Original article

β-catenin expression pattern in primary oral squamous cell carcinoma

CAI Zhi-gang, SHI Xiao-jian, GAO Yan, WEI Ming-jie, WANG Cun-yu and YU Guang-yan

Keywords: *β-catenin; squamous cell carcinoma; immunohistochemical staining*

Background β-catenin, a 92 kDa protein that binds to the cytoplasmic tail of E-cadherin, has an essential role in intercellular adhesion and signal transduction. Aberrant expression of β-catenin has been associated with progression and metastasis of various human cancers. The aim of this study was to elucidate the expression pattern of β-catenin in primary oral squamous cell carcinoma and examine the correlation between β-catenin expression and tumor differentiation, histological grade and lymph node status as well as its clinical significances.

Methods Seventy-six patients with oral squamous cell carcinoma and sixteen metastatic lymph nodes were studied. The β-catenin expression was determined by immunohistochemical staining. The correlation with clinical, histological data was analyzed statistically.

Results Normal oral epithelium showed strong β-catenin expression at the cell membrane, but no cytoplasmic or nuclear expression. Different degrees of reduced expression of β-catenin at the cell membrane were found in 54 cases with squamous cell carcinoma (71%). Cytoplasmic β-catenin expression was found in 17 tumors (22.4%). Three cases were found with nuclear β-catenin expression. In sixteen lymph nodes with metastatic squamous cell carcinoma, negative β-catenin expression at the cell membrane was seen in 13 tumors (81.2%) and weak expression in 3 tumors (18.8%). Statistical analysis showed that there was an inverse correlation between β-catenin expression and lymph node status and histological grade of tumors.

Conclusions Reduced β-catenin expression at the cell membrane is clearly associated with lymph node metastasis. A reduced expression of β-catenin may constitute a hallmark of aggressive biological behavior of squamous cell carcinoma.

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The most common type of oral cancers is squamous cell carcinoma, which accounts for approximately 90% of oral malignancies. Postoperative life quality of the patients with oral squamous cell carcinoma has improved in recent years. However, the 5-year survival rate has not been raised significantly. About 30% of patients with early stage disease will eventually die as a result of their tumors. In addition, 30%–40% of patients without evidence of nodal disease at resection eventually die from metastatic spread.[1] Thus, early diagnosis and appropriate treatment are important. Although many prognostic parameters, such as CT and MRI, have been investigated for their accuracy in predicting the prognosis of squamous cell carcinoma, the results have been conflicting. Currently, the clinical staging system is considered as a classic criterion to guide treatments and evaluate prognosis. Unfortunately tumor heterogeneity and individual differences influence accurate estimation.[2] Therefore, developing a "molecular staging" system based on biomarkers and clinical parameters may help to improve our strategy for cancer treatment.[3]

The main characteristics of oral squamous cell carcinoma are invasive growth and the tendency to metastasize. Escape from the primary tumor is the first step of invasion and metastasis of cancer. This involves disruption of normal cell-cell adhesion. The loss of E-cadherin is thought to influence this cell dissociation.[4,5] Cadherins are transmembrane cell adhesion molecules that mediate cell-cell interactions and are important for maintenance of epithelial cell integrity.[6] This function is dependent on an indirect interaction between the cytoplasmic domain of the cadherin molecule with α-, β-, and γ-catenin. Growing evidence suggests that alterations in cadherin or catenin expression or function play an important role in the development of an invasive or metastatic phenotype.[7]

The aim of this study was to elucidate the expression of immunohistochemical localization of β-catenin in primary oral squamous cell carcinoma and examine the correlation between β-catenin expression and tumor differentiation, clinical stage (primary tumor, lymph node, and distant metastasis, TNM stage), and lymph node status.

Department of Oral and Maxillofacial Surgery (Cai ZG, Shi XJ and Yu GY), Department of Oral Pathology (Gao Y and Wei MJ), Peking University School and Hospital of Stomatology, Beijing 100081, China

Division of Oral Biology and Medicine, UCLA School of Dentistry, Los Angeles, CA 90095, USA (Wang CY)

Correspondence to: Prof. WANG Cun-yu, Division of Oral Biology and Medicine, UCLA School of Dentistry, Los Angeles, CA 90095, USA (Tel: 01-310-8254415. Fax: 01-310-7947109. Email: cwang@dentistry.ucla.edu); Prof. YU Guang-yan, Department of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology, Beijing 100081, China (Tel: 86-10-62191099. Fax: 86-10- 62173402. Email: gyyu@263.net)

METHODS

Seventy-six cases of oral squamous cell carcinoma were selected from the Department of Oral Pathology, Peking University School of Stomatology. There were 47 male and 29 female patients, mean age 62 years. Clinical data were retrieved from the register of medical records of Department of Oral and Maxillofacial Surgery, including age, gender, location, TNM stage, histopathological grade, and lymph node status of the tumors. Clinical stage was assigned using the TNM classification of the International Union Against Cancer (UICC). There were 14, 27, 22, and 13 cases for T1, T2, T3, and T4, respectively. The histological grade of tumors was evaluated on the basis of the World Health Organization (WHO) criteria. There were 34 cases classified as grade I, 31 cases as grade II and 11 cases as grade III. The locations of the tumor are shown in Table 1. All patients with radiotherapy or chemotherapy before operation were excluded from this study. Sixteen lymph nodes with squamous cell carcinoma metastasis were also studied in this research. The pertinent data on the patients are summarized in Table 1.

Table 1. Clinical and pathological data on the patients

Clinical and pathological data	Patients (*n*)
Number of patients	76
T category	
T1	14
T2	27
T3	22
T4	13
TNM stage	
Stage I	12
Stage II	24
Stage III	24
Stage IV	16
Histological grade	
Grade I	34
Grade II	31
Grade III	11
Lymph node metastasis	
Yes	44
No	32
Location of the tumors	
Tongue	24
Floor	16
Gingiva	14
Buccal	11
Lip	4
Hard palate	3
Maxillary sinus	2
Mandible	2

Immunohistochemistry

The paraffin sections of 5 μm were deparaffinized, rehydrated in graded alcohols and washed twice for 5 minutes with phosphate buffered saline (PBS; pH 7.2-7.4). Fresh 3% hydrogen peroxidase in methanol was used to remove endogenous peroxides activity for 10 minutes and washed 3 times with PBS. The sections were heated in a microwave oven in 0.1 mmol/L citrate buffer for two 5 minutes, washed with PBS after they were cold. After incubation with normal goat serum to block non-specific binding, the tissue sections were incubated overnight at 4°C with a primary mouse monoclonal anti-β-catenin antibody (Transduction Laboratories, USA) at a dilution of 1: 500. After another washing step, the sections were treated with secondary antibody and streptavidin-biotin complex (SABC) for 20 minutes at room temperature. 3, 3-diaminobenzidine tetrahydrochloride (DAB) was used as chromogen. Finally, the samples were slightly counterstained with haematoxylin, dehydrated, cleared and mounted. In each staining batch, normal epithelium served as a positive control. In negative controls, primary antibody was omitted.

Evaluation of staining

The expression of β-catenin in cancer cells was compared with that of normal epithelial cells in the same sample. Tumors in which > 75% of the cancer cells stained were considered strong stain. When between 25% and 75% positively stained, the tumor cells were considered to be weakly stained. The tumor cells that stained positively less than 25% or accompanied with cytoplasm or nuclear staining were considered to have negative staining. The original haematoxylin and eosin (HE) stained sections were reviewed and pathology grade classification I, II, and III were scored.

Statistical analysis

The chi-square and Fisher's exact tests, where appropriate, were used to analyze the statistical significance of the relationship between β-catenin expression pattern and the clinicopathological variables. Multivariate analysis was done using the Logistic regression. SPSS 10.0 program package was used for statistical analysis. *P* value less than 0.05 was regarded as statistically significant.

RESULTS

Expression of β-catenin in normal epithelium

Normal epithelium showed strong β-catenin at the membrane and the intercellular junctions of the cells (Figure 1). Expression of these molecules was more marked in the prickle cell layer. The membranous staining in basal cells bounded in submucosa was not found. No cytoplasmic or nuclear staining of β-catenin was seen in normal epithelium.

Expression of β-catenin in squamous cell carcinoma

The reduced expression of β-catenin was found in 54 tumors (71%). Cytoplasmic β-catenin expression was seen in 14 tumors (18.4%). The nucleus staining (Figure 2) was rare and only 3 cases were seen in the total 76 cases. The cytoplasm and nuclear β-catenin expression was only seen in the tumors with poor differentiation (Figure 3). In well differentiated cancer nests, keratin pearls or keratinizing cells in the middle showed weak or negative β-catenin staining (Figure 4). However, negative β-catenin expression was also found in the edge of poor differentiated cancer nests (Figure 5). In sixteen lymph nodes with metastatic

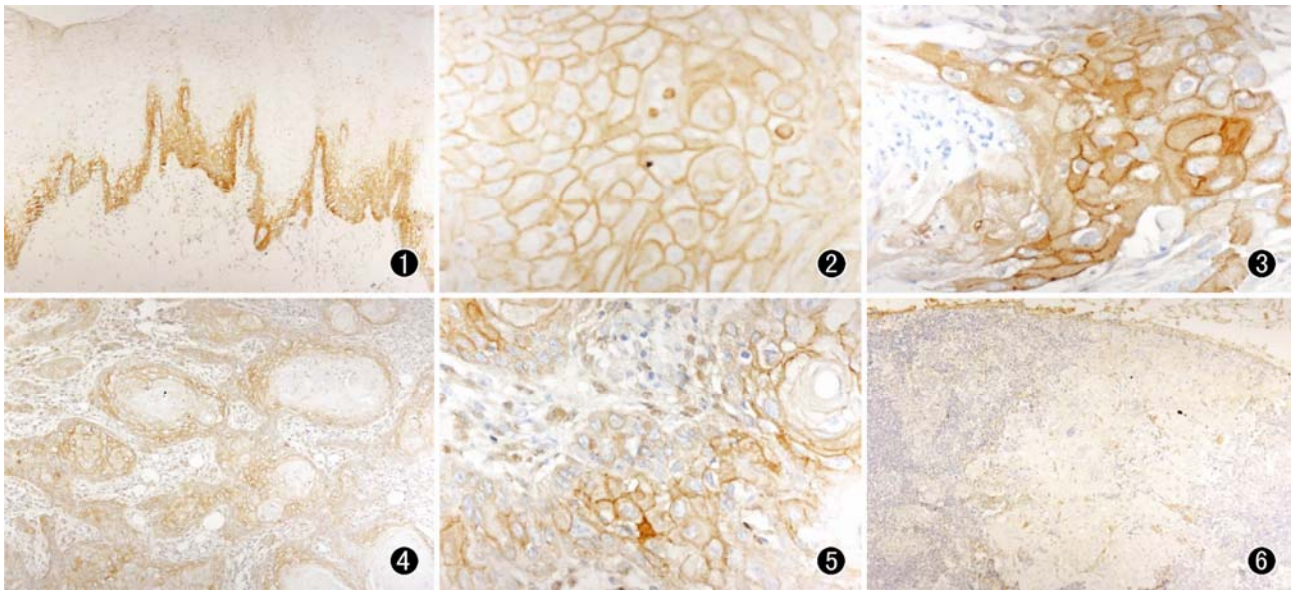

Figure 1. Normal epithelium showed strong β-catenin expression at the membrane and the intercellular junctions of the cells (HE staining, original magnification × 100).
Figure 2. Well differentiated carcinoma, nuclear β-catenin expression (HE staining, original magnification × 200).
Figure 3. Poor differentiated carcinoma, cytoplasmatic β-catenin expression (HE staining, original magnification × 200).
Figure 4. Well differentiated cancer nests, strong expression of β-catenin, keratin pearls or keratinizing cells in the middle showed weak or negative β-catenin staining (HE staining, original magnification × 100).
Figure 5. Poor differentiated carcinoma, weak expression of β-catenin, the margin of the cancer nests showed negative staining (HE staining, original magnification × 200).
Figure 6. Metastatic lympho node, negative β-catenin expression (HE staining, original magnification × 100).

squamous cell carcinoma, negative β-catenin expression was seen in 13 tumors (81.2%) and weak expression in 3 tumors (18.8%). No strong expression of β-catenin was found in these lymph nodes (Figure 6). Table 2 shows the relationship between clinical, histopathologic, lymph nodes status and expression of β-catenin.

Table 2. Relationship between clinical, histopathologic, lymph nodes metastasis and expression of β-catenin

Variables	Patients (*n*)	β-catenin expression (*n*)		
		Negative	Weak	Strong
T category				
T1	14	4	3	7
T2	27	9	10	8
T3	22	9	9	4
T4	13	3	7	3
TNM stage				
Stage I	12	3	2	7
Stage II	24	8	9	7
Stage III	24	10	10	4
Stage IV	16	4	8	4
Histological grade				
Grade I	34	5	14	15
Grade II	31	14	11	6
Grade III	11	6	4	1
Lymph node metastasis				
Yes	44	17	22	5
No	32	8	7	17
Metastatic lymph nodes	16	13	3	0

Relationship between β-catenin expression and clinical, histopathologic variables

For statistical purposes, reduced and preserved β-catenin expression classification seemed more useful in assessment the correlation between the clinico-pathological variables of tumors and the β-catenin expression pattern. No significant differences were found among age, gender, location of tumor, T category, TNM stage and the variable of β-catenin expression. A significant inverse correlation existed between the β-catenin expression and histological grade (χ^2=7.300, P=0.026, Table 3) and lymph node metastasis (χ^2=20.114, P<0.001, Table 4).

Table 3. Relationship between β-catenin expression and histological grade*

Histological grades	Reduced	Preserved	Total
Grade I	19	15	34
Grade II	25	6	31
Grade III	10	1	11

*Pearson chi-square: value: 7.300, df: 2, Sig. (2-sided): 0.026.

Table 4. Relationship between β-catenin expression and lymph node status*

Lymph node metastasis	Negative	Weak	Strong	Total
Yes	17	22	5	44
No	8	7	17	32

*Pearson chi-square: value: 16.049, df: 2, Sig. (2-sided): 0.000.

Multivariate analysis was performed by means of the Logistic regression by entering the following variables: T category of the tumors, TNM stage, histological grade, lymph node status and β-catenin expression. The selected variable was β-catenin expression. The result showed that only lymph node status had an inverse correlation with β-catenin expression (P=0.0129, Table 5).

Sixteen lymph nodes with metastatic squamous cell carcinoma were studied. Compared to primary carcinomas,

Table 5. Multivariate Logistic regression (dependent variable: reduced, preserved β-catenin expression)

Variables	B	SE	Wald	df	Sig.	R	Exp (B)
T	1.0795	0.6755	2.5544	1	0.1100	0.0771	2.9433
S	–0.9124	0.6936	1.7306	1	0.1883	0.0000	0.4015
G	0.849	0.5022	2.8632	1	0.0906	0.0962	2.3390
L	–1.7817	0.7162	6.1893	1	0.0129	0.2120	0.1684

there was negative expression of β-catenin in 13 metastatic lymph nodes out of 16 cases. Three lymph nodes revealed weak expression and no sample showed strong staining pattern. The frequency of β-catenin loss in metastatic lymph nodes versus primary carcinomas was statistically significant (χ^2 =13.545, *P*=0.001, Table 6).

Table 6. The frequency of β-catenin loss in lymph node metastases versus primary carcinomas*

Lesion	Negative	Weak	Strong	Total
Primary carcinomas	25	29	22	76
Metastatic lymph nodes	13	3	0	16
Total	38	32	22	92

*Pearson chi-square: value: 13.545, df: 2, Sig. (2-sided): 0.001.

DISCUSSION

The aim of this study was to investigate the expression pattern of β-catenin in primary oral squamous cell carcinoma and examine the relationship between β-catenin expression and clinicopathologic variables. We studied the location, T status, TNM stage, histologic grade, lymph node status, β-catenin expression of tumors in 76 cases.

The expression of E-cadherin and β-catenin has been examined in several human carcinomas, including oesophagus, stomach, colon and breast. In these carcinomas, β-catenin expression was often reduced compared with normal epithelium.[8] It was reported that 88% of head and neck squamous cell carcinomas showed reduced membranous β-catenin expression.[9] We observed 71% of tumors had reduced β-catenin expression in our 76 cases. The difference may be due to the semi-quantitative of immunohistochemical analysis, the specific antibody used, techniques in tissue procurement.

The clinical significance of β-catenin expression in oral cancer is controversial. Several investigators have reported that reduced expression of β-catenin has correlated with poorly differentiated tumors, advanced stage, and poor survival.[10] Decreased expression of β-catenin was found to be significantly correlated with lymph node status of the patients by some studies[11,12] and not by others.[13,14] We studied the relationship between β-catenin expression and clinicopathological variables.

The important finding is that the β-catenin expression has an inverse correlation with lymph node metastasis (*P* <0.0001) and histological grade (*P*=0.026). In multivariate regression analysis revealed that only lymph node status was correlated with reduced β-catenin expression (*P*=0.0129). Bánkfalvi et al[15] also pointed out that decreased β-catenin expression was a predictive marker for lymph node metastases. Their results are in line with our present study. As lymph node metastasis is one of the most important prognostic factors in squamous cell carcinoma, it is not very surprising that we find a significant prognostic value for β-catenin expression in squamous cell carcinoma.

β-catenin plays an important role in the interactions between cadherins and other transmemberane receptor proteins and regulated cellular differentiation and proliferation. Reduced and absent expression of β-catenin in tumors results in cell proliferation, migration, and invasion, and is associated with poor prognosis.[10,16,17] Jawhari et al[18] reported that reduced expression of catenin might be a useful prognostic marker, independent of tumor type, grade or stage.

The results from this study indicate that reduced β-catenin protein in oral squamous cell carcinoma may disrupt stability and integrity of the E-cadherin/catenin complex and disturb cellular adheren junctions, resulting in the dissociation of the tumor cells from primary sites, thereby allowing metastases. The other main characteristic of malignant tumors is invasive growth. Down regulation of β-catenin expression is associated with increased invasiveness.[19,20] This viewpoint is supported by our study. A low histological grade has an inverse correlation with β-catenin expression of tumors, although the correlation was of negative significance in the multivariate analysis. In sixteen studied metastatic lymph nodes, the frequency of β-catenin loss in lymph node metastases is higher than that of primary carcinomas (*P*=0.001). The reduced β-catenin expression was often found in the margin of the tumor nests, in which it is believed the tumor cells are more invasive.

β-catenin is a multifunctional protein involved in two apparently independent processes: cell-cell adhesion and signal transduction. In addition to its role in regulating E-cadherin-mediated cell adhesion, β-catenin is a transcription cofactor in the wingless (Wnt) signaling pathway and a target of the adenomatosus polyposis coil (APC) gene product, which is implicated in the initiation of different human cancers.[13,21] Disruption of these two β-catenin pathways could be important in tumor development and progression. APC gene mutations lead to the accumulation of cytoplasmic and nuclear β-catenin. Deregulated expression of β-catenin, which may result from APC defects, activating mutations in the β-catenin gene itself or other alterations in the Wnt pathway, has been implicated as an important step in carcinogenesis.[22] Pirinen et al[23] reported that nuclear staining of β-catenin was found in 16 (7%) cases in his study and indicated that a reduction in the nuclear expression of β-catenin is a sign of more aggressive tumor behavior. Pukkila et al[24] studied 161 patients with primary oropharyngeal and hypo-pharyngeal squamous cell carcinoma. Nuclear β-catenin expression was positive in 23% of the tumors. In

univarite analysis, patients whose tumors had nuclear β-catenin expression had shorter overall survival than patients with no nuclear expression. In the present study, we found that 3 cases have nuclear β-catenin expression and 17 cases with cytoplasmic β-catenin expression. No statistically significant difference was found between the patients with nuclear or cytoplasm β-catenin expression and patients without such expression, but further studies of the nuclear β-catenin expression in oral squamous cell carcinoma should be conducted. Importantly, we found β-catenin expression has an inverse correlation with lymph node status, which is consistent with other researches,[11,12] and suggesting that assessment of β-catenin expression is clinically useful, especially for selecting patients that need neck dissection. Close follow-up or comprehensive treatment is necessary for patients with low β-catenin expression.

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