Review Article

Ovarian Cancer Stem Cells: A New Target for Cancer Therapy

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Ovarian cancer is a highly lethal disease among all gynecologic malignancies and is the fifth leading cause of cancer-related death in women. Although the standard combination of surgery and chemotherapy was initially effective in patients with ovarian cancer, disease relapse commonly occurred due to the generation of chemoresistance. It has been reported that cancer stem cells (CSCs) are involved in drug resistance and cancer recurrence. Over the past decades, increasing studies have been done to identify CSCs from human ovarian cancer cells. The present paper will summarize different investigations on ovarian CSCs, including isolation, mechanisms of chemoresistance, and therapeutic approaches. Although there are still numerous challenges to translate basic research to clinical applications, understanding the molecular details of CSCs is essential for developing effective strategies to prevent ovarian cancer and its recurrence.

1. Introduction

Ovarian cancer, the fifth leading cause of cancer-related death in women, is a highly lethal disease among all gynecologic malignancies. It is estimated that 22,280 women are diagnosed with ovarian cancer and 15,500 women will die of this disease in 2012 in the United States. From 2005 to 2009, the median age at diagnosis for ovarian cancer in women was 63 years. Based on incidences from 2007 to 2009, one in seventy-two women will be diagnosed with ovarian cancer during their lifetime. The overall five-year relative survival rate was 43.7% from 2002 to 2008 [1].

Ovarian cancer is a heterogeneous disease composed of different types of tumors [2]. Based on different histological features, most tumors of the ovary contain three major types of cells: surface epithelial stromal cells, sex cord stromal cells (including granulose, theca, and hilus cells), and germ cells (oocytes) [3]. Epithelial ovarian carcinoma (EOC) is the major form of the disease and accounts for about 90% of ovarian tumors [4]. According to distinctive morphology and molecular genetic background, epithelial ovarian cancer can be further categorized into eight subtypes, including serous, mucinous, endometrioid, clear cell, transitional cell tumors (Brenner tumors), carcinosarcoma, mixed epithelial tumor, and undifferentiated carcinoma [5]. Various subtypes of epithelial ovarian cancers can be also simply divided into two groups named type I and type II by Kurman and Shih in 2010 [6]. Type I tumors are clinically indolent and genetically stable, including low-grade serous, low-grade endometrioid, clear cell, and mucinous and transitional (Brenner) carcinomas. Type II tumors are more aggressive and genetically unstable, including high-grade serous, high-grade endometrioid, carcinosarcoma, mixed epithelial tumor, and undifferentiated carcinomas [7].

Over the past decades, the combination of surgery and platinum-based chemotherapy was the standard treatment for advanced ovarian cancer [8]. Although numerous molecular targeting agents have been developed due to deeper understanding of the disease progression, recurrence still commonly occurs in 70% of patients who underwent the frontline treatment within 18 months. The five-year survival rate of those patients with advanced ovarian cancer is only 30.6% [9, 10]. Thus it is crucial to develop effective strategies to attack cancer cells that become resistant to current chemotherapy.

Recently, scientists have proposed that the existence of cancer stem cells was one of the reasons for disease relapse [11, 12]. Traditional chemotherapy can kill the majority of cancer cells, while failing to target cancer stem cells. Moreover, initial treatment increased the proportion of drug-resistant cancer stem cells, resulting in recurrence of disease [13]. In
this paper, we will summarize the studies on ovarian CSCs, including the isolation, their roles in chemoresistance, and the therapeutic approaches.

2. Cancer Stem Cells of Ovaries

The terms cancer stem cells (CSCs) or cancer initiating cells (CICs) are a very small subgroup of tumor cells with the ability to self-renew, differentiate, and form secondary/tertiary tumors after serial xenotransplantation into immune-compromised animal models [14, 15]. Actually, the reason for 90% of tumors arising from ovary surface epithelium is that stem cells reside in the area. In early stage of ovarian cancer, the number of EOC stem cells can be used to predict progression of the disease [16].

Understanding the origin of cancer cells may have clinical significance. It has been reported that both luminal and basal epithelial cells are cells of origin for prostate cancer [17, 18]. In the case of CSCs, it originated not only from adult stem cells that underwent oncogenic transformation, but also from downstream progenitor or differentiated cells with acquired stem cell-like characteristics [19]. However, limited evidence suggested that adult stem cells were the originator of ovarian cancer. Tumors arising from CSCs usually contain a mixed population of cells due to asymmetric division of CSCs. Such cell division can produce one daughter cell that retains the feature of parent cell and another that continually divides to form the bulk of tumor [20].

In 1997, Bonnet and Dick first isolated the cancer stem cells in leukemic cells expressing stem cell marker CD34 [21]. Later, many other types of CSCs were also identified, including ovarian CSCs [22]. The first evidence of ovarian stem cells was the isolation of the ascites from a patient with ovarian cancer [23]. One of the properties for stem cells is to exclude harmful dyes, thus containing less cytoplasmic dyes compared to the rest of the cell population by fluorescence-activated cell sorting (FACS) analysis [24]. Ovarian cancer stem cells can be successfully isolated via distinctive efflux of the DNA binding dye Hoechst 33342. These ovarian CSCs are also called "side population" (SP) stem cells that have the capacity of self-renewal and differentiation in comparison with the non-SPs [25]. However, there is no universal single marker for ideally isolating the ovarian CSCs. In 2009, Gao and his colleagues have isolated SP cells from OVCAR-3, a human ovarian cancer cell line. However, these cell fractions only accounted for 0.9% of the total cell populations [26]. Another study successfully established stable SP cells and ALDH1A1 positive cell populations from the ovarian cancer cell line A2780. Those SP cells exhibited partial resistance to the chemotherapy drug platinum. Nevertheless, it should be noted that a cancer stem cell population may not be a group of cells with a single feature, but may contain overlapping cell fractions with mixed stem-like markers [27].

3. Chemoresistance of Ovarian CSCs

Although the standard combination of surgery and chemotherapy can effectively reduce tumor mass, most patients with residual ovarian CSCs eventually acquire chemoresistance. Hence, recurrent cancer is inevitable in the vast majority of cases [28, 29]. Such phenomenon attracts researchers' attention to decipher the molecular mechanisms involved in escaping the chemotherapy for cancer stem cells.

3.1. Glutathione (GSH) System. The GSH system can suppress oxidative stress and maintain cellular redox homeostasis [30]. The contribution of GSH and GSH-related enzymes to chemoresistance has been demonstrated in different types of tumor, including ovarian cancer and brain tumor [31, 32]. GSH is also involved in the detoxification of various xenobiotics [33]. Upon metabolism of chemotherapeutic agents, the enzymes of glutathione-S-transferase (GST) family could prompt the formation of GSH-drug conjugates. Many chemotherapeutic agents have been shown to conjugate with GSH, including chloroethylnitrosoureas (CENUs), platinum compounds, and other alkylating agents. The resulting GSH-drug conjugates are more water soluble and less active than the compounds themselves. They are thus exported from the cell via the transporter-mediated system [34]. These findings reasonably support the application of antioxidant inhibitors, in combination with standard chemotherapy in patients.

3.2. Overexpression of Bmi-1. Bmi-1, a member of the polycomb group (PcG) family, participates in the self-renewal and maintenance of CSCs [35]. As an oncogene, Bmi-1 could enable cancer cells to escape apoptosis by modulating multiple growth signaling pathways [36]. Thus, its overexpression in cancer cells could be used as a survival marker. The role of Bmi-1 in chemoresistance has been addressed recently. For example, Bmi-1 could allow the resistance of glioma cells to chemotherapy drug such as doxorubicin and bis-chloroethylnitrosourea (BCNU) [37]. It can also prompt chemoresistance, invasion and tumorigenesis in pancreatic cancer cells [38]. For ovarian cancer cells, silencing of Bmi-1 gene could promote sensitivity to cisplatin and induction of apoptosis [39].

3.3. Loss and Localization of p53. The tumor suppressor gene p53 plays a critical role in cell proliferation and apoptosis by controlling several signaling pathways. Loss of p53 function could cause multidrug resistance in many types of tumors, including ovarian cancer [40]. In addition, the control of intracellular localization of p53 is also associated with the regulation of apoptosis and chemosensitivity in human ovarian cancer cells [41]. The p53-associated Parkin-like cytoplasmic (PARC) protein is critical for p53 subcellular localization and function. It has been demonstrated that a low level of PARC could increase p53 accumulation in nucleus, thus inducing apoptosis [42]. Downregulation of Ca^{2+}-dependent PARC could enhance cisplatin-induced apoptosis in chemosensitive but not in chemoresistant human ovarian cancer cells [43]. The detailed molecular mechanism affecting PARC/p53 interaction between chemosensitive and chemoresistant cancer cells remains to be determined. However, it is vital to note that p53 is not an absolute indicator for the resulting response to
chemotherapy because not all drugs induce cell death via p53 in cancer cells [44].

3.4. Drug Effluxion. The development of multidrug resistance is also associated with the failure of drug uptake. The export of drugs is mediated by transmembrane polysubstrate efflux pumps, which prevent drugs from entering their intracellular targets [45]. These drug transporters are composed of four domains, including two nucleotide-binding domains (NBD) and two transmembrane domains (TMD). The TMD recognizes and translocates substrates, while the NBD is required for conformational changes [46].

Mammalian P-glycoprotein is a transmembrane transporter related with resistance of hydrophobic anticancer drugs. It belongs to one of the ATP-binding cassette (ABC) transporter families [47]. For decades, other efflux transporters in the ABC transporter family have been also identified. For example, ABCC2 encoding for MRP2 (multidrug resistance protein) was involved in effluxion of cisplatin-derived compound in ovarian cancers [48]. ABCG2 (breast cancer resistance protein or BCRP) permitted effluxion of cellular DNA-binding dye Hoechst. Thus, Hoechst can be used to isolate stem-like cells in a variety of tissues, including bone marrow, skeletal muscle, mammary epithelium [49,50], and ovarian carcinomas [51]. Moreover, ABCG2/BCRP was considered as a drug-resistant marker, which involved in transport of substances and cellular products by using ATP as energy source [52]. In addition to the ABC family, some other transporters have been described such as copper transporter proteins (CTR), organic cation transporters (OCTs), copper-transporting ATPases, and multidrug and toxin extrusion (MATE) [53].

Wender and his colleagues recently conjugated a known drug (Taxol) to oligoarginine, which is a guanidinium-rich molecular transporter responsible for delivery of attached molecule into cells. Such Taxol-oligoarginine conjugates may overcome drug efflux-based resistance through prolonging the half-life of the drug and increasing the drug stability in human ovarian carcinoma cells [54].

3.5. Quiescence of Ovarian CSCs. Mammalian adult stem cells are known to maintain in a quiescent, nondividing, or G0 state [55]. CSCs also demonstrated the similar property. This is also one of the reasons for their resistance to chemotherapy since most anticancer drugs preferentially target dividing cancer cells. Thus, intensive understanding of quiescent mechanism of CSCs is important to improve clinical outcome for cancer patients.

Recent studies have suggested that several genes played key roles in maintaining quiescence of normal stem cells and CSCs. For example, p53 expression was increased and could promote quiescence in hematopoietic stem cells (HSCs) [56]. Necdin, a growth-suppressing protein, as well as a p53 target gene, has been recently identified to improve hematopoietic stem cells quiescence [57]. Nonetheless, the loss of zinc-finger repressor Gfi-1 (growth factor independent 1) enabled HSCs high proliferation [58]. Cited2, a transcriptional modulator, could maintain HSCs quiescence via both HIF-1 (a negative regulator) dependent and independent pathways. Deletion of Cited2 could improve HSCs apoptosis and loss of quiescence. Moreover, its deletion could increase cycling in conditional knockout mice [59]. In addition, the reduced miRNAs (miR-31 and let-7) were demonstrated to keep the balance between lung cancer stem-like side population (SP) cells and nonside population (non-SP) cells. Inhibition of let-7 could prompt growth of both SP and non-SP cells by accelerating G1 to S phase transition, while repression of miR-31 could cause cell cycle arrest in G0/G1 phase in both of SP and non-SP cells [60].

4. Therapeutic Approaches of Ovarian CSCs

The elimination of ovarian CSCs has been challenging in part due to heterogeneity. Thus the efficacy of any single drug was limited for cancer patients. Combined treatments that target CSCs will be a new direction in the future. Nevertheless, drug treatment for CSCs may increase the risk of toxicity since CSCs share common features with normal stem cells. The current therapeutic strategies in ovarian CSCs are discussed below.

4.1. Cell Surface and Nonsurface Markers. Cell surface markers (i.e., CD molecules, short for cluster of differentiation) have been widely used to isolate putative CSCs through flow cytometry. Most types of CSCs share the identical biomarkers, including ovarian cancer stem cells. To activate the immune system to clear cancer cells in patient body, antibody-based therapy for cancer has been developed for decades. Moreover, the strategy of antibody-drug conjugates has achieved considerable success in recent years [61]. Indeed, development of specific therapies that target biomarkers of ovarian CSCs could improve clinical outcome and patient's survival [62].

4.1.1. CD133. CD133, a transmembrane glycoprotein, is one of the most widely described ovarian CSCs markers [63]. Its expression level is higher in advanced serous ovarian cancer than that in normal ovaries and benign tumors [62]. Tumor cells carrying CD133 marker (often abbreviated as CD133+) displayed greater resistance to chemotherapy [63]. In addition, CD133+ ovarian CSCs have hyperactivity in migration and invasion due to the activation of chemokine (c-c motif) ligand 5 (CCL5) [64]. In 2009, Baba and his colleagues found that methylation in promoter region could regulate the expression of CD133 in ovarian cancers, implying that epigenetic modification might be involved in the induction of stemness of tumor [65]. In addition, the combination of a murine derived anti-human CD133 antibody and a cytotoxic drug (monomethyl auristatin F, MMAF) significantly inhibited the cell growth in hepatocellular and gastric cancers [66].

4.1.2. CD44. CD44, another CSC surface transmembrane glycoprotein, is a receptor for hyaluronic acid (HA) involved in cell-cell and cell-matrix interactions. It will ultimately affect cellular growth, differentiation, and motility [67,68]. CD44 is highly expressed in many types of cancer, including
ovarian CSCs. The CD44⁺/CD24⁻ ovarian cancer cells were correlated with invasion and chemoresistance [69]. Several antibodies against isoforms of CD44 have been developed, and some of them have entered into clinical trials for the patients with head and neck squamous cancers [70]. VFF18 and BIWA-1 were two murine IgG1 monoclonal antibodies that recognized human CD44 variant exon 6 (CD44v6). They were evaluated for their targeting potential in squamous cell carcinoma (SCC) and head and neck SCC (HNSCC), respectively. To avoid human anti-mouse antibody response in patients, humanized forms of such antibodies were developed, such as BIWA-2, BIWA-4 and BIWA-8 [71]. The phase I clinical trial of BIWA-4 (bivatuzumab) has been carried out to evaluate its safety, tumor-targeting potential, pharmacokinetics, and immunogenicity in patients with HNSCC [72]. However, these clinical outcomes still need further confirmation. Except for antibody-based therapy, scientists also proposed other approaches in recent years. Casagrande and his colleagues reported that a toxin called clostridium perfringens enterotoxin (CPE) could eradicate CD24⁺ ovarian CSCs [99]. A novel class of ALDH inhibitor (Aldi) discovered recently could further confirmation. Except for antibody-based therapy, scientists also proposed other approaches in recent years. Casagrande and his colleagues reported that a toxin called clostridium perfringens enterotoxin (CPE) could eradicate CD24⁺ ovarian CSCs [99]. A novel class of ALDH inhibitor (Aldi) discovered recently could

4.1.3. CD24. CD24 is a glycosylphosphatidylinositol-linked cell surface protein expressed in various solid tumors [75]. Gao et al. have successfully isolated CD24⁺ CSCs from ovarian tumor specimens and identified CD24 as a putative CSC marker in ovarian cancer [76]. Expression of CD24 affected metastasis and represented poor prognosis in ovarian cancer [77]. A study demonstrated that CD24 could localize in the cytoplasm of ovarian serous tumors, while normal epithelium and serous cystadenomas expressed CD24 marker in the apical membrane. Thus, the cytoplasmic expression of CD24 could be used as a specific marker to predict the survival rates and recurrence of cancer [78]. The depletion and over-expression of CD24 could regulate the phosphorylation of STAT3 and FAK by affecting Src (nonreceptor tyrosine kinases) activity. SWA11, an antibody against CD24 tyrosine kinase involved in cell signal transduction. It activates Wnt/β-catenin-ATP-binding cassette G2 pathway was required for cisplatin/paclitaxel-based chemoresistance caused by CD117 in ovarian CSCs [85]. A potent CD117 specific inhibitor (Imatinib Mesylate) has been used in the clinical trials for the treatment of many types of cancer, including persistent epithelial ovarian cancer [86]. Patel and his colleagues demonstrated that Imatinib Mesylate involved in complex cellular processes, including metabolic pathways, cell cycle, cell proliferation, apoptosis, and signal transduction through mass spectrometry-based proteomics method in human ovarian cancer cell line A2780 [87].

4.1.5. EpCAM. The epithelial cell adhesion molecule EpCAM is a glycosylated membrane protein. It is highly expressed in different tumor types, including colon, lung, pancreas, breast, head and neck, and ovary [88]. EpCAM was found to be hyperglycosylated and frequently associated with cytoplasmic staining in carcinoma tissues [89, 90]. EpCAM is comprised of an extracellular domain (EpEX), a single transmembrane domain and a short 26-amino acid intracellular domain (EpICD). Among them, EpEX is a potential target for cancer therapy. Catumaxomab, a monoclonal antibody against EpCAM is a trifunctional antibody, which can bind three different cell types, including tumor cells, T cells, and accessory cells (dendritic cell, macrophages, and natural killer cells) [93]. It is now used in phase III clinical trials in patients with malignant ascites [94]. The investigation of its efficacy and safety was also entered in phase II clinical trials on advanced ovarian cancer patients who had experienced complete chemotherapy. Based on both preclinical and clinical outcomes, EpCAM may be served as a possible therapeutic target against epithelial ovarian cancer.

4.1.6. Aldehyde Dehydrogenase (ALDH) Isozymes. ALDH proteins are a superfAMILY containing 19 enzymes that protect cells from carcinogenic aldehydes [95]. ALDH1A1 was identified as a putative cancer stem cell marker, and it was associated with chemoresistance in the ovarian CSC [96]. Besides ALDH1A1, other ALDH isozymes such as ALDH1A3, ALDH3A2, and ALDH7A1 also had high expression level in ovarian tumors when compared to normal ovarian tissues [97]. The dual positivity of ALDH1 and CD133 ovarian cells had higher ability to regenerate tumor in mice than single ALDH¹ or CD133⁺ ovarian cancer cells [98]. These findings suggest that ALDH can be used as a reliable marker to study ovarian cancer stem cells.

Recently, clinical trials have been initiated using disulfiram (an ALDH inhibitor). The combination of disulfiram with a drug named gemcitabine had a synergistic effect on cytotoxicity in glioblastoma multiforme cells [99]. A novel class of ALDH inhibitor (Aldi) discovered recently could
endow lung cancer cell line A549 with higher sensitivity to mafosfamide [100].

Other two stem cell markers, Lin28 and Oct4, are also served as new molecular targets due to their roles in the maintenance of pluripotency in ovarian cancer [101]. In addition, high expression of the Müllerian inhibiting substance (MIS) type II receptor has been reported in ovarian cancer cell lines [102]. MIS could significantly inhibit the cell population with stem-like characteristics in ovarian cancer cell lines [103].

4.2. Differentiation of Ovarian CSCs. Current methods to eliminate CSCs cannot be successfully applied in all clinical situations. One way to eradicate CSCs is to induce their differentiation, resulting in loss of their stemness property [104]. Thus, the understanding of regulation of differentiation processes is necessary for designing new agents to eliminate CSCs. In 2012, Yin and his colleagues observed that TWIST-1 (a basic helix-loop-helix transcription factor) played a key role in triggering differentiation of epithelial ovarian cancer (EOC) [105]. Jain et al. recently reported that p53 capable for regulating molecular networks can activate two miRNAs (miR-34a and miR-145). These miRNAs were then shown to prompt differentiation of human embryonic stem cells [106]. Indeed, emerging evidence indicated that miRNAs were involved in self-renewal and differentiation of normal and cancer stem cells. It is suggested that such miRNAs should be a new therapeutic target for cancer treatment [107].

Retinoic acid (a vitamin A metabolite) and its analogs are the most common differentiation agents. They are also the only agents used in clinical trials [108]. The all-trans-retinoic acid (ATRA) can inhibit the proliferation and induce the differentiation via inhibition of Wnt/β-catenin pathway in head and neck squamous carcinoma CSC [109]. The clinical study of ATRA has shown an increased survival rate of patients with acute promyelocytic leukemia. However, successful cases are limited in solid tumors [110]. Recently, Whitworth and his colleagues effectively reduced the growth of ovarian CSC via a drug (Carboplatin) combined with three novel retinoid compounds [111]. In addition, specific unsaturated fatty acids (palmitoleic, oleic, and linoleic acids) can trigger adipocyte-like differentiation in many types of cancer cells, including ovarian cancer cell line SKOV3 [112]. However, more detailed regulation of differentiation remains to be determined.

4.4. MicroRNAs (miRNAs). MiRNAs are a group of small noncoding RNAs with 20–28 nucleotides in length. They could regulate gene expression at posttranscriptional level. Thus, miRNAs are involved in diverse biological processes, such as development and tumorigenesis [119]. The expression profile of miRNAs was different between normal stem cells and CSCs [120, 121]. MiR-214 was highly expressed in ovarian CSCs and endowed the property of self-renewal and chemoresistance in ovarian CSCs via repressing p53-Nanopathway [122]. MiR-199a significantly rescued the sensitivity of ovarian CSCs to some chemotherapy agents, including cisplatin, paclitaxel, and Adriamycin. Moreover, miR-199a prevented tumorigenesis in xenograft model via downregulating expression of CSCs marker CD44 [123]. In addition, the expression of miR-200a could reduce migrating ability of CD133+ ovarian CSCs. This was because miR-200a inhibited E-cadherin and ZEB2, two genes critical for migration process [124]. However, some miRNAs own oncogenic property, such as miR-125, miR-9, miR-30, miR-21, and miR-215 [125, 126]. In conclusion, miRNAs have become a potential target for ovarian cancer treatment.

5. Conclusion

Understanding the roles of CSCs in cancer therapy may markedly improve the survival rate of ovarian cancer patients. However, it is impossible to cure patients with advanced ovarian cancer in all cases. One possible reason is the heterogeneity of ovarian CSCs, which leads to different sensitivities to the therapy used for one subset of CSCs. Thus combinative therapy will be the major direction for ovarian cancer treatment in the future. In addition, personalized medicine dependent on different genomic background of individuals will become a more effective therapeutic method. Current technological advances, such as next-generation DNA sequencing and mass spectrometry-(MS-) based proteomics, would facilitate implementation of personalized medicine. The establishment of comprehensive gene/protein network from cancer patients could provide more accurate platform for clinical prognosis [127, 128]. In 2012, Vathipadiekal and his colleagues have reported the gene expression profile in ovarian cancer stem cells by affymetrix microarray and identified the activation of Notch signaling pathway, as well as several other genes unique to ovarian CSCs [129].

In brief, we have highlighted the recent advances on ovarian CSCs, including isolation, mechanisms of chemoresistance, and therapeutic strategies. It is easy to imagine that understanding of the CSCs will be helpful to guide medical decision. Basic research is also fundamental to develop new
agents for patients. It is our hope that therapies that target ovarian CSCs will result in better clinical outcomes.

**Abbreviations**

CSCs: Cancer stem cells  
EOC: Epithelial ovarian carcinoma  
SP: Side population  
GSH: Glutathione  
PARC: p53-associated Parkin-like cytoplasmic  
ABC: ATP-binding cassette  
MRP: Multidrug resistance protein  
BCRP: Breast cancer resistance protein  
HSCs: Hematopoietic stem cells  
HA: Hyaluronic acid  
SCC: Squamous cell carcinoma  
HNSCC: Head and neck SCC  
CPE: Clostridium perfringens enterotoxin  
EpCAM: Epithelial cell adhesion molecule  
EMT: Epithelial mesenchymal transition  
ALDH: Aldehyde dehydrogenase  
MSC: Mesenchymal stem cells.

**Conflict of Interests**

The authors declare that they have no conflict of interests.

**References**


