Oral Oncology 49 (2013) 598–603

Contents lists available at SciVerse ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Podoplanin expression in oral leukoplakia: Tumorigenic role

Juan Carlos de Vicente [a,c,*], Juan Pablo Rodrigo [b,c], Tania Rodriguez-Santamarta [a], Paloma Lequerica-Fernández [c], Eva Allonca [c], Juana María García-Pedrero [c]

[a] Department of Oral and Maxillofacial Surgery, Facultad de Medicina, Universidad de Oviedo, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain
[b] Department of Otolaryngology, Facultad de Medicina, Universidad de Oviedo, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain
[c] Instituto Universitario de Oncología del Principado de Asturias, Oviedo, Asturias, Spain

ARTICLE INFO

Article history:
Received 11 December 2012
Received in revised form 6 February 2013
Accepted 14 February 2013
Available online 7 March 2013

Keywords:
Oral leukoplakia
Epithelial dysplasia
Oral cancer
Squamous cell carcinoma
Malignant transformation
Podoplanin
Immunohistochemistry

SUMMARY

Objectives: Recent studies have identified podoplanin, a mucin-type transmembrane glycoprotein, as a biomarker for oral cancer risk in patients with oral leukoplakia (OPL). The aim of this study was to investigate the potential association between podoplanin and the risk of malignant transformation of OPL with epithelial dysplasia.
Materials and methods: In this retrospective study, podoplanin immunoexpression was analyzed in 58 patients with oral leukoplakia that showed epithelial dysplasia. Lesions with podoplanin expression in the basal and suprabasal layers of oral epithelium at one area or showing suprabasal expression at two or more areas were considered as positive. Association between podoplanin expression and oral cancer development was analyzed.
Results: Twenty-two of the 58 lesions (38%) were classified as podoplanin-positive, and the remaining 36 (62%) lesions were considered podoplanin-negative. The expression of podoplanin was correlated with the grade of dysplasia ($p < 0.0005$), and with the risk of progression to oral cancer ($p < 0.0005$). In multivariate survival analysis, only premalignant oral lesions displaying positive podoplanin expression showed a significantly increased risk of developing an oral squamous cell carcinoma (hazard ratio = 8.738, $p = 0.007$).
Conclusion: Podoplanin could be a valuable biomarker for risk assessment of malignant transformation in patients with OPL along with histological assessment.

Introduction

The majority of head and neck cancers are squamous cell carcinomas of the upper aerodigestive tract. Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the head and neck region,[1] and it is frequently preceded by premalignant lesions. Leukoplakia (OPL) is the most commonly diagnosed preneoplastic lesion in the oral cavity[2] and shows a rate of malignant transformation between 17% and 24% with a median follow-up more than 7 years, and during periods of up to 30 years.[3–5] Although dysplastic features are thought to be the most important indicator for risk of oral cancer, its progression ranges from 6% to 36%.[4] Consequently, the majority of oral cancers develop from lesions that lack dysplastic changes.[3,4] Furthermore, at present, histologic assessment of epithelial dysplasia is the gold standard for determining the malignant transformation risk of OPL; however, the accuracy of the histopathologic assessment of epithelial dysplasia depends on the quality of the tissue, the site at which a biopsy is taken, and it is also largely subjective, with interobserver and intraobserver variability.[6] Therefore, the identification of biologic and molecular factors that may serve to reliably identify high-risk lesions is needed. Recently, Kawaguchi et al.[7] have demonstrated the potential of podoplanin as a biomarker for oral cancer risk assessment in patients with OPL, and analogous results have been obtained by analyzing a series of laryngeal premalignancies.[8]

Podoplanin is a mucin-like transmembrane glycoprotein that is highly and specifically expressed in lymphatic endothelial cells, but not in blood endothelium.[9] Although its biological function is not yet clearly understood, it has been suggested that podoplanin may act as a mediator of tumor cell invasion and metastasis.[10] Podoplanin is also expressed in hyperplastic and dysplastic areas adjacent to the primary tumors,[11] indicating that its abnormal expression occurs early in oral tumorigenesis. Moreover, in oral premalignant lesions there are molecular genetic traits in common with OSCC even in the absence of histologically defined dysplasia.[12]

The purpose of this study was to investigate the potential association between podoplanin and the risk of malignant transformation of oral leukoplakia with epithelial dysplasia.

* Corresponding author. Address: Department of Oral and Maxillofacial Surgery, Facultad de Medicina, Universidad de Oviedo, Hospital Universitario Central de Asturias, c/Catedrático José Serrano s/n, 33006 Oviedo, Asturias, Spain. Tel.: +34 985 103638; fax: +34 985 103673.
E-mail address: jvicente@uniovi.es (J.C. de Vicente).

1368-8375/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.oraloncology.2013.02.008

Material and methods

Patients and tissue specimens

Surgical tissue specimens from patients who were diagnosed with oral mucosa dysplasia at the Hospital Universitario Central de Asturias between 2000 and 2005 were retrospectively collected. Patients with oral leukoplakia included in this study had to meet the following criteria: (i) pathological diagnosis of oral epithelial dysplasia; (ii) no previous history of head and neck cancer, (iii) complete excisional biopsy of the lesion; and (iv) a minimum follow-up of 5 years (or until progression to malignancy occurred). Fifty-eight patients who met these criteria were included in this study. Patients were followed-up every 2 months for the first 6 months after completing the treatment, every 3 months until the second year, and every 6 months thereafter.

Representative tissue sections were obtained from archival, paraffin-embedded blocks. The premalignant lesions were classified into the categories of mild, moderate or severe dysplasia following the WHO classification.[13] Tumor blocks were also obtained from those patients who developed an invasive OSCC. Alveolar mucosa obtained from unerupter third molars surgery was used as control. All patients gave their consent to excise this normal tissue.

Immunohistochemistry

The formalin-fixed, paraffin-embedded tissues were cut into 4-μm sections and dried on capillary-gap glass slides (DakoCytomation). The sections were deparaffined with standard xylene and hydrated through graded alcohol into water. Antigen retrieval was performed by heating 5 min in a pressure cooker with citrate buffer (pH 6.0). Tissue slides were incubated overnight at 4 °C in a humid chamber with mouse IgG anti-podoplanin monoclonal antibody (clone D2-40, Covance Inc. formerly Signet Catalog No. 730-01) at 1:100 dilution and staining was done by using the DakoCytomation Envision Plus peroxidase mouse system and diaminobenzidine chromogen as substrate (DakoCytomation). Counterstaining with hematoxylin was the final step. After staining, the slides were dehydrated through graded alcohols and mounted with a cover slip using a standard medium. Expression of podoplanin in lymphatic endotelial cells within the stroma served as an internal positive control. A negative control with an omission of the antiserum from the primary incubation was also included. The slides were analyzed randomly by three of the authors, blinded to the clinical data. Immunostaining was scored using a similar scoring system to that described by Kawaguchi et al.[7] as follows: (0) if no expression was observed in any part of the epithelium, (1) expression restricted to the basal layer of the epithelium, (2) expression in the basal and suprabasal layers at one area, and (3) suprabasal layer expression at two or more areas. According to Yuan et al.[14] lesions with scores $\geqslant 2$ were considered as podoplanin-positive based on the notion that lesions with extensive podoplanin expression beyond the basal layer may reflect clonal expansion and are more prone to progression.

Statistical analysis

χ^2 and Fisher's exact test were used for comparison between categorical variables. The effect of age was determined by Student́s *t* test. Correlations were analyzed by Spearman's test. For time-to-event analysis, Kaplan–Meier curves were plotted. Differences between survival times were analyzed by the log-rank test. Cox proportional hazards models were used for univariate and multivariate analyses. The hazard ratios (HRs) with their 95% confidence intervals (CIs) and *p* values were reported. All tests were two-sided. *p* Values $\leqslant 0.05$ were considered statistically significant.

Results

Patient characteristics

Twenty-seven patients (46.6%) were men and the remaining 31 women, with a mean age of 64 years (SD 12.9, range 39–87 years). Thirty-five of 58 patients (60%) were smokers and 28 (48%) were habitual alcohol drinkers. The mean tobacco consumption was 20 cigarettes a day. Forty-three of 58 patients (74.1%) were classified as mild dysplasia, 7 (12.1%) as moderate dysplasia, and the remaining 8 (13.8%) as severe dysplasia/carcinoma *in situ*. During the follow-up period, 13 (22.4%) of 58 patients developed an invasive OSCC. The baseline characteristics of study participants are presented in Table 1.

Podoplanin expression in oral tumorigenesis

Immunohistochemical analysis of podoplanin expression was performed on a set of 58 oral mucosal dysplasias. The sections selected for study also contained normal epithelia as internal controls. Podoplanin expression was consistently detected in endotelial cells of lymphatic vessels, as expected according to its well-established role as lymphatic marker. In contrast, podoplanin expression in normal oral epithelium was negligible or restricted to small clusters of cells within the basal layer that showed mainly membranous and cytoplasmic staining (Fig. 1A). Podoplanin expression in dysplastic oral epithelium was highly variable, yielding a predominant membranous pattern at the basal layer (Fig. 1B–E). In some cases, the expression extended to suprabasal layer or above at one or multiple areas (Fig. 1D–E).

Among the 58 dysplastic lesions analyzed, 18 cases (31%) showed no detectable podoplanin expression in the epithelium (scored as 0, Fig. 1B), 18 (31%) showed podoplanin expression only in certain basal cells (scored as 1, Fig. 1C), 7 (12%) podoplanin expression extending to suprabasal layer at one area (scored as 2, Fig. 1D), and 15 (26%) at two or more areas (scored as 3, Fig. 1E). Table 2 presents the association between podoplanin expression and the evolution of OPL. Twenty-two of the 58 lesions (38%) were classified as podoplanin-positive (scores 2 and 3), and the remaining 36 (62%) lesions were considered podoplanin-negative (scores

Table 1
Patient baseline characteristics.

	Unstransformed oral epithelial dysplasia	Malignant transformed oral epithelial dysplasia	*P*
Age (years)			
Mean (SD)	63.8 (12.7)	64.08 (13.8)	0.36
Range	39–87	46–83	
Gender, number (%)			
Female	22 (49)	9 (69)	0.37
Male	23 (51)	4 (31)	
Smoking, number (%)			
Yes	29 (64.4)	6 (46.2)	0.39
No	16 (35.6)	7 (53.8)	
Ethanol intake, number (%)			
Yes	25 (55.5)	3 (23)	0.25
No	20 (44.5)	10 (77)	
Epithelial dysplasia			
Mild	41 (91)	2 (15.4)	
Moderate	2 (4.5)	5 (38.5)	<0.0005
Severe	2 (4.5)	6 (46.2)	