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REVIEW ARTICLE

# The WNT/β-catenin signaling in the context of odontogenic cysts and tumors: an update

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## Abstract:

The Wnt/β-catenin signaling pathway is tightly controlled under typical physiological conditions, being involved in teeth developmental processes. Its dysregulation and the abnormal activation of downstream target genes can result from mutations in essential pathway components. These dysregulations might ultimately foster odontogenic cysts and tumors onset and progression. In this review, we retrieve the main alterations reported in the Wnt/β-catenin signaling pathway in the context of odontogenic cysts and tumors. Increasing evidence supports the involvement of the Wnt/β-catenin pathway disruption in the biology of calcifying odontogenic cyst, odontoma, adenoid ameloblastoma, and ghost cell-containing odontogenic tumors. A deeper understanding of these molecular mechanisms may contribute to improved diagnostic and therapeutic strategies in the future.

**Keywords:** WNT signaling pathway; Odontogenic cysts; Odontogenic tumors; Immunohistochemistry; Molecular biology.

## INTRODUCTION

The wingless-type MMTV integration site family (WNT) pathway is a highly conserved signaling shared between vertebrates and invertebrates, and plays fundamental roles in embryogenesis and homeostasis processes, regulating events such as stem cell differentiation, cell proliferation, and tissue regeneration[1]. The formation of dental tissues is intimately associated with the participation of several Wnt signaling components, including Wnt ligands, receptors, transducers, transcription factors, and antagonists[2]. Upon Wnt receptor activation, three different pathways can be activated, namely the canonical Wnt/β-catenin cascade, the noncanonical planar cell polarity (PCP) pathway, and the Wnt/Ca2+ pathway. Among these three pathways, the canonical one consists of four segments: extracellular signaling, membrane segment, cytoplasmic segment, and nuclear segment, and it is also the most extensively studied, being the central topic of this review[1].

The β-catenin protein, encoded by the *CTNNB1* gene, is the main component of the canonical Wnt/β-catenin molecular pathway[1]. The canonical Wnt/β-catenin signal transduction system is mainly activated by Wnt1, Wnt2, and Wnt3 ligands, causing the unphosphorylated β-catenin to accumulate in the cytoplasm, enter the nucleus, and complex with lymphoid enhancer factor/T cell factor (LEF-1/TCF)[1]. When the pathway is inactive by the absence of Wnt ligands, β-catenin is phosphorylated by a protein destruction complex, made up of kinases [glycogen synthase kinase 3β (GSK3β) and casein kinase 1α (CK1α)], adenomatosis polyposis coli (APC) and AXIN, and subsequently degraded in the proteasome, impeding its functions as a transcription factor and in cell-cell interaction[1].

**Statement of Clinical Significance**
The Wnt/β-catenin signaling pathway plays a critical role in normal tooth development and its dysregulation has been implicated in the biology of odontogenic cysts and tumors. Increasing evidence links Wnt/β-catenin pathway alterations to calcifying odontogenic cysts, odontomas, adenoid ameloblastomas, and ghost cell-containing odontogenic tumors.

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The schematic diagram of the canonical Wnt/β-catenin signal pathway is shown in Figure 1.

Abnormal activation of the WNT pathway is associated with the pathogenesis of several types of cancer, including solid tumors, hematological malignancies, and sarcoma[1]. Alterations related to the canonical Wnt/β-catenin pathway have been linked to odontogenic cysts and tumors, which are lesions derived from tooth apparatus[2-4]. With increasing evidence supporting the involvement of the Wnt/β-catenin pathway disruption in the pathogenesis of calcifying odontogenic cyst (COC), odontoma, ameloblastoma adenoid (AA), and ghost cell-containing odontogenic tumors[5-8], understanding its role in tumor initiation, growth, and expression in a tumor-type-specific manner would be universally beneficial. With this in mind, here we review the main alterations reported in the Wnt/β-catenin pathway components in the context of the odontogenic cysts and tumors, focusing on their molecular profile.

## LITERATURE REVIEW

### Search strategy and selection criteria

References used in this review were identified through searches of Medline/PubMed (US National Library of Medicine, Bethesda, USA) and SCOPUS (Elsevier, Amsterdam, The Netherlands) with the search terms "odontogenic cysts", "odontogenic tumors", "molecular", "genetics", "mutation", "Wnt/β-catenin signaling pathway", "*CTNNB1*", "immunohistochemistry" linked with Boolean operators 'AND' or 'OR'. Hand searches were also undertaken by cross-checking the reference lists of the included articles. Studies needed to be in English. Articles investigating Wnt/β-catenin signaling pathway in odontogenic lesions were included. Exclusion criteria were articles in which the data could not be extracted, letters to the editor, and expert opinions/comments, unless any of these types of articles provided sufficient and detailed data on Wnt/β-catenin signaling pathway in odontogenic lesions.

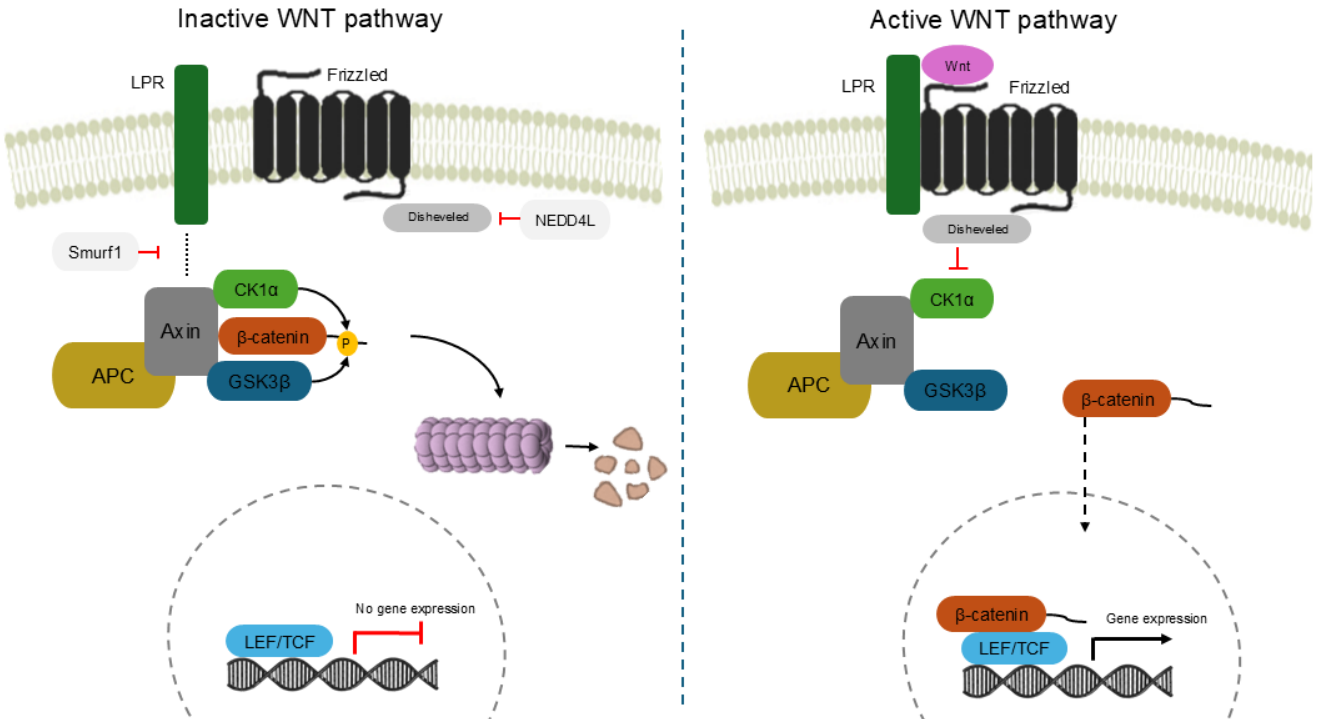


**Figure 1.** Schematic diagram depicting Wnt pathway. (A) In the absence of extracellular Wnt signals, a destruction complex — consisting of AXIN, adenomatous polyposis coli (APC), glycogen synthase kinase 3β (GSK3β), and casein kinase 1α (CK1α) — phosphorylates β-catenin, targeting it for ubiquitylation and proteasomal degradation. (B) WNT binding to Frizzled and LRP (low-density lipoprotein receptor-related protein) co-receptors activates Disheveled (DVL), which results in inhibition of the destruction complex. As a result, β-catenin translocates to the nucleus, where it bridges with lymphoid enhancer factor/T cell factor (LEF-1/TCF) transcription factors to drive gene expression.

## Role of Wnt/β-catenin signaling in odontogenesis

Extensive research into tooth morphogenesis has highlighted the involvement of Wnt/β-catenin signaling during epithelial–mesenchymal interactions in odontogenesis[2]. However, the precise role of each Wnt family member in tooth development remains unclear, as knockout models of certain Wnt genes result in early lethality, failure to initiate odontogenesis, or disruption of tooth development progression[3]. Inhibition of Wnt signaling via Dkk1 expression (a Wnt inhibitor) or epithelial-specific β-catenin deletion arrests tooth development at the bud stage and reduces the expression of key regulators like Shh, Bmp4, Msx1, and Lef1[3]. Some Wnt genes are expressed exclusively in the dental epithelium, others solely in the mesenchyme, and a subset exhibit dynamic expression patterns that shift from early developmental stages to postnatal phases, demonstrating a spatial and temporal specificity in Wnt function[2,3]. For example, recent studies have shown that during the bud stage of tooth development, Wnt10a is expressed only in the epithelium, while Dkk1 and Sost, both Wnt modulators, are restricted to the mesenchyme[2]. As tooth development progresses, Wnt10a expression appears in the mesenchyme, and in the postnatal stage, Wnt10a, Dkk1, and Sost are detected in both terminally differentiating and secretory odontoblasts. Following dentin and enamel mineralization, Wnt10a expression becomes limited to odontoblasts[2].

## Altered Wnt/β-catenin signaling in odontogenic lesions

Wnt/β-catenin signaling components can generally be classified as either positive or negative regulators. In tumoral lesions, the negative regulators — primarily responsible for suppressing tumorigenesis — are often mutated or exhibit loss-of-function alterations, while the positive regulators tend to be activated[1,3]. Alterations in this molecular pathway can be related to WNT inhibitor silencing, frizzled receptors, LEF-1/TCF components, and *AXIN*, *APC* and *CTNNB1* mutations[1]. *CTNNB1* mutations are one of the main events related to alteration in Wnt/β-catenin pathway. *CTNNB1* dysregulation produce conformational changes in the protein that prevent its phosphorylation, resulting in its stabilization and cytoplasmic accumulation and subsequent translocation to the nucleus, from where it exerts its action through (LEF-1/TCF) transcriptional factors, allowing the expression of genes such as *CCND1* and *MYC*, encoding the cyclin D1 and c-myc proteins, respectively, stimulating cell proliferation and thus enabling tumorigenic development[1].

Cysts and tumors derived from the odontogenic apparatus exhibit a wide spectrum of histopathological patterns, ranging from benign to malignant, and often display local invasiveness[9]. Among these lesions, several demonstrate alterations in components of the Wnt/β-catenin signaling pathway. Table 1 summarizes immunhohistochemical and molecular findings in odontogenic cyst and tumors presenting alterations in Wnt/β-catenin signaling. In odontogenic cysts, only a limited subset — primarily syndromic or recurrent odontogenic keratocysts and COC — show complete nuclear translocation and/or cytoplasmic accumulation of β-catenin, indicating pathway activation and concurrent reduction in membranous expression[4]. Nuclear β-catenin translocation has also been observed in odontogenic tumors, particularly in AA, dentinogenic ghost cell tumor (DGCT), ghost cell odontogenic carcinoma (GCOC), odontogenic carcinoma with dentinoid (OCD), and ameloblastoma[4–8]. In some of these lesions, cytoplasmic and/or nuclear β-catenin immunopositivity is associated with identifiable mutations in the Wnt/β-catenin pathway[4–8]. The Wnt/β-catenin mutations observed in some of these odontogenic lesions are displayed in Figure 2.

## Calcifying odontogenic cyst

Calcifying odontogenic cyst (COC) is a developmental odontogenic cyst characterized by ameloblastomatous epithelial lining containing focal accumulations of ghost cells, juxtaepithelial dentinoid and calcifications[9]. Studies have established a clear association between the activation of the Wnt/β-catenin pathway and the pathogenesis of COC[5,10]. Mutations in the *CTNNB1* gene, which encodes β-catenin protein, were evidenced in approximately 90% of COC cases included in molecular studies[10]. The study by Hassanein et al. found cytoplasmic and nuclear β-catenin staining in tumors with shadow cells, including COC samples, and provided the foundation for subsequent research implicating *CTNNB1* in COC biogenesis[11]. In line with this, Sekine et al. were the first authors to describe COC cases (9/10) harboring β-*catenin* hotspots mutations — involving codons 32, 33, 34, 37 and 41 of exon 3 — through single-gene analysis[10]. Of note, these codon regions in exon 3 are serine-threonine phosphorylation sites for GSK-3β and these mutations inhibit GSK-3β resulting in β-catenin accumulation[10]. Additional evidence also supports the identification of *CTNNB1* mutations in codons 4 and 5 in one case of COC[5]. Through a next generation sequencing panel covering 50 cancer genes, Sousa et al. confirmed *CTNNB1* mutation in the hotspot codon 33

**Table 1.** Immunohistochemical and molecular analysis of odontogenic cysts and tumors exhibiting alterations in Wnt/β-catenin signaling.

| Reference | Number of cases | Genes/Proteins detected | Results | |
|---|---|---|---|---|
| | | | Immunohistochemistry | Molecular studies (mutation) |
| **Calcifying odontogenic cyst** | | | | |
| Sekine et al.[10] | 11 | *CTNNB1* β-catenin | cytoplasmic and nuclear staining (11/11) | Asp **32** Asn (2 cases)<br>Asp **32** Tyr<br>Ser **33** Phe<br>Ser **33** Cys<br>Gly **34** Arg<br>Gly **34** Val<br>Ser **37** Cys<br>Tyr **41** Ile (2 cases)<br>(9/11) |
| Hassanein et al.[11] | 6 | *CTNNB1* β-catenin | strong nuclear and cytoplasmic staining (6/6) | NE |
| Ahn et al.[5] | 3 | *CTNNB1* β-catenin | cytoplasmic and nuclear expression (3/3) | Thr **3** Ser<br>Gln **4** His<br>Ala **5** Val<br>Val **57** Ala<br>(3/3) |
| Bilodeau et al.[15] | 11 | *CTNNB1* β-catenin | NI (9/11) | NE |
| | | LEF-1 | NI (7/11) | NE |
| Sousa et al.[12] | 3 | 50 tumor suppressor genes and oncogenes, including *CTNNB1* β-catenin | NE | Ser **33** Phe<br>(2/3) |
| Dutra et al.[14] | 6 | β-catenin | cytoplasmic and nuclear expression (5/6). Undetectable in the ghost cells | NE |
| | | Wnt1 | lining epithelium (6/6), ghost cells (5/6) | |
| | | Wnt5a | lining epithelium, ghost cells (6/6) | |
| Yukimori et al.[13] | 11 | *CTNNB1* β-catenin | nuclear staining (11/11) | Asp **32** Gly (2 cases)<br>Ser **33** Cys (4 cases)<br>Ser **33** Phe (2 cases)<br>Ser **37** Phe (2 cases)<br>(10/11) |
| | | *APC* | NE | Pro **1433** Leu<br>(1/11) |
| **Dentinogenic ghost cell tumor** | | | | |
| Kim et al.[16] | 1 | *CTNNB1* β-catenin | nuclear, cytoplasmic, and membranous expression. Undetectable in the ghost cells | Thr **3** Ser |
| Ahn et al.[5] | 1 | *CTNNB1* β-catenin | cytoplasmic and nuclear expression | Thr **3** Ser |

Continue...

**Table 1.** Continuation.

| Reference | Number of cases | Genes/Proteins detected | Results | |
|---|---|---|---|---|
| | | | Immunohistochemistry | Molecular studies (mutation) |
| Soares et al.[19] | 1 | *CTNNB1* β-catenin | weak positivity in the cell membrane and nuclear expression | NE |
| Oh et al.[17] | 3 | CTNNB1 β-catenin | nuclear expression (3/3) | WT in codons 32, 33, 34, 37, 41, and 45 |
| | | *APC* | NE | Q**1080** (1 case) |
| Xue et al.[18] | 15 | *CTNNB1* β-catenin | nuclear expression (15/15) | NE |
| **Adenoid ameloblastoma** | | | | |
| Bastos et al.[6] | 9 | *CTNNB1* β-catenin | nuclear expression (7/9) | Ser **33** Cys (2/9)<br>Gly **34** Arg<br>Ser **37** Phe (4/9) |
| Xue et al.[18] | 13 | *CTNNB1* β-catenin | nuclear expression (13/13) | Gly **34** Arg (1/13) |
| Oh et al.[17] | 2 Typical AA | *CTNNB1* β-catenin | nuclear expression (2/2) | Y**333**fs (1 case) |
| | | *APC* | NE | E**789**fs (1 case) |
| | 2 AA-DGCT | *SMURF1* | NE | L**424**fs (1 case) |
| | | *NEDD4L* | NE | N**846**fs (1 case) |
| **Odontoma** | | | | |
| Fujii et al.[7] | 21 | *CTNNB1* β-catenin | cytoplasmic and nuclear expression (15/21) | WT (2 cases) |
| | | *APC* | NE | WT (2 cases) |
| Tanaka et al.[23] | 69 | *CTNNB1* β-catenin | membrane, cytoplasmic and nuclear labeling in odontogenic epithelial cells some of the ghost cells weakly positive | NE |
| | | Lef-1 | nuclear labeling in odontogenic epithelial cells some of the ghost cells weakly positive | NE |
| França et al.[21,24] | 23 Compound odontoma | *CTNNB1* β-catenin | membrane of the odontogenic lining epithelium and ectomesenchyme | NE |
| | | WNT-1 | membrane of odontogenic epithelium | |
| | 21 Complex odontoma | *CTNNB1* β-catenin | nuclear in the odontogenic epithelium and ectomesenchyme | NE |
| | | WNT-1 | membrane of odontogenic epithelium | |

Continue...

**Table 1.** Continuation.

| Reference | Number of cases | Genes/Proteins detected | Results | |
|---|---|---|---|---|
| | | | Immunohistochemistry | Molecular studies (mutation) |
| **Ghost cell odontogenic carcinoma** | | | | |
| Ahn et al.[5] | 1 | *CTNNB1* β-catenin | cytoplasmic and nuclear expression | Ser **33** Tyr |
| Rappaport et al.[25] | 1 | *CTNNB1* | NE | Ser **33** Cys |
| Bose et al.[26] | 1 | *APC* | NE | V**123**fs |
| Ohata et al.[28] | 1 | *CTNNB1* β-catenin | nuclear expression | Ser **33** Cys |
| Seki-Soda et al.[27] | 1 | *CTNNB1* β-catenin | nuclear expression | Ser **33** Cys |
| | | Lef-1 | nuclear expression | NE |
| Sakamoto et al.[29] | 1 | *CTNNB1* β-catenin | nuclear expression | Asp **32** Val |
| **Odontogenic carcinoma with dentinoid** | | | | |
| Gondak et al.[8] | 2 | *CTNNB1* β-catenin | cytoplasmic and nuclear expression (2 cases) | Ser **33** Ala (1 case) |
| | | *APC* | NE | p.R**876**fs.2626 C>T (1 case) |

Fs: frameshift mutations; NE: not evaluated; WT: wild type.

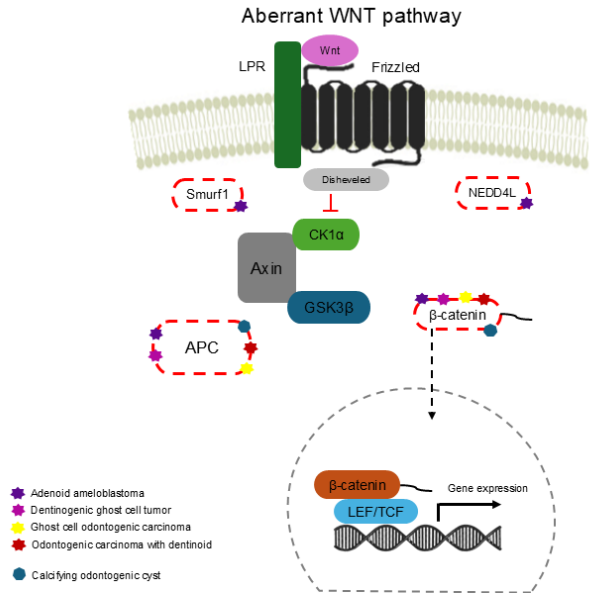


**Figure 2.** Members of Wnt/β-catenin signaling that show alterations in the odontogenic lesions included in this review. Mutations in *APC*, β-*catenin*, and in E3 ubiquitin ligases, *SMURF1* and *NEDD4L*, identified in some lesions reviewed here. As result, β-catenin interacts with LEF-1/TCF transcription factors to drive gene expression.

as the only pathogenic mutation in 2/3 cases of COC[12]. Yukimori et al., using the same cancer hotspot panel in 11 cases of COC, observed *CTNNB1* mutations in 10 of them, which mainly occurred in codon 33[13]. An additional APC mutation of uncertain significance was also detected in this study[13]. These molecular findings often coincide with the cytoplasmic and nuclear immunohistochemical (IHC) expression of β-catenin observed in the cystic epithelial lining and in transition cells, while undetectable in ghost cells[5,10,13,14]. The expression of β-catenin is consistent with its activated state and an essential step to its translocation into the nucleus to activate the TCF/LEF, causing a downstream to activate WNT target genes. The coexpression of β-catenin and other members of the WNT signaling, such the nuclear transcription factor LEF-1, Wnt1 and Wnt5a, was also demonstrated in the epithelium in COC[14,15]. Regarding the expression in the ghost cells, Wnt1 and Wnt5a were detectable in the ghost cells of most COCs[14].

## Dentinogenic ghost cell tumor

Dentinogenic ghost cell tumor (DGCT) is defined as a benign but locally infiltrative odontogenic tumour characterized by a conventional ameloblastoma-like epithelium with the presence of ghost cells and

dentinoid deposition[9]. Only a few cases of DGCT have been molecularly studied in the literature, making a comprehensive molecular characterization of these tumors more challenging. However, all the samples until now showed alteration in genes and proteins related to the Wnt/β-catenin pathway. Ahn et al. and Kim et al. by single-gene analysis were the first studies that reported the same missense mutation in codon 3 of the *CTNNB1* in one case of DGCT each, resulting in the substitution of threonine by serine[5,16]. More recently, the group of Oh et al. did not show mutation in hotspot codons 32, 33, 34, 37, 41 and 45 of *CTNNB1* in the 3 samples investigated[17]. On the other hand, a nonsense mutation of *APC* gene was reported in one case of DGCT[17]. The presence of *CTNNB1* hotspot mutations had also been investigated by Xue et al. in a cohort of 14 cases of DGCT via DNA direct sequencing and none of the samples harbored *CTNNB1* hotspot mutations[18]. Immunohistochemical analyses of β-catenin expression in DGCT demonstrated consistent positivity across all cases, with pronounced nuclear, cytoplasmic, and membrane accumulation of β-catenin in tumor cells, while ghost cells lacked expression[5,16-19]. The existence of wild-type *CTNNB1* cases but with cytoplasmic and nuclear accumulation of β-catenin suggests the possibility of mutations in other genes involved in the WNT pathway, such as *APC*, *Axin1* and *Axin2*, whose proteins are part of the β-catenin degradation complex; or a large deletion involving exon 3[10].

### Adenoid ameloblastoma

Adenoid ameloblastoma (AA) is a newly recognized epithelial odontogenic tumor, classified as a distinct entity in the 5th WHO Classification of Head and Neck Tumors updated in 2022. To date, approximately 40 cases have been reported worldwide[9]. Microscopically, AA exhibits ameloblastoma-like proliferation, duct-like spaces, cribriform architecture and cellular condensations called morules. In addition, focal ghost cells, dentinoid deposition, and clear cells serve as desirable diagnostic criteria[9].

The discovery that mutations in the *CTNNB1* gene were associated with a subset of AA made by Bastos et al. provided considerable impetus to the aberrant activation of Wnt/β-catenin signaling[6]. The authors identified *CTNNB1* hotspot mutations in the exon 3 by Sanger sequencing in 4/9 AAs: two cases in codon 33, one in codon 34 and the remaining one at codon 37, which are codons related to phosphorylation sites by GSK3 and CK1 comprising the β-catenin destruction complex[6]. Notably, the protein product of *CTNNB1* gene, β-catenin, was also found in 7/9 AA cases, showing intense nuclear immunoexpression identified by IHC[6]. Another study of Xue et al. identified *CTNNB1* mutation in only one case of AA (1/13), despite detecting nuclear overexpression of β-catenin in all thirteen cases by IHC[18]. Oh et al. reported no *CTNNB1* hotspot mutations in codons 33, 37, 41, and 45 in AA[17]. However, they reported *CTNNB1* frameshift insertion leading to a premature stop codon in one case of AA (1/4) identified by whole-exome sequencing, and nuclear overexpression of β-catenin protein by IHC in all analyzed cases[17]. Genetic evidence also supports the existence of mutations in other components of the WNT pathway, such as *APC*, *SMURF1* and *NEDD4L* mutations, in AA[17]. More specifically, loss of function mutation in the *APC* gene leading to a premature stop codon was found in one case of AA (1/4). Loss of function mutations in E3 ubiquitin ligases, *SMURF1* and *NEDD4L*, were also identified in one case of AA each (1/4) by whole-exome sequencing[17].

Based on these data it is reasonable to propose that *CTNNB1* hotspot mutations are detected in AA at a relatively low frequency (<50%)[6]. It seems that loss of function alterations in genes related to β-catenin destruction complex or encoding E3 ubiquitin ligases may be an event modeling Wnt/β-catenin signaling in AA, which could also explain the accumulation of β-catenin in the nucleus of tumor cells.

### Odontoma

Odontoma is a hamartomatous odontogenic lesion with compound and complex subtypes and the most common odontogenic tumors. Histologically, odontoma is composed of calcified dental tissues of both epithelial and ectomesenchymal origin, displaying different levels of organization, including tooth-like structures[9,20]. The importance of Wnt/β-catenin signaling during embryonic development of the teeth is well established[2,20], and it also seems that aberrant regulation of Wnt/β-catenin processes could contribute to odontoma initiation and progression[7,20,21]. For instance, an experimental animal model demonstrated that the activation of the Wnt/β-catenin pathway in SOX2-positive embryonic progenitors enables ectopic expression of signals promoting odontogenesis throughout the oral cavity. The odontogenic potential of epithelial dental stem cells persists postnatally, and targeted Wnt/β-catenin hyperactivation within these cells is sufficient to drive the formation of ectopic dental malformations resembling supernumerary teeth or odontomas[20]. Furthermore, certain odontomas are associated with genetic

disorders, such as familial adenomatous polyposis, caused by *APC* mutations, another critical component of the WNT pathway[22].

In cases of human odontomas, Fujii et al. detected β-catenin accumulation in the nucleus/cytoplasm in 15 out of 21 cases and in the cell membrane in 6 out of 21 cases in odontogenic epithelial cells within odontoma[7]. To elucidate whether the activation of the β-catenin pathway was associated with genetic mutations of the *CTNNB1* or *APC* genes, the authors direct sequenced two odontomas with accumulation of β-catenin in nucleus/cytoplasm for exon 3 of the *CTNNB1* gene, and the exon 15 of the *APC* gene, and found no mutations of *CTNNB1* or *APC*, suggesting that another mechanism of β-catenin accumulation without genetic mutations may be present in odontomas[7]. The Wnt/β-catenin signaling cascade has also been implicated in odontomas with different phases of development and with ghost cells[23]. Tanaka et al. examined 69 odontomas with ghost cells, detecting positive reaction for β-catenin in the nucleus, cytoplasm and membrane of odontogenic epithelial cells around the ghost cells by IHC. Likewise, the expression of the transcription factor related to Wnt/β-catenin, Lef-1, was seen in the nucleus of odontogenic epithelial cells. Some of the ghost cells were focally and weakly positive for β-catenin and Lef-1[23]. In addition, compound and complex odontomas subtypes showed different β-catenin immunoexpression pattern by IHC[21]. In compound odontoma, the β-catenin had higher immunoexpression in the membrane of the odontogenic lining epithelium and ectomesenchyme, while nuclear β-catenin was observed in the odontogenic epithelium and ectomesenchyme next to ghost cells in complex odontoma[21,24]. Focal immunoexpression of Wnt-1 was also found in islands of odontogenic epithelium in compound and complex odontoma[21]. Altogether, these findings highlight potential differences in Wnt/β-catenin signaling involvement during odontoma development.

### Ghost cell odontogenic carcinoma

Ghost cell odontogenic carcinoma (GCOC) is a malignant odontogenic neoplasm with ghost cells and occasionally dentinoid deposition, arising either de novo or from a benign precursor, such as COC or DGCT[9]. So far, molecular studies on GCOC have been restricted to a single case-report or the analysis of multiple samples from the same tumor, often in cases where benign and malignant components coexist or when the tumor is recurrent or metastatic[5,25-29]. In order to better characterize these few samples tested, next generation sequencing was applied by three studies[25-27]. By direct DNA sequencing or next generation tools, *CTNNB1* Ser 33 Cys mutation in codon 33 was the most frequent alteration harbored by four cases[5,25-28]. One tumor showed *CTTNB1* Asp 32 Val mutation in codon 32[29]. An integrative genomic and transcriptomic investigation showed a homozygous frameshift *APC* V123fs mutation, which might result in truncated non-functional protein[26]. In addition, transcriptomic analysis in GCOC found overexpression of several members of WNT signaling, such as WNT5a, WNT4, FZD10 and GSK3β, contrasting to downexpression of APC, likely due to the truncating mutation in APC detected[26]. In all investigated cases, IHC demonstrated β-catenin localization in the cytoplasm and/or nucleus of tumor cells[5,27-29]. LEF-1 immunoexpression was also evidenced in nuclei of tumor cells in one study[27].

### Odontogenic carcinoma with dentinoid

Odontogenic carcinoma with dentinoid (OCD) is a rare and controversial condition, not included in the 2022 WHO classification of odontogenic lesions. To date, only a few cases reported in the literature fulfill the diagnostic criteria for OCD[8,30]. Microscopically, OCD exhibits epithelial proliferation composed of eosinophilic, pale, or clear cells, often associated with abundant dentinoid material deposition. Focal areas with microcystic pattern, duct-like structures, ameloblast-like differentiation and cellular atypia can be observed[8,30]. From the OCD reported in the literature, only one study searched for mutations in this tumor and showed that OCD carries WNT pathway mutations[8]. Gondak et al. by using next-generation sequencing approach identified pathogenic mutations *CTNNB1* Ser 33 Ala in codon 33 and *APC* R876fs in one case of OCD each[8]. Consistent with WNT-signaling activation, both tumors showed strong β-catenin accumulation in the cytoplasm and in the nuclei of tumor cells[8].

## CONCLUSION

This review retrieves the main molecular and immunohistochemical findings that signalize the involvement of Wnt/β-catenin signaling in the molecular biology of calcifying odontogenic cyst, dentinogenic ghost cell tumor, odontoma, adenoid ameloblastoma, ghost cell-odontogenic carcinoma and odontogenic carcinoma with dentinoid. Mutation in *CTNNB1* gene — involving codons 32, 33, 34, and 37 of exon 3 — was almost universally seen in the analyzed lesion. However, the existence

of wild-type *CTNNB1* cases but with cytoplasmic and nuclear accumulation of β-catenin suggests mutations in other genes involved in the WNT pathway, such as *APC*, *Axin* and E3 ubiquitin ligases, *SMURF1* and *NEDD4L*, whose proteins are part of the β-catenin degradation complex. Although ghost cell-containing tumors frequently harbor *CTNNB1* mutations, their role in ghost cell formation is not yet fully understood. Finally, although molecular analysis is a valuable tool for gaining deeper insights into tumor characteristics, these genetic features are not diagnostic and cannot replace a meticulous histopathological and clinicoradiographic evaluation for the diagnosis of odontogenic cysts and tumors.

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## AUTHORS' CONTRIBUTIONS

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