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Ovarian Cancer Stem Cells: Are They Real and Why are they Important?

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Abstract

The cancer stem cell hypothesis has been put forward as a paradigm to describe varying levels of aggressiveness in heterogeneous tumors. Specifically, many subpopulations have been clearly demonstrated to possess increased tumorigenicity in mice, broad differentiating capacity, and resistance to therapy. However, it is still not clear the extent to which these experimental findings are potentially clinically significant. This review will describe the principles of this emerging hypothesis, ways in which it may be appropriate in ovarian cancer based on the clinical course of the disease, and how we might exploit it to improve outcomes in ovarian cancer patients.

Introduction – What is the Cancer Stem Cell Hypothesis?

Ovarian cancer is the most lethal gynecologic malignancy in the United States. While recent advances in chemotherapy agents, administration, and dosing have yielded modest improvements in overall survival and quality of life, durable cures have not significantly increased (1). Although deadly, ovarian cancer is actually one of the more chemosensitive solid malignancies. A patient presenting with advanced stage disease will have a 50-70% chance of having a complete clinical response after surgery and chemotherapy, and a 5-year survival rate of 40-50%. By comparison, rarely do patients with advanced stage colorectal or pancreatic cancer have a complete clinical response after surgery and chemotherapy and just 28% and 2% of these patients will survive 5 years, respectively (2, 3). The clinical course of ovarian cancer and the increasing knowledge about the heterogeneity of malignant cells (4) emphasize that there are distinct cellular populations within a tumor. While most ovarian cancer cells are initially chemosensitive, there is a population of cells that survives initial therapy, only to later grow to a clinical recurrence. Although responses are frequently seen even in the setting of recurrence, virtually all patients will ultimately succumb to disease that has become resistant to all known cytotoxic or biologic therapies. If durable cures are to be

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achieved in ovarian cancer, there will be a need to determine what allows these small subpopulations to survive.

One model by which to explore ovarian cancer tumor heterogeneity is the cancer stem cell hypothesis. This idea proposes that within a heterogeneous ovarian tumor, there are small populations (generally less than 2% of cells) that have increased tumorigenicity and differentiating capacity than other cells (5). This subpopulation, like normal stem cells, gives rise to more differentiated progeny that comprise most of the ovarian tumor mass and are more responsive to chemotherapy. Cancer stem cell populations have been shown in some respects to be more tumorigenic in multiple experimental systems, including increased rates of tumorigenicity, metastasis, invasion, angiogenic stimulation, and resistance to therapy (6, 7). While variability of nomenclature within the field has led to some confusion, with cells often (appropriately) described by their function, such as tumor initiating cells (TICs), cancer initiating stem cells (CICs), “stem-cell-like” cells, or therapy-resistant cells (TRCs), this population will be referred to as cancer stem cells (CSCs) for consistency. The latter term has evolved to hold greater meaning than the purely experimentally-derived former terms, specifically that CSCs not only carry increased tumorigenicity, but also may be responsible for tumor initiation, metastatic disease, and resistance to therapy. Although numerous experiments demonstrate such a privileged population can be isolated, the scope of their importance is sometimes speculated upon. Excitement regarding the cancer stem cell hypothesis lies in its potential: if indeed the population surviving initial therapy (CSCs or other) can be isolated, perhaps the biologic pathways on which they depend for resistance can be identified and targeted, killing all – instead of most – malignant cells with primary therapy. But such excitement may need to be tempered with the current limitations of the hypothesis, including the methods in which they are often studied, and the scope of populations identified.

Why Do Cancer Stem Cells in Ovarian Cancer Make Sense?

The concept of ovarian cancer as a stem-cell disease is logical for many reasons. The most compelling argument is the most important – the clinical course. Most cells in a presenting advanced tumor are chemosensitive, but almost all patients will recur after growth of a resistant population. The fact that most patients at first recurrence will respond to secondary therapy implies that this recurrent tumor is again composed of a heterogeneous population of chemosensitive and chemoresistant cells, suggesting a differentiating capacity in the initial surviving population. Beyond the clinical course, additional pathologic characteristics support the hypothesis. The ovarian surface epithelium (OSE) is genetically more mesenchymal and less differentiated than other epithelial cells, as evidenced by the preferential expression of N-cadherin over E-cadherin (8, 9). Bowen and colleagues demonstrated through gene expression profiling that human OSE exists in an arrested state, similar to somatic stem cells (10). This global mesenchymal state may explain how ovarian epithelial stem cells can be present in the normal ovarian surface epithelium despite a lack of convincing evidence that the OSE gives rise to more differentiated structures in the normal ovary (in contrast to normal stem cells of the breast and colonic epithelium). The CSC hypothesis may be even more applicable if the fallopian tube is the origin of many ovarian cancers, since the epithelial lining of the fallopian tube certainly represents tissue in which

there is high turnover and normal stem cells giving rise to differentiated (ciliated) cells would be expected. In fact, prominent expression of stem cell genes has been identified in both OSE (11) and fallopian tube epithelium (12). Additionally, epithelial ovarian cancer encompasses numerous histologic phenotypes, including papillary serous, endometrioid, clear cell, and mucinous subtypes, suggesting a multipotent differentiating capacity. While in many cases these subtypes may have different cells of origin, such as fallopian tube giving rise to papillary serous tumors and endometriotic implants producing endometrioid or clear cell carcinomas, these lineages are not likely absolute. The high rate of multiple “mixed” histologies within the same tumor suggests either a common cell of origin with capacity to differentiate into several phenotypes or multiple CSC phenotypes.

Although we know that tumor heterogeneity is profound, it is still unclear if all of the resistant clones are present within the original tumor and initially identifiable, if they are induced by administration of cytotoxic therapy and change in microenvironment, or a combination of both of these mechanisms. Numerous groups have isolated putative cancer stem cells from primary tumor specimens and demonstrated chemoresistance and self-renewal of this population, suggesting that these cells are present before chemotherapy is given. Lineage tracing experiments done in colonic adenomas support this hypothesis, demonstrating that stem cells exist in a quiescent state but stochastically expand in response to evolution or microenvironment stressors (13). Although some models have shown that “stemness” can be induced by stressors such as chemotherapy, it seems unlikely that full chemotherapy resistance is induced by administration of primary therapy. In other gynecologic malignancies, such as gestational trophoblastic disease and ovarian germ cell tumors, durable cures are frequently obtained with similarly aggressive and toxic chemotherapeutic regimens. If the population of cells that causes recurrence were induced by chemotherapy, it follows that relapses would be noted at a similar rate in all treated malignancies. While it is possible that cells responsible for recurrence arise from a mutagenic effect of therapy, this does not explain why most recurrent tumors are histologically and genetically very similar to the primary tumor.

Alternatively, there is growing evidence that cell plasticity allows non-CSCs to gain a CSC phenotype. Cobaleda and colleagues found that mature B cells dedifferentiated to an aggressive lymphoma composed of pro-B cells when Pax5, a gene associated with lineage commitment, was deleted in a murine model (14). Xie et al. described the reprogramming of mature B cells into macrophages by retroviral-forced expression of C/EBP α , a transcription factor involved in lymphoid differentiation (15). While the majority of work has been done in hematopoietic cells, emerging data suggests that differentiated respiratory epithelial cells exhibit similar plasticity in the presence of injury (16).

Whether cancer stem cells are a very small, but present, population of cells prior to any therapy, develop in response to internal or external stimuli, or are a population comprised of cells derived from both etiologies is unclear. Regardless of origin, the preponderance of evidence suggests that they are associated with recurrence, therefore agents specifically targeting these cells may need to be included in upfront or maintenance therapy or both to minimize the risk of recurrence.

Early Evidence and Definitions of the Cancer Stem Cell

Lapidot and colleagues first isolated a tumorigenic stem cell population in acute myeloid leukemia in 1994, where a single cell was found to completely reinitiate leukemia in mice (5). Interest was kindled in solid tumors when populations with increased tumorigenicity were isolated from breast and GBM cancers (17, 18). Multiple experimental models have been used to potentially isolate putative cancer stem cells (Table 1). (19) Bapat and colleagues were one of the first groups to demonstrate heterogeneous growth properties in ovarian cancer cells in 2005 (20). They isolated unsorted ascitic cells from a patient and developed 19 spontaneously immortalized clones through low-density culturing. Of these, only two could be passaged sequentially into nude mice. The tumors that formed from these single clones closely resembled the tumor from which they were originally isolated, and had increased expression of stem cell mediators (Nestin, Nanog, and Oct4). Growth in spheroids, whereby single cells are plated in serum-free media (preventing differentiation) and agar-coated or charged plates (preventing attachment and laying down of an extracellular matrix) creates 3-dimensional balls of cells that have multipotentiality. (21) While these spheroids often are more resistant to cytotoxic therapies, it has been difficult to delineate to what degree this is due to the properties of the cells themselves, or other factors such as decreased penetration of drug or increased production of survival factors mediated by tightly-bound cells. It is now generally agreed upon that a cancer stem cell must have certain characteristics: 1) increased tumorigenicity in xenograft models, whereby isolated CSCs require 100-1000-times fewer cells injected in a mouse to establish a tumor; 2) unlimited self-renewal, generally demonstrated by maintenance of tumorigenicity after multiple passages; and 3) pluripotency, whereby tumors that form after CSC injection are composed of both marker-positive and marker-negative cells (22). It is preferable that cells initially isolated and sorted by the marker of interest are directly from a patient sample. This has proven challenging, as the process of separating a solid tumor into single cells by mechanical and/or chemical dissociation can be traumatic to the cells if not performed carefully and with well-organized coordination with clinicians, especially when the cells of interest comprise a small percentage of the total tumor.

Which Populations Represent Ovarian Cancer Stem Cells?

Attempts at identification of the CSC population began by isolating cells by markers shown to have CSC properties in other tumors. The evidence behind these markers has been well-described in other review articles (23) and is summarized in Table 2. Some markers are based on functional assays, such as the “side population” (SP), a population of cells expressing ABCG2 (aka breast cancer resistance protein-1); or the ALDEFLUOR assay, which identifies cells with active ALDH1A1 enzyme. This same ALDEFLUOR assay is commonly used to isolate bone marrow stem cells for stem cell transplants (23). Other isolated populations are based on surface expression of proteins, such as CD133, CD44, and c-kit. CD44 is a receptor for the extracellular matrix component hyaluronic acid, which after binding activates several intracellular survival pathways (24). C-kit is the receptor for stem cell factor (SCF) that has represented one of the more significant treatment successes in cancers with clonal overexpression of c-kit (25). Interestingly, the protein most consistently identifying a CSC across different tumor types, CD133, is perhaps least well understood in

its functionality. In some cases, increased expression of these populations is associated with poor survival (26-28), but this has not consistently been the case (29, 30). These studies have been conducted in a variety of cell types including patient tumor samples, patient ascitic samples and cancer cell lines, which may contribute to the heterogeneity of the results. It should be noted that it is unknown if timing of CSC isolation (ie, before or after surgery or other therapy) affects the ability to isolate, or even the very characteristics of, the population. Indeed, exposure to such stressors of tissue processing or exposure to chemotherapy may induce some stem cell pathways as survival mechanisms. It is for this reason that studies in multiple settings (ie use of both in vitro and ex vivo methods) and by several investigators allows the greatest confidence that a putative population does have stem cell properties.

Clinical Significance of Cancer Stem Cells

Investigators have successfully identified several cell subpopulations that exhibit aggressive features in experimental *ex vivo* conditions, but mediators of tumorigenicity in immunocompromised mice are likely different than mediators of chemoresistance in women with ovarian cancer. An important question remains: are these populations also playing a role in outcomes and chemoresistance in patient tumors?

A few studies have examined patient samples to begin to address this question. Correlation between density of CSCs and poor outcome have been observed in the side population (31) and with expression of CD44 (30), CD133(27, 29), or ALDH1 (26). Aktas et al. compared blood samples from healthy patients and patients with metastatic breast cancer, looking for circulating tumor cells (CTC) positive for markers of epithelial-mesenchymal transition (EMT) and ALDH1 (32). They theorized that circulating tumor cells are likely responsible for metastasis and that they have characteristics similar to cancer stem cells. They found that healthy women were negative for circulating breast tumor cells with expression of ALDH1, the EMT marker EpCam, MUC-1, and HER2 transcripts. Not all breast cancer samples were positive for CTCs, but in those that were, there was a correlation between expression of EMT markers and ALDH1 with response to treatment. 74% of women who did not respond to treatment were found to have EMT markers, ALDH1 or both, while only 10% of responders had the same profile. This suggests that circulating tumor cells with stem cell markers have prognostic value. Iinuma and colleagues performed a similar analysis on CTCs in patients with colorectal cancer, using the markers CEA, cytokeratin 19, cytokeratin 20, and CD133 (33). They found that expression of one or several of these markers positively correlated with increasing Dukes' stage as well as colon cancer recurrence after adjuvant chemotherapy. While definitive conclusions regarding the relationship between CTC and cancer stem cells cannot be made from these data, they strongly suggest that a subpopulation of CTCs with stem cell markers exists and elevated levels correlate with poorer clinical outcome.

In ovarian cancer, Rizzo and colleagues examined cells in malignant ascites for the percentage of side-population cells. Ascites from patients who had recurred after first-line platinum therapy was enriched with side-population cells as compared to ascites from chemonaïve patients. Sequential ascitic samples from 3 patients who recurred showed a

progressive increase in the percentage of side population cells, implying that cytotoxic agents select for side-population cells (31).

One method to examine a subpopulation for an association with chemoresistance is to compare pre- and post-treatment specimens for changes with the exposure. Kulkarni-Datar showed that CD133/Sca-1 cells persist in tumors treated with carboplatin and paclitaxel treatment, and maintain their tumor-initiating properties (34). Steg et al. measured expression of stem cell markers in a cohort of matched primary and recurrent tumor patient samples, on the premise that if CSCs are an important population to target, they should represent a denser population within recurrent chemoresistant tumors (35). Tumors taken at the time of primary cytoreductive surgery showed significant heterogeneity in expression of ALDH1A1, CD44, and CD133, but persistent tumors collected soon after treatment with chemotherapy showed a much higher density of these markers. Other pathways postulated to be important in maintenance of “stemness” such as TGF-beta, Notch, Wnt, and Hedgehog were also upregulated in the recurrent samples as compared to their matched primary samples. While the recurrent patient samples did not show uniform increases in all stem cell markers, all samples showed an increase in at least one marker, once again suggesting that the ovarian CSC population may be quite heterogeneous between patients. Interestingly, in recurrent tumors that were collected at the time of a first clinically apparent recurrence, the density of the CSC populations was similar to density in the original primary untreated tumor, supporting the hypothesis that these populations can give rise to more differentiated, marker-negative populations that comprise the bulk of the tumor mass. Of note, while the recognized CSC populations were higher in chemoresistant tumors, they still did not make up greater than 75% of the tumor mass, suggesting that we have still not discovered the absolute population that survives traditional treatment.

Targeting Cancer Stem Cells

Given emerging data that cancer stem cells contribute to chemoresistance and clinical outcome, efforts are being made to specifically target this subpopulation. Szotek found that the dye-effluxing side-population isolated from a mouse ovarian cancer cell line was growth-inhibited after treatment with Mullerian-inhibiting substance (36). Wei and colleagues separated human ovarian cancer cells into unsorted, non-side population, and side-population groups; then treated each with Mullerian-inhibiting substance, doxorubicin and cisplatin. They found that treating unsorted cells with cytotoxic agents enriched the population for side population cells; they also saw that non-side population cells were sensitive to chemotherapy and that side population cells were not. Interestingly, side population cells were inhibited at a much lower dose than non-side population cells when treated with Mullerian-inhibiting substance, giving rationale for exploring a two-pronged treatment strategy in ovarian cancer: one targeted towards the larger, non-CSC population, and the other towards the more quiescent, traditionally chemoresistant CSC population (37).

The hyaluronic acid receptor CD44 has also been explored as a therapeutic target. Bourguignon et al. have demonstrated that the CD44-hyaluronan complex activates Nanog, an embryonic stem cell transcription factor important in maintaining self-renewal and pluripotency (38). Downstream effects of Nanog include activation of the drug efflux pump

MDR1, giving a plausible link between stem-cell marker and chemoresistance. Slomiany and colleagues separated CD44⁺ cells into CD133-high- and -low expression groups (39). They found that while the total levels of CD44 were not different between the two groups, the CD133-high cells were associated with high levels of receptor tyrosine kinases, drug and lactate transporters, and emmprin (a matrix metalloproteinase inducer). Interfering with the CD44- hyaluronan complex with small hyaluronan oligosaccharides diminished drug-effluxing capacity and tumorigenicity. Using CD44 to identify cells with high claudin-4 expression, Casagrande et al. exploited claudin-4's high affinity for *Clostridium perfringens* enterotoxin to selectively kill CD44-positive cells in vitro and in vivo (40). Impressively, 50% of mice treated with sublethal doses of the enterotoxin had a complete, durable response after 8 treatments. Nanoparticle delivery of siRNA to mediate downregulation of both CD44 and ALDH1A1 expression reduced tumor growth either alone or in combination with chemotherapy (26, 41).

If aggressive subpopulations of cells are identified by markers of stemness, it follows that targeting pathways mediating stem cell biology may also be effective, such as Notch, Hedgehog, Wnt, and TGF-beta pathways. The Notch pathway is a highly conserved cell-fate pathway important in embryogenesis and angiogenesis. Park and colleagues found that the Notch pathway, particularly Notch3, was upregulated in approximately 20% of ovarian cancers and may function as an oncogene (42). They demonstrated increased apoptosis and decreased cellular proliferation by targeting Notch with siRNA and a pan-Notch inhibitor. McAuliffe et al. also found that ovarian CSCs could be specifically targeted with gamma-secretase inhibitors or Notch3-specific siRNA, and when combined with cisplatin the entire pool of CSCs was eliminated (43). Steg et al. showed that targeting Jagged1, a Notch ligand, restored chemosensitivity in taxane-resistant cells, in part through anti-angiogenic effects (35). Surprisingly, they also found that the mechanism of chemoresistance through Jagged1 is likely Notch-independent and is mediated at least in part through crosstalk with the Hedgehog transcription factor Gli-2. Similar restoration of chemosensitivity has also been demonstrated by targeting the Hedgehog pathway with clinically available Smoothed inhibitors (44), and is effective in reducing spheroid-forming capacity of cells in differentiating-inhibiting media (45). The Wnt pathway contributes to platinum resistance and can also be used to sensitize cells to platinum agents, either by downregulation of individual components of the pathway such as Wnt2B (46) or by using the c-kit inhibitor imatinib, which blocks Wnt signaling (47). Indeed many targeted therapies that failed as single agents may have more efficacy if used in combination with chemotherapy, as chemoresistance pathways are reduced. The complexity of interactions between these developmental pathways remains to be elucidated, but may prove clinically useful.

One of the defining characteristics of cancer stem cells is quiescence, which renders them relatively insensitive to traditional chemotherapy agents that depend on rapid cell cycling to induce cell death. Wnt5A was found to induce senescence in ovarian cancer cells (48). Bioenergetics, the study of mitochondrial metabolism, is an emerging field of inquiry that looks at an alternate route to apoptosis besides DNA damage, which may allow specific targeting of cancer stem cells. Alvero and colleagues used a novel isoflavone, NV-128, to depress mitochondrial function in CD44⁺/MyD88⁺ ovarian cancer stem cells (49). This led to inhibition of the mTOR pathway and loss of mitochondrial membrane potential that in

turn led to caspase- independent cell death. The same group had previously shown decreased tumor growth in a murine model with NV-128, in which apoptosis was induced in both the general cancer population and the cancer stem cell population, without significant murine toxicity (50).

A major control on normal stem cell differentiation lies in epigenetic regulators. Controls on methylation of CpG islands on gene promoters, acetylation of histones to control chromatin unwinding, and gene regulation through microRNA production are important for understanding tumorigenesis and potential targets for therapy. Excellent reviews on this topic as it applies to ovarian cancer are available (51, 52), but epigenetics may play a particularly important role in targeting the cancer stem cell population. A comprehensive analysis of methylation profiles found the sonic hedgehog pathway to be of particular importance(53), and methylation of several genes in the Notch superfamily are prominent and correlate with poor survival in the TCGA dataset(54). Recent work by Matei et al. showed that hypomethylation via low-dose decitabine can restore chemosensitivity in platinum-resistant ovarian cancer, suggesting reversal of drug evasion characteristic of cancer stem cells (55). Targeting ovarian cancer epigenetic processes both globally and at the individual gene level has shown promise in numerous preclinical studies (55-57).

Immunotherapy has garnered much attention in the treatment of cancer. Interferon alpha has both anti-viral and anti-tumor effects and has had varying success in the treatment of leukemias and solid tumors, including gynecologic cancers (58, 59). Moserle has demonstrated that interferon-alpha selectively targets side population cells(60). Kobayashi found that the side population was increased in paclitaxel- resistant ovarian cancer cell lines, and that interferon-alpha strongly induced apoptosis in those cells, but not in cells that were paclitaxel-sensitive (61). With new insights into the biology of ovarian cancer stem cells, interferon-alpha may be rationally reintroduced as targeted therapy against such side population cells.

Another form of immunotherapy that has gained traction is specific cancer stem cell-targeted dendritic cell vaccination. Pellegatta and colleagues demonstrated that dendritic cells exposed to glioma neurospheres cured up to 80% of mice injected with the same cell line(62). Xu et al. loaded dendritic cells with glioblastoma cancer stem-like cells and found that cytotoxic T-cell response against the CSCs was increased, prolonging survival in mice (63). Weng applied similar techniques when looking at immunoresponse to dendritic cells fused with ovarian cancer stem-like cells. Using CD44 and CD24 as sorting antigens, this group isolated CSCs then fused them with dendritic cells. These fusion cells were incubated with T-lymphocytes, which in turn were incubated with unsorted ovarian cancer cells and CD44+ cells from the same line, resulting in preferential large-scale lysis of the CD44+ population(64). In conjunction with current investigations to develop individualized *in vivo* ovarian cancer models, inducing a vaccine-generated response against ovarian cancer stem cells is a new treatment prospect in the quest for personalized medicine.

The most effective method of targeting the CSC population still remains to be elucidated. As described above, there are numerous points in CSC biologic pathways that can serve as therapeutic targets. If the CSC population changes in response to treatment, or if there are

multiple populations with stem-cell properties, it may be that a multi-agent approach would be required. Some have argued that cells that gain stem cell properties in response to stressors do not truly fit the definition of an inherent “stem cells.” Like the epithelial-to-mesenchymal transition postulated to contribute to cancer metastasis, CSC pathways may be inducible, allowing certain cells to survive therapy. Regardless, if these cells are responsible for recurrence, specifically targeting that population (or populations) should be an effective therapeutic strategy. It is yet unknown when is the best timing of administering biologic drugs that specifically treat CSC. More research is needed to determine if these therapies are most effective when used in combination with traditional cytotoxic chemotherapy, or if they are most effective in a maintenance role. Novel trial designs will be needed to incorporate new therapeutics in the scenario at which targeting these populations is most likely to provide significant clinical benefit – that being at the time of primary therapy, or when these population are at their minimal volume, at the completion of primary therapy.

Conclusion

Evidence is accumulating that stem cell pathways are important drivers of carcinogenesis in ovarian cancer and potential targets for therapy. The natural course of this disease suggests that there is a small subpopulation of cells that is chemotherapy-resistant and is able to repopulate the tumor. As more is discovered regarding ovarian cancer stem cell biology, more can be applied in the clinical setting. However, significant challenges remain. Cancer stem-like cells comprise a very small percentage of the total tumor burden, making them challenging to identify. Most translational studies examine only tissue specimens taken at the time of primary surgery, in which the vast majority of cells are going to be killed by primary chemotherapy, and thus not the population we need to be targeting. Small but important populations can easily be missed. It would be exceedingly valuable to incorporate biopsies of recurrent specimens into clinical trials and studies attempting to develop personalized therapies, since by definition the recurrent tumor is more densely composed of chemoresistant cells. There is no consensus regarding a universal marker (or set of markers) that can identify the treatment-resistant population in ovarian cancer (or any malignancy). Indeed, given the number of genes that are dysregulated in ovarian cancer, it is possible that there are numerous combinations of markers that vary not only by histology, but in individual patients. By their very nature, cancer stem cells evade therapy through mechanisms such as the ABCG2 transporter and ALDH1A1 enzyme, which makes chemical targeting of these cells quite difficult. The importance of pathways stem cells preferentially use, such as Wnt, Notch, and Hedgehog, is under clinical investigation. While the most chemoresistant population may not be absolutely identified with markers currently recognized, it appears clear that stem cell pathways contribute to survival, and that these populations must be targeted in order to achieve durable cures in this deadly disease.

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Research highlights

- * Growing evidence supports the presence of a chemoresistant cancer cell population that relies on stem cell pathways for enhanced survival.
- * Examination of patient specimens confirms enrichment of stem cell pathways in surviving tumors.
- * Targeting ovarian cancer stem cells is a promising approach to overcoming chemotherapy resistance in this subpopulation of heterogeneous tumors.

Table 1
***Ex vivo* characteristics of cancer stem cells**

<ul style="list-style-type: none">• Increased tumorigenicity in xenograft models• Clonogenic• Unlimited self-renewal• Pluripotency• Ability to recapitulate parent tumor• Chemoresistance• Radiation resistance• Form spheroids in suspension
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Table 2
Putative ovarian cancer stem cell markers with enhanced tumorigenicity

Marker	Endpoint	Reference
CD133+	Increased tumorigenic efficiency (cell lines, primary tumor, ascites, xenograft tumor)	Baba(65), Curley(66)
	Enhanced vasculogenesis (cell lines, primary tumor, ascites, xenograft tumor)	Kusumbe(67), Silva(27)
CD44+/MyD88+	Increased tumorigenesis; spheroid formation; chemoresistance (ascites, cell lines)	Alvero(68)
CD44+/CD117+	Increased tumorigenesis; chemoresistance (primary tumor, xenograft tumors)	Zhang(69)
CD44+/CD24-	Spheroid formation; recapitulate parental tumor (cell lines)	Shi(70)
CD44+/CD24+	Increased tumorigenesis (primary tumor, xenograft tumor)	Gao(71)
ALDH1A1+	Increased tumorigenesis; pluripotency (cell lines)	Landen(26)
ALDH1A1+/CD133+	Increased tumorigenesis; chemoresistance (cell lines, primary tumor, xenograft tumor)	Silva(27) Kryczek(72)
	Increased tumorigenesis; self-renewal (primary tumor)	
ABCG2 (Side pop'n)	Increased tumorigenesis (cell lines (murine and human), ascites)	Szotek(36)
	Increased tumorigenesis; chemoresistance (cell lines, ascites, xenograft tumor)	Hu(73)
	Increased tumorigenesis; chemoresistance; self-renewal (cell lines, xenograft tumor)	Dou(74)
	Chemoresistance (cell lines)	Kobayashi(61)