Expression of Bone Matrix Proteins in Malignant Myoepithelioma with Extensive Osteoid Formation Occurring in The Maxilla

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We report here a rare case of malignant myoepithelioma occurring in the maxilla, accompanied by extensive osteoid formation. The patient was a 20-year-old Japanese man with a rapidly swelling mass in the hard palate. Expressions of bone sialoprotein (BSP) and osteopontin (OPN) were examined by immunohistochemistry, in situ hybridization (ISH) and RT-PCR. The mass showed invasive growth of atypical spindle and polygonal cells with abundant osteoid tissue in the stroma. These cells retained immunohistochemical features of myoepithelium, characterized by positive reactivity for cytokeratin (WS), cytokeratin 14, S-100 protein, vimentin, smooth muscle myosin heavy chain and calponin. In addition, malignant myoepithelium noted in and around the osteoid tissue showed expression of BSP on immunohistochemical analysis and ISH, but with no expression of OPN. The normal salivary gland tissue was negative for BSP and OPN. These results suggested that BSP might play a significant role in osteoid development in the present case.

Key word: Bone sialoprotein, Osteopontin, Malignant myoepithelioma

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Introduction
Malignant myoepithelioma (MME) is a very rare tumor, defined as a malignant tumor composed of atypical myoepithelial cells characterized by a lack of ductal or acinar differentiation, infiltrative growth, cellular pleomorphism and increased mitotic activity (1). Most MMEs occur in the parotid gland, while the minor salivary gland MMEs are usually located in the hard palate (2). The mean age of patients with MME is over 50 years old. However, some cases in younger subjects have also been reported (2). MMEs have been considered to arise from de novo or malignant transformation of myoepithelioma or benign mixed tumors (3). It has been reported that MMEs tend to be low-grade when they arise in a benign mixed tumor, and high-grade when they arise de novo; however, there is disagreement among various authors concerning the biological behavior (2, 3). Savera et al. (2) categorized MME into five cell types: epithelioid, spindle, clear, plasmacytoid and mixed cellular. Myxoid matrix and extensive hyalinization are typically formed in the stroma of MMEs. Metaplastic changes, including squamous, chondroid and sebaceous changes, are occasionally seen. Osteoid or chondroid hard tissues formed in salivary gland tumors have been almost exclusively observed in benign mixed tumors of the parotid gland, although with a very low frequency (4).

We encountered a rare case of MME arising in a young adult male in the right maxilla, characterized by extensive osteoid formation. In this case, immunohistochemistry, in situ hybridization (ISH) and RT-PCR were used to analyze the expression of the bone matrix proteins, bone sialoprotein (BSP) and osteopontin (OPN), from the differential viewpoint of osteosarcoma. Immunohistochemical analysis for several useful markers was also performed. Our results suggest that BSP expression was only implicated in the characteristic osteoid formation in this case.

Case report
A 20-year-old Japanese man was admitted to the Department of Oral Surgery, Tokai University Hospital, in March 1999, due to a rapidly swelling mass in the right
side of the hard palate. This lesion had been present for more than 2 years, but the patient had not sought medical attention because the tumor had been painless. The part of the mass protruding into the oral cavity measured approximately 2 \( \times \) 1.5 cm. On preoperative examination, MRI showed that the cortex of the maxillary bone on the right side was destroyed and the inferior portion of the orbit was invaded by the tumor. Furthermore, the right maxillary and ethmoid sinuses were filled with the tumor. Laboratory examination indicated elevation of serum alkaline phosphatase activity. Under a probable diagnosis of malignant myoepithelioma (MME) with osteoid stroma arising from the minor salivary gland in the biopsy, the subject underwent wide resection of the right maxilla and total dissection of neck lymph nodes. The subject died in December 1999 because of a locally recurrent tumor that extensively involved the brain base.

**Pathology**

On observation of the cut surface of resected material, the mass appeared gray-white in color with hemorrhagic foci and measured 7 \( \times \) 6 cm (Fig. 1). Both maxillary and ethmoid sinuses were expanded due to the mass involvement. The boundary was mostly well demarcated. The tumor was composed of epidermoid and polygonal cells with hyperchromatic and enlarged nuclei, which were arranged in solid sheet or reticular pattern. Spindle shaped cells growing as a sheet with high cellularity were also observed (Fig. 2). Extensive myxoid and osteoid matrix with focal calcification was predominantly noted in the stroma (Fig. 3a). The mass was multifocally involved in necrosis and excessive hyalinization. Abnormal mitotic figures were frequently observed. No apparent duct-like or acinar structures were present. In the stroma, the mononuclear cells, which were present around the osteoid matrix, appeared to be osteoblasts (Fig. 3b). These cells displayed hyperchromatic and enlarged nuclei with prominent nucleoli. The mononuclear cells embedded in the osteoid resembled osteocytes. There was no evidence of chondrogenesis or endochondral ossification. No benign counterparts of mixed tumor or myoepithelioma were noted. Foci of normal minor salivary gland tissue remained in the vicinity of the tumor. No regional lymph node metastasis was observed.

**Immunohistochemistry**

Specimens taken from the tumor mass were fixed in 10% buffer formalin for 24 h without decalcification and embedded in paraffin. Immunohistochemical analysis was performed using the streptavidin-biotinylated immunoperoxidase method. Monoclonal antibodies used were as follows: anti-cytokeratin 14 (CK 14), anti-EMA (epithelial membrane antigen), anti-S-100 protein, vimentin, anti-SMA (smooth muscle actin), anti-SMMS-1 (smooth muscle myosin heavy chain), anti-calponin, and anti-GFAP (glial fibrillary acid protein). A polyclonal antibody against cytokeratin (CK) was also used. Immunohistochemical detection of BSP and OPN was performed using rabbit polyclonal antibodies against LF83 and LF123, respectively, which were kindly provided by Dr. L.W. Fisher (Bone Research Branch, National Institute of Dental Research, NIH, Bethesda, MD, U.S.A.).

The majority of tumor cells were positive for CK, CK 14, S-100 protein, vimentin, SMMS-1 and calponin but were negative for GFAP, EMA and SMA. The osteoblast-like cells with atypia, which were present around the osteoid tissue, were also stained positively for CK, CK 14 (Fig. 3c), S-100 protein (Fig. 3d), calponin (Fig. 3e), SMMS-1 (Fig. 3f) and vimentin. The osteocyte-like cells showed expression patterns similar to those of osteoblast-like cells. Immunoreactivity for BSP protein was detected predominantly in osteoblast-like cells around the osteoid matrix (Fig. 3g), but little or no expression was detected in the osteoid matrix or other atypical cells away from the osteoid (Table 1). The osteocyte-like cells in the osteoid matrix also showed BSP expression (Fig. 3g). No OPN expression was noted in any tumor component. The normal salivary gland was negative for BSP and OPN.

**RT-PCR**

Total RNA was isolated from the tumor using ISOGEN (Nippon Gene, Toyama, Japan). Reverse transcription (RT) and PCR were performed using an RNA LA PCR TM kit (AMV) Ver.1.1 (Takara, Tokyo, Japan) according to the manufacturer’s instructions. The human BSP oligonucleotide primer was 5’-ATCGGGATCCAAAGCAGAGGATTCTGAAG-3’ and 5’-
Fig. 2: A sheet-like arrangement of atypical spindle-shaped and polygonal cells was noted (original magnification 100).

Fig. 3: (a) Extensive osteoid formation and focal mineralized deposits were observed (original magnification 50). (b) Osteoblast-like tumor cells were seen in and around the osteoid tissue (original magnification 150). (c) Immunohistochemical expression of CK 14 (original magnification 600), (d) S-100 protein (original magnification 600), (e) calponin (original magnification 600), (f) SMMS-1 (original magnification 600) and (g) BSP protein (original magnification 600) in the tumor cells. (h) BSP mRNA signals were detected in the tumor cells by ISH (original magnification 600).
CCTGAAGCTTGCATCTCCAGCCTTCTTGGG-3'  Prim-
ers for detection of human OPN were 5'-
CCAAGTAAGTCCAACGAAAG-3' and 5'-
ATGTCTGCTCCTGTAGTGG-3' (5).  Human
b-actin was
amplified as an internal control.  The PCR products were
electrophoresed on 1.5% TBE agarose gels and stained
with ethidium bromide.

Amplified products corresponding to BSP tran-
scripts were detected in RT-PCR samples derived from
the tumor, but no OPN was detected (Fig. 4).

In situ hybridization

The human BSP and OPN primers used for PCR
were also used to prepare BSP and OPN cRNA probes for
ISH (6).  Digoxigenin (DIG)-11-UTP-labeled single-
stranded cRNA probes for human BSP and OPN were
prepared with a DIG labeling kit (Boehringer-Mannheim
Biochemica, Mannheim, Germany) according to the
manufacturer's instructions.  The procedures of ISH have
been previously described in detail.  mRNA was detected
colorimetrically with a DIG-non-radioactive nucleic acid
detection kit (Boehringer-Mannheim).

Strong BSP mRNA expression was demonstrated
in osteoblast-like cells, indicating active synthesis of BSP
limited to these cells (Fig. 3b).  However, no OPN mRNA
expression was noted.  These results corresponded with
those of immunohistochemical analysis (Table 1).  The
normal salivary gland was negative for expression of these
mRNAs.

Discussion

Based on these histopathological findings, includ-
ing the results of immunohistochemical, RT-PCR and ISH
analyses, the tumor was diagnosed as malignant myo-
epithelioma (MME) of minor salivary gland origin, char-
acterized by considerable osteoid matrix.  The important
differential diagnoses include osteosarcoma due to their
histological resemblance.  Asai et al. (7) reported that
strong emphasis should be placed on the importance of
discrimination between osteosarcoma and malignant sali-
vary gland tumors complicated by osteogenesis from the
chemotherapeutic viewpoint because of the poor respons-
siveness of malignant salivary gland tumors to chemo-
therapy.

Therefore, immunohistochemical analysis of myo-
epithelial markers is considered to be essential for differ-
ential diagnoses.  Immunohistochemically, myoepithelial
markers such as S-100 protein, vimentin, SMA and
calponin are of little help in distinguishing MME from
osteosarcoma as these proteins are also commonly ex-
pressed in osteosarcoma (8,9).  However, calponin has been
reported to be a new highly sensitive marker of myoepi-
thelial differentiation in salivary gland tumors (10).  Cells
positive for SMMS-1, which is useful for detecting smooth
muscle myosin filaments, have been identified not only
in the normal myoepithelium of the salivary and mam-
mary glands but also in the neoplastic myoepithelium
(11).  SMMS-1 expression was demonstrated in 3 of 10
MMEs (12).  Our immunohistochemical study in three
cases of osteosarcoma arising from the jaw showed nega-
tive reactivity for SMMS-1 (data not shown).  It is well
established that normal or neoplastic myoepithelium is
consistently expressing CK.  Immunoexpression of CK14
is specific in the myoepithelium of the normal salivary
gland (12).  Savera et al. (2) reported that wide spectrum
CK and CK14 were positive in 100% and 53% of MMEs.
Nago et al. (12) demonstrated better sensitivity for CK14
(8/10).  In contrast, according to Hasegawa et al. (13), one
case of the osteoblastic type of 18 osteosarcomas was posi-
tive for wide spectrum CK.  There are large differences in
positive ratio of CK expression between MME and os-
teosarcoma.  Absence of CK expression would suggest a
diagnosis of osteosarcoma rather than MME.  Usually,
following decalcification, tissue would often fail to express
CK.  Therefore, CK false-negative findings may be a pos-
sible diagnostic pitfall between MME with osteoid and
osteosarcoma arising from the maxilla.

Interestingly, the neoplastic myoepithelium mani-

Table 1: Result of BSP and OPN Expression

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<th>BSP</th>
<th>OPN</th>
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<tr>
<td>osteoblast-like</td>
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</tr>
<tr>
<td>osteocyte-like</td>
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<td>--</td>
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<td>other atypical cell</td>
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<td>osteoid matrix</td>
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Abbreviations: BSP, bone sialoprotein; OPN, osteopontin; +, positive; -, negative.
thelial tumors, including myoepithelioma (15), benign (16-18) or malignant mixed tumors (7), and malignant adenomyoepithelioma of the breast (14). Even osteosarcomatous components of the carcinosarcoma are characterized by proliferation of neoplastic myoepithelium (19). Thus, neoplastic myoepithelium is considered to have the potential to develop osteoid tissue. However, the mechanism of bone or osteoid formation observed in salivary gland tumors remains to be clarified. BSP and OPN are major non-collagenous proteins in bone and other mineralized tissues. Generally, it has been shown that both proteins contribute to the initiation and regulation of mineralization (6). The distributions of BSP mRNA and protein are known to be relatively restricted in the hard tissue forming cells, compared to other non-collagenous bone matrix proteins such as osteonectin or OPN (6). In addition, BSP also enhances the attachment activity of fibroblasts, osteoblasts and osteoclasts to plastic surfaces and has an affinity to collagen (20). Our immunohistochemical study detected the presence of BSP protein in the neoplastic cells in and around the osteoid tissue, but little or no expression was noted in extracellular osteoid matrix or other neoplastic cells. Compared to the immunohistochemical profiles of BSP, cells expressing BSP mRNA were fewer in number. These results suggested that expression of BSP might play a significant role in the osteoid development.

OPN also expresses in variety of soft tissues and involves developmental processes, wound healing and immunological response (21). Kusafuka et al. (22) reported that OPN was localized in the calcification-unrelated myxoid or hyaline area of the salivary benign mixed tumor by immunohistochemical analysis. OPN is unlikely associated with hard tissue formation of the salivary gland tumors. On the other hand, OPN is frequently expressed in osteosarcoma (23). Total absence of OPN expression may support the salivary gland origin of the present case.

In conclusion, we reported here a rare case of malignant myoepithelioma of minor salivary gland origin, mimicking osteosarcoma. Co-expression of CKs, S-100 protein, vimentin, calponin and SMMS-1 helped confirm the differential diagnosis. BSP may have played an important role in the characteristic osteoid development, whereas unlike osteosarcoma OPN was not associated with the characteristics of this tumor. We would like to emphasize the importance in making a differential diagnosis between malignant salivary gland tumor with osteoid/bone formation and osteosarcoma arising from the oral region.

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References


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