

# Regulatory Signaling Pathways in Ovarian Cancer Stem Cells: Their Role in Tumor Progression and Therapeutic Strategies

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## ABSTRACT

In ovarian cancer, OCSCs not only contribute to tumor formation but also drive the progression, metastasis, and chemoresistance. The defining properties of OCSCs are sustained through the Wnt/ $\beta$ -catenin, Notch, and Hedgehog signaling pathways, which can synergize rather than act independently to form a triad that enhances key functional capabilities of OCSCs. Various therapeutic agents have been studied to target these pathways in OCSCs, including  $\gamma$ -secretase inhibitors (niraparicicab), SMO inhibitors (vismodegib and sonidegib), and Wnt/ $\beta$ -catenin inhibitors (PRI-724 and ipafriccept). While these agents have demonstrated antitumor activity in preclinical and early clinical studies, their clinical use remains challenging due to compensatory interactions among signaling pathways, which diminish the efficacy of single-agent treatments. To improve treatment outcomes, strategies involving combination approaches and personalized treatments need to be explored. This review aims to summarize current evidence on the role of Wnt/ $\beta$ -catenin, Notch, and Hedgehog signaling pathways in OCSCs and their therapeutic implications in ovarian cancer. **DOI: 10.61882/ibj.5158**

**Keywords:** Hedgehogs, Ovarian neoplasms, Therapeutics, Wnt signaling pathway

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## List of abbreviations

**ABC:** ATP-binding cassette; **ADAM17:** a disintegrin and metalloproteinase domain-containing protein 17; **ALDH:** aldehyde dehydrogenase; **ALDH1A1:** aldehyde dehydrogenase 1 family member A1; **APC:** adenomatous polyposis coli; **AXIN:** axis inhibition protein; **BRAF:** B-Raf proto-oncogene, serine/threonine kinase; **CCEPR:** carcinoma expressed PCNA regulatory; **CCND1:** cyclin D1; **COP1:** constitutive; photomorphogenic protein 1; **CSC:** cancer stem cell; **CTNNB1:** catenin *beta* 1; **DAPT:**  $\gamma$ -secretase inhibitor; **DHH:** desert hedgehog; **DLL1:** delta-like ligand 1; **DVL:** dishevelled; **EMT:** epithelial-mesenchymal transition; **EOC:** epithelial ovarian cancer; **FGF9:** fibroblast growth factor 9; **FOXO1:** forkhead box O1; **FZD:** frizzled; **FZD7:** frizzled class receptor 7; **GANT61:** GLI1 inhibitor; **GATA1:** GATA binding protein 1; **GLI1:** GLI family zinc finger 1; **Globocan:** Global Cancer Observatory; **GnT-III:** N-acetylglucosaminyltransferase III; **GPR137:** G protein-coupled receptor 137; **GPX4:** glutathione peroxidase 4; **GSK3 $\beta$ :** glycogen synthase kinase 3 beta; **HER2:** human epidermal growth factor receptor 2; **HES:** hairy and enhancer of split; **HEY1:** hairy/enhancer-of-split with YRPW motif 1; **HGSC:** high-grade serous ovarian cancer; **IHH:** Indian hedgehog; **ISYNA1:** inositol-3-phosphate synthase 1; **JAG:** Jagged; **KAT6A:** lysine acetyltransferase 6a; **LEF:** lymphoid enhancer-binding factor; **LGR6:** leucine-rich repeat-containing G protein-coupled receptor 6; **lncRNAs:** long noncoding RNA; **MAML:** mastermind-like; **MGP:** matrix gla protein; **miRNA:** microRNA; **MMP-7:** matrix metalloproteinase 7; **MYC:** myc proto-oncogene; **Nanong:** nanog homeobox; **NF- $\kappa$ B:** nuclear factor kappa B; **NICD:** Notch intracellular domain; **Notch1:** Notch receptor 1; **OCSC:** ovarian cancer stem cell; **OS:** overall survival; **pp30:** E1A-binding protein p300; **PTCH1:** Patched 1; **RAV8A:** ras-related protein Rab-8A; **RBPJ:** recombination signal binding protein for immunoglobulin kappa J; **RUNX3:** runt-related transcription factor 3; **SFRP4:** secreted frizzled-related protein 4; **SHH:** sonic hedgehog; **SMO:** smoothened; **SNORA72:** small nucleolar RNA 72; **SOX:** SRY-box transcription factor; **SUFU:** suppressor of fused; **TCF:** T-cell factor; **TME:** tumor microenvironment; **TUSC7:** tumor suppressor candidate 7; **Twist1:** twist family BHLH transcription factor 1; **Zeb2:** zinc finger E-box binding homeobox 2

## INTRODUCTION

Ovarian cancer (OC) ranks among the most prevalent cancers in women and is associated with one of the poorest prognoses, with a five-year survival rate of only 50%. In 2022, the Globocan reported 324,603 newly diagnosed cases and 206,956 deaths globally<sup>[1,2]</sup>. High mortality is associated with the largely asymptomatic nature of the disease, which frequently results in late-stage diagnosis and limited curative potential<sup>[3]</sup>. Although initial responses to platinum- and taxane-based chemotherapy are often notable, relapse after treatment is the main cause of death. Approximately 75-80% of patients diagnosed at advanced stages experience recurrence, which contributes to the persistently low survival rates<sup>[4]</sup>.

CSCs are a small subset of tumor cells that can indefinitely replicate and generate phenotypic diversity, leading to the formation of distinct tumor clonal proliferations. These cells, in turn, drive tumor initiation, continuous propagation, and heterotypic cellular interactions within the TME, thereby contributing to interstitial cellular heterogeneity. While conventional chemotherapy focuses primarily on the bulk of tumor cells, it often fails to eradicate CSCs, leading to an increased risk of relapse and metastasis<sup>[5]</sup>. In OC, these CSCs are known as OCSCs and are identified by the expression of *CD44*, *CD117*, *CD133*, and *CD24*, elevated *ALDH* activity, and the expression of pluripotent transcription factors *Sox2*, *Oct4*, and *Nanog*<sup>[6]</sup>. Unlike solid tumors that originate from the normal tissue of origin, OCSCs may arise from the fallopian tube epithelium and the ovarian epithelium, particularly secretory epithelial cells and intermediate populations expressing the transcription factors *RUNX3* and *SOX17*. OCSCs display remarkable plasticity and can stochastically switch between stem-like and differentiated states. In addition, the absence of anatomical barriers facilitates transcoelomic metastasis into the peritoneal cavity<sup>[7,8]</sup>.

CSCs employ multiple strategies to evade therapeutic interventions. These strategies include ABC efflux proteins, ALDH overactivity, enhanced DNA repair, and activation of certain intracellular signaling pathways<sup>[9]</sup>. Moreover, it has been presented that various molecular signaling pathways, including Wnt/ $\beta$ -catenin, Notch, and Hedgehog, are engaged in the regulation of OCSCs. These pathways also control EMT, the metastatic process, and the maintenance of stem-like characteristics of CSC<sup>[10]</sup>. Thus, targeting CSCs offers a promising approach to overcoming therapeutic resistance. In addition, it provides insights into resistance and elucidates the molecular pathways that regulate CSC characteristics and contribute to drug resistance<sup>[11]</sup>.

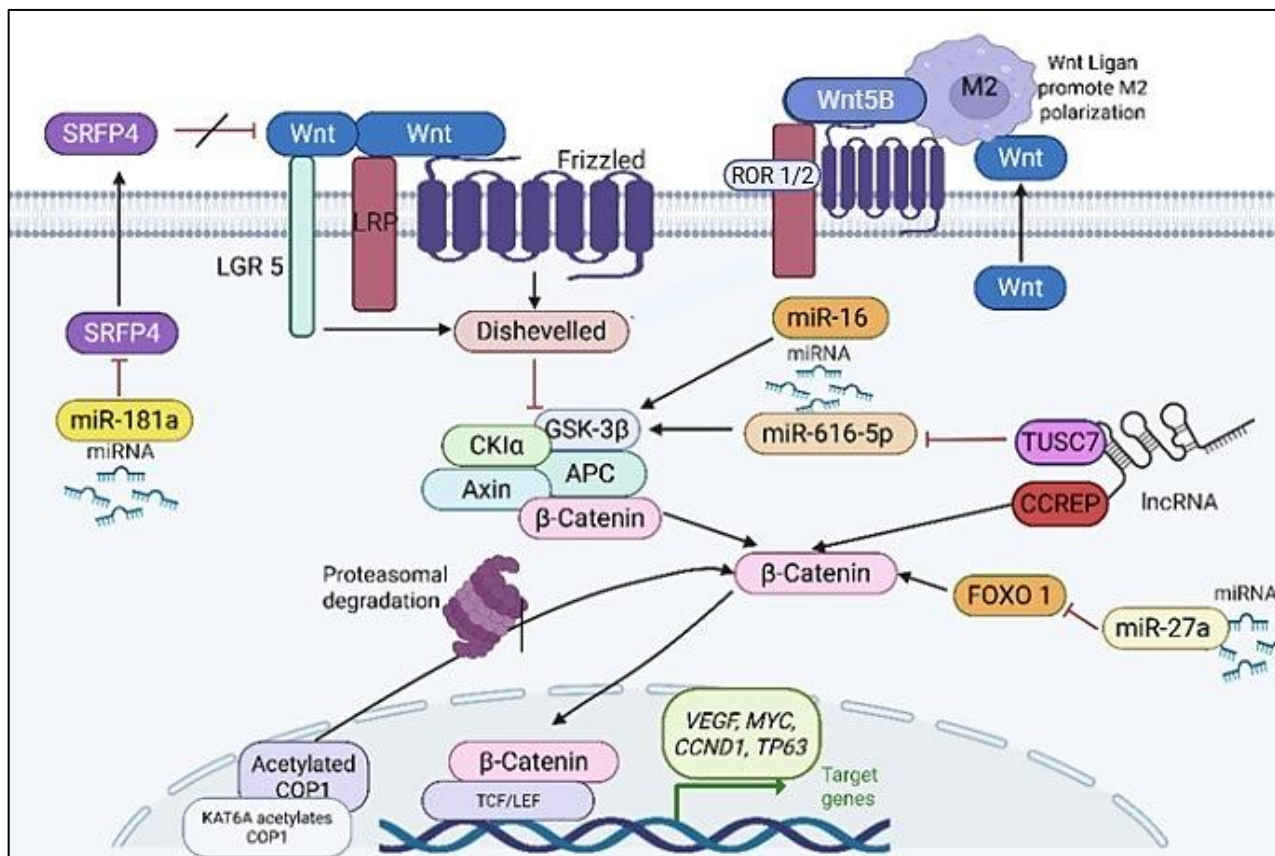
Despite growing interest in OCSC-directed therapies, translating preclinical findings into clinical applications is challenging. The major barriers include insufficient characterization of tumor heterogeneity, complex signaling crosstalk, and the lack of selective inhibitors that preserve normal stem cells. This review examines how signaling pathways regulate OCSCs and influence tumor growth and their potential as therapeutic targets to improve treatment outcomes.

## Critical signaling pathways in OCSCs driving tumor progression

### Wnt/ $\beta$ -catenin signaling

The Wnt/ $\beta$ -catenin pathway regulates cell differentiation and behavior and fuels the development and maintenance of CSCs<sup>[12]</sup>. Different mechanisms associated with Wnt/ $\beta$ -catenin activity contribute to the preservation of stem-like characteristics (Fig. 1). In the absence of Wnt signaling,  $\beta$ -catenin is phosphorylated by a destruction complex consisting of GSK3 $\beta$ , CK1 $\alpha$ , AXIN, and APC, leading to its degradation<sup>[13]</sup>. Following ligand interaction with FZD receptors and low-density lipoprotein receptor-related Protein 5/6, phosphorylation of LRP6 promotes the recruitment of DVL and inhibits the destruction complex. This process enables  $\beta$ -catenin accumulation and nuclear translocation, where it activates TCF/LEF-directed transcription of vascular endothelial growth factor, MYC, and CCND1<sup>[14,15]</sup>.

In EOC, this pathway could promote multiple CSC properties, including self-renewal, metastasis, drug resistance, angiogenesis, and immune escape. However, it is unclear whether these effects are consistent across different EOC subtypes, suggesting the need for subtype-specific validation. Mutations in *CTNNB1*, *AXIN*, or *APC* are predominantly found in endometrioid and mucinous subtypes. However, HGSC is linked to several abnormalities, including ligand stimulation, receptor activation, disruption of the  $\beta$ -catenin destruction complex, impaired  $\beta$ -catenin/E-cadherin interactions at the cell membrane, and elevated  $\beta$ -catenin/TCF-driven transcription<sup>[16]</sup>. These alterations may drive tumor progression and contribute to poor clinical outcomes in EOC. lncRNAs and miRNAs are non-coding RNAs that lack protein-coding ability but are involved in gene regulation. lncRNA may target Wnt/ $\beta$ -catenin signaling and regulate CSC properties either as an activator or repressor of transcription through interaction with regulatory RNAs or transcription-related proteins<sup>[17]</sup>. In OC, lncRNA CCEPR shows elevated expression and facilitates cell invasion and progression to higher International Federation of Gynecology and Obstetrics stages, resulting in poorer OS through modulation of signaling pathways that lead to increased expression of



**Fig. 1.** Wnt/ $\beta$ -catenin signaling in OCSCs (Created by Biorender). Wnt ligand binding to the FZD-LRP5/6 complex inhibits the  $\beta$ -catenin destruction complex, stabilizing  $\beta$ -catenin and promoting transcription of Wnt target genes that support OCSC stemness and chemoresistance. Wnt/ $\beta$ -catenin signaling is controlled by several regulatory mechanisms. IncRNA CCREP stimulates the pathway, elevating Cyclin D1, c-Myc, MMP-7, and  $\beta$ -catenin. However, IncRNA TUSC7 suppresses this signaling by sponging miR-616-5p, keeping GSK3 $\beta$  active and limiting  $\beta$ -catenin accumulation. miR-27a indirectly activates Wnt/ $\beta$ -catenin via FOXO1 repression, while miR-16 inhibits it by boosting GSK3 $\beta$ -mediated  $\beta$ -catenin degradation. In chemoresistant OCSCs, miR-181a inhibits the Wnt antagonist SFRP4, and KAT6A acetylates COP1 to prevent  $\beta$ -catenin breakdown. Activation of FZD7 enhances  $\beta$ -catenin, tumor protein p63 (TP63), and GPX4, protecting OCSCs from chemotherapy-induced oxidative stress. LGR6, on the other hand, stabilizes  $\beta$ -catenin through Wnt ligand-triggered disheveled signaling. Within the TME, OCSCs release Wnt ligands that induce M2-like macrophages to secrete *Wnt5B*. This secretion subsequently activates non-canonical Wnt signaling in OCSCs, forming a feedback loop that sustains stemness and chemoresistance.

subsequent targets such as *CCND1*,  $\beta$ -catenin, *MYC*, and *MMP-7*<sup>[18]</sup>. Conversely, downregulation of IncRNA *TUSC7* increases cancer cell aggressiveness, as *TUSC7* normally functions as a sponge for *miR-616-5p* to suppress GSK3 $\beta$  and  $\beta$ -catenin signaling<sup>[19]</sup>. Unlike IncRNAs, miRNAs primarily act by binding to target mRNAs, preventing their translation into protein. MiR-27a enhances motility, promotes EMT, and facilitates OC cell link by targeting FOXO1 within the main signaling pathway. Additionally, miR-16 raises GSK3 $\beta$  activity<sup>[20,21]</sup>. Aberrant Wnt signaling underpins chemotherapy resistance in OC. In HGSC, *miR-181a* suppresses *SFRP4*, a natural Wnt inhibitor. In parallel, KAT6A, a MYST-type histone acetyltransferase, acetylates COP1, thereby disrupting its E3 ligase activity and resulting in increased  $\beta$ -catenin stabilization

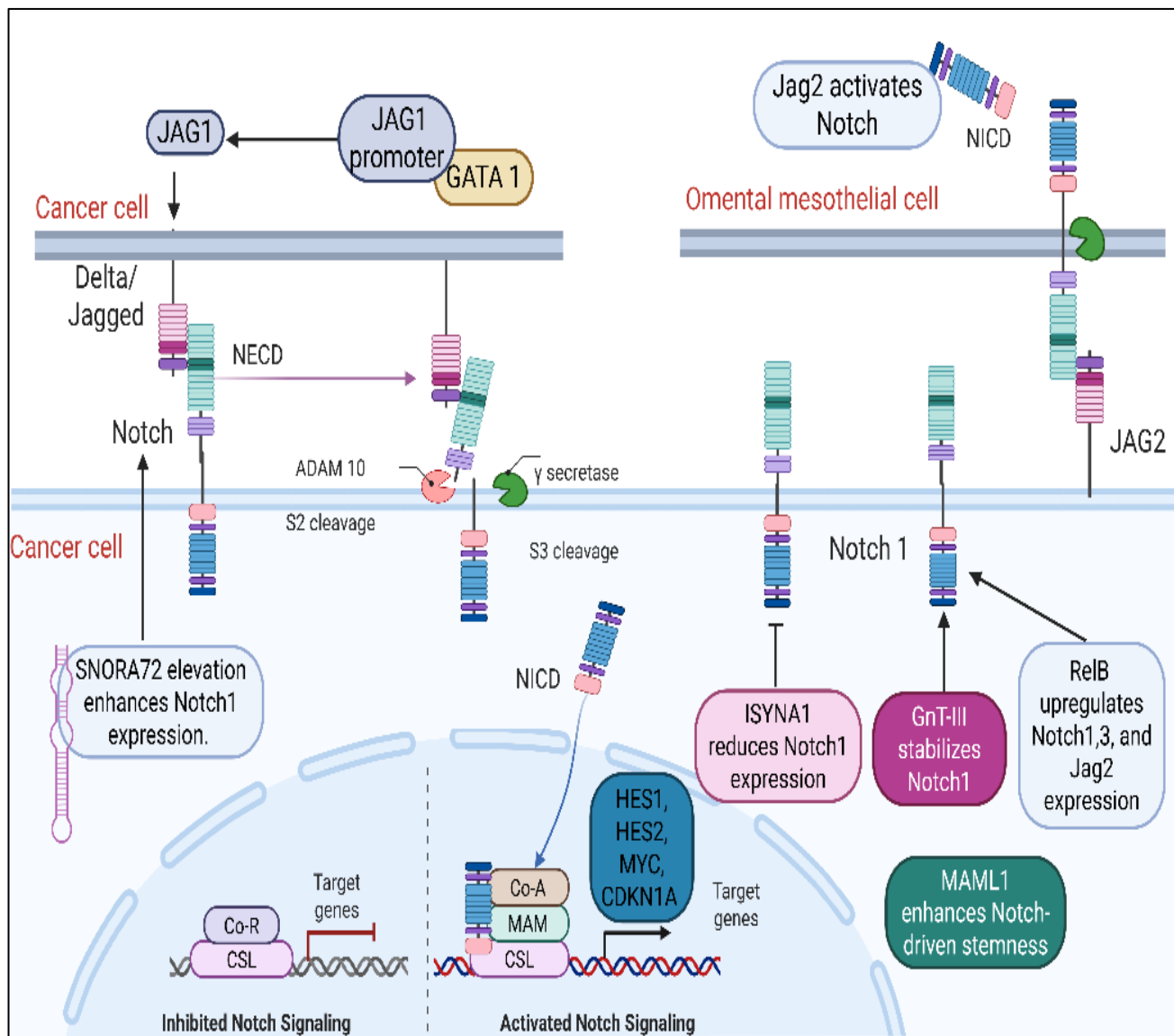
and transcriptional activity. Both mechanisms enhance Wnt/ $\beta$ -catenin signaling, thereby supporting the maintenance of CSC stemness and promoting resistance to cisplatin<sup>[22,23]</sup>. Tumors that are tolerant to platinum have more ALDH (+) cells, more stem cell-related transcription factors, and high levels of Wnt receptor FZD7. Activation of FZD7 triggers  $\beta$ -catenin signaling, which upregulates the tumor protein p63 and enhances the expression of the cytoprotective enzyme GPX4, and protects cells from oxidative damage during chemotherapy<sup>[24]</sup>. Moreover, the inhibition of GPX4 increases drug sensitivity. Similarly, LGR6 increases Wnt/ $\beta$ -catenin signaling and promotes the acquisition of CSC-like features and chemoresistance. Silencing *LGR6* attenuates this signal; however, reactivation of  $\beta$ -catenin through *S33Y* mutation maintains the aggressive

phenotype even when *LGR6* is knocked down<sup>[25]</sup>. Taken together, these studies demonstrate that various upstream regulators act convergently on the effector pathways to promote OC initiation, progression, and chemoresistance in tumors, highlighting the therapeutic potential of targeting these pathways in ovarian tumorigenesis. OC spreads transcoelomically into the peritoneal cavity, a process facilitated by the absence of physical compartmental barriers. By increasing vascular permeability and obstructing lymphatic vessels, OC cells cause ascites accumulation. Malignant ascites not only enables tumor spread but also shapes the TME by providing tumor-promoting pro-inflammatory cytokines and immune modulators<sup>[26]</sup>. OCSCs release Wnt ligands to the TME, which stimulate immune polarization toward an M2-like phenotype. These immune cells increase *Wnt5B* gene expression, which promotes the release of Wnt ligands that further increase ALDH activity in OCSCs. However, the process triggers a positive feedback loop that sustains chemoresistance and cellular invasion. Of note, the inhibition of *Wnt5B* in macrophages has been linked to Wnt signaling suppression in OCSCs<sup>[8]</sup>.

### Notch signaling

Studies have previously shown that Notch signaling plays a role in maintaining CSC characteristics while also contributing to proliferation, differentiation, angiogenesis, metastasis, and immune evasion<sup>[27]</sup>. The mammalian Notch signaling has four receptors on the cell membrane: Notch1–4 and five ligands, which are JAG1 and 2 and DLL1, 3, and 4. When the ligands bind, the  $\gamma$ -secretase enzyme mediates the cleavage of NICD, where it enters the nucleus and binds CBF1, the suppressor of hairless lag-1. This complex subsequently recruits coactivators MAML and the E1A-binding protein p300, and then activates target genes such as *HES*, *MYC*, *HER2*, *NF- $\kappa$ B*, and *CCND1*<sup>[28,29]</sup>. Various triggers and sustained activation of the Notch pathway can promote OC progression (Fig. 2). Notch-mediated signaling pathways also alter the characteristics of OCSCs, including differentiation, stem-like maintenance, and survival dynamics. Differential expression of Notch-related genes in OC correlates with poor outcomes. Specifically, high levels of *Notch2*, *Notch3*, and *ADAM17* predict reduced OS, and *Notch2*, *Notch3*, *MAML1*, and *ADAM17* indicate worse disease-free survival<sup>[30]</sup>. *SNORA72* is a small nucleolar RNA that is overexpressed in OCSC-like OC spheres. Functional studies have demonstrated that the stemness of OCSCs is, in part, attributed to *SNORA72*, which activates the Notch1/c-Myc signaling pathway<sup>[31]</sup>. Also, increased *JAG1* expression, transcriptionally regulated

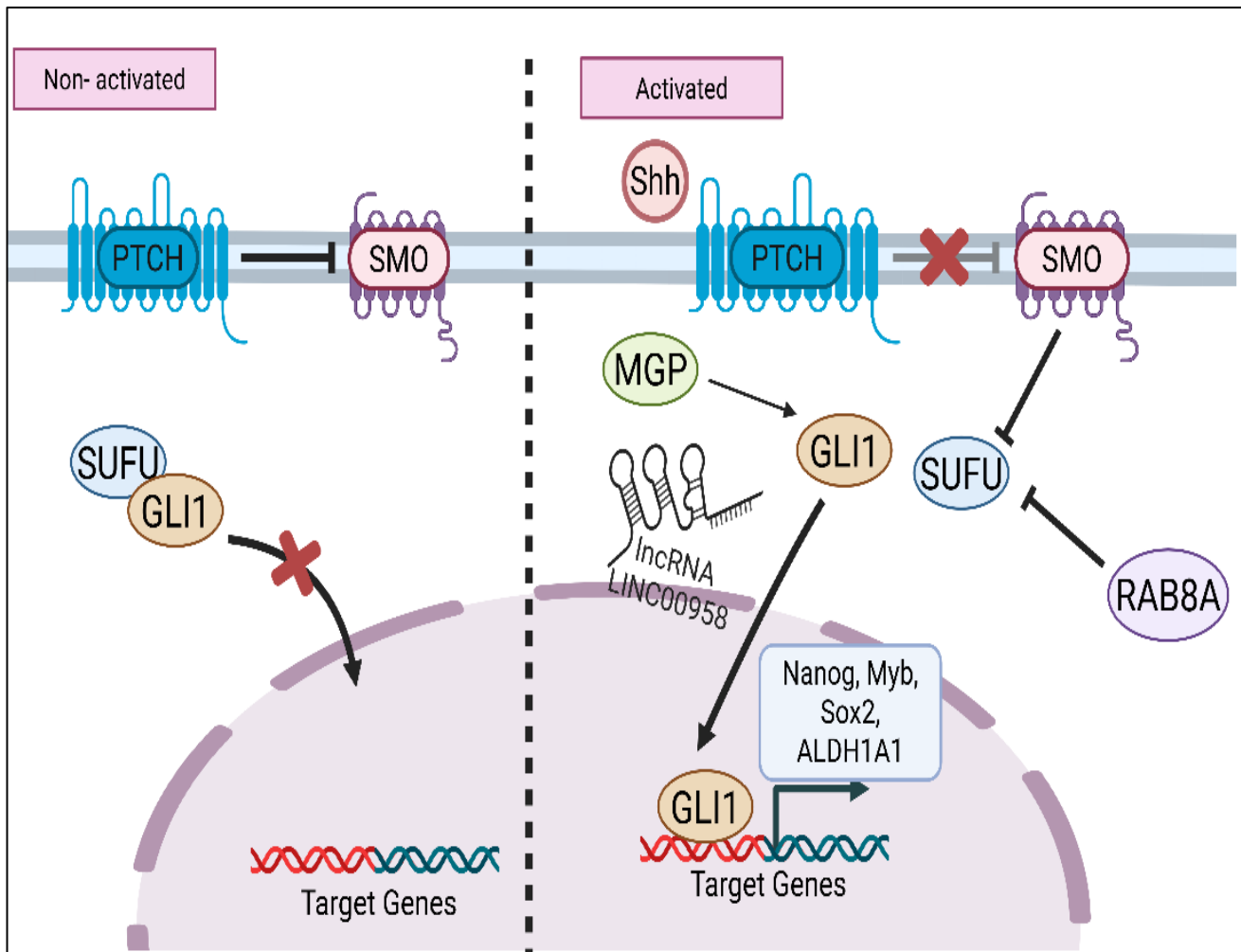
by GATA1, enhances Notch signaling and sustains the OCSC phenotype. However, *JAG1* knockdown reduces Notch activation and diminishes stem-like features, indicating its role in cancer progression and chemoresistance<sup>[32]</sup>. The downstream factor MAML1 has been identified as a component that connects. Higher expression levels of *MAML1* in CD44<sup>+</sup> OCSCs prevent the cells from differentiation and self-renewal by upregulating critical Notch targets such as *HES1*, *HEY1*, and *HEY2*. MAML1 also promotes the EMT by upregulating Snail, Slug, Twist1, fibronectin, and Zeb2 or downregulating regulatory miRNAs such as miR-200c and miR-34a<sup>[33]</sup>. Collectively, the above findings demonstrate that MAML1 drives the association of Notch signaling, stemness, and EMT in OCSCs, which are likely involved in chemoresistance, invasion, and metastasis in OC. Previous studies have elucidated the modulatory influence of enzymatic regulators on OCSCs<sup>[34,35]</sup>. *ISYNA*, an enzyme that regulates myo-inositol biosynthesis, acts as an inhibitory modulator of OCSC phenotypes. Its decreased expression is associated with poor prognosis and enhanced stem-like potential, as indicated by elevated ALDH activity in CD44<sup>+</sup>/CD117<sup>+</sup> subpopulations. Deficiencies in *ISYNA1* increase the stemness and malignancy of OCSCs by reducing suppression of Notch1 signaling. Conversely, *ISYNA1* ectopic expression decreases proliferative potential, migratory capacity, invasiveness, and stem-like characteristics<sup>[34]</sup>. On the other hand, the glycosyl-transferase enzyme GnT-III, which is overexpressed in EOC, promotes OCSC expansion by activating the Notch signaling pathway through aberrant glycosylation. GnT-III inhibition has been shown to suppress Notch signaling more potently than  $\gamma$ -secretase inhibition by redistributing receptors to lysosomes<sup>[35]</sup>. Recent evidence suggests that OCSCs demonstrate enhanced Notch signaling via Notch1, Notch3, and their ligand JAG2, which facilitates progression and metastasis. JAG2, produced by tumor cells, activates Notch signaling in the omental mesothelium and facilitates the formation and amplification of metastatic tumors in the omentum. Ablation experiments have indicated that the omentum resection is associated with decreased tumor engraftment. JAG2-induced Notch signaling corresponds with heightened CSC properties in omental metastases and fosters proliferation, spherogenesis, and expansion, as well as relative chemoresistance at secondary sites. Moreover, within ovarian CSC subsets, the conventional ligands DLL1 and DLL3 are diminished, whereas downstream mediators like *CDKN1A* are elevated, signifying persistent Notch signaling. This pathway is simultaneously enhanced by the atypical NF- $\kappa$ B



**Fig. 2.** Notch signaling in OCSCs (created by Biorender). Multiple regulatory axes converge on Notch signaling in OCSCs. SNORA72 upregulates Notch1. However, ISYNA1, an enzyme involved in myo-inositol biosynthesis, suppresses Notch1, attenuating pathway activity. GnT-III stabilizes Notch1 at the plasma membrane via bisecting glycosylation. The transcription factor GATA1 binds the JAG1 promoter, driving JAG1 expression to activate Notch signaling in neighboring cells. Tumor-derived JAG2 interacts with Notch on omental mesothelial cells, promoting colonization and metastatic outgrowth. The non-canonical NF- $\kappa$ B component RelB enhances the expression of Notch1, Notch3, and JAG2, sustaining CSCs' survival and enabling tumor relapse post-chemotherapy. MAML1 sustains self-renewal in OCSCs through the activation of Notch targets *Hes1*, *Hey1*, and *Hey2*. It also promotes EMT via the upregulation of Snail, Slug, Twist1, Fibronectin, and Zeb2, and through the modulation of miR-200c/miR-34a.

component RelB, which increases the expression of Notch1, Notch3, and JAG2, thus maintaining the persistence of CSCs and promoting tumor re-formation following chemotherapy<sup>[36,37]</sup>. Using combined Notch-targeted therapies is likely to help overcome resistance; however, the lack of specificity will continue to be a problem, as the normal functions of Notch are essential for the preservation of tissue homeostasis. Conversely,

GANT61, LDE225, and GDC0449, which are all CSC-targeted inhibitors are effective in reducing CSC populations, motility, migration, and clonogenic proliferation. The exosomal lncRNA, LINC00958, by forming a Hedgehog signaling complex with GLI1, promotes OCSC maturation, M2 macrophage polarization, and tumor progression.

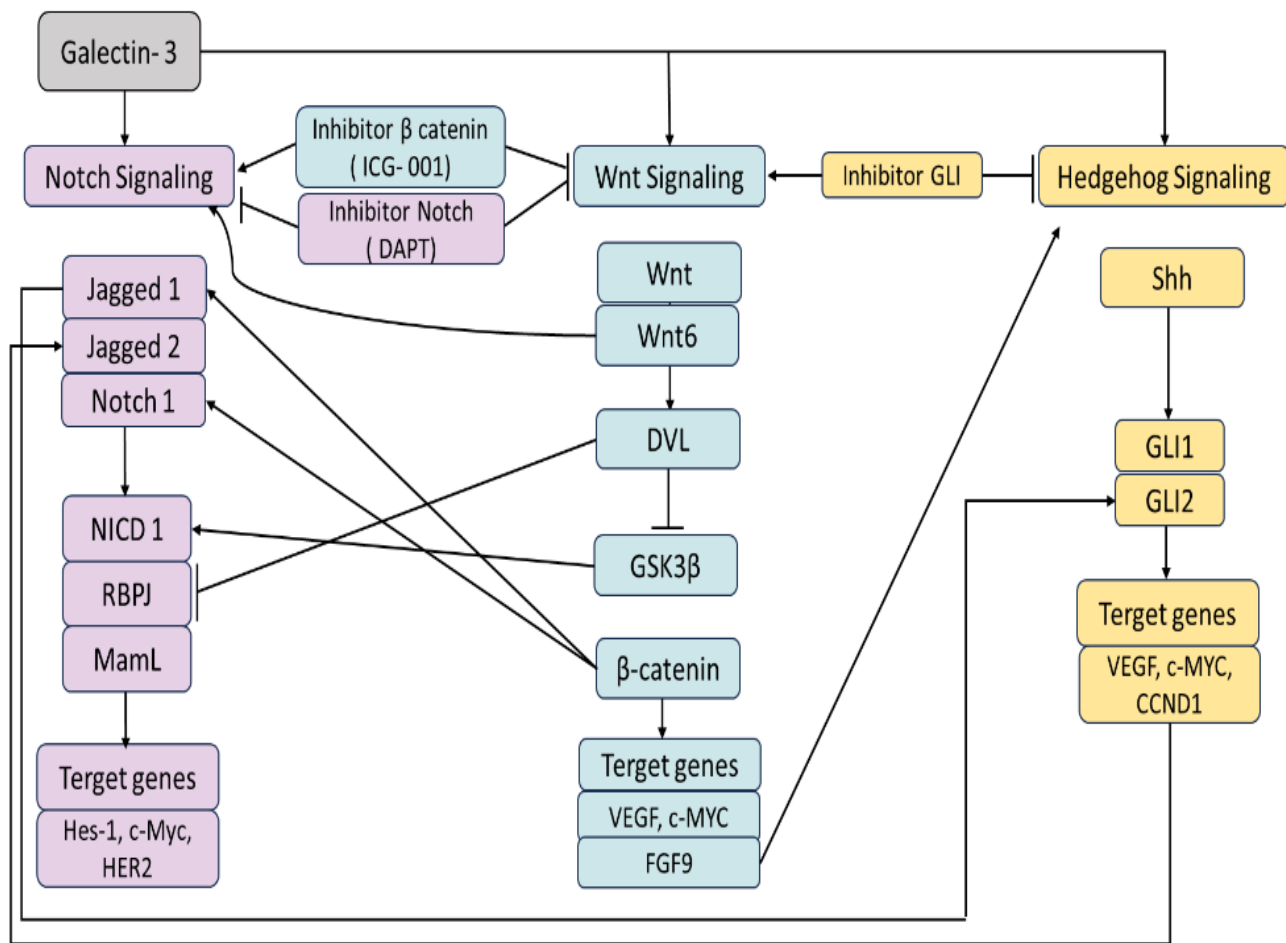


**Fig. 3.** Hedgehog signaling pathway in OCSCs (created by Biorender). SHH, IHH, or DHH ligands bind to PTCH1, relieving SMO inhibition and releasing SUFU from GLI1 and GLI2, allowing GLI (GLI1, GLI2, and GLI3) to enter the nucleus and activate target genes such as *Myc*, *Sox2*, and *Nanog*. This pathway also upregulates OCSC markers ALDH1A1 and *Nanog*. Additionally, MGP induced by peritoneal interactions enhances *Nanog* expression via GLI1. The exosomal lncRNA LINC00958 binds to GLI, promoting OCSC maturation, M2 macrophage polarization, and tumor progression, while the GPR137–RAB8A axis disrupts the GLI–SUFU complex, releasing GLI1 and reinforcing the aggressive cancer cell phenotype.

### Hedgehog signaling

The Hedgehog–GLI signaling pathway regulates embryogenesis, cell proliferation, lineage specification, and stemness maintenance (Fig. 3). In mammals, this pathway is regulated by three ligands: SHH, IHH, and DHH. In the absence of a ligand, PTCH1, a transmembrane protein, prevents SMO from interacting with the primary cilium. As a result, GLI2 and GLI3 act as transcriptional repressors, leading to the suppression of target gene expression. Ligand binding relieves this repression by activating GLI transcription factors, which in turn activate the expression of several target genes, including *Myc*, *Sox2*, and *Nanog*. GLI1 functions as a pathway amplifier. For normal development,

precise spatiotemporal control is essential; however, its inappropriate reactivation plays a part in tumorigenesis<sup>[38]</sup>. For OC, particularly HGSC, CSCs are maintained via aberrant Hedgehog–GLI signaling. Hedgehog signaling activation through SHH stimulates GLI1 transcription, which leads to the increased expression of ALDH1A1 and the formation of CSC spheroids. Conversely, GANT61, LDE225, and GDC0449, which are all CSC-targeted inhibitors, are effective in reducing CSC population, motility, migration, and clonogenic proliferation<sup>[39]</sup>. The exosomal lncRNA, LINC00958, promotes OCSC maturation, M2 macrophage polarization, and tumor progression by forming a Hedgehog signaling complex



**Fig. 4.** Signaling crosstalk of Notch, Wnt, and Hedgehog pathways in ovarian tumor development (created by Biorender). Galectin-3 activates all three pathways: Wnt/ $\beta$ -catenin, Notch, and Hedgehog.  $\beta$ -catenin stabilizes Notch1, while GSK3 $\beta$  phosphorylates NICD1 to activate the pathway and NICD2 to inhibit it. Wnt inhibition via ICG-001 activates Notch, while Notch inhibition using DAPT reduces  $\beta$ -catenin levels. WNT6 activates  $\beta$ -catenin and increases JAG1 expression, promoting proliferation and stemness. Additionally, FGF9, a target of Wnt signaling, also activates Hedgehog signaling. Inhibition of Hedgehog via cyclopamine or GLI inhibitor upregulates Wnt genes. Crosstalk between Hedgehog and Notch signaling occurs through the upregulation of JAG2 by Hedgehog signaling, while silencing JAG1 suppresses the expression of GLI2, a key mediator in Hedgehog signaling.

with GLI1<sup>[40]</sup>. A mechanism through which exosomal lncRNAs influence the TME. Hedgehog signaling also influences the prognosis of OC. Analysis of a patient cohort indicated that the overexpression of *SHH*, *PTCH1*, *PTCH2*, and *GLI1*, markers of active Hedgehog signaling, was associated with better PFS and OS. In contrast, higher levels of *GLI3* and *SUFU* were associated with worse outcomes, especially in serous EOC<sup>[41]</sup>. The Hedgehog–GLI–Nanog signaling pathway is involved in preserving stemness and chemoresistance in OC. When the Hedgehog signaling pathway becomes hyperactive, GLI1 and GLI2 exert enhanced transcriptional control, leading to the upregulation of stemness markers, including *Nanog*. Consequently, the stemness-associated signaling pathway perpetuates the CSC-like phenotype and aids in cisplatin resistance<sup>[42]</sup>.

MGP also promotes OCSC characteristics through the activation of the Hedgehog–GLI1–Nanog pathway. Through peritoneal interactions, MGP is upregulated and promotes the proliferation, invasion, and adhesion of OC cells<sup>[43]</sup>. Targeting this signaling axis may help suppress OCSC-driven relapses. Moreover, *GPR137* is reported to be highly expressed in OC tissues. *RAB8A*, a downstream target of *GPR137*, is upregulated by *GPR137* through mRNA stabilization. *RAB8A* then promotes OC progression through the Hedgehog regulatory axis by interfering with the GLI–SuFu complex, creating a positive feedback loop between Hedgehog and *GPR137* activity and establishing a self-reinforcing loop that maintains the aggressive characteristics of cancer cells<sup>[44]</sup>. Together with Wnt and Notch, dysregulated Hedgehog signaling contributes to

the maintenance of OCSCs and is implicated in therapy resistance, immune tolerance, and angiogenesis<sup>[7]</sup>. Future therapies must address pathway crosstalk, which may require combinatorial inhibition approaches and personalized treatment strategies.

### Signaling crosstalk of Notch, Wnt, and Hedgehog pathways in ovarian tumor development

Crosstalk between Notch, Wnt/ $\beta$ -catenin, and Hedgehog pathways sustains cancer cell stemness, proliferation, migration, and therapy resistance (Fig. 4). Rather than acting in isolation, these pathways interact dynamically with each other, with effects varying according to tumor context.

#### Wnt and Notch signaling

$\beta$ -catenin stabilizes Notch1 by preventing its ubiquitination, while GSK3 $\beta$  differentially phosphorylates NICD1 (activating) and NICD2 (inhibiting). DVL physically interacts with RBPJ, the primary transcription factor involved in Notch signaling, resulting in diminished activity of transcriptional RBPJ. This interaction exemplifies a complex, bidirectional regulatory relationship between the two pathways, which is crucial for the retention of stem-like traits and the proliferative potential of tumor cells<sup>[45]</sup>. Bocchicchio et al. have shown how OC cells grow and spread through the interplay of these pathways<sup>[46]</sup>. Wnt inhibition via ICG-001 activates the Notch pathway, and DAPT-driven Notch inhibition reduces  $\beta$ -catenin levels. However, the lack of an additive suppression effect because of dual inhibition suggests a one-way compensatory system, in which Notch compensates for the loss of Wnt signaling. In contrast, Wnt does not compensate for Notch inhibition. Other work has highlighted the role of Wnt6 in this pathway. Wnt6 is overexpressed in ovarian malignancies and promotes tumor progression through  $\beta$ -catenin, which is linked to poor outcomes. Interestingly, the suppression of Wnt6 and  $\beta$ -catenin expression also results in a significant reduction in *Notch1* expression, suggesting a functional linkage between the Wnt/ $\beta$ -catenin and Notch signaling networks<sup>[47]</sup>. In one of the earlier studies using in vitro and in vivo models of OC, Chen et al. demonstrated that Notch3 and  $\beta$ -catenin cooperate to regulate *JAG1* levels, leading to increased growth<sup>[48]</sup>. Knockdown of either component reduced *JAG1* levels, and this effect was more pronounced in the combined knockdown. Moreover, galectin-3, through the promotion of NICD1 processing and *Hes1*, *Hey1*, and Hedgehog/Wnt target genes, activates all three pathways: Notch, Wnt, and Hedgehog, thus enhancing OCSC stemness and therapeutic resistance<sup>[49]</sup>. The interaction of Wnt and

Notch pathways has also been documented in other cancers. For instance, in renal CSCs, autocrine Wnt activation (e.g., WNT10A) triggers downstream Notch signaling<sup>[50]</sup>. A comparable interaction in OC may support CSC maintenance, self-renewal, and EMT, suggesting a Wnt-driven activation of Notch signaling.

#### Wnt and Hedgehog signaling

Although the Wnt-Hedgehog interaction has been explored in the context of OC proliferation and differentiation, existing studies are scarce. *FGF9*, a target of the Wnt pathway, also activates the Hedgehog pathway, and in some analyzed tumors, both pathways are co-activated in serous OC<sup>[51]</sup>. Inhibition of the Hedgehog pathway with cyclopamine or GLI inhibitors in SKOV3 and IGROV cells increased expression of Wnt pathway genes *AXIN2* and *FGF9*, with the most pronounced effect observed in IGROV, indicating a compensatory activation of Wnt signaling<sup>[52]</sup>. Wang et al. examined another cancer type, oral squamous cell carcinoma, and showed that the Wnt and Hedgehog pathways were also aberrantly bidirectionally regulated<sup>[53]</sup>. In this case, hyperactivation of the Wnt/ $\beta$ -catenin pathway, driven by unregulated Gli2-mediated Hedgehog signaling, facilitates EMT, enhancing the proliferation, invasion, and metastatic spread of cancer cells to distant organs. This is the first indication of the potential role of the interaction between Wnt and Hedgehog pathways in the tumorigenesis across multiple cancer types. Definite conclusions about the mechanisms and their potential for targeted therapy remain elusive due to the currently limited data.

#### Hedgehog and Notch signaling

There is crosstalk between Notch and Hedgehog signaling. These pathways are essential for stem cell maintenance and cancer development. In cancer, the Hedgehog pathway activation induces Notch signaling through JAG2 ligand expression<sup>[54]</sup>. In this regard, Chang et al. have studied the expression and mutations of 72 Notch and Hedgehog pathway genes across 21 cancer types (18,484 patients), focusing on 13 key genes<sup>[55]</sup>. With this approach, the study integrated disparate genomic, transcriptomic, and clinical datasets to define tumor types with hyperactive Notch-Hedgehog signaling. These tumor types exhibited clinically significant stemness and hyperactivity during tumor progression. The combined influence of hypoxia and mortality. In OC, the crosstalk between Notch and Hedgehog remains incompletely understood. However, an early study by Steg et al. has demonstrated that *JAG1* silencing reduced the viability of OC cells and resistance to taxane treatments, partly through the suppression of

**Table 1.** Summary of treatments modulating OCSC signaling pathways in ovarian cancer

Signaling pathway	Therapeutic agents	Mechanism of action	Clinical phase	Preclinical/clinical outcomes	Ref.
Wnt/ β-catenin	5-Aza	Alleviating the methylation on the <i>sFRP</i> gene promoter and reinstating the expression of <i>sFRP</i> mRNA, particularly <i>sFRP4</i>	Preclinical	Inhibition of OCSCs' proliferation and survival	[67]
	Ginsenoside-Rb1	Reducing the transcriptional regulation and expression of target genes, such as <i>ABCG2</i> and <i>P-gp</i>	Preclinical	Suppression of the regenerative capacity and increased sensitivity to cisplatin and paclitaxel	[68]
	TF3	Inhibiting the expression of <i>β-catenin</i> , <i>LEF-1</i> , <i>c-Myc</i> , and <i>CCND1</i> in ALDH <sup>+</sup> cells	Preclinical	Inhibition of ovarian CSC growth	[69]
	Trimebutine maleate	Dual inhibition of ion channels, namely VGCC and BKCa, thus reducing the activity of the Wnt/β-catenin pathway	Preclinical	Reduced proliferation, decreased stemness, and induction of cell death in OCSCs	[70]
	Inhibitor GPX4	Increasing the sensitivity of FZD7(+) cells to ferroptosis	Preclinical	Targeting platinum-tolerant OC cells (FZD7 <sup>+</sup> ) via GPX4 inhibition-induced ferroptosis	[24]
	Ipafricept	Binding to Wnt ligands	Phase 1B	Improved response to taxane-platinum combination therapy, with limitations due to bone toxicity	[71]
	WNT974	Inhibiting the porcupine enzyme, which was involved in the process of Wnt ligand secretion	Preclinical	Reduction in tumor size and ascites volume, with increased CD8 <sup>+</sup> T-cell infiltration.	[72]
Notch	Pyruvium	Increasing axin	Preclinical	Induction of apoptosis, inhibition of cell proliferation, and enhanced chemosensitivity	[73]
	γ-secretase inhibitor (RO4929097)	Blocking the activation of the NICD by inhibiting its release	Phase II	Limited efficacy of RO4929097 as monotherapy in platinum-resistant OC	[74]
	γ-secretase inhibitor (DAPT)	Reducing the levels of <i>JAG1</i> expression	Preclinical	Suppression of cell growth, induction of programmed cell death, and inhibition of tumor progression by γ-secretase inhibition	[75]
	Navicixizumab	Binding to DLL4	Phase 1B	Therapeutic response to paclitaxel-navicixizumab combination with manageable toxicity	[76]
	Tarextumab (OMP-59R5)	Attaching to Notch2 and Notch3	Phase 1B	Tarextumab tolerability at doses <2.5 mg/kg weekly and 7.5 mg/kg every 2–3 weeks, with manageable dose-limiting diarrhea and Notch pathway inhibition.	[77]
Hedgehog	Eugenol	Combining cisplatin and eugenol inhibits the Notch-Hes1 pathway	Preclinical	Reduction in the population of OCSCs resulted in increased sensitivity of cells to treatment and extended disease-free survival in animal models	[78]
	Sonidegib (LDE225)	Inhibiting SMO	Phase 1B	Acceptable tolerability of paclitaxel combination below the HDT, with limited clinical efficacy (6.7% partial response).	[79]
	Vismodegib	Reducing Hedgehog ligand and <i>GLII</i> expression	Preclinical	In vitro sensitivity with limited in vivo efficacy	[80]
	GANT61	Inhibiting <i>GLII</i> expression	Preclinical	<ul style="list-style-type: none"> <li>• Induction of genetic stress and DNA damage through <i>FANCD2</i> downregulation</li> <li>• Inhibition of tumor proliferation, reversal of cisplatin resistance, and reduced <i>Nanog</i> expression</li> <li>• Increased <i>ALDH1A1</i> expression following GANT61 treatment</li> </ul>	[39,42,81]

VGCC: voltage-gated calcium channels; 5-Aza: 5-azacytidine; *P-gp*: *P-glycoprotein*; TF3: theaflavin-3, 3'-digallate; BKCa: big potassium channels activated by calcium; NICD: notch intracellular domain; DLL4: delta-like ligand 4; ORR: overall response rate; EOC: epithelial ovarian cancer; HDT: highest dose tolerated;

*GLI2*<sup>[56]</sup>. More recent evidence further supports a functional interaction between these pathways in other malignancies. For instance, in colorectal cancer with *BRAF* mutations, activation of Hedgehog–GLI signaling maintains Notch1 activity. Altogether, these pathways trigger EMT, a key mechanism underlying tumor invasiveness and metastatic spread<sup>[57]</sup>.

Understanding and modulating key signaling pathways in ovarian cancer for therapeutic advancement. The last few years have seen considerable progress in understanding the Wnt/ $\beta$ -catenin, Notch, and Hedgehog signaling pathways, allowing the development of novel and more targeted therapies. Understanding these pathways is critical to maintain stemness, promote therapy resistance, and cause tumor recurrence and metastasis<sup>[58]</sup>. Thus, ongoing research is exploring how targeting these pathways might help overcome resistance, improve treatment outcomes, and prevent recurrence in OC. In November 2023, the FDA approved nirogacestat, a  $\gamma$ -secretase inhibitor of Notch signaling, for the treatment of desmoid tumors after the DeFi trial<sup>[59,60]</sup>. Based on evidence of efficacy in phase II/III studies, vismodegib and sonidegib, both SMO inhibitors targeting the Hedgehog pathway, have gained FDA approval for basal cell carcinoma<sup>[61,62]</sup>. Although not yet approved for OC, these inhibitors have shown potential to suppress OCSC growth in preclinical studies<sup>[11]</sup>. Table 1 below provides an overview of the main therapeutic agents that target OCSC signaling pathways. Targeting OCSC-related signaling pathways has shown potential in slowing tumor progression. However, limitations such as toxicity and limited sample sizes remain. Proposed strategies to address these challenges include targeted drug delivery systems, dose optimization, and biomarker-based patient stratification. Nanoparticle-based delivery approaches may further enhance therapeutic efficacy while reducing systemic adverse effects. The use of polymers, lipids, and metals for the production of nanoparticles improves not only the bioavailability of the chemotherapeutic agents but also the specificity of these agents<sup>[63]</sup>. Moreover, optimizing drug dosages based on the genetic differences among patients is also an essential step to reduce toxicity. For instance, cytochrome P450 2D6 plays a role in tamoxifen metabolism, while glucose-6-phosphate dehydrogenase and thiopurine S-methyltransferase affect responses to rasburicase and thiopurine, respectively<sup>[64]</sup>. The incorporation of molecular biomarkers into cancer treatment has been shown to increase therapeutic efficacy and decrease toxicity by selecting the most suitable therapy based on the biological features of the patient's tumor<sup>[65]</sup>. Additionally, to manage gastrointestinal side effects associated with some treatments, augmenting the

immune system with supplementation of glutamine and increased production of glutathione to mitigate toxicity has also been considered. Nutritional screening tools such as the Geriatric Nutritional Risk Index and Mini Nutritional Assessment can assist in identifying malnutrition or cachexia, enabling early intervention before toxicity worsens<sup>[66]</sup>. There are several limitations to evaluating the signaling pathways involved in OCSCs. First, many investigations have been performed mostly at the preclinical or early-phase clinical trial level. Second, small sample sizes limit the ability to draw definitive conclusions regarding the effectiveness and clinical relevance of therapies targeting these pathways. Third, human patients have a complex and diverse range of clinical comorbidities. Hence, results from a laboratory and an in vivo animal experiment do not linearly translate to human medical care. Although the potential signaling pathways Wnt/ $\beta$ -catenin, Notch, and Hedgehog are implicated in OCSCs' stem and resistance signaling, the mechanisms of their interactions are unclear. Furthermore, not all the interactions have been thoroughly explored, and clinical trials of OC drugs are rare. Thus, clinical data require suitable methods to enhance clinical practice.

## CONCLUSION

Considering the role of CSCs in the initiation, growth, metastasis, and resistance in the treatment of OC, they are probable targets for immunotherapy. The Wnt/ $\beta$ -catenin, Notch, and Hedgehog signaling pathways are involved in the regulation of stemness and are interconnected through complex regulatory interactions that sustain OCSC persistence. Agents such as nirogacestat ( $\gamma$ -secretase inhibitor), vismodegib and sonidegib (SMO inhibitors), and PRI-724 (Wnt/ $\beta$ -catenin blocker) have shown promise in preclinical studies for targeting these pathways. However, specific challenges persist, primarily because multiple signaling pathways can substitute for one another and undergo compensatory activation, which limits the effectiveness of monotherapy. The lack of clinical success appears due to the adoption of therapeutic lines that fail to integrate inhibitors in a rational, sequential, or combination manner. The main challenges for clinical applicability of the preclinical test include the safe application of these multi-targeted ideas and other factors such as dosing, tumor heterogeneity, and clinical grade. Moreover, focusing on the role of non-coding RNAs and the TME may provide new approaches to disrupt OCSC-driven progression, with the potential to improve therapeutic efficacy. Regarding clinical application, the proposed strategies need to undergo

clinical trials to assess their feasibility and effectiveness on human subjects. Overcoming the challenges, such as appropriate patient selection based on molecular and clinical tumor characteristics, the identification of reliable biomarkers to assess treatment response, and the safe clinical implementation of combination therapies, will be essential. Thus, the use of personalized medicine based on the proposed approaches of multiple targets is likely to provide sustained therapeutic benefits, improving the quality of life and the life expectancy of patients suffering from OC.

## DECLARATIONS

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### Ethical approval

Not applicable.

### Consent to participate

Not applicable.

### Consent for publication

All authors reviewed the results and approved the final version of the manuscript.

### Authors' contributions

DA: conceptualization, literature review, formal analysis, visualization, and writing—review & editing; FF: clinical interpretation, resources, and writing—review & editing; AA: conceptualization, supervision, project administration, and writing—review & editing.

### Data availability

All relevant data can be found within the manuscript.

### Competing interests

The authors declare that they have no competing interests.

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