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Odontogenic Gingival Epithelial Hamartoma; with Reference to the Expression of Ameloblastin Gene by *in situ* Hybridization and Immunohistochemistry

Na Rae Kim · Yeon-Lim Suh[1]
Je G. Chi[2] · Young Joon Lee[3]
Suk Keun Lee[3] · Jae Il Lee[4]
Chang-Yun Lim[4] · Ji-Young Park[5]

Departments of Pathology and [5]Pharmacology, Gachon Medical School Gil Medical Center, Incheon; [1]Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul; [2]Department of Pathology, Ilsanpaik Hospital, College of Medicine Inje University, Koyang; [3]Department of Oral Pathology, College of Dentistry, Kangnung National University, Kangnung; [4]Department of Oral Pathology, College of Dentistry, Seoul National University, Seoul, Korea

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Corresponding Author
Yeon-Lim Suh, M.D.
Department of Pathology, Samsung Medical Center
50 Ilwon-dong, Kangnam-gu, Seoul 135-710, Korea
Tel: 02-3410-2761
Fax: 02-3410-0025
E-mail: ylsuh@smc.samsung.co.kr

Odontogenic gingival epithelial hamartoma (OGEH) is an extremely rare lesion characterized by an abnormal proliferation of odontogenic epithelium. This lesion is thought to arise from the rest of the dental lamina lying dormant in the gingival tissue after odontogenesis. Distinguishing OGEH from the granular cell variant of ameloblastoma and central odontogenic fibroma is important. To date, only eleven cases have been reported, and its pathogenesis remains unclear. We report here on a case of OGEH, where the epithelial strands in the lesion were conspicuously positive for the antisera of cytokeratin 19 and ameloblastin. Tumor cells intensely expressed ameloblastin mRNA by *in situ* hybridization. To the best of our knowledge, this is the first case of OGEH to which ameloblastin immunohistochemical stain and *in situ* hybridization were applied. Although our study is limited to a single case, the coexpression of cytokeratin 19 and ameloblastin might indicate the origin and specific cytodifferentiation of OGEH is quite different and unique, when contrasted to other odontogenic tumors.

Key Words : Odontogenic Gingival Epithelial Hamartoma–Ameloblastin–Cytokeratin 19

Odontogenic gingival epithelial hamartoma (OGEH) is a rare benign lesion and eleven cases of OGEH have been reported.[1-6] However, its cellular origin remains uncertain and there are no definitive immunohistochemical application. Different types of cytokeratin are expressed at different stages of odontogenesis,[7] and all epithelial elements including fetal oral epithelium, tooth germ, and a variety of odontogenic tumors, express cytokeratin 19. Ameloblastin is a tooth specific gene and cytokeratin 19 is regarded as specific to odontogenic epithelium.[8,9] Here, we describe an additional case of OGEH and we performed immunohistochemistry for cytokeratin and ameloblastin as well as *in situ* hybridization for the ameloblastin gene in order to elucidate the cellular origin for this lesion.

CASE REPORT

A 51-year-old woman presented with a gingival swelling in the left molar area of the mandible for several years. A panoramic radiographic view revealed a well-defined unilocular cystic lesion at the alveolar socket in the left mandibular molar area (Fig. 1). Intraoperatively, the lesion was well circumscribed and located

within the gingiva at the left third molar area, in which no tooth was found. Excision and curettage of the underlying alveolar ridge of the mandible were done.

The excised specimen was fixed with 10% neutral formalin. Paraffin sections prepared were stained with hematoxylin and eosin. Upon gross examination, the excised mass showed an oval pinkish-gray glistening firm nodule with no attached bone, and it measured 8.0×6.0×5.0 mm. The external surface was smooth and the cut surface was soft, homogeneous tan. Histologically, the specimen showed nests, bands, cords and a trabecular pattern of odontogenic epithelial cells with intervening hyalinized myxoid stroma (Fig. 2A). The neoplastic cells were arranged in cords of two to three-cell layers and there were peripheral cuboidal to columnar cells and central small cells resembling basal layer cells (Fig. 2B). The epithelial cells had large vesicular nuclei and there was scanty to moderate amounts of granular cytoplasm (Fig. 2C, left). Squamous metaplastic cells with intercellular bridges were also occasionally found. Some cells showed a hydropic degeneration that formed a pseudoglandular pattern. There were no destroyed bony fragments but there was some tiny calcification (Fig. 2C, right). At the periphery of the lesion, it was well delineated from its fibrous pseudocapsule. PAS-positive granules were demonstrated in the cytoplasm of the epithelial cells. Immunohistochemistry using antibodies were performed by the avidin-biotin complex methods. The antibodies we used were as follows: pancytokeratin (AE1/AE3, DAKO, Glostrup, Denmark, 1:80 dilution), cytokeratin 19 (DAKO, 1:80), cytokeratin 8 (DAKO, 1:80), smooth muscle actin (HHF35, DAKO, 1:100), S-100 protein (DAKO, 1:1,200), glial fibrillary acidic protein (Biogenesis, Newfield, UK, 1:3,000) and fibronectin (DAKO, 1:100). Immunohistochemistry for ameloblastin using an avidin-biotin-peroxidase complex method was done as follows. A synthetic peptide, YEYSLPVHPPPLPSQ, encoding for the human exon 3a of the ameloblastin (370-414, NM_016519) was also used to produce a polyclonal antibody from a rabbit. For *in situ* hybridization of the ameloblastin gene, the full length base pair of ameloblastin Y224 was cloned into the pBluscript SK(-) vector. The plasmids were linealized by EcoRI or XhoI for antisense or sense probe production, respectively. The single-stranded antisense and sense RNA probes were labeled with digoxigenin-UTP, and then they were generated by using T3 and T7 RNA polymerases (Boehringer Mannheim, Indianapolis, IN). The detection of *in situ* hybridization was carried out using the Genius Detection system (Boehringer Mannheim, Indianapolis, IN), in which the specific transcripts were detected with an anti-digoxigenin antibody conjugated to alkaline phosphatase. Immunohistochemically, the epithelial cells were strongly reactive for pancytokeratin, ameloblastin and they were focally reactive for cytokeratin 19 (Fig. 3A) but they were negative for cytokeratin 8, smooth muscle actin, S-100 protein, or glial fibrillary acidic protein. Myxoid stroma was neg-

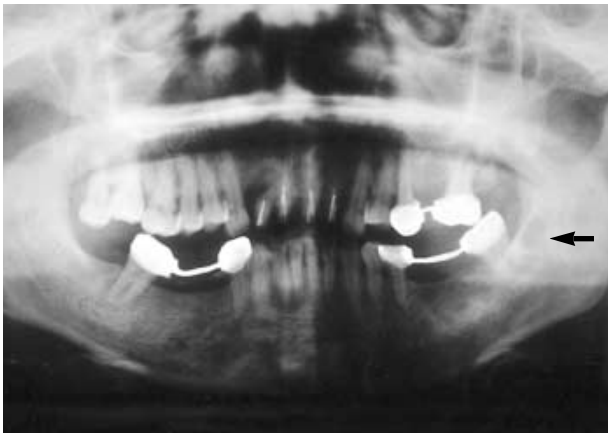

Fig. 1. A panoramic radiograph shows a radiolucent area (arrow) in the left molar region of the mandible.

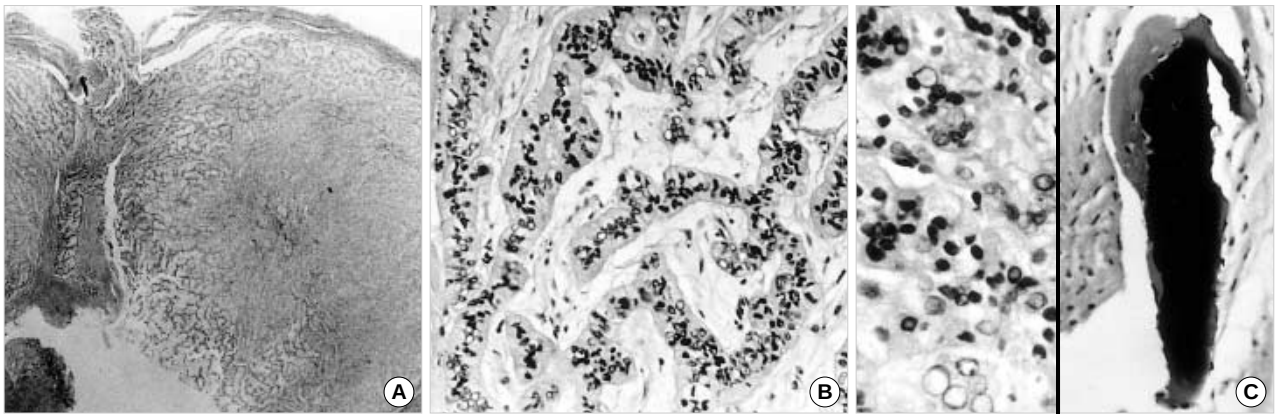

Fig. 2. (A, B) A well circumscribed mass shows proliferation of epithelial cells forming cords or plexiform arrangements. (C) The tumor cells have large vesicular nuclei and granular cytoplasm with myxoid stroma (left), and a focus of calcification within the mass is also found (right).

ative for fibronectin or PAS-stainability. *In situ* hybridization for ameloblastin revealed that ameloblastin was expressed in the epithelial glands of the lesion (Fig. 3B). The diagnosis we arrived at was odontogenic gingival epithelial hamartoma (OGEH), and this lesion was confined within the alveolar socket of the mandible, i.e. the intraosseous variant. During the thirty months of follow-up, she was in good health without recurrence.

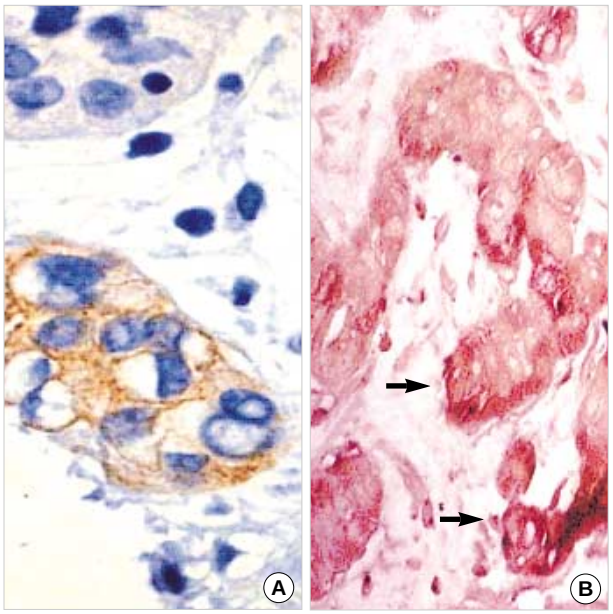

Fig. 3. (A) Immunoreactivity for cytokeratin 19 in the epithelial cells (cytokeratin 19 immunostain). (B) *in situ* hybridization for ameloblastin showing strong positivity in the nuclei of epithelial glands (arrows).

DISCUSSION

The granular epithelial cells and myxoid stroma of the present case initially gave the impression of salivary gland tumor. However, the histologic findings such as nuclear polarization of granular epithelial cell nests with myxoid stroma, imply that this lesion had an odontogenic origin. Granular cells are usually found in OGEH, granular cell ameloblastoma and plexiform granular cell odontogenic tumor.[10] Other differential diagnoses could include central odontogenic fibroma, granular cell variant (WHO type), calcifying epithelial odontogenic tumor and choristomatous salivary glands of the gingiva. Granular cell ameloblastoma can be differentiated from OGEH by the absence of stellate reticulum as well as pseudoencapsulation. A calcifying epithelial odontogenic tumor shows the proliferation of large polygonal odontogenic epithelial cells containing psammoma bodies in the amyloid like stroma.[11] A pathological diagnosis of choristomatous salivary glands of the gingiva was excluded not only by the histology

Table 1. Clinicopathologic summary of twelve cases of odontogenic gingival epithelial hamartoma

Authors (yr)	Sex/Age (yr)	Site	Radiology	Size (mm)	Continuity with oral epithelium	Calcification	Procedure	Clinical outcome
Baden, *et al.* (1968)[1]	F/59	Gingiva, mandible	No bone resorption	Pea size	+	+	Mass excision & adjacent bone excision	NER 2 yr F/U
	M/55	Gingiva, maxilla	No bone resorption	2.5	+	–	Wide excision	NER 2 yr F/U
	F/65	Gingiva, mandible	No bone resorption	0.7	+	–	Mass excision	NER 2 yr F/U
Baden, *et al.* (1973)[2]	F/60	Gingiva, mandible	Cupping of interdental alveolar bone	9×7	–	–	Wide excision	NER 16 mo F/U
Gardner (1973)[3]	F/50	Gingiva, mandible	No bone resorption	3×2	–	–	Hemimandibulectomy*	NER 23 yr F/U
Sciubba, *et al.* (1978)[4]	F/40	Gingiva, mandible	NR	NR	–	–	Wide excision	NR
Moskow, *et al.* (1989)[5]	NR	NR	NR	NR	–	–	NR	NR
	NR	NR	NR	NR	–	–	NR	NR
	NR	NR	NR	NR	–	+	NR	NR
	NR	NR	NR	NR	–	+	NR	NR
Kitano, *et al.* (1991)[6]	F/63	Gingiva, mandible	No bone resorption	9×8	–	+ (tiny calcification)	Mass excision	NER 2 yr F/U
Kim, *et al.* (2004)	F/51	Gingiva, alveolar socket of mandible	Well-defined radiolucency	8×6	–	+	Mass excision	NER 30 mo F/U

*: This case was initially misdiagnosed as ameloblastoma. yr, year; mo, months; NER, no evidence of recurrence; F/U, follow up; NR, not recorded.

studies, but also by the immunohistochemical results. Central odontogenic fibroma of the granular cell variant (WHO type) differs from OGEH in that the epithelium of the former plays a minor role compared to the fibrous component.[12]

The entity of OGEH has provoked some controversy,[13,14] and it's possible that a small number of the cases reported in the literature might the result of an improperly defined classification of odontogenic tumor or the variably reported names that were used; there improper classification would include central odontogenic fibroma of WHO type, or ameloblastoma.[1,14] There are only eleven total cases of OGEH that have been reported in the literature.[1-6] We summarized twelve cases (including the present case) in Table 1. The eight cases in which precise data were available showed a female preponderance (7 females and 1 male). All OGEH presented as a small nodular mass in late adulthood, and all the lesions have occurred at the premolar area except for the present case. Radiologically, all the cases except for three including the present case, were located in the gingiva without the resultant destruction of the adjacent jaw bone. A well-circumscribed cystic radiological finding is a distinguishing point from intraosseous ameloblastomas, as the latter lesion shows locally invasive destructive growth. None of the lesions showed recurrence. Upon examination by light microscope, OGEH's showed similar features including the well-defined circumscription by a fibrous pseudocapsule, pseudoglandular arrangements, hydropic degeneration and squamous metaplasia in the myxoid stroma. Three cases had continuity between the lesions and the overlying rete ridges of the oral epithelium, while in the remaining seven no such connections could be found. Deposition of a dentinoid or predentin-like material or tiny calcification in the stromal layer was also found.[1,5] In reviewing the cases of OGEH reported by Moskow and Baden,[5] we found that the illustrations on epithelial nests and concentric calcification were atypical displays for OGEH. Therefore, Moskow and Baden's four cases are not formally accepted as typical examples of the lesion in question, and this is due to a complete lack of data on gender, age and clinicoradiologic features. However, Moskow and Baden's paper is interesting from another point of view, because these authors have described two types of odontogenic epithelial hamartoma, i.e. a peripheral or gingival type which was called OGEH, and an intraosseous or central variant. The cases reported prior to their report were all located in the gingiva, but Moskow and Baden suggested that such lesions are likely to occur wherever epithelial residues from the dental lamina persist. In addition, they found hamartoma in four out of 325 jaw specimens, accounting for an incidence of 1.2%. Considering the somewhat common clinical incidence, the real incidence including tiny foci of asymptomatic cases, may be much higher than the cases actually diagnosed. Therefore, their observations suggest that OGEH's can occur wherever epithelial residues from the dental lamina persist and a much higher incidence of asymptomatic small hamartomas may exist and remain dormant throughout one's life than has been reported.

There are two suggested hypotheses for the pathogenesis of this benign lesion. One is the origin from a dental lamina nest, the so-called "glands of Serres" after odontogenesis is started. Because odontogenesis is a highly complex process of interplay between epithelial and mesenchymal tissues (ectomesenchyme),[15] errors are commonly encountered. Abnormally displaced, supernumerary tooth germs were pinched off during development, and these isolated foci failed to differentiate into a fully formed tooth, but rather they proceed to develop into OGEH. Gingival nests containing glands of Serres are encountered throughout the entire dentition, and they can regress or stay dormant as vestigial structures in children. The other theory is that OGEH arises from the proliferation of a basal layer of the surface epithelium after various provoking stimuli, such as inflammation, trauma and so on. The fact that the reported cases occurred in older adults suffering from chronic irritation on gingiva supports the latter theory. Sciubba *et al*.[4] even suggested that this lesion is in a transitional status between a true odontogenic neoplasm and a developmental anomaly. In our opinion, the former theory is more plausible, i.e. OGEH originated from the pinched-off remnant of dental lamina rather than the basal layer of gingival epithelium. The supporting facts of the former are as follows; first, the site of the lesion generally shows the frequent absence of connection between the lesion and the overlying epithelium. Second, OGEH can occur in the intraosseous portion of the jawbone, where gingival epithelium is not a normal component, although Moskow and Baden's cases are not typical examples of OGEH. Third, it frequently occurs in the third molar area, where remnants of the dental lamina may persist in adults,[16] as well as in the premolar gingiva. Fourth, the immunohistochemical results in the present case support the dental lamina origin. The expression of cytokeratin 19 and ameloblastin may reflect that tumor cells retain an immature phenotype of odontogenic differentiation.[8,9,17] The question about the cell of origin should not always be seen only from the viewpoint of cytokeratin immunohistochemistry, a specific type of cytokeratin is expressed in different stages of odontogenesis; all epithelial elements including fetal oral epithelium, tooth germ, as well as a variety of odontogenic tumors, express cytokeratin 19.[8] Compared to the cytokeratin

19, ameloblastin is more specific to enamel epithelium of the tooth's organ.[8,9] The expression of both cytokeratin 19 and ameloblastin in this study may reflect that tumor cells retain immature phenotype of odontogenic differentiation. In particular, the present study used a novel antibody against the epitope of ameloblastin exon 9, which is specific to the precursor ameloblastin in the cytoplasm of enamel epithelium, this is, however, excluding the degraded products of ameloblastin in the extracellular tissue. In our opinion, these results might support that OGEH is a benign tumor composed of relatively well-differentiated odontogenic epithelium that was derived from the pinched-off remnant of dental lamina.

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