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REVIEW

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A comprehensive overview of ovarian cancer stem cells: correlation with high recurrence rate, underlying mechanisms, and therapeutic opportunities

Hadi Alizadeh[1†], Parastoo Akbarabadi[1†], Alireza Dadfar[1], Mohammad Reza Tareh[1] and Bahram Soltani[1*]

Abstract

Ovarian cancer is one of the most lethal gynecological malignancies, with a recurrence rate of 70–80%, particularly in patients diagnosed at advanced stages (stage III or IV), where the five-year survival rate falls below 30%. A key driver of this recurrence is the presence of cancer stem cells (CSCs), which exhibit resistance to chemotherapy and possess the capacity for self-renewal, plasticity, and tumor regeneration. The tumor microenvironment (TME) plays a crucial role in maintaining ovarian cancer stem cells (OCSCs) by providing nutrient and oxygen gradients, extracellular matrix (ECM) interactions, immune cell modulation, and support from cancer-associated fibroblasts (CAFs). CAFs secrete growth factors, cytokines, and ECM components that create a pro-tumorigenic niche, promoting CSC maintenance, invasion, and chemoresistance. Additionally, dysregulation of critical signaling pathways, including WNT, NOTCH, PI3K/AKT/mTOR, TGF-β, JAK/STAT, Hedgehog, NF-κB, and Hippo, supports CSC stemness, plasticity, maintenance, and adaptability, thereby increasing their survival and progression. Numerous inhibitors targeting these pathways have shown promise in preclinical studies. This review discusses the molecular mechanisms underlying CSC-mediated recurrence in ovarian cancer and highlights emerging therapeutic strategies. Particular emphasis is placed on the potential of combination therapies involving routine platinum or taxane based regimens with OCSC inhibitors to overcome chemoresistance, reduce recurrence rates, and improve survival outcomes for patients with advanced-stage ovarian cancer.

Keywords Ovarian cancer, Cancer stem cells, Cancer-associated fibroblasts, Cancer stem cell inhibitors, Combination therapy

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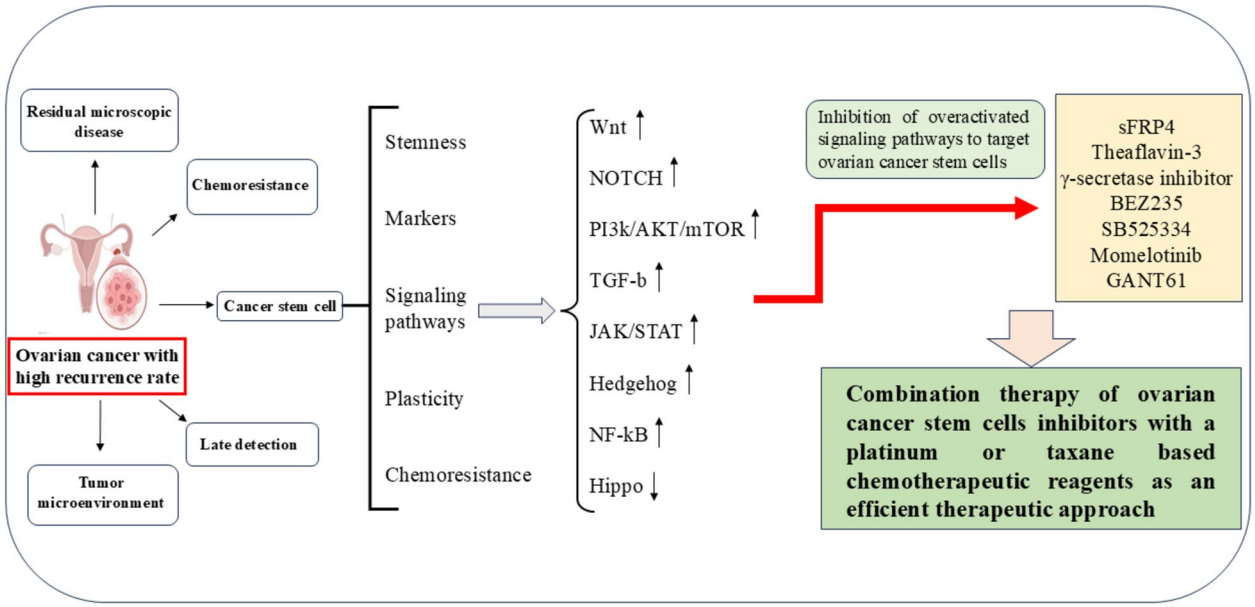
Graphical Abstract



Introduction

Ovarian cancer remains the most lethal gynecological malignancy worldwide, presenting a significant public health challenge. It is the eighth most common cancer among women, with over 210,000 new cases and approximately 200,000 deaths annually [1]. The overall 5-year survival rate is about 50%, but when detected at early stages, survival improves dramatically to over 90% [2, 3]. However, more than 70% of cases are diagnosed at advanced stages, largely due to nonspecific symptoms such as bloating, abdominal pain, and urinary changes, making early detection particularly challenging [4–6]. Despite advances in treatment, including surgery and platinum-based chemotherapy, ovarian cancer has a high recurrence rate, with over 80% of advanced-stage cases relapsing within two years [7].

Tumor relapse is driven by multiple interconnected factors, including chemoresistance, genetic mutations, and the TME. While BRCA1 DNA repair associated (*BRCA1*), BRCA2 DNA repair associated (*BRCA2*), and tumor protein p53 (*TP53*) mutations can enhance initial treatment responses, they also contribute to resistance through secondary mutations and enhanced DNA repair mechanisms [8, 9]. Additionally, the TME harbors immune-suppressive cells and inflammatory mediators, supporting cancer cell survival and metastasis even after therapy [10]. Microscopic residual disease and quiescent tumor cells further contribute to relapse, making effective treatment particularly difficult [11].

Among these factors, OCSCs are emerging as a key driver of recurrence. OCSCs represent a rare subpopulation of tumor cells with self-renewal capacity, chemotherapy resistance, and tumorigenic potential, allowing them to evade treatment and regenerate tumors [12]. Their survival is closely linked to the evolution of ovarian cancer, which progresses through genomic instability, clonal selection, and adaptation to environmental pressures. Early *TP53* mutations promote high-grade serous ovarian cancer (HGSOC) progression [8], while platinum-based chemotherapy exerts selective pressure, eliminating sensitive clones and enriching therapy-resistant populations. Over time, CSC-like populations marked by CD133 (*PROM1*) and aldehyde dehydrogenase 1 family member A1 (*ALDH1 A1*) become dominant, driving disease recurrence and drug resistance [13]. Clonal evolution in ovarian cancer is highly dynamic, with fewer than 2% of somatic mutations persisting at relapse, highlighting the tumor's adaptability. Acquired mutations in *BRCA1*, *BRCA2*, and tumor protein p53 binding protein 1 (*TP53BP1*) further enhance resistance to PARP inhibitors (PARPi) and platinum therapies, reinforcing CSC survival [14]. Additionally, epithelial-to-mesenchymal transition (EMT) in circulating tumor cells (CTCs) enhances

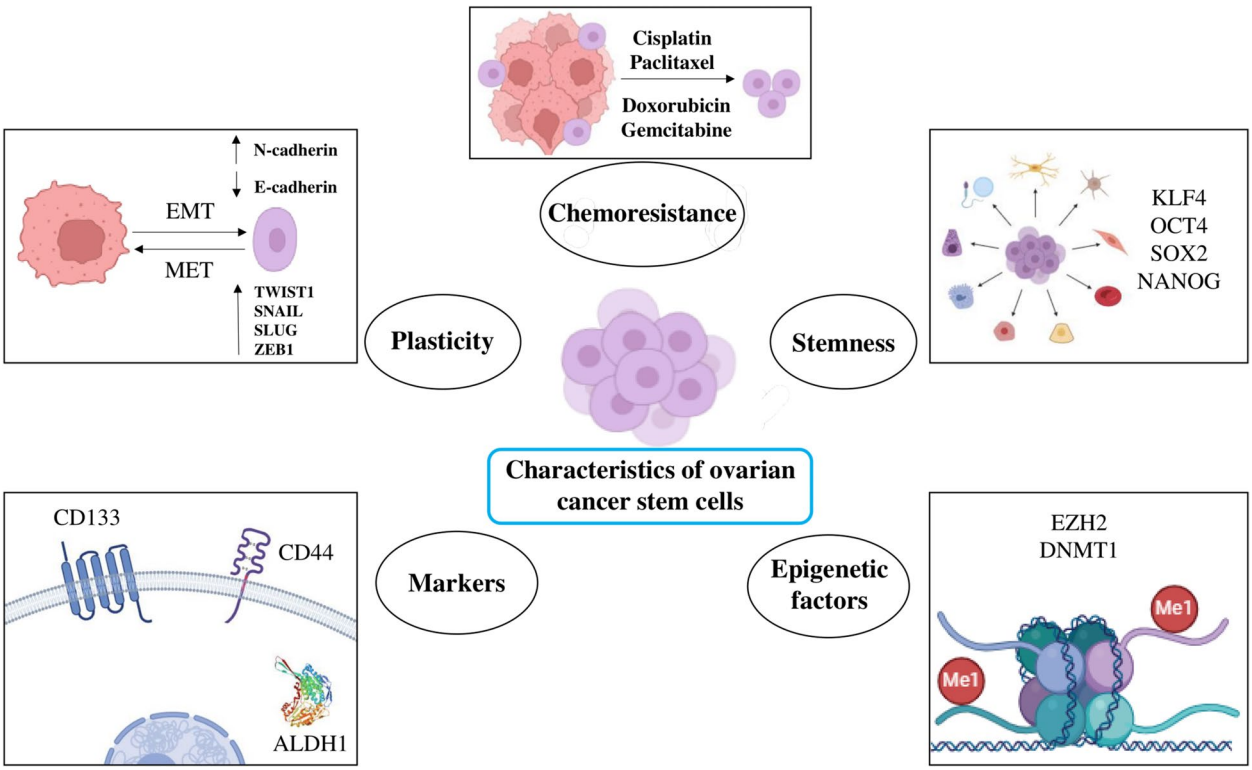


Fig. 1 Characteristics of ovarian cancer stem cells. This figure highlights the defining characteristics of ovarian cancer stem cells. One of the key features of these cells is chemoresistance, where they display resistance to common chemotherapies such as cisplatin, gemcitabine, doxorubicin, and paclitaxel. These cells also exhibit plasticity, which allows them to undergo EMT, and MET, processes that are marked by changes in cadherin expression, specifically N-cadherin and E-cadherin; and closely related to transcription factor such as TWIST1, SNAIL, SLUG, and ZEB1. Stemness, a crucial property of cancer stem cells, is regulated by a network of transcription factors including KLF4, OCT4, SOX2, and NANOG, which maintain their ability to self-renew and differentiate. Additionally, several markers such as CD133, CD44, and ALDH1 are commonly used to identify and isolate ovarian cancer stem cells. The role of epigenetic factors, particularly enzymes like EZH2 and DNMT1, is also significant, as they mediate modifications to the genome that promote stem cell properties and contribute to tumorigenesis. These characteristics underscore the nature of ovarian cancer stem cells and their potential role in tumor progression and recurrence

invasiveness and therapy resistance. Post-chemotherapy, EMT-like CTCs become more prevalent, demonstrating the tumor's ability to adapt. Notably, PI3 Kα/TWIST-expressing CTCs further support EMT's role in OCSC evolution, with emerging therapies targeting the PI3 K/AKT/mTOR pathway showing promise in overcoming this resistance [15] (Fig. 1).

Given the pivotal role of OCSCs in tumor relapse and drug resistance, targeting CSCs has become a critical focus in ovarian cancer research [16]. Advances in single-cell analysis and molecular profiling have identified CSC-specific markers, offering new therapeutic opportunities [17–19]. Understanding the biology and behavior of OCSCs will not only help develop novel targeted therapies but also enhance the efficacy of existing treatments, ultimately reducing recurrence rates and improving patient outcomes.

Characteristics of ovarian cancer stem cells

Origin and plasticity of OCSCs

OCSCs are key drivers of tumor initiation, progression, and recurrence, contributing to the marked heterogeneity observed in ovarian cancer. Tumor heterogeneity, defined by diverse cellular subpopulations with distinct functions, growth rates, and drug sensitivities, complicates treatment and fosters chemotherapy resistance. Clonal evolution, driven by genetic and epigenetic changes, further amplifies this complexity by enabling the selective expansion of aggressive subclones with stem-like properties. This dynamic process fuels tumor progression and relapse. Advancements in single-cell RNA sequencing (scRNA-seq) have transformed the study of ovarian cancer heterogeneity by providing transcriptomic insights at single-cell resolution. scRNA-seq analysis of HGSOC has identified key stemness-associated genes, including Thy-1 cell surface antigen (*THY1*),

epithelial cell adhesion molecule (*EPCAM*), and *CD44*, defining distinct OCSC subpopulations. The fallopian tube epithelium has been recognized as a primary origin site for OCSCs, particularly secretory epithelial cells and intermediate populations expressing RUNX family transcription factor 3 (*RUNX3*) and SRY-box transcription factor 17 (*SOX17*), which play a crucial role in neoplastic transformation. Additionally, scRNA-seq studies have identified a cellular communication network factor 1 (*CCN1*)-positive (CYR61^{+})"stress"subpopulation within primary ovarian tumors, which drives metastasis and therapy resistance. These relapse-initiating cells highlight the dynamic nature of tumor evolution. Integrative approaches combining bulk RNA-seq with scRNA-seq have further identified stemness-related genes, including lymphocyte cytosolic protein 2 (*LCP2*), Fc gamma receptor IIIa (*FCGR3 A*), collagen type I alpha 1 chain (*COL1 A1*), and mitochondrially encoded cytochrome b (*MT-CYB*), providing a molecular signature for OCSCs [20–23].

Lineage tracing is critical for understanding OCSC roles in tumor initiation, progression, and relapse. Evidence suggests that rare pluripotent stem cells, such as very small embryonic-like stem cells (VSELs) and germline-derived stem cells, reside within the ovarian surface epithelium (OSE) and ovarian cortex. These cells express key pluripotency markers such as POU class 5 homeobox 1 (*POU5 F1*) also known as *OCT4*, Nanog homeobox (*NANOG*), SRY-box transcription factor 2 (*SOX2*) and germline markers including DEAD-box helicase 4 (*DDX4*) also known as *VASA* and developmental pluripotency associated 3 (*DPPA3*), linking them to both normal ovarian stem cell function and tumorigenesis. The co-expression of CSC markers such as *CD44* and leucine rich repeat containing G protein-coupled receptor 5 (*LGR5*) with *VASA* suggests trans-differentiation or dedifferentiation processes contributing to CSC heterogeneity. Lineage tracing studies using immunofluorescence, genetic labeling, and single-cell transcriptomics have revealed the plasticity of OCSCs, demonstrating their ability to transition between progenitor and CSC states in response to microenvironmental cues. This supports the hypothesis that ovarian cancer may arise from aberrant reprogramming of resident ovarian stem cells, emphasizing the need for targeted strategies to disrupt OCSC-driven tumor progression and metastasis [24]. The debate over whether ovarian cancer follows a hierarchical/stem cell model or a stochastic model remains unresolved, especially in primary human epithelial tumors. One study employed a microfluidic single-cell culture system to analyze the differentiation potential of distinct ovarian cancer cell populations while reducing contamination issues associated with fluorescence-activated cell sorting (FACS). The study identified a branched differentiation hierarchy in ovarian cancer cells, though rare stochastic events were also observed, indicating some degree of plasticity. Additionally, bone morphogenetic protein 2 (*BMP2*) expression varies across ovarian cancer cell populations, playing a crucial role in CSC regulation. *BMP2* promotes CSC expansion while inhibiting the proliferation of more differentiated cells, potentially serving as a feedback mechanism for CSC maintenance. While *BMP2* suppresses cancer cell growth in vitro, it enhances tumor progression and chemoresistance in vivo, reinforcing the need for therapies that specifically target CSCs rather than overall tumor cell populations. *BMP2* expression is upregulated in ovarian cancer and correlates with poor prognosis, highlighting its complex role in tumorigenesis [25].

Recent advances in spatial transcriptomics have provided unprecedented insights into the early molecular changes occurring in serous tubal intraepithelial carcinoma (STIC), the precursor lesion of HGSOC. High-resolution gene expression mapping has revealed key stemness-associated pathways, including insulin-like growth factor (IGF) signaling and the epigenetic regulation of insulin like growth factor binding protein 2 (*IGFBP2*). Notably, *IGFBP2* upregulation in STICs appears to result from epigenetic relaxation, suggesting a mechanism by which precursor cells acquire stem-like properties that facilitate malignant transformation. These findings underscore the significance of epigenetically reprogrammed stem-like cells in the initiation and progression of HGSOC [26]. Recent single-cell transcriptomic studies have identified a distinct epithelial subpopulation, E0, that plays a pivotal role in platinum resistance in HGSOC. This subpopulation is significantly enriched in chemo-resistant tumors and exhibits high tumor purity, low immune infiltration, and activation of EMT, cellular stress responses, and stem cell differentiation pathways. These characteristics suggest that E0 cells possess stem-like properties that enable them to evade chemotherapy-induced cytotoxicity. CSCs, or OCSCs in this context, are a subset of tumor cells with the ability to self-renew, differentiate, and resist standard therapies, contributing to recurrence and treatment failure. Spatial transcriptomic analyses have further elucidated the tumor microenvironment's role in sustaining these resistant cells, demonstrating that E0 interacts with fibroblasts and endothelial cells via fibroblast growth factor (FGF), laminin, midkine (MK), secreted phosphoprotein 1 (SPP1), and semaphorin 3 (SEMA3) signaling, fostering an immunosuppressive and protective niche. A key molecular regulator of this resistant phenotype is tumor associated calcium signal transducer 2 (*TACSTD2*), a

highly expressed gene within E0, which has been linked to poor prognosis and increased platinum resistance. Mechanistically, *TACSTD2* enhances chemoresistance by modulating the Rap1/PI3 K/AKT pathway, promoting AKT phosphorylation and tumor cell survival. Functional studies have shown that *TACSTD2* knockout significantly reduces stemness features and restores cisplatin sensitivity, reinforcing its potential as a therapeutic target. These findings highlight the significance of spatial transcriptomic approaches in resolving tumor heterogeneity and underscore the central role of OCSCs in driving platinum resistance, providing a foundation for developing targeted strategies against chemo-resistant ovarian cancer [27].

The plasticity of OCSCs is closely tied to their ability to undergo EMT. EMT allows OCSCs to dynamically shift between stem-like and differentiated states in response to microenvironmental stressors such as hypoxia, nutrient deprivation, and immune pressure, helping them adapt to various challenges. This process is governed by critical signaling pathways like WNT/β-catenin, NOTCH, Hedgehog, and PI3 K/AKT, which promote CSC self-renewal and tumor heterogeneity. However, this plasticity is not entirely deterministic; rather, OCSCs exhibit random plasticity, where stochastic fluctuations in gene expression and signaling pathway activity drive unpredictable transitions between cellular states. During EMT, ovarian cancer acquires mesenchymal traits such as increased motility, invasiveness, and resistance to apoptosis, which contribute to metastasis and survival at distant sites. EMT is marked by downregulation of epithelial markers such as E-cadherin and upregulation of mesenchymal markers including N-cadherin, vimentin, with transcription factors like SNAIL, SLUG (SNAI2), zinc finger E-box binding homeobox 1 (ZEB1), and TWIST driving these changes. Additionally, the TME supports EMT through factors like hypoxia and immune evasion, reinforcing the mesenchymal phenotype and facilitating detachment, invasion, and survival as circulating tumor cells. At metastatic sites, OCSCs may revert to an epithelial state through MET, forming secondary tumors [28–30]. Importantly, random plasticity introduces a layer of unpredictability to this process, allowing OCSCs to escape strict regulatory control and adopt diverse phenotypic states independent of deterministic cues. This stochastic variability enhances their adaptability, enabling them to survive therapeutic pressure and seed metastases in heterogeneous microenvironments. The dynamic interplay between EMT, MET, and OCSC plasticity further reinforces tumor adaptability and therapy resistance [31–33].

Markers, related genes, and transcription factors of OCSCs

OCSCs are regulated by specific surface markers, genes, transcription factors, and epigenetic regulators that sustain their stem-like properties and contribute to tumor progression, metastasis, and chemoresistance.

Main OCSC markers

CD44, a transmembrane glycoprotein and receptor for hyaluronic acid, promotes tumorigenicity, migration, invasion, and resistance to chemotherapy through activation of pathways such as PI3 K/AKT [34, 35]. CD133 enhances tumor initiation, metastasis, and drug resistance by facilitating adhesion to metastatic niches, leading to disease recurrence and poor prognosis [36]. ALDH1, an intracellular aldehyde-metabolizing enzyme involved in detoxification and oxidative stress protection, is strongly associated with poor clinical outcomes due to its role in tumor aggressiveness and resistance to platinum-based chemotherapies [37, 38]. CD24, a glycosylphosphatidylinositol (GPI)-anchored glycoprotein, contributes to immune evasion and tumor progression by enhancing spheroid formation, stemness, and tumorigenicity [39, 40]. CD117 (*c-KIT),* a receptor tyrosine kinase, regulates stem cell maintenance and differentiation, with CD117-positive ovarian cancer cells exhibiting increased tumorigenicity, apoptosis resistance, and metastatic potential via activation of the MAPK and PI3 K/AKT pathways [41]. EpCAM is another crucial surface marker that facilitates cell adhesion, proliferation, and differentiation, contributing to tumor initiation and progression. Its overexpression is linked to higher metastatic potential and chemoresistance, making it a promising therapeutic target [42]. Similarly, C-X-C motif chemokine receptor 4 (CXCR4), a chemokine receptor, plays a pivotal role in tumor cell migration, invasion, and metastasis by interacting with the C-X-C motif chemokine ligand 12 (CXCL12) to promote cancer cell dissemination and therapy resistance [36]. Collectively, these markers and molecular regulators define the core characteristics of OCSCs and present potential therapeutic targets for overcoming ovarian cancer resistance and recurrence [43–45].

OCSC-related genes

LGR5, also known as G protein-coupled receptor 49 (GPR 49) is a critical stem cell marker in OCSCs, playing a dual role in tumor development and progression. It is enriched in fallopian tube secretory cells, supporting the theory that HGSC originates from *LGR5*$^{+}$ progenitors, while also contributing to stromal remodeling in low-grade serous carcinoma [46]. *LGR5* enhances ovarian cancer cell proliferation, tumorigenicity, and EMT via the NOTCH1 signaling pathway [47]. Its expression

in early-stage ovarian cancer and loss in advanced stages suggest a dynamic role in tumor initiation and progression [48].

THY1 also known as CD90, which is a GPI-anchored cell surface glycoprotein primarily involved in cell–cell and cell–matrix interactions, plays a crucial role in OCSCs, driving tumor recurrence and chemoresistance. High *THY1* expression is associated with reduced progression-free survival, particularly in patients with endometrioid ovarian cancer, where patients face a threefold higher recurrence risk. *THY1*-expressing CSCs exhibit enhanced proliferation, self-renewal, and resistance to platinum-based chemotherapy. Notably, *THY1* knockdown reduces these aggressive traits, highlighting its potential as a therapeutic target [49].

Activated leukocyte cell adhesion molecule (*ALCAM*) also known as CD166, plays a key role in maintaining OCSCs by promoting adhesion, migration, drug resistance, and tumorigenicity. CD166 enhances CSC-like properties through interactions with CD9, focal adhesion kinase (FAK) signaling, and activation of epidermal growth factor receptor (EGFR), AKT, and YAP pathways. Silencing *ALCAM* reduces stemness markers and impairs proliferation, adhesion, and chemoresistance [50].

Also, cisplatin treatment induces an increase in the CSC state in ovarian cancer, which correlates with the expression of CD49f (also known as Integrin alpha-6, encoded by the *ITGA6* gene). CD49f is a cell surface receptor involved in cell adhesion, migration, and interaction with the extracellular matrix. It plays a crucial role in maintaining stemness, self-renewal, and therapy resistance in cancer stem cells. In cisplatin-resistant ovarian cancer cells, CD49f expression effectively distinguishes populations with higher expression of CSC transcription factor and enhanced self-renewal capacity [51].

Important transcription factors in OCSCs

SOX2 plays a pivotal role in the formation and maintenance of OCSCs, driving tumor-initiating capacity, spheroid formation, and chemoresistance. As a master transcription factor, SOX2 regulates pluripotency, quiescence, and long-term self-renewal, which are key characteristics of CSCs. Compared to other stemness-associated factors like OCT4 and NANOG, SOX2 expression is more consistently elevated in 3D spheroid cultures and HGSOC cell lines, suggesting its stronger involvement in sustaining OCSC properties. Knockdown of SOX2 significantly impairs spheroid formation efficiency, reinforcing its essential role in CSC maintenance. Additionally, SOX2 is highly expressed in chemotherapy-treated cells and recurrent tumors, implicating it in tumor relapse. Through the downregulation of cell cycle regulators such as cyclin D1 and Cyclin-dependent kinase 4 (CDK4), SOX2 may promote a quiescent phenotype, enabling CSCs to evade cytotoxic treatments. Furthermore, its expression is enriched in a minority population of $CD117^+$ or $ALDH^+/CD133^+$ CSCs, highlighting its specificity as a marker for aggressive, relapse-prone ovarian cancer cells. In contrast to OCT4 and NANOG, SOX2 is significantly elevated in recurrent ovarian cancer based on TCGA datasets, underscoring its potential as a key driver of disease progression [52].

OCT4, a pivotal transcription factor in maintaining stemness, plays a crucial role in the EMT process in OCSCs, contributing to tumor progression and chemoresistance. As a member of the POU family, OCT4 is essential for the self-renewal and pluripotency of embryonic and cancer stem cells. In the context of ovarian cancer, its aberrant expression correlates with poor prognosis and aggressive tumor behavior. OCT4 facilitates EMT by downregulating epithelial markers like E-cadherin and upregulating mesenchymal markers such as N-cadherin, promoting cell motility, invasion, and metastatic potential. Additionally, OCT4 is intricately linked with the PI3K/AKT/mTOR signaling pathway, a key driver of tumorigenesis and resistance to therapy. Studies suggest that OCT4 interacts with phosphorylated AKT, enhancing pathway activation and further supporting the EMT phenotype [53].

NANOG, a key transcription factor in OCSCs, drives tumor progression by promoting EMT, enhancing migration, invasion, and chemoresistance. It interacts with the AMPK/mTOR pathway, where high NANOG and low phosphorylated AMPK levels correlate with poor prognosis. NANOG knockdown activates AMPK, inhibiting mTOR signaling and suppressing EMT [54]. Additionally, the androgen receptor signaling axis upregulates NANOG, sustaining OCSC self-renewal and tumorigenicity. CRISPR/Cas9-based tracking of NANOG-expressing OCSCs highlights its role in stemness maintenance [55].

KLF4, alongside SOX2, OCT4, and NANOG, plays a key role in maintaining cancer stemness in various cancers. Recently, KLF4 has been shown to increase cisplatin resistance through the activation of mTORC1, suggesting its involvement in chemotherapy resistance mechanisms [56].

FOXM1, FOXK2, and FOXP1 from Forkhead box (FOX) family of transcription factors play key roles in regulating OCSCs and driving tumor progression, chemoresistance, and metastasis, particularly in HGSOC. FOXM1 expression is induced by the peritoneal TME through FAK signaling upon OCSC adhesion to the peritoneal niche, promoting OCSC survival and metastatic potential. Inhibition of FOXM1 reduces peritoneal seeding and restores chemosensitivity to cisplatin

Table 1 Important markers, genes, and transcription factors of OCSCs

Category	Marker/Gene/ Transcription factor	Type	Function and effect	Detection Methods	Experimental models and samples	References
Main OCSC Markers	**CD44**	Transmembrane glycoprotein, receptor for hyaluronic acid	Promotes tumorigenicity, migration, invasion, and chemotherapy resistance via PI3 K/AKT pathway, enhancing OCSC properties and therapy resistance	Meta-analysis/IHC, FACS	Ovarian cancer tissue samples/Peripheral blood samples	[34, 35, 60]
	CD133 (PROM1)	Cell surface glycoprotein	Facilitates tumor initiation, metastasis, and drug resistance by aiding adhesion to metastatic niches, contributing to disease recurrence and poor prognosis	IHC, FACS, MACS	Human primary endometrial cancer	[36]
	ALDH1	Intracellular aldehyde-metabolizing enzyme	Participates in detoxification, oxidative stress protection, and chemoresistance, increasing tumor aggressiveness and resistance to platinum-based chemotherapy	ALDEFLUOR assay and FACS	A2780 cell line	[37, 38]
	CD24	GPI-anchored glycoprotein	Supports immune evasion, spheroid formation, and tumor progression, enhancing stemness and tumorigenicity	FACS	Human ovarian tumor tissue/CAOV3, OV-90, SKOV3 cell lines	[39, 40]
	CD117 (c-KIT)	Receptor tyrosine kinase	Regulates stem cell maintenance, apoptosis resistance, and metastasis via MAPK & PI3 K/AKT pathways, increasing tumorigenicity and metastatic potential	IHC	Resected tumor tissues from epithelial ovarian cancer	[41]
	EpCAM	Cell adhesion molecule	Facilitates cell adhesion, proliferation, and differentiation, increasing metastatic potential and chemoresistance	IHC and FACS	Ovarian cancer tissues/A2780, SKOV3, OVCA3, SW626, and ES-2 cell lines/ C57BL/6 mice	[42]
	CXCR4	Chemokine receptor	Promotes tumor cell migration, invasion, and metastasis via CXCL12 interaction, enhancing therapy resistance and cancer dissemination	IHC, FACS, and MACS	Human primary endometrial cancer	[36]
OCSC-Related Genes	**LGR5 (GPR49)**	G protein-coupled receptor	Enhances proliferation, tumorigenicity, and EMT via NOTCH1 pathway, supporting tumor initiation, progression, and stromal remodeling	IHC, ISH, and Western blotting	Benign and carcinoma ovarian samples/CaoV-3, NIH OVCAR-3, SNU-8, SNU-119 cell lines	[46–48]
	Thy-1 (CD90)	GPI-anchored glycoprotein	Drives tumor recurrence, self-renewal, and chemoresistance, correlating with poor prognosis and higher recurrence risk	FACS, qRT-PCR, Western blotting	high-grade serous ovarian cancer patients/A2780, TOV211D cell lines	[49]
	ALCAM (CD166)	Cell adhesion molecule	Maintains OCSC properties through CD9, FAK, EGFR, AKT, and YAP signaling, enhancing migration, adhesion, and drug resistance	FACS	tumor tissues of ovarian cancer patients/A2780 cell line/BALB/c-nu/nu (nude) mice	[50]
	CD49f (ITGA6)	Integrin, cell surface receptor	Involved in cell adhesion, migration, and ECM interaction, maintaining stemness, self-renewal, and cisplatin resistance	qPCR	A2780 cell line	[51]

Table 1 (continued)

Category	Marker/Gene/ Transcription factor	Type	Function and effect	Detection Methods	Experimental models and samples	References
Important Transcription Factor	**SOX2**	Transcription factor	Regulates pluripotency, quiescence, and self-renewal, driving spheroid formation, chemoresistance, and tumor initiation	FACS, qRT-PCR	ACI23 cell line/female athymic Nu/Nu mice	[52]
	OCT4 (POU5 F1)	Transcription factor	Facilitates EMT, invasion, and metastatic potential, correlating with poor prognosis and therapy resistance	IHC, Western blotting, qRT-PCR, Immunofluorescence assay	SKOV3, A2780, OVCA433, SKOV3-IP1, HEY, HEY A8, ES-2, and OVCA429 cell lines/BALB/c nude mice	[53]
	NANOG	Transcription factor	Drives EMT, migration, and invasion, enhancing tumor progression and chemoresista	IHC, Western blotting	417 samples of ovarian cancer tissues/ SKOV-3 and A2780 cell lines	[54, 55]
	KLF4	Transcription factor	Increases cisplatin resistance via mTORC1 activation, contributing to chemotherapy resistance	IHC, Western blotting	Ovarian cancer samples of 68 patients/A2780, SKOV3 cell lines/ BALB/c nude mice	[56]
	FOXM1	Transcription factor	Induced by peritoneal TME via FAK signaling, promoting metastasis and chemoresistance	FACS, Western blotting	HGSOC patients/TYK-nu cell line	[57]
	FOXK2	Transcription factor	Regulates UPR via IRE1α and activates oncogenic pathways, enhancing CSC survival and therapy resistance	FACS, Western blotting, IHC	Human HGSOC Tumors and Malignant Ascites/ OVCAR5, OVCAR3, CAOV3, OV90, COV362, Kuramochi, OVCAR4, OVCAR8 cell lines/Female nude mice	[58]
	FOXP1	Transcription factor	Upregulates stemness genes (OCT4, NANOG, SOX2, ABCG2), supporting proliferation, spheroid formation, and drug resistance	FACS, Western blotting, RT-PCR	A2780 and SKOV3 cell lines/BALB/c-nude mice	[59]

and paclitaxel, with the FOXM1 inhibitor Thiostrepton showing enhanced efficacy when combined with the PARP inhibitor Olaparib [57]. FOXK2 supports CSC survival by regulating the unfolded protein response (UPR) through endoplasmic reticulum to nucleus signaling 1 (*ERN1*), which encodes the UPR sensor (inositol-requiring enzyme 1 alpha) IRE1α, leading to increased splicing of X-box binding protein 1 (*XBP1*) and enhanced cellular adaptation to stress. FOXK2 also activates oncogenic pathways like PI3 K/AKT and WNT/β-catenin, driving CSC maintenance and chemoresistance [58]. FOXP1 enhances CSC-like properties, including spheroid formation, proliferation, and drug resistance, by upregulating stemness genes such as OCT4, NANOG, SOX2, and ATP binding cassette subfamily G member 2 (ABCG2), a drug efflux transporter that protects CSCs from chemotherapy. Targeting FOXM1, FOXK2, and FOXP1 presents a promising strategy to eliminate CSCs and improve treatment outcomes in ovarian cancer [59] (Table 1).

OCSCs and chemoresistance

CSCs play a crucial role in ovarian cancer chemoresistance, driving tumor recurrence and therapeutic failure. While chemotherapy reduces the bulk of cancer cells, a subpopulation of CSCs survives, leading to relapse. These cells exhibit distinct resistance mechanisms, including reduced apoptosis, increased drug efflux, and enhanced antioxidative defenses [61]. CSCs are also characterized by resistance to radiation due to their advanced DNA repair mechanisms, mediated by elevated MDM2 activity, a transcriptional target of p53 [62]. Additionally, a recent study suggests that CSCs share traits with drug-tolerant"persister"cells, such as elevated stemness factors, EMT genes, and antioxidant enzymes like glutathione peroxidase 4 (GPX4), which protect them from oxidative stress and ferroptosis [63]. Also, enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*) plays a pivotal role in OCSC chemoresistance by transcriptionally upregulating checkpoint kinase 1 (*CHK1*) expression, thereby enhancing CSC survival and tumorigenicity. Through direct binding to the *CHK1* promoter, *EZH2* promotes G2/M checkpoint maintenance and DNA damage repair, ultimately conferring resistance to cisplatin-induced apoptosis. Notably, CRISPR-mediated *EZH2* knockout significantly reduced *CHK1* expression, impairing CSC self-renewal, sphere formation, and chemoresistance, further highlighting the functional dependency of ovarian CSCs on the EZH2/CHK1 axis [64]. Further, it has been shown that translesion synthesis contributes to OCSC chemoresistance by enabling DNA polymerase eta (Pol η) to bypass cisplatin-induced DNA damage. The 3′-UTR of Pol η mRNA is targeted by *miR-93*, and reduced *miR-93* increases Pol η expression, enhancing OCSC survival [65].

TME of OCSCs

The survival and maintenance of OCSCs are tightly regulated by TME, a dynamic and complex network of stromal and immune cells, ECM components, and soluble factors. This specialized niche also plays a crucial role in promoting immune evasion. Among the key elements of the TME, hypoxia, tumor-associated macrophages (TAMs), and CAFs play central roles in sustaining OCSC properties and shaping the tumor's aggressive behavior.

Role of CAFs in TME of OCSCs

CAFs play a crucial role in ovarian cancer recurrence and chemoresistance by sustaining the OCSC population through WNT5a-mediated paracrine signaling. CAF-derived WNT5a enhances symmetric OCSC division, inhibits differentiation, and induces the dedifferentiation of bulk ovarian cancer cells, thereby promoting tumor plasticity. Notably, WNT5a expression is significantly higher in CAFs than in ovarian cancer cells, and its secretion is further upregulated by ovarian cancer cell interactions and carboplatin treatment, indicating a cancer-driven enrichment of WNT5a-expressing CAF subpopulations. Also, WNT5a activates the non-canonical ROR2/PKC/CREB1 pathway, which maintains OCSC properties [66]. In addition, platinum-based chemotherapy induces interleukin 6 (IL-6) signaling, which promotes OCSC enrichment through autocrine and paracrine mechanisms. CAFs in the tumor microenvironment secrete IL-6 in a paracrine manner, supporting the conversion of non-CSCs to CSCs. IL-6 signaling activates signal transducer and activator of transcription 3 (STAT3), leading to increased *ALDH1 A1* expression and the maintenance of OCSCs traits [67].

Role of TAMs in OCSCs

The interplay between TAMs and OCSCs play a crucial role in shaping the TME and driving chemoresistance. Emerging evidence highlights the IL-6/STAT3 signaling axis as a key mediator of this crosstalk, particularly through the activity of M2-polarized macrophages. M2 macrophages enhances OCSC maintenance within hetero-spheroids by secreting IL-6, which in turn activates STAT3 phosphorylation and promotes the enrichment of $ALDH^+$ CSC population. Also, pharmacological inhibition of this axis using Ruxolitinib, SC144, or Tocilizumab significantly attenuates $ALDH^+$ enrichment. Also, TAMs sustain OCSCs through WNT5B signaling. Macrophage-derived WNT5B enhances $ALDH^+$ CSC enrichment. Inhibiting WNT5B reduces OCSC maintenance. Additionally, WNT5B-driven WNT-β-catenin

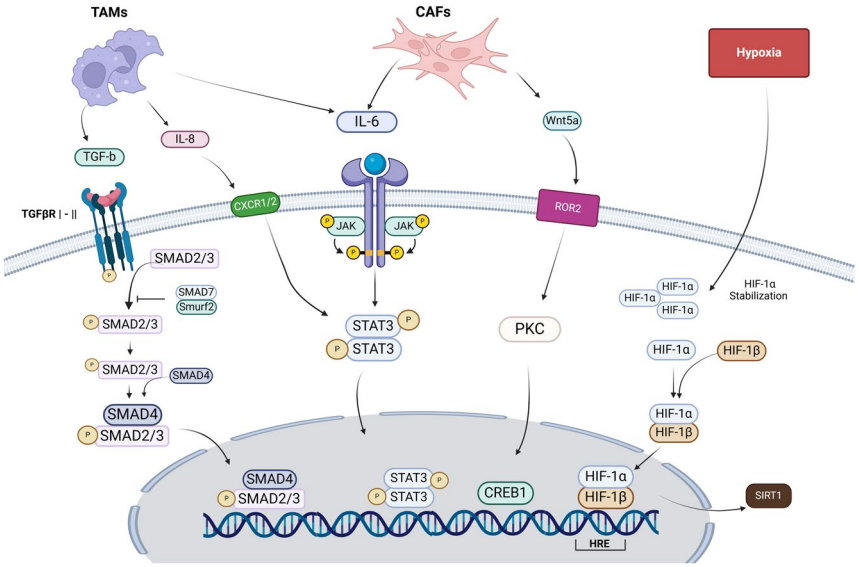


Fig. 2 Crosstalk between TME and intracellular signaling pathways involved in ovarian cancer stem cell maintenance. This schematic illustrates how the ovarian TME facilitates the maintenance and survival of OCSCs through multiple signaling axes. Hypoxia stabilizes HIF-1α, which is translocated to the nucleus and cooperates with HIF-1β to regulate HRE, promoting stemness and therapy resistance.bCAFs secrete IL-6 and WNT5a, activating JAK/STAT3 and ROR2/PKC pathways, respectively, which drive transcriptional programs supporting cancer cell stemness and survival. TAMs produce TGF-β and IL-8, activating SMAD2/3-SMAD4 signaling and enhancing STAT3 phosphorylation through CXCR1/2 activation. Collectively, these converging pathways sustain OCSCs by promoting plasticity, survival, and adaptation to the hostile tumor microenvironment

signaling fosters EMT, invasion, and immune evasion by excluding T-cells [68]. Also, the interaction and crosstalk between macrophages and ovarian cancer cells enhance stemness, driven by IL-8/STAT3 signaling. In SKOV3 cells, recombinant human IL-8 (rhIL-8) induces sphere and colony formation and increases CD133 and CD44 levels. Furthermore, macrophages derived from THP-1 cells polarize toward the M2 phenotype when co-cultured with ovarian cancer stem-like cells (OCSLCs), further promoting SKOV3 stemness through IL-8 secretion and STAT3 activation in macrophages. Additionally, macrophages secrete IL-10, vascular endothelial growth factor (VEGF), and matrix metalloproteinase-9 (MMP-9), fostering an immunosuppressive and tumor-supportive microenvironment, while decreased IL-12 and nitric oxide (NO) levels weaken anti-tumor immunity [69]. IL-8 exerts its effects by binding to CXCR1 and CXCR2, leading to STAT3 activation, which reinforces the pro-tumorigenic functions of macrophages and enhances cancer stemness [70]. Also, TGF-β, secreted by TAMs in the TME, plays a crucial role in increasing stemness and maintaining CSCs. This effect, observed in various cancers, suggests that further research is needed to explore its role in OCSCs [71].

Hypoxic effect on promoting OCSCs

Hypoxia plays a crucial role in the maintenance and progression of OCSCs by inducing the expression of hypoxia-inducible factor 1-alpha (HIF-1α). HIF-1α is a transcription factor that regulates cellular response to low oxygen levels and has been closely linked to tumor invasion, metastasis, poor prognosis, and resistance to therapy in ovarian cancer. Studies have shown that HIF-1α expression is more frequent in malignant ovarian tumors compared to benign ones, and is particularly elevated in patients with poor survival. In ovarian cancer stem cells, HIF-1α promotes CSC-like properties, including enhanced expression of CSC markers, increased chemoresistance, tumorigenesis, and EMT. Furthermore, HIF-1α regulates the expression of sirtuin 1 (*SIRT1*), a NAD^+-dependent histone deacetylase involved in cell proliferation, survival, and differentiation. SIRT1 expression is elevated under hypoxic conditions and is mediated by HIF-1α through the activation of the NF-κB signaling pathway. Silencing *SIRT1* reduces the CSC-like traits promoted by HIF-1α [72] (Fig. 2).

Immune evasion and immune checkpoints in OCSCs

Programmed death-ligand 1 (PD-L1) is a key regulator of immune evasion in ovarian cancer, driving tumor

progression, metastasis, and therapy resistance. Its expression is closely associated with the TME, particularly tumor-infiltrating lymphocytes (TILs) and CSCs, and it is frequently co-expressed with CSC markers such as CD44 and LGR5, suggesting a role in maintaining stem-like properties [73, 74]. Also, HIF-1α is often overexpressed in ovarian cancer and linked to poor prognosis, and induces CSC-like traits by upregulating stem cell markers in ovarian cancer [72]. It has been shown that under hypoxic conditions, both HIF-1α and HIF-2α stabilize and enhance PD-L1 levels in cancer cells and myeloid-derived suppressor cells (MDSCs), facilitating immune evasion through T-cell exhaustion. Targeting HIF-1α has been shown to downregulate PD-L1, restore TIL function, and promote cytotoxic T-cell infiltration [75]. Additionally, one study on endometrioid cancer stem cells revealed that hypoxia-induced HIF activation directly upregulates PD-L1 by binding to the hypoxia response element (HRE) within its promoter region, suggesting a similar mechanism may operate in OCSCs [76]. Moreover, targeting PD-L1 using CRISPR/Cas9 has shown promise in reversing immune suppression. A novel photoactivated CRISPR/Cas9 system enhances gene editing efficiency by using light to trigger nanoparticle degradation, delivering CRISPR plasmids to cells. This approach has demonstrated improved PD-L1 knockout in CSCs, potentially restoring immune function and eliminating cancer stem cells [77].

Expanding on these findings, OCSCs actively modulate the tumor microenvironment by upregulating immunosuppressive molecules such as C–C motif chemokine ligand 5 (CCL5), C-X-C motif chemokine ligand 2 (CXCL2), and indoleamine 2,3-dioxygenase 1 (IDO1), thereby evading immune surveillance. Moreover, OCSCs have been found to downregulate tumor-associated

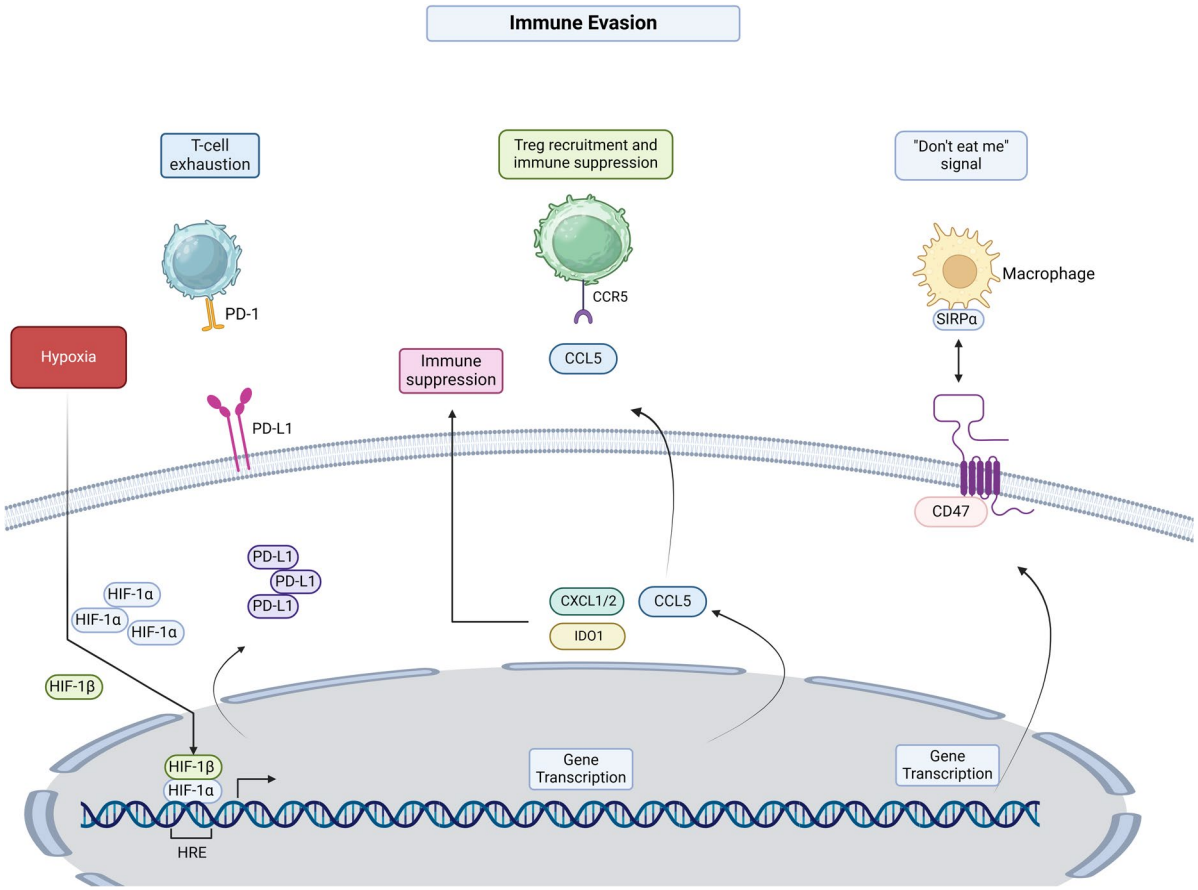


Fig. 3 Mechanisms of immune evasion driven by the OCSC TME. This figure illustrates the key pathways involved in immune evasion within the ovarian tumor microenvironment. Hypoxia stabilizes HIF-1α, which transcriptionally induces PD-L1 expression, leading to T-cell exhaustion through PD-1/PD-L1 interaction. Additionally, hypoxia and tumor-derived signals upregulate immune-suppressive factors such as CXCL1/2, CCL5, and IDO1, enhancing recruitment of regulatory T cells and promoting immunosuppressive gene programs. The CD47–SIRPα signaling axis acts as a "don't eat me" signal, inhibiting macrophage-mediated phagocytosis and further enabling tumor immune escape. Together, these pathways create an immunosuppressive niche that sustains immune evasion, OCSC maintenance, and tumor progression

antigens (TAA) and major histocompatibility complex (MHC) molecules, further reducing their recognition by immune cells. A particularly significant finding is the ability of OCSCs to recruit regulatory T cells (Tregs) via CCL5 expression, a chemokine implicated in cancer invasion and metastasis. Tregs, which express high levels of C–C motif chemokine receptor 5 (CCR5)—the receptor for CCL5—are preferentially recruited to the tumor microenvironment, where they contribute to immune suppression. This interaction fosters an immunosuppressive niche that promotes tumor progression by inhibiting T-cell responses and enhancing immune evasion. Additionally, OCSCs have been shown to increase IL-10 secretion in Tregs, reinforcing their immunosuppressive activity. In turn, Tregs further facilitate tumor invasion by inducing MMP9 expression, a protease associated with metastasis and tumor progression [78].

Besides PD-L1, several other immune checkpoints have been proven to play a role in immune evasion in various CSCs, though they have not yet been reported in ovarian CSCs. Cluster of Differentiation 47 (CD47) encodes a transmembrane protein that interacts with signal regulatory protein α (SIRPα) on macrophages, delivering a"don't eat me"signal that protects CSCs from phagocytosis, thereby promoting immune evasion. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a negative regulator of T cell activation, expressed on Tregs, and its interaction with B7 ligands on antigen-presenting cells suppresses anti-tumor immune responses, indirectly benefiting CSCs. T Cell Immunoglobulin and Mucin-Domain Containing-3 (TIM-3) is a checkpoint receptor expressed on exhausted T cells and innate immune cells, and its activation leads to T cell dysfunction, helping CSCs evade immune surveillance. Lymphocyte Activation Gene-3 (LAG-3) functions as an inhibitory receptor binding to MHC class II molecules, suppressing T cell proliferation and contributing to immune escape mechanisms in CSCs. These immune checkpoints, through their distinct mechanisms, reinforce CSC-mediated immunosuppression and tumor persistence, but their role in ovarian CSCs remain unexplored. Investigating these checkpoints in ovarian CSCs could provide valuable insights into tumor immune evasion mechanisms and offer new therapeutic targets for overcoming immune resistance [79] **(**Fig. 3**)**.

OCSCs metabolism

OCSCs exhibit a distinct metabolic phenotype that sets them apart from non-CSCs. Unlike differentiated tumor cells, CSCs prefer oxidative phosphorylation (OXPHOS) over glycolysis, as indicated by reduced pyruvate dehydrogenase kinase 1 (PDHK1) and Phospho-Pyruvate Dehydrogenase α1 (phospho-PDH) expression, suggesting an escape from the Warburg effect. Metabolomic analyses reveal that CSCs actively engage the tricarboxylic acid (TCA) cycle, with increased mitochondrial respiration, elevated reactive oxygen species (ROS) production, and a metabolic shift characterized by higher citric acid accumulation and reduced lactate production. High glucose uptake and pentose phosphate pathway (PPP) activity help maintain ROS homeostasis, supporting CSC survival and resistance to therapeutic stress. Also, 3D spheroid culture models confirm these metabolic adaptations, showing increased expression of stemness markers and a reliance on amino acid metabolism, particularly the serine and glutamine pathways. This highlights the metabolic heterogeneity of tumors and suggests that targeting CSC metabolism with OXPHOS inhibitors like metformin, which disrupts mitochondrial respiration, could enhance therapeutic efficacy [80, 81]. Additionally, metabolic reprogramming plays a crucial role in ovarian cancer chemoresistance by promoting CSC adaptation to the TME and enhancing their ability to withstand chemotherapy-induced stress. A key mechanism in this resistance is the enrichment of $ALDH^+$ CSCs following platinum-based treatment, which drives tumor relapse and recurrence. One study has demonstrated that OXPHOS and *SIRT1* contribute to this process, with platinum treatment increasing mitochondrial activity and *SIRT1* expression, thereby supporting CSC survival. Inhibiting OXPHOS using IACS-010759 effectively blocks the enrichment of $ALDH^+$ CSCs, suggesting a potential strategy to overcome platinum-resistant ovarian cancer [82]. A recent study has highlighted the pivotal role of peroxisome proliferator-activated receptors (PPARs), particularly PPARα, in the metabolic regulation of OCSCs. PPARα has emerged as a critical modulator of metabolic reprogramming in CSCs, enabling their survival under stressful conditions such as hypoxia and nutrient deprivation. The inhibition of PPARα has been shown to exert profound effects on the viability and metabolic function of OCSCs. Pharmacological inhibition using the antagonist GW6471, as well as gene silencing approaches, significantly reduced cell viability, spheroid formation, and mitochondrial function in spheroids derived from A2780 ovarian cancer cells. The metabolic shift from glycolysis to OXPHOS observed in CSCs are closely regulated by PPARα, which enhances fatty acid oxidation through the regulation of lipid metabolism genes. This metabolic adaptation ensures efficient ATP production under nutrient-limited and hypoxic conditions. PPARα inhibition resulted in a marked decrease in mitochondrial function, including basal respiration, maximal respiration, spare respiratory capacity, and ATP production. Furthermore, the disruption of PPARα activity led to reduced reliance on fatty acid, glucose, and

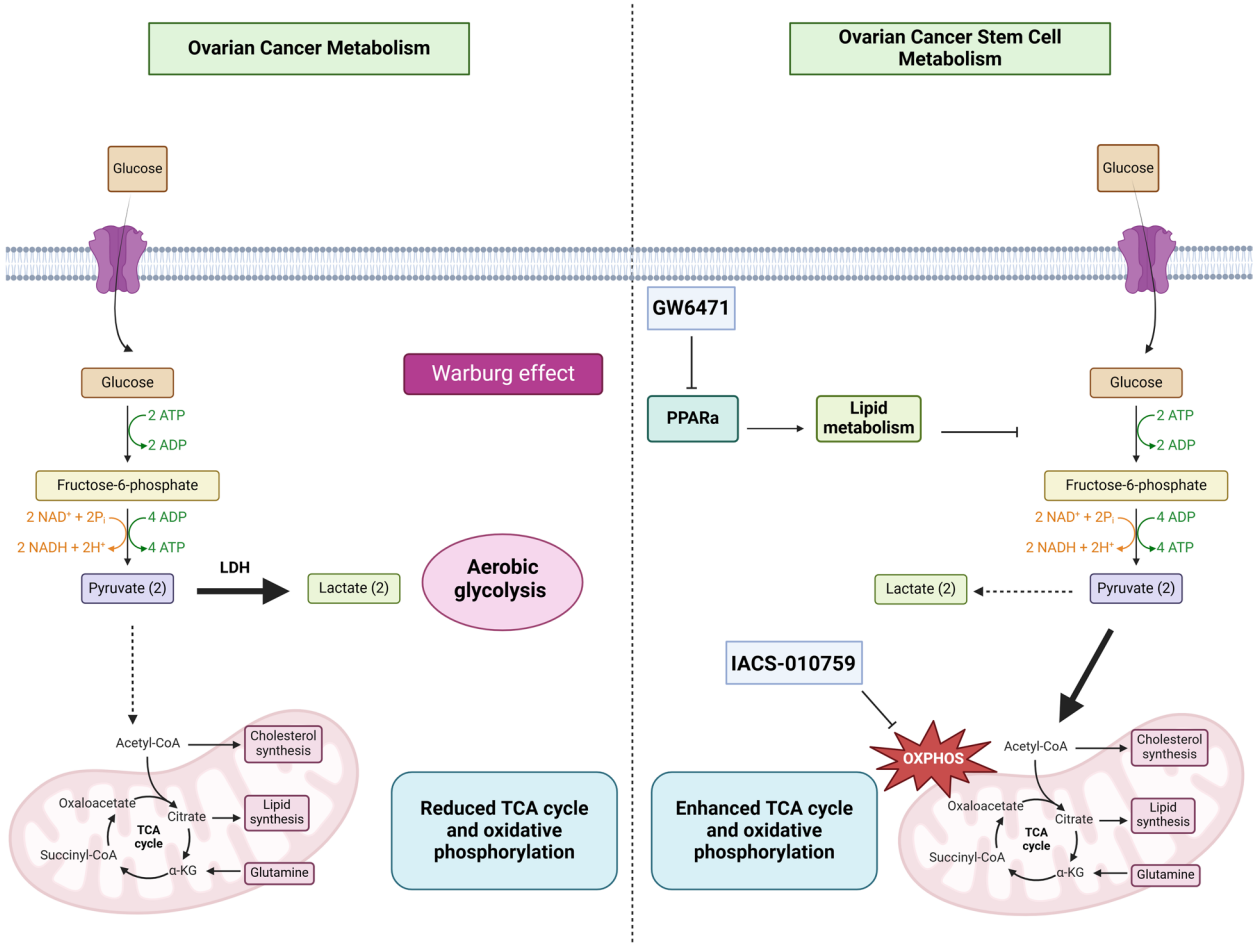


Fig. 4 Metabolic reprogramming of ovarian cancer stem cells promotes therapy resistance. OCSCs exhibit metabolic flexibility by shifting between glycolytic and oxidative phosphorylation pathways. On the left, ovarian cancer cells primarily rely on aerobic glycolysis (Warburg effect), converting pyruvate to lactate via LDH enzyme, leading to reduced TCA cycle and mitochondrial oxidative metabolism. In contrast, OCSCs, shown on the right, display enhanced mitochondrial function with increased TCA cycle and OXPHOS activities. This metabolic shift is partly regulated by PPARα-mediated lipid metabolism and is further influenced by mitochondrial pyruvate utilization. Pharmacological inhibition of these pathways using agents such as GW6471 and IACS-010759 impairs mitochondrial metabolism and may sensitize CSCs to chemotherapy. The metabolic plasticity of CSCs contributes to their survival under therapeutic stress and drives tumor recurrence

glutamine metabolism, driving the cells into a state of metabolic stress and ultimately inducing apoptosis. These findings underscore the central role of PPARα in maintaining CSCs metabolic plasticity and survival. Notably, high levels of PPARα expression have been correlated with poor prognosis and reduced survival rates in ovarian cancer patients, reinforcing the significance of PPARα as both a prognostic biomarker and a potential therapeutic target. These insights suggest that PPARα inhibition could serve as a promising strategy for improving ovarian cancer treatment outcomes, particularly in targeting CSC-driven tumor recurrence and resistance [83] (Fig. 4).

CRISPR Screening in OCSCs

CRISPR-based screening has proven valuable for studying OCSCs, enabling precise genetic modifications to investigate gene function. Several studies have employed CRISPR/Cas9 to explore the roles of specific genes in OCSCs. One study used CRISPR/Cas9-mediated knockout in ovarian cancer cell lines to demonstrate that suppressing DAB2 interacting protein (*DAB2IP*) gene sustains the malignant phenotype and stemness of OCSCs. In contrast, *DAB2IP* overexpression reduced stemness, reinforcing its role as a tumor suppressor. Similar findings in renal, prostate, and colorectal cancers suggest that *DAB2IP* acts as a CSCs suppressor [84].

Another study examined the relationship between androgen receptor signaling and NANOG in OCSCs, focusing on their impact on tumorigenicity and stemness. Researchers employed CRISPR/Cas9 to label NANOG with a GFP marker, ensuring precise expression tracking while avoiding issues associated with viral vectors. The findings revealed that androgen receptor signaling enhances NANOG expression, promoting cancer cell proliferation, migration, and stem-like differentiation. This suggests that the androgen receptor-NANOG axis plays a critical role in ovarian cancer progression. By leveraging CRISPR/Cas9 technology, the study established a stable and efficient model for investigating gene regulation in OCSCs [55]. Additionally, a study highlighted the inverse relationship between cancer stemness and immune checkpoint inhibitor (ICI) response in ovarian cancer. Using CRISPR-based cell line data and multi-omics analysis, researchers identified key stemness-related genes, with small nuclear ribonucleoprotein polypeptide E (SNRPE) emerging as a critical regulator of immune evasion. A Cancer Stemness Index (CSI) was developed, demonstrating superior accuracy in predicting ICI response across multiple datasets. CSI

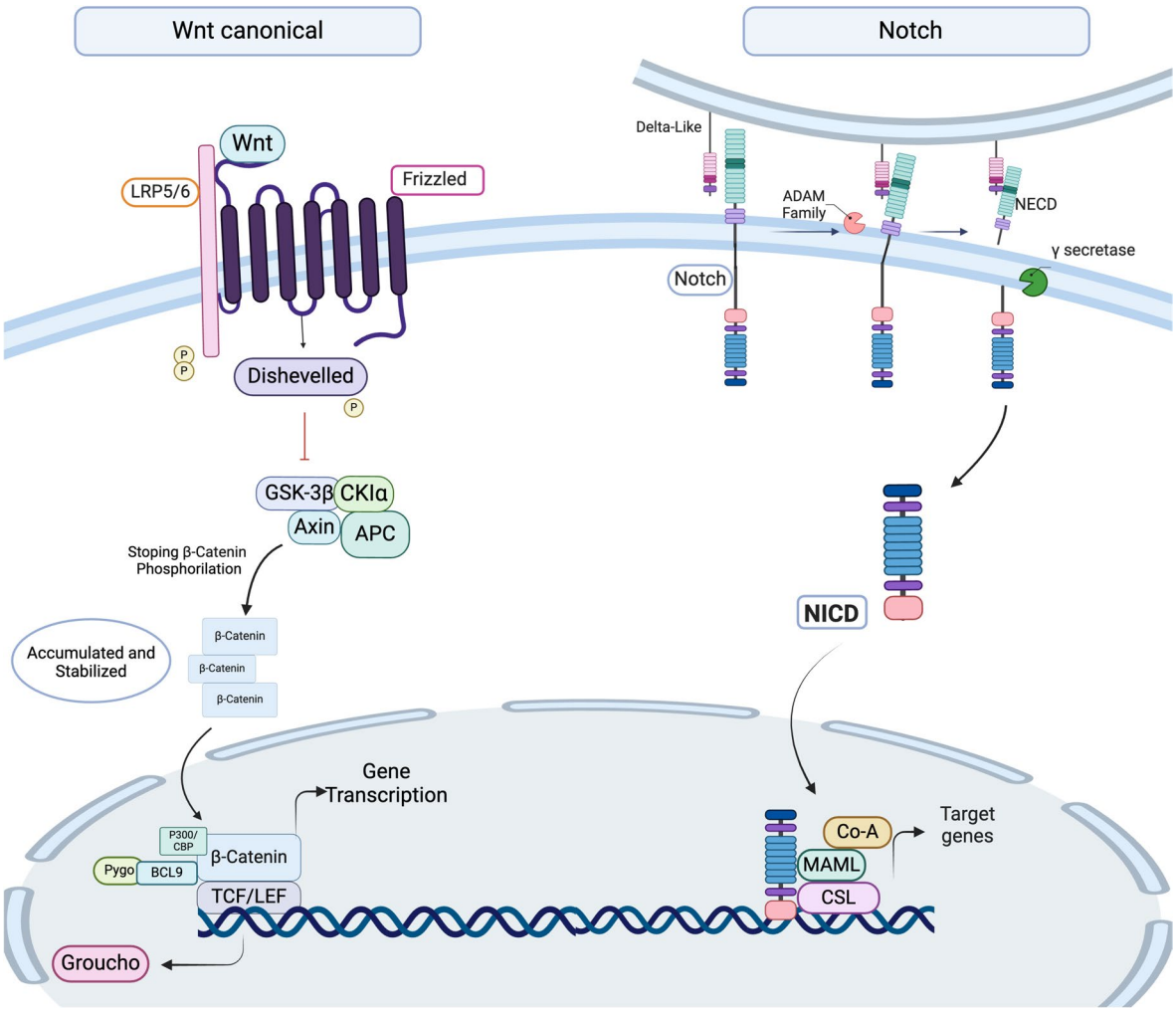


Fig. 5 Canonical WNT/β-catenin and NOTCH signaling pathways involved in ovarian cancer stem cell maintenance. This figure illustrates two pivotal signaling pathways that regulate OCSC self-renewal and survival. Left panel: The canonical WNT pathway is initiated by WNT ligand binding to Frizzled and LRP5/6 co-receptors, leading to Dishevelled activation and inhibition of the β-catenin destruction complex (GSK-3β, Axin, APC). Stabilized β-catenin translocates into the nucleus where it interacts with TCF/LEF transcription factors and co-activators such as CBP/p300 to drive stemness-associated gene expression. Right panel: The NOTCH pathway is activated through ligand (Delta-like) binding, leading to sequential cleavage by ADAM proteases and γ-secretase, releasing the NICD. NICD translocates to the nucleus and binds to CSL transcription factor and co-activators (MAML, Co-A) to regulate target genes involved in stemness and proliferation

outperformed existing models, underscoring its potential as a biomarker for immunotherapy outcomes [85].

Key signaling pathways in ovarian cancer stem cells

Signaling pathways play a critical role in maintaining CSC properties. In OCSCs, key pathways such as WNT/β-catenin, NOTCH, PI3 K/AKT/mTOR, TGF-β, JAK/STAT, Hedgehog, NF-κB, and Hippo are frequently dysregulated.

WNT/β-Catenin Pathway

The WNT/β-catenin pathway regulates OCSCs by promoting proliferation, self-renewal, survival, and chemoresistance. Its aberrant activation enhances plasticity, allowing transitions between quiescent and proliferative states. Additionally, WNT signaling interacts with other pathways, further driving tumor progression and therapy resistance. Also, TAMs enhance OCSC stemness, chemoresistance, and invasiveness via WNT5B-induced IL-6 secretion [68, 86]. Additionally, environmental pollutants bisphenol A (BPA) and polychlorinated biphenyls 126,153 (PCB126, PCB153) activate WNT/β-catenin signaling in ovarian cancer cell line, increasing sphere formation and stemness markers SOX2, NANOG, and OCT4 [87]. Further, WNT/β-catenin inhibitors such as ginsenoside Ginsenoside-Rb1 and its metabolite compound K (IC50: 125 nM) suppress OCSC sphere formation and reduce resistance mediated by ABCG2 and P-glycoprotein [88]. Moreover, non-canonical WNT signaling, particularly WNT5a, plays a crucial role in OCSC maintenance. CAFs secrete WNT5a, activating the ROR2/PKC/CREB1 pathway, which promotes symmetric OCSC division and chemoresistance [66]. Additionally, *LINC00115* sponges *miR-30a*, upregulating *SOX9* and WNT/β-catenin activity, which enhances stemness properties. Silencing *LINC00115* reduces stemness markers and promotes OCSC apoptosis [89]. Collectively, these findings emphasize the central role of WNT/β-catenin signaling in OCSC regulation, chemoresistance, and tumor progression.

NOTCH Pathway

The NOTCH signaling pathway is critical in regulating OCSC self-renewal, differentiation, and survival. Dysregulation of NOTCH receptors (e.g., NOTCH1 and NOTCH3) and ligands (Jagged and Delta-like) enhances chemoresistance, tumor progression, and poor prognosis. NOTCH activation, particularly through Hes1 upregulation, enriches OCSC population and promotes resistance to platinum-based chemotherapy [90]. Hypoxia further activates NOTCH signaling via HIF-1α, enhancing OCSC characteristics through the NOTCH1-SOX2 axis. Pharmacological inhibition of NOTCH with γ-secretase inhibitors reduces stemness, depletes OCSC population, and sensitizes cells to platinum therapies [91]. Also, glycosylation mediated by GnT-III regulates NOTCH intracellular domain (NICD) production and downstream NOTCH signaling. Suppression of GnT-III enhances the efficacy of γ-secretase inhibitors, which block NICD generation [92]. Additionally, the NOTCH1-c-MYC axis, amplified by small nucleolar RNA, H/ACA box 72 (SNORA72), promotes OCSC metastasis and chemoresistance. Targeting SNORA72 or glycosylation, along with NOTCH inhibition, offers potential therapeutic strategies for overcoming chemoresistance in ovarian cancer [93] (Fig. 5).

PI3 K/AKT/mTOR Pathway

The PI3 K/AKT/mTOR signaling pathway is a central regulator of cellular survival, growth, and metabolism in OCSCs. Its hyperactivation drives CSC self-renewal, proliferation, and resistance to apoptosis, contributing to tumor progression and chemoresistance. The pathway plays a pivotal role in EMT, characterized by decreased E-cadherin, increased N-cadherin, and upregulation of EMT transcription factors, promoting invasiveness [94]. Additionally, in HGSC, gamma-glutamylcyclotransferase gene (GGCT) enhances PI3 K/AKT/mTOR activation, driving EMT and CSC-related traits, while silencing GGCT suppresses these processes [95]. Also, it has been shown that inhibition of the PI3 K/AKT/mTOR pathway disrupts OCSC proliferation, migration, angiogenesis, and mitochondrial bioenergetics, while also impairing tumor-stromal interactions critical for OCSC survival. Targeting this pathway sensitizes platinum-sensitive and resistant ovarian cancer cells to cisplatin [96–98].

TGF-β Pathway

TGF-β signaling plays a crucial role in ovarian cancer by regulating EMT, MET, and CSC traits. In ovarian cancer spheroids, TGF-β promotes EMT during spheroid formation and MET upon reattachment, enhancing cell motility, invasion, and metastasis [99]. TGF-β signaling also maintains CSC traits and chemoresistance through activation of paired like homeodomain 2 A/B (PITX2 A/B) isoforms via SMAD-dependent and non-SMAD pathways, which upregulate the drug resistance transporter ATP binding cassette subfamily B member 1 (ABCB1) [100]. Additionally, TGF-β1 triggers EMT, driving epithelial cell dedifferentiation into stem-like mesenchymal cells with increased tumorigenic potential. The TGF-β1-ERK1/2 axis induces key stemness markers such as ZEB1, SOX2, and NANOG while enhancing ABC transporter

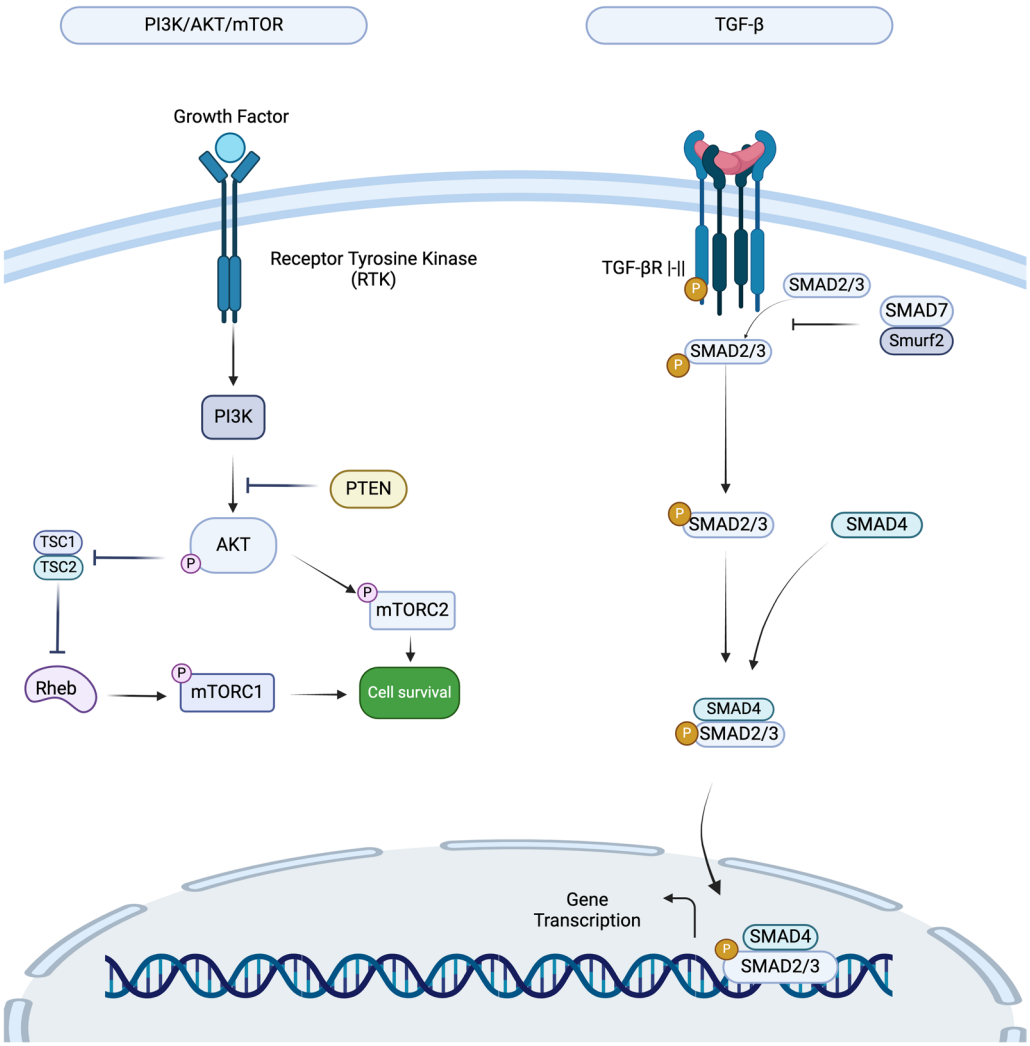


Fig. 6 PI3 K/AKT/mTOR and TGF-β/SMAD signaling pathways contributing to ovarian cancer stem cell maintenance and survival. The left panel illustrates the PI3 K/AKT/mTOR pathway, which is activated upon growth factor binding to receptor tyrosine kinases, leading to PI3 K-mediated activation of AKT. Activated AKT phosphorylates and inhibits TSC1/2, resulting in Rheb-mediated mTORC1 activation, which promotes cell survival and proliferation. AKT also enhances mTORC2 activity, further supporting survival signaling. PTEN serves as a negative regulator of this pathway by inhibiting PI3 K signaling. Right panel: The TGF-β pathway, upon ligand binding, triggers SMAD2/3 phosphorylation, allowing complex formation with SMAD4 and subsequent nuclear translocation to regulate gene transcription. Negative regulators such as SMAD7 and Smurf2 limit excessive signaling. Both pathways are critically involved in the regulation of ovarian cancer stem cell plasticity, survival, and therapy resistance

expression, contributing to chemoresistance. These CSC population also exhibit pluripotent potential, expressing endothelial and hematopoietic markers like CD31 and CD45 [101] (Fig. 6).

JAK/STAT Pathway

The JAK/STAT signaling pathway is crucial for maintaining the stemness and tumorigenic potential of OCSCs. Activated by cytokines and growth factors like IL-6, EGF, and leptin, this pathway primarily involves STAT3, translocated to the nucleus to promote the transcription of genes linked to self-renewal, survival, and chemoresistance [102, 103]. Aberrant STAT3 activation enhances OCSC stemness, immune evasion, and metastatic behavior by regulating stemness markers such as NANOG, CD133, and CD24, and by promoting extracellular matrix degradation through MMP-9 expression. STAT3 also drives EMT, contributing to tumor progression, metastasis, and chemoresistance [104, 105]. Suppressor of cytokine signaling (SOCS) proteins, particularly SOCS3, normally regulate JAK/STAT activity, but SOCS3 suppression in ovarian cancer leads to unchecked STAT3 activation, amplifying OCSC traits and migratory

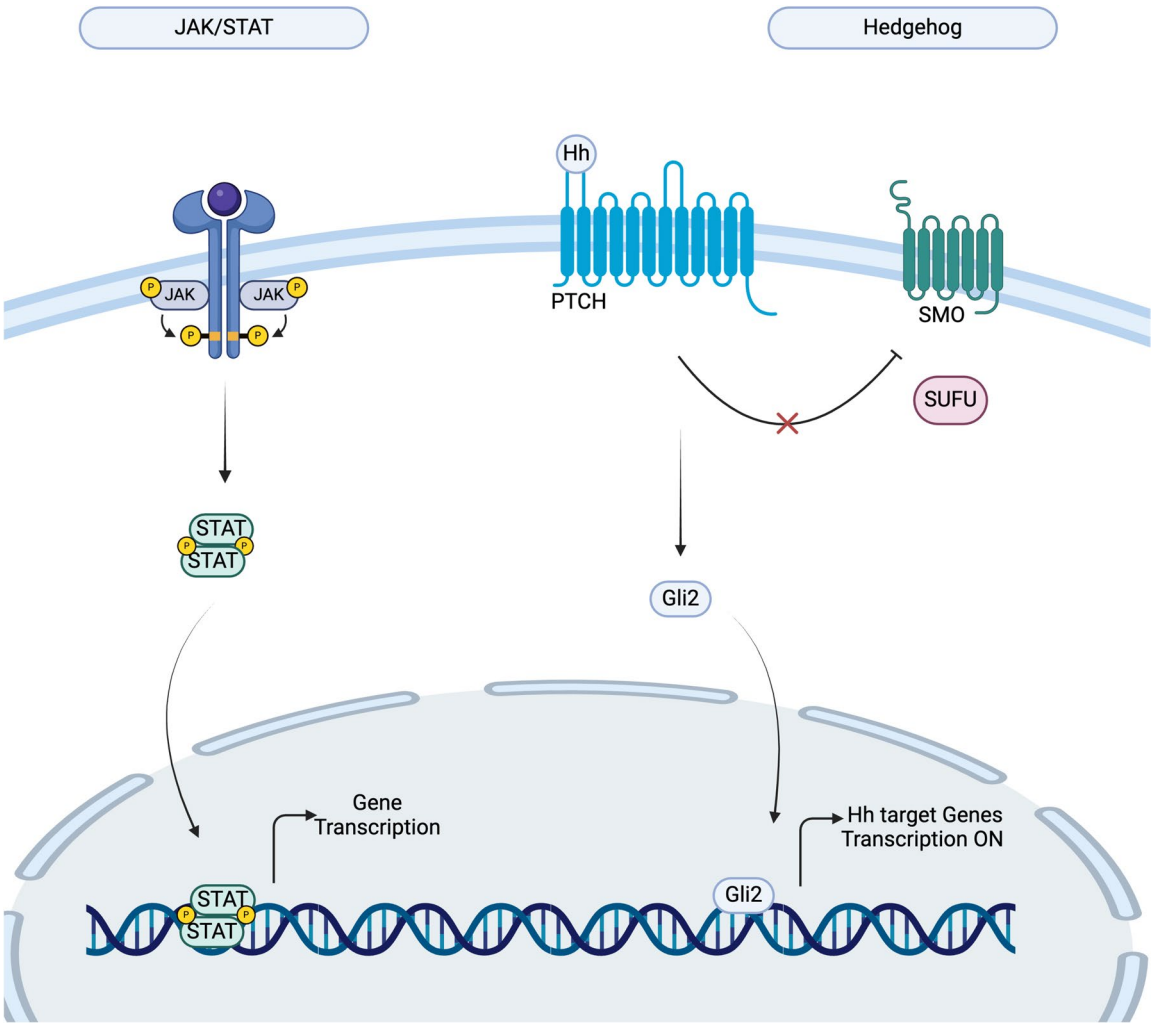


Fig. 7 JAK/STAT and Hedgehog signaling pathways in the regulation of ovarian cancer stem cell properties. This figure illustrates two pivotal signaling cascades involved in the maintenance and function of OCSCs. Left panel: The JAK/STAT pathway is activated upon cytokine binding to its receptor, resulting in JAK kinase phosphorylation and subsequent activation of STAT proteins. Phosphorylated STATs translocate into the nucleus to regulate transcription of target genes associated with stemness, proliferation, and survival. Right panel: The Hedgehog pathway is triggered by ligand binding to PTCH, relieving SMO inhibition. Activated SMO promotes Gli2 nuclear translocation, where it regulates the expression of Hh target genes essential for CSC maintenance, EMT, and chemoresistance

capacity. Cytokines like IL-6 and LIF, secreted by cancer-associated mesenchymal stromal cells (CA-MSCs), further enhance JAK2/STAT3 signaling, increasing $ALDH^+$ CSC population and tumorigenicity [106].

Hedgehog Pathway

The Hedgehog (Hh) signaling pathway plays a critical role in maintaining OCSCs and driving ovarian cancer progression. Dysregulation of components like Sonic Hedgehog (SHH), Patched (PTCH), and Smoothened (Smo) enhances OCSC self-renewal, EMT, invasion, and chemoresistance in HGSOC. Hedgehog inhibitors such as GANT61 effectively reduce OCSC population, suppress tumor growth, and downregulate key Hh components [107]. Also, CD24 acts downstream of Hh signaling and mediates Shh-induced cell migration and invasion. Increased CD24 expression correlates with advanced stages and poor prognosis in ovarian cancer [108]. Additionally, Gli transcription factors, especially Gli2, are pivotal in Hh-mediated chemoresistance. Gli2 is a more reliable marker of platinum resistance than Gli1, and targeting Gli with GANT61 enhances cisplatin sensitivity and reduces CSC-like properties. Both canonical and non-canonical Hh signaling pathways contribute to ovarian cancer progression and therapy resistance [109] (Fig. 7).

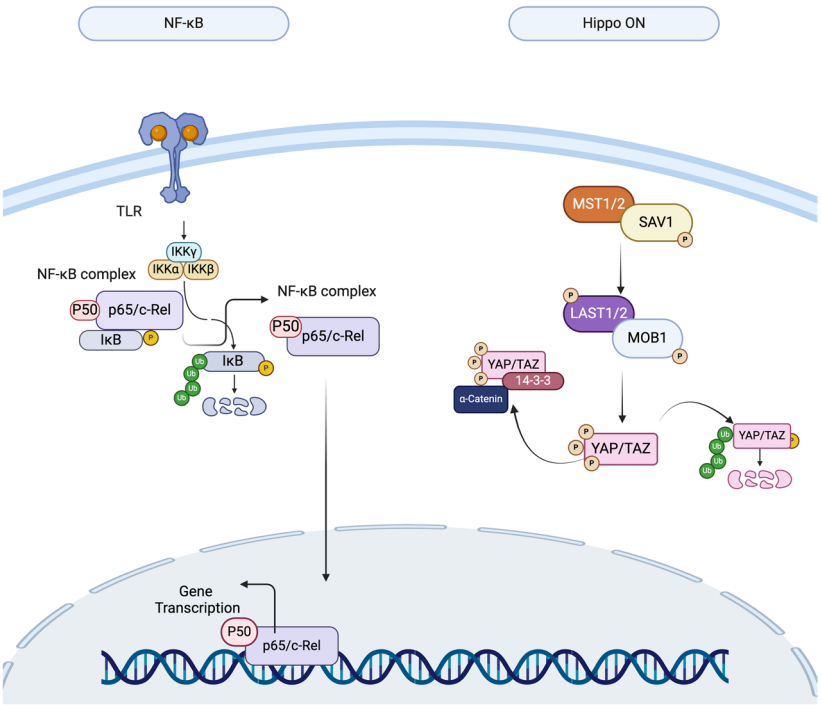


Fig. 8 NF-κB and Hippo signaling pathways regulating ovarian cancer stem cell plasticity and therapeutic resistance. This figure illustrates two critical signaling pathways involved in OCSC regulation. Left panel: The NF-κB pathway is activated by inflammatory cytokines and stress signals, leading to activation of the IKK complex, degradation of IkB, and nuclear translocation of NF-κB dimers (p50/p65) to drive the expression of pro-survival and pro-inflammatory genes. Right panel: The active Hippo pathway, through MST1/2-SAV1-mediated activation of LATS1/2 and MOB1, promotes phosphorylation of YAP/TAZ, resulting in their cytoplasmic sequestration via 14–3-3 binding or proteasomal degradation. In contrast, YAP/TAZ activation promotes dedifferentiation into CSC-like phenotypes, tumor growth, and resistance to platinum-based chemotherapy. Collectively, Together, these pathways contribute to the malignant properties and therapeutic resistance of ovarian cancer stem cells

NF-κB

The NF-κB signaling pathways, both canonical and non-canonical, are essential in regulating OCSCs. The canonical pathway promotes OCSC proliferation, chemoresistance, and EMT via RELA and p50, which are highly expressed in $CD44^{+}$ OCSCs. It also upregulates stemness genes like NANOG and CD44, and its inhibition reduces OCSC population, ovosphere formation, and colony growth. The non-canonical pathway contributes to OCSC self-renewal by regulating ALDH1 A2. Elevated RELB and IKKa (also known as component of inhibitor of nuclear factor kappa B kinase complex (CHUK)) levels highlight its importance, with IKKa required for both NF-κB pathways and maintaining key stemness markers [110, 111]. Additionally, inflammation-driven EMT and CSC traits are mediated by NF-κB/p65 and TWIST1, forming the NF-κB/TWIST axis. This axis is disrupted by the flavonoid chrysin (ChR), which suppresses EMT and CSC features by downregulating NF-κB/p65 and TWIST1 [112].

Hippo Pathway

The Hippo signaling pathway, mediated by YAP and TAZ, plays a crucial role in OCSCs by regulating self-renewal, metastatic potential, and chemoresistance. Dysregulation of the Hippo pathway, often through myosin phosphatase target subunit 1 (MYPT1) downregulation, leads to pathway inactivation, increased NF2/Merlin phosphorylation, and YAP/TAZ activation. Activated YAP/TAZ promote tumor growth, dedifferentiation into CSC-like states, and resistance to platinum-based chemotherapy, while MYPT1 upregulation restores Hippo activity, suppressing YAP/TAZ and reducing CSC traits [113]. AT-rich interaction domain 1 A (ARID1 A), a chromatin remodeling factor, enhances upstream Hippo kinase activity, suppressing YAP/TAZ and mitigating OCSC maintenance and EMT. ARID1 A loss, common in ovarian cancer, increases tumor progression, chemoresistance, and OCSC maintenance. Targeting TAZ shows promise in ARID1 A-deficient cancers [114]. Additionally, the miR-520e/HLF/YAP1 axis is critical in OCSC regulation. *miR-520e* downregulation leads to HLF upregulation,

activating YAP1 and enhancing OCSC proliferation, metastasis, and carboplatin resistance [115] (Fig. 8).

Cross-talking and synergistic effect of signaling pathways

The maintenance and progression of OCSCs are governed by intricate crosstalk between key signaling pathways, such as PI3 K-AKT-mTOR, NF-κB, NOTCH1, and c-MYC, which operate synergistically to support CSC survival, self-renewal, and therapy resistance. The PI3 K-AKT-mTOR pathway drives CSC proliferation and metabolic flexibility, while NF-κB creates an inflammatory niche that protects OCSCs from apoptosis. Feedback loops involving angiogenic factors like VEGF further amplify CSC traits, complicating therapeutic targeting, as inhibition of one pathway often activates compensatory mechanisms [98]. In ovarian cancer, NOTCH1 signaling promotes metastasis and resistance, with its downstream effector, c-MYC, acting as a master regulator of OCSC stemness and differentiation. The interplay between NOTCH1 and c-MYC is reinforced by small nucleolar RNAs (snoRNAs), such as *SNORA72*, which upregulate stemness markers like CD133, NANOG, and OCT4, bolstering the NOTCH1/c-MYC axis. This complex network of interactions underscores the need for combinatorial therapeutic strategies to disrupt OCSC signaling, prevent tumor progression, and reduce the risk of relapse [93].

Therapeutic approaches and inhibitors of ovarian cancer stem cells

In recent years, significant progress has been made in developing inhibitors specifically targeting OCSCs. These inhibitors are designed to address the unique characteristics and survival mechanisms of CSCs, which contribute to tumor progression, recurrence, and resistance to conventional therapies. Some of these inhibitors act by directly targeting key signaling pathways essential for the maintenance and self-renewal of OCSCs. Others work indirectly by modulating the tumor microenvironment or interfering with cellular processes that support OCSC function. Additionally, several inhibitors focus on the suppression of specific genes or proteins known to play critical roles in sustaining the stem-like properties of OCSCs. The goal of these therapeutic strategies is to diminish the OCSC population within tumors, thereby reducing their capacity for tumor initiation, chemoresistance, metastasis, and recurrence. Below, we discuss each of these inhibitors in detail, exploring their mechanisms of action and their potential impact on ovarian cancer treatment.

WNT inhibitors

The WNT signaling pathway is critical in regulating OCSCs, which are known for their resistance to conventional therapies and contribution to cancer recurrence. Several inhibitors targeting this pathway have shown promise in enhancing chemosensitivity and reducing OCSC self-renewal. The secreted frizzled-related protein 4 (sFRP4) disrupts spheroid formation, decreases OCSC survival, and induces apoptosis by inhibiting the WNT pathway. This mechanism reduces the expression of stemness markers and survival proteins such as Cyclin D1 and BCL-XL while increasing pro-apoptotic markers like Bax, enhancing the efficacy of cisplatin and offering potential as a chemo-sensitizing agent in ovarian cancer therapy [116]. Epigenetic modification of the WNT signaling pathway has also been explored, with 5-Azacytidine, a DNA methyltransferase inhibitor, showing the ability to demethylate sFRP4, thereby reducing WNT signaling and associated molecules like β-catenin. This epigenetic regulation, including histone modifications, contributes to the inhibition of OCSC self-renewal and tumor progression, highlighting the potential of targeting DNA methylation as a therapeutic strategy in ovarian cancer [117]. Additionally, Theaflavin-3 (TF3), a polyphenolic compound derived from black tea, inhibits the proliferation of OCSCs by targeting the WNT/β-catenin pathway. It downregulates β-catenin, lymphoid enhancer-binding factor 1 (LEF-1), c-MYC, and cyclin D1, impairing OCSC viability and tumor-sphere formation. Notably, β-catenin overexpression attenuates these effects, highlighting TF3 as a promising candidate for eradicating OCSCs and improving treatment outcomes [86]. Furthermore, ginsenoside Rb1 and its metabolite compound K selectively target OCSCs through the inhibition of the WNT/β-catenin pathway and suppression of EMT, which are essential for OCSC maintenance and tumor progression. Compound K, in particular, disrupts drug efflux transporters such as ABCG2 and P-glycoprotein, which enhances its anti-cancer effects and reduces chemoresistance, positioning these compounds as potential non-toxic therapeutic agents for ovarian cancer [88]. Finally, Trimebutine Maleate (TM) targets OCSCs by inhibiting BKCa and Ca2 + channels, which are critical for maintaining CSC properties. While TM does not directly target the WNT/β-catenin pathway, its blockade of calcium channels results in downstream suppression of WNT signaling, reducing stemness-related transcription factors such as OCT3/4 and SOX2. These findings emphasize TM's potential as a selective and effective therapeutic agent for ovarian cancer, warranting further clinical investigation [118].

NOTCH inhibitors

The NOTCH signaling pathway plays a critical role in maintaining the self-renewal and stemness of OCSCs, primarily through regulating transcription factors such as OCT4 and SOX2. Dysregulation of this pathway is associated with aggressive cancer phenotypes and chemoresistance. The γ-secretase inhibitor DAPT effectively blocks NOTCH signaling by preventing the activation of NICD and HES1 proteins, resulting in significant inhibition of OCSC self-renewal and proliferation. DAPT treatment reduces the expression of key surface markers CD44, CD117, and CD133 and pluripotency-associated genes (OCT4 and SOX2), thereby decreasing the stem-like subpopulation and promoting differentiation, highlighting its potential as a therapeutic agent to disrupt OCSC stemness and self-renewal [119]. Also, OMP-59R5 (Tarextumab), a NOTCH2/3-specific monoclonal antibody, exhibits significant antitumor activity in ovarian cancer by inhibiting NOTCH signaling in both tumor and stromal cells. In the OMP-OV38 serous ovarian cancer xenograft model, OMP-59R5 demonstrated efficacy as a single agent and in combination with chemotherapy, reducing tumor growth and delaying recurrence after chemotherapy cessation. Mechanistically, OMP-59R5 downregulated key NOTCH pathway genes, including HES1, NOTCH2, and NOTCH3, disrupting CSC maintenance and tumor progression. These findings highlight the potential of OMP-59R5 as a therapeutic strategy in ovarian cancer, particularly for overcoming chemoresistance and enhancing treatment efficacy [120].

PI3 K/AKT/mTOR inhibitors

The PI3 K/mTOR signaling pathway is critical in the proliferation and survival of OCSCs, contributing to chemoresistance and tumor recurrence. Several inhibitors, such as VS-5584, BEZ235, Compound C (dorsomorphin), and, have shown potential in targeting this pathway to suppress OCSCs. VS-5584, a dual PI3 K–mTOR inhibitor, significantly reduces OCSC population characterized by $CD44^+/CD117^+$ markers, positioning it as a promising candidate for maintenance therapy to prevent tumor recurrence [121]. BEZ235 effectively inhibits the PI3 K/AKT/mTOR pathway, reverses EMT, and reduces OCSC markers CD133 and CD44, sensitizing cisplatin-resistant epithelial ovarian cancer (EOC) cells to chemotherapy and enhancing chemosensitivity through apoptosis and oxidative stress induction [94]. Compound C (dorsomorphin) inhibits the PI3 K-AKT-mTOR-NF-κB signaling axis, suppressing malignant phenotypes and mitochondrial bioenergetics while synergizing with cisplatin to enhance cytotoxicity and reduce tumor vascularization by inhibiting VEGF expression and also can be used for targeting OCSCs [98]. Further, Ropivacaine, a local anesthetic, inhibits ovarian cancer cell growth and OCSC characteristics by targeting $ALDH^+$ cells and promoting ferroptosis through PI3 K/AKT pathway inactivation, highlighting its potential as a novel therapeutic agent against ovarian cancer [16].

TGF-β inhibitors

The TGF-β signaling pathway plays a crucial role in cancer progression by promoting EMT, enhancing CSC properties, and contributing to drug resistance, particularly in ovarian cancer. Targeting this pathway offers a strategic approach to mitigate metastasis and chemoresistance. SB525334, a selective inhibitor of the TGF-β pathway, demonstrates significant efficacy in impairing OCSCs by disrupting critical processes such as self-renewal, clonality, migration, and invasion. It achieves this by reducing Smad2/3 phosphorylation and enhancing Smad4 expression, thereby suppressing EMT markers like SNAIL and Vimentin while increasing E-cadherin levels. This dual impact on TGF-β/Smad signaling and EMT underscores SB525334's potential to reduce tumor metastasis and recurrence [122]. Similarly, cordycepin, a bioactive compound from Cordyceps species, targets TGF-β-induced EMT and CSC activity in ovarian cancer cells. By restoring E-cadherin expression and countering EMT, cordycepin sensitizes cells to cisplatin, effectively overcoming chemoresistance. Its mechanism involves disrupting mitochondrial function and modulating estrogen-related receptor pathways, positioning cordycepin as a promising agent for addressing CSC-driven chemoresistance in ovarian cancer [123].

JAK/STAT inhibitors

The SRC/STAT3 and JAK/STAT pathways are pivotal in regulating OCSCs, promoting their survival, drug resistance, and metastatic potential. These pathways are often activated in ovarian cancer, contributing to the aggressive behavior and chemoresistance of OCSCs. Epigallocatechin gallate (EGCG) and Momelotinib are promising agents that target these critical signaling cascades to suppress OCSC function and improve treatment outcomes. EGCG, a polyphenol derived from green tea, inhibits the SRC/STAT3 pathway, reducing the expression of stemness markers NANOG and CD133, which are crucial for OCSC maintenance and EMT. Additionally, EGCG downregulates anti-apoptotic proteins like BCL-2 and promotes apoptosis through markers such as cPARP and BAX, leading to a reduction in OCSC population and enhanced sensitivity to chemotherapy [104]. Similarly, Momelotinib, a selective JAK1/2 inhibitor, targets

the JAK2/STAT3 pathway by inhibiting JAK2 phosphorylation and reducing downstream STAT3 activation mouse model of human ovarian cancer. This disruption decreases the expression of CSC-like markers and alleviates chemotherapy resistance, offering a promising therapeutic strategy to reduce tumor burden, metastasis, and recurrence in ovarian cancer [124].

Hedgehog inhibitors

The Hedgehog signaling pathway plays a critical role in regulating OCSCs, ensuring their self-renewal, plasticity, and tumorigenic potential. In HGSOC, aberrant activation of the Hedgehog pathway has been implicated in the maintenance of OCSCs, contributing to chemotherapy resistance and tumor recurrence. The Hedgehog pathway inhibitor GANT61 has emerged as a promising therapeutic candidate due to its ability to inhibit Gli1-mediated transcriptional activity, a crucial effector in the Hedgehog signaling cascade. By impairing Gli1 activity, GANT61 downregulates the expression of key CSC markers, stem cell transcription factors, and associated signaling pathways such as WNT and NOTCH, which are also involved in the regulation of OCSC properties. In vitro studies and xenograft models have demonstrated that GANT61 treatment significantly reduces spheroid formation, a hallmark of OCSC activity, as well as tumor initiation and growth in ALDH1 $A1^+$ CSCs. Furthermore, Hedgehog pathway blockade using the anti-SHH antibody 5E1 has been shown to reduce OCSC population by preventing SHH binding to its receptor, patched, thus inhibiting downstream signaling events that promote OCSC maintenance. These findings underscore the potential of Hedgehog inhibitors, particularly GANT61, as targeted therapies for eradicating CSCs in HGSOC. Moreover, the combinatory use of GANT61 with WNT and NOTCH pathway inhibitors offers a promising approach to overcome pathway crosstalk and enhance therapeutic outcomes, potentially improving the effectiveness of treatments in targeting the OCSC compartment and overcoming resistance mechanisms in ovarian cancer [107].

Other inhibitors

Beyond targeting major signaling pathways, several compounds reduce OCSCs through alternative mechanisms. These inhibitors disrupt metabolic dependencies, stemness factors, and epigenetic regulators, offering new strategies against chemoresistance and recurrence. LY500307, an estrogen receptor beta (ERβ) agonist, significantly reduces the viability, sphere formation, and self-renewal capacity of OCSCs. By activating tumor suppressor genes like ferredoxin reductase (*FDXR*) and cyclin dependent kinase inhibitor 1 A (*CDKN1 A*) also known as p21, LY500307 induces apoptosis and cell cycle arrest, effectively diminishing the tumor-initiating potential of OCSCs, particularly in mouse xenograft models [125]. Celastrol, a natural compound, exerts its anti-cancer effects by suppressing peptidylprolyl cis/trans isomerase, NIMA-interacting 1 (*PIN1*), a key regulator of oncogenic pathways. It inhibits stem cell markers CD44, NANOG, KLF4, and OCT4, while reducing the population of OCSCs. Celastrol induces apoptosis and cell cycle arrest by downregulating cyclins and increasing pro-apoptotic factors like Caspase-3 and Bax. Additionally, Celastrol interferes with critical signaling pathways, including IL-6/STAT3, NF-κB, JNK/P38, and AKT, essential for OCSC maintenance, thus positioning it as a promising lead compound for overcoming ovarian cancer recurrence and metastasis [126]. 7-Difluoromethoxyl-5,4'-di-n-octylgenistein (DFOG), a synthetic genistein analogue, inhibits the self-renewal and proliferative activity of OCSLCs derived from the SKOV3 cell line. DFOG treatment induces apoptosis by inactivating key signaling pathways such as FOXM1, NF-κB, and AKT, highlighting its potential as a therapeutic strategy to inhibit OCSC activity and improve ovarian cancer treatment outcomes [127]. Further, Gossypol acetate (GAA) targets OXPHOS in OCSCs by inhibiting the LRPPRC protein, a critical regulator of mitochondrial OXPHOS. This disruption impairs mitochondrial activity, ATP production, and cellular energy metabolism, triggering a metabolic shift and reducing tumor stem cell viability. GAA treatment decreases tumorigenicity and tumor sphere formation, with evidence showing its potential to target OCSCs and inhibit tumor growth. Moreover, GAA's action on LRPPRC suggests a novel mechanism for suppressing OCSC maintenance and overcoming drug resistance, particularly in cisplatin-resistant ovarian cancer cells [128]. Another study identifies receptor tyrosine kinase like orphan receptor 1 (*ROR1*) as a key driver of OCSC properties, promoting tumor recurrence and chemoresistance. High *ROR1* expression correlates with poor prognosis and CSC-related gene signatures, including stem cell markers and EMT factors, enhancing tumor migration, spheroid formation, and xenograft tumorigenicity. Silencing *ROR1* or using the monoclonal antibody UC-961 suppresses CSC traits, reducing spheroid formation, migration, and tumor engraftment. UC-961 also decreases the expression of CSC marker ALDH1, impairing tumor re-engraftment and highlighting its potential as a therapeutic strategy against chemotherapy-resistant ovarian cancer [129]. Also, CM37, a selective ALDH1 A1 inhibitor, shows promise in targeting OCSCs by inhibiting spheroid growth, inducing ROS accumulation, and causing DNA damage. Its consistent effects across cell lines highlight ALDH1 A1's role

Table 2 Ovarian cancer stem cells inhibitors

Inhibitor Type	Drug Name	Function/Result	Experimental Models and samples	References
WNT inhibitors	**sFRP4**	Inhibits spheroid formation, decreases OCSC survival, induces apoptosis, reduces Cyclin D1 and BCL-XL while increasing Bax	Cell line: A2780	[116]
	5-Azacytidine	An epigenetic therapy approach, Demethylates sFRP4, reduces WNT signaling and β-catenin expression, decreases OCSC self-renewal and tumor progression	Cell line: A2780	[117]
	Theaflavin-3	Reduces β-catenin, LEF-1, c-MYC, and cyclin D1 levels, impairing CSC viability and tumorsphere formation, with effects attenuated by β-catenin overexpression	Cell lines: A2780/OVCAR3	[86]
	Ginsenoside Rb1 and compound K	suppresses EMT, decreases stemness, reduces chemoresistance, also disrupts drug efflux transporters (ABCG2, P-glycoprotein)	Cell lines: SKOV-3/HEYA8 Tissue: Primary human ovarian cancer samples In vivo: nude mice	[88]
	Trimebutine Maleate	Inhibits BKCa and Ca^{2+} channels, disrupts calcium homeostasis, and reduces stemness, indirectly suppresses WNT signaling, decreases stemness factors OCT3/4 and SOX2, enhances its therapeutic efficacy	Cell line: A2780	[118]
NOTCH inhibitors	**γ-secretase inhibitor DAPT**	Inhibits NICD and HES1 activation, reduces OCSC self-renewal, proliferation, stemness markers (CD44, CD117, CD133), and pluripotency genes (OCT4, SOX2), while promots differentiation	Cell lines: SKOV3/HO8910	[119]
	OMP-59R5 (Tarextumab)	Blocks NOTCH2/3 signaling, reduces CSC frequency and EMT gene expression. Enhances tumor regression, modulates angiogenesis by downregulating Rgs5, normalizes tumor vasculature, and improves drug delivery, particularly in pancreatic cancer models	Tissue: surgically removed patient tumors In vivo: NOD/SCID mice	[120]
PI3 K/AKT/mTOR inhibitors	**VS-5584**	Inhibits class I PI3 K isoforms, mTORC1, and mTORC2, reduces CSC population ($CD44^+/CD117^+$) and self-renewal potential. It demonstrates 10- to 30-fold greater sensitivity against CSCs compared to non-CSCs, effectively suppressing tumor-initiating capabilities and delaying recurrence	Tissue: human ovarian tumor In vivo: NOD-SCID mice	[121]
	BEZ235	Reverses EMT by restoring E-cadherin, reduces mesenchymal markers (N-cadherin, Vimentin), and suppresses CSC markers (CD133, CD44). It sensitizes cisplatin-resistant EOC cells to chemotherapy by inducing apoptosis and oxidative stress, overcoming CSC-associated chemoresistance	Cell lines: A2780/ IGROV1	[94]
	Compound C (Dorsomorphin)	Inhibits NF-κB (p65RelA) activation, impairs mitochondrial bioenergetics, targets CSCs, enhances cisplatin cytotoxicity, and reduces tumor vascularization by inhibiting VEGF and angiogenesis	Cell lines: SKOV3/CAOV3/OVCAR3/ IGROV1 In vivo: Athymic nude mice	[98]
	Ropivacaine	Targets ALDH-positive ovarian CSCs, reducing stemness markers (OCT4, NANOG) and promoting ferroptosis via PI3 K/AKT pathway inactivation, disrupts tumor progression and chemoresistance mechanisms	Cell lines: SKOV3/OVCAR-3 In vivo: Nude female BALB/c-nu mice	[16]

Table 2 (continued)

Inhibitor Type	Drug Name	Function/Result	Experimental Models and samples	References
TGF-β inhibitors	**SB525334**	Inhibits TGF-β signaling by reducing Smad2/3 phosphorylation and enhances Smad4 expression, disrupts EMT by decreasing SNAIL and Vimentin levels and increases E-cadherin expression. Reduces OCSC self-renewal, migration, and invasion, inhibits tumor metastasis and recurrence	Cell line: SKOV-3	[122]
	Cordycepin	Suppresses TGF-β-mediated stemness, restores E-cadherin to counter EMT, reverses cisplatin resistance, and disrupts mitochondrial activity while modulating estrogen-related receptor pathways	Cell line: SKOV-3	[123]
JAK/STAT inhibitors	**Epigallocatechin gallate (EGCG)**	Suppresses the Src/STAT3 pathway, reducing CSC markers (NANOG, CD133) and EMT traits. It inhibits anti-apoptotic proteins (BCL-2) and induces apoptotic markers (cPARP, BAX), diminishing CSC population and enhancing chemotherapy sensitivity	Cell line: ES-2	[104]
	Momelotinib	Suppresses paclitaxel-induced JAK2/STAT3 signaling by inhibiting JAK2 phosphorylation and reducing STAT3 activation, overcomes chemotherapy resistance, Decreases CSC-like markers	Cell lines: HEY/ TOV21G In vivo: Female Balb/c nu/nu mice	[124]
Hedgehog inhibitor	**GANT61**	Blocks Gli1-mediated transcription, reduces CSC markers, stem cell factors, and WNT/NOTCH pathways. Decreases spheroid formation, tumor initiation, and growth in ALDH1 $A1^+$ CSCs	Cell lines: OVCAR3,4,5/PEO1,4/CaOV2,3/SKOV3/A2780 Tissue: Malignant ascites-derived HGSOC cells In vivo: CrTac: NCr Foxn1nu (NCRNU-F) female nude mice	[107]
Estrogen receptor beta (ERβ) inhibitor	**LY500307**	Activates tumor suppressor genes (*FDXR*, *CDKN1 A*), reduces viability, sphere formation, and self-renewal, and decreases tumor-initiating potential in xenograft models	Cell lines: SKOV3/ OV90/ ES2/ OVSAHO/ A2780 In vivo: NCr/SCID female mice	[125]
Pin1 inhibitor	**Celastrol**	Inhibits stem cell markers (CD44, NANOG, KLF4, OCT4) and reduces the $CD44^+CD24^-$ CSC population. It induces apoptosis, G2/M cell cycle arrest, and modulates key signaling pathways (IL-6/STAT3, NF-κB, JNK/P38, AKT), critical for CSC maintenance	Cell lines: A2780/SKOV3/OVCAR3	[126]
Genistein analogue	**7-Difluoromethoxyl-5,4′-di-n-octylgenistein (DFOG)**	Inactivates FOXM1, NF-κB, and AKT signaling, inhibits self-renewal and proliferation, induces apoptosis, and improves treatment outcomes	Cell lines: SKOV3/A2780	[127]
Oxidative phosphorylation (OXPHOS) inhibitor	**Gossypol acetate (GAA)**	Reduces LRPPRC expression, impairing OXPHOS-related proteins, mitochondrial activity, ATP production, and energy metabolism. triggers a metabolic shift, increasing glycolysis and reducing OXPHOS activity, targets OCSCs, overcomes cisplatin-resistant ovarian cancer	Cell lines: A2780/SKOV3 Tissue: Ovarian cancer tissues from 107 patients In vivo: BALB/c nude mice	[128]
ROR1 inhibitor	**UC-961 (anti-ROR1 mAb)**	Reduces spheroid formation, migration, and tumor engraftment, decreases ALDH1 expression, suppresses CSC traits, improves chemotherapy response	Tissue: Primary ovarian-tumor specimens In vivo: Immune-deficient mice	[129]

Table 2 (continued)

Inhibitor Type	Drug Name	Function/Result	Experimental Models and samples	References
ALDH1 inhibitor		Inhibits spheroid growth, induces ROS accumulation, causes DNA damage, Disrupts ALDH1 A1-mediated detoxification and retinoid signaling, reducing stemness and chemoresistance	Cell lines: COV362/OVCAR5/SKOV3/OVCAR3/OV90 Tissue: High-grade serous Ovarian cancer and primary peritoneal carcinomatosis In Vivo: Foxn1nu nude mice	[130]

in maintaining CSC characteristics. However, functional redundancy among ALDH isoforms may limit efficacy, suggesting broader-spectrum inhibitors could enhance outcomes. Despite CM37's potent in vitro activity, its short half-life and low bioavailability pose challenges for clinical use [130]. *HOTAIR*, an oncogenic lncRNA, drives chemoresistance and cancer stem cell persistence in high-grade serous ovarian cancer. CRISPR-based knockout of *HOTAIR* resensitized cells to platinum chemotherapy, reduced CSC population, and impaired stemness-related phenotypes. It also altered oncogenic pathways, including NF-κB, by recruiting EZH2 for H3 K27 trimethylation. In vivo, combining *HOTAIR* and EZH2 inhibitors with chemotherapy reduced tumors and improved survival. Targeting *HOTAIR* with epigenetic therapy has shown promise and may help overcome resistance and prevent ovarian cancer progression [131, 132].

These studies highlight the therapeutic potential of targeting specific pathways and molecular markers to eradicate ovarian cancer stem cells, offering novel approaches to improve treatment outcomes and prevent recurrence in ovarian cancer (Table 2).

Combination therapy

As discussed earlier, CSCs significantly complicate ovarian cancer treatment, contributing to a high rate of recurrence and therapy resistance. Despite ongoing investigations into the characteristics and signaling pathways of OCSCs, common treatments like cisplatin, paclitaxel, and PARP inhibitors fail to effectively target these cells. Worse, evidence suggests these therapies may inadvertently induce or enrich the OCSC population within tumors, marked by cells expressing CD133 and CD117 [43]. This enrichment fosters tumor relapse, increased aggressiveness, and resistance due to CSCs enhanced DNA repair capabilities, metabolic adaptability, and activation of inflammatory signaling pathways such as NF-κB and IL6 secretion. Additionally, factors like high mobility group AT-hook 1 (HMGA1)-driven Nicotinamide phosphoribosyl-transferase (NAMPT) expression and elevated NAD^+ levels exacerbate CSC phenotypes, further highlighting the limitations of conventional therapies [121, 133, 134].

To address these challenges, combination therapy represents a promising strategy. By integrating OCSC-targeted inhibitors with standard chemotherapeutic agents, both bulk tumor cells and OCSCs can be effectively targeted, mitigating recurrence and resistance. In this section, we explore the rationale and therapeutic potential of combination approaches in ovarian cancer, emphasizing their impact on OCSCs and their role in improving clinical outcomes.

Rb1 and Compound K with Cisplatin and Paclitaxel

The combination of ginsenoside Rb1 and its metabolite, compound K, holds significant promise for targeting OCSCs and enhancing the efficacy of cisplatin and paclitaxel. These compounds primarily exert their anti-CSCs effects by inhibiting the WNT/β-catenin signaling pathway and EMT, with compound K demonstrating a more potent effect than Rb1. By amplifying the cytotoxic response to lower doses of chemotherapeutic agents, Rb1 and compound K present a viable strategy to overcome chemoresistance while reducing associated toxicity. Additionally, their ability to inhibit drug efflux transporters such as ABCG2 and P-glycoprotein underscores their potential in combating drug resistance [88].

Eugenol with Cisplatin

Pairing of cisplatin with Eugenol presents another promising strategy to overcome cisplatin resistance in ovarian cancer by specifically targeting OCSCs. While cisplatin alone tends to enrich OCSC population and contribute to drug resistance, Eugenol enhances its cytotoxic and pro-apoptotic effects, thereby reducing tumor proliferation and invasion both in vitro and in vivo. This combination significantly suppresses OCSC population, particularly those marked by $CD44^+$ and $ALDH^+$ expression, and inhibits the self-renewal capacity of tumor-initiating cells. Furthermore, Eugenol mitigates the cisplatin-induced upregulation of drug efflux pumps, thereby improving drug retention and efficacy. Notably, the combination therapy exerts its effects by inhibiting the NOTCH signaling pathway, specifically downregulating Hes1, a critical driver of stemness and resistance [90].

Withaferin with DOXIL Efficacy

One another approach involves the use of DOXIL, a liposomal formulation of doxorubicin, combined with withaferin A (WFA), a bioactive compound from Withania somnifera. While DOXIL is favored for its reduced cardiotoxicity compared to doxorubicin and effectively targets proliferating cancer cells, its efficacy against OCSCs remains limited. These OCSCs, marked by ALDH1, CD24, and CD44, are critical contributors to tumor recurrence and chemoresistance. WFA, with its anticancer and anti-inflammatory properties, enhances DOXIL's therapeutic effect by effectively targeting CSCs. At the molecular level, this combination suppresses ALDH1 expression and inhibits the NOTCH1 signaling pathway, which is essential for OCSC self-renewal. The synergistic action of DOXIL and WFA not only reduces spheroid formation and tumor growth in both in vitro and in vivo models but also minimizes side effects by enabling the use of lower DOXIL doses. This dual-targeting strategy

provides a robust approach to combating ovarian cancer chemoresistance [135].

Gamma-Secretase Inhibitor with Cisplatin

Also, utilizing a gamma-secretase inhibitor (GSI) to block NOTCH signaling, particularly NOTCH3, effectively depletes OCSC population and sensitizes cells to cisplatin. OCSCs often evade this cisplatin damages by enhancing DNA repair mechanisms, such as ATM and BRCA2 activation. GSI-mediated inhibition of the NOTCH pathway disrupts these repair processes, heightens DNA damage (as indicated by γ-H2 AX phosphorylation), and induces G2/M cell-cycle arrest, thereby amplifying the efficacy of cisplatin. Synergistic interactions between GSI and cisplatin have been demonstrated in preclinical models, successfully eliminating both OCSCs and non-CSC tumor cells, reducing tumor burden, and prolonging disease-free survival. This dual-targeted approach offers significant potential for overcoming chemoresistance, particularly in patients with high NOTCH3 expression, by combining the cytotoxic effects of cisplatin on bulk tumor cells with OCSCs depletion through NOTCH pathway inhibition [136].

BET inhibitors (JQ1) with Cisplatin

In addition, BET inhibitors, such as JQ1, by downregulating the *ALDH1 A1* gene through inhibition of a BRD4-regulated super-enhancer and its associated enhancer RNA (eRNA). This suppression reduces OCSC proliferation and tumor relapse, which are major challenges in EOC. While cisplatin, effectively induces apoptosis in bulk tumor cells, it has limited efficacy against OCSCs. The addition of BET inhibitors enhances cisplatin's cytotoxic effects by selectively impairing OCSC function through bromodomain containing 4 (BRD4) inhibition, thereby preventing CSC-mediated tumor regrowth [137].

Pan-ALDH1 A Inhibitor 673 A with Cisplatin

Another effective combination is Pan-ALDH1 A inhibitor 673 A with cisplatin. 673 A selectively induces necroptosis in $ALDH^+/CD133^+$ OCSCs, a key population responsible for tumor initiation, recurrence, and resistance. Mechanistically, 673 A disrupts ALDH1 A activity, initiating the formation of necroptosomes, high mobility group box 1 (HMGB1) cytoplasmic translocation, and mixed lineage kinase domain like pseudokinase (MLKL) membrane localization, the hallmarks of necroptosis. These events lead to reduced ATP production and calcium-dependent programmed necrosis, further amplified by the upregulation of uncoupling protein 1 (UCP1) and UCP3, which promote autophagic death and metabolic reprogramming. Importantly, 673 A sensitizes chemo-resistant tumors to cisplatin, by eradicating OCSCs and reversing ALDH1 A-driven resistance [138].

SGI-110 with Carboplatin

In one study it has shown that the use of SGI-110, a DNA methyltransferase inhibitor (DNMTI), in combination with carboplatin effectively targets $ALDH^+$ OCSCs, a population enriched in platinum-resistant ovarian cancer. ALDH1 A1 expression in these cells underscores their stem-like properties, driving resistance and recurrence. SGI-110 induces global DNA hypomethylation, reprogramming OCSCs by repressing stem-cell-associated gene expression and reducing their viability, sphere formation, and tumorigenic potential. This epigenetic modulation disrupts the methylation of key genes involved in stemness and resistance, likely mediated by interactions between DNA methylation and histone modifications, including EZH2 and ALDH1 A1. By resensitizing OCSCs to carboplatin, the combination therapy delays tumor progression, providing a comprehensive approach to reduce recurrence and enhance therapeutic outcomes in ovarian cancer [139].

BEZ235 with Cisplatin

Additionally, the dual PI3 K/mTOR inhibitor BEZ235 has shown potential in addressing these challenges, particularly in cisplatin-resistant EOC cell lines (A2780-cis and IGROV1-cis), which exhibit multidrug resistance along with enhanced EMT and OCSC marker expression. BEZ235 effectively inhibits the PI3 K/AKT/mTOR pathway, reverses EMT, reduces OCSC marker expression, and restores sensitivity to cisplatin, leading to increased apoptosis and reduced cell viability. Furthermore, the combination of BEZ235 with cisplatin demonstrates synergistic effects, including greater suppression of oxidative stress and OCSC characteristics compared to either treatment alone [94].

GW280264X with Cisplatin

In ovarian cancer, combination therapy targeting both CSCs and bulk tumor cells with A disintegrin and metalloprotease 17 (ADAM17) inhibitors and cisplatin offers a promising strategy to overcome chemoresistance. ADAM17, a sheddase involved in activating key signaling pathways such as EGFR, ERK, PI3 K/AKT, STAT3, and JNK, plays a pivotal role in promoting cancer cell proliferation, survival, and resistance to apoptosis. While cisplatin, effectively induces apoptosis in tumor cells, it also inadvertently activates ADAM17, triggering survival pathways that contribute to chemoresistance. Inhibiting ADAM17 with GW280264X disrupts this compensatory

mechanism by reducing ligand shedding and receptor tyrosine kinase activation, thereby enhancing apoptotic signaling. This dual-targeting strategy simultaneously disrupts the OCSC niche and tumor bulk, addressing the molecular drivers of chemoresistance [140].

Calcium channel blockers (CCBs) with Cisplatin

Further, the combination of CCBs with cisplatin has shown success in reducing OCSCs. CCBs, including manidipine, lacidipine, benidipine, and lomerizine, target L- and T-type voltage-gated calcium channels that are highly expressed in OCSCs. By inhibiting these channels, CCBs disrupt key signaling pathways such as PI3 K/AKT and ERK, crucial for maintaining OCSC stemness and survival. This leads to the downregulation of stemness markers OCT3/4, NANOG, SOX2 and induces apoptosis through caspase activation. Cisplatin complements this action by causing DNA damage in the rapidly dividing bulk cancer cells. The combined therapy enhances drug sensitivity, reduces tumor growth, and decreases recurrence likelihood, offering a comprehensive strategy to combat ovarian cancer effectively [141].

CYT387 with Paclitaxel

Another study demonstrates that paclitaxel treatment in ascites-derived tumor cells activates JAK2/STAT3, leading to enhanced OCSC markers. Notably, the JAK2 inhibitor CYT387 effectively suppresses STAT3 phosphorylation, reducing OCSC characteristics and tumor burden in mouse models. This dual treatment approach shows promise in targeting both the bulk tumor and the resistant OCSC population, potentially mitigating recurrence and improving patient outcomes by addressing the JAK2/STAT3 pathway's role in maintaining CSCs and resistance to therapy [142].

Nicotinamide phosphoribosyl transferase inhibitors with cisplatin

Also, targeting NAMPT, a pivotal enzyme in NAD + biosynthesis, emerges as a promising approach to suppress therapy-induced senescence-associated CSCs (TI-CSCs). NAMPT inhibitors, such as FK866 and GMX1778, effectively deplete the NAD + pool, thereby disrupting NF-κB signaling and the senescence-associated secretory phenotype (SASP), both of which are crucial for OCSC proliferation and maintenance. The upregulation of HMGA1, a chromatin architectural protein abundant in OCSCs, has been implicated in the transcriptional activation of NAMPT expression, promoting OCSC survival post-platinum-based chemotherapy. Combining NAMPT inhibitors with cisplatin has demonstrated efficacy in delaying tumor relapse and extending survival in vivo. Notably, NAMPT inhibitors alone show limited effectiveness, reinforcing their specific role in mitigating chemotherapy-induced CSC expansion rather than directly reducing tumor burden [134].

CPI-613 with Carboplatin/Paclitaxel/PARP inhibition

Finally, the metabolic inhibitor CPI-613 (devimistat), which targets key mitochondrial enzymes such as pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase, has been shown to reduce CSC-associated markers, including $CD133^{+}$ and $CD117^{+}$, as well as diminish sphere-forming capacity and tumorigenicity. These findings suggest that CPI-613 disrupts the critical energy sources for OCSC survival, making them more vulnerable to conventional therapies. CPI-613's potential to enhance the efficacy of carboplatin, paclitaxel, and PARP inhibitors, such as olaparib, is particularly significant. Conventional treatments often result in the enrichment of OCSC population, which exhibit resistance to standard chemotherapy and PARP inhibition. By integrating CPI-613 with these agents, a substantial reduction in OCSC population is achieved, indicating that metabolic inhibition can mitigate OCSC resistance mechanisms.

These combination strategies (Table 3) not only address the chemoresistance inherent to OCSCs but also hold promise for reducing tumor recurrence and enhancing overall treatment efficacy in ovarian cancer. These findings advocate for targeting both OCSCs and bulk tumor cells, to potentially improve clinical outcomes for patients with advanced ovarian cancer [133].

Clinical trials targeting CSCs in ovarian cancer

Clinical trials investigating CSC-focused approaches in ovarian cancer have demonstrated their potential to significantly improve treatment outcomes for patients with recurrent EOC. One pivotal study introduced a functional cytotoxicity assay utilizing live tumor cells and CSCs derived from patient biopsies or malignant fluid aspirates to identify chemotherapy agents with optimal efficacy. By targeting both CSCs and bulk tumor cells, this assay enables a more personalized approach to chemotherapy selection. In a cohort of 45 patients with poor-prognosis recurrent EOC (3rd–5 th relapse), assay-guided treatments led to remarkable improvements in progression-free survival (PFS) and overall survival, with median PFS reaching 11 months compared to 5.6 months in historical controls at the third relapse. Importantly, patients who did not respond to assay-identified treatments faced over 30 times the hazard of death compared to responders. Beyond its clinical efficacy, this approach also demonstrated cost-effectiveness by improving

Table 3 Combination therapy

Combination Therapy	Mechanism of action	Key findings/Effects	Experimental models and samples	References
Rb1 and Compound K with Cisplatin and Paclitaxel	Inhibits WNT/β-catenin signaling, EMT, and drug efflux transporters (ABCG2, P-glycoprotein)	Enhances chemotherapeutic efficacy, overcomes chemoresistance, reduces toxicity. Potent anti-CSCs effects, particularly with compound K	Cell lines: SKOV-3/HEYA8 Tissue: Primary human ovarian cancer samples In vivo: nude mice	[88]
Eugenol with Cisplatin	Inhibits NOTCH pathway (Hes1), reduces cisplatin-induced drug efflux pump expression	Reduces OCSC population ($CD44^+$, $ALDH^+$), suppresses the self-renewal, improves cisplatin efficacy by inhibiting drug efflux pumps	Cell lines: SKOV3/OV2774 In vivo: Female Nu/J mice	[90]
Withaferin with DOXIL	Suppresses ALDH1 expression, inhibits NOTCH1 signaling	Effective against $ALDH1^+$ CSCs, synergistic effect with DOXIL, reduces spheroid formation, tumor growth, and side effects	Cell lines: A2780/CaOV3 In vivo: SCID mice	[135]
Gamma-Secretase Inhibitor (GSI) with Cisplatin	Blocks NOTCH3 signaling, disrupts DNA repair (ATM, BRCA2), induces G2/M arrest	Sensitizes cells to cisplatin, depletes CSCs, reduces tumor burden, prolongs the disease-free survival	Cell lines: OVCAR3,5/SKOV3 Tissue: Primary lines from ovarian cancer patient In vivo: SCID mice	[136]
BET inhibitors (JQ1) with Cisplatin	Suppresses ALDH activity through BRD4 inhibition	Enhances cisplatin efficacy, reduces recurrence, targets both CSCs and bulk tumor cells	Cell lines: OVCAR3/A2780 In vivo: Female (NSG) mice	[137]
Pan-ALDH1 A Inhibitor (673 A) with Cisplatin	Inhibits all three ALDH1 A family members, with partial inhibition of ALDH2 or ALDH3	Sensitizes chemo-resistant tumors to cisplatin, reducing tumor burden, delaying relapse, targeting CSCs effectively, inducing necroptosis in ALDH +/CD133 + CSCs	Cell lines: PEO1,4/OvCar8/Kuramochi/A2780/Ovsaho/OVCAR5/SKOV3 Tissue: Primary cells from HGSC patients In vivo: Female (NSG) mice	[138]
DNMTI SGI-110 with Carboplatin	Induces DNA hypomethylation (DNA Methyltransferase Inhibitor)	Resensitizes CSCs to carboplatin, reduces tumor progression, enhances therapeutic outcomes, represses stem-cell-associated gene expression	Cell lines: A2780/SKOV3 Tissue: Biopsies from chemotherapy naïve HGSOC patients In vivo: Female nude, athymic, BALB/c-nu/nu mice	[139]
BEZ235 with Cisplatin	Inhibits PI3 K/AKT/mTOR pathway, reverses EMT	Restores cisplatin sensitivity, reduces of CSC characteristics, increases apoptosis, suppresses oxidative stress and OCSC characteristics	Cell lines: A2780/IGROV1	[94]
ADAM17 Inhibitor GW280264X with Cisplatin	Inhibits ADAM17, disrupts EGFR/ERK/PI3 K/AKT pathways, enhances apoptotic signaling	Enhances cisplatin efficacy, targets both CSCs and bulk tumor cells, overcoming resistance mechanisms, disrupts CSC niche, enhances apoptosis	Cell lines: HEY IGROV-1/SKOV-3/OVCAR-8/A2780 Tissue: Primary ascites cells from advanced-stage ovarian cancer patients	[140]
Calcium Channel Blockers (CCBs) with Cisplatin	Targets L- and T-type voltage-gated calcium channels, disrupts PI3 K/AKT and ERK pathways	Reduces CSC markers (OCT3/4, NANOG, SOX2), induces apoptosis, enhancing cisplatin sensitivity, reducing tumor recurrence	Cell line: A2780 In vivo: Female BALB/c-nu/nu mice	[141]
CYT387 with Paclitaxel	Inhibits JAK2/STAT3 pathway	Suppresses of STAT3 phosphorylation, reduces tumor burden, improves therapeutic outcomes by targeting CSCs, reduces CSCs markers	Cell line: HEY Tissue: Ascites collected from patients diagnosed with Stages IIa-IV serous ovarian carcinoma and adenocarcinom In vivo: Female Balb/c *nu/nu* mice	[142]
NAMPT Inhibitors (FK866/GMX1778) with Cisplatin	Depletes NAD + pool, disrupts NF-κB signaling, and mitigating senescence-associated CSC expansion	Delays tumor relapse, extends survival, addresses CSC-driven recurrence, synergistic effect with cisplatin	Cell lines: OVCAR3/OVCAR5 Tissue: Ovarian tumor samples In vivo: NSG mice	[134]
CPI-613 with Carboplatin/Paclitaxel/PARP inhibition	Targets mitochondrial oxidative phosphorylation, inhibits pyruvate dehydrogenase and α-ketoglutarate dehydrogenase	Reduces CSC population, enhances carboplatin/paclitaxel efficacy, mitigating resistance mechanisms in platinum-resistant ovarian cancer, reduces CSC markers	Cell lines: UWB1.289 WT and MUT/PEO1/OVCAR3,4 In vivo: NOD/SCID mice	[133]

outcomes while reducing healthcare expenditures. Although limited by its small sample size and lack of randomization, the study provides a robust proof of concept for integrating CSC cytotoxicity assays into precision medicine strategies. Ongoing multi-center randomized trials are expected to validate these findings and facilitate the broader adoption of CSC-directed therapies in routine clinical practice [143].

Another clinical trial provides strong evidence supporting Metformin as a promising adjuvant therapy for EOC, specifically targeting CSCs. This study uniquely assessed the effect of Metformin in a nondiabetic ovarian cancer population and demonstrated a significant reduction in ALDH1-positive and CD133-positive CSC population alongside improved overall survival. Metformin enhanced chemotherapy sensitivity by reducing CSC-driven platinum resistance and disrupting the chemoresistance-promoting effects of CA-MSCs. Moreover, metformin-induced DNA methylation changes in CA-MSCs appeared to reduce their ability to drive chemoresistance, potentially contributing to immunomodulatory effects that strengthen antitumor activity. While this nonrandomized trial was limited in design, its outcomes compare favorably to landmark clinical trials, suggesting metformin's ability to extend overall survival, particularly for stage II–III ovarian cancer patients. These findings highlight the need for further investigation through randomized phase III trials to confirm metformin's efficacy as a CSC-targeting agent and adjuvant therapy in EOC [144].

Despite these promising findings, CSC-targeting clinical trials in ovarian cancer remain sparse, with significant limitations in trial design. One major challenge is the lack of validated biomarkers and CSC-specific endpoints, making it difficult to accurately assess treatment efficacy. Additionally, the heterogeneity of CSC markers across patients complicates patient stratification and response evaluation. Future studies should focus on refining CSC identification methods and incorporating more precise CSC-specific endpoints into clinical trial designs. Furthermore, innovative approaches such as bispecific antibodies, CSC-directed immunotherapies, and high-throughput assays combining chemotherapy with metabolic inhibitors should be explored to enhance treatment efficacy. These strategies hold promise in overcoming current limitations and improving overall survival rates. Expanding research efforts in these areas will be crucial for translating CSC-targeted therapies into routine clinical practice and achieving durable responses in ovarian cancer patients.

Conclusion

The high recurrence rate in ovarian cancer underscores the need for a deeper understanding of the mechanisms driving this phenomenon. A key factor in recurrence is the maintenance and survival of OCSCs, which exhibit resistance to conventional therapies such as platinum-based drugs, taxanes, and PARP inhibitors. Understanding the unique features of OCSCs is essential for developing targeted therapies to improve clinical outcomes.

Targeting EMT and stemness pathways offers a promising strategy to combat recurrence. Critical signaling pathways, such as NOTCH and WNT, regulate self-renewal, differentiation, and survival of OCSCs. Inhibition of these pathways, along with EMT-related processes, can reduce stemness, decrease OCSC survival, and sensitize tumors to conventional therapies. The strong link between OCSCs and EMT suggests that drugs targeting EMT can effectively reduce OCSC population and improve treatment outcomes. For example, ginsenoside Rb1 and its metabolite compound K inhibit both WNT/β-catenin signaling and EMT, targeting OCSCs and enhancing chemotherapy efficacy, highlighting their potential as therapeutic agents.

Additionally, the tumor microenvironment, particularly CAFs, plays a crucial role in ovarian cancer chemoresistance and OCSC maintenance. Recent studies highlight the importance of CAF-derived WNT5a-mediated paracrine signaling in sustaining OCSC population. Targeting WNT5a, possibly through receptor antagonists, may disrupt the crosstalk between CAFs and OCSCs, providing a promising therapeutic strategy to reduce recurrence and improve chemotherapy outcomes.

While conventional therapies have shown efficacy against bulk tumor cells, they can inadvertently promote the expansion of OCSCs, increasing their population and allowing them to dominate over the cancer cells. This highlights the necessity for combination therapies, as they can address both OCSCs and bulk tumor cells, overcoming the resistance posed by OCSCs and improving treatment outcomes.

Despite the potential of OCSC-targeted therapies, there is a lack of clinical trials specifically focused on CSCs in ovarian cancer, with the exception of the Metformin trial. This gap highlights the need for more research into the role of OCSCs in recurrence and treatment resistance. Overcoming challenges such as developing reliable ovarian tumor models with CSC markers, addressing long timelines for recurrence studies, and overcoming translational limitations of stem-like models will be crucial for advancing these therapies.

In conclusion, the pursuit of combination therapies targeting both bulk tumors and OCSCs, alongside

approaches like EMT, NOTCH, and WNT inhibition, offers hope for tackling recurrence in ovarian cancer. Additionally, designing high-throughput assays that combine chemotherapies with metabolic inhibitors could potentially improve overall survival outcomes. Expanding clinical trials in this area is essential to translating these strategies into effective treatments, ultimately offering hope to patients with this aggressive disease.

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Authors' contributions
H.A: Writing main manuscript text, Conceptualization, Supervision, Visualization, Editing P.A: Writing main manuscript text, Visualization, Tables, Editing (H.A and P.A contributed equally to this manuscript) A.D: Writing main manuscript text, Tables, Editing M.T: Writing main manuscript text B.S: Supervision, Corresponding author.

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