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PLEOMORPHIC ADENOMA WITH NUCLEAR PALISADING ARRANGEMENT OF MODIFIED MYOEPITHELIAL CELLS: HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL STUDY

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Abstract

Pleomorphic adenomas with a nuclear palisading arrangement of spindle-shaped modified myoepithelial cells (MMCs), suggesting the appearance of palisading leiomyoma or Antoni's A type of neurilemmoma, are quite rare, and its cytologic nature has been poorly understood. This paper reports histologic and immunohistochemical findings of palisading MMCs in two cases of pleomorphic adenoma. Histologically, foci of spindle-shaped MMCs with nuclei in a palisading arrangement were scattered in the myxoid areas. Near the large foci of spindle-shaped MMCs with nuclear palisading arrangements, tiny foci of spindle-shaped MMCs forming nuclear palisading or rosette-like arrangements were seen. Such nuclear palisading arrangements of MMCs were suggestive of differentiation or transformation of MMCs into cells that were more smooth muscle in nature, supported by occasional existence of palisading leiomyoma in the myometrium and gastrointestinal tract. However, immunohistochemical findings of palisading MMCs in pleomorphic adenoma were similar to those of non-palisading MMCs, and showed no evidence of smooth muscle differentiation; neither palisading nor non-palisading MMCs in pleomorphic adenoma expressed desmin, muscle specific actin (HHF-35), alpha smooth muscle actin, or myoglobin.

The biologic significance and formative mechanism of nuclear palisading arrangement of MMCs in pleomorphic adenoma could not be determined in the present study. However, if the MMCs with nuclear palisading arrangements in pleomorphic adenoma, presented here, are aspirated for cytologic diagnosis or are included in a small biopsy specimen, the correct diagnosis of pleomorphic adenoma may be confused by a suspicion of myogenic or neurogenic tumor.

Key words: Pleomorphic adenoma—Salivary gland tumor—Palisading arrangement—Modified myoepithelial cells

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INTRODUCTION

Pleomorphic adenomas are the most common benign salivary gland tumors, constituting 40 to 70% of such tumors, and are renowned for their cytomorphologic and architectural diversity. Despite their protean histopathology, each tumor shares with others the essential diagnostic features of being composed of both epithelial and mesenchymal-like tissues. Epithelial cells of pleomorphic adenoma basically originate from duct epithelial cells and myoepithelial cells. Tumor cells of duct epithelium origin are cuboidal in shape, and line duct-like structures which vary in size and shape; tumor cells of myoepithelium origin, termed modified myoepithelial cells (MMCs), are polygonal, spindle, or plasma cell-like in shape, form sheet-, clump-, or strand-structure, and are admixed with myxoid or myxo-chondroid components. It is well known that MMCs in pleomorphic adenoma occasionally form interlacing fascicles composed principally of spindle-shaped cells, and these findings are often more suggestive of a neurogenic or myogenic tumor than an epithelial one. However, pleomorphic adenomas with nuclear palisading arrangements of spindle-shaped MMCs, suggesting the appearance of palisading leiomyoma or Antoni’s A type of neurilemmoma, are quite rare, and their cytologic nature has been poorly understood. This paper reports histologic and immunohistochemical findings of palisading MMCs found in pleomorphic adenomas.

MATERIALS AND METHODS

Surgically excised specimens of two cases of pleomorphic adenomas with nuclear palisading arrangements of MMCs were submitted for histologic and immunohistochemical examination. Case 1 was a 62-year-old Japanese male who had a painless and slow-growing parotid mass of 10 months’ duration. Case 2 was a 48-year-old Japanese female with a painless swelling of the hard palate of 3 months’ duration. Histopathologic examination of biopsy specimens in both cases revealed the lesions to be pleomorphic adenoma with a nuclear palisading arrangement.

The surgically excised tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for routine histopathologic examination. For immunohistochemical ex-

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### Table 1 Antibodies used in the present study and immunohistochemical results

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Dilution</th>
<th>Source</th>
<th>Pleomorphic adenoma*</th>
<th>Leiomyoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Palisading</td>
<td>Non-palisading</td>
</tr>
<tr>
<td>vimentin (M)</td>
<td>1:1,000</td>
<td>Dako, USA</td>
<td>≤</td>
<td>+</td>
</tr>
<tr>
<td>desmin (M)</td>
<td>1:50</td>
<td>Dako, USA</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>HHF-35α (M)</td>
<td>1:100</td>
<td>Dako, USA</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>αSMAα (M)</td>
<td>1:200</td>
<td>Dakopatts, Denmark</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>myoglobin (P)</td>
<td>1:1,000</td>
<td>Dakopatts, Denmark</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>GFAP (P)</td>
<td>1:2,000</td>
<td>Dako, USA</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>NSEα (P)</td>
<td>1:2,000</td>
<td>Dako, USA</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>S-100 (P)</td>
<td>1:2,000</td>
<td>Dako, USA</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>cytokeratin, S’ (M)</td>
<td>1:100</td>
<td>Immunon, USA</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>cytokeratin, NS’ (M)</td>
<td>1:100</td>
<td>Immunon, USA</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

α, muscle specific actin; β, alpha smooth muscle actin; γ, glial fibrillary acidic protein; δ, non-specific enolase; ε, squamous; η, non-squamous

M, monoclonal antibody; P, polyclonal antibody

+ , strong positive (diffuse or focal); ≤ , weak positive (diffuse or focal); − , negative

* pleomorphic adenoma with spindle-shaped modified myoepithelial cells
amination, a streptavidin-biotin peroxidase
complex technique was employed for forma-
lin-fixed paraffin-embedded sections. The
series of primary antibodies and their work-
ing dilutions are listed in Table 1. Two
leiomyomas, one an ordinary leiomyoma aris-
ing in the myometrium and the other a pali-
sading leiomyoma in the gastric wall, were
compared with palisading and non-palisading
tumor cells of the present pleomorphic
adenoma by immunohistochemical staining.
Staining results were scored as follows: +,
strong positive (diffuse or focal); ±, weak
positive (diffuse or focal); and −, negative.

RESULTS

1. Histopathologic findings

Case 1. Surgically excised material showed
a pleomorphic adenoma with a typical histo-
logic appearance. Some areas contained
cuboidal tumor cells arranged in tubular
structures, and proliferating tumor cells in
strands or sheets around these tubular struc-
tures; in other areas, the tumor cells were
stellate, polyhedral, or spindle form in shape.
Myxoid and fibrous tissues constituted more
than half of the tumor mass, and small areas
of chondroid or myxo-chondroid tissue
were occasionally seen. Furthermore, foci of
spindle-shaped tumor cells showing the
nuclei in a palisading arrangement, very
similar to that of certain benign myogenic or
neurogenic tumors, were scattered in myxoid
areas (Fig. 1, 2). Near the large foci of spindle-
shaped tumor cells with nuclear palisading
arrangements, tiny foci of spindle-shaped
tumor cells forming nuclear palisading ar-
rangements or rosette-like structures were
seen (Fig. 3).

Case 2. Histologic findings of the surgically
excised specimen were similar to those of
Case 1, but richer in myxoid areas with no
formation of chondroid tissue. Various-sized
foci of fusiform tumor cells with nuclear pali-
sading arrangements were scattered in the
myxoid areas (Fig. 4).

Fig. 1 Scattered foci of spindle-shaped tumor cells with the nuclear palisading arrangement in
pleomorphic adenoma of Case 1 (H&E staining ×15).
Fig. 2  (a) A focus of spindle-shaped tumor cells with the nuclei in a palisading arrangement in myxoid areas of pleomorphic adenoma of Case 1 (H-E staining ×40).  (b) Palisading arrangement of spindle-shaped tumor cells showing a very similar patterns to that of certain benign myogenic or neurogenic tumors (H-E staining ×200).

Fig. 3  (a, b) Near the large foci of spindle-shaped tumor cells with a nuclear palisading arrangement, scattered tiny foci of spindle-shaped tumor cells forming nuclear palisading arrangement or rosette-like structure in myxoid areas (H-E staining ×400).
Fig. 4  (a) A focus of spindle-shaped tumor cells with the nuclei in a palisading arrangement in myxoid areas of pleomorphic adenoma of Case 2 (H-E staining ×40).  (b) Palisading arrangement of spindle-shaped tumor cells showing very similar to that of certain benign myogenic or neurogenic tumors (H-E staining ×150).

Fig. 5  Immunohistochemical staining of palisading spindle-shaped tumor cells (upper half) and non-palisading cells (lower half) for vimentin (×150). Non-palisading cells show a strongly positive reaction, while palisading cells are faintly positive.
2. Immunohistochemical findings

The palisading spindle-shaped cells in pleomorphic adenoma of both cases stained weakly with vimentin, neuron specific enolase (NSE), and S-100 protein (Table 1). Non-palisading spindle-shaped cells showed staining results similar to those of palisading ones, but stainability of vimentin and S-100 protein was more intensive in non-palisading cells (Fig. 5). In addition, non-palisading cells stained weakly with cytokeratin and glial fibrillary acidic protein (GFAP). In contrast, tumor cells of both ordinary- and palisading-types of leiomyoma were strongly positive for vimentin, desmin, muscle specific actin (HHF-35), and alpha smooth muscle actin. GFAP, NSE, S-100 protein, myoglobin and cytokeratin were negative in these leiomyomas.

DISCUSSION

Two distinct cell types are generally considered to be involved in the histogenesis of pleomorphic adenoma in the salivary glands; one is the intercalated duct cell and the other is the myoepithelial cell located around the acini and intercalated ducts. The typical histopathologic feature of pleomorphic adenoma includes tubular, ductal and duct-like structures composed of a double layer of cells, with the inner layer of tumor cells probably of ductal cell origin and the outer layer of myoepithelial cell origin. The luminal layer cells are occasionally found to undergo squamous differentiation or metaplasia, and outer layer cells proliferate as outgrowths which usually show myxoid or hyaline alterations with or without the induction of chondroid tissues. Thus, the direction of histologic differentiation of two types of cells in pleomorphic adenoma is different; luminal tumor epithelial cells of duct cell origin probably undergo squamous differentiation, finally forming solid structures. On the other hand, the outer tumor cells of myoepithelial cell origin proliferate and transform into cells with myofibrous or more mesenchymal in nature, termed modified myoepithelial cells (MMCs). MMCs in pleomorphic adenoma are polygonal, spindle, or plasma cell-like in shape, form sheet-, clump-, or strand-structures, and are admixed with myxoid or myxo-chondroid components. Furthermore, it is well known that MMCs in pleomorphic adenoma occasionally form interlacing fascicles composed principally of spindle-shaped cells; these findings are often more suggestive of myogenic, neurogenic, or fibroblastic tumors than epithelial ones. However, pleomorphic adenoma with a nuclear palisading arrangement of spindle-shaped MMCs, suggesting the appearance of palisading leiomyoma or Antoni’s A type of neurilemmoma, is quite rare\(^1,4,9\). In the present examination, two of 164 pleomorphic adenomas showed multiple foci of spindle-shaped MMCs with a nuclear palisading arrangement in myxoid areas. To examine one possibility, we postulated that such palisading arrangement of MMCs might be suggestive of differentiation or transformation of MMCs into cells more smooth muscle in nature, because of the palisading leiomyoma occasionally seen in the myometrium and gastrointestinal tract\(^2,5,7,12\). However, immunohistochemical findings of palisading MMCs in pleomorphic adenoma were similar to those of non-palisading MMCs\(^3,10,11\), and no evidence of the smooth muscle differentiation reported in spindle cell stromal tumors\(^2,5,12\). Neither palisading nor non-palisading MMCs in pleomorphic adenoma expressed desmin, muscle specific actin (HHF-35), alpha smooth muscle actin, or myoglobin. Although the biologic significance and formative mechanism of nuclear palisading arrangement could not be determined in the present study, formation of nuclear palisading arrangement of spindle MMCs began multifocally in myxoid areas, and tiny foci of MMCs with nuclear palisading arrangements gradually increased in size to form large foci.

Neoplasms which must be considered by the pathologist confronted with spindle tumor cells with nuclear palisading arrangements from a lesion of a salivary gland include
pleomorphic adenoma and some mesenchymal tumors, because nuclear palisading arrangement of tumor cells can be identified in certain myogenic and neurogenic tumors. Non-epithelial tumors of salivary glands are rare; they accounted for 4 to 5% of all salivary gland tumors\(^\text{(1,15)}\). Of these, neurogenic tumors appear to be the most common non-epithelial tumors in salivary glands in adults; they formed 8% of the non-epithelial tumors in the British Salivary Gland Tumour Panel (BSGTP) material\(^\text{(1)}\), and 17.5% of the material presented by Seifert et al.\(^\text{(13)}\). Three cases of 11 neurogenic tumors in BSGTP material and 9 of 21 in material of Seifert et al. were neurilemmomas\(^\text{(1,13)}\). The present spindle cell aggregates with nuclear palisading arrangement in pleomorphic adenoma can be histopathologically differentiated from Antoni’s A type of neurilemmoma by routine HE-staining alone for diagnosis of surgically excised materials, because of the gradual transition between non-palisading cells and palisading ones, and the lack of a fibrous capsule surrounding foci of palisading spindle cells in pleomorphic adenoma.

Fine needle aspiration cytology (FNAC) is an increasingly important diagnostic procedure used to evaluate salivary gland lesions and to assist in their preoperative management and overall therapy. Pleomorphic adenomas are the tumors most frequently encountered in FNAC of the salivary gland tumors\(^\text{(4,4)}\). Although it has great histologic diversity, the cytologic diagnosis of pleomorphic adenoma in most cases is easy because of its typical cytologic image; the combination of bland epithelial cells and ground substance with spindle-shaped cells is very characteristic of this tumor. However, in some cases, major variations from this common cytologic pattern exist, resulting in erroneous cytologic diagnoses in many cases. For example, the absence of myxoid elements and a predominance of spindle-shaped cells may lead to erroneously diagnosing a pleomorphic adenoma as a benign spindle cell mesenchymal tumor. In fact, the cytological similarities between pleomorphic adenoma and neurilemmoma have been described previously\(^\text{8,10}\). Furthermore, if MMCs with nuclear palisading arrangements in pleomorphic adenoma, as presented here, are aspirated for cytologic diagnosis or are included in a small biopsy specimen, the correct diagnosis of pleomorphic adenoma may be confused by a suspicion of myogenic or neurogenic tumor.

**REFERENCES**


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