

## WNT signalling pathway in oral lesions

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### Abstract

Wingless-Integrated/Beta-catenin (WNT/ $\beta$ -catenin) signalling pathway is one of the principal intercellular signalling pathways in humans. It plays an intrinsic role in the cellular proliferation, differentiation and regeneration along with many other cellular functions. Epigenetic deoxyribonucleic acid methylations and silencing of WNT signalling pathway genes have a significant role in malignant transformation of oral lesions such as oral submucous fibrosis, oral leukoplakia, oral lichen planus and erythroplakia. The increase in WNT inhibitory proteins along with inflammatory factors cause bone loss in periapical lesions, such as chronic apical periodontitis. This review discusses the molecular genetics of potentially malignant oral lesions, sheds light on our understanding of WNT/ $\beta$ -catenin signalling in bone loss pertaining to periapical lesions, and alteration of this pathway for therapeutic benefits.

**Keywords:** Bone loss, Malignant transformation, Oral lesions, Periapical lesions, WNT signalling.

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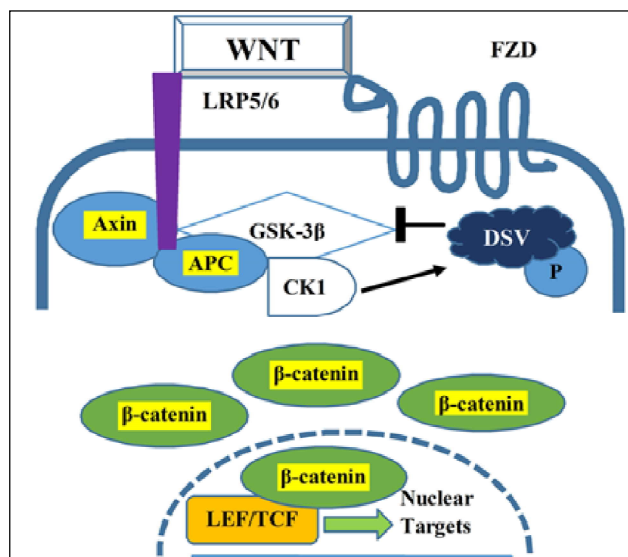
### Introduction

Wingless-Integrated/Beta-catenin (WNT/ $\beta$ -catenin) signalling pathway is one of the fundamental mechanisms that regulate various biological processes such as cell division, migration and fate determination during embryonic development, cell regeneration and maintenance in adults. Wingless-Integrated (WNT) pathway has been characterised into non-canonical (beta-catenin-independent) pathway and canonical (beta-catenin-dependent) pathway. WNT/ $\beta$ -catenin pathway comprises of family of ligands, receptors and co-receptors which are associated with signal transduction across the cell and induce various intracellular responses. WNT pathway ligands comprise a diverse family of glycolipoproteins having about 350-400 amino acids such as WNT1, WNT2 and WNT3 etc.<sup>1</sup> WNT protein initiates signalling when it binds to Frizzled receptor (transmembrane receptor). In addition to WNT

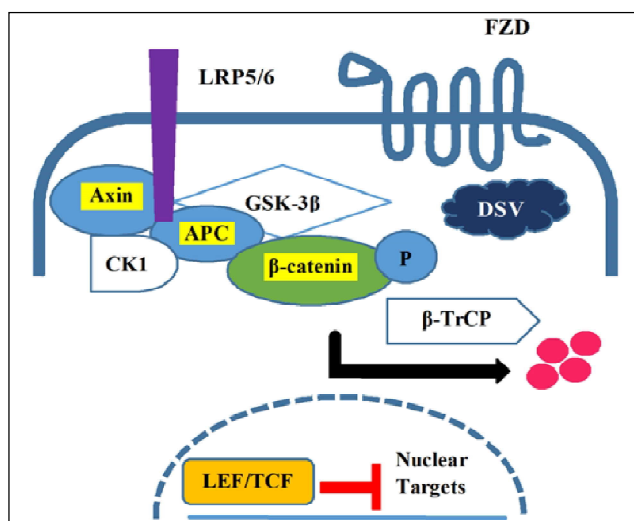
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ligand and receptor, co-receptors are required such as lipoprotein receptor-related protein 5/6 (LRP-5/6) to promote interaction between WNT protein and (Frizzled (Fz)) receptor. The signal is then transmitted to dishevelled (DVL) protein inside the cell which regulates the expression of associated gene. The canonical WNT signalling is associated with cytoplasmic accumulation of beta-catenin ( $\beta$ -catenin) and ultimately its translocation into the nucleus, where it interacts with the transcription factors i.e., T-cell factor/lymphocyte enhancer factor (TCF/LEF) (Figure 1A).  $\beta$ -catenin is subjected to degradation in the absence of WNT signalling through the WNT pathway destruction complex. This destruction complex includes the following proteins: glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ), protein phosphatase 2A (PP2A), axis inhibition protein 2 (Axin2), adenomatosis polyposis coli (APC) and casein kinase 1 $\alpha$  (CK1 $\alpha$ ).<sup>2,3</sup> The destruction complex phosphorylates  $\beta$ -catenin which is eventually degraded by ubiquitin-mediated proteasomal proteolysis (Figure 1B). In addition to excitatory pathway, another pathway which regulates the proper functioning of WNT signalling has been

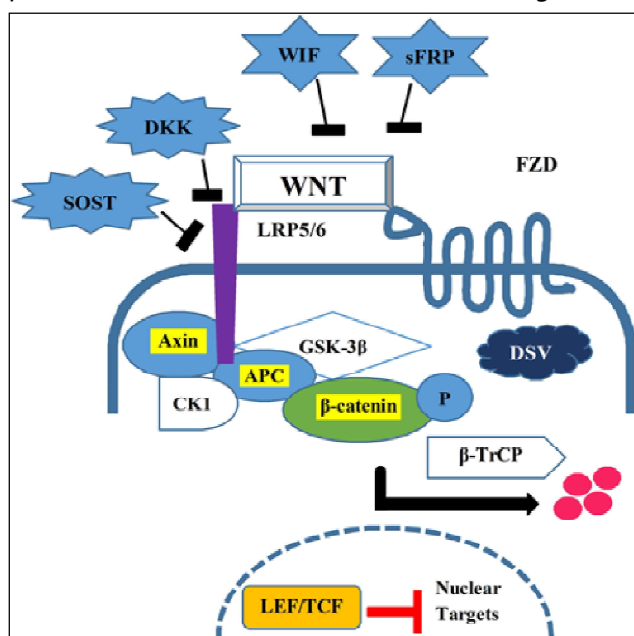


**Figure-1 (A):** Schematic presentation of Wnt/ $\beta$ -catenin signalling pathway. In the presence of a WNT ligand, phosphorylation and degradation of  $\beta$ -catenin are inhibited, allowing it to accumulate in the cytoplasm and translocate into the nucleus. Nuclear  $\beta$ -catenin interacts with LEF/TCF family transcription factors to initiate transcription of target genes.



**Figure-1 (B):** In the absence of a WNT ligand, cytoplasmic  $\beta$ -catenin is associated with APC and Axin, phosphorylated by GSK3 $\beta$  and CK1, and ubiquitinated by the  $\beta$ TrCP complex, targeting it for proteasomal degradation; under these conditions, LEF/TCF transcription factors repress the transcription of Wnt target genes in the nucleus.

characterised as WNT inhibitory pathway and may be classified into two types: (1) WNT antagonists that antagonise and neutralise the WNT ligand i.e., Secreted frizzled-related proteins 1-5 (SFRP 1-5) and WNT inhibitory factor-1 (WIF1), and (2) WNT antagonists that antagonize the WNT receptors i.e., Dickkopf-related proteins 1-4 (DKK1-4) and Sclerostin (SOST) (Figure 1C).<sup>4</sup>



**Figure-1 (C):** In the presence of Wnt antagonists, Wnt ligand and receptor are inhibited,  $\beta$ -catenin is phosphorylated and targeted for proteasomal degradation.

Misregulation of the WNT/ $\beta$ -catenin pathway causes various skeletal defects, fibrotic diseases, inflammatory disorders and malignant transformations of multiple cancers. Micro-environmental factors, such as inflammatory factors and cytokines, can cause impairment of WNT pathway which results in the development of various pathologies.<sup>5-7</sup> Various oral lesions have been characterised as potentially malignant oral diseases (PMODs), such as oral submucous fibrosis (OSMF), erythroplakia, oral leukoplakia (OL) and oral lichen planus (OLP).<sup>8</sup> A number of molecular mechanisms have been reported so far in the pathogenesis and malignant transformation of the above-mentioned oral lesions. In addition, WNT/ $\beta$ -catenin signalling plays a fundamental role in bone biology, and aberrations in WNT pathway play a critical role in bone destruction related to periapical and periodontal lesions. The current literature review was planned to elucidate molecular genetics that regulate WNT signalling in potentially malignant oral lesions and to analyse the recent advancements that will improve our understanding in the identification of therapeutic biomarkers for early recognition and effective treatment of such diseases. Also, it is hoped that this review would provide knowledge regarding WNT signalling in bone pathology to illustrate the pathological process of bone destruction in periapical diseases and how the regulation of WNT/ $\beta$ -catenin signalling may impart better therapeutic aids to restrict bone loss in endodontic disease. In this review data is compiled regarding role of WNT antagonists, such as DKK3, WIF1 and SFRP in malignant transformation of OSMF. Similarly, the role of other WNT molecules such as WNT3, Axin2 and  $\beta$ -catenin in malignant transformation of leukoplakia, lichen planus and erythroplakia is also discussed. The effects of agonist and antagonist of WNT signalling pathway on bone loss in periapical and periodontal lesions are explained. Electronic literature databases such as PubMed and Google scholar were searched using the Medical Student Headlines (MeSH) terms, such as WNT signalling, OSMF, OL, OLP, erythroplakia, potentially malignant oral lesions, periapical lesions, bone loss, WNT agonists and WNT antagonists in various combinations. All studies in English language having full-texts available and those which investigated the role and implications of WNT signalling within potentially malignant oral and periapical lesions were included. The papers that were published in

language other than English and whose full texts were not available were excluded.

### **Malignant transformation in oral submucous fibrosis and WNT pathway**

Oral submucous fibrosis (OSMF) is a complex, insidious, chronic disease of the oral cavity, first recognized in the early 1950s, characterised by progressive scarring and juxta-epithelial hyalinization of lamina propria. The progression of OSMF ultimately leads to stiffness of oral mucosa and eventually limited mouth-opening.<sup>9</sup> It can affect any part of the oral cavity i.e., anterior and posterior buccal mucosa, tongue, soft palate and sometimes the upper oesophageal tract. OSMF is one of the pre-malignant lesions of oral squamous cell carcinoma (SCC).<sup>10,11</sup>

Dickkopf (DKK) antagonists inhibit the intracellular molecular cascade by binding to WNT receptor. The DKK family consists of a number of members i.e., DKK1-4 which have different impact on WNT/ $\beta$ -catenin canonical pathway. Aberrant promoter 5'-Cytosine-phosphate-Guanine-3' (CpG) methylation of DKK3 along with increased levels of DKK3 in different stages of OSMF have revealed its oncogenic role in malignant progression of OSMF. The co-expression of other genes, like zinc finger protein 415 (ZNF415), testis-specific Y-like protein 5 (TSPYL5), zinc finger protein 107 (ZNF107) and leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), along with DKK3 provide evidence that various molecular mechanisms regulate the pathogenesis of OSCC. DKK3 also inhibits the fundamental process of apoptosis through mitochondrial and calcium ( $\text{Ca}^{2+}$ ) dependent pathway. The early identification of DKK3 may be used as a potential biomarker and reintroduction of DKK3 may provide effective treatment approach.<sup>12</sup>

WNT inhibitory factor-1 (WIF1) is a WNT antagonist and functions as a tumour suppressor gene. It binds to WNT protein and inhibits the WNT/ $\beta$ -catenin signalling pathway. Aberrant promoter methylation of tumour suppressor gene is a key step in malignant transformation of OSMF. WIF1 expression is down-regulated not only at protein level but also at messenger ribonucleic acid (mRNA) level in the carcinogenesis of OSMF. Epigenetic modification of WIF1 by promoter CpG methylation and gradual reduction in its expression in early, moderately advanced and advanced stages of OSMF till the malignant transformation suggest that WIF1 is a potential

tumour marker for early detection of oral pre-cancerous lesions.<sup>13</sup>

Secreted frizzled-related protein (SFRP) is another family of WNT antagonists that regulates the WNT pathway by attaching directly to the WNT ligand and inhibiting the receptor. SFRP family comprises five secreted extracellular signalling glycoproteins i.e., SFRP 1-5. Misregulation of SFRP1 and SFRP5 by promoter methylation leads to increased  $\beta$ -catenin accumulation in the cell and ultimately progression and malignancy of OSMF. The gradual reduction in protein expression of SFRP1 and SFRP5 at all stages of OSMF suggests a possible role in carcinogenesis of OSMF.<sup>14</sup>

### **Malignant transformation in oral leukoplakia and WNT pathway**

According to World Health Organisation (WHO), oral leukoplakia (OL) is defined as a "white patch or plaque that cannot be characterised clinically or histopathologically as any other disease, and is not associated with any physical or chemical causative agent except the use of tobacco".<sup>15</sup> It develops commonly on the buccal mucosa, tongue and the floor of oral cavity in response to constant irritation to the mucous membrane of oral mucosa. OL presents clinically in three different forms i.e., homogenous, non-homogenous and verrucous types. The causative factors include exposure to smoking, cheek biting, alcohol consumption, ill-fitting dentures, and vitamin deficiency. The estimated rate of malignant transformation is about 1-20% of patients over a period of 5-30 years.<sup>16</sup>

WNT3 glycolipoprotein, although not present in healthy oral epithelium, is strongly expressed on epithelial cell membrane in OL. The aberrant WNT3 expression has a strong correlation with nuclear expression of  $\beta$ -catenin. In healthy oral mucosa,  $\beta$ -catenin expression is located in the cell membrane and the expression gradually shifts from cytoplasm to the nucleus with the increasing grades of dysplasia. This nuclear accumulation of  $\beta$ -catenin plays an important role in malignant transformation of OL. The downstream target proteins such as cellular-Myc (c-Myc) (a proto-oncogene), and cyclin dependent protein 1 (cyclin D1) (a protein required for progression during G1 phase of cell cycle) expression increases with progression of severity in OL.<sup>17</sup> A study reported WNT3-positive and negative cytoplasmic with negative nuclear expression in OL whereas in proliferative verrucous

leukoplakia, the expression of WNT3 was nuclear only.<sup>18</sup>

Axis inhibition protein 2 (Axin2), a component of WNT/ $\beta$ -catenin destruction complex, phosphorylates  $\beta$ -catenin along with GSK3 $\beta$  and results in stabilisation of  $\beta$ -catenin. Axin2 is also important for nuclear export of GSK3 $\beta$  which results in increased nuclear snail. Activation of WNT canonical pathway is important for stabilization of nuclear snail.<sup>19,20</sup> The increased cytoplasmic expression of Axin2 and increased cytoplasmic as well as nuclear expression of Snail in OL lesions is associated with malignancy of these lesions. Axin2 serves as an oncogene of tumour progression, and the increased epithelial expression of Axin2 is strongly associated with epithelial mesenchymal transformation which ultimately leads to progression and malignant transformation of OL lesions.<sup>21</sup>

DKK3 expression increases with the severity of the OL. The positive cytoplasmic expression of DKK3 in mild dysplasia is less than the severe dysplasia where the expression is increased although not significant compared to its expression in OSMF. The increased cytoplasmic expression may be justified through inactivation of DKK3 by promoter CpG methylation and thus contributes to the malignant progression of OL.<sup>22</sup>

#### **Malignant transformation in oral lichen planus and WNT pathway**

WHO defined oral lichen planus (OLP) as "a benign, chronic autoimmune lesion which presents with white, papular, characteristic striated pattern typically on the buccal mucosa" OLP has three major subtypes i.e., reticular, erosive and ulcerative lesions which are present infrequently.<sup>23</sup> The highest malignant potential occurs in erosive or ulcerative OLP cases. The malignant potential of OLP transformation into oral SCC is less than 1%.<sup>24</sup> WNT3 in OLP has been characterised by positive cytoplasmic expression and lack of nuclear staining. This positive aberrant cytoplasmic and nuclear WNT3 expression suggests its role in the disease process and the expression is similar during the dysplastic change within OLP.<sup>18</sup>

#### **Malignant transformation in erythroplakia and WNT pathway**

Oral erythroplakia is defined as "discrete, velvety or plaque-like lesion, commonly found on the soft palate and buccal mucosa, which cannot be differentiated

clinically or histopathologically as any other recognisable condition".<sup>25</sup> A study showed that increased nuclear expression of  $\beta$ -catenin is directly proportional to greater severity of oral dysplasia with erythroplakia and leukoplakia as increased expression of  $\beta$ -catenin in lower epithelial strata is associated with cell proliferation and malignant transformation of these oral dysplastic lesions.<sup>26</sup>

#### **Bone loss in periapical lesions and WNT pathway**

WNT/ $\beta$ -catenin signalling pathway has been known for a long period to regulate normal bone development and maintain homeostasis through osteoblast recruitment from mesenchymal stem cells. WNT pathway also regulate bone resorption by altering the ratio of osteoprotegerin (OPG) and receptor activator of nuclear factor-kappa B ligand (RANKL) and increasing OPG production through osteoblast differentiation which blocks RANKL-induced osteoclastogenesis.<sup>27</sup>

Oral inflammatory diseases in which jaw bone loss occurs include periapical and periodontal lesions. Periapical lesions develop as a result of bacterial contamination of the dental pulp and eventual release of inflammatory factors cause deregulation of triad having receptor for RANK-ligand (RANK), RANKL and OPG. Apical periodontitis is one of the periapical lesions and occurs in response to bacterial infection in dental pulp and causes bone resorption surrounding the root apex.<sup>28</sup>

Liposaccharide (LPS), produced by bacteria in periapical infections, is a well-studied bone destruction molecule as it mediates inhibition of osteoblast differentiation, promote osteoclast formation and reduce the osteogenic differentiation of bone marrow mesenchymal stem cells (BMMSC) through inhibition of WNT/ $\beta$ -catenin signalling. A study supplemented the cultured rat BMMSCs with *Porphyromonas-gingivalis* lipopolysaccharide (Pg-LPS) and found the direct association of bacterial infection with decreased GSK3 $\beta$  phosphorylation in BMMSC, decreased  $\beta$ -catenin nuclear accumulation and translocation. Lithium chloride, a powerful GSK3 $\beta$  inhibitor, has shown very promising results by stimulating WNT/ $\beta$ -catenin pathway resulting in increased bone formation and improved healing in bone lesions. Contrary to it, transient exposure to WNT3a, an osteogenic differentiation regulator, rescued the osteoblastic maturation inhibition from LPS whereas chronic exposure prevented the differentiation process.<sup>29</sup>

One study verified increased DKK1 expression in rat model of periapical lesion using immunohistochemistry. Increased DKK1 expression was consistent with bony lesion expansion between day 7 and 21 i.e., active phase of bone resorption, whereas DKK1 expression decreased after day 21 i.e., chronic phase associated with the slow expansion. The co-localisation of DKK1 expression with RANKL-positive cells indicate DKK1 participation in bone resorption in periapical disease by inducing the expression of RANKL-protein.<sup>30</sup>

Another study used pre-osteoblastic cell lines conditioned with *Escherichia* (E.) coli lipopolysaccharide (LPS) and rat model of chronic apical periodontitis to access the role of DKK1 in bone destruction and restoration respectively. Initially at day 0 and 1, DKK1 expression levels raised in response to elevated pro-inflammatory cytokines interleukin 6 (IL6) and tumour necrosis factor-alpha (TNF- $\alpha$ ), reduced phosphorylation of GSK3 $\beta$  and decreased nuclear  $\beta$ -catenin protein levels.  $\beta$ -catenin reduction resulted in decreased osteogenic differentiation and addition of DKK1 inhibitor rescued the inhibition of osteogenic differentiation. At later stages of periodontitis i.e., 7, 14 and 21, DKK1 expression decreased which prevented the process of osteoblastic differentiation into osteocytes. The failed maturation of osteoblast increased the RANKL expression and activated nuclear-factor kappa-light-chain-enhancer of activated-B cells (NF- $\kappa$ B) signalling, but decreased the  $\beta$ -catenin expression. The addition of DKK1 recombinant protein rescued the inhibition at late stage of osteogenic differentiation.<sup>31</sup>

Up-regulation of WNT antagonists DKK1 and SOST in the gingival tissues of patients of chronic apical periodontitis has been demonstrated. This molecular up-regulation suggests a possible role of increased pro-inflammatory cytokines, like TNF- $\alpha$  and interleukin 1 $\beta$  (IL-1 $\beta$ ), release along with WNT antagonists in periodontal diseases. In addition, the up-regulation of TNF- $\alpha$  and SOST levels in the serum of healthy chronic periodontitis patients proposed the possible role of SOST in biological functions at the periodontal as well as at the systemic levels in periodontitis individuals.<sup>32</sup>

A study on a rat model of periapical lesion caused by occlusal pulp exposure and through immunohistochemistry found a rapid increase of GSK3 $\beta$ -positive cells from day 14-28. Conversely, the ratio of phosphorylated GSK3 $\beta$  (p-GSK3 $\beta$ )/GSK3 $\beta$  increased,

**Table:** Expression levels and localisation of Wnt signaling pathway molecules in oral lesions.

| Oral lesions  | Wnt Molecules                      | Expression Levels                                            |
|---------------|------------------------------------|--------------------------------------------------------------|
| OSMF          | DKK3                               | Increase <sup>12</sup>                                       |
| OSMF          | WIF-1                              | Decrease <sup>13</sup>                                       |
| OSMF          | SFRP1                              | Decrease <sup>14</sup>                                       |
| OSMF          | SFRP5                              | Decrease <sup>14</sup>                                       |
| OL            | DKK3                               | Increase <sup>21</sup>                                       |
| OL            | Wnt3                               | Increase epithelial membrane expression <sup>16</sup>        |
| OL            | $\beta$ -catenin                   | Increase nuclear expression <sup>16</sup>                    |
| OL            | Wnt3                               | +ve and -ve cytoplasmic, -ve nuclear <sup>17</sup>           |
| OL            | Axin2                              | Increase cytoplasmic expression <sup>20</sup>                |
| PVL           | Wnt3                               | Increase nuclear expression <sup>17</sup>                    |
| OLP           | Wnt3                               | +ve cytoplasmic and -ve nuclear expression <sup>17</sup>     |
| Erythroplakia | $\beta$ -catenin                   | Increase nuclear expression <sup>25</sup>                    |
| CAP           | DKK1                               | Increase expression up to day 21 then decrease <sup>29</sup> |
| CAP           | SOST                               | Increase <sup>31</sup>                                       |
| CAP           | DKK1                               | Increase <sup>31</sup>                                       |
| CAP           | GSK3 $\beta$                       | Increase <sup>28</sup>                                       |
| CAP           | p-GSK3 $\beta$ (Ser9)/GSK3 $\beta$ | Increase up to day 21 then decrease <sup>29</sup>            |

OSMF, oral submucous fibrosis; OL, oral leukoplakia; PVL, proliferative verrucous leukoplakia; CAP, chronic apical periodontitis; DKK3, Dickkopf 3; WIF-1, Wnt inhibitory factor-1; SFRP, Secreted frizzled-related protein; SOST, sclerostin; GSK3 $\beta$ , glycogen synthase kinase-3 $\beta$ ; p-GSK3 $\beta$ , phosphorylated glycogen synthase kinase-3 $\beta$ .

initially up to day 4, and later declined from day 14-28. The immunofluorescence report further confirmed the co-expression of p-GSK3 $\beta$  and RANKL in rat periapical lesions from day 7-28. These results suggested the active involvement of p-GSK3 $\beta$  in RANKL-induced osteoclastogenesis and bone loss in periapical lesions (Table).<sup>33</sup>

## Limitations

The current narrative review focused on WNT signalling in potentially malignant oral and periapical lesions but did not encompass WNT signalling in malignant oral lesions.

## Conclusion

WNT pathway agonists and antagonists play a pivotal role in pathogenesis of potentially malignant oral lesions and pathological bone loss in periapical lesions. The early identification of CpG methylated WNT antagonists in OSMF can be used as a potential biomarkers for early recognition of this pre-malignant lesion as well as re-introduction of WNT antagonists can be an effective treatment option for it. Similarly, regulation of WNT signalling pathway in periapical lesions, through therapeutic aids such as addition of DKK1 recombinant protein and administration of lithium chloride, a powerful

GSK-3 $\beta$  inhibitor, can restrict the pathological bone loss in periapical and periodontal lesions.

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**Conflict of Interest:** None.

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