Ovarian Cancer Stem Cells: Characterization and Role in Tumorigenesis

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10.1 Introduction

Ovarian cancer originates from the female organ responsible for producing eggs. This cancer most often remains undetected until it has spread locally within the pelvis. In the early stage, patient usually remains asymptomatic. Although in later stages, patients develop symptoms such as anorexia, loss of weight which are nonspecific, causing more confusion. Moreover, there is no better way to detect cancer at an early stage. Late detection is primarily the most important factor contributing to difficult-to-treat cases and increased fatality [1].

Ovarian cancer is the most lethal gynecological cancer in women worldwide. According to World Ovarian Cancer Coalition, ovarian cancer is the fifth most common cause of death from cancer. Approximately 295,000 women are diagnosed with ovarian cancer every year worldwide and account for more than 184,000 deaths per year. The 5-year survival rate is approximately 30% compared to 80% in case of breast cancer. By the year 2035, the diagnosis of new cases of ovarian cancer is expected to increase by 55% and the number of deaths would increase by 70% [2]. In the United States, out of 21,750 new cases estimated in 2020, 13,940 women will die with it within 5 years of diagnosis [3]. Despite substantial improvement in technology, lack of early diagnosis remains a clinical problem and contributes to the highest mortality among female gynecological cancers.

Ovary comprises of different cell types such as surface epithelial cells, germ cells, and sex cord-stromal cells. All these different cell types can give rise to different tumors. If we consider the 5-year survival rate of different stages of the disease, it has been observed that if it can be diagnosed at an early stage, it is highly curable. Moreover, if the disease is confined to the ovary, the survival rate is approximately 90% while it drops significantly as it proceeds further higher stages. Unfortunately, ovarian cancer patients frequently present with advanced disease and hence survival rate drops to about 40% after 5 years of diagnosis [2].

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Ovarian cancer is a heterogeneous disease with variable clinicopathological and molecular mechanisms of carcinogenesis, progression, metastasis, response to oncotherapy, and manifests as different histotypes when examined under microscope [4]. The most frequently presented subgroup is epithelial ovarian cancer which originates from the surface of the ovary and among this group there are different histotypes described briefly as follows: Germ cell tumor is seen in younger age group and stromal cell tumors appear in midlife. Not every ovarian cancer is the same. Different subtypes have different prognoses. Median survival rate varies with the type of ovarian cancer [5]. Hence, it is pertinent to identify and stratify the tumor type/subtype so as to administer appropriate treatment to the patient depending upon their differential response to the chemotherapy regimen.

Primary cytoreductive surgery combined with chemotherapy is initially effective treatment in the annihilation of bulk of tumor thus retaining the cells with stemness properties (self-renewal and quiescence) also termed as cancer stem cells (CSCs) which may get enriched leading to therapy recalcitrance and disease relapse [6, 7]. Chemoresistance is a crucial hindrance to achieve success in ovarian cancer therapy and is a major factor for stage-wise (I, II, III) progression of tumorigenesis [8]. Histopathologically epithelial ovarian cancer is classified as high-grade serous carcinoma, low-grade serous carcinoma, endometrioid clear cell carcinoma, and mucinous carcinoma. Modulation and cross-talk of various signaling pathways (Wnt, Shh, Notch1, etc.) might be implicated in this CSC-mediated therapeutic resistance [7, 8]. Recent report (utilizing genetically engineered mouse models and organoids) implicated both fallopian tube and ovarian surface epithelium (OSE) as the origin of high-grade serous ovarian carcinoma [9]. In addition, intratumoral heterogeneity of CSCs may be responsible for chemoresistance which could be probed to study at single-cell RNA transcriptome level with respect to tumor stage, patient-specific treatment regimen, and clinical outcome to establish corelation and thus effective therapies.

Tumor represents a complex ecosystem comprising of varied subclones differing in their genetic and epigenetic constitution (i.e., mutational burden and promoter hypermethylation) [intrinsic factors] and those from its surrounding microenvironment [extrinsic factors] constituting the spatiotemporal variations within the stromal cells, extracellular matrix components, immune, and endothelial cells [10]. This heterogeneity is a by-product of either or both of the situations defined by the clonal selection and stochastic model of CSCs, respectively. CSCs represent heterogeneity/plasticity in terms of the spectrum provided by their variability from stemness towards differentiation pathway thus providing a hierarchy of cells within a single tumor. Oncogenic transformation of cells culminating into CSCs rendering them with self-renewal ability and hence tumor aggressiveness and therapy resistance represents their genetic/epigenetic and also functional plasticity [11, 12].

Oncogenic transformation of OSE cells have been reported and implicated in epithelial ovarian cancer however there exists a gap in the knowledge about novel oncogenes and their molecular mechanisms [13]. Recently, Securin also known as pituitary transforming gene (PTTG) has been reported to be responsible for the transformation of normal cells to cancer cells. PTTG1, first cloned from ovary and testis is basically involved as a regulator of sister chromatid separation during cell cycle during normal physiology. It is a multi-domain proto-oncogene with pleiotropic functional significance due to its overexpression in different tumor types such as pituitary, thyroid, and breast besides ovarian cancer [14–16]. Recently PTTG1 was represented as a novel candidate which could demarcate the normal stem cells and ovarian CSC compartments (within the OSE layer and cortex region) in benign, borderline, and high-grade human ovarian tumors and ascites derived CSCs in comparison to normal ovaries by its co-expression with CSC-specific markers. Silencing of PTTG1 by gene-specific siRNA, or adenovirus vector expressing PTTG1 siRNA in ovarian cancer cells abrogated and enhanced the expression of PTTG1 leading to suppression of tumor progression and metastasis.

In addition, self-renewal, Wnt/B-Catenin, Notch1, and EMT pathway-specific markers were differentially regulated signifying a definitive role of PTTG1 in CSC self-renewal and EMT [17]. Hence, some fundamental concepts are described below.

10.2 Normal Cell Versus Cancer Cell

NCCP guideline for ovarian cancer 2019

There are three characteristically different features between normal and cancer cells.

1. Unlike normal cells, cancer cells grow in an uncontrolled manner without contact inhibition resulting in the development of tumor.
2. Cancer cell can invade other tissues which are known as invasion. Normal cells lack such property.
3. Unlike normal cells, cancer cells can propagate to a different part of the body by implanting or seeding, or via blood or lymphatic vessels.

10.3 Stem Cells Versus Cancer Stem Cells

10.3.1 Stem Cell

It is a special type of cell that possesses the ability to renew itself through cell division and differentiate into cells of multiple lineages. This cell may be of three types: adult stem cells, embryonic stem cell (ESC), and induced pluripotent stem cells (iPSCs).

(a) *Mesenchymal stem cells (MSCs)* are non-hematopoietic, multipotent adult stem cells and possess a varying degree of propensity to differentiate into Mesodermal lineage. Moreover, it can transdifferentiate into ectodermal and endodermal lineages. Later on, it was observed that these cells are present in almost all tissues such as adipose tissue, amniotic fluid and membrane, dental tissue, endometrium, limb bud, peripheral blood, placenta and fetal membrane, salivary gland, skin and foreskin, sub amniotic umbilical cord lining membrane, synovial fluid, Wharton's jelly, and menstrual blood [18].

(b) *Embryonic stem cells (ESCs)* are pluripotent in nature and have ability to differentiate into any type of somatic cells derived from an embryo. As a result of this, ESCs can be used as a promising biological tool for exploring the complex mechanism of development of multiple organ structures. First time, the embryonic stem cell line was derived successfully from mouse embryo in 1981 [19, 20]. Later on, Thompson and coworkers in 1998 generated the first stable ESC line from human embryos produced by in vitro fertilization [21].

(c) *Induced pluripotent stem cells (iPSCs)*: Despite the highest therapeutic potentiality of human ESC (hESC) in translational medicine, their use was limited to be so popular because of its controversy related to ethical issue. In order to overcome this problem, scientists have developed induced pluripotent stem cells (iPSCs) by introducing specific gene into already specialized mouse adult cells and thus overexpressing the transcription factors such as Oct3/4 (octamer-binding transcription factor 3/4), Sox2 (sex-determining region Y)-box 2, Klf4 (Kruppel-like factor 4), and c-Myc (Avian Myelocytomatosis virus oncogene cellular homolog) [22].

10.3.2 Cancer Stem Cells (CSCs)

CSCs represent a specialized group of cells but a minuscule fraction (~0.1–0.8%, maximum of 30%) within most of the tumors (solid and liquid) capable of initiation of tumor. These cells possess cellular and molecular heterogeneity and capable of self-renewal and reflect pluripotency. Recent experimental and clinical evidence both implicate CSCs in cancer initiation, progression, metastasis, and recurrence, as well as radio- and chemotherapeutic resistance [23, 24]. Expression

of several surface/non-surface markers, transcription factors related to stemness and self-renewal, other cellular properties such as autofluorescence [25] and dye-efflux mechanisms for Side population cells [26, 27] exhibiting stemness potential have been utilized to identify them (summarized in Table 10.1).

Although CSCs initially referred as tumor stem cells express distinct cell surface markers, their origin per se is still debatable. Lapidot and group first reported CD34+/CD38− subpopulation in primary acute myeloid leukemia with tumorigenic potential upon transplantation in SCID mice. A recent review by Nimmakayala and group [54] summarized the dynamics of CSCs from origin to metastasis whereby they explained cell fusion, horizontal gene transfer, and mutations driving cellular transformation and reprogramming into CSCs and metabolic shifts from glycolytic to oxidative phosphorylation or vice versa implicated in cancer stemness. Upon extensive reconciliation of the literature, it was interestingly proposed in this review that CSC populations with specific phenotypes, metabolic profiles, and clonogenic potential may metastasize to specific organs.

According to one theory, the tissue stem cells undergo mutation and behave as a cancer stem cell. Another theory is that the cancer cells acquire stemness following oncogenic hit [54–56] (Fig. 10.1). This detailed hierarchy could not be defined in solid tumor since there are subpopulations of cells residing within the same tumor such as "resident cancer stem cells" which can initiate the tumor and "migrating stem cells" which are responsible for propagation of tumor growth and metastasis [59]. Thus, the alternative model of carcinogenesis has been originated and states that tumor is composed of heterogeneous clones of cells resulting from different types of mutation and accounts for different phases of tumor development [60].

CSCs should be considered as one of the main targets of novel experimental and therapeutic strategy such as tissue repair in various clinical fields such as cardiac, orthopedic, plastic, and breast surgery [61–63]. CSC targeted therapies include drugs targeting cell surface, signaling pathways, chief components of tumor microenvironment, those aimed at reversing drug resistance, those focussed upon differentiation of CSCs, and other miscellaneous cellular features of CSCs [64]. However, several therapeutic interventions targeting CSCs per se are still immature and clinical trial outcomes are yet in pipeline to conclude constructively.

Similar to CSCs targeting, the origin of CSCs is enigmatic because as per the Clonal or Stochastic model all the cells may possess tumor-initiating properties, whereas the Hierarchical or CSC model suggests the persistence of a small fraction of CSCs [65, 66]. A tumor as an entity as such may reflect complex hierarchy in terms of the CSC profile because the normal tissue resident stem cells may acquire mutations and exhibit transformed phenotype and subsequently altered key cellular properties; or the progenitor cells, a progeny of stem cells may acquire mutations or their terminally differentiated progeny, in turn, may exhibit mutated version of cancer/tumor cells thus implicating self-renewal, differentiation, and proliferation as key mechanisms guiding the putative origin of CSCs [67].

10.4 Genetics of Ovarian Cancer

Ovarian cancer contributes to nearly 3% of all cancers among women. In 2035, it will account for more than 200,000 deaths all over the world [2]. Considering the facts, it is of crucial importance to identify women who are at enhanced risk of developing ovarian cancer so that preventive measures can be ensured. Early onset of menarche, late menopause, and being nulliparous are considered as well-known risk factors for ovarian cancer [68, 69]. However, presence of family history of ovarian cancer especially in first-degree relative has been found to elevate the lifetime risk of developing ovarian cancer. Hereditary ovarian cancer contributes to 20% of all ovarian cancer and results from mutation in BRCA1 and BRCA2 genes [70, 71]. This mutation varies with ethnicity giving rise to the higher prevalence of ovarian cancer in certain ethnic populations such as Polish, French, Canadian, and Ashkenazi Jews

Table 10.1 Cancer stem cell markers in ovarian cancer

Marker		Experimental design	Outcome	Authors [References]
CD24	Transmembrane glycoprotein	Ovarian serous tumor from patients	Presence of CD24 in the cytoplasm independently predicts poor survival	Choi et al. [28]
CD24	Transmembrane glycoprotein	Human ovarian cancer cell line Caov3	CD 24 is responsible for metastasis and chemoresistance through the induction of epithelial to mesenchymal transition via Akt -ERK signaling mechanism	Nakamura et al. [29]
CD44+/CD24−	CD44: Hyaluronate receptor	Ovarian cancer cell line (SKOV3 and OV90), Cancer cell isolated from ascites of ovarian cancer patients	These markers are predictor of chemoresistance, relapse, and poor prognosis	Meng et al. [30]
CD117/c-kit	Receptor/Oncoprotein having tyrosine kinase activity	Paraffin-embedded specimens of human serous ovarian carcinoma	Indicative of chemoresistance	Raspollini et al. [31]
CD133 (Prominin-1)	Transmembrane glycoprotein	Flow cytometric analysis of various Cancer cell lines and cells isolated from ascitic fluid of ovarian cancer patients	CD133 is an indicator of tumorigenicity and its expression is modulated by epigenetics	Baba et al. [32]
ALDH1A1	Intracellular enzyme, one of 17 isoforms of ALDH	Several cancer cell lines and primary xenograft developed from omental tissue of metastatic ovarian cancer patients	Predictor of tumor initiation, identification of chemoresistant cells	Landen et al. [33]
CD44+/CD117+	CD117: Stem cell factor receptor	Xenograft experiment	Indicative of greater tumorigenicity	Zhang et al. [34]
SP cells	Having dye exclusion property	H2B-GFP transgenic mice models	Identification and characterization of ovarian cancer stem cells	Szotek et al. [26]
CD133	Transmembrane glycoprotein	Primary tumor and cells isolated from ascitic fluid of ovarian cancer	Contributes to angiogenesis for driving metastasis	Kusumbe and Bapat [35]
CD44+, MyD88+	MYD88: Innate immune signal transduction adaptor	Ascites sample from advanced ovarian cancer patients	Maintenance of cell survival and chemoresistance via TLR4-MyD88 and NF kappa B pathway	Alvero et al. [36]
CD34+	Transmembrane phosphoglycoprotein	Xenograft tumor	Role in angiogenesis	Alvero et al. [37]

(continued)

Table 10.1 (continued)

Marker		Experimental design	Outcome	Authors [References]
CD105, CD44, CD106	CD105: Type I membrane glycoprotein; CD106: vascular cell adhesion molecule-1 (VCAM-1)	Human ovarian cancer cell line OVCAR3	Significant association with progression of disease, relapse, and chemoresistance	Zhang et al. 2019 [38]
Epithelial cell adhesion molecule (EpCAM)	Type I transmembrane glycoprotein	In vitro study in human ovarian cancer cell line and in vivo study in C57BL/6 mice and finally clinical correlation	Role in chemoresistance and prognostication	Tayama et al. [39]
CD44-EpCAM		Ovarian cancer cell line OVCAR8, SKOV3, OCC1, ES2, and HEK293	Effects on tumor growth	Zheng et al. [40]
SOX2 (SRY-box transcription factor 2)	Transcription factor	Human epithelial ovarian cancer line SKOV3 and HO8910	Required for maintenance of ovarian cancer stem cells	Wen et al. [41]
ROR1 (receptor-tyrosine-kinase-like orphan receptor 1)	Transmembrane protein from receptor tyrosine kinase family	ROR1 expression investigated in ovarian cancer patients	Independently prognosticated with disease-free survival	Zhang et al. [42]
NANOG	Homeobox transcription factor	Expression checked in ovarian cancer cell line e.g., SKOV3 and ovarian cancer patients	Prognostic factor of ovarian cancer and chemoresistance	Lee et al. [44]
OCT4	Transcription factor	Side population cells isolation from ovarian cancer cell lines (SKOV3 and A2780) by Hoechst dye exclusion method	Responsible for tumor progression via JAK/STAT pathway	Ruan et al. [45]
MYC	Oncogenic transcription factor	Ovarian cancer cell line SKOV3 and OVCAR and tissues from ovarian cancer patients	Expression of c-Myc has significant association with disease progression	Ning et al. [46]
ABCG2	Member of the ATP-binding cassette (ABC) transporter	Ovarian cancer cell line A2780	Related to drug resistance	Duo et al. [47]
PTTG1 (Securin)	Oncogene	Ovarian cancer cell line A2780 and CSCs isolated from ascitic fluid of ovarian cancer patients	Relation with disease progression via regulation of EMT	Parte et al. [17]

(continued)

Table 10.1 (continued)

Marker		Experimental design	Outcome	Authors [References]
LGR5 (leucine-rich repeat-containing G-protein coupled receptors)	Transmembrane receptors	Mice model	Identification of stem cells/ progenitor cells	Schindler et al. [48]; Ng et al. [49]
VASA	ATP-dependent RNA helicases	Ovarian cancer cell line and tissues from epithelial ovarian cancer patients	Impact in disease progression by abrogation of DNA damage-induced G2 checkpoint	Hashimoto et al. [50]
NANOG, SOX2, SSEA4 (stage-specific embryonic antigen-4)	SSE4: glycosphingolipid	Paraffin-embedded ovarian tissue from high-grade serous ovarian carcinoma	Involved with tumorigenesis	Virant-Klun et al. [51]

Note: Information were collected from Zuber et al. [7], Bapat [52], Padilla et al. [53]

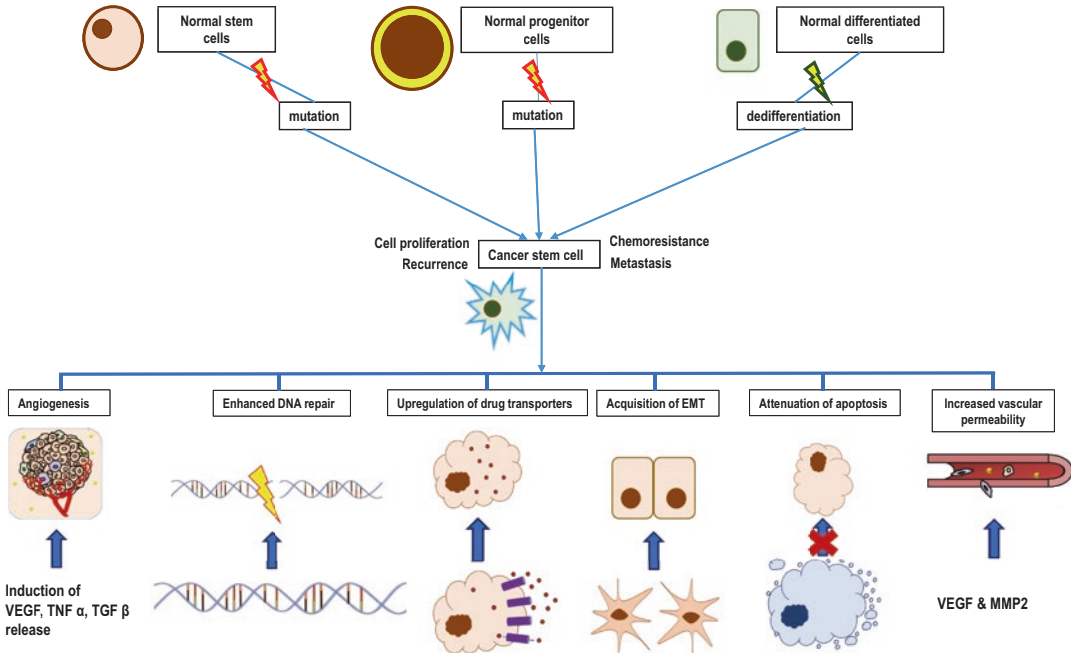

Fig. 10.1 Potential mechanisms of generation of cancer stem cells and their functions in tumor initiation, progression, and metsatsis. (Adopted from [6, 56–58])

[72, 73]. Since ovarian cancer is strongly associated with BRCA1/2 mutation, it is quintessential to carry out genetic testing and counseling of all ovarian cancer cases irrespective of age at onset and presence or absence of family history. BRCA1/2 gene mutations are responsible for earlier age of onset of ovarian cancer compared to women without mutation [70, 74]. It has been reported that BRCA1 gene mutation has a higher contribution nearly 48% compared to BRCA2 gene (nearly 29%) in elevating the lifetime risk of ovarian cancer [75]. BRCA1/2 associated OC

primarily originates from surface epithelium. They belong to high-grade serous histotype and are invasive in nature [76]. However, a recent large pooled cohort analysis revealed that OC with BRCA mutation carriers showed a better prognosis in terms of 5-year survival rate compared to that with noncarriers [77]. This could be explained by the better response of BRCA mutation to treatment with conventional platinum-based chemotherapy [78, 79].

Other contributing genes include BARD1, BRIP1, C14EK2, MRE11, MSH6, NBN, PALB2, RAD50, RAD51c, and TP53. RAD51C and RAD51D (paralog of RAD51) originate from the same ancestral gene of RAD51 and intrinsic component of homologous recombination-mediated double-strand break repair pathway. As opposed to BRCA1/2 linked OC, the average age at onset is older in RAD51C carrier [80–82]. Ovarian cancer related to RAD51C and RAD51D are found to originate from surface epithelial cells. Given the fact of their predisposition to develop OC, the role of RAD51 needs to be validated in mutation positive larger cohorts in order to determine whether germline mutation has any clinical implications as well as to establish screening and therapeutic strategies [82, 83].

Recent advanced gene testing technologies would help us in understanding the pathogenesis of ovarian cancer in a better way and thus would help in identifying women who are at risk of developing OC before the development of the disease and in turn help in implementing preventive strategies in time.

10.5 Identification of Ovarian CSCs

CSCs are identified by their tissue-specific expression of proteins known as biomarkers [84]. The stemness and tumorigenicity of isolated CSCs are validated by spheroid forming assay and limiting dilution assay, respectively on experimental models [85–87]. Ovarian CSC (OCSC) was detected first time from ascites of an ovarian cancer having the capacity of tumorigenicity in mice for several generations [88]. Ovarian CSCs express two types of markers: cell surface and nonsurface markers which are used either alone or in combination to identify and isolate the CSCs from the primary tumor and metastasized colony. The cell surface marker which was identified first time on ovarian CSC was CD117, a tyrosine kinase receptor [89–91] while more commonly documented ovarian CSC surface marker is CD133, transmembrane glycoprotein [32, 92–94]. Other reported surface markers are CD44, epithelial cell adhesion molecule (EpCAM), ROR1, and CD24 [28, 29, 95, 96]. These surface markers are significantly associated with tumor initiation, cancer propagation, prognosis, drug resistance, and recurrence of the disease [28, 32, 89, 90]. The nonsurface marker detected in ovarian CSC is aldehyde dehydrogenase family 1A2 (ALDH1A2). In an animal model, attenuation of ALDH1 improved the sensitivity of the cells to therapy [33]. Several studies have documented the association of the enzyme with the promotion of cell proliferation, facilitation of propagation, chemoresistance, unfavorable prognosis, and survival [97–99]. Some transcription factors, such as NANOG, OCT4, and SOX2, which are crucial for maintaining the stemness of embryonic stem cells [100], are also identified in ovarian CSCs [41]. Ovarian CSC biomarkers and their significance in various preclinical and clinical experiments have been presented in Table 10.1.

A special type of CSCs present in ovary having capacity to efflux the DNA binding dye are known as SP "side population" cells [101]. These cells are heterogeneous in nature because of differential expression of surface markers such as higher expression of Oct4, CD117, and CD44 compared to others. This heterogeneity in one tumor as well as difference between individual patients makes some ovarian cancer more chemoresistant and difficult to treat by universal treatment [102]. Hence, the concept of personalized therapy should be adopted to obtain effective outcome. Functional significance of variability and heterogeneity in expression of various CSC markers have been reported for example ALDH+/CD133+ possess higher tumorigenic potential than ALDH+/CD133− population, whereas

CD133+ CSCs induced CD133− cells to undergo EMT and metastatic potential via CCL5 and NFKB signaling. Similarly, ALDH1 and CD44 coexpressing cells may elicit a chemotherapeutic response and poor clinical outcome in patients rather than ALDH1+ only populations [23]. Despite encouraging results, clinical trials reveal only limited success attributed to intra- and inter-tumor heterogeneity among CSC and non-CSC compartments. In another study, Parte et al. [103, 104] extensively studied the expression of CSC surface markers in Benign, Borderline, and High-Grade ovarian tumors compared to normal ovaries which co-expressed with germline stem cell-specific markers initially implicated in ovarian stem cells. Similarly, they also explored and characterized actively dividing cell marker Ki67 with CSC populations distributed across the OSE and cortex regions of the tumorous ovaries. Novel insights about the tumor stage- and cellular compartment-specific distribution of ovarian CSCs co-expressing germline stem cell and proliferation markers were first demonstrated in these studies. Knowledge about the marker expression of tumor-initiating populations coupled with molecular mechanisms could improve their effective targeting in future and prevention of metastatic dissemination into peritoneum. Against this background several researchers and oncologists are striving hard to achieve success by addressing the bottlenecks and much remains to be researched. Recently Udoh et al. [105] and Carter et al. [106] during independent studies explored the potential of a fungal metabolite from *Myrothecium verrucaria* known as Verrucarin J (VJ) to target lung and ovarian CSCs via inhibition of the Wnt/β-Catenin and Notch1 stemness related signaling pathways. Similarly, Kakar and his group in the last couple of years have pronounced the effect of a herbal supplement Withaferin A (WFA) from *Withania somnifera* aka Ashwagandha plant extract and delineated its significant role in inhibiting ovarian ALDH1+ CSC populations while combining with standard chemotherapeutic drug Cisplatin [107]. ALDH1+ CSCs are differentially distributed within Benign, Borderline, and High-Grade ovarian tumors compared to normal ovaries within the OSE and cortex compartments [108, 109]. Further WFA while acting in synergy with Doxil exhibited a phenomenal inhibition of tumorigenic potential of ovarian CSCs thus attenuating the side effects of higher dose of Doxil and thus revealing potential against curbing recurrence by destroying the CSC population. Even in in vitro (A2780 cells) and in vivo models (mice treated with Cisplatin and WFA either alone or in combination), revealed significant reduction of CSC markers and thus proving the efficacy of WFA with conventional chemotherapy.

10.6 Tumor Microenvironment and OCSCs

One of the crucial contributing factors for cancer progression is interaction between ovarian cancer stem cells and tumor microenvironment (TME) which involves the following:

(a) *Extracellular matrix* which includes cytokines, chemokines, matrix metalloproteinases (MMP), and integrins [110].
(b) *Cancer-associated fibroblasts* derived from mesenchymal cells or as a result of transdifferentiation of pericytes and epithelial cells following exposure to various growth factors such as vascular endothelial growth factor (VEGF) [111]. They promote tumor growth via increased expression of CXCL14, IL-6, STAT3, and promote dissemination via neovascularisation, and immune suppression through intrusion of regulatory T lymphocytes. They modulate chemosensitivity and induce recurrence through higher expression of fibroblast activation protein alpha. They can remain in a quiescent state as well as in the active state. Cancer cells through the release of cytokines can activate CAFs which in turn can control the activity of immune cells [112]. This interaction between CSCs and other components of TME through various signaling pathways (TGF-β, Hedgehog, JAK) promotes tumorigenesis. Hence, targeting CAF might be promising therapeutic

approach for the prevention of disease progression [113].

(c) *Endothelial cells* comprise of the blood vessels and are primarily linked to neo-angiogenesis which are activated by VEGF, TGF-β, TNF-α, prostaglandin E2 and inhibited by angiopoietin, thrombospondin1 [114]. In presence of hypoxia, VEGF acts through its receptors present on endothelial cells and co-receptors neuropilins while angiopoietin attenuated signal transduction via modulation of tyrosine phosphorylation [115].

(d) *Immune cells* comprise of macrophages, dendritic cells, myeloid-derived suppressor cells (MDSC), and lymphocytes. Macrophages present within the tumor microenvironment known as tumor-associated macrophages (TAM). In the presence of interferon gamma and lipopolysaccharide, these macrophages suppress tumor growth through its cytotoxic effect by the release of IL-1, IL-12, and TNF-α. However, in presence of IL-4 and IL-10, TAM can promote tumorigenesis via immune suppression through inhibition of T cell multiplication [116]. MDSCs are GR1 and CD11b expressing myeloid cells, that facilitate tumorigenesis through exhaustion of nutrients required for the survival of T lymphocytes such as L-arginine and L-cysteine and induction of T cell apoptosis [117]. MDSCs further promote neo-angiogenesis in the presence of ischemia through the release of VEGF and EGF2 as well as activation of STAT3. They can also promote dissemination via the release of MMP 9. Therefore, targeting the molecular pathways involved in the activation of various components of TME might be promising for inhibition of tumorigenesis, invasion, metastasis, and recurrence of ovarian cancer [118].

(e) *Extracellular vesicles* released from ovarian cancer stem cells form a part of "premetastatic niche" and helps in communication with other components of the microenvironment for example stromal cells and extracellular matrix via its biological content such as lipids, proteins, ds-DNA, mRNA, and micro RNA [119]. This exosome model explains the metastatic role of ovarian cancer stem cells in transportation of biological material including CD44 along the bloodstream to recipient cells of distant organs. Exosomes act as vehicles for transferring the miR222-3p to the macrophages giving rise to Tumor-associated macrophages (TAM). Moreover, exosomes containing miR-21, miR-103, miR-205, miR-200 are linked to adverse outcomes in OC patients. Having its immense diagnostic and therapeutic potential, an extracellular vesicle is considered as one of the recently investigated target for the treatment of refractory ovarian cancers [119].

10.7 Epithelial to Mesenchymal Transition (EMT)

Following downregulation of factors responsible for intercellular adhesion such as E-cadherin, Epcam, occludin, claudin and upregulation of vimentin, fibronectin, and MMPs, epithelial cells reversibly convert to mesenchymal cells having spindle shape. This process is known as EMT which passes through a more aggressive intermediate dynamic stage having both the properties of epithelial and mesenchymal cells [120]. EMT is a characteristic feature for embryogenesis, wound healing, and tumor progression. This process makes the cancer cell mobile and acts as a driving force for maintaining the stemness of the cancer cells thus resulting in the migration, invasion, and resistance to chemotherapy and immunotherapy. The controlling transcription factors involved in EMT include zinc-finger E-box-binding homeobox factors Zeb1 and Zeb2, Snail (SNAI1), Slug (SNAI2), Twist1, and Twist2 [121]. Various experimental studies documented that chemoresistant cancer cells acquire the features of mesenchymal cells, indicating the implication of EMT in refractoriness to therapy [122]. EMT could induce chemoresistance by altered expression of class III beta tubulin, increase in drug efflux via overexpression of ATP-binding cassette transporters [123], accentuating DNA repair mechanism by Sirtuin6-mediated activation of poly

ADP ribose polymerase (PARP) enzyme [124] attenuation of p-53 regulated apoptosis and modulation of the expression of various microRNA (miR200b represses [125] and miR20a induces EMT [126]). EMT causes immune-resistance through upregulation of programmed cell death-ligand 1(PD-L1) [127]. Hence, EMT could be a potential target to regain the sensitivity to chemo and immunotherapy. Atezolizumab and Bevacizumab (anti-PDL1 and Anti-VEGF) therapy might improve sensitivity symbiotically to cisplatin targeting EMT via attenuation of STAT3 phosphorylation [128]. Recent studies on administration of TWIST-targeted siRNA and miR15a and miR-16 in the form of nanoparticles could alleviate the drug resistance in an experimental animal model [129].

Since it is challenging to target the effector molecules of EMT, inhibitors targeting various metabolic pathways involved in EMT would be promising. Hence, recently various clinical trials are on-going to repurpose various metabolic inhibitors such as phosphodiesterase 4 (PDE4) inhibitor—Rolipram, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor—Simvastatin, Heparinase-inhibitor—Suramin for prevention of cancer progression in combination with already established standard therapy [130].

10.8 Targeted Therapy for OCSC

Even after satisfactory response to traditional chemotherapy, almost 70% of ovarian cancer patients return within 5 years with features suggestive of recurrence and chemoresistance. Hence, in order to improve the survival rate of advanced ovarian cancers, there is a critical need to find out novel therapeutic approaches to target specific molecular pathways and their complex interplay responsible for carcinogenesis. This is known as targeted therapy having lesser toxic effects compared to conventional chemotherapy which can also affect normal dividing cells because of its DNA damaging effects. Potential therapeutic targets have been depicted in Fig. 10.2. Very recent preclinical study including patient-derived xenograft model documented the significant role of Axitinib, tyrosine kinase inhibitor, in inhibition of tumor growth via modulation of VEGFR signaling pathway indicating the crucial role of angiogenesis along with overexpression of VEGF in ovarian cancer progression [133]. Hence, targeting angiogenesis could be one effective mode of therapy for the prevention of disease recurrence.

(a) *Angiogenesis Inhibitor*

Four double-blind, placebo-controlled phase III trials were conducted on chemotherapy with or without monoclonal VEGF antibody, Bevacizumab. In (GOG-0218) [134] and International Standard Randomised Controlled Trial, (ISRCTN91273375) [135] treatment was given to newly diagnosed advanced ovarian cancer patients, while in OCEANS [136] and AURELIA [137], treatment was given to platinum-sensitive and resistant recurrent epithelial ovarian cancer cases, respectively. Another, VEGF-independent angiogenesis pathway targeted, double-blind phase III trial (TRINOVA-1) conducted on trebananib which inhibits binding of angiopoietin to its receptor Tie2 for recurrent ovarian cancer reported a significant improvement in progression-free survival [138]. Various phase II/III clinical trials on multiple tyrosine kinase inhibitors such as Pazopanib, Nintedanib (*BIBF 1120*), Cediranib, Sunitinib targeting VEGF receptors, PDGF receptors, C-Tyrosine kinase, and FMS-like tyrosine kinase-3 (c-KIT) in combination showed promising results [139].

(b) *Poly-adenosine-diphosphate-ribose-polymerase (PARP) Inhibitor*

PARP inhibitors convert single-strand DNA damage to double-strand break in ovarian cancer patients with a mutation in BRCA 1/2 tumor suppressor protein which contributes to double-strand DNA repair and thus produces synthetic lethality to the cells. Various phase III clinical trials as maintenance therapy of PARP inhibitors either alone or in combination such as SOLO1 (Olaparib), PRIMA (Niraparib), PAOLA

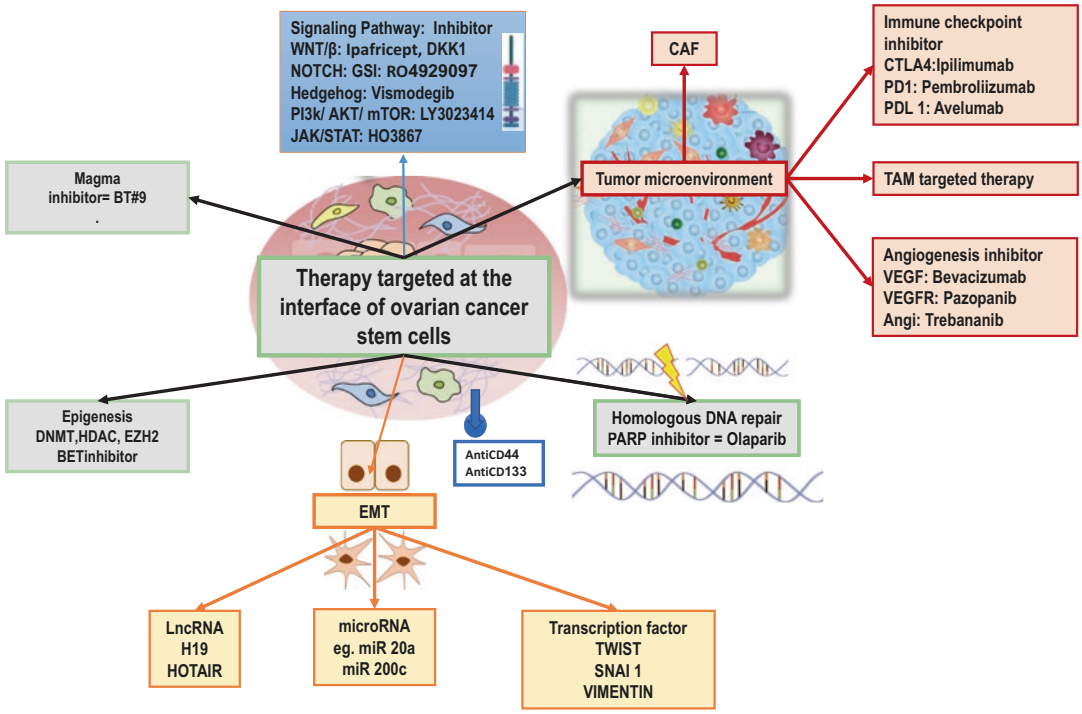

Fig. 10.2 Potential targets for the elimination of ovarian cancer stem cells. *CAF* cancer-associated fibroblast, *VEGF* vascular endothelial growth factor, *VEGFR* VEGF receptor, *EMT* epithelial to mesenchymal transition, *Magma* mitochondrial associated granulocyte macrophage colony stimulating factor, *LncRNA* long noncoding RNA, *EZH2* enhancer of zeste homolog 2, *TAM* tumor-associated macrophage, *PD1* programmed death 1, *PDL1* programmed death-1 (PD-1) ligand1, *PARP* poly (ADP-ribose) polymerase 1, *DKK1* Dickopf 1, *GSI* gamma secretase inhibitor. (Sources: [6, 52, 57, 110, 131, 132])

(Olaparib + bevacizumab) for newly diagnosed advanced ovarian cancer [140] and SOLO2/ENGOT-Ov21 on Olaparib as maintenance therapy for BRCA mutant relapsed ovarian cancer patients showed significant improvement in progression-free survival (PFS) [141].

(c) *Targeting Underlying Signaling Mechanisms for OCSC*

Cumulating evidence suggested that various signaling pathways such as Wnt/β, Hedgehog, Notch, JAK-STAT play an important role in the proliferation of ovarian CSCs and initiation of metastasis. Hence, various clinical studies on signaling pathway blockers are in process to validate their clinical implications in the elimination of the OCSCs and in turn prevention of recurrence.

A phase I, dose-escalation trial (NCT01608867) on Ipafricept a recombinant fusion protein targeting Wnt signaling pathway, was found to have better tolerance in combination with conventional chemotherapy for advanced ovarian cancer patients [142]. The interplay between Notch and Wnt-β catenin signaling pathways is attributed to tumor growth through the survival of CSCs. Enoticumab (REGN421), a Delta-like Ligand 4 (Dll4) monoclonal antibody was documented to be a safe drug in phase I, human study (NCT00871559) conducted on advanced ovarian cancer patients [143]. Various phase II clinical trials on mTOR inhibitor Temsirolimus either alone or in combination with Bevacizumab were found to be reasonably tolerated in advanced ovar-

ian cancer cases (https://clinicaltrials.gov/ct2/home).

(d) *Immune Checkpoint Inhibitors for OCSC*

Considering the sensible role of immune checkpoint pathways in maintaining stem related features of ovarian CSCs, various phase I/II clinical trials were conducted on immune checkpoint inhibitors such as anti-PDL1 [Avelumab (NCT01772004) and Atezolizumab (NCT01375842)], anti-PD-1 Pembrolizumab (NCT02674061) for recurrent, advanced ovarian cancer cases. However, preliminary results obtained were far from being satisfactory. In order to improve the outcome, various phase III clinical trials (NCT03598270, NCT02891824, NCT02659384) are on-going as a combination of immune checkpoint inhibitor and PARP inhibitor and/or anti-VEGF drugs [144]. Preliminary results are awaited.

(e) *Epigenetic Therapy for Annihilation of OCSCs*

Dysregulation of epigenesis contributes to the survival and gain in plasticity resulting in the development of metastatic features of ovarian CSCs. Guadecitabine (SGI-110) effectively helps in the differentiation of ALDH+ OCSCs and thus regained the chemosensitivity in ovarian cancer cell lines [145]. Moreover, Histone deacetylase inhibitors could restore the differentiation of epithelial cells via attenuation of gene expression of HIF-1α, Notch-1, and STAT3 [146]. Similarly, Bromodomain and Extra-terminal inhibitor JQ1 could inhibit tumorigenesis by suppression of ALDH activity [147]. These preclinical studies emphasize the role of epigenetic reprogramming in OCSC aggressiveness and implicate as OCSCs differentiation strategy.

Gening et al. [148] documented that circulating long noncoding RNA (LncRNA), MALAT and HOTAIR, were significantly associated with recurrence-free periods in ovarian cancer patients indicating their role in the prognosis of the disease. Silencing of LncRNA, LINC00152, could regress tumor growth via modulation of miR-125b-mediated mitochondrial apoptosis [149] as well as regain the sensitivity towards Cisplatin in ovarian cancer cell line. Moreover, LncRNA HotairM1 attributes to a critical role in maintaining the stemness properties of CSCs through downstream effector HOXA1-Nanog [150]. These studies provide the evidence of a cross-talk between epigenetic regulation via LncRNA and ovarian cancer progression indicating the role of LncRNA as a potential therapeutic target along with conventional therapy to prevent recurrence in ovarian cancer through the elimination of CSCs.

10.9 Concluding Remarks/ Foresights

It is surmised hence that a comprehensive understanding of various cellular events, types, and dynamics of cellular functions and activities of the tumor bulk cells as well as CSCs within a tumor in context of patient tumor grade, oncotherapy administrated to the patient, and the interaction of cancer cells and CSCs with each other, and that of a tumor as an entity with its surrounding microenvironmental components are very pertinent aspects that require to be understood in greater details to further develop effective therapies targeting CSCs. Collectively, the most recent developments of clinical trials (vividly covered in this chapter) reflect remarkable improvements on this front. Nevertheless worth noting are the dismal setbacks which rather serve as newer milestones/targets to be achieved by cancer researchers and oncologists alike, to improvise on the translational front thus providing greater hopes for better clinical management of patients which remains the ultimate goal.

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