PROSPECTS
Journal of Cellular Biochemistry 114:21–34 (2013)

Journal of Cellular Biochemistry

Cancerous Ovarian Stem Cells: Obscure Targets for Therapy but Relevant to Chemoresistance

Nuzhat Ahmed,[1,2,3,4]* Khalid Abubaker,[1,3] Jock Findlay,[1,2,4] and Michael Quinn[1,2]

[1]*Women's Cancer Research Centre, Royal Women's Hospital, Melbourne, Australia*

[2]*Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, Australia*

[3]*Department of Surgery, University of Melbourne, Melbourne, Australia and* [4]*Prince Henry's Institute of Medical Research, Melbourne, Australia*

ABSTRACT

Chemotherapy with platinum and taxanes is the first line of treatment for all epithelial ovarian cancer (EOC) patients after debulking surgery. Even though the treatment is initially effective in 80% of patients, recurrent cancer is inevitable in the vast majority of cases. Emerging evidence suggests that some tumor cells can survive chemotherapy by activating the self-renewal pathways resulting in tumor progression and clinical recurrence. These defined population of cells commonly termed as ''cancer stem cells'' (CSC) may generate the bulk of the tumor by using differentiating pathways. These cells have been shown to be resistant to chemotherapy and, to have enhanced tumor initiating abilities, suggesting CSCs as potential targets for treatment. Recent studies have introduced a new paradigm in ovarian carcinogenesis which proposes in situ carcinoma at the fimbrial end of the fallopian tube to generate high-grade serous ovarian carcinomas, in contrast to ovarian cortical inclusion cysts (CIC) which produce borderline and low grade serous, mucinous, endometrioid, and clear cell carcinomas. This review summarizes recent advances in our understanding of the cellular origin of EOC and the molecular mechanisms defining the basis of CSC in EOC progression and chemoresistance. Using a model ovarian cancer cell line, we highlight the role of CSC in response to chemotherapy, and relate how CSCs may impact on chemoresistance and ultimately recurrence. We also propose the molecular targeting of CSCs and suggest ways that may improve the efficacy of current chemotherapeutic regimens needed for the management of this disease. J. Cell. Biochem. 114: 21–34, 2013.

KEY WORDS: OVARIAN CARCINOMA; STEM CELL MARKERS; CHEMORESISTANCE; RECURRENCE

Epithelial ovarian cancer (EOC) is the sixth major cause of cancer mortality in women [Jemal et al., 2009]. Nearly 200,000 new cases of ovarian cancer are reported worldwide each year [Ozols et al., 2004], and more than 60% die within 5 years [Society, 2007]. The high mortality rate in ovarian cancer patients results from the diagnosis at a late-stage when the cancer has spread into the peritoneal cavity and metastasized to vital organs [Lengyel, 2010]. One of the great challenges in detecting and treating ovarian cancer is its heterogeneous nature. The term ''ovarian cancer'' refers to a diverse group of cancers that affect the ovaries [Karst and Drapkin, 2010]. Ovarian malignancy may develop from one of the three types of cells: epithelial cells, sex cord-stromal cells (including granulose, theca, and hilus cells), or germ cells (oocytes) [Auersperg et al., 2001]. However, 90% of all ovarian malignancies are epithelial and include a heterogeneous group of neoplasms with diverse tumor morphologies and varying genetic alterations and clinical manifestations [Auersperg et al., 2001].

Current classification for ovarian cancers are however more simple and divide malignancies into type 1 tumors which are low-grade, slow growing, generally confined to the ovary at diagnosis, and develop from well established precursor lesions that are termed ''borderline tumors'' [Kurman and Shih Ie, 2008; Levanon et al., 2008; Karst and Drapkin, 2010] and type 2 tumors, which, are high-grade and rapidly progressing for which well-defined precursor lesions have not been described [Kurman and Shih Ie, 2008]. Gene expression studies have shown high-grade tumors to cluster separately from low-grade and borderline tumors, suggesting that the two groups of tumors have a different genetic makeup [Saad

The authors declare that they have no competing interests.

Grant sponsor: Women's Cancer Foundation and National Health and Medical Research Council of Australia 441101.

*Correspondence to: Dr. Nuzhat Ahmed, Women's Cancer Research Centre, Royal Women's Hospital, 20 Flemington Road, Parkville, Vic 3052, Australia. E-mail: nuzhat.ahmed@thewomens.org.au

Manuscript Received: 26 June 2012; Manuscript Accepted: 31 July 2012

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 10 August 2012

DOI 10.1002/jcb.24317 • © 2012 Wiley Periodicals, Inc.

et al., 2010]. Moreover, low-grade ovarian neoplasms have been shown to be associated with mutations in KRAS, BRAF, PTEN, and CTNNB1/β-catenin, in contrast to type 2 tumors in which these mutations are rarely seen [Kurman and Shih Ie, 2008; Karst and Drapkin, 2010]. Tumors that remain confined to the ovaries belong to type 1 group and account for 25% of all ovarian cancers [Kurman and Shih Ie, 2008]. The vast majority of ovarian cancer are type 2 which spread to extra-ovarian sites, specifically, the peritoneum and the fallopian tube early in their development, and involve the ovary at a later stage [Kurman and Shih Ie, 2008; Karst and Drapkin, 2010].

Type 1 tumors include the major histotypes endometrioid (cells resembling the endometrium), mucinous (cells resembling endocervical glands) and low-grade serous (cells resembling glandular epithelium of fallopian tube), while type 2 tumors are all high-grade serous carcinomas, undifferentiated carcinomas or carcinosarcomas [Kurman and Shih Ie, 2008]. These tumors are composed of large masses of cells with large multinucleated nuclei [Kurman and Shih Ie, 2008]. They have high mitotic activity and the majority have active DNA damage repair mechanism (DDR) and mutated or ineffective p53 function commonly known as a "p53 signature" [Kurman and Shih Ie, 2008; Levanon et al., 2008]. These tumors may also exhibit gene amplification and over expression of the HER2/neu (10–20%) and AKT2 (10–20%) oncogenes [Kurman and Shih Ie, 2008].

TWO MODELS OF OVARIAN CANCER EVOLUTION

Until recently, the vast majority of EOC was thought to arise from the malignant transformation of the ovarian surface epithelium (OSE) [Auersperg et al., 2001; Saad et al., 2010]. OSE is a flat-to cuboidal uncommitted mesothelial layer of cells which covers the exterior surface of the ovaries. During ovulation which involves follicular rupture and oocyte release, physical trauma is induced, creating a wound in the OSE which must be repaired for subsequent ovulatory cycles [Murdoch and Martinchick, 2004; Ahmed et al., 2006]. Over the course of a woman's reproductive life this process of wounding and repair confers plasticity in OSE, which facilitates the expression of both epithelial and mesenchymal genes needed for tissue remodelling [Auersperg et al., 1994; Ahmed et al., 2006]. In addition to this physical trauma, OSE is also subjected to the exposure of ovulation induced inflammatory cytokines and reactive oxygen species that results in DNA damage [Murdoch and Martinchick, 2004]. Accrual of these DNA damaging events over time may result in the neoplastic transformation of OSE. In addition, impairment in the repair of OSE may result in the invaginations of OSE which may trap the wounded released OSE into the ovarian corticol stroma, forming circular OSE-lined structures termed "cortical inclusion cysts (CICs)" [Auersperg et al., 2001]. Inside the ovary, CICs are exposed to several hormones that have growth-promoting and differentiation properties resulting in a state of metaplasia [Folkins et al., 2009]. If the cells within the CICs harbor DNA damage induced by the trauma or the inflammatory microenvironment, they may be the prime targets for neoplastic transformation, eventually giving rise to ovarian carcinomas [Karst and Drapkin, 2010]. This OSE–CIC model can account for many important features of ovarian cancer such as the cystic nature of benign tumors and the presence of borderline tumors within the cortical stroma of the ovary [Karst and Drapkin, 2010]. This model is also consistent with epidemiological data suggesting a correlation between the decrease in ovulatory cycles (due to preganacy, lactation and contraceptive pills) and the decreased risk of ovarian cancer [Permuth-Wey and Sellers, 2009]. However, this model does not explain the origin of the two diverse types of ovarian cancer (types 1 and 2), the clear genetic differences that exist between them, and the presence of extraovarian peritoneal carcinomas which are identical to serous ovarian carcinomas but do not involve the ovaries.

These questions were recently addressed in a series of studies which investigated sections of fallopian tubes in women with germline BRCA1,2 inherited gene mutations with familial ovarian and breast cancer syndromes which accounts for ~11–15% of ovarian carcinomas [Wooster and Weber, 2003; Risch et al., 2006]. Many women with BRCA1, 2 mutations opt to undergo risk-reducing bilateral salpingo-oophorectomy (ovary and fallopian tube removal), after which their ovaries are examined for evidence of occult cancer [Karst and Drapkin, 2010]. Until recently, the fallopian tubes were not examined following such surgery and early stage tubal cancer was rarely reported. In 2001–2003, attention was drawn to the histology of fallopian tubes from BRCA1, 2 mutation patients, and a high incidence of fallopian tube dysplasia was reported [Piek et al., 2001, 2003; Agoff et al., 2002]. The fallopian tube epithelium (FTE) is a columnar layer of cells composed of secretory and ciliated cell types. Histological examination of the dysplastic region of FTE from BRCA1,2 patients revealed a complete loss of ciliated cells with a shift towards a secretory population of cells with acquisition of high proliferative index (Ki67 staining) [Piek et al., 2001]. Later studies, which involved histological examination of fimbria uncovered a high incidence of serous tubal intraepithelial carcinomas (STIC) in the fallopian tube and serous ovarian carcinomas in the fimbriated end of the fallopian tube, suggesting that fimbria is the preferred site of serous ovarian carcinoma origin in BRCA1,2 mutation women [Medeiros et al., 2006; Kindelberger et al., 2007]. The fimbriae lies in close proximity to OSE, are a continuous part of the peritoneum and are exposed to the same inflammatory environment during ovulation. It is possible that the transformed FTE during early stages of transformation migrates to the ovarian surface or to the peritoneum with minimum ovarian involvement [Karst and Drapkin, 2010]. During the course of these studies, it was observed that "p53 signatures" occur more frequent where STICs were present, and were common in secretory cells and were characterized with the DNA damage marker γ-H2AX [Lee et al., 2007], a phosphorylated form of histone protein activated by DNA damage sensing kinases ATM and ATR at sites of DNA damaged double strand break [Rogakou et al., 1999]. These observations suggested a common origin of STICs and serous ovarian carcinomas. Evidence that supports these findings were further supported by clinical studies which have shown that 38% of BRCA1,2 mutation women undergoing salpingo-oophorectomy have an early lesion (STIC) in the fallopian tube but not in the ovaries, and 80% of these carcinomas appeared exclusively in the fimbriated end of the fallopian tube, indicating that the fimbria is

the preferred site of serous carcinogenesis in women harboring *BRCA*1,2 mutations [Medeiros et al., 2006; Karst and Drapkin, 2010]. These observations suggest that carcinomas of the ovaries, FT and peritoneum share a common origin.

In light of these observations, a model of ovarian cancer development was developed [Karst and Drapkin, 2010]. This model suggests that that there are two distinct pathways leading to ovarian tumorigenesis. The first is the traditional OSE-CIC pathway in which OSE is entrapped in CICs and induced to undergo Mullerian metaplasia (differentiation) giving rise to mostly endometrioid, mucinous and serous borderline and low grade tumors. This pathway leads to the formation of type 1 tumors. The second pathway combines fallopian tube fimbria, where a combination of "p53 signature" and genotoxic stress in the form of DNA damage response leads to the clonal expansion of fallopian tube secretory epithelial cells forming a neoplastic precursor lesion which with additional mutational hits (due to environmental cues such as inflammatory cytokines, reactive oxygen species, etc) gives rise to type 2 tumors. This new model of ovarian carcinogenesis accounts for 70% of high grade serous carcinomas and has recently been evaluated in vitro [Levanon et al., 2010; Jazaeri et al., 2011] and in vivo mouse models [Jazaeri et al., 2011; Karst et al., 2011]. The schematic image of these two models of ovarian cancer progression is depicted in Figure 1.

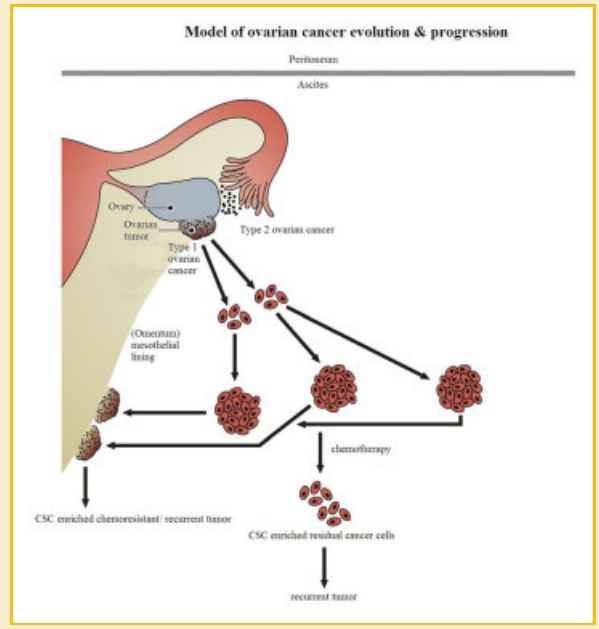

Fig. 1. A model of CSC-mediated ovarian cancer evolution and progression [adapted from Ahmed et al., 2007]. EOC originates from CSCs derived from either OSE or FTE which forms niches for ovarian CSCs. During the course of tumor progression there is a shedding of tumor cells into the peritoneum where they survive as cellular aggregates or spheroids. These spheroids undergo microenvironment-induced changes in the ascites until they find a secondary attachment sites on the peritoneum. Both the ascites and the metastatic peritoneum sites serve as niches for CSCs. During chemotherapy treatment the bulk of the tumor cells are eradicated leaving behind CSC enriched cells that facilitate recurrence by facilitating the growth of residual tumor.

CANCER STEM CELLS OF THE OVARIES

The cancer stem cell (CSC) hypothesis postulates that the tumorigenic potential of CSCs is confined to a very small subset of tumor cells and is defined by their ability to self-renew and differentiate leading to the formation of a tumor mass [Mimeault and Batra, 2008]. The observation that cancers can arise long after initial exposure to carcinogens [Sell, 2004], implies that the carcinogenic event may have occurred in the long-lived slowly proliferating stem cell population which in many cases may have been triggered by unknown mechanism(s) (e.g., DNA damage, exposure to inflammatory cytokines and reactive oxygen species, etc) after being dormant for an indefinite length of time ranging from months to years. These early cancer cells or CSCs would then give rise to generations of cells resulting in tumor masses. CSCs are not necessarily transformed adult stem cells but they may be progenitor cells or differentiated cells that have acquired stem cell like characteristics [Barker and Clevers, 2007]. Although there is evidence in some tumor types (such as melanoma) normal adult stem cells are the initial precursor cells to neoplastic transformation [Staud and Pavek, 2005; Schatton et al., 2008], definite evidence of these adult stem cells as the originator of ovarian cancer has been lacking. The term "cancer stem cells" usually refers to a defined population of tumor cells that express a distinct set of cell surface or intracellular markers which are universally expressed in many tissues. The term "cancer initiating cells" or "tumor initiating cells" have been used with CSCs but neither of these terms define the cells that initiate the tumor. CSCs are usually characterized by their ability to renew and give rise to a progeny of cells that have high proliferative and invasive capacity. This phenomenon often described in the literature as "asymmetric division" defines a process whereby one daughter cell on division retains the characteristics of the parent cell while the other may not necessarily have the parental traits [Guddati, 2012]. Hence, tumors that arise from CSCs consist of CSCs and a mixed population of cells which creates the full heterogeneous phenotype of the tumor. Within the tumor CSCs possess several key properties which includes, (i) unlimited proliferative potential; (ii) ability to renew indefinitely in an undifferentiated state; (iii) resistance to therapies; (iv) high DNA repair capacity; and (v) the ability to drive the expansion of tumor by cells that are deregulated at various stages of differentiation [Al-Hajj et al., 2003]. These properties of CSCs represent a critical target for new cancer therapy. Nonetheless creating and designing therapies against ovarian CSCs has proven complex because, (i) there are no CSC marker for ovarian cancer that can be specifically targeted and (ii) ovarian CSCs are protected by resistance mechanisms that make them less susceptible to conventional therapies.

The first description of stem cells in ovarian cancer was reported in the ascites of an ovarian cancer patient, derived from a single cell which could sequentially propagate tumors over several generations [Bapat et al., 2005]. CSCs have been isolated from ovarian tumors and cell lines based on their abilities to differentially efflux the DNA binding dyes [Szotek et al., 2006]. This population of cells normally

termed as ''side population'' (SP) stem cells displayed the classical stem cell property in tumorigenicity assays and have been shown to be resistant to chemotherapy [Vathipadiekal et al., 2012]. However, side populations have been shown to have heterogeneity with some cells retaining more for stem cell markers such as Oct4, CD117, and CD44 more than other cells in the same population. The ATP-binding cassette (ABC) transporter family of protein has also been shown to be over expressed in side populations [Zhou et al., 2001]. A correlation was observed with the expression of ABCB1 and chemoresistance to cisplatin and paclitaxel in ovarian cancer [Hu et al., 2010; Hosonuma et al., 2011]. In the same context, side population cells extracted from the ascites of ovarian cancer patients were found to be enriched for ABCB1 and histone methyltransferase, EZH2 after chemotherapy [Rizzo et al., 2011]. Gene expression analysis showed that the side population cell signature was enriched in patients with early recurrence (1–12 months) compared to those with a later (13–24 months) recurrence [Hosonuma et al., 2011]. The presence of side population cells in ovarian cancer patients also correlated with worse prognosis [Hosonuma et al., 2011]. Hence, utilizing side population cells may be helpful in prospectively isolating ovarian CSCs.

In recent studies several cell surface and non-surface markers have been ''borrowed'' from solid malignancies to isolate ovarian CSCs. CSCs in these studies have been isolated depending on the distinct pattern of surface markers (i.e., CD44, EpCAM, CD133, CD117, Thy1, CD24) [Ferrandina et al., 2008; Zhang et al., 2008; Alvero et al., 2009; Gao et al., 2010] and non-surface markers (aldehyde dehydrogenase) [Landen et al., 2010]. Even though CSCs sorted on the basis of these markers have shown the potential to have ''CSC characteristics'' (ability to self renew, resistance to therapy, develop tumors in very small numbers ~100 cells, etc), none of them have shown any significance in a clinical setting. Most of the studies used to demonstrate the tumorigenicity of the isolated CSC enriched tumor cells have used the subcutaneous mouse xenograft model in immunocompromised mice which does not truly represent human ovarian carcinomas [Zhang et al., 2008]. These models lack the true metastatic feature of ovarian cancer in vivo which is uniquely localized in the peritoneum and occurs directly from the ovaries to adjacent organs (extraovarian pelvic organs, colon, bladder, liver for example), or by the attachment of exfoliated cancer cells which survive as spheroids and are carried by the peritoneal tumor fluid (ascites) to surrounding organs in the peritoneal cavity [Ahmed et al., 2007; Lengyel, 2010] (Fig. 1). Hence, the use of mouse xenograft models used for tumorigenicity assays for marker-defined CSC population has raised several questions [Hill, 2006; Kelly et al., 2007]. Moreover, a very high frequency of the tumor cells without CSC characteristics can also develop tumor in immunocompromised mouse models, suggesting that the tumor-initiating and sustaining abilities are not only confined to the so called ''CSC population'' and the xenotransplantation model currently used for CSC studies may select for population of cells suited for the foreign (mouse) microenvironment. Some of the short comings of these animal models for CSC studies have been evaluated in a recent paper [Curley et al., 2011].

CANCER STEM CELLS AND PROGRESSION OF OVARIAN CANCER

Current literature strongly suggests that the microenvironment provided by the CSC niche is critical for carcinoma progression [Borovski et al., 2011]. As such, one would assume that the niche of ovarian CSC will reside at the origin of carcinoma initiation and would gradually change as the tumor moves to distant sites. Under these circumstances, the parenchyma and stroma of the tumor containing distinct cell types such as cancer associated fibroblasts, endothelial cells, inflammatory cells will determine the behavior of cancer by establishing a bidirectional link in support and preservation of CSC niche [Schauer et al., 2011]. Hence, it can be hypothesized that the ''stemness'' genes in primary tumors may not be the same or expressed at the same level as in distant metastases. This is consistent with studies reporting a differential expression of genes in primary ovarian tumors compared to metastatic lesions on the omentum [Bignotti et al., 2007; Dressman et al., 2007]. It is possible that a set of differentially expressed genes in metastases are induced as a result of tumor-stromal or tumor-microenvironment interaction in the new tumor niches. Hence, one would assume that CSCs derived from the primary ovarian tumors would display a different expression profile than those of intermediate and distant metastases, even though both populations are supposedly derived from the same lineage. Moreover, tumor spread has been shown to be facilitated by epithelial–mesenchymal transition (EMT), where the disseminated cancer cells acquire self-renewal properties, similar to those exhibited by stem-cells [Huber et al., 2005]. These studies have been supported by the observation of frequent expression of stem cells and EMT markers in circulating tumor cells of breast cancer patients [Aktas et al., 2009], and the observation of a mesenchymal phenotype in residual breast cancer cells after conventional chemotherapy [Creighton et al., 2009], suggesting that cellular plasticity (EMT) and CSCs are not only involved with cancer progression but also with recurrence. As ovarian cancer cells can be induced to undergo EMT [Huber et al., 2005; Ahmed et al., 2010] and generate CSCs in response to drug treatment [Latifi et al., 2011], one can speculate that there is a close association between cellular plasticity and CSC in ovarian cancer progression and recurrence. Recent literature suggests that after metastatic spread, disseminated cells interact with the associated parenchyma and undergo a secondary transformation termed as mesenchymal epithelial reverting transition (MErT) which is responsible for the growth and sustenance of secondary disease [Chao et al., 2010].

Chemotherapy resistance in ovarian cancer cells is usually manifested in multicellular aggregates present as epithelial cell adhesion molecule (EpCAM) rich spheroids in circulating ascites (tumor fluid) of patients presented with recurrent advanced-stage disease. In this scenario, the metastatic cancer cells surviving the insult of chemotherapy may represent an ectopic tumor mass with a certain degree of EMT and some representation of the parent tumor (Fig. 1). One can speculate on the process of recurrence as a colonization process which may be driven by a ''hybrid'' of CSCs with traits of EMT and/or MErT phenotype [Haviv and Thompson, 2012]. Molecular targeting of these distinct CSC sub-populations

and the associated cellular plasticity signaling may improve the current efficacy of standard chemotherapy protocols, much needed for recurrent ovarian cancer. Given the present uncertainty of the true phenotype of CSCs it is critical to understand the differences/similarities between stem cells in primary and in metastatic tumors.

The recent discovery of FTE as the origin of most high grade serous carcinomas, and OSE as the originator of borderline and low grade mucinous, endometrioid, and serous carcinomas, suggests that FTE and OSE may be the site where ovarian CSC may originally reside and should be investigated. Somatic stem cell and stem/progenitor cell characteristics have been identified in OSE [Szotek et al., 2008; Virant-Klun et al., 2008]. The genes and pathways previously shown to be associated with adult stem cell maintenance have been shown to be highly expressed in OSE [Bowen et al., 2009], suggesting that OSE can serve as an originator of ovarian cancer. A recent study has reported the isolation of multipotent mesenchymal stem cells from the fallopian tubes [Jazedje et al., 2009]. In addition, ex vivo cultures of primary FTE with characteristics of ovarian cancer cells have been reported [Levanon et al., 2010]. These studies suggest that both FTE and OSE may be the potential origin of ovarian CSCs. In this context, it has also been shown that endometrial implants on the ovaries can produce endometriosis which on malignant transformation can produce endometrioid and clear cell ovarian cancer [Kuhn et al., 2012], suggesting that endometrium can be a potential CSC source for endometrioid and clear cell ovarian cancer. Hence, we can speculate that types 1 and 2 ovarian tumors may have distinct sub-set of CSCs or there may be overlapping CSCs shared between the two groups. Detailed studies on control populations (BRCA mutation negative) are needed to explore this concept further with regard to ovarian CSC characterization. It is possible that the current set of ''borrowed CSC markers'' routinely used for ovarian cancer (CD44, CD117, CD133, etc) may only be involved as ''oncogenic markers'' without having a distinct role as CSC markers, and as such there is an urgent need to identify bona fide ovarian CSCs.

CHEMOTHERAPY AND OVARIAN CANCER STEM CELLS

Current treatment strategies for advanced stage ovarian cancer patients consists of aggressive surgery (cytoreduction or tumor ''debulking'') followed by chemotherapy to eradicate any residual disease [Bristow et al., 2002]. Postoperatively, all women, except those diagnosed with stage 1 well differentiated tumors are given platinum (cisplatin or carboplatin) and taxane based chemotherapy, resulting in remission in up to 80% of patients. Unfortunately, the majority of these patients relapse within 2 years, resulting in a 5-year survival rate of only 10–30% [Ozols, 2006]. This low survival rate is largely due to the ability of residual tumor cells to evade chemotherapy associated cytotoxicity resulting in acquired chemoresistance. Platinum based chemotherapy is extremely efficient in removing the bulk of the tumor mass. However, as recently shown, platinum treatment leaves behind a core of CSC-like cells which are not only very invasive but are able to cause relapse of the cancer [Oliver et al., 2010; Latifi et al., 2011]. Recurrent ovarian tumors are enriched with CSCs and stem cell pathway mediators, suggesting that CSCs may contribute to recurrent disease [Steg et al., 2012a]. Current studies have also shown that residual cells after chemotherapy treatment secrete soluble factors that provide a favorable microenvironment to facilitate the growth of residual cells [Bose et al., 2011]. This close relationship between chemotherapy surviving CSC-like cells and their secretory microenvironment represent a potential target for cancer therapy. Metastatic and drug resistant recurrent ovarian tumors have been shown to have a significantly higher IL6 expression compared to the matched primary tumors [Guo et al., 2010]. Furthermore, a monoclonal anti-IL6 antibody, siltuximab (CNTO 328) has been shown to suppress IL-6 induced STAT3 phosphorylation and nuclear translocation, and also decrease the expression of STAT3 downstream proteins and sensitize paclitaxel resistant EOC cell lines to chemotherapy [Guo et al., 2010]. These data form a novel therapeutic intervention strategy for chemoresistance.

OVARIAN CSCs AS THERAPEUTIC TARGETS

CELL SURFACE MARKERS

Elimination of ovarian CSCs has been challenging. This has possibly been due to the non-specific nature of CSC markers used to identify ovarian CSCs, or to the presence of multiple subsets of CSCs present in a single tumor which are not equally sensitive to a therapy designed for one subset of CSC. The most common ovarian cancer CSCs described in the current literature and their use as therapeutic targets is discussed below:

CD44. CD44 is a cell surface transmembrane glycoprotein involved in cell-cell, cell-matrix interactions that affect cellular growth, differentiation and motility [Marhaba et al., 2008]. CD44 positive cells have been shown to be present in primary and metastatic ovarian tumors [Alvero et al., 2009]. Its expression has been associated with poor prognosis and resistance to chemotherapy [Meng et al., 2012]. CD44 positive cells have been shown to express high levels of other stem cell markers such as Oct4 and nestin, show enhanced NFKβ activity and an inflammatory cytokine expression profile which includes high expression of IL1β, IL6, and IL8 [Alvero et al., 2009]. These CD44-mediated characteristics might influence the response of patients to chemotherapy resulting in negative prognosis. Several antibodies have been designed against different isoforms of CD44, and phase I clinical trials in head and neck squamous cell carcinoma were initiated using CD44 antibodies [Heider et al., 2004; Aguilar-Gallardo et al., 2012]. However, these clinical trials did not provide positive outcomes. In an alternative approach, a recent study has reported the development of a bio-conjugate of hylauronic acid (an extracellular matrix that binds CD44) and paclitaxel for future intraperitoneal treatment of ovarian cancer [Orian-Rousseau, 2010]. This conjugate has been tried on mouse xenograft models with promising results but its application in humans is yet to be determined.

EpCAM. The epithelial cell adhesion molecule (EpCAM; CD326) is a glycosylated membrane protein with oncogenic signaling properties which results in cell proliferation and tumor formation [van der Gun et al., 2010]. EpCAM is highly expressed in ovarian carcinomas and has been shown to be an independent prognostic

marker for reduced survival [Spizzo et al., 2006]. Metastatic and recurrent tumors were found to express significantly higher levels of EpCAM when compared with primary carcinomas [Bellone et al., 2009]. EpCAM expressing cells have also been described to possess a tumor-initiating role with stem/proginetor-like features [Yamashita et al., 2009]. Anti-EpCAM antibodies (Adrecolomab, Adecatumumab, and MT201) have been in phase III and phase I clinical trials for the last 10 years but have shown inconsistent cytotoxic action [Kurtz and Dufour, 2010]. Recently, the European Medicines Agency has approved the use of catumaxomab a trifunctional monoclonal antibody (anti-EpCAM × anti-CD3) to treat ovarian cancer patients with ascites [Seimetz et al., 2010]. The use of catumaxomab in this clinical scenario is encouraging but a smaller and more effective EpCAM targeting molecule is needed for ovarian cancer therapy.

CD133. CD133 is a transmembrane glycoprotein which has been shown to be overexpressed in ovarian carcinomas and associated with poor prognosis of the disease [Ferrandina et al., 2008]. High expression of CD133 has been correlated with resistance to chemotherapy, shorter disease-free and overall survival [Ferrandina et al., 2008]. CD133 expression in ovarian cancer is directly regulated by epigenetic modification [Baba et al., 2009]. A monoclonal murine anti-human CD133 antibody conjugated to monomethyl auristatin F, a potential cytotoxic drug, has shown promising growth inhibitory effects on gastric and hepato cellular cancer cells in vitro [Smith et al., 2008].

CD117. CD117 is commonly known as c-kit, is a receptor tyrosine kinase and is a good target for small molecule kinase inhibitors. Expression of c-kit has been observed in 40% of ovarian carcinomas and has been correlated with resistance to conventional chemotherapy [Luo et al., 2011]. Ovarian cancer cells with positive CD117 expression have been shown to possess CSC-like properties including self-renewal, differentiation, a high tumorigenic potential and chemoresistance [Zhang et al., 2008; Luo et al., 2011], making CD117 an attractive target for therapy. Several phase II clinical trials have been tried with imatinib mesylate (Gleevec), a potent CD117 specific tyrosine kinase inhibitor, in persistent and recurrent ovarian cancer [Schilder et al., 2008; Huh et al., 2010]. Some of these trials were conducted in the presence of taxane-base chemotherapy [Mundhenke et al., 2008]. Even though imatinib mesylate on its own or in combination with taxane was well tolerated by the patients, few patients showed sustained responses or stable disease [Matei et al., 2008].

CD24. CD24 is a small heavily glycosylated glycosylphosphatidylinositol-linked cell surface protein, which is expressed in hematological malignancies as well as in a large variety of solid tumors. In univariate survival analysis of all invasive ovarian carcinomas, a highly significant association of increased CD24 expression with shortened patient survival was demonstrated [Kristiansen et al., 2002]. One recent study has demonstrated CD24 as a new CSC phenotype by successfully isolating $CD24^+$ cells from ovarian tumor specimens [Gao et al., 2010]. In this study, $CD24^+$ cells were shown to proliferate slowly, were more resistant to chemotherapy, and possessed enhanced tumorigenicity potential compared to $CD24^-$ cells. In a follow-up, the same group demonstrated that tumor clones generated from different regions of the tumor (front or rear zone) showed phenotypically and genetically distinct populations of cells [Choi et al., 2011]. Clones from the tumor front zone were relatively rich in side population cells with accumulated genetic, transcriptional and gene product alterations, $CD24^+$ and $CD117^+$ expression and were resistant to chemotherapy [Choi et al., 2011]. This suggests that the expression of CSC-like molecules may regulate the intratumoral heterogeneity, where tumor cells in the frontal region may respond to environmental changes and adapt to these changes by facilitating the expression of stem cell-like characteristics [Choi et al., 2011]. These observations have been supported by some studies that have demonstrated a distinct population of migratory stem cells at the invasive front of the cancer [Hermann et al., 2007].

Aldehyde dehydrogenase 1 (ALDH1). The role of ALDH1 as a CSC marker has recently been reported in ovarian cancer [Landen et al., 2010]. More than 50% of EOC patients were shown to have higher ALDH1 expression and that correlated with poor overall survival [Wang et al., 2012]. In another contradictory study, however, ALDH1 on its own was associated with a better prognosis in ovarian cancer [Chang et al., 2009]. Some recent studies have shown that the expression of ALDH1 combined with CD44 ($ALDH1^+CD44^+$) [Wang et al., 2012] or CD133 ($ALDH1^+CD133^+$) [Silva et al., 2011; Kryczek et al., 2012] in primary tumor specimens correlated with reduced disease-free and overall survival in ovarian cancer patients.

In addition to the above markers, embryonic stem cell markers such as Oct4 and Lin28 were found to be expressed at high levels in ovarian tumors [Peng et al., 2010]. Lin 28 has been shown to positively regulate Oct4 and ALDH1 [Yang et al., 2010], suggesting that CSCs may evolve through an embryonic program whereby the expression of embryonic stem cells may balance the expression of consecutive subtypes of CSCs.

Signaling pathways as potential targets for CSCs. Wnt, Sonic Hedgehog (Shh), and Notch signaling have been implicated with the self-renewal and tumorigenic aspect of CSCs [García Campelo et al., 2011]. Of these the Wnt and hedgehog signaling pathways are the driving force behind several carcinomas including ovarian cancer [Chen et al., 2007; Gatcliffe et al., 2008]. Wnt signaling plays a key role in the embryonic development of the ovary, and is involved in normal follicular development and ovarian function [Ricken et al., 2002]. In ovarian cancer aberrant regulation of Wnt signaling is not clearly defined but there is compelling evidence implicating this pathway in cancer development [Rask et al., 2003]. Mutations in the human CTNNB1 gene encoding β-catenin have been observed in 30% of endometrioid tumors [Palacios and Gamallo, 1998; Wright et al., 1999; Lee et al., 2003], while a significant correlation between nuclear β-catenin expression and high-grade serous carcinomas has been implicated with decreased survival in some patients [Lee et al., 2003]. One of the common Wnt and Hedgehog target genes, leucine-rich G protein-coupled receptor 5 (Lgr5, also known as Gpr49), has been shown to mark rapidly cycling stem cells in the small intestine and hair follicles [Morris et al., 2004; Barker and Clevers, 2007]. Lgr5 is an orphan seven-transmembrane domain receptor with similarity to thyroid-stimulating hormone, follicle-stimulating hormone and luteinizing hormone receptors. Selective up regulation of Lgr5 has been reported in ovarian, colon and heptocellular carcinomas [McClanahan et al., 2006], and its role in tumorigenesis has been

supported by the induction of transformation when expressed in NIH3T3 cells [McClanahan et al., 2006] and tumor formation in nude mice transplanted with Lgr5 expressing HaCaT cells [Tanese et al., 2008]. Moreover, knock down of Lgr5 by siRNA has been shown to induce apoptosis in colon cancer cells [McClanahan et al., 2006]. Even though the molecular function of Lgr5 is unknown, its close association with "stemness" [Barker et al., 2007] is consistent with its role in the control of ovarian CSCs behavior and therefore offers potential as a therapeutic target.

In a recent clinical trial in breast cancer, targeting the $CD44^{+}/CD24^{-/low}$ population on CSCs by the glycogen synthase kinase drug Tykerb has resulted in a complete disappearance of the disease [Bates, 2008]. Association between Nanog and CD44 has been shown to activate signal transducer and activator of transcription protein 3 (STAT3) in ovarian cancer cells [Bourguignon et al., 2008]. This results in multidrug resistant gene expression with concomitant chemoresistance. We have recently shown activation of the STAT3 pathway in ovarian tumors [Colomiere et al., 2009]. Hence, targeting STAT3 signaling pathways in CSCs of ovarian tumors may represent a novel approach to overcome CSC-mediated chemo-resistance. Moreover, researchers at the laboratory of Genomic Diversity, NCI-Frederick are investigating how molecules such as cyclopamine (a naturally occurring alkaloid found in corn lily) can control the self-renewal of CSCs by inhibiting Hedgehog signaling [Bates, 2008]. Cyclopamine has recently been demonstrated to inhibit Hedgehog-dependent Lgr5 expression as well as the growth of basal cell carcinomas [Tanese et al., 2008]. Cyclopamine has also been shown to reverse taxane resistance in ovarian cancer cell lines [Steg et al., 2012b]. A recent study has shown that silencing of jagged 1, a Notch ligand, to sensitize ovarian cancer cell lines to taxane through cross talk with the Hedgehog pathway [Steg et al., 2011], suggesting that the Hedgehog pathway represents an important target to eradicate ovarian tumorigenesis. Studies concentrating on the in situ elimination of CSCs have been initiated by OncoMed Pharmaceuticals (antibody that target CSCs), Geron Corporation (phase I/II clinical trial with GRN163L to inhibit TERT), and GlaxoSmithKline (Tykerb in breast cancer) [Bates, 2008]. It is believed that result from these studies may offer a strategy for the eradication of cancer through the elimination of CSCs.

CHEMOTHERAPY TREATMENT FACILITATES GENERATION OF OVARIAN CSCs: A STUDY ON OVARIAN HEY CELL LINE AS AN EXPERIMENTAL MODEL

The human ovarian HEY cell line (originally derived from a peritoneal deposit of a patient diagnosed with papillary cystadenocarcinoma of the ovary) [Buick et al., 1985] was treated with cisplatin (1 μg/ml) or paclitaxel (2 ng/ml) or cisplatin and paclitaxel (1 μg/ml and 1 ng/ml) (combination) for 3 days. Almost all residual surviving cells displayed enhanced expression of ERCC1 (cisplatin and combination treatment) or β-tubulin type 3 (TUBB3; paclitaxel and combination treatment), suggesting a chemoresistant phenotype of residual cells to the respective drug treatment [Scheil-Bertram et al., 2010; Gao et al., 2012] (Fig. 2). Treatment with chemotherapy (cisplatin or paclitaxel or combination) resulted in enhanced expression of CD44, CD24, CD133, CD117, and EpCAM in residual cells compared to parental untreated cells (Fig. 3). A similar result was observed in other ovarian cancer cell lines (OVCA 433, SKOV3, and OVCAR5) in response to cisplatin or paclitaxel or combination of both (Abubaker et al., unpublished data). In

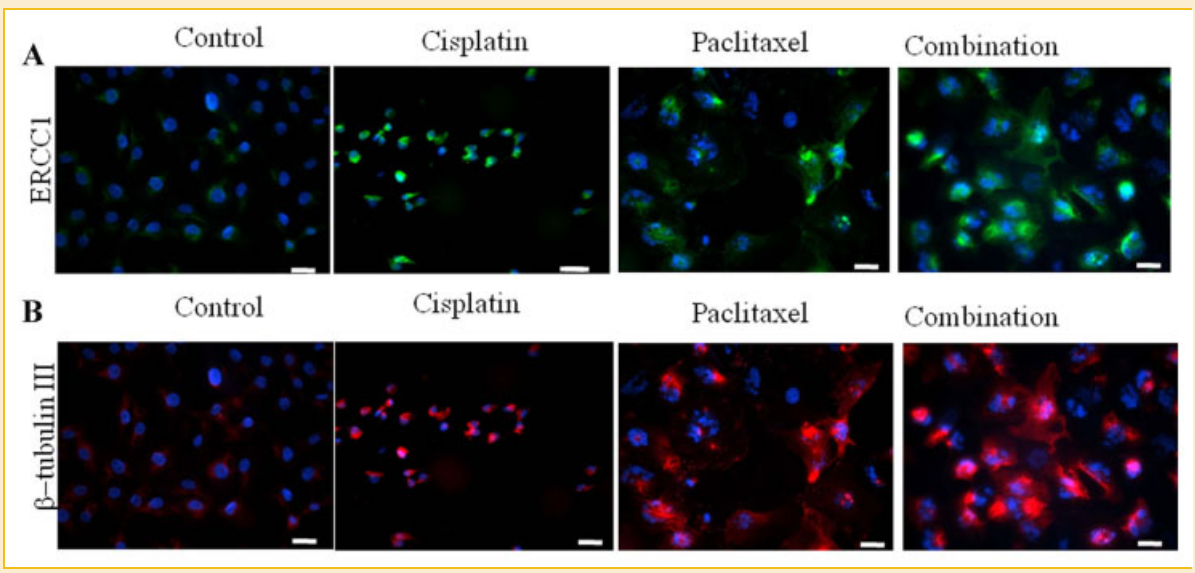

Fig. 2. Expression and immunolocalization of ERCC1 and TUBB3 in response to cisplatin (1 μg/ml), paclitaxel (2 ng/ml) and combination of both [cisplatin and paclitaxel (1 μg/ml and 1 ng/ml)] in HEY cells for 3 days. The images were evaluated by using rabbit polyclonal antibodies (red) or mouse monoclonal antibodies (green) as described previously [Latifi et al., 2011]. Cellular and nuclear staining were visualized by using secondary Alexa 488 (green) or 590 (red) fluorescent labeled antibody and DAPI (blue). Magnification 100×; scale bar = 50 μm.

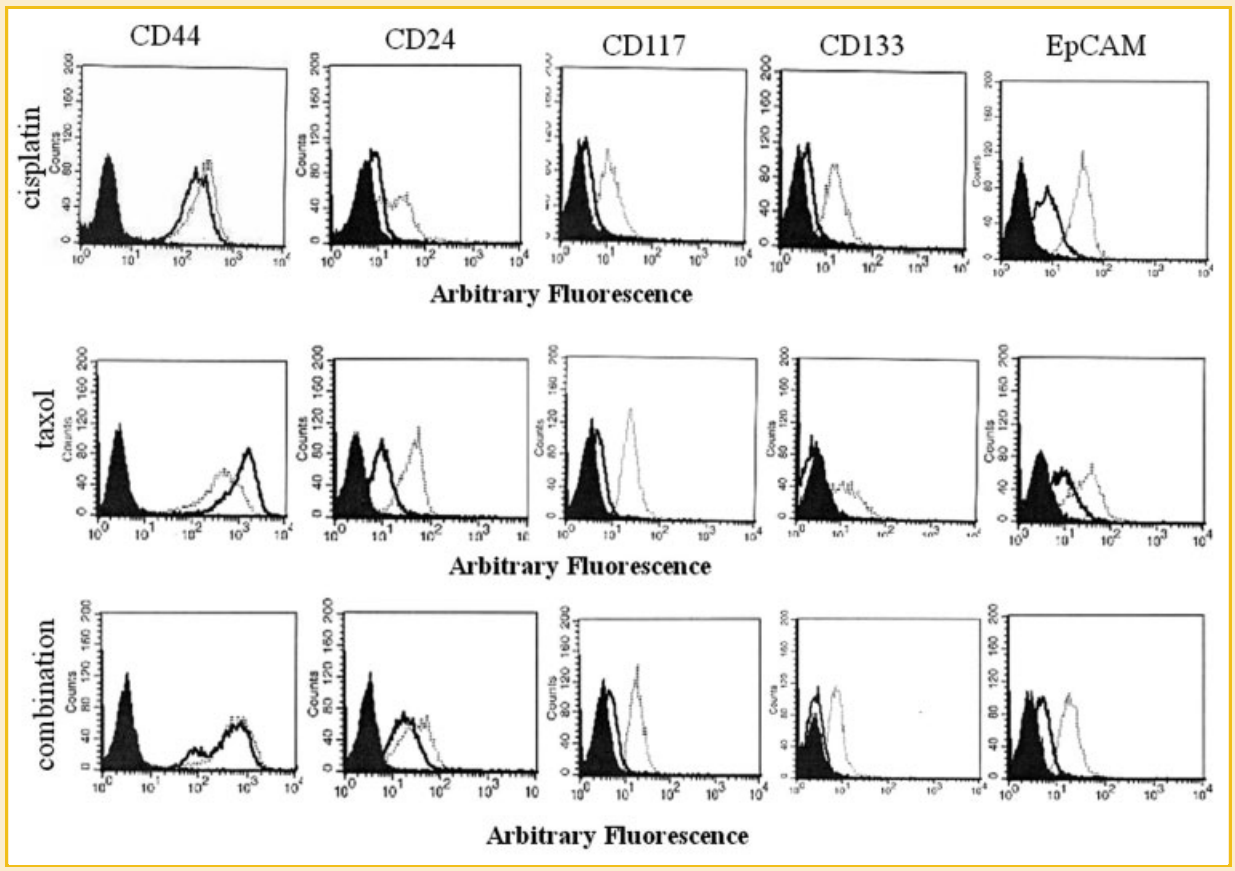

Fig. 3. Effect of cisplatin, paclitaxel and combination of both on the expression of CSC-like markers in HEY cells. The expression of CSC was assessed by Flow cytometry as described previously [Latifi et al., 2011]. Untreated or chemotherapy treated cells were incubated with either control IgG or relevant primary antibodies against the respective CSC-like markers followed by secondary goat anti-mouse IgG conjugated with phycoerythrin. The filled histogram in each figure is control IgG, black lines indicate protein expression in control cells while broken lines demonstrate protein expression in chemotherapy treated cells. Results are representative of three independent samples.

addition, our recent published data also demonstrates co-ordinated up regulation of CSC markers and activation of extracellular signal-regulated kinase (ERK)1/2 and STAT3 in response to cisplatin treatment in ovarian cancer cells, as well as tumor cells isolated from primary ovarian tumors and ascites of advanced-stage ovarian cancer patients [Ahmed et al., 2010; Latifi et al., 2011]. These data suggest that chemotherapy treatment prompts a cascade of events which not only enhances the expression of CSC markers but also triggers the associated signaling pathways in ovarian tumors. These events may be crucial for the survival of residual chemoresistant tumor cells, suggesting that targeted inhibition of these pathways during the course of chemotherapy may circumvent chemoresistance. However, this enhancement in CSC-like characteristics can be maintained in the cells only in the presence of chemotherapy and is lost with continuous passage of cells (Abubaker et al., unpublished data), suggesting that chemotherapy selects for a population of cells with enhanced CSC-like characteristics and that property is lost with propagation possibly due to asymmetric division of CSC-enriched cells. Such a model provides an understanding about the survival and dissemination of residual disease after chemotherapy treatment. This reservoir of residual cells with induced stem cell-like functions is the likely cause of recurrence and mortality in 70% of ovarian cancer patients.

NEW THERAPEUTIC APPROACHES

DIFFERENTIATING PATHWAYS AS POTENTIAL TARGETS FOR CSCs

Anticancer approaches to eliminate CSC by various means so far have not been successful. As described above, these approaches usually selects for residual cancer cells that retain the CSC-like characteristics and regenerate the tumors. One way to treat cancer cells would be to induce differentiation which would result in the loss of the CSCs self renewal property [Sell, 2004; Aguilar-Gallardo et al., 2012]. Differentiation of one cell type into cells of other lineages, commonly known as trans-differentiation, has recently received attention. Trans-differentiation of fibroblast into functional cardiomyocytes [Ieda et al., 2010] or neurons [Vierbuchen et al., 2010] by enforced expression of a cocktail of relevant genes are good examples. Another example is the use of all trans-retinoic acid for terminal differentiation of acute promyelocytic leukemia cells into mature granulocytes. This therapeutic approach has been in use for leukemia for last one decade and has provided clinical benefit [Soignet et al., 1998]. However, this treatment has not proven useful for solid cancers. A recent study has demonstrated that specific unsaturated fatty acids, such palmitoleic, oleic and linoleic acid can trigger adipocyte differentiation in human cancer cell lines, including ovarian cancer [Ruiz-Vela et al., 2011]. These cells

demonstrate massive production of lipid droplets and up regulation of the adipogenic nuclear regulator PPARγ, which belong to the Peroxisome Proliferator-Activated Receptor (PPARs) superfamily [Ruiz-Vela et al., 2011]. We have demonstrated over expression of PPARγ in ovarian carcinomas [Zhang et al., 2005], suggesting that this adipogenic trans-differentiation may be feasible in a certain sub-set of ovarian carcinomas. In addition, PPARγ ligands, drug such as pioglitazone, troglitazone and cigglitazone have been shown to modulate PPARγ activity by effecting the proliferation of ovarian cancer cells [Vignati et al., 2006]. These drugs were originally used as anti-diabetic drugs for their involvement in lipid homeostasis and energy metabolism [Semple et al., 2006]. Another classical anti-diabetic drug shown to have anti-proliferative and anti-metastatic effects on ovarian cancer cells is metformin an m-TOR inhibitor [Gotlieb et al., 2008]. This drug also inhibited ovarian cancer dissemination by inhibiting angiogenesis [Liao et al., 2012]. These differentiation strategies represent promising non-cytotoxic methods of decreasing tumor burden but how such approaches will impact on the CSC pool and activity yet remains to be determined.

Alternative approaches to target CSC. The most promising new therapies in cancer therapeutics is the introduction of nanoparticles whereby small molecules drugs, proteins, peptides either on its own or in combination with a drug delivery polymers or lipids can be introduced into targeted cells [Aguilar-Gallardo et al., 2012].

Aptamers. Aptamers are chemically synthesized, stable, non-immunogeneic and non-cytoxic short single-stranded DNA or RNA isolated by the SELEX (systematic evolution of ligands by exponential enrichment) method without minimal batch variation [Ellington and Szostak, 1990]. They fold into a 3D structure and are capable of binding to target molecules with high specificity [Pu et al., 2010]. The cost of manufacturing large quantities of aptamers is relatively low, and as they are 10–20 times smaller than antibodies and can penetrate tissues effectively, they are therefore advantageous over antibodies in targeting cancer regulating molecules. Several aptamers are currently in clinical trials [Das et al., 2009; Missailidis and Hardy, 2009], and have been approved by US Food and Drug administration [Lee et al., 2005]. Nuclease-resistant aptamer has previously been shown to bind specifically to EpCAM in breast and colon cancer cell lines [Shigdar et al., 2011]. Aptamer-conjugated drugs are actively internalized in cancer cells which enables the release of conjugated chemotherapy drugs to targeted cells with minimal killing of normal cells [Shigdar et al., 2011]. Hence, use of these nuclease-resistant aptamers which specifically bind to defined groups of CSCs (e.g., EpCAM, CD44, etc) should be developed as prognostic tools to evaluate ovarian cancer disease progression and treatment monitoring.

MicroRNA (miR). These are a class of naturally occurring small non-coding RNA that regulate gene expression at the post-transcriptional level. Since 2006, few studies have shown that the miRNA profile is different in normal ovaries compared to primary and recurrent ovarian tumors [van Jaarsveld et al., 2010]. It has also been suggested that it may be possible to detect free circulating miRNAs in the serum of cancer patients as an aid to detect early-stage cancer [Taylor and Gercel-Taylor, 2008; Resnick et al., 2009]. miR-214, miR-30a, miR-27a, and miR-451 have been associated with chemoresistance in ovarian cancer [van Jaarsveld et al., 2010]. Let 7a and miR-200 families have been shown to be deregulated in ovarian pathogenesis [van Jaarsveld et al., 2010]. Additional studies have associated the expression of miR-200 with chemotherapy treatment response and survival outcome [Leskela et al., 2010; Hu et al., 2009]. Ectopic expression of miR-200a in ovarian cancer cell lines has been shown to inhibit EMT by targeting E-cadherin repressor ZEB2 in $CD133^{+}$ ovarian CSCs, suggesting that miR-200a has the capacity to reverse the invasive functions of ovarian CSCs [Wu et al., 2011]. In addition, a recent paper has demonstrated the ability of let7a, miR-125, miR-9, and miR-30 to regulate the expression of lin28, a pluripotent stem cell factor which has been shown to act as an oncogene promoting factor in ovarian cancer cells [Zhong et al., 2010]. In this context, miRNAs associated with CSCs can be suggested as potential targets for therapy. Viral delivery of let7 to suppress the tumor growth in a mouse model of lung adenocarcinoma has recently been demonstrated [Kumar et al., 2008]. This study is further supported by the commercial availability of miRNA mimics (Sigma–Aldrich, Life Technology) which can regulate the expression of specified miRNAs in vitro studies [Thorsen et al., 2012; Xu et al., 2012ab]. These studies suggest that targeting miRNAs in ovarian cancer is feasible and might lead to new therapeutic strategies.

Personalized medicine and CSCs. In the last decade, there has been an increasing interest in personalized medicine which relies on the use of information from the genome of individuals as a guide in medical decision making. The interrogation of DNA and RNA sequence variation can identify individual risk for patients and guide clinical decision making for patient management [Ginsburg and Willard, 2009]. This may form a basis for informed and effective treatment approaches. Recent studies have demonstrated that therapy resistant and therapy responsive cancers to manifest distinct patterns of genes associated with stemness/differentiation pathways [Glinsky, 2007; Glinsky, 2008]. These differences have recently been exploited to develop a stemness cancer therapy outcome predictor, an algorithm that combines the scores of stemness signatures that have been shown to provide a superior prognostic accuracy in retrospective supervised analysis of large cohorts of breast, prostate, lung and ovarian cancer patients [Glinsky, 2008]. These analyses suggest that stemness genomics may govern the clinical behavior of cancer relapse/recurrence. Hence, future studies are needed to validate this concept in a clinical setting.

CONCLUSION

The outlook for patients with ovarian cancer may be markedly improved by identifying disease-specific CSCs which are relevant to the development of each subtype of cancer. The involvement of CSCs in chemoresistance and recurrence opens a new avenue to develop new CSC-specific drug-delivery conjugates in the form of aptamers, differentiating agents, miRNA mimics or targeting peptides/nucleotides which could be given to patients intraperitoneally to tackle specifically the chemoresistance-associated CSCs. In addition, the application of personalized medicine in the form of a genomic signature (DNA or RNA), even though not yet standardized

and integrated into the health system for clinical consideration, may facilitate individual-specific drug and dose selection resulting in better ovarian cancer diagnosis and prognosis. These strategies may result in the reduction and or/eradication of post-chemotherapy residual cancer resulting in better patient outcomes.

ACKNOWLEDGMENTS

The authors wish to thank Women's Cancer Foundation and National Health and Medical Research Council of Australia (JKF, RegKey #441101) for supporting this work. KA is a recipient of an Australian Postgraduate Award.

REFERENCES

Agoff SN, Mendelin JE, Grieco VS, Garcia RL. 2002. Unexpected gynecologic neoplasms in patients with proven or suspected BRCA-1 or -2 mutations: Implications for gross examination, cytology, and clinical follow-up. Am J Surg Pathol 26:171–178.

Aguilar-Gallardo C, Rutledge EC, Martinez-Arroyo AM, Hidalgo JJ, Domingo S, Simon C. 2012. Overcoming challenges of ovarian cancer stem cells: Novel therapeutic approaches. Stem Cell Rev 8:994–1010.

Ahmed N, Maines-Bandiera S, Quinn MA, Unger WG, Dedhar S, Auersperg N. 2006. Molecular pathways regulating EGF-induced epithelio-mesenchymal transition in human ovarian surface epithelium. Am J Physiol Cell Physiol 290:C1532–C1542.

Ahmed N, Thompson EW, Quinn MA. 2007. Epithelial–mesenchymal interconversions in normal ovarian surface epithelium and ovarian carcinomas: An exception to the norm. J Cell Physiol 213:581–588.

Ahmed N, Abubaker K, Findlay J, Quinn M. 2010. Epithelial mesenchymal transition and cancer stem cell-like phenotypes facilitate chemoresistance in recurrent ovarian cancer. Curr Cancer Drug Targets 10:268–278.

Aktas B, Tewes M, Fehm T, Hauch S, Kimmig R, Kasimir-Bauer S. 2009. Stem cell and epithelial–mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. Breast Cancer Res 11:R46.

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. 2003. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 100:3983–3988.

Alvero AB, Chen R, Fu HH, Montagna M, Schwartz PE, Rutherford T, Silasi DA, Steffensen KD, Waldstrom M, Visintin I, Mor G. 2009. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. Cell Cycle 8:158–166.

Auersperg N, Maines-Bandiera SL, Dyck HG, Kruk PA. 1994. Characterization of cultured human ovarian surface epithelial cells: Phenotypic plasticity and premalignant changes. Lab Invest 71:510–518.

Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC. 2001. Ovarian surface epithelium: Biology, endocrinology, and pathology. Endocr Rev 22:255–288.

Baba T, Convery PA, Matsumura N, Whitaker RS, Kondoh E, Perry T, Huang Z, Bentley RC, Mori S, Fujii S, Marks JR, Berchuck A, Murphy SK. 2009. Epigenetic regulation of CD133 and tumorigenicity of CD133+ ovarian cancer cells. Oncogene 28:209–218.

Bapat SA, Mali AM, Koppikar CB, Kurrey NK. 2005. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Res 65:3025–3029.

Barker N, Clevers H. 2007. Tracking down the stem cells of the intestine: Strategies to identify adult stem cells. Gastroenterology 133:1755–1760.

Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H. 2007. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449:1003–1007.

Bates J. 2008. Cancer stem cells-emerging therapeutic, diagnostic and market opportunities. in Report.

Bellone S, Siegel ER, Cocco E, Cargnelutti M, Silasi DA, Azodi M, Schwartz PE, Rutherford TJ, Pecorelli S, Santin AD. 2009. Overexpression of epithelial cell adhesion molecule in primary, metastatic, and recurrent/chemotherapy-resistant epithelial ovarian cancer: Implications for epithelial cell adhesion molecule-specific immunotherapy. Int J Gynecol Cancer 19:860–866.

Bignotti E, Tassi RA, Calza S, Ravaggi A, Bandiera E, Rossi E, Donzelli C, Pasinetti B, Pecorelli S, Santin AD. 2007. Gene expression profile of ovarian serous papillary carcinomas: Identification of metastasis-associated genes. Am J Obstet Gynecol 196:245 e1-e11.

Borovski T, De Sousa EMF, Vermeulen L, Medema JP. 2011. Cancer stem cell niche: The place to be. Cancer Res 71:634–639.

Bose D, Zimmerman LJ, Pierobon M, Petricoin E, Tozzi F, Parikh A, Fan F, Dallas N, Xia L, Gaur P, Samuel S, Liebler DC, Ellis LM. 2011. Chemoresistant colorectal cancer cells and cancer stem cells mediate growth and survival of bystander cells. Br J Cancer 105:1759–1767.

Bourguignon LY, Peyrollier K, Xia W, Gilad E. 2008. Hyaluronan-CD44 interaction activates stem cell marker Nanog, Stat-3-mediated MDR1 gene expression, and ankyrin-regulated multidrug efflux in breast and ovarian tumor cells. J Biol Chem 283:17635–17651.

Bowen NJ, Walker LD, Matyunina LV, Logani S, Totten KA, Benigno BB, McDonald JF. 2009. Gene expression profiling supports the hypothesis that human ovarian surface epithelia are multipotent and capable of serving as ovarian cancer initiating cells. BMC Med Genomics 2:71.

Bristow RE, Tomacruz RS, Armstrong DK, Trimble EL, Montz FJ. 2002. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: A meta-analysis. J Clin Oncol 20: 1248–1259.

Buick RN, Pullano R, Trent JM. 1985. Comparative properties of five human ovarian adenocarcinoma cell lines. Cancer Res 45:3668–3676.

Chang B, Liu G, Xue F, Rosen DG, Xiao L, Wang X, Liu J. 2009. ALDH1 expression correlates with favorable prognosis in ovarian cancers. Mod Pathol 22:817–823.

Chao YL, Shepard CR, Wells A. 2010. Breast carcinoma cells re-express E-cadherin during mesenchymal to epithelial reverting transition. Mol Cancer 9:179.

Chen X, Horiuchi A, Kikuchi N, Osada R, Yoshida J, Shiozawa T, Konishi I. 2007. Hedgehog signal pathway is activated in ovarian carcinomas, correlating with cell proliferation: It's inhibition leads to growth suppression and apoptosis. Cancer Sci 98:68–76.

Choi YP, Shim HS, Gao MQ, Kang S, Cho NH. 2011. Molecular portraits of intratumoral heterogeneity in human ovarian cancer. Cancer Lett 307: 62–71.

Colomiere M, Ward AC, Riley C, Trenerry MK, Cameron-Smith D, Findlay J, Ackland L, Ahmed N. 2009. Cross talk of signals between EGFR and IL-6R through JAK2/STAT3 mediate epithelial-mesenchymal transition in ovarian carcinomas. Br J Cancer 100:134–144.

Creighton CJ, Li X, Landis M, Dixon JM, Neumeister VM, Sjolund A, Rimm DL, Wong H, Rodriguez A, Herschkowitz JI, Fan C, Zhang X, He X, Pavlick A, Gutierrez MC, Renshaw L, Larionov AA, Faratian D, Hilsenbeck SG, Perou CM, Lewis MT, Rosen JM, Chang JC. 2009. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proc Natl Acad Sci USA 106:13820–13825.

Curley MD, Garrett LA, Schorge JO, Foster R, Rueda BR. 2011. Evidence for cancer stem cells contributing to the pathogenesis of ovarian cancer. Front Biosci 16:368–392.

Das M, Mohanty C, Sahoo SK. 2009. Ligand-based targeted therapy for cancer tissue. Expert Opin Drug Deliv 6:285–304.

Dressman HK, Berchuck A, Chan G, Zhai J, Bild A, Sayer R, Cragun J, Clarke J, Whitaker RS, Li L, Gray J, Marks J, Ginsburg GS, Potti A, West M, Nevins JR, Lancaster JM. 2007. An integrated genomic-based approach to individual-

ized treatment of patients with advanced-stage ovarian cancer. J Clin Oncol 25:517–525.

Ellington AD, Szostak JW. 1990. In vitro selection of RNA molecules that bind specific ligands. Nature 346:818–822.

Ferrandina G, Bonanno G, Pierelli L, Perillo A, Procoli A, Mariotti A, Corallo M, Martinelli E, Rutella S, Paglia A, Zannoni G, Mancuso S, Scambia G. 2008. Expression of CD133-1 and CD133-2 in ovarian cancer. Int J Gynecol Cancer 18:506–514.

Folkins AK, Saleemuddin A, Garrett LA, Garber JE, Muto MG, Tworoger SS, Crum CP. 2009. Epidemiologic correlates of ovarian cortical inclusion cysts (CICs) support a dual precursor pathway to pelvic epithelial cancer. Gynecol Oncol 115:108–111.

Gao MQ, Choi YP, Kang S, Youn JH, Cho NH. 2010. CD24+ cells from hierarchically organized ovarian cancer are enriched in cancer stem cells. Oncogene 29:2672–2680.

Gao S, Zhao X, Lin B, Hu Z, Yan L, Gao J. 2012. Clinical implications of REST and TUBB3 in ovarian cancer and its relationship to paclitaxel resistance. Tumour Biol Jun 10 [Epub ahead of print].

García Campelo MRACG, Aparicio Gallego G, Grande Pulido E, Antón Aparicio LM. 2011. Stem cell and lung cancer development: Blaming the Wnt, Hh and Notch signalling pathway. Clin Transl Oncol 13:77–83.

Gatcliffe TA, Monk BJ, Planutis K, Holcombe RF. 2008. Wnt signaling in ovarian tumorigenesis. Int J Gynecol Cancer 18:954–962.

Ginsburg GS, Willard HF. 2009. Genomic and personalized medicine: Foundations and applications. Transl Res 154:277–287.

Glinsky GV. 2007. Stem cell origin of death-from-cancer phenotypes of human prostate and breast cancers. Stem Cell Rev 3:79–93.

Glinsky GV. 2008. ''Stemness'' genomics law governs clinical behavior of human cancer: Implications for decision making in disease management. J Clin Oncol 26:2846–2853.

Gotlieb WH, Saumet J, Beauchamp MC, Gu J, Lau S, Pollak MN, Bruchim I. 2008. In vitro metformin anti-neoplastic activity in epithelial ovarian cancer. Gynecol Oncol 110:246–250.

Guddati AM. 2012. Ovarian cancer stem cells: Elusive targets for chemotherapy. Med Oncol May 26 [Epub ahead of print].

Guo Y, Nemeth J, O'Brien C, Susa M, Liu X, Zhang Z, Choy E, Mankin H, Hornicek F, Duan Z. 2010. Effects of siltuximab on the IL-6-induced signaling pathway in ovarian cancer. Clin Cancer Res 16:5759–5769.

Haviv I, Thompson EW. 2012. Soiling the seed: Microenvironment and epithelial mesenchymal plasticity. Cancer Microenviron 5:1–3.

Heider KH, Kuthan H, Stehle G, Munzert G. 2004. CD44v6: A target for antibody-based cancer therapy. Cancer Immunol Immunother 53:567–579.

Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ, Heeschen C. 2007. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 1:313–323.

Hill RP. 2006. Identifying cancer stem cells in solid tumors: Case not proven. Cancer Res 66:1891–1895; discussion 1890.

Hosonuma S, Kobayashi Y, Kojo S, Wada H, Seino K, Kiguchi K, Ishizuka B. 2011. Clinical significance of side population in ovarian cancer cells. Hum Cell 24:9–12.

Hu X, Macdonald DM, Huettner PC, Feng Z, El Naqa IM, Schwarz JK, Mutch DG, Grigsby PW, Powell SN, Wang X. 2009. A miR-200 microRNA cluster as prognostic marker in advanced ovarian cancer. Gynecol Oncol 114:457–464.

Hu L, McArthur C, Jaffe RB. 2010. Ovarian cancer stem-like side-population cells are tumourigenic and chemoresistant. Br J Cancer 102:1276–1283.

Huber MA, Kraut N, Beug H. 2005. Molecular requirements for epithelial–mesenchymal transition during tumor progression. Curr Opin Cell Biol 17:548–558.

Huh WK, Sill MW, Darcy KM, Elias KM, Hoffman JS, Boggess JF, Alvarez RD, Long HJ, O'Malley DM, Birrer MJ. 2010. Efficacy and safety of imatinib mesylate (Gleevec) and immunohistochemical expression of c-Kit and PDGFR-beta in a Gynecologic Oncology Group Phase II Trial in women with recurrent or persistent carcinosarcomas of the uterus. Gynecol Oncol 117:248–254.

Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D. 2010. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell 142:375–386.

Jazaeri AA, Bryant JL, Park H, Li H, Dahiya N, Stoler MH, Ferriss JS, Dutta A. 2011. Molecular requirements for transformation of fallopian tube epithelial cells into serous carcinoma. Neoplasia 13:899–911.

Jazedje T, Perin PM, Czeresnia CE, Maluf M, Halpern S, Secco M, Bueno DF, Vieira NM, Zucconi E, Zatz M. 2009. Human fallopian tube: A new source of multipotent adult mesenchymal stem cells discarded in surgical procedures. J Transl Med 7:46.

Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. 2009. Cancer statistics, 2009. CA Cancer J Clin 59:225–249.

Karst AM, Drapkin R. 2010. Ovarian cancer pathogenesis: A model in evolution. J Oncol 2010:932371.

Karst AM, Levanon K, Drapkin R. 2011. Modeling high-grade serous ovarian carcinogenesis from the fallopian tube. Proc Natl Acad Sci USA 108:7547–7552.

Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A. 2007. Tumor growth need not be driven by rare cancer stem cells. Science 317:337.

Kindelberger DW, Lee Y, Miron A, Hirsch MS, Feltmate C, Medeiros F, Callahan MJ, Garner EO, Gordon RW, Birch C, Berkowitz RS, Muto MG, Crum CP. 2007. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. Am J Surg Pathol 31:161–169.

Kristiansen G, Denkert C, Schluns K, Dahl E, Pilarsky C, Hauptmann S. 2002. CD24 is expressed in ovarian cancer and is a new independent prognostic marker of patient survival. Am J Pathol 161:1215–1221.

Kryczek I, Liu S, Roh M, Vatan L, Szeliga W, Wei S, Banerjee M, Mao Y, Kotarski J, Wicha MS, Liu R, Zou W. 2012. Expression of aldehyde dehydrogenase and CD133 defines ovarian cancer stem cells. Int J Cancer 130: 29–39.

Kuhn E, Kurman RJ, Shih IM. 2012. Ovarian cancer is an imported disease: Fact or fiction? Curr Obstet Gynecol Rep 1:1–9.

Kumar MS, Erkeland SJ, Pester RE, Chen CY, Ebert MS, Sharp PA, Jacks T. 2008. Suppression of non-small cell lung tumor development by the let-7 microRNA family. Proc Natl Acad Sci USA 105:3903–3908.

Kurman RJ, Shih Ie M. 2008. Pathogenesis of ovarian cancer: Lessons from morphology and molecular biology and their clinical implications. Int J Gynecol Pathol 27:151–160.

Kurtz JE, Dufour P. 2010. Adecatumumab: An anti-EpCAM monoclonal antibody, from the bench to the bedside. Expert Opin Biol Ther 10:951–958.

Landen CN, Jr., Goodman B, Katre AA, Steg AD, Nick AM, Stone RL, Miller LD, Mejia PV, Jennings NB, Gershenson DM, Bast RC, Jr., Coleman RL, Lopez-Berestein G, Sood AK. 2010. Targeting aldehyde dehydrogenase cancer stem cells in ovarian cancer. Mol Cancer Ther 9:3186–3199.

Latifi A, Abubaker K, Castrechini N, Ward AC, Liongue C, Dobill F, Kumar J, Thompson EW, Quinn MA, Findlay JK, Ahmed N. 2011. Cisplatin treatment of primary and metastatic epithelial ovarian carcinomas generates residual cells with mesenchymal stem cell-like profile. J Cell Biochem. 112:2850–2864.

Lee CM, Shvartsman H, Deavers MT, Wang SC, Xia W, Schmandt R, Bodurka DC, Atkinson EN, Malpica A, Gershenson DM, Hung MC, Lu KH. 2003. beta-catenin nuclear localization is associated with grade in ovarian serous carcinoma. Gynecol Oncol 88:363–368.

Lee JH, Canny MD, De Erkenez A, Krilleke D, Ng YS, Shima DT, Pardi A, Jucker F. 2005. A therapeutic aptamer inhibits angiogenesis by specifically targeting the heparin binding domain of VEGF165. Proc Natl Acad Sci USA 102:18902–18907.

Lee Y, Miron A, Drapkin R, Nucci MR, Medeiros F, Saleemuddin A, Garber J, Birch C, Mou H, Gordon RW, Cramer DW, McKeon FD, Crum CP. 2007. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. J Pathol 211:26–35.

Lengyel E. 2010. Ovarian cancer development and metastasis. Am J Pathol 177:1053–1064.

Leskela S, Leandro-Garcia LJ, Mendiola M, Barriuso J, Inglada-Perez L, Munoz I, Martinez-Delgado B, Redondo A, de Santiago J, Robledo M, Hardisson D, Rodriguez-Antona C. 2010. The miR-200 family controls beta-tubulin III expression and is associated with paclitaxel-based treatment response and progression-free survival in ovarian cancer patients. Endocr Relat Cancer 18:85–95.

Levanon K, Crum C, Drapkin R. 2008. New insights into the pathogenesis of serous ovarian cancer and its clinical impact. J Clin Oncol 26:5284–5293.

Levanon K, Ng V, Piao HY, Zhang Y, Chang MC, Roh MH, Kindelberger DW, Hirsch MS, Crum CP, Marto JA, Drapkin R. 2010. Primary ex vivo cultures of human fallopian tube epithelium as a model for serous ovarian carcinogenesis. Oncogene 29:1103–1113.

Liao H, Zhou Q, Gu Y, Duan T, Feng Y. 2012. Luteinizing hormone facilitates angiogenesis in ovarian epithelial tumor cells and metformin inhibits the effect through the mTOR signaling pathway. Oncol Rep 27:1873–1878.

Luo L, Zeng J, Liang B, Zhao Z, Sun L, Cao D, Yang J, Shen K. 2011. Ovarian cancer cells with the CD117 phenotype are highly tumorigenic and are related to chemotherapy outcome. Exp Mol Pathol 91:596–602.

Marhaba R, Klingbeil P, Nuebel T, Nazarenko I, Buechler MW, Zoeller M. 2008. CD44 and EpCAM: Cancer-initiating cell markers. Curr Mol Med 8:784–804.

Matei D, Emerson RE, Schilder J, Menning N, Baldridge LA, Johnson CS, Breen T, McClean J, Stephens D, Whalen C, Sutton G. 2008. Imatinib mesylate in combination with docetaxel for the treatment of patients with advanced, platinum-resistant ovarian cancer and primary peritoneal carcinomatosis: A Hoosier Oncology Group trial. Cancer 113:723–732.

McClanahan T, Koseoglu S, Smith K, Grein J, Gustafson E, Black S, Kirschmeier P, Samatar AA. 2006. Identification of overexpression of orphan G protein-coupled receptor GPR49 in human colon and ovarian primary tumors. Cancer Biol Ther 5:419–426.

Medeiros F, Muto MG, Lee Y, Elvin JA, Callahan MJ, Feltmate C, Garber JE, Cramer DW, Crum CP. 2006. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. Am J Surg Pathol 30:230–236.

Meng E, Long B, Sullivan P, McClellan S, Finan MA, Reed E, Shevde L, Rocconi RP. 2012. CD44+/CD24-ovarian cancer cells demonstrate cancer stem cell properties and correlate to survival. Clin Exp Metastasis May 18 [Epub ahead of print].

Mimeault M, Batra SK. 2008. Targeting of cancer stem/progenitor cells plus stem cell-based therapies: The ultimate hope for treating and curing aggressive and recurrent cancers. Panminerva Med 50:3–18.

Missailidis S, Hardy A. 2009. Aptamers as inhibitors of target proteins. Expert Opin Ther Pat 19:1073–1082.

Morris RJ, Liu Y, Marles L, Yang Z, Trempus C, Li S, Lin JS, Sawicki JA, Cotsarelis G. 2004. Capturing and profiling adult hair follicle stem cells. Nat Biotechnol 22:411–417.

Mundhenke C, Weigel MT, Sturner KH, Roesel F, Meinhold-Heerlein I, Bauerschlag DO, Schem C, Hilpert F, Jonat W, Maass N. 2008. Novel treatment of ovarian cancer cell lines with Imatinib mesylate combined with Paclitaxel and Carboplatin leads to receptor-mediated antiproliferative effects. J Cancer Res Clin Oncol 134:1397–1405.

Murdoch WJ, Martinchick JF. 2004. Oxidative damage to DNA of ovarian surface epithelial cells affected by ovulation: Carcinogenic implication and chemoprevention. Exp Biol Med (Maywood) 229:546–552.

Oliver TG, Mercer KL, Sayles LC, Burke JR, Mendus D, Lovejoy KS, Cheng MH, Subramanian A, Mu D, Powers S, Crowley D, Bronson RT, Whittaker CA, Bhutkar A, Lippard SJ, Golub T, Thomale J, Jacks T, Sweet-Cordero EA. 2010. Chronic cisplatin treatment promotes enhanced damage repair and tumor progression in a mouse model of lung cancer. Genes Dev 24:837–852.

Orian-Rousseau V. 2010. CD44, a therapeutic target for metastasising tumours. Eur J Cancer 46:1271–1277.

Ozols RF. 2006. Systemic therapy for ovarian cancer: Current status and new treatments. Semin Oncol 33:S3–S11.

Ozols RF, Bookman MA, Connolly DC, Daly MB, Godwin AK, Schilder RJ, Xu X, Hamilton TC. 2004. Focus on epithelial ovarian cancer. Cancer Cell 5:19–24.

Palacios J, Gamallo C. 1998. Mutations in the beta-catenin gene (CTNNB1) in endometrioid ovarian carcinomas. Cancer Res 58:1344–1347.

Peng S, Maihle NJ, Huang Y. 2010. Pluripotency factors Lin28 and Oct4 identify a sub-population of stem cell-like cells in ovarian cancer. Oncogene 29:2153–2159.

Permuth-Wey J, Sellers TA. 2009. Epidemiology of ovarian cancer. Methods Mol Biol 472:413–437.

Piek JM, van Diest PJ, Zweemer RP, Jansen JW, Poort-Keesom RJ, Menko FH, Gille JJ, Jongsma AP, Pals G, Kenemans P, Verheijen RH. 2001. Dysplastic changes in prophylactically removed Fallopian tubes of women predisposed to developing ovarian cancer. J Pathol 195:451–456.

Piek JM, Torrenga B, Hermsen B, Verheijen RH, Zweemer RP, Gille JJ, Kenemans P, van Diest PJ, Menko FH. 2003. Histopathological characteristics of BRCA1- and BRCA2-associated intraperitoneal cancer: A clinic-based study. Fam Cancer 2:73–78.

Pu Y, Zhu Z, Liu H, Zhang J, Liu J, Tan W. 2010. Using aptamers to visualize and capture cancer cells. Anal Bioanal Chem 397:3225–3233.

Rask K, Nilsson A, Brannstrom M, Carlsson P, Hellberg P, Janson PO, Hedin L, Sundfeldt K. 2003. Wnt-signalling pathway in ovarian epithelial tumours: Increased expression of beta-catenin and GSK3beta. Br J Cancer 89:1298–1304.

Resnick KE, Alder H, Hagan JP, Richardson DL, Croce CM, Cohn DE. 2009. The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol 112:55–59.

Ricken A, Lochhead P, Kontogiannea M, Farookhi R. 2002. Wnt signaling in the ovary: Identification and compartmentalized expression of wnt-2, wnt-2b, and frizzled-4 mRNAs. Endocrinology 143:2741–2749.

Risch HA, McLaughlin JR, Cole DE, Rosen B, Bradley L, Fan I, Tang J, Li S, Zhang S, Shaw PA, Narod SA. 2006. Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: A kin-cohort study in Ontario, Canada. J Natl Cancer Inst 98:1694–1706.

Rizzo S, Hersey JM, Mellor P, Dai W, Santos-Silva A, Liber D, Luk L, Titley I, Carden CP, Box G, Hudson DL, Kaye SB, Brown R. 2011. Ovarian cancer stem cell-like side populations are enriched following chemotherapy and overexpress EZH2. Mol Cancer Ther 10:325–335.

Rogakou EP, Boon C, Redon C, Bonner WM. 1999. Megabase chromatin domains involved in DNA double-strand breaks in vivo. J Cell Biol 146:905–916.

Ruiz-Vela A, Aguilar-Gallardo C, Martinez-Arroyo AM, Soriano-Navarro M, Ruiz V, Simon C. 2011. Specific unsaturated fatty acids enforce the transdifferentiation of human cancer cells toward adipocyte-like cells. Stem Cell Rev 7:898–909.

Saad AF, Hu W, Sood AK. 2010. Microenvironment and pathogenesis of epithelial ovarian cancer. Horm Cancer 1:277–290.

Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, Zhan Q, Jordan S, Duncan LM, Weishaupt C, Fuhlbrigge RC, Kupper TS, Sayegh MH, Frank MH. 2008. Identification of cells initiating human melanomas. Nature 451:345–349.

Schauer IG, Sood AK, Mok S, Liu J. 2011. Cancer-associated fibroblasts and their putative role in potentiating the initiation and development of epithelial ovarian cancer. Neoplasia 13:393–405.

Scheil-Bertram S, Tylus-Schaaf P, du Bois A, Harter P, Oppitz M, Ewald-Riegler N, Fisseler-Eckhoff A. 2010. Excision repair cross-complementation group 1 protein overexpression as a predictor of poor survival for high-grade serous ovarian adenocarcinoma. Gynecol Oncol 119:325–331.

Schilder RJ, Sill MW, Lee RB, Shaw TJ, Senterman MK, Klein-Szanto AJ, Miner Z, Vanderhyden BC. 2008. Phase II evaluation of imatinib mesylate in the treatment of recurrent or persistent epithelial ovarian or primary peritoneal carcinoma: A Gynecologic Oncology Group Study. J Clin Oncol 26:3418–3425.

Seimetz D, Lindhofer H, Bokemeyer C. 2010. Development and approval of the trifunctional antibody catumaxomab (anti-EpCAM x anti-CD3) as a targeted cancer immunotherapy. Cancer Treat Rev 36:458–467.

Sell S. 2004. Stem cell origin of cancer and differentiation therapy. Crit Rev Oncol Hematol 51:1–28.

Semple RK, Chatterjee VK, O'Rahilly S. 2006. PPAR gamma and human metabolic disease. J Clin Invest 116:581–589.

Shigdar S, Lin J, Yu Y, Pastuovic M, Wei M, Duan W. 2011. RNA aptamer against a cancer stem cell marker epithelial cell adhesion molecule. Cancer Sci 102:991–998.

Silva IA, Bai S, McLean K, Yang K, Griffith K, Thomas D, Ginestier C, Johnston C, Kueck A, Reynolds RK, Wicha MS, Buckanovich RJ. 2011. Aldehyde dehydrogenase in combination with CD133 defines angiogenic ovarian cancer stem cells that portend poor patient survival. Cancer Res 71:3991–4001.

Smith LM, Nesterova A, Ryan MC, Duniho S, Jonas M, Anderson M, Zabinski RF, Sutherland MK, Gerber HP, Van Orden KL, Moore PA, Ruben SM, Carter PJ. 2008. CD133/prominin-1 is a potential therapeutic target for antibody-drug conjugates in hepatocellular and gastric cancers. Br J Cancer 99:100–109.

Society AC. 2007. Cancer facts and figures. www.cancer.org.

Soignet SL, Benedetti F, Fleischauer A, Parker BA, Truglia JA, Ra Crisp M, Warrell RP, Jr. 1998. Clinical study of 9-cis retinoic acid (LGD1057) in acute promyelocytic leukemia. Leukemia 12:1518–1521.

Spizzo G, Went P, Dirnhofer S, Obrist P, Moch H, Baeuerle PA, Mueller-Holzner E, Marth C, Gastl G, Zeimet AG. 2006. Overexpression of epithelial cell adhesion molecule (Ep-CAM) is an independent prognostic marker for reduced survival of patients with epithelial ovarian cancer. Gynecol Oncol 103:483–488.

Staud F, Pavek P. 2005. Breast cancer resistance protein (BCRP/ABCG2). Int J Biochem Cell Biol 37:720–725.

Steg AD, Katre AA, Goodman B, Han HD, Nick AM, Stone RL, Coleman RL, Alvarez RD, Lopez-Berestein G, Sood AK, Landen CN. 2011. Targeting the notch ligand JAGGED1 in both tumor cells and stroma in ovarian cancer. Clin Cancer Res 17:5674–5685.

Steg AD, Bevis KS, Katre AA, Ziebarth A, Dobbin ZC, Alvarez RD, Zhang K, Conner M, Landen CN. 2012a. Stem cell pathways contribute to clinical chemoresistance in ovarian cancer. Clin Cancer Res 18:869–881.

Steg AD, Katre AA, Bevis KS, Ziebarth A, Dobbin ZC, Shah MM, Alvarez RD, Landen CN, Jr. 2012b. Smoothened Antagonists Reverse Taxane Resistance in Ovarian Cancer. Mol Cancer Ther 11:1587–1597.

Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, Dombkowski D, Preffer F, Maclaughlin DT, Donahoe PK. 2006. Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian Inhibiting Substance responsiveness. Proc Natl Acad Sci USA 103:11154–11159.

Szotek PP, Chang HL, Brennand K, Fujino A, Pieretti-Vanmarcke R, Lo Celso C, Dombkowski D, Preffer F, Cohen KS, Teixeira J, Donahoe PK. 2008. Normal ovarian surface epithelial label-retaining cells exhibit stem/progenitor cell characteristics. Proc Natl Acad Sci USA 105:12469–12473.

Tanese K, Fukuma M, Yamada T, Mori T, Yoshikawa T, Watanabe W, Ishiko A, Amagai M, Nishikawa T, Sakamoto M. 2008. G-protein-coupled receptor GPR49 is up-regulated in basal cell carcinoma and promotes cell proliferation and tumor formation. Am J Pathol 173:835–843.

Taylor DD, Gercel-Taylor C. 2008. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol 110: 13–21.

Thorsen SB, Obad S, Jensen NF, Stenvang J, Kauppinen S. 2012. The therapeutic potential of MicroRNAs in cancer. Cancer J 18:275–284.

van der Gun BT, Melchers LJ, Ruiters MH, de Leij LF, McLaughlin PM, Rots MG. 2010. EpCAM in carcinogenesis: The good, the bad or the ugly. Carcinogenesis 31:1913–1921.

van Jaarsveld MT, Helleman J, Berns EM, Wiemer EA. 2010. MicroRNAs in ovarian cancer biology and therapy resistance. Int J Biochem Cell Biol 42:1282–1290.

Vathipadiekal V, Saxena D, Mok SC, Hauschka PV, Ozbun L, Birrer MJ. 2012. Identification of a potential ovarian cancer stem cell gene expression profile from advanced stage papillary serous ovarian cancer. PLoS ONE 7:e29079.

Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M. 2010. Direct conversion of fibroblasts to functional neurons by defined factors. Nature 463:1035–1041.

Vignati S, Albertini V, Rinaldi A, Kwee I, Riva C, Oldrini R, Capella C, Bertoni F, Carbone GM, Catapano CV. 2006. Cellular and molecular consequences of peroxisome proliferator-activated receptor-gamma activation in ovarian cancer cells. Neoplasia 8:851–861.

Virant-Klun I, Zech N, Rozman P, Vogler A, Cvjeticanin B, Klemenc P, Malicev E, Meden-Vrtovec H. 2008. Putative stem cells with an embryonic character isolated from the ovarian surface epithelium of women with no naturally present follicles and oocytes. Differentiation 76:843–856.

Wang YC, Yo YT, Lee HY, Liao YP, Chao TK, Su PH, Lai HC. 2012. ALDH1-bright epithelial ovarian cancer cells are associated with CD44 expression, drug resistance, and poor clinical outcome. Am J Pathol 180:1159–1169.

Wooster R, Weber BL. 2003. Breast and ovarian cancer. N Engl J Med 348: 2339–2347.

Wright K, Wilson P, Morland S, Campbell I, Walsh M, Hurst T, Ward B, Cummings M, Chenevix-Trench G. 1999. beta-catenin mutation and expression analysis in ovarian cancer: Exon 3 mutations and nuclear translocation in 16% of endometrioid tumours. Int J Cancer 82:625–629.

Wu Q, Guo R, Lin M, Zhou B, Wang Y. 2011. MicroRNA-200a inhibits CD133/1+ ovarian cancer stem cells migration and invasion by targeting E-cadherin repressor ZEB2. Gynecol Oncol 122:149–154.

Xu K, Liang X, Shen K, Cui D, Zheng Y, Xu J, Fan Z, Qiu Y, Li Q, Ni L, Liu J. 2012a. MiR-297 modulate multidrug resistance in human colorectal carcinoma by down-regulating MRP-2. Biochem J 446:291–300.

Xu K, Liang X, Shen K, Sun L, Cui D, Zhao Y, Tian J, Ni L, Liu J. 2012b. MiR-222 modulate multidrug resistance in human colorectal carcinoma by down-regulating ADAM-17. Exp Cell Res 318:2168–2177.

Yamashita T, Ji J, Budhu A, Forgues M, Yang W, Wang HY, Jia H, Ye Q, Qin LX, Wauthier E, Reid LM, Minato H, Honda M, Kaneko S, Tang ZY, Wang XW. 2009. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. Gastroenterology 136: 1012–1024.

Yang X, Lin X, Zhong X, Kaur S, Li N, Liang S, Lassus H, Wang L, Katsaros D, Montone K, Zhao X, Zhang Y, Butzow R, Coukos G, Zhang L. 2010. Double-negative feedback loop between reprogramming factor LIN28 and microRNA let-7 regulates aldehyde dehydrogenase 1-positive cancer stem cells. Cancer Res 70:9463–9472.

Zhang GY, Ahmed N, Riley C, Oliva K, Barker G, Quinn MA, Rice GE. 2005. Enhanced expression of peroxisome proliferator-activated receptor gamma in epithelial ovarian carcinoma. Br J Cancer 92:113–119.

Zhang S, Balch C, Chan MW, Lai HC, Matei D, Schilder JM, Yan PS, Huang TH, Nephew KP. 2008. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Res 68:4311–4320.

Zhong X, Li N, Liang S, Huang Q, Coukos G, Zhang L. 2010. Identification of microRNAs regulating reprogramming factor LIN28 in embryonic stem cells and cancer cells. J Biol Chem 285:41961–41971.

Zhou S, Schuetz JD, Bunting KD, Colapietro AM, Sampath J, Morris JJ, Lagutina I, Grosveld GC, Osawa M, Nakauchi H, Sorrentino BP. 2001. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 7:1028–1034.