RESEARCH ARTICLE

Increased Serum S-TRAIL Level in Newly Diagnosed Stage-IV Lung Adenocarcinoma but not Squamous Cell Carcinoma is Correlated with Age and Smoking

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Abstract

Background: Lung cancer is the leading cause of cancer mortality in the world. Many factors can protect against or facilitate its development. A TNF family member TRAIL, has a complex physiological role beyond that of merely activating the apoptotic pathway in cancer cells. Vitamin D is converted to its active form locally in the lung, and is also thought to play an important role in lung health. Our goal was to investigate the possible clinical significance of serum sTRAIL and 1,25-dihydroxyvitamin D(3) levels in patients with non-small cell lung cancer (NSCLC). Materials and Methods: Totals of 18 consecutive adenocarcinoma and 22 squamous cell carcinoma patients with stage-IV non-small cell lung cancer referred to our institute were included in this study. There were 12 men and 6 women, with ages ranging from 38 to 97 (mean 60.5) years with adenocarcinoma, and 20 men and 2 women, with ages ranging from 46 to 80 (mean 65) years with squamous cell carcinoma. Serum levels of sTRAIL and 1,25-dihydroxyvitamin D(3) were measured in all samples at the time of diagnosis. Results: sTRAIL levels in NSCLC patients were higher than in the control group. Although there was no correlation between patient survival and sTRAIL levels, the highest sTRAIL levels were correlated with age and cigarette smoking in the adenocarcinoma patients. sTRAIL level in healthy individuals were correlated with serum 1,25-dihydroxyvitamin D(3). Conclusions: Serum sTRAIL concentrations were increased in NSCLC patients, and correlated with age and smoking history, but not with overall survival.

Keywords: Soluble TRAIL - non-small cell lung cancer - adenocarcinoma - squamous cell carcinoma

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Introduction

Lung cancer is the leading cause of cancer mortality in the world accounting for 31% (for men) and 26% (for women) of all cancer deaths. Trends in lung cancer incidence and mortality reflect smoking habits and/or exposure to other environmental or occupational carcinogens. The incidence rate in men is 34.9 per 100,000 with the highest rates observed in more developed countries, while in women the incidence rates are lower (11.1 per 100.000) (Greenlee et al., 2000; Parkin, 2001; 2004). Lung cancers can be grouped into two major histological types, namely non-small cell and small cell lung cancer (NSCLC and SCLC respectively), a reflection of their different clinical behaviours and sensitivity to chemo- and radiotherapy. NSCLC accounts for 75-85% of lung cancer patients and consists of several subtypes, predominantly squamous cell carcinomas, adenocarcinomas and large cell carcinomas. In all cases surgery is the mainstay of treatment. Many factors can influence lung cancer incidence, including, but not limited to, cigarette smoking, passive smoking and age (Schiller, 2001; Spira and Ettinger, 2004; Stupp et al., 2004).

TNF-related apoptosis inducing ligand (TRAIL) is a TNF family member expressed as either a type II transmembrane protein or, similarly to other membrane-bound ligands of the TNF superfamily, as a soluble protein, which is detectable in the serum under physiological conditions. Although the best characterized biological activity of TRAIL, also known as Apo2 ligand, is associated with a potent induction of apoptosis in a variety of cancer cell types, the wide expression of TRAIL and TRAIL receptors in many normal tissues suggests that the physiological role of TRAIL is more complex than merely activating the

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apoptotic pathway in cancer cells (Sanlioglu et al., 2006; Terzioglu et al., 2007; Griffith and Lynch, 2008; Aydin et al., 2010). In general, TRAIL has been studied in tumour immunology settings. It selectively induces apoptosis in transformed cells while leaving non-transformed cells unaffected (Ashkenazi et al., 1999; Walczak et al., 1999). Although it has been shown that TRAIL can activate both pro-apoptotic and anti-apoptotic pathways, the factors regulating which one of these pathways are triggered are not well understood (McGrath, 2011).

Vitamin D is a steroid hormone that has been shown to possess anti-tumorigenic and immune-modulatory effects in vitro and in vivo. Its potential in cancer prevention and treatment is currently under detailed investigation. Evidence from animal model studies and in vitro cell culture suggest that vitamin D may play a beneficial role in pulmonary inflammation. Vitamin D status may be an important issue for lung cancer prevention (Beumer et al., 2012; Norton and O'Connell, 2012; Zhang et al., 2012).

With the prospect of more effective therapeutic options for advanced stage disease, the characterization of new methodologies for monitoring patients' pre-/post- therapy is of interest. In the present study, we investigated the concentrations of soluble TRAIL in the peripheral blood of adenocarcinoma and squamous cell carcinoma patients as well as in healthy controls, and performed a preliminary analysis of the relationship of those levels to disease outcome.

Materials and Methods

Patients

Subjects consisted of 18 patients with adenocarcinoma and 22 patients with squamous cell carcinoma. Clinical specimens were obtained from NSCLC patients seen at the Department of Oncology with a new diagnosis of stage IV disease. Clinical data for the patients was also recorded. 20 healthy age- and sex-matched healthy individuals served as a control group.

Written informed consent was obtained from all participants according to the Declaration of Helsinki. The study was approved by Antalya Education and Research Hospital Local Committee on Ethics.

ELISA analysis

TRAIL and 1,25-dihydroxyvitamin D(3) concentrations in serum samples from 40 NSCLC patients and 21 healthy donors were analyzed using a TRAIL/APO2L ELISA kit (Diaclone, France) and Roche kit for 1,25-dihydroxyvitamin D(3) according to the manufacturer's instructions. The absorbance values in all assays were measured using a spectrophotometer set at 450 nm, and the concentrations of sTRAIL (pg/ml) and 1,25-dihydroxyvitamin D(3) (ng/ml) calculated from OD readings of recombinant standards.

Statistical analysis

Data were analyzed using GraphPad Software (Prism 5.0, San Diego, CA) and were expressed as mean±standard error of the mean (SEM). GraphPad Software was used to plot data for all three groups (control, adenoC and SCC). Comparison of parameters between the three groups used a one-way ANOVA (Tukey's multiple comparison).

Correlations between the patient characteristics and TRAIL concentrations were analyzed by Spearman correlation analysis. Values of $p<0.05$ were considered to indicate statistical significance.

Results

The demographics of the NSCLC patients were as follows: Adenocarcinoma group: mean age, 60.5±13.3 years; squamous cell carcinoma group: mean age, 65±11.7 years. Forty five percent of all patients were previous or ongoing smokers, and all patients were classified as stage IV.

Serum sTRAIL levels were 1660±41.7 pg/ml (adenocarcinoma group); 1520±38.7 pg/ml (squamous cell carcinoma group); and 634±18.0 pg/ml (control group) (Table 1).

1,25-dihydroxyvitamin D(3) levels were 15.9±2.1 ng/ml (adenocarcinoma group); 16.9±1.3 ng/ml (squamous cell carcinoma group); and 20.0±2.0 ng/ml (control group) (Table 1).

Figure 1 illustrates the serum sTRAIL distribution for NSCLC and control groups. Both adenocarcinoma and squamous cell carcinoma patients had significantly higher serum levels of sTRAIL than the healthy controls, with a significant difference between the two patient populations.

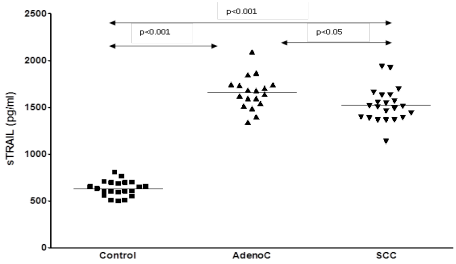


Figure 1. Scatter Dot Plots. Peripheral blood samples from 21 healthy controls, 18adenocarcinoma and 22 squamous cell carcinoma patients showing the sTRAIL levels (pg/ml)by ELISA

Table 1. sTRAIL Levels and 1,25-dihydroxyvitamin D(3) levels of Control, Adeno Ca and Squamous Cell Carcinoma Groups

Parameters	Control	AdenoCa	SCC
sTRAIL levels of control, adeno Ca and squamous cell carcinoma groups			
Subjects (n)	21	18	22
sTRAIL (pg/ml)	633.8±18.03	1657±41.65 a: p<0.001	1521±38.69 b: p<0.001 c: p<0.05
1,25-dihydroxyvitamin D(3) levels of control, adeno carcinoma (AdenoC) and squamous cell carcinoma (SCC) groups			
Vitamin D (ng/ml)	19.97±2.02	15.91±2.10 a: p>0.05	16.88±1.33 b: p>0.05 c: p>0.05

Table 2. Healthy Individuals' Correlation Analyses

	sTRAIL & Vitamin D	Age & Vitamin D
Spearman r	-0.4836	-0.3429
95% confidence interval	-0.7687 to -0.03817	-0.6894 to 0.1314
p value	0.0154	0.0694
Is the correlation significant? (alpha=0.05)	Yes	No

Investigation of potential correlations between levels of circulating TRAIL and other parameters, such as age, smoking status, survival and 1,25-dihydroxyvitamin D(3) in NSCLC patients are presented in supplementary Tables 1-3. Although the serum TRAIL concentrations were not correlated with survival in NSCLC patients, there was a correlation with age (p=0.016) and cigarette smoking (p=0.022) seen for adenocarcinoma patients but not squamous cell carcinoma patients (Supp. table 1 and supp. table 2). In addition, there was a correlation between the circulating sTRAIL and 1,25-dihydroxyvitamin D(3) levels seen in the control group (Table 2).

Discussion

Tumor marker measurements, which may potentially provide sensitive and cost-effective early detection of recurrence, are becoming increasingly important in assessing the efficacy of novel therapy. Our study demonstrates that circulating sTRAIL levels were significantly higher in untreated NSCLC patients than in healthy controls although there was no correlation between sTRAIL levels and patient survival. One advantage to our study is that patients were newly diagnosed stage-IV NSCLC and none had undergone any treatment, so that sTRAIL levels were not influenced by previous drug treatment. However, the small size of the study population may in itself have been a contributing factor to our failure to establish a link between sTRAIL levels and disease outcome.

Recent studies have reported on serum sTRAIL levels in many disorders including cancer, cardiac, renal and even autoimmune disease (Liabeuf et al., 2010; Bisgin et al., 2012a; 2012b; Deftereos et al., 2012; Yalcin et al., 2012). A correlation has also been reported between sTRAIL levels and response to chemotherapy, anti-Ig E therapy in some cases (Bisgin et al., 2012; Yalcin et al., 2012).

Age is a major determinant of cancer risk with adenocarcinoma diagnosed at younger ages (Charloux et al., 1997; Greenlee et al., 2000). Interestingly, we observed that serum concentrations of sTRAIL increased with age in adenocarcinoma patients.

Smoking obeys a dose–response relationship with risk for all types of lung cancer, (Curran et al., 2011), and approximately half of the patients, particularly those with adenocarcinoma, in our study reported a history of smoking. The connection between smoking and lung cancer was most evident among patients with squamous and SCLC, and weaker for adenocarcinoma (Barbone et al., 1997; Brambilla et al., 2001; Radzwikowska et al., 2002). We found a significant correlation between sTRAIL levels and smoking status.

Many studies have demonstrated a relationship between vitamin D and cancer, including malignancies of the breast, colorectum, prostate and lung (Pazdiora et al., 2011; Woloszynska-Read et al., 2011; Zhang et al., 2012). Vitamin D or its analogs, alone or in combination with cytotoxic drugs, may have some efficacy in the treatment of lung cancer (Ramnath et al, 2011). Vitamin D is converted to its active form locally in the lung, consistent with the idea that it plays a role in lung health. We observed a correlation between sTRAIL and 1,25-dihydroxyvitamin D(3) levels in healthy individuals, although no such correlation was seen in NSLC patients.

In conclusion, this is the first study to assess sTRAIL levels in NSCLC patients and to evaluate the relationship of those levels with clinical outcome. Our study demonstrates that sTRAIL levels are higher in newly diagnosed stage-IV NSCLC than in healthy controls. Further studies are needed to investigate whether the TRAIL system has a causal role in clinical outcome in adenocarcinoma, and its value as a marker for monitoring patients with NSCLC.

Acknowledgements

AB participated in the design and execution of the study and drafted the manuscript. AK followed up the patients and collected clinical data. ADY conceived of the study, and participated in its design and coordination. SG performed the statistical analysis and drafted figures. AB and ES carried out the immunoassays and helped to draft the figures. BK did the literature search. All authors read and approved the final manuscript.

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