

# Molecular Profiling of Odontogenic Tumors - Pilot Study

## SUMMARY

**Background/Aim:** In the pathogenesis of odontogenic tumors which arise from the rests of the dental apparatus in the jaw, several molecular pathways have been shown to play critical roles such as genetic alterations in the hedgehog, BRAF/Ras/MAPK, epidermal growth factor receptor. Next generation genomic sequencing has identified gene mutations in many different tumors. **Materials and Methods:** Here we report four types of odontogenic tumor including six cases in which five had mutation according to next generation sequencing analysis from archival paraffin blocks that diagnosed previously as ameloblastoma (solid), ameloblastoma (unicystic-mural), ameloblastic fibroma, squamous odontogenic tumor, and adenomatoid odontogenic tumor. **Results:** All ameloblastomatous tumors were shown BRAF mutation and adenomatoid odontogenic tumors were KRAS mutation. **Conclusion:** This evidence may highlight the poorly understood pathogenesis of odontogenic tumors. Further comparisons need to be made with other benign and malignant odontogenic tumors so that unique odontogenic features may be found.

**Key words:** Odontogenic Tumors, Next Generation Sequencing, Mutation

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ORIGINAL PAPER (OP)  
Balk J Dent Med, 112-115

## Introduction

Odontogenic tumors represent a spectrum of lesions ranging from malignant and benign neoplasms to dental hamartomas, all arising from epithelial or mesenchymal cells of the dental apparatus in the jaw or oral mucosa. Odontogenic tumors may pose both diagnostic and prognostic challenges due to overlapping histology and high propensity for local recurrence of some of them, even they are considered as benign<sup>1</sup>.

Current treatment options for odontogenic tumors 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. Moreover, patients with some odontogenic tumors such as ameloblastoma, should be followed up for a lifetime due to unpredictable biological behavior of the tumor<sup>1,2</sup>.

Development of non-invasive therapies has been precluded by a lack of understanding of the molecular background of odontogenic tumors pathogenesis. A

number of molecular pathways have been shown to play critical roles in the pathogenesis of them. Its frequent association with a wide spectrum of mutations in genes encoding various signal transducers are often linked to therapy response leads developing of molecular profiling analysis<sup>2-5</sup>.

The term molecular profiling is used to collectively describe molecular approaches that concomitantly measure the expression of multiple genes on tissue or other biological samples. Next generation genomic sequencing (NGS) is the comprehensive molecular profiling method, which has identified gene mutations or single nucleotide polymorphisms associated with many of the tumors. The NGS based genetic alteration analyses remain open area of investigation for odontogenic tumors and may need to be identified for potential future tailored treatment approach<sup>5,6</sup>.

The main objectives of this study is to identify a comprehensive molecular profiling of benign odontogenic tumors and to analyze their association with clinical parameters.

## Materials and Methods

### Sample selection

The study was conducted on previously diagnosed six odontogenic tumor specimens from the archives of paraffin-embedded diagnostic tissues of Department of Oral Pathology, Faculty of Dentistry, Gazi University, Ankara, Turkey.

The reevaluation and reclassification was done by an oral pathologist on hematoxylin-eosin (H&E) stained slides under light microscope according to World Health Organization (WHO) classification<sup>1</sup>.

The data regarding age, gender and localization was recorded. The study protocol was approved by the institutional review board of the Faculty of Dentistry at the Gazi University, Ankara and Faculty of Medicine at Cologne University.

### Molecular analysis

A total of six tumor samples were sequenced at the molecular pathology laboratory of Institute for Pathology University Cologne Cologne Germany, using the Ion Torrent PGM and the 30 gene AmpliSeq Library Kit from 10ng of extracted DNA.

Six sections of 10- $\mu$ m thickness were cut from FFPE tissue blocks. Sections were deparaffinised and the tumor areas were macrodissected from unstained slides using a marked H&E stained slide as a reference. After proteinase K digestion, the DNA was isolated with the Maxwell<sup>®</sup> 16 FFPE Plus Tissue LEV DNA Purification Kit (Promega, Mannheim, Germany) on the Maxwell<sup>®</sup> 16 (Promega) following manufacturer's instructions. The DNA content was measured using a real-time qPCR-based method.

For multiplex PCR-based target enrichment, the isolated DNA (10 ng each) was amplified with two customized GeneRead DNaseq Targeted Panel V2 (Qiagen, Hilden, Germany), targeting 17 lung cancer and

17 melanoma relevant genes, and the GeneRead DNaseq Panel PCR Kit V2 (Qiagen) according to the GeneRead DNaseq Gene Panel Handbook (Qiagen). These two panels comprise a subset of cancer relevant genes including: ARAF, BRAF, CDK4, CDKN2A, CTNNB1, DDR2, EGFR, ERBB2, FGFR2, FGFR3, GNA11, GNAQ, HRAS, IDH1, KEAP1, KIT, KNSTRN, KRAS, MAP2K1, MET, NFE2L2, NRAS, OXA1L, PDGFRA, PIK3CA, PTEN, RAC1 and TP53.

Libraries were constructed using the Gene Read DNA Library I Core Kit and the Gene Read DNA I Amp Kit (Qiagen). After End-Repair and adenylation, NEXTflex DNA Barcodes were ligated (Bio Scientific, Austin, TX, USA). Library products were quantified with Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) on the Qubit 2.0 Fluorometer (Thermo Fisher Scientific), diluted and pooled in equal amounts. Finally, 12 pM of the constructed libraries were sequenced on the MiSeq (Illumina, San Diego, CA, USA) with a MiSeq reagent kit V2 (300-cycles) (Illumina) following the manufacturer's recommendations.

Data were exported as FASTQ files. Alignment and annotation was done using a modified version of a previously described method.<sup>7</sup> BAM files were visualized in the Integrative Genomics Viewer (<http://www.broadinstitute.org/igv/>, Cambridge; USA). A 5% cutoff for variant calls was used and results were only interpreted if the coverage was >200x.

## Results

Table 1 shows the demographic, histologic and genomic data of the cases. The distribution of the tumors are as follows: two adenomatoid odontogenic tumors (AOT), two ameloblastomas, one ameloblastic fibroma (AF) and one squamous odontogenic tumor (SOT) (Figure 1).

Case #	Diagnosis	Age	Sex	Site	Mutation	Size	Teeth relation
1	AOT	33	M	Right-both	KRAS	6.5x4.5x0.5 cm	Yes
2	AOT	23	F	Anterior	KRAS	1.5x1.2x1 cm	No
3	SOT	56	M	Right- posterior	Unavailable	2x1x1 cm	No
4	AF	15	F	Left-posterior gingiva	BRAF	4x3x2cm	Yes
5	Ameloblastoma (Solid)	68	M	Anterior	BRAF	4.3x2.2x2 cm	No
6	Ameloblastoma (Unicystic mural)	29	F	Left-posterior	BRAF	3.4x2.8x2.5 cm	Yes

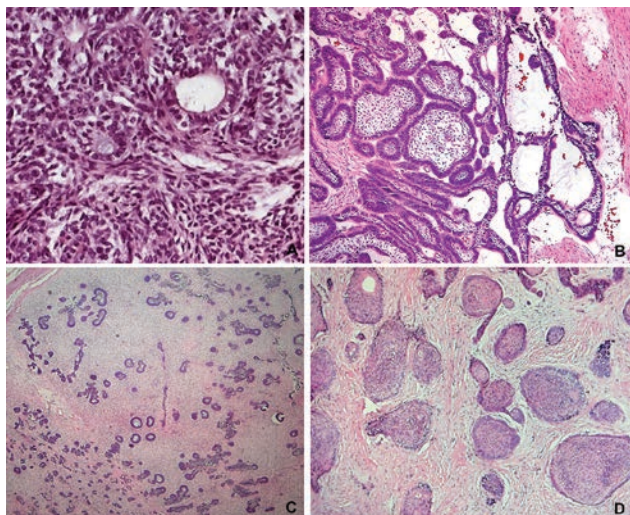


Figure 1. A) Hyperchromatic columnar cells with adenomatoid arrangements in adenomatoid odontogenic tumor (Hematoxylin and eosin, x200). B) Follicular ameloblastoma with palisading basal cells and stellate reticulum like cells (Hematoxylin and eosin, x100). C) Cellular loose connective tissue with islands of odontogenic epithelium in ameloblastic fibroma (Hematoxylin and eosin, x40). D) Squamous odontogenic tumor exhibiting typical epithelial islands in fibrous stroma (Hematoxylin and eosin, x100).

The mean age of the patients was 37.3 and gender distribution was equal. All the tumors located in mandible. AOT cases were located in the anterior, other tumors mostly located at the posterior of the mandible. Half of the tumors (three out of six) had relation with the unerupted teeth (Figure 2).

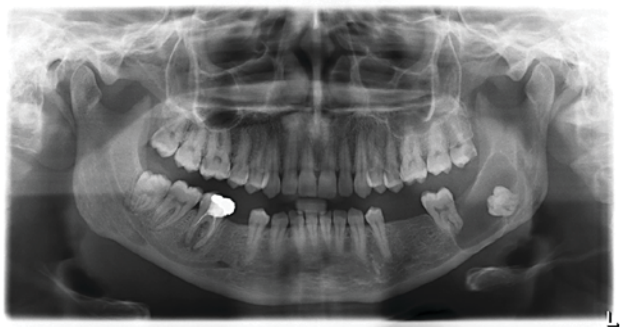


Figure 2. The unicystic ameloblastoma case had the relation with unerupted third molar.

The total five out of six cases were performed comprehensive analysis by NGS. In one case sequence analysis failed due to massive degradation of DNA. Mutations were identified in all five odontogenic tumors. Two types of gene mutation were detected; BRAF V600E was seen in ameloblastoma and ameloblastic fibroma, both AOT cases showed KRAS mutations. The age of the patients with BRAF mutated tumors are higher (mean: 37.3) than KRAS mutated ones (mean:28).

## Discussion

Odontogenic tumors are relatively rare tumors with challenging diagnosis and prognosis. The clinical course, imaging technique and both histological and immunohistochemical evaluations have defined the correct diagnosis of odontogenic tumors in the past. The practice of pathology is currently undergoing significant change, due to advances in the field of molecular pathology. In recent couple of years, an emerging technology- molecular genomic profiling by means of NGS- may provide clinicians with an additional tool to understand, better classify and develop new treatment modalities of the odontogenic tumors. In the present study NGS technologies has allowed us to generate a comprehensive molecular profiling of six benign odontogenic tumors, which adds additional new information to the field, limited with five studies since 2014<sup>2-5,8</sup>.

The results of the study displayed BRAF (V600E) or KRAS mutations in all of the sequenced tumors. BRAF mutations were found in two ameloblastoma and one ameloblastic fibroma cases. BRAF is a potent activator of the mitogen activated protein kinase (MAPK) cascade, which is capable to induce proliferation and to promote proliferation. Our findings are in concordance with the studies, which showed the high frequency of driver mutation in BRAF in the ameloblastomas<sup>2-6</sup>. In our knowledge, the present study is the second research that showed the BRAF mutation in ameloblastic fibroma. Previously, Brown et al., who performed the molecular analysis in two ameloblastic fibromas, identified it<sup>3</sup>. Depending these findings, we may speculate that this tumor is pathogenetically associated to the ameloblastoma and moreover, it may represent the early phase of the ameloblastoma. Ameloblastic fibromas are generally found in younger age group and exhibit less aggressive behavior. On the other hand, these tumors are histologically very similar to conventional ameloblastoma<sup>1</sup>. Overall, these findings suggest that ameloblastic tumors may be a distinct group of odontogenic tumors with the common genetic alterations<sup>8,9</sup>. This may give rise to use BRAF mutations as a potential diagnostic marker for ameloblastic odontogenic tumors.

The other notable finding of our study is, both AOT cases displayed KRAS mutation. KRAS, is one of the oncogene belongs to the RAS protein family which activates the BRAF protein and is commonly mutated in cancers<sup>10</sup>. There has been limited studies that utilized deep sequencing analysis on odontogenic tumors which were mostly focused on ameloblastomas. Only one study reported KRAS mutation in the seven out of nine AOT cases<sup>11</sup>. These findings reveal that MAPK pathway plays critical role in the development of benign odontogenic tumors.

## Conclusion

In conclusion, we have performed a comprehensive molecular profiling of benign odontogenic tumors in order to identify novel genomic alterations that will lead to recognition of molecular classification and influence the new therapeutic approaches. However, a similar study design should be applied to a larger and more varied group of odontogenic tumors to confirm the present results, and further comparisons need to be made with other malignant tumors so that unique odontogenic features may be found.

**Note:** The results of this paper were presented as a part of an invited lecture at the 22<sup>nd</sup> BaSS Congress.

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**Received on March 15, 2017.**

**Revised on May 2, 2017.**

**Accepted on May 29, 2017.**

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