CLINICAL SIGNIFICANCE OF TRAIL AND TRAIL RECEPTORS IN PATIENTS WITH HEAD AND NECK CANCER

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Abstract: *Background.* Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a death ligand currently under clinical trials for cancer. The molecular profile of TRAIL and TRAIL receptors has not yet been mapped for patients with laryngeal squamous cell carcinoma (SCC) or patients with oral cavity squamous cell carcinoma (OCSCC).

Methods. Paraffin-embedded tissues from 60 patients with laryngeal SCC and 14 patients with OCSCC were retrospectively analyzed using immunohistochemistry.

Results. An increase in decoy-R1 (DcR1) but a decrease in decoy-R2 (DcR2) expression were observed in patients with laryngeal SCC and in patients with OCSCC compared with control individuals with benign lesions. Clinical and pathologic grading revealed distinctive TRAIL and TRAIL receptor profiles in patients with squamous cell carcinoma of the head and neck (SCCHN).

Conclusions. TRAIL and a TRAIL receptor expression profile might be useful to follow-up disease progression by virtue of its connection with clinical staging and pathologic grading in patients with laryngeal SCC. © 2010 Wiley Periodicals, Inc. *Head Neck* **33:** 1278–1284, 2011

Keywords: TRAIL; TRAIL receptor; molecular diagnostics; clinical and pathologic staging; head and neck cancer

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a novel member of the TNF superfamily, which has currently been tested in a gene therapy clinical trial.[1,2] Five different receptors have been identified to interact with TRAIL: TRAIL-R1 (DR4), TRAIL-R2 (DR5), TRAIL-R3 (DcR1), TRAIL-R4 (DcR2), and osteoprotegerin.[3] DR4 and DR5 function as authentic death receptors, whereas DcR1 and DcR1 are unable to induce such signaling but can serve as decoy receptors.[4] Interestingly, TRAIL does not cause any harm to normal cells but can selectively induce apoptosis in cancer cells.[5,6] The main reason for TRAIL resistance was initially attributed to the presence of decoy receptors (DCR1 and DcR2) that compete with apoptosis-inducing TRAIL death receptors (DR4 and DR5) for binding to TRAIL.[7] In this scenario, it is believed that decoy receptors either function to dilute out TRAIL ligands (like DcR1) or supply anti-apoptotic signals (like DcR2) to cells. As reported previously, DcR2 binding activated the anti-apoptotic NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling pathway, leading to the blockade of TRAIL-induced apoptosis.[8,9] Likewise, a panel of squamous cell carcinoma of the head and neck (SCCHN) cell lines was highly resistant to TRAIL-induced apoptosis because of activation of NF-κB-mediated cell survival pathways, and inhibition of NF-κB rendered SCCHN cells sensitive to TRAIL.[10] Intriguingly, more than half of the human cancers tested display TRAIL resistance,[11] and elevated expression of the TRAIL decoy receptor in apoptosis-resistant cells was suggested for the observed phenotype.[12] Likewise, down-regulation of decoy receptor gene expression by small interfering RNA (siRNA) strategy sensitized normally apoptosis-resistant cancer cells to TRAIL.[13–15] Thus, knowing the TRAIL receptor expression profile in patients with SCCHN may help us to better predict the extent to which these cancer cells have a survival advantage and/or their responsiveness to TRAIL.

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Molecular screening of tissue samples from patients with SCCHN has been designed with the ultimate goal of improving early diagnosis and treatment.[16] In this scenario, particular emphasis has been given to identifying molecular markers useful for determining preoperative prognosis through routine tumor biopsy. Although a rare loss of function mutation of DR5[17] and a nucleotide substitution in DR4[18] have been associated with head and neck cancer, the mechanism of how TRAIL and TRAIL receptors contribute to SCCHN carcinogenesis remains unknown. Revelation of TRAIL and TRAIL receptor profile in SCCHN will be essential to resolve this issue. Interestingly, laryngeal cancer has been implicated to be 1 of the rare cancer types in which past treatment modalities did not increase 5-year survival rates of patients with laryngeal SCC over the last 30 years.[19] Thus, the deployment of molecular markers for screening has been advised as a new avenue in the clinical management of laryngeal cancer. By this token, we investigated the expression profile of TRAIL and TRAIL receptors in tissues from 60 patients with laryngeal SCC for the purpose of expanding the current marker library useful for the prognostic assessment. In addition, tumor tissues from 14 patients with oral cavity squamous cell carcinoma (OCSCC) were used for comparison purposes.

MATERIALS AND METHODS

Clinical Assessment of Patients with SCCHN. After investigating many patients previously admitted to the Head and Neck Surgery Department of Baskent University Hospitals and Clinics, paraffin-embedded tissues from 60 patients with laryngeal SCC and from 14 patients with OCSCC were selected from the pathology archives for our study since these patients had proper diagnostic and clinical follow-up information suitable for retrospective analysis. In addition, we included 14 patients with benign laryngeal lesions and 12 patients with benign oral cavity lesions for comparison purposes. Written informed consent in accord with the Declaration of Helsinki was obtained from all patients and the study was approved by the Baskent University local committee on ethics. Demographic data of the patients are given in Table 1.

Disease in all patients was staged in accord with the T/N/M Classification of American Joint Committee on Cancer guidelines.[20,21] Although some patients were followed as long as 113 months, the median disease-free survival time and overall survival time for patients with laryngeal SCC and patients with OCSCC were about 32 months. Eight of 60 patients with laryngeal SCC and 3 of 14 patients with OCSCC had distant metastasis. Five of the patients with laryngeal SCC and 3 patients with OCSCC were lost during the follow-up.

Dedicated personnel from the head and neck surgery staff conducted patient care and follow-up procedures throughout the study. When there was no lymph node involvement, T1 laryngeal SCC tumors (stage 1) were treated by radiotherapy alone. Patients with stage 2 and stage 3 laryngeal SCC underwent surgery involving partial or total laryngectomy, neck dissection, and/or radiation. For patients with stage 4 laryngeal SSC, surgery followed by radiation and/or chemoradiation was indicated. With regard to patients with OSCC, when lymph node involvement was absent, surgery was performed for patients whose disease was classified as T1, whereas the rest of the OSSC tumors were removed through dissection of the neck and other relevant sites. When lymph node involvement was evident, radiation and chemotherapy were administered in combination.

Table 1. Patient characteristics.

Characteristic	No. of patients with oral cavity tumors (%) ($n = 14$)	No. of patients with laryngeal tumors (%) ($n = 60$)	All patients, no. (%) ($n = 74$)
Age, y			
≤50	3 (21.4)	4 (6.7)	7 (9.5)
>50	11 (78.6)	56 (93.3)	67 (90.5)
Sex			
Male	7 (50.0)	58 (96.7)	65 (87.8)
Female	7 (50.0)	2 (3.3)	9 (12.2)
Smoking			
No	3 (21.4)	6 (10.0)	9 (12.2)
Yes	11 (78.6)	54 (90.0)	65 (87.8)
Alcohol			
No	8 (54.5)	52 (86.7)	60 (81.1)
Yes	6 (45.5)	8 (13.3)	14 (18.9)

Immunohistochemistry. All primary antibodies used for immunohistochemistry were obtained from Alexis Biochemicals (Lausen, Switzerland): anti-human TRAIL monoclonal antibody (mAb) (III6F; ALX-804-326-C100), anti-human DR4 mAb (HS101; ALX-804-297A-C100), anti-human DR5 pAb (ALX-210-743-C200), anti-human DcR1 polyclonal antibody (pAb) (ALX-210-744-C200), and anti-human DcR2 mAb (HS402; ALX-804-299A-C100). Negative controls included samples that were stained only with the appropriate secondary antibody. Since TRAIL and TRAIL receptors were predominantly expressed in lymphoid tissues, lymph node sections (regardless of disease) were used as positive controls to optimize primary antibody titers.

Immunohistochemical analysis was carried out for TRAIL and TRAIL receptors as described previously.[22] Benign and malignant tissues were stained using the above-stated primary antibodies at 1/50 dilution. Specifically, 5-μm-thick sections were deparaffinized, rehydrated, and rinsed in distilled water. Endogenous peroxidase activity was quenched with 3% H_2O_2 for 8 minutes at room temperature. The slides were then rinsed and immersed in boiling citrate buffer. For the immunolocalization of TRAIL and TRAIL receptors, the slides were treated with

the antibody of interest in a humidified chamber overnight. They were subsequently rinsed in phosphate-buffered saline (PBS), and incubated with a secondary antibody for 1 hour at room temperature. Visualization of antibody location was accomplished using a substrate chromogen solution of 3,3′-diaminobenzidine (DAB) for 10 minutes. The slides were counterstained with hematoxylin and then coverslipped.

Semiquantitative Scoring of TRAIL and TRAIL Receptor Expressions for Immunohistochemical Analysis. Specimen analyses were performed by 2 independent pathologists with no prior knowledge of the clinical data performed. Both intensity and marker distribution (percentage of the positively stained epithelial cells) were used for the calculation of the immunostaining scores in benign and malignant tissues.[22–25] Intensity of the staining was scored as follows: 0 = negative; 1 = weak; 2 = moderate; and 3 = strong. Similarly, the marker distribution was scored as follows: 0 = <10% of the epithelial cells stained on the sections; 1 = 10% to 40%; 2 = 40% to 70%; and 3 = >70%. A final combined immunostaining score was then calculated by adding both intensity and marker distribution scores for each patient.

Statistical Analysis. SPSS 13.0 software for Windows (SPSS Inc., Chicago, IL) was used for the statistical analyses. Statistical differences were evaluated at the 5% probability level ($p < .05$). The standard error of the mean (±SE) is provided as error bars for all data points in all figures. The normality test was conducted using the Kolmogorov–Smirnov (K-S) test. Because a normal distribution was not detected, Mann–Whitney *U* tests were used to statistically compare the 2 independent groups. The correlation between TRAIL/TRAIL receptor profile and clinical staging or pathologic grading was revealed by Spearman rho analysis. Gleason scoring is a pathologic grading system used to evaluate the prognosis of patients with prostate cancer that is based on tumor pattern.

RESULTS

Confirmation of TRAIL and TRAIL Receptor Expression for Immunohistochemistry Analysis of SCCHN Sections. Before the analyses of TRAIL and TRAIL receptor expression profiles of patients with SSCHN, optimization of the primary antibody concentra-tions was performed using lymph node sections. Primary antibodies specific for TRAIL and the different TRAIL receptors generated a good staining pattern on cervical lymph node sections (data not shown). Conversely, treatment of lymph node sections with the secondary antibody alone (negative control) did not yield any detectable staining. These results suggested that the immunohistochemical staining procedure could be used to detect TRAIL and TRAIL receptor expression on SCCHN sections.

Patients with Laryngeal SCC Displayed Increased DcR1 but Decreased DcR2 Expression Compared with Patients with Benign Laryngeal Lesion. To determine the TRAIL and TRAIL receptor profiles in patients with laryngeal SCC, 60 paraffin-embedded tissues were analyzed by immunohistochemistry using antibodies specific for TRAIL and the different TRAIL receptors. In addition, 14 patients with benign laryngeal lesion were used as controls. Representative immunohistochemical staining images of patients with benign laryngeal lesion (top panels) and patients with laryngeal SCC (bottom panels) are shown in Figure 1A, and Figure 1B shows comparative TRAIL and TRAIL receptor expression profiles of these patient groups. In patients with laryngeal SSC, high TRAIL death receptor gene expression was detected on average in contrast to low decoy receptor gene expression. To determine statistical differences between the patients with laryngeal SCC and patients with benign laryngeal lesion, first the K-S test ($n = 100$) was used to determine whether the patient groups were normally distributed. Because a Gaussian distribution was not detected, the statistical difference between the groups was assessed using Mann–Whitney *U* test. Up-regulation in DcR1 expression ($p = .009$) but down-regulation in DcR2 expression ($p = .028$) were the only statistically significant findings observed between patients with laryngeal SCC and patients with benign laryngeal lesion (Figure 1B).

Patients with OCSCC Displayed TRAIL and TRAIL Receptor Expression Profiles Similar to Those of Patients with Laryngeal SCC. To compare and contrast TRAIL and TRAIL receptor profiles of patients with laryngeal SCC to those of patients with OCSCC, 14 paraffin-embedded tissues were analyzed by immunohistochemistry as described earlier. Moreover, tissues from 12 patients with benign oral cavity lesion were included in the study for comparison. Representative immunohistochemical staining images of these patients are given in Figure 2A. Similar to the observation made in patients with laryngeal SCC, patients with OCSCC displayed higher levels of death receptor expression compared with decoy receptor expression on average (Figure 2B). Mann–Whitney *U* test was used to reveal statistical differences between benign oral cavity lesion and OCSCC. Compared with patients with benign oral cavity lesion, patients with OCSCC displayed higher DcR1 ($p = .022$) but lower DcR2 expression ($p = .027$), an observation similar to that described earlier between laryngeal SCC and benign laryngeal lesion.

TRAIL and TRAIL Receptor Expression Profiles in Patients with SCCHN. We next statistically evaluated control groups and cancer groups among themselves. Tissues from 14 patients with benign laryngeal lesion and tissues from 12 patients with

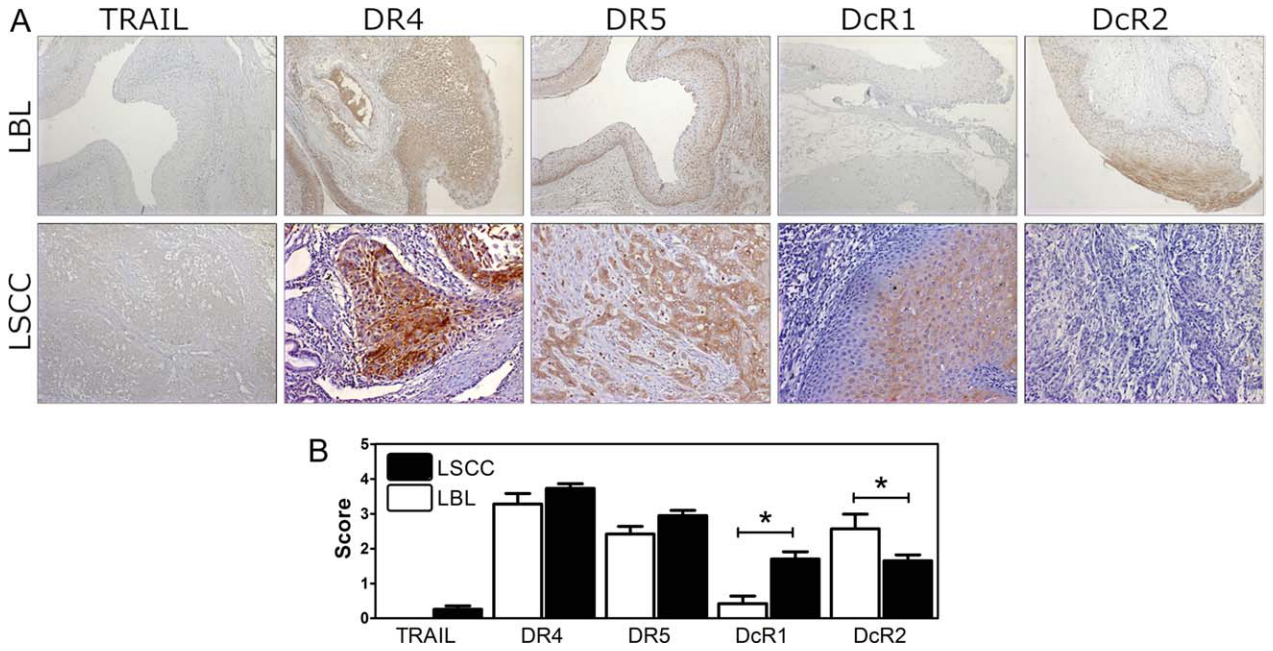

FIGURE 1. **(A)** Immunohistochemical staining of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and TRAIL receptors in 60 patients with laryngeal SCC (bottom panel) and 14 patients with benign laryngeal lesion (top panel). Representative images (magnification $\times$100) are provided from different patients. TRAIL and TRAIL receptor subtypes are listed above the each image, and each image represents a single patient. Brown precipitate indicates positive staining. **(B)** Semiquantitative analysis of the immunohistochemical staining in patients with laryngeal SCC (solid bars) or benign laryngeal lesion (open bars). Immunohistochemical scoring (mean $\pm$ SE) was performed as described in Materials and Methods. "*" represents statistically significant difference. SCC, squamous cell carcinoma. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

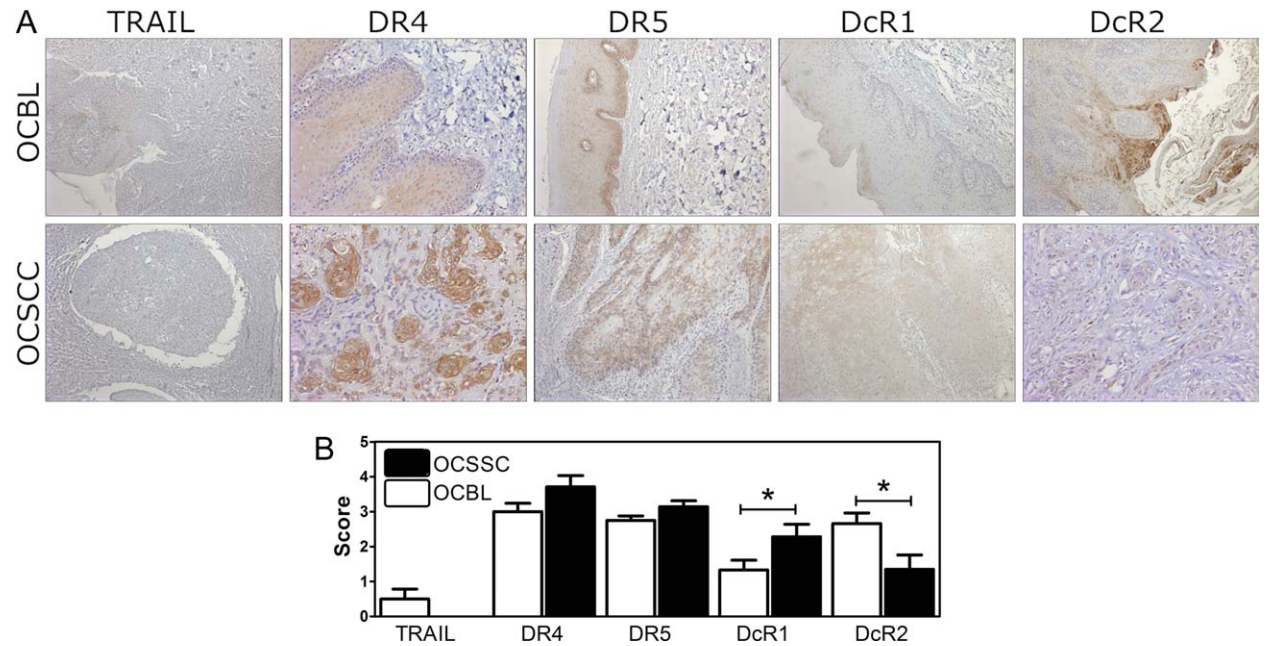

FIGURE 2. **(A)** Immunohistochemical staining of TRAIL and TRAIL receptors in 14 patients with OCSCC and 12 patients with benign oral cavity lesion. Representative images (magnification $\times$100) in duplicates are provided from different patients. **(B)** Semiquantitative analysis of the immunohistochemical staining of patients with OCSSC (solid bars) or benign oral cavity lesion (open bars). Immunohistochemical scoring (mean $\pm$ SE) was performed as described in Materials and Methods. "*" represents statistically significant difference. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; OCSCC, oral cavity squamous cell carcinoma. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

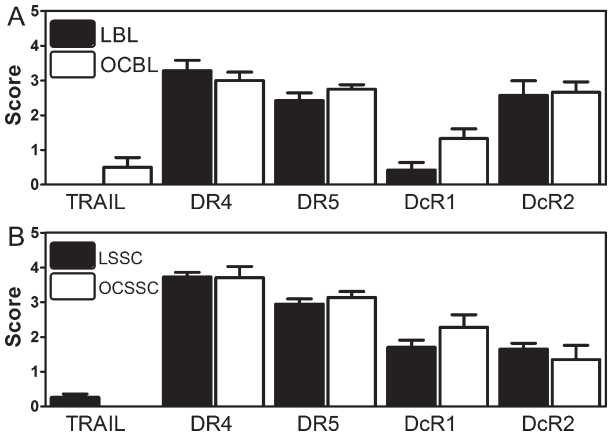

FIGURE 3. Distinctive TRAIL and TRAIL receptor expression profile in patients with benign versus malignant lesions of the head and neck. **(A)** The comparative analysis of TRAIL and TRAIL receptor profile in patients with benign laryngeal lesion (solid bars) versus benign oral cavity lesion (open bars). Differences in TRAIL and its receptor profile between laryngeal SCC (solid bars) and OCSSC (open bars) are given in **(B)**. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; OCSCC, oral cavity squamous cell carcinoma.

benign oral cavity lesion were compared based on TRAIL and TRAIL receptor expression profiles as shown in Figure 3A. Mann–Whitney *U* test was administered to reveal any statistical significance between the 2 groups, but no difference was noted. Similarly, comparison between tissues from patients with laryngeal SCC ($n = 60$) and tissues from patients with OCSCC ($n = 14$) did not result in any statistical difference in terms of TRAIL and TRAIL receptor expression profiles, as shown in Figure 3B.

Alteration in DR5 Expression Profile Correlated with the Clinical Staging of Patients with SCCHN. One of the parameters that have been used to follow disease progression of patients with SCCHN is clinical staging. Based on clinical staging, patients with laryngeal SCC were categorized as follows: 14 patients with stage 1, 14 patients with stage 2, 16 patients with stage 3, and 16 patients with stage 4. In addition, clinical staging of patients with OCSCC showed that 9 patients had stage 1, 4 patients had stage 2, and 1 patient had stage 3. Spearman rho correlation test was implemented to reveal any correlation between the clinical staging of patients and TRAIL/TRAIL receptor expression profile. As shown in Table 2, only the alteration in DR5 expression correlated with the clinical staging of patients with laryngeal SCC and patients with OCSCC.

The Significance of TRAIL and TRAIL Receptor Profile in Connection with Pathologic Grading. Another important parameter influencing disease progression is the cell differentiation status of cancer cells, known as histopathologic tumor grading. Based on this parameter, among patients with laryngeal SCC 10 cases were grade 1, 40 cases were grade 2, and 10 cases were grade 3. In addition, 9 tissue samples from patients with OCSCC were categorized as grade 1, whereas 5 tissue samples from patients with OCSCC were grade 2. Spearman rho correlation test was used to reveal any statistical difference between TRAIL/TRAIL receptor profile and the cellular differentiation of patients with laryngeal SCC and patients with OCSCC. Interestingly, alteration in TRAIL and DR4 expression correlated only with the pathologic grading of patients with laryngeal SCC, but not of patients with OCSSC (Table 3).

Table 2. Comparative analysis of TRAIL/TRAIL receptor profile and clinical staging in patients with laryngeal SCC or OCSCC.

Spearman's rho correlation (clinical stage)	Laryngeal SCC	OCSCC
TRAIL		
Correlation coefficient	−.054	—
Significance (2-tailed)	.682	—
n	60	14
DR4		
Correlation coefficient	.073	.278
Significance (2-tailed)	.579	.336
n	60	14
DR5		
Correlation coefficient	−.335(**)	.567(*)
Significance (2-tailed)	.009	.034
n	60	14
DcR1		
Correlation coefficient	.103	.420
Significance (2-tailed)	.434	.135
n	60	14
DcR2		
Correlation coefficient	−.071	.138
Significance (2-tailed)	.592	.638
n	60	14

Abbreviations: TRAIL, TNF (tumor necrosis factor) related apoptosis- inducing ligand; SCC, squamous cell carcinoma; OCSCC, oral cavity SCC; DR4, TRAIL-R1; DR5, TRAIL-R2; DcR1, Decoy-R1; DcR2, Decoy-R2.
** Correlation is significant at the .05 level (2-tailed).*
*** Correlation is significant at the .01 level (2-tailed).*

DISCUSSION

There are 2 main purposes of knowing TRAIL and TRAIL receptor expression profile in cancer. Recent studies suggest that TRAIL receptor expression profile is 1 of the factors that determine the extent to which cancer cells may respond to TRAIL treatment. Based on our previous research, prostate cancer cell lines with high levels of TRAIL death receptor expression but low levels of decoy receptor expression were sensitive to TRAIL.[26] Consequently, tissues from patients with laryngeal SCC and tumor samples from patients with OCSSC exhibited high levels of TRAIL death receptor expression but only low levels of decoy receptor expression. Therefore, it is reasonable to assume that cancer cells of these patients should naturally be sensitive to TRAIL treatment. The fact that antibodies specific for DR5 eradicate radioresistant human laryngeal squamous cell carcinoma cells supports this notion.[27]

Table 3. Alteration in TRAIL/TRAIL receptor profile based on pathologic grading in patients with laryngeal SCC or OCSCC.

Spearman's rho correlation (pathologic grade)	Laryngeal SCC	OCSCC
TRAIL		
Correlation coefficient	.276(*)	—
Significance (2-tailed)	.033	—
n	60	14
DR4		
Correlation coefficient	−.302(*)	.000
Significance (2-tailed)	.019	1.000
n	60	14
DR5		
Correlation coefficient	−.227	−.166
Significance (2-tailed)	.081	.570
n	60	14
DcR1		
Correlation coefficient	−.214	−.214
Significance (2-tailed)	.100	.463
n	60	14
DcR2		
Correlation coefficient	−.155	−.260
Significance (2-tailed)	.236	.369
n	60	14

Abbreviations: TRAIL, TNF (tumor necrosis factor) related apoptosis- inducing ligand; SCC, squamous cell carcinoma; OCSCC, oral cavity SCC; DR4, TRAIL-R1; DR5, TRAIL-R2; DcR1, Decoy-R1; DcR2, Decoy-R2.
** Correlation is significant at the .05 level (2-tailed).*

Second, since benign and malignant tumors appeared to possess different TRAIL and TRAIL receptor expression profiles, the change in cancer behavior might be reflected on the distribution of TRAIL and its receptors. For example, TRAIL and TRAIL receptor expression profile could be used to separate patients with benign prostatic hyperplasia from patients with prostate cancer,[25] and high DcR2 expression in patients with prostate cancer correlated with a poor clinical outcome such as high Gleason scores, prostate specific antigen recurrence, and decreased survival.[28] In accord with our findings, TRAIL and TRAIL receptor profile could be used to separate patients with laryngeal SCC from patients with benign laryngeal lesion. Similarly, patients with OCSSC can be distinguished from patients with benign oral cavity lesion based on differences in TRAIL decoy receptor expression profile. In addition, SCCHN tumors display similar clinical and molecular pathologies. Likewise both patients with laryngeal SCC and patients with OCSCC displayed similar TRAIL and TRAIL receptor expression profiles. Considering that laryngeal cells and cells of the oral cavity originate from the endoderm during embryonic development, these similarities in TRAIL and TRAIL receptor expression profiles of patients with SSCHN should not come as a surprise.

Taking clinical staging into account as a prognostic criterion, the alteration in DR5 expression was the most prominent feature both in patients with laryngeal SCC and in patients with OCSCC. The fact that DR5 expression decreased as clinical staging progressed in patients with laryngeal SCC might mean that laryngeal SCC cells might increase their chance of survival by down-regulating 1 of the TRAIL death receptors. In OCSSC, however, we observed an increase rather than a decrease in DR5 expression as clinical stage advanced. This finding may support a previous report demonstrating the connection of high DR5 expression to a large tumor size in patients with OCSCC.[29]

Pathologic grading is another prognostic marker used in patients with SCCHN. Evaluation of TRAIL and TRAIL receptor profile in connection with tumor cell differentiation revealed that TRAIL and DR4 expression have been correlated with the pathologic grading in patients with laryngeal SCC. Here, an increase in TRAIL expression but a decrease in DR4 expression was suggestive of poor prognosis. TRAIL is an apoptosis-inducing protein and a molecule important in inhibiting cellular immunity.[23,30–32] Similarly, cancer cells use TRAIL to evade the antitumor immune response.[33] Interestingly, an OCSCC cell line (CAL27) induced apoptosis of tumor invading cytotoxic T lymphocytes exclusively through a TRAIL-mediated mechanism,[34] suggesting laryngeal SCC cells may use TRAIL for the metastatic spreading and/or, as in our case, for halting tumor cell differentiation. Additionally, it has been suggested that DR4 expression in patients with breast cancer is important for the transition from a low-grade to a high-grade tumor.[24] Based on this finding, decrease in DR4 expression in our study might lead to a poor cell differentiation, worsening the prognosis of patients with laryngeal SCC. The fact that genetic polymorphisms found in *DR4* were associated with an increased risk for SCCHN by way of TRAIL resistance supports our hypothesis.[35] Intriguingly, 3 of 5 patients with OCSCC with grade 2 tumors died as a result of disease progression.

Early diagnosis necessary for the early treatment of cancer is truly the only effective way of increasing patient survival. Thus, identifying the molecular events affecting cellular behavior through screening becomes a powerful tool in decreasing tumor-related mortality. Our data suggest the expression profile of TRAIL and TRAIL receptor could be used to classify patients with benign lesions (benign laryngeal lesion and benign oral cavity lesion) from malignant ones (laryngeal SCC and OCSCC). The fact that the alteration in DR5 expression correlated with the clinical staging and the pathologic grading correlated with TRAIL and DR4 expression also suggest that these markers could be useful to follow disease progression. Knowing patient prognosis is important in the design and administration of the most effective treatment regime to increase patient survival. Mass spectrometry techniques, such as matrix-assisted laser desorption/ionization time of flight spectroscopy (MALDI-TOF) or surface-enhanced laser desorption/ionization time of flight spectroscopy (SELDI-TOF), are more quantitative and sensitive analytical methods to screen for a protein of interest or a panel of markers compared

with the routinely used immunohistochemical staining methods. Thus, TRAIL and TRAIL receptor expression profiles might best be quantitatively revealed by mass spectroscopy using biopsy material for prospective screening purposes.

CONCLUSIONS

TRAIL and TRAIL receptor expression profile might be useful to follow up disease progression by virtue of their connection to clinical staging and pathologic grading in patients with laryngeal SCC, whereas these patients appear to be ideal targets for a TRAIL-mediated gene therapy approach.

Because of the short follow-up time and the infrequent number of events (disease recurrence or death), we were not in the position of determining any correlation between TRAIL/TRAIL receptor profile and disease-free or overall survival. Thus, longer follow-up time is needed to test any putative correlation as the survival time being the end point.

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