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Cancer stem cells, epithelial-mesenchymal transition, and drug resistance in high-grade ovarian serous carcinoma

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Summary

Although epithelial ovarian cancer cells are eliminated by debulking surgery and chemotherapy during initial treatment, it is believed that only a subset of cancer cells, that is, cancer stem cells, may be an important source of tumor recurrence and drug resistance. This review highlights our current understanding of high-grade serous carcinoma, ovarian cancer stem cells, common methods for enrichment of ovarian cancer stem cells, mechanisms involved in drug resistance, and potential strategies for overcoming drug resistance, with associated potential controversies and pitfalls. We also review the potential relationship between epithelial-to-mesenchymal transition and cancer stem cells and how we can induce cancer cells to differentiate into benign stromal fibroblasts in response to certain chemotherapy drugs.

Keywords

High-grade ovarian serous cancer; Cancer stem cell; Epithelial-mesenchymal transition

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1. Introduction

Epithelial ovarian carcinoma (EOC) accounts for about 90% of all ovarian malignancies, and most patients with EOC (70% or more) have advanced-stage disease at presentation [1-2]. Although approximately 80% of individuals with EOC respond to primary neoadjuvant or adjuvant chemotherapy, 60%–80% of those who initially respond subsequently experience disease recurrence and a poor treatment response rate (~15%) [3]. In most cases, the recurring disease becomes resistant to chemotherapy, after which patients typically survive only 16–21 months, with death a consequence of metastatic spread [4].

Rather than existing as a single entity, EOC appears to be a group of distinct diseases that can be further classified into the following five histological subtypes: serous, endometrioid, mucinous, clear cell, and undifferentiated carcinomas [5-7]. Among these subtypes, high-grade serous carcinoma (HGSC) usually represents the archetypical EOC and is responsible for the highest fatality rate. This review highlights our current understanding of epithelial-to-mesenchymal transition (EMT) and ovarian cancer stem cells (CSCs) in the context of cancer biology and drug resistance in HGSCs.

2. Origin of ovarian HGSC

It has been long accepted that all EOCs are derived from the ovarian surface epithelium (mesothelium) (Fig. 1A) and that subsequent metaplastic changes lead to the development of the various cell types—serous, endometrioid, and mucinous cells—that morphologically resemble the epithelium of the fallopian tube, endometrium, gastrointestinal tract, or endocervix, respectively. However, this theory has a number of limitations [8]. Evidence from the past decade suggests that some human HGSCs may originate from secretory epithelial cells of the fallopian tube (Fig. 1B). Almost all HGSCs often have a mutation in the *TP53* gene and mutation and functional loss in approximately 50% of *BRCA1* and *BRCA2* genes [9-11] but rarely harbor *BRAF*, *KRAS* that are common in low grade serous carcinoma. Until recently, early cancerous or precancerous lesions in the fallopian tubal fimbria—serous tubal intraepithelial carcinoma (STIC)—had been identified in women harboring the *TP53* mutation as well as *BRCA1* or *BRCA2* dysfunction in sporadic pelvic serous carcinoma (Fig. 1C-1F) [10, 12-15]. On the basis of these studies, the fallopian tube has emerged as an important source for ovarian or pelvic HGSC (Fig. 2A-2D). Several excellent reviews on this topic have been published, and readers are advised to refer to them for details [15-16].

3. Definition of cancer stem cells

The concept that a rare population of cells with embryonic stem cell–like properties is the source of tumors or of post-therapeutic tumor recurrence was proposed a century ago [17]. The finding that only certain cells are capable of initiating, maintaining, and promoting the development of tumors strongly supports the CSC hypothesis. The modern revival paradigm of the CSC hypothesis was suggested by Spangrude and colleagues [18] who isolated multipotent hematopoietic progenitor cells and demonstrated that not all human cancer cells xenografted into immunocompromised animals had the same ability to initiate tumors. At present, CSC properties, as postulated by the American Association for Cancer Research, include tumorigenic or self-renewal capacity, the potential for multilineage differentiation, the ability to participate in serial passage, and expression of a unique repertoire of surface markers that allows for their reliable identification and purification [19].

The origin of the CSC has long been debated, particularly whether CSCs are somatic stem cells that have undergone malignant change or whether they are more differentiated cells that return to “stem” status as part of malignant transformation. A common misperception

that continues to pervade both oral and written discussions is that the CSC must have arisen from a normal stem cell. Normal stem cells are the likely targets of mutagenesis, leading to the formation of CSCs, since they already possess active self-renewal pathways, whereas induction of self-renewal genes is required to transform differentiated cells. Furthermore, normal stem cells are the only cells with a lifespan long enough to accumulate the genetic mutations that lead to tumorigenesis. However, experimental cancer models have clearly determined that CSCs are not necessarily derived from normal stem cells [20]. Growing evidence suggests that there are two potential pathways: loss of growth regulation in a normal stem cell and a mature somatic cell (non-stem cell) acquiring the properties of self-renewal [21].

3. Common methods for enriching CSCs

Current methods for determining which cell populations from solid tumors are CSCs have varied across laboratories. Presently, the single most critical factor in identifying CSCs is their ability to regenerate the parental tumor upon generating a tumor on a secondary site, as demonstrated by serial passage in xenotransplantation models. On the other hand, the most frequently used methods are fluorescence-activated cell sorting (FACS) and/or magnetic-activated cell sorting (MACS) using specific antibodies directed against one or several stem cell-like surface biomarkers [22-24]. Hoechst dye efflux sorting of the side population is also commonly used for CSC selection [25-27]. The subpopulation of cells with side population features demonstrated self-renewal activity, differentiated progeny production, tumorigenicity and invasiveness, as well as the capability to express CSC markers and the presence stem cell genes [28-30]. The side population phenotype was associated with overexpression of ATP-binding cassette (ABC) transporters that can pump several types of endogenous and exogenous metabolites and drugs out of the cell [31-32].

Another interesting characteristic of CSCs is that they can be identified in tumors by their ability to grow in spheres, also known as tumor spheres. CSCs from epithelial organs can be expanded as sphere-like cellular aggregates in serum-free medium containing epidermal growth factor and basic fibroblast growth factor [33-40], which is a method derived from the culturing of neural stem/progenitor cells [41]. In these spheres, only 4% to 20% of the cells are stem cells; the others are progenitor cells in various phases of differentiation [42-43]. Even though enriching the CSC population by sphere formation may not be suitable for many solid tumors, this method is applicable to ovarian CSCs [44-47]. CSCs have been identified in hematopoietic and solid malignancies including those of the breast, central nervous system, lung, prostate, pancreas, colon, and liver and in melanoma and primary squamous cell carcinoma of the head and neck [35-36, 48-54].

4. Surface markers for enrichment of CSCs and ovarian CSCs

CD133 has been considered a normal stem cell marker and has been widely used as a CSC marker in many solid malignancies [55]; however, researchers disagree about its ability to identify CSCs in colon cancer [37, 51, 55-56], central nervous system tumors [54, 57-58], and other malignancies. It has been shown that CD133-glioma cells were tumorigenic by implanting these cells into the brains of immunodeficient mice [59], which resulted in tumors containing CD133+ cells. Furthermore, it has been demonstrated that C6 gliomas contained only a few CD133+ and NESTIN+ cells, but these cells were composed mainly of CSCs [60-61]. Other studies confirmed that both CD133+ and CD133- subpopulations contained CSCs in lung cancer [62] and melanoma [63-65]. Indeed, there is great disagreement about the usefulness and reproducibility of some common CSC markers for various tumor types.

Ovarian CSCs have been found to be associated with the following phenotypes: CD133+, ALDH+, CD44+/CD117+, CD44+/MyD188+, and CD24- [66]. It has also been reported that CD133+ cells derived from ovarian tumors were capable of self-renewal and were associated with increased tumor aggression in xenografts [67]. Another study found that expression of CD133 was higher in primary ovarian cancer than in normal ovaries or benign ovarian tumors, but was low in metastatic ovarian tumors [68]. Therefore, CD133-expressing tumors had no effect in defining the functional criteria of CSCs although CD133+ ovarian tumor cells were found to have higher proliferative and clonogenic capabilities than did CD133- cells.

Other investigators have used ALDH as a potential marker for ovarian CSCs. It has been shown that ALDH+ cells were chemoresistant and preferentially grew tumors, compared with ALDH-cells in ovarian cancer cell lines [69]; furthermore, ALDH+, CD133+, and ALDH+CD133+ cells formed three-dimensional spheres more efficiently and developed larger tumors more rapidly than did their negative counterparts [45]. This property was preserved in the xenografted mice model. Although CD133 initially may have appeared to be a weak candidate marker for ovarian CSCs, increasing evidence has suggested that CD133+ combined with ALDH+ cells may have increased sensitivity for enriched ovarian CSCs.

CD44 is another commonly used CSC marker that has been combined with other CSC markers for enrichment of ovarian CSCs. It has been shown that only 100 CD44+CD117+ cells could serially propagate their original tumors, whereas 1×10^5 CD44-CD117- cells remained nontumorigenic in a xenografted mouse model [70]. It has also been shown that 1×10^3 CD117+ ovarian cancer cells were able to regenerate tumors in a mouse model and that these cells had 100 times more tumorigenic potential than did CD117- cells [71]. A combination of CD44+ and CD24- has been used as a potential marker to select potential ovarian CSCs. It has been reported that CSCs isolated from ascites and solid tumors were characterized by CD44+, MyD88+, constitutive NF- κ B activity, and cytokine and chemokine production [72]. CD44+/CD24- cells were shown to have a 60 times higher level of matrigel invasion in both the SKOV3 and OV90 cell lines than did other phenotypes [73]. This phenotype also had 71% to 93% higher resistance to all chemotherapy agents in all cell lines compared with baseline and was more likely to recur (83% vs 14%; $P = 0.003$), with shorter median progression-free survival while crossing the threshold of 25% CD44+/CD24- ovarian cancer cells.

In our laboratory, we performed immunohistochemical staining of human ovarian carcinoma samples for some common CSC markers including CD133, sex-determining region Y-box 2 (SOX2), ALDH1, and CD44, with use of tissue microarray. We found that expression of CD133 and SOX2 was associated with HGSC and advanced disease stage as well as shorter overall and/or disease-free survival durations. Moreover, CD133 expression was associated with non-response to chemotherapy [74-75] (Fig. 1G, 1H). Similarly, CD44 expression was associated with HGSC and advanced disease stage, but not with survival time (Zhang et al., Human Path, in press). Our data suggested that the combined biomarkers to purify CSCs in ovarian carcinoma may be more reliable than the use of a single antibody. However, we did not find that ALDH1 expression alone was correlated with worse prognosis in patients with ovarian cancer [76]. It remains to be determined if ALDH1 in combination with other markers can increase the specificity of other markers in the enrichment of cancer stem cells.

Taken together, the existing data have shown conflicting results regarding the specificity of markers used in defining CSCs. Thus far, no CSC markers are available for specific identification of CSCs in all solid tumors. Inconsistent results have been reported from various laboratories that have used a variety of methods to separate ovarian CSCs from non-

stem cells, and the lack of precise information on specific biomarkers of CSCs remains a major challenge in the field of ovarian CSC research. The current major methodologies for human ovarian CSC propagation or isolation are summarized in Table 1.

6. Ovarian CSCs

Despite of popular assays in using markers for enrichment of CSCs, functional assays have been used to demonstrate CSCs in ovarian cancer. Bapat and colleagues [66] identified clonogenic cells isolated from ascites from a patient with EOC without using surface markers. This small subpopulation of cells possessed stem cell-like properties including propagating as multilayered spheroids in serum-containing media, expressing several markers of pluripotency, generating differentiated progeny *in vitro*, forming xenograft tumors *in vivo*, and serially transplanting in nude mice. Other investigators have isolated CSCs through drug selection [77]. Using cisplatin and paclitaxel, two widely used chemotherapeutic drugs, a subpopulation forming nonadherent spheres was isolated from the SKOV3 human ovarian cancer cell line and characterized by microarray analysis with respect to tumorigenic potential, expression of cell surface antigens, and of a panel of stem cell genes. The self-renewing isolated sphere cells were found to display stem cell properties (e.g., expressing high levels of several stem cell genes, such as Nanog, Oct4, SOX2, nestin, ABCG2, CD133, and CD117), to be highly tumorigenic, and remarkably, to be resistant not only to cisplatin and paclitaxel but also to adriamycin and methotrexate. This resistance property renders the sphere cells a suitable assay with which to test chemotherapeutic protocols in order to identify an efficient strategy for killing them. In fact, as recently reviewed [78], CSC isolation and characterization from ovarian cancer is extremely important to developing therapies against this malignancy. In particular, the isolation of ovarian CSCs may facilitate the search for mechanisms of intrinsic drug resistance [78].

7. Mechanisms of drug resistance in CSCs

Several mechanisms have been reported to generate resistance to therapy in ovarian CSCs. Drug penetration resulting in further resistance to DNA damage is one such mechanism of resistance in ovarian CSCs. The DNA integrity defense system in normal stem cells is the relatively high expression of efflux transporters from the ATP-binding cassette (ABC) gene family. These pumps allow normal stem cells to preserve their genome more effectively against chemical mutagens in an attempt to prevent carcinogenesis. Ovarian CSCs, however, may derive resistance to DNA damage from the preexisting DNA repair systems in normal stem cells; CSCs may also derive resistance to chemical mutagens (e.g., chemotherapy) through the expression of drug efflux pumps in normal stem cells from which they were derived [79-80]. In one experiment, Piwil2, alias mili in mouse or Hili in human, played an important role in stem cell self-renewal, RNA silencing, and translational regulation in various organisms and was a key factor in regulating chromatin modifications, especially in response to cisplatin. The overexpression of Piwil2 in some cancers could lead to cellular cisplatin resistance, possibly due to enhanced chromatin condensation affecting normal DNA repair [81].

Another mechanism involved in generating resistance to therapy is ovarian CSCs' tendency to resist apoptosis. It has been shown that ovarian CSCs are resistant to tumor necrosis factor alpha-mediated apoptosis [72] and that EMT is required for the development of resistance to paclitaxel in ovarian cancer cells [82]. This process is orchestrated through the acquisition of a novel subset of gene targets that is repressed under conditions of stress, effectively inactivating p53-mediated apoptosis and acquiring a stem cell-like phenotype in ovarian cancer cells. The transcriptional repressors snail and slug are critical to cancer cells' acquisition of stem cell characteristics, specifically those enabling resistance to

chemotherapy-mediated cellular stress, and this may be a determinative aspect of aggressive cancer metastases.

The microenvironment of ovarian CSCs contributes to drug resistance. Oxygen is a well-known chemotherapy-sensitizing agent because of its ability to form chemotherapy-induced reactive oxygen species that can damage DNA. Unexpectedly, it was discovered that CSCs reside along perivascular areas [83] and are thus likely to be well oxygenated [84]. Lis et al. [85] demonstrated that both cancer-associated and bone marrow-derived mesenchymal stem cells displayed a strong protective effect by inducing thermotolerance in ovarian cancer cells. Specifically, stroma-derived factor 1 alpha (SDF-1/CXCL12) induced this thermotolerance, and inhibition of CXCL12/CXCR4 interaction restored cytotoxicity of hyperthermia in co-culture experiments. Tumor-associated and bone marrow-derived mesenchymal stem cells were co-cultured with CXCL12-induced thermotolerance. It was found that exosomes from ovarian cancer cells induced the myofibroblastic phenotype and functionality in adipose tissue-derived mesenchymal stem cells by activating an intracellular signaling pathway [86], although the activated pathway differed from exosome to exosome. Ovarian cancer-derived exosomes contributed to the generation of tumor-associated myofibroblasts from mesenchymal stem cells in tumor stroma.

8. EMT as a potential mechanism for generating ovarian CSCs but also benign stromal fibroblasts

EMT, a basic process in the morphogenesis of various fetal tissues during embryogenesis [87], is defined as the loss of epithelial traits by the former epithelial cells with the acquisition of mesenchymal characteristics, such as invasive motility and the presence of vimentin and myosin [88]. EMT has been recently associated with the generation of cancer stem cell-like cells [89]. The EMT characteristic [90-93] of increasing cell motility and invasion [91] that results in tumor metastasis [94], drug resistance [95], and tumor recurrence is tightly linked with CSC biology [96-103]. A series of studies determined that induction of EMT could generate cells with the properties of stem cells [89, 104]. On this point, there is a high degree of plasticity between CSCs and non-CSCs, with the possibility that CSCs generate non-CSCs and vice versa. The EMT processes in malignant cells are triggered by various signals, some of which are released by the tumor stromal cells [105]. Transforming growth factor-beta, platelet-derived growth factor, fibroblast growth factor, hypoxia, and noncoding RNAs can act independently or in concert to trigger the EMT processes [106-110]. In the tumorigenic cell population obtained by Morel et al. [111] from premalignant breast cells, the stem cell-like phenotype was associated with features reminiscent of EMT, establishing a link between CSCs and EMT.

It has been shown that transforming growth factor-beta induced a tissue transglutaminase 2-regulated EMT process, formation of spheroids, and induction of ovarian cancer metastasis [112]. Knockdown of tissue transglutaminase 2 in ovarian cancer cells enhanced ovarian tumor metastasis by decreasing the number of cells with a CSC phenotype (CD44+/CD117+). It has also been shown that hyperactive epidermal growth factor receptor signaling prompted ovarian cancer cells to resist cisplatin and to recur because of the EMT processes caused by activation of both Stat3 and Jak-Stat3 [113]. Cisplatin-induced EMT and its associated increase in residual OVCA 433 cell migration has been found to increase the expression of CSC markers (e.g., CD44, α 2 integrin subunit, CD117, CD133, EpCAM, Nanog, and Oct4) and mesenchymal markers (e.g., N-cadherin and vimentin) and decrease the expression of epithelial markers (e.g., E-cadherin) [114]. Moreover, additional studies demonstrated the important roles of the pituitary tumor transforming gene, disabled homolog 2, semaphorin 3E, miR-187 and 429, endothelin-1/endothelin A receptor in EMT processes associated with enhancement of mammosphere-forming ability, tumor-initiating

capacity, the outgrowth of tumor *in vivo*, metastases, and drug resistance of ovarian cancer [115-120].

These findings strongly suggest that the induction of EMT could generate ovarian cancer stem cell-like cells, although the detailed molecular mechanisms are not fully understood. It has been shown that high-mobility group AT-hook 2 (HMGA2) exhibited overexpression and coexpression with p53 in more than 50% of patients with HGSC [105]. The introduction of *HMGA2* overexpression was sufficient to induce ovarian surface epithelial transformation *in vitro* and further tumor formation in the xenografts of nude mice. Silencing HMGA2 resulted in decreased aggressiveness in the ovarian cancer phenotype. HMGA2-mediated tumorigenesis was associated with EMT-related target genes and microRNAs. Therefore, it is possible that HMGA2 contributes to the formation of HGSC through the modulation of EMT genes and that EMT may play a key role in the formation, migration, and location of serous tubal intraepithelial carcinoma and its transformation into invasive serous carcinoma [119, 121].

Despite the generally accepted wisdom that EMT is associated with not only tumor invasion, dissemination, and metastasis, and resistance to therapy, our recent data suggest that EMT may also play a role in generating benign fibroblasts as part of the stroma in response to the chemotherapy drug paclitaxel. We showed that SKOV3 cells with paclitaxel can differentiate into benign fibroblasts that have a totally different morphology from their parental cells (SKOV3 cells); we also showed that SKOV3 cells may behave like benign fibroblasts, displaying slow proliferation, low invasion, and weak tumor-formation ability via the PI3K/Akt pathway [122] (Fig. 3), suggesting that EMT may play an important role in generating both a malignant and a benign phenotype of cancer cells. We further demonstrated that stem cell-like cells can be generated through formation of polyploidy giant cancer cells (PGCCs) through an evolutionally conserved cell life cycle [123], these PGCCs are highly resistant to hypoxia-induced stress and showed increased resistance to cisplatin and therefore offer new therapeutic targets for intervention.

9. Potential strategies for drug-resistant CSCs

Although many questions remain regarding CSCs, the isolation and further characterization of these cells may open up new perspectives in clinical oncology. Isolation of CSCs from cancer cell lines after drug management can mimic what happens *in vivo* during chemotherapy, thus making selected cells particularly appropriate for investigating drug-resistance mechanisms and for testing new therapeutic agents. A future challenge for the use of CSCs cultured *in vitro* will be the development of culture conditions favoring the maintenance of their stem cell-like properties and, ideally, simulating the growth and differentiation inhibitory signaling provided by the microenvironment.

If CSCs play a critical role in chemotherapy resistance, an appropriate strategy for screening CSC markers may be comparing the differences in primary and chemotherapy-resistant specimens [125-128]. Our data provide another possibility as well—that SKOV3 cells that are resistant to paclitaxel are a benign phenotype and should receive more attention (Fig. 3). The drug-resistance and recurrence-associated markers should be examined cautiously. Successful cytoreductive surgery and chemotherapy can destroy a niche of CSCs, thus preventing recurrence to a certain extent, and together form the basis of CSC targeted therapy [122]. This combination of treatments, which target both subpopulations, would therefore be essential to preventing tumor regrowth and relapse. Maintaining or consolidating CSC targeted therapy may provide a treatment option in future trials for patients after primary therapy that includes cytoreductive surgery and standard chemotherapy.

10. Conclusions

The high recurrence rate of EOC suggests that there is a subpopulation in which tumors are able to repopulate by cytoprotective mechanisms, allowing tumor cells to survive and eventually to resist therapy. Gaining an understanding of CSCs opens a new path in tumor treatment. Today, CSCs as a novel drug target may be designed to the signaling pathways associated with self-renewing, niche factors, drug transporters, surface functional molecules, and differentiation of ovarian CSCs into drug-sensitive descendants. Identification of specific EOC molecular marker targets suggests the promise of personalized therapy of this highly deadly disease. However, considering the elusive nature of CSCs and lack of specific methods to define them, this task may represent a challenge. More profound studies and more practical experimental models are needed to resolve the significant gaps in CSCs and clinical fields.

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Fig. 1. Hematoxylin and eosin (H&E) staining in normal ovarian (A), normal fallopian tube (B), ovarian high-grade serous carcinoma (HGSC) (C), HGSC in situ in the fallopian tubal lumen (D, *black arrows*), and HGSC in situ in the fallopian tube (E). Immunohistochemical staining shows strongly positive expression of p53 in the fallopian tubal epithelial cells with HGSC in situ (F), CD133-positive expression (G), and SOX2-positive expression (H) in ovarian HGSC tissues.

Fig. 2.

Proposed tumorigenesis of human ovarian high-grade serous carcinoma (HGSC), and related molecular alterations: (A) fallopian tube secretory epithelial (FTSE) cells; (B) serous tubal intraepithelial carcinoma (STIC; most STIC was found in the distal ends of the fallopian tubes [fimbria]), known as precursor lesions; (C) invasive papillary serous carcinoma (PSC) from fimbria; (D) dissemination of ovarian carcinoma into peritoneal cavity.

Fig. 3. Epithelial-mesenchymal transition (EMT) promotes both malignant and benign phenotypes together with the associated pathways. (A) Traditional view of EMT associated with aggressive malignant phenotype; (B) the gain of potential benign characteristic through EMT.

Table 1

Current methods for isolation of human ovarian cancer stem cells

Selection method	Original source	Surface marker	Selection method	Characteristics of isolated CSCs	Chemo-sensitivity	Reference
Sphere formation	Ovary, ascites	Not tested	Sphere formation; xenograft; serial transplantation	Increased tumor-initiation ability	Not tested	(66)
	Cell line	CD44+	Sphere formation	Up-regulation of genes associated with EMT, decreased susceptibility to apoptosis	Not tested	(44)
	Ovary tumors	CD133+/CD117+	Sphere formation; xenograft; serial transplantation	Genes related to cell communication, cell-cell adhesion, cellular development, and extracellular matrix expressed differently	Drug resistance	(47)
Cell line	CD44+/CD24-	Sphere formation; xenograft; serial transplantation	Increased expression of stemness	markers; increased tumor-initiation ability	Drug resistance	(124)
	Ovary	CD44+/CD117+	Sphere formation; xenograft; serial transplantation	Increased tumor-initiation ability	Drug resistance	(125)
	Cell line	CD133-	Sphere formation; xenograft; serial transplantation	Increased expression of stemness markers; increased tumor-initiation ability; karyotype alterations	Not tested	(126)
Side population sorting	Cell line	Not tested	Serial transplantation	Increased tumor-initiation ability and extensive cell proliferation	Drug resistance	(80)
	Ovary, ascites, cell line	Not tested	Sphere formation; xenograft; serial transplantation	increased tumor-initiation ability	Sensitive to interferon alfa	(127-128)
Stemness cell surface markers	Ovary, ascites	CD133/CD 44/CD24/EpCam	Sphere formation; xenograft; serial transplantation	Increased tumor-initiation ability	Drug resistance	(23)
	Ovary	CD117/CD 44	Sphere formation; xenograft; serial transplantation	Increased tumor-initiation ability; resistance to chemotherapy	Drug resistance	(70)

Selection method	Original source	Surface marker	Selection method	Characteristics of isolated CSCs	Chemo-sensitivity	Reference
	Ovary, ascites	CD133	Sphere formation; xenograft; serial transplantation	Neoangiogenesis	Not tested	(129)
	Ovary, ascites, cell line	CD44/Myd88	Sphere formation; xenograft; serial transplantation	Neoangiogenesis	Drug resistance	(72, 130)
	Ovary	CD133	Sphere formation; xenograft; serial transplantation	Increased tumor-initiation ability; fewer metastases	Not tested	(68)
	Cell line	CD133	Sphere formation; xenograft; serial transplantation	Increased tumor-initiation ability	Drug resistance	(67)
	Cell line	ALDH	Sphere formation; xenograft; serial transplantation	Resistant to chemotherapy; poor clinical outcomes	Drug resistance	(131)
	Cell line	ALDH	Xenograft; serial transplantation	Increased tumor initiation ability; chemoresistance; poor outcome	Drug resistance	(132)