RESEARCH ARTICLE

Association between Laryngeal Squamous Cell Carcinoma and Polymorphisms in Tumor Necrosis Factor Related Apoptosis Induce Ligand (TRAIL), TRAIL Receptor and sTRAIL Levels

Aysegul Verim[1], Saime Turan[2], Ammad Ahmad Farooqi[3], Ozlem Timirci Kahraman[2], Cigdem Tepe-Karaca[1], Yemliha Yildiz[2], Barıs Naiboglu[1], Nazli Ezgi Ozkan[2], Arzu Ergen[2], Gulbu Aydinoglu Isitmangil[4], Ilhan Yaylim[2]*

Abstract

The laryngeal squamous cell carcinoma (LSCC) is one of the most common malignant tumors occurring in the head and neck. Tumor necrosis factor related apoptosis induce ligand (TRAIL) and TRAIL-receptors (DR4, DR5, DcR1, DcR2) are known as important members of TRAIL-mediated biochemical signaling pathway. Associations between polymorphisms in these genes and clinicopathological characteristics of human laryngeal carcinoma are not well defined. This study therefore aimed to investigate a possible relationship among the TRAIL and TRAIL-DR4 polymorphisms and sTRAIL levels in the risk or progression of LSCC. A total of 99 patients with laryngeal cancer and 120 healthy subjects were enrolled in the study. DR4 C626G and TRAIL 1595 C/T genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis and sTRAIL levels were measured by ELISA. There were significant differences in the distribution of DR4 C626G genotypes and frequencies of the alleles between laryngeal cancer patients and controls ($p<0.001$) but not in TRAIL 1595 C/T. We found the increased frequency of the DR4 C626G homozygote CC genotype in patients than in controls ($p<0.001$). Haplotype analysis revealed that there was also a statistically significant relationship between TRAIL and TRAIL-DR4 polymorphisms and laryngeal cancer. Serum sTRAIL levels in the laryngeal patients with CC genotype who had advanced tumour stage were lower than those of patients with early tumor stage ($p=0.014$). Our findings suggest that DR4 C626G genotypes and sTRAIL levels might be associated with progression of laryngeal cancer in the Turkish population.

Keywords: Laryngeal cancer - TRAIL - DR4 - sTRAIL - risk - progression - polymorphism - Turkey

Asian Pac J Cancer Prev, **15 (24)**, 10697-10703

Introduction

Larynx cancer is a multifactorial disease that environmental and genetic factors play role in its etiology and has high incidence among the types of head and neck cancers. Well-known risk factors are smoking and consumption of alcohol. Dietary, papilloma virus infection, and exposure to toxic substances are also other risk factors for larynx cancer. In addition to environmental factors, accumulation of many mutations may alter the balance between cell proliferation and apoptosis, and also may lead to development of cancer (Manjarrez et al., 2006; Braakhuis et al., 2012). Extrinsic pathway of apoptosis in cells triggers by tumor necrosis factor (TNF)-related apoptosis-inducing factor (TRAIL) which is a key effector molecule of cellular signal mechanism (Wang, 2008). TRAIL has emerged as an attractive and deeply studied ligand that selectively induced apoptosis in cancer cells while leaving normal cells intact. There is a rapidly increasing evidence that substantiates the fact that TRAIL induced apoptosis in cancer cells through death receptors (DR4 and DR5). TRAIL induced formation of a Death Inducing Signaling Complex (DISC) at Death receptor that consisted of FADD and Pro-caspase-8. Caspase-8 upon activation further activated its downstream effectors including caspase-3 (Oikonomou et al., 2013). TRAIL is capable of binding to four transmembrane receptors out of the soluble receptor which is called as osteoprogenator. These transmembrane receptors are classified in two groups which are agonist (TRAIL-R1 or DR4 and TRAIL-R2 or DR5) and antagonist (Dobson et al., 2009). DR4 receptors are composed of two extracellular cysteine-

*[1]Department of Otorhinolaryngology/Head and Neck Surgery, [4]Laboratory of Immunology, Haydarpasa Numune Education and Research Hospital, [2]Department of Molecular Medicine, Istanbul University, Institute of Experimental Medicine, Istanbul, Turkey, [3]Laboratory for Translational Oncology and Personalized Medicine, Rashid Latif Medical College, Lahore, Pakistan *For correspondence: ilhanyaylim@gmail.com*

rich, ligand-binding pseudo-repeats (50s and 90s loops), a single transmembrane helix and a cytoplasmic death domain engaged with adaptor protein such as FADD, which activates effector caspase 3 by interacting with initiator procaspases 8 or 10 (Xu et al., 2007).

Gene polymorphism studies may be acceptable a crucial marker to determine the risk and prognosis of cancer. SNPs in the TRAIL and DR4 gene have been reported in different human cancer types such as lung cancer, head and neck cancer (Fisher et al., 2001), bladder cancer (Hazra et al., 2003), breast cancer (Frank et al., 2005; Yildiz et al., 2010) prostat cancer (Mi et al., 2011) etc.

TRAIL gene contains four introns and five exons in which five SNPs was found in the 3'UTR at 1525, 1588 and 1595 whereas the other two were at positions 192 and 912 for healthy individuals (Gray et al., 2001). Fisher et al found two missense alterations in the ectodomain of DR4. One of them is at nucleotide 626. It can change a cytosine to a guanine (C626G) and leads to a substitution of an arginine for threonine. The other is at nucleotide 422. There is another substitution from a guanine to adenine (G422A) and results in a substitution of a histidine for arginine. It has been reported that these alterations result in amino acid changes in or near the ligand-binding domain of DR4. According to the based on the crystal structure of DR5, it shows a homology with DR4 and possess the potential to affect TRAIL binding to DR4 (Fisher et. al, 2001). Increased soluble TRAIL (sTRAIL) concentrations are shown to be related with cancer (Yildiz et al., 2010; Bisgin et al., 2012) and also different disease states such as multiple sclerosis (Wandinger et al., 2003), hepatisis B virus (HBV) infection (Han et al., 2002), fatty liver disease (Yan et al., 2009).

In our previous study a correlation between sTRAIL concentrations, 1595 C/T polymorphism and colorectal cancer was shown in the patients with distant metastasis (Yaylim et al., 2012).

Despite many investigations, the associations between DR4, TRAIL polymorphisms and the risk of human cancers is incompletely understood because of inter/intra ethnic variability. In this study, we aimed to investigate the polymorphisms of DR4 C626G and TRAIL 1595 C/T and sTRAIL levels and also possible effects of these parameters on laryngeal carcinoma susceptibility or progression.

Materials and Methods

Characteristics of subjects

In the present study, we determined TRAIL 1595 C/T and TRAIL-DR4 C626G polymorphisms in 99 Lx SCC patients and 120 controls. The demographic details and clinical characteristic of the study groups, patients and control groups, show similar age (60.8±9.1 years for patients and 57.3±12.5 years for controls), gender (all of them are male), family history (no history for any kind of cancer for controls) , smoking (selected controls are no-smokers) and alcohol consumption (selected controls have no alcohol consumption) status (Table 1). Patients' questionnaires, pathology records and laryngoscopy findings were collected from the medical charts of the patients to confirm the diagnosis and cancer staging. The control subjects, who were not taking any regular medication by that time, were randomly selected among healthy volunteers. The blood samples were collected after pathological diagnosis prior to any surgical, chemotherapeutic or radiation therapy from those who had not undergone blood transfusion. Clinical and pathological information on all larynx SCC diagnoses were confirmed by manual review of the pathology reports and endoscopic findings of Otorhinolaryngology Department. Stage of the laryngeal cancers were defined according to the American Joint Committee on Cancer (AJCC) TNM classification. Glottic and supraglottic tumors were categorized in T1, T2, T3 and T4 subclasses according to the localization of the tumor. Nodal status was categorized as no regional lymph nodes affected (N0), metastasis in a single ipsilateral lymph node, ≤3 cm in greatest dimension (N1) or metastasis in a single ipsilateral lymph node, >3cm but ≤6cm in greatest dimension or multiple lymph nodes ipsi or contralaterally (N2a,b,c).

DNA Isolation

Genomic DNA was extracted from peripheral whole blood containing EDTA according to salting-out technique. DNA was isolated from the blood leukocytes in 10 ml EDTA by the method of Miller et al. based on sodium dodecyl sulphate lysis, ammonium acetate extraction, and ethanol precipitation (Miller et al., 1988).

SNP Detection

TRAIL and DR4 polymorphisms were determined by polymerase chain reaction (PCR) and restriction lenght polymorhism (RFLP) analysis. A previously described method was used for TRAIL1595 gene region determination. The primers for polymerase chain reaction (PCR) for this region were: Forward: 5'-TGA GCA CTA CAG CAA ACA TGA-3' and reverse primer 5'-GCA CCA CTA AAA GAT CGC AGT-3'. To amplify TRAIL-1595 region, 25µl PCR mixture was prepared containing approximately 100ng of template DNA, 0.5µl of each primer, all four deoxyribonucleoside 5' triphosphates (each at 0.2 mM), 2.5mM MgCl2 and 1U Taq polymerase in 1X reaction buffer (Fermentas, Lithuania). PCR conditions were: an initial melting step of 45s at 95˚C; followed by 35 cycles of 45s at 94˚C, 45s at 56˚C and 45s at 72˚C; and a final elongation step of 5min at 72˚C. The 391-base pair PCR product was digested by RsaI (MBI Fermantas) at 37°C for 2.5h. After enzyme digestion the homozygous individuals for C allele (CC genotype) were identified by the presence of 59 and 332bp. The homozygous individuals for the T allele (TT genotype) were identified by the presence of 59, 146, 186 bp fragments. Digestion products were visualized under ultraviolet light after agarose gel electrophoresis.

A previously described method was used to amplify the DR4-E4 gene region to evaluate the polymorphism in this region. The primers used for this procedure were: Forward: 5'- AAGGTCAAGGGACACGTCAGG -3' and reverse: 5'- GCTTCTGTGGTTTCTTTGAGG -3'. Total reaction mixture for PCR amplification was 25µl containing about

100 ng of template DNA, 0.5 µl of each primer, all four deoxyribonucleoside 5' triphosphates (each at 0.2mM), 2.5mM MgCl2 and 1U of Taq polymerase in 1X reaction buffer (Fermentas, Lithuania). The PCR reaction was carried out with an initial melting step of 45s at 95˚C; followed by 35 cycles of 45s at 94˚C, 45s at 59˚C, 45s at 72˚C; and a final elongation step of 5min at 72˚C. The 220-base pair PCR product was digested by DRA III (MBI Fermantas) at 37°C for 2h. After enzyme digestion the homozygous individuals for C allele (CC genotype) were identified by the presence of 164 and 56bp. The homozygous individuals for the G allele (GG genotype) were identified by the presence of 220bp fragments. Digestion products were visualized under ultraviolet light after agarose gel electrophoresis.

TRAIL assay

Fresh-blood samples were immediately centrifuged at 3000 rpm for 5min to separate serum and samples were kept frozen at -20°C until the study. Serum TRAIL levels were determined with commercially available sandwich ELISA kit (Platinium ELISA, Bender MedSystems GmbH, Vienna).

Statistical analysis

Statistical analyses were performed using the SPSS version 11.0 for Windows (SPSS Inc. Chicago, IL, USA). Differences in the frequencies of the TRAIL 1595 C/T and TRAIL-DR4 C626G polymorphisms between Lx SCC patients and the control group and between clinical data within the Larynx cancer subgroup were analyzed using the Chi-square test. The Hardy-Weinberg equilibrium was tested for all polymorphisms. Differences in sTRAIL serum levels between patients and control subjects were examined using the Mann-Whitney U test. Odds ratios (ORs) and 95% confidence intervals (95%CI) were calculated to estimate the risk for larynx cancer. The threshold for significance was $p<0.05$. Haplotype frequencies D' and r^2 were calculated using Haploview 4.2 programme.

Results

Table 1 shows the demographic characteristics of our Lx SCC patients. Genotypes and allele frequencies for TRAIL 1595 C/T and TRAIL-DR4 C626G polymorphisms in larynx cancer patients and controls are given in Table 2. Genotype distributions for TRAIL 1595 C/T polymorphism in both groups were in agreement with Hardy-Weinberg equilibrium (for patients $\chi^2=0.001$, $p=0.97$; for controls $\chi^2=1.95$, $p=0.16$). Genotype distributions for TRAIL-DR4 C626G polymorphism in patient group were in agreement with Hardy–Weinberg equilibrium ($\chi^2=2.21$, $p=0.137$) but not in control group ($\chi^2=5.36$, $p=0.02$).

TRAIL 1595 C/T genotype and allele frequencies between larynx cancer patients and controls were not statistically significant ($p>0.05$). When the TRAIL-DR4 C626G polymorphism was compared between larynx and controls, it was found that there was a statistically significant difference. TRAIL-DR4 C626G genotype and allele frequencies between larynx cancer patients and controls were statistically significant ($p<0.001$). The frequency of CC homozygote wild type genotype were found 3.737 fold higher in larynx cancer patients than those with controls ($p<0.0001$; OR: 3.737; CI% 2.063-6.771). Moreover, patients having CG genotype showed significantly 0.720 fold lower risk for larynx cancer when compared with other genotypes ($p=0.018$; OR=0.720; Cl% 0.544-0.953).

Regarding distributions of TRAIL 1595 C/T and TRAIL-DR4 genotypes according to clinical parameters and tumor characteristics of larynx cancer patients no

Table 1. Characteristics of Patients with Lx Squamous Cell Carcinomas

Parameters		Larynx Cancer Patients	
		n	%
Smoking status	Before age 18	56	61.5
	After age 18	35	38.5
Reflux	Yes	44	47.8
	No	48	52.2
Alcohol consumption	Yes	51	53.1
	No	45	46.9
Family history	Yes	33	34.7
	No	62	65.3
Tumor location	Glottic	72	76.6
	Supraglottic	22	23.4
Tumor stage	T1	7	7.2
	T2	15	15.5
	T3	44	45.4
	T4	31	32.5
Lymph node	N0	53	54.6
	N1	36	37.1
	N2	6	6.2
	N3	2	2.1
Metastasis	Yes	3	3.1
	No	94	96.9
Diferentiation	Poor	11	18.3
	Medium	41	68.3
	Well	8	13.3
Perineural invasion	Yes	77	79.4
	No	9	14.3
Tumor recurrence	Yes	9	14.3
	No	54	85.7

Table 2. Genotype and Allele Frequencies of the TRAIL-1595 C/T and TRAIL-DR4-E4 C626G Polymorphisms among the Lx SCC and Controls

Genotypes and Alleles	Larynx Cases (n=99)	Controls Cases (n=120)	p-value
	Frequency (%)	Frequency (%)	
TRAIL-1595 C>T			
CC	56 (56.6)	67 (58.8)	p>0.05
TT	6 (6.1)	4 (3.3)	
CT	37 (37.1)	49 (40.8)	
C Allele	149 (75.3)	183 (76.2)	p>0.05
T Allele	49 (24.7)	57 (23.8)	
DR4-E4			
CC	37 (37.4)	12 (10)	p<0.001*
GG	21 (21.2)	39 (32.5)	
CG	41 (41.4)	69 (57.5)	
C Allele	115 (58.1)	83 (38.7)	p<0.001*
G Allele	83 (41.9)	147 (61.3)	

*Chi-square test was used to compare genotypes in the study group. Lx SCC; n, number of individuals

Table 3. The sTRAIL levels, TRAIL 1595 C T Genotypes of our Patients Groups and Some Tumor Characteristics of Lx SCC Patients

	sTRAIL levels (pg/ml)					
	TRAIL 1595 C/T			TRAIL-DR4-Ex4 C626G		
Tumor parameters	CC	CT	TT	CC	CG	GG
Patients with advanced tumor stage (T_3+T_4)	893±136	946±106	945±211	853±86a	762±110	1349±309
Patients with early tumor stage (T_1+T_2)	958±241	338±239	2051	1469±310	976±310	806
Lenf node (n_1,n_2,n_3)	912±180	1123±131	945±211	1046±153	793±166	1394±192
Lenf node (n_0)	906±156	913±147	2051	908±135	834±155	1236±422

*Values are represented as X ± SE (Srandard Deviation). *comparisons TRAIL levels between study groups by using Student T-test.. ap = 0.014; compare between the patients with advanced tumor stage and early tumor stage.

Table 4. Haplotype Frequencies of Lx SCC Cases and Controls

Block	Haplotype	Case. Control Ratio Counts	Chi Square	p value
Block 1			X^2	
GC		0.325, 0.492	12.322	0.0004
CC		0.438, 0.270	13.32	0.0003

Haplotype consist of rs1131580 and rs20575. p-values of haplotype association were calculated using Haploview 4.2;*p values less than 0.05 denoted statistical significance

statistically significant association was found between TRAIL, TRAIL-DR4 genotypes and clinical parameters including tumor location, tumor T stage, lymph node, metastasis, perineural invasion and tumor differentiation, presence of reflux and alcohol consumption in Lx SCC patients.

Among larynx cancer patients, there were no significant association for sTRAIL levels between the patients and healthy controls. Any other association was not found between sTRAIL levels and the clinical parameters of Larynx patients or genotypes. sTRAIL levels in Larynx patients with the early tumor stage and with CC genotype of TRAIL-DR4 were lower (853±86 pg/ml) than those with advanced tumor stage (1469±310) (p=0.014) (Table 3).

In addition, TRAIL and TRAIL-DR4 haplotypes were evaluated for association with Lx SCC. Haplotype analysis revealed that there was a statistically significant relationship between TRAIL and TRAIL-DR4 polymorphisms and Lx SCC (for Lx SCC cases: p=0.0004; X2: 12.322 LOD: 3, and *r*-squared: 0.8) (Table 4).

Discussion

Apoptosis induction via the extrinsic pathway, where TRAILR1 and TRAIL play a role, is recently investigated as an anticancer theraphy (Hollander et al., 2013). It is known that the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL or Apo2L) is a important molecule which induces the death of cancer but not normal cells. It has been suggested its potential use as a tumor-specific antineoplastic agent. TRAIL have a capable of binding to the proapoptotic death receptors DR4 and the p53-regulated proapoptotic KILLER/DR5 and also to the decoy receptors TRID and TRUNDD (Kim et al., 2000). TRAIL receptors are known as a candidate tumor suppressor genes, because their inactivation leads to result a defective apoptotic signaling (Fisher et al., 2001).

Accumulating evidence has indicated that genetic variants of DR4 gene especially chromosome 8p21-22 is a frequent site of allelic deletions in many cancer types such as colorectal cancer (Frank et al., 2006), bladder cancer (Hazra et al., 2003), gastric cancer (Kuraoka et al., 2005), head and neck cancer, lung cancer (Fisher et al., 2001) and non-Hodgkin's lymphoma (NHL) (Lee et al., 2001). It is also reported that somatic mutation of TRAIL-R2 has an important role in the pathogenesis of some non-small cell lung cancers (NSCLC) and that the TRAIL-R2 gene is one of the genes relevant to the frequent loss of chromosome 8p21-22 in NSCLC (Lee et al., 1999).

The C-G single nucleotide polymorphism in exon 4 of DR4 gene is the most studied polymorphism which has recently been linked with cancer in Caucasian susceptibility (Chen et al., 2014).

A C-->G polymorphism at amino acid 626, located immediately 3' to one of the main receptor ligand interface regions, are known as a threonine-->arginine change in DR4 exon. Hazra et al. reported that wild-type genotype CC was related to increased risk of bladder cancer in Caucasians (Hazra et al., 2003). In another study Frank et al. have reported that DR4 heterozygous carriers have decreased risk for colorectal cancer (Frank et al., 2006).

Dechant et al suggested that homozygous genetic alterations within the DR4 gene may be an important role in the formation of osteosarcoma (Dechant et al., 2004). It has also reported that TRAIL receptor I (DR4) polymorphisms C626G is associated with an increased risk for hepatocellular carcinoma (HCC) in HCV-infected patients (Korner et al., 2012). Kuraoka et al showed an association between the DR4 polymorphism and the risk of gastric cancer. But, there was no association between the genotypes and clinicopathological characteristics (depth of invasion, lymph node metastasis, distant metastasis, stage and grade of differentiation) of gastric carcinoma patents. When patients were investigated by immunohistochemistry, DR4 was constantly expressed in gastric carcinoma, but not in non-neoplastic gastric epithelium. According to their data, this single nucleotide polymorphism (a Thr to Arg) in the extracellular domain of DR4 could not be related to the development and progression of gastric cancer (Kuraoka et al., 2005).

Horak et al. (2005) showed that DR4 gene do not lead to clinically relevant ovarian cancer predisposition and are therefore most unlikely to contribute to familial ovarian cancer. It has also suggested that TRAIL-R-associated

mechanisms may result in immune-modulatory effects on tumor-infiltrating polymorphonuclear cells. Moreover, the significant association of somatic TRAIL-R1 genetic polymorphisms in squamous cell carcinoma of the head and neck (HNSCC) suggests a potential association between constitutive TRAIL-R1 polymorphisms and development of HNSCC (Teng et al., 2005). Fisher et al evaluated 19 head and neck squamous cell cancer patients and detected forty-seven % of the patients was homozygous for C626G and this difference was statistically significant (Fisher et al., 2001).

In our study, we have found a significant association between CC homozygote wild type carriers and increased larynx cancer risk. Also, compared with other genotypes, patients having CG genotype have showed significantly lower risk for larynx cancer than those with controls.

There was a meta-analysis include nine studies, among which eight articles including 2941 cases and 3358 controls had described C626G genotypes. They reported that there was no association between C626G polymorphism and the risk of cancer in all genetic models when all the eligible studies were pooled into the meta-analysis (Chen et al., 2009). But, in a recent meta-analysis, it has been reported that TRAIL DR4 C626G and A683C polymorphisms were indeed associated with cancer risk (Chen et al., 2014).

Another SNP analysis for TRAIL C1595T have been studied in fatty liver disease (Yan et al., 2009), breast cancer (Yildiz et al., 2010), sporadic breast tumor (Pal et al., 2011) and prostate cancer (Mi et al., 2011). We have investigated TRAIL C1595T polymorphism and its relation to breast cancer and colorectal cancer in our previous studies (Yildiz et al., 2010; Yaylim et al., 2012).

Wang et al have reported that 1595T (CT+TT) was significantly lower in gastric cancer than in healthy controls. When they did a stratification analysis, 1595T (CT+TT) carriers were associated with poorly-differentiated gastric cancer (Wang et al., 2013).

We had reported a significant data between the colorectal cancer patients with advanced stage tumor for TRAIL 1595 genotypes, but found no significant difference in the distribution of TRAIL 1595 genotypes between control and the patient group (Yaylim et al., 2012).

It has been reported that several cytokines induced Increased TRAIL gene transcription killing selectively various cancer cells without resulting in toxicity to many normal cells (Allen et al., 2012).

Recent studies have reported that sTRAIL levels are associated with some cancer types such as colorectal cancer (Bisgin et al., 2012), lung cancer (Kargi et al., 2013) or oral cavity cancer (Jablonska et al., 2008). Confluence of information suggests that interferon α regulated expression of TRAIL in neutrophils in chronic myelogenous leukemia patients. It is worth mentioning that interferon α treated neutrophils displayed an increase in intracellular expression of TRAIL and there was an increase in secretion of sTRAIL in the culture supernatant (Tanaka et al., 2007). Subsequent study in oral cavity cancer patients revealed that serum levels of sTRAIL were increased in patients in Stage II before treatment and decreased in the same patients after treatment Jablonska et al. (2008). Kim et al reported that serum TRAIL levels were significantly lower in women with endometriosis than in women without endometriosis, but they didn't find any association between sTRAIL levels for early and advanced endometriosis (Kim et al., 2012).

Yıldız et al. (2010) reported that during pretreatment by Bevacizumab-based chemotherapy, sTRAIL and sDR4 levels were similar to the levels of controls. They showed that there were no significant difference in sTRAIL, sDR4, and sDR5 levels in metastatic colorectal cancer patients before and after treatment.

It is interesting to note that in bevacizumab-treated metastatic colon cancer, serum sTRAIL levels were found to be associated with patient survival (Bisgin et al., 2014). It has been shown that infecting lung adenocarcinoma cells with sTRAIL expressing rAAV2/5 vector resulted in secretion of sTRAIL and notable apoptosis in cancer cells. Transducing tumor bearing mice with sTRAIL expressing rAAV2/5 vector resulted in regression of tumor growth (Shi et al., 2005). On the other hand, Le et al. (1999) indicated that higher level of sTRAIL expression is correlated with liver damage. They suggest that apoptosis induced by sTRAIL may be one of the mechanisms of liver damage in HBV (Zhonghua et al., 2005). However, there are few data for sTRAIL association with larynx cancer. TRAIL induced apoptosis has been studied in human laryngeal squamous carcinoma Hep-2 cell line (Yao et al., 2011). It is becoming progressively more understandable that TRAIL induced apoptosis in Hep-2 cancer cells can be improved via synergistically treating cancer cells with TRAIL and chemotherapeutic drugs including cisplatin and paclitaxel. Increased apoptosis was noted through increased expression of DR4 and DR5 (Zhang and Zhou, 2009). Increasingly it is being recognized that different cancer cells are resistant to TRAIL mainly due to inactivation of extrinsic, intrinsic pathway or downregulation of death receptors. Non-availability of DRs on cell surface has opened new dimension for basic and clinical scientists to improve the efficacy of TRAIL via combining it with natural agents and chemotherapeutic drugs that promoted expression and cell surface appearance of DRs.

Therefore, in this study, we compared the sTRAIL levels of larynx cancer patients with those of healthy controls. We have detected some differences in sTRAIL levels among patients having different stage tumors. Our study has suggested a hypothesis that low sTRAIL levels may be related with disregulation of apoptosis in advanced stage larynx cancer (Dobson et al., 2009; Yildiz et al., 2010; Kargi et al., 2012; Kargi et al., 2013).

There is a recent report that suggests an increase in decoy-R1 (DcR1) but a downregulation in decoy-R2 (DcR2) (Yoldas et al., 2011). In line with this approach, it has previosuly been reported that level of the DR5, DcR2 related with the degree of LSCC histological differentiation ($p<0.05$) (Yao et al., 2006).

On a similar note, monoclonal antibody (TRA-8) to human DR5 also induced apoptosis in irradiated cancer cells via upregulation of DR5 (Wu et al., 2009). Tecchio et al demonstrated that therapeutic concentrations of

interferon alpha (IFNalpha) can stimulate the expression of high levels of TRAIL mRNA and the release of elevated amounts of a soluble bioactive form of TRAIL (sTRAIL) in both human neutrophils and monocytes (Tecchio et al., 2004).

It is known that The TRAILR1/TRAIL system participates in the induction of the extrinsic apoptotic pathway and constitutes an emerging target in cancer therapeutics (Heredia-Galvez et al., 2014). The mechanism underlying the effect of the C626G polymorphism on the sTRAIL levels is not clear yet. However, we found a significant difference between sTRAIL levels and TRAIL DR4 genotypes for early tumor stage and advanced tumor stage.

In our study, we investigated the relation among TRAIL and TRAIL-DR4 polymorphisms, sTRAIL levels and larynx cancer. Our results suggest that the altered TRAIL, DR4 alleles and sTRAIL levels may be associated with some other potential biomarkers for laryngeal cancer. Further studies with larger patient groups should be done to investigate additionally as these kind of potential markers for, predisposition and progression to common malignancies.

References

Allen JE, El-Deiry WS (2012). Regulation of the human TRAIL gene. *Cancer Biol Ther*, **13**, 1143-51.

Bisgin A, Kargi A, Yalcin AD et al (2012). Increased serum sTRAIL levels were correlated with survival in bevacizumab-treated metastatic colon cancer. *BMC Cancer*, **12**, 58.

Braakhuis BJ, Brakenhoff RH, Leemans CR (2012). Treatment choice for locally advanced head and neck cancers on the basis of risk factors: biological risk factors. *Ann Oncol*, **23**, 173-7.

Chen B, Liu S, Wang XL, et al (2009). TRAIL-R1 polymorphisms and cancer susceptibility: an evidence-based meta-analysis. *Eur J Cancer*, **45**, 2598-605.

Chen W, Tang WR, Zhang M, Chang K, Wei YL (2014). Association of DR4 (TRAIL-R1) polymorphisms with cancer risk in Caucasians: an updated meta-analysis. *Asian Pac J Cancer Prev*, **15**, 2889-92.

Dechant MJ, Fellenberg J, Scheuerpflug CG, Ewerbeck V, Debatin KM (2004). Mutation analysis of the apoptotic "death-receptors" and the adaptors TRADD and FADD/MORT-1 in osteosarcoma tumor samples and osteosarcoma cell lines. *Int J Cancer*, **109**, 661-7.

den Hollander MW, Gietema JA, de Jong S, et al (2013). Translating TRAIL-receptor targeting agents to the clinic. *Cancer Lett*, **332**, 194-201.

Dobson CL, Main S, Newton P, et al (2009). Human monomeric antibody fragments to TRAIL-R1 and TRAIL-R2 that display potent *in vitro* agonism. *MAbs*, **1**, 552-62.

Fisher MJ, Virmani AK, Wu L, et al (2001). Nucleotide substitution in the ectodomain of trail receptor DR4 is associated with lung cancer and head and neck cancer. *Clin Cancer Res*, **7**, 1688-97.

Frank B, Hemminki K, Shanmugam KS, et al (2005). Association of death receptor 4 haplotype 626C-683C with an increased breast cancer risk. *Carcinogenesis*, **26**, 1975-7.

Frank B, Shanmugam KS, Beckmann L, et al (2006). Death receptor 4 variants and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev*, **15**, 2002-5.

Gray HL, Sorensen EL, Hunt JS, et al (2001). Three polymorphisms in the 3' UTR of the TRAIL (TNFrelated apoptosis-inducing ligand) gene. *Genes Immun*, **2**, 469-70.

Han LH, Sun WS, Ma CH, et al (2002). Detection of soluble TRAIL in HBV infected patients and its clinical implications. *World J Gastroenterol*, **8**, 1077-80.

Hazra A, Chamberlain RM, Grossman HB, et al (2003). Death receptor 4 and bladder cancer risk. *Cancer Res*, **63**, 1157-9.

Heredia-Galvez B, Ruiz-Cosano J, Torres-Moreno D, et al (2014). Association of polymorphisms in TRAIL1 and TRAILR1 genes with susceptibility to lymphomas. *Ann Hematol*, **93**, 243-7.

Horak P, Pils D, Roessler M, et al (2005). Common death receptor 4 (DR4) polymorphisms do not predispose to ovarian cancer. *Gynecol Oncol*, **97**, 514-8.

Jablonska E, Jablonski J, Marcinczyk M, et al (2008). The release of soluble forms of TRAIL and DR5 by neutrophils of oral cavity cancer patients. Folia *Histochem Cytobiol*, **46**, 177-83.

Kargi A, Bisgin A, Yalcin AD, et al (2013). Increased serum S-TRAIL level in newly diagnosed stage-IV lung adenocarcinoma but not squamous cell carcinoma is correlated with age and smoking. *Asian Pac J Cancer Prev*, **14**, 4819-22.

Kargi A, Yalcin AD, Erin N, et al (2012). IL8 and serum soluble TRAIL Lev-els following Anti-VEGF monoclonal antibody treat-ment in patients with metastatic colon cancer. *Clinical Laboratory*, **5-6**, 501-5.

Kim H, Ku SY, Suh CS, et al (2012). Association between endometriosis and polymorphisms in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), TRAIL receptor and osteoprotegerin genes and their serum levels. *Arch Gynecol Obstet*, **286**, 147-53.

Kim K, Fisher MJ, Xu SQ, et al (2000). Molecular determinants of response to TRAIL in killing of normal and cancer cells. *Clin Cancer Res*, **6**, 335-46.

Korner C, Riesner K, Kramer B, et al (2012). TRAIL receptor I (DR4) polymorphisms C626G and A683C are associated with an increased risk for hepatocellular carcinoma (HCC) in HCV-infected patients. *BMC Cancer*, **12**, 85.

Kuraoka K, Matsumura S, Sanada Y, et al (2005). A single nucleotide polymorphism in the extracellular domain of TRAIL receptor DR4 at nucleotide 626 in gastric cancer patients in Japan. *Oncol Rep*, **14**, 465-70.

Lee SH, Shin MS, Kim HS, et al (1999). Alterations of the DR5/TRAIL receptor 2 gene in non-small cell lung cancers. *Cancer Res*, **59**, 5683-6.

Lee SH, Shin MS, Kim HS, et al (2001). Somatic mutations of TRAIL-receptor 1 and TRAIL-receptor 2 genes in non-Hodgkin's lymphoma. *Oncogene*, **20**, 399-403.

Manjarrez ME, Ocadiz R, Valle L, et al (2006). Detection of human papillomavirus and relevant tumor suppressors and oncoproteins in laryngeal tumors. *Clin Cancer Res*, **12**, 6946-51.

Mi YY, Li JM, Shao N, et al (2011). TRAIL gene polymorphism and genetic susceptibility to prostate cancer in the Chinese Han population of Nanjing. *Zhonghua Nan Ke Xue*, **17**, 242-6.

Miller SA, Dykes DD, Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*, **16**, 1215.

Oikonomou E, Pintzas A (2013). The TRAIL of oncogenes to apoptosis. *Biofactors*, **39**, 343-54.

Pal R, Gochhait S, Chattopadhyay S, et al (2013). Functional implication of TRAIL-716 C/T promoter polymorphism on its *in vitro* and *in vivo* expression and the susceptibility to sporadic breast tumor. *Breast Cancer Res Treatment*, **126**, 333-43.

Shi J, Zheng D, Liu Y, et al (2005). Overexpression of soluble

TRAIL induces apoptosis in human lung adenocarcinoma and inhibits growth of tumor xenografts in nude mice. *Cancer Res*, **65**, 1687-92.

Tanaka H, Ito T, Kyo T, et al (2007). Treatment with IFNalpha *in vivo* up-regulates serum-soluble TNF-related apoptosis inducing ligand (sTRAIL) levels and TRAIL mRNA expressions in neutrophils in chronic myelogenous leukemia patients. *Eur J Haematol*, **78**, 389-98.

Tecchio C, Huber V, Scapini P, et al (2004) IFNalpha-stimulated neutrophils and monocytes release a soluble form of TNF-related apoptosis-inducing ligand (TRAIL/Apo-2 ligand) displaying apoptotic activity on leukemic cells. *Blood*, **103**, 3837-44.

Teng MS, Brandwein-Gensler MS, Teixeira MS, et al (2005). A study of TRAIL receptors in squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg*, **131**, 407-12.

Wandinger KP, Lunemann JD, Wengert OA, et al (2003). TNF-related apoptosis inducing ligand (TRAIL) as a potential response marker for interferon-beta treatment in multiple sclerosis. *Lancet*, **361**, 2036-43.

Wang C, Xu S, Yi F, et al (2013). Tumor necrosis factor-related apoptosis inducing ligand gene polymorphisms are correlated with gastric cancer. in central China. *Pharm Res*, [Epub ahead of print]

Wang S (2008). The promise of cancer therapeutics targeting the TNF-related apoptosis-inducing ligand and TRAIL receptor pathway. *Oncogene*, **27**, 6207-15.

Wu F, Hu Y, Long J, et al (2009). Cytotoxicity and radiosensitization effect of TRA-8 on radioresistant human larynx squamous carcinoma cells. *Oncol Rep*, **21**, 461-5.

Xu G, Shi Y (2007). Apoptosis signaling pathways and lymphocyte homeostasis. *Cell Res*, **17**, 759-71.

Yan X, Xu L, Qi J, et al (2009). sTRAIL levels and TRAIL gene polymorphisms in Chinese patients with fatty liver disease. *Immunogenetics*, **61**, 551-6.

Yao H, Ji W, Guan C, et al (2006). The expression of tumor necrosis factor related apoptosis induce ligand in human laryngeal squamous cell carcinoma. *Lin Chuang Er Bi Yan Hou Ke Za Zhi*, **20**, 1105-8.

Yao H, Xiao H, Wang C, et al (2011). Apoptosis inducing effect by TRAIL on human laryngeal squamous carcinoma Hep-2 cells. *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*, **25**, 127-9.

Yaylim I, Ozkan N, Turan S et al (2012). sTRAIL serum levels and TRAIL 1595 genotypes: associations with progress and prognosis of colorectal carcinoma. *J Cancer Therapy*, **3**, 941-7.

Yildiz R, Benekli M, Buyukberber S, et al (2010). The effect of bevacizumab on serum soluble FAS/FASL and TRAIL and its receptors (DR4 and DR5) in metastatic colorectal cancer. *J Cancer Res Clin Oncol*, **136**, 1471-6.

Yildiz Y, Yaylim-Eraltan I, Arikan S, et al (2010). Is there any correlation between TNF-related apoptosis-inducing ligand (TRAIL) genetic variants and breast cancer? *Arch Med Sci*, **6**, 932-6.

Yoldas B, Ozer C, Ozen O, et al (2011). Clinical significance of TRAIL and TRAIL receptors in patients with head and neck cancer. *Head Neck*, **33**, 1278-84.

Yue FE, Liu DY, Song J, et al (2005). Soluble tumor necrosis factor related apoptosis inducing ligand (sTRAIL) and clinical outcome of HBV infection in human. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*, **19**, 146-8.

Zhang M, Zhou L (2009). Synergistic effects and mechanisms of combined tumor necrosis factor-related apoptosis-inducing ligand and chemotherapeutic drugs or radiotherapy in killing laryngeal squamous carcinoma cells *in vitro*. *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*, **44**, 565-70.