A Histopathological and Immunohistochemical Study of Cartilage-Like Tissue Formation in Pleomorphic Adenoma
—Comparative Study of the Major and Minor Salivary Gland Adenomas—

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Abstract
Pleomorphic adenomas are the most commonly occurring salivary gland tumors, which are histopathologically characterized by both the presence of epithelial cell proliferation and the formation of myxomatous or cartilage-like tissue. Given their variability, to date, there have been numerous morphological and immunohistochemical studies of the tumors, but most have focused on the major salivary gland tumors and minor salivary gland tumors have yet to be sufficiently investigated. The present study is a comparative histopathological and immunohistochemical study conducted with the aim of focus on the formation of cartilage-like tissue in pleomorphic adenomas occurring in the major and minor salivary glands. Histopathologically, the tumor parenchyma findings consisted of double-layered tubular (inner cells and outer cells), sheet-like, and cord-like proliferation of epithelial cells in all pleomorphic adenomas of the major salivary glands (11 cases) and minor salivary glands (50 cases). The outer cells of the tubular proliferation were dissociated and myxomatous or cartilage-like tissue interposed with matrix was observed. All cases (100%) of the major salivary gland tumors showed hyaline cartilage-like tissue formation that showed metachromasia on Tb staining at pH 7.0, pH 4.1 and pH 2.5. On the other hand, in the minor salivary gland tumors, chondrogenesis was present in 4 of 50 cases (8%), which was predominantly in the form of fibrocartilage-like tissue that presented metachromasia with Tb staining at pH 7.0 and pH 4.1. Immunohistochemical investigations revealed keratin-positive cells in both the inner cells and outer tumor cells in the area of double-layered tubular proliferation. In addition, vimentin- and S-100 protein-positive findings were observed in the outer cells and dissociated cells. The cartilage-like matrix in the major salivary gland tumors was remarkably positive for type II collagen, while the cartilage-like matrix in the minor salivary gland tumors was primarily positive for type I collagen. Markedly positive findings were observed in the hyaline cartilage-like tissue of the major salivary gland tumors for bone morphogenetic protein (BMP)-2, BMP-4 and BMP-6, fibroblast growth factor-2 and chondromodulin-1, which were associated with chondrogenesis. The results suggest that cartilage-like tissue forms more readily in the major salivary gland tumors than in the minor salivary gland tumors. Neoplastic myoepithelial cells are involved in the formation of cartilage-like tissue, and it can be inferred that due to the quantity of cartilage-inducing factors, the formation of cartilage-like tissue is more typical in the major salivary gland tumors than in the minor salivary gland tumors.

Keywords:
pleomorphic adenoma, minor salivary gland, major salivary gland, neoplastic myoepithelial cells, cartilage-like tissue formation
Introduction

Pleomorphic adenomas are painless and slow to develop, and are the most common tumor of the salivary glands. Consequently, numerous clinical and pathological studies have focused on these tumors. Anatomically, salivary glands are broadly classified as the major and minor salivary glands. The former consists of the parotid gland, submandibular gland, and sublingual gland, while the latter includes the palatal gland, buccal gland, labial gland, lingual gland, gland in the floor of the mouth, and retromolar gland. In addition, pleomorphic adenomas occur in both the major and minor salivary glands; in the major salivary glands, most frequently in the parotid gland, and in the minor salivary glands, most frequently in the palatal gland (1–3).

Most of the information and working research on these tumors are on the major salivary gland tumors; at present, there are a few detailed studies of the minor salivary gland tumors. However, in dental clinical practice, the tumors are encountered more frequently in the minor salivary glands than in the major salivary glands, and the minor salivary gland tumors are more frequently malignant (1–4).

Histopathologically, with pleomorphic adenoma, not only does epithelial tumor cell proliferation occur, but mesenchyme–like tissue components are also present with the formation of myxomatous and cartilage–like tissue, and neoplastic myoepithelial cells are known to be involved in the formation of these mesenchyme–like tissues. However, there is little information comparing the major and minor salivary gland tumors in detail with regard to the formation of cartilage–like tissues in the tumor.

Therefore, the present study conducted histopathological, histochemical and immunohistochemical examinations for the purpose of observation on the morphological characteristics of pleomorphic adenomas with a particular focus on the differences between the major and minor salivary glands in the formation of cartilage–like tissue.

Materials and Methods

Materials

The materials used in the present study were collected for the purpose of diagnosis and treatment at the Nihon University Hospital at Matsudo. Sixty–one cases of pleomorphic adenoma histopathologically diagnosed [11 cases of the major salivary glands (parotid gland, 4 cases; submandibular gland, 7 cases) and 50 cases of the minor salivary glands (palatal gland, 47 cases; labial gland, 2 cases; buccal gland, 1 case)] were included.

The present study was conducted in accordance with the guidelines for clinical research of the Nihon University School of Dentistry at Matsudo (Ethics Committee Approval No.: EC08–022).

Methods

1) Preparation of histopathologic tissue samples

According to conventional procedures, the excised tissues were immersed and fixed in 10% neutral formalin solution, embedded in paraffin blocks, and cut into thin sections (4 μm) with a microtome, then subjected to hematoxylin and eosin double staining (HE), toluidine blue (Tb) staining (at pH 2.5, pH 4.1 and pH 7.0), and immunohistochemical staining.

2) Immunohistochemical staining

Primary antibodies used in the immunohistochemical staining were: anti–keratin rabbit polyclonal antibody (keratin; dilution, 1: 200; Dako Cytomation, Glostrup, Denmark); anti–vimentin mouse monoclonal antibody (vimentin; dilution, 1: 50; Dako Cytomation, Glostrup, Denmark); anti–S–100 protein rabbit polyclonal antibody (S–100; dilution, 1: 100; Dako Cytomation, Glostrup, Denmark); anti–type I collagen rabbit polyclonal antibody (type I collagen; dilution, 1: 200; Abcam, Cambridge Science Park, UK); anti–type II collagen rabbit polyclonal antibody (type II collagen; dilution, 1: 200; Abcam, Cambridge Science Park, UK); anti–bone morphogenetic protein–2 mouse monoclonal antibody (BMP–2; dilution, 1: 50; Abcam, Cambridge Science Park, UK); anti–BMP–4 rabbit polyclonal antibody (BMP–4; dilution, 1: 50; Abcam, Cambridge Science Park, UK); anti–BMP–6 mouse
monoclonal antibody (BMP–6; dilution, 1:50; Abcam, Cambridge Science Park, UK); anti–FGF–2 rabbit polyclonal antibody (FGF–2; dilution, 1:50; Abcam, Cambridge Science Park, UK); and anti–chondromodulin–I rabbit polyclonal antibody (ChM–I; dilution, 1:50; Abcam, Cambridge Science Park, UK). Immunohistochemical staining was conducted using the EnVision polymer technique (Dako Cytomation, Glostrup, Denmark). The procedure was described below.

After deparaffinization and rehydration, sections were incubated in 0.01 M citrate buffer solution (121 °C, 10 min), were allowed to cool to room temperature after processing, and were then reacted with each of the primary antibodies listed above for 1 hour at room temperature. To block endogenous peroxidase, sections were exposed to a mixed solution of 0.3% hydrogen peroxide solution and methanol for 20 minutes, and after washing with 10 mM phosphate buffer solution, were exposed to the EnVision polymer for 30 minutes. After developing for 2 minutes with 3% hydrogen peroxide containing 3,3’-diaminobenzidine tetrahydrochloride, counterstaining was performed using Mayer’s hematoxylin solution, and then dehydration, washing, and mounting were performed and sections were subjected to observation by light microscopy.

Positive control tests were performed for each primary antibody using positive findings in cartilage and bone lesion tissues from files of Department of Oral Pathology, Niho University School of Dentistry at Matsudo. Negative controls were conducted using rabbit serum solution for keratin, S–100, type I collagen, type II collagen, BMP–4, FGF–2 and ChM–I, and mouse IgG solution for vimentin, BMP–2 and BMP–6 instead of the primary antibodies.

**Results**

**Histopathological findings**

1) HE

Histopathological investigations revealed tubular, cyst-like, solid nest-like, and cord-like proliferations of relatively small cubic, polygonal, and round cells in the tumor parenchyma in both the major and minor salivary gland tumors (Fig. 1a, b). Regions of tubular proliferation showed a double-layered structure, the inside of which comprised cubic duct–like cells encircling a lumen (inner cells), and on the outside, polygonal to round cells were proliferating in a sheet–like to nest–like pattern (outer cells). In addition, intercellular dissociation occurred in the outermost region of these outer cells, these cells were referred to as dissociated cells, which were migrating to myxomatous tissue through mucoid matrices slightly stained by hematoxylin.

In all cases of the major salivary gland tumors (100%), contiguous with the myxomatous tissue, mixed regions of hematoxylic or eosinophilic substance were present, and the formation of hyaline cartilage–like tissue with matrices and lacunar structures was observed, with cells present within these lacunae (chondrocyte–like cells). In 4 of the 50 cases (8%) of the minor salivary gland tumors, fibrocartilage–like tissue with eosinophilic matrices and lacunar structures was observed, with cells present within these lacunae (chondrocyte–like cells). In the minor salivary gland tumors, plasmacytoid cells with eosinophilic cytoplasm and eccentric nuclei were observed on the outside of the tumor parenchyma (Fig. 1c, d). In 1 of the 50 cases, a minor salivary gland tumor accompanied by bone–like tissue formation was found, around the periphery of which an arrangement of osteoblast–like cells was seen, with cells present in the lacunae (osteocyte–like cells) (Fig. 1e, f), whereas no bone–like tissues was observed in the major salivary gland tumors.

2) Tb

Table 1 shows the findings of metachromasia with Tb.

Myxomatous matrix showed strong level of metachromasia (a condition in which Tb, normally a blue dye, stains reddish–violet) when stained at pH 7.0, and moderate metachromasia at pH 4.1. With staining at pH 2.5, no metachromasia was present, or there were areas in which metachromasia of slight level was seen. The hyaline cartilage–like matrix observed in the major salivary gland tumors showed a strong level of metachromasia at pH 7.0 and pH 4.1,
Fig. 1. Histopathological findings
(a) The major salivary gland tumor consists of proliferation of double-layered tubular structure with myxomatous and cartilage-like tissues. (HE, original magnification ×200) (bar, 100 μm)
(b) The minor salivary gland tumor comprises proliferation of double-layered tubular components with myxomatous and fibrous connective tissues. (HE, original magnification ×200) (bar, 100 μm)
(c) The major salivary gland tumor shows hyaline cartilage-like tissues (HC) and myxomatous tissues (Mx). (HE, original magnification ×200) (bar, 100 μm)
(d) The minor salivary gland tumor indicates fibrocartilage-like tissues (FB) and myxomatous tissues (Mx). (HE, original magnification ×200) (bar, 100 μm) (in-set, plasmacytoid cells)
(e) Bone-like tissue is observed in a case of the minor salivary gland tumors. (HE, original magnification ×400) (bar, 100 μm)
(f) Osteoblast-like cells are observed in periphery of the bone-like tissue. Osteocyte-like cells are involved in the lacuna of the matrix. (HE, original magnification ×400) (bar, 100 μm)
(g) Matrix of cartilage-like tissues in the major salivary gland tumor shows remarkable metachromasia with Tb pH 2.5. (original magnification ×200) (bar, 100 μm)
(h) Unremarkable metachromasia with pH 2.5 is observed in the minor salivary gland tumor. (original magnification ×200) (bar, 100 μm)

<table>
<thead>
<tr>
<th>pH</th>
<th>Myxomatous tissues</th>
<th>Hyaline cartilage-like matrix</th>
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<tbody>
<tr>
<td>7.0</td>
<td>++</td>
<td>+</td>
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<tr>
<td>4.1</td>
<td>+</td>
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<tr>
<td>2.5</td>
<td>~ ±</td>
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and moderate to strong level at pH 2.5 (Fig. 1g). The fibrocartilage-like matrix that appeared in the minor salivary gland tumors showed a strong level of metachromasia at pH 7.0, and moderate level at pH 4.1, but metachromasia of slight level was observed in some areas at pH 2.5 (Fig. 1h).

**Table 1. Findings of metachromasia with Tb**

<table>
<thead>
<tr>
<th>Major salivary gland</th>
<th>Minor salivary gland</th>
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<td>pH</td>
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<tr>
<td>Myxomatous tissues</td>
<td>Fibrocartilage-like matrix</td>
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<td>++</td>
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<td>+</td>
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</table>

+++ : strong level, + : moderate level, ± : slight level, - : no metachromasia

Immuno histochemical findings

The results of immunohistochemical staining are summarized in Table 2.

1) keratin

In both the major and minor salivary gland tumors, the inner cells in the double-layered tubular tissue showed strong immunoreactivity, while the outer cells showed slight immunoreactivity; the dissociated cells in the myxomatous tissue showed slight immunoreactivity, while chondrocyte-like cells showed negative findings or slight immunoreactivity, and the mucoid matrix and the cartilage-like matrix showed slight immunoreactivity (Fig. 2a, b). In addition, negative findings or slight to moderate immunoreactivity for keratin were observed in the osteoblast-like cells and osteocyte-like cells in the tumors in which bone-like tissue was found, and negative findings were detected in the bone-like matrix.

2) vimentin

In both the major and minor salivary gland tumors, the inner cells showed negative findings and the outer cells indicated strong immunoreactivity, were observed in the dissociated cells and chondrocyte-like cells, and negative findings or slight immunoreactivity were found in the mucoid and cartilage-like matrices (Fig. 2c, d). In bone-like tissues, moderate immunoreactivity was seen in the osteoblast-like cells (Fig. 2e).

3) S-100

In both the major and minor salivary gland tumors, negative findings or slight immunoreactivity were observed in the inner cells, and moderate immunoreactivity was observed in the outer cells. In addition, the dissociated cells of the myxomatous tissue presented localized moderate immunoreactivity, while the chondrocyte-like cells showed...
Table 2. Immunohistochemical findings

<table>
<thead>
<tr>
<th>Primary antibody</th>
<th>double-layered tubular tissues</th>
<th>Myxomatous tissues</th>
<th>Cartilage-like tissues</th>
<th>Bone-like tissue</th>
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<td>Outer cells</td>
<td>Dissociated cells</td>
<td>Mucoid matrix</td>
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<td>± ± ±</td>
<td>±</td>
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<tr>
<td></td>
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<td>± ± ±</td>
<td>±</td>
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<td>± ± ±</td>
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<td></td>
<td>Minor</td>
<td>− L ±</td>
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<tr>
<td>FGF-2</td>
<td>Major</td>
<td>− ± ±</td>
<td>− ± ±</td>
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<td></td>
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<td>− ± ±</td>
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<tr>
<td>ChM-I</td>
<td>Major</td>
<td>± ± ±</td>
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<td></td>
<td>Minor</td>
<td>− ± ±</td>
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</table>

Major: Major salivary gland, Minor: Minor salivary gland
++: strong immunoreactivity, +: moderate immunoreactivity, ±: slight immunoreactivity, −: negative findings, L: localized

Moderate immunoreactivity and the mucoid matrix showed negative or slight immunoreactivity. In the cartilage-like matrix, slight immunoreactivity was observed in the minor salivary gland tumors, and negative findings or positive findings of slight immunoreactivity were detected in the minor salivary gland tumors (Fig. 3a, b). Moderate immunoreactivity was found in the osteoblast-like cells in the tumor tissue in which bone-like tissue was seen, moderate immunoreactivity was found in osteocyte-like cells, and slight immunoreactivity was detected in the bone matrix (Fig. 3c).

4) type I collagen

In the major salivary gland tumors, negative findings or slight immunoreactivity were found in the outer cells and dissociated cells, moderate immunoreactivity was seen in the mucoid matrix, slight to moderate immunoreactivity was observed in the cartilage-like matrix, and negative findings were observed in the chondrocyte-like cells. In the minor salivary gland tumors, slight immunoreactivity was seen in the inner cells, cartilage-like matrix and outer cells, while moderate immunoreactivity was observed in the dissociated cells, chondrocyte-like cells and the mucoid matrix (Fig. 3d, e).

5) type II collagen

In the major salivary gland tumors, slight immunoreactivity was observed in the inner cells, while moderate immunoreactivity was observed in the outer cells, dissociated cells and cartilage-like
Fig. 2. Immunohistochemical findings of keratin and vimentin
(a) Strongly positive immunoreactivity for keratin is observed in the inner cells of the major salivary gland tumor. (original magnification ×200) (bar, 100 μm)
(b) Strong immunoreactivity for keratin is also found in the inner cells of the minor salivary gland tumor. (original magnification ×200) (bar, 100 μm)
(c) Strong immunoreactivity for vimentin is identified in the outer cells of the major salivary gland tumor. (original magnification ×200) (bar, 100 μm)
(d) Strong immunoreactivity for vimentin is also seen in the outer cells of the minor salivary gland tumor. (original magnification ×200) (bar, 100 μm)
(e) Positive immunoreactivity for vimentin is observed in the osteoblast-like cells (original magnification ×400) (bar, 100 μm)
matrix, and slight to moderate immunoreactivity was observed in the chondrocyte-like cells, while negative findings or slight immunoreactivity were found in the mucoid matrix. On the other hand, in the minor salivary gland tumors, slight immunoreactivity was only seen in the outer cells and cartilage-like matrix (Fig. 3f, g).

6) BMP-2

In the major salivary gland tumors, slight immunoreactivity for BMP-2 was observed in the outer cells, slight to moderate immunoreactivity was seen in the dissociated cells, and moderate immunoreactivity was found in the chondrocyte-like cells; negative findings or slight immunoreactivity were observed in the mucoid and cartilage-like matrices. In the minor salivary gland tumors, negative findings or slight immunoreactivity were detected in the outer cells and cartilage-like matrix, and moderate immunoreactivity was observed in the chondrocyte-like cells (Fig. 4a, b). In the bone-like tissue, moderate immunoreactivity was seen in the both osteoblast-like cells and osteocyte-like cells (Fig. 4c).

7) BMP-4

In the major salivary gland tumors, slight to moderate immunoreactivity was observed in the inner cells, and moderate immunoreactivity was found in the dissociated cells; strong immunoreactivity was seen in the chondrocyte-like cells, and negative findings or slight immunoreactivity were observed in the mucoid and cartilage-like matrices. On the other hand, in the minor salivary gland tumors, slight immunoreactivity was observed in the outer cells and cartilage-like matrix, negative findings or slight immunoreactivity were seen in the dissociated cells, slight to moderate immunoreactivity was observed in the chondrocyte-like cells and mucoid matrix (Fig. 4d, e). In the bone-like tissue, osteoblast-like cells and osteocyte-like cells showed strong immunoreactivity, whereas the matrix indicated negative founding or slight reactivity (Fig. 4f).

8) BMP-6

In the major salivary gland tumors, slight to moderate immunoreactivity was observed in the inner cells, while slight immunoreactivity was found in the dissociated cells and chondrocyte-like cells, and negative findings or slight immunoreactivity were observed in the mucoid and cartilage-like matrices. In the minor salivary gland tumors, localized slight immunoreactivity was seen in the outer cells and dissociated cells, and negative findings or slight immunoreactivity were observed in the mucoid and cartilage-like matrices. In areas of the bone-like tissue, osteoblast-like cells showed strong immunoreactivity, and osteocyte-like cells presented slight to moderate immunoreactivity (Fig. 5c).

9) FGF-2

In the major salivary gland tumors, negative findings or slight immunoreactivity were observed in the inner cells, outer cells, and mucoid and cartilage-like matrices, slight immunoreactivity was found in the dissociated cells, and strong immunoreactivity was seen in the chondrocyte-like cells. In the minor sali-
vary gland tumors, negative findings or slight immunoreactivity were observed in the inner cells, outer cells and cartilage-like matrix, but moderate immunoreactivity was observed in the chondrocyte-like cells (Fig. 5d, e). In the bone-like tissues osteoblast-like cells presented moderate immunoreactivity (Fig. 5f).

10) ChM-I

In the major salivary gland tumors, moderate immunoreactivity was observed in the inner cells and the cartilage-like matrix, slight immunoreactivity was found in the outer cells and dissociated cells, strong immunoreactivity was observed in the chondrocyte-like cells, and negative findings or slight immunoreactivity were seen in the mucoid matrix. In the minor salivary gland tumors, negative findings or slight immunoreactivity were observed in the dissociated cells and the mucoid matrix, moderate immunoreactivity was seen in the chondrocyte-like cells, slight to moderate immunoreactivity was found in the cartilage-like matrix (Fig. 6a, b). In the bone-like tissue, slight to moderate immunoreactivity was observed in the osteoblast-like cells (Fig. 6c).

Discussion

Neoplastic myoepithelial cells, which have multilineage differentiation potential for formation of myxomatous or cartilage-like tissue, a histopathological characteristic of pleomorphic adenoma, are connected to and are believed to have a mechanism similar to that of the epithelial-mesenchymal interaction (2, 5). A general characteristic of fibrous connective tissue and bone and mesenchymal tissue such as cartilage is the formation of an intercellular or extracellular matrix, but each of these tissues differs in the constituent components of this matrix, and each tissue also has unique properties (6). In all cases of the major salivary gland tumors, a matrix of hyaline cartilage-like tissue was observed, which presented metachromasia on Tb staining at pH 7.0, pH 4.1 and pH 2.5. In general, in the major salivary glands, the tumors become large due to the size or the course, as well as the fact that space for tumor growth is assured as there is no physical irritation. As a result, a cartilaginous matrix that is more typical of pleomorphic adenoma is frequently observed.

On the other hand, in the minor salivary gland tumors, a few cases of fibrocartilage-like tissue were observed. This is believed to be because the course is shorter than in the major salivary gland tumors, as space for tumor growth is limited in the minor salivary glands, which are also affected by external physical factors due to irritation, or because these tumors are located at sites where they are recognized at an early stage; thus, differentiation into cartilage is not readily seen. The matrix in these tumors showed metachromasia with Tb at pH 7.0 and pH 4.1, but was only partially observed with staining at pH 2.5. Metachromasia is a finding in which mucopolysaccharides of the matrix are stained a reddish violet color in reaction to staining with a blue basic aniline dye such as Tb.

Cartilage is categorized as hyaline cartilage, fibrocartilage or elastic cartilage, and different constituent components are seen in the matrix of each of
Fig. 5. Immunohistochemical findings of BMP-6 and FGF-2
(a) Positive immunoreactivity for BMP-6 is observed in inner and outer cells of the major salivary gland tumor. (original magnification ×200) (bar, 100 μm)
(b) Positive immunoreactivity for BMP-6 is also found in inner and outer cells of the minor salivary gland tumor. (original magnification ×200) (bar, 100 μm)
(c) Strongly positive immunoreactivity for BMP-6 is seen in osteoblast-like and osteocyte-like cells. (original magnification ×400) (bar, 100 μm)
(d) Positive immunoreactivity for FGF-2 is identified in chondrocyte-like cells of the major salivary gland tumor. (original magnification ×200) (bar, 100 μm)
(e) Positive immunoreactivity for FGF-2 is also observed in chondrocyte-like cells of the minor salivary gland tumor. (original magnification ×200) (bar, 100 μm)
(f) Positive immunoreactivity for FGF-2 is seen in osteoblast-like cells. (original magnification ×400) (bar, 100 μm)

Fig. 6. Immunohistochemical findings of ChM-I
(a) Strongly positive immunoreactivity for ChM-I is identified in chondrocyte-like cells of the major salivary gland tumor. (original magnification ×200) (bar, 100 μm)
(b) Positive immunoreactivity for ChM-I is also observed in chondrocyte-like cells and cartilage-like matrix of the minor salivary gland tumor. (original magnification ×200) (bar, 100 μm)
(c) Positive immunoreactivity for ChM-I is seen in osteoblast-like cells. (original magnification ×400) (bar, 100 μm)

these types. Hyaline cartilage is the most widely distributed among them, and its matrix is primarily composed of type II collagen and proteoglycans. On the other hand, fibrocartilage is rich in collagen with relatively little proteoglycan. Elastic cartilage is rich in elastic fibers comprised of elastin with collagen proteins and proteoglycans mixed in. In the results of the present study, when the cartilage-like tissue appearing in the pleomorphic adenomas was compared to the cartilage subtypes of normal tissue, the cartilage-like matrix that appeared in the major salivary gland tumors resembled the hyaline carti-
lage in that it had an affinity for hematoxylin, presents metachromasia with Tb at pH 7.0, pH 4.1 and pH 2.5, and was positive for type II collagen. On the other hand, the results suggested that the cartilage-like matrix in the minor salivary gland tumors corresponded to the fibrocartilage, which is eosinophilic, showed metachromasia with Tb at pH 7.0 and pH 4.1, and reacted positively to type I collagen.

Developmentally, the structural morphology of the hyaline cartilage is classified as follows, from the exterior to the interior: (A) resting chondrocyte layer; (B) proliferating chondrocyte layer; (C) mature chondrocyte layer; (D) hypertrophic chondrocyte layer; and (E) calcifying chondrocyte layer (6). In addition, comparing the mechanisms of development and formation to the corresponding degree of differentiation, the degree of differentiation is believed to increase as development progresses from (A) to (E) (7, 8). Consequently, based on the findings showing that the cartilage-like tissue appearing in the major salivary gland tumors was similar to hyaline cartilage, the results of the present study suggested a relatively high level of differentiation to cartilage. Although the formation of fibrocartilage-like tissue was observed in the minor salivary gland tumors, the majority of the fiber components corresponded to those of the outer layers (A) to (B) in the layered structures (A) through (E) discussed above; thus, presumably, there is less differentiation to cartilage in the minor salivary gland tumors than in the major salivary gland tumors.

Fibrocartilage is normally present in the sacroiliac joint, temporomandibular joint and articular discs, pubic symphysis, sternoclavicular joints, articular meniscus and intervertebral discs. Although the significance of this localization is unclear, it has been suggested that fibrocartilage is present in areas that require physical strength, and the matrix is composed primarily of type I collagen (6). Consequently, comparing the major and minor salivary gland tumors, the fact that the minor salivary gland tumors developed adjacent to the surface of mucous membranes, an environment susceptible to physical irritation due to oral functions such as mastication, was also presumed to be a factor in the formation of type I collagen in the fibrocartilage-like tissue formed in the minor salivary gland tumors.

BMPs, on the other hand, are liquid factors belonging to the TGF-β superfamily, and are cartilage- and bone-inducing factors. BMP-2 and BMP-4 are involved in chondrocytic differentiation; the former is localized in the hypertrophic cartilage layer, and the latter in the outermost layer of cartilage (perichondrium) (8-10). BMP-6 is localized in the hypertrophic cartilage layer, and induces ossification by calcification in the layer below it. When the main bones of the cranium are formed by fibrous tissues, BMP-6 has the function of inducing direct osteogenesis without endochondral ossification (11). Normally, as is indicated by the information above, chondrogenesis and osteogenesis are known to be closely related to osteogenesis mediated by the functions of BMP-2 and BMP-4. In addition, FGF-2 is not only an angiogenic factor, but together with BMPs, synergistically promotes the proliferation, differentiation and maturation of chondrocytes (12-14). ChM-I also promotes chondrocyte proliferation and matrix synthesis (15).

The results of the present study, based on the positive findings for BMP-2, BMP-4, FGF-2 and ChM-I in the major and minor salivary gland tumors, reveal possibility or potential of these tumors for chondrogenesis. In addition, immunoreactivities tended to be stronger in the major salivary glands than in the minor salivary glands. Consequently, based on the expression levels of these molecules, it is believed that in the major salivary gland tumors, highly differentiated hyaline cartilage-like tissue formation is promoted, which, when compared to the layered differentiation of chondrogenesis described above, would be equivalent to the hypertrophic chondrocyte layer, while in the minor salivary gland tumors, fibrocartilage-like tissue is formed, which has a relatively low level of differentiation corresponding to the resting chondrocyte layer.

On the other hand, although pleomorphic adenomas are characterized by the formation of
cartilage-like tissue, osteogenesis is very rare. With regard to this phenomenon, Kusafuka et al. (16–18), reported that osteogenesis is inhibited in these tumors due to the expression of an angiogenesis inhibitory factor by ChM-I, and the coexpression of FGF-2 and FGF receptors.

Immunohistochemical findings revealed that in the minor salivary gland tumors in which bone-like tissue formation was observed, this bone-like tissue presented a positive reaction to S-100, and was also positive for vimentin, which suggested that dissociated cells from mesenchymal neoplastic myoepithelial cells played a role. In addition, osteoblast-like tissues showed strong positive reactions to BMP-4 and BMP-6. The strong expression of BMP-4 is observed in salivary gland cancer cell lines with chondrogenic potential, which induces osteoblast and chondrocytic differentiation (19–22). Based on the above, it is possible that in addition to the dissociated cells, osteocytes and osteoblasts could also have been derived from the proliferating neoplastic myoepithelial cells. Moreover, given the high level of BMP-6 expression, when compared to pleomorphic adenoma in which myxomatous and cartilage-like tissue have formed, it is believed that osteogenesis is induced directly without undergoing endochondral ossification.

In addition, there were results that corresponded to immunohistochemical findings of cartilage-like tissue in the dissociated cells in the myxomatous tissue that immunohistochemically indicated a progression to cartilage-like tissue. These findings suggest that the dissociated cells may have “blast cell” or precursor-like properties in the hyaline cartilage-like tissue of the major salivary gland tumors, and in the cartilage-like tissue in the minor salivary gland tumors.

Based on these findings, neoplastic myoepithelial cells, which were closely involved in the formation of cartilage-like tissue, appeared to be formed by differentiation-inducing factors centering on BMPs. In addition, positive findings for BMPs were more severe in the major salivary gland tumors than in the minor salivary gland tumors. The major salivary gland tumors also have an environment more conducive to hyaline cartilage-like tissue formation, and it is believed that the formation of hyaline cartilage-like tissue occurs as chondrocyte-like cells are formed and maintained, due to differentiation-inducing factors such as BMP-2, BMP-4, BMP-6, FGF-2 and ChM-I. On the other hand, in the minor salivary gland tumors, the expression of differentiation-inducing factors such as BMP is weak, and histopathological findings showed that a transition to myxomatous tissue and fibrocartilage-like tissue had occurred, due in part to physical irritation in the oral cavity.

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