

# Molecular Drivers and Epigenetic Regulation in the Pathogenesis of Ameloblastoma: A Comprehensive Review

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## ABSTRACT

Ameloblastomas molecular landscape is defined by recurrent mutations in key signaling pathways, primarily the MAPK and Hedgehog pathways, which drive tumorigenesis and influence clinical behavior. BRAF V600E mutations are the most prevalent, particularly in mandibular tumors, and are associated with diagnostic specificity and potential responsiveness to BRAF/MEK inhibitors. In contrast, SMO mutations are more common in maxillary ameloblastomas and often co-occur with alterations in KRAS, NRAS, HRAS, PTEN, correlating with aggressive subtypes and higher recurrence risk. Mutations in FGFR2 and RAS family genes also converge on the MAPK pathway, and their mutual exclusivity suggests these as early driver events in Ameloblastoma pathogenesis. Rarely, mutations in CTNNB1(Wnt pathway) and SMARCB1 may contribute to tumor progression. The advances in throughput sequencing methods have revealed co-occurring mutations that necessitate the need to map the associated mutations to their corresponding signaling pathways. Understanding this co-mutational landscape will eventually direct towards the rationale for pathway-specific therapeutic interventions. Among the epigenetic alterations, the role of microRNA(miRNAs) remains largely unexplored, though the literature review reveals the differential expression of miRNAs in ameloblastoma, their clinical relevance and their use in terms of therapeutic planning in ameloblastoma is under-evaluated, warranting further research. Studies predicting miRNA target genes and analysing their expression profiles may largely open avenues for the development of non-surgical management of recurrent or unresectable cases.

**Keywords :** Ameloblastoma; Molecular pathogenesis; BRAF V600E; SMO mutations; MAPK pathway; Hedgehog signaling; MicroRNAs; Epigenetic Modifications; Co-mutations; Odontogenic tumors

## INTRODUCTION

Odontogenic tumors are the most common tumors of the head and neck region and occupy around 3% to 9% of all biopsied specimens<sup>1</sup>. These lesions arise from or are associated with the odontogenic apparatus, their derivatives, or their remnants. Among the odontogenic tumors, ameloblastoma is considered to be the most common odontogenic tumor after odontoma in the Asian population<sup>2</sup>. It is a tumor of the enamel organ type tissue, which does not undergo differentiation to the point of enamel formation. Robinson defined ameloblastoma as “unicentric, nonfunctional, intermittent in growth, anatomically benign, and clinically persistent”<sup>3</sup>. Despite its benign nature, ameloblastoma exhibits locally invasive behavior, leading to significant facial disfigurement and functional issues if left untreated. The tumor predominantly occurs in the mandible, with about 80% of cases located there, often presenting as a slow-growing, painless swelling<sup>4</sup>.

According to the 2022 World Health Organization (WHO) Classification of Head and Neck Tumors, ameloblastomas are classified under benign epithelial odontogenic tumors<sup>5</sup>. The current classification has introduced a new entity called Adenoid ameloblastoma, while maintaining previously established categories. The classification organizes odontogenic tumors based on their biological behavior into benign and

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malignant categories. Within benign tumors, they are further divided according to their histogenetic origin into three major groups: epithelial, mesenchymal, and mixed types. Ameloblastomas are broadly classified into several subtypes based on clinical, radiographic, histopathologic, and behavioral as-

pects. These include conventional (solid/multicystic), unicystic, peripheral ameloblastomas and Metastasizing ameloblastomas, although histologically benign, this type can metastasize.

Histopathologically, conventional ameloblastomas are further divided into six subtypes: follicular, plexiform, acanthomatous, basal cell, granular, and desmoplastic.<sup>2</sup> The desmoplastic subtype has a distinct site of occurrence and radiographic appearance. It is not uncommon to observe mixtures of different histological patterns within a single lesion, often classified based on the predominant pattern present.

The management of ameloblastoma primarily involves extensive surgical resection, due to its aggressive local invasion and high recurrence rate and poses a significant challenge despite its benign nature<sup>6</sup>.

The molecular pathogenesis of ameloblastoma involves several key genetic mutations and signaling pathways. Studying the molecular aspects of ameloblastoma is essential for several reasons, including aiding in developing more accurate diagnostic tools. It may further provide insights into tumor proliferation and aggressiveness, thereby predicting clinical outcomes and identifying the potential therapeutic targets, reducing the need for extensive surgery, thereby improving patient quality of life. This review aims to explore the key genetic mutations, signalling pathways, and molecular alterations of ameloblastoma.

Molecular analysis techniques used to detect genetic alterations in ameloblastoma include simple targeted methods and advanced sequencing technologies. Targeted methods such as Sanger sequencing are employed to identify known point mutations in genes like BRAF, SMO, and KRAS. Commonly, immunohistochemistry (IHC) is used to detect mutation-associated protein expression, such as BRAF V600E. Real-Time PCR (qPCR) and Digital PCR (dPCR) offer sensitive detection and quantification of specific genetic alterations and are widely used because of its availability and simplicity of the technique. In contrast, advanced sequencing technologies like Next-Generation Sequencing (NGS) and pyrosequencing allow for the analysis of many genes at once, and also helps to find identify both rare and new mutations. These methods collectively contribute to the molecular characterization and understanding of ameloblastoma pathogenesis.<sup>7-11</sup>

### Molecular aspects of ameloblastomas

Molecular pathogenesis of benign but locally aggressive odontogenic tumor, Ameloblastoma, is driven by a combination of genetic mutations and epigenetic modifications that dysregulate key signaling pathways. These alterations contribute to tumorigenesis, tumor progression, and recurrence.

Genetic mutations such as BRAF, SMO, RAS, and FGFR2 contribute to the dysregulation of critical signaling pathways, notably the MAPK/ERK, Hedgehog, and Wnt/ $\beta$ -catenin pathways. The BRAF V600E mutation is particularly prevalent, occurring in approximately 57% of cases, and is a primary driver of MAPK pathway activation<sup>8,9</sup>. Mutations in RAS genes (KRAS, NRAS, HRAS) and FGFR2 are also identified, often in a mutually exclusive manner with BRAF mutations, further implicating MAPK pathway dysregulation in tumor development<sup>9-14</sup> while SMO mutations are independent of other muta-

tions and activate the Hedgehog pathway. In addition to the genetic dysregulation or mutations, the epigenetic modifications contribute to tumor progression and recurrence, underscoring the complexity of ameloblastoma pathogenesis<sup>8,11</sup>. Understanding this molecular interplay is crucial for developing targeted therapies and improving patient outcomes.

### Genetic alterations in Ameloblastoma

#### BRAF V600E Mutation and MAPK Pathway Activation in Ameloblastoma

Ameloblastoma is driven by a complex interplay of multiple genetic mutations, with BRAF V600E being the most prominent, found in approximately 60-70% of mandibular ameloblastomas, while less frequently associated with maxillary ameloblastomas, where other mutations like SMO are more prevalent<sup>15-17</sup>.

There is no clear consensus on whether BRAF V600E directly influence tumor size or gender, although ameloblastoma itself more prevalent in male irrespective of the mutations presented. This mutation directly activates the MAPK/ERK pathway by phosphorylating and activating MEK, which in turn phosphorylates and activates ERK. This activation is crucial for cell cycle progression and survival, promoting cell growth and proliferation<sup>16,18</sup>. The MAPK/ERK pathway can also be activated by other mutations, such as RAS and FGFR2, although these are generally mutually exclusive with BRAF mutations.<sup>19</sup>

The BRAF V600E mutation does not significantly affect the recurrence rate of ameloblastoma, making BRAF inhibitors a potential alternative to extensive surgery while in contrast, BRAF wild-type (unmutated form of BRAF gene) may not respond to BRAF inhibitors<sup>18,20</sup>.

#### RAS mutations and activation of MAPK/ERK pathway activation:

RAS mutations, including KRAS, NRAS, and HRAS are identified in up to 20% of ameloblastomas. The MAPK/ERK pathway is a critical signalling cascade that transmits signals from cell surface receptors to the nucleus, regulating cell proliferation, differentiation, and survival. RAS proteins act as molecular switches and when mutated leads to consecutive activation of RAS signalling, even in the absence of external signals<sup>15</sup>. These mutations contribute to the activation of the MAPK/ERK pathway, driving tumor growth and aggressiveness<sup>9,13</sup>. Unlike BRAF mutations, which are more common and associated with specific clinicopathological features (mandibular ameloblastomas), RAS mutations provide an alternative pathogenetic mechanism and that may guide the use of targeted therapies, such as MEK inhibitors, which target the MAPK pathway downstream of RAS.

#### FGFR2 mutations and activation of MAPK/ERK and PI3K/AKT signaling pathways

FGFR2 mutations in ameloblastoma are known to activate both the MAPK/ERK and PI3K/AKT signaling pathways. The FGFR2 mutations C382R and V395D are specific alterations in the FGFR2 gene that have been identified in a subset of ameloblastoma cases. These mutations occur in the transmembrane domain of the FGFR2 receptor and lead to constitutive



activation of the MAPK pathway, similar to their role in other cancers like endometrial carcinoma<sup>9,19</sup>. The activation of the MAPK pathway promotes cell proliferation and survival, while the PI3K/AKT pathway enhances cell survival and resistance to apoptosis. FGFR2 mutations are found in approximately 6% to 18% of ameloblastomas and are generally mutually exclusive with BRAF and RAS mutations, suggesting that each mutation can independently activate the MAPK pathway<sup>19,20</sup>. Targeted therapies against FGFR2, such as erdafitinib, have shown promise in treating cases with these mutations, offering a potential alternative to surgery. These pathways are critical in various cancers, including breast and sarcoma, where FGFR2 mutations can lead to increased signaling and tumor progression<sup>22</sup>.

**Mutual Exclusivity and Convergence of BRAF, RAS, and FGFR2 Mutations on the MAPK/ERK Pathway in Ameloblastoma**

The mutual exclusivity and convergence of BRAF, RAS, and FGFR2 mutations on the MAPK/ERK pathway in ameloblastoma highlight the complex molecular landscape of this tumor (Fig 1). Despite their distinct mechanisms of action, of each mutations can independently activate the MAPK pathway, making additional mutations unnecessary, thereby making targeted therapies a promising approach against specific mutations. Further research into these co-mutation pathways and understanding their cross-talks will be essential for developing effective therapeutic strategies tailored to the molecular profiles of individual tumor.(Fig 2A)

**SMO mutations and activation of Hedgehog pathway**

The Sonic Hedgehog (SHH) pathway is a key signaling cascade involved in embryonic development, tissue homeostasis,

and cell proliferation, regulating cell differentiation, growth, and survival. The pathway is activated when SHH protein binds to the Patched (PTCH) receptor, relieving its inhibition on Smoothed (SMO). This allows SMO activation, which in turn triggers GLI transcription factors (GLI1, GLI2, GLI3) to regulate target genes responsible for cell proliferation and survival<sup>23</sup> (Fig 2B).

Dysregulation of the Hedgehog (HH) pathway can occur through mutations or alterations in its regulatory components, leading to constitutive SMO activation, which leads to uncontrolled cell proliferation, inhibition of apoptosis, and tumorigenesis. Such dysregulations are observed in various cancers, including basal cell carcinoma, medulloblastoma, and ameloblastoma<sup>23-25</sup>.

In ameloblastoma, aberrant Hedgehog (HH) signaling occurs due to PTCH1 mutations and SMO activation, leading to increased cell proliferation, inhibition of apoptosis, and enhanced tumor cell survival, thereby contributing to its extensive invasion and aggressiveness<sup>26</sup>. These mutations define a distinct molecular subclass of ameloblastoma with unique histological and clinical features compared to BRAF mutated cases. The focus on comparing BRAF and SMO mutations arises from their high prevalence, mutual exclusivity, distinct molecular pathways, while other mutations, such as those in RAS, FGFR2, PTEN, and PIK3CA, have also been identified but occur less frequently or as secondary alterations and actionable therapeutic targets. Below table compares BRAF and SMO-mutated ameloblastomas<sup>25-27,9</sup>.(Table 2)

**CTNNB1 Mutations and Wnt/ $\beta$ -Catenin Pathway Dysregulation in Ameloblastoma**

Table 1 - ABBREVIATIONS

BRAF- <i>v-raf murine sarcoma viral oncogene homolog B1</i>
SMO (gene)- Smoothed gene
PTCH1- <i>Patched homolog 1</i>
PTEN(gene)- <i>Phosphatase and tensin homolog</i>
RAS - <i>Rat sarcoma</i>
HRAS -Harvey- RAS
KRAS- Kirsten - RAS
NRAS- Neuroblastoma- RAS
FGFR2- Fibroblast Growth Factor Receptor 2
MAPK- Mitogen-Activated protein Kinase
SHH- Sonic Hedge Hog
ERK- Extracellular signal-regulated kinase
CTNNB1 -Catenin 1 (also known as $\beta$ -catenin)
PI3KT/AKT- Phosphatidylinositol 3-kinase/Protein Kinase B
Wnt- Wingless-related integration site
SMARCB1- SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily B, member 1

Table 2: Comparison of SMO-Mutated and BRAF-Mutated Ameloblastomas

Feature	SMO-Mutated Cases	BRAF-Mutated Cases
Location	Predominantly Maxillary	Predominantly Mandibular
Radiographic features (Less documented )	May present differently	Often unilocular radiolucencies in unicystic cases
Histology	Plexiform subtype	unicystic /Follicular subtypes
Age/Gender	No specific predilection	More common in younger patients
TUMOR size	Variable; may be larger in maxillary cases	Variable; often smaller in unicystic cases
Recurrence	Possible earlier recurrence	Recurrence rates not significantly affected by mutation status
Therapy	Hedgehog inhibitors (e.g., ATO)	BRAF inhibitors (e.g., vemurafenib)



In ameloblastoma, CTNNB1 mutations are rare and are likely secondary to primary driver mutations like BRAFV600E or SMO<sup>19</sup>. Their presence has been implicated in tumorigenesis through nuclear/cytoplasmic accumulation of beta-catenin. The CTNNB1 gene encodes beta-catenin, a key protein involved in the Wnt/ $\beta$ -catenin signaling pathway which regulates cell proliferation, differentiation, and tissue homeostasis. Mutations in CTNNB1 can lead to constitutive activation of beta-catenin, resulting in dysregulated Wnt signalling<sup>9,13,16,28,29</sup>. Nuclear localization of beta-catenin has been linked to aggressive tumor behavior in various cancers, including odontogenic lesions. In ameloblastoma, this translocation may contribute to increased aggressiveness, particularly in solid ameloblastoma subtypes and malignant odontogenic tumors.<sup>30-32</sup>

Rarity of CTNNB1 mutations in ameloblastoma along with studies from literature suggests the potential crosstalk of the Wnt and Hedgehog pathways in ameloblastoma, while Kumamoto and Ooya (2008), found that a subset of ameloblastomas displayed both nuclear  $\beta$ -catenin accumulation (showing Wnt activation) and molecular markers of HH pathway activation, therefore confirming a dual pathways involvement<sup>29,31</sup>. (Fig 2C)

Mutations in genes such as BRAF, SMO, RAS, and FGFR2 are associated with key signalling pathways—including MAPK/ERK, Hedgehog, PI3K/AKT, and Wnt/ $\beta$ -catenin—that regulate tumor growth, invasiveness, and cellular differentiation in ameloblastoma. Nevertheless recent studies using high-throughput sequencing methods such as NGS and pyrosequencing have identified co-occurring mutations along with BRAF, suggesting the mutational landscape is more complicated than previously thought. Understanding the biological behaviour of the tumor with its associated signalling pathway may demand pathway-specific therapeutic interventions. The associated signalling pathways, mutation frequencies, and their clinical implications are summarised in Table 3.

### Epigenetic modification in ameloblastoma

Epigenetics refers to the dysregulation occurring to gene activity (altering the cellular function and phenotype) without altering the gene sequences. The most common forms would be DNA methylation, histone modifications and the involvement

of non-coding RNAs<sup>32,33</sup>.

Epigenetic modifications play an important role in the pathogenesis of ameloblastoma. Although expanded studies have been done on genetic mutations, there is a lack of epigenetic modification studies which can offer insights into tumor biology and may open avenues for potential therapeutic targets. These epigenetic changes are reversible and can be aimed at modulating gene expression without altering the DNA sequences.

### DNA Methylation in Ameloblastoma

DNA methylation is a process that involves the addition of a methyl group to the 5th carbon of cytosine residues in CpG dinucleotides. This modification is catalysed by DNA methyltransferases (DNMTs), which include DNMT1, DNMT3A, and DNMT3B. It is crucial for regulating gene expression and maintaining genomic stability. Aberrant DNA methylation, including hypermethylation, can lead to silencing of tumor suppressor genes, while hypomethylation can activate oncogenes. Both these dysregulations have been implicated in the pathogenesis of various tumors, including ameloblastoma, by influencing their tumor behaviour and progression.

Hypermethylation of CpG islands in tumor suppressor genes like p16 and E-cadherin lead to silencing of genes, leading to altered cell cycle regulation and cell adhesion, which in turn promotes uncontrolled proliferation, reduced apoptosis, and increased invasiveness, thereby contributing to tumor progression and potential malignant transformation.

Similarly, apoptosis-related genes such as BCL2L1 (BIM) exhibit altered methylation patterns in ameloblastomas, where hypermethylation suppresses apoptosis, allowing tumor cells to survive and grow unchecked. Also, an integrative analysis has identified thousands of differentially methylated CpG sites in ameloblastomas, correlating with altered expression of genes like FGFR2, NID2 and PAK6, which are implicated in tumor invasion and pathology<sup>34</sup>.

Cancer cells often exhibit global hypomethylation alongside localized hypermethylation. In ameloblastoma, hypomethylation has been observed in certain oncogenes such as Matrix Metalloproteinases (MMPs) which promotes extra cellular ma-

**Table 3:** An overview of these mutations and their association with signaling pathways with clinical relevance is provided in the following table.

Gene Mutation	Signaling Pathway	Frequency in Ameloblastoma	Clinical Implications
BRAF V600E	MAPK/ERK	46% to 90%	Tumor growth and proliferation <sup>12,13</sup>
SMO	Hedgehog	10.6%	Tumor progression and recurrence <sup>12,14</sup>
RAS (KRAS, NRAS, HRAS)	MAPK/ERK	Up to 20%	Tumor aggressiveness and recurrence <sup>8,14</sup>
FGFR2	MAPK/ERK and PI3K/AKT	6% to 18%	Tumor growth and invasion <sup>8</sup>
CTNNB1	Wnt/ $\beta$ -catenin	Lower frequency	Tumor progression and differentiation <sup>8</sup>
PIK3CA	PI3K/AKT	Lower frequency	Tumor growth and survival <sup>8</sup>
PTEN	PI3K/AKT	Lower frequency	Tumor growth and survival <sup>12</sup>
SMARCB1	SWI/SNF complex	Lower frequency	Tumor progression and aggressiveness <sup>12</sup>



trix degradation and thereby enhances tumor invasiveness.

DNMTs play a role in both maintaining existing methylation patterns (DNMT1) and establishing new ones (DNMT3A and DNMT3B). Over-expression of DNA methyltransferases (DNMTs), particularly DNMT3B, has been associated with the aggressiveness and recurrence of ameloblastomas<sup>35</sup>. The differentially methylated genes could serve as biomarkers for early diagnosis or predicting recurrence risk. Likely the epigenetic therapies targeting DNMTs or reversing aberrant methylation patterns may offer novel treatment strategies for ameloblastoma.<sup>34-35</sup>

**Histone modifications in Ameloblastoma**

Histone modifications have been linked with the pathogenesis of various tumors, including ameloblastoma. This modification may lead to the overexpression of oncogenes or silencing of tumor suppressor genes contributing to tumor progression. H3K9 refers to a specific site on the histone H3 lysine 9 protein, where modifications such as methylation((H3K9 methylation)), acetylation, (H3K9 acetylation) and ubiquitination (H2A monoubiquitination) are methods of histone modification<sup>33,34,36,37</sup>. Histone modifications often work alongside DNA methylation to regulate gene expression, tumor suppressor genes silencing thereby potentially influencing tumor behavior and aggressiveness<sup>34</sup>.

Various studies have shown that histone H3K9 methylation is differentially expressed in various odontogenic lesions, including ameloblastoma. H3K9 methylation is found to be associated with transcriptional repression leading to gene silencing and heterochromatin formation. The trimethylated H3K9 levels are found to vary significantly among different types of odontogenic tumors. Interestingly, multilocular ameloblas-

toma and increased recurrence rates are linked with elevated Histone H3Lysine 9 Trimethylation (H3K9me3) levels<sup>36</sup>.

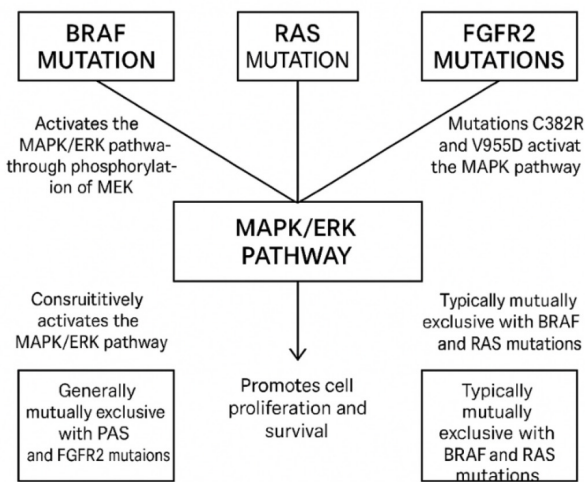
Conversely, H3K9 acetylation is linked to active gene transcription, as acetylation reduces histone-DNA interactions, making chromatin more accessible to transcription factors. Increased levels of H3K9Ac have been observed even in normal dental follicles. However, increased levels of H3K9Ac have been observed in more aggressive forms like ameloblastic carcinomas(AC), suggesting a potential role in malignancy. Similarly, H2A monoubiquitinating (H2Aub1) involves the addition of a single ubiquitin molecule to histone H2A and has been found in association with ameloblastoma. However, its specific role is not extensively studied and requires further exploration<sup>32,37</sup>.

**Non-coding RNAs in ameloblastoma**

Non-coding RNAs (ncRNAs) are RNA molecules that do not code for proteins but play a crucial role in regulating gene expression. Their distinct role in the pathogenesis and progression are recent topic of interest, with a focus on identifying signature ncRNA through comprehensive RNA profiling. Studies have highlighted the dysregulation of specific ncRNAs, particularly long non-coding RNAs (lncRNAs), small nucleolar RNAs (snoRNAs), and microRNAs (miRNAs), emphasizing their potential as therapeutic targets. However, other ncRNAs remain less explored, warranting further investigation<sup>38,39</sup>.

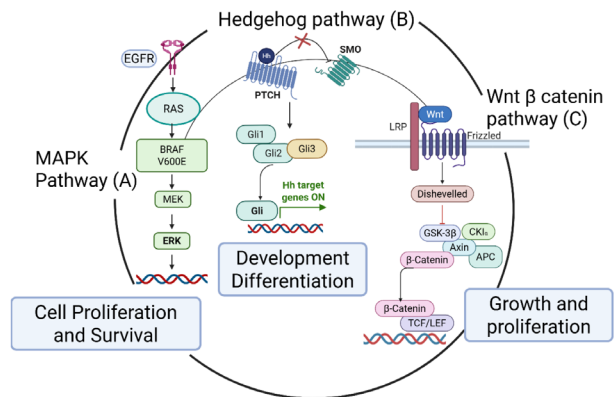
**Long non coding RNAs (lncRNAs) in ameloblastoma**

lncRNAs can act as both oncogenes or tumor suppressors and influence the tumor development and progression through transcriptional regulation and chromatin remodelling. Interestingly, its mechanism is independent of common mutations like BRAF-V600E and SMO-L412F, which opens the scope for lncRNA to act as a biomarker. Various studies reveal lnc340 and KIAA0125 are associated with ameloblastoma. The overexpression of LINC340 has been evidenced and linked to tumor size, while upregulated KIAA0125 was observed in ameloblas-



**FIG. 1**  
Mutual Exclusivity and Convergence of BRAF, RAS and FGFR2 Mutations on the MAPK/ERK Pathway in Ameloblastoma

**Fig. 1:** Schematic diagram showing the BRAF, RAS, and FGFR2 mutations’ mutual exclusivity and convergence on the MAPK/ERK pathway in ameloblastoma



**Fig. 2:** Schematic diagram representing three major signaling pathways in molecular pathogenesis of ameloblastoma. (A) the MAPK/ERK pathway, (B) the Hedgehog pathway, and (C) the Wnt/β-catenin pathway.

tomas compared to dental follicles, confirming its role in tumor pathobiology and potential therapeutic applications<sup>39,40</sup>.

### Small nucleolar RNAs (snoRNAs) in Ameloblastoma

Recent studies have identified aberrant snoRNA (SNORD116-25, SNORA11, SMORA21, SNORA47, SNORA65) expression in ameloblastoma and may contribute to altered ribosome biogenesis, disrupted protein synthesis, and tumor cell proliferation. Similar to lncRNAs, these snoRNA are independent of common mutation like BRAF and SMO, identifying it as a potent target. However, its specific implication in ameloblastoma requires further exploration<sup>33,39</sup>.

### MicroRNAs(miRNAs) in Ameloblastoma

MicroRNAs (miRNAs) are unique non-coding RNAs with distinct characteristics and functions. They are small, typically 21–23 nucleotides in length, and form small-loop structures during their biogenesis. Predominantly localized in the cytoplasm, miRNAs interact with target messenger RNAs (mRNAs), leading to either translational repression or mRNA degradation. By binding to the 3' untranslated region (3' UTR) of target mRNAs, miRNAs play a crucial role in post-transcriptional gene regulation and RNA silencing. Thus they can regulate key cancer hallmarks, including proliferation, apoptosis resistance, invasion, metastasis, and angiogenesis.

A literature review identified 40 differentially expressed miRNAs in ameloblastoma, with distinct expression patterns between the solid (SA) and unicystic (UA) subtypes. Notably, miR-489 distinguishes SA from UA<sup>40</sup>, while miR-31, miR-135B, miR-592, and miR-944 are overexpressed in both subtypes, suggesting their involvement in tumor progression<sup>41</sup>. Several miRNAs have been implicated in the molecular pathogenesis of ameloblastoma, like miR-29a highlighted as a molecular marker influencing invasion and metastasis via CTNNBIP1 suppression and Wnt/ $\beta$ -catenin pathway activation, while miR-1-3p exhibits tumor-suppressive properties by downregulating LAMP2 (lysosome-associated membrane protein 2), thereby inhibiting autophagy. Similarly, miR-516b plays a tumor suppressive role by inhibiting the MYCBP/c-myc axis and upregulating RECK gene, thereby suppressing proliferation, invasion, and metastasis. Its downregulation in tumors contributes to aggressive behaviour, thereby making it a promising target for therapeutic intervention, while miR-524-5p, another tumor suppressor marker, regulates the tumor microenvironment via the IL-33/ST2 pathway<sup>42</sup>.

These miRNAs can be detected and quantified using various experimental techniques, including quantitative real-time PCR (qRT-PCR), microarray analysis, and next-generation sequencing (NGS). Bioinformatics tools further aid in predicting miRNA target genes and analyzing expression profiles; however, their full potential in studying ameloblastoma remains unexplored.

Availability of miRNAs in cytoplasmic location and their role in post-transcriptional regulation of many genes, makes it a preferable tool to study the molecular alterations in tumors, offering a more targeted approach compared to other non-coding RNAs.

### MicroRNAs and Ameloblastoma: Pathways and Research Gaps

The relationship between microRNAs (miRNAs) and FGFR2-RAS-BRAF-MAPK signaling pathways involved in ameloblastoma is supported by research in other cancers. Despite their significance, research on miRNA alterations in odontogenic tumors, particularly ameloblastomas, remains limited. Specific studies on miRNA-target interactions may facilitate the development of miRNA-based therapeutic strategies for ameloblastoma management.

While miRNAs like miR-21<sup>43</sup> and miR-29b<sup>4</sup> are known to regulate MAPK signaling in other cancers, however there is a lack of experimentally studies explicitly connecting them to the FGFR2-RAS-BRAF-MAPK pathway in ameloblastoma. If further studies are done, the interactions with key signaling molecules pertaining to ameloblastoma can be explored, and it may lead in identifying definitive miRNAs that can regulate the FGFR2-RAS-BRAF-MAPK pathway. And this could provide novel biomarkers for ameloblastoma diagnosis and prognosis. Further, combining miRNA-based therapies with MAPK pathway inhibitors (e.g., BRAF or MEK inhibitors) may enhance efficacy and reduce resistance to such an aggressive tumor.

### CONCLUSION

The genetic and molecular landscape of ameloblastoma is complex, with BRAF, SMO, PTCH1, FGFR2, KRAS, and CTNNB1 mutations playing key roles in tumor development and progression. Understanding these molecular markers is essential for predicting the clinical behaviour of ameloblastomas and developing targeted therapeutic strategies. Additionally, miRNAs have emerged as significant biomarkers for ameloblastoma diagnosis, prognosis, and therapy. Studies with interactions of these markers with signalling pathways aligning with advances in genomic and transcriptomic profiling may provide valuable insights into tumor biology, paving the way for novel therapeutic approaches.

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In the MAPK pathway, activation of EGFR or RAS mutations, including BRAF V600E, leads to downstream activation of MEK and ERK, promoting cell proliferation and survival. The Hedgehog pathway is activated upon binding of Hedgehog ligands (Hh) to the PTCH receptor, relieving inhibition of SMO, which in turn activates GLI transcription factors (Gli1, Gli2, Gli3) to regulate genes involved in development and differentiation. The Wnt/ $\beta$ -catenin pathway is initiated by Wnt ligand binding to Frizzled and LRP receptors, leading to inhibition of the  $\beta$ -catenin destruction complex (GSK-3 $\beta$ , CK1 $\alpha$ , APC, Axin) and stabilization of  $\beta$ -catenin, which translocates to the nucleus to activate transcription of genes involved in cell growth and proliferation. Together, these pathways contribute to tumor initiation, progression, and potential therapeutic targets in ameloblastoma.

