Original Article

p16 Expression in Odontogenic Cysts

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ABSTRACT

***Background*:** p16 protein acts as a tumor suppressor and its functional loss seems to be one of the most frequent genetic alterations in human tumors. Because there is a lack of information, the expression of p16 is examined in different odontogenic cysts to clarify the possible role of this factor to determine the biological and clinical behavior of these lesions.

***Methods*:** Eighteen radicular cysts (RC), 9 follicular cysts (FC), and 15 keratocystic odontogenic tumors (KCOT) were immunohistochemically evaluated. Chi-square test was used to compare data.

***Results*:** The cysts showed a different pattern of p16 expression according to the histologic type. All RCs or FCs were strongly or moderately positive for p16, whereas all KCOT cases exhibited loss of p16 expression or a low immunoreactivity.

***Conclusions*:** KCOT is characterized by an aggressive potential of growth and recurrences after removal. Here, a dysregulation of the p16 pathway in KCOT was demonstrated. This lack of expression of positivity for p16 in KCOT could help explain the differences in the clinical and pathological behavior of KCOT and could be related to the increased aggressive behavior, invasiveness and high frequency of recurrences found in KCOT.

***Keywords*:** Cell cycle proteins, immunohistochemistry, odontogenic tumors, p16 genes.

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Introduction

p16 protein is the product of the CDKN2 gene and it acts as a tumor suppressor.[1,2] It may act as a cyclin dependent kinase (CDK) inhibitor by binding competitively to CDK4 thus preventing the interaction between CDK4 and cyclin D1.[3,4] It plays a key role in cell cycle control at the G1-S checkpoint, inactivating the CDK that phosphorylates the tumor-suppressor protein pRB, leading to a deceleration of the cell cycle.[2,5] Loss of p16 occurs by deletion, point mutation and promoter hypermethylation[3], and it seems to be one of the most frequent genetic alterations in human tumors.[6] Abnormalities of the p16/cyclin D/Rb pathway have been reported in many kinds of human tumors.[6,7] Loss of p16 was found to be correlated with melanoma progression, with lower expression in more advanced lesions.[8] On the other hand, overexpression of p16 has been reported in some tumor tissues.[2] In oral squamous cell carcinoma (OSCC) p16 frequently increased according to the increasing severity of the lesions from dysplasia to SCC, and a tendency for overexpression of p16 was reported from normal tissues to oral premalignant lesions and to nonmetastatic OSCC.[2] A decreased p16 intensity was, however, described in metastatic OSCC and in metastatic lymph nodes.[2] There is a rather complex picture regarding the role of p16 in oral carcinoma and a lack of information regarding odontogenic tumors and cysts. The aim of the present study was to examine the expression of p16 in

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different odontogenic cysts to clarify the possible role of this factor in the biological and clinical behavior of these lesions.

Materials and Methods

The tissues of 18 radicular cysts (RC), 9 follicular cysts (FC), and 15 keratocystic odontogenic tumors (KCOT) were evaluated in the present study.[9] The tissues were retrieved from the archives of the Department of Pathologic Anatomy and Histopathology of the University "Politecnica delle Marche" Ancona, Italy. The patients comprised 22 females aged 18 to 42 years (mean 34 years) and 20 males aged 23 to 39 years (mean 31 years). All specimens had been immediately fixed in 10% neutral buffered formalin and embedded in paraffin. Three μm sections were subsequently obtained with a Leitz 1512 microtome and stained with Hematoxylin-Eosin. The diagnoses were checked and confirmed, and the slides for the immunohistochemical evaluations were selected. The immunohistochemical staining of p16 (DakoCytomation, Glostrup, DK, Denmark) was performed using the strep-ABC-peroxidase (Streptavidin-Biotin-Peroxidase) method. Three μm sections were cut and mounted on poly-L-lysine-coated slides. Paraffin sections were dewaxed by xylene, rehydrated and finally washed in PBS (pH 7.4) for ten minutes. In order to unmask the antigens, a microwave oven and a 2.1% content of citric acid was used related to the antibody p16. The subsequent steps were optimized by automatic staining (Optimax, Bio Genex San Ramon, CA, USA). Sections were incubated with primary antibody for 30 minutes at room temperature. Slides were rinsed in buffer, and immunoreaction was completed with the Strep-ABC-Peroxidase method, applying the "Super sensitive immunodetection" kit (DakoCytomation, Glostrup, DK, Denmark) and utilizing a multi-link as a secondary biotinylated antibody. After incubation with a chromogen employing "liquid DAB substrate pack" (BioGenex, San Ramon, CA, USA), the specimens were counterstained with Mayer's hematoxylin and coverslipped. A prevalently cytoplasmatic staining of epithelial cells was observed and the cells were evaluated in peripheral or central areas. To evaluate the p16 expression, mean percentage of positive cells was determined, derived from the analysis of 100 cells in ten random areas at x 400 magnification. Immunostaining for p16 protein was classified as follows: 1) negative (-), 5% or less of the epithelial cells were positive; 2) weakly positive (+), 5% to less than 25% of the epithelial cells were positive; 3) moderately positive (++), 25% to less than 50% of the epithelial cells were positive; and 4) strongly positive (+++), 50% or more of the epithelial cells were positive. As a control, specimens of 10 normal, healthy, non-inflamed gingivae, retrieved during removal of impacted wisdom teeth, were used.

Results

Positive staining for p16 was detected in all 10 (100%) basal and parabasal cells of the control normal gingiva. The cysts showed a different pattern of p16 expression according to the histologic type (Table 1). All RCs or FCs were strongly or moderately positive for p16 (Figures 1 and 2), and none of them was negative or weakly positive, whereas all KCOT cases exhibited loss of p16 expression or a low immunoreactivity (Figure 3). In particular, in the negative cases, a focal p16 expression was detected in areas of orthokeratinization of the wall lining. p16 expression was statistically lower in KCOT cases compared with FC and RC ones ($P < 0.0005$ for both comparisons, chi-square test).

Table 1. Correlation between p16 expression and histopathologic features of odontogenic cyst type.

Odontogenic cyst type	p16 immunoreactivity			
	-	+	++	+++
Radicular cyst	0	0	6	12
Follicular cyst	0	0	3	6
Keratocystic Odontogenic tumor	10	5	0	0
Total	10	5	9	18

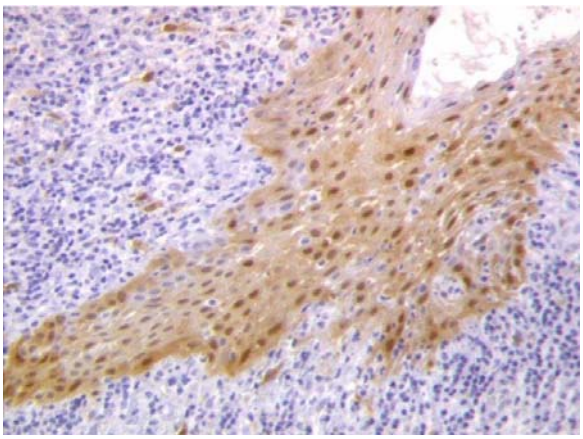

Figure 1. Radicular cyst: high positivity of all epithelial layers (p16 immunostain 100 X).

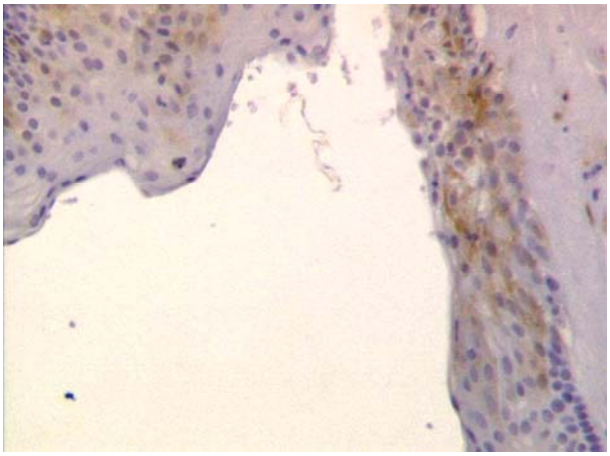

Figure 2. Follicular cyst: positivity of squamous epithelium of parabasal-intermediate layers (p16 immunostain 100 X).

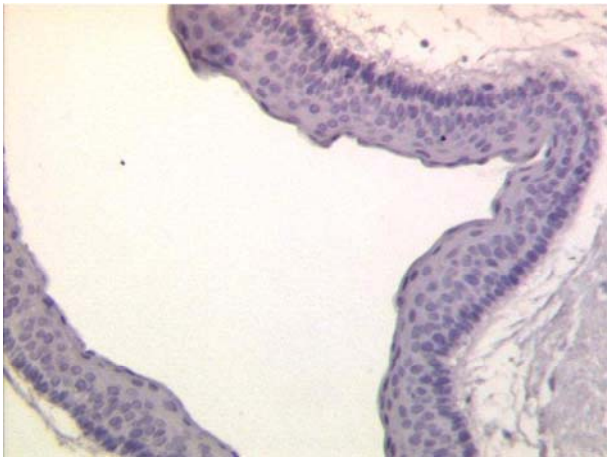

Figure 3. Keratocyst: negativity of the cells of squamous epithelium (p16 immunostain 100 X).

Discussion

Odontogenic keratocyst is a lesion with a characteristic histological appearance and a clinical behavior, characterized by an aggressive potential of growth and recurrences after removal.[10-24] The World Health Organization working group has, for these reasons, recommended the use of the term "keratocystic odontogenic tumour" (KCOT) that better reflects the neoplastic nature of these lesions.[9] The epithelium of KCOT could have an abnormal control of the cell cycle.[11,12] An altered expression of tumor-suppressor genes and oncogenes in KCOT has been reported recently.[14-16] Mutations of the PTCH gene, responsible for the nevoid basal cell carcinoma syndrome (NBCCS), have been shown to occur in sporadic KCOT.[14-16] Approximately 5% of all KCOT are associated with NBCCS.[14] An allelic imbalance of tumor suppressor genes in KCOTs could point to a neoplastic rather than a developmental origin.[14-17] Orthokeratinized KCOTs are less aggressive and have a lower rate of recurrence. Reed et al.[25] found that the alteration of p16 gene occurred in 83% of the head and neck tumors by immunohistochemistry. Yuen et al.[26] found a decreased p16 expression in 48% of their cases with head and neck squamous cell carcinoma, while Lang et al.[27] found a p16-specific gene alteration in 65% of patients. The results of the present study showed that while in RC and FC there was a positivity to p16, in KCOT cases there was a complete negativity of all the elements of the cyst wall thickness or a low positivity in all cases. This fact could certainly point to a dysregulation of the p16 pathway in KCOT that has recently been reclassified as a benign tumor. Its underexpression or loss can lead to failure to inactivate the cyclin D-dependent kinases and probably represents a compromise of regulated cell proliferation. Downregulation of p16 can contribute to cell proliferation that results locally in a more advanced tumor.[26] This lack of expression of positivity for p16 in KCOT could help explain the differences in the clinical and pathological behavior of KCOT and, according to what seems to be the pattern in several types of epithelial tumors, could be related to the increased aggressive behavior, invasiveness and high frequency of recurrences found in KCOT.

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