

Combined overexpression of cadherin 6, cadherin 11 and cluster of differentiation 44 is associated with lymph node metastasis and poor prognosis in oral squamous cell carcinoma

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Abstract. Oral squamous cell carcinoma (OSCC) is a highly invasive lesion that frequently metastasizes to the cervical lymph nodes and is associated with a poor prognosis. Several adhesion factors, including cadherin 6 (CDH6), cadherin 11 (CDH11) and cluster of differentiation 44 (CD44), have been reported to be involved in the invasion and metastasis of multiple types of cancer. Therefore, the aim of the present study was to determine the expression of CDH6, CDH11 and CD44 in tumor tissues from patients with OSCC, and whether this was associated with the metastasis and survival of OSCC. The mRNA expression of the human tumor metastasis-related cytokines was examined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) in OSCC tumors with or without lymph node metastasis (n=10/group). The expression of CDH6, CDH11 and CD44 in 101 OSCC and 10 normal oral mucosa samples was examined by immunohistochemical staining. The association between overall and disease-specific survival times of patients with OSCC and the expression of these three proteins was evaluated using Kaplan-Meier curves and the log-rank test. RT-qPCR results indicated that the mRNA expression of CDH6, CDH11 and CD44 was increased in OSCC patients with lymph node metastasis (2.93-, 2.01- and

1.92-fold; $P<0.05$). Overexpression of CDH6, CDH11 and CD44 was observed in 31/35 (89%), 25/35 (71%) and 31/35 (89%) patients, respectively. The number of OSCC patients with lymph node metastasis exhibiting CDH6, CDH11 and CD44 overexpression was significantly higher than the number of patients without lymph node metastasis exhibiting overexpression of these proteins ($P=0.017$, $P=0.038$ and $P=0.007$, respectively). OSCC patients with high co-expression of CDH6, CDH11 and CD44 exhibited lower disease-specific survival times ($P=0.047$; $\chi^2=3.933$) when compared with OSCC patients with low co-expression of these adhesion factors. CDH6, CDH11 and CD44 serve important roles in OSCC metastasis and the combined use of these factors as biomarkers may improve the accuracy of the prediction of cancer metastases and prognosis.

Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common subtypes of all malignant head and neck tumors worldwide (1). OSCC is a highly invasive lesion that frequently metastasizes to cervical lymph nodes (2). Metastasis to regional lymph nodes and distant sites occurs in ~40% of patients with oral cancer and is associated with a poor prognosis (3,4). At present, only 25-40% of patients with lymph node metastasis (LNM) achieve 5-year survival, compared with ~90% of patients without metastasis (5).

Metastasis is the result of complicated events, in which the dysfunction of epithelial cell-cell adhesion is known to be critical for tumor invasion and metastasis (6). Cadherins, as a major class of cell-cell adhesion molecules, regulate the adhesion and migration of cells in a calcium-dependent manner (7). Reduction of E-cadherin expression predicts the loss of epithelial cell adhesion (8), which is a prognostic factor for head and neck squamous cell carcinoma, according to a systematic review with meta-analysis (9). Cadherin 6 (CDH6) is a class II cadherin and its upregulation reportedly promotes the motility and invasion of several cancer cells (10-12). Cadherin 11 (CDH11), a mesenchymal cadherin, is upregulated during prostate cancer (PCa) progression and bone metastasis (13). CDH11 expression increased the migration and invasion of PCa cells and enabled PCa cells to intercalate into an osteoblast

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Abbreviations: OSCC, oral squamous cell carcinoma; LNM, lymph node metastasis; CDH6, cadherin 6; CDH11, cadherin 11; PCa, prostate cancer; IHC, immunohistochemical; SABC, streptavidin-biotin complex; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; MMPs, matrix metalloproteinases; EMT, epithelial-mesenchymal transition; Akt, protein kinase B; PI3K, phosphatidylinositol 3-kinase; MAPK, mitogen-activated protein kinase; STAT, signal transducer and activator of transcription

Key words: cadherin 6, cadherin 11, cluster of differentiation 44, oral squamous cell carcinoma, metastasis, prognosis

monolayer (14). However, the roles of these two proteins in mediating cell migration in OSCC remain unclear. CD44 is a transmembrane glycoprotein and a cell adhesion molecule that serves a crucial role in the differentiation, invasion and metastasis of various types of tumor cell (15,16). CD44 is also involved in the epithelial-mesenchymal transition (EMT), which is associated with metastasis.

Recent studies have suggested that the increase in CD44 expression in OSCC is associated with increased metastasis and decreased survival (17,18). However, the association between CDH6, CDH11 and CD44 expression in OSCC has not previously been investigated. Therefore, the aim of the present study was to determine whether these adhesion factors may serve as potential prediction factors for OSCC metastasis and prognosis, in order to better understand the underlying pathogenesis of this malignancy and potentially to improve the prognosis and treatment outcome of patients with OSCC.

Materials and methods

Patients and tumor procurement. A total of 101 patients with OSCC who were treated at the Department of Oral and Maxillofacial Surgery, Peking Union Medical College Hospital (Beijing, China) between March 2006 and December 2010 were retrospectively enrolled in the present study. Tumors were pathologically staged according to the American Joint Cancer Committee staging system (19) Within 30 min after surgical extirpation, tissues were frozen in liquid nitrogen, and the study was approved by the Ethics Committee of Peking Union Medical College. The clinicopathological information of the patients from whom the tissues were obtained is presented in Table I.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Tumor tissue samples with or without LNM (n=10/group) were homogenized, and total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and was further purified using the RNeasy RNA Extraction Mini kit (Qiagen GmbH, Hilden, Germany). RNA samples were converted into first strand cDNA using an RT² First Strand kit, according to the manufacturer's protocols (SABiosciences; Qiagen GmbH). Quantitative gene expression was analyzed by qPCR, performed on an ABI Prism 7900 HT (Applied Biosystems; Thermo Fisher Scientific, Inc.). The optimized parameters for PCR were: 95°C for 2 min, 94°C for 10 sec, 61.5°C for 30 sec and 72°C for 40 sec for 40 cycles). The assay used an RT² Profiler PCR array system (RT² ProfilerTM PCR Array Human Tumor Metastasis (PAHS-028Z); SABiosciences; Qiagen GmbH) that contained a panel of 84 genes and SYBRGreen qPCR detection method and was performed according to the manufacturer's protocols. Quality controls were all within the recommended range. Data were analyzed by the 2^{-ΔΔC_q} method (20).

Immunohistochemical (IHC) staining. IHC staining was performed by the streptavidin-biotin complex (SABC) method using 4% formalin-fixed (fixed for 48 h at 25°C), paraffin-embedded 4-μm tissue sections. Tissue sections were heat-immobilized at 60°C for 30 min and deparaffinized in

Table I. Clinicopathological characteristics in patients with oral squamous cell carcinoma (n=101).

Variables	No. patients (%)
Age, years	
>60	53 (52.5)
<60	48 (47.5)
Sex	
Male	65 (85.1)
Female	36 (14.9)
Location	
Tongue	45 (44.6)
Gingiva	23 (22.8)
Floor of mouth	17 (16.8)
Buccae	14 (13.9)
Hard palate	2 (1.9)
Lymph node metastasis	
Yes	35 (34.7)
No	66 (65.3)
Tumor stage	
I	25 (24.8)
II	34 (33.6)
III	19 (18.8)
IV	23 (22.8)
Differentiation	
Well	38 (37.6)
Moderate	50 (49.5)
Poor	13 (12.9)
Smoking	
Yes	70 (69.3)
No	31 (30.7)
Alcohol	
Yes	65 (64.4)
No	36 (35.6)

xylene and rehydrated through a descending ethanol series (100, 95, 90, 80 and 70%) at room temperature for 5 min at each concentration. Following the blocking of endogenous peroxidase activity with 0.3% hydrogen peroxide for 10 min, the sections were blocked for 1 h at room temperature with 5% bovine serum albumin, prior to being incubated with primary rabbit anti-CDH6 antibody (dilution, 1:100; cat. no. ab64917; Abcam, Cambridge, MA, USA), mouse anti-CDH11 antibody (dilution, 1:50; cat. no. ab151446; Abcam) or rabbit anti-CD44 antibody (dilution, 1:100; cat. no. ab51037; Abcam) for 24 h at 4°C. The slides were rinsed with phosphate-buffered saline (PBS) three times and were incubated with a goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (dilution, 1:500; cat. no. ZB-2010; OriGene Technologies, Inc., Beijing, China) or a goat anti-mouse secondary antibody (cat. no. ZB-2305; OriGene Technologies, Inc.) for 30 min at room temperature. Following incubation with SABC for 20 min at room temperature, the sections were exposed to

Table II. Expression profiling of human tumor metastasis-related gene in oral squamous cell carcinoma tissues with or without metastasis, as determined by reverse transcription-quantitative polymerase chain reaction. Fold-change was calculated using the $2^{-\Delta\Delta C_q}$ method. n=10/group.

Gene abbreviations	Full gene name	Fold-change	P-value
Upregulated			
APC	Adenomatous polyposis coli	1.25	0.035 ^a
CD44	CD44 molecule	1.92	0.011 ^a
CDH6	Cadherin 6	2.93	0.007 ^b
CDH11	Cadherin 11	2.01	0.009 ^b
CTNNA1	Catenin α 1	1.49	0.034 ^a
FAT1	FAT tumor suppressor homolog 1	1.76	0.032 ^a
ITGA7	Integrin, α 7	2.14	0.022 ^a
FGFR4	Fibroblast growth factor receptor 4	1.34	0.040 ^a
MDM2	Mdm2 p53 binding protein homolog	1.92	0.009 ^b
MMP7	Matrix metalloproteinase 7 (matrilysin)	2.36	0.029 ^a
MMP9	Matrix metalloproteinase 9	5.11	0.001 ^b
MMP13	Matrix metalloproteinase 13	2.51	0.008 ^b
CCL2	Chemokine (C-C motif) 2(MCP-1)	1.98	0.013 ^a
CCL3	Chemokine (C-C motif) 3(MIP-1A)	2.16	0.021 ^a
CCL5	Chemokine (C-C motif) 5(RANTES)	2.71	0.004 ^a
CCL7	Chemokine (C-C motif) 7(MCP-3)	1.74	0.023 ^a
CXCR4	Chemokine (C-X-C motif) receptor 4	1.72	0.019 ^a
MTA1	Metastasis-associated 1	1.32	0.061
TNFSF10	Tumor necrosis factor superfamily, member 10	1.13	0.081
TP53	Tumor protein p53	1.43	0.021 ^a
TSHR	Thyroid stimulating hormone receptor	1.65	0.018 ^a
Downregulated			
CDH1	E-cadherin (epithelial)	-1.13	0.083 ^a
BRMS1	Breast cancer metastasis suppressor 1	-1.42	0.021 ^a
TIMP3	TIMP metalloproteinase inhibitor 3	-0.92	0.102
GNRH1	Gonadotropin-releasing hormone 1	-0.61	0.050 ^a
HPSE	Heparanase	-0.66	0.034 ^a

^aP<0.05, ^bP<0.01, Student's t-test.

3,3'-diaminobenzidine for 1 min, counterstained with hematoxylin at room temperature for 15 sec, mounted in neutral gum and images were captured using a Nikon Eclipse E600 microscope (Nikon Corporation, Tokyo, Japan). PBS was used instead of the primary antibody as a negative control.

Evaluation of immunohistochemistry was quantified by two pathologists, who were blinded to all patient diagnoses. The staining intensity was estimated in a 4-step scale as previously reported (0, no staining; 1, weak intensity; 2, moderate intensity; and 3, strong intensity) (21). The fraction of stained cells was scored according to the following criteria: Score 0, <10% positive cancer cells; score 1, 11-50% positive cancer cells; score 2, 51-80% positive cancer cells; and score 3, >80% positive cancer cells. The final staining score was assigned based on the multiplication of the staining intensity and the percentage of positive cells, and was graded as follows: 0, 0; 1, 1-3; 2, 5-6; and 3, 7-9. Scores of 0 or 1 were classed as low expression, and scores 2 or 3 were classed as high expression for further analysis.

Statistical analysis. All experiments were repeated at least three times. Data were analyzed using SPSS software (version 20.0; SPSS, Inc., Chicago, IL, USA) and GraphPad prism software (GraphPad Software, Inc., La Jolla, CA, USA). Significance values were assigned using Student's t-tests and the χ^2 test was used to compare the results between the two groups and the associations with clinicopathological features, including age, sex, histopathological grade, LNM and tumor stage. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. The patient cohort included 65 males and 36 females, with a median age of 62 years (range, 34-85 years). Primary OSCC tumors were most frequently identified on the tongue (45/101; 44.6%), followed by the gingiva (23/101; 22.8%), the floor of the mouth (17/101; 16.8%), the buccal (14/101; 13.9%) and the hard palate (2/101; 1.9%).

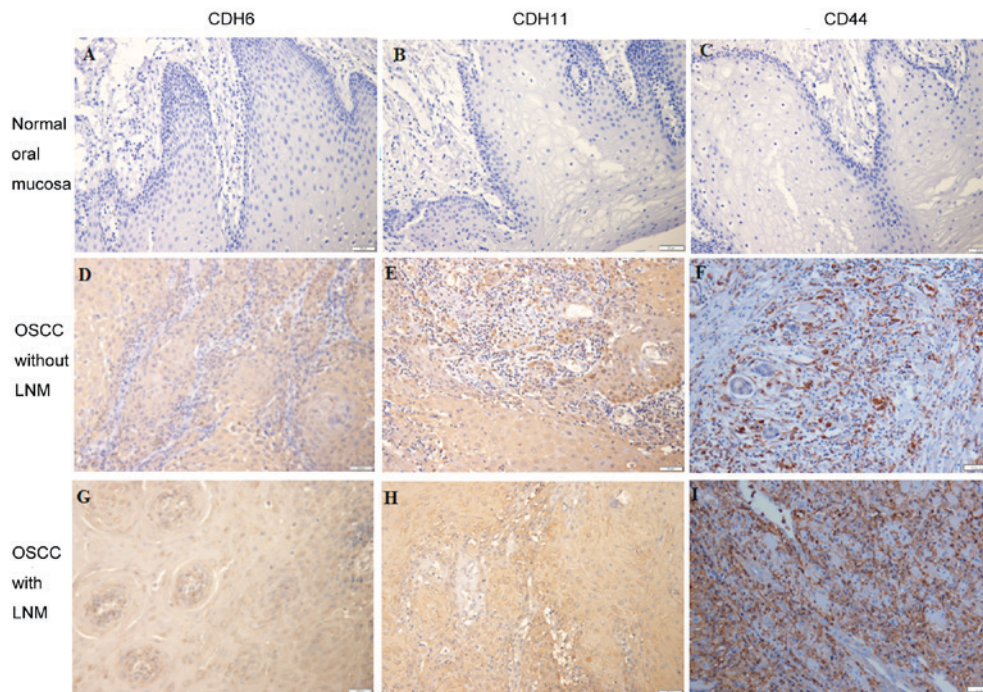


Figure 1. Expression of CDH6, CDH11 and CD44 in normal tissue and in OSCC with and without LNM. Photomicrographs demonstrate representative examples of (A) CDH6, (B) CDH11 and (C) CD44 expression in normal oral mucosa. Representative examples of (D) CDH6, (E) CDH11 and (F) CD44 expression in OSCC without LNM. Representative examples of (G) CDH6, (H) CDH11 and (I) CD44 expression in OSCC with LNM. Magnification, x200; scale bar, 50 μ m. CDH, cadherin; CD44, cluster of differentiation 44; OSCC, oral squamous cell carcinoma; LNM, lymph node metastasis.

LNM occurred in 35/101 patients (34.7%). Further patient characteristics are presented in Table I.

Expression of tumor metastasis cytokines involved in cell adhesion and inflammation is increased in OSCC tumors with LNM. The expression of 84 tumor metastasis cytokines was analyzed using RT-qPCR analysis. CDH6, CDH11 and CD44 mRNA expression was significantly increased in the OSCC patients with LNM compared with the OSCC patients without LNM (2.93-, 2.01- and 1.92-fold; $P < 0.05$), which was assessed by Student's t-test. Including CDH6, CDH11 and CD44, 21 genes were upregulated and 5 others were downregulated in OSCC tumors with LNM compared with OSCC tumors without LNM (Table II). Of the genes that were upregulated, 7 were associated with cell-cell adhesion (adenomatous polyposis coli, CD44, CDH6, CDH11, catenin $\alpha 1$, FAT tumor suppressor homolog 1 and integrin subunit $\alpha 7$). The expression of 3 matrix metalloproteinases (MMPs) was increased (MMP7, MMP9 and MMP13), which was reported to be associated with tumor LNM (22-24). The majority of the upregulated chemokines belong to the chemokine ligand subfamily and are involved in macrophage recruitment [monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein-1 α , regulated on activation, normal T cell expressed and secreted and MCP-3] (25), while the other three are chemokine receptors (C-X-C chemokine receptor type 4, fibroblast growth factor receptor 4 and thyroid-stimulating hormone receptor). Three genes involved in apoptosis (metastasis-associated 1, tumor necrosis factor superfamily member 10 and tumor protein p53) were also upregulated. In contrast to the increased expression of MMPs, two extracellular matrix proteins (heparanase

precursor and tissue inhibitor of metalloproteinase-3), which are MMP inhibitors, were downregulated. The expression of gonadotrophin-releasing hormone 1, a growth factor included in the array, was decreased (Table II).

CDH6, CDH11 and CD44 expression, detected by IHC staining, exhibited a significant positive association with cervical LNM in tumor tissues. The expression of CDH6, CDH11 and CD44 in specimens from patients with OSCC was determined using IHC staining. As demonstrated by Fig. 1, there was almost no staining in normal mucosa for CDH6 (Fig. 1A), CDH11 (Fig. 1B) or CD44 (Fig. 1C). However, these adhesion factors were localized to the nucleus and the cytoplasm of tumor cells, and high expression of CDH6 (44/66, 66%; Fig. 1D), CDH11 (33/66, 50%; Fig. 1E) and CD44 (37/66, 56%; Fig. 1F) was observed in 66 OSCC cases without LNM (Table III). Additionally, the expression of CDH6 (31/35, 89%; Fig. 1G), CDH11 (25/35, 71%; Fig. 1H) and CD44 (31/35, 89%; Fig. 1I) was significantly increased in tumor cells with LNM, compared with those without LNM ($n=35$; $P < 0.001$; Table III). These results indicated that high expression of CDH6, CDH11 and CD44 was significantly associated with LNM in OSCC (Table III).

Associations between CDH6, CDH11 and CD44 expression and clinicopathological factors. The associations between CDH6, CDH11 and CD44 protein expression and clinicopathological data were assessed using the χ^2 test and are summarized in Table IV. CDH6, CDH11 and CD44 protein expression was significantly associated with LNM ($P=0.017$, $P=0.038$ and $P=0.001$, respectively). OSCC cases with LNM

Table III. Immunohistochemical analysis of CDH6, CDH11 and CD44 expression in OSCC. Association between expression of CDH6, CDH11 or CD44 and tumor metastasis was analyzed using SPSS software.

Variables	No. of patients (%)		P-value
	High	Low	
CDH6			
OSCC without LNM	44 (66)	22 (34)	0.017 ^a
OSCC with LNM	31 (89)	4 (11)	
CDH11			
OSCC without LNM	33 (50)	33 (50)	0.038 ^a
OSCC with LNM	25 (71)	10 (29)	
CD44			
OSCC without LNM	37 (56)	29 (44)	0.001 ^b
OSCC with LNM	31 (89)	4 (11)	

^aP<0.05, ^bP<0.01, as determined by the χ^2 test. OSCC, oral squamous cell carcinoma; CDH, cadherin; CD44, cluster of differentiation 44; LNM, lymph node metastasis.

exhibited higher rates of elevated CDH6, CDH11 and CD44 protein expression. In addition, tumor stage was significantly associated with these adhesion factors (P=0.002, P=0.016 and P=0.018, respectively). However, no significant differences were identified between the expression of these proteins and the degree of tumor differentiation, age or sex (P>0.05).

Associations among CDH6, CDH11 and CD44 protein expression in OSCC. In the present study, the associations among the protein expression of CDH6, CDH11 and CD44 were assessed using the χ^2 test. As demonstrated by Table V, 56/101 patients with OSCC exhibited high expression and 14 exhibited low expression of CD44 and CDH6. The association between CD44 and CDH6 expression was statistically significant ($r=0.266$; P=0.008). Similarly, there was a statistically significant association between the expression of CD44 and CDH11 ($r=0.254$; P=0.011), with 45/101 patients with OSCC exhibiting a high expression. These results indicated the co-association between CDH6, CDH11 and CD44.

Association between CDH6, CDH11 and CD44 expression and survival in patients with OSCC. No significant associations between the 5-year overall survival rate of patients with OSCC, and CDH6, CDH11 or CD44 expression were identified (Fig. 2A-C). However, the 5-year disease-specific survival rate of OSCC patients with high CDH6 and CD44 expression was significantly decreased when compared with those exhibiting low expression [CDH6 (P=0.018; $\chi^2=5.534$) and CD44 (P=0.031; $\chi^2=4.622$); Fig. 2D and F]. As CDH6, CDH11 and CD44 were revealed to be associated with LNM in the present study, the association between patient survival and the co-expression of these factors was also investigated. The results revealed that the co-expression of CDH6, CDH11 and CD44 was not significantly associated with overall survival

(Fig. 3A). However, the 5-year disease-specific survival rate of patients with high co-expression of these proteins was decreased when compared with that of patients exhibiting low co-expression (P=0.047; $\chi^2=3.933$; Fig. 3B).

Discussion

The primary cause of mortality in OSCC is metastasis, occurring primarily via the lymphatic system. The impact of this depends on the size and site of the primary tumor, the Tumor-Node-Metastasis (TNM) stage (19), the depth of invasion, perineural invasion, patient compliance, biological tumor markers and tumor grade (26). Intercellular adhesion is mediated by a family of glycoproteins known as cadherins, which serve an important role in the migration and dissemination of cells during tumor progression and metastasis (27). It is well-known that loss of E-cadherin and increased expression of N-cadherin is associated with tumor cell invasion in oral and ovarian cancer (28,29). By contrast, other mesenchymal-associated cadherins and adhesion factors, including CDH6, CDH11 and CD44, which have been proven to be able to interfere with epithelial cell-cell adhesion and to promote cancer cell invasion and metastasis, are often overexpressed in ovarian cancer (30-32). Until now, however, the precise role of the three factors in OSCC has not received a great deal of attention. The present study investigated the role of three factors in the metastasis of OSCC using IHC staining and RT-qPCR. It was revealed that CDH6, CDH11 and CD44 expression in tumor cells was associated with LNM in patients with OSCC.

In the present study, the mRNA expression of 84 potential human tumor metastatic factors, including CDH6, CDH11 and CD44, was observed in OSCC tissues with or without LNM. CDH6, CDH11 and CD44 mRNA expression was significantly increased in the OSCC patients with LNM, and 18 other upregulated and 5 downregulated cytokines were observed. CDH6, CDH11 and CD44 protein expression was subsequently examined in 10 normal oral mucosae and 101 OSCC tissues, including 35 cases with LNM, using IHC staining. These results demonstrated that no cases of normal oral mucosa exhibited high protein expression of these factors. However, high protein expression of CDH6, CDH11 and CD44 was observed in 89, 71 and 89% of 35 cases, respectively, of OSCC with LNM compared with 66, 50 and 56% of 66 OSCC cases, respectively, without LNM. The association between CDH6, CDH11 or CD44 protein expression and several clinicopathological indicators was assessed. The overexpression of these factors was associated with aggressive histopathological features, including LNM and advanced TNM stages, whereby OSCC patients with an advanced TNM stage (III-IV) and LNM exhibited higher expression of these proteins. However, there was no association between protein expression and age, sex or tumor differentiation. Furthermore, the present study observed that the prognostic value may be largely enhanced by co-evaluating the expression of these three proteins in patients with OSCC. These results were in line with those of previous studies in other types of cancer (29-31), indicating that CDH6, CDH11 and CD44 may be either directly or indirectly involved in the metastasis of OSCC. Therefore, it is reasonable to combine the expression of these three proteins as predictive parameters for improved evaluation of the progression of OSCC.

Table IV. Association between expression of CDH6, CDH11 or CD44 and clinicopathological characteristics in patients with oral squamous cell carcinoma (n=101).

Variable	CDH6			CDH11			CD44		
	High	Low	P-value	High	Low	P-value	High	Low	P-value
Patient number	75	26		58	43		68	33	
Age, years									
>60	40	13	0.769	32	21	0.528	37	16	0.576
<60	35	13		26	22		31	17	
Sex									
Male	47	18	0.547	40	25	0.261	40	25	0.096
Female	28	8		18	18		28	8	
Lymph node metastasis									
Yes	31	4	0.017 ^a	25	10	0.038 ^a	31	4	0.001 ^b
No	44	22		33	33		37	29	
Tumor (AJCC) stage									
>II	38	4	0.002 ^b	30	12	0.016 ^a	33	9	0.018 ^a
<II	37	22		28	31		33	26	
Differentiation									
Well	26	12	0.067	23	15	0.859	29	9	0.853
Moderately	35	15		26	24		30	20	
Poorly	10	3		9	4		9	4	
Smoking									
Yes	52	18	0.992	42	28	0.278	47	23	0.953
No	23	8		15	16		21	10	
Alcohol									
No	45	15	0.836	33	27	0.551	38	22	0.301
Yes	30	11		25	16		30	11	

^aP<0.05, ^bP<0.01, as determined by the χ^2 test using SPSS software. CDH, cadherin; CD44, cluster of differentiation 44; AJCC, American Joint Committee on Cancer.

Table V. Associations between CD44 protein expression and the expression of CDH6 and CDH11 in patients with oral squamous cell carcinoma (n=101).

Protein expression (no.)	CD44		rΦ	P-value
	High (n=68)	Low (n=33)		
CDH6			0.266	0.007 ^a
High (75)	56	19		
Low (26)	12	14		
CDH11			0.254	0.01 ^a
High (58)	45	13		
Low (43)	23	20		

^aP<0.01, as determined by the χ^2 test using SPSS software. CD44, cluster of differentiation 44; CDH, cadherin.

CDH6 and CDH11 expression have been revealed to be associated with aggressive tumor cell migration and a poorer

patient outcome (30-32). However, the underlying mechanisms of CDH6 and CDH11 in the progression of several types of cancer are not completely understood. CDH6 has been suggested to promote EMT during tumor development and progression by mediating pro-EMT signals (33). CDH6 affects the activity of F-actin and Rho GTPase, which is important for cell motility and EMT (34). EMT is a complex change in the cell phenotype that is important for cell migration and carcinoma metastasis. A previous study also demonstrated that CDH6 is a novel transforming growth factor (TGF)- β target gene during EMT in thyroid tumors and that its expression is controlled by TGF- β pathway molecules, including the Smad pathway or the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway (35).

During EMT, cadherin expression changes (cadherin switching), including E- to N-cadherin switching, is associated with cancer metastatic occurrence (36). CDH6 to CDH11 switching is also involved in the EMT (37). The present study also indicated that CDH6 is associated with CDH11, and that overexpression of CDH11 is associated with LNM. Although high expression of CDH11 alone was not associated with a low overall survival rate in the OSCC cohort of the present study, it

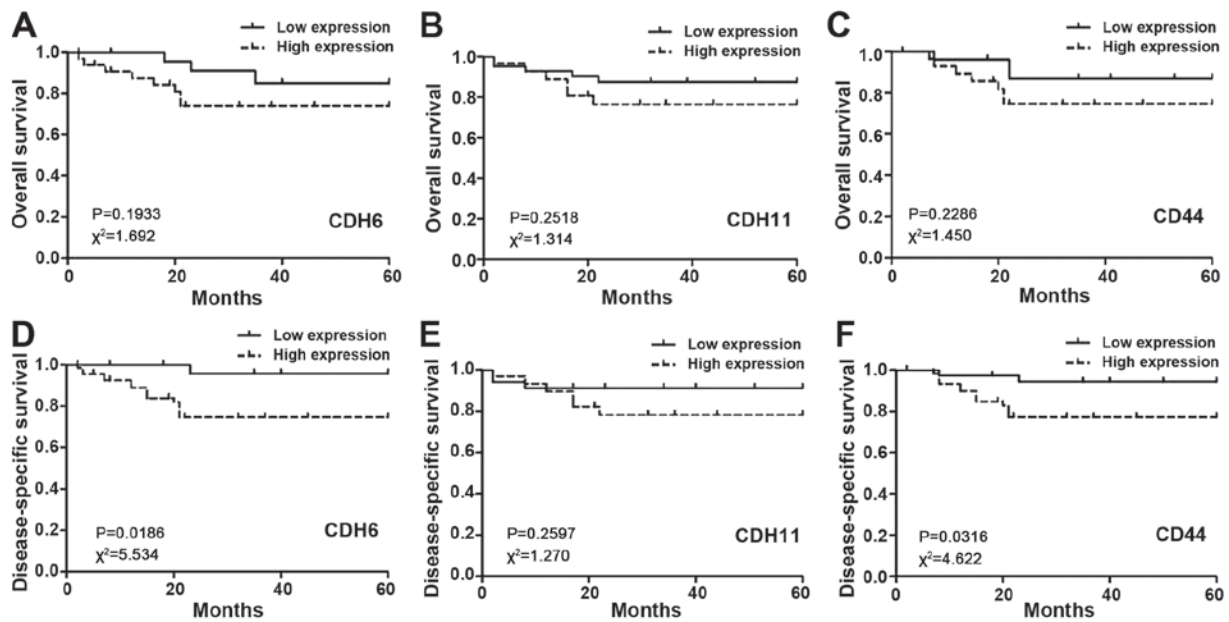


Figure 2. Association between CDH6, CDH11 or CD44 expression and survival in OSCC patients. Overall survival rate of OSCC patients with high or low expression of (A) CDH6, (B) CDH11 and (C) CD44. Disease-specific survival rate of OSCC patients with high or low expression of (D) CDH6, (E) CDH11 and (F) CD44 were analyzed using Prism software. P-values and χ^2 values demonstrate statistically significant differences. CDH, cadherin; CD44, cluster of differentiation; OSCC, oral squamous cell carcinoma.

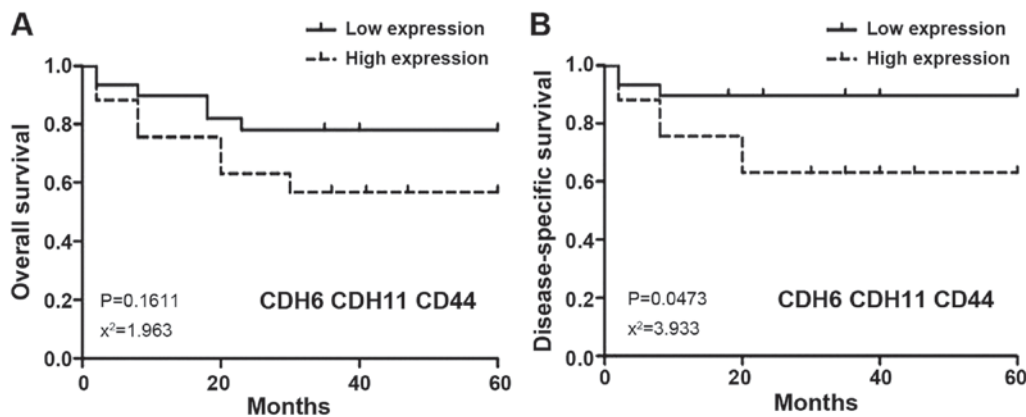


Figure 3. Association between co-expression of CDH6, CDH11 and CD44, and survival in patients with OSCC. (A) Overall and (B) disease-specific survival rates of patients with OSCC exhibiting high or low co-expression of CDH6, CDH11 and CD44 were analyzed using Prism software. P-values and χ^2 values demonstrate statistically significant differences. CDH, cadherin; CD44, cluster of differentiation 44; OSCC, oral squamous cell carcinoma.

was a statistically significant prognostic factor in several types of cancer, through its participation in EMT (38). However, the prognostic value was enhanced by co-evaluating the expression of CDH6, CDH11 and CD44 in the present study. Previous studies have reported that CDH11 interacts with angiomin (Amot) to promote the migration of cancer cells. Amot is known to be involved in cell polarity and migration (39,40). The Amot p80 isoform was revealed to regulate apical polarity by interacting with Rich1 (39), a small GTPase-activating protein, and to affect proliferation (41). CDH11 also regulates α -catenin turnover at the adherens junctions, facilitating the dynamic remodeling of cell-cell interactions, which are important for inter-cellular invasion (42,43). CDH11 was associated with signal transduction molecules, including the PI3K/Akt pathway (44).

In the present study, CD44 was revealed to be significantly associated with LNM, suggesting that CD44 is also involved in OSCC metastasis. These results are consistent with those of previous studies, which revealed that upregulation of CD44 represents a crucial event in the development of metastasis and that silencing CD44 expression suppressed its tumorigenic effects, including proliferation, migration and invasion in ovarian cancer and breast cancer cells (45,46). CD44 interacts with c-Src kinase to regulate the mitogen-activated protein kinase (MAPK), PI3K and signal transducer and activator of transcription 3 pathways (47). The MAPK and PI3K/Akt/mechanistic target of rapamycin pathways have been demonstrated to promote cancer cell proliferation and to enhance invasiveness and angiogenesis (18). In addition, a previous study revealed that CD44 expression is associated

with EMT and LNM in patients with OSCC (48). The present study revealed that CD44 was significantly associated with cadherins, including CDH6 and CDH11, and that its expression was also associated with the PI3K/Akt pathway. Therefore, it may be beneficial to combine these adhesion factors as metastasis-predictive parameters in order to improve their prognostic value, as multiple signal pathways, including the MAPK and PI3K/Akt pathways, may be involved in the metastasis of OSCC.

In conclusion, the present study demonstrated that the high expression of CDH6, CDH11 and CD44 in OSCC cells was significantly associated with an increased propensity to develop LNM and an advanced tumor stage. A positive association between CD44, CDH6 and CDH11 protein expression in OSCC patients was also observed in the present study. The combined use of these factors may improve the accuracy of metastasis and prognosis prediction. The results of the present study highlighted the important role of these adhesion factors in the progression of OSCC, which may provide a novel perspective in the prediction and prevention of metastasis in OSCC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CM drafted the article, designed the study and analyzed the data. JZ, RL, LZ, YC and LY collected and analyzed the data. TS, MW, ML and YL performed the experiments and collected the data. TZ designed the study, secured the funding and performed critical revision of the article.

Ethics statement and consent to participate

The study was approved by the Ethics Committee of Peking Union Medical College.

Consent for publication

All patients provided written informed consent for the publication of associated data and accompanying images.

Competing interests

The authors declare that they have no competing interests.

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