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Immunohistochemical Detection of Proliferating Cell Nuclear Antigen (PCNA) in 23 Cases of Ameloblastoma

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Ameloblastoma is the most frequent odontogenic tumour. It occurs mainly in the mandible and grows expansively. The treatment of ameloblastoma, which influences the prognosis, is decided in consideration of many factors, especially the age and size of the tumour. Conservative treatment sometimes leads to the recurrence of tumours and poor prognosis, but the relationships between the prognosis and the cytological features of tumour cells are still unclear. In the present study, we examined the immunohistochemical detection of proliferating cell nuclear antigen (PCNA) in 23 cases of ameloblastoma and evaluated the correlation between the positive index of PCNA and the clinical and histological character. Our results revealed the higher the age of the patient the greater was the incidence of a positive index of PCNA. It was also shown that the mean positive PCNA index in the follicular type (34.56 ± 14.00 S.D.) was higher than that of the plexiform type (24.436 ± 15.74 S.D., $P < 0.10$). The cystic type showed a low positive PCNA index (14.75 ± 8.41 S.D.). In the follicular type, the localisation of PCNA-positive cells was different according to the histological patterns of tumours. Additionally, the positive indices of the same patient differed at different periods of treatment.

Key words: ameloblastoma, dredging technique, PCNA

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INTRODUCTION

Because of its complex biological character, the treatment of ameloblastoma varies according to the opinion of the individual institution. We have employed radical resection of the tumour and also, for the morphological requirement, deflating or dredging techniques as far as the condition of the patient permits. The dredging technique is a conservative surgical method, in which after deflation and enucleation or only enucleation, repeated dredging was applied to accelate new born formation by removing the scar tissue from the bony cavity [1, 2]. Employment of conservative treatment sometimes leads to the recurrence of the tumour many years after surgery [3, 4]. Some reports showed malignant transformation of ameloblastoma after the recurrence of benign ameloblastoma [5–7]. Although the possibility of malignant transformation of ameloblastoma exists, little is known about the biological character and the mechanism of the malignant transformation of the tumour cells. Proliferating cell nuclear antigen (PCNA) is a co-factor of DNA polymerase δ, which participates in the replication of the leading- and lagging-chains of DNA, and in repair of damaged DNA [8, 9]. PCNA has been utilized for the evaluation of proliferation ability of many types of tumours [10, 11]. Some reports showed the immunohistochemical detection of PCNA in odontogenic tumours [11, 12]. However, the correlation between the localisation of proliferating cells and the morphology of the tumour island has not been discussed. Additionally, no reports revealed the influence of surgical treatment on the ability of tumour cells to proliferate. In the present study, 23 cases of ameloblastoma were investigated by immunohistochemical detection of PCNA and the correlation between the positive index, localisation of PCNA and the clinical and histological character was studied.

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MATERIALS AND METHODS

Tumours

For the present study, 23 cases of well-stocked ameloblastoma, which were excised at the Dental Hospital of the Hokkaido University School of Dentistry from 1984 to

Table 1. Clinical and histological findings

	Plexiform	Follicular	(Cystic)	Total
Number of cases	15	5	(3)	23
Gender (male:female)	12:3	3:2	(2:1)	17:6
Average age	28.2	42	(27.3)	31.1
Treatment				
Only enucleation	6	1	(1)	8
Dredging technique	7	4	(2)	13
Others	2			2

1994, were subjected to the histological and immunohistochemical investigations described below.

Histological and immunohistochemical examinations

Surgically extirpated materials were fixed in 10% neutral formalin. Paraffin-embedded tissues were sectioned at a thickness of 3 μm and stained with haematoxylin–eosin. Immunohistochemical studies were carried out as follows. A monoclonal anti-PCNA antibody (PC 10, Dako Japan, Kyoto, Japan) was applied at 37°C for 60 min. After washing with PBS, a second antibody was applied at room temperature for 40 min. Subsequently, materials were washed with PBS and stained with strepto-avidin–biotin conjugated horseradish-peroxidase (SABC-HRP, Dako Japan), at room temperature for 30 min. Visualisation was carried out with diamino-benzidine (DAB), and counterstaining with haematoxylin. The cells positive for PCNA within at least 1000 tumour cells were counted and the positive index (PI) was calculated. The two-tailed unpaired *t*-test was used for statistical evaluation of the positive index of PCNA.

RESULTS

Clinical and histological findings

We divided the tumours into the plexiform and the follicular types according to the International Histological Classification of Tumours of the World Health Organization [13]. We observed three histologically identifiable cases which showed single cyst formation macroscopically. In this study, we categorised these three cases as the cystic type. The clinical and histological findings are summarised in Table 1.

Correlation between the age of patient and the PI of PCNA

For investigation of the correlation between the age of the patient and the PI of PCNA, we used biopsy specimens to avoid the influence of surgical treatment on PI. Figure 1 shows that the higher the age of the patient the greater the PI of PCNA. However, a remarkably high PI was also seen in two teenage patients.

Correlation between the histological type and the PI of PCNA

The PI of the plexiform cases was 24.4% on average, ranging from 3.3 to 53.1% (S.D. = 15.74). The PI of the follicular type was 29.7% on average, ranging from 5.2 to 42.9% (S.D. = 14.00). The PIs of the cystic type (3 cases) were 5.2%, 8.8% and 20.7% (Fig. 2).

Localisation of PCNA-positive cells

Plexiform pattern tumours. The cells positive for PCNA were mainly basal cells or suprabasal cells of the tumour. We could scarcely find positive inner stellate cells of the tumour island. The positive cells were scattered among basal cells of tumours, and they were also concentrated in some other areas of tumours. We observed that the more positive inner cells there were, the higher the PI of PCNA. In addition, relatively many positive cells were observed in the area showing squamous metaplasia (Fig. 3).

Follicular pattern tumours. PCNA-positive cells were mainly observed among basal cells of the tumour island just as in the plexiform type. We could find different types of localisation of positive cells according to the form of the tumour island. First, in the tumours which were comparatively round, scattered positive cells were observed in the

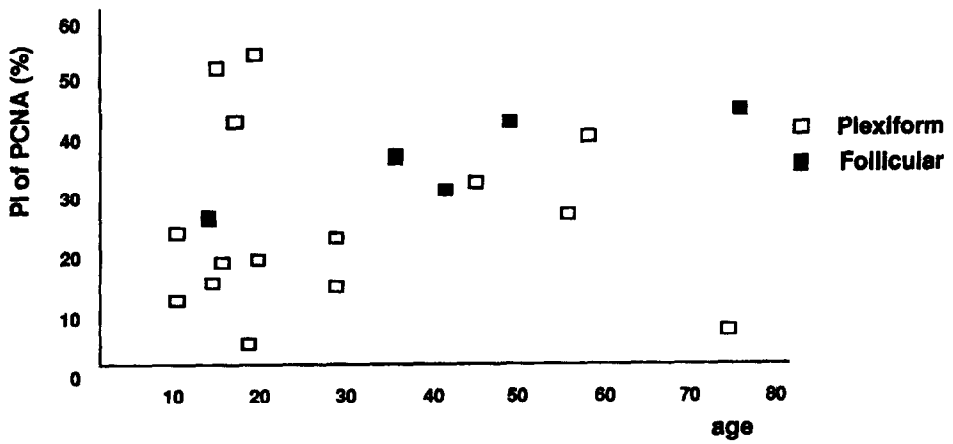

Fig. 1. The correlation between patient age and the positive index of PCNA. The higher the age of the patient the greater the positive index of PCNA.

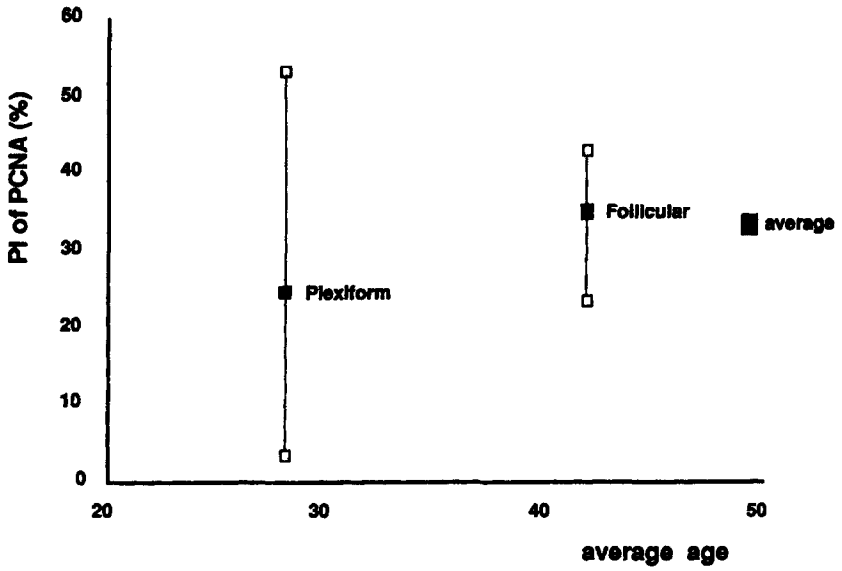

Fig. 2. The positive indices of two types of ameloblastoma. The follicular type had a relatively high index.

entire tumour island. Second, when the form of the tumour island was irregular, positive cells were concentrated in certain areas. In addition, in small nests, relatively many positive cells were observed (Fig. 4).

Changes in the PI with the period of treatment

Comparison of the PIs of biopsy specimens and materials extirpated later, that is materials obtained by operation or dredging, revealed a 33.1% difference, indicating a difference between the PI in the period of treatment and the tumour in the same patient (Fig. 5).

DISCUSSION

Ameloblastoma is considered to be the most common odontogenic tumour, representing about 10% of odontogenic tumours [14]. Approximately 80% of ameloblastomas occur in the mandible and the remaining 20% occur in the maxilla or other parts of the oral cavity [15, 16]. Robinson reported that the average age of patients at the time of initial diagnosis was 30.1 years [17]. No significant difference in the incidence of tumours by sex is reported. Reichart *et al.* reported that plexiform (30.2%) and follicular (33.9%) ameloblastomas constituted the more common histological subtypes. The acanthomatous type (11.3%) was also relatively frequent, whereas the basal cell and the granular cell types of ameloblastoma were rare [13]. Ueno *et al.* reported, as concerns the incidence of recurrence, a better prognosis in younger patients when comparing patients aged less than 20 years old and those aged 20 years or more [18]. Our results revealed a higher positive index of PCNA in aged patients than in younger patients, suggesting a possible correlation between the prognosis and patient age, although in aged patients the treatment procedure is limited by consideration of the many physical factors connected to aging.

Regezi *et al.* [14] and Kramer [19] reported that there is no correlation between histological subtypes, clinical symptoms and biological behavior. In contrast, Ueno *et al.* reported that the recurrence rate was significantly higher in the follicular type (56.8%) than in the plexiform one (32.3%). In addition, recurrence was seen more frequently

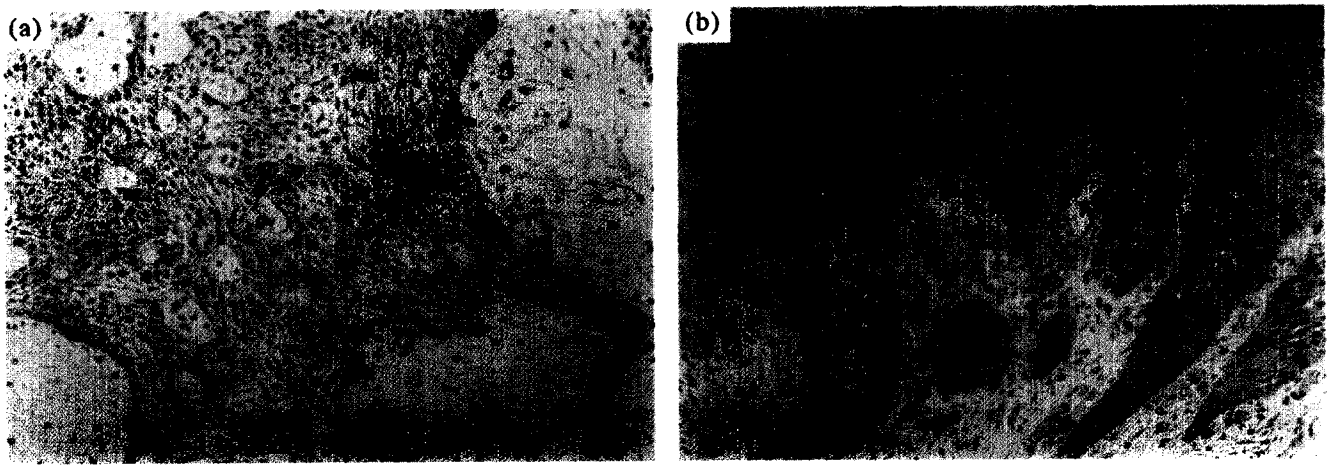

Fig. 3. Results of immunohistochemical study of PCNA in plexiform ameloblastoma. Positive cells were seen in the basal and suprabasal layers (a), × 180. Relatively many PCNA-positive cells were observed in the area showing squamous metaplasia (b), ×180.

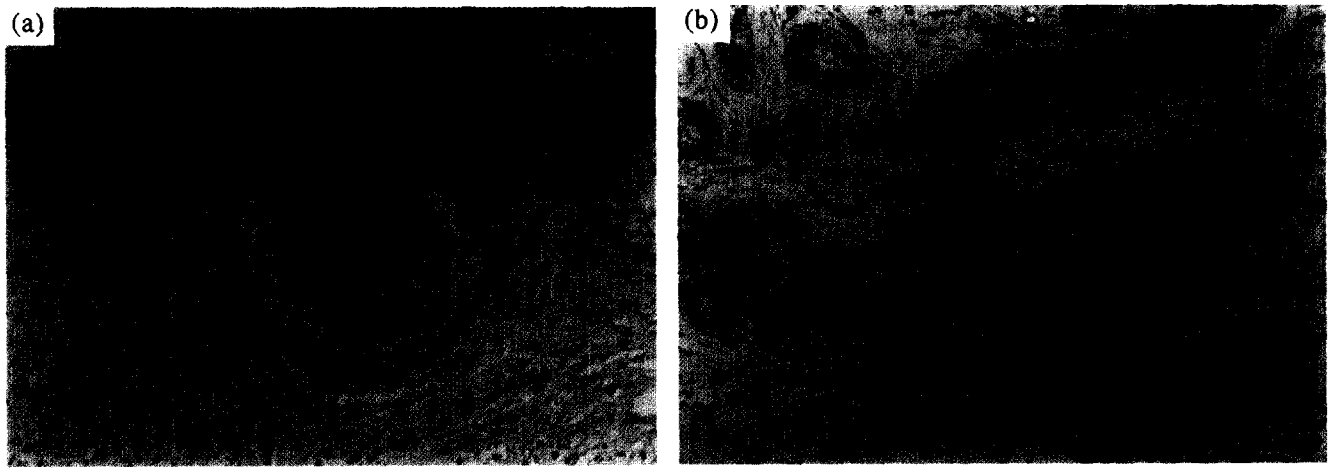

Fig. 4. Localisation of PCNA-positive cells in the follicular type of ameloblastoma. Scattered positive cells were seen in the basal cell layer of the tumour island having a relatively round form (a), ×180. In contrast, in tumour islands having an irregular form, concentrated PCNA-positive cells were seen in various areas (b), × 180.

in the multilocular or soap bubble type (60.7%) than in the unilocular type (35.0%) [18]. Our results revealed higher rates of the positive index of PCNA in the follicular type compared to plexiform ($P < 0.10$). These results suggested that, as concerns the proliferation ability, the follicular type of ameloblastoma may grow more quickly than the plexiform type. The cystic type showed low rate for the positive index of PCNA.

While Stenman *et al.* reported that columnar cells account for the main proliferative capacity of ameloblastomas *in vitro* and most likely also *in vivo* [20], little is reported about the correlation between the morphology of the tumour island and the localisation of proliferating cells. We observed proliferating cells mainly in the basal and suprabasal cell layers of the tumour island, as did Li *et al.* [12], and found some differences in the localisation of proliferating cells according to the form of the tumour island in the follicular type of ameloblastoma. Proliferating cells were observed frequently in bud-like lesions of the tumour island, suggesting that the morphology of the follicular type of ameloblastoma may result from the concentrated localisation of proliferating cells in a certain area of the tumour island. However, no correlation between the morphology of the tumour nest and the localisation of proliferating cells of the plexiform of ameloblastoma was observed.

There are some reports of rare cases of malignant transformation of benign ameloblastomas [7, 21] after several episodes of surgery. These reports indicate the possibility that surgical damage might affect induction of malignant transformation of tumour cells. However, the mechanisms of the malignant transformation of benign ameloblastoma cells are unclear. In our institute, in spite of employment of conservative treatment—the dredging technique—which requires repeated surgery [1, 2], no malignant transformation was observed. In addition, no established cell line, which indicates the immortalisation or transformation of tumour cells, has been reported, although many cases of cell culture of ameloblastoma have been reported [20, 22, 23].

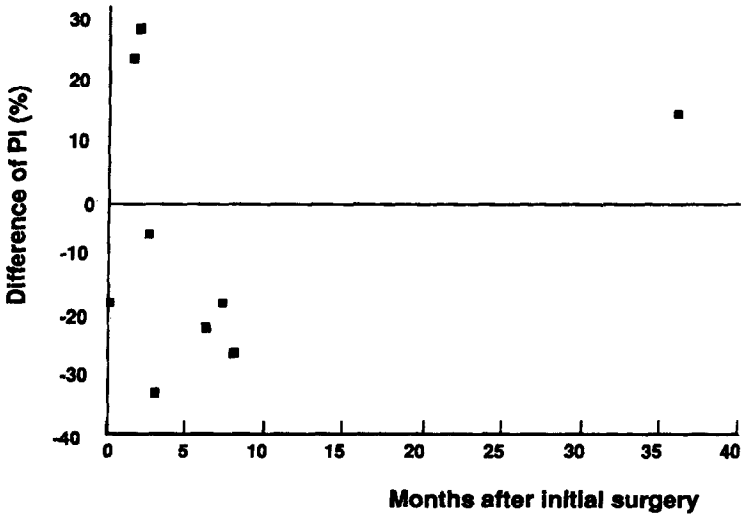

Fig. 5. Comparison of the positive index of PCNA according to the time course of treatment in the same patient. The positive indices obtained from the probed material and material extirpated later are compared. The PCNA-positive index of the probed materials represents 0%.

Our results revealed that the positive index of PCNA changed with the period of treatment. However, clear correlation, for instance, the increase of positive index of PCNA with surgery was not observed. This indicates that the biological character, including proliferative ability, of tumour cells cannot be evaluated from a single examination of the tumour. In summary, we conclude that older patients and those with the follicular type have a higher incidence of PCNA-positive cells. However, for evaluation of the biological character, including proliferative ability, of tumour cells, various materials from different periods of treatment or locations of the tumour have to be studied. In addition, the complex morphological growth pattern of the follicular type, which is likely to lead to microremnants of excised tumours, may be one of the reasons for the poor prognosis compared to the plexiform.

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