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High Concentration of Serum Soluble Fas in Patients with Head and Neck Carcinoma: A Comparative Study Before and After Surgical Removal of Tumor

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Abstract

Background: Alternative splicing of the Fas transcript can produce a natural secreted isoform of this molecule. Some cancer cells can also produce soluble Fas (sFas) which may have suppressive effects on the immune system's anti-tumor response. Elevated concentrations of sFas have been detected in the sera of patients with different malignancies.

Materials and Methods: The concentrations of sFas in sera of patients with head and neck carcinoma (HNC, n=98) and healthy individuals (n=30) were measured by Sandwich ELISA and compared to values obtained six months after surgical removal of the tumor (n=48). Data were correlated with different clinical findings of the patients.

Results: sFas concentrations in the sera of HNC patients were found to be significantly higher in patients with different tumor stages. sFas concentration did not correlate with age or tumor invasiveness, however a higher concentration of sFas was found in the sera of patients who had higher tumor grades. Surgical removal of tumors in patients resulted in a substantial decrease in sFas concentration.

Conclusion: The initial rise in sFas concentration in the sera of HNC patients and its consequent decrease could be regarded as a sign of tumor suppressive mechanisms. Additional studies are needed to fully elucidate this mechanism however these findings might show the prospective use of such biomarkers to determine disease prognosis and even immunotherapeutic applications.

Keywords: Fas, Head and ceck carcinoma, Soluble Fas

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Introduction

The early stages of head and neck squamous cell carcinomas (HNC) are mostly presented with non-specific symptoms and diagnosis happens only at later stages of the disease. Therefore, identification of new diagnostic biomarkers is highly desirable.[1-4] Fas is one of the major members of the tumor necrosis factor receptor (TNFR) superfamily which is considered to be a main mediator for induction of the apoptosis cascade. In contrast to the broad expression of Fas on the surface of many different cells expression of its ligand (FasL) in normal tissues is limited to activated T-lymphocytes, natural killer cells, and a few immunoprivileged tissues such as the brain, eyes, testes and placenta.[5,6] A soluble form of Fas (sFas) could be generated by alternative mRNA splicing and it is believed to antagonize FasL-mediated cell killing.[7] The presence of sFas both systemically and locally within the tumor microenvironment in cancer patients has been shown before.[8,9] It is suggested that serum sFas may suppress apoptosis of cancer cells by blocking FasL on lymphocytes, and therefore can affect tumor progression.[10] Such elevated levels of sFas have been reported in hematopoietic and non-hematopoietic tumors.[11-17]

It is now well shown in many cancer models that immune T cells are engaged to kill cancer cells by apoptosis mechanisms. However, induction of apoptosis in lymphocytes could also be regarded as an escape mechanism for tumor cells. Therefore, due to the importance of the TNF/TNFR superfamily members and regulatory effects of soluble forms of these molecules in this study the concentration of sFas was measured in the sera of HNC patients and correlated with different criteria of the disease before and after surgical removal of the tumor.

Materials and Methods

Patients and samples

In this study 98 patients with histopathological diagnoses of HNC were enrolled. None of the patients had a history of radiotherapy or chemotherapy. Autoimmune diseases, immunodeficiency disorders and active infectious disease were also ruled out. The first blood sample of 3-5 ml was taken from patients on the day of surgery and serum samples were snap frozen at -80ºC. Six months after surgery, a second blood sample was available from only 48 patients during the post-surgery follow-up.

An additional 30 healthy individuals matched for age and sex were selected as controls. The healthy volunteers also had no familial history of malignancies and immune disorders.

Data on tumor differentiation, tumor staging and metastasis in addition to smoking habits were collected for all patients.

Written informed consents were obtained from all patients and healthy volunteers after the approval of the study by the Ethical Committee of Shiraz University of Medical Sciences.

Measurement of soluble forms of Fas by ELISA

Commercial Sandwich ELISA kits (Bendermed Systems, Austria) were used to quantify serum content of sFas. The sensitivity of the Fas detection kit was 13.2 pg/ml and the kit was designed to detect and measure the reactants in different biological samples, such as serum and plasma. All procedures were performed according to the manufacturer's instructions. The kit provided standard and control samples with defined concentrations of the measuring reactants. These were used to plot standard curves to estimate the concentration of reactants in the patients' samples.

Statistical analysis

Mann-Whitney U test was used to compare serum contents of the patients and healthy controls. In order to evaluate differences in measurements before and after surgery, the Wilcoxon paired t-test was used. Correlation of measurements to clinical data was estimated by the Pearson and Spearman tests. Statistical analysis was done by SPSS version 11.5 (IL, USA) and data were plotted by Graphpad prism (CA, USA). All data were presented as mean±standard deviation. *P* value of <0.05 was considered to be statistically significant.

Results

Patients' information

In this study, the concentrations of sFas in sera of 72 men and 26 women with HNC were measured and compared to corresponding concentrations in 30 healthy individuals matched for age and sex. The mean ages of patients and healthy individuals were 60±9 and 59.2±7 years, respectively. Additional data have been summarized in Figure 1.

sFas concentration before and after surgery

Commercial ELISA kits were used to measure the concentration sFAS in the sera of HNC patients. The initial serum samples from all patients were collected on the day of surgery while the second samples from 48 patients were collected six months following surgery. The mean concentration of sFas in the initial HNC samples was 355.30±124.60 pg/ml and 123.20±71.34 pg/ml in the sera of healthy individuals (Figure 2). The concentration of sFas in the sera of patients and healthy individuals ranged between 107-711pg/ml and 0-294pg/ml, respectively. A comparison of the mean concentrations shows a significant increase of this molecule in the sera of patients (P=0.01). However, there was not a significant correlation between sFas concentration and the ages or sex of patients (r=0.07, P=0.52). No correlation was found between sFas concentration and tumor size, either (r=0.03, P=0.93). As shown in Figure 2, the mean serum concentration of sFas in patients with tumor stages I, II, III and IV showed a gradual increase, of which all were significantly higher than sFas in healthy individuals (P=0.045). Despite the rise in sFas concentration in patients of stages I to IV, there were no statistically significant differences amongst these stages. Both groups of patients with invasive and non-invasive tumors had similar serum contents of sFas (283.5±23 pg/ml vs. 291.3±34 pg/ml, respectively). However, comparison of sFas concentrations showed significant differences among patients with various tumor differentiation states (P=0.03). Patients with well differentiated tumors had 175.2±76.31 pg/ml of sFas while this concentration increased to 256.6±91.34 pg/ml and 423.0±72.11 pg/ml in patients with moderate differentiated or non-differentiated tumors, respectively. The concentrations of sFas were compared in the sera of patients who were smokers and those who were non-smokers, which showed no significant statistical difference between these two groups (315.9±46 pg/ml vs. 273.8±61 pg/ml).

sFas after surgical removal of tumor

The mean concentration of sFas in the sera of 48 patients, six months after surgery, was 219.9±91.31 pg/ml which showed a significant decrease compared to the original concentration prior to surgery (355.30±124.60 pg/ml; P=0.001). However, the mean concentration of sFas was still higher than its concentration in the sera of

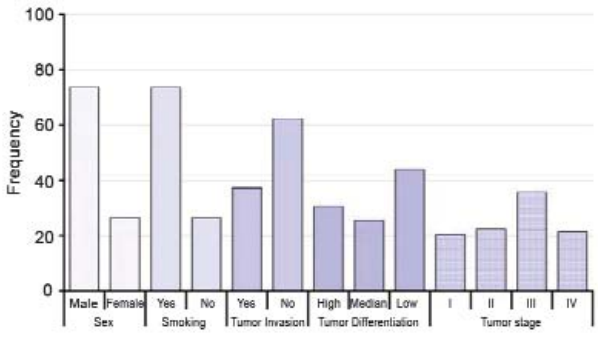

Figure 1. Distribution of patients with head and neck cancer according to different criteria.

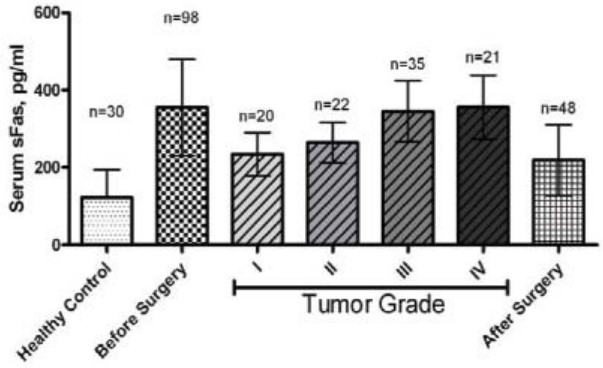

Figure 2. Serum concentration of sFas in healthy individuals compared to patients with head and neck carcinoma before surgery, as a whole or when patients were divided according to their tumor stages and after tumor excision.

healthy control individuals ($P<0.05$). The decrease in sFas concentration among patients was not similar ($P<0.03$) and the amount of decrease did not correlate with the original size of tumor or tumor stage (r=0.04, $P=0.96$).

Discussion

In this study we showed an elevated concentration of sFas in the sera of HNC patients which decreased six months after surgery. The concentrations of sFas in patients' sera following surgery were still higher than in healthy individuals. Several studies have reported the role of the Fas/FasL pathway in tumor suppression and have shown the presence of soluble forms of these molecules in cancer patients. [8-10] Demonstration of higher concentrations of sFas in patients' sera, even from early stages of the disease, has shown a possible use of this molecule as a prognostic biomarker for the disease. Konno et al. have used the same soluble factor as a prognostic marker in gynecological malignancies.[18] Mouawad et al. have shown significantly elevated sFas and sFasL plasma levels in metastatic melanoma patients when compared with healthy controls.[19] Higher concentrations of sFas in the sera of patients with metastatic melanoma (stage IV) as compared with those in the control groups or with patients in stages I and II has also been reported elsewhere.[20] In our study we were able to demonstrate a gradual, but significant increase in serum sFas concentration with increasing tumor stage; however immunohistochemical studies on oral squamous cell carcinoma by Muraki et al. have shown a reduction of Fas expression on the surface of tumor cells.[21] Such dissimilarity could be explained by the different techniques that have been used in these two studies. Moreover, we measured the soluble form of the molecule which has a different mechanism of expression.[13] In a study by Somma et al., a correlation between Fas/FasL expression and tumor invasiveness was demonstrated.[22] However, Fas concentration in the tested patients in our study did not show a significant association with tumor invasion.

Fas/FasL interaction is an immunological mechanism that has been employed by the immune system to induce apoptosis in tumor cells. However, production of the soluble form of Fas by tumor cells can confer an escape mechanism for these cells.[23,24] Increased levels of sFas have been found to be associated with poor prognosis in patients suffering from hepatoma and renal cell carcinoma.[25] In fact, cancer cells might escape Fas mediated cell apoptosis by different ways. First, the loss of cell-surface Fas would render cancer cells resistant to FasL mediated apoptosis by immune cells. Second, neutralization of FasL by sFas would prevent ligation. A decrease in serum content of sFas after surgical removal of tumor tissue might lead to this suggestion. In this regard, our results are in accordance with data from Pignataro et al. who have shown decreased serum concentrations of sFas as early as two weeks following surgery.[26] Similar results have been reported in patients with nonhematological malignancies as well as hepatocellular carcinoma.[27, 28] However, the mean serum concentration of sFas in our study remained higher than healthy volunteers. This observation might suggest the presence of other sources of sFas production with other activities. Activated T cells could be considered as a source for this production.[28]

Overall, our data re-emphasizes possible escape mechanisms employed by tumors through Fas/FasL in head and neck carcinomas which would be part of a "counterattack mechanism",[29,30] and supports the idea of using sFas as a relatively reliable prognostic biomarker in head and neck carcinomas.

Our data shows a significant increase of sFas in patients with head and neck carcinoma with substantial recovery six months after tumor excision. These findings suggest that the Fas/FasL pathway and their related soluble forms may be linked with induction or progression of this type of carcinoma; as such an association has been shown in other diseases. More studies should be carried out to examine Fas, its ligand and other members of the TNF/TNF receptor superfamily

in order to reveal their exact mechanism of action and prove their role as a prognostic marker.

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