

Immunoexpression of cytokeratin-19 in the oral lichen planus and related oral squamous cell carcinoma

Gian Paolo Bombeccari DDS, PD, MS
Aldo Bruno Gianni MD
Francesco Spadari MD

Maxillo-Facial and Dental Unit (Head Prof. AB Gianni)
Fondazione Ca' Granda IRCCS Ospedale Maggiore
Policlinico Milan, Italy - Department of Biomedical,
Surgical and Dental Sciences, University of Milan,
Italy

Corresponding author:

Gian Paolo Bombeccari
Department of Biomedical, Surgical, and Dental Sciences, Unit of Oral Pathology and Medicine, University of Milan
Via Della Commenda 10
20122 Milan, Italy
E-mail: gpbombeccari@libero.it

Summary

Aims. Cytokeratin 19 (CK-19) is an epithelium-specific intermediate filament protein that has been investigated in oral lichen planus (OLP) lesions but has not been compared with the expression of CK-19 in the OLP-related oral squamous cell carcinoma (OSCC). The aim of the present study has been to objectively compare the immunohistochemical expression of the CK-19 in OLP lesions and subsequent OSCC lesions that developed over time, to evaluate the change of the staining pattern among OLP and the grades of differentiation in OSCC.

Methods. Thirty-six formalin-fixed tissues of 18 OLP patients (18 samples from OLP lesion and 18 samples from OLP-related OSCC lesion) were included. The monoclonal antibody for CK-19 was used at 1:100 dilution for the immuno-staining on 4- μ m thick sections. Staining pattern of CK-19 was graded into a 4-point scale: (1) no staining, (2) only few cells staining, (3) less than 50% of the cells stained, and (4) 50% or more of the cells stained. Microslides were examined under the light microscope using objective lenses magnifications of 4x, 10x, and 20x.

Results. The CK19 positive rate in OLP tissues was 33% (6 out of 18) and 56% (10 out of 18) in OSCC tissues. The CK19 positive score in OSCC tissues was significantly higher than that in the

corresponding OLP tissues (Mann-Whitney test, $P=0.02$). Well-differentiated OSCC showed significantly lower of C-K19 scores than those moderately differentiated grades (Mann-Whitney, $P=0.007$).

Conclusions. The quantity and distribution of CK-19 staining in OSCCs showed relevant difference in comparison with OLP lesions. The increased of CK19 protein expression in OSCC tissues correlates significantly with the pathologic differentiation grade.

Key words: cytokeratin, oral cancer, oral squamous cell carcinoma, oral lichen planus, head and neck cancer.

Introduction

Oral lichen planus (OLP) is a chronic inflammatory disorder with a prevalence accepted to be approximately 1% of the general population (1).

The alleged potentially malignant character of the oral lichen planus has been matter of debate for several decades. In a seven-year follow-up study of 327 OLP patients the annual malignant transformation rate amounted to less than 0.5% (2).

However, when the incidence of oral cancer is set at 5 per 100.000 per year, then an annual risk of malignant transformation in oral lichen planus patients of 0.5% is a hundred times increased risk (1).

Cytokeratins (CKs) are epithelium-specific intermediate filament proteins that maintain cellular integrity and participate in cell-to-cell attachments (3).

Cytokeratins are the main differentiation markers of the stratified epithelium, and 19 subclasses of CKs can be classified in according to their molecular weights, and distinguished by the site-specific distribution in the epithelium (4).

Cytokeratin-19 (CK-19) has been identified as a useful marker of cellular atypia, associated with pre-malignant lesions in the oral epithelium (5).

Also, CK-19 expression has been regarded as an important clue in the initial events during oral carcinogenesis (6).

Although a few studies demonstrated positive CK-19 staining in the basal layer of some dysplastic and malignant oral lesions (5, 7, 8), other studies have not shown changes in the CK-19 expression in presence of oral dysplastic lesions, and/or any correlation between the histological parameters and CK-19 staining pattern in OLP lesions (9-11).

Since the available data are discordant and the pro-

duction of Ck-19 in the suprabasal cells of the oral mucosa – usually produced by cells in the basal layer- may- indicate alteration in cell behavior and probable premalignant changes (5, 12, 13), more data are needed to establish a correlation between CK-19 expression pattern and the oral malignant transformation.

The aim of the present study is to objectively analyze the immunohistochemical expression of the CK-19 in OLP lesions undergoing malignant transformation and subsequent OLP-related oral squamous cell carcinoma (OSCC), and to compare the change of the staining pattern among OLP and the grades of differentiation in OSCC.

Materials and methods

Specimen selection

The study was conducted using the oral mucosa biopsy samples of 18 Caucasian patients that developed an oral squamous cell carcinoma (OSCC), within a group of 683 subjects with clinical and pathologic diagnosis of OLP. The entire cohort of OLP patients has been followed from March 2001 through November 2015, with a frequency established on the basis of the clinical features and need for topical OLP therapy.

The OLP diagnosis was defined according to revised and modified World Health Organization diagnostic criteria (14).

For each of the 18 OLP-OSCC patients 2 tissue samples were taken: the first sample from OLP lesion and second sample from OLP-related OSCC lesion. Clinical criteria for biopsy sampling in OLP-OSCC suspected lesions were the evidence of a loss of keratotic homogeneity associated with red areas of granular appearance and an increased consistency of the OLP lesions. The criteria of the American Joint Committee on Cancer (AJCC) have been adopted to determine the clinical cancer stage (15). The Local Ethical Committee approval was obtained by the Institutional Board, and each of the patients, once thoroughly informed, provided oral and written (signed) informed consent.

Histological examination

The oral biopsy samples of OLP-OSCC group patients were fixed in 10% formalin, paraffin-embedded and processed. Haematoxylin and eosin stained slides of 6 µm were cut for diagnostic procedures. The OLP biopsies were reviewed by an expert oral pathologist and were deemed to be eligible when the following histopathologic features were observed: 1) the presence of one or more well defined band-like zone of cellular infiltration, which was confined to the superficial part of connective tissue; 2) evidence of liquefaction degeneration in the basal cell layer; and 3) absence of epithelial dysplasia. The localization of the OLP lesions in the oral mucosa and site of primary OSCC arising from OLP were recorded.

Immunohistochemistry and evaluation of immunostaining

For immunohistochemical staining 4-µm thick sections from formalin-fixed, paraffin-embedded tissue blocks were cut. The monoclonal antibody for CK-19 (DAB-Ventana-Roche, Benchmark-XT system, Tucson, AZ, USA) were used at 1:100 dilution. The incubation period for antibody was 12 hours. The expression level of CK-19 was analyzed using Biotin free Multimer Ultraview. As regard CK-19, a positive reaction was considered as clear staining within the epithelium (basal and/or suprabasal) of OLPs and OSCCs sections. The staining pattern of CK-19 was graded into a 4-point scale: (1) no staining, (2) staining of only few cells, (3) less than 50% of the cells stained, and (4) 50% or more of the cells stained. All immunostained microslides were examined under the light microscope (Olympus model U-MDO10B, USA) equipped with objective lenses magnifications of 4x, 10x, and 20x.

Statistical analysis

Frequency tables were analyzed using the Chi-square test in order to assess the relevance of the correlation among the categorical variables. Mann-Whitney *U* test were applied with the objective of comparing the means of the grades of immunoeexpression of CK-19 for each diagnostic group (OLP group and OSCC group). In each analyze the probability values less than 0.05 ($p < 0.05$) were regarded as significant. Statistical calculations were performed with the use of STATA 11.1 (College Station, TX, USA).

Results

Out of 683 OLP patients prospectively followed for 19-128 months in the mean observation period of 89.7 months (SD 13.9), 18 of them developed an OSCC. The group of 18 OLP-OSCC patients was composed of 12 women (67%) and 6 men (33%), with the women's mean age of 65 years (range 60-71) and the men's mean age of 62 years (range 55-68). The length of follow-up period before malignant transformation event ranged from 24 to 78 months [mean value 47.3 months (SD 12.90)].

The anatomic sites most commonly involved in subsequent malignant transformations of the OLP lesions were the tongue [8/18 patients, (44%)] and oral buccal mucosa [6/18 patients, (33%)].

The observed clinical form of OLP that more frequently underwent malignant transformation was the atrophic and/or erosive form, with erosive OLP being detected in 9 out of the 18 cases (50.0%) of OSCC and the keratotic form in 6 out of 18 patients (33%).

Histologically, 13 out of 18 cases (72.0%) of OSCC presented a well differentiated grade with a microinvasive pattern of infiltration. The remaining 5 out of 18 cases (28.0%) displayed a moderate degree of differentiation.

Microscopic evaluation of the immunostained sections

Thirty-six biopsy samples (18 OLP samples and 18 OLP-related OSCC) were studied for CK-19 immunostaining (Figs. 1-3). For each patient, the staining pattern of CK-19 is shown in Table 1. Cytokeratin-19 expression was positive in 6/18 of the OLP biopsy

more marked in the OSCC samples *versus* the OLP specimens (Mann-Whitney, $P=0.02$). Of the OLP positive samples, 6/18 (33%) were classified in category 2 (only few cells staining), with CK-19 staining confined to the basal cell layer of the oral epithelium. Of the 10/18 OSCC samples, 5/10 (50%) were categorized of grade 2 and 5/10 (50%) of grade 3 (less than

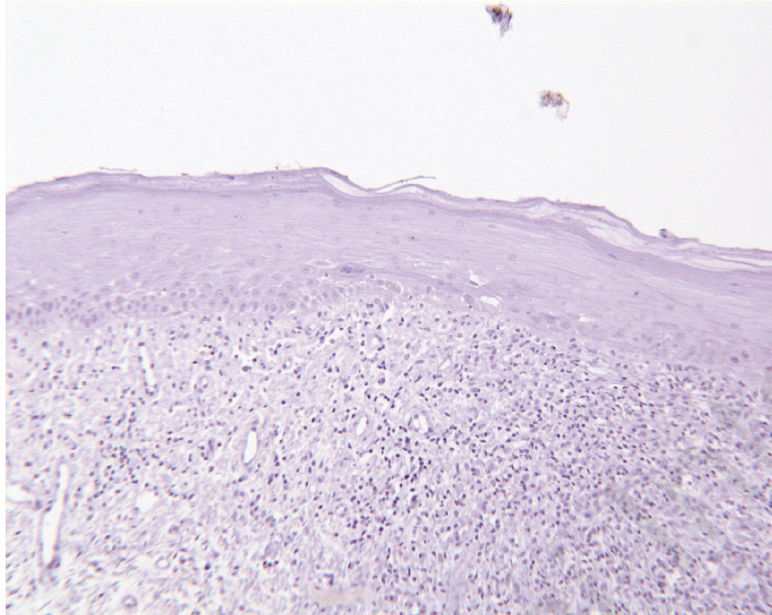


Figure 1. Oral lichen planus immuno-histochemistry: complete negativity for CK-19 staining in the basal and suprabasal layer (10x, CK19).

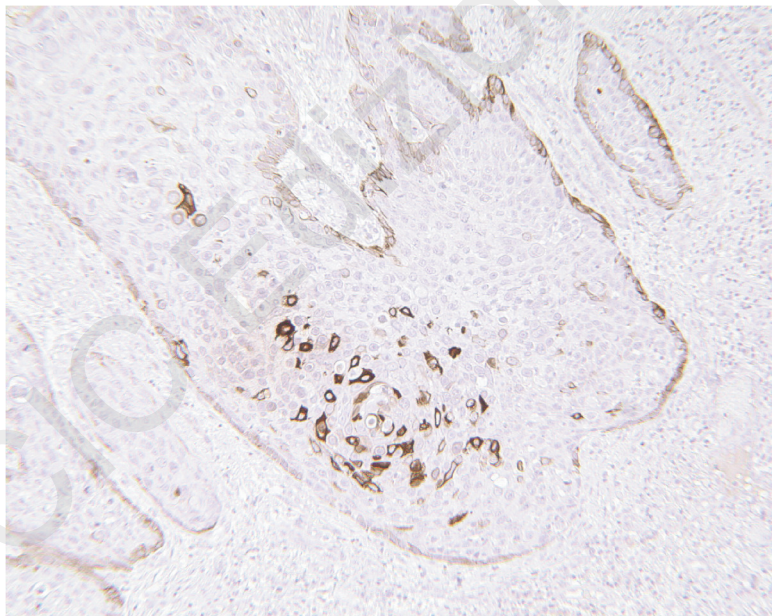


Figure 2. Well-differentiated OSCC: positivity for CK-19 in the basal and suprabasal layer with a score of 3 in the staining pattern (10x, CK19).

samples (33%), and 10/18 of the OLP-related OSCC biopsy samples (56%), with no significant difference between the samples of OLP and OSCC (Mann-Whitney, $P>0.10$).

Concerning to the grading of the tissues staining into a 4-point scale CK-19, it was statistically significantly

50% of the cells stained), with CK-19 expressed in basal-parabasal layer and focally on the superficial epithelium (Tab. 2). In the paired comparisons of CK-19 staining scoring, well-differentiated OSCC showed significantly lower C-K19 scores than those moderately differentiated grades (Mann-Whitney, $P=0.007$)

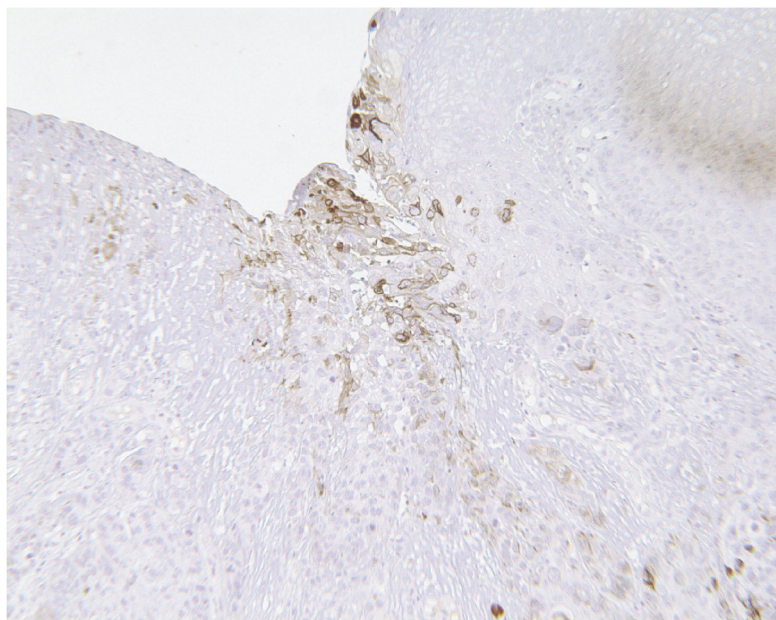


Figure 3. Moderate-differentiated OSCC: positivity for CK-19 in the basal layer and superficial layer with a score of 3 in the staining pattern (10x, CK19).

Table 1. Clinical-epidemiological data, oral localization of the lesions and comparative values of CK-19 score for each patient: (1) no staining, (2) only few cells staining, (3) less than 50% of the cells stained, and (4) 50% or more of the cells stained.

Sample	sex	age	Lesions site	Clinical form of OLP	Expression of CK-19	
					OLP	OSCC
BS	F	60	Dorsum of the tongue	Atrophic, Erosive	2	3
CN	F	67	Lateral margin of the tongue	Keratotic	1	1
DD	M	55	Lateral margin of the tongue	Atrophic, Erosive	1	1
FF	F	69	Maxillary gingiva	Atrophic, Erosive	2	3
PS	M	67	Buccal mucosa	Keratotic	1	1
PM	M	58	Lateral margin of the tongue	Mixed form	1	2
RA	F	71	Buccal mucosa	Keratotic	1	1
ZL	F	63	Buccal mucosa	Atrophic, Erosive	1	2
TV	F	61	Buccal mucosa	Atrophic, Erosive	1	2
BM	F	67	Lateral margin of the tongue	Keratotic	1	1
BT	F	62	Ventral tongue mucosa	Atrophic, Erosive	2	3
CS	M	68	Buccal mucosa	Keratotic	1	1
BG	M	64	Lateral margin of the tongue	Atrophic, Erosive	2	3
CA	F	68	Lateral margin of the tongue	Atrophic, Erosive	1	1
PG	M	60	Dorsum of the tongue	Keratotic	2	3
PE	F	64	Lateral margin of the tongue	Mixed form	2	2
LM	F	70	Buccal mucosa	Atrophic, Erosive	1	1
SE	F	61	Mandibular gingiva	Mixed form	1	2
<i>P-value</i>			<i>0.045</i> (Tongue)	<i>0.11</i>	<i>0.10</i> (OLP vs OSCC)	
(Expression of CK-19)					<i>0.02</i> (OSCC+ vs OLP+)	

CK-19, cytokeratin 19; OLP, Oral Lichen Planus; OSCC, Oral Squamous Cell Carcinoma

Table 2. Distribution and statistical comparison of CK-19 scores between OLP lesions and OLP-related OSCCs.

CK-19 staining pattern	Specimens	OLP	OSCC	W-D OSCC	M-D OSCC
	No (%)	No (%)	No (%)	No (%)	No (%)
No staining (grade 1)	20 (56)	12 (67)	8 (44)	8 (62)	0
Only few stained cells (grade 2)	11 (30)	6 (33)	5 (28)	4 (30)	1 (20)
<50% of the cells stained (grade 3)	5 (14)	0	5 (28)	1 (8)	4 (80)
>50% of the cells stained (grade 4)	0	0	0	0	0
Total number of specimens	36	18	18	13	5
<i>P-value</i> (CK19- vs CK19+)	0.12	0.15	0.7	0.007 (W-D vs M-D OSCC)	

CK-19, cytokeratin 19; OLP, Oral Lichen Planus; OSCC, Oral Squamous Cell Carcinoma; W-D, Well-Differentiated OSCC; M-D, Moderately-Differentiated OSCC

(Tab. 2). The CK-19 staining pattern was significantly more frequent in the tongue tissues than in other localizations of the oral mucosa (Chi-square test $P=0.045$). Regarding the different types of OLP, the CK-19 expression has not been statistically significant in relation with any clinical form of OLP among those reported in Table 1 (Chi-square test $P=0.11$).

Discussion

The purpose of the present study was to examine the expression of cytokeratin 19 (CK-19) in both oral lichen planus (OLP) biopsy samples and those of oral squamous cell carcinoma (OSCC) developed in the OLP lesions. Outcomes showed that CK-19 staining was heterogeneously expressed in the 33% of the OLP lesions and the 56% of the OLP-related OSCCs. Data collected on the immunostaining OLP lesions are consistent with the results of the study by Mattila et al., which reported a 29% rate of CK-19 marked OLP specimens (10). As for the findings of CK-19 positivity in the OSCC samples our data are coherent with a previous study by Safadi et al. which reports an overall percentage of 66,7% of stained sections (8). Also, additional evidence showed that the grading of CK-19 staining in the OSCC sections was significantly more marked in comparison with the OLP sections. In the current study, the aberrant expression and localization – basal and suprabasal cells layer – of CK-19 staining observed in the OSCCs could be interpreted as a potential increased progression correlated with disturbed stem cells distributions, which are normally present in the basal cell layer (6, 16). In a previous study of 33 OSCCs cases, Zhong et al. reported that the CK-19 protein expression in OSCCs tissues correlate significantly with a pathologic differentiation grade of the malignant lesions, with an increasing m-RNA CK-19 level from well-differentiated cancerous tissues in comparison with those with poorly grade of differentiation (13). In the present study, the high prevalence (72%) of well-differentiated OSCCs lesions could explain the medium scoring

- between 2 and 3 - of the observed staining pattern in the tissue sections. Furthermore, a significantly higher CK-19 score in moderately differentiated OSCCs than well-differentiated ones was reported. Previous studies examined the rate of CK-19 expression in relation to the grades of pathological differentiation in the OSCCs, detecting a positive association (6, 8, 13, 17, 18). The achieved findings are consistent with the results of those studies and could be related to the progressive increased expression of precursor-cell keratin – as cytokeratin-19 – in less-differentiated grades of OSCCs (6, 8, 13). The staining pattern of CK-19 was detected as significantly more frequent in the tongue of the OLP and OSCC patients than in other sites of the oral mucosa. This trend is in line with a previous study by Mattila et al. concerning the topographic expression of CK-19 in OLP lesions (10). As a matter of fact, the tongue appears to be the preferential site of primary neoplasia OLP-related, in accordance with previously reported in literature (2, 19).

Although the data achieved in the present study are consistent with other reports, the most significant limitation of this single-center study is the relatively small number of investigated cases, also due to the low rate of malignant transformation that occurring on average in the OLP patients. Another limitation can be considered the absence in our cases of poorly differentiated OSCCs, that has not made possible a CK-19 staining comparison on all histopathological differentiation grades in the OSCCs. Despite of the all above mentioned, it could be stated that the positive staining of CK-19 has resulted to be intermittent within both OLP and OSCCs lesions. The marking pattern for CK-19 in OLP lesions has been categorized as scarce and confined to the basal cell layer of the epithelium in comparison to what observed in OSCCs lesions. The supplementary immunostaining with monoclonal antibody for CK-19 in the OLP-related OSCCs could contribute to better define their histopathological differentiation grade.

The findings obtained in the present study suggest that the amount and distribution of CK-19 staining have significantly increased in OSCCs in comparison

with OLP-related lesions.

This CK-19 staining pattern is significantly more marked in moderately-differentiated OSCCs than well-differentiated ones.

Conflict of interest statement

We have no conflicts of interest connected with this work. We declare that we have no competing financial interest.

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None.

Authors' contributions

GPB has conceived and drafted the manuscript. FS and ABG have participated in the design and coordination of this study.

All Authors read and approved the final manuscript.

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