RESEARCH ARTICLE

β-Catenin Alterations in Squamous Cell Carcinoma of the Lip

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Abstract

This study aimed to investigate the correlation between ß-catenin immunoexpression and histopathological grades of lower lip squamous cell carcinoma (LSCC). β-Catenin abnormal expression was found in 29% of the squamous cells of well differentiated LSCC, 63% of moderately differentiated and 86% of poorly differentiated, and therefor was significantly associated with histological grade (p=0.000). Nuclear β-catenin expression appeared in 5% of the cells and was also correlated with the histological grades (p=0.000). In 14.7% of the cells it was localized in the cytoplasm, again correlating with histology (p=0.002). According to this study the expression of β-catenin is an independent prognostic factor for histological grade and to the tumor differentiation. This appears to reflect a structural association and the role of β-catenin in tumor progression.

Keywords: Lip squamous cell carcinoma - β-catenin - immunoexpression

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Introduction

Head and neck cancers are the sixth most common malignancy in the world, accounting for more than 500,000 new cases every year (Zhu et al., 2015). Oral squamous cell carcinoma is the most common type of cancer in the oral cavity, representing more than 90% of all oral cancers - composes About 95% of oral cancers in India (Krishna et al., 2014) and The prevalence of oral cancers is high in Asian countries, especially in South and Southeast Asia (Krishna Rao et al., 2013; Kuo et al., 2015)-The characterization of altered molecules in oral cancer is essential to understand molecular mechanisms underlying tumor progression as well as to contribute to cancer biomarker and therapeutic target discovery. The most important risk factors for oral SCC are: use of tobacco or betel quid and the regular drinking of alcoholic beverages. However, infection with high-risk human papillomavirus (HPV) genotypes, and a diet low in fresh fruits and vegetables have also recently been implicated in the aetiopathogenesis of oral SCC (El-Mofty, 2014). The considerable variation in the pattern of oral and of oropharyngeal cancer incidence in different parts of the world reflects differences in the prevalence of specific risk factors.

Histologically the lip can be divided in three different parts. The cutaneous lip goes over the vermilion or dry mucosa of the lip, which forms a transition zone between the skin and the wet oral mucosa. Its lateral limits are about 1cm from the lip commissures: the upper limits are the naso-labial groove at the skin and the gingivo-labial groove at the mucosa side; the lower limits are the mento-labial and the lower gingivo-labial groove. Its lymphatic drainage goes to the sub-mental, sub-mandibulary and sub-digastric lymph nodes. The submandibular nodes are the most frequently involved. Most lip cancers are squamous cell cancers (90%). The remaining 10% are basal cell cancer (starting from the cutaneous area). Sarcomas, cylindromas, melanomas are very rare.

Pre-cancerous conditions, such as actinic cheilitis, leucoplakia, Bowen's disease, are frequently associated. They often constitute a diagnostic and an additional therapeutic problem. In general these associated lesions can be adequately treated by radiotherapy but form rather a contraindication to limited surgery. All patients affected with tumours reaching the median line are at high risk for bilateral nodal involvement. The mean age of a patient suffering from lip cancer is over 65 years. (Pigneux et al., 1979; Orecchia et al., 1991; Tombolini et al., 1998)

Farm labourers and fishermen have a higher risk of lip cancer, as they are likely to be exposed to these risk factors. A high incidence of lip cancer is found among white races exposed to solar radiation in Ausralia and Portugal (Ariyawardana and Johnson, 2013; Monteiro et al., 2013). Despite advancements in prevention and multimodality treatments, oral cancer is still characterized by poor prognosis and a low survival rate (da Silva et al., 2011).

Catenin (cadherin-associated protein), beta 1, 88kDa (the HUGO-approved official symbol, CTNNB1; HGNC ID,HGNC:2514), also called beta-catenin (or β-catenin), is a dual function protein, regulating the coordination of cell–cell adhesion and gene transcription. In humans, the CTNNB1 protein is encoded by the CTNNB1 gene (MacDonald et al., 2009).

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The junctional portion of ß-catenin links E-cadherin to alpha-catenin and consequently to the structure of the actin microfilaments of the cytoskeleton; playing this way a very representative role with respect to cell adhesion(Akdeniz et al., 2013). Corroborating this importance, several studies have shown that deregulation of cadherin-catenin complex, in addition to reduced expression of ß-catenin, is present in several types of malignancies, especially those of a more aggressive nature and a higher metastatic potential (Beavon, 2000; Kurtz et al., 2006).

On the other hand, non-junctional portion of ß-catenin is rapidly phosphorylated and degraded in the cytoplasm. However, the activation of the Wnt induces the stabilization of cytoplasmic ß-catenin, facilitating its translocation to the nuclei. In the nuclei, the protein binds to members of the family of transcription factors T-cell factor/ lymphoid enhancer factor - TCF/LEF (Beavon, 2000). Such deposits can contribute to the development of malignancies(Gao et al., 2005). Wnt/β-catenin is one of the intracellular signaling pathways that may control transcription factor binding to the c-myc promoter. Interestingly, the Wnt/β-catenin pathway has been reported to be a target of COX-2 inhibitors.

Thus, this study was to evaluate the immunohistochemical expression of ß-catenin in squamous cell carcinoma of the lower lip with in order to establish a correlation between the expression of this protein and the histopathological features of the tumor.

Materials and Methods

Sample selection

To perform this study, 29 cases of lower lip SCC were selected from the archive of the department of oral histology and pathology in the faculty of dentistry-Damascus University. The cases were divided into three groups according to the histological differentiation : grade I (n=11) and grade II (n=9) and grade III (n=9).

Five samples containing oral normal epithelium obtained from patients who did not have cancer, were used as a control group.

Immunohistochemistry

Histological sections of 3µm in thickness were obtained from tissue samples fixed in 10% formalin and embedded in paraffin. The specimens were processed by the immunohistochemical technique by the streptavidin-biotin method, using anti-ß-catenin polyclonal antibodie at a dilution of 1:500 (Ab-1 clone, LabVision/Neomarkers). The sections were subjected to antigen retrieval through steamer treatment, for 25 minutes, in citric acid (pH 6.0). After incubation with primary, secondary antibodies and streptavidin-biotin complex (DAKO, A/S, Glostrup, Denmark), revelation of the sections using a diaminobenzidine chromogen solution and a counter-staining with Mayer's hematoxylin were conducted.

Based on the histological grading proposed by Bryne et al. (1989), two observers identified the front of tumor invasion (light microscopy-Olympus CX-31) and at a 400x magnification and analyzed the presence or absence of immunostaining in the cell membrane, cytoplasm, nucleus or a combination of these.

Statistical analysis

One-way Anova and Tukey's test were used for comparison and correlation between the different grades of lip SCC. Pearson correlation coefficient was used to establish the correlation between the expressions of both strainers.

Results

Data related to ß-catenin immunoexpression are shown in (Table 1). It was observed a variable expression in relation to the topography of ß-catenin, which was detected in cytoplasmic membrane, cell nucleus or both (Figure 1, 2), the expression was aberrant in 58.6% of the cells (29% of WDLSCC, 63.7% of MDLSCC and 86.5% of PDLSCC), there was a significant correlation ($p<0.05$) of β-catenin aberrant expression and the histological degrees , whereas in normal epithelia The staining

Table 1. β-catenin Abnormal Expression

Grade	β-catenin expression			
	Highest value	Lower value	Standard deviation	median
WDLSCC	38.8%	15.6%	6.1%	29.1%
MDLSCC	74.5%	51.7%	8.2%	63.8%
PDLSCC	93.9%	76.9%	6.3%	86.6%
Total	93.9%	15.6%	25.5%	58.6%

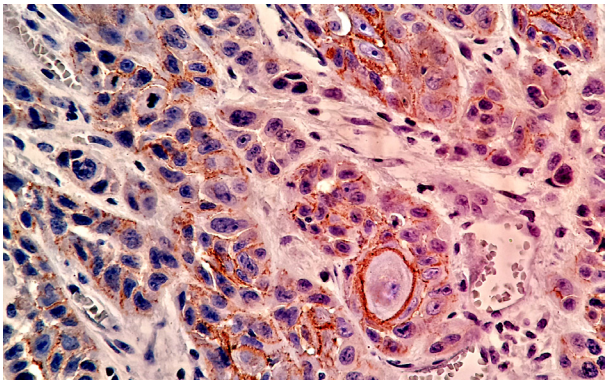

Figure 1. β-catenin Expression in LSCC. a) the normal membranous expression; b) absence of β-catenin expression in squamous cells

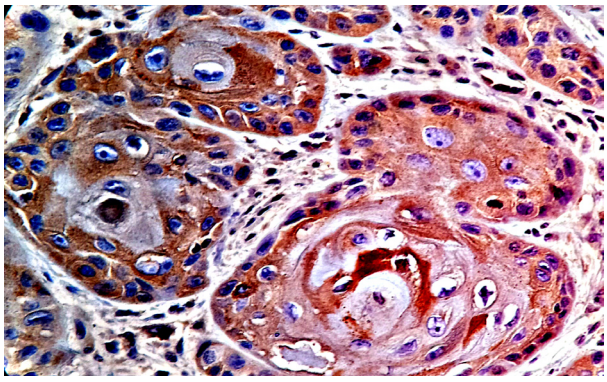

Figure 2. β-catenin Expression in LSCC. a) the cytoplasmic expression; b) the nuclear expression

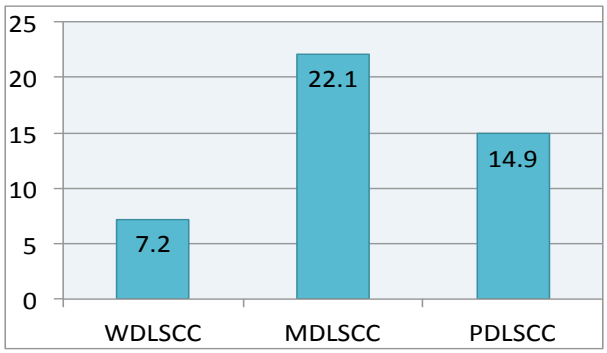

Figure 3. The Distribution of Cytoplasmic Expression of β-catenin

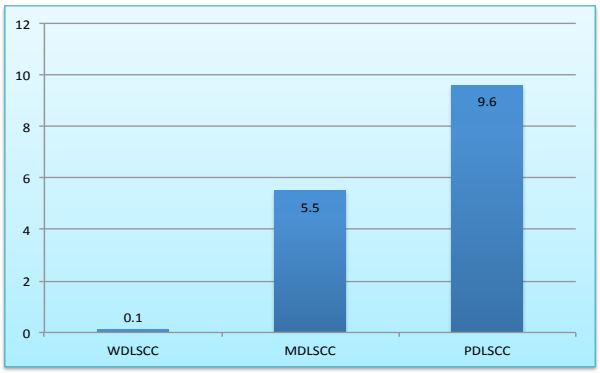

Figure 4. The Distribution of Nuclear Expression of β-catenin

patterns and localization were identical. the marker brightly decorated the epithelium in a circumferentially membranous fashion (basolateral and glycocalyceal). No cytoplasmic or nuclear staining was noted. In all 5 control cases, the basal and parabasal cells displayed the greatest intensity of staining, whereas the most external and differentiated layers were not stained.

14.7% of the cells exhibited a cytoplasmic expression (7.2% of WDLSCC, 22.1% of MDLSCC and 14.9% of PDLSCC) (Figure 3), there was a significant correlation between WDLSCC and MDLSCC (p=0.001) also between WDLSCC and PDLSCC (p=0.041) and no significant correlation between MDLSCC and PDOSCC (p=0.421) .The nuclear expression for β-catenin appeared in 5% of the cells (0.1% of WDLSCC, 5.5% of MDLSCC and 9.6% of PDLSCC) (Figure 4), there was a significant correlation (p<0.05) of β-catenin expression and the histological degrees.

Discussion

It has been shown a close association between the reduction of the intercellular adhesion and the loss of cell Differentiation which affects the histopathological classification of the squamous cells carcinoma (Beavon, 2000), One of the molecules related to cell adhesion is ß-catenin, with a dichotomous effect: it is able not only to actively participate in the process of intercellular adhesion but also related to intracellular signals transduction.

In our study, a clear increasing of ß-catenin abnormality was observed. More than 56% of the tumor cells in the whole sample showed abnormal expression for ß-catenin , in 14.7% a cytoplasmic expression was noticed whereas the nuclear expression appeared in 5% of the cells. these results are in line with other studies of oral SCC, as has been observed reduced expression of ß-catenin molecule (Andrews et al., 1997; Chow et al., 2001; Zaid, 2014). except the relation between MDLSCC and PDOSCC (p=0.421). the cytoplasmic accumulation of β-catenin in the cytoplasm increases its chances to enter the nucleus where it play his role in activating the tumoral genes transcription (Zaid, 2014).

the statistical analysis showed a significant differences between ß-catenin expression between the groups (p<0.05).

These results is in concordance with some studies which were able to establish a statistically significant relationship between the decreased ß-catenin expression with the histologic differentiation of SCC(Odajima et al., 2005; Cai et al., 2008) and with (Yothaisong et al., 2014) who found that Wnt/β-catenin signaling pathway expression increased along with carcinogenesis processes.

but not in concordance with(Fadare et al., 2005) who studied a different anatomical site-cervical carcinoma- and that may be the reason behind this difference. some factors limited our ability to compare data with previous studies cause we analyzed only cases of OSCC in Lip, since the anatomical location of oral squamous cell carcinoma influences the biological behavior of this tumor(Bankfalvi et al., 2002). It is also possible that the loss of intercellular adhesion is only one of the stages required for the occurrence of metastases. Soon, there could be a need for other phenomena, such as loss of cell adhesion to the extracellular matrix, which can be caused by the metaloproteinases expression(Lopes et al., 2009).

In conclusions, the results of the present study proved that the aberrant expression of β-catenin is a significant factor in predicting the histological grade in patients with Lip SCC. Thus, we believe that analyzing the expression patterns and locations of β-catenin is useful with other previously determined clinicopathologic indices.

References

Akdeniz O, Akduman D, Haksever M, et al (2013). Relationships between clinical behavior of laryngeal squamous cell carcinomas and expression of VEGF, MMP-9 and E-cadherin. *Asian Pac J Cancer Prev*, **14**, 5301-10.

Andrews NA, Jones AS, Helliwell TR, et al (1997). Expression of the E-cadherin-catenin cell adhesion complex in primary squamous cell carcinomas of the head and neck and their nodal metastases. *Br J Cancer*, **75**, 1474-80.

Ariyawardana A, Johnson NW (2013). Trends of lip, oral cavity and oropharyngeal cancers in Australia 1982-2008: overall good news but with rising rates in the oropharynx. *BMC Cancer*, **13**, 333.

Bankfalvi A, Krassort M, Buchwalow IB, et al (2002). Gains and losses of adhesion molecules (CD44, E-cadherin, and beta-catenin) during oral carcinogenesis and tumour progression. J Pathol, 198, 343-51.

Beavon IR (2000). The E-cadherin-catenin complex in tumour metastasis: structure, function and regulation. *Eur J Cancer*, **36**, 1607-20.

Bryne M, Koppang HS, Lilleng R, et al (1989). New malignancy

grading is a better prognostic indicator than Broders' grading in oral squamous cell carcinomas. *J Oral Pathol Med*, **18**, 432-7.

Cai ZG, Shi XJ, Gao Y, et al (2008). beta-catenin expression pattern in primary oral squamous cell carcinoma. *Chin Med J (Engl)*, **121**, 1866-70.

Chow V, Yuen AP, Lam KY, et al (2001). A comparative study of the clinicopathological significance of E-cadherin and catenins (alpha, beta, gamma) expression in the surgical management of oral tongue carcinoma. *J Cancer Res Clin Oncol*, **127**, 59-63.

da Silva SD, Ferlito A, Takes RP, et al (2011). Advances and applications of oral cancer basic research. *Oral Oncol*, **47**, 783-91.

El-Mofty SK (2014). Histopathologic risk factors in oral and oropharyngeal squamous cell carcinoma variants: an update with special reference to HPV-related carcinomas. *Med Oral Patol Oral Cir Bucal*, **19**, 377-85.

Fadare O, Reddy H, Wang J, et al (2005). E-Cadherin and beta-Catenin expression in early stage cervical carcinoma: a tissue microarray study of 147 cases. *World J Surg Oncol*, **3**, 38.

Gao S, Eiberg H, Krogdahl A, et al (2005). Cytoplasmic expression of E-cadherin and beta-Catenin correlated with LOH and hypermethylation of the APC gene in oral squamous cell carcinomas. *J Oral Pathol Med*, **34**, 116-9.

Krishna A, Singh RK, Singh S, et al (2014). Demographic risk factors, affected anatomical sites and clinicopathological profile for oral squamous cell carcinoma in a north Indian population. *Asian Pac J Cancer Prev*, **15**, 6755-60.

Krishna Rao SV, Mejia G, Roberts-Thomson K, et al (2013). Epidemiology of oral cancer in Asia in the past decade--an update (2000-2012). *Asian Pac J Cancer Prev*, **14**, 5567-77.

Kuo CH, Lin YW, Chen RS (2015). Lipopeptides extract from bacillus amyloliquefaciens induce human oral squamous cancer cell death. *Asian Pac J Cancer Prev*, **16**, 91-6.

Kurtz KA, Hoffman HT, Zimmerman MB, et al (2006). 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*, **134**, 142-6.

Lopes FF, da Costa Miguel MC, Pereira AL, et al (2009). Changes in immunoexpression of E-cadherin and beta-catenin in oral squamous cell carcinoma with and without nodal metastasis. *Ann Diagn Pathol*, **13**, 22-9.

MacDonald BT, Tamai K, He X (2009). Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell*, **17**, 9-26.

Monteiro LS, Antunes L, Bento MJ, et al (2013). Incidence rates and trends of lip, oral and oro-pharyngeal cancers in Portugal. *J Oral Pathol Med*, **42**, 345-51.

Odajima T, Sasaki Y, Tanaka N, et al (2005). Abnormal beta-catenin expression in oral cancer with no gene mutation: correlation with expression of cyclin D1 and epidermal growth factor receptor, Ki-67 labeling index, and clinicopathological features. *Hum Pathol*, **36**, 234-41.

Orecchia R, Rampino M, Gribaudo S, et al (1991). Interstitial brachytherapy for carcinomas of the lower lip. results of treatment. *Tumori*, **77**, 336-8.

Pigneux J, Richaud PM, Lagarde C (1979). The place of interstitial therapy using 192 iridium in the management of carcinoma of the lip. *Cancer*, **43**, 1073-7.

Tombolini V, Bonanni A, Valeriani M, et al (1998). Brachytherapy for squamous cell carcinoma of the lip. the experience of the institute of radiology of the university of Rome "La Sapienza". *Tumori*, **84**, 478-82.

Yothaisong S, Thanee M, Namwat N, et al (2014). *Opisthorchis viverrini* infection activates the PI3K/ AKT/PTEN and Wnt/ beta-catenin signaling pathways in a cholangiocarcinogenesis model. *Asian Pac J Cancer Prev*, **15**, 10463-8.

Zaid KW (2014). Immunohistochemical assessment of E-cadherin and beta-catenin in the histological differentiations of oral squamous cell carcinoma. *Asian Pac J Cancer Prev*, **15**, 8847-53.

Zhu DW, Yuan YX, Qiao JK, et al (2015). Enhanced anticancer activity of a protein phosphatase 2A inhibitor on chemotherapy and radiation in head and neck squamous cell carcinoma. *Cancer Lett*, **356**, 773-80.