

Peripheral Ameloblastoma of the Lower Molar Gingiva: A Case Report and Immunohistochemical Study

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(Received February 2, 2012; Accepted April 16, 2012)

Peripheral ameloblastoma (PA) is a rare extraosseous odontogenic tumor with histological characteristics similar to those of the common intraosseous ameloblastoma. Two questions regarding PA remain: its histogenic origin and how to differentiate between PA and intraoral basal cell carcinoma. We describe a patient with PA. The result of immunohistochemistry showed cytokeratin (CK) 7, CK14⁺, CK19⁺, AE1/AE3⁺, CAM5.2⁺, 34 β E12⁺, epithelial membrane antigen, Ber-EP4, p53, p63⁺, and low Ki-67, that was similar to those of 4 cases of intraosseous ameloblastoma. Our results suggest that a PA originates from odontogenic epithelial remnants, rather than from the oral epithelium.

Key words: peripheral ameloblastoma, intraosseous ameloblastoma, intraoral basal cell carcinoma, immunohistochemistry, odontogenic tumor

INTRODUCTION

Ameloblastomas are the most common odontogenic tumor and they are clinically and histologically diverse. These tumors have several distinct clinical types, including solid, multicystic, unicystic, desmoplastic, and peripheral ameloblastomas (PAs). Each has a specific biological behavior and consequently a different prognosis and treatment [1]. Although ameloblastomas are subclassified as follicular, plexiform, granular, basal cell, acanthomatous, and desmoplastic types in histology [2], different histological patterns can coexist in the same lesion. This diversity may reflect the complex development of dental structures. Ameloblastomas generally occur in the intraosseous region of the jaws. However, PA is a rare variant of ameloblastoma that occurs in extraosseous regions such as the gingiva and oral mucosa covering the alveolus. PAs comprise 1.3–10% of all ameloblastomas and frequently occur in the fifth through seventh decades [2, 3]. The mean age of patients with PA (males 53 years, females 51 years) is significantly higher than for the intraosseous counterpart, which has a mean age of 37 years [2]. The male:female ratio is 1.9:1 and the mandible: maxilla ratio is 2.4:1 [2]. Clinically, a PA is a painless, firm, exophytic growth with a smooth, granular, pebbly, warty, or papillary surface [2].

Two questions concerning PA remain: its histogenic origin and how to differentiate PA from intraoral basal cell carcinoma (BCC) [4]. Typical ameloblastomas are tumors of intraosseous odontogenic epithelial origin [5]. By contrast, PAs are thought to be derived from

the extraosseous remnants of odontogenic epithelium within the underlying connective tissue or from the basal cell layer of the oral epithelium, which is believed to have odontogenic potential [2, 6]. Continuity between the tumor nests and oral epithelium is reported in about 50% of cases [5], suggesting a basal cell origin for PA. However, the tumor may simply extend into and involve the oral epithelium [4]. The histopathological features of follicular PA are similar to those of intraoral BCC [4, 7, 8]. Furthermore, both PA and intraoral BCC can arise from the surface epithelium [9]. Therefore, PA and intraoral BCC may be the same entity [8]. However, distinguishing between these two neoplasms is important because their biological behavior and treatment differ [8].

This paper reports a patient with PA and the results of immunohistochemical studies for cytokeratins (CK7, 14, 19, AE1/AE3, CAM5.2, and 34 β E12), epithelial membrane antigen (EMA), Ber-EP4, p53, p63, and Ki-67 to further evaluate the two above-mentioned questions regarding PA.

CASE REPORT

A 46-year-old man was referred to the Department of Oral and Maxillofacial Surgery, National Hospital Organization Shizuoka Medical Center with a painless mass of 5 months' duration on the lingual gingival region of the lower left third molar.

There was no swelling of the left cheek or associated lymph node enlargement. On intraoral examination, the mass was a 1.5-cm-diameter, firm, exophytic growth with a papillary surface (Fig. 1). Plain radiographs



Fig. 1 Intraoral view of a 1.5-cm-diameter peripheral ameloblastoma on the lingual gingiva of the lower left third molar. The lesion showed exophytic growth with a papillary surface.



Fig. 2 **A)** Panoramic radiograph showing no bone resorption. **B)** CT image showing no bone resorption.

and computed tomography (CT) showed no bone resorption (Fig. 2). There was no history of dental or other medical problems. The rest of the clinical and laboratory examination was within normal limits. With the provisional diagnosis of a gingival tumor, the biopsy was performed. The pathological examination showed a follicular type ameloblastoma and confirmed the diagnosis of peripheral ameloblastoma. The mass was excised with a small margin of normal tissue under general anesthesia. Although the surface of the denuded bone showed no resorption, the surface was removed. Healing was uneventful and there has been no sign of recurrence for 2 years and 3 months.

Histopathologically, the tumor demonstrated an exophytic growth pattern and consisted of proliferating squamous-like cells. The tumor had the bulbous rete ridges of an ameloblastoma. These ridges consisted of peripheral rows of palisaded columnar cells and a central area of cuboidal or stellate cells. The tumor nests were continuous with the surface epithelium (Fig. 3).

We performed immunohistochemical studies for cytokeratins (CK7, 14, 19, AE1/AE3, CAM5.2, and 34 β E12), EMA, Ber-EP4, p53, p63, and Ki-67 to further evaluate the PA. Biopsy and operative specimens of four typical intraosseous ameloblastomas (IAs) and the PA from this case were examined. The IA specimens were obtained from the mandibles of two males and two females ranging in age from 17 to 53

years (mean age 39.5 years). Details of the primary antibodies and their use, and the results are presented in Tables I and II, respectively. The routine indirect immunoperoxidase method was used for immunohistochemistry. After deparaffinization, the sections were immunostained in an autoanalyzer (BenchMark[®] XT; Roche Diagnostics K.K., Tokyo, Japan). After activating the antigen, monoclonal antibodies were used as the primary antibody and an iView DAB Detection kit (Roche Diagnostics K.K.) was used for the autoimmunostaining reagent. Dehydration and mounting were performed after nuclear staining with hematoxylin. All cases were positive for CK14, CK19, AE1/AE3, 34 β E12, and p63, while negative for CK7, CAM5.2, EMA, Ber-EP4, and p53. (Fig. 4). The Ki-67 labeling index (Ki-67 LI) in the PA and IAs was 2.22% and 1.37% (mean), respectively (Fig. 5).

DISCUSSION

PA is difficult to diagnose based only on clinical findings, and it can be confused clinically with an epulis, fibroma, gingival tumor, or carcinoma including intraoral BCC [3]. PA is frequently diagnosed after histological examination of specimens from a resected tumor or biopsy. Histologically, a PA consists of proliferating ameloblastic epithelium underlying mucosal epithelium [3]. In our case, we were not able to diagnose PA based only on clinical findings.

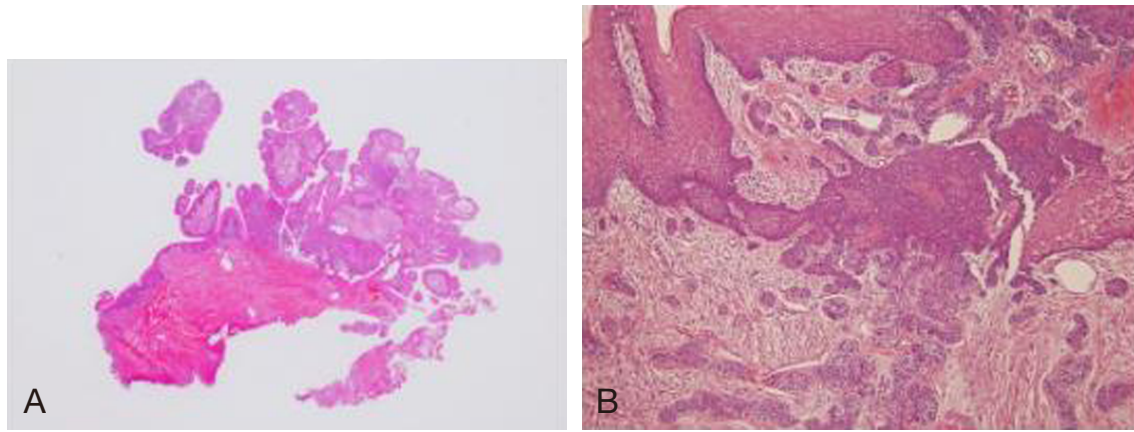


Fig. 3 Photographs showing pathological findings. **A)** A low-power view of the tumor shows papillary proliferation of epithelial tissue (H&E, $\times 4$). **B)** A medium-power view of the tumor shows the bulbous rete ridges of ameloblastoma. The tumor nests are continuous with the surface epithelium (H&E, $\times 100$).

Table I Primary antibodies used immunohistochemical staining in this study

Antigen (Antibody)	Clonality	dilution	source
Cytokeratin7 (OV-TL)	mouse IgG	1:1000	Dako
Cytokeratin14 (LL002)	mouse IgG	1:40	Novocastra
Cytokeratin19 (Ks19.1)	mouse IgG	1:50	Progen
Cytokeratin 8 (CAM5.2)	mouse IgG	1:10	Becton Dickin
Cytokeratin1-8,10,14-16,19 (AE1/AE3)	mouse IgG	1:1	Roche
Cytokeratin 1,5,10,14 (34 β E12)	mouse IgG	1:1000	Dako
Epithelial membrane antigen (E29)	mouse IgG	1:200	Dako
p53 (DO7)	mouse IgG	1:2000	Diyatoron
p63(4A4)	mouse IgG	1:10	Dako
Ber-EP4	mouse IgG	1:200	Dako
Ki-67 (MIB-1)	mouse IgG	1:1000	Dako

Radiographically, a PA is superficial to cortical bone with no sign of bone involvement. Therefore, a PA is considered less aggressive than an IA, which does invade bone [2]. A few cases of PA have shown some bone involvement, which has been referred to as cupping or saucerization due to the depression resulting from the pressure of the tumor on bone [6, 10]. In our case, no bone resorption was seen.

The treatment of PA consists of surgical excision down through the periosteum. The recurrence rate of PA is much lower (19%) than that of IA (33%) [11]. Recurrence is rare even with conservative resection; however, some cases of malignant transformation of PA have been reported. Moreover, PA and intraoral BCC may be the same entity [8] Therefore, long-term follow-up is essential [3, 12].

We performed immunohistochemical studies to further evaluate the two issues regarding PA. CKs consist of 20 polypeptides, and are divided into acidic (type I) and basic (type II) subfamilies [13, 14]. The expression pattern varies with the epithelial cell type [14, 15]. In previous studies, CK14 and CK19 are positive in all types of ameloblastoma cell; CK19 is a marker of odontogenic origin and CK14 is the major cytoskeletal polypeptide in ameloblastomas [14, 16]. In our case, acidic CKs including CK14 and CK19 were expressed moderately or strongly, whereas basic CKs including CK7 and CK8 (CAM5.2) were not consistently

expressed. AE/AE3 and 34 β E12, which include two types, were expressed moderately in all cases. These results suggest that the expression of CKs is associated with classification using the isoelectric point. EMA, which labels normal and neoplastic epithelium, were not expressed in all cases, as in previous reports [3, 14, 17]. p53 protein is a tumor suppressor and the p53 antibody used in our study reacts with wild and mutant types of p53 protein. The p63 protein, which is a p53 homolog, plays an essential role in epithelial development [18]. In our case, all samples were negative for p53, whereas p63 protein was expressed moderately in all samples. The expression of p53 has been reported in ameloblastoma [19], although p53 protein was not enhanced in some previous studies [20]. The opposite result may be the cause of the diversity in ameloblastoma. Regarding the difference between PA and BCC, previous studies have shown that PA and IA are positive for CK19 and negative for Ber-EP4, which labels most epithelial cells and is expressed in neoplastic basal cells, whereas the opposite is true for BCC [21, 22]. In this study, all cases were negative for Ber-EP4, as in previous reports. Regarding proliferative activity, the mean Ki-67 LI of the IAs (1.37%) and PA (2.22%) indicated their low growth potential, supporting the benign biological behavior of these lesions.

Immunohistochemical reactivity may be associated with oncogenesis and cytodifferentiation of the tumor.

In this study, the immunohistochemical reactivity of the PA was similar to that of IAs. Although continuity between the tumor nests and oral epithelium was seen in our case, these findings suggest that PA and IA are tumors with a common origin, and that PA is derived from odontogenic epithelial remnants, rather than from basal cells of the oral epithelium.

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Table II Immunohistochemical results

Marker substance	Age/Sex	Histologic feature	CK7	CK14	CK19	CAM5.2	AE1/AE3	34 β E12	EMA	p53	p63	Ber-EP4	Ki-67
PA	46/M	Follicular	-	++	+	-	+	+	-	-	+	-	LI: 2.22%
IA1	17/F	Plexiform	-	++	++	-	+	++	-	-	+	-	LI: 3.65%
IA2	44/F	Follicular	-	++	++	-	+	+	-	-	+	-	LI: 0.49%
IA3	44/M	Plexiform	-	+	++	-	+	+	-	-	+	-	LI: 0.24%
IA4	53/M	Follicular	-	++	++	-	+	+	-	-	+	-	LI: 1.1%

PA: peripheral ameloblastoma, IA: intraosseous ameloblastoma

++: strong-positive reaction, +: moderate-positive reaction, ±: weak-positive reaction, -: non-reaction

LI: labeling index

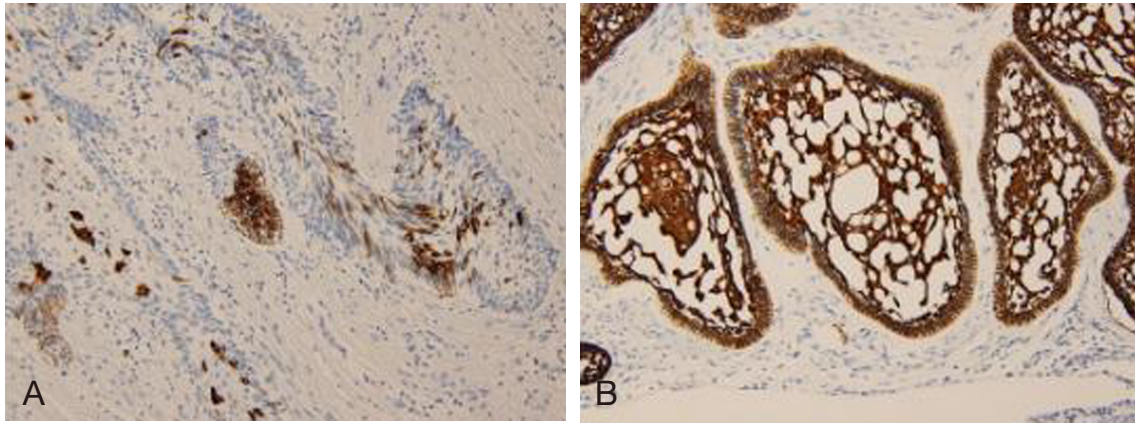


Fig. 4 The expression of CK19: **A)** moderate immunoreactivity for CK19 in PA; and **B)** strong immunoreactivity for CK19 in IA (case 2) ($\times 200$).

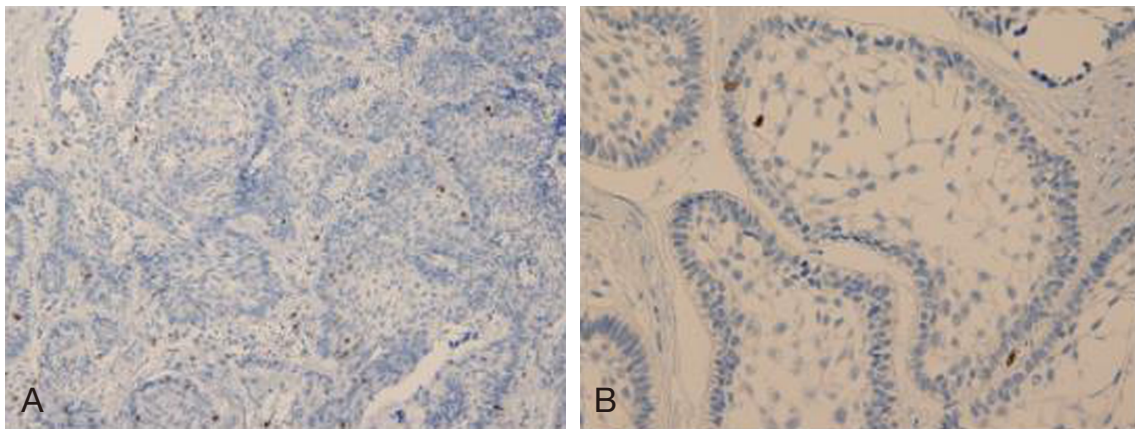


Fig. 5 The Ki-67 labeling index is **A)** 2.22% in PA; and **B)** 0.49% in IA (case 2) ($\times 200$).

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