Dental Research Journal

Original Article

CD44 and CD74: The promising candidates for molecular targeted therapy in oral squamous cell carcinoma

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ABSTRACT

Background: Considering molecular target therapy concept in the treatment of oral squamous cell carcinoma (OSCC), many attempts have been performed to introduce an effective molecular marker during recent years. Several investigations have emphasized on the role of CD44 in various cancers and few studies have mentioned CD24 and CD74. The purpose of this study was to investigate the relationship between CD44, CD24 and CD74 expressions and several clinical or histopathological factors in OSCC patients.

Materials and Methods: In our analytical cross-sectional study, forty primary OSCC specimens were immunohistochemically stained for CD44, CD24, and CD74 proteins. Then, the relationship between their expressions and age, sex, lymph node metastasis, and histopathologic grading was statistically analyzed using Mann-Whitney nonparametric and *t*-test. Furthermore, $P < 0.05$ was considered as significant.

Results: CD44 and CD74 proteins were significantly over-expressed in OSCC patients with high grade ($P = 0.001$ and $P = 0.001$) as compared to those with low grade. Furthermore, CD74 immunoreactivity showed significantly higher expression in patients with lower age ($P = 0.039$). Considering lymph node metastasis, we observed significant overexpression of CD74 in patients with no lymph node involvement ($P = 0.033$).

Conclusion: Our observations support the significant role of membranous CD44 protein in progression of OSCC and also introduce CD74 protein as a probable interfering factor in different aspects of OSCC.

Key Words: Antigen, CD24, CD44, carcinoma, immunohistochemistry, squamous cell of head and neck

Received: November 2013
Accepted: March 2014

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is one of the most common cancers with more than 4,00,000 new cases and 2,00,000 deaths worldwide annually.[1] Oral squamous cell carcinoma (OSCC), as a subgroup of HNSCC and the most common oral cancer, is estimated to be the eighth most common cancer in human population.[2] Despite recent advances in treatment and early diagnosis of OSCC, the overall 5 year survival rate is still <50%.[3]

Although several histologic and clinical criteria have been proposed to evaluate the prognosis of OSCC, the most important prognostic factor is still clinical stage of the tumor.[4] Also recently, there is much interest in novel molecular markers for predicting patient prognosis and estimating of overall survival rate.[5]

CD24 is a heavily glycosylated mucin-like surface protein which has been proposed as a specific

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ligand for P-selectin.[6] Although in breast cancer, ambiguity exists about the role and expression pattern of CD24, as a cancer stem cell (CSC) associated marker,[7,8] several studies have shown that CD24 is highly expressed in some human malignancies[9] and its higher level of expression might enhance the metastatic potential of tumor cells and lead to a poor prognosis.[10] Therefore, CD24 provides a novel molecular target for therapeutic strategies.[11]

CD44 is a transmembrane glycoprotein that forms a member of cell adhesion molecules and provides its crucial roles, including cell proliferation, migration and survival.[12] CD44 is the first marker used to introduce CSC theory,[13] which describes a small population of tumor cells, as cancer-initiating cells, demonstrate stem cell properties and they are responsible for progression in some malignant tumors.[14,15] Therefore, many recent studies propose this marker in both CSCs detection and molecular cancer therapy.[16,17]

Also a combined evaluation of CD24/CD44 immunohistochemical expression has been proposed in some recent studies to estimate the presence of CSCs in a tumor and predict the prognosis of patients.[18,19]

CD74 is a transmembrane glycoprotein that associates with the major histocompatibility complex (MHC) Class II and plays many important roles in the immune system.[20,21] CD74 expression has been found on HLA Class II-positive normal cells including B cells, macrophages and dendritic cells.[22] Also, in some hematologic[23] and non-hematologic malignancies, including gastric[24] and renal cell[25] carcinomas, CD74 is expressed. Furthermore, recent studies have suggested that CD74 can be used as a prognostic factor and eventually a potential therapeutic target in CD74+ malignancies.[26,27]

During recent years, many investigations have focused on the novel methods for treatment of OSCC to increase the efficacy of therapeutic procedures and reduce the consequent morbidity.[28] The aim of this study was the evaluation of immunohistochemical expression of three suggested prognostic markers (CD24, CD44, CD74) and also potentially introduced molecular target for cancer therapy in OSCC and analyze their correlations with several clinicopathological factors.

MATERIALS AND METHODS

Patients

In this analytical cross-sectional study, forty primary OSCC patients, consisting of 17 specimens from tongue and 23 specimens from other sites of the oral cavity (7 labial mucosa, 5 floor of the mouth, 4 gingiva or edentulous ridge, 3 hard palate, 3 buccal mucosa and 1 soft palate) with no prior chemo-radiotherapy were included in this study. The mean age of the patients, consisting of 20 males (ranging from 26 to 79 with a mean age of 50.5) and 20 females (ranging from 28 to 80 with a mean age of 60.5) was 55. Tissue specimens of the patients were provided from archival 10% formalin-fixed paraffin blocks of the Pathology laboratory of Cancer Institute (Tehran University of Medical Sciences) between 2007 and 2011 and the tumors were histopathologically graded by two independent pathologists using Bryne's Grading System (16 Grade I, 13 Grade II, 11 Grade III).[29] In controversial cases, the lesions were graded by the third pathologist to confirm one of the previous viewpoints. This study was approved by the Ethical Committee of Babol University of Medical Sciences (30/5720).

Immunohistochemistry

Anti-CD24 (mouse monoclonal, clone SN3b, Bio care Medical LLC, CA, USA), Anti-CD44 (mouse monoclonal, clone VFF-327 v3, Leica Bio systems Ltd, Newcastle, UK) and Anti-CD74 (mouse monoclonal, clone LN2, Sky Tek Laboratories Inc., UT, USA) were applied to deparaffinized and hydrated 4 μm thick tissue sections. Prior to antibody application, endogenous peroxidase activity was blocked using 5% (v/v) H_2O_2 in methanol. Slides were then washed with Tris-buffered saline and heated for 15 min at 100°C in 10 mM sodium citrate buffer (pH 6.0) and boiled in a microwave oven (700 W) for antigen retrieval. Also, nonspecific binding was blocked by incubation of slides with 1% bovine serum albumin for 1 h. It was followed by application of secondary streptavidin-biotin-peroxidase complex method and then primary antibody was visualized with diaminobenzidine. Finally, they were counter-stained with Mayer's hematoxylin and mounted with DPX mountant.

Negative control samples were processed in parallel to test samples by replacing the primary antibody with phosphate buffer saline. As a positive control, we used colorectal carcinoma, lymph node and normal

tonsil for CD24, CD74 and CD44, respectively (based on the manufacturer's instructions).

For immunohistochemical analysis, membranous staining for CD44 and cytoplasmic and/or membranous staining for CD24/CD74were blindly and independently evaluated by two observers using a semi-quantitative system[30,31] [Table 1]. Expression index was determined based on the mean percentage of stained neoplastic cells in 10 representative fields.

Statistical analysis

Data were analyzed using SPSS V16.0 (SPSS Inc., Chicago, IL, USA).

Mann-Whitney nonparametric test was conducted to determine the differences between the antibody expressions. Spearman correlation was applied to determine the association between the variables. Significant variable differences were identified using *t*-test. $P < 0.05$ was considered as significant.

RESULTS

As shown in Table 2, the CD44 and CD74 proteins were significantly over-expressed in OSCC patients with high grade (Grade III) ($P = 0.001$ and $P = 0.001$) [Figures 1 and 2] as compared to those with low grade (Grade I). Also, CD44 immunoreactivity showed significantly higher expression in patients with lower age (<50 years) ($P = 0.039$). Considering lymph node metastasis, we observed significant overexpression of CD74 in OSCC patients with no lymph node involvement ($P = 0.033$).

Furthermore, CD24 expression [Figure 3] revealed no significant association with the mentioned clinicopathologic criteria [Table 2].

DISCUSSION

We found a significant association between immunohistochemical expression of CD44 and CD74

Table 1: The system used for evaluation of the markers (mean percentage of stained neoplastic cells in 10 representative fields)

Score	Marker		
	CD24	CD44	CD74
I	0	0-24	0
II	1-10	25-49	1-10
III	11-50	50-75	11-50
IV	50<	75<	50<

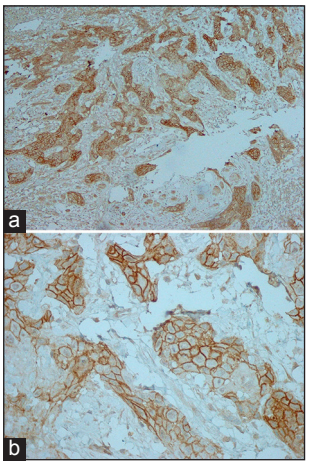

Figure 1: High immunohistochemical reactivity for CD44 (Score IV) in oral squamous cell carcinoma (Grade II); (a) original magnification ×100; (b) original magnification ×400

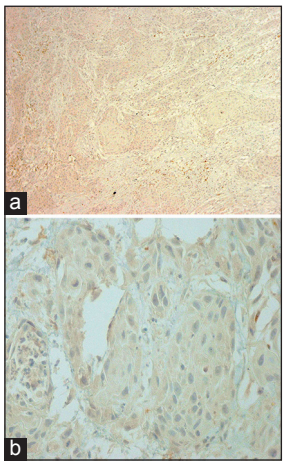

Figure 2: High immunohistochemical reactivity for CD74 (Score III) in oral squamous cell carcinoma (Grade II); (a) original magnification ×100; (b) original magnification ×400

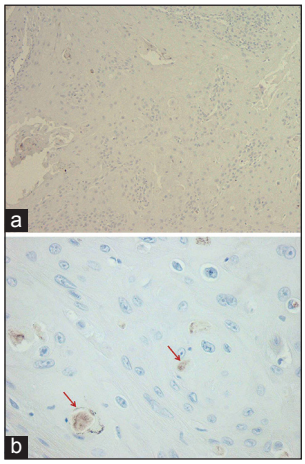

Figure 3: Low immunohistochemical reactivity for CD24 (Score II) in oral squamous cell carcinoma (Grade I); (a) original magnification ×100; (b) original magnification ×400. Scattered stained cells (arrows) are seen

Table 2: Correlation of CD24, CD44 and CD74 expression with clinicopathological variables in OSCC

Clinicopathologic factors	Cases	CD24 expression			CD44 expression			CD74 expression		
		Low[a] *n* (%)	High[b] *n* (%)	*P* value	Low *n* (%)	High *n* (%)	*P* value	Low *n* (%)	High *n* (%)	*P* value
Age										
>50	29	29 (100)	0	0.187	14 (48)	15 (52)	0.569	25 (86)	4 (14)	0.039*
50≥	11	11 (100)	0		4 (36)	7 (64)		7 (64)	4 (36)	
Sex										
Male	26	26 (100)	0	0.875	10 (39)	16 (61)	0.790	21 (81)	5 (19)	0.976
Female	14	14 (100)	0		8 (58)	6 (42)		11 (78)	3 (22)	
Grade										
I	16	16 (100)	0	0.576	15 (93)	1 (7)	0.001*	15 (94)	1 (6)	0.001*
II	13	13 (100)	0		1 (8)	12 (92)		11 (85)	2 (15)	
III	11	11 (100)	0		2 (18)	9 (82)		6 (54)	5 (46)	
Lymph node**										
Positive	20	20 (100)	0	0.846	9 (45)	11 (55)	0.584	19 (95)	1 (5)	0.033*
Negative	13	13 (100)	0		6 (46)	7 (54)		9 (69)	4 (31)	

*$P < 0.05$ was considered as significant; **Lymph node involvement in seven cases was not apparent; [a]Low: Score I + Score II; [b]High: Score III + Score IV. OSCC: Oral squamous cell carcinoma

and several clinicopathological criteria in OSCC specimens.

Over recent years, the clinicians have been interested in molecular target cancer therapy. This preference is mainly derived from the cancer cell resistance to routine chemotherapeutic agents in many malignancies and also the substantial morbidity of patients suffering from the side-effects and complications related to conventional cancer treatments.[32,33] Considering oral cancer therapy, several other advantages of this modality of treatment have been described as little accumulation of the agents, rare bone marrow suppression, etc.[34]

Due to several aberrant signaling pathways introduced in the pathogenesis of OSCC, the combination of various molecular targeted agents is highly recommended to attain the maximum effectiveness of treatment outcomes and reduction of therapeutic complications.[35] Therefore, beside conventional targets for immunotherapy such as epidermal growth factor and cyclooxygenase-2,[36] novel promising combinations of molecular targets have been attracted much attention from investigators.

In addition to CD44, a well-known CSC marker, we proposed CD74 as a novel target for cancer therapy in OSCC. A significant association between CD74 overexpression and tumor grade has previously been revealed in renal cell carcinoma, which represents this marker as a potential therapeutic target.[37] The role of CD74 in regulating Class II MHC folding and its related functions may depict this marker in close relation with underlying inflammatory process described in the grading of OSCC. Therefore, we highlight the role of inflammatory cells and probably associated cytokines in evolution and progression of OSCC. Although it should be mentioned that this role may be tumor dependent, such as B-cell neoplasms,[23] and may significantly differ between various patients; therefore, it is recommended that before any therapeutic intervention, the clinicians evaluate the immunohistochemical expression of CD74 in the tumoral tissue.

Although recent investigations have focused on CD44/CD24 expression in several carcinomas,[18,19] in our study, CD24 expression revealed no relation with clinicopathological criteria. This difference may be associated with the type of carcinomas and consequently their various molecular pathways, which affect the pathogenesis of the tumors. Also, our findings confirmed the results of several recent studies[14] in which CD44 expression has shown a significant correlation with tumor grade and consequently introduced it as a CSC marker in HNSCC. In recent decades, this subpopulation of tumoral cells has been depicted as the cells with capabilities of sustaining neoplastic growth and many investigations have been performed to clarify the detectable molecular characteristics of these cells.[38] It seems to achieve a more effective treatment and minimizing tumor recurrence, CSCs should be considered as a potential target and therefore CD44 could be a reliable marker in HNSCC.[14,39]

Also, the role of CD44 in the initiation and progression of OSCC is mainly emerges from

physiologic function of this membranous protein in cellular adhesion and homing, which conceptually describe metastatic migration of the neoplastic cells.[12]

CONCLUSION

The significant increase of CD44 and CD74 expressions in high grade OSCC supports the role of these proteins in the progression of OSCC and introduces them as targets in molecular therapy. Also, we observed that lymph node metastasis and increasing patients' age led to a significant decrease of CD74 protein expression, which the former may be associated with a limited number of the cases and the latter could present age of the patients as an independent prognostic factor considering the role of CD74 in the pathogenesis of OSCC.

Therefore, it is encouraged to follow these markers utilizing advanced experimental methods to sustain enough documents to represent them as an effective molecular target in OSCC therapy.

ACKNOWLEDGMENTS

This work was financially supported by Babol University of Medical Sciences (Grant no. 9032326). We would like to thank Dr. Jahanzad (Cancer Institute of Tehran University of Medical Sciences) for his kind support in providing the specimens.

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How to cite this article: Shakib PA, Ensani F, Abdirad A, Valizadeh B, Seyedmajidi M, Sum S. CD44 and CD74: The promising candidates for molecular targeted therapy in oral squamous cell carcinoma. Dent Res J 2015;12:181-6.

Source of Support: This work was financially supported by Babol University of Medical Sciences (Grant no. 9032326). **Conflict of Interest:** None declared.