IMMUNOHISTOCHEMICAL EVALUATION OF PCNA, P53 AND SURVIVIN IN AMELOBLASTOMA

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ABSTRACT

The purpose of this study was carried out to examine the expression of PCNA, P^{53} and survivin in different types of ameloblastoma. The cases of ameloblastoma consisted of solid type tumors and histologic arrangements of different subtypes were observed. In some cases, more than one histologic subtype was identified in the same lesion, and each tumor was categorized according to the predominant cell pattern. PCNA and survivin immunohistochemical expression revealed stronger staining for the follicular ameloblastoma, while for p^{53} protein the strongest stain was detected in the plexiform type. The results indicated that the ameloblastoma has great proliferative potential, which can contribute to explain its aggressiveness and invasive characteristics.

INTRODUCTION

Odontogenic tumors are remarkable among oral lesion because of their clinic and histologic heterogenicity [(4)]. Ameloblastoma is the most frequently encountered tumor arising from odontogenic epithelium and is characterized by a benign but locally invasive behavior, with high risk of recurrence [(16)]. Histologically, ameloblastoma shows considerable variation, including follicular, plexiform, acanthomatous, granular cell, basal cell and desmoplastic types [(8)].

Determination of epithelial proliferative activity is a potentially useful means of investigating differences in biologic behavior between tumors. Assessment of cell proliferation in many types of tumors is an important adjunct to histologically based tumor classification and has potential relevance as an indicator of tumor behavior, treatment response and relapse. Immunohistochemical assessment of cell proliferation has advantage over the other techniques such as titrated thymidine incorporation and flow cytometry, because the tissue architecture remains intact and proliferating cells can be visualized in relation to other histologic characteristics [(17)].

PCNA is one of several cell cycle-related nuclear proteins that are maximally elevated in the late G1 and S - phases of proliferating cells. The current importance of PCNA as a marker of cellular proliferation relates to its ability to be reproducibly detected in routinely fixed and processed tissues. It is more convenient marker than other proliferation

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markers, such as DNA polymerase alpha and bromodeoxyuridine [18].

Apoptosis, -also known as programmed or physiologic cell death-, plays divers roles in embryogenesis and normal homeostasis, as well as oncogenesis. Apoptotic process is modulating by various factors which have inhibitory or stimulatory effects [14]. The inhibitor of apoptosis protein (IAP) family proteins are characterized by a novel domain and this domain is capable of inhibiting caspases and thus suppressing apoptosis [6].

Survivin is an IAP member, whose gene is located in chromosome 17q25 and is a unique bifunctional protein which suppresses apoptosis by inhibiting caspases 3 and 7 and regulates the G2/M phase of the cell cycle by associating mitotic spindle microtubules [21]. Expression of survivin is largely absent from normal adult tissues, but is highly identified in developing fetal tissues and many common types of neoplasms [13].

p^{53} is a nuclear protein that is essential for cell cycle control, DNA repair and induction of apoptosis by many stresses [19]. Mutations in the p^{53} tumor suppressor gene are among the most common abnormalities in human cancer. Expression of p^{53} has been observed in normal epithelia, including oral mucosa and has also been detected in tissues of developing teeth. In tumor derived and transformed cell lines the level of p^{53} are often elevated and several investigators have suggested that inactivation of the p^{53} gene confers a selective advantage for the development of the tumorigenic phenotype with its subsequent impact on changing cellular activity [7].

In the present study, expression of PCNA, p^{53} and survivin in ameloblastoma was examined using immunohistochemistry, as they are known to play important roles in cell proliferation and tumorigenesis, to clarify the possible role of these proteins in ameloblastoma and their association with the varied histologic subtypes of ameloblastoma.

MATERIALS AND METHODS

Fifteen formalin fixed paraffin embedded blocks of ameloblastoma were retrieved from the biopsy files of the Pathology Department, National cancer institute, Cario, Egypt. The tumors were grouped as follows; follicular ameloblastoma (n=5), plexiform ameloblastoma (n=4) granular ameloblastoma (n=3), acanthomatous+follicular (n=4) and basal cell ameloblastoma (n=1). The sections were cut at 4 um thick from paraffin blocks and stained with hematoxyline and eosin (H&E) for the confirmation of the histopathologic examination and diagnosis.

Immunohistochemistry

For immunohistochemistry, 5 um thick sections were cut and transferred on to the slide, deparafinized in xylene, and hyderated in a decreasing ethanol series. Endogenous peroxidase activity was blocked by immersing the slides in 0.3% hydrogen peroxide solution. Antigenecity were retrived by microwave heating (3 cycles of 5 min) in acetate buffer (pH 6). The sections were incubated with the following primary antibodies at the dilution specified (PCNA diluted 1: 100 overnight, p53 diluted 1:300 overnight, survivin Ab-6 diluted 1:50). Incubation in secondary serum, streptoavidin –biotin complex for 30 min at room temperature, development of the reaction with diaminobenzidine (DAB). Between stages, the material was immersed in phosphate buffer saline (PBS). After development of the reaction, the material was counterstained with Mayers hematoxyline (2 min) and mounted.

The slides submitted to immunohistochemical reaction were observed by optical microscopy both for analysis of the presence of the reaction and for quantitative assessment of the areas corresponding to the histologic patterns of ameloblastoma. Immunohistochemical staining intensity was evaluated on a qualitative 2 point scale described previously [7]. According to the classification used in this study, a positive staining would mean the presence of a dark, brownish, intranuclear precipitate. A negative reac-

tion would not show any brownish staining. For quantization of the state of PCNA, p^{53} and survivin proteins expression, the mean percentage of positive cells was determined in at least five random fields at 400x magnification in each section. The intensity of the immunoreactions then was scored as follows: 1+, weak; 2+, moderate; 3+, intense.

RESULTS

Immunohistochemical reactivity for PCNA

PCNA was stained in all tissue sections mainly in the nuclei of the outer layer cells of ameloblastoma, and only a few positive cells were seen in the inner layer. In general, intense PCNA staining was found in the follicular ameloblastoma (fig. 1) than plexiform subtype. The basal cell ameloblastoma showed the weakest staining. In acanthomatous ameloblastoma, only the nuclei of outer layers of epithelial component were positively stained while the inner layers were weak to negative (fig. 2).

Immunohistochemical reactivity for P^{53}

Immunohistochemical reactivity for p^{53} was more concentrated in the nuclei of peripheral columnar or cuboidal cells. The peripheral epithelial cells of the islets presented moderate to weak staining (fig. 3). Plexiform ameloblastomas exhibited higher p^{53} expression than follicular ameloblastomas. Keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas were not with anti-p^{35} antibody. Basal cell ameloblastomas showed p^{53} reactivity in scattered neoblastic cells.

Immunohistochemical reactivity for survivin

Ameloblastomas showed survivin reactivity in all peripheral columnar and central polyhedral cells (fig. 4). Keratinizing cells showed no reactivity for survivin in acanthomatous ameloblastomas while granular cells showed intense reactivity for survivin in granular cell ameloblastomas (fig. 5). Expression of survivin protein tended to be higher in follicular ameloblastomas than in plexiform ameloblastomas. Basal cell ameloblastoma showed survivin reactivity in most neoblastic cells.

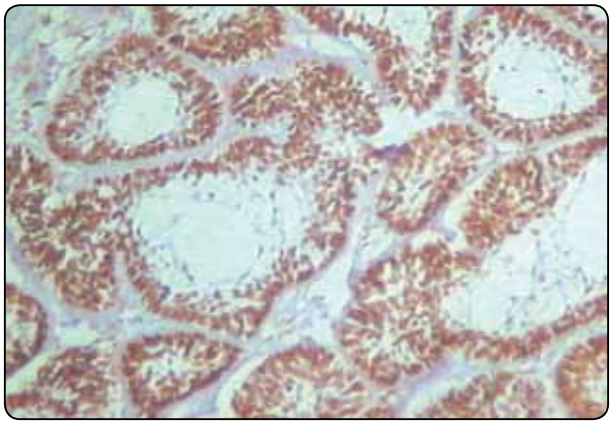

FIG. (1) : PCNA expression of peripheral columnar or cuboidal cells and central polyhedral cells (I7 X10).

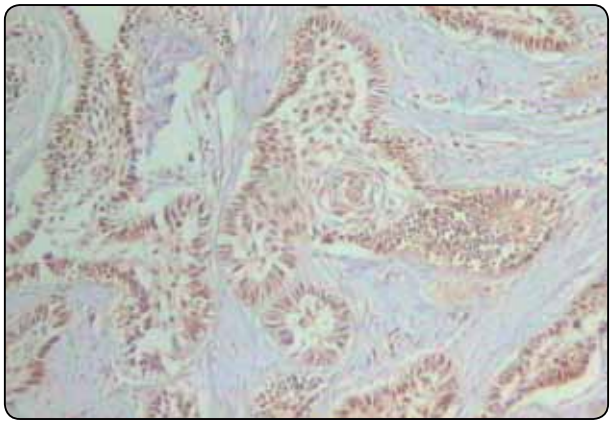

FIG. (2): PCNA expression of peripheral columnar or cuboidal cells and central polyhedral cells (18 X10)

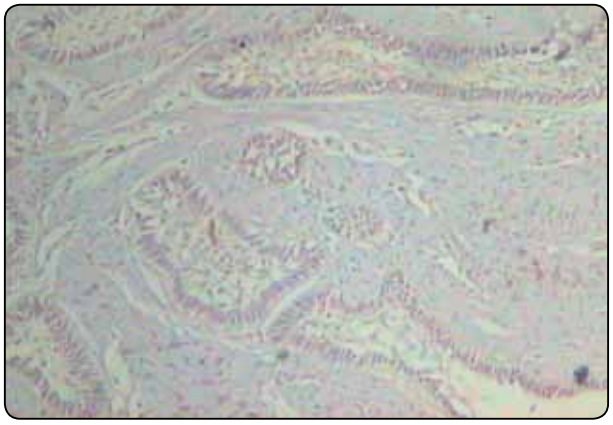

FIG. (3): p53 expression showing weak staining in peripheral columnar or cuboidal cells (15 x 10)

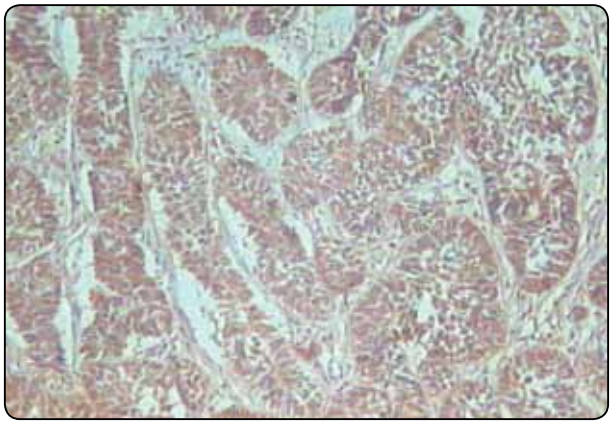

FIG. (4): survivin expression of peripheral columnar or cuboidal cells and central polyhedral cells (1 x 10)

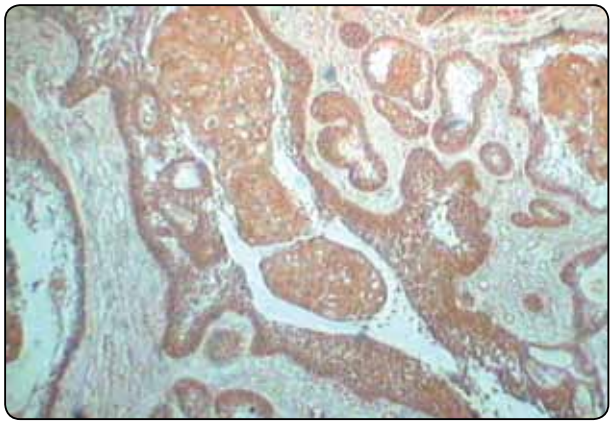

FIG. (5): survivin expression of peripheral columnar or cuboidal cells and central granular cells (S 9 x 3.2)

DISCUSSION

Ameloblastomas are the most common human odontogenic neoplasia and although typically considered locally invasive and benign, they frequently recur subsequent to surgical resection (55-90%) and can even occasionally undergo malignant transformation [3,22].

The proliferating tumor may infiltrate the cancellous marrow spaces without causing bone destruction. It tends to expand the bone rather than perforate it. The expansion may become extremely disfiguring and local extension to the base of the skull is life-threatening [1].

A possible correlation between the biologic behavior of ameloblastoma 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. The sample we examined in this study consisted of solid ameloblastomas and the following histologic subtypes were identified: follicular, plexiform, acanthomatous, granular and basal cell. In some cases, more than one histologic subtype was found in the same lesion.

In the present study, PCNA expression was found to be strong in most of the cases. Strong PCNA staining may indicate higher cellular proliferation, which would explain the aggressive biologic behavior of the ameloblastoma. Because most of the tissue sections did not contain positive inner layer cells, and outer layer cells are known to reflect the growth activity of ameloblastoma. Taking into account the various histologic subtypes of ameloblastoma recognized in the sample, the 15 cases included in this study were categorized according to the predominant cell pattern. The follicular ameloblastoma presented the strongest PCNA staining, while the basal cell ameloblastoma showed the weakest staining. In acanthomatous ameloblastoma, the nuclei of outer layers of epithelial component were positively stained while the inner layers were negative. This suggested that inner layer cells of acanthomatous ameloblastoma were in a mature stage and not actively proliferating. But there were no differences among the various histologic subtypes of ameloblastoma. These findings are consistent with those of **Kim and Yook,**[9] who did not find differences in the proliferative activity among the different histologic types of solid ameloblastoma and **Carvalhais et al.,**[5] who did not find significant difference between the follicular and plexiform ameloblastoma. Nevertheless, these findings diverge from those of **kumamoto et al.,**[10] stated that the basal cell ameloblastoma possesses more proliferative activity than other types of ameloblastoma**.**

All cases in this study presented p53 positive staining. The cases of ameloblastoma presented, in general, moderate to weak p53 staining. Considering the different histologic pattern of ameloblastoma, it was observed that the plexiform subtype had the strongest stain, followed by the follicular subtype. Higher reactivity for p53 in plexiform type than in follicular type suggesting that tissue structuring of ameloblastoma might be affected by p53 expression. The results of this study were agreement with the previous results of **Slootweg** [15]**, Kumamoto**[12] **and Barboza**[4].

Survivin function as a suppressor of apoptotic cell death and is also involved in regulation of cell division. Survivin is highly expressed during embryonic and fetal development, but is not detected in terminally differentiated adult tissues, except for placenta and thymus [2]. Although expression of survivin is very restricted in normal adult tissues, high expression of surviving is found in many common human malignancies [2,11,13].

In the present study, Ameloblastomas showed survivin reactivity in all peripheral columnar and central polyhedral cells. Keratinizing cells showed no reactivity for survivin in acanthomatous ameloblastomas these features suggest that survivin protein might have a role in terminal differentiation of these neoplastic cells. Expression of survivin protein tended to be higher in follicular ameloblastomas than in plexiform ameloblastomas. These results suggest that survivin protein may be involved in tissue structuring of ameloblastomas. These results were in accordance with **Kumamoto et al.,**[10] who reported that ameloblastomas expressed survivin protein chiefly in neoplastic cells neighboring the basement membrane.

In conclusion, in this study, the morphologic analysis of ameloblastoma cases identified the presence of more than one histologic pattern in the same lesion. PCNA and survivin immunohistochemical expression revealed strong staining for the follicular ameloblastoma while for p^{53} protein the strong staining was detected in the plexiform type. The results indicating that the follicular type of ameloblastoma was found to be the most actively proliferating cell type. This study will be useful to understand the behavior of ameloblastoma**.**

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