Eur Arch Otorhinolaryngol
DOI 10.1007/s00405-014-3095-2

HEAD AND NECK

Low blood levels of sTWEAK are related to locoregional failure in head and neck cancer

Francesc Xavier Avilés-Jurado · Ximena Terra · David Gómez · Joan Carles Flores · Antoni Raventós · Elsa Maymó-Masip · Xavier León · Vicente Serrano-Gonzalvo · Joan Vendrell · Enric Figuerola · Matilde R. Chacón

Received: 6 February 2014 / Accepted: 7 May 2014

Abstract Identifying serum pre-treatment molecular markers that can predict response to therapy is of great interest in head and neck oncology and is required to develop personalized treatments that maximize survival while minimizing morbidity. The main aim was to investigate the potential prognostic significance of tumor necrosis factor-like weak inducer of apoptosis (TWEAK), and its receptors, fibroblast growth factor-inducible 14 (Fn14) and CD163, in head and neck squamous cell carcinoma (HNSCC). The study comprised 37 consecutive patients with pathologically confirmed, untreated HNSCC. Serum and tissue samples from these patients were available for study. We determined sTWEAK and sCD163 levels in serum from 37 HNSCC patients by ELISA. *TWEAK, CD163, Fn14* and *TNF-α* gene expression were detected by real-time RT-PCR in 111 matched tissue samples (tumoral, adjacent and distal/normal mucosa). Our results showed a significant relationship between low sTWEAK levels and poor locoregional control of the disease. Kaplan–Meier curves indicated that the locoregional recurrence-free survival rate in patients with low sTWEAK circulating levels was significantly lower than in patients with high levels, and that high *CD136/TWEAK* expression ratio in tumors was also related to poor prognosis. sTWEAK pre-treatment serum levels might be used as prognostic non-invasive biomarkers for locoregional control in patients with HNSCC. Future investigations are warranted to determine the potential prognostic significance of this non-invasive biomarker

Francesc Xavier Avilés-Jurado and Ximena Terra have contributed equally to this work.

F. X. Avilés-Jurado (✉) · J. C. Flores · E. Figuerola
Otorhinolaryngology Head-Neck Surgery Department. Hospital Universitari de Tarragona Joan XXIII. IISPV, Universitat Rovira i Virgili, Mallafré Guasch, 4, 43007 Tarragona, Catalonia, Spain
e-mail: fxavilesj@gmail.com

F. X. Avilés-Jurado · X. Terra · E. Figuerola
HJ23 Otolaryngology Disease Research Group, Insitut d'Investigació Sanitària Pere Virgili, Universitat Rovira i Virgili, Tarragona, Spain

X. Terra
MoBioFood Research Group, Departament de Bioquímica i Biotecnologia, Universitat Rovira i Virgili, Tarragona, Spain

D. Gómez
Radiation Oncology Department, Hospital Sant Joan de Reus, Reus, Tarragona, Catalonia, Spain

A. Raventós
Pathology Department, Hospital Universitari de Tarragona Joan XXIII, Universitat Rovira i Virgili, Tarragona, Catalonia, Spain

E. Maymó-Masip · J. Vendrell · M. R. Chacón
Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Hospital Universitari Joan XXIII de Tarragona, Insitut d'Investigació Sanitària Pere Virgili, Universitat Rovira i Virgili, Tarragona, Catalonia, Spain

X. León
Otorhinolaryngology Head-Neck Surgery Department, Hospital de la Santa Creu i Sant Pau and Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN, MICINN, ISCIII), Universitat Autònoma de Barcelona, Barcelona, Spain

V. Serrano-Gonzalvo
Anetesiology and Reanimation Department, Hospital Universitari de Tarragona Joan XXIII. IISPV, Universitat Rovira i Virgili, Tarragona, Catalonia, Spain

M. R. Chacón (✉)
Research Unit, Hospital Universitari de Tarragona Joan XXIII, Mallafré Guasch, 4, 43007 Tarragona, Catalonia, Spain
e-mail: mrodriguez.hj23.ics@gencat.cat

Published online: 26 May 2014

in the rapid discrimination according to the locoregional control achieved in patients who received a non-surgical organ preservation treatment.

Keywords sTWEAK · Non-invasive biomarker · Locoregional control · Treatment · Prognosis

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. HNSCC is associated with severe disease and treatment-related morbidity, with 5-year survival rates of approximately 50 % [1]. Treatments include the use of radiotherapy or chemoradiotherapy, surgery, or a combination of these procedures. At present, prognostic factors that can efficiently predict the outcome after treatment are not available. Identifying pretreatment molecular markers that can predict response to therapy is of great interest in head and neck oncology and is required to develop personalized treatments that maximize survival while minimizing morbidity.

The role of tumor necrosis factor-α (TNF-α) in HNSCC has been investigated previously [2, 3]. In contrast, tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK), another member of the TNF superfamily, has never been studied in HNSCC. TWEAK is a multifunctional cytokine that, through its receptor fibroblast growth factor-inducible 14 (Fn14), controls many cellular activities, including proliferation, migration, differentiation, apoptosis, angiogenesis and inflammation [4, 5]. Fn14 has been reported as the signal transducer receptor that controls several TWEAK-associated activities such as proliferation of endothelial cells and angiogenesis [6]. However, the full biological effects of TWEAK on cancer remain largely unknown because cells lacking Fn14 have also been shown to be TWEAK sensitive [7, 8]. On that point, CD163 has been reported as a receptor for sTWEAK [9]. This protein belongs to a scavenger receptor cysteine-rich family exclusively expressed on the surface of monocyte/macrophages. Also, a soluble form of CD163 is constitutively present in serum and is generated by a proteolytic cleavage of the membrane CD163. Its circulating levels are higher in diseases associated with macrophage activation, including cancer [7].

In most cancers, TWEAK expression is higher in tumor tissues than in normal ones. TWEAK-Fn14 up-regulates VEGF expression to foster ovarian cancer cell metastasis [10] and regulates breast cancer cell invasive capacity [11, 12]. Furthermore, elevated *Fn14* expression promotes gastric cancer growth [13]. The expression of *Fn14* is often increased in malignant tumors, so it may be considered as a tumor-specific cell surface biomarker and a tumor cell regulatory molecule [14]. However, the expression of TWEAK/Fn14/CD163 and their potential function in HNSCC has not been addressed yet. Only Nakayama et al. [15] have studied the role of TWEAK in human oral squamous cell carcinoma HSC3 cells. Their results indicate that TWEAK acts in HSC3 cells through caspase activation, leading to cell death by apoptosis. In agreement with these findings, other researchers have also found that TWEAK may have anti-tumor effects [4, 15–18].

On the basis of recent research, we hypothesized that TWEAK, CD163, and Fn14 might may be involved in HNSCC cancer development and affect the survival rate, so we carried out this preliminary approach to investigate whether sTWEAK and sCD163 serum levels and the *TWEAK, CD163* and *Fn14* gene expression might predict locoregional control in patients with HNSCC and therefore, may serve as biological markers of outcome in this type of carcinoma.

Materials and methods

Patients

The study comprised 37 consecutive patients with pathologically confirmed, untreated squamous cell carcinomas. The institution's cancer committee evaluated all of our patients, and the decision to treat with radiotherapy or chemoradiotherapy was based on the centre's organ preservation protocol. In general, all patients with stage I–II tumors, and some patients with stage III tumors, depending on the location of the primary tumor, received radiotherapy (RT) or chemoradiotherapy (ChRT). Patients with advanced tumors (stages III–IV) were treated with chemoradiotherapy (ChRT).

External beam radiotherapy was administered in total doses of 65–74 Gy to the primary site, 50 Gy to the neck in all patients with N0 nodes except those with T1N0 glottic carcinomas, and 70 Gy to the neck in patients with clinical metastatic neck nodes (N+). Treatment was administered by continuous-course radiotherapy 5 days a week, 2 Gy per session in normo-fractionated treatments, and 1.2 Gy twice daily in hyper-fractionated treatments. Chemoradiotherapy consisted of radiotherapy at the same doses plus 3 cycles of cisplatin at a dose of 100 mg/m^2 on day 1 every 3 weeks.

Routine follow-up consisted of an evaluation of symptoms and locoregional examinations at 2-month intervals during the first year, 3-month intervals in the second year, and 4-month intervals over years 3–5. The median follow-up of the patients included in the study was 2.9 years (95 % CI 1.6–4.1 years). The University Hospital Joan XXIII Ethics Committee approved the study, and all patients gave informed consent. Table 1 shows the characteristics of the patients included in the study.

Table 1 Characteristics of the patients included in the study

Characteristics	Number of patients (%)
Age (years)	
<50	6 (16.2)
50–60	16 (43.2)
60–70	13 (35.1)
>70	2 (5.4)
Sex	
Male	35 (94.6)
Female	2 (5.4)
Tobacco consumption	
Never	1 (2.7)
<20 cigarettes per day	2 (5.4)
>20 cigarettes per day	34 (91.9)
Alcohol consumption	
Never	4 (10.8)
Mild–moderate	5 (13.5)
Severe	28 (75.5)
ECOG index	
0	13 (35.1)
1	14 (37.8)
2	7 (18.9)
3–4	3 (8.1)
Tumor location	
Oral cavity–oropharynx	12 (32.4)
Larynx–hypopharynx	25 (67.6)
T category	
T1–T2	15 (40.5)
T3–T4	22 (59.5)
N category	
N0	15 (40.5)
N+	22 (59.5)
Stage	
I–II	11 (29.7)
III–IV	26 (70.3)
Tumor differentiation	
Good	7 (18.9)
Moderate	25 (67.6)
Poor	5 (13.5)

Quantification of sTWEAK and sCD163 circulating levels

Pre-treatment blood samples were obtained from 37 patients after an overnight fast. Blood was drawn in a 10 mL vacutainer tube from an antecubital vein. Within 1 h of drawing, the serum was separated by centrifugation at 1,500×*g* for 15 min at 4 °C. Serum concentrations of sTWEAK and sCD163 were determined in duplicate by ELISA using the commercially available human TWEAK/TNFSF12 kit #DY1090, and human CD163 kit #DY1607 (R&D Systems Europe, Abingdon, Oxon, UK), respectively.

Analysis of mRNA expression

We analyzed the gene expression pattern of 111 matched mucosa samples from 37 patients. The biopsy specimens were taken from: (1) the primary site of the tumor, (2) an adjacent location, and (3) distal from the tumor, as control mucosa. A sample aliquot was used for the pathologic diagnosis of the malignancy, and another aliquot was immediately stabilized by inclusion in RNAlater (Qiagen GmbH, Hilden, Germany) and stored at −80 °C until processing. Total RNA was isolated from 30 mg of tissue, according to the manufacturer's protocol RNeasy mini kit (Qiagen). The cDNA was prepared by reverse transcribing 1 µg RNA with the High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Real-time quantitative PCR was carried out in duplicate on a 7900HT Fast Real-Time PCR System using commercial Taqman Assays (Applied Biosystems). Ct values for each sample were normalized with the ratio of two reference genes *GAPDH* (Hs99999905_m1) and *PPIA* (Hs99999904_m1). The pre-designed assay probes (Applied Biosystems) used for the detection of the selected genes were: *TNFα* (Hs99999043_m1), *TWEAK* (Hs00611242_m1), *CD163* (Hs00174705_m1) and *Fn14* (Hs00171993_m1).

Statistical analysis

The bivariate Pearson correlation tests were used in the correlation studies. One-way ANOVA analysis was used to compare the gene expression between the different mucosae. Circulating levels and mRNA expression according to the clinico-pathological variables were compared using Student's t test. As proposed by Chiesa et al. [19] for studies on predictive factors in HNSCC, we evaluated the outcome by the locoregional control with a follow-up of at least 2 years. The locoregional recurrence-free survival counted only deaths from HNSCC as events. Patients who had HNSCC but died of other causes contributed to follow-up until the time of death, as censored cases. Chi-Squared Test was used to analyze the relationship between categorical variables. The continuous value of the circulating levels or mRNA expression of the variables studied was categorized according to the locoregional control of the disease with the classification and regression tree (CRT) method. The locoregional recurrence-free survival according to each categorized variable was calculated by the Kaplan–Meier method. Differences in survival rates were compared using the log-rank test. Multivariate analysis was made with a Cox regression analysis considering the locoregional recurrence-free survival as the dependent variable, and location

of the tumor, local and regional extension, stage, type of treatment, and the categorized variables as the independent variables. All statistical analyses were made using SPSS software v. 20.0 (IBM).

Results

Gene expression analysis of *TWEAK, CD163, Fn14*, and *TNF-α* in HNSCC tissue samples

Figure 1a shows the gene expression of *TWEAK*, the receptors *CD163* and *Fn14*, and *TNF-α* in three mucosae: control, adjacent and tumoral mucosa. We found that these genes were expressed in all mucosae. Interestingly, a gradual significant increase in the expression pattern of these molecules from control to cancerous tissues was observed. The results indicate that in all cases the gene expression in the tumoral mucosa was higher than the expression in normal or adjacent mucosa. In addition, adjacent mucosa was also significantly different from normal mucosa.

Interestingly, circulating levels of sTWEAK correlated with its expression in tumoral mucosa ($r = 0.504$, $p = 0.002$), but not with the expression in adjacent ($r = -0.013$, $p = 0.945$) or normal mucosa ($r = 0.100$, $p = 0.650$).

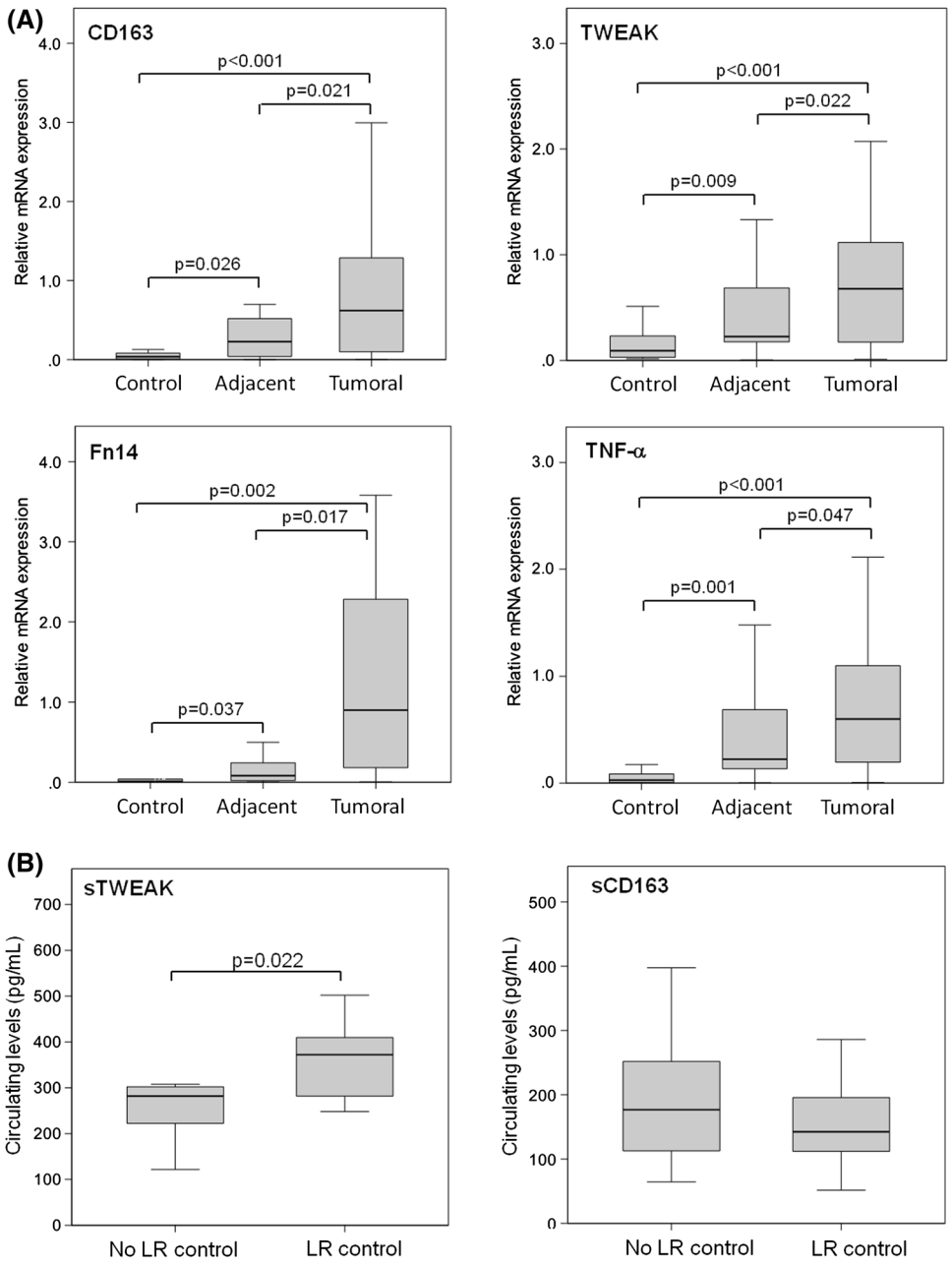

Fig. 1 *CD163, TWEAK, Fn14* and *TNF-α* gene expression in different mucosae and sCD163 and sTWEAK circulating levels. **a** *Box plot* analysis showing *CD163, TWEAK, Fn14* and *TNF-α* gene expression in control, adjacent and tumor tissue samples. Differences between tissue samples were calculated using One-way ANOVA analysis. **b** *Box plot* analysis showing sCD163 and sTWEAK circulating levels according to the locoregional control achieved. *LR* locoregional. Differences between samples were calculated using Student's *t* test for independent samples. Differences were considered statistically significant at $p < 0.05$

Relationship between sTWEAK and sCD163 circulating levels and the clinico-pathological parameters

To investigate these relationships, we studied 37 patients with full records including histological grade, primary site, clinical stage, tumor size, lymph node metastasis, age and outcome. Figure 1b shows the mean and standard deviation of sTWEAK and sCD163 circulating levels according to the locoregional control of the disease. We found that patients with a better locoregional control of the disease presented higher sTWEAK levels than patients with poor prognosis.

Levels of sTWEAK or sCD163 were not significantly associated with the other clinico-pathological parameters tested, such as clinical stage, tumor size or lymph node metastasis, neither were the expression levels of *TWEAK, CD163, Fn14*, and *TNF-α* (data not shown).

Relationship between sTWEAK categories and the locoregional control of the tumor

We categorized sTWEAK levels according to the locoregional control of the disease by use of the cut-off value obtained in the previous classification trees created (sTWEAK = 323.3 pg/mL) (Fig. 2a). Two groups of patients were defined, one with low levels ($n = 21$) and the other with high values of sTWEAK ($n = 16$).

Table 2 shows the locoregional recurrence-free survival values according to the location of the primary tumor, the local extension of the tumor, and the type of treatment according to the categories of sTWEAK levels. The locoregional recurrence-free survival rate was higher in the category of patients with higher levels of sTWEAK, reaching statistical significance for patients with late-stage tumors (T3–4, N+, Stage III–IV) and tumors located at the larynx–hypopharynx.

Table 2 also shows the locoregional recurrence-free survival values according to the categories of CD163/TWEAK mRNA tumoral expression ratio. The cut-off value of 1.03 was obtained in the previous classification trees (Fig. 2b). Two groups of patients were defined, one with low ($n = 23$) and the other with high ratios ($n = 14$). The locoregional recurrence-free survival rate was higher in the category of patients with lower ratios of CD163/TWEAK mRNA tumoral expression for patients with late-stage tumors (T3–4, Stage III–IV), and tumors located at the larynx-hypopharynx.

COX regression analysis

Table 3 shows the results of the multivariate study considering the locoregional control as the dependent variable. Categorical (binary) variables included in the analysis were the stage, T and N categories, primary site and, on one hand, sTWEAK circulating levels, and on the other hand *CD163/TWEAK* expression ratio in tumoral tissues. According to the results, only sTWEAK and the ratio *CD163/TWEAK* categories were significantly related to the locoregional control of the disease. Considering patients with a low level of sTWEAK as the reference category, those with a higher level of sTWEAK had a lower risk of locoregional failure of the tumor after treatment. On the contrary, patients with high mRNA ratios of *CD163/TWEAK* had a higher risk of locoregional failure. Neither sCD163 levels nor the ratio *Fn14/TWEAK* mRNA expression in tumoral tissues significantly predicted the locoregional failure risk.

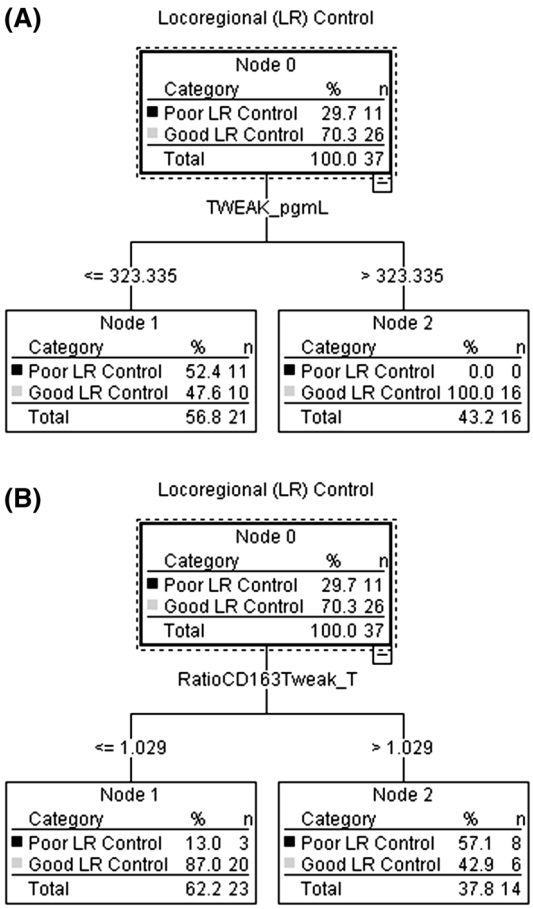

Fig. 2 Classification and regression tree for locoregional control. Variables entered were: the primary location, the stage, T and N categories, the ECOG index, and sTWEAK circulating levels (**a**) or the ratio *CD163/TWEAK* gene expression in tumors (**b**)

Kaplan–Meier curves

Figure 3 shows the locoregional recurrence-free survival curves according to the category of sTWEAK, the *CD163/TWEAK* expression ratio or the *Fn14/TWEAK* expression ratio in tumoral tissue. The locoregional recurrence-free survival rate for patients with low sTWEAK levels was

Table 2 Locoregional recurrence-free survival in function of clinical variables according to the category of sTWEAK circulating levels or tumoral mRNA expression ratio of *CD163/TWEAK*

Clinico-pathological variables	sTWEAK levels			*CD163/TWEAK* expression ratio		
	Low (%)	High (%)	*p* Value	Low (%)	High (%)	*p* Value
Stage						
I–II	83.3	100.0	0.338	100.0	50.0	0.200
III–IV	35.7	100.0	0.001*	84.6	41.7	0.041*
T category						
T1–T2	75.0	100.0	0.155	100.0	75.0	0.308
T3–T4	33.3	100.0	0.002*	83.3	30.0	0.027*
N category						
N0	85.7	100.0	0.268	100.0	66.7	0.214
N+	30.8	100.0	0.002*	80.0	36.4	0.080
Tumor location						
Oral cavity–oropharynx	42.9	100.0	0.091	80.0	40.0	0.524
Larynx–hypopharynx	53.8	100.0	0.007*	93.8	37.5	0.007*

Differences between groups were calculated using the Chi-Squared test

* $p < 0.05$, statistically significant

Table 3 Results of the multivariate Cox regression analysis

Variables	Categories	HR	95 % CI	*p* Value
Model 1				
Ratio *CD163/TWEAK*	High versus Low	16.90	1.14–249.40	**0.040***
Stage	III–IV versus I–II	0.00	0.00–840.00	0.938
T category	T3–4 versus T1-2	30.49	0.00–517.18	0.962
N category	N+ versus N0	13.49	0.00–645.96	0.921
Tumor location	LH versus OCO	1.59	0.15–17.29	0.702
Model 2				
sTWEAK levels	High versus Low	0.07	0.01–0.90	**0.041***
Stage	III–IV versus I–II	0.00	0.00–217.52	0.968
T category	T3–4 versus T1–2	0.79	0.07–935.23	0.850
N category	N+ versus N0	40.04	0.00–363.48	0.959
Tumor location	LH versus OCO	1.38	0.17–11.24	0.763

Dependent variable: Locoregional control. Cox regression with backward exclusion method was applied. Binary variables included in each model were: Model 1. Stage, T and N categories, primary site and the ratio *CD163/TWEAK* mRNA expression in tumors; Model 2. Stage, T and N categories, primary site and sTWEAK levels

HR hazard ratio, *CI* confidence interval, *LH* larynx–hypopharynx, *OCO* oral cavity–oropharynx

* $p < 0.05$ was considered to be statistically significant

42.5 % (95 % CI 20.0–76.0), and it was 100.0 % (95 % CI 73.0–100.0) for patients with high sTWEAK circulating levels. The survival rate for patients with high *CD163/TWEAK* ratio was 41.3 % (95 % CI 20.0–78.0), and it was 90.5 % (95 % CI 76.0–100.0) for patients with a low *CD163/TWEAK* ratio. Finally, the expression ratio *Fn14/TWEAK* in tumors did not discriminate between patients according to the locoregional control ($p = 0.738$).

Discussion

In our study, we analyzed the gene expression pattern of *TWEAK, Fn14, CD163* and *TNF-α* in 111 matched samples of normal, adjacent and tumoral mucosa obtained from 37 patients with HNSCC. As far as we know, it is the first time that the TWEAK pathway has been studied in this type of cancer. The results show a gradual and significant increase in the expression pattern of these genes from control to cancerous tissues. The differential expression found between adjacent and control mucosa in all the genes analyzed highlights the fact that, although there are no neoplasic cells in adjacent tissue, this tissue is contributing to the development of a favorable microenvironment for tumor progression [4, 20, 21].

Fn14, the well-known TWEAK receptor, was reported to be over-expressed in most solid tumors relative to control non-tumor tissues, such as in oesophageal [22], liver [23], breast [12], gynecologic [24] and brain cancer [25],

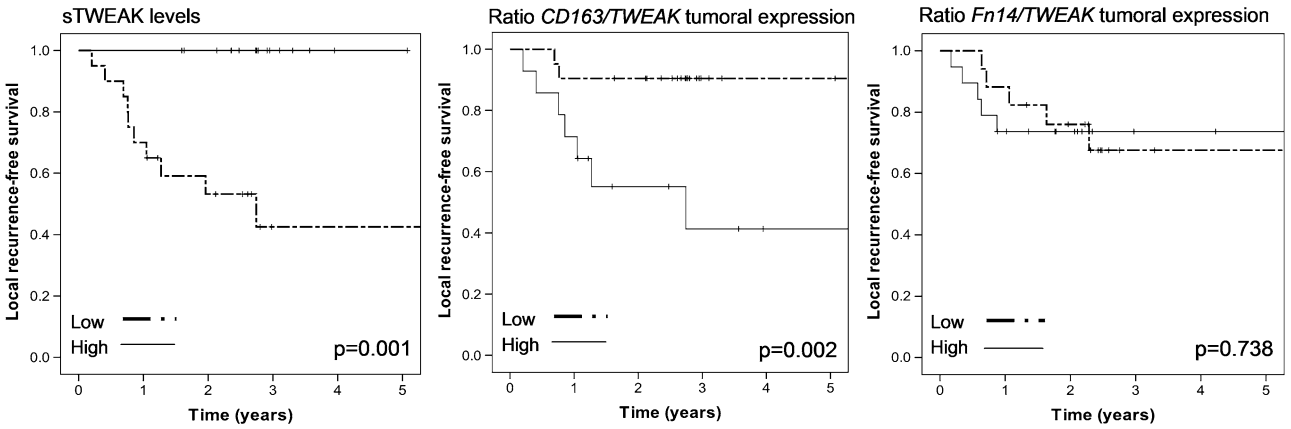

Fig. 3 Locoregional recurrence-free survival according to the categorized levels of sTWEAK circulating levels or the ratio of *CD163/TWEAK* or *Fn14/TWEAK* gene expression in tumors

although it has never been addressed in HNSCC. In line with these findings, we have found that the expression of *Fn14* is significantly up-regulated in tumors compared with that in adjacent or normal mucosa. In adjacent tissue, its expression is more than six times lower than in tumors and in the control mucosa it is almost undetectable. The specific and homogeneous expression of *Fn14* in tumoral mucosa might indicate that it also has potential as a malignant tumor marker in HNSCC [23]. Our results indicate that *Fn14* expression, although being higher in tumoral tissue, is not related to patients' clinical subtype/stages, pathological features or the outcome, in contrast to what would be expected.

The role of TWEAK on tumor cells is inconclusive, as some studies show that TWEAK alone is able to promote cancer cell proliferation [26, 27] while others show that TWEAK promotes cell death (cell apoptosis and/or necrosis) [8, 15, 24, 28, 29]. In concrete, Alaoui et al. [29] demonstrated the direct pro-apoptotic effect of TWEAK in human keratinocytes. They hypothesized that TWEAK plays a role in immune and/or remodeling reactions in healthy and in pathological skin, such as psoriasis and squamous cell carcinoma. There is evidence that the different, even opposed, actions of TWEAK could be determined by the cells present in the tumor microenvironment and the TWEAK receptor they have [24].

Fn14 is mainly expressed by cancer cells [30] whereas CD163 is a highly specific macrophage marker. This marker is expressed primarily by M2 macrophages, which, when infiltrated in tumors, express elevated levels of tumor-promoting cytokines and growth factors. In our study, the scavenger receptor *CD163* has been found to be over-expressed in HNSCC tumors. It has been proposed that CD163 macrophages may scavenge sTWEAK within human tissues [7]. Therefore, the ratio *CD163/TWEAK* may reflect the relative amount of each molecule in the tumor microenvironment. Interestingly, the analysis performed reveals that *CD136/TWEAK* expression ratio in tumoral tissue is inversely related to the survival rate. The results indicate that patients with a high *CD163/TWEAK* ratio may have a poorer prognosis than those in the low ratio. Most importantly, the Kaplan–Meier curves indicate that high *CD136/TWEAK* expression ratio is related to poor prognosis.

Because most cancer research done so far on TWEAK pathway has been related to the *Fn14* receptor, there is no data on the effects of TWEAK/CD163 activation on cancer development. Bover et al. [9] demonstrated that monocytes can sequester TWEAK from supernatants thus preventing tumor cell apoptosis. Also, the same authors obtained data consistent with the hypothesis that CD163 either acts as a sTWEAK scavenger in pathological conditions or serves as an alternate receptor for sTWEAK in cells lacking the *Fn14* receptor [9, 31].

On the basis of this preceding literature, it could be that CD163 prevents sTWEAK from exerting its biological functions by sequestering it from the tumor microenvironment. Then the possible CD163-mediated sTWEAK clearance might be deleterious in this type of cancer.

Finally, our results indicate that blood sTWEAK levels are linked to *TWEAK* gene expression in tumors, but not with adjacent or control mucosa expression. Although *TWEAK* is expressed in many tissues, these results suggest that the tumoral tissue in these patients might drive the differences found in the serum levels. This finding, together with the possible predictive usefulness of sTWEAK, suggests that pre-treatment blood sTWEAK levels might become a personalized non-invasive biomarker of outcome for patients with HNSCC. This issue has never been suggested before in head and neck cancer; however, other authors obtained similar survival data in metabolic diseases as atherosclerosis [31], coronary and peripheral artery disease [32, 33] type 2 diabetes or end-stage renal disease [34].

The present study is the first to explore the correlation between the TWEAK/Fn14/CD163 axis and the locoregional recurrence-free survival in patients with HNSCC. Notwithstanding this fact, there are some limitations to be considered. Our preliminary results together with other previously reported findings support the hypothesis that TWEAK has a protective role, maybe producing cell apoptosis alone or via the *Fn14* receptor, and that CD163 is opposing these effects by sequestering sTWEAK. However, we have not carried out any mechanistic studies that might confirm this hypothesis. Another limitation is the relatively small number of patients included in the study, which might limit the statistical power of our analysis. The usefulness of sTWEAK needs to be confirmed in a larger cohort in order to sub-classify patients according to the clinico-pathological parameters. However, all the laboratory analyses performed suggest that sTWEAK might be a promising non-invasive tumor-progression biomarker. Finally, it is worthy to mention again that, apart from the blood sample, we obtained matched tissue samples from three different mucosae.

In conclusion, this study suggests that the TWEAK pathway may play an important role in the regulation of the locoregional control in HNSCC. We found that the gene expression of *TWEAK* increased in HNSCC. Moreover, there is a close correlation between low blood levels of sTWEAK and low locoregional control rates, which highlights the potential prognostic significance of this non-invasive biomarker in the rapid discrimination according to the locoregional control achieved in patients who received a non-surgical organ preservation treatment. Future investigations are warranted to determine the role and mechanism of action of TWEAK/Fn14/CD163 in the development of HNSCC, and to confirm in a larger and independent cohort of patients the potential role of sTWEAK as a prognostic circulating marker. Further research in this area would drive oncologists towards more personalized medicine.

Acknowledgments This study was supported by the Institut d'Investigació Sanitària Pere Virgili, Tarragona, Catalonia (IISPV) and by the Instituto de Salud Carlos III, Ministerio de Economia y Competitividad, Spain (Grant Numbers PI11/00049 to MRCH, PI11/00085 to JV and PI11/02380 to XL). MRCH is supported by the Research Stabilization Programme of the Instituto de Salud Carlos III (ISCIII) co-financed by Institut Català de Salut (ICS) in Catalonia, Spain. We express our gratitude to Meritxell Cortés and Jessica Capera for their assistance in sample collection.

Conflict of interest None.

References

1. Quintana A, Avilés FX, Terra X et al (2013) Overexpression of the nuclear factor-kappa B (p65) in association with local failure in patients with head and neck carcinoma undergoing radiotherapy or chemoradiotherapy. Head Neck 35:370–375
2. Jackson-Bernitsas D, Ichikawa H, Takada Y et al (2006) Evidence that TNF-TNFR1-TRADD-TRAF2-RIP-TAK1-IKK pathway mediates constitutive NF-kB activation and proliferation in human head and neck squamous cell carcinoma. Oncogene 26:1385–1397
3. Duffey DC, Crowl-Bancroft C, Chen Z et al (2000) Inhibition of transcription factor nuclear factor-kB by a mutant inhibitor-kB attenuates resistance of human head and neck squamous cell carcinoma to TNF-a caspase-mediated cell death. Br J Cancer 83:1367–1374
4. Winkles JA (2008) The TWEAK-Fn14 cytokine-receptor axis: discovery, biology and therapeutic targeting. Nat Rev Drug Discov 7:411–425
5. Llauradó G, González-Clemente JM, Maymó-Masip E, Subías D, Vendrell J, Chacón MR (2012) Serum levels of TWEAK and scavenger receptor CD163 in type 1 diabetes mellitus: relationship with cardiovascular risk factors. A Case-Control Study. PLoS One 7:e43919
6. Burkly LC, Michaelson JS, Zheng TS (2011) TWEAK/Fn14 pathway: an immunological switch for shaping tissue responses. Immunol Rev 244:99–114
7. Tang X (2013) Tumor-associated macrophages as potential diagnostic and prognostic biomarkers in breast cancer. Cancer Lett 332:3–10
8. Polek TC, Talpaz M, Darnay BG, Spivak-Kroizman T (2003) TWEAK mediates signal transduction and differentiation of RAW264.7 cells in the absence of Fn14/TweakR: evidence for a second TWEAK receptor. J Biol Chem 278:32317–32323
9. Bover LC, Cardó-Vila M, Kuniyasu A et al (2007) A previously unrecognized protein–protein interaction between TWEAK and CD163: potential biological implications. J Immunol 178:8183–8194
10. Dai L, Gu L, Ding C, Qiu L, Di W (2009) TWEAK promotes ovarian cancer cell metastasis via NF-kB pathway activation and VEGF expression. Cancer Lett 283:159–167
11. Gaudineau B, Fougère M, Guaddachi F, Lemoine F, de la Grange P, Jauliac S (2012) Lipocalin 2, the TNF-like receptor TWEAKR and its ligand TWEAK act downstream of NFAT1 to regulate breast cancer cell invasion. J Cell Sci 125:4475–4486
12. Willis AL, Tran NL, Chatigny JM et al (2008) The fibroblast growth factor-inducible 14 receptor is highly expressed in HER2-positive breast tumors and regulates breast cancer cell invasive capacity. Mol Cancer Res 6:725–734
13. Kwon O, Park S, Kang T et al (2012) Elevated fibroblast growth factor-inducible 14 expression promotes gastric cancer growth via nuclear factor-κB and is associated with poor patient outcome. Cancer Lett 314:73–81
14. Michaelson JS, Kelly R, Yang L, Zhang X, Wortham K, Joseph IBJK (2012) The anti-Fn14 antibody BIIB036 inhibits tumor growth in xenografts and patient derived primary tumor models and enhances efficacy of chemotherapeutic agents in multiple xenograft models. Cancer Biol Ther 13:812–821
15. Nakayama M, Ishidoh K, Kayagaki N et al (2002) Multiple pathways of TWEAK-induced cell death. J Immunol 168:734–743
16. Zou H, Wang D, Gan X et al (2011) Low TWEAK expression is correlated to the progression of squamous cervical carcinoma. Gynecol Oncol 123:123–128
17. Wang D, Fung JNT, Tuo Y, Hu L, Chen C (2010) TWEAK/Fn14 promotes apoptosis of human endometrial cancer cells via caspase pathway. Cancer Lett 294:91–100
18. Kaduka Y, Takeda K, Nakayama M, Kinoshita K, Yagita H, Okumura K (2005) TWEAK mediates anti-tumor effect of tumor-infiltrating macrophage. Biochem Biophys Res Commun 331:384–390
19. Chiesa F, Mauri S, Tradati N et al (1999) Surfing prognostic factors in head and neck cancer at the millennium. Oral Oncol 35:590–596

20. Varilla V, Atienza J, Dasanu CA (2013) Immune alterations and immunotherapy prospects in head and neck cancer. Expert Opin Biol Ther 13:1241–1256
21. Douglas WG, Tracy E, Tan D et al (2004) Development of head and neck squamous cell carcinoma is associated with altered cytokine responsiveness. Mol Cancer Res 2:585–593
22. Wang S, Zhan M, Yin J et al (2006) Transcriptional profiling suggests that Barrett's metaplasia is an early intermediate stage in esophageal adenocarcinogenesis. Oncogene 25:3346–3356
23. Jakubowski A, Ambrose C, Parr M et al (2005) TWEAK induces liver progenitor cell proliferation. J Clin Invest 115:2330–2340
24. Gu L, Dai L, Cao C et al (2013) Functional expression of TWEAK and the receptor Fn14 in human malignant ovarian tumors: possible implication for ovarian tumor intervention. PLoS ONE 8:e57436
25. Tran NL, McDonough WS, Donohue PJ et al (2003) The human Fn14 receptor gene is up-regulated in migrating glioma cells in vitro and overexpressed in advanced glial tumors. Am J Pathol 162:1313–1321
26. Kawakita T, Shiraki K, Yamanaka Y et al (2004) Functional expression of TWEAK in human hepatocellular carcinoma: possible implication in cell proliferation and tumor angiogenesis. Biochem Biophys Res Commun 318:726–733
27. Ho DH, Vu H, Brown SAN, Donohue PJ, Hanscom HN, Winkles JA (2004) Soluble tumor necrosis factor-like weak inducer of apoptosis overexpression in HEK293 cells promotes tumor growth and angiogenesis in athymic nude mice. Cancer Res 64:8968–8972
28. Schneider P, Schwenzer R, Haas E et al (1999) TWEAK can induce cell death via endogenous TNF and TNF receptor 1. Eur J Immunol 29:1785–1792
29. Sabour Alaoui S, Dessirier V, de Araujo E et al (2012) TWEAK affects keratinocyte G2/M Growth arrest and induces apoptosis through the translocation of the AIF protein to the nucleus. PLoS ONE 7:e33609
30. Zhou H, Hittelman WN, Yagita H et al (2013) Antitumor activity of a humanized, bivalent immunotoxin targeting Fn14-positive solid tumors. Cancer Res 73:4439–4450
31. Urbonaviciene G, Martin-Ventura JL, Lindholt JS, Urbonavicius S, Moreno JA, Egido J, Blanco-Colio LM (2011) Impact of soluble TWEAK and CD163/TWEAK ratio on long-term cardiovascular mortality in patients with peripheral arterial disease. Atherosclerosis 219:892–899
32. Jelic-Ivanovic Z, Bujisic N, Spasic S et al (2009) Circulating sTWEAK improves the prediction of coronary artery disease. Clin Biochem 42:1381–1386
33. Moreno JA, Dejouvencel T, Labreuche J et al (2010) Peripheral artery disease is associated with a high CD163/TWEAK plasma ratio. Arterioscler Thromb Vasc Biol 30:1253–1262
34. Kralisch S, Ziegelmeier M, Bachmann A et al (2008) Serum levels of the atherosclerosis biomarker sTWEAK are decreased in type 2 diabetes and end-stage renal disease. Atherosclerosis 199:440–444