Patterns of Expression of Intermediate Filaments and S-100 Protein in Desmoplastic Ameloblastoma

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Abstract

Seventeen cases of desmoplastic ameloblastoma were examined immunohistochemically. Immunoperoxidase techniques were applied for detection of keratin, desmin, vimentin and S-100 protein expression in these tumors. The tumor epithelium of desmoplastic ameloblastoma exhibited weak, focal, inconstant keratin staining, weak, variable expression of S-100 protein, desmin immunoreactivity of mild to moderate intensity and vimentin non-reactivity. The pertinent literature on the immunohistochemistry of ameloblastomas is briefly reviewed.

Introduction

Ameloblastoma with pronounced desmoplasia was first described by Eversole et al.[¹] in 1984, who reported three cases of this unusual variant. Histologically, the tumor is characterized by an abundance of stromal collagen and presence of atypical, odontogenic epithelial islands. Radiographically, it appears as an ill-defined radiolucent-radiopaque lesion suggestive of a benign fibro-osseous entity. Subsequent to the initial description, one serial study of 14 cases² and three case reports³⁵ have been documented. A review of the English language literature disclosed that the immunohistochemical characteristics of this variant have not been described previously.

The purpose of this study was primarily to determine the patterns of expression of intermediate filaments (IF) such as keratin, desmin and vimentin, and S-100 protein in 17 cases of desmoplastic ameloblastoma retrieved from the files of the Division of Stomatology, Institute for Medical Research, Kuala Lumpur.

Materials and Methods

Seventeen cases of the desmoplastic variant of ameloblastoma that occurred between 1967 and 1991 were retrieved from the files of the Division of Stomatology, Institute for Medical Research, Kuala Lumpur. The criteria for selection included representative morphologic characteristics and adequate tumor mass.

There were twelve female and five male patients. Age at diagnosis ranged between 21 and 60 years, with an average of 36.6 years. Ten of the tumors arose from the mandible and seven from the maxilla. The majority of the lesions were located in the anterior or premolar regions of either jaw.

All specimens were processed routinely, cut into 5-µm-thick sections for conventional staining with hematoxylin and eosin, and for immunohistochemistry.

Immunostaining was carried out with the peroxidase-antiperoxidase (PAP) technique and the indirect method. The staining protocols are detailed in Table 1. Known positive controls

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were included for all antisera. Negative control staining was obtained by substituting the primary antibody with non-immune serum.

Commercially available antibodies such as polyclonal anti-human keratin, polyclonal anti-bovine S-100 protein, monoclonal anti-porcine vimentin and anti-human desmin obtained from Dakopatts, Copenhagen, Denmark, were used. According to the specifications from Dakopatts: (a) keratin proteins were purified from human stratum corneum (foot pad), (b) S-100 protein was purified from ox brain, (c) vimentin was purified from porcine eye lens, and (d) desmin was purified from human muscle.

### Results

**Histopathology**

The tumor tissue in all 17 cases was characterized histologically by the presence of an abundant coarsely collagenous stroma containing scattered islands of atypical odontogenic epithelium (Fig.1). The latter commonly exhibited two main cell types: spindle-shaped and squamatoid. These were arranged compactly in fascicles or whorls (Fig.2). Some tumor islands

<table>
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<tr>
<th>Table 1 Immunohistochemical staining methods</th>
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<td>PAP technique</td>
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<tr>
<td>1. Deparaffilization</td>
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<td>2. Trypsin pretreatment (except vimentin):</td>
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<td>0.01% trypsin/PBS (pH 7.6) 30 min</td>
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<td>3. Inactivation of endogenous peroxidase:</td>
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<td>0.3% H₂O₂/methanol soln. 30 min</td>
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<td>4. Background blocking:</td>
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<td>Normal swine serum 1:20 30 min</td>
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<tr>
<td>5. 1st layer:</td>
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<tr>
<td>S–100 protein 1:200 30 min</td>
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<tr>
<td>Keratin 1:200 30 min</td>
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<tr>
<td>6. 2nd layer:</td>
</tr>
<tr>
<td>Anti-rabbit Ig G swine serum 1:40 30 min</td>
</tr>
<tr>
<td>7. 3rd layer:</td>
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<tr>
<td>(HRP, rabbit anti–peroxidase 1:100 30 min)</td>
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<tr>
<td>8. Visualization of peroxidase activity:</td>
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<td>0.05 M Tris buffer solution (pH 7.6)</td>
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<tr>
<td>9. Counterstain, dehydrate and mount</td>
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were bounded by a peripheral row of preameloblast-like cells (Fig. 2).

The tumors in all 17 cases were non-encapsulated. Rather, towards the peripheral parts of the lesions, tumor islands infiltrating the cancellous spaces of intact trabecular bone were evident (Fig. 3).

**Immunohistochemical findings**

The tumor epithelium in all 17 cases of desmoplastic ameloblastoma showed focal, weak and inconstant keratin expression (Fig. 4), weak and focal S-100 protein staining (Fig. 5) and limited areas of desmin immunoreactivity of mild to moderate intensity (Fig. 6). Within the

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**Fig. 3** Peripheral portion of lesion with tumor islands (arrows) invading cancellous spaces of intact trabecular bone (hematoxylin and eosin stain, x40)

**Fig. 4** Tumor islands showing weak, focal and inconstant keratin expression (peroxidase-antiperoxidase method, x100)

**Fig. 5** Variable and weak S-100 protein staining of tumor epithelial cells of desmoplastic ameloblastoma (peroxidase-antiperoxidase method, x100)

**Fig. 6** Tumor island with epithelial cells exhibiting moderate desmin immunoreactivity (indirect method, x100)
tumor elements, no reactivity for vimentin was noted.

Discussion

In recent years, considerable attention has been focused on establishing the immunocytological characteristics of ameloblastoma and its variants. Nakajima et al. [6], in their study on the distribution of S-100 protein in normal and neoplastic tissues, listed their only case of ameloblastoma as S-100-negative. In 1984 Thesleff and Ekblom found that keratin was expressed by all types of epithelial cells in ameloblastoma, and that these keratin-positive epithelial islands were surrounded by a continuous line of laminin[7]. Subsequently, studies on granular cell ameloblastomas established that the granular cells express antigenic determinants for keratin, filamin, α1-antitrypsin and myosin[8–12]. These same cells may variably coexpress cytokeratin and vimentin[8,12]. Desmin and S-100 protein positivity in these cells was also focal and variable[8,9,11]. However no reaction was reported for prekeratin, epithelial membrane antigen, actin, lysozyme, lactoferrin, transferrin, neuron-specific enolase or glial fibrillary acidic protein[8,9,11–13].

The immunoprofile of the desmoplastic variant of ameloblastoma has not, to our knowledge, been described previously. Our results demonstrated that as with ameloblastomas of other histologic types, the tumor cell types of desmoplastic ameloblastoma showed variable expression of S-100 protein and desmin[8,9,11]. However, unlike previous reports [7,10], keratin immunoreactivity was inconstant and confined to tumor cells showing squamous differentiation. Vimentin was not expressed by either squamatoid or spindle-shaped cells. The differences or variations in the expression of these antigens among ameloblastomas of various histologic types may be attributed to diverse factors such as dedifferentiation or the rate of proliferation of the neoplastic cells, inherent cellular potentials or extracellular mediators[8,13].

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