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Oral and Maxillofacial Pathology

A comparative study of epithelial cell proliferation between the odontogenic keratocyst, orthokeratinized odontogenic cyst, dentigerous cyst, and ameloblastoma

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OBJECTIVES: The aim of the present study was to compare the proliferation index of the epithelial cells between odontogenic keratocysts (OKC), orthokeratinized odontogenic cysts (OOC), dentigerous cysts (DC), and ameloblastomas.

MATERIALS AND METHODS: The proliferation index, employing a novel cell proliferation marker IPO-38, was studied by the immunohistochemical technique in 10 OKC, seven OOC, eight DC and 10 ameloblastomas.

RESULTS: The ameloblastoma had no higher labeling index (LI) of IPO-38 than the OKC ($P = 0.910$) but had higher LI than the OOC ($P = 0.001$) and DC ($P = 0.000$); the OKC had higher LI than the OOC ($P = 0.002$) and DC ($P = 0.000$); and the OOC had higher LI than the DC ($P = 0.011$). IPO-38-positive cells in the OKC and OOC were located principally in the suprabasal cell layers while the ameloblastoma were found in the peripheral portion in particularly, the follicular and plexiform types.

CONCLUSION: These findings support previous studies that the proliferation indices are useful in predicting the different biological behavior of the odontogenic lesions and the OKC should be regarded as a benign tumor rather than simply an odontogenic cyst.

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Keywords: ameloblastoma; dentigerous cyst; IPO-38; odontogenic keratocyst; orthokeratinized odontogenic cyst

Introduction

Different odontogenic cysts and tumors have variable clinical and biological behaviors. The ameloblastoma regardless of histopathologic variants is well recognized to behave locally aggressively (Neville *et al*, 1995). Similarly, the odontogenic keratocyst (OKC) is an aggressive cystic lesion that has a tendency to recur if not adequately removed. The recurrence rates have been documented with variable results from 3 to 60% (Shear, 1992). Although the dentigerous cyst (DC) can become a large lesion and expand the cortical bone of the maxilla or mandible, the mechanism of expansion of the DC is considered passive occurring by accumulation of fluid in the lumen. Orthokeratinized odontogenic cyst (OOC) also known as OKC was suggested to have clinicopathologic differences to the classic OKC (Wright, 1981). The recurrence rates of the OKC and OOC were studied and the results showed that the OKC recurred in at least 42.6%, compared with only 2.2% for the OOC (Crowley, Kaugars and Gunsolley, 1992). These data suggested the importance of distinguishing between the OKC and OOC.

IPO-38 antigen, also known as the non-lineage 116 antigen (N-L116), is an antigen of 14–16 kD whose expression is constant through most stages of the cell cycle except during mitosis where a 400-fold increase in concentration has been observed (Sidorenko *et al*, 1990; Mikhalap *et al*, 1998). IPO-38 antigen is expressed at this high concentration earlier than Ki67 antigen at the beginning of the cell cycle. Moreover, IPO-38 does not block the binding of Ki67 antibodies, further substantiating IPO-38 as a novel nuclear antigen of proliferating cells. IPO-38 expressed in malignant tumors including breast cancer, colorectal cancer and poorly differentiated stomach cancer. While in normal cells of these tissues, the expression was markedly reduced. IPO-38 was suggested to have potential prognostic value as a marker of cellular proliferation and in monitoring of tumor progression (Mikhalap *et al*, 1998).

The objective of the present study was to compare the proliferation index of the epithelial cells between the OKC, OOC, DC, and ameloblastoma by using a novel cellular proliferation marker, IPO-38.

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Materials and methods

Sample collection and immunohistochemistry

Ten cases of the OKC, seven cases of the OOC, eight cases of the DC, and 10 cases of the ameloblastoma (follicular type, $n = 2$; plexiform type, $n = 4$; desmoplastic type, $n = 4$) were collected from the archives of the Oral Pathology Laboratory, Faculty of Dentistry, Chiang Mai University, Thailand and the Unit of Stomatology, Cancer Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia.

The study of the epithelial cell prolifeartion was conducted by a technique of immunohistochemistry with the use of the mouse monoclonal anti-IPO-38 (Clone IPO-38; Novocastra, Newcastle, UK) antibody. The specificity of the clone IPO-38 has previously been examined on different human and murine lymphoid and non-lymphoid cell lines (Sidorenko *et al*, 1990; Mikhalap *et al*, 1998). Briefly, deparaffinized sections were immersed in 3% hydrogen peroxide solution for 15 min to block endogenous peroxidase activity. Subsequently, sections were submitted to a water bath treatment in Antigen Retrieval Solution® (Dako, Glostrup, Denmark) for 40 min, allowed to cool down for 20 min, washed in Tris-buffered saline (TBS), and incubated with 5% normal serum for 10 min to block non-specific binding. Sections were then incubated with the mouse monoclonal anti-IPO-38 antibody (1:10 dilution) overnight at 4°C. On the following day, sections were washed in TBS, incubated with biotinylated anti-mouse secondary antibody and ABC by using Vectastain® ABC Kit (Vector, Burlingame, CA, USA). Chromogen was developed by using DAKO® AEC substrate system (Dako) for 15 min. Sections were counterstained with hematoxylin, and mounted. The slides were viewed and photographed under an epifluorescence microscope (Olympus, Tokyo, Japan). Negative control sections were processed identical to experimental sections except that the primary antibody was omitted and replaced with normal serum or buffer. Positive control tissue was an oral squamous cell carcinoma.

Quantitative and statistical analyses

The labeling index (LI) of IPO-38 was determined by the numbers of positive nuclear profiles per 100 epithelial cells. The LI of IPO-38 of the OKC, OOC, DC and ameloblastoma per 10 fields at the magnification of ×400 were counted under an epifluorescence microscope, and the mean was determined. To compare the mean LI of IPO-38 between OKC, OOC, DC, and ameloblastoma, we used Mann–Whitney test. *P*-values of < 0.05 were regarded as significant.

Results

The staining of IPO-38 showed variable patterns in the OKC, OOC, DC, and ameloblastoma. The epithelial lining of the OKC (Figure 1) and OOC (Figure 2) demonstrated nuclear labeling of IPO-38 particularly in the suprabasal cell layers while that of the DC showed staining mainly in the basal and parabasal cell layers (Figure 3). The follicular (Figure 4) and plexiform types (Figure 5) of the ameloblastoma exhibited predominant staining pattern in the nuclei of the peripheral cells whereas the desmoplastic variant of the ameloblastoma showed intense staining in both the peripheral and inner cells (Figure 6).

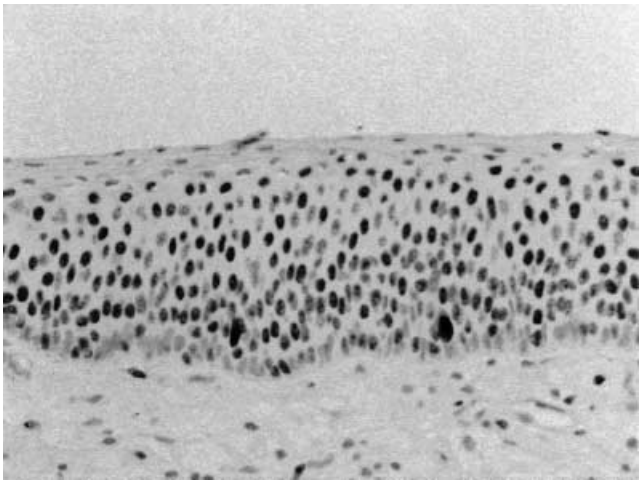

Figure 1 The epithelial lining of the odontogenic keratocyst shows nuclear staining of IPO-38 predominantly in the suprabasal cells (ABC technique, original magnification, ×200)

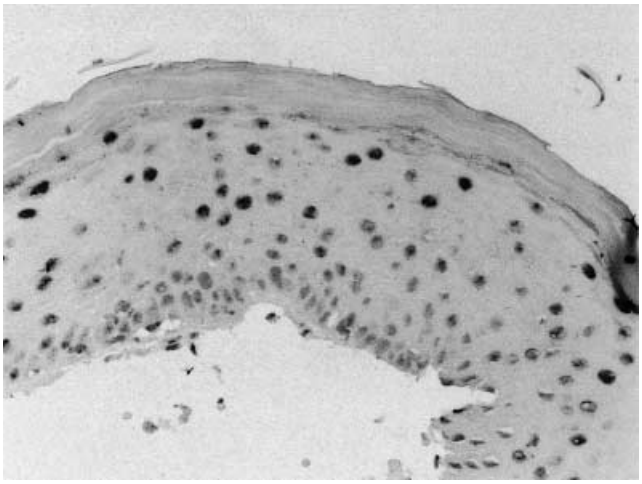

Figure 2 The orthokeratinized odontogenic cyst shows occasional suprabasal nuclear staining of IPO-38 (ABC technique, original magnification, ×200)

The mean LI of IPO-38 positive epithelial cells in the ameloblastoma, OKC, OOC and DC were 76.1 ± 14.6, 75.8 ± 18.7, 32.9 ± 21.1 and 5.5 ± 6.5 cells/100 cells, respectively. By using Mann–Whitney analysis, the IPO-38 LI was not significantly higher in the ameloblastoma than in the OKC ($P = 0.910$). The IPO-38 LI was significantly higher in the ameloblastoma than in the OOC ($P = 0.001$) and DC ($P = 0.000$). The IPO-38 LI was significantly higher in the OKC than in the OOC ($P = 0.002$) and DC ($P = 0.000$). The IPO-38 LI was significantly higher in the OOC than in the DC

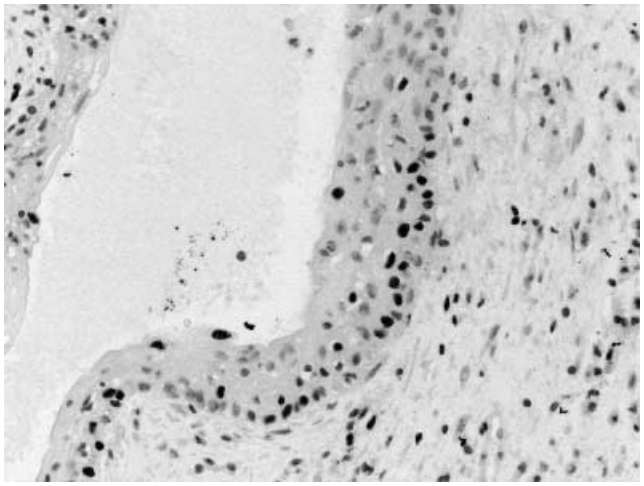

Figure 3 A thin epithelial lining of the DC exhibits nuclear staining of IPO-38 particularly in the basal and parabasal cell layers (ABC technique, original magnification, ×200)

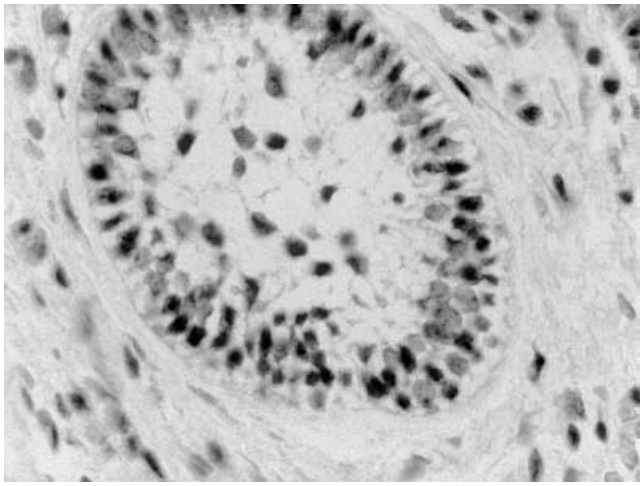

Figure 4 Intense nuclear staining of IPO-38 is mainly demonstrated in the peripheral cells of the follicle of the ameloblastoma (ABC technique, original magnification, ×400)

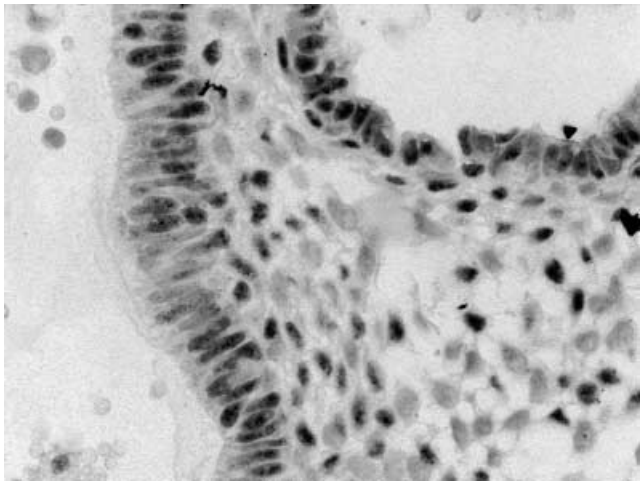

Figure 5 The plexiform type of the ameloblastoma shows nuclear staining of IPO-38 in the peripheral and inner cells (ABC technique, original magnification, ×400)

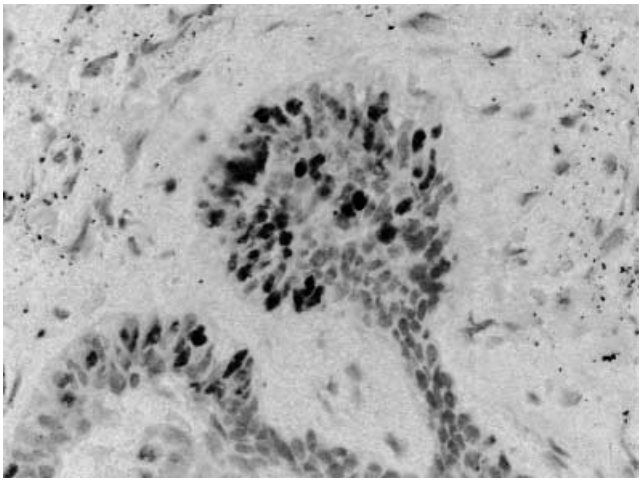

Figure 6 Condensed cells of the desmoplastic variant of the ameloblastoma exhibit nuclear labeling of IPO-38 (ABC technique, original magnification, ×400)

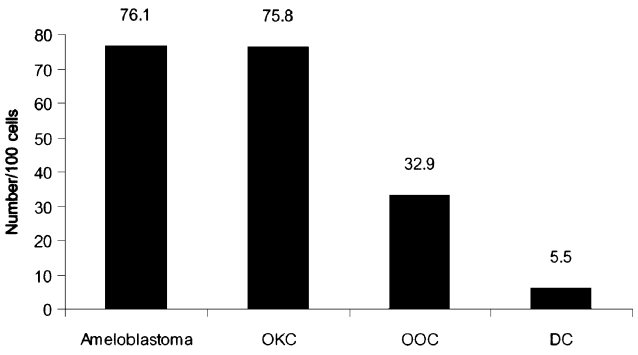

Figure 7 The comparative histograms of the mean labeling indices (LI) of IPO-38 of the ameloblastoma, odontogenic keratocysts (OKC), orthokeratinized odontogenic cysts (OOC), and dentigerous cysts (DC)

($P = 0.011$). The mean LI of IPO-38 of the ameloblastoma, OKC, OOC, and DC are graphically shown in Figure 7.

Discussion

Cell proliferation molecules and related factors including Ki67, proliferating cell nuclear antigen (PCNA), p53 and nucleolar organizer regions (AgNORs) have previously been used to indicate the biological behavior of odontogenic tumors and cysts (Shear, 2002). In the present investigation, we used IPO-38 as a cell proliferation marker and found that the LI of IPO-38 was higher in the ameloblastoma than in the OOC and DC and was higher in the OKC than in the OOC and DC. Although the LI of IPO-38 was higher in the ameloblastoma than in the OKC, the values of IPO-38 between the ameloblastoma and OKC were not significantly different. Previous studies have also shown a similar trend in the OKC and ameloblastoma that had higher proliferation indices of Ki67 and/or PCNA than in

other kinds of odontogenic cysts including the DC and radicular cysts (Matthews, Mason and Browne, 1988; Li, Browne and Matthews, 1994, 1995; Slootweg, 1995; Piattelli *et al*, 1998; Wang, Yu and Gao, 1998). When the OKC and ameloblastoma were compared, it was found that the percentage of PCNA-positive cells determined by point counting was significantly lower in the ameloblastoma than in the OKC but the percentage of PCNA-positive cells in the epithelial lining of the OKC was not significantly different from those in the peripheral cells of follicular and plexiform patterns of the ameloblastoma (Takahashi *et al*, 1998). Collectively, these data helped to explain the biological behavior of the OKC that should be considered to be a benign odontogenic tumor rather than merely a cyst, as its proliferation indices were comparable with those of the ameloblastoma and significantly higher than in other kinds of odontogenic cysts.

In the present study, we observed the predominant suprabasal distribution of IPO-38-positive cells in contrast to the lower number of IPO-38-positive basal cells in the OKC epithelial lining. These results were consistent with previous studies with the use of Ki67 or PCNA markers (Matthews *et al*, 1988; Li *et al*, 1994, 1995; Slootweg, 1995; Piattelli *et al*, 1998). Interestingly, the expression of p53 was more frequently observed in the OKC than in other odontogenic cysts (Slootweg, 1995; Li *et al*, 1996; Piattelli *et al*, 2001) and the distribution of p53-positive epithelial cells in the OKC was also predominantly suprabasal (Li *et al*, 1996) or parabasal (Piattelli *et al*, 2001). Data of the distributions of cell proliferation markers and p53 of previous and our studies supported a view that the OKC may have unique processes of cell proliferation and differentiation.

Several attempts have been performed to clinically, histologically, and biologically distinguish the OKC from the OOC. A large series of the study revealed that the OOC was more often associated with an impacted tooth than the OKC and the OKC recurred in at least 42.6%, compared with only 2.2% for the OOC (Crowley *et al*, 1992). However, there were no significant differences between the OOC and OKC when age, race, sex, presenting symptoms, and the clinical impression were compared. Histologically, the OKC has a thin wall unless there is inflammation (Kramer, Pindborg and Shear, 1992). The basal layer of the epithelium is well defined and is composed of either columnar or cuboidal cells that are arranged in a palisaded pattern. The luminal surface, often corrugated, is typically covered with parakeratin. The OOC has a thin, uniform epithelial lining with a luminal surface of orthokeratin and a well-developed granular layer (Wright, 1981). The basal cells of the OOC are much less developed than those in the OKC. They tend to be cuboidal or squamous and show little tendency to polarize or palisade. It was suggested that care must be taken to distinguish between keratin metaplasia in otherwise non-keratinized odontogenic cysts and the OOC (Wright, 1981). Keratin metaplasia is usually characterized by parakeratosis. Although there can be orthokeratin metaplasia, it is invariably a focal process. Moreover, the keratinization in the OOC is more extensive. These observations are very helpful especially for non-pathologists to better understand the differences between these two lesions.

By the use of the scanning electron microscope, the OOC showed to have different ultrastructural configurations compared with the OKC (Philipsen *et al*, 1992). Recently, the immunohistochemical profiles of cytokeratins 10, 13, and 14 and extracellular matrix proteins, fibronectin, types I and III collagen, and tenascin, indicated that the OOC presented a well-formed cystic enveloping, whereas the OKC profile was compatible with a more aggressive biological behavior (da Silva *et al*, 2002). In the present study, we attempted to compare the cellular proliferation between the OOC and OKC by measuring the LI of IPO-38 and found that the OOC had significantly lower LI than in the OKC. Similar results were also shown in a previous study of the proliferative activity between the OOC and OKC (Li *et al*, 1998). Taken together, these findings suggested that the OOC is clinicopathologically and biologically different from the OKC and should be regarded as a distinct clinical entity.

In conclusion, the findings of the present study indicated that the proliferation index of IPO-38 was useful in predicting the different biological behavior of the odontogenic lesions. Moreover, the OKC should be regarded as a benign tumor while the OOC as a non-aggressive cystic lesion.

Acknowledgements

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