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P53, Bcl-2 and Ki67 immunoexpression in follicular solid ameloblastomas

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Abstract

The study included a total of 17 ameloblastomas diagnosed in a range of 15 years. Clinical data processing was followed by histopathological and immunohistochemical analysis, following the expression of p53, Bcl-2 and Ki67 in the tumor epithelial compartments. Lesions predominated in the age range 20–40 years, to male gender, being located mainly in the mandible (88.2%). Typical follicular ameloblastoma was present in 70.6% of analyzed tumors. P53 and Bcl-2 immunoexpression was identified especially in the peripheral cells, the number of marked cells being over 50%, while the percentage of positive cells from the stellate reticulum was below 10%. The Ki67 positivity index was below 10% in both compartments. Squamous and granular cells reveal no stain for the investigated markers. Peripheral columnar cells are active compartment of ameloblastomas being involved in the processes of proliferation–apoptosis.

Keywords: ameloblastoma, p53, Bcl-2, Ki67.

Introduction

Ameloblastoma represent the most common odontogenic neoplasia of the mandible, being included by the WHO in the group of tumors derived from odontogenic epithelium, with mature fibrous stroma [1]. It has a wide variety of histological forms and unpredictable biological behavior. Conventional intraosseous form is generally considered benign, but locally invasive, with high relapse rate, which varies between 15–75% depending on the type of surgery (conservative *vs.* radical) and which may exceptionally metastasis (2–5%) despite its benign histological features [2–5].

Biologic behavior cannot be predicted based on morphological appearance, but metastases usually occur late, after multiple relapses [6, 7].

Molecular and genetic changes associated with the development and progression of odontogenic tumors include oncogenes, tumor suppressor genes, oncoviruses, growth factors, telomerase, control of cell cycle factors, factors related to apoptosis, cell adhesion molecules, matrix proteinases, angiogenic factors and osteolytic cytokines [8].

It is hoped that a better understanding of related molecular mechanisms will contribute to understanding the evolution of odontogenic tumors and lead to the development of new concepts of therapeutic management.

The aim of this study was to evaluate the immunohistochemical expression of p53, Bcl-2 and Ki67 in ameloblastomas, being known their role in cell proliferation and tumorigenesis.

Materials and Methods

The study included a total of 17 ameloblastomas diagnosed in the Laboratory of Pathology, Emergency County Hospital, Craiova, and selected within 15 years from 1996–2011.

The biological material was represented by tumor resection pieces obtained from patients hospitalized in the Clinic of Oral and Maxillo-Facial Surgery from the same Hospital.

Surgical pieces were processed by common histopathological technique using 10% formalin fixation, paraffin embedding and Hematoxylin–Eosin stain. The histopathological diagnosis was done in conformity with criterions established in 2005 by IARC nominated work group for odontogenic tumors within WHO [9].

Immunohistochemical analysis was performed on serial sections using monoclonal antibodies mouse antihuman p53 (clone DO-7, dilution 1:50, Dako), antihuman Bcl-2 (clone 124, dilution 1:100, Dako), respectively antihuman Ki67 (clone MIB-1, dilution 1:100, Dako).

As antigen retrieval, we used Heat-Induced Epitope Retrieval (HIER) Techniques at microwave for 21 minutes in Tris-EDTA buffer pH 9 for p53 and Bcl-2 and in citrate buffer pH 6.1 for Ki67. Immunohistochemical reactions were performed according to technical specifications LSAB™+Kit/HRP (Dako, code K0679) and DAB (diaminobenzidine tetrahydrochloride) was used for the visualization.

Immunohistochemical analysis followed the semi-quantitative markers expression in the epithelial compartments of tumor. It was calculated an index of

positivity (IP) for each used biomarker by reporting the number of marked cells by the total number of cells identified at magnification of 400×, followed by multiplying the result by 100, for each case counting 500 epithelial cells in each compartment (basal, stellate reticulum, squamous, granular). Also, the intensity of reaction was assessed as low, moderate or intense [10].

To validate the reactions were used negative external controls and positive external controls represented by breast carcinoma (p53, Ki67) and tonsil (Bcl-2) (data not shown).

Statistical analysis used the Pearson index, ANOVA test and *chi*-square test performed automatically using software SPSS16.

The acquisition of the images was done with Nikon Eclipse E600 and software program Lucia 5.

Results

The study included 17 cases of ameloblastomas who presented various clinical and pathological aspects (Table 1).

Table 1 – ***Clinico-morphological parameters of the ameloblastomas***

Clinico-morphological parameters		No. of cases	%
Age [years]	20–40	10	58.9
	40–60	5	29.4
	>60	2	11.7
Gender	Female	6	35.3
	Male	11	64.7
Location	Mandible	15	88.2
	Maxillary	2	11.8
Extension	Bone	2	11.7
	Tegument	1	5.9
Histopathology	Typical follicular ameloblastoma	12	70.6
	Acanthomatous ameloblastoma	3	17.6
	Granular cell ameloblastoma	2	11.8
Immunohistochemistry	P53	9	52.9
	Bcl-2	15	88.2
	Ki67	14	82.3

Investigated cases belonged to patients aged between 27–71 years, predominantly males. Tumors were located predominantly in the posterior region of the mandible. In two cases, the tumors have invaded the bone and in one case, the lesion extended up to the oral mucosa. Histopathologically, the tumors corresponded in all cases to solid ameloblastomas, with typical follicular growth pattern in 12 cases (Figure 1, a and b), acanthomatous in three cases or with granular cells in two cases.

Immunohistochemical study noted the expression of p53, Bcl-2 and Ki67 in the compartments of epithelial tumors parenchyma, namely peripheral columnar cells, stellate reticulum cells, squamous cells and granular cells (Table 2).

P53 nuclear immunoexpression was present in nine of the 17 investigated ameloblastomas (52.9%). This was observed with low or moderate intensity, predominantly in columnar cells in the periphery of neoplastic islands (Figure 1, c and d). For five of the tumors, we noted the p53 positivity also with low intensity on rare stellate reticulum cells but not in areas with squamous cell or granular cell.

Bcl-2 protein immunoexpression analysis indicated its presence in 15 of investigated ameloblastomas (88.2%). It was expressed cytoplasmic, constant and intense by the columnar cells, in periphery of tumor islands and rare cells of the stellate reticulum (Figure 1, e and f). In contrast, cells of the inner layer as the stellate reticulum, but also groups of granular or squamoid cells were negative.

Investigation of Ki67 index revealed positivity in 14 cases (82.3%). Cells presented nuclear stain and were located predominantly in the peripheral area of tumor islands, but also in cells of the central stellate reticulum (Figure 1, g and h). Squamous and granular cells did not express Ki67.

Medium index of positivity for Bcl-2 and p53 presented values above 50% in the peripheral cells (67.7% and 83.8%), unlike stellate reticulum compartment where the medium index was below 10% (2.2%, respectively 1.5%). Positivity index of Ki67 had medium values below 10% in both compartments, both in peripheral columnar cells (5.2%) and the stellate reticulum (1.1%).

Statistical analysis indicated significant differences in reactivity for p53, Bcl-2 and Ki67 in the peripheral columnar cells ($p<0.001$, ANOVA), the stain values distribution being antibody specific (in each case, the Pearson index <0.3). The comparing test for means of analyzed markers indicated the lack of significant differences in the stellate reticulum ($p>0.05$, ANOVA). *Chi*-square test revealed no association between biomarkers expression and histological type of solid ameloblastomas ($p>0.05$, *chi*-square).

Table 2 – ***Distribution of cases and median index of positivity (IP) for p53, Bcl-2 and Ki67 in epithelial compartments of ameloblastomas***

	Columnar cells			Stellate reticulum cells			Squamous cells			Granular cells		
No. of cases/ Median IP	***<10***	***10–50***	***>50***	***<10***	***10–50***	***>50***	***<10***	***10–50***	***>50***	***<10***	***10–50***	***>50***
P53	0	0	9/67.7	5/2.2	0	0	0	0	0	0	0	0
Bcl-2	0	0	15/83.8	15/1.5	0	0	0	0	0	0	0	0
Ki67	14/5.2	0	0	14/1.1	0	0	0	0	0	0	0	0

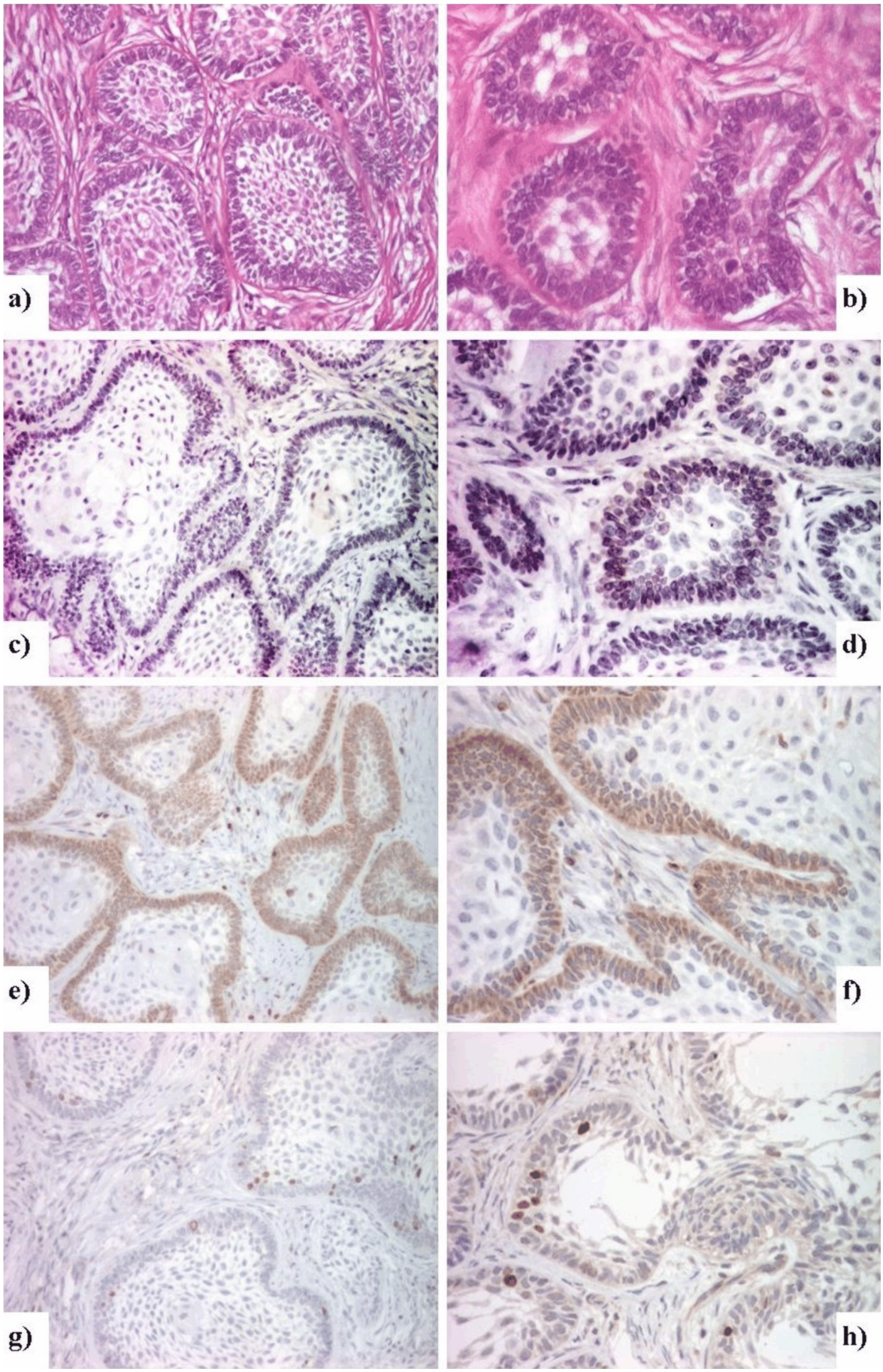

Figure 1 – *(a and b) Ameloblastoma, follicular type, HE stain, ×100, ×200; (c–h) Ameloblastoma, follicular type, positivity of the peripheral columnar basal cells and central stelate reticulum cells: p53 stain, ×100, ×200 (c and d); Bcl-2 stain, ×100, ×200 (e and f); Ki67 stain, ×100, ×200 (g and h).*

Discussion

Currently, it is considered for a variety of human tumors that neoplastic transformation is a multistage accumulation of genetic events in a large region of the genome [11]. P53 tumor suppressor gene mutations are among the most common abnormalities that occur in human cancers [12, 13]. P53 proto-oncogene product is a protein expressed at low levels in untransformed cells and acts as a negative regulator of cell division. The malignant cells are often elevated levels of p53 and several authors have suggested that p53 gene inactivation confers a selective advantage for tumor phenotype development with subsequent impact on cellular activity change [14, 15]. Assessment of p53 expression could provide a better understanding of neoplastic transformation process in these lesions and may be useful in optimizing their treatment [16].

Analysis of p53 expression revealed weak or moderate intensity positivity in less than half of the tumors (52.9%). P53 protein expression does not necessarily imply an association with malignant disease, but tend to be expressed in a quantitative and qualitative increase manner, involves a more aggressive behavior of cancers. Therefore, p53 overexpression can promote cell proliferation in odontogenic lesions [17]. Most similar studies communicate the reduced p53 positivity in solid ameloblastomas, in less than half of the analyzed casuistry [16, 18, 19]. Moderate or weak p53 expression could be due to slow and expansive growth of tumors. In these cells can be initiated changes in DNA followed by the appearance of mutant forms of p53 that will negatively regulate expression of p53 wild form, in the sense of p53 mutant–p53 wild complex formation, the effect being to mutual inactivation of the two proteins [15].

Kumamoto H *et al.* (2004) found that the p53 pathway aberrations (p14ARF–MDM2–p53 cascade) of the control system of the cell cycle are correlated with neoplastic transformation [8]. Although ameloblastomas and metastasizing ameloblastomas have very similar nuclear expression of p53 gene, ameloblastic carcinomas have a high p53 reactivity suggesting malignant transformation of odontogenic epithelium.

Also, several studies have been concerned about the role of apoptosis-proliferation balance in different tumor types and subtypes. Bcl-2 is an antiapoptotic protein and a marker of epithelial cell differentiation [20]. Suppression of apoptosis *via* Bcl-2 can block apoptosis and lead to tumor development [21].

In this study, Bcl-2 expression was present in 88.23% of the investigated ameloblastomas, predominantly in columnar cells from the peripheral zone. Similar studies in literature communicates that around 90% of ameloblastomas are positive for Bcl-2, which indicates that Bcl-2 expression may be related to differentiation and proliferation of odontogenic epithelium, and Bcl-2 overexpression may be associated with the ameloblastomas development [21–23]. These results indicate that in ameloblastomas Bcl-2 protein could function primarily as anti-apoptotic factor, which reflects the proliferative activity of neoplasms [19]. Also, the expression of Bcl-2 suggests the aggressive nature of odontogenic tumor and these results will be beneficial in the differential diagnosis of odontogenic tumors and other tumors that occur in the mouth [20]. In addition, it is estimated that the Bcl-2 protein may play a role in maintaining stem cell population in peripheral layers of tumor islands of which are recruited proliferating cells [24].

There is a significant difference in Ki67 expression between central cells and peripheral cells of tumor islands, peripheral areas being considered proliferative areas [25]. Ki67 index indicates quite variable proliferative activity of ameloblastomas. Sandra F *et al.* (2001) communicate correlation of Ki67 and PCNA index with age, the lowest values being present in young patients and the highest in the elderly [26].

Other studies have failed to demonstrate correlations of proliferation markers with ameloblastomas clinical parameters [27]. In a study conducted in 2006, by Migaldi M *et al.*, it was demonstrated the presence of a low proliferation index for ameloblastomas without correlation with histological type, but dependent on the presence of relapses [28]. Bello IO *et al.* found in another study, in 2009, that the proliferative activity of ameloblastic carcinomas and ameloblastomas is hard to compare using only Ki67 index, because its values were close [29]. Also, although the number of mitoses in ameloblastic carcinoma is low, the number of cells that can proliferate is very large, one explanation being the Notch translocation to nucleus and cell cycle arrest during cell activation induced by Notch 1 pathway [30].

Conclusions

The performed immunohistochemical study revealed the presence of two compartments of the tumor, one active, with increased reactivity for markers of proliferation–apoptosis, represented by peripheral columnar cells, the other inactive, with diminished (stellate reticulum cells) or absent reactivity (squamous and granular cells) for the same antibodies.

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