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Review

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Role of Wnt ligands and receptors in oral squamous cell carcinoma

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Abstract: Oral squamous cell carcinoma (OSCC) poses significant challenges in terms of diagnosis and treatment, with high rates of morbidity and mortality. Emerging evidence highlights the critical involvement of Wnt ligands and receptors in OSCC pathogenesis. Dysregulated Wnt signaling pathways' contribution to tumor initiation, progression, and therapy resistance is characterized by their ability to promote cellular proliferation, epithelial–mesenchymal transition (EMT), and the maintenance of cancer stem cells (CSCs). Targeting Wnt signaling presents a promising therapeutic avenue, yet its complex interplay with other signaling pathways requires a deeper understanding to implement effective intervention. This study sheds light on the current knowledge of the role of Wnt ligands and receptors in OSCC, emphasizing their potential as diagnostic biomarkers and therapeutic targets. Future research directions involve elucidating context-specific Wnt signaling dynamics and exploring combination therapies to improve clinical outcomes for OSCC patients.

Key words: Oral squamous cell carcinoma; Wnt signaling; Molecular mechanisms; Tumorigenesis; Therapeutic targets

1 Introduction

The Wnt signaling pathway is crucial in oral cancer development, affecting cell survival, migration, polarity, and proliferation. Oral cancer cells express *Wnt* genes, notably *Wnt5a* and Frizzled receptor 5 (Fzd5), stimulating migration and invasion. Dysregulated Wnt components like *Wnt5a* can be markers for oral carcinogenesis. The activated Wnt pathway highlights the need for therapeutic targets and prognostic markers in oral cancer transformation. The Wnt signaling pathway is crucial in cancer, governing proliferation, differentiation, apoptosis, and migration. It is involved in tumor initiation, growth, senescence, differentiation, and metastasis. Research has focused on Wnt as a cancer treatment target, with clinical trials testing small molecules like LGK974 and biological agents like OMP-18R5. A deeper understanding of gene functions has advanced targeted tumor *therapie*s (Zhang et al., 2020).

Understanding the role of Wnt ligands and receptors in OSCC requires a comprehensive exploration of their functions and interactions within the complex tumor microenvironment (TME). The ligands, which comprise Wnt family members like *Wnt1*, *Wnt3a*, and *Wnt5a*, function as signaling molecules that bind to receptors on the cell surface, initiating signaling cascades downstream. The receptors involved in Wnt signaling, including Frizzled (FZD) receptors and co-receptors such as low-density lipoprotein receptor-related protein 5/6 (LRP5/6), mediate the transduction of Wnt signals into the cell, regulating gene expression and cellular behavior (Noguti *et al.*, 2012, Reyes *et al.*, 2020).

In OSCC, abnormal levels of Wnt ligands and receptors play a significant role in different stages of cancer

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development, such as tumor formation, EMT (Bai *et al.*, 2020, Krisanaprakornkit and Iamaroon, 2012), angiogenesis (Farrapo *et al.*, 2022), and metastasis (Tan *et al.*, 2023). In addition, the interaction between Wnt signaling and other cancer-causing pathways adds complexity to our comprehension of how OSCC progresses. For example, Wnt signaling can enhance the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway through β-catenin stabilization, promoting cell survival, while AKT activation, in turn, can increase β-catenin activity. Additionally, Wnt signaling can modulate *PTEN*, influencing PI3K/AKT pathway regulation (He and Gan, 2023, Yu *et al.*, 2021). This phenomenon is well-known in other cancers, such as colorectal cancer (Fleming-de-Moraes *et al.*, 2022, Prossomariti *et al.*, 2020), but studies related to OSCC are lacking.

This review aims to provide a comprehensive overview of the role played by Wnt ligands and receptors in OSCC, synthesizing the existing literature to elucidate their multifaceted functions and underlying mechanisms. By examining the impact of Wnt signaling dysregulation on OSCC pathogenesis, we aim to identify potential therapeutic targets and strategies for the management of this devastating disease. By enhancing our understanding of Wnt signaling in OSCC, we endeavor to make a valuable contribution to the advancement of innovative therapeutic interventions, ultimately leading to improved patient outcomes and quality of life.

2 Wnt Ligands in OSCC

2.1 Canonical Wnt Ligands in OSCC

2.1.1 Wnt1

Canonical Wnt ligands are crucial regulators of various cellular processes, including proliferation, differentiation, and migration, and they play significant roles in the progression of OSCC (Table 1). Wnt1 plays an important role in OSCC tumorigenesis, having elevated expression levels in OSCC tissues compared to normal oral mucosa, indicating its crucial involvement in OSCC progression (Cierpikowski *et al.*, 2023). Mechanistically, *Wnt1* activates the canonical Wnt pathway by interacting with FZD receptors and LRP5/6 co-receptors, leading to the stabilization of β-catenin and its translocation into the nucleus. This nuclear translocation of β-catenin enables its binding to transcription factors like T-cell factor/lymphoid enhancer factor (TCF/LEF), thereby initiating the transcription of target genes essential for cell cycle progression and proliferation (Cadigan and Waterman, 2012, Xie *et al.*, 2021). One of the key target genes activated by *Wnt1* signaling is *cyclin D1*, a critical regulator of cell cycle progression from G1 to S phases, promoting uncontrolled cell proliferation and tumor growth in OSCC. In addition, the activation of the canonical Wnt pathway by *Wnt1* results in the elevation of *c-Myc*, a transcription factor recognized for its role in promoting cell proliferation and survival (Ma *et al.*, 2017, Zhang *et al.*, 2014). The concurrent influence of *cyclin D1* and *c-Myc* emphasizes *Wnt1's* capacity to facilitate the proliferation and advancement of OSCC tumors by promoting cell cycle progression and survival.

Table 1 Roles of canonical Wnt ligands in OSCC progression

Wnt Ligand	***Role in OSCC***	***Mechanism of Action***
Wnt1	-Elevated expression in OSCC tissues, indicating significant involvement in OSCC progression	-Canonical Wnt pathway activation via FZD and LRP5/6 co-receptors -β-catenin stabilization and nuclear translocation, activating genes for cell cycle progression and survival
Wnt3a	-Heightened expression in OSCC tissues, particularly in advanced stages. Implicated as a key driver of OSCC pathogenesis	-Canonical Wnt signaling activation via FZD and LRP5/6 co-receptors. -β-catenin stabilization and nuclear translocation -Complex formation with TCF/LEF, activating genes for proliferation, survival, and EMT -Promotes EMT in OSCC, enhancing invasiveness and metastasis
Wnt8a	-Vital function in regulating diverse cellular activities, including self-renewal, proliferation, differentiation, and motility	-Plausible impact on OSCC pathogenesis through the canonical Wnt signaling pathway, similar to other Wnt family members

	-Specific role in OSCC progression yet to be fully elucidated	
Wnt8b	-Identified as an independent prognostic marker for nasopharyngeal carcinoma -Role in OSCC progression not extensively documented	-Promotion of cell proliferation and migration through activation of cyclin D1 and c-Myc expression, suggesting potential implications for OSCC malignancy
Wnt10a	-Implicated in the progression of OSCC	-Regulates proliferation, stemness, pluripotency, and cell fate via WNT/β-catenin signaling -Upregulated in late-stage OSCC, indicating a role in tumor progression
Wnt10b	-Significantly involved in OSCC progression	-Regulates bone metabolism by controlling osteoblast differentiation and interacting with WNT signaling transcription factors -Acts as a suppressor of adipocyte differentiation, potentially affecting the osteoblast-adipocyte balance in the OSCC tumor microenvironment

Wnt1-inducible signaling pathway protein 1 (WISP-1), part of the *Wnt1*-inducible signaling pathway proteins, plays a role in cell growth, differentiation, and wound healing. A recent study found elevated WISP-1 levels in OSCC tissues, which was linked to treatment failure and lower 5-year survival rates. Experiments confirmed that WISP-1 promotes invasive behavior and inhibits apoptosis in OSCC cells. These findings highlight WISP-1 as a potential therapeutic target for combating aggressive behavior in oral cancer (Jung *et al.*, 2017). A study on *WISP1*, which is linked to carcinogenesis, examined *WISP1* SNPs in 900 OSCC patients and 1200 cancer-free individuals. Carriers of the *WISP1* rs2929970 polymorphism, especially with a G allele, were more susceptible to OSCC. Non-smokers with specific *WISP1* variants had late-stage, larger tumors, while betel quid chewers with certain variants had a lower risk of lymph node metastasis. These findings suggest that *WISP1* polymorphisms, along with smoking and betel nut chewing, influence OSCC risk and could be useful for marker identification and therapeutic targeting (Lau *et al.*, 2017).

A recent study investigated the prognostic significance of *WNT1*, *NOTCH1*, *PDGFRβ*, and *CXCR4* in OSCC. Immunohistochemistry of 60 OSCC samples revealed the presence of *WNT1*, *NOTCH1*, *PDGFRβ*, and *CXCR4* in 51.7%, 25.0%, 63.3%, and 70.0% of patients, respectively. *WNT1* correlated with *NOTCH1* and *CXCR4*, and *NOTCH1* correlated with *CXCR4*. *WNT1* and *PDGFRβ* expression were linked to decreased overall survival. Multivariate analysis identified *WNT1* and *CXCR4* as independent prognostic factors. The study suggested that WNT and NOTCH signaling pathways contribute to OSCC angiogenesis, with *WNT1* and *CXCR4* as potential prognostic indicators (Cierpikowski, Lis-Nawara and Bar, 2023).

Quercetin, a compound with potential benefits in various carcinomas, including OSCC, affects the WNT/β-catenin pathway and miRNA regulation. Research has assessed its impact on miR-22 and the WNT1/β-catenin pathway in OSCC. CCK-8 and flow cytometry analyses showed quercetin and miR-22 overexpression decreased cell viability and increased apoptosis in OSCC. *WNT1* was identified as a direct target of miR-22 through bioinformatics and luciferase assays. Quercetin enhanced miR-22 expression and inhibited *WNT1* and β-catenin in OSCC cells, but this effect was negated when miR-22 was inhibited. In vivo studies confirmed that quercetin inhibited OSCC tumor growth by upregulating miR-22 and suppressing the WNT1/β-catenin pathway. These findings highlight quercetin's potential as a therapeutic agent in OSCC treatment (Zhang *et al.*, 2019).

2.1.2 Wnt3a

Wnt3a has emerged as a pivotal player in the progression of OSCC, as evidenced by its heightened expression levels in OSCC tissues, particularly in the advanced stages of the disease (Purwaningsih *et al.*, 2021). This elevated expression of *Wnt3a* implicates it as a key driver of OSCC pathogenesis.

Mechanistically, *Wnt3a* exerts its effects by activating the canonical Wnt signaling pathway (Purwaningsih, Khor, Nik Mohd Rosdy and Abdul Rahman, 2021, Reyes *et al.*, 2020). *Wnt3a* binds to FZD receptors and LRP5/6 co-receptors to initiate the canonical Wnt pathway, stabilizing β-catenin and allowing it to move into

the nucleus, where it complexes with TCF/LEF transcription factors. This complex activates genes related to cell proliferation, survival, and EMT. The activation of these genes by *Wnt3a* enhances OSCC cell survival, helping them evade apoptosis and persist in the tumor microenvironment (Reyes, Flores, Betancur, Peña-Oyarzún and Torres, 2020).

Furthermore, *Wnt3a*-mediated signaling contributes to the induction of EMT in OSCC cells. This process gives cancer cells invasive and migratory properties, enabling them to disseminate from the primary tumor site and invade surrounding tissues, ultimately facilitating metastasis (Purwaningsih, Khor, Nik Mohd Rosdy and Abdul Rahman, 2021, Reyes, Flores, Betancur, Peña-Oyarzún and Torres, 2020). Therefore, *Wnt3a's* activation of the canonical Wnt signaling pathway plays a crucial role in the progression of OSCC, facilitating tumor growth, survival, and metastasis (Shiah *et al.*, 2015, Xie, Huang, Lu and Zheng, 2021). For example, a recent study found that CDK5RAP2 expression is elevated in OSCC and regulated by the Wnt signaling pathway. Downregulating CDK5RAP2 disrupts spindle orientation, significantly impeding OSCC progression and altering the cancer stem cell (CSC) signature of OSCC. These findings highlight CDK5RAP2's role in OSCC progression and its potential as a CSC marker and therapeutic target (Shen *et al.*, 2023). Understanding the intricate mechanisms underlying Wnt3a-mediated signaling provides valuable insights into potential therapeutic targets for OSCC treatment, offering avenues for the development of targeted therapies aimed at mitigating OSCC progression and improving patient outcomes.

2.1.3 Wnt8a and Wnt8b

Wnt8a, as a constituent of the Wnt family, assumes a vital function in regulating diverse cellular activities, encompassing self-renewal, proliferation, differentiation, and motility (Ngernsombat *et al.*, 2021). The specific role of *Wnt8a* in OSCC remains to be fully elucidated. However, given the established functions of Wnt family members in promoting or suppressing tumor progression via the canonical Wnt signaling pathway, it is plausible that *Wnt8a* may similarly impact OSCC pathogenesis.

Wnt8b, another member of the Wnt family, has been identified as an independent prognostic marker for nasopharyngeal carcinoma (Ngernsombat, Prattapong, Larbcharoensub, Khotthong and Janvilisri, 2021). While the direct involvement of *Wnt8b* in OSCC progression is not extensively documented in the literature, its role in other cancers suggests potential implications for OSCC (Ngernsombat *et al.*, 2021, Nie *et al.*, 2020). Studies have shown that *Wnt8b* can promote cell proliferation and migration through the activation of *cyclin D1* and *c-Myc* expression (Ma, Ren, Zhang, Kong, Wang, Shi and Bu, 2017). This mechanism may enhance OSCC malignancy by regulating cell cycle progression and invasion. While the specific roles of *Wnt8a* and *Wnt8b* in OSCC are not well-detailed, their known functions in cell regulation suggest they could significantly influence OSCC pathogenesis, affecting cell proliferation, migration, and other cancer-related pathways. Further research on these Wnt proteins in OSCC could reveal their precise contributions to tumor growth and metastasis.

2.1.4 Wnt10a and Wnt10b

Wnt10a is implicated in OSCC progression and plays crucial roles in various tissues, including bone, adipocytes, teeth, skin, hair, the immune system, muscle, the placenta, and the heart. Abnormal *Wnt10a* signaling is linked to diseases like cancer, obesity, and osteoporosis. In OSCC, Wnt10a significantly regulates proliferation, stemness, pluripotency, and cellular fate by activating the WNT/β-catenin signaling pathway (Ma, Ren, Zhang, Kong, Wang, Shi and Bu, 2017). Furthermore, the high levels of *Wnt10a* expression observed in the advanced stages of OSCC indicate its potential role in tumor progression. The upregulation of *Wnt10a* in late-stage OSCC suggests its involvement in promoting tumorigenesis and disease advancement (Kalinke *et al.*, 2016).

Wnt10b, a member of the Wnt family, activates the canonical WNT/β-catenin signaling pathway to regulate various cellular functions in different tissues. It is crucial for osteoblast differentiation and the induction of osteoblast genes. In OSCC, *Wnt10b* plays a significant role in influencing cell proliferation, differentiation, and potentially metastasis. *Wnt10b* suppresses adipocyte differentiation, indicating enhanced osteoblastogenesis

and reduced adipogenesis, which may impact the balance between osteoblasts and adipocytes in the OSCC tumor microenvironment. Dysregulation of *Wnt10b* affects OSCC progression. Additionally, a study found that restoring miR-148a levels in cancer-associated fibroblasts (CAFs) impaired OSCC cell migration and invasion by targeting *WNT10B* (Min *et al.*, 2016).

Overall, both *Wnt10a* and *Wnt10b* significantly contribute to OSCC progression by impacting crucial cellular processes related to tumor growth and metastasis. Their roles in regulating tissue-specific functions and disease states highlight their potential as therapeutic targets for OSCC management. Investigating the precise mechanisms by which these Wnt proteins influence OSCC pathogenesis could offer valuable insights for developing targeted treatment strategies.

2.2 Non-canonical Wnt Ligands

The non-canonical Wnt pathway, unlike the canonical Wnt/β-catenin pathway, does not stabilize β-catenin or activate its associated transcriptional programs. Instead, it primarily regulates cell movement and polarity through pathways such as planar cell polarity (PCP) and Wnt/Ca^{2+} signaling, which are crucial for processes like migration and cytoskeletal rearrangement—key factors in cancer progression. In OSCC, non-canonical Wnt ligands such as Wnt5a/b and Wnt7a/b play significant roles in tumor progression. Wnt5a is frequently upregulated in OSCC and is linked to increased cell migration and invasion (Prgomet *et al.*, 2017), possibly through the activation of Rho and Rac signaling pathways, which are essential for cytoskeletal dynamics and motility (Bueno *et al.*, 2022). Similarly, *Wnt7a/b* has been associated with modulation of the tumor microenvironment, promoting cancer cell proliferation and invasion by activating downstream effectors like JNK and Ca^{2+}/NFAT, contributing to a more aggressive tumor phenotype. These non-canonical pathways result in distinct cellular outcomes compared to the canonical Wnt pathway, which primarily drives cell proliferation through β-catenin signaling. Understanding these differences is vital for elucidating the metastasis and tumor progression mechanisms in OSCC and underscores the potential for targeted therapies that could specifically disrupt the non-canonical Wnt signaling pathways involved in cancer progression.

2.2.1 Wnt5a and Wnt5b

Non-canonical Wnt ligands such as *Wnt5a*, *Wnt5b*, *Wnt7a*, and *Wnt7b* contribute to OSCC progression by promoting cell proliferation, migration, invasion, and metastasis (Table 2). *Wnt5a* is described as the "most important Wnt protein activating the non-canonical Wnt pathway" in OSCC (Prgomet *et al.*, 2013). Studies have shown that *Wnt5a* can regulate various cellular processes that are crucial for cancer progression, including proliferation, differentiation, migration, adhesion, and polarity (Prgomet, Lindberg and Andersson, 2013). It has been specifically reported through various studies that *Wnt5a* can enhance the migration and invasion of OSCC cells by activating non-canonical Wnt signaling pathways, including the Wnt/Ca2+/PKC pathway. A recent article states that *Wnt5a* activates this pathway, which promotes migration and invasion in OSCC (Xie, Huang, Lu and Zheng, 2021). Additionally, the overexpression of *Wnt5a* is correlated with an unfavorable prognosis and aggressive demeanor in patients with OSCC. It is proposed that increased expression of *WNT5A* protein in OSCC is correlated with an unfavorable clinical prognosis (Prgomet *et al.*, 2017). According to a recent study, *SOX2* and *OCT4* indicate proliferative potential, while *WNT5A* signifies invasiveness. Immunohistochemistry on 20 carcinoma, 20 dysplasia, and 25 normal tissue specimens revealed higher *SOX2* levels in carcinoma, minimal *OCT4* expression, and increasing *WNT5A* expression from normal to dysplastic to carcinoma tissue. *SOX2* alone may serve as a proliferation marker, while *WNT5A* may indicate oral squamous cell carcinoma invasiveness (Vijayakumar *et al.*, 2020). Furthermore, a study used immunohistochemistry to examine Wnt5a and β-Catenin expression levels in OSCC tissues. Most cases showed widespread cytoplasmic expression of both proteins. Statistical analysis revealed a positive correlation between *Wnt5a* expression and OSCC differentiation, while cytoplasmic β-Catenin expression was inversely correlated with differentiation. Cytoplasmic β-Catenin accumulation was associated with lymph node metastasis and loss of β-Catenin on the cell membrane was inversely correlated with differentiation. These findings underscore the involvement of the Wnt/β-Catenin

pathway in OSCC tumorigenesis, metastasis, and prognosis, and *Wnt5a* is also linked to histological grade (Zhu *et al.*, 2005). Another study investigated the influence of ΔNp63β, a variant associated with EMT, on cell motility in tongue SCC. DNA microarray analysis reveals that *Wnt5a* is significantly down-regulated upon ΔNp63β overexpression in tongue SCC cells displaying an EMT phenotype. Wnt5a–Ror2 signaling was implicated in enhancing tongue SCC cell aggressiveness and promoting MMP-2 production, highlighting potential therapeutic targets for oral cancer treatment (Sakamoto *et al.*, 2017). These studies show that *Wnt5a* plays a crucial role in OSCC progression by activating non-canonical Wnt signaling pathways, promoting cell migration, invasion, and other cancer-related processes. Overexpression of *Wnt5a* is associated with poor prognosis and aggressive behavior in OSCC patients, suggesting it as a potential therapeutic target for this cancer type.

Table 2 Roles of Non-Canonical Wnt Ligands in OSCC Progression

Wnt Ligand	***Role in OSCC***	***Mechanism of Action***
Wnt5a	-Activation of non-canonical Wnt signaling pathways promoting migration, invasion, and potentially other cancer-related cellular processes in OSCC -Correlation with poor prognosis and aggressive behavior in OSCC patients	-Activation of Wnt/Ca2+/PKC pathway boosts OSCC cell migration and invasion -Linked to OSCC invasiveness and histological grade -Down-regulated by ΔNp63β overexpression, increasing cell aggressiveness and MMP-2 production
Wnt5b	-Promotion of migration and invasion of OSCC cells, contributing to metastatic behavior	-Increased mRNA expression in highly metastatic OSCC cells - Suppression of Wnt5b reduces migratory capacity; its application enhances migration and promotes filopodia-like protrusions in OSCC cells
Wnt7a	-Notable upregulation in OSCC tissues compared to adjacent normal tissues -Correlation with increased tumor growth, lymph node metastasis, advanced tumor staging, and shorter recurrence-free survival times -Involvement in promoting EMT process, enhancing OSCC cell migration and invasion	-Interaction with various signaling pathways including EGF/PI3K/AKT/WNT7A/β-catenin/MMP9 pathway. -Sensitization of OSCC cells to cisplatin treatment, enhancing apoptotic responses -Stimulation of OSCC cells by CAFs to produce WNT7A, contributing to tumor progression
Wnt7b	-Gradual upregulation in OSCC tissues, suggesting involvement in tumor development and advancement -Implicated in the promotion of tumor invasion and impacting resistance to anticancer therapies	-Activation of canonical Wnt signaling pathways, regulating downstream genes like MMP1, augmenting cancer cell's invasive capabilities

Wnt5b, closely related to *Wnt5a*, shares 80.5% total amino acid identity. Recent studies reveal its significant role in promoting the migration and invasion of OSCC cells. Increased *Wnt5b* mRNA levels were observed in highly metastatic OSCC cell lines compared to less metastatic ones. Suppression of *Wnt5b* expression led to decreased migratory capacity, while its application enhanced migration. *Wnt5b* also facilitates the development of filopodia-like protrusions in OSCC cells, which are associated with increased motility and invasion (Takeshita *et al.*, 2014). This observation underscores *Wnt5b's* role in controlling cytoskeletal dynamics and cell motility, essential for OSCC metastasis. *Wnt5b* is pivotal in OSCC advancement, enhancing migration and invasion, likely by activating non-canonical Wnt signaling pathways and regulating cytoskeletal dynamics. These findings emphasize *Wnt5b's* significance in OSCC metastatic behavior.

2.2.2 Wnt7a and Wnt7b

The emergence of *Wnt7a* as a key player in OSCC progression highlights its pivotal role in the cancer's

aggressiveness. Comprehensive analysis reveals significant upregulation of *Wnt7a* in OSCC tissues at both mRNA and protein levels. This overexpression correlates with various critical aspects of OSCC advancement, including increased tumor growth, lymph node metastasis, and advanced tumor staging, indicating a poorer prognosis for patients. Elevated *Wnt7a* levels also correlate with shorter recurrence-free survival times. Additionally, *Wnt7a's* involvement in promoting the EMT process elucidates its role in enhancing OSCC cell migration and invasion, crucial steps in metastasis (Jia *et al.*, 2019). For example, analysis of *Wnt7a* mRNA and protein levels in tongue squamous cell carcinoma (TSCC) tissues showed a notable increase compared to adjacent non-cancerous tissues. Clinical data analysis revealed a correlation between *Wnt7a* expression and T classification, lymph node metastasis, and pathological differentiation, suggesting a likelihood of shorter recurrence-free survival in TSCC patients. Suppressing *Wnt7a* expression inhibited cell proliferation, migration, and invasion and reversed EMT in TSCC cell lines. These results suggest *Wnt7a's* role as an oncogene and a potential therapeutic target in managing TSCC (Jia, Qiu, Chu, Sun, Xu, Zhao and Zhao, 2019).

Recent studies highlight the multifaceted role of *WNT7A* in OSCC progression, demonstrating its complex interaction with various signaling pathways. For instance, *WNT7A's* interaction with other signaling cascades significantly contributes to its oncogenic potential in OSCC. A recent study demonstrates that EGF stimulation increases *WNT7A* mRNA and protein levels in OSCC cells, with p-AKT mediating this induction. Inhibiting AKT activation prevents the upregulation of *WNT7A* and MMP9 expression caused by EGF, along with the translocation of β-catenin from the cytoplasm to the nucleus. Histological examination of OSCC specimens shows a significant association between *WNT7A* expression and unfavorable clinical prognosis. These findings reveal a novel signaling pathway, PI3K/AKT/WNT7A/β-catenin/MMP9, implicated in the EGF-induced migration of OSCC cells (Xie *et al.*, 2020). In a study involving 42 OSCC patients, *WNT7A* mRNA was notably upregulated. Subsequent experiments on OSCC cell line KB cells showed that *WNT7A* knockdown increased sensitivity to cisplatin treatment, with reduced nuclear β-catenin levels and increased cleaved caspase-3 and cleaved PARP expression. In an in vivo mouse model, *WNT7A* knockdown reduced tumor weight and volume, alongside increased apoptotic cell counts after cisplatin treatment. These findings suggest that inhibiting *WNT7A*-β-catenin signaling could enhance cisplatin sensitivity in OSCC, providing insights for molecular diagnostics and treatment strategies (Tian *et al.*, 2018).

The TME comprises cellular and non-cellular components that profoundly influence OSCC progression. Cancer cells interact with stromal cells, immune cells, extracellular matrix components, and signaling molecules within the TME, shaping tumor behavior and therapeutic responses. Stromal cells like CAFs, endothelial cells, and immune cells contribute to tumor growth, invasion, metastasis, angiogenesis, and immune evasion. For instance, a recent study investigated CAFs' impact on OSCC progression by exploring Wnt signaling activation. It found that CAFs stimulate OSCC cells to produce *WNT7A*, enhancing tumor cell migration and invasion. High *WNT7A* expression correlated with poor prognosis and downregulation of *CLDN1*, a tumor suppression gene. Additionally, *WNT7A* activation of AKT signaling contributed to *CLDN1* downregulation. These findings highlight the importance of targeting the TME in OSCC therapy (Kayamori *et al.*, 2023). Hence, these findings solidify *Wnt7a* as a central orchestrator of OSCC aggressiveness, shedding light on its potential as a therapeutic target and prognostic marker in managing this challenging malignancy.

Emerging evidence of the role of *Wnt7b* in cancer progression underscores its significance in tumor development and resistance to anticancer therapies. Positioned within the intermediately transforming or non-transforming members of the Wnt family, *Wnt7b's* association with cancer development and resistance mechanisms is increasingly evident (Kumar *et al.*, 2021). Recent investigations have revealed a gradual upregulation of *Wnt7b* in oral lichen planus (OLP) and OSCC tissues, suggesting its potential involvement in oral inflammation and cancer progression. Notably, its heightened expression in OSCC tissues compared to adjacent normal tissues suggests a pivotal role in tumor development and advancement. Mechanistically, *Wnt7b* has been implicated in promoting tumor invasion by regulating downstream genes like MMP1, thereby augmenting cancer cells' invasive capabilities. This facilitation of invasion is attributed to *Wnt7b's* activation of canonical Wnt signaling pathways, which regulate key genes associated with cancer progression (Chen *et al.*, 2022). In

summary, *Wnt7b* has emerged as a critical player in OSCC, influencing tumor invasion and potentially impacting resistance to anticancer therapies. Its upregulation in OSCC tissues and its link to oral inflammation underscore its significance in the pathogenesis and progression of this cancer type. Further elucidation of the precise mechanisms by which Wnt7b modulates OSCC development and therapy resistance holds promise for the development of targeted therapeutic interventions.

Additionally, it is imperative to underscore the significance of other non-canonical Wnt ligands—including *Wnt4*, *Wnt6*, *Wnt9a*, *Wnt9b*, *Wnt11*, and *Wnt16*—in driving the progression of OSCC. Understanding the intricate interplay of these non-canonical Wnt ligands is crucial for unraveling the complex molecular landscape of OSCC and identifying novel therapeutic targets for more effective management of this aggressive malignancy.

3 Wnt Receptors in OSCC

3.1 Frizzled Receptors in OSCC

The Wnt signaling pathway, implicated in a range of cellular processes like cell proliferation, differentiation, and migration, relies heavily on the FZD receptors. These receptors consist of a family of 10 transmembrane receptors. The development and progression of OSCC and other cancer types have been linked to the activity of these receptors (Smith *et al.*, 2021, Zeng *et al.*, 2018). The FZD receptors, which belong to the G-protein-coupled receptor (GPCR) family, are distinguished by the presence of a cysteine-rich domain (CRD) in their extracellular N-terminus. This CRD plays a crucial role in the binding of Wnt ligands. Upon the binding of Wnt ligands to the CRD of FZD receptors, downstream signaling cascades are activated, consisting of the canonical Wnt/β-catenin pathway and the non-canonical Wnt/planar cell polarity (PCP) and Wnt/Ca2+ pathways (Smith, Sompel, Elango and Tennis, 2021, Zeng, Chen and Fu, 2018).

A number of studies have reported the dysregulation of FZD receptors in OSCC, suggesting their involvement in the pathogenesis of this disease (Umar *et al.*, 2022). For example, FZD8 has been shown to be upregulated in head and neck carcinoma, and its overexpression is associated with increased expression of CSC markers and the activation of the ERK/c-fos signaling axis, leading to increased CSC activity and chemotherapeutic resistance (Sompel *et al.*, 2021). Similarly, FZD7 is overexpressed in OSCC, and is linked to EMT for cancer spread. A study on miR-27b's impact on cisplatin sensitivity in OSCC found lower expression in drug-resistant tissues. Overexpressing miR-27b inhibited proliferation and migration, and boosted apoptosis in drug-resistant OSCC cells by suppressing FZD7/β-catenin signaling (Liu *et al.*, 2019). In another investigation, miR-27b plays a crucial role in regulating OSCC cell proliferation by targeting FZD7 and the Wnt signaling pathway. Reduced miR-27b levels in OSCC cell lines contrasted with those of controls. Overexpressing miR-27b notably hindered OSCC cell proliferation, suggesting its potential as a therapeutic target to disrupt key signaling pathways in cancer progression (Liu *et al.*, 2017).

Given the important role of FZD receptors in the pathogenesis of OSCC, they have emerged as potential therapeutic targets (Fig. 1). Several small-molecule inhibitors and monoclonal antibodies targeting FZD receptors, such as G007-LK, G244-LM, and OMP-18R5, are currently under investigation for the treatment of OSCC and other cancers (Umar, Dong, Nihal and Chang, 2022, Zeng, Chen and Fu, 2018). Furthermore, studies are currently investigating the efficacy of ICG-001, an inhibitor of the Wnt/β-catenin pathway that specifically targets the interaction between β-catenin and the transcriptional coactivator CREB-binding protein, for the treatment of OSCC (Kartha *et al.*, 2018). Overall, FZD receptors play a crucial role in the pathogenesis of OSCC, and targeting these receptors or their associated signaling pathways holds promise as a potential therapeutic strategy for this disease.

Fig. 1 A comprehensive explanation of key components illustrating the role Frizzled (FZD) receptors play in oral squamous cell carcinoma (OSCC). It begins by showing FZD receptors, highlighting the cysteine-rich domain (CRD) crucial for binding Wnt ligands. The binding of Wnt ligands to the CRD is responsible for the initiation of downstream signaling cascades. Dysregulation of FZD receptors in OSCC is emphasized, along with the implications for OSCC progression, such as enhanced cancer cell strength and treatment resistance.

3.2 LRP5 and LRP6

The LRP5 and LRP6 co-receptors are essential components of the canonical Wnt/β-catenin signaling pathway, which is widely recognized for its significant involvement in the development and progression of diverse cancer types (Roslan *et al.*, 2019). LRP5 shows significant expression in the liver, pancreas, prostate, placenta, and small intestine, with lower levels in the ovary, thymus, skeletal muscle, colon, spleen, kidney, testis, heart, and lung. In contrast, LRP6 is highly expressed in the ovary, heart, brain, placenta, lung, kidney, pancreas, and spleen while having lower levels in the liver, skeletal muscle, prostate, colon, and peripheral blood leukocytes. Despite distinct tissue expression, LRP5 and LRP6 share 71% amino acid sequence identity, indicating structural similarity (Joiner *et al.*, 2013, Wang *et al.*, 2018).

Both LRP5 and LRP6 contain an extracellular domain with YWTD-type β-propeller domains, EGF-like domains, and LDLR type A domains, as well as an intracellular domain with PPPSP motifs that are essential for

Wnt signaling. These structural features allow them to function as co-receptors, binding to Wnt ligands and facilitating the activation of the canonical Wnt/β-catenin pathway (Ren *et al*., 2021, Wang, Luo, Xu, Zhou and Zhang, 2018). For example, in OSCC, *FAT1* expression changes significantly, impacting patient prognosis. Activated LRP5 signalosome links to poor outcomes. Loss of *FAT1* function inhibits cell growth and metastasis in OSCC. Inhibiting *FAT1* enhances cisplatin sensitivity in resistant OSCC cells by disrupting the LRP5/WNT2/GSS pathway. *FAT1* shows promise as a therapeutic target to enhance cisplatin treatment effectiveness in OSCC patients (Hsu *et al.*, 2019). In another study, researchers used MALDI-IMS proteomics to study OSCC development, revealing LRP6 upregulation in OSCC tissues linked to clinicopathologic factors. Inhibiting LRP6 suppressed OSCC cell growth. A positive correlation was found between LRP6 expression and the oncogene FGF8 in OSCC cells (Yuan *et al.*, 2017).

LRP5 and LRP6's involvement in the Wnt pathway suggests a key role in OSCC development and progression. Dysregulation of Wnt signaling is common in OSCC, with LRP5 and LRP6 potentially influencing tumor initiation, growth, and spread. Further research is essential to uncover their precise contributions and potential as therapeutic targets or biomarkers in OSCC. Understanding these co-receptors could offer crucial insights into OSCC's molecular basis and aid in developing targeted therapies.

4 Clinical Implications of Wnt Ligands and Receptors in OSCC

4.1 Diagnostic biomarkers

The Wnt signaling pathway plays a crucial role in OSCC pathogenesis, with abnormal expression of its components observed in OSCC tissues versus normal oral mucosa. Dysregulated Wnt pathway activation can lead to uncontrolled cell growth, increased cell survival, and the promotion of EMT, key factors in cancer development. Studies have noted changes in Wnt ligands (*Wnt1*, *Wnt3*, *Wnt5a*) and receptors (FZD, LRP) in OSCC tissues compared to normal oral tissues, shedding light on the molecular mechanisms underlying this disease (Purwaningsih, Khor, Nik Mohd Rosdy and Abdul Rahman, 2021, Xie, Huang, Lu and Zheng, 2021).

The dysregulation of the Wnt signaling pathway has been closely linked to the development and progression of OSCC. For example, the *WISP-1* has been found to be involved in the progression of OSCC (Xie, Huang, Lu and Zheng, 2021). Additionally, the non-canonical Wnt/Ca2+/PKC pathway, activated by *Wnt5a*, has been shown to promote migration and invasion in OSCC cells (Xie, Huang, Lu and Zheng, 2021). The identification and validation of Wnt-related biomarkers for OSCC could have significant clinical implications. These biomarkers could be used as diagnostic tools to detect OSCC at an early stage, enabling timely intervention and improving patient outcomes. Furthermore, the understanding of Wnt signaling in OSCC pathogenesis may lead to the development of targeted therapies that could be integrated into the management of this disease (Bai, Sha and Kanno, 2020, Xie, Huang, Lu and Zheng, 2021).

One of the notable advantages of utilizing Wnt ligands and receptors as diagnostic biomarkers in OSCC is their detectability in bodily fluids, particularly serum and saliva. For example, a study found that salivary miR-30c-5p, which targets genes in the Wnt signaling pathway, was downregulated in OSCC patients compared to healthy controls. This miRNA showed good diagnostic performance with an AUC of 0.82 (Mehterov *et al.*, 2021). Another review discussed the potential of using salivary protein biomarkers like CA-125, which is involved in the Wnt pathway, for OSCC diagnosis and prognosis. The review also mentioned that the crosstalk between EGFR and Wnt pathways in OSCC could have diagnostic and prognostic value when assessed through salivary biomarkers (Pekarek *et al.*, 2023).

Circulating levels of Wnt signaling components can be measured through minimally invasive blood-based assays or salivary tests, offering convenient and non-invasive approaches for early cancer detection (Sajeev *et al.*, 2023). Studies have demonstrated promising diagnostic accuracy of Wnt ligands and receptors in discriminating OSCC patients from healthy individuals or those with benign oral lesions, underscoring their potential clinical utility in diagnostic settings.

Additionally, incorporating Wnt signaling components into biomarker panels alongside other markers or clinical data can enhance OSCC diagnostic accuracy, particularly in high-risk groups. Panels, including Wnt ligands, receptors, and relevant molecules, may improve sensitivity and specificity for OSCC detection, enabling early intervention and personalized treatments. For instance, a study on a salivary autoantibody panel showed enhanced sensitivity (63.8%) and specificity (90%) for early-stage OSCC detection compared to individual markers, underscoring the potential of combining Wnt-related biomarkers with other molecules to boost diagnostic performance (Hsueh *et al.*, 2022). Additionally, longitudinal monitoring of circulating Wnt ligands and receptors during OSCC treatment and follow-up can provide valuable insights into treatment response and disease recurrence, guiding clinical decision-making and improving patient outcomes (Table 3). However, several challenges remain in the translation of Wnt signaling components into routine clinical practice as diagnostic biomarkers for OSCC (Table 3). Standardization of assays and validation in large, multicenter cohorts are imperative to establish their reliability and reproducibility.

Table 3 Diagnostic biomarkers for OSCC: role of Wnt signaling components

Diagnostic Biomarkers	Description
Atypical Expression of Wnt Signaling Components	Altered Wnt ligands (Wnt1, Wnt3, Wnt5a) and receptors (Frizzled, LRP) in OSCC drive unchecked proliferation, increased cell viability, and EMT, contributing to pathogenesis.
WISP-1 and Non-Canonical Wnt/Ca2+/PKC Pathway	WISP-1 and the Wnt/Ca2+/PKC pathway activated by Wnt5a drive OSCC migration and invasion. Understanding these could aid in finding biomarkers and developing targeted therapies.
Detectability in Bodily Fluids	Wnt ligands and receptors in bodily fluids enable non-invasive OSCC detection. Salivary biomarkers like miR-30c-5p and CA-125 distinguish OSCC patients from healthy individuals.
Blood-Based Assays and Salivary Tests	Blood and saliva tests for Wnt signaling components offer minimally invasive, accurate options for early OSCC detection and differentiation from benign lesions.
Biomarker Panels	Combining Wnt signaling components with other biomarkers improves OSCC detection accuracy and facilitates early intervention and personalized treatment, especially in high-risk populations.

4.2 Prognostic indicators

In OSCC, prognostic indicators play a pivotal role in predicting disease trajectory, guiding treatment strategies, and ultimately determining patient outcomes. Wnt ligands and receptors have emerged as significant prognostic indicators in OSCC, reflecting the intricate interplay between molecular signaling pathways and tumor behavior (Table 4).

Table 4 Prognostic indicators for OSCC: role of dysregulated Wnt signaling components

Prognostic Indicators	Description
Dysregulated Wnt Ligands and Receptors	Dysregulated Wnt ligands (e.g., Wnt1, Wnt3, Wnt5a) correlate with aggressive OSCC, advanced stage, larger tumors, and lymph node metastasis, leading to worse prognosis and survival.
Clinicopathological Correlations	Wnt ligands and receptors correlate with OSCC tumor stage, grade, and invasion. Combining Wnt biomarkers with conventional parameters improves prognostic accuracy and risk stratification.
Therapeutic Resistance Mechanisms	Dysregulated Wnt signaling causes resistance to chemotherapy and radiotherapy in OSCC. Evaluating Wnt biomarkers helps identify patients at risk of resistance and recurrence, guiding personalized treatment and surveillance.
Targeting the Wnt Pathway	Targeting the Wnt pathway, alone or with standard therapies, can improve OSCC treatment outcomes and overcome resistance. Evaluating Wnt components aids in personalized treatment strategies.

Dysregulated Wnt ligands and receptors in OSCC show a strong correlation with aggressive tumor behavior and negative clinical outcomes. Higher levels of specific Wnt proteins (Wnt1, Wnt3, Wnt5a) are linked to advanced tumor stage, larger size, and lymph node metastasis. Wnt signaling activation drives EMT, crucial

for tumor spread. Aberrant Wnt signaling accelerates invasive traits in OSCC, leading to poorer prognosis and lower survival rates. Upregulated Wnt ligands in squamous cell carcinomas, like *Wnt-7A*, highlight the pathway's significance in OSCC progression, notably the Wnt/β-catenin pathway (Xie *et al.*, 2020). In addition, Wnt signaling, especially the Wnt/β-catenin pathway, actively fuels processes like EMT, which is crucial for tumor invasion and metastasis in head and neck squamous cell carcinoma (HNSCC). Wnt ligands like *Wnt5a* promote migration, invasion, and metastasis in HNSCC (Xie, Huang, Lu and Zheng, 2021). This dysregulated Wnt signaling in laryngeal squamous cell carcinoma accelerates invasion and metastasis, worsening prognostic outcomes. Targeting Wnt signaling is crucial for managing OSCC.

The expression levels of Wnt ligands and receptors are significantly correlated with various clinicopathological features of OSCC, including tumor stage, grade, and lymphovascular invasion (Bueno, Saad and Roversi, 2022, Tan, Wang, Xu, Li, Huang, Qin, Nice, Tang and Huang, 2023). Assessing Wnt-related biomarkers with conventional parameters improves prognostic accuracy for OSCC. This integrated approach aids in tailored treatment plans and patient management.

Furthermore, dysregulated Wnt signaling has been implicated in therapeutic resistance mechanisms in OSCC (Xie, Huang, Lu and Zheng, 2021). Tumors with aberrant Wnt pathway activation may display resistance to standard treatments such as chemotherapy and radiotherapy, resulting in treatment failure and disease recurrence (Xie, Huang, Lu and Zheng, 2021). Evaluating Wnt ligands and receptors helps identify OSCC patients at risk of treatment resistance and recurrence. Targeting the Wnt pathway, alone or with standard therapies, shows promise for improving outcomes and overcoming resistance.

5 Experimental models

5.1 In vitro models used to study Wnt signaling in OSCC

In cancer research, both in vitro and in vivo models offer distinct advantages and limitations (Fig. 2). In vitro models are indispensable in elucidating the intricate mechanisms underlying various biological processes, including the Wnt signaling pathway implicated in OSCC. OSCC-derived cell lines are cell lines that have been established from tumors or lesions of OSCC patients; they are valuable tools for studying the biology, behavior, and response of oral cancer cells to various treatments in a controlled laboratory setting (Dong *et al.*, 2015, Lee *et al.*, 2002). The utilization of OSCC-derived cell lines, such as SCC-9, SCC-15, CAL 27, and HSC-3, in OSCC is well-documented in the scientific literature. These cell lines are highly valuable due to their accessibility, ease of cultivation, and amenability to genetic modifications, making them ideal for studying the impact of Wnt ligands, inhibitors, and downstream effectors on various aspects of OSCC, such as proliferation, migration, invasion, and stemness properties (Xie, Huang, Lu and Zheng, 2021). Cell lines are crucial for understanding cancer stem cell traits and the role of Wnt signaling in OSCC, helping us explore how Wnt pathway components affect OSCC behavior and revealing intricate development mechanisms. For instance, *Wnt5b* elevation in metastatic OSCC cells is linked to increased migration, countered by gene silencing. Conversely, *Wnt5b* stimulation enhances protrusion formation in SAS-LM8 cells (Takeshita *et al.*, 2014, Xie, Huang, Lu and Zheng, 2021). In addition, non-canonical Wnt signaling pathways, such as the Wnt/Ca2+/PKC pathway, are activated by *Wnt5a*, promoting migration and invasion in OSCC (Xie, Huang, Lu and Zheng, 2021).

Fig. 2 Comparison of in vitro and in vivo models for studying the Wnt signaling pathway in OSCC. Strengths (green) and weaknesses (red) of each model are highlighted. In vitro models offer controlled environments for studying specific aspects of the Wnt pathway but may lack the complexity of the in vivo tumor microenvironment. In vivo models provide a more physiologically relevant setting but can be more challenging to manipulate and may be limited in terms of accessibility and reproducibility.

Three-dimensional (3D) cell culture refers to growing cells in an environment that mimics the 3D structure and conditions found in living organisms more closely than traditional two-dimensional (2D) cell cultures (Dalir Abdolahinia and Han, 2023, Urzì *et al*., 2023). In 3D cell culture, cells are cultured within a scaffold or matrix, which allows them to interact with neighboring cells and the surrounding environment in a manner more akin to their natural state. Conventional monolayer cell cultures fail to recapitulate the complex microenvironment encountered by OSCC cells in vivo. To address this limitation, researchers increasingly employ 3D cell culture models, such as spheroids and organoids (Lee *et al*., 2023, Wanigasekara *et al*., 2023). For example, recent research has created a biobank of 110 HNC organoid models, which retain key tumor DNA alterations and reflect patient responses to treatments. Organoids have shown potential for guiding treatment decisions, particularly in radiotherapy, where their response correlates with clinical outcomes. The validation of cisplatin and carboplatin as radio-sensitizers, along with the identification of cetuximab's radioprotective effects, underscores the utility of organoids in evaluating therapeutic efficacy. Additionally, targeted treatments on organoids have suggested new therapeutic options and possible treatment stratification. CRISPR-Cas9-based gene editing has further enhanced the role of organoids in biomarker discovery and validation (Millen *et al.*, 2023). These models better mimic the architecture and cell–cell interactions within OSCC tumors. Culturing OSCC cells in 3D environments enables researchers to more accurately study how Wnt signaling affects tumor growth, invasion, and response to therapy. Models like spheroids and organoids mimic OSCC tumor architecture and cell interactions, providing valuable insights into Wnt's role in disease progression and treatment response.

Researchers can manipulate the expression levels of key Wnt signaling components using techniques like transient transfection, lentiviral transduction, or CRISPR/Cas9-mediated gene editing, which enables researchers to delineate the functional consequences of aberrant Wnt signaling in OSCC (Ai *et al.*, 2020, Shen, Chen, Lin, Li, Liu, Zhang, Wang, Chan, Mak, Kahn, Qi and Yang, 2023). The overexpression of Wnt components in OSCC cell lines like SCC-9, SCC-15, CAL 27, and HSC-3 helps us to explore their roles in cancer progression. Conversely, targeted knockdown allows for assessing tumor-suppressive functions and therapeutic

potential. These accessible, easily cultivable cell lines facilitate in vitro studies on the impact of Wnt signaling on OSCC proliferation, migration, invasion, and stemness.

Co-culture systems involve culturing two or more different cell types together in the same environment, allowing them to interact with each other. These systems can mimic the complex cell–cell interactions and microenvironment found in vivo, offering valuable insights into various biological processes, disease mechanisms, and therapeutic responses (Ge *et al.*, 2023, Nguyen *et al.*, 2023). Co-culture systems, wherein OSCC cells are cultured alongside stromal cells such as fibroblasts, endothelial cells, or immune cells, provide a more physiologically relevant platform upon which to study Wnt signaling in the context of tumor microenvironment interactions (Takabatake *et al.*, 2020, Zhang *et al.*, 2017). These models facilitate the investigation of how Wnt signaling modulates crosstalk between OSCC cells and various stromal components, thereby influencing tumor growth, angiogenesis, immune evasion, and therapy resistance. For example, the co-culture of OSCC cells with CAFs has been shown to activate pro-inflammatory signaling pathways, including the upregulation of cytokines and chemokines like IL-8 and CXCL5, which are regulated by Wnt signaling (Arebro *et al.*, 2023). Additionally, the co-culture of OSCC cells with monocyte-derived macrophages can lead to the upregulation of *CCL2* and *CCR2* expression, which is mediated by the Wnt signaling regulator *Kif4A* (Zhang, Liu, Qu, Wang, Zhang, Jing, Li, Wei and Qu, 2017).

High-throughput screening (HTS) is a method used in drug discovery and chemical biology to rapidly test large numbers of compounds or molecules for their biological activity or therapeutic potential. The goal of HTS is to identify lead compounds that could serve as starting points for drug development or further optimization (Cadena *et al.*, 2023, Verrelle *et al.*, 2024). HTS assays enable the rapid and systematic evaluation of large compound libraries to identify novel regulators of Wnt signaling (Grimaldi *et al.*, 2018). Utilizing reporter gene assays, such as TOPFlash or TCF/LEF luciferase assays, researchers can assess the transcriptional activity of Wnt-responsive genes in response to small molecule inhibitors, activators, or natural compounds. These HTS platforms facilitate the discovery of potential therapeutic agents targeting aberrant Wnt signaling pathways in OSCC. For example, a high-throughput screen for Wnt/β-catenin signaling pathway modulators identified pyrithione zinc (PYZ) as a potent inhibitor of Wnt/β-catenin signaling in OSCC cells (Srivastava *et al.*, 2015). PYZ was found to decrease the expression of key Wnt signaling components, including β-catenin, TCF1, LEF1, cyclin D1, and c-Myc, leading to the inhibition of OSCC cell proliferation and tumor growth (Srivastava, Matta, Fu, Somasundaram, Datti, Walfish and Ralhan, 2015). An HTS cell model for OSCC, utilizing a TOPFlash reporter assay, quantifies Wnt/β-catenin transcriptional activity and aids in identifying therapeutic targets and compounds to regulate Wnt signaling in OSCC. Combined with reporter gene assays, 3D cultures, and co-culture systems, these platforms provide a comprehensive approach to studying Wnt's role in OSCC and discovering potential therapies.

5.2 In vivo models and their relevance to understanding Wnt signaling in OSCC

In vivo models are essential for comprehensively understanding the relevance of Wnt signaling in OSCC. Xenograft models are a type of preclinical animal model used in cancer research to study the growth and behavior of human tumors in vivo. In xenograft models, human tumor cells or tissues are implanted or injected into immunodeficient mice or other animals, allowing researchers to study various aspects of tumor biology, progression, and response to treatment. Xenograft models involve the transplantation of human OSCC cells into immunocompromised mice—typically subcutaneously or orthotopically into the oral cavity (Menon, 2017). These models enable the study of tumor growth, invasion, and metastasis in vivo. By modulating the expression of Wnt signaling components in OSCC cells prior to transplantation, researchers can assess the impact of aberrant Wnt signaling on tumor behavior and response to therapy (Hou *et al.*, 2024, Liu *et al.*, 2018). For example, a study using a xenograft model demonstrated that overexpression of the Wnt target gene *CDK5RAP2* in OSCC cells led to increased tumor growth, invasion, and stemness properties. Conversely, knockdown of *CDK5RAP2* inhibited these malignant behaviors, highlighting the crucial role of Wnt signaling in OSCC progression (Shen *et al.*, 2023). Additionally, xenograft models are used to evaluate Wnt-targeted therapies' efficacy against OSCC

progression. Researchers employ these in vivo models to test inhibitors like small molecules or antibodies and gauge their potential as OSCC treatments. These models offer a comprehensive strategy for understanding how Wnt signaling drives OSCC development and identifying potential therapeutic targets within the pathway.

Genetically engineered mouse models (GEMMs) involve mice that have been genetically modified to carry specific mutations or alterations in their genome, often to mimic human diseases such as cancer. These models are valuable tools in biomedical research for studying the underlying mechanisms of disease, testing potential therapeutics, and exploring gene function in vivo (Walrath *et al.*, 2010). GEMMs provide a valuable in vivo platform for studying Wnt signaling in OSCC by mimicking the stepwise accumulation of genetic alterations observed in human OSCC (Li *et al.*, 2020, Walrath *et al.*, 2010). These models allow researchers to investigate the role of Wnt signaling in tumor initiation, progression, and metastasis within an intact immune system and tumor microenvironment. For example, K14-cre; Ctnnb1^(ex3)^/+ mice harbor activating mutations in β-catenin, which leads to the development of OSCC with features such as hyperplasia, hyperkeratosis, severe epithelial dysplasia, and cancer (Tasoulas *et al.*, 2023). GEMMs provide a faithful representation of human OSCC development, facilitating the study of the impact of aberrant Wnt signaling on tumor behavior and therapy response. GEMMs enable preclinical testing of Wnt-targeted therapies and help with understanding the mechanisms of therapy resistance. They offer insights into the interplay between Wnt signaling, the immune system, and the tumor microenvironment in OSCC development and progression.

Patient-derived xenografts (PDX) are preclinical models used in cancer research that involve the transplantation of tumor tissue directly from cancer patients into immunodeficient mice. These models aim to replicate the biological characteristics and heterogeneity of human tumors more accurately than traditional cell line-based xenograft models (Invrea *et al.*, 2020, Zanella *et al.*, 2022). PDX models are valuable in vivo tools for translational research in OSCC, involving the transplantation of patient-derived OSCC tumor tissues directly into immunocompromised mice, preserving the histological and molecular heterogeneity of the original patient tumors (Silveira *et al.*, 2023). By engrafting OSCC tumors with known Wnt signaling alterations, such as mutations in β-catenin or APC, researchers can investigate their impact on tumor behavior and response to therapy in vivo. PDX models may facilitate the identification of patient-specific therapeutic vulnerabilities and the evaluation of personalized treatment strategies targeting aberrant Wnt signaling pathways. These models maintain the original tumor architecture, allowing for the study of tumor growth, invasion, and metastasis in a more physiologically relevant context compared to cell lines (Liu *et al.*, 2023). Researchers can utilize PDX models to study the impact of Wnt signaling alterations on tumor behavior and response to therapy in vivo, providing valuable insights into the potential therapeutic targets and personalized treatment strategies for OSCC patients.

Orthotopic implantation models are in vivo models. This model can be used to study Wnt signaling in OSCC. These models involve the inoculation of OSCC cells directly into the oral cavity of immunocompromised mice, mimicking the anatomical site of human OSCC development. By modulating Wnt signaling in OSCC cells prior to implantation, researchers can investigate how dysregulated Wnt signaling influences tumor–stroma interactions, angiogenesis, and immune evasion in vivo (Chaves *et al.*, 2023, Vahle *et al.*, 2012). Orthotopic implantation models provide a platform for evaluating novel therapeutic strategies targeting the Wnt signaling pathway in the context of primary OSCC tumors. These models enable the study of tumor growth, invasion, and metastasis within the context of the oral microenvironment, providing valuable insights into the impact of Wnt signaling alterations on tumor behavior and response to therapy in vivo (Bais *et al.*, 2015, Bais *et al.*, 2015).

Metastasis models are preclinical models used in cancer research to study the process by which cancer cells spread from the primary tumor to distant sites in the body, forming secondary tumors. Understanding the mechanisms of metastasis is crucial for developing effective treatments to prevent or target metastatic disease, which is the primary cause of cancer-related mortality (Zhang *et al.*, 2021). These models are vital for understanding and predicting metastasis, a significant factor affecting patient prognosis. The studies discuss combining variables like gene profiles, clinical parameters, and molecular factors to create accurate prediction

models for nodal metastasis in OSCC. Notably, the combination of CDKN2A, PLAU, T stage, and pathological grade has been highlighted as an effective predictive model for lymph node metastasis in OSCC (Xu *et al.*, 2023). In the context of in vivo metastasis models for OSCC, the search results primarily focus on orthotopic mouse models. These models involve injecting tumor cells into the tongues of mice to induce local tumor growth and metastasis. The models using non-metastatic CAL27 cells and metastatic UMSCC2 cells have been developed to reflect tumor growth and metastasis in OSCC. These models provide valuable insights into the mechanisms of OSCC growth and metastasis, aiding in evaluating potential therapeutics (Bais, Kukuruzinska and Trackman, 2015). Overall, the combination of predictive models based on gene profiles, clinical parameters, and molecular factors, along with the development of orthotopic mouse models, plays a crucial role in advancing our understanding of OSCC metastasis and developing effective therapeutic strategies to combat this challenging aspect of the disease.

6 Therapeutic targets and strategies

Exploring therapeutic targets and strategies involving the Wnt signaling pathway in OSCC is essential for developing effective treatments. By targeting specific components of the Wnt signaling pathway and employing diverse therapeutic strategies, researchers aim to develop innovative treatments that effectively inhibit OSCC progression and improve patient outcomes (Fig. 3).

Fig. 3 Various therapeutic strategies for treating OSCC. Precision medicine tailors treatments based on molecular profiling, optimizing therapy for individual patients. Combination therapies involve combining Wnt pathway inhibitors with EGFR-targeted therapies or immune checkpoint inhibitors to enhance treatment efficacy in OSCC. β-Catenin inhibition using ICG-001 and E7386 offers the potential to suppress Wnt-driven oncogenic processes. Targeting LRP5/6 receptors disrupts Wnt signaling, inhibiting OSCC progression. Inhibiting FZD receptors with monoclonal antibodies or small-molecule inhibitors is crucial for inhibiting OSCC progression. In OSCC, LGK974 inhibits porcupine, blocking Wnt ligand palmitoylation and secretion, suppressing tumor growth by reducing Wnt signaling.

6.1 Wnt ligands inhibition

Targeting Wnt ligands directly represents a promising therapeutic strategy for disrupting the intricate signaling pathways involved in OSCC progression. Wnt ligands are pivotal components of the Wnt signaling pathway, orchestrating cellular processes critical for tumor growth and metastasis. In OSCC, dysregulated Wnt ligand secretion fuels autocrine and paracrine signaling loops, fostering malignant transformation and disease progression (Patel *et al.*, 2019). Therefore, inhibiting Wnt ligand secretion is a viable therapeutic approach to impede OSCC progression.

Porcupine is an essential enzyme for Wnt ligand secretion and catalyzes the post-translational modification of Wnt ligands necessary for their secretion and activation. Small molecule inhibitors targeting porcupine have demonstrated efficacy in preclinical models of OSCC (Peña-Oyarzún *et al.*, 2024). Notably, LGK974 is a potent and selective inhibitor of porcupine that has shown promise in inhibiting Wnt ligand secretion and downstream signaling cascades. By inhibiting porcupine activity, LGK974 effectively disrupts the secretion of Wnt ligands, thus attenuating Wnt signaling activation and suppressing tumor growth in OSCC. The mechanism of action of porcupine inhibitors like LGK974 involves binding to porcupine's active site, which blocks its enzymatic activity and prevents the palmitoylation of Wnt ligands. Palmitoylation is a lipid modification critical for the secretion and proper functioning of Wnt ligands (Liu *et al.*, 2013). By inhibiting porcupine-mediated palmitoylation, LGK974 effectively impedes the secretion of Wnt ligands into the extracellular environment, disrupting both autocrine and paracrine Wnt signaling loops in OSCC. Preclinical studies evaluating the efficacy of porcupine inhibitors in OSCC have shown promising results. Treatment with LGK974 has been shown to inhibit cell proliferation, induce apoptosis, and suppress tumor growth in xenograft models (Paluszczak, 2020, Reyes *et al.*, 2020).

While porcupine inhibitors such as LGK974 hold promise as OSCC therapies, further research is needed into their safety, pharmacokinetics, and side effects in clinical use. Biomarker-driven trials are required to optimize treatment strategies and identify the patient groups that benefit the most. Targeting Wnt secretion via porcupine inhibition offers a promising approach to disrupting Wnt signaling and combating OSCC progression.

6.2 Frizzled receptor inhibition

FZDs are crucial mediators of the Wnt signaling pathway, which plays a pivotal role in the pathogenesis of OSCC (Sompel *et al.*, 2021, Zeng, Chen and Fu, 2018). Aberrant activation of the Wnt/FZD signaling axis has been implicated in the development and progression of OSCC, making FZDs attractive therapeutic targets (Smith, Sompel, Elango and Tennis, 2021, Xie, Huang, Lu and Zheng, 2021).

Blocking the interaction between FZDs and their cognate Wnt ligands can effectively inhibit downstream signaling cascades, thereby preventing the malignant transformation and proliferation of OSCC cells (Zeng, Chen and Fu, 2018, Zhang *et al.*, 2015). One promising approach is the use of monoclonal antibodies that specifically target individual FZD receptors. These antibodies can bind to the extracellular domain of FZDs, preventing Wnt ligand binding and subsequent activation of the Wnt signaling pathway (Smith, Sompel, Elango and Tennis, 2021, Zeng, Chen and Fu, 2018).

In addition to monoclonal antibodies, small molecule inhibitors that interfere with the FZD receptor–ligand interaction have also been explored as potential therapeutic strategies for OSCC. These small molecules can disrupt the formation of the FZD–Wnt complex, effectively blocking the initiation of Wnt signaling and its downstream effects on cell proliferation, migration, and invasion (Sompel, Elango, Smith and Tennis, 2021, Xie, Huang, Lu and Zheng, 2021).

6.3 LRP receptor inhibition

The LRPs, particularly LRP5 and LRP6, are essential co-receptors in the Wnt signaling pathway, which is aberrantly activated in OSCC. The formation of the Wnt–Frizzled–LRP complex is a critical step in the initiation of Wnt signaling, making LRP5/6 receptors attractive therapeutic targets for OSCC. Inhibiting the activity

of LRP5/6 receptors can effectively disrupt the Wnt–Frizzled–LRP complex, preventing the downstream activation of Wnt signaling cascades. This approach can lead to the suppression of various malignant phenotypes associated with OSCC, such as uncontrolled cell proliferation, migration, and invasion (Hsu, Huang, Huang, Huang, Yeh, Chao and Bamodu, 2019, Yuan, Xie, Jiang, Wei, Wang, Chen, Li, Sun, Zhao, Zeng, Jiang, Zhou, Dan, Feng, Liu, Wang and Chen, 2017).

Several strategies have been explored to target LRP receptors in OSCC. Small molecule inhibitors that bind to and block the extracellular domain of LRP have shown promising results in preclinical studies (Robert *et al.*, 2018). These inhibitors can effectively interfere with the interaction between Wnt ligands and LRP receptors, preventing the activation of Wnt signaling.

6.4 β-Catenin inhibition

β-Catenin is a central mediator of the canonical Wnt signaling pathway, which plays a crucial role in the pathogenesis of OSCC (Robert, Dakshinamoorthy, Ganapathyagraharam Ramamoorthy, Dhandapani, Thangaiyan, Muthusamy, Nirmal and Prasad, 2018). As a key transcriptional regulator, β-catenin drives the expression of target genes involved in various oncogenic processes, such as cell proliferation, survival, and metastasis (Kim *et al.*, 2019, Wang *et al.*, 2021).

The inhibition of β-catenin activity has been identified as a promising therapeutic approach for OSCC. Preclinical studies have demonstrated encouraging findings regarding small molecule inhibitors that selectively focus on β-catenin or impede its interaction with transcriptional co-activators, including CREB-binding protein (CBP) (Chandler *et al.*, 2020, Reyes, Flores, Betancur, Peña-Oyarzún and Torres, 2020).

One such small molecule inhibitor, ICG-001, has been found to effectively inhibit the β-catenin/CBP interaction, leading to the suppression of EGFR oncogenic activity in OSCC cells. By disrupting this critical signaling axis, ICG-001 can reduce the malignant phenotype of OSCC, including cell proliferation, migration, and invasion. Similarly, another small molecule inhibitor, E7386, has also been shown to target the β-catenin/CBP interaction, resulting in increased expression of fucosyltransferases and enhanced EGFR N-glycan antennary fucosylation. This modification of EGFR glycosylation can potentially impact its oncogenic signaling and contribute to the anti-tumor effects of β-catenin inhibition (Chandler, Alamoud, Stahl, Nguyen, Kartha, Bais, Nomoto, Owa, Monti, Kukuruzinska and Costello, 2020).

These studies highlight the therapeutic potential of targeting the β-catenin signaling axis in OSCC. By disrupting the aberrant activation of β-catenin and its downstream transcriptional programs, small molecule inhibitors can effectively suppress Wnt-driven oncogenic processes and offer a promising approach for the management of this disease.

6.5 Combination therapies

Emerging evidence suggests that combining Wnt pathway inhibitors with standard chemotherapeutic agents, targeted therapies, or immunotherapies may enhance treatment efficacy in OSCC (Meng *et al.*, 2021, Silva *et al.*, 2023). The aberrant activation of the Wnt signaling pathway is a key driver of OSCC pathogenesis, and targeting this pathway in combination with other therapeutic modalities has shown promising results in preclinical studies.

One potential combination strategy could be the use of Wnt pathway inhibitors alongside EGFR-targeted therapies. EGFR is a crucial signaling node in OSCC, and its inhibition can effectively suppress tumor progression. However, resistance to EGFR-targeted therapies often develops, and the combination with Wnt pathway inhibitors may help overcome this challenge. Preclinical studies have demonstrated that the inhibition of Wnt signaling—for example, by targeting FZD receptors or β-catenin—can sensitize OSCC cells to EGFR-targeted therapies, leading to enhanced anti-tumor effects (Silva, Pinto, Monteiro, Silva and Bousbaa, 2023).

Another promising combination approach is using Wnt pathway inhibitors in conjunction with immune checkpoint inhibitors. The Wnt/β-catenin pathway has been implicated in the modulation of the TME and

immune evasion in OSCC. By combining Wnt inhibitors with immune checkpoint blockade, researchers have observed synergistic effects in preclinical models, leading to improved tumor control and increased immune cell infiltration.

Furthermore, the combination of Wnt pathway inhibitors with standard chemotherapeutic agents, such as cisplatin or 5-fluorouracil, has also been explored in OSCC. Preclinical studies have shown that the inhibition of Wnt signaling can sensitize OSCC cells to the cytotoxic effects of these chemotherapeutic drugs, resulting in enhanced anti-tumor activity and reduced drug resistance.

These combination approaches targeting the Wnt pathway in conjunction with other therapeutic modalities have the potential to improve treatment outcomes in OSCC. However, further research and clinical trials are necessary to fully evaluate the efficacy and safety of using these combination therapies in the management of this disease.

6.6 Precision medicine approaches

Precision medicine strategies offer significant potential for managing OSCC by using molecular profiling of tumors to inform personalized treatment choices (Zhong *et al.*, 2018). The aberrant activation of the Wnt signaling pathway has been widely implicated in the pathogenesis of OSCC. Molecular profiling of OSCC tumors can identify patients with specific genetic or epigenetic alterations in Wnt pathway components, such as dysregulated expression of Wnt ligands, FZD receptors, LRP co-receptors, or β-catenin. By recognizing these molecular subtypes of OSCC, clinicians can select targeted therapies that specifically inhibit the Wnt signaling axis, potentially optimizing therapeutic efficacy and minimizing adverse effects (Deepa Jatti and Rakesh, 2021, Silva, Pinto, Monteiro, Silva and Bousbaa, 2023).

Biomarker-driven clinical trials evaluating the efficacy of Wnt pathway inhibitors in molecularly defined subgroups of OSCC patients are essential for validating the therapeutic potential of these targeted approaches. These trials can assess the clinical benefit of Wnt inhibitors, such as FZD receptor antagonists, LRP inhibitors, or β-catenin inhibitors, in patients whose tumors exhibit specific Wnt pathway alterations. By identifying biomarkers that predict treatment response, these studies can help guide the selection of those OSCC patients most likely to benefit from Wnt-targeted therapies (Deepa Jatti and Rakesh, 2021, Silva, Pinto, Monteiro, Silva and Bousbaa, 2023).

Furthermore, the integration of Wnt pathway inhibitors with other treatment modalities, such as chemotherapy, EGFR-targeted agents, or immunotherapy, may enhance the efficacy of precision medicine approaches in OSCC. Combination therapies that simultaneously target multiple oncogenic signaling pathways can potentially overcome therapeutic resistance and improve clinical outcomes for patients with Wnt-driven OSCC.

Overall, the molecular profiling of OSCC tumors and the development of biomarker-driven clinical trials that evaluate Wnt pathway inhibitors represent crucial steps toward implementing precision medicine for this disease. By tailoring treatments to the individual tumor characteristics, clinicians can optimize therapeutic strategies and improve the quality of care for OSCC patients.

7 Challenges and future perspectives

Current research on the role of Wnt ligands and receptors in OSCC faces several limitations that hinder the translation of findings into clinical practice. One major challenge is the inherent heterogeneity of OSCC, both intertumoral and intratumoral. This diversity complicates targeted therapy development as treatments may not effectively address all subtypes of OSCC, leading to inconsistent treatment responses and limited clinical efficacy (Patel *et al.*, 2019). Additionally, the lack of robust biomarkers for OSCC further impedes progress in this field. Many studies rely on small sample sizes and lack validation in larger patient cohorts, undermining the reliability and generalizability of findings.

Therapeutic resistance poses another significant obstacle in OSCC treatment (Sa *et al.*, 2024, Zhao *et al.*,

2024). Despite advancements in targeting Wnt pathways, resistance mechanisms remain poorly understood. This lack of understanding limits the effectiveness of targeted therapies and necessitates further investigation into alternative treatment modalities and combination therapies. In addition, the translation of preclinical findings into clinical applications is hindered by the limitations of existing preclinical models, which often fail to fully replicate the complex tumor microenvironment and molecular heterogeneity of OSCC. This highlights the need for more representative and clinically relevant model systems to improve the predictive value of preclinical studies.

Emerging technologies and methodologies offer promising solutions to overcome the limitations of current research on Wnt ligands and receptors in OSCC. Single-cell analysis, for instance, provides a comprehensive understanding of cellular heterogeneity within OSCC tumors, facilitating the identification of rare cell populations and signaling pathways (Sun *et al.*, 2023, Yang *et al.*, 2023). The integration of omics technologies, such as genomics, transcriptomics, proteomics, and metabolomics, enables a holistic view of the molecular landscape of OSCC, aiding in the identification of dysregulated Wnt signaling pathways and potential therapeutic targets. Additionally, CRISPR/Cas9 genome editing allows for precise manipulation of the genome to elucidate the functional significance of Wnt pathway alterations in OSCC progression.

Furthermore, patient-derived organoid models offer a valuable platform for drug screening and personalized medicine approaches. These models faithfully recapitulate the histological and molecular features of OSCC tumors, enabling the evaluation of therapeutic responses in a patient-specific context. Organoid models hold promise for identifying effective treatment strategies tailored to individual patient profiles, thereby advancing personalized medicine in OSCC management (Farshbaf *et al.*, 2023).

Targeting Wnt ligands and receptors presents significant opportunities for the development of targeted therapies in OSCC. Small molecule inhibitors and monoclonal antibodies directed against key components of the Wnt pathway demonstrate efficacy in preclinical models and hold potential for clinical translation. Biomarker-guided therapy, integrating molecular biomarkers into clinical practice, enables personalized treatment selection and optimization of therapeutic regimens based on individual patient characteristics. This approach enhances treatment efficacy and minimizes unnecessary treatment-related toxicities.

Additionally, immunotherapeutic approaches such as immune checkpoint inhibitors have shown promise in OSCC treatment. Modulation of the immune microenvironment by targeting Wnt signaling pathways may enhance the efficacy of immunotherapy and overcome resistance mechanisms. Precision oncology trials incorporating comprehensive molecular profiling and targeted therapeutic interventions are essential for advancing personalized medicine in OSCC. These trials enable the real-time adaptation of treatment strategies based on genomic data, biomarker analysis, and therapeutic response monitoring, which maximizes therapeutic efficacy and improves patient outcomes in OSCC.

8 Conclusions

In conclusion, the role of Wnt ligands and receptors in OSCC is increasingly recognized as pivotal in driving tumorigenesis and progression. Through intricate signaling cascades, the aberrant activation of Wnt pathways contributes to key hallmarks of cancer, including proliferation, invasion, and metastasis in OSCC. In addition, dysregulation of Wnt signaling has been implicated in the maintenance of cancer stem cell populations, further emphasizing its significance in OSCC pathogenesis. Targeting Wnt signaling components presents a promising therapeutic strategy for OSCC treatment, with the potential for both direct inhibition of tumorigenic pathways and synergistic effects with existing therapies. However, the complexity of Wnt signaling networks underscores the need for a comprehensive understanding of context-specific interactions and feedback mechanisms within OSCC microenvironments. Future research efforts should focus on elucidating the precise roles of individual Wnt ligands and receptors in OSCC progression, as well as exploring combinatorial therapeutic approaches to effectively target Wnt signaling and improve clinical outcomes for OSCC patients.

Abbreviations

OSCC Oral squamous cell carcinoma
EMT Epithelial-mesenchymal transition
CRC Colorectal cancer
NSCLC Non-small cell lung cancer
CSC Cancer stem cell
PCP Planar cell polarity
PKC Protein kinase C
CaMKII Calcium/calmodulin-dependent protein kinase II
LRP5/6 Lipoprotein receptor-related protein 5/6
WISP1 WNT1-inducible signaling pathway protein 1
SNPs Single-nucleotide polymorphisms
TCF/LEF T-cell factor/lymphoid enhancer factor
CAFs Cancer-associated fibroblasts
3D Three-dimensional
HTS High-throughput screening
GEMMs Genetically engineered mouse models
PDX Patient-derived xenografts
TME Tumor microenvironment
GPCRs G-protein coupled receptors
CRD Cysteine-rich domain

Data availability statement

Not applicable.

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Authors contribution

Muhammad Tufail: Conceptualization, Original Drafting, Visualization, Writing - Review & Editing. **Cai-Yun He**: Conceptualization, Visualization, Revisions, Editing. **Can-Hua Jiang**: Visualization. **Ning Li**: Supervision, reviewed, and editing. All authors have reviewed and approved the final manuscript for publication. Muhammad Tufail and Cai-Yun He contributed equally to this manuscript. All authors have reviewed and approved the final manuscript for publication.

Ethics declarations

Muhammad Tufail, Cai-Yun He, Can-Hua Jiang, and Ning Li declare that they have no conflict of interest. This review does not contain any studies with human or animal subjects performed by any of the authors.

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