seen in the serum samples of healthy women or EOC patients who provided postoperative serum samples. These data lead us to hypothesize that epithelial ovarian tumors concomitantly affect sErbB1 and EGF levels in the circulatory system.

In conclusion, this retrospective analysis of sErbB1 and EGF levels in advanced stage EOC patients demonstrates that: (a) serum samples from patients with advanced stage EOC contain p110 sErbB1; (b) serum sErbB1 and EGF (postoperative only) levels differ significantly between healthy women and patients with stage III or IV EOC; (c) serum levels of sErbB1 and EGF change temporally in some surgically debulked EOC patients during the course of chemotherapy; and (d) serum sErbB1 and EGF levels are positively associated only in EOC patients prior to cytoreductive surgery. These data suggest that altered and/or changing serum sErbB1 and EGF levels may be important diagnostic and/or prognostic tumor biomarkers for patients with EOC and that a larger prospective study of serum sErbB1 and EGF levels in patients with stage III and IV EOC is warranted. This study is the first report of serum sErbB1 and EGF levels in patients with EOC. Future studies will allow us to determine whether these molecules alone or in combination can provide clinically useful diagnostic and/or prognostic information to physicians and their patients, and hence affect the management of EOC.

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References

Serum sErB1 and Epidermal Growth Factor Levels As Tumor Biomarkers in Women with Stage III or IV Epithelial Ovarian Cancer

Andre T. Baron, Jacqueline M. Lafky, Cecelia H. Boardman, et al.

Cancer Epidemiol Biomarkers Prev 1999;8:129-137.

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