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Factors affecting the accuracy of anti-BRAF V600E immunohistochemistry results in ameloblastomas

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Abstract

Background: There are still some controversies about the results of anti-BRAF V600E-specific antibody immunohistochemistry in ameloblastomas. This study aimed to examine the accuracy of V600E-specific antibody immunohistochemistry in detection of BRAF V600E mutation in ameloblastoma tissue sections of different ages.

Methods: The BRAF V600E status of 64 ameloblastoma specimens was assessed using both Sanger sequencing and V600E-specific antibody immunohistochemistry, and the sensitivity, specificity, positive predictive value, and negative predictive value were calculated. The difference in V600E-specific antibody immunohistochemistry staining intensity among the three groups of ameloblastoma tissue blocks of different ages was evaluated by chi-square test. The consistency between V600E-specific antibody immunohistochemistry and DNA sequencing results and the V600E-specific antibody immunohistochemistry staining intensity of 15 paired newly-cut and 3-month storage sections of the same 15 ameloblastomas were also compared.

Results: For detection of BRAF V600E mutation, the V600E-specific antibody immunohistochemistry had high sensitivity (98.21% 55/56), specificity (87.5% 7/8), positive predictive value (98.21% 55/56), and negative predictive value (87.5% 7/8). Heterogeneity of the staining intensity was observed in the same tissue section, but all or none expression pattern was noticed in the solid tumor nests. The storage time of paraffin tissue blocks ranging from 2 to 14 years did not affect the V600E-specific antibody-positive staining intensity. However, the three-month storage sections showed a significant diminishment of V600E-specific antibody-positive staining signals.

Conclusions: The BRAF V600E-specific antibody immunohistochemistry is suitable for routine detection of BRAF V600E mutation in ameloblastomas. The all or none expression pattern suggests the BRAF V600E mutation may be an early event in the pathogenesis of ameloblastoma.

KEYWORDS
ameloblastoma, BRAF V600E immunohistochemistry, DNA sequencing

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1 | INTRODUCTION

Since 2014, several studies revealed that activating MAPK pathway (FGFR2-RAS-BRAF) mutations play a predominant role in the pathogenesis of ameloblastoma.[1–4] Moreover, the most common mutation identified is BRAF V600E mutation, which is almost mutually exclusive from RAS and FGFR2 mutations, and the combined incidence from the initial studies is about 62.7% (96/153).[1–4] In addition to ameloblastoma, numerous neoplasms possess BRAF V600E mutation at different percentages, including melanoma,[5] papillary thyroid carcinoma,[6] colorectal cancer,[7] non-small cell lung cancer,[8] and so forth. The detection of BRAF V600E mutation is helpful in diagnosis and evaluation of the feasibility for BRAF inhibitor-targeted therapeutics in these patients with BRAF V600E mutation.

The common approaches to identify BRAF V600E mutation can be divided into two ways: (1) molecular methods, such as Sanger sequencing,[5] real-time PCR,[9] and next generation sequencing,[10] and (2) immunohistochemistry (IHC) using monoclonal antibody against BRAF V600E-mutated protein (clone VE1).[11,12] Many studies have compared the consistency between molecular and VE1 IHC assays to detect BRAF V600E mutation in various tumors. Good concordance for assessment of BRAF V600E mutation status is observed between DNA sequencing and VE1 IHC results in melanoma,[13] colorectal cancer,[12] papillary thyroid carcinoma,[14] and lung cancer.[15] Due to the high sensitivity and specificity, the VE1 IHC is considered as a reliable technique for the routine detection of the BRAF V600E mutation in the aforementioned diseases.

However, there are still some controversies about the performance of VE1 IHC in ameloblastomas. The initial VE1 IHC study done by Brown et al. in 50 cases of ameloblastoma showed 100% concordance between allele-specific PCR and VE1 IHC results.[2] However, some discrepancies are reported by the later studies. One false-negative VE1 IHC case was observed by Kurppa et al. in 20 solid/multicystic ameloblastoma cases.[3] Pereira et al. further stated inconsistency between VE1 IHC and Sanger sequencing results in ameloblastomas based on three false-positive cases out of eight ameloblastomas in their study.[16] Recent two studies related to the diagnostic utility of VE1 IHC in ameloblastomas showed 100% specificity and 83.8% sensitivity in one study[17] and 100% specificity and 95% sensitivity in the other study.[18]

This study aimed to test the feasibility of the VE1 IHC in ameloblastomas by evaluating the sensitivity and specificity of 64 ameloblastoma tissue specimens in which both VE1 IHC and Sanger sequencing were performed. To facilitate clinical application, we used the fully-automated BenchMark system and VENTANA BRAF V600E (VE1) assay from Roche Molecular Diagnostics (Rotkreuz, Switzerland) which has been proved for in vitro diagnostic (IVD) use. A DNA sequencing-proved *BRAF V600E* mutated papillary thyroid carcinoma and a *BRAF V600* wild-type dental follicle were used as positive and negative controls, respectively. In order to find out the best VE1 IHC condition for ameloblastoma, we used formalin-fixed and paraffin-embedded (FFPE) cell pellets of two ameloblastoma cell lines, AM-1 and AM-3, which are *BRAF* mutant and wild type, respectively, for adjusting staining protocol first. Moreover, considering the BRAF protein might degrade with time, we wondered the decline in BRAF V600E protein level might have a negative influence on the sensitivity of VE1 antibody in ameloblastoma. Therefore, the examination of staining intensity of VE1 IHC in the 64 FFPE ameloblastoma specimens according to the years of diagnosis was performed. Moreover, 15 paired newly-cut and 3-month storage sections from DNA sequencing-proved *BRAF V600E*-mutant FFPE ameloblastoma samples were also used to compare the VE1 IHC results.

2 | MATERIALS AND METHODS

2.1 | Tumor specimens

Sixty-four non-decalcified FFPE tissue specimens of ameloblastoma were included. The 64 specimens consisted of 25 follicular, 15 plexiform, 23 unicystic, and one granular cell ameloblastomas. All tissue blocks were obtained from the Department of Oral Pathology, National Taiwan University Hospital (NTUH), Taipei, Taiwan, from 2005 to 2017. The diagnosis was based on histological examination of hematoxylin and eosin-stained tissue sections by three board-certified oral pathologists (Chun-Pin Chiang, Julia Yu Fong Chang, and Yi-Ping Wang). The detailed epidemiologic data were summarized in Table S1. One dental follicle and one DNA sequencing-proved papillary thyroid carcinoma were used as negative and positive control, respectively. This study was approved by the Research Ethics Committee of NTUH (No. 201412058RINA, 201608088RINA, and 201901034RIND).

2.2 | DNA extraction, PCR, and Sanger sequencing

Two to five 10 μm-thick FFPE tissue sections were used for macrodissection of tumor components for DNA extraction. More than 80% of the tumor components of ameloblastoma were obtained in the dissected-off tissue samples. DNA extraction, PCR, and Sanger sequencing was performed as described previously.[19] Then, the sequencing data were analyzed with sequence alignment software (Bioedit Ltd., Manchester, UK).

2.3 | Cell lines and cell culture

Ameloblastoma cell lines (AM1 and AM3) were generous gifts from Dr. Shosei Kishida from Kagoshima University in Japan. These cells were cultured in KSFM (Defined Keratinocyte serum-free medium; Gibco, Billings, MT; Thermo Fisher Scientific) in a humidified incubator with 5% CO_2 at 37°C.

2.4 | BRAF VE1 immunohistochemistry

IHC was performed on 4 μm tissue sections using Ventana BenchMark GX autostainer (Ventana, Tucson, AZ). This automated process

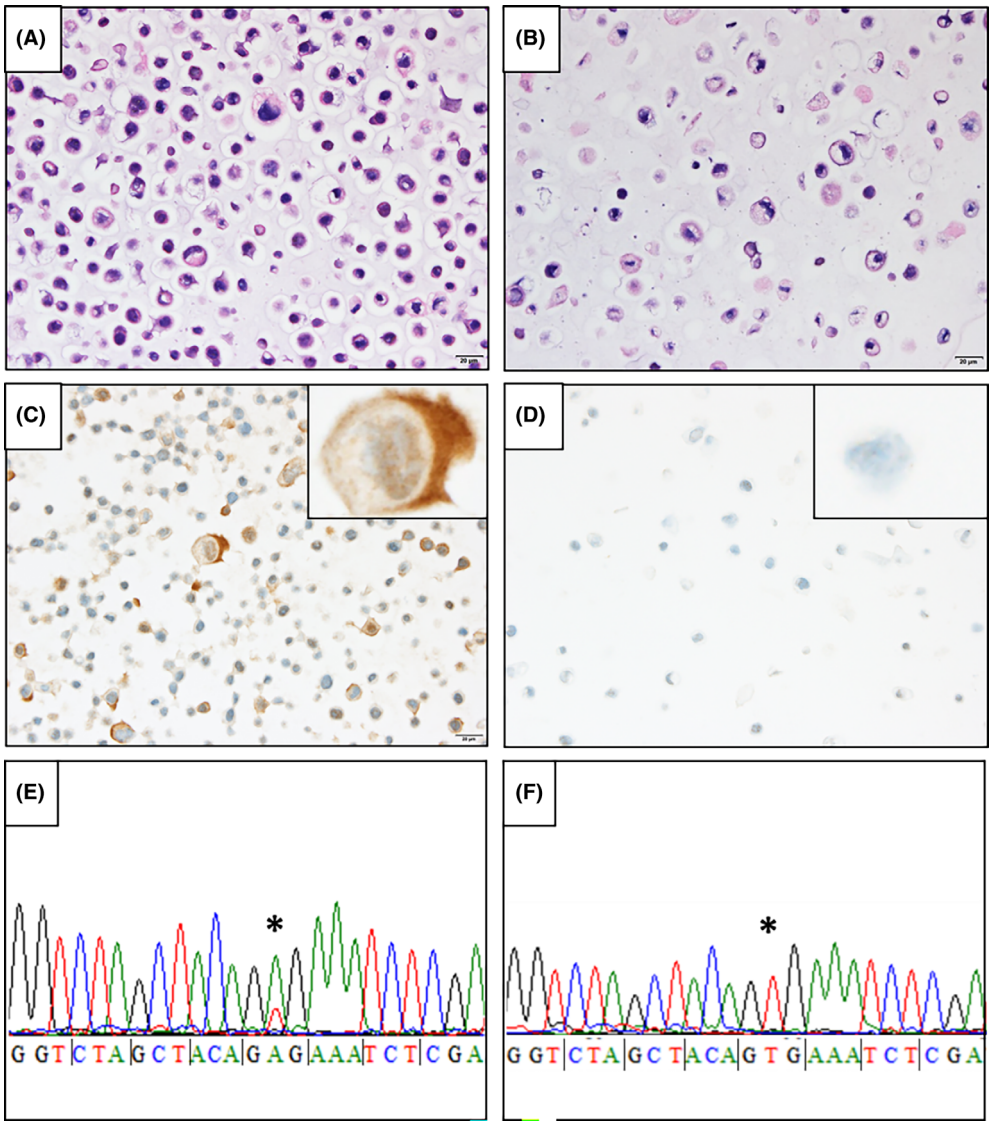

FIGURE 1 The histologic, BRAF VE1 immunohistochemistry, and *BRAF* DNA sequencing of ameloblastoma cell lines. (A and B) Hematoxylin and eosin-stained section of AM1 (A) and AM3 (B) cell pellet; scale bar = 20 μm. (C and D) BRAF VE1 immunohistochemistry for AM1 (C) and AM3 (D) cell pellet; scale bar = 20 μm. (E and F) *BRAF* DNA sequencing results for AM1 (E) and AM3 (F)

included deparaffinization by EZ prep (Ventana) and a CC1-based antigen retrieval using Cell Condition 1 solution (CC1; Tris-EDTA buffer [pH 8.0]) (Ventana) for 64 min. The slides were incubated with anti-BRAF V600E (clone VE1, Roche Molecular Diagnostics) ready-to-use antibody for 16 min. The VE1-positive cytoplasmic staining intensity was recorded as 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong) cytoplasmic staining. Labeling was detected with the OptiView DAB Detection Kit (Ventana) and then the immunostained sections were counterstained with hematoxylin.

2.5 | Double immunohistochemistry for BRAF VE1 and SOX2

The double immunohistochemical stains for the BRAF VE1 and SOX2 using the above staining protocol to stain BRAF VE1 first, and then a rabbit polyclonal antibody against the SOX2 protein (1:100 dilution; 3579 S; Cell Signaling, Danvers, MA) and EnVision Doublestain System (DAB+/Permanent Red) were used in Ventana BenchMark GX autostainer (Ventana) on the same sections.

2.6 | Statistical analysis

Assuming *BRAF V600E* sequencing is the gold standard for the identification of the gene mutation, the analysis of concordance between VE1 IHC and DNA sequencing was measured as sensitivity, specificity, positive predictive value, and negative predictive value. The association of categories of ameloblastoma subtypes (follicular, plexiform, and unicystic) and BRAF V600E mutation status was evaluated by chi-square test. The VE1 IHC staining intensity in three tissue block groups of different years including 2005–2008, 2009–2012, and 2013–2017 was also evaluated with chi-square test. All analyses were performed using SPSS, version 20 (IBM Corp., Armonk, NY). A p-value <0.05 was considered significant.

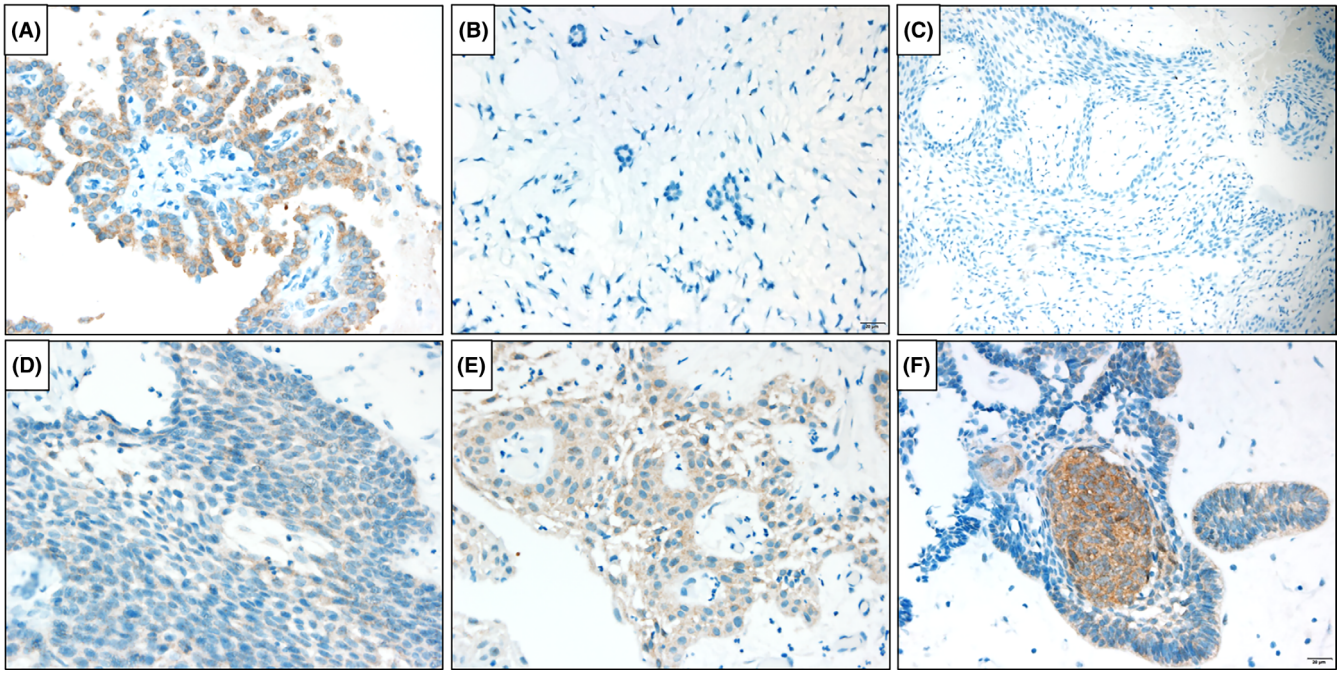

FIGURE 2 Representative microphotographs for BRAF VE1 immunohistochemistry. (A) Papillary thyroid carcinoma as positive control. (B) Dental follicle as negative control. (C) VE1-negative stain. (D) Weak VE1-positive cytoplasmic stain (1+). (E) Moderate VE1-positive cytoplasmic stain (2+). (F) Strong VE1-positive cytoplasmic stain (3+) (scale bar = 20 μm)

3 | RESULTS

3.1 | Establishment and verification of BRAF V600E automated immunohistochemistry protocol

As recommended by IVD level of BRAF V600E diagnostic kit, establishment and verification of the BRAF V600E automated IHC protocol was required for each laboratory and instrument. We first used Sanger sequencing results as the gold standard, *BRAF V600E* mutated ameloblastoma cell line AM1 and wild-type BRAF ameloblastoma cell line AM3 cell pellets as positive and negative controls, respectively, to verify the staining protocol of the diagnostic kit (Figure 1). Later, we used a DNA sequencing-proved *BRAF V600E* mutated papillary thyroid carcinoma as positive control and a *BRAF* wild-type dental follicle as negative control to further verify the staining efficacy (Figure 2A,B). Only the granular cytoplasmic staining was interpreted as VE1-positive staining. No background staining in the stromal tissue in our protocol was seen. Thus, even weak VE1-positive staining could still be identified and interpreted as positive result without doubt. Representative immunostained microphotographs for negative, weak, moderate, and strong BRAF V600E staining patterns were shown in Figure 2C–F.

3.2 | Comparison of BRAF V600E immunohistochemistry and DNA sequencing

The 64 FFPE samples of ameloblastoma were examined for BRAF V600E status using Sanger sequencing, and 56 (87.5%) of the 64 samples were identified with *BRAF V600E* gene mutation. The mutation rate was 88.0% (22/25) for the follicular type, 86.7% (13/15) for the plexiform type, 87.0% (20/23) for the unicystic type, and 100% (1/1) for the granular cell type ameloblastomas. No statistical difference in the mutation rate among the three main subtypes (follicular, plexiform, and unicystic) was noted ($p > 0.05$; Table S1).

Almost identical results were observed between VE1 IHC and *BRAF V600E* sequencing. Among the 56 sequencing-positive cases, 55 cases were correctly recognized by the VE1 IHC with the sensitivity of 98.21% (55/56). In the eight sequencing-negative cases, seven cases were correctly diagnosed by the VE1 IHC; therefore, the specificity was 87.5% (7/8). The positive predictive value and negative predictive value were 98.21% (55/56) and 87.5% (7/8), respectively (Table S2).

3.3 | Heterogeneity in VE1-positive staining intensity and all or none staining pattern

Due to the heterogeneity in VE1-positive staining pattern that might raise the concern of resistance to target therapy in the future, we particularly paid attention to the heterogeneity in VE1-positive staining intensity and pattern. Among 55 (because there was one false-negative) VE1 IHC positive cases, 40 (40/55, 72.7%) cases displayed heterogeneity in their staining intensities, but all of these cases showed all or none staining pattern (Figures 2C–F and 3A–F).

Some strong VE1-positive staining areas seemed to show budding features (Figure 3A,B). To elucidate whether these strong

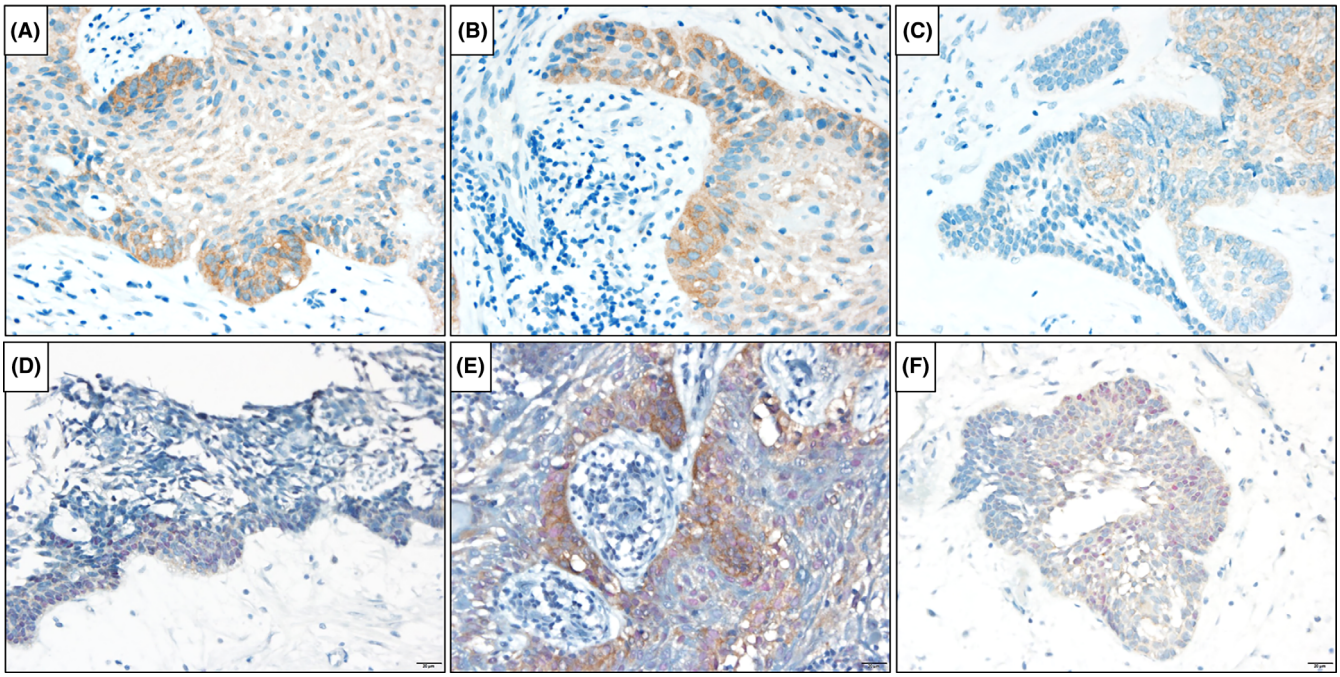

FIGURE 3 Representative microphotographs for BRAF VE1 immunohistochemistry demonstrating heterogeneous intensities. (A–C) BRAF VE1-positive staining sections with heterogeneous intensities. (D-F) BRAF VE1 (brown cytoplasmic stain)/Sox2 (red nuclear stain) double immunohistochemical staining sections with heterogeneous intensities (scale bar = 20 μm)

VE1-positive staining budding areas also harbor stem cell property, double staining of SOX2 and BRAF V600E were performed. Interestingly, these stronger VE1-positive staining areas also demonstrated strong SOX2-positive staining (Figure 3D,E).

We also recognized the histopathologic patterns frequently displaying heterogeneous staining intensity, such as the tumor cells of mural nests showed stronger staining than luminal lining cells (Figure 4A,B), the ameloblast-like cells showed weaker VE1-positive staining intensity than immature cells or other cell types (Figure 4C,D, arrows) and tumor cells in inflamed areas (Figure 4E) or in areas fused with mucosal surface epithelium showed weaker VE1-positive staining intensity (Figure 4F). Notably, smudged cells easily lost or reduced their VE1-positive staining (Figure 5A,B) and might affect the interpretation. Occasionally, VE1-positive nuclear staining areas were observed (Figure 5C, arrow), but only the granular VE1-positive cytoplasmic staining was interpreted as positive based on the manufacturer's instruction.

3.4 | The correlation between VE1-positive staining intensity and the year of FFPE ameloblastoma samples

The 64 FFPE ameloblastoma samples were divided into three groups according to the diagnosis year of these cases, including group I: 2005–2008, group II: 2009–2012, and group III: 2013–2017. The VE1-positive staining intensity of the three groups was shown in Figure 6. The distribution of the four grades of VE1-positive staining intensity (0, 1, 2, and 3) was similar in these three groups and there was no statistical difference among the three groups.

3.5 | The influence of timing of section cutting on VE1-positive staining intensity in ameloblastomas

The 15 paired newly-cut sections and 3-month storage sections from the same paraffin blocks had distinct VE1 IHC results. The 15 newly-cut sections all revealed positive VE1 IHC results, but 9 (60%) of the 15 3-month storage sections showed negative cytoplasmic staining and 6 VE1-positive staining cases displayed much weaker staining compared to their counterpart newly-cut sections.

4 | DISCUSSION

Ameloblastoma is a locally aggressive odontogenic neoplasm and has a high risk for recurrence, so surgical resection is often required to avoid the subsequent operations. A definitive diagnosis of the biopsy specimen is important for doing a proper treatment. So far, *BRAF V600E* mutation is thought to be the most frequent genetic alteration in ameloblastomas, although some other odontogenic tumors with ameloblastic features also have been reported to harbor *BRAF V600E* mutations.[20] Detection of *BRAF V600E* status is also beneficial in differential diagnosis of odontogenic lesions, especially in small biopsy or severe inflamed cases in which the histopathological pattern is disturbed. Moreover, with several studies bringing insight into the

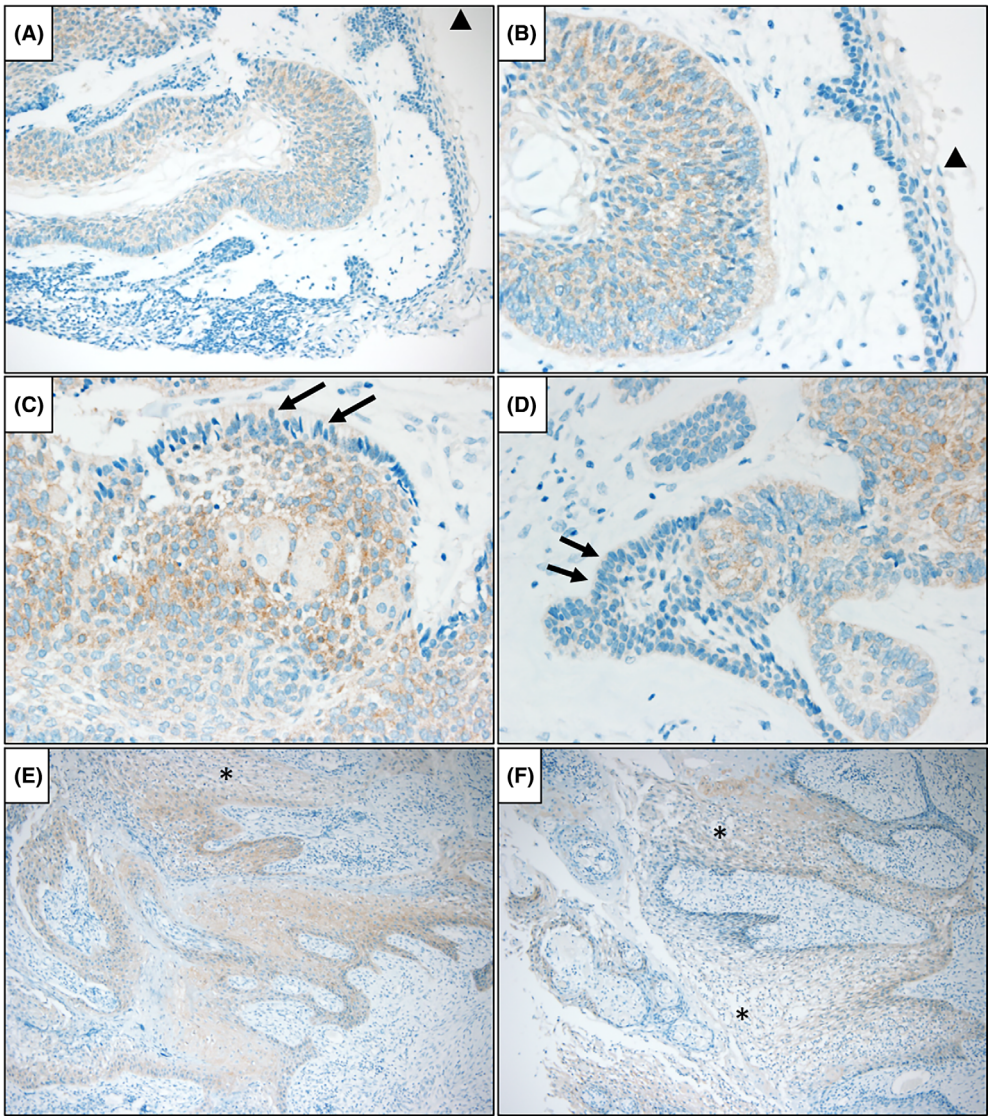

FIGURE 4 Representative microphotographs for BRAF VE1 immunohistochemistry demonstrating heterogeneous intensities in different conditions. (A and B) Moderate VE1-positive staining in the tumor cells of mural nests and weak VE1-positive staining in luminal (arrow head) epithelial cells; A: 200×, B: 400×. (C and D) Weak VE1-positive staining in secretory ameloblast-like cells (arrows), C and D, 200×. (E and F) Weak VE1-positive staining in (E) inflamed area (star) and (F) areas fused with mucosal surface epithelium (stars), E and F, 100×.

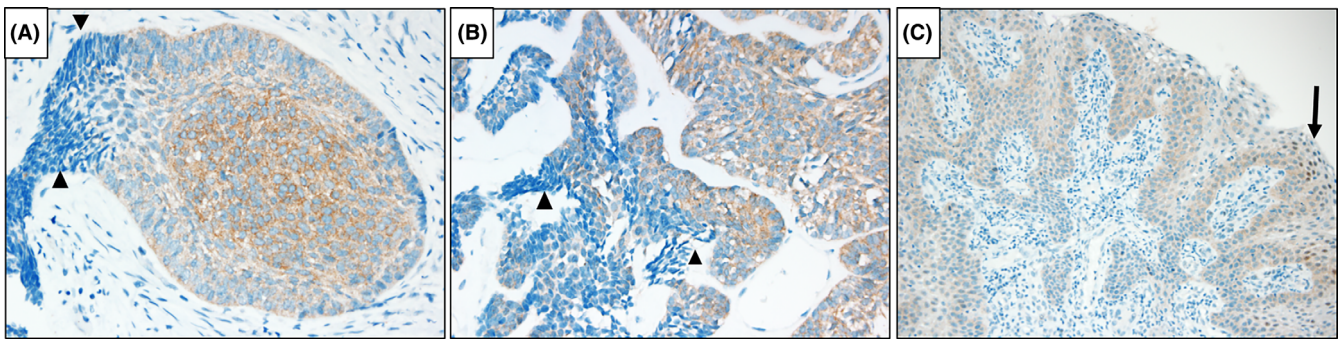

FIGURE 5 Representative microphotographs for BRAF VE1 immunohistochemistry demonstrating loss of stain and nuclear staining pattern. (A and B) VE1-negative staining in areas with smudged cells and nuclei (arrow heads). (C) VE1-positive nuclear staining pattern (arrow). (A–C, 400×)

molecular pathogenesis of ameloblastoma recently, the development of molecule-targeted therapy is foreseeable. Clinically, to examine the feasibility of BRAF-targeted therapy for ameloblastoma patients, the first step is to investigate whether there is *BRAF V600E* mutation in the ameloblastoma. Therefore, to find out a credible method for routinely identifying *BRAF* mutation status in ameloblastoma is critical.

Some studies have compared the VE1 IHC and *BRAF* DNA sequencing in ameloblastomas.[1–3,17,21–23] The highest sensitivity and

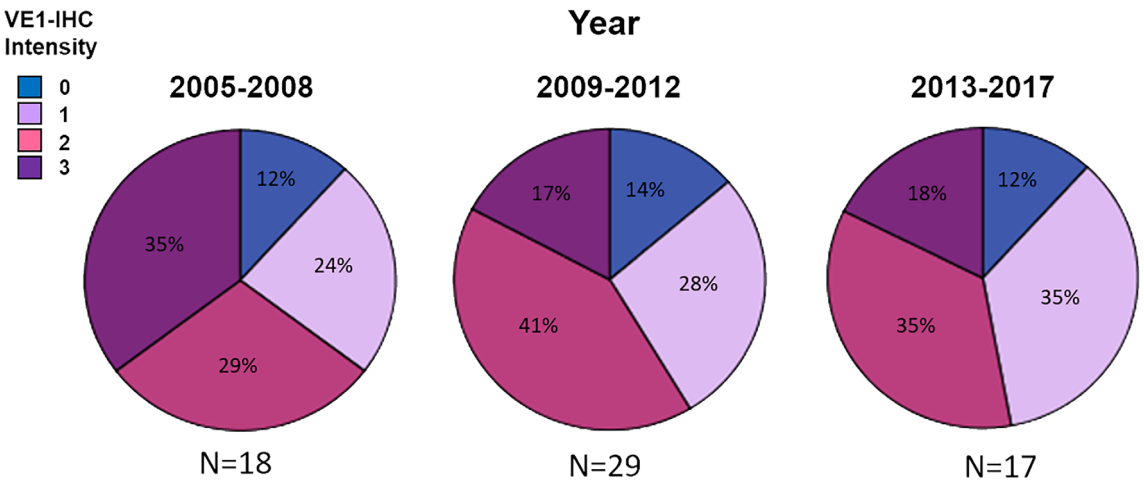

FIGURE 6 The illustration of BRAF VE1 immunohistochemical results in formalin-fixed and paraffin-embedded tissue sections with different ages. 0—negative staining; 1—1+ weak; 2—2+ moderate; 3—3+ strong staining intensities

specificity can reach 100%,[2] however, VE1-positive staining was observed in DNA sequencing-negative cases of ameloblastoma and even in a case of dentigerous cyst in one report.[16] The concordance between VE1 IHC and *BRAF* DNA sequencing was extensively examined and discussed in the initial verification of VE1 IHC in melanomas, thyroid papillary carcinoma, and colorectal carcinomas.[12,24–26] The results of these investigations indicate that rigorous in-house antibody validation is required in each laboratory before using the VE1 IHC for confirmation of *BRAF* gene mutation in clinical practice. Therefore, we first established and validated the staining protocol and evaluated the concordance of VE1 IHC and *BRAF* DNA sequencing. Due to frequent weaker VE1-positive staining in ameloblastomas comparing to the papillary thyroid carcinomas, which might interfere with the interpretation if any background existed, our setting was none of background staining in the normal thyroid follicular cells, and connective tissue parts in thyroid, dental follicle, and ameloblastoma. Thus, we could still clearly recognize and interpret the VE1-positive staining without doubt even in cases with very weak VE1-positive staining intensity.

In this study, considering DNA sequencing was the gold standard method for identification of *BRAF V600E* mutation, we observed that the VE1 IHC result was quite consistent with *BRAF V600E* DNA sequencing result. The high sensitivity (98.21% 55/56) and specificity (87.5% 7/8) were discovered. Only two mismatched cases were found, including one false-negative and one false-positive sample. The false-negative case was a follicular ameloblastoma. Prominent crushed artifact was noted in this section, and this might affect the accuracy of VE1 IHC result. The false-positive case was a unicystic ameloblastoma. Due to the difficulty in macro-dissection and low tumor content, the DNA sequencing signal from the scanty epithelial component might be overridden by stromal negative signal. In addition to low tumor content, Sanger sequencing is known as an assay with lower sensitivity than pyrosequencing and allele-specific PCR assays.[12,27] Thus, this might result in spurious false-positive results on VE1 IHC, which in turn to cause an inaccurately low measurement of specificity.

Based on the manufacturer's initial investigation and instruction,[12] the VE1 IHC would be interpreted as positive when unequivocal diffuse cytoplasmic staining in more than 85% of tumor cells. In this study, the VE1-positive staining intensity expressed in tumor cells was scored in a 0–3 scale. Our study showed common heterogeneity in VE1-positive staining intensity with frequent weak staining, but all cases demonstrated all or none expression pattern in ameloblastomas, suggesting that *BRAF V600E* mutation may be an early event in the pathogenesis of ameloblastomas. One of our cases displayed both nuclear and cytoplasmic VE1-positive staining patterns in some tumor cells. We interpreted the VE1-positive nuclear staining as negative. However, because of the existence of VE1-positive cytoplasmic staining, *BRAF V600E* mutation was confirmed in this case. Based on the manufacturer's instruction, VE1-positive nuclear staining was sometimes observed in normal or neoplastic colonic epithelial cells, and this VE1-positive nuclear staining was considered to be negative.[21] The VE1-positive nuclear staining pattern has also been reported in some ameloblastomas.[16,17,28] One case has solely VE1-positive nuclear staining without VE1-positive cytoplasmic staining,[17] which might cause some difficulties in interpretation. The significance of VE1-positive nuclear staining pattern is unclear.[12] Therefore, further molecular studies to confirm the status of *BRAF* mutation in these cases with solely VE1-positive nuclear staining pattern are mandatory before starting the targeted therapy.

In this study, we noted some common factors affecting the VE1-positive staining intensity: (1) tumor cells of the mural nests showed stronger VE1-positive staining than luminal lining cells, (2) the ameloblast-like cells revealed weaker VE1-positive staining than immature cells or other cell types, and (3) tumor cells in inflamed areas or in areas fused with mucosal surface epithelium displayed weaker VE1-positive staining intensity. Interestingly, some tumor cell budding areas which showed strong VE1-positive staining were also enriched in the SOX2 expression. Our previous study also demonstrated the enrichment of Sox2-positive cells in BRAF V600E mutated and recurrent ameloblastoma.[19] Future studies to investigate whether the

ameloblastoma is started with Sox2-positive cells which subsequently acquire *BRAF V600E* mutation may be beneficial to understand the pathogenesis of ameloblastoma.

We also tried to understand the factors affecting the accuracy of VE1 IHC results. Notably, smudged cells mainly caused by crushed or cautery artifacts easily lost or reduced their VE1-positive staining, and this might affect the accuracy of VE1 IHC. Previous report also mentioned the absence of VE1-positive staining in areas with desmoplastic phenotype.[17] We further analyzed whether the age of the tissue blocks and the 3-month storage tissue sections would affect the accuracy of VE1 IHC. Interestingly, we found that the age of the paraffin tissue blocks did not affect the VE1-positive staining intensity, but the 3-month storage tissue sections showed significantly effaced or reduced VE1-positive staining intensity. It is possible that because our environment was humid and hot, and thus the antigens in thin sections might be easily destroyed. Therefore, the use of freshly-cut tissue sections to perform VE1 IHC is recommended. Furthermore, the manufacturer's original article has examined the fixation condition for proper VE1 IHC results and suggests that the tissue specimen within 2 h of cold ischemic time should be fixed in 10% neutral buffered formalin for 12–24 h.[12]

In conclusion, our study showed good concordance of VE1 IHC with DNA sequencing. Vigorous in-house antibody validation is required in each laboratory before using the VE1 IHC for determination of the presence of BRAF V600E mutation for clinical practice. The VE1 IHC can be used as an initial screening test for determination of the presence of BRAF V600E mutation in ameloblastomas. Freshly-cut tissue sections are needed for performing the VE1 IHC. Areas with smudged tumor cells and nuclei can affect the accuracy of VE1 IHC staining and should be interpreted with caution. The VE1-negative staining or nuclear staining cases should be further evaluated using the molecular assays to detect the BRAF V600E mutation in ameloblastomas.

AUTHOR CONTRIBUTIONS

Julia Yu Fong Chang: Conception and design of study; supervision; methodology; data curation; drafting and revising the manuscript; resources. **Pei Hsuan Lu:** Methodology; data curation; writing – original draft. **Chih-Huang Tseng:** Validation; data curation. **Yi-Ping Wang**, **Jang-Jaer Lee:** Formal analysis; data curation. **Chun-Pin Chiang:** Conception and design of study; supervision; data curation; revising the manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest relevant to this article.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

All data included in this study appear in the article, and are available upon request by contact with the corresponding author.

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REFERENCES

1. Brown NA, Betz BL. Ameloblastoma: a review of recent molecular pathogenetic discoveries. *Biomark Cancer*. 2015;7(Suppl 2):19-24.
2. Brown NA, Rolland D, McHugh JB, et al. Activating FGFR2-RAS-BRAF mutations in ameloblastoma. *Clin Cancer Res*. 2014;20:5517-5526.
3. Kurppa KJ, Caton J, Morgan PR, et al. High frequency of BRAF V600E mutations in ameloblastoma. *J Pathol*. 2014;232:492-498.
4. Sweeney RT, McClary AC, Myers BR, Biscocho J, Neahring L. Identification of recurrent SMO and BRAF mutations in ameloblastomas. *Nat Genet*. 2014;46:722-725.
5. Gorden A, Osman I, Gai W, et al. Analysis of BRAF and N-RAS mutations in metastatic melanoma tissues. *Cancer Res*. 2003;63:3955-3957.
6. Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA. High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. *Cancer Res*. 2003;63:1454-1457.
7. Yuen ST, Davies H, Chan TL, et al. Similarity of the phenotypic patterns associated with BRAF and KRAS mutations in colorectal neoplasia. *Cancer Res*. 2002;62:6451-6455.
8. Ding L, Getz G, Wheeler DA, et al. Somatic mutations affect key pathways in lung adenocarcinoma. *Nature*. 2008;455:1069-1075.
9. Jarry A, Masson D, Cassagnau E, Parois S, Laboisse C, Denis MG. Real-time allele-specific amplification for sensitive detection of the BRAF mutation V600E. *Mol Cell Probes*. 2004;18:349-352.
10. Sakai K, Tsurutani J, Yamanaka T, et al. Extended RAS and BRAF mutation analysis using next-generation sequencing. *PLoS One*. 2015;10:e0121891.
11. Capper D, Preusser M, Habel A, et al. Assessment of BRAF V600E mutation status by immunohistochemistry with a mutation-specific monoclonal antibody. *Acta Neuropathol*. 2011;122:11-19.
12. Dvorak K, Aggeler B, Palting J, McKelvie P, Ruszkiewicz A, Waring P. Immunohistochemistry with the anti-BRAF V600E (VE1) antibody: impact of pre-analytical conditions and concordance with

DNA sequencing in colorectal and papillary thyroid carcinoma. *Pathology*. 2014;46:509-517.
13. Manfredi L, Meyer N, Tournier E, et al. Highly concordant results between immunohistochemistry and molecular testing of mutated V600E BRAF in primary and metastatic melanoma. *Acta Derm Venereol*. 2016;96:630-634.
14. Sun Y, Shi C, Shi T, Yu J, Li Z. Correlation between the BRAF(v600E) gene mutation and factors influencing the prognosis of papillary thyroid microcarcinoma. *Int J Clin Exp Med*. 2015;8:22525-22528.
15. Gow CH, Hsieh MS, Lin YT, Liu YN, Shih JY. Validation of immunohistochemistry for the detection of BRAF V600E-mutated lung adenocarcinomas. *Cancers (Basel)*. 2019;11:866.
16. Pereira NB, Pereira KM, Coura BP, et al. BRAFV600E mutation in the diagnosis of unicystic ameloblastoma. *J Oral Pathol Med*. 2016;45:780-785.
17. Mendez LD, Wolsefer NS, Asa SL, Wasman J, Yoest JM, Stojanov IJ. The diagnostic utility of BRAF VE1 mutation-specific immunohistochemistry in ameloblastoma. *Mod Pthol*. 2022;35:1570-1577.
18. Martins-de-Barros AV, Anjos RSD, Silva CCG, Silva E, Araujo F, Carvalho MV. Diagnostic accuracy of immunohistochemistry compared with molecular tests for detection of BRAF V600E mutation in ameloblastomas: systematic review and meta-analysis. *J Oral Pathol Med*. 2022;51:223-230.
19. Tseng CH, Lu PH, Wang YP, Chang JYF. Enrichment of SOX2-positive cells in BRAF V600E mutated and recurrent ameloblastoma. *J Pers Med*. 2022;12:77.
20. Brunner P, Bihl M, Jundt G, Baumhoer D, Hoeller S. BRAF p.V600E mutations are not unique to ameloblastoma and are shared by other odontogenic tumors with ameloblastic morphology. *Oral Oncol*. 2015;51:e77-e78.
21. Heikinheimo K, Huhtala JM, Thiel A, et al. The mutational profile of unicystic ameloblastoma. *J Dental Res*. 2019;98:54-60.
22. Oh KY, Cho SD, Yoon HJ, Lee JI, Hong SD. Discrepancy between immunohistochemistry and sequencing for BRAF V600E in odontogenic tumours: comparative analysis of two VE1 antibodies. *J Oral Pathol Med*. 2021;50:85-91.
23. da Silva Marcelino BMR, Parise GK, do Canto AM, et al. Comparison of immunohistochemistry and DNA sequencing for BRAF V600E mutation detection in mandibular ameloblastomas. *Appl Immunohistochem Mol Morphol*. 2021;29:390-393.
24. Adackapara CA, Sholl LM, Barletta JA, Hornick JL. Immunohistochemistry using the BRAF V600E mutation-specific monoclonal antibody VE1 is not a useful surrogate for genotyping in colorectal adenocarcinoma. *Histopathology*. 2013;63:187-193.
25. Bledsoe JR, Kamionek M, Mino-Kenudson M. BRAF V600E immunohistochemistry is reliable in primary and metastatic colorectal carcinoma regardless of treatment status and shows high intratumoral homogeneity. *Am J Surg Pathol*. 2014;38:1418-1428.
26. Kuan SF, Navina S, Cressman KL, Pai RK. Immunohistochemical detection of BRAF V600E mutant protein using the VE1 antibody in colorectal carcinoma is highly concordant with molecular testing but requires rigorous antibody optimization. *Human Pathol*. 2014;45:464-472.
27. Ritterhouse LL, Barletta JA. BRAF V600E mutation-specific antibody: a review. *Semin Diagn Pathol*. 2015;32:400-408.
28. Seki-Soda M, Sano T, Ito K, Yokoo S, Oyama T. An immunohistochemical and genetic study of BRAF(V600E) mutation in Japanese patients with ameloblastoma. *Pathol Int*. 2020;70:224-230.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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