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Immunohistochemical Study on Proliferating Cell Nuclear Antigen Expression in Ameloblastomas

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The aim of this study is to evaluate the proliferating activity of ameloblastomas and its correlation to the biological behaviour according to each histological type. 38 cases of solid and unicystic ameloblastomas were reviewed including 1 case of ameloblastic carcinoma and 1 recurrent case. An anti-PCNA antibody, PC10(Dako), was applied for the detection of proliferating cell nuclear antigen (PCNA) in paraffin embedded tissue sections. Also 20 cases of dentigerous cysts were reviewed. In conclusion, we observed that there was no difference between the proliferating activities of the different histological types of solid ameloblastomas. Because 1 case of ameloblastic carcinoma and one recurrent case revealed remarkably high PCNA reactivity, we believe that PCNA would be useful in showing the differentiation between benign and malignant ameloblastomas. In unicystic ameloblastomas, plexiform intraluminal growth was considered to be an important feature in tumorous transformation of cystic epithelium.
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INTRODUCTION

AMELOBLASTOMAS, THE most common epithelial odontogenic tumour, are known to originate from residual odontogenic epithelial cells, cystic epithelial cells, basal cells of mucosal epithelium and enamel organ of developing tooth germs [1, 2]. Ameloblastomas are histologically characterised by structures simulating enamel organ. In spite of a benign cytological feature, the infiltrative growth still results in a high recurrency [2–4].

This high recurrency in ameloblastomas can be explained in two ways. One group explained that the aggressiveness of these tumours depends mainly on their histological types such as granular cell or follicular types [5, 6]; while another group emphasised that an incomplete surgical removal plays a main role in the recurrency [3, 7].

As a specific type of ameloblastoma, unicystic ameloblastoma deserves its own diagnostic category because of its lower recurrence rate compared with conventional types [8, 9]. To distinguish neoplastic proliferation from reactive cystic epithelium histologically has been a diagnostic problem in the cystic lesion. Also, the differentiation between unicystic ameloblastoma and odontogenic cyst has not been easy to diagnose clinically or radiologically.

The identification of proliferating activity in tumours can be a useful tool to distinguish neoplastic from non-neoplastic conditions and to predict their biological behaviour in malignant tumours [10]. The advent of DNA flow cytometry made it possible to secure information about kinetics of tumour cells [11]. Furthermore, immunohistochemical techniques for cell cycle related antigens are of particular interest in that proliferative fractions can be observed on the basis of morphological architectures. The proliferating cell nuclear antigen (PCNA), as one of the cell cycle related antigens, is particularly useful for retrospective evaluation with routinely formalin fixed paraffin embedded tissues, while Ki-67 only works on frozen tissues [10, 12]. PCNA, an auxillary protein of DNA polymerase delta, is known to be an excellent marker representing DNA synthetic phased cells [12–14]. The recent development of antibodies for PCNA has been providing prognostic information in some malignant tumours [15–17].

Accordingly, we performed the present study for two purposes. One was to evaluate the proliferating activity of ameloblastomas according to the histological types and to correlate this with their biological behaviour. The second was to determine the histological characteristics for the purpose of diagnosing unicystic ameloblastomas with reference to proliferating activity.

MATERIALS AND METHODS

38 cases of ameloblastomas, 25 cases of solid types including one case of ameloblastic carcinoma and 1 recurrent case, and 13 cases of unicystic types were obtained from the file in the department of oral pathology of Yonsei University, Dental College. Additionally, 20 cases of dentigerous cysts were observed.

Most of the cases belonged to plexiform or follicular types, of which there were 9 and 8 cases, respectively. Also, included were 3 cases of the acanthomatous type, 2 cases of the basal cell type, and 1 case of the granular cell type.

Unicystic ameloblastomas were diagnosed by the histological criteria proposed by Vickers and Gorlin [18], and

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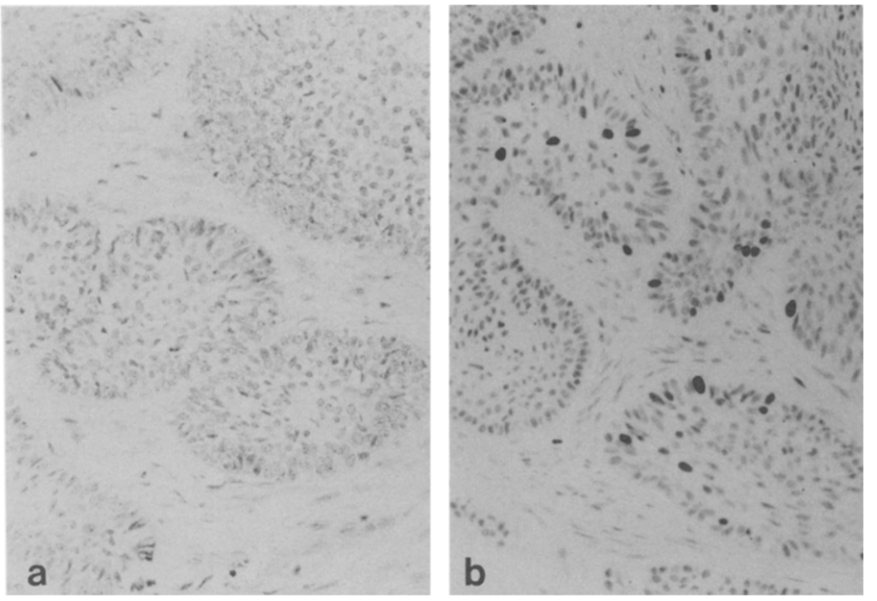

Fig. 1. Follicular ameloblastoma. (a) Haematoxylin eosin, × 66. (b) Most of the positive nuclei are seen in the periphery of the tumour. PC10, × 50.

Robbins and Martinez [9]. Plexiform unicystic ameloblastomas were diagnosed by the criteria reported by Gardner [19, 20].

We used immunoperoxidase staining by the avidin–biotin complex method. The method was as follows: 10% neutralised buffered formalin fixed and paraffin embedded tissues were used for an immunohistological stain, 5 μm sectioned tissues were deparaffinised and immersed with 0.3% methanol containing hydrogen peroxide for 30 min. To analyse the proliferating activity, anti-proliferating cell nuclear antigen antibody, PC10 (DAKO), which was diluted to 1:30, was applied for 30 min and stained by avidin–biotin method with LSAB kit (DAKO). Sections were developed with 3-amino-9-ethylcarbazole (AEC kit, Biomeda) and then counterstained with Meyer's haematoxylin.

Five fields were randomly selected in each case. At high magnification PCNA labelled cells were counted per 2000 tumour cells. The Mann–Whitney test was used for statistical analysis.

RESULTS

Dentigerous cysts

Of the 20 cases of dentigerous cysts studied, only 11 cases demonstrated PCNA positive cells. The labelled cells were counted from 8.6 to 21.3 cells per 2000 epithelial cells in the positive cases.

Conventional ameloblastomas

In solid ameloblastomas, there was no significant difference in each histological type. Each histological type, such as follicular, plexiform, acanthomatous, granular cell and basal cells showed less than 100 positive cells per 2000 tumour cells (Figs 1, 2). In this study, we could not find any evidence to support that the granular cell type was more aggressive than other types in terms of proliferating activity (Table 1).

The fraction of positive cells was remarkably high in an ameloblastic carcinoma and a recurrent ameloblastoma; 379.1 and 240.3, respectively (Table 1, Figs 3–5).

PCNA reactivity showed a variable pattern in every case and area. In follicular ameloblastomas, PCNA positivity was found mainly in the peripheral layered cells (Fig. 1). On the other hand, the positive cells were found not only in the peripheral layer, but also in the central areas in the plexiform type (Fig. 2). Basal cell types revealed relatively even distribution of positive cells throughout. No immunoreactivity was noted in the granular cells of the ameloblastoma.

Unicystic ameloblastomas

The observation was done by studying the histological types of the epithelium lining and the growth pattern of the tumours. The epithelium lining was divided into three types: cystic epithelium without proliferation (Fig. 6), proliferative thickened epithelium without ameloblastic change, and epithelium showing ameloblastic differentiation suggested by Vickers and Gorlin [18]. The growth pattern was divided into two types: invasive growth and intraluminal plexiform growth (Fig. 7).

Again we set our criteria at 2000 cells. The proliferative epithelium showed higher immunoreactivity than the area of the flat cystic epithelium lining; however, there was no statistical significance. Also the epithelium which fit into ameloblastic change showed no significant difference compared to the epithelium without ameloblastic change. The area of plexiform intraluminal growth showed the highest proliferating activity among unicystic ameloblastomas, but there was a wide range in the differentials (Table 2, Fig. 7).

DISCUSSION

Since PCNA was discovered by Miyachi *et al.* in 1978 [21] and then reported as an excellent marker representing DNA synthetic phase cells [12], it has been widely assessed as a useful marker of proliferating index in malignant tumours. In several studies [15–17], PCNA expression correlated well with histological grade and prognosis, although a good correlation may not be seen between PCNA index and S-phase fraction as measured by flow cytometry.

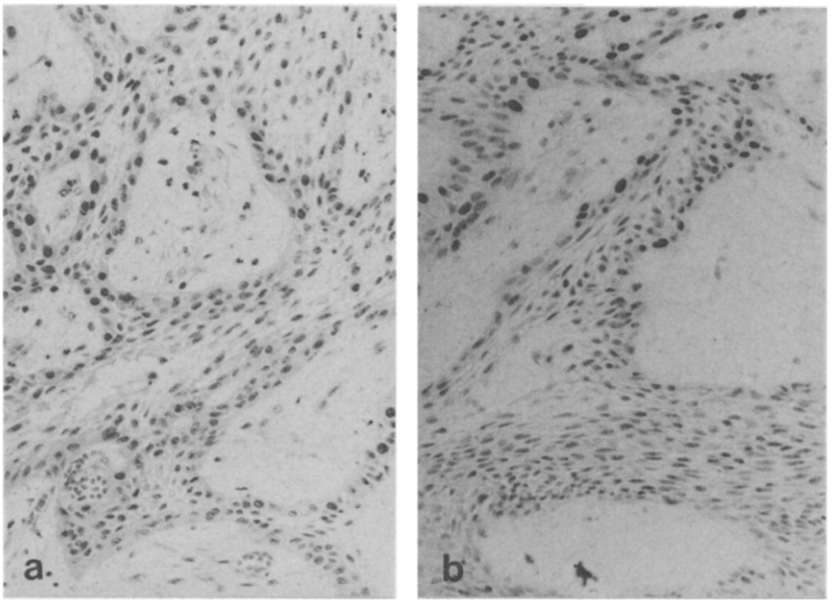

Fig. 2. Plexiform ameloblastoma. (a) Haematoxylin eosin, ×66. (b) Positive nuclei for PC10 are found in the peripheral cells. PC10, ×50.

Table 1. PCNA expression in conventional ameloblastomas

Histological types	No. of cases	Mean score ± S.D. (/2000 cells)
Follicular	8	69.1 ± 25.0
Plexiform	9	76.5 ± 21.9
Acanthomatous	3	77.6 ± 42.3
Basal cell	2	70.4
Granular cell	1	78.9
Ameloblastic ca.	1	379.1
Recurrent amelo.	1	240.3

ca. = carcinoma. amelo. = ameloblastoma.

In our studies, there was no significant difference of proliferative activity between each histological type of solid ameloblastomas. Each histological type, such as follicular, plexiform, acanthomatous and basal cells showed less than 100 positive cells per 2000 tumours cells. In terms of PCNA reactivity, granular cells showed no immunoreactivity. We could not find any evidence to support that the granular cell or the follicular type are more aggressive than other types. To verify the aggressiveness of these types, it is necessary to investigate other factors determining their prognosis.

Malignancy of ameloblastoma, a very uncommon tumour, was defined by WHO in 1972 [22] as a neoplasm, in which the features of an ameloblastoma are observed by the primary and metastatic growth, focusing on the metastasis of this tumour. As has been indicated [23, 24], the definition by WHO had two

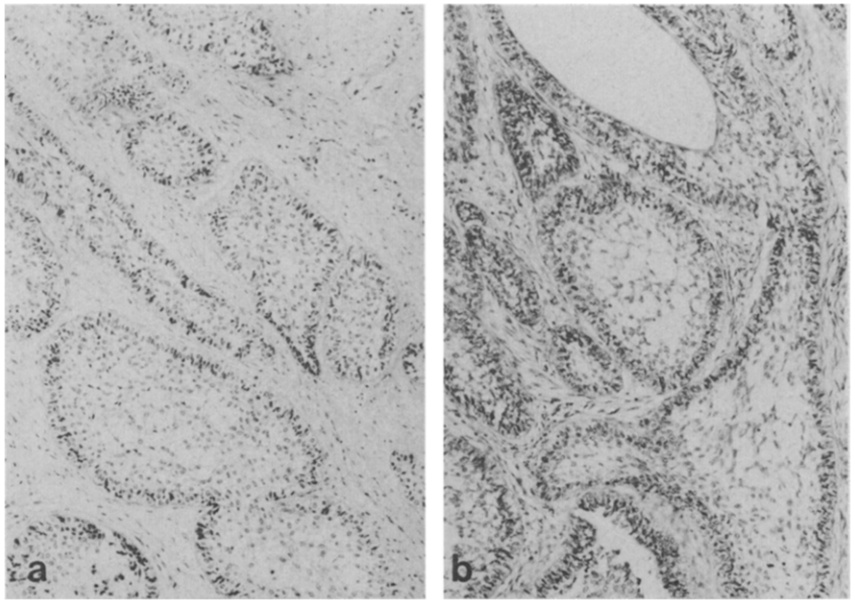

Fig. 3. Recurrent ameloblastoma: both primary (a) and recurrent (b) cases of ameloblastoma show no histological atypia. Haematoxylin eosin, ×25 (a), ×50 (b).

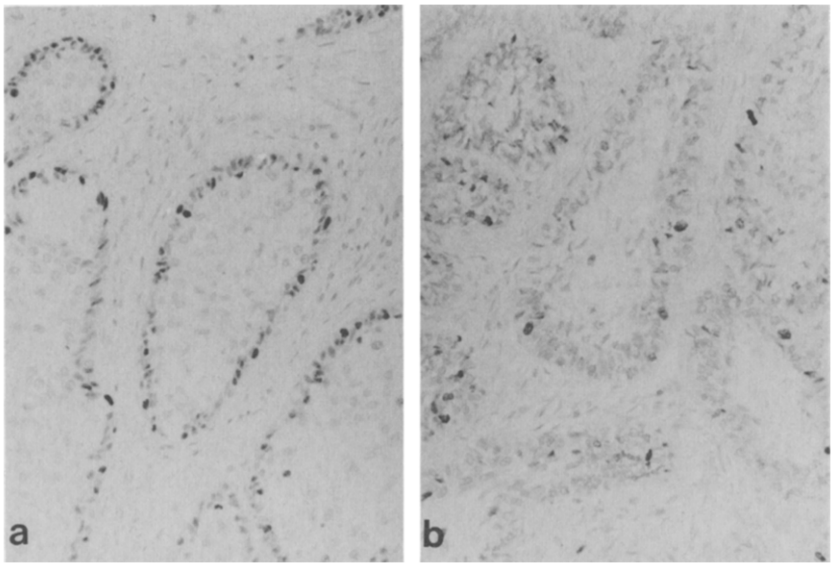

Fig. 4. Recurrent ameloblastoma: primary tumour (a) shows the similar frequency of positive nuclei to that of the recurrent case (b). PC10, × 50.

disadvantages. First, the diagnosis of malignant ameloblastoma could not be made until metastasis could be proven clinically and/or histologically. Secondly, the tumours showing histological malignancy, without proven metastasis, could not be treated as malignant tumours because of the lack of histological description. Further studies by Shafer *et al.* made a distinction between malignant ameloblastoma and ameloblastic carcinoma [2]. They defined ameloblastic carcinoma, as tumours in which there had been histologically malignant transformation. Malignant ameloblastomas were confined to tumours revealing benign features in both primary and metastatic lesions. Slootweg and Muller [23] and Corio *et al.* [24] have further defined ameloblastic carcinoma on the basis of histological malignancy regardless of whether it had metastasised or not. In our studies, we also made a diagnosis of ameloblastic carcinoma, based on histological features showing cellular anaplasia and tumour necrosis. Interestingly, PCNA reactivity was remarkably high in one case of ameloblastic carcinoma, which we found to be statistically significant. Based on this result, PCNA reactivity appeared to be useful in confirming ameloblastic carcinoma.

We observed one case of ameloblastoma with several recurrences. This tumour showed the typical follicular and basal cell pattern of ameloblastoma devoid of the evidence of histological malignancy in both primary and recurrent lesions. Histologically no presumptive evidence of aggressive behaviour was present. In our studies, this recurrent tumour showed noticeably high immunoreactivity in both primary and recurrent cases. PCNA reactivity could be of practical importance in predicting the biological behaviour in ameloblastomas;

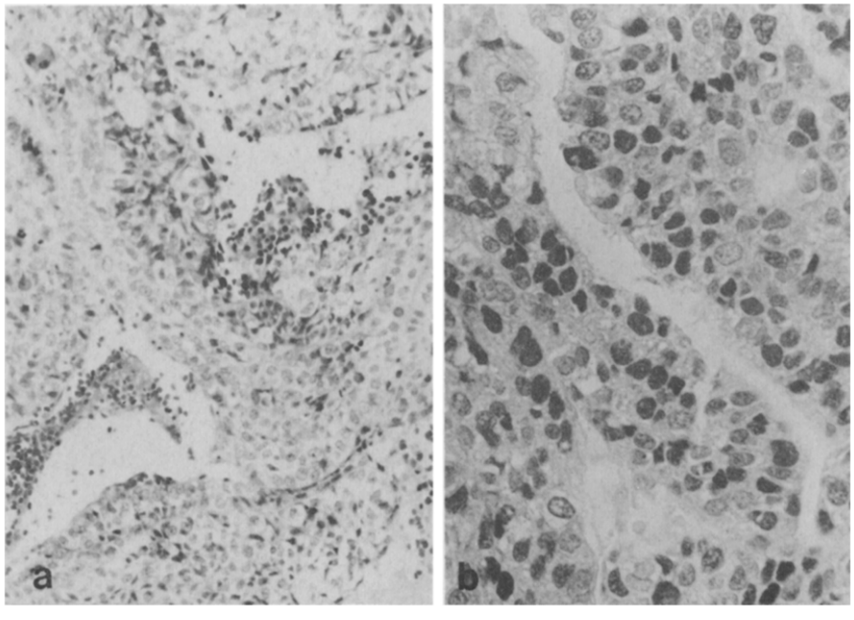

Fig. 5. Ameloblastic carcinoma. (a) Tumour cells show nuclear pleomorphism and tumour necrosis. Haematoxylin eosin, × 40. (b) Most of the cells show positive nuclear staining. PC10, × 80.

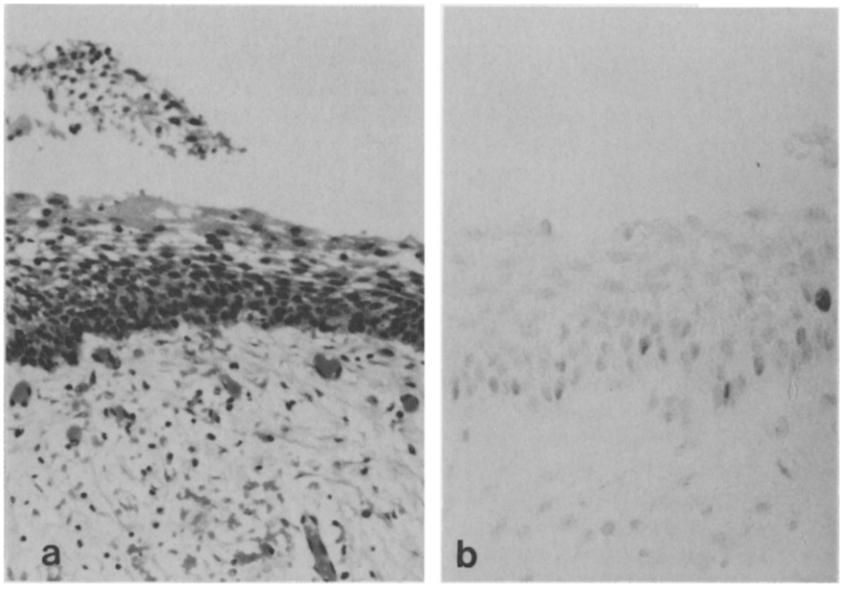

Fig. 6. Unicystic ameloblastoma. (a) The epithelial lining cells without ameloblastic change. Haematoxylin eosin, × 50. (b) A few positive nuclei are seen. PC10, × 100.

however, we need to do further investigation on other cases to be able to accurately use this method for prognostic evaluation.

As a specific variant of ameloblastoma, unicystic ameloblastoma was one of the more difficult lesions to diagnose, because the histological criteria as well as its terminology is still questionable.

Both Vickers and Gorlin [18] and Robinson and Martinez [9] focused on the ameloblastic differentiation of cystic epithelium as a histological marker of the transformation of ameloblastomas from odontogenic cysts; while the latter set two additional criteria; one being invasive growth, the other being intraluminal nodules showing columnar cells with stellate reticulum. Furthermore in their studies, Ackermann *et al.* [25] showed that if there was invasive growth in the connective tissue wall then this needed to be treated more aggressively.

As a different criteria, Gardner reported on plexiform unicystic ameloblastoma [19, 20]. Although no ameloblastic change was shown in the epithelium lining, he regarded the area of intraluminal plexiform proliferation to be a variant of unicystic ameloblastomas.

We found that the epithelium lining in dentigerous cysts showed no or very low PCNA reactivity. But hyperplastic areas accompanied by chronic inflammation revealed higher reactivity than areas without inflammation.

Although both proliferative epithelium with or without ameloblastic change showed higher immunoreactivity than the area of the flat epithelium lining, there was still no statistical

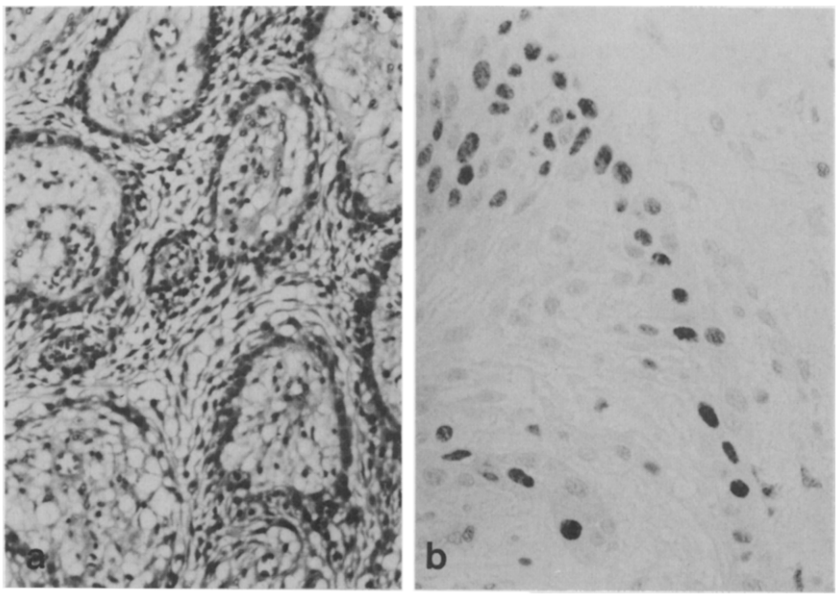

Fig. 7. Unicystic ameloblastoma. (a) Intraluminal growth showing plexiform pattern. Haematoxylin eosin, × 50. (b) The frequency of the positive nuclei is similar to that of conventional ameloblastoma. PC10, × 100.

Table 2. PCNA expression in unicystic ameloblastomas

Histological types	No. of cases	Mean score ± S.D. (/2000 cells)
Epithelium lining		
Non-prol. ep.	11	16.1 ± 10.4
Prol. non-amelo. ep.	8	48.3 ± 21.7
Amelo. ep.	5	39.8 ± 30.1
Growth pattern		
Intraluminal growth	11	84.0 ± 73.4
Invasive growth	7	33.1 ± 29.0

Prol. ep. = proliferative epithelium.
Amelo. ep. = ameloblastic epithelium.

significance found. The ameloblastic epithelium, which fit into histological criteria suggested by Vickers and Gorlin [18], showed no significant difference compared to the epithelium without ameloblastic change. This result showed that PCNA reactivity might be independent of the cytological differentiation.

The area of plexiform intraluminal growth showed the highest proliferating activity among unicystic ameloblastomas, lending support to the idea that plexiform growth represents one pattern of unicystic ameloblastomas [19, 20]. But it is doubtful that PCNA expression accurately represents its proliferating activity in a variety of benign cystic lesions, judging from the wide range of the differentials. The long half life of PCNA and the low proliferating potential of the lesion should be regarded as the reason for the wide range of the differentials. Hall *et al.* [26] showed that there was a poor correlation between PCNA expression and cell proliferation in breast and gastric cancers, due to the long half life of PCNA protein and deregulation of gene expression in those tumours. Although PCNA expression was found to have no correlation to S-phase fraction in some tumours, it was found to correlate well with histological grading in some malignant tumours [15–17]. We can extrapolate that PCNA might be a reliable marker for cell proliferation, even for benign tumours with low proliferating potential, if a good relation between proliferating and nonproliferating normal cells were present with respect to PCNA expression [10].

In conclusion, we could not see any difference in the proliferating activity in each histological type of conventional ameloblastomas, but the recurrency and malignancy can be predicted from PCNA reactivity. We think that plexiform intraluminal growth might be an important feature in tumorous transformation of cystic epithelium. We feel strongly that further studies will validate the usefulness of PCNA as an indicator for the prognostic evaluation of ameloblastomas and for the histological delineation of unicystic ameloblastomas.

1. Lucas RB. *Pathology of Tumours of the Oral Tissues*. Edinburgh, Churchill Livingstone, 1984, 31–51.
2. Shafter WG, Hine MK, Levy BM, Tomich CE. *A Textbook of Oral Pathology*. Philadelphia, J.B. Saunders, 1983, 276–285.
3. Gardner DG, Pecak AMJ. The treatment of ameloblastoma based on pathologic and anatomic principles. *Cancer* 1980, **46**, 2514–2519.
4. Mehlisch DR, Dahlin DC, Masson JK. Ameloblastoma: a clinicopathologic report. *J Oral Surg* 1972, **30**, 9–22.
5. Hartman KS. Granular-cell ameloblasoma. A survey of twenty cases from the Armed Forces Institute of Pathology. *Oral Surg Oral Med Oral Pathol* 1974, **38**, 241–248.
6. Ueno S, Mushimoto K, Shirasu R. Prognostic evaluation of ameloblastoma based on histologic and radiographic typing. *J Oral Maxillofac Surg* 1989, **47**, 11–15.
7. Ikemura K, Tashiro H, Fujino H, Ohbu D, Nakajama K. Ameloblastoma of the mandible with metastases to the lungs and lymph nodes. *Cancer* 1972, **29**, 930–940.
8. Gardner DG, Corio RL. Plexiform unicystic ameloblastoma. A variant of ameloblastoma with a low-recurrence rate after enucleation. *Cancer* 1984, **53**, 1730–1735.
9. Robinson L, Martinez MG. Unicystic ameloblastoma. A prognostically distinct entity. *Cancer* 1977, **40**, 2278–2285.
10. Robbins BA, Vega D, Ogata K, Tan EM, Nakamura RM. Immunohistochemical detection of proliferating cell nuclear antigen in solid human malignances. *Arch Pathol Lab Med* 1987, **111**, 841–845.
11. Landberg G, Tan EM, Roos G. Flow cytometric multiparameter analysis of proliferating cell nuclear antigen/cyclin and Ki-67 antigen: a new view of the cell cycle. *Exp Cell Res* 1990, **187**, 111–118.
12. Dierenconck JH, Eijsman JH, Keijzer R, Velde CJH, Cornelisse CJ. Cell-cycle-related staining patterns of anti-proliferating cell nuclear antigen monoclonal antibodies. *Am J Pathol* 1991, **138**, 1165–1172.
13. Bravo R, Frank R, Blundell PA, MacDonald-Bravo H. Cyclin/PCNA is the auxillary protein of DNA polymerase-delta. *Nature* 1987, **326**, 515–517.
14. Bravo R. Synthesis of the nuclear protein cyclin (PCNA) and its relationship with DNA replication. *Exp Cell Res* 1986, **163**, 287–293.
15. Kamel OW, LeBrun DP, Davis RE, Berry GJ, Warnke RA. Growth fraction estimation of malignant lymphomas in formalin-fixed paraffin embedded tissue using anti-PCNA/cyclin 19A2; correlations with Ki-67 labelling. *Am J Pathol* 1991, **138**, 1471–1477.
16. Yu CC-W, Hall PA, Eletcher CDM, *et al.* Hemangiopericytoma: the prognostic value of immunohistochemical staining with a monoclonal antibody to proliferating cell nuclear antigen (PCNA). *Histopathology* 1990, **19**, 29–33.
17. Woods AL, Hall PA, Shepherd NA, *et al.* The assessment of proliferating cell nuclear antigen (PCNA) immunostaining in primary gastrointestinal lymphomas and its relationship to histological grade, S + G2 + M phase fraction (flow cytometric analysis) and prognosis. *Histopathology* 1991, **18**, 21–27.
18. Vickers RA, Gorlin RJ. Ameloblastoma: delineation of early histopathologic features of neoplasia. *Cancer* 1970, **26**, 699–710.
19. Gardner DG. Plexiform unicystic ameloblastoma: a diagnostic problem in dentigerous cysts. *Cancer* 1981, **47**, 1358–1363.
20. Gardner DG, Corio RL. The relationship of plexiform unicystic ameloblastoma to conventional ameloblastoma. *Oral Surg Oral Med Oral Pathol* 1983, **56**, 54–60.
21. Miyachi K, Fritsler MJ, Tan EM. Autoantibody to a nuclear antigen proliferation cells. *J Immunol* 1978, **121**, 2228–2234.
22. Pindborg JJ, Kramer IRM. Histologic typing of Odontogenic Tumours, Cysts and Allied Lesions. Geneva. World Health Organization, 1971, 53.
23. Slootweg PG, Muller H. Malignant ameloblastoma or ameloblastic carcinoma. 1984, **57**, 168–179.
24. Corio RL, Goldblatt LI, Edwards PA, Hartman KS. Ameloblastic carcinoma: a clinicopathologic study and assessment of eight cases. *Oral Surg Oral Med Oral Pathol* 1987, **64**, 570–576.
25. Ackermann GL, Altini M, Shear M. The unicystic ameloblastoma: a clinicopathological study of 57 cases. *J Oral Pathol* 1988, **17**, 541–546.
26. Hall PA, Levison DA, Wood AC, Yu CC-W, Kellook DB, Watkins JA. Proliferating cell nuclear antigen (PCNA) immunolocalization in parraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. *J Pathol* 1990, **162**, 285–294.