Serum Soluble Fas Level as a Prognostic Factor in Patients with Gynecological Malignancies

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ABSTRACT

The Fas-Fas ligand system is important in apoptosis mediated by CTLs and natural killer cells. The suppression of apoptosis contributes to carcinogenesis, as well as to a resistance to chemotherapy and radiotherapy. Circulating soluble Fas (sFas), which is generated by alternative mRNA splicing, can antagonize cell-surface Fas function. We investigated sFas levels in 64 patients with gynecological malignancies (28 cervical carcinomas, 18 endometrial carcinomas, and 18 ovarian carcinomas) and in 24 healthy female donors by using a Fas-specific ELISA. In each carcinoma group, serum sFas demonstrated a statistically significant elevation relative to levels in normal controls (P < 0.0001). Levels of serum sFas in patients with advanced cancer (FIGO stages III and IV) significantly exceeded those in patients with localized cancer (FIGO stages I and II) or those in normal control subjects (P < 0.0001). We divided the patients into two groups based on the level of serum sFas and examined the relationship between serum sFas levels and survival. No deaths occurred in the groups with cervical and endometrial cancer with a serum sFas level <1.5 ng/ml. Survival rates in groups with cervical carcinoma, endometrial carcinoma, and ovarian carcinoma with a serum sFas level <1.5 ng/ml exceeded those in groups with sFas levels of ≥1.5 ng/ml (P < 0.001, P = 0.128, and P = 0.012, respectively). Proportional hazard models demonstrated that serum sFas level was a statistically significant factor (P = 0.0196) for survival, as well as histological grade (P = 0.0168) in ovarian carcinoma.

INTRODUCTION

Fas/APO-1/CD95 is a type I membrane protein and a prototypic member of the nerve growth factor/tumor necrosis factor-R4 superfamily that induces apoptosis (1). The Fas-FasL system is a cytotoxic effector of NK cells and CTLs. The interaction between FasL and cell-surface Fas induces apoptosis in sensitive cells (1). Fas can occur both as a cell-surface protein and as a soluble protein. Cell-surface Fas is anchored by a single membrane-planning domain and is widely expressed in normal and malignant cells (1–4). sFas, which is generated by alternative mRNA splicing and lacks a transmembrane domain, is thought to inhibit Fas-FasL binding and blocks Fas-mediated apoptosis (5–8). This apoptotic Fas-FasL pathway may play a role in tumorigenesis and disease progression (6, 9).

sFas has been identified in the supernatants of activated human lymphocytes (5–8) and in several tumor cell lines, including hepatoma (8), osteosarcoma (6), and T- and B-cell leukemias and lymphomas (7, 10). Elevated levels of sFas have also been observed in the serum of patients with solid tumors (11, 12) and hematopoietic malignancies (10). The clinical significance of serum sFas levels has not yet been clarified. The molecular mechanisms underlying resistance to apoptosis mediated by the Fas-FasL system are complex and involve both postreceptor and prereceptor events (1, 13). Prereceptor resistance can be mediated by sFas, which antagonizes both anti-Fas- and FasL-mediated cell lysis in a dose-dependent manner (5–8). Because a loss of Fas function has been implicated in the pathogenesis of tumor progression (14), the production of sFas may be involved in the pathogenesis of malignant disease.

The present study evaluates the circulating sFas levels using ELISA in patients with gynecological malignancies, compares these levels with those of normal controls, and examines the relationship between sFas levels, the stage of malignant disease, and survival rate. We hypothesize that elevated serum sFas levels in patients diagnosed with gynecological cancer may be used as a prognostic factor.

MATERIALS AND METHODS

Patients. We evaluated serum sFas levels in patients diagnosed with gynecological malignancies after informed consent was obtained from each subject. Pretreatment serum samples were obtained from 64 patients (range, 27–69 years old; mean ± SD, 48.2 ± 11.1) with gynecological malignancies at the Department of Obstetrics and Gynecology, Tohoku University Hospital. Twenty-eight patients diagnosed with cervical carcinoma, 18 with endometrial carcinoma, and 18 with ovarian carcinoma were used as subjects for the present study. Tumors were classified according to FIGO classification. Analysis between stages was classified into two categories: local disease as stages I and II versus advanced disease as stages III and IV. As
a normal control, sera were obtained from 24 healthy female volunteers (range, 27–58 years old; mean ± SD, 40.5 ± 6.9). Patients and their medical history, including cancer stage, treatment, histological type, grade of histological differentiation, and survival rate, are presented in Table 1. Blood samples were kept at 4°C, centrifuged at 10,000 rpm for 15 min, and then immediately frozen at −80°C until assayed.

**Fas-specific ELISA.** A double antibody sandwich ELISA was constructed to detect sFas in sera using a sFas (S) ELISA Kit (Medical & Biological Laboratories Co., LTD., Nagoya, Japan). This assay uses Fas antibodies against two different epitopes. One antibody was a polyclonal antibody that recognizes the intracellular domain (amino acids 305–319), whereas the other was a monoclonal antibody that recognizes the extracellular domain (amino acids 110–120).

Samples to be measured or the standards were incubated in wells coated with anti-Fas polyclonal antibody. After washing, a peroxidase-conjugated anti-Fas monoclonal antibody was added to each microwell and incubated. After another washing, the peroxidase substrate was mixed with the chromogen and allowed to incubate for an additional period of time. An acid solution was then added to each well to terminate the enzyme reaction and to stabilize the developed color. The absorbance of each well was then measured at 450 nm using a microplate reader. The concentration of sFas was calibrated from a dose-response curve based on reference standards.

**Statistical Analysis.** Data are shown as mean ± SD. Differences between sFas levels among the patients and the normal controls were determined using the two-sided Mann-Whitney U test. Correlation between age and serum sFas level was evaluated with Pearson’s correlation test. A nonparametric Kruskal-Wallis test was used in evaluating the differences between the stages of subgroups, and when appropriate, Scheffe’s F test was used as a post hoc test. Life-table estimates were calculated according to the method of Kaplan and Meier. Survival differences between groups were evaluated with the log-rank test. Comparison between factors contributing for survival was performed with the use of regression models (proportional hazard model) with a stepwise variable selection method. A P < 0.05 was considered statistically significant. Statistical analysis was performed on Statview 4.51.1 software (Abacus Concepts, Inc., Berkley, CA).

**RESULTS**

sFas serum levels in the groups with either cervical carcinoma, endometrial carcinoma, or ovarian carcinoma were significantly elevated when compared with those of normal controls (Table 1).

There was a significant correlation between age and sFas levels in normal controls (Fig. 1; r = 0.436, P = 0.0330). However, serum sFas levels obtained from either cervical, endometrial, or ovarian carcinoma patient groups demonstrated no significant correlation with age (P = 0.6630, P = 0.3348, and P = 0.4337, respectively).

In patients diagnosed with cervical carcinoma (Fig. 2), serum sFas levels in those patients with advanced cancer significantly exceeded levels observed in patients with local cancer.

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**Table 1** Patients, their history, and serum sFas levels

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Control</th>
<th>Cervical carcinoma</th>
<th>Endometrial carcinoma</th>
<th>Ovarian carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>24</td>
<td>28</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Stage I</td>
<td>40.5 ± 6.9</td>
<td>47.2 ± 13.5</td>
<td>55.0 ± 13.9</td>
<td>52.4 ± 9.8</td>
</tr>
<tr>
<td>Treatment</td>
<td>Surgery 13</td>
<td>Surgery 9</td>
<td>Surgery + chemotherapy 3</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>Surgery 10 (1*)</td>
<td>Surgery 3</td>
<td>Surgery + chemotherapy 1</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>Surgery 4</td>
<td>Radiotherapy 3</td>
<td>Radiotherapy + chemotherapy 1</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>Radiotherapy 3</td>
<td>Surgery 1</td>
<td>Surgery + chemotherapy 1</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>3 (2*)</td>
<td>Radiotherapy + chemotherapy 1</td>
<td>Probe + chemotherapy 1</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>Radiotherapy 2</td>
<td>Surgery + radiotherapy 1</td>
<td>Probe + chemotherapy 1</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>2 (2*)</td>
<td>Surgery + chemotherapy 1</td>
<td>Chemotherapy 2</td>
<td></td>
</tr>
<tr>
<td>Histological type</td>
<td>Squamous cell 28</td>
<td>Endometrioid 18</td>
<td>Serous 8</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>11</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival (%)</td>
<td>23 (82.1)</td>
<td>15 (83.3)</td>
<td>11 (61.1)</td>
<td></td>
</tr>
<tr>
<td>Serum sFas levels (ng/ml)</td>
<td>0.944 ± 0.262^c,d</td>
<td>1.877 ± 1.678</td>
<td>1.661 ± 0.499^d</td>
<td>1.660 ± 0.609^d</td>
</tr>
</tbody>
</table>

^c Case in which a patient died.
^d Ovarian carcinoma versus control, P < 0.0001.
Serum sFas in Gynecological Malignancies

Serum sFas levels in patients with local cancer did not differ from those in the control group ($P = 0.1747$). In patients diagnosed with endometrial carcinoma (Fig. 2), it was found that when sFas levels were compared among the subgroups of patients and normal controls, patients with advanced disease showed significantly higher sFas levels when compared with those with localized disease or those individuals in the normal control group. Serum sFas levels in patients with local disease also exceeded those of normal controls. Patients with ovarian carcinoma demonstrated sFas levels of advanced disease that were significantly higher than those of normal controls (Fig. 2).

We next examined the relationship between sFas levels and survival rate in patients with gynecological malignancies. Kaplan-Meier survival curves (Fig. 3) are shown for patients with cervical carcinoma, endometrial carcinoma, and ovarian carcinomas. Mean follow-up periods of patients diagnosed with cervical carcinoma, endometrial carcinoma, and ovarian carcinoma were 32.1 months (range, 2.4–55.5), 24.0 months (range, 3.1–55.7), and 28.6 months (range, 2.1–51.9), respectively. Patients were then divided into two groups based on their serum sFas levels. Because the mean ($\pm$ SD) of sFas level in patients with malignant tumors was $>1.5$ ng/ml, as shown in Table 1, the survival rates were compared between patients with sFas levels of $\geq 1.5$ ng/ml and those showing levels $<1.5$ ng/ml for each diagnostic group.

In patients diagnosed with cervical carcinoma, no deaths occurred in the group with a sFas level $<1.5$ ng/ml, and the survival rates significantly exceeded in those patients in which the serum levels of sFas were $\geq 1.5$ ng/ml. In patients with endometrial carcinoma, no deaths occurred in the group showing a sFas level $<1.5$ ng/ml, and the survival rate exceeded that in the group showing levels of $\geq 1.5$ ng/ml, but not to a statistically significant extent. In patients diagnosed with ovarian carcinoma, the survival rate was significantly higher in the group showing a sFas level $<1.5$ ng/ml than in the group showing a level of $\geq 1.5$ ng/ml.

Proportional hazard models demonstrated that serum sFas level was a statistically significant factor ($P = 0.0196$) for survival, as well as histological grade ($P = 0.0168$) in ovarian carcinoma (Table 2). On the other hand, stage was more statistically significant in the patients with both cervical carcinoma and endometrial carcinoma than sFas level.

DISCUSSION

It is known that sFas is lacking in the transmembrane domain of the full-length Fas, yet exists as a result of alternate mRNA splicing. FasL is highly expressed in NK and CTLs and is an important component of cytotoxic effector cell function (1). The biological role of sFas is thought to involve the binding and neutralization of either soluble or cell-surface FasL. It is possible that sFas forms complexes with cell-surface Fas to prevent or alter signal transduction (7, 8). sFas production has been shown to be present in normal individuals, as well as in patients with rheumatoid arthritis, systemic lupus erythematosus, and B- and T-cell leukemia (5, 10).

Moller et al. (15) demonstrated that Fas expression is markedly reduced in colon carcinomas compared with normal adjacent tissues, and that the loss of Fas expression in primary tumors correlated with disease progression or metastasis. The first experimental evidence that the loss of Fas can enhance tumor development has recently been reported (14). Because sFas can functionally antagonize FasL to effectively inactivate cell-surface Fas function, it is conceivable that elevated sFas production may promote tumorigenesis and disease progression.

The literature on serum sFas levels in patients with malignant tumor(s) is limited. Previous reports have shown that serum sFas levels are elevated in patients with leukemia, colon cancer, breast cancer, bladder cancer, and several other types of cancers (10–12). Concerning gynecological malignancies, one report investigated only 10 cases of ovarian cancer (11). The present
study is the first to evaluate survival rate and disease stage in patients with gynecological malignancies.

In our study, serum sFas levels were elevated in all groups of patients with cervical cancer, endometrial cancer, and ovarian cancer when compared with those of healthy control subjects. Moreover, the serum sFas level was elevated in patients with advanced cancer, compared with those patients with local cancer, and in every diagnostic group. Our results agree with those obtained with other solid tumors (11, 12). This finding suggests that serum sFas levels are elevated in patients with more advanced cancers, unrelated to the type of cancer.

A previous study of patients with breast cancer demonstrated a decrease in serum sFas levels after surgical resection, compared with levels obtained before surgery (11). This indicates that the serum level of sFas may be a useful tumor marker in that it may reflect cancer status. We cannot evaluate the therapeutic modality in patients diagnosed with gynecological malignancies because of the small number of patients in the present study. It is necessary to investigate the follow-up of sequential changes in serum sFas levels during the course of therapy.

We observed a better survival rate in patients with a serum sFas level <1.5 ng/ml before therapy than in those with a level of ≥1.5 ng/ml (Fig. 3). This difference was statistically significant in patients with cervical cancer and ovarian cancer. Moreover, analysis of survival rate using proportional hazard models with a stepwise variable selection method suggests that an early stage of cancer was the most statistically significant factor for both cervical carcinoma and endometrial carcinoma. On the other hand, serum sFas level was a significantly prognostic factor in ovarian carcinoma as well as histological grade. A study by Mizutani et al. (12) describes the relationship between serum sFas level and survival rate in patients with bladder cancer. Their report resembles the present study in that they found elevated levels of sFas and its association with a poor prognosis (12). Mizutani et al. (12) also demonstrated an inverse correlation between the level of serum sFas and antiauxologous tumor cytotoxicity in patients with malignant disease. Apoptosis mediated by the Fas-FasL system is suggested to be involved in the progression of malignant tumors and the response to therapy (11). It is reasonable to assume that the relationship between a high level of serum sFas and a worse prognosis in patients with ovarian carcinoma may be dependent on a mechanism for the escape of tumor cells from the immune surveillance of NK cells and CTLs. It has been suggested that serum sFas level could be

**Table 2** Analysis of prognostic factors using a proportional hazard model with stepwise variable selection method

<table>
<thead>
<tr>
<th>Factors</th>
<th>Step 0</th>
<th>Step 1</th>
<th>Step 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P$</td>
<td>$P$</td>
<td>$P$</td>
</tr>
<tr>
<td></td>
<td>Exp.$a$</td>
<td>Score statistic</td>
<td>Exp.$a$</td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum sFAS &lt;1.5 ng/ml vs. ≥1.5 ng/ml</td>
<td>0.0247</td>
<td>0.2422</td>
<td></td>
</tr>
<tr>
<td>Stage III/IV vs. stage I/II</td>
<td>&lt;0.0001</td>
<td>0.0029</td>
<td>0.035</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum sFAS &lt;1.5 ng/ml vs. ≥1.5 ng/ml</td>
<td>0.1570</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage III/IV vs. stage I/II</td>
<td>0.0077</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2, 3 vs. grade 1</td>
<td>0.2649</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum sFas &lt;1.5 ng/ml vs. ≥1.5 ng/ml</td>
<td>0.0128</td>
<td>0.0034</td>
<td>$P = 0.0093$</td>
</tr>
<tr>
<td>Stage III/IV vs. stage I/II</td>
<td>0.0974</td>
<td>0.0566</td>
<td></td>
</tr>
<tr>
<td>Grade 2, 3 vs. grade 1</td>
<td>0.0093</td>
<td>0.0213</td>
<td>0.169</td>
</tr>
</tbody>
</table>
| $a$ Exp, exponent.

**Fig. 3** Survival rate in patients diagnosed with cervical cancer, endometrial cancer, and ovarian cancer determined by the Kaplan-Meyer method. Patients were divided into two groups based on the level of serum sFas. The survival rate was compared between patient groups with a sFas level of ≥1.5 ng/ml and in those patients with levels <1.5 ng/ml. $P$s evaluated by the log-rank test were $P < 0.001$(*), $P = 0.128$ (**), and $P = 0.012$ (***), in cervical carcinoma, in endometrial carcinoma, and in ovarian carcinoma, respectively.
used as a prognostic predictive marker in patients with ovarian carcinoma.

This clinical study did not identify the source of serum sFas. The mechanism of sFas secretion remains to be clarified, although it may involve either an autocrine or paracrine interaction, or both (5, 10). We believe that a "tug-of-war" between tumor cells and NK cells, or activated CTLs, regulates the amount of sFas secreted. Feasibility of developing molecular therapy aimed at controlling this mechanism may be very useful in patients diagnosed with a malignancy.

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