Ameloblastoma: A Review of Recent Molecular Pathogenetic Discoveries

Supplementary Issue: Signaling Pathways as Biomarkers

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ABSTRACT: Ameloblastoma is an odontogenic neoplasm whose molecular pathogenesis has only recently been elucidated. The discovery of recurrent activating mutations in *FGFR2, BRAF*, and *RAS* in a large majority of ameloblastomas has implicated dysregulation of MAPK pathway signaling as a critical step in the pathogenesis of this tumor. Some degree of controversy exists regarding the role of mutations affecting the sonic hedgehog (SHH) pathway, specifically Smoothened (SMO), which have been postulated to serve as either an alternative pathogenetic mechanism or secondary mutations. Here, we review recent advances in our understanding of the molecular pathogenesis of ameloblastoma as well as the diagnostic, prognostic, and therapeutic implications of these discoveries.

KEYWORDS: ameloblastoma, MAPK, *BRAF, RAS*, odontogenic tumor, therapy

Introduction

Ameloblastoma is an uncommon, locally invasive odontogenic neoplasm arising in the jaw. The average age of diagnosis is 36 years, with equal incidence in men and women.[1] Most ameloblastomas (up to 80%) occur in the posterior mandible, with fewer tumors arising in the maxilla.[1] Patients typically present with painless swelling of the jaw, and the vast majority of ameloblastomas are slow-growing neoplasms without metastatic potential. However, ameloblastic tumors with cytologic atypia are classified as ameloblastic carcinoma and have a propensity for rapid growth and metastasis.[2] Rarely, ameloblastic neoplasms metastasize in spite of a benign histologic appearance, and they are classified as metastasizing ameloblastoma. Current treatment options for ameloblastoma include both conservative treatment (enucleation or curettage) and resection. The former is associated with high rates of recurrence, while the latter results in significant facial deformity and morbidity.[3]

Ameloblastoma is thought to arise from cells of the dental lamina[4] and resembles structures of the cap/bell stage of the developing tooth.[5] In the 2005 World Health Organization (WHO) classification, ameloblastomas include four subtypes based on location and histopathology: solid/multicytic (91%), unicystic (6%), extra-osseous (2%), and desmoplastic (1%).[6] Histologically, most tumors display a follicular pattern characterized by islands of epithelium within fibrous stroma that lacks inductive capacity to form hard tissue. The epithelium is comprised of columnar, preameloblast-like, palisaded cells with reverse polarization at the periphery, and loosely arranged cells resembling the stellate reticulum in the center (Fig. 1). However, some tumors display a plexiform pattern of epithelium with inconspicuous stellate reticulum.

While the molecular pathogenesis of ameloblastoma was largely unknown prior to 2014, there was mounting evidence suggesting that activation of the mitogen-activated protein kinase (MAPK) pathway plays a prominent role. Several studies demonstrated activation of components of the MAPK pathway in an ameloblastoma cell line (AM-1) under various circumstances, including stimulation with tumor necrosis factor alpha (TNF α)[7] and fibroblast growth factors 7 and 10.[8] In addition, transgenic mice expressing v-Ha-Ras under the zeta-globin promoter were shown to develop odontogenic tumors resembling ameloblastoma.[9]

MAPK Pathway Mutations

In 2014, three separate reports identified recurrent MAPK mutations in ameloblastoma.[10–12] The most common and first mutation identified was *BRAF* V600E. Two of these reports found *BRAF* mutations at a similar frequency (64% and 63%; 54/84 and 15/24),[10,12] while a third demonstrated a lower frequency (46%; 13/28).[11] A more recent study reported *BRAF* mutations in 82% (14/17) of cases.[13] The combined incidence from all four studies is 62.7% (96/153).
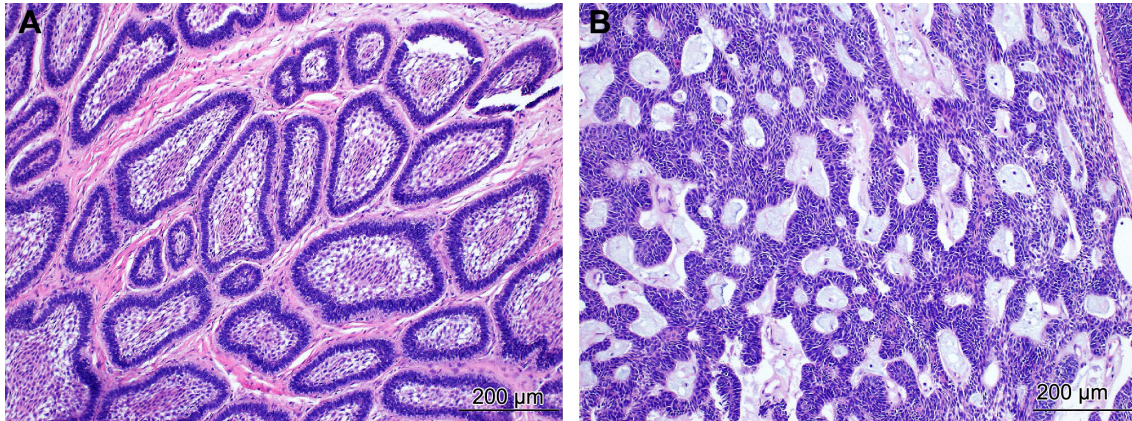
Figure 1. Histopathology of ameloblastoma. (**A**) The follicular pattern with islands of odontogenic epithelium within fibrous stroma. The epithelium consists of peripheral palisading cells showing reverse polarization and central loosely arranged cells resembling the stellate reticulum. H&E staining (×100). (**B**) The plexiform pattern with anastomosing strands of basal cells, delicate stroma, and inconspicuous stellate reticulum. H&E staining (×100).

BRAF is a serine-threonine kinase within the MAPK pathway. The V600E mutation is present in numerous neoplasms including melanoma,[14] hairy cell leukemia,[15] papillary thyroid carcinoma,[16] Langerhans cell histiocytosis,[17] and colorectal cancer.[18] This mutation results in constitutive activation of the BRAF protein and downstream MEK and ERK signaling, enhancing cell proliferation, survival, and ultimately neoplastic transformation.[19] Both Brown et al[12] and Sweeney et al[11] also identified the *BRAF* V600E mutation in the ameloblastoma cell line AM-1, and demonstrated evidence of in vitro activation of MAPK signaling that was blocked by BRAF inhibition.

In addition to *BRAF*, two studies identified mutations affecting other genes in the MAPK pathway upstream of *BRAF* (Fig. 2).[11,12] The BRAF protein is normally activated by the G-protein RAS. *RAS* mutations were identified in up to 20% of ameloblastomas, including *KRAS*, *NRAS*, and *HRAS*.[12] All *RAS* mutations occurred at sites commonly mutated in other neoplasms (codons 12 and 61) and are known to lead to constitutive activation of RAS signaling. The activation of RAS and the remainder of the MAPK pathway is normally triggered by the activation of a growth factor receptor in response to a growth factor. Fibroblast growth factor receptor 2 (FGFR2) is one of several receptors
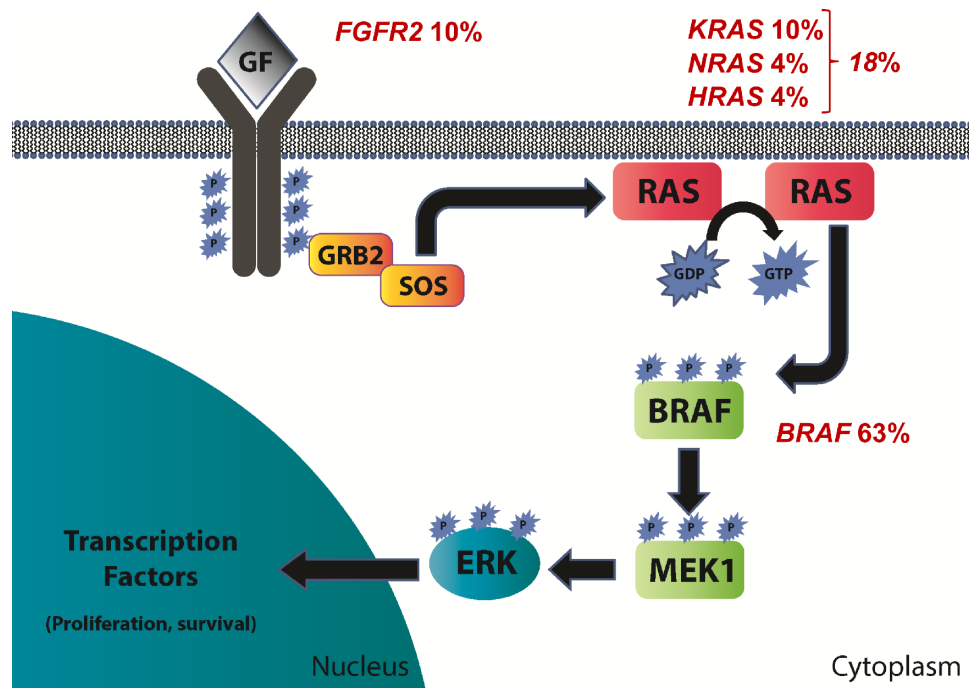
Figure 2. Schematic of the mitogen activated protein kinase (MAPK) pathway with mutation frequencies in ameloblastoma based on all studies in which each gene was evaluated.[10–13]

that activate MAPK signaling. *FGFR2* mutations were identified in 6%–18% of ameloblastomas,[11,12] occurring in either the transmembrane (C382R and V395D) or kinase domain (N549K) of the receptor. These mutations have been described in both endometrial carcinoma and craniosynostosis and are known to result in constitutive MAPK pathway activation that is abrogated by treatment with FGFR inhibitors.[20–23] Together, *FGFR2*, *RAS*, and *BRAF* mutations are present in 78%–88% of ameloblastomas. Importantly, mutations affecting these genes were mutually exclusive in all 65 cases described except one (Fig. 3). This case from Sweeney et al[11] demonstrated concomitant mutations of *BRAF* and *FGFR2*. The high prevalence and near-complete mutual exclusivity of these mutations suggests that activation of the MAPK pathway likely represents a critical event that occurs early in the pathogenesis of ameloblastoma.

SMO and Other Mutations

Several mutations were identified within genes not involved in the MAPK pathway. These included *SMO*, *CTNNB1*, *PIK3CA*, and *SMARCB1*. Of these, *SMO* mutations were the most frequent, occurring in 16%–39% of cases.[11,12] *SMO* mutations included W535L and L412F, which have been previously described in basal cell carcinoma[24,25] and meningioma,[26,27] as well as a novel mutation G416E. The Smoothened (SMO) protein is a nonclassical G-protein-coupled receptor that mediates sonic hedgehog (SHH) signaling and is normally repressed by patched (PTCH1) in the absence of the Hedgehog ligand.[28] Polymorphisms and deleterious germline mutations within *PTCH1* have been shown to affect the risk of ameloblastoma.[29,30] Sweeney et al[11] demonstrated increased sonic hedgehog signaling activity in *Smo*^{−/−} mouse embryonic fibroblasts carrying SMO L412F. Furthermore, the effect of this mutation was inhibited by pharmacologic inhibitors of SHH signaling, including KAAD-cyclopamine and arsenic trioxide.

It is unclear whether MAPK and the Hedgehog pathway mutations represent two molecular subclasses of ameloblastoma, as suggested by Sweeney et al,[11] or whether *SMO* mutations function as secondary events with MAPK pathway activation

being the essential driver of pathogenesis, as suggested by Brown et al.[12] *BRAF* and *SMO* were the two most frequently mutated genes in both studies, and mutations in these genes were mutually exclusive with one another in all but three instances (16% of *SMO* mutated cases). However, *SMO* mutations frequently co-occurred with *RAS* mutations (37% of *SMO* mutated cases) and *FGFR2* mutations (32% of *SMO* mutated cases). Sixteen percent of *SMO* mutations occurred in the absence of any MAPK pathway mutations, accounting for 4% of ameloblastomas overall.

Brown et al[12] also identified mutations in several other genes at a lower frequency. These included *CTNNB1*, *PIK3CA*, and *SMARCB1* present in 4%, 6%, and 6% of cases, respectively. These mutations were not mutually exclusive with one another or with MAPK pathway or *SMO* mutations. All mutations have previously been described in other neoplasms. It is unclear precisely what role these mutations play in the pathogenesis of ameloblastoma.

MAPK Mutations in Other Odontogenic Tumors

Two studies investigated the pathogenetic specificity of MAPK pathway mutations, particularly *BRAF* V600E, by evaluating other odontogenic tumors. In one study, *BRAF* mutations were identified in 2 ameloblastic fibromas and 1 ameloblastic fibrodentinoma but were not identified in 37 other odontogenic tumors. These included ameloblastic carcinoma, odontoameloblastoma, clear cell odontogenic carcinomas, adenomatoid odontogenic tumor, keratocystic odontogenic tumor, calcifying cystic odontogenic tumor, calcifying epithelial odontogenic tumor, odontogenic fibroma, and odontogenic myxoma.[12] A subsequent study identified *BRAF* V600E mutations in 3/8 (38%) ameloblastic carcinomas and 1/1 clear cell odontogenic tumor, but found no mutations in either of the two ghost cell odontogenic carcinomas.[13] The presence of *BRAF* mutations in ameloblastic carcinoma and ameloblastic fibroma/fibrodentinoma suggests that these tumors may be pathogenetically related to ameloblastoma. Some ameloblastic carcinomas appear to arise from a pre-existing, benign ameloblastoma and are therefore designated dedifferentiated ameloblastic carcinoma.[2] While ameloblastic fibromas and fibrodentinomas
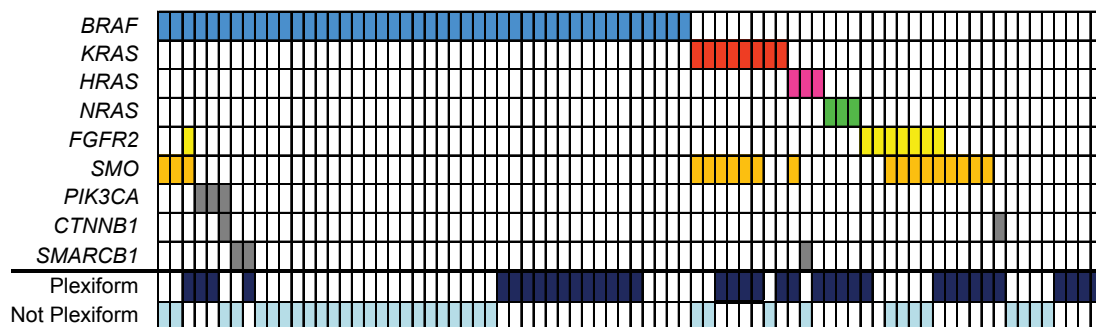
Figure 3. Summary of *BRAF*, *KRAS*, *HRAS*, *NRAS*, *FGFR2*, *SMO*, *PIK3CA*, *CTNNB1*, and *SMARCB1* mutations in ameloblastoma based on two studies in which all of these genes were evaluated.[11,12] Colored boxes indicate the presence of mutations in the indicated genes (rows) and samples (columns). The histologic pattern (plexiform versus non-plexiform) is also indicated (if known).

tend to occur in younger individuals and exhibit less locally aggressive behavior, these tumors are histologically very similar to classic ameloblastoma, differing primarily in the appearance of the stroma surrounding odontogenic epithelium.[31] Overall, these findings suggest that ameloblastic tumors are a distinct group of odontogenic tumors with characteristic genetic abnormalities. These findings also implicate the *BRAF* V600E mutation as a potential diagnostic marker in the work-up of odontogenic tumors that is specific for ameloblastic tumors. Notably, Brown et al[10] observed perfect concordance between VE1 immunohistochemistry and the molecular detection of *BRAF* V600E mutations, demonstrating that both techniques may be useful in the diagnosis of ameloblastic tumors.

Clinicopathologic Associations

The mutation profile of ameloblastomas correlates with histopathology, location (Fig. 4), age at diagnosis, and prognosis (Fig. 5). As stated earlier, Sweeney et al[11] postulated that *BRAF*-mutated and *SMO*-mutated tumors represent two distinct molecular subtypes with ameloblastoma with different clinicopathologic features including location, histologic pattern (follicular versus plexiform), and possibly prognosis. The former two showed statistically significant associations with genotype (*BRAF*-mutated vs *SMO*-mutated). However, in the larger series from Brown et al,[12] these associations correlated better with the presence or absence of the *BRAF* mutation rather than the presence of *SMO* mutations, which were found in only a minority of *BRAF* wild-type tumors (37%). *BRAF* mutations were shown to occur much more frequently in the mandible and only rarely in the maxilla (5.6%), while 43% of *BRAF* wild-type tumors arose in the maxilla. This trend was not specific for *SMO*-mutated tumors; indeed, 64% of *BRAF* wild-type, *SMO* wild-type tumors also arose in the maxilla.

Sweeney et al[11] observed that 80% of ameloblastomas with the plexiform histologic pattern were *BRAF* wild-type, *SMO* mutant ($P < 0.02$). Brown et al[10] did not comment on the relationship of follicular/plexiform pattern and genotype. However, a review of their data shows that, while there was no statistically significant association between plexiform histology and *SMO* mutations (50% frequency in *SMO*-mutated compared to 43% frequency in *SMO* wild-type), plexiform histology was significantly more common among *BRAF* wild-type tumors (62%) compared with *BRAF*-mutated tumors (35%; $P < 0.02$). Similarly, when data from these two studies are combined (Fig. 3), plexiform histology remains significantly more common among *BRAF* wild-type tumors (62% versus 36%; $P = 0.026$), while *SMO* status shows no significant association.

Brown et al[12] also found that *BRAF* mutations occurred in younger patients with a mean age at diagnosis of 34.5 years compared to 53.6 in *BRAF* wild-type cases ($P < 0.0001$). Similarly, the mean age at diagnosis among *BRAF* wild-type, *SMO* wild-type cases was 57.2 years. In addition, this study showed that *BRAF* V600E is an independent predictor of recurrence-free survival with *BRAF* wild-type tumors recurring earlier ($P = 0.046$). No statistically significant association was observed between recurrence and *SMO* mutation status.

Overall, several clinicopathologic features correlate with the presence or absence of *BRAF* mutations and are not specific for *SMO* mutations. These findings are analogous to *BRAF* V600E mutations in melanoma, which also occur in younger patients and have a different anatomic distribution compared with *NRAS* and other mutations.[32–35] In melanoma, different anatomic distributions are thought to result from differences in ultraviolet light exposure. It is unclear why the anatomic distribution differs between *BRAF* V600E and *BRAF* wild-type ameloblastomas.
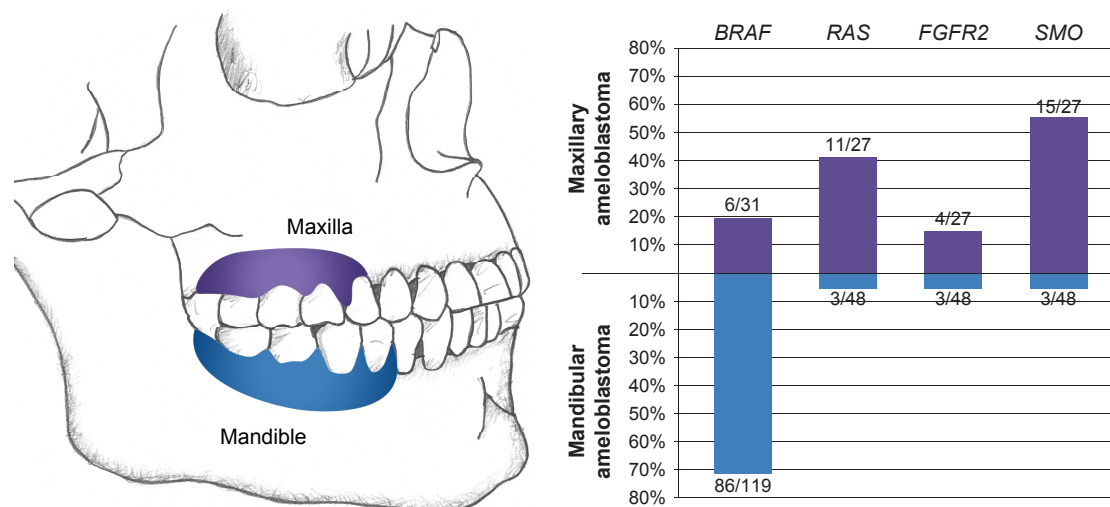
Figure 4. Relationship between the anatomic location and mutation frequency in ameloblastoma based on all studies in which *BRAF*, *RAS*, *FGFR2*, and *SMO* were evaluated.[10–13,40]
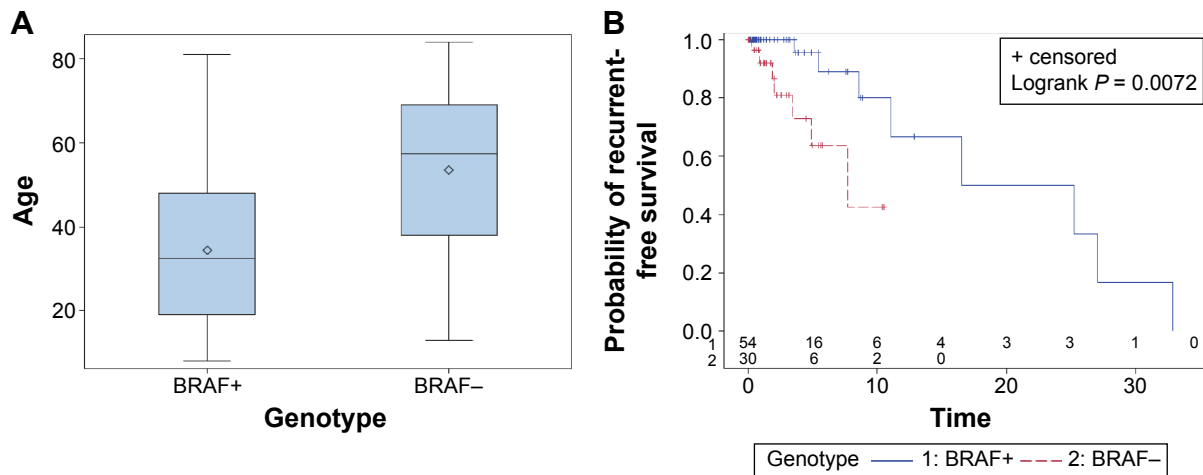
Figure 5. Clinical and prognostic significance of the *BRAF* V600E mutation in ameloblastoma adapted from Brown et al.[12] (**A**) Box plot showing a statistically significant difference in age distribution for *BRAF* V600E-mutated and *BRAF* wild-type ameloblastomas ($P = 0.0007$). Diamond indicates the mean, middle horizontal line indicates the median, box indicates 25th and 75th percentiles, and whiskers indicate minimum and maximum. (**B**) Recurrence-free survival (in years) for *BRAF* V600E-mutated and *BRAF* wild-type ameloblastomas using the Kaplan–Meier method.

Implications for Therapy

Several small-molecule inhibitors targeting BRAF and MEK are FDA-approved or in clinical trials for the treatment of neoplasms with activating MAPK pathway mutations, principally *BRAF* V600E-mutated melanoma. Two separate studies showed that ameloblastoma cells harboring the *BRAF* V600E mutation are sensitive to the BRAF inhibitor vemurafenib in vitro.[11,12] More recently, Kaye et al[36] reported a patient with multiply recurrent ameloblastoma in the mandible and metastatic ameloblastoma in the lung that was found to harbor a *BRAF* V600E mutation. This patient was treated with a combination of dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor) and achieved a dramatic response after 8 weeks of therapy.

These findings suggest a potential role for BRAF and MEK inhibitors in ameloblastoma treatment. While ameloblastoma is typically treated surgically, surgical resection often results in significant facial deformity and recurrences are common. In addition, pharmacological treatment may be particularly useful in metastatic and locally aggressive cases and in patients who are poor surgical candidates. A clinical trial investigating the utility of dabrafenib in *BRAF*-mutated melanoma is currently under way.

Other mutations identified in ameloblastoma may also be targetable. MEK inhibitors are currently being investigated as potential treatment for *NRAS*-mutated melanoma and may therefore also be useful in ameloblastomas with *RAS* mutations.[37] The utility of FGFR inhibitors is also under investigation,[38] as is SHH pathway inhibition with medications such as itraconazole and arsenic trioxide.[39]

Conclusion

Great strides have recently been made in our understanding of the underlying molecular pathogenesis of ameloblastoma.

Mutations affecting several genes within the MAPK pathway are now known to occur in a large majority of cases. The biologic importance of these mutations is highlighted by their high frequency and pattern of mutual exclusivity. The *BRAF* V600E mutation is the most common mutation, occurring in approximately two-thirds of cases. The presence or absence of this mutation correlates with several clinicopathologic features including location, age at diagnosis, histology, and prognosis. This mutation has also been shown to be specific for ameloblastic tumors, suggesting a potential role as a diagnostic marker. Somatic mutations affecting the Hedgehog pathway, specifically *SMO*, are also fairly common. It is currently unclear whether MAPK and Hedgehog pathway mutations represent two molecular subclasses of ameloblastoma, or whether *SMO* mutations function as secondary events with MAPK pathway mutations being the essential driver of pathogenesis. However, the higher frequency of MAPK mutations, the lack of mutual exclusivity of Hedgehog with MAPK pathway mutations, and the lack of clinicopathologic associations with *SMO* that are independent of *BRAF* status would argue against viewing *SMO*-mutated tumors as a truly distinct subclass of ameloblastoma. Finally, both in vitro and anecdotal clinical data implicate MAPK pathway inhibition as a promising future treatment option for ameloblastoma.

Author Contributions

Conceived and designed the experiments: NAB, BLB. Analyzed the data: NAB, BLB. Wrote the first draft of the manuscript: NAB, BLB. Contributed to the writing of the

manuscript: NAB, BLB. Agree with manuscript results and conclusions: NAB, BLB. Jointly developed the structure and arguments for the paper: NAB, BLB. Made critical revisions and approved final version: NAB, BLB. Both authors reviewed and approved of the final manuscript.

REFERENCES

1. Reichart PA, Philipsen HP, Sonner S. Ameloblastoma: biological profile of 3677 cases. *Eur J Cancer B Oral Oncol*. 1995;31B(2):86–99.
2. Sciubba JJ, Eversole LR, Slootweg PJ. Odontogenic/ameloblastic carinomas. In: Barnes L, Eveson JW, Reichart P, Sidransky D, eds. *World Health Organization Classification Head and Neck Tumours*. Lyon: IARC Press; 2005:287–289.
3. Mendenhall WM, Werning JW, Fernandes R, Malyapa RS, Mendenhall NP. Ameloblastoma. *Am J Clin Oncol*. 2007;30(6):645–648.
4. Heikinheimo K, Kurppa KJ, Laiho A, et al. Early dental epithelial transcription factors distinguish ameloblastoma from keratocystic odontogenic tumor. *J Dent Res*. 2015;94(1):101–111.
5. Melrose RJ. Benign epithelial odontogenic tumors. *Semin Diagn Pathol*. 1999; 16(4):271–287.
6. Gardner DG, Heikinheimo K, Shear M, Philipsen HP, Coleman H. Ameloblastomas. In: Barnes L, Eveson JW, Reichart P, Sidransky D, eds. *World Health Organization Classification Head and Neck Tumours*. Lyon: IARC Press; 2005: 287–289.
7. Hendarmin L, Sandra F, Nakao Y, Ohishi M, Nakamura N. TNFalpha played a role in induction of Akt and MAPK signals in ameloblastoma. *Oral Oncol*. 2005; 41(4):375–382.
8. Nakao M, Mitsuyasu T, Kawano S, Nakamura N, Kanda S, Nakamura S. Fibroblast growth factors 7 and 10 are involved in ameloblastoma proliferation via the mitogen-activated protein kinase pathway. *Int J Oncol*. 2013;43(5): 1377–1384.
9. Cardiff RD, Leder A, Kuo A, Pattengale PK, Leder P. Multiple tumor types appear in a transgenic mouse with the ras oncogene. *Am J Pathol*. 1993;142(4): 1199–1207.
10. Kurppa KJ, Catón J, Morgan PR, et al. High frequency of BRAF V600E mutations in ameloblastoma. *J Pathol*. 2014;232(5):492–498.
11. Sweeney RT, McClary AC, Myers BR, et al. Identification of recurrent SMO and BRAF mutations in ameloblastomas. *Nat Genet*. 2014;46(7):722–725.
12. Brown NA, Rolland D, McHugh JB, et al. Activating FGFR2-RAS-BRAF mutations in ameloblastoma. *Clin Cancer Res*. 2014;20(21):5517–5526.
13. Diniz MG, Gomes CC, Guimarães BV, et al. Assessment of BRAFV600E and SMOF412E mutations in epithelial odontogenic tumours. *Tumour Biol*. 2015; 36(7):5649–5653.
14. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. *N Engl J Med*. 2005;353(20):2135–2147.
15. Tiacci E, Trifonov V, Schiavoni G, et al. BRAF mutations in hairy-cell leukemia. *N Engl J Med*. 2011;364(24):2305–2315.
16. Puxeddu E, Moretti S, Elisei R, et al. BRAF(V599E) mutation is the leading genetic event in adult sporadic papillary thyroid carcinomas. *J Clin Endocrinol Metab*. 2004;89(5):2414–2420.
17. Badalian-Very G, Vergilio JA, Degar BA, et al. Recurrent BRAF mutations in langerhans cell histiocytosis. *Blood*. 2010;116(11):1919–1923.
18. Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. *Nature*. 2002;418(6901):934.
19. Niault TS, Baccarini M. Targets of Raf in tumorigenesis. *Carcinogenesis*. 2010; 31(7):1165–1174.
20. Byron SA, Gartside M, Powell MA, et al. FGFR2 point mutations in 466 endometrioid endometrial tumors: relationship with MSI, KRAS, PIK3CA, CTNNB1 mutations and clinicopathological features. *PLoS One*. 2012;7(2): e30801.
21. Konecny GE, Kolarova T, O'Brien NA, et al. Activity of the fibroblast growth factor receptor inhibitors dovitinib (TKI258) and NVP-BGJ398 in human endometrial cancer cells. *Mol Cancer Ther*. 2013;12(5):632–642.
22. Dutt A, Salvesen HB, Chen TH, et al. Drug-sensitive FGFR2 mutations in endometrial carcinoma. *Proc Natl Acad Sci U S A*. 2008;105(25):8713–8717.
23. Pollock PM, Gartside MG, Dejeza LC, et al. Frequent activating FGFR2 mutations in endometrial carcinomas parallel germline mutations associated with craniosynostosis and skeletal dysplasia syndromes. *Oncogene*. 2007;26(50): 7158–7162.
24. Xie J, Murone M, Luoh SM, et al. Activating smoothened mutations in sporadic basal-cell carcinoma. *Nature*. 1998;391(6662):90–92.
25. Von Hoff DD, LoRusso PM, Rudin CM, et al. Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. *N Engl J Med*. 2009;361(12):1164–1172.
26. Clark VE, Erson-Omay EZ, Serin A, et al. Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. *Science*. 2013; 339(6123):1077–1080.
27. Brastianos PK, Horowitz PM, Santagata S, et al. Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. *Nat Genet*. 2013;45(3): 285–289.
28. Stone DM, Hynes M, Armanini M, et al. The tumour-suppressor gene patched encodes a candidate receptor for Sonic hedgehog. *Nature*. 1996;384(6605): 129–134.
29. Kawabata T, Takahashi K, Sugai M, et al. Polymorphisms in PTCH1 affect the risk of ameloblastoma. *J Dent Res*. 2005;84(9):812–816.
30. Dalati T, Zhou H. Gorlin syndrome with ameloblastoma: a case report and review of literature. *Cancer Invest*. 2008;26(10):975–976.
31. Slootweg PJ. Ameloblastic fibroma/fibrodentinoma. In: Barnes L, Eveson JW, Reichart P, Sidransky D, eds. *World Health Organization Classification Head and Neck Tumours*. Lyon: IARC Press; 2005:308.
32. Edlundh-Rose E, Egyházi S, Omholt K, et al. NRAS and BRAF mutations in melanoma tumours in relation to clinical characteristics: a study based on mutation screening by pyrosequencing. *Melanoma Res*. 2006;16(6):471–478.
33. Bauer J, Büttner P, Murali R, et al. BRAF mutations in cutaneous melanoma are independently associated with age, anatomic site of the primary tumor, and the degree of solar elastosis at the primary tumor site. *Pigment Cell Melanoma Res*. 2011;24(2):345–351.
34. Bucheit AD, Syklawer E, Jakob JA, et al. Clinical characteristics and outcomes with specific BRAF and NRAS mutations in patients with metastatic melanoma. *Cancer*. 2013;119(21):3821–3829.
35. Platz A, Egyhazi S, Ringborg U, Hansson J. Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. *Mol Oncol*. 2008;1(4):395–405.
36. Kaye FJ, Ivey AM, Drane WE, Mendenhall WM, Allan RW. Clinical and radiographic response with combined BRAF-targeted therapy in stage 4 ameloblastoma. *J Natl Cancer Inst*. 2014;107(1):378.
37. Ascierto PA, Schadendorf D, Berking C, et al. MEK162 for patients with advanced melanoma harbouring NRAS or Val600 BRAF mutations: a non-randomised, open-label phase 2 study. *Lancet Oncol*. 2013;14(3):249–256.
38. Powell MA, Sill MW, Goodfellow PJ, et al. A phase II trial of brivanib in recurrent or persistent endometrial cancer: an NRG Oncology/Gynecologic Oncology Group Study. *Gynecol Oncol*. 2014;135(1):38–43.
39. Kim J, Aftab BT, Tang JY, et al. Itraconazole and arsenic trioxide inhibit Hedgehog pathway activation and tumor growth associated with acquired resistance to smoothened antagonists. *Cancer Cell*. 2013;23(1):23–34.
40. McClary AC, West RB, McClary AC, et al. Ameloblastoma: a clinical review and trends in management. *Eur Arch Otorhinolaryngol*. 2015.