Pathology – Research and Practice 207 (2011) 448–451

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.de/prp

Original article

Correlation between E-cadherin and CD44 adhesion molecules expression and cervical lymph node metastasis in oral tongue SCC: Predictive significance or not

Leila Vazifeh Mostaan[a], Mohammad Taghi Khorsandi[b], Shahriar-Mohammad Reza Sharifian[a], Fatemeh Homee Shandiz[a], Fatemeh Mirashrafi[b], Homa Sabzari[a], Reza Badiee[a], Hasti Borghei[b], Nasrin Yazdani[b,*]

[a] Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Otorhinolaryngology Research Center, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 9 November 2010
Received in revised form 27 March 2011
Accepted 11 April 2011

Keywords:
Tongue
Squamous cell carcinoma
Lymph node metastasis
E-cadherin
CD44

ABSTRACT

The aim of this article was to evaluate the expression of E-cadherin and CD44 adhesion molecule in oral tongue squamous cell carcinoma (SCC) since inappropriate expression of adhesion molecules raises the metastatic ability of the tumor cells.

Biopsy specimens from 92 patients with tongue SCC were examined for the expression of E-cadherin and CD44 by immunohistochemistry. The relationship of immunoreactivity with tumor stage and cervical lymph node metastasis was then analyzed.

Sixty-one patients (66.3%) had reduced or negative staining for CD44. Weak or absent staining for E-cadherin was seen in 14 patients (15.21%). Cervical lymph node metastasis is associated with decreased or negative staining for CD44, but no association was found between E-cadherin immunoreactivity and nodal metastasis.

Our study reveals that reduced expression of CD44 could be an indicator of high invasiveness of tumor by increasing cervical lymph node metastasis.

© 2011 Elsevier GmbH. All rights reserved.

1. Introduction

Oral cancer is the eighth most common cancer in the world and the third in developing countries. Among oral cancers, tongue is the most rampant subsite involved. It has a geographic predilection, with a higher incidence reported in Southeast Asia and Brazil [1–4].

It is not uncommon to see loco-regional metastasis in patients with early stages of the disease. The most important prognostic factor is the presence of cervical lymph node metastasis due to rich lymphatic vessels, which is the main culprit for tumor recurrence [4–7]. The ability to predict tumor invasiveness and lymph node metastasis with clinicopathological or immunohistochemical parameters can improve the decision-making and the patient outcome [6,8–10]. Evaluation for cervical lymph node metastasis is the key point to determine the surgical approach and possible use of adjuvant chemotherapy in patients with oral or oropharyngeal cancer [11].

Although TNM classification can accurately predict prognosis in some patients, loco-regional relapse after standard treatment in early stage diseases does produce conflicts. This calls to investigate for new biomarkers which may be helpful to determine oral cavity cancer behavior and to predict the prognosis [4,12–15].

CD44 was first described by Dalchau et al. as a molecule present on the surface of T-lymphocytes, granulocytes, and cortical thymocytes [16]. Human CD44 is a transmembrane hyaluronan-binding glycoprotein [17] that can bind to hyaluronic acid, an extracellular matrix, and regulate a variety of cellular functions, such as cell migration, proliferation, cell–cell interaction, and apoptosis. These cellular functions of CD44 imply that a disorder of CD44 expression plays a crucial role in the behavior of a malignant tumor [18]. Some published studies have demonstrated that loss of CD44 expression was significantly associated with greater loss of expression in the tumor tissue [2]. Moreover, irregular staining of CD44 predicted more advanced disease and shortened survival of the patients [7].

On the other hand, E-cadherin is a cell membrane-associated protein involved in cell–cell adhesion, and loss of expression of the cadherin/catenin complex has been described in various human malignancies [13]. The cell–cell adhesion molecule E-cadherin has been shown to suppress invasive growth of epithelial cells *in vitro*, and loss of its expression is thought to be related to metastatic

* Corresponding author at: Otorhinolaryngology Research Center, AmirAlam Hospital, North Saadi Ave., P.O. Box 11457-65111, Tehran, Iran. Tel.: +98 21 66760269; fax: +98 21 66760269.
E-mail address: n_yazdani@sina.tums.ac.ir (N. Yazdani).

0344-0338/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.prp.2011.04.001

Table 1
Patients' characteristics and tumor features of the tongue squamous cell carcinoma study population.

Characteristics	*N* (%)
Age (years)	
Mean	57.8
Range	23–84
Sex	
Male	49 (53.26%)
Female	43 (46.73%)
Risk factor (alcohol use or cigarette smoking)	
Positive	24 (26.08%)
Negative	68 (73.91%)
Stage	
I	16 (17.39%)
II	20 (21.73%)
III	26 (28.26%)
IVa	30 (32.6%)
Tumor differentiation	
WD	22 (23.91%)
MD	56 (60.86%)
PD	14 (15.21%)
E-cadherin	
0	3 (3.26%)
1	11 (11.95%)
2	47 (51.08%)
3	31 (33.69%)
CD44	
Group 1	31 (33.69)
Group 2	39 (42.39%)
Group 3	22 (23.91%)

potential of epithelial tumors *in vivo*. It has been described that membranous E-cadherin expression has prognostic importance in patients with head and neck SCC [8].

It seems that other factors, such as biological behavior of the tumor cells, may affect the course of disease. Thereby, recognition of such behavior and other prognostic factors could help to plan a more effective treatment protocol [8,10,14].

This multi-central study evaluated the expression of the two tumor markers "CD44" and "E-cadherin" in oral tongue SCC and their correlation with cervical lymph node metastasis in two of our major referral hospitals.

2. Materials and methods

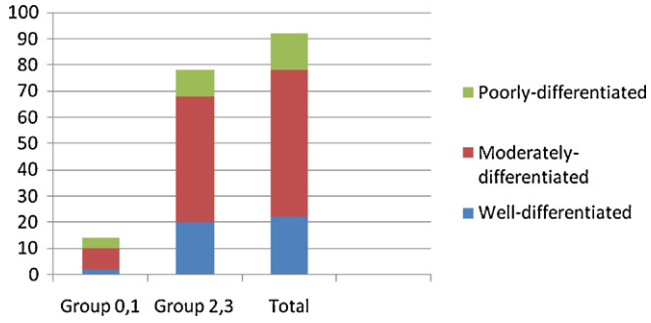
2.1. Tissue samples and clinical data

Biopsy specimens of 92 patients with oral tongue SCC were evaluated. The patients were 49 males and 43 females. The mean age was 57.8 (ranging from 23 to 84). Thirty-seven (40.2%) of the patients had cervical lymph node metastasis at the time of the initial diagnosis. None of them had received previous treatment. All of these patients underwent surgical resection of the primary tumor followed by cervical lymph node dissection. Positive risk factors of cigarette smoking, opium usage, and alcohol consumption were found among 26% of the patients. The clinicopathological characteristics of the patients are summarized in Table 1.

2.2. Immunohistochemistry

Paraffin-embedded tissue sections were cut and mounted on polylysine-coated slides and then dried at 56 °C for 30 min. Specimens were de-paraffinized in xylene and rehydrated through graded ethanol solutions. Target antigen retrieval was performed with a 95 °C solution of 0.01 M sodium citrate buffer (pH 6). After neutralization of the endogenous peroxidase in the tissue sections, incubation with a protein block, and a washing in TBS (Tris Buffer Saline), sections were incubated with the primary antibody against CD44 (Lyophilized Novacastra Variant 3 Company) and E-cadherin

Table 2
Correlation between tumor differentiation and E-cadherin expression.



(Concentrrated Novacastra Company with 1/50 dilution). The final steps of staining were then performed as recommended by the manufacturer. Positive tissue control and negative tissue control were observed for each staining process.

The staining results were evaluated by a single qualified pathologist who was blinded to sample characteristic.

The semi-quantitative immunoreactivity of E-cadherin was classified into four group based on the work of Feritas [19]. According to this classification, group 0 indicates no staining; group +1 (weak), staining in less than 10% of cells; group +2 (moderate), moderate homogenous or intense localized staining in 10–75% of cells; and group +3 (intense), intense homogenous staining in >75% of cells. The immunostaining pattern of CD44 was classified as follows: group1 for distinct positivity except for those cells in the central part of cancer cell nests; group 2, reduced staining in the peripheral cells of one or more cancer nests; and group 3, completely negative staining in one or more nests according to Sato's study [9].

The correlation between E-cadherin immunoreactivity and CD44 immunoexpression with clinicopathological findings was analyzed by Pearson's Chi-square test and Fisher's exact test for qualitative data, and independent *t*-test for quantitative data.

3. Results

T stage was T1 in 17 patients (18.47%), T2 in 29 patients (31.52%), T3 in 30 patients (32.6%), and T4 in 16 patients (17.39%). N stage was N0 in 45 patients (48.91%), N1 in 18 patients (19.56%), and N2b in 29 patients (31.52%).

Based on TNM staging, 16 patients (17.39%) were in stage I, 20 patients (21.73%) in stage II, 26 patients (28.26%) in stage III, and 30 patients (32.6%) were in stage IV at the time of diagnosis.

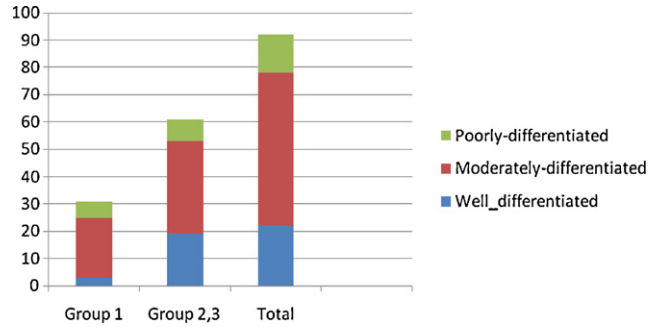
Degree of tumor differentiation was well differentiated (WD) in 22 (23.91%), moderately differentiated (MD) in 56 (60.86%), and poorly differentiated (PD) in 14 patients (15.21%).

The immunoreactivity of E-cadherin within tumors was negative in 3 patients (3.26%), 1^+ in 11 patients (11.95%), 2^+ in 47 patients (51.08%), and 3^+ in 31 patients (33.69%). Weak or absent staining was seen in 14 patients (15.21%) and moderate to intense staining in 78 patients (84.78%).

According to above mentioned criteria for CD44 immunoreactivity, 31 patients (33.69%) were in group 1, 39 patients (42.39%) in group 2, and 22 patients (23.91%) in group 3. In other words, 61 patients (66.3%) had reduced or negative staining for CD44.

Data analysis revealed that the age of the patients ($p = 0.01$), positive history of risk factors ($p = 0.002$), and T stage ($p < 0.0005$) correlated significantly with cervical lymph node metastasis. However, tumor differentiation did not show any significant correlation with nodal metastasis ($\chi^2 = 4.654$, $p = 0.098$), E-cadherin expression (Table 2; $\chi^2 = 2.613$, $p = 0.271$), and CD44 (Table 3; $\chi^2 = 5.271$, $p = 0.072$) immunoreactivity.

Table 3
Correlation between tumor differentiation and CD44 expression.



Negative or reduced staining for CD44 had a significant correlation with cervical lymph node metastasis (Table 4; $\chi^2 = 15.204$, $p = 0.000$). Reduced E-cadherin expression has not shown any significant correlation with cervical lymph node metastasis (Table 5; $\chi^2 = 1.562$, $p = 0.211$). Likewise, no correlation was found between E-cadherin (Table 2) and CD44 (Table 3) immunoreactivity and tumor differentiation.

4. Discussion

In the recent years, there has been an impressive progression in the treatment of cancers. However, even in early stages of head and neck cancers, a poor outcome is not unexpected due to locoregional involvement. The 5-year survival of these patients with nodal metastasis still remains at 53%. Cervical lymph node and distant metastasis are the major causes of mortality in these patients [4,8,9,15,20].

Tongue is the most common subsite of involvement in head and neck cancers. Cervical lymph node metastasis (even delayed metastasis in initial N0) is not unusual and is often accompanied by a poor prognosis [9,12].

Metastasis is a multistep process consisting of cell detachment from the primary tumor, invasion to the extracellular matrix, and extravasation into the blood [5–7,13,21]. Recently, biological markers have been an area of interest to predict tumor invasiveness and metastatic ability [12]. Change in the expression of the different molecules involved in each step of metastasis is a point of interest for investigators. There has been extensive research conducted to explain the expression of these molecules in head and neck cancer and their relationship to tumor progression and patient outcome. However, our research has produced contradictory results.

As mentioned, the first step in metastasis is cell detachment for which changes in the expression of different adhesion molecules might be responsible and can theoretically be used as prognostic markers [8,12].

Table 4
Correlation between CD44 expression and cervical lymph node metastasis.

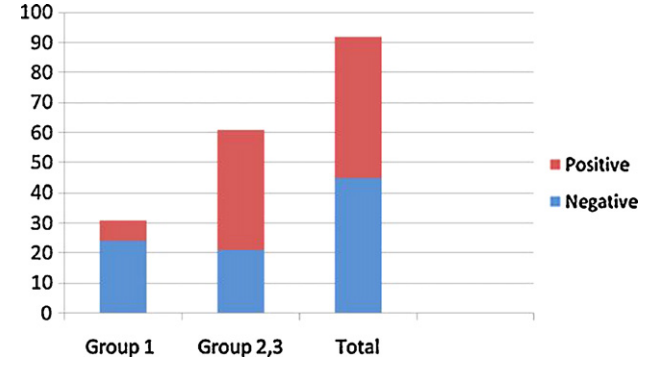
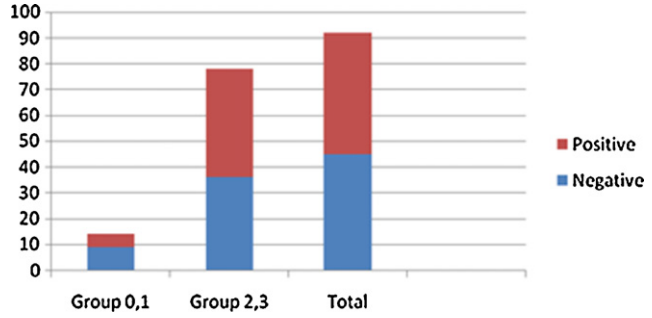


Table 5
Correlation between E-cadherin expression and cervical lymph node metastasis.



E-cadherin, a part of the adherens junction, is a transmembrane calcium-dependent epithelial cell-specific cell adhesion molecule. It may play a role in epithelial differentiation and tissue architecture. Decreased expression of this cell surface molecule can promote tumor invasiveness and metastasis [4,5,8,12,21–24].

CD44 is another transmembrane hyaluronan-binding glycoprotein. Epithelial cells express isoforms that also act as cell–cell adhesion molecules [4,9,17,20,23,25].

Some clinical studies show that reduced expression of E-cadherin in oral SCC is associated with an increased incidence of poor differentiation and cervical lymph node metastasis [7,8,22,26]. Nevertheless, studies indicating no significant association between E-cadherin and poor prognosis in oral SCC are also available [12].

Mattijessen et al. found no correlation between E-cadherin expression and T or N stages in their case series of patients with oral cavity and laryngeal carcinoma. However, they found a significant relation between E-cadherin expression and tumor differentiation in these patients [24]. In this study, we could not find any correlation between E-cadherin expression and nodal metastasis or tumor differentiation.

Although our final results were based on staging and grading, some studies published their result upon survival rate. For instance, Kurtz found a significant decrease in disease-free and overall survival among head and neck tumor patients with a low expression of E-cadherin which was parallel to the Freitas study on patients with tongue SCC [19,27]. Furthermore, Stoll et al., in their case series of oral and oropharyngeal SCC, reported a 39.4% decreased expression of CD44 isoforms. Even though they could not find any direct correlation between cervical lymph node metastasis and CD44 expression, the survival and recurrence-free survival of these patients were significantly poorer than of those patients with positive results of CD44 expression [20].

These controversial results may be contributed to the heterogeneous immunoreactivity of tumor cells, which may be due to the effect of tumor microenvironment [7,22]. However, when immunoreactivity is specifically evaluated in invasive tumor front (ITF), it may statistically show a significant relationship with the invasive front grading score, primary tumor pathology, tumor thickness, and poor survival [22].

Kusunen et al. showed that decreased expression of CD44 correlated with poor tumor differentiation, advanced clinicopathological stage, and an unfavorable outcome in patients with oral SCC [10]. As Okamoto mentioned, CD44 can probably stabilize its connection with extracellular matrix by binding with hyaluronan, so he concluded that a decreased level of CD44 in tumor cells may cause high metastatic potential and shortened survival [10]. These results are parallel to the studies of Tracey, Massano, and Lyons [4,17,23].

In the present study, reduced expression of CD44 has a significant correlation with cervical lymph node metastasis though it has no correlation with tumor differentiation.

Patients with head and neck squamous cell carcinoma who demonstrate loss of E-cadherin expression will experience a worse outcome compared to patients whose tumors have not lost this tumor suppressor.

5. Conclusion

Our study revealed that reduced expression of CD44 is an indicator of high invasiveness of tumor by increasing the rate of cervical lymph node metastasis. Consequently, CD44 could be assumed as a useful tumor marker to predict the invasive behavior of tongue SCC and patient's outcome.

References

[1] M.D. Freitas, T.G. Caballero, J.A. Lopez, et al., Reduced E-cadherin expression is an indicator of unfavourable prognosis in oral squamous cell carcinoma, Oral Oncol. 42 (2006) 190–200.
[2] M.A. González-Moles, M. Bravo, I. Ruiz-Ávila, F. Esteban, A. Bascones-Martínez, S. González-Moles, Adhesion molecule CD44 expression in non-tumour epithelium adjacent to tongue cancer, Oral Oncol. 40 (2004) 281–286.
[3] D. Guttman, Y. Stern, T. Shpitzer, et al., Expression of MMP-1, TIMP-1, CD34 and factor-8 as prognostic markers for squamous cell carcinoma of the tongue, Oral Oncol. 40 (2004) 798–803.
[4] B.E.K. Hagen, J.R. Simon, Comparative study of the expression of p53, Ki67, E-cadherin and MMP-1 in verrucous hyperplasia and verrucous carcinoma of the oral cavity, Head Neck Pathol. 1 (2007) 118–122.
[5] E.J.M. Hannen, J. Van der Laak, J. Manni, et al., Improved prediction of metastasis in tongue carcinoma, combining vascular and nuclear tumor parameters, Cancer 92 (2001) 1881–1887.
[6] A. Jarvinen, R. Autio, S. Kilpinen, High-resolution copy number and gene expression microarray analyses of head and neck squamous cell carcinoma cell lines of tongue and larynx, Genes. Chromosomes Cancer (2008) 500–509.
[7] A. Kosunen, R. Pirinen, K. Ropponen, M. Pukkila, J. Kellokoski, J. Virtaniemi, R. Sironen, M. Juhola, E. Kumpulainen, R. Johansson, J. Nuutinen, V.-M. Kosma, CD44 expression and its relationship with MMP-9, clinicopathological factors and survival in oral squamous cell carcinoma, Oral Oncol. 43 (2007) 51–59.
[8] V. Mattijssen, H.M. Peters, Schalkwijk l, et al., E-cadherin expression in head and neck squamous-cell carcinoma is associated with clinical outcome, Int. J. Cancer 4 (1993) 580–585.
[9] Sh. Nakayama, A. Sasaki, H. Mese, et al., Establishment of high and low metastasis cell lines derived from a human tongue squamous cell carcinoma, Invasion Metast. 18 (1998/1999) 219–228.
[10] M. Okamoto, M. Nishimine, M. Kishi, et al., prediction of delayed neck metastasis in patients with stage I/II squamous cell carcinoma of the tongue, J. Oral Pathol. Med. 31 (2002) 227–233.
[11] S.M. Olsen, E.J. Moore, C.A. Koch, J.L. Kasperbauer, K.D. Olsen, Oral cavity and oropharynx squamous cell carcinoma with metastasis to the parotid lymph nodes, Oral Oncol. 47 (2011) 142–144.
[12] Y. Kudo, Sh. Kitajiama, I. Ogawa, et al., Invasion and metastasis of oral cancer cells require methylation of E-cadherin and/or degradation of membranous β-catenin, Clin. Cancer Res. 10 (2004) 54–55.
[13] K.A. Kurtz, H.T. Hoffman, M.B. Zimmerman, et al., Decreased E-cadherin but not beta-catenin expression is associated with vascular invasion and decreased survival in head and neck squamous carcinomas, Otolaryngol. Head Neck Surg. 1 (2006) 142–146.
[14] A.J. Lyons, J. Jones, Cell adhesion molecules, the extracellular matrix and oral squamous carcinoma, Int. J. Oral Maxillofac. Surg. 36 (2007) 671–679.
[15] J. Massano, F. Regateiro, G. Januano, Oral squamous cell carcinoma: review of prognostic and predictive factors, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 102 (2006) 67–76.
[16] R. Dalchau, J. Kirkley, J.W. Fabre, Monoclonal antibody to a human leukocyte-specific membrane glycoprotein probably homologous to the leukocyte-common (L-C) antigen of the rat, Eur. J. Immunol. 10 (1980) 737–744.
[17] S. Sato, M. Miyauchi, T. Takekoshi, et al., Reduced expression of CD44 variant 9 is related to lymph node metastasis and poor survival in squamous cell carcinoma of tongue, Oral Oncol. 36 (2000) 545–549.
[18] Y. Takamune, T. Ikebe, O. Nagano, H. Nakayama, K. Ota, T. Obayashi, H. Saya, M. Shinohara, ADAM-17 associated with CD44 cleavage and metastasis in oral squamous cell carcinoma, Virchows Arch. 450 (2007) 169–177.
[19] S.W. Pyo, M. Hashimoto, Y.S. Kim, et al., Expression of E-cadherin, P-cadherin and N-cadherin in oral squamous cell carcinoma: correlation with clinicopathologic features and patients outcome, J. Cranio. Maxillofac. Surg. 35 (2007) 1–9.
[20] T. Sakaki, I. Tamura, H. Kadota, K. Kakudo, Changing expression of E- and P-cadherin during rat tongue carcinogenesis induced by 4-nitroquinoline1-oxide, J. Oral Pathol. Med. 32 (2003) 530–537.
[21] T.J.H. Siebers, M.A.W. Merkx, P.J. Slootweg, et al., No high-risk HPV detected in SCC of the oral tongue in the absolute absence of tobacco and alcohol – a case study of seven patients, Oral Maxillofac. Surg. 12 (2008) 185–188.
[22] C. Stoll, G. Baretton, F. Soost, Prognostic importance of the expression of CD44 splice variants in oral squamous cell carcinoma, Oral Oncol. 35 (1999) 484–489.
[23] S. Syrjanen, Human papillomavirus (HPV) in head and neck cancer, J. Clin. Virol. 32S (2005) 59–61.
[24] A.M. Tracey, G. Harrison, R.E. Mansel, The role of the CD44/ezrin complex in cancer metastasis, Crit. Rev. Oncol./Hematol. 46 (2003) 165–186.
[25] P.K. Tsantoulis, N.G. Kastrinakis, A.D. Tourvas, et al., Advances in the biology of oral cancer, Oral Oncol. 43 (2007) 523–534.
[26] M.J. Veness, Tongue cancer in younger patients, Austr. Radiol. 43 (1999) 76–81.
[27] X. Wang, J. Zhang, M. Fan, et al., The expression of E-cadherin at the invasive tumor front of oral squamous cell carcinoma: immunohistochemical and RT-PCR analysis with clinicopathological correlation, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 107 (2009) 547–554.