The *BRAF* p.V600E mutation is a common event in ameloblastomas but is absent in odontogenic keratocysts

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Objective. Odontogenic keratocysts (OKCs) are jaw lesions with a tendency to recur. *PTCH1* gene mutations are common events in most OKCs; however, other genetic alterations underlying OKC pathogenesis have not yet been elucidated. *BRAF* p.V600E mutations have recently been detected in some odontogenic tumors, such as ameloblastoma and ameloblastic fibroma, although their involvement in OKC is still unclear. In this study we aimed to clarify the presence and/or frequency of *BRAF* p.V600E mutations in OKCs.

Study Design. Thirty-five cases of OKCs, 13 of which were associated with Gorlin syndrome, were evaluated for *BRAF* p.V600E mutations by direct sequencing of the formalin-fixed, paraffin-embedded, and frozen tissue samples. Seventeen cases of ameloblastoma and six cases of dentinogenic ghost cell tumor were also included in this study for comparative purposes.

Results. *BRAF* p.V600E mutations were not detected in any of the OKCs or dentinogenic ghost cell tumors. In contrast, 14 of 17 cases of ameloblastoma (82.35%) were proven to harbor *BRAF* p.V600E mutations.

Conclusion. *BRAF* p.V600E mutations were common in ameloblastomas, as previously reported, but were absent in OKCs and dentinogenic ghost cell tumors. These results further confirmed the noninvolvement of *BRAF* in OKCs and suggested different pathogenic mechanisms involved in various odontogenic lesions. (Oral Surg Oral Med Oral Pathol Oral Radiol 2020;129:229−235)

Odontogenic keratocyst (OKC), also known as keratocystic odontogenic tumor, is a common odontogenic cyst often found in the mandible.[1] Some OKCs occur as part of Gorlin syndrome (also known as nevoid basal cell carcinoma syndrome [NBCCS]), and in these syndromic cases OKCs are likely to be multiple and found in younger patients.[2,3] Patched 1 *(PTCH1)* tumor suppressor gene mutations are commonly associated with OKCs.[4] *PTCH1* gene product acts as ligand for sonic hedgehog (SHH) receptor; mutations in the *PTCH1* gene could result in constitutive activation of hedgehog signaling, which can be a potential risk factor for the development of OKC.[5] According to our previously published data, *PTCH1* mutation frequency in sporadic OKC could be up to 84% based on analyzing samples of epithelial linings separated from the connective tissues, which is close to the frequency detected in syndromic OKCs, leaving 15%−20% of OKCs with unknown genetic alterations.[6,7]

B-Raf is a member of the rapidly accelerated fibrosarcoma kinase family (*ARAF, BRAF,* and *CRAF*) and is a key regulator of the mitogen-activated protein kinase (MAPK) pathway.[8] *BRAF* mutation has been detected in a wide range of benign and malignant lesions, such as melanomas, papillary thyroid and colon cancers, and Langerhans cell histiocytosis.[9-11] Previous studies have found that *BRAF* p.V600E mutations are common in ameloblastomas and some odontogenic tumors with ameloblastic morphology.[12-14] Recently 2 contrasting opinions came from 2 research groups regarding the frequencies of *BRAF* p.V600E mutations in OKC samples.[15,16] Considering the potential risk for dealing with OKCs in BRAF-targeted therapy, we expanded the number of OKC cases to further evaluate whether OKC samples harbored *BRAF* p.V600E mutations or not.

In addition, as a rare odontogenic tumor, dentinogenic ghost cell tumor (DGCT) also has ameloblastoma-like epithelium infiltrating into the mature connective tissues.[1] Therefore we selected some cases to assess the mutation rate of *BRAF* p.V600E in DGCT at the same time.

MATERIALS AND METHODS

Patients and samples

From the database of the Department of Oral Pathology at Peking University Hospital of Stomatology, we

Statement for Clinical Relevance

Our results indicated that *BRAF* p.V600E mutations were absent in odontogenic keratocysts and dentinogenic ghost cell tumors but occurred often in ameloblastomas, suggesting different pathogenic mechanisms might be involved in various odontogenic lesions. Thus different therapeutic targets might be implicated in this group of lesions.

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selected 35 recent Chinese patients with OKC, among whom 13 patients met the diagnostic criteria of the NBCCS (also known as Gorlin syndrome) based on the World Health Organization classification; one sample was from a recurrent lesion. All 22 other OKC samples were from patients with sporadic OKC, among which 13 harbored *PTCH1* mutations and the other nine had no *PTCH1* mutations based on our published or unpublished data.[6] The 13 NBCCS-associated samples were from the same patients as those in our previously published study, with *PTCH1* mutations detected in patients' blood and fresh tissues.[17,18] In this study we also enrolled 6 cases of dentinogenic ghost cell tumors because they harbor areas of odontogenic epithelium similar to ameloblastoma. Additionally, 17 cases of ameloblastoma were included to validate the experimental procedures in this study. All diagnoses were reviewed and confirmed by 2 oral pathologists. None of the samples were dropped out because of failures. This study was approved by the Peking University Health and Science Center's ethics committee.

DNA extraction and *BRAF* p.V600E mutation analysis

For sporadic OKC samples, we used the same DNA from epithelial linings with our previous published data or unpublished data; these DNA samples had been stored at −80°C.[6] For all other samples (NBCCS OKC, ameloblastoma, and dentinogenic ghost cell tumor), we selected cases with a prominent epithelial component and extracted genomic DNA using a Universal DNA Purification kit (Tiangen, Beijing, China) from formalin-fixed paraffin-embedded tissues. Polymerase chain reaction (PCR) was performed by using the following primer sequences to detect *BRAF* p.V600E mutations: Forward 3'-TCATAATGCTTGCTCTGATAGGA-5' and reverse 3'-CCAAAAATTTAATCAGTGGA-5'. One hundred nanograms of template DNA were used per reaction, and the reaction volume was 50 microliters. The thermal cycling protocol consisted of initial denaturation at 94°C for 5 minutes, followed by 35 cycles of amplification at 94°C for 30 seconds, 64−65°C for 30 seconds, and 72°C for 30 seconds. A final extension was performed at 72°C for 10 minutes. All PCR products were directly sequenced to confirm the results on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. All PCR results were validated by reverse sequencing and more than 2 independent experiments. The DNA extraction procedures and subsequent PCR experiments were performed simultaneously for all samples.

RESULTS

Of all the 22 sporadic OKC cases that we selected, 6 cases were from our previous data and the other 16 cases were new. The median age of the sporadic patients was 35.8 years old (range 1−68 years); the male/female ratio was 15:7. Six of the sporadic cases occurred in the maxilla, and 16 of the lesions were found in the mandible. Of them, 13 samples harbored *PTCH1* mutation in the epithelial components; the other 9 samples did not. The median age of the NBCCS patients was 31 years (range 13−62); the male/female ratio was 11:2. All of them had multiple lesions. All 6 DGCT patients were male, and the median age was 42.3 years (range 18−60); 3 of the lesions were from the mandible, and 3 of the lesions were from the maxilla. The median age of the ameloblastoma patients in this study was 29.2 years old (range 8−45), with a male/female ratio of 7:10. All the ameloblastoma lesions were from the mandible. The clinical information is summarized in Table I.

In this study none of the OKC samples harbored *BRAF* p.V600E mutation, but we detected a high mutation frequency of approximately 82.4% in ameloblastoma cases (Fig. 1). Although DGCT have areas resembling ameloblastoma, no *BRAF* p.V600E mutation was detected in DGCT samples (Table I).

DISCUSSION

As a *RAS*-regulated serine-threonine kinase, *BRAF* serves as an activator of the MAPK signaling cascade. The majority of *BRAF* mutations are found in exon 15 with a thymine to adenine transversion mutation at nucleotide 1799 position, which is responsible for valine to glutamic acid substitution at amino acid 600 position (V600E).[19] This mutation alters kinase activity of BRAF, which constitutively activates the MAPK pathway.[20] Heterozygous mutations in codon

Table I. Summary of *BRAF* p.V600E mutation detections in odontogenic tumors reported in English literatures

	Mean age	*Male: female*	*PTCH1 mutation*	*BRAF p. V600E mutations*
Sporadic-OKC	36.6	15:7	13/22 (59%)	0/22 (0%)
NBCCS-OKC	26.3	11:2	13/13 (100%)	0/13 (0%)
Dentinogenic ghost cell tumor	42.3	6:0	NA	0/6 (0%)
Ameloblastoma	29.2	7:10	NA	14/17 (82.35%)

OKC, odontogenic keratocyst; NBCCS, nevoid basal cell carcinoma syndrome; NA, not available.
Data on *PTCH1* mutations were from some of our unpublished data and previous studies.[6,17,18]

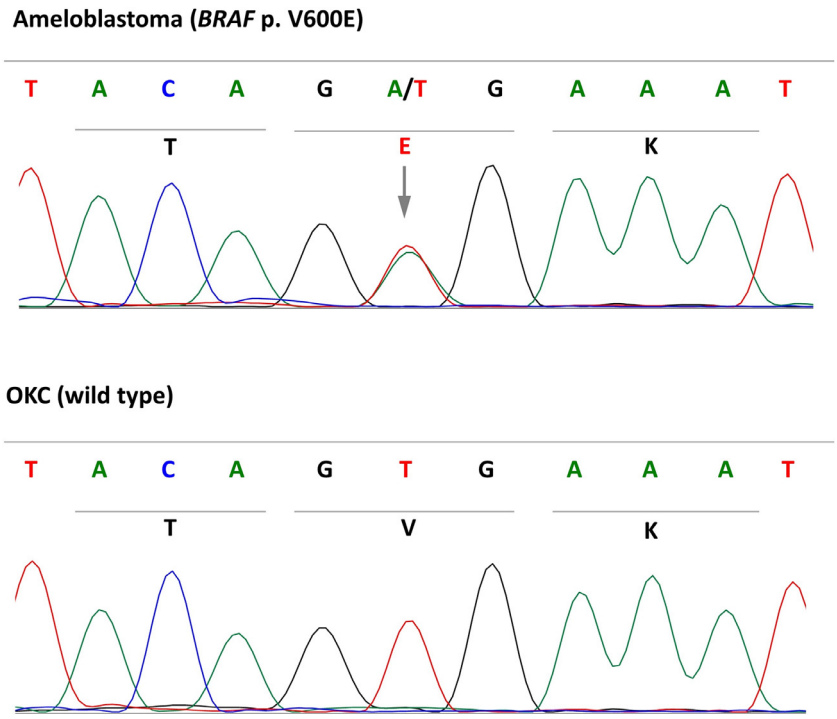

Fig. 1. Example of DNA sequence analysis showing the presence of *BRAF* p.V600E mutation in an ameloblastoma but absence of the mutation in the detached epithelial layer of an odontogenic keratocyst (OKC).

600 are sufficient to transform the activity of BRAF kinase, which results in the continuous stimulation of cell proliferation and inhibits programmed cell death.[21,22] Mutations in the *BRAF* gene have been identified in a variety of lesions, including thyroid cancers, melanoma, and colorectal cancers.[9,10] In addition to a high frequency of *BRAF* mutations in ameloblastomas, which have been reported since 2014 by 3 separate research groups,[12,13,23] *BRAF* p.V600E mutations were also detected in some benign and malignant odontogenic tumors, including clear cell odontogenic carcinoma, ameloblastic fibroma, ameloblastic fibrodentinoma, ameloblastic fibro-odontoma, and ameloblastic carcinoma (Table II).[12-15,23-26] These findings may provide possibilities for application of *BRAF* inhibitors to odontogenic tumors harboring *BRAF* mutations.

There has been a reversal of the nomenclature of OKC in the new World Health Organization classification of head and neck tumors, reflecting the uncertainty over the nature of this lesion.[1,2] Clinically, OKC shares some features with ameloblastoma, such as a high risk of recurrence; however, OKC has characteristic histopathologic and radiologic features such as parakeratinized epithelium (Fig. 2A and 2a, 2C and 2c), a well-demarcated radiolucent lesion, and rare resorption of involved roots.[1] At the molecular level, the significant changes in OKCs are mutations of the *PTCH1* gene; more than 80% of both NBCCS-associated and sporadic OKCs harbor *PTCH1* mutations.[6,7] Although some other mutations have also been detected in OKCs, such as *PTCH2, SUFU*, and *SMO*, but these are not common mutational events.[27,28] With the detection of *BRAF* p.V600E mutations in ameloblastomas and some other odontogenic tumors, many researchers started to evaluate *BRAF* p.V600E mutation status in OKCs. Brown et al.[13] reported an absence of *BRAF* p.V600E mutations in OKCs (0/19 cases) using allele-specific PCR. Brunner et al.[14] reported a single case of OKC without *BRAF* p.V600E mutation. Cha et al.[15] was the first to report the high frequency of *BRAF* p.V600E mutation in OKCs (24 of 38 cases, 63.2%) and suggested that it could be a potential therapeutic target for OKC patients. Not long after, Franca et al.[16] argued that *BRAF* p.V600E mutations were rare in OKCs by finding 1 case with *BRAF* p.V600E mutation out of 28 patients. In our study we selected 35 OKC cases, 22 of which were sporadic cases, and the remaining 13 OKCs were associated with NBCCS. The DNA samples of sporadic cases were extracted from the epithelial linings separated from the connective tissue capsules, a procedure that could avoid the masking effect of the fibrous capsules during sequencing analysis of OKC cyst walls, and ameloblastoma samples served as positive controls.[6] We detected a high frequency of *BRAF* p.V600E mutation in ameloblastoma cases (82.35%, 14/17), but no positive *BRAF* p.V600E mutation in any of the OKC cases. Additionally, in a

Table II. Summary of relevant clinico-pathological data and *BRAF* p.V600E mutations in OKC, dentinogenic ghost cell tumors, and ameloblastomas

		Mean age (range)	*Male:female*	*Site*		*BRAF p.V600E mutation*	*Reference*
				Mandible	*Maxilla*		
Ameloblastoma (n = 264)		36.5 (22−58)	3:1	4	0	3/4 (75%)	Cha et al., 2017[15]
		43.47 (16−87)	12:7	NA	NA	12/19 (63.16%)	Soltani et al., 2018[24]
		41.3 (8−84)	26:58	68	16	54/84 (64.29%)	Brown et al., 2014[13]
		45.75 (14−84)	15:9	24	0	15/24 (62.5%)	Kurppa et al., 2014[23]
		NA	NA	13	11	13/24 (54.17%)	Sweeney et al., 2014[12]
		34.7	35:38	63	10	34/73 (46.58%)	Fregnani et al., 2017[25]
		39.5 (13−88)	12:7	17	2	14/19 (73.68%)	Brunner et al., 2015[14]
		30.2 (8−75)	9:8	13	4	14/17 (82.35%)	Diniz et al., 2015[26]
Ameloblastic fibroma (n = 7)		NA	NA	NA	NA	2/2 (100%)	Brown et al., 2014[13]
		20.2 (8−39)	1:4	4	1	2/5 (40%)	Brunner et al., 2015[14]
Ameloblastic fibrodentinoma (n = 17)		NA	NA	NA	NA	1/1 (100%)	Brown et al., 2014
		10.2 (1−18)	13:5	11	7	6/18 (33.3%)	Brunner et al., 2015[14]
Ameloblastic Carcinoma (n = 13)		NA	NA	NA	NA	0/1 (0%)	Brown et al., 2014[13]
		49.8 (33−68)	3:1	3	1	1/4 (25%)	Brunner et al., 2015[14]
		48 (21−65)	6:2	6	2	3/8 (37.5%)	Diniz et al., 2015[26]
Odontogenic keratocyst (n = 86)	Sporadic	38.5 (11−76)	9:11	16	4	1/20 (5%)	Franca et al., 2018[16]
	NBCCS	17 (14−29)	1:1	6	2	0/8 (0%)	
	Sporadic	30.36 (10−69)	23:13	20	16	22/36 (61.11%)	Cha et al., 2017[15]
	NBCCS	23.5 (15−32)	1:1	1	1	2/2 (100%)	
		NA	NA	NA	NA	0/19 (0%)	Brown et al., 2014[13]
		32 (32)	0:1	1	0	0/1 (0%)	Brunner et al., 2015[14]
Orthokeratinized odontogenic cyst (n = 11)		31.82 (16−56)	6:5	9	2	1/11 (9.09%)	Cha et al., 2017[15]
Dental follicles (n = 4)		11 (6−16)	3:1	4	0	0/4 (0%)	Brunner et al., 2015[14]
Dentigenrous cysts (n = 22)		47.7 (15−72)	18:4	20	2	0/22 (0%)	Brunner et al., 2015[14]
Odontogenic fibroma (n = 2)		NA	NA	NA	NA	0/2 (0%)	Brown NA. et al., 2014[13]
Odontogenic myxoma (n = 5)		NA	NA	NA	NA	0/5 (0%)	Brown et al., 2014[13]
Calcifying cystic odontogenic tumor (n = 14)		NA	NA	NA	NA	0/2 (0%)	Brown et al., 2014[13]
		49.9 (12−77)	8:04	7	5	0/12 (0%)	Brunner et al., 2015[14]
Ademomatoid odontogenic tumor (n = 2)		NA	NA	NA	NA	0/2 (0%)	Brown et al., 2014[13]
Ghost cell odontogenic carcinoma (n = 2)		42 (27−57)	2:0	1	1	0/2 (0%)	Diniz et al., 2015[26]
Clear cell odontogenic carcinoma (n = 2)		64	1:0	1	0	1/1 (100%)	Diniz et al., 2015[26]
Odontoameloblastoma (n = 1)		NA	NA	NA	NA	0/1 (0%)	Brown et al., 2014[13]
Odontogenic carcinoma (n = 5)		NA	NA	NA	NA	0/5 (0%)	Brown et al., 2014[13]
Intraosseous carcinoma (n = 4)		63 (57−73)	2:2	2	2	0/4 (0%)	Brunner et al., 2015[14]

OKC, odontogenic keratocyst; *NA,* not available; *NBCCS,* nevoid basal cell carcinoma syndrome.

separate ongoing study, we conducted whole-exome sequencing in 5 OKC cases to identify novel mutations; *BRAF* mutation was also absent (unpublished data).

The absence of *BRAF* p.V600E mutations in OKCs and frequent detection in ameloblastomas further suggested that these 2 lesions might have different pathogenic mechanisms. Normal odontogenesis is regulated by signaling pathways such as Bone Morphogenetic Protein, SHH, WNT, MAPK; these signaling pathways are involved at different developmental stages.[29-32] Most pathogenic gene mutations that have been identified in odontogenic tumors are present downstream of these signaling pathway genes. *PTCH1*-mediated SHH signaling plays a pivotal role in early odontogenesis, stimulating the proliferation of epithelial cells and the formation of tooth bud, whereas MAPK cascade, in which *BRAF* gene is involved, regulates the bell stage of tooth development.[32,33] Thus it is interesting to speculate that OKCs may arise from epithelial remnants formed during the early stages of odontogenesis, such as the dental lamina, a widely accepted tissue origin for OKC. Ameloblastoma consists of ameloblast-like cells with reversely polarized nuclei and stellate reticulum−like cells; it tends to be a lesion of the enamel organ.[34] The contrasting different mutation frequencies of *BRAF* in OKC and ameloblastoma could be helpful in differential diagnosis between unicystic variant of ameloblastoma and OKC, particularly when the biopsied tissue is too small or severely inflamed.

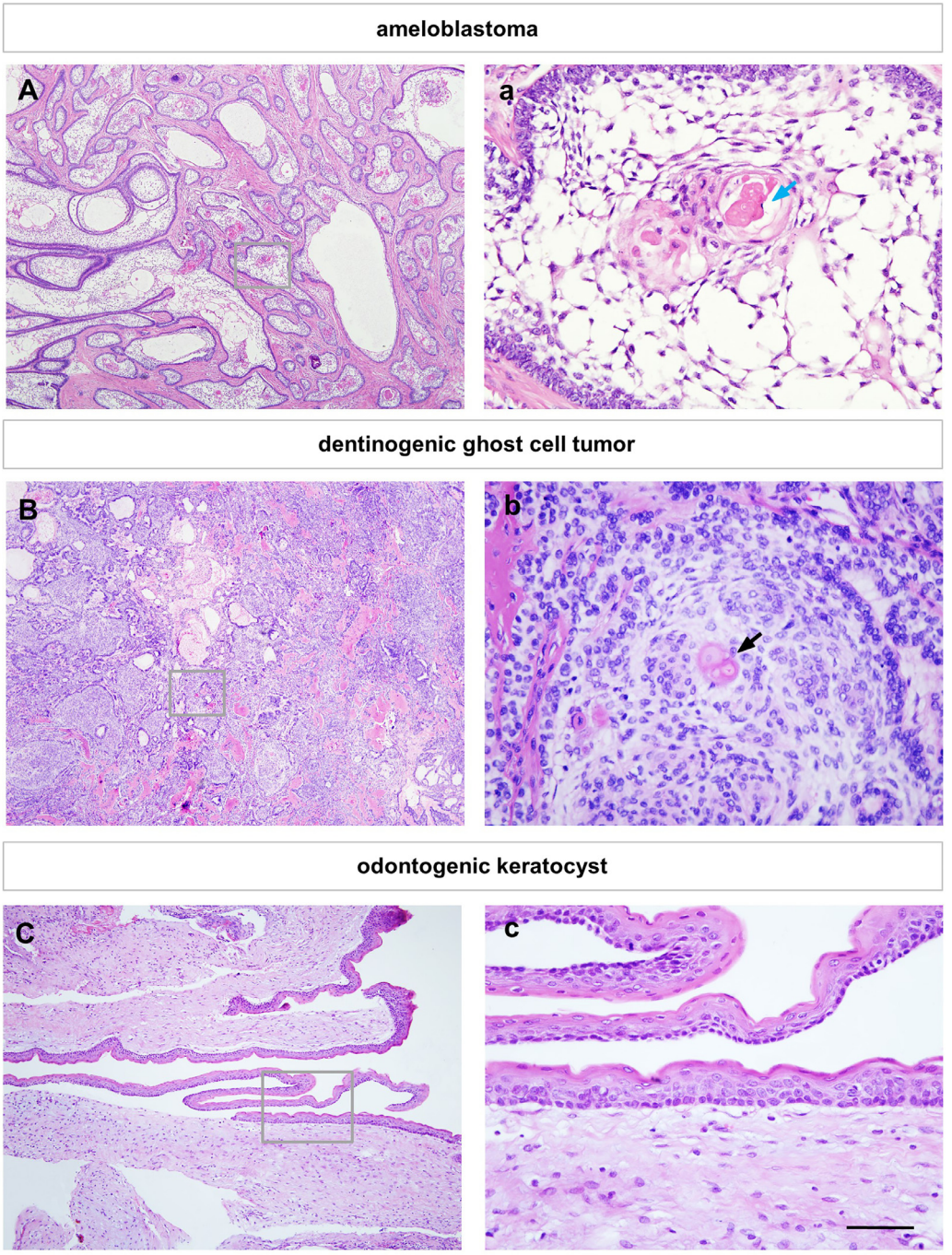

Fig. 2. Histopathologic images of an ameloblastoma (A, a), a dentinogenic ghost cell tumor (DGCT) (B, b) and an odontogenic keratocyst (OKC) (C and c). Images a−c are the higher-magnification views of the boxed areas in A-C, respectively. Ameloblastoma is made of islands of odontogenic epithelium and stellate reticulum−like cells (A, a). The DGCT consists of odontogenic epithelium resembling ameloblastoma; the distinct feature is the presence of ghost cells (B and b, arrow). Typical histologic characteristics of OKC include the presence of a parakeratin layer and palisading basal cells with hyperchromatic nuclei (C and c). Scale bar: 250 μm (A, B), 100 μm (C), 25 μm (a, b, c).

Moreover, in some cases, OKC and ameloblastoma can occur simultaneously in a patient.[35] In such situations, *BRAF* could be a good molecular target to be examined.

In addition, *BRAF* p.V600E mutations were also absent in dentinogenic ghost cell tumors. Histologically the epithelial components of this tumor resemble that of ameloblastoma, but with additional features of dentinoid deposits and foci of ghost cells (Fig. 2A and 2a, 2B and 2b). Thus detection of *BRAF* p.V600E mutations could be

used for distinguishing atypical cases such as ameloblastoma with suspicious features of ghost cells or dentinoid.[36]

To summarize, our results indicated that *BRAF* p.V600E mutations were absent in OKCs and dentinogenic ghost cell tumors but occurred commonly in ameloblastomas. These results suggest that different pathogenic mechanisms might be involved in various odontogenic lesions. Thus different therapeutic targets might be implicated in this group of lesions.

REFERENCES

1. Adel K, EI-Naggar JKCC, Grandis JR, Takata T, Slootweg PJ. WHO Classification of Head and Neck Tumours. 4th ed Geneva, Switzerland: World Health Organization; 2017.
2. Brannon RB. The odontogenic keratocyst. A clinicopathologic study of 312 cases. Part II. Histologic features. *Oral Surg Oral Med Oral Pathol*. 1977;43:233-255.
3. Noy D, Rachmiel A, Zar K, Emodi O, Nagler RM. Sporadic versus syndromic keratocysts: can we predict treatment outcome? A review of 102 cysts. *Oral Dis*. 2017;23:1058-1065.
4. Li TJ. The odontogenic keratocyst: a cyst, or a cystic neoplasm? *J Dent Res*. 2011;90:133-142.
5. Hoyos Cadavid AM, Kaminakagura E, Rodrigues MFSD, Pinto CAL, Teshima THN, Alves FA. Immunohistochemical evaluation of Sonic Hedgehog signaling pathway proteins (Shh, Ptch1, Ptch2, Smo, Gli1, Gli2, and Gli3) in sporadic and syndromic odontogenic keratocysts. *Clin Oral Investig*. 2018;7.
6. Qu J, Yu F, Hong Y, et al. Underestimated PTCH1 mutation rate in sporadic keratocystic odontogenic tumors. *Oral Oncol*. 2015;51:40-45.
7. Guo YY, Zhang JY, Li XF, Luo HY, Chen F, Li TJ. PTCH1 gene mutations in Keratocystic odontogenic tumors: a study of 43 Chinese patients and a systematic review. *PLoS One*. 2013;8:e77305.
8. Kim HS, Jung M, Kang HN, et al. Oncogenic BRAF fusions in mucosal melanomas activate the MAPK pathway and are sensitive to MEK/PI3 K inhibition or MEK/CDK4/6 inhibition. *Oncogene*. 2017;36:3334-3345.
9. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature*. 2002;417:949-954.
10. Jones JC, Renfro LA, Al-Shamsi HO, et al. (Non-V600) BRAF mutations define a clinically distinct molecular subtype of metastatic colorectal cancer. *J Clin Oncol*. 2017;35:2624-2630.
11. Bodnar M, Burduk P, Antosik P, Jarmuz-Szymczak M, Wierzbicka M, Marszalek A. Assessment of BRAF V600 E (VE1) protein expression and BRAF gene mutation status in codon 600 in benign and malignant salivary gland neoplasms. *J Oral Pathol Med*. 2017;46:340-345.
12. Sweeney RT, McClary AC, Myers BR, et al. Identification of recurrent SMO and BRAF mutations in ameloblastomas. *Nat Genet*. 2014;46:722-725.
13. Brown NA, Rolland D, McHugh JB, et al. Activating FGFR2-RAS-BRAF mutations in ameloblastoma. *Clin Cancer Res*. 2014;20:5517-5526.
14. Brunner P, Bihl M, Jundt G, Baumhoer D, Hoeller S. BRAF p.V600 E mutations are not unique to ameloblastoma and are shared by other odontogenic tumors with ameloblastic morphology. *Oral Oncol*. 2015;51:e77-e78.
15. Cha YH, Cho ES, Kang HE, et al. Frequent oncogenic BRAF V600 E mutation in odontogenic keratocyst. *Oral Oncol*. 2017;74:62-67.
16. Franca JA, de Sousa SF, Diniz MG, et al. Absence of BRAFV600 E mutation in odontogenic keratocysts. *J Oral Pathol Med*. 2018;47:186-191.
17. Hong YY, Yu FY, Qu JF, Chen F, Li TJ. Fibroblasts regulate variable aggressiveness of syndromic keratocystic and non-syndromic odontogenic tumors. *J Dent Res*. 2014;93:904-910.
18. Hong Y, Zhang J, Zhang H, et al. Heterozygous PTCH1 mutations impact the bone metabolism in patients with nevoid basal cell carcinoma syndrome likely by regulating SPARC expression. *J Bone Miner Res*. 2016;31:1413-1428.
19. Roskoski Jr R. Targeting oncogenic Raf protein-serine/threonine kinases in human cancers. *Pharmacol Res*. 2018;135:239-258.
20. Ji H, Wang Z, Perera SA, et al. Mutations in BRAF and KRAS converge on activation of the mitogen-activated protein kinase pathway in lung cancer mouse models. *Cancer Res*. 2007;67:4933-4939.
21. Koh HY, Kim SH, Jang J, et al. BRAF somatic mutation contributes to intrinsic epileptogenicity in pediatric brain tumors. *Nat Med*. 2018;24:1662-1668.
22. Wang HG, Miyashita T, Takayama S, et al. Apoptosis regulation by interaction of Bcl-2 protein and Raf-1 kinase. *Oncogene*. 1994;9:2751-2756.
23. Kurppa KJ, Caton J, Morgan PR, et al. High frequency of BRAF V600 E mutations in ameloblastoma. *J Pathol*. 2014;232:492-498.
24. Soltani M, Tabatabaiefar MA, Mohsenifar Z, et al. Genetic study of the BRAF gene reveals new variants and high frequency of the V600 E mutation among Iranian ameloblastoma patients. *J Oral Pathol Med*. 2018;47:86-90.
25. Fregnani ER, Perez DE, Paes de Almeida O, et al. BRAF-V600 E expression correlates with ameloblastoma aggressiveness. *Histopathology*. 2017;70:473-484.
26. Diniz MG, Gomes CC, Guimarães BV, et al. Assessment of BRAFV600 E and SMOF412 E mutations in epithelial odontogenic tumours. *Tumour Biol*. 2015;36:5649-5653.
27. Sun LS, Li XF, Li TJ. PTCH1 and SMO gene alterations in keratocystic odontogenic tumors. *J Dent Res*. 2008;87:575-579.
28. Shimada Y, Katsube K, Kabasawa Y, et al. Integrated genotypic analysis of hedgehog-related genes identifies subgroups of keratocystic odontogenic tumor with distinct clinicopathological features. *PLoS One*. 2013;8:e70995.
29. Wang Y, Li L, Zheng Y, et al. BMP activity is required for tooth development from the lamina to bud stage. *J Dent Res*. 2012;91:690-695.
30. Seppala M, Fraser GJ, Birjandi AA, Xavier GM, Cobourne MT. Sonic hedgehog signaling and development of the dentition. *J Dev Biol*. 2017;5.
31. Duan P, Bonewald LF. The role of the wnt/beta-catenin signaling pathway in formation and maintenance of bone and teeth. *Int J Biochem Cell Biol*. 2016;77:23-29.
32. Greenblatt MB, Kim JM, Oh H, et al. p38 alpha MAPK is required for tooth morphogenesis and enamel secretion. *J Biol Chem*. 2015;290:284-295.
33. Cobourne MT, Miletich I, Sharpe PT. Restriction of sonic hedgehog signalling during early tooth development. *Development*. 2004;131:2875-2885.
34. Diniz MG, Gomes CC, de Sousa SF, Xavier GM, Gomez RS. Oncogenic signalling pathways in benign odontogenic cysts and tumours. *Oral Oncol*. 2017;72:165-173.
35. Neuman AN, Montague L, Cohen D, Islam N, Bhattacharyya I. Report of two cases of combined odontogenic tumors: ameloblastoma with odontogenic keratocyst and ameloblastic

fibroma with calcifying odontogenic cyst. *Head Neck Pathol*. 2015;9:417-420.
36. Ledesma-Montes C, Gorlin RJ, Shear M, et al. International collaborative study on ghost cell odontogenic tumours: calcifying cystic odontogenic tumour, dentinogenic ghost cell tumour and ghost cell odontogenic carcinoma. *J Oral Pathol Med*. 2008;37:302-308.

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