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Proliferating Cell Nuclear Antigen (PCNA) and p53 Protein Expression in Ameloblastoma and Adenomatoid Odontogenic Tumor

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In this study, proliferating cell nuclear antigen (PCNA) and p53 protein expressions were analyzed in 16 cases of ameloblastoma and 8 cases of adenomatoid odontogenic tumor (AOT). The cases of ameloblastoma consisted of solid type tumors and histologic arrangements of different subtypes were observed. In some specimens, more than one histologic subtype was identified in the same lesion, and each tumor was categorized according to the predominant cell pattern. The odontogenic tumors were grouped as follows: follicular ameloblastoma (n=7), plexiform ameloblastoma (n=4), acanthomatous + follicular ameloblastoma (n=3), basal cell ameloblastoma (n=2), adenomatoid odontogenic tumor (n=8). PCNA immunohistochemical expression revealed stronger quantitative labeling index for the follicular ameloblastoma, while for p53 protein the strongest quantitative labeling index was detected in the plexiform type. Nevertheless, statistical analysis using ANOVA and Tukey's test did not detect significant differences ($p>0.05$) among the histologic subtypes of ameloblastoma. The findings of this study suggest that the different histologic patterns of ameloblastoma did not show a direct correlation with their clinical behavior and consequently with the prognosis of the cases. The results also indicated that the ameloblastoma has greater proliferative potential than the AOT, which can contribute to explain its more aggressive and invasive characteristics.

Key Words: ameloblastoma, adenomatoid odontogenic tumor, PCNA, p53 protein.

INTRODUCTION

Odontogenic tumors are remarkable among oral lesions because of their clinic and histologic heterogeneity. This diversity reflects in the complex development of dental structures because odontogenic tumors derive from aberrations in odontogenesis. The ameloblastoma deserves special attention, not only because of its particular biologic behavior, exhibiting great infiltrative potential, high recurrence rate and capacity to metastasize, but also due to the relatively high frequency that it is diagnosed among odontogenic tumors.

The adenomatoid odontogenic tumor (AOT), on the other hand, in spite of sharing a common origin (epithelial tissue) with the ameloblastoma, causes less pain and can be treated with conservative surgery, with extremely few reports of recurrence or metastasis.

Three clinically distinct types of ameloblastoma are recognized: solid or multi-cystic, unicystic and peripheral, each of them presenting a specific biologic behavior and thus different prognosis and treatment (1). From a histologic standpoint, the ameloblastoma exhibits distinct microscopic characteristics and a variable histologic pattern, according to which they are classified as follicular, plexiform, granular, basal cell, acanthomatous and desmoplastic (2). Despite this histologic diversity, the cellular patterns can coexist in the same lesion.

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Divergent opinions are found in the literature with respect to the possible relation between the histologic type and the clinical behavior of a lesion. It has been asserted (3) that the follicular ameloblastoma recurs more often than the plexiform type and that the unicystic ameloblastoma shows lower recurrence rate than the solid type.

Knowledge of the biologic behavior of pathologic entities affecting the oral cavity, including the odontogenic tumors, is essential for rendering the most appropriate therapeutic approach and establishing a prognosis for each case. This has led several oral pathologists to investigate different aspects related to the molecular biology of cell populations in tumors, in an attempt to elucidate many points that still remain unclear, resorting to a variety of methodologies. Among these are the tritiated thymidine ([3H]-thymidine) incorporation followed by autoradiography; bromodeoxyuridine incorporation followed by anti-BrdUimmunohistochemistry; flow cytometry analysis; and immunohistochemistry using specific monoclonal anti-bodies that recognize antigens related to the dynamics of the cell cycle (4).

Several studies (5-8) have tried to explain the mechanisms of proliferative activity of various lesions with an oral origin. Regarding the ameloblastoma, variable results have been shown (9-13) with respect to its histologic subtypes.

The purpose of this immunohistochemical study was to investigate PCNA and p53 protein expressions in ameloblastoma and adenomatoid odontogenic tumor, as they are known to play important roles in cell proliferation and tumorigenesis.

MATERIAL AND METHODS

The material used in this study consisted of 16 cases of ameloblastoma and 8 cases of adenomatoid odontogenic tumor obtained from the files of the Department of Oral Pathology of the Federal University of Rio Grande do Norte, Natal, RN, Brazil.

The paraffin-embedded material was prepared in 5-µm-thick slices, which were stained with hematoxylin and eosin, mounted on microscope slides and examined by optical microscopy to evaluate the morphologic aspects of the sample.

The cases of ameloblastoma consisted of solid type tumors and histologic arrangements of different subtypes were observed. In some specimens, more than one histologic subtype was identified in the same lesion, and each tumor was categorized according to the predominant cell pattern. The odontogenic tumors were grouped as follows: follicular ameloblastoma (n=7), plexiform ameloblastoma (n=4), acanthomatous + follicular ameloblastoma (n=3), basal cell ameloblastoma (n=2), adenomatoid odontogenic tumor (n=8).

For the immunohistochemical reaction, 3-µm-thick slices were obtained from the paraffin-embedded material and submitted to the following methodology: paraffin removal; hydration in a decreasing ethanol series; removal of formalin pigment with 10% ammonium hydroxide in 95% ethanol; treatment in a microwave oven (3 cycles of 5 min at maximum power of 700 watts); blocking of endogenous peroxidase using a hydrogen peroxide solution; incubation with primary anti PCNA antibody (PC-10, diluted 1:80; Biogenex, San Ramon, CA, USA) for 18 h and anti-p53 antibody (DO-7, diluted 1:50; DAKO, Carpinteria, CA, USA) for 18 h; incubation in secondary serum; streptoavidin-biotin complex (SABC) for 30 min at room temperature; development of the reaction with diaminobenzidine.

Between stages, the material was immersed in a Tris phosphate buffer solution, pH 7.4 (Vetec, Rio de Janeiro, RJ, Brazil). After development of the reaction, the material was counterstained with Mayers hematoxylin (Vetec) in Permount (Fisher Scientific, New Jersey, NY, USA).

The slices submitted to immunohistochemical reaction were observed by optical microscopy both for analysis of the presence of a reaction and for quantitative assessment of the areas corresponding to the histologic patterns of ameloblastoma and AOT.

The quantitative analysis for PCNA and p53 expressions was undertaken using the index of positivity (IP), calculated by the relation between the number of PCNA and p53 positive cells per 1000 cells counted for each case studied, multiplied by 100 [(PCNA- and p53-positive cells/1000 cells) X 100]. Counting was undertaken using optical microscopy with X1000 magnification.

RESULTS

All examined lesions were positive for the antibodies used (PCNA and p53), positivity being considered as the presence of a cell exhibiting any nuclear

labeling.

In the cases of ameloblastoma, the peripheral cylindrical epithelial cells of the islets presented, in general, strong PCNA labeling index (Fig. 1) and moderate to weak p53 protein labeling index (Fig. 2). Some cells in the central area of the follicles resembling the stellate reticulum of the enamel organ also presented positive PCNA labeling index. In the cases of AOT, the PCNA positive cells showed strong labeling (Fig. 3) while the cells labeled with anti-p53 antibody showed weak labeling (Fig. 4).

The results of the quantitative analysis of the PCNA and p53 positive cells are given in Table 1.

PCNA and p53 protein labeling indices were compared in the five groups of lesions using one-way ANOVA. The results showed no statistically significant difference ($p>0.05$) among the groups for PCNA, whereas significant difference ($p<0.05$) was detected for p53 protein. Multiples comparisons using Tukey's test showed significant difference ($p<0.05$) between the follicular ameloblastoma and the AOT regarding the indices of positivity for the p53 protein.

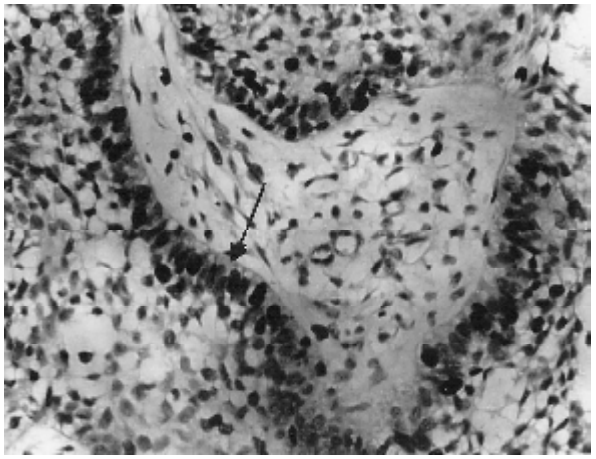

Figure 1. Histologic section of the follicular ameloblastoma showing strong nuclear staining for PCNA (arrow). SABC. (original magnification X 200).

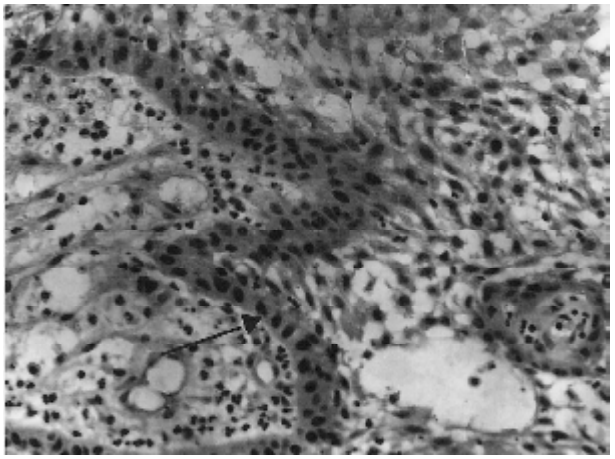

Figure 2. Histologic section of the follicular ameloblastoma showing p53 positive cells (arrow). SABC. (original magnification X 200).

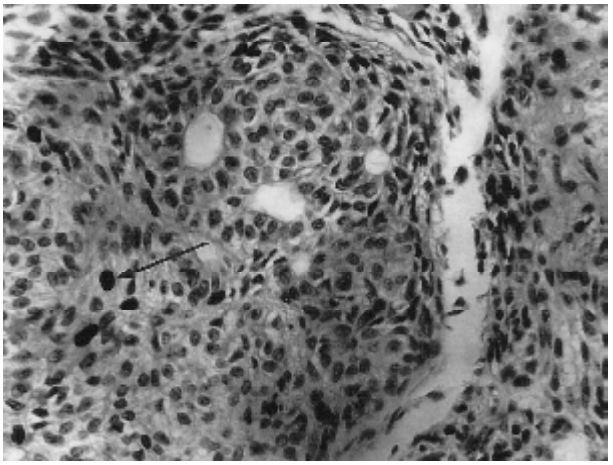

Figure 3. Histologic section showing positive cells in AOT with moderate staining for PCNA (arrow). SABC. (original magnification X 200).

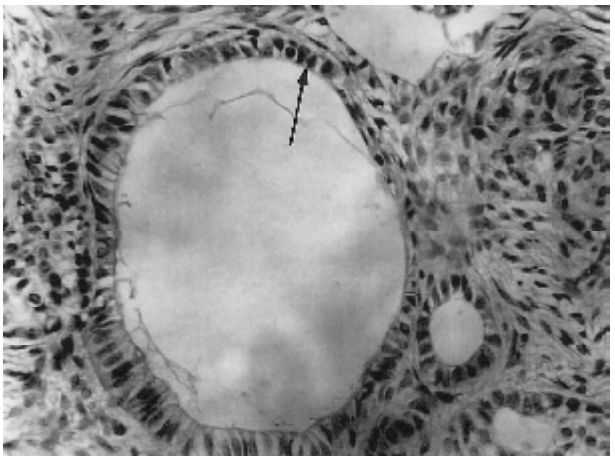

Figure 4. Histologic section showing p53 positive cells in AOT (arrow). SABC. (original magnification X 200).

Table 1. Statistical results for PCNA and p53 indices of positivity in the odontogenic tumors, according to histologic subtype.

	n	Mean ± SD	Minimum	Maximum
PCNA index				
Follicular ameloblastoma	7	78.4 ± 15.9	59.7	95.7
Plexiform ameloblastoma	4	74.3 ± 16.9	51.0	91.3
Follicular + acanthomatous ameloblastoma	3	77.5 ± 8.2	68.9	85.3
Basal cell Ameloblastoma	2	69.7 ± 23.5	53.1	86.3
Adenomatoid odontogenic tumor	8	56.6 ± 18.4	28.9	82.0
p53 index				
Follicular ameloblastoma	7	42.4 ± 14.1*	23.6	59.7
Plexiform ameloblastoma	4	46.0 ± 15.9	26.8	64.2
Follicular + acanthomatous ameloblastoma	3	31.6 ± 27.8	3.8	59.3
Basal cell Ameloblastoma	2	26.1 ± 16.9	14.1	38.0
Adenomatoid odontogenic tumor	8	19.5 ± 11.1*	7.2	34.9

Data from the Postgraduate program in Oral Pathology, UFRN. n = number of cases. *Statistically significant difference ($p<0.05$; ANOVA and Tukey's test).

DISCUSSION

A possible correlation between the biologic behavior of ameloblastomas and their histologic appearance has been investigated over the years in an attempt to establish histologic criteria that could be helpful not only in the treatment but also in the establishment of a prognosis for these lesions. According to Gardner (1), it is important to recognize the clinical types of ameloblastomas because the unicystic and peripheral tumors have better prognosis after conservative resection than the solid ameloblastomas. The sample we examined in this study consisted of solid ameloblastomas and the following histologic subtypes were identified: follicular, plexiform, acanthomatous, basal cell and desmoplastic. In some cases, more than one histologic subtype was found in the same lesion, as reported in the literature. Histologic patterns of granular cells and clear cells were not observed.

Studies (1,14) have postulated that there is no correlation between the histologic types of ameloblastomas and the clinical behavior (and consequent prognosis) of these lesions because more than one cellular configuration can be seen in a single lesion. On the other hand, Ueno et al. (15) stated that it is possible to establish a correlation between both the patient's age and the radiographic/histologic aspects of a tumor and its clinical behavior. They also reported that the ameloblastoma with a follicular histologic pattern presents higher recurrence rate, which is consistent with the findings of Reichart et al. (3), who reported that the follicular ameloblastoma recurs more often than the plexiform ameloblastoma.

Studies using the AgNOR technique (5,12) have found no differences in the cellular activity of the ameloblastoma and the adenomatoid odontogenic tumor, but statistically significant differences were observed (5) between the follicular and plexiform ameloblastoma subtypes. In the present study, there was statistically significant higher incidence of PCNA labeling in the cases of ameloblastoma (mean 76.0%) than in the cases of AOT (mean 56.6%). Higher PCNA labeling index may indicate higher cellular proliferation rate, which would explain the more aggressive biologic behavior of the ameloblastoma compared to the adenomatoid odontogenic tumor. However, PCNA expression probably results not only form cellular proliferation, but also from other sources, including DNA repair (16) and factors influencing the increase of autocrine and paracrine rates in messenger-RNA of the proliferating cell nuclear antigen (17). Because of these high levels, PCNA can be found in cells that are not in the cellular cycle. According to Scott et al. (18), another important issue that should be considered is that PCNA has a considerably longer half-life (approximately 20 h) compared to the rapid cell cycle time.

Taking into account the various histologic subtypes of ameloblastoma recognized in the sample, the 16 cases included in this study were categorized according to the predominant cell pattern. The follicular ameloblastoma presented the strongest PCNA labeling index (mean 78.4%), while the basal cell ameloblastoma showed the weakest labeling index (mean 69.7%). One-way analysis of variance did not detect statistically significant differences either among the histologic subtypes of ameloblastoma or between the cases of ameloblastoma and AOT. These findings are consistent with those of Kim and Yook (6), who did not find differences

in the proliferative activity among the different histologic types of solid ameloblastoma and Carvalhais et al. (19) who did not find significant difference between the follicular and the plexiform ameloblastoma. Nevertheless, these findings diverge from those of Funaoka et al. (9), who reported higher PCNA labeling index for the follicular than for the plexiform ameloblastoma, and Kumamoto et al. (10), who stated that the basal cell ameloblastoma possesses more proliferative activity than other types of ameloblastoma. An alternative statistical analysis using the Student's t-test, which is less conservative, was also done but no difference was found among the various histologic subtypes of ameloblastoma. Statistically significant difference was detected only between the follicular ameloblastoma and the AOT, the first presenting greater proliferative potential. According to Reichart et al. (3) and Ueno et al. (15), this could explain the higher recurrence rate of follicular ameloblastomas.

All lesions in this study presented p53 positive cell labeling, the mean labeling index being 37.2% among the 16 cases of ameloblastoma and 19.5% among the 8 cases of AOT. The cases of ameloblastoma presented, in general, moderate to weak p53 labeling index, while the cases of AOT presented weak p53 labeling index. Considering the different histologic pattern of ameloblastoma, it was observed that the plexiform subtype had the strongest labeling index (mean 46.0%), followed by the follicular subtype (mean 42.4%). Comparing the different histologic patterns of ameloblastoma among each other and with the AOT, one-way ANOVA detected significant differences between the groups of lesions. Tukey's test for multiple comparisons identified significant difference between the follicular ameloblastoma and the AOT. An alternative statistical analysis using the Student's t-test, which is less conservative, detected significant difference between the plexiform ameloblastoma and the AOT. The cases of plexiform ameloblastoma having the strongest p53 labeling index could be indicative of the higher level of cellular activity. However, we believe that comparison of our results for p53 labeling index to those of other studies may not be useful to assess the kinetic cellular events in cellular kinetic tumors because variations in the methodology, such as undertaking or not antigenic recuperation in a microwave oven, can considerably alter the labeling index, as shown by Dowell and Ogden (20).

PCNA and p53 protein immunodetection results showed that the ameloblastoma has greater proliferative potential than the AOT, which can be helpful to explain the more aggressive biologic behavior of ameloblastomas. However, our results differ from those obtained using AgNOR technique (5,12), in which differences between the ameloblastoma and the AOT were established in terms of cellular activity.

In this study, the morphologic analysis of ameloblastoma cases identified the presence of more than one histologic pattern in the same lesion. PCNA immunohistochemical expression revealed stronger quantitative labeling index for the follicular ameloblastoma, while for p53 protein the strongest quantitative labeling index was detected in the plexiform type. Nevertheless, statistical analysis did not detect significant differences among the histologic subtypes of ameloblastoma. The findings of this study suggest that the different histologic patterns of ameloblastoma did not show a direct correlation with their clinical behavior and consequently with the prognosis of the cases. The results also indicated that the ameloblastoma has greater proliferative potential than the AOT, which can contribute to explain its more aggressive and invasive characteristics.

RESUMO

Nesse estudo, a expressão do antígeno nuclear de proliferação celular (PCNA) e da proteína p53, foi analisada em 16 casos de ameloblastoma e 8 casos de tumor odontogênico adenomatóide (TOA). Os casos de ameloblastoma eram do tipo sólido e, do ponto de vista morfológico, apresentavam-se nos diferentes subtipos histológicos. Em alguns casos, contudo, havia mais de um subtipo histológico na mesma lesão. As lesões foram então categorizadas em função da predominância dos achados histológicos, tendo sido identificados 7 casos de ameloblastoma com padrão folicular, 4 plexiformes, 3 foliculares + acantomatosos e 2 de células basais.Todas as lesões exibiram positividade para o PCNA e para a proteína p53, embora a expressão imunoistoquímica para o PCNA tenha sido mais forte nos casos de ameloblastoma folicular, enquanto a expressão da proteína p53 tenha se apresentou mais forte em ameloblastomas do subtipo plexiforme. A análise estatística (ANOVA e Teste de Tukey) não revelou diferença signficante ($p>0.05$) entre os tipos histológicos do ameloblastoma. Estes achados sugerem que os padrões histológicos do ameloblastoma não apresentaram correlação direta com o comportamento clinico e, conseqüentemente, com o prognóstico destas lesões. Os resultados também indicam que o ameloblastoma tem maior potencial proliferativo do que o TOA, o que contribui para explicar sua característica mais agressiva e invasiva.

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