Immunohistochemical Demonstration of Antiapoptotic Proliferating Protein Bcl-2 in the Ameloblastomas

Mohsin Ghanchi, Dhaval Jani

ABSTRACT

The Bcl-2 (B-cell lymphoma) gene is known through its product, Bcl-2 protein, which protects the cell by blocking postmitotic differentiation from apoptosis. As a critical regulator of apoptosis, Bcl-2 plays an important role in the early stages of oral tumor formation. Because Bcl-2 prevents cell death, its occurrence in odontogenic tissues is helpful in identifying the cell population from which the odontogenic tumors may arise. The aim of this study is to observe the expression of Bcl-2 protein in the different variants of ameloblastomas. In the present study, Bcl-2 expression was observed in 12 different cases of ameloblastomas using a monoclonal antibody against antihuman Bcl-2 oncoprotein. Bcl-2 expression was observed in all the cases of ameloblastomas predominantly in the outer layer, while being negative in the inner cell layer. Based on the study, conclusion has been made that the Bcl-2 protein is thought to play a role in maintaining the stem cell population in the peripheral layers of the tumor nest from which proliferating cells can be recruited.

Keywords: Ameloblastoma, Bcl-2, Immunohistochemistry, Marker, Protein

INTRODUCTION

The odontogenic epithelium is responsible for tooth development under physiologic conditions, and can give rise to tumors or cysts in the jaws. Ameloblastoma is the most frequently encountered tumor arising from the epithelium of the odontogenic apparatus or its derivative or remnant tissues and exhibits considerable histological variation. Ameloblastoma cells have various proliferating activities depending on the histological type and cytological pattern. Both apoptosis and the proliferating activity of the cell are implicated in the development of the ameloblastoma.

Apolipoprotein, also known as programmed cell death or physiologic cell death, plays diverse roles in embryogenesis and normal homeostasis as well as in oncogenesis. Apoptotic processes are modulated by several gene-encoded products, including the Bcl-2 family proteins, which have inhibitory or stimulatory effects.

Bcl-2 derives its name from B-cell lymphoma 2, as it is the second member of a range of proteins initially described as a reciprocal gene translocation in chromosomes 14 to 18 in follicular lymphomas. They govern mitochondrial outer membrane permeabilization and can be either proapoptotic (Bax, BAD, Bak, and Bok, among others) or antiapoptotic (including Bcl-2 proper, Bcl-xL, and Bcl-w, among an assortment of others). There are a total of 25 genes in the Bcl-2 family known to date. The Bcl-2 gene product protects cells by blocking postmitotic differentiation from apoptosis, thus maintaining a stem cell pool.

In the present study, the expression of the Bcl-2 protein was examined immunohistochemically (IHC) in different types of ameloblastomas to clarify the possible roles of this Bcl-2 protein in oncogenesis and cytodifferentiation of tumors derived from odontogenic epithelium.

MATERIALS AND METHODS

Samples and Procedures

The study was conducted in the Department of Oral Pathology, College of Dental Science, Amargadh, Bhavnagar. A total 35 cases, 10 each of follicular and plexiform ameloblastomas, while 5 each of acanthomatous, granular, and unicystic ameloblastomas with data including clinical characteristics, radiographic interpretations, and confirmed histopathological diagnosis, were retrieved from the archives of the department. Evaluation was done for IHC expression of Bcl-2 (DAKO, monoclonal mouse antihuman, RTU).

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissues sectioned at 4 μm thickness were obtained from each block and subjected to IHC staining by using the polymer horseradish peroxidase (poly-HRP) detection system. This system offers great advantages, such as “minimal background noise” and “minimal incubation time.” Antigen retrieval was carried out by “heat-induced antigen retrieval method” in which tissue sections were placed in a pressure cooker.

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along with 10 mM aqueous citrate buffer (pH 6.0) and the pressure cooker operated at 120°C with full pressure. Tissue sections were then immersed in 3% hydrogen peroxidase for 10 minutes to block endogenous peroxidase and subsequently incubated with antibody to CK18 and CK19 overnight at 4°C. The HRP-labeled rabbit antimouse antibody was added to the tissue sections at room temperature for 1 hour. Reaction product was developed by adding 3,3′-diaminobenzidine tetrahydrochloride to the tissue sections. Tissue sections were then counterstained with hematoxylin and eosin stains and evaluated under light microscope (LABOMED, CXR5) at 100- and 250-fold magnifications. The presence of a brown end product at the site of target antigen indicated positive immunoreactivity and absence of staining indicated negative immunoreactivity. Tissue sections of breast carcinoma were taken as positive control. The cytokeratin expressions were graded as negative, mild, moderate, and intense as given in Table 1.

Statistical Analysis

Expressions of Bcl-2 in ameloblastoma subtypes were analyzed with chi-square test according to their intensity using Statistical Package for the Social Sciences (SPSS) software (version 17, IBM Corporation, US). A p-value less than 0.05 was considered to be significant.

RESULTS

Expression of Bcl-2 Protein

Almost all tissue sections were stained by anti-Bcl-2 antibody (Table 2). Intense positive expression was found predominant in all the histological types. Very few cases reported with mild and moderate expression patterns (Tables 3 and 4). Bcl-2 was detected mainly in the outer layers of epithelial tissues in ameloblastomas, and only a few cells were positively stained in the inner layers.

Positive staining appeared in the perinuclear portion and cytoplasm of the tumor cells. In follicular ameloblastomas, the Bcl-2 protein expression was found in the outer layer of the epithelial component, whereas inner stellate reticulum-like cells were negative (Fig. 1). In some cases, squamoid cells (acanthomatous ameloblastoma) were observed in inner layers, but these were negative. Slightly flattened cells occurring between outer layer cells and inner cells, and termed intermediate cells, were also Bcl-2 positive.

The Bcl-2 protein expression pattern of the plexiform ameloblastoma showed outer cuboidal cells and intermediate cells were positive and inner cells (stellate reticulum-like cells) were negative. A similar expression pattern was seen in the mixed columnar and cuboidal cell types (Fig. 2).

In unicystic ameloblastomas, positive reactions were found in the outer layer columnar cells and inner stellate reticulum-like cells were negative (Fig. 3). In granular cell ameloblastoma, reactivity for the Bcl-2 protein in the

### Table 1: Bcl-2 intensity grading in ameloblastoma

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>(-)</td>
<td>Negative</td>
<td>Fewer than 5% positive or no staining</td>
</tr>
<tr>
<td>(+)</td>
<td>Mild</td>
<td>5-24% positive</td>
</tr>
<tr>
<td>(++)</td>
<td>Moderate</td>
<td>25-50% positive</td>
</tr>
<tr>
<td>(+++)</td>
<td>Intense</td>
<td>More than 50% positive</td>
</tr>
</tbody>
</table>

### Table 2: Bcl-2 expression in ameloblastoma

<table>
<thead>
<tr>
<th>Total cases of ameloblastoma</th>
<th>No. of positive</th>
<th>No. of negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 (100%)</td>
<td>33 (94%)</td>
<td>2 (6%)</td>
</tr>
</tbody>
</table>

### Table 3: Bcl-2 expression patterns based on intensity in ameloblastoma

<table>
<thead>
<tr>
<th>Total no. of cases</th>
<th>Positive expression</th>
<th>Negative (–) expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ (Mild)</td>
<td>4 (11%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>++ (Moderate)</td>
<td>8 (23%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>+++ (Intense)</td>
<td>21 (60%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Total</td>
<td>33 (94%)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: Bcl-2-positive expression pattern in follicular ameloblastoma staining outer layer cells

Fig. 2: Bcl-2-positive expression pattern in plexiform ameloblastoma staining outer cuboidal cells and intermediate cells
peripheral columnar and central polyhedral cells was fundamentally similar to that of follicular and plexiform ameloblastoma (Fig. 4).

**DISCUSSION**

Just as individual cells balance anabolic and catabolic reactions, multicellular organisms must equalize the rates of cell generation and cell death to maintain a constant size. An organism must also remove damaged or abnormal cells that could interfere with organ function or develop into tumors.2

The family of Bcl-2-related proteins constitutes one of the biologically most relevant classes of apoptosis regulatory gene products. Bcl-2, a 26-kDa protein, was first identified in non-Hodgkin’s follicular B-cell lymphomas, encoded by the Bcl-2 oncogene in the molecular analysis of the t (14;18) chromosome translocation. The Bcl-2 gene, located on chromosome 18q21, has emerged as a critical regulator of programmed cell death in a variety of physiological and pathological contexts. Immunoreactivity for the Bcl-2 product is present mainly in cell populations that are long lived and/or with high proliferation ability.2

Expression of the Bcl-2 protein has been reported in many sites during development, especially those characterized by epithelial–mesenchymal interactions.6 Slootweg and de Weger7 demonstrated that the epithelial component (inner and outer enamel epithelium, stratum intermedium) of human tooth germ and dental lamina expressed the Bcl-2 protein from the bud stage to the bell stage. The study of Mitsuyasu et al8 shows in all 25 cases of ameloblastomas, the Bcl-2 protein was found in the outer layer of tumor cells, whereas the inner cells were negative.

Sandra et al2 performed immunohistochemistry to determine the apoptotic behavior of ameloblastomas by analyzing the role of Bcl-2 family proteins in ameloblastomas, from 32 patients, and the location of terminally apoptotic cells in the ameloblastoma tissues. They found ameloblastoma has much more apoptosis-inhibiting (Bcl-2) protein than the apoptosis-modulating protein. Our study has shown clearly that in all ameloblastomas, which are the basis of the present report, the Bcl-2 protein was detected in outer layer tumor cells and in intermediate cells, which is consistent with the findings of Mitsuyasu et al8 and Sandra et al.2

In the present study, no expression of the Bcl-2 family proteins was found in keratinizing areas or granular cell clusters in acanthomatous or granular cell ameloblastomas, which is similar to the findings of Kumamoto and Ooya1 and Sandra et al,2 suggesting terminal differentiation of tumor cells.

The participation of Bcl-2 could be affected in three ways. Firstly, Bcl-2 maintains cells in a stem cell pool by inhibiting postmitotic differentiation terminating in

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**Table 4: Bcl-2 expression patterns within different histological types of ameloblastomas**

<table>
<thead>
<tr>
<th>Histological pattern</th>
<th>Total no. of cases</th>
<th>Positive expression</th>
<th>+++ (Intense)</th>
<th>++ (Moderate)</th>
<th>+ (Mild)</th>
<th>Total</th>
<th>Negative (-) expression</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular ameloblastoma</td>
<td>10 (100)</td>
<td>00 (0)</td>
<td>2 (20)</td>
<td>8 (80)</td>
<td>10 (100)</td>
<td>00 (0)</td>
<td></td>
<td>0.017</td>
</tr>
<tr>
<td>Plexiform ameloblastoma</td>
<td>10 (100)</td>
<td>01 (10)</td>
<td>03 (30)</td>
<td>05 (50)</td>
<td>09 (90)</td>
<td>01 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acanthomatous ameloblastoma</td>
<td>05 (100)</td>
<td>00 (0)</td>
<td>01 (20)</td>
<td>04 (80)</td>
<td>05 (100)</td>
<td>00 (0)</td>
<td></td>
<td>0.017</td>
</tr>
<tr>
<td>Granular cell ameloblastoma</td>
<td>05 (100)</td>
<td>01 (20)</td>
<td>01 (20)</td>
<td>02 (40)</td>
<td>04 (80)</td>
<td>01 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unicystic ameloblastoma</td>
<td>05 (100)</td>
<td>02 (40)</td>
<td>01 (20)</td>
<td>02 (40)</td>
<td>05 (100)</td>
<td>00 (0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 3:** Bcl-2-positive expression pattern in unicystic ameloblastoma staining outer layer columnar cells

**Fig. 4:** Bcl-2-positive expression pattern in unicystic ameloblastoma staining outer layer cells
cell death; secondly, Bcl-2 keeps differentiated cells in a functional stage by preventing cell death; and, thirdly, Bcl-2 seems to contribute to the formation of cell condensations at sites of epithelial–mesenchymal interaction by decreasing the focal rate of cell death relative to mitosis.9

From these data, we conclude that in ameloblastomas, the Bcl-2 family proteins might function primarily as antiapoptotic factors, reflecting proliferative activity. The outer layer cells in ameloblastoma not only have proliferative activity but also have inhibited cell death in the same manner as an epithelial component of the tooth germ.

REFERENCES