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Histopathological Variation of Primary Mucosa-associated Lymphoid Tissue Lymphoma of the Oral Cavity

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Primary mucosa-associated lymphoid tissue (MALT) type lymphoma arising in the oral cavity is rare. We examined histopathologic, immunohistological and genotypic findings of seven cases of intraoral MALT lymphoma using formalin-fixed paraffin-embedded tissues. Histologically, two variants have been delineated. (i) In four cases of minor salivary gland type, the lymphoid follicles were surrounded by centrocyte-like (CCL) cells with occasional follicular colonization. The CCL cells invaded the residual salivary gland duct resulting in a lymphoepithelial lesion. CCL cells frequently showed plasmacytic differentiation. (ii) In three cases of follicular growth type, the lesion was characterized by follicular growth pattern resulting from prominent follicular colonization. CCL cells showed minimal plasma cell differentiation. There was no residual epithelial component detected even by cytokeratin immunostaining. There were no Epstein-Barr virus-encoded small RNA-positive cells detected by *in situ* hybridization. API2-MALT1 fusion transcript does not appear to be associated with either histological variant of primary intraoral MALT lymphoma. (Pathology Oncology Research Vol 13, No 4, 345–349)

Key words: mucosa-associated lymphoid tissue lymphoma, oral cavity, follicular colonization, immunohistochemistry

Introduction

Malignant lymphomas of the oral cavity are uncommon and account for 3.5% of all oral malignancies.[1] The majority of primary malignant lymphomas of the oral cavity are diffuse large B-cell lymphomas, and intraoral marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) type appears rarely.[2,3] Thus, only a few cases of primary MALT lymphoma arising from oral cavity have been described in the literature.[4-8] To clarify further histopathologic findings of intraoral MALT type lymphomas, histopathologic, immunohistochemical and genotypic findings of seven such cases were studied, and two histological varieties are delineated.

Materials and Methods

Seven cases were collected from a series treated between 1992 and 2006 by one of the authors (MK). Medical records of seven cases were extensively reviewed. One case (no. 4) have been reported previously.[8]

Tissue specimens were fixed in formalin, routinely processed and embedded in paraffin. For light microscopic examination, the sections were stained with hematoxylin-eosin (HE).

Immunohistochemistry was performed on paraffin sections using a Ventana automated (BenchMark™) stainer according to the manufacturer's directions. A panel of

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Table 1. **Summary of clinical findings in seven patients**

	Age/ gender	*Site of disease*	*Tumor size (cm)*	*Concurrent tumor*	*Therapy and outcome*
1	23/F	Rt buccal mucosa	1.5	Orbita	RT, 3 m lung recurrence, Chem+PBSCT, 68 m A (–)
2	51/M	Soft palate	1.5	–	Resection, 120 m A (–)
3	60/M	Lt buccal mucosa	3	–	Resection, RT, 168 m A (–)
4	64/F	Soft palate	2	–	Resection several times of recurrences, 180 m A (+)
5	77/F	Soft palate	1.5	Parotid gland	Chem, 41 m A (–)
6	78/F	Rt gingiva	3	–	RT, 14 m A (–)
7	83/M	Rt buccal mucosa	3.5	–	Chem, PR, 18 m A (+)

F, female; M, male; Rt, right; Lt, left; Bil, bilateral; RT, radiotherapy; Chem, combination chemotherapy; PR, partial response; PBSCT, autologous peripheral blood stem cell transplantation; m, months; A, alive; (–), without disease; (+), with disease

antibodies included those against IgD (Novocastra, Newcastle, UK), IgM (Dako A/S, Glostrup, Denmark), CD3 (PS-1; MBL, Nagoya, Japan), CD5 (4C7; Novocastra), CD10 (56C6; Novocastra), CD20 (L26; Dako), CD23 (1B12; Novocastra), CD43 (DFT-1; Dako), bcl-2 (124; Dako), cyclin D1 (5D4, MBL), bcl-6 (polyclonal; Dako), bcl-10 (151; Dako) and cytokeratin AE1/3 (Dako). Replacement of primary antibodies by normal mouse- or rabbit serum was used as a negative control.

In situ hybridization (ISH) with Epstein-Barr virus (EBV)-encoded small RNA (EBER) oligonucleotides was performed to test for the presence of EBV small RNA in formalin-fixed paraffin-embedded sections using a Ventana automated (BenchMark™) stainer.

In four cases (nos. 4-7), the API2-MALT1 fusion transcript was examined using formalin-fixed and paraffin-embedded tissue according to the method we recently described.[9]

Results

The main clinicopathologic findings are shown in *Tables 1* and *2*.

Clinical findings

The patients, four females and three males, ranged in age from 23 to 83 years with a median age of 64 years. In three patients (nos. 2, 4 and 5), the tumor was located in the soft palate, in three (nos. 1, 3 and 7) in the buccal mucosa and in the remaining one (case no. 6), the tumor originated in the gingiva. None of the patients had systemic symptoms or history of autoimmune disease, including Sjögren's syndrome. Patient 1 had concurrent MALT lymphoma in the orbita, and patient 5 in the parotid gland. In both patients, extraoral MALT type lymphoma was discovered simultaneously with intraoral lymphoma. An analysis of patients' life styles did not suggest any risk factors for human immunodeficiency virus type-1 (HIV-1) infection, although serological findings for anti-HIV-1 antibody were available in two cases (no. 1 and 5).

Three patients (nos. 1, 3 and 6) were treated with radiotherapy, two patients (nos. 5 and 7) received combination chemotherapy, and two (case no. 2 and 4) did not receive any medication after surgery.

Follow-up data were obtained for all seven patients. The median follow-up period (from diagnosis to last follow-up)

Table 2. **Summary of pathological, EBV and genotypic findings in seven patients**

	Histology	*PCD*	*FC*	*LEL*	*sIgM*	*sIgD*	*cIg*	*CD43*	*Bcl-2*	*Bcl-10*	*EBER*	*API2-MALT1*
1	MSGT	++	+	++	P	N	Kappa	N	P	N	N	ND
2	MSGT	++	++	++	P	N	Kappa	N	P	N	N	ND
3	FG	+	+++	–	P	P	N	ND	P	N	N	ND
4	MSGT	++	+	+	P	N	Kappa	ND	ND	N	N	N
5	MSGT	+	++	+	ND	ND	Lambda	P	ND	N	N	N
6	FG	+	+++	–	P	N	N	P	P	N	N	N
7	FG	+	+++	–	P	P	N	ND	P	N	N	N

PCD, plasma cell differentiation; FC, follicular colonization; LEL, lymphoepithelial lesion; sIg, surface immunoglobulin; cIg, cytoplasmic immunoglobulin; EBER, Epstein-Barr virus-encoded small RNA, MSGT, minor salivary gland type; FG, follicular growth type, –, negative or absent; +, scattered or mild; ++, moderate; +++, numerous or prominent; N, negative; P, positive; ND, not determined

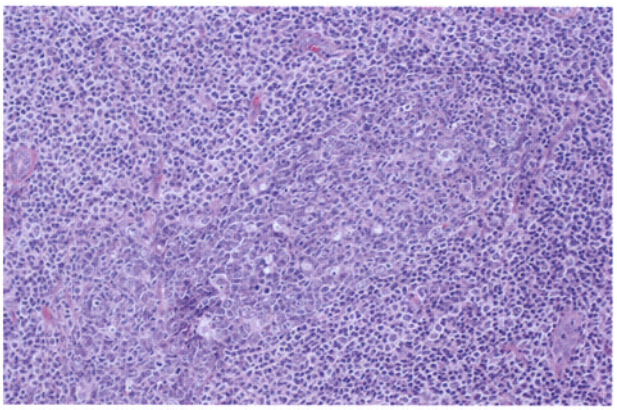

Figure 1. *On low-power field, a colonized lymphoid follicle with indistinct mantle zone surrounded by neoplastic cells. HE x25, Case 5*

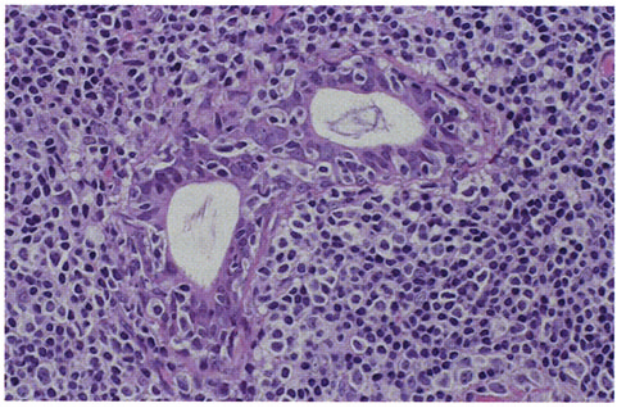

Figure 2. *On high-power field, medium-sized lymphocytes with round or indented nuclei and moderate to abundant cytoplasm invaded the salivary gland duct. Tumor cells exhibited plasma cell differentiation. HE x100, Case 5*

was 68 months with a range of 14 months to 180 months. One patient (no. 1) showed a relapse in the bilateral lungs after 3 months, but was managed using combination chemotherapy and autologous peripheral blood stem cell transplantation. Case 4 had multiple intraoral recurrences of the tumor. All seven cases were alive at last follow-up.

Pathological, immunohistochemical and EBV findings

Two distinct morphological patterns have been delineated.

I) MALT lymphoma arising from minor salivary gland (minor salivary gland type) (n=4; cases 1, 2, 4 and 5)

The lymphoid follicles were surrounded by centrocyte-like (CCL) cells with occasional follicular colonization (*Fig.1*). The CCL cells were medium-sized, and usually had round or indented nuclei and moderate to abundant cytoplasm (*Fig. 2*). The CCL cells invaded the residual salivary gland duct resulting in lymphoepithelial lesion. Occasionally large cells resembling centroblasts or immunoblasts were also identified. Plasmacytoid differentiation of tumor cells was frequent (*Fig. 2*), and in one case (no. 4), it was the predominant feature of neoplastic cells. In one lesion (no. 4), the majority of the lesion was occupied by amyloid deposition.

The tumor cells were CD5-, CD10-, CD20+, CD23-, CD43-, bcl-2+, bcl-6-, bcl-10-, cyclin D1-, surface IgM+/-, surface IgD-. Monoclonal intracytoplasmic immunoglobulin was detected in all four cases (nos. 1, 2, 4: kappa, 5: lambda).

II) Prominent follicular colonization pattern (follicular growth type) (n=3; cases 3, 6 and 7)

Histologically, at low-power field, the lesion was characterized by follicular and diffuse pattern with a marginal zone component (*Fig. 3*). Interstitial fibrosis compartmentalized the lesion. Occasionally, mantle cells invaded into the neoplastic follicles colonized by the tumor cells, and somewhat resembled the "floral variant" follicular lymphoma in two cases (nos. 6 and 7) (*Fig. 3*). The CCL cells exhibited minimal plasma cell differentiation (*Fig. 4a*). Occasionally, large cells resembling centroblasts were also identified. The colonized germinal centers were occupied by neoplastic cells with various numbers of residual follicular center cells and mantle cells (*Fig. 4b*).

The tumor cells were CD5-, CD10-, CD20+, CD23-, CD43+, bcl-2+ (*Fig. 5a*), bcl-6- (*Fig. 5b*), bcl-10-, cyclin D1-, surface IgM+, surface IgD+/- and cytoplasmic immunoglobulin-. Residual non-neoplastic germinal center cells were CD10+, CD20+, CD43-, bcl-2- (*Fig. 5a*), bcl-6+ (*Fig. 5b*) and bcl-10+. CD23 immunostaining demonstrated disrupted follicular dendritic cell pattern characteristics of follicular colonization in MALT lymphoma (*Fig. 5c*). Cytokeratin AE1/3 immunostaining did not demonstrate any residual epithelial component.

There was no Epstein-Barr virus encoded small RNA was detected by the ISH in any of the seven cases exam-

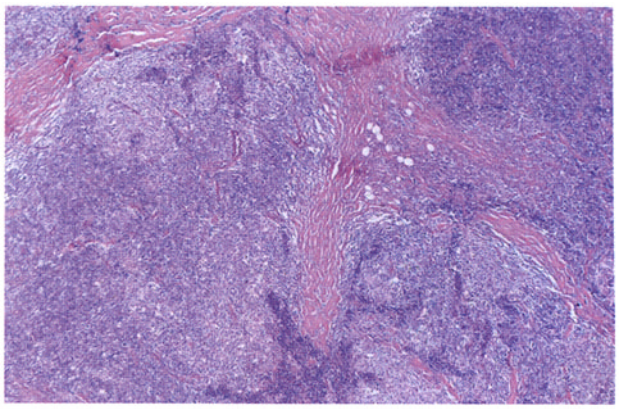

Figure 3. *On low-power field, neoplastic follicles colonized by the tumor cells somewhat resembled "floral variant" follicular lymphoma. Interstitial fibrosis compartmentalized the lesion. HE x10, Case 6*

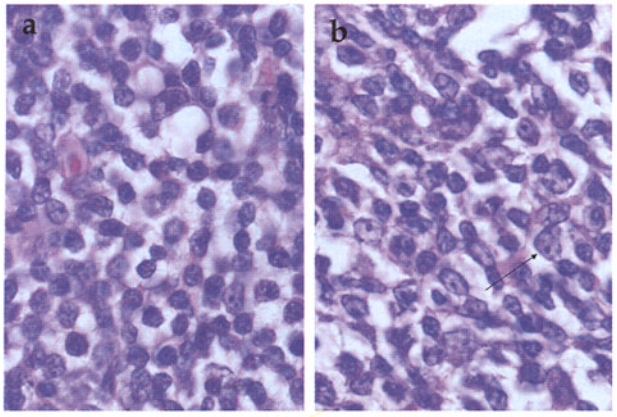

Figure 4. (a) On high-power field, the tumor cells in the interfollicular area were composed of medium-sized lymphocytes with scant cytoplasm, indented or round nuclei and absent or small nucleoli. Note the large cells resembling centroblasts. Tumor cells exhibited minimal plasma cell differentiation. (b) Colonized follicle was occupied by medium-sized neoplastic cells with residual germinal-center cells (arrow). HE x250, Case 6

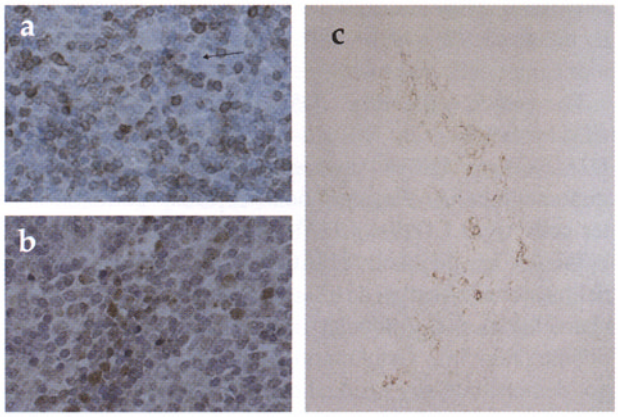

Figure 5. (a) In a colonized lymphoid follicle, tumor cells were bcl-2+, but the residual germinal center cells were bcl-2- (arrow). x100 (b) In a colonized lymphoid follicle, tumor cells were bcl-6-negative. Note the bcl-6-positive residual germinal center cells. x100 (c) CD23 immunostaining demonstrating a disrupted follicular dendritic cell pattern characteristic of follicular colonization. x25, Case 6

ined. In only one case (no. 7), scattered reactive T cells were EBER+.

There was no API2-MALT1 fusion transcript detected in any of the four cases examined (nos. 4-7).

Discussion

Mucosa-associated lymphoid tissue (MALT) type lymphoma arising in the oral cavity is rare.[2,3] To our knowledge, only seven cases of primary intraoral MALT type lymphoma have been reported.[4-8] The previously reported cases appeared to be MALT type lymphoma arising from minor salivary gland in the oral cavity (minor salivary gland type). The histopathologic, immunohistochemical and genotypic findings of MALT type lymphoma of the minor salivary gland type have been well described.[4-8, 10, 11] Histopathologically, these cases were characterized by the marginal zone distribution pattern of CCL cells, the presence of colonized follicles and lymphoepithelial lesions, and CCL cells representing occasional plasma cell differentiation.[10,11] Four of our seven cases were MALT lymphomas of the minor salivary gland type.

MALT lymphomas occasionally contain numerous follicles and may exhibit a prominent follicular growth pattern resulting from follicular colonization.[10,12] The histopathological findings of the remaining three cases in our series appear quite different from those of the MALT lymphoma of the minor salivary gland type. Histologically, these three lesions were characterized by a prominent follicular growth pattern resulting from follicular colonization, absence of lymphoepithelial lesions, CCL cells representing minimal plasma cell differentiation (follicular growth type). Moreover, mantle cells invaded the neoplastic follicles colonized by tumor cells, resembling follicular lymphoma, particularly the "floral variant".[13] Floral variant sometimes exhibit marginal zone differentiation.[13] However, CD10, CD43, bcl-2 and bcl-6 immunostaining clearly separated the residual non-neoplastic germinal center cells from CCL cells.[13-15] Moreover, CD23 immunostaining demonstrated a disrupted follicular dendritic cell pattern characteristic of follicular colonization in MALT lymphoma.[10, 12] In these three cases, the marginal zone nature of the CCL cells was most recognizable by immunohistochemistry, although the histologic appearance alone may cause some diagnostic problems.

Histopathological findings of these two types of intraoral MALT lymphoma appear quite different. However, the present study indicates that API2-MALT1 fusion transcript does not appear to be associated with intraoral MALT lymphoma as previously indicated.[5,7] Recently, Du et al. indicated strong bcl-10 nuclear staining by immunohistochemistry, suggesting the presence of t(1;14)(q21;q21).[16] However, there was no strong bcl-6 nuclear staining in any of our seven cases.

Previously, Solomides et al found EBER+ tumor cells in a portion of MALT type lymphomas.[2] However, there was no EBER+ cells in any of our seven cases.

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