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ORIGINAL ARTICLE

Immunohistochemical evaluation of intermediate filament proteins in squamous papilloma and oral verrucous carcinoma

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OBJECTIVE: Cytokeratins (CKs) are the intermediate filament proteins of the epithelium cells, which have become important markers of normal and abnormal cell differentiation. The goal of the present study was to investigate the expression pattern of CK 10, 13, 14 and 16 in oral verrucous carcinoma (OVC) and oral squamous papilloma (OSP).

MATERIAL AND METHODS: Formalin-fixed paraffin-embedded sections from eight cases of each lesion were assessed. Immunohistochemistry was carried out using streptoavidin-biotin complex method.

RESULTS: In OVC, CK 10 was expressed in suprabasal to superficial layers whereas in OSP mainly in superficial layer. CK 13 was detected in prickle and superficial cells in most cases of OVC and in suprabasal to superficial cells of OSP. All the cell layers of OVC reacted positively for CK 14 while basal and suprabasal layers of OSP were more pronounced for CK 14. Finally, CK 16 was observed in suprabasal to superficial layer in OVC and the majority cases in OSP showed only superficial reactive cells.

CONCLUSIONS: CK 10, 13, 14 and 16 immunohistochemical profile emphasis the biological behavior of the studied lesions and confirm the use of these proteins as markers of differentiation.

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Keywords: oral verrucous carcinoma; oral squamous papilloma; cytokeratin; immunohistochemistry

Introduction

Oral squamous papilloma (OSP) is a benign exophytic growth of stratified squamous epithelium arranged in papillary projections, giving it a clinical 'cauliflower' appearance. In regard to its nature, several controversies exist as to whether OSP is a neoplasm or a reactive lesion (Yamaguchi *et al*, 1998).

Oral verrucous carcinoma (OVC) represents a remarkably innocuous variant of the oral squamous cell carcinoma that has typical clinic and histopathologic presentation. In spite of the malignant phenotype, it has a slow growth pattern, generally with no metastasis, and demonstrates lateral primary dissemination causing local destruction. It usually appears as a superficial and exophytic white lesion due to the prominent production of keratin (Spiro, 1998; Yeh, 2003).

Cytokeratins (CKs) constitute the major component of the cytoskeleton of all epithelium; these intermediate filaments are encoded by a large multigene family and they are differentially expressed in specific epithelium and in distinctive epithelium cells types within a given tissue. Several cellular alterations occur in the cytoskeleton during oncogenic development that can be assessed through the expression of these proteins. For example, neoplastic cells may express new filaments not present in normal cells or downregulate filaments expressed in physiological tissues. Hence, the expression of CKs may lead to a better knowledge concerning the biological mechanisms associated to the behavior of diverse pathoses (Chu and Weiss, 2002).

To date, few studies have investigated the inherent aspects of OSP and OVC using immunohistochemical methods. The goal of the present study was to analyze the immunohistochemical expression of CK intermediate filaments in order to provide data for a better understanding on the biological behavior of these lesions and also to confirm the accuracy of CK proteins as markers of differentiation.

Materials and methods

Tissue specimens

Eight cases of OSP and eight cases of OVC fixed in 10% buffered formalin and embedded in paraffin blocks were retrieved from the files of the Department of Oral

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Pathology of the Federal University of Rio Grande do Norte. The samples were selected independently on the anatomical localization and all cases were diagnosed on the basis of clinic and histopathologic features. Strict histopathologic criteria for OSP was as follows: squamous epithelium arrayed in finger-like projections with fibrovascular connective tissue cores. The epithelium showed a normal maturation pattern and the lesions were hyperparakeratotic. In addition, koilocytosis was seen in all cases and basilar hyperplasia was a frequent finding. Concerning OVC, the following characteristics were taken into account: wide and elongated rete ridges, prominent hyperparakeratosis and a papillary or verruciform surface. The clefts between the surface projections were filled with parakeratin. Epithelium cells usually showed a normal maturation pattern with no significant degree of cellular atypia. Furthermore, there was a frequent intense infiltrate of chronic inflammatory cells in the subjacent connective tissue. Lesions resembling other pathoses such as condylomas and verruca vulgaris were excluded. The Bioethics Committee of the Federal University of Rio Grande do Norte approved this experiment.

Immunohistochemical methods

From each case 3-μm thick sections were cut and mounted on glass silanesed microscope slides previously cleaned (3-aminopropyltriethoxysilane, Sigma Chemical Co., Carpinteria, CA, USA, dilution 1:150). The expression of CKs 10, 13, 14 and 16 was assessed immunohistochemically using streptoavidin-biotin complex method. The antibodies used and its specifications are listed on Table 1.

Immunostained slides were examined with a light microscope by three oral pathologists. The evaluation parameters were as follows: presence (P) or absence (A) of immunostaining, topographic localization of the immunostaining in neoplastic and in adjacent normal epithelium. The normal epithelium was used either as a comparative parameter or as an intern positive control for antibodies. In all cases, parakeratinization was seen in normal epithelium. After data collection, a descriptive statistical analysis was performed.

Results

All cases of OSP and OVC were immunopositive for CK 10, 13, 14 and 16 (Figures 1–8). In OVC, CK 10 was expressed in suprabasal to superficial layers whereas in OSP mainly in superficial layer. CK 13 was detected in prickle and superficial cells in most cases of OVC and in suprabasal to superficial cells of OSP. All the cells layers of OVC reacted positively for CK 14 while basal and suprabasal layers of OSP were more pronounced for CK

Table 1 Primary antibodies specifications

Antibody specificity	*Code*	*Dilution*	*Incubation period (h)*	*Antigen retrieval treatment*
Cytokeratin 10 (Dako*)	LHP1	1:50	1	Trypsin 0.1% 30′
Cytokeratin 13 (Dako*)	KS-1^{A}3	1:150	1	Citrate buffer 20′
Cytokeratin 14 (Dako*)	LL002	1:20	1	Citrate buffer 20′
Cytokeratin 16 (Dako*)	LL025	1:30	1	Citrate buffer 20′

*Dako Corporations, Carpinteria, CA, USA

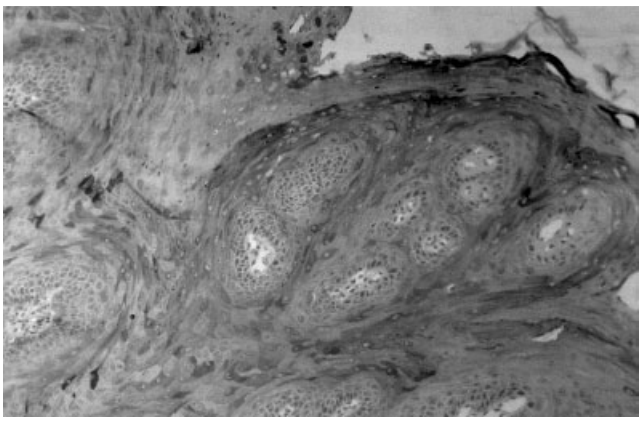

Figure 1 Oral squamous papilloma: CK10 immunostaining in prickle to superficial layers (SABC-100×)

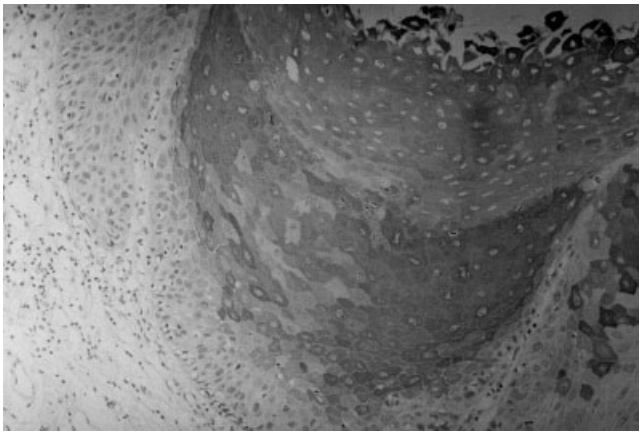

Figure 2 Oral verrucous carcinoma: CK 10 immunostaining since suprabasal to superficial layer (SABC-100×)

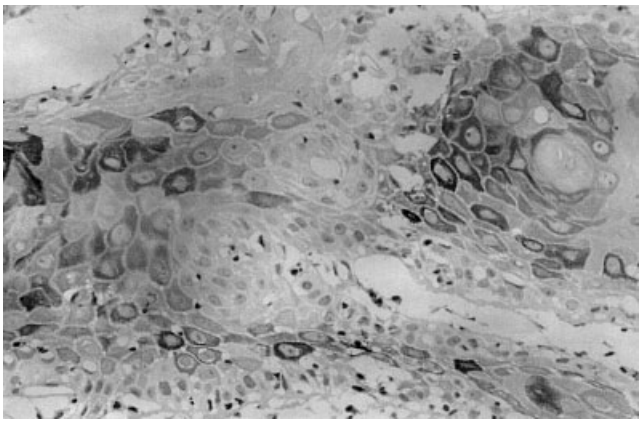

Figure 3 Oral squamous papilloma: immunoreactivity for CK 13 in prickle and superficial layers (SABC-200×)

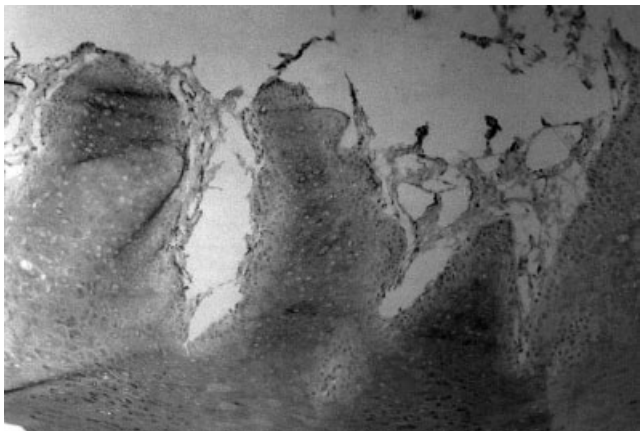

Figure 4 Oral verrucous carcinoma: immunoreactivity for CK 13 in suprabasal to superficial layers (SABC-100×)

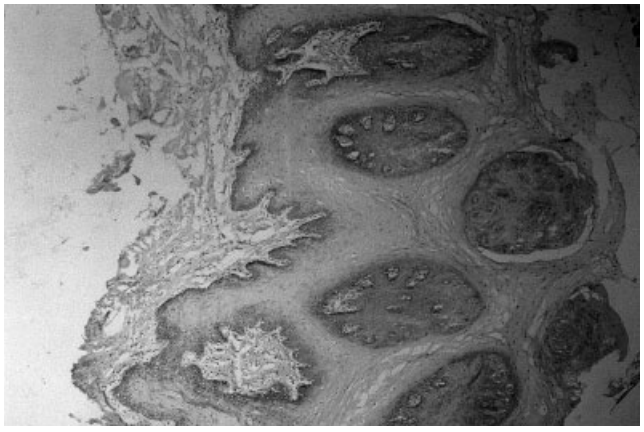

Figure 5 Oral squamous papilloma: CK 14 expression in basal and suprabasal cells (SABC-40×)

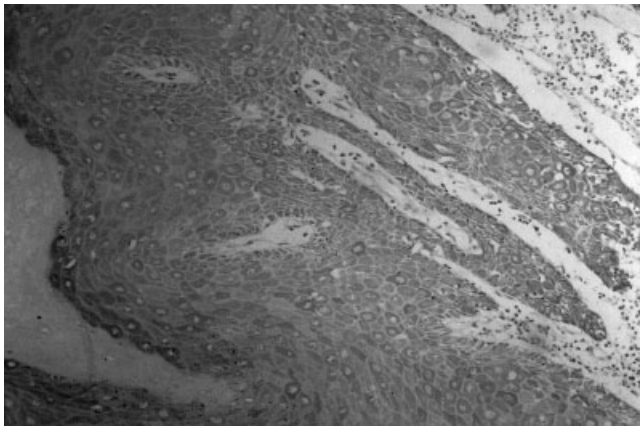

Figure 6 Oral verrucous carcinoma: CK 14 expression in all epithelium layers (SABC-200×)

14. Finally, CK 16 was observed in suprabasal to superficial layer in OVC and the majority cases in OSP showed only superficial reactive cells. The immunohistochemical findings are listed on Tables 2 and 3.

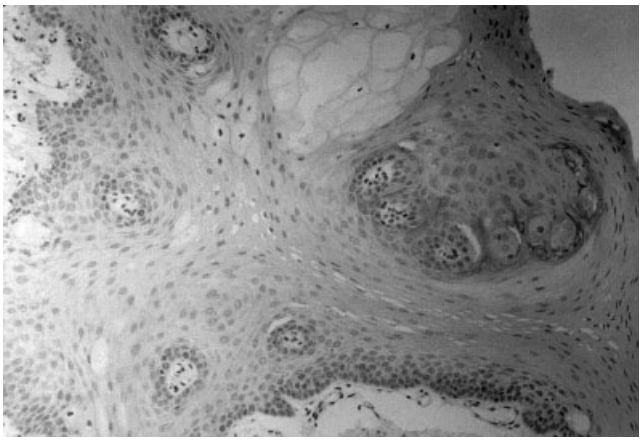

Figure 7 Oral squamous papilloma: immunostaining for CK16 in superficial cells (SABC-200×)

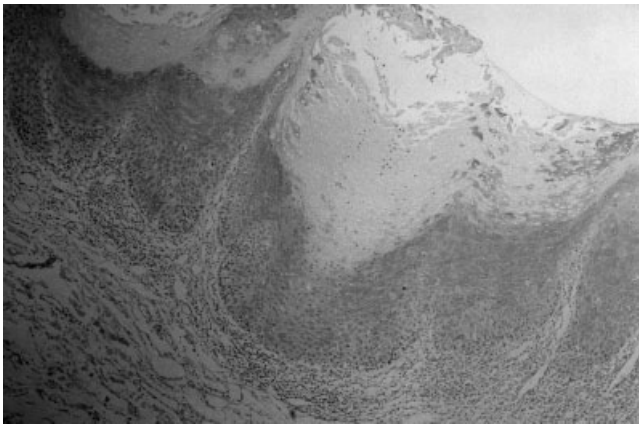

Figure 8 Oral verrucous carcinoma: immunostaining for CK16 in suprabasal to superficial layers (SABC-40×)

Table 2 Immunohistochemical detection of CK 10, 13, 14 and 16 in oral squamous papilloma

	Epithelium layers				
	Basal–suprabasal	*Prickle–superficial*	*Suprabasal–superficial*	*Superficial*	*All layers*
CK 10	–	1	2	5	–
CK 13	–	–	8	–	–
CK 14	7	–	–	–	1
CK 16	–	2	1	5	–

Table 3 Immunohistochemical detection of CK 10, 13, 14 and 16 in oral verrucous carcinoma

	Epithelium layers				
	Basal–suprabasal	*Prickle–superficial*	*Suprabasal–superficial*	*Superficial*	*All layers*
CK 10	–	–	8	–	–
CK 13	–	6	2	–	–
CK 14	–	–	–	–	8
CK 16	–	–	7	–	1

Discussion

Of the several types of papillomas, the one occurring in the mouth and oropharynx is almost always the squamous papilloma. It represents approximately 2.5% of all biopsied oral soft tissue lesions (Sulkowska *et al*, 2001). Several controversies exist as to whether OSP represents a neoplasm or a reactive lesion. According to Yamaguchi *et al* (1998) the majority of the lesions may be developed as a result of reactive growths due to constant traumatic injuries in the oral mucosa. In contrast, Paparotto Lopes and Meeks (2001) and Reszec *et al* (2002) state that OSP is a true benign neoplasm probably associated with human papilloma virus infection.

Oral verrucous carcinoma is considered a low-grade and uncommon variant of oral squamous cell carcinoma. Few studies have been performed attempting the CKs expression profile in these lesions despite the knowledge of the cytoskeleton disturbances associated with the neoplastic development. Such alterations can be assessed through the expression of CKs proteins, which can contribute to a better understanding of some mechanisms implicated with the behavior of diverse neoplasms (Spiro, 1998; Yeh, 2003).

In the present experiment, CK 10, 13, 14 and 16 expression profile in OSP and OVC was assessed and compared with the profile observed in normal epithelium. Furthermore, our results were also compared with those of other CKs studies in the literature, especially in oral squamous cell carcinoma. OVC is a low-grade variant of the oral squamous cell carcinoma, hence it is coherent to match data from these neoplasms. Whilst a number of previous studies have reported the CKs profiles in oral squamous cell carcinoma, this would appear to be the first report examining CKs profile in OVC.

CK 10 is found among the suprabasal layer of keratinized epithelium. Some studies have demonstrated that CK 10 is strongly expressed in well-differentiated oral squamous cell carcinoma whereas it is not present in poorly or undifferentiated lesions (Morgan and Lan, 1994; Bongers *et al*, 1996; Vaidya *et al*, 1996; Depondt *et al*, 1999; Silva, 1999). Giving support to these data, we found an increased CK10 immunolabeling in OVC.

van der Velden *et al* (1999) reported an overexpression of CK10 in hyperkeratinized benign lesions. In our study, increased CK 10 immunostaining was observed in OSP compared with the normal epithelium. Furthermore, greater CK 10 immunoexpression was found in OVC than in OSP. This finding could be related to the fact that OVC is a malignant neoplasm, hence a more significant alteration in the cell architecture is expected.

According to Boisnic *et al* (1995) and Chu and Weiss (2002), CK 13 is a stratification marker expressed in the suprabasal layers of non-keratinized epithelium. Our results in the normal epithelium support this data. Moreover, most of the cases of OVC showed CK 13 expression in prickle to the superficial layers. The immunostained cells showed a similar pattern to normal stratified epithelium, which suggests a significant degree of maturation in OVC.

The present CK 13 findings in OSP are not in agreement with the earlier results of van der Velden *et al* (1999). We found an intense immunoexpression in suprabasal to superficial layers in OSP, whereas van der Velden observed absence of CK 13 expression in hyperkeratinized benign lesions. We also identified a higher CK 13 immunostaining in OSP compared with OVC. Our data confirms the benign nature of OSP and its higher degree of cellular maturation.

It has been postulated that CK 14 is normally present in basal cells of keratinized and non-keratinized stratified epithelium (Heyden *et al*, 1992; Depondt *et al*, 1999). CK 14 profile of the OSP revealed similarity with the profile observed in normal epithelium, which indicates that the cytoskeleton disturbances were unable to modify the expression of this protein.

In previous studies performed by Su *et al*. (1996), Morgan and Lane (1996) and Guirado *et al* (1998) CK 14 expression was detected regardless of the differentiation compartment. In this study, all epithelium layers in OVC exhibited strong immunostaining for CK 14. It must be emphasized that the inflammatory infiltrate beneath the epithelium may have a role in the overexpression of CK 14 (Mackenzie and Gao, 1993). In fact, we found an intense inflammatory mononuclear infiltrate close to epithelium in all cases of OVC.

CK 16 is characteristically expressed in hyperproliferative epithelium and is considered a CK of fast cell turnover (Morgan and Lan, 1994; Dalbesteen *et al*, 1998; Depondt *et al*, 1999). A higher CK 16 immunostaining was expected in OSP than in normal epithelium. Nonetheless, a weak CK 16 expression restricted to the superficial layers was found in OSP. This finding is difficult to interpret. A plausible explanation for this is that OSP is a hyperproliferative but not extensive lesion. OSP may stops its growth due to unknown reasons, hence shows a decrease in the CK 16 immunoexpression.

In conclusion, the CK 10, 13, 14 and 16 profile emphasis the biological behavior of the studied lesions, especially the well-differentiated pattern of OVC as the CKs profile was similar to the CKs profile in well-differentiated oral squamous cell carcinoma reported in the literature. Moreover, it confirms the use of these proteins as markers of differentiation.

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