Circulating Soluble Fas in Patients with Breast Cancer

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Abstract. It has been suggested that circulating soluble Fas (sFas) contributes to tumor progression. However, little is known about the role of sFas in breast cancer. This study was designed with the aim of elucidating the possible relation between sFas and breast cancer. A series of 57 consecutive patients with invasive breast cancer undergoing surgery were prospectively included in the study and evaluated. Venous blood samples were collected before surgery. Sera were obtained by centrifugation and stored at −70°C until assayed. The control group consisted of 12 patients with benign breast tumors (6 with fibrocystic disease, 6 with fibroadenoma). Serum concentrations of sFas were measured by the quantitative sandwich enzyme immunoassay technique. The data on primary tumor staging, age, estrogen receptor status, lymph node status, tumor grading, and TNM staging were reviewed and recorded. The mean value of circulating sFas in patients with invasive breast cancer was 794.2 ± 183.0 pg/ml and that of the control group 582.1 ± 62.8 pg/ml; the difference was significant (p = 0.021). In the multivariate analysis, TNM stage (p = 0.020) and in those with a more advanced TNM stage (p = 0.021) appeared to be an independent factor for significantly higher circulating sFas in patients with invasive breast cancer. Thus circulating sFas levels may reflect the severity of invasive breast cancer. Hence the possible prognostic value of sFas for breast cancer deserves further elucidation and evaluation with long-term patient follow-up.

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1. Soluble Fas (sFas) arises from alternatively spliced mRNA, leading to protein with deletion or disruption of the single membrane-spanning domain. Five alternatively spliced Fas mRNAs have been described [6, 7]. Soluble Fas can inhibit Fas-mediated apoptosis by neutralizing FasL or anti-Fas antibody [8]. Elevated sFas has been noted in various malignant diseases including leukemia, lymphoma, bladder cancer, and hepatocellular carcinoma [9–12]. Elevated levels of sFas were found to be associated with a poor prognosis in patients with bladder cancer [12]. Therefore it was postulated that malignant cells might up-regulate or induce the production of sFas, which may protect malignant cells from Fas-mediated apoptosis [13]. This study was designed with the aim of elucidating the possible relation between sFas and breast cancer.

Materials and Methods

From November 1998 to March 2000 a series of 57 consecutive patients with invasive breast cancer were studied. Venous blood samples were collected before the surgery; and the sera were obtained by centrifugation and stored at −70°C until assayed. All 57 patients were women with ages ranging from 32 to 79 years (mean 51 years). All of the patients underwent modified radical mastectomy, and the diagnosis of breast cancer was confirmed by histologic examination. Invasive breast cancer was defined as carcinoma, regardless of origin (duct or lobule), with invasion to or beyond the basement membrane [14]. The data for primary tumor staging, age, estrogen receptor status, lymph node status, tumor grade, and TNM stage were collected. Thorough physical examination, chest radiography, serum alkaline phosphatase level, and mammography were part of the preoperative routine for all patients.

Bone scans and abdominal ultrasonography were performed for all the patients with provisional clinical stage 3 to rule out the presence of distant metastases. Regardless of the provisional clinical stage, all the patients with elevated serum alkaline phosphatase levels, special complaints such as bone pain, or any specific findings indicating the possibility of distant metastases such as hepatomegaly also underwent bone scanning and abdominal ultrasonography to determine if there were distant metastases.
Estrogen receptor status was determined by an immunohistochemical staining method [15–17]. Tumors were graded according to the criteria described by Bloom and Richardson [18]. Twelve patients with benign breast tumor (6 with fibrocystic disease, 6 with fibroadenoma) comprised the control group.

**Measurement of Circulating Soluble Fas**

The enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) was used for quantitatively determining the serum concentration of sFas. In brief, each serum sample was diluted and added to microtiter wells precoated with a monoclonal antibody specific for Fas, followed by an additional incubation with an enzyme-linked polyclonal antibody specific for Fas. Color development was performed using a tetramethyl benzidine-H$_2$O$_2$ mixture and stopped by sulfuric acid. The absorbance of each well was determined using a spectrophotometer. The minimum detectable dose of sFas is typically less than 20 pg/ml. The intraassay coefficient of variation is 2.9% to 4.6%.

**Statistical Analysis**

Student’s t-test was used to assess the significance of difference in the levels of circulating sFas between the patient and control groups. The following clinicopathologic variables were first entered into the univariate analysis by Student’s t-test or ANOVA: primary tumor staging, age, estrogen receptor status, lymph node status, and TNM stage. These clinicopathologic variables were then assessed by the multiple linear regression (stepwise) method. A value of $p < 0.05$ was accepted as significant. Results were in picograms per milliliter and were expressed as the mean ± SD.

**Results**

The levels of circulating sFas were compared for the patient and control groups. The mean value of circulating sFas in patients with breast cancer was 794.2 ± 183.0 pg/ml, and that of the control group was 582.1 ± 62.8 pg/ml; the difference was significant ($p < 0.001$). The levels of circulating sFas in patients with clinicopathologic variables calculated by univariate analysis are shown in Table 1. The serum levels of sFas for the control and patient groups are presented as box plots (Fig. 1).

The older patients (age ≥ 50) ($p = 0.020$) and the patients with more advanced TNM staging ($p = 0.021$) were shown to have significantly higher levels of circulating sFas (Table 1). In the multivariate analysis, TNM staging ($p = 0.005$) appeared to be an independent factor regarding the significantly higher circulating sFas levels.

**Discussion**

Evaluating 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, the physician must consider all available prognostic data [15–17, 19–21]. We conducted this study to investigate if there is any correlation between the serum sFas and clinicopathologic variables and, furthermore, to elucidate a possible relation between sFas and breast cancer. We included patients with invasive breast cancer only, as intraductal, noninvasive breast cancer usually has a quite different, better clinical course.

The origin of sFas in patients with breast cancer remains unclear. There are three possibilities. First, sFas may be derived from the tumor itself, corresponding to previous findings in tumor cell lines [13, 22]. Second, sFas may be derived from peripheral blood lymphocytes [9]. Third, the surrounding stromal tissue may produce sFas in response to tumor or immune activation [23].

The function of sFas is not fully understood, although several reports suggest that it plays a role in tumor progression. sFas can inhibit Fas-mediated apoptosis by neutralizing FasL or anti-Fas antibody [8]. Thus sFas is thought to be one of the mechanisms for