Adenoid Ameloblastoma Shares Clinicopathologic, Immunohistochemical, and Molecular Features With Dentinogenic Ghost Cell Tumor

A Comparative Analysis

Jiang Xue, MD, PhD,†‡ Wenyi Zhang, MD,†§ Jianyun Zhang, MD, PhD,*†‡ Jiaying Bai, BDS,*†‡ Aobo Zhang, MD, PhD,*†‡ Xiaowen Guo, DDS,*†‡ Lisha Sun, PhD,‡†‖ and Tiejun Li, MD, PhD*†‡*

Abstract: The updated classification of odontogenic tumors by the World Health Organization (WHO) has included adenoid ameloblastoma (AA) as a distinct entity. However, distinguishing between AA and dentinogenic ghost cell tumor (DGCT) can still be challenging due to their significant morphologic similarities. In this study, we aimed to compare the clinicopathologic, immunohistochemical, and molecular characteristics of AA and DGCT to aid in their differentiation and to shed light on their pathologic mechanisms. Thirteen cases of AA and 14 cases of DGCT (15 samples) were analyzed, along with 11 cases of adenomatoid odontogenic tumor (AOT) and 18 cases of conventional ameloblastoma (AM) for comparative purposes. The study found that AA and DGCT shared a similar long-term prognosis. Immunohistochemically, all cytokeratins detected, except CK8/18, were not statistically significant in differentiating AA and DGCT, while there was a statistically significant difference in the immunophenotype of CK7 and CK10/13 between AA and AM. Nuclear β-catenin accumulation were detected in all cases of AA and DGCT, while AOTs and AMs exhibited cytoplasmic β-catenin. Molecularly, *CTNNB1* hotspot mutations were found in only 1 case of AA (1/13), but not found in the other 3 types of tumors. *BRAF p.V600E* mutation was positive in 2/13 (15%) AA, 1/15 (7%) DGCT, and 2/11 (18%) AOT cases. In comparison, conventional AM was positive for *BRAF p.V600E* mutation in 94% (17/18) of cases, while *KRAS* mutations were detected in 63% (7/11) of AOT cases. The study suggests that the so-called AA is a rare benign tumor that exhibits clinical, immunohistochemical, and molecular features similar to DGCTs. Based on these findings, AA should not be categorized as a standalone entity solely based on the presence of whorls/morules and cribriform/duct-like structures. Further studies are needed to investigate the pathologic mechanisms of these tumors and to identify potential therapeutic targets.

Key words: adenoid ameloblastoma, dentinogenic ghost cell tumor, immunohistochemistry, β-catenin, WNT pathway

(*Am J Surg Pathol* 2023;47:1274–1284)

From the Departments of *Oral Pathology; §Oral and Maxillofacial Radiology; ‖Central Laboratory, Peking University School and Hospital of Stomatology; †National Center of Stomatology & National Clinical Research Center for Oral Diseases & National Engineering Research Center of Oral Biomaterials and Digital Medical Devices; and ‡Research Unit of Precision Pathologic Diagnosis in Tumors of the Oral and Maxillofacial Regions, Chinese Academy of Medical Sciences (2019RU034), Beijing, P. R. China.

T.L. and L.S. developed the study concept and design. J.X. contributed to the development of methodology and writing, review and revision of the paper. J.X., J.Z., and W.Z. provided acquisition, analysis, and interpretation of data. T.L., L.S., J.B., A.Z., and X.G. provided technical and material support.

Conflicts of Interest and Source of Funding: Supported by Research Unit of Precision Pathologic Diagnosis in Tumors of the Oral and Maxillofacial Regions, Chinese Academy of Medical Sciences (2019RU034). The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Correspondence: Lisha Sun, PhD, Central Laboratory, Peking University School and Hospital of Stomatology, Beijing 100081, P. R. China (e-mail: lisa_sun@bjmu.edu.cn); Tiejun Li, MD, PhD, Department of Oral Pathology, Peking University School and Hospital of Stomatology, Beijing 100081, P. R. China (e-mail: litiejun22@vip.sina.com).

Supplemental Digital Content is available for this article. Direct URL citations are provided in the HTML and PDF versions of this article on the journal's website, www.ajsp.com.

Adenoid ameloblastoma (AA) is an epithelial odontogenic tumor that has been recently classified by the World Health Organization (WHO). Diagnostic features for AA include conventional ameloblastoma (AM)-like epithelium, duct-like structures, cribriform architecture, and cellular condensations such as morules or whorls. Dentinoid, clear cells, and focal ghost cell keratinization are desirable diagnostic criteria for AA.[1] Recent research findings indicate the presence of β-catenin mutations in AA.[2] Dentinogenic ghost cell tumor (DGCT), another locally infiltrative odontogenic tumor, shares clinical similarities and histologic features with AA, such as ameloblastic epithelium and the presence of ghost cells and dentinoid.[1,3,4] Previously, DGCT was differentiated from AA based on the presence (AA) or absence (DGCT) of cribriform or duct-like structures. However, a recent meta-analysis has shown the presence of cribriform or duct-like structures in several DGCT cases.[5] Furthermore, the existence of "mixed" tumors that contain a combination of

AA and DGCT histopathologic patterns has been reported,[4] leading to consideration for their reclassification. Nuclear β-catenin accumulation has also been noted in DGCT cases,[5,6] which is a commonality in clinical, histologic, and molecular aspects further undermine the rationale for distinguishing the 2 entities.

Conventional AM and adenomatoid odontogenic tumor (AOT) are other histopathologic differential diagnoses for AA, with AM resembling AA's histologic epithelial features and AOT resembling its adenoid elements. However, AA has been found to have *CTNNB1* hotspot mutations and nuclear β-catenin accumulation,[2] which are not present in AM or AOT.[7] The inclusion of odontoameloblastoma, which presents mixed epithelial and mesenchymal odontogenic tumor, in previous WHO classifications, has also created obstacles in distinguishing between the 2.[8–13] The distinction with AOT is not very problematic, since AOT lacks an AM-like component but displays rosette-like structures.[14] Conversely, AA presents an AM-like component but does not exhibit rosette-like structures. In addition, AA has been found to harbor neither *BRAF* nor *KRAS* mutations,[7] which are hallmarks of AM and AOT, respectively.[15,16] Instead, AA has *CTNNB1* hotspot mutations and nuclear β-catenin accumulation,[2] which are also present in DGCT.[6] However, the evidence for these conclusions is insufficient due to the small number of reported cases. In this study, we conducted quantitative histologic and immunohistochemical analysis, molecular profiling using Sanger sequencing, and statistical analysis of accumulated clinical data to compare characteristics between AA and DGCT. We also retrieved cases of AOT and AMs for comparative purposes to clarify the features of these histologic mimics. The findings of this study can contribute to a better understanding and distinguishing of AA from other similar tumors, ultimately improving diagnosis and treatment outcomes.

MATERIALS AND METHODS

Patients and Tissue Samples

A retrospective search was conducted to identify cases diagnosed as AA or DGCT between 2009 and 2022 in the Department of Oral Pathology, Peking University Hospital of Stomatology. The archived hematoxylin-and-eosin-stained slides of the identified cases were reviewed by 3 oral pathologists based on the WHO Classification of Head and Neck Tumors. Thirteen AA cases and 15 samples of DGCT from 14 cases were included in this study. Two samples were derived from a single DGCT patient who developed 2 recurrent tumors with a 6-month interval after curettage of the primary tumor. For comparative purposes, 11 cases of AOT and 18 cases of AB were also retrieved. The analysis was performed on formalin-fixed paraffin-embedded (FFPE) tissues. Clinical data were collected from medical and pathology records. Follow-up information was obtained by telephone follow-up or by reviewing the medical records of the patients.

Histologic Analysis

According to the new WHO classification,[1,3] there are considerable overlapping histologic features between AA and DGCT, such as ameloblastic epithelium and the presence of ghost cells and dentinoid. The primary distinction between these 2 tumors is based on the presence (AA) or absence (DGCT) of cribriform or duct-like structures. However, duct-like or cribriform structures have been described in several DGCT cases too,[5] warranting reevaluation of the following overlapping histologic features[1]: duct-like or cribriform structures,[2] ghost cells,[3] dentinoid,[4] morules. Duct-like or cribriform structures were defined as cuboidal to columnar basal ameloblast-like cells arranged in a cribriform or duct-like pattern, some of which contain mucin. The proportion of the area showing these structures to the total tumor area was calculated for each case. "Ghost cells" were defined as cells with eosinophilic cytoplasm containing a central hole suggestive of loss of the nucleus. The proportion of ghost cells to the total tumor cells was calculated for each case. "Dentinoid" was defined as eosinophilic dentin or osteodentin-like material in proximity to epithelial cells. Its relative amount was categorized into none, rare, focal, and diffuse.

Immunohistochemistry and Immunostaining evaluation

Four-millimeter-thick serial sections were cut for all cases except for 2 (case #10 in AOT and case #23a in DGCT) due to the limited amount of tissue. Immunohistochemical staining was performed using the BOND-MAX autostainer (Leica Biosystems) following the manufacturer's instructions. The primary monoclonal antibodies included CK5/6, CK7, CK8/18, CK10/13, CK14, CK18, CK19, CK20, β-catenin, and Ki-67. Details of the primary antibodies used are listed in Supplementary Table S1 (Supplemental Digital Content 1, http://links.lww.com/PAS/B604). Two independent blinded observers evaluated the staining intensity and ratio of positive cells semiquantitatively. Reactivity was determined based on the percentage of positive cells: 0 (up to 1%), 1 (2% to 25%), 2 (26% to 50%), 3 (51% to 75%), and 4 (over 75%). Intensity was graded as follows: 0 (negative), 1 (weakly positive), 2 (moderately positive), and 3 (strongly positive). The final score was obtained by multiplying the reactivity and intensity scores and classified as negative (0 to 1, −), weak (2 to 4, +), moderate (5 to 8, ++), and strong (9 to 12, +++).[17] According to the immunostaining score, negative and weak staining were considered as low expression, while moderate and strong staining were defined as high expression (overexpression).

Mutation Analysis after DNA Extraction

Genomic DNA was isolated from FFPE samples using the QIAamp DNA FFPE Tissue Kit (Qiagen), following the manufacturer's instructions. The DNA concentration and quality were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Direct DNA sequencing of the polymerase chain

reaction (PCR)-amplified target sequence of *CTNNB1*, *BRAF*, and *KRAS* was performed on all samples. PCR was carried out using Q5 High-Fidelity 2× Master Mix (New England Biolabs Inc.) according to the manufacturer's instructions. Primers were designed to amplify a 239 bp fragment of *CTNNB1*, including the Asp32, Ser33, Gly34, Ser37, Thr41, and Ser45 codons,[6] a 163 bp sequence of *KRAS*, including the Gly12 codon; and a 173 bp sequence of *BRAF*, including the Val600 codon. The following primer sequences were used: for *CTNNB1*, forward, 5′-ATGGCTACTC AAGCTGATTTGATGGAG-3′ and reverse, 5′-GCTA CTTGTTCTTGAGTGAAGGACTGAG-3′; for *KRAS*, forward, 5′-GGCCTGCTGAAAATGACTGAA-3′ and reverse, 5′-GGTCCTGCACCAGTAATATGC-3′; and for *BRAF*, forward, 5′-TGCTTGCTCTGATAGGAAAAT G-3′ and reverse, 5′-CCACAAAATGGATCCAGACA-3′. PCR products were analyzed by electrophoresis and purified using a DNA purification system (Promega). Bidirectional DNA sequencing was performed and the samples were run on an ABI3730XL DNA Analyzer (Applied Biosystems).

Statistical Analysis

All analyses were conducted using SPSS 16.0. The Fisher exact and unpaired *t* tests were performed to compare variables between groups for immunostaining data. Recurrence-free survival time was defined as the duration from the date of surgery to either recurrence or follow-up cutoff date (March 31, 2023). Probability of recurrence rates were estimated using the Kaplan-Meier method and compared using log-rank tests. All tests were 2-sided and a *P*-value ≤ 0.05 was considered statistically significant.

RESULTS

Table 1 summarizes the clinical, histologic, and molecular features of the AA and DGCT cases included in this study. Tables 2 and 3 list the clinical and molecular features of 11 cases of AOT and 18 cases of AM, respectively, retrieved for comparative purposes.

Clinical Features

Table 1 summarizes the clinical data from the 13 AAs included in this study. The patients' age ranged from 17 to 51 years (mean, 34 y), with a male-to-female ratio of 5.5:1 (11 males, 2 females). The mandible was the most frequently affected site (69%), and 4 occurred in the maxilla. The duration of symptoms ranged from 2 to 168 months, with a median of 73 months. About half of the patients (6 of 13) underwent segmental or total maxillectomy or mandibulectomy and 7 patients underwent curettage. After experiencing multiple recurrences, a patient underwent surgery followed by radiotherapy. Local recurrence after initial treatment occurred in 10 cases, including 7 after curettage and 3 after the maxillectomy or segmental mandibulectomy, with the number of recurrences ranging from 1 to 6. Follow-up data were available for all patients ranging from 8 to 192 months, with a median of 94 months.

Table 1 also summarizes the clinical data from the 14 DGCT cases included in this study. The patients included 9 males and 5 females, with an age range of 9 to 61 years (mean, 36 y). The tumors were mainly located in the maxilla (64%) with 5 cases occurring in the mandible. The duration of symptoms ranged from 5 months to 288 months, with a median of 89 months. Six patients underwent curettage and 8 of 14 were treated with total maxillectomy or mandibulectomy. Due to multiple recurrences, 2 of them received radiotherapy after surgery. Six patients are alive with no evidence of disease after initial curettage. Local recurrence after initial treatment occurred in 7 cases, including 4 after curettage and 3 after the maxillectomy, with the number of recurrences ranging from 1 to 5. Follow-up data were available for 13 of 14 patients ranging from 7 to 296 months, with a median of 85 months. The cumulative probabilities of recurrence between AA and DGCT were not statistically significant ($P = 0.4940$), either in curettage groups ($P = 0.2810$) nor in osteotomy groups ($P = 0.7679$) (Fig. 1). Table 2 summarizes the clinical data from the 11 cases of AOT. The cases included 7 males and 4 females, with an age range of 11 to 30 years (mean, 17 y). Six cases affected the mandible, and 5 occurred in the maxilla. The duration of symptoms ranged from 0.5 to 60 months, with a median of 9.6 months. All patients underwent curettage and 8 patients are alive with no evidence of disease after initial curettage. Follow-up data were available for 8 patients ranging from 4 to 138 months with a median of 40 months. Table 3 presents a comprehensive summary of clinical data obtained from the analysis of 18 cases of AM. The AM cases included 10 males and 8 females, with an age range of 17 to 77 years (mean, 36 y). The mandible was the most commonly affected site (94%), with 4 cases occurring in the maxilla. The duration of symptoms ranged from 1 to 552 months, with a median of 76 months. Ten patients received segmental or total maxillectomy or mandibulectomy, while 8 patients underwent curettage. As of the time of the analysis, 12 patients were alive and showed no signs of the disease. Follow-up data were available for 15 patients, ranging from 5 to 552 months, with a median of 60 months. Local recurrence occurred in 5 cases after initial treatment, with the number of recurrences ranging from 1 to 4.

Pathologic Findings

All AAs and DGCTs met the essential diagnostic features outlined in the latest WHO classification. The so-called AA cases exhibited a duct-like or cribriform phenotype with cellular condensations or morules, whereas DGCT cases were characterized by conventional AM-like epithelium with varying proportion of ghost cells and dentinoid. Representative histologic images of each type are presented in Figure 2. All the so-called AAs demonstrated the duct-like or cribriform structures with a proportion from 10% to 100% (Figs. 2E, F). Notably, 5 cases in DGCT showed varying proportions of duct-like or cribriform structures ranging from 5% to 20% (Figs. 2A, B). Except for 4 AA cases, all cases studied displayed varying amounts of ghost cells (Figs. 2D, H). And except for 1 AA case,

TABLE 1. Summary of the Clinical, Histologic, and Molecular Features of So-called AA and DGCT Included in This Study

	Clinical						Histologic				Immunohistochemical and mutation assessment			
Case no.	Age	Sex	Site	Duration (mo)	Initial treatment	Status/ mo†	Duct-like/ cribriform structures (%)	GC (%)	DN‡	Cellular condensations§ (morules)	Nuclear β-catenin‖	*CTNNB1*	*BRAF*	*KRAS*
1*	41	M	Left mandible	60	Segmental mandibulectomy	Rec/60; NED/ 120	90	1	++	+	+++	WT	WT	WT
2*	29	M	Right mandible	96	Curettage	Rec/3; Rec/84	70	0	+++	+	+++	WT	WT	WT
3*	29	F	Right maxilla	168	Curettage/RA	Rec/72; Rec/132; Rec/144; Rec/156; Rec/168; Rec/176	90	0	−	+	+++	WT	WT	WT
4*	43	M	Right maxilla	60	P-maxillectomy	Rec/12; NED/49	100	5	++	+	++	GGA > AGA (G34R)	WT	WT
5*	51	M	Left mandible	132	Curettage	Rec/18; Rec/36; NED/84	40	1	++	+	+++	WT	WT	WT
6*	23	F	Left mandible and right mandible (anterior)	36	P-mandibulectomy	NED/84	50	1	++	+	+++	WT	WT	WT
7*	29	M	Right mandible	2	Curettage	Rec/12; NED/60	90	1	++	+	+++	WT	WT	WT
8*	33	M	Left mandible	96	Curettage	Rec/84; NED/ 192	90	1	+	+	+++	WT	GTG > GAG (V600E)	WT
9*	27	M	Right mandible	5	P-mandibulectomy	NED/ 106	90	1	++	+	+++	WT	WT	WT
10*	17	M	Right mandible	24	Curettage	Rec/11; Rec/20; NED/ 123	90	1	+	+	+++	WT	GTG > GAG (V600E)	WT
11*	39	M	Left maxilla	84	P-maxillectomy	Rec/24; NED/ 108	40	1	+	+	+++	WT	WT	WT
12*	35	M	Right mandible	36	Curettage	Rec/24	20	0	++	+	+++	WT	WT	WT
13*	46	M	Right maxilla (invading left maxilla)	144	P-maxillectomy	NED/8	10	0	+++	+	+++	WT	WT	WT
14	31	M	Left maxilla	30	P-maxillectomy	NED/7	20	5	+++	+	+++	WT	WT	WT
15	53	F	Left mandible	216	Curettage	Rec/216; NED/ 296	0	20	+	+	++	WT	GTG > GAG (V600E)	WT
16	36	M	Left maxilla	84	P-maxillectomy	NA	0	10	+	+	+++	WT	WT	WT
17	35	M	Left maxilla	24	Curettage	Rec/19	5	20	+++	+	+++	WT	WT	WT
18	50	M	bilateral mandibular	36	P-mandibulectomy	NED/98	0	5	+	−	+++	WT	WT	WT
19	52	F	Right maxilla	168	P-maxillectomy	NED/ 129	0	10	+	+	+++	WT	WT	WT
20	9	F	Left mandible	6	Curettage	NED/ 127	0	10	+	+	+++	WT	WT	WT
21	32	F	Left maxilla	132	P-maxillectomy	Rec/11; NED/43	0	1	++	+	+++	WT	WT	WT
22	61	M	Right maxilla	12	P-maxillectomy/ RA	Rec/14; Rec/35; Rec/78	5	5	+	−	+++	WT	WT	WT
23a	27	M	Right maxilla	5	Curettage	Rec/5; Rec/10;	0	20	+	−	NA	WT	WT	WT
23b	27	M	Right maxilla	15	—	−	0	20	+	−	+++	WT	WT	WT
24	29	F	Right mandible	144	P-mandibulectomy	NED/21	10	1	++	+	+++	WT	WT	WT
25	56	M	Left maxilla	288	P-maxillectomy/ RA	Rec/12; Rec/60;	0	5	+++	−	+++	WT	WT	WT

TABLE 1. (*continued*)

	Clinical						Histologic				Immunohistochemical and mutation assessment			
Case no.	Age	Sex	Site	Duration (mo)	Initial treatment	Status/ mo†	Duct-like/ cribriform structures (%)	GC (%)	DN‡	Cellular condensations§ (morules)	Nuclear β-catenin‖	*CTNNB1*	*BRAF*	*KRAS*
26	45	M	Right maxilla	84	Curettage	Rec/51; Rec/72; Rec/78; Rec/83; Rec/97; NED/ 128	0	15	++	−	+++	WT	WT	WT
27	18	M	Left mandible	84	Curettage	Rec/12; Rec/24; Rec/84	5	1	++	+	+++	WT	WT	WT

*So-called AA included in the study.
†Follow-up period or time from the initial treatment to recurrence.
‡−, none; +, rare; ++, focal; +++, diffuse.
§+, present; −, none.
‖++, focal; +++, diffuse.
DN indicates dentinoid; F, female; GC, ghost cell; L, left; M, male; Man, mandibular; Max, maxillary; NA, not available; NED, no evidence of disease; P, partial; R, right; RA, radiotherapy; Rec, recurrence; WT, wild-type.

all cases showed varying amounts of dentinoid (Figs. 2C, G). Interestingly, the presence of epithelial pearls or morules, which is one of the essential diagnostic criteria for AA, also appeared in large quantities in DGCT (9/15, 60%). Furthermore, multiple blood-filled cavities and collagen were observed within the DGCT (Fig. 2B), which may degenerate and drop out leaving out a duct-like space.

Immunohistochemical Findings

Immunostaining was performed on a total of 55 samples, comprising of 13 AAs, 14 DGCTs, 10 AOTs, and 18 conventional AMs. CK5/6 overexpression was detected in all cases, while CK20 overexpression was not observed (data not shown). The diagnostic utilities of cytokeratin expressions in differentiating AAs from other mimics were analyzed and presented in Supplementary Table S2

TABLE 2. The Clinical, Immunohistochemical, and Molecular Features of AOT Included in This Study

	Clinical						Immunohistochemical and Mutation Assessment			
Case no.	Age (y)	Sex	Site	Duration (mo)	Initial treatment	Status/ mo*	Nuclear β-catenin	*CTNNB1*	*BRAF*	*KRAS*
1	13	M	Left mandible	2	Curettage	NED/9	—	WT	WT	GGT > CGT (G12R)
2	20	F	Left mandible	4	Curettage	NED/16	—	WT	WT	WT
3	13	M	Right maxilla	16	Curettage	NED/22	—	WT	WT	GGT > GTT (G12V)
4	30	M	Left mandible	0.5	Curettage	NED/30	—	WT	WT	GGT > CGT (G12R)
5	14	M	Right mandible	NA	Curettage	NA	—	WT	WT	GGT > GTT (G12V)
6	13	M	Right maxilla	2	Curettage	NA	—	WT	GTG > GAG (V600E)	GGT > GTT (G12V)
7	26	M	Right maxilla	0.5	Curettage	NED/57	—	WT	WT	GGT > GTT (G12V)
8	16	F	Left mandible	1	Curettage	NED/4	—	WT	WT	WT
9	11	F	Right mandible	1	Curettage	NED/45	—	WT	WT	WT
10	24	F	Left maxilla	60	Curettage	NA	NA	WT	WT	GGT > CGT (G12R)
11	11	M	Left maxilla	9	Curettage	NED/138	—	WT	GTG > GAG (V600E)	WT

*Follow-up period or time from the initial treatment to recurrence.
— indicates negative; F, female; L, left; M, male; Man, mandibular; Max, maxillary; NA, not available; NED, no evidence of disease; R, right; WT, wild-type.

TABLE 3. Summary of the Clinical, Immunohistochemical, and Molecular Features of Conventional AM Included in This Study

	Clinical						Immunohistochemical and mutation assessment			
Case no.	Age	Sex	Site	Duration (mo)	Initial treatment	Status/mo*	Nuclear β-catenin	*CTNNB1*	*BRAF*	*KRAS*
1	37	F	bilateral mandible	108	P-mandibulectomy	NED/6	—	WT	GTG > GAG (V600E)	WT
2	18	M	Left mandible	2	segmental mandibulectomy	NED/8	—	WT	GTG > GAG (V600E)	WT
3	42	M	Right mandible	12	P-mandibulectomy	NED/6	—	WT	WT	WT
4	77	M	Right maxilla	24	P-maxillectomy	NA	—	WT	GTG > GAG (V600E)	WT
5	28	F	Left mandible	24	Curettage	Rec/19	—	WT	GTG > GAG (V600E)	WT
6	30	M	Left mandible	3	Curettage	NA	—	WT	GTG > GAG (V600E)	WT
7	32	M	Left mandible	60	P-mandibulectomy	Rec/57	—	WT	GTG > GAG (V600E)	WT
8	29	F	Right mandible	1	P-mandibulectomy	NED/6	—	WT	GTG > GAG (V600E)	WT
9	62	F	Left mandible	1	Segmental mandibulectomy	NA	—	WT	GTG > GAG (V600E)	WT
10	23	M	Left mandible	108	P-mandibulectomy	NED/5	—	WT	GTG > GAG (V600E)	WT
11	21	M	Right mandible	24	P-mandibulectomy	NED/8	—	WT	GTG > GAG (V600E)	WT
12	37	F	Right mandible	180	Curettage	Rec/12; Rec/60	—	WT	GTG > GAG (V600E)	WT
13	17	M	Left mandible	84	Curettage	NED/8	—	WT	GTG > GAG (V600E)	WT
14	56	M	Right mandible	144	Curettage	Rec/72; NED/144	—	WT	GTG > GAG (V600E)	WT
15	23	F	Right mandible	1	Curettage	NED/5	—	WT	GTG > GAG (V600E)	WT
16	24	F	Left mandible	12	Curettage	NED/6	—	WT	GTG > GAG (V600E)	WT
17	69	F	Right mandible	552	Curettage	Rec/120; Rec/324; Rec/468; Rec/516; NED/552	—	WT	GTG > GAG (V600E)	WT
18	22	M	Right mandible	24	P-mandibulectomy	NED/8	—	WT	GTG > GAG (V600E)	WT

*Follow-up period or time from the initial treatment to recurrence.
— indicates negative; F, female; L, left; M, male; Man, mandibular; Max, maxillary; NA, not available; NED, no evidence of disease; P, partial; R, right; Rec, recurrence; WT, wild-type.

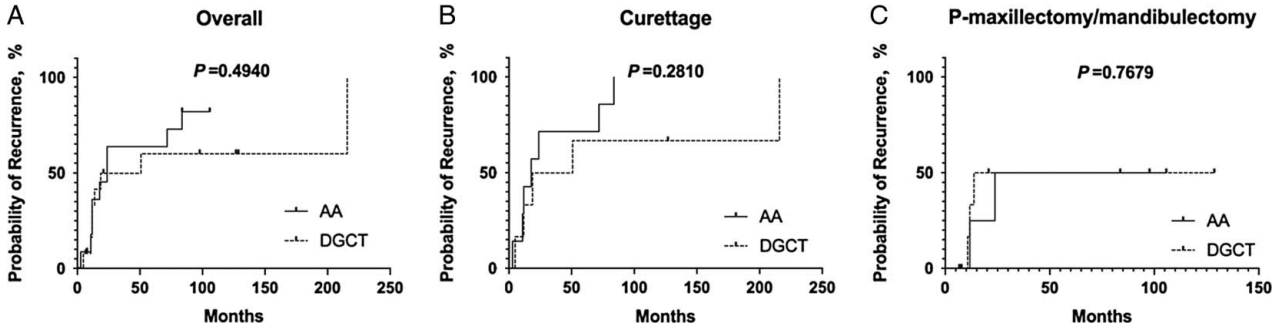

FIGURE 1. Cumulative recurrence rate categorized by groups. A, Kaplan-Meier analysis shows the overall cumulative recurrence rate based on AA and DGCT. B, The recurrence rate for patients with AA or DGCT undergoing curettage. C, The recurrence rate for patients with AA or DGCT undergoing radical surgery.

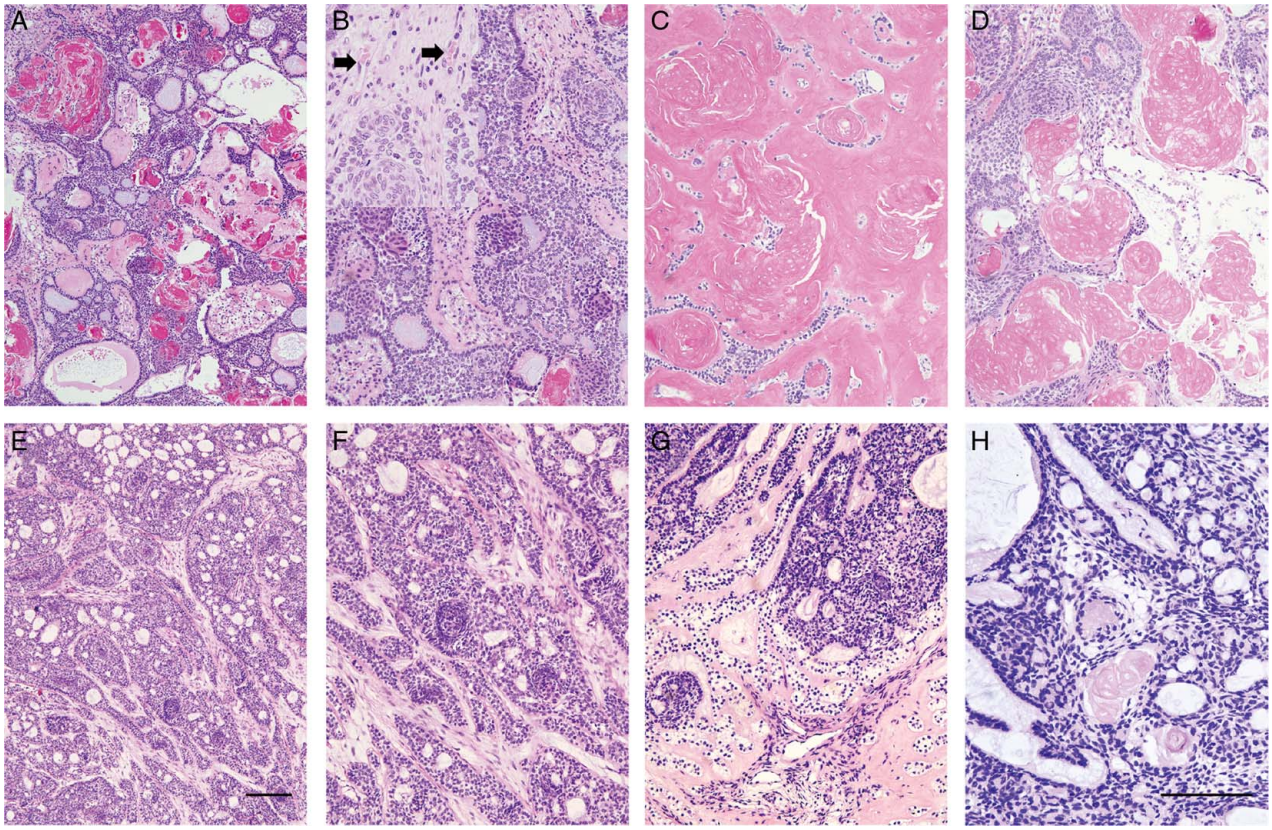

FIGURE 2. Representative histologic features observed in AA and DGCT. A–D show DGCT. E–H show AA. Cribriform arrangement of the AM-like epithelial component, duct-like spaces (A, E), whirling or morules structures (B, F) were observed, some multiple blood-filled cavities and collagen are found in the stroma (arrow in the upper left corner of B). C and G, Clear-cell clusters and dentinoid matrix deposits were frequently observed. DGCT exhibited a high occurrence of ghost cells (D), whereas their presence was comparatively infrequent in AA (H). Scale bar: 200 μm. Hematoxylin-eosin stains.

(Supplemental Digital Content 2, http://links.lww.com/PAS/B605) and Figure 3. Among the cytokeratins, CK8/18 was the only marker that exhibited a statistically significant difference between DGCTs and AAs, with DGCTs showing a higher proportion of CK8/18 overexpression (Figs. 3B, F). High proportions of CK14 and CK19 overexpression were observed in both DGCTs and AAs, while the positive rate of CK10/13 and CK18 was low. The overexpression of CK7 was 54%, 21%, and 40% in so-called AAs, DGCTs, and AOTs, respectively, whereas the positive rate for conventional AMs was only 6% (Figs. 3A, E, I, M). Statistically significant differences in the immunophenotype of CK7 and CK10/13 were found between AA and AM. All samples of so-called AAs and DGCTs showed focal positive nuclear immunoexpression of β-catenin (Fig. 3, Table 1), whereas all AOTs and conventional AMs showed only cytoplasmic or cytomembrane expression (Figs. 3K, O). The Ki-67 index was no > 3% in all AOTs, whereas it ranged from 2% to 15% in so-called AAs, DGCTs and conventional AMs. Notably, the overexpression for CK7 in AAs were associated with the diffusing dentinoid materials (Fig. 4), although statistical analysis could not be performed due to the limited sample size.

Mutational Profilings

Despite consistent nuclear β-catenin accumulation in so-called AA and DGCT, only one so-called AA sample tested positive for *CTNNB1* mutation, specifically at codons 34 (p.Gly34Arg) (Table 1). The other 3 tumors were found to be wild-type for *CTNNB1* mutation. Notably, *BRAF p.V600E* mutation was detected in 2 out of 13 AAs, 1 out of 15 DGCTs, and 2 out of 11 AOTs (Tables 1, 2). However, 17 out of 18 conventional AMs were found to harbor *BRAF p.V600E* mutation (Table 3). In addition, 7 out of the 11 cases in AOTs that were tested positive for *KRAS* mutation, specifically at codons 12 (p.G12V or p.G12 R). None of the other 3 tumors were found to harbor *KRAS* (codon 12) mutations (Tables 1,3).

DISCUSSION

Ever since AA was first included as a distinct entity in the new edition of WHO classification,[1] there has been persistent controversy and confusion regarding the relationship between AA and DGCT, which share significant histomorphologic features.[4,18] Herein, we presented a detailed analysis of the clinicopathologic, immunohistochemical, and molecular features of 27 Chinese patients who meet the

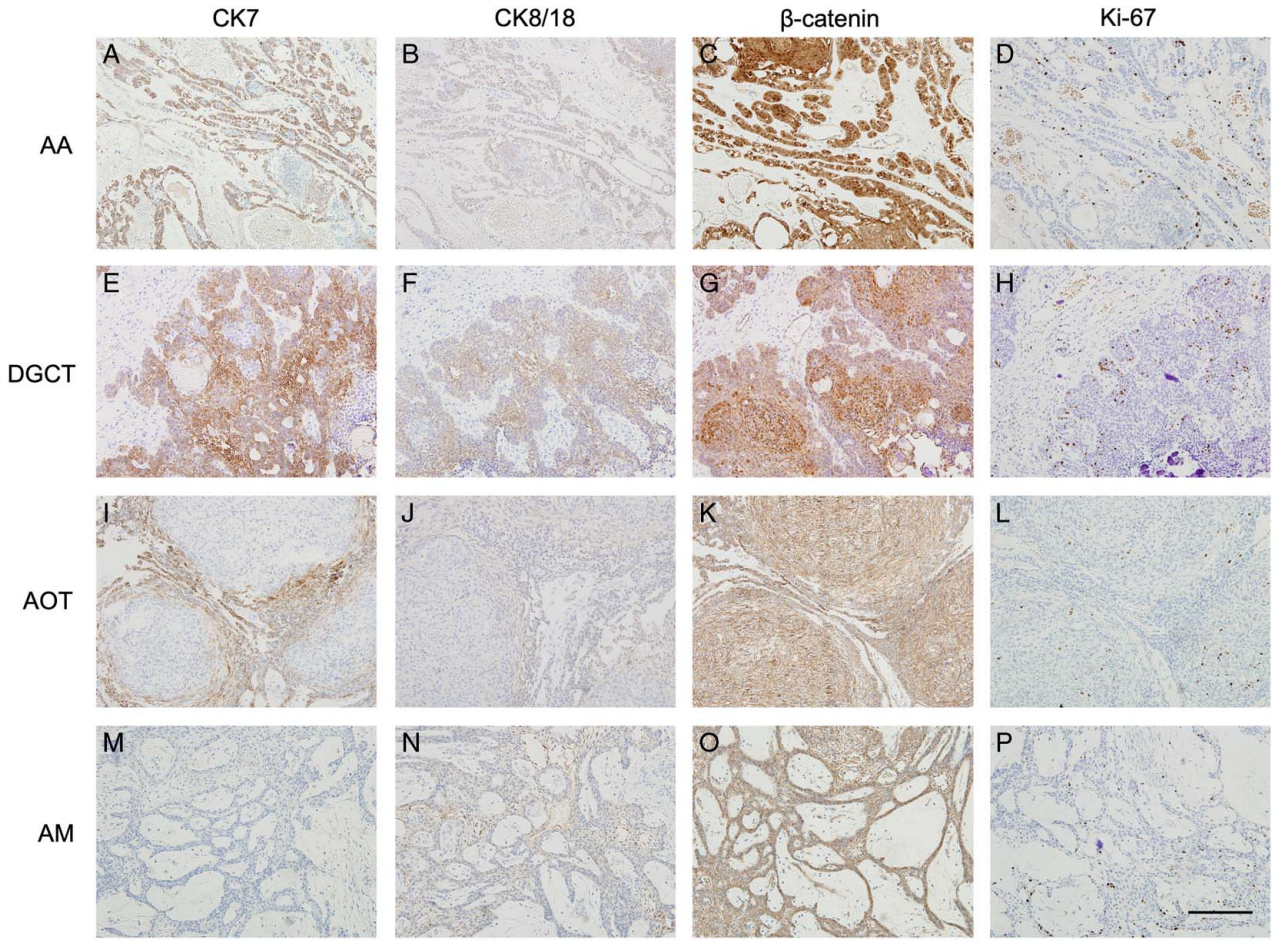

FIGURE 3. Representative immunohistochemical staining for CK7, CK8/18, β-catenin, and Ki-67 in AA and DGCT. Negative CK7 staining in AM (M), diffusely positive CK7 staining at other 3 tumors (A, E, I). Mild positive CK8/18 staining in AA (B), AOT (J), and AM (N), diffusely positive CK8/18 staining in DGCT (F). Diffuse nuclear β-catenin staining is observed in AA and DGCT (C, G), while cytoplasmic β-catenin staining in AOT and AM (K, O). A higher Ki-67 index in AA (D), DGCT (H), and AM (P) compared with AOT (L). Scale bar: 200 μm.

diagnostic criteria of AA and DGCT according to the new WHO classification.[1,3] In addition, we provided follow-up data for these patients. To enhance the study's comparative value, we also identified 2 histologic mimics. A literature analysis revealed clinical similarities between the previously reported cases of AA and DGCT.[4] Both diseases presented similar mean ages, with AA patients having a mean age of 39.0 years and central DGCT patients having a mean age of 38.8 years, despite the wide age ranges. There were no significant differences in sex distribution, tumor site, and recurrence rate between AA and central DGCT. The current study found that the median age of patients with AA and DGCT was in general agreement with previous reports, with AA showed a more obvious male preference (male: female = 5.5:1 in AA and male:female = 1.8:1 in DGCT). The mandible was the most frequently affected site (69%) in AA whereas the DGCT were mainly located in the maxilla (64%). The cumulative probabilities of recurrence were not statistically significant ($P = 0.4940$) between AA and DGCT, either in curettage groups ($P = 0.2810$) or osteotomy groups ($P = 0.7679$), which were inconsistent with previous reports.[4,5,19] Regarding the comparison between AA and AM, AA presents with demographic similarities to conventional AM, but exhibited distinct histopathologic differences and a higher rate of multiple recurrences, indicating its biological aggressiveness.[20]

Upon microscopic examination, AA is defined as a distinct entity characterized by epithelium resembling conventional AM, duct-like or cribriform architecture, and cellular condensations called morules or whorls. However, it can be challenging to distinguish it from dentinoid ghost cell tumor based solely on the cribriform or duct-like structures which could exhibit in epithelial odontogenic tumors from time to time, such as DGCT in the current series (36%, 5/14). It is believed that the glandular spaces are not a differentiation phenomenon, but rather a result of the degeneration of blood vessels and collagen, leaving behind a duct-like space. Such spaces could be due to cystic degeneration of the stroma resulting from self-strangulation rather than the differentiation of

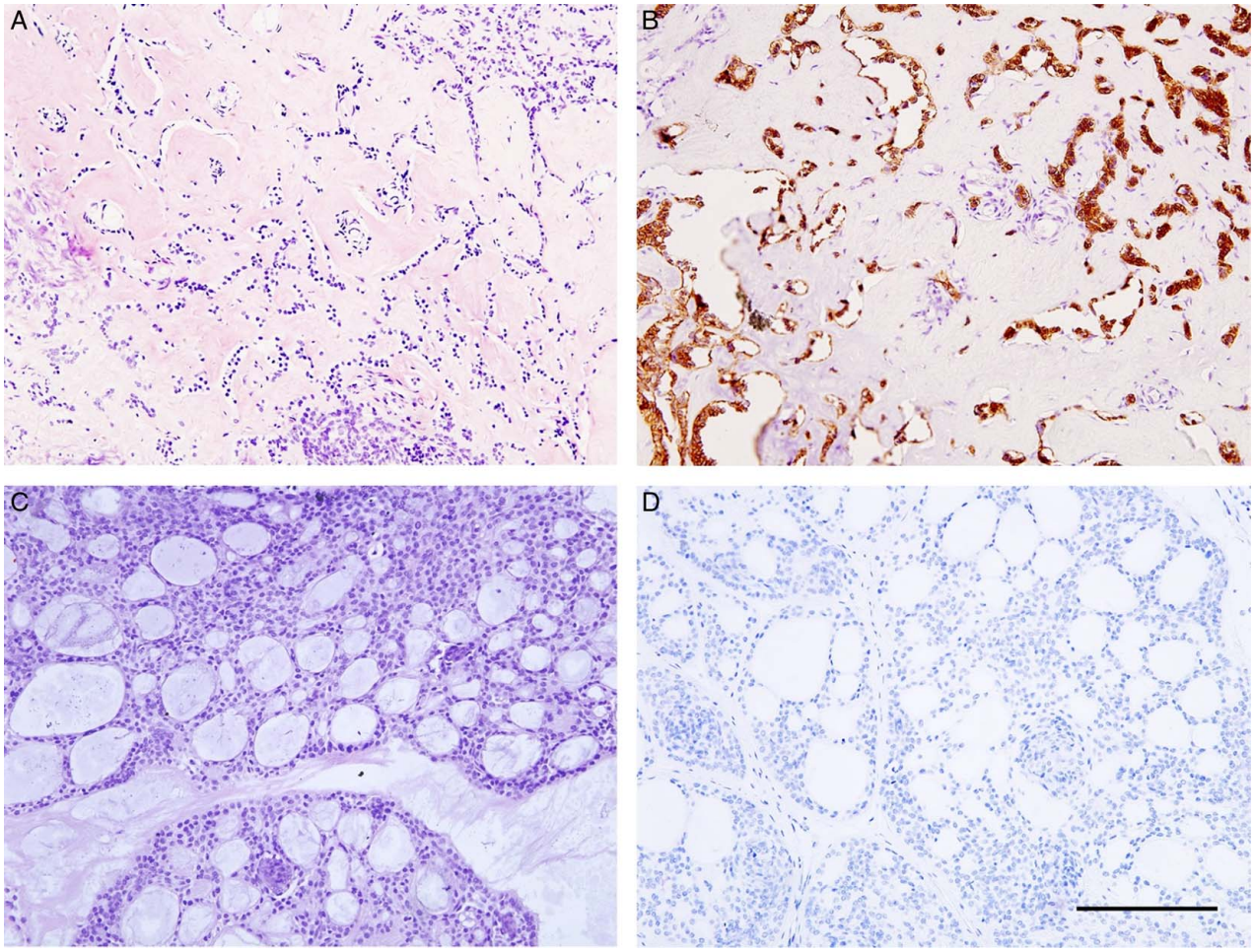

FIGURE 4. The association between histologic features and immune phenotype in AA. Cases with abundant dentinoid materials, which show positive CK7 (A, B), whereas cases with rare dentinoid materials are CK7 negative (C, D). Scale bar: 200 μm. Hematoxylin-eosin stains (A, C); immunohistochemistry stains (B, D).

the neoplastic epithelium. Thus, making a diagnosis depending upon such elements is not practical. Practically, an oral surgeon is never interested in the detailed histology of any lesion; all he wants is a straightforward diagnosis on which the entire treatment plan relies upon.[21] Dentinoid and ghost cells may or may not be present in these tumors, resulting in significant histologic overlap between AA and DGCT. Epithelial pearls, which are the essential diagnostic features of AA, can also be found in cases of DGCT (9/15, 60%). Therefore, the proportion of duct-like structures and ghost cells in DGCT and AA played a decisive role in their diagnosis. However, the proportion of duct-like structures cannot be clearly defined in the diagnostic criteria of AA due to the limitations of the specimens. There is a possibility that the areas of ghost cells and duct-like regions may differ to some extent from the actual condition of the tumor. In addition, the presence of duct-like structures in DGCT also indicates the overlap and difficulty in fully distinguishing between these 2 types of tumors histologically. The differential diagnosis between AA and AM is primarily based on the presence of cribriform features and dentinoid, as dentinoid is typically absent in AM. However, it should be noted that the occasional occurrence of ghost cells and ameloblastic epithelial can cause some overlap between the 2 entities.

CK expression has been widely recognized as a valuable tool in identifying different epithelial types and origins. Loyola et al.[14] detected 5 AA cases which stained focally positive for CK7, 8, 14, and 18 and diffusely positive for CK19. However, Adorno-Farias et al[22] showed high expression for CK14 (n = 6) and CK19 (n = 3) and all cases (n = 8) were negative for CK7. In our study, the expression of CK7, CK14, and CK19, which was previously reported in germinal dental tissues and other odontogenic tumors, was observed in both AA and DGCT cases, reinforcing their odontogenic origin.[23] All cytokeratins markers were detected, but CK8/18 was not statistically significant in differentiating AA from DGCT and CK8/18 was slightly higher in DGCT compared with AA. Nuclear β-catenin were identified in all AA and DGCT cases analyzed, and Ki-67 index ranging from 2% to 13% was detected in both types of tumors,

consistent with previous reports.[5,19] Regarding the distinction between AA and AM, dentinoid is typically absent in AM, although the occasional presence of ghost cells in AM, indicating some overlap between them. However, based on the observed genetic mutations and nuclear β-catenin reactivity of AA, it is not exactly what one would expect if this were a variant of AM.

In a recent report, it was revealed that AA shares similar demographic characteristics with conventional AM but has distinctive histopathologic features and a higher incidence of multiple recurrences, indicating its aggressive biological behavior. The authors suggest adding AA as a subtype of AM in the next WHO classification of odontogenic tumors.[20] In the current study, *BRAF* mutation had been detected in 2 cases in AA and 1 case in DGCT. However, 17 out of 18 conventional AMs were found to harbor *BRAF p.V600E* mutation, which suggested that AA may have a closer association with DGCTs than with AM. AAs were first incorporated into the new classification as a separate entity due to the absence of *BRAF* mutation together with the detection of β-catenin mutation. In addition, *CTNNB1* hotspot mutations are detected in AA at a relatively low frequency ($< 50\%$)[2] and not detected in another analysis.[4] *CTNNB1* hotspot mutations have been reported in only 1 case of central DGCT to date.[6] However, in the current study, *CTNNB1* hotspot mutations were only detected in only 1 case of AA (1/13), and no *CTNNB1* mutations were detected in DGCT. Nevertheless, nuclear accumulation of β-catenin, which is a hallmark of WNT pathway activation, was observed in both AA and DGCT cases. Activation of the WNT pathway leads to the transcription of genes involved in numerous cellular processes. However, mutations in components of the β-catenin destruction complex, such as the APC or Axin tumor suppressor proteins, can result in the activation of the pathway due to the accumulation of β-catenin. This can contribute to the development of various types of cancer. In addition, crosstalk between the WNT pathway and other signaling pathways can also activate the pathway.[24] A whole-exome sequencing analysis conducted on 5 cases, including 2 typical AA, 2 cases consisting of a mixture of AA and DGCT histopathologic patterns, and 1 typical DGCT, showed that all 5 cases analyzed had a significant mutation in WNT pathway components, which suggested that these tumors represent a histologic spectrum of WNT pathway-altered benign odontogenic tumors instead of being 2 distinct entities.[4] Therefore, from our point of view, considering the relatively low frequency of *CTNNB1* mutation, prognostic, immunohistochemical, and molecular similarities in AAs and DGCTs, together with the significant morphologic overlapping indicated that current evidence is insufficient to propose that AA represent a standalone entity.

The study revealed that the so-called AAs shares similar prognostic, immunohistochemical, and molecular features with DGCTs. Therefore, it should not be considered a distinct entity solely based on the presence of whorls/morules and cribriform/duct-like structures, which may be attributed to stromal degeneration. This finding suggests that the so-called AA could be interpreted as a possible variation of adenoid differentiation in DGCTs, with no significant differences in their biological behavior. Further studies are necessary to investigate the pathologic mechanisms of these tumors and to identify potential therapeutic targets.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Chinese Academy of Medical Sciences for their generous funding of this research through grant number 2019RU034. They are also grateful to the Regional Ethical Review Board of Peking University School and Hospital of Stomatology for approving this study and ensuring that the study was conducted in accordance with the Declaration of Helsinki.

REFERENCES

1. Thavaraj S, Bilodeau EA. Adenoid ameloblastoma. In: WHO Classification of Tumours Editorial Board, ed. *Head and Neck Tumours*. International Agency for Research on Cancer; 2022. https://tumourclassification.iarc.who.int/welcome/
2. Bastos VC, Coura BP, Guimarães LM, et al. Adenoid ameloblastoma harbors beta-catenin mutations. *Mod Pathol*. 2022;35:1562–1569.
3. Carlos R, Vered M. Dentinogenic ghost cell tumour. In: WHO Classification of Tumours Editorial Board, ed. *Head and Neck Tumours*. International Agency for Research on Cancer; 2022. https://tumourclassification.iarc.who.int/welcome/
4. Oh KY, Hong SD, Yoon HJ. Adenoid ameloblastoma shares clinical, histologic, and molecular features with dentinogenic ghost cell tumor: the histologic spectrum of WNT pathway-altered benign odontogenic tumors. *Mod Pathol*. 2023;36:100051.
5. de Souza Vieira G, de Pinho Montovani P, Rozza-de-Menezes RE, et al. Comparative analysis between dentinogenic ghost cell tumor and ghost cell odontogenic carcinoma: a systematic review. *Head Neck Pathol*. 2021;15:1265–1283.
6. Kim SA, Ahn SG, Kim SG, et al. Investigation of the beta-catenin gene in a case of dentinogenic ghost cell tumor. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2007;103:97–101.
7. Coura BP, Dos Santos JN, Fonseca FP, et al. Adenoid ameloblastoma with dentinoid is molecularly different from ameloblastomas and adenomatoid odontogenic tumors. *J Oral Pathol Med*. 2021;50:1067–1071.
8. Rai HK, Pai SM, Dayakar A, et al. Adenoid ameloblastoma with dentinoid: a rare hybrid variant. *J Oral Maxillofac Pathol*. 2017;21:319.
9. Ide F, Mishima K, Saito I, et al. Diagnostically challenging epithelial odontogenic tumors: a selective review of 7 jawbone lesions. *Head Neck Pathol*. 2009;3:18–26.
10. Ghasemi-Moridani S, Yazdi I. Adenoid ameloblastoma with dentinoid: a case report. *Arch Iran Med*. 2008;11:110–112.
11. Evans BL, Carr RF, Phillipe LJ. Adenoid ameloblastoma with dentinoid: a case report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2004;98:583–588.
12. Matsumoto Y, Mizoue K, Seto K. Atypical plexiform ameloblastoma with dentinoid: adenoid ameloblastoma with dentinoid. *J Oral Pathol Med*. 2001;30:251–254.
13. Slabbert H, Altini M, Crooks J, et al. AMeloblastoma with dentinoid induction—dentinoameloblastoma. *J Oral Pathol Med*. 1992;21:46–48.
14. Loyola AM, Cardoso SV, de Faria PR, et al. Adenoid ameloblastoma: clinicopathologic description of five cases and systematic review of the current knowledge. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2015;120:368–377.
15. Kurppa KJ, Catón J, Morgan PR, et al. High frequency of BRAF V600E mutations in ameloblastoma. *J Pathol*. 2014;232:492–498.
16. Groesser L, Herschberger E, Ruetten A, et al. Postzygotic HRAS and KRAS mutations cause nevus sebaceous and Schimmelpenning syndrome. *Nat Genet*. 2012;44:783–787.

17. Li YY, Zhou CX, Gao Y. Moesin regulates the motility of oral cancer cells via MT1-MMP and E-cadherin/p120-catenin adhesion complex. *Oral Oncol*. 2015;51:935–943.
18. Bastos VC, Guimarães LM, Gomes CC, et al. Adenoid ameloblastoma vs dentinogenic ghost cell tumor: are they related entities? *Mod Pathol*. 2023;36:100132.
19. Sachdev SS, Chettiankandy TJ, Sardar MA, et al. Adenoid ameloblastoma with dentinoid: a systematic review. *Sultan Qaboos Univ Med J*. 2022;22:325–338.
20. Jayasooriya PR, Abeyasinghe WAMUL, Liyanage RLPR, et al. Diagnostic enigma of adenoid ameloblastoma: literature review based evidence to consider it as a new sub type of ameloblastoma. *Head Neck Pathol*. 2022;16:344–352.
21. Pandiar D, Gopinath D. Adenoid ameloblastoma: the Histological Paradox. *Head Neck Pathol*. 2022;16:538–539.
22. Adorno-Farias D, Muniz VRVM, Soares AP, et al. Ameloblastoma with adenoid features: a series of eight cases. *Acta Histochem*. 2018; 120:468–476.
23. Crivelini MM, de Araújo VC, de Sousa SO, et al. Cytokeratins in epithelia of odontogenic neoplasms. *Oral Dis*. 2003;9:1–6.
24. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. *Oncogene*. 2017;36:1461–1473.