Histol Histopathol (2010) 25: 113-120

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

The role of cancer stem cells and the side population in epithelial ovarian cancer

Miranda Y. Fong and Sham S. Kakar

Department of Physiology and Biophysics, James Graham Brown Cancer Center, University of Louisville, Louisville, USA

Summary. Ovarian cancer is the most lethal cancer of the female reproductive tract, accounting for ~15,000 deaths per year according to the National Cancer Institute and American Cancer Society. This review article covers risk factors for the development of ovarian cancer, current detection strategies, prognostic markers, treatment strategies, etiology of tumorigenesis, and ovarian somatic stem cells. While the etiology of ovarian cancer is still unknown, several theories have been proposed as the mechanism of carcinogenesis. One theory states that the surface epithelium undergoing invagination and forming inclusion cysts that are exposed to growth factors and cytokines. The "gonadotropin theory" has also been proposed. Other reigning models for tumorigenesis include the stochastical model where a distinct population of cells acquires somatic mutations leading to metastasis, and the hierarchical model where the tumor is initiated by cancer stem cells (CSCs). CSCs isolated from primary tumors have the ability to regenerate the tumor and reconstitute the original tumor phenotype with as few as 100 cells. CSCs from ovarian carcinomas display the cell surface markers $CD44^+CD117^+CD133^+$. CSCs are also thought to account for chemotherapy resistance through the expression of highly selective transporters ABCG2 and MDR1 and activation of TLR4/MyD88. The side population has been characterized by their ability to efflux lipophilic substrates, including the dye Hoechst 33342 and many chemotherapy agents. This ability has been attributed to the expression of the transporters ABCG2 and MDR1.

Key words: Cancer stem cells, Side population, Ovarian cancer etiology, CD133, CD44, CD117

Offprint requests to: Sham S. Kakar, Professor, Department of Physiology and Biophysics, 580 South Preston Street, Baxter II, Room 321E, University of Louisville, Louisville, KY 40202. USA. e-mail: sskaka01@louisville.edu

Introduction

Ovarian cancer (OCA) is the eighth most common and the fifth cause of cancer death in women, responsible for ~15,000 deaths per year with over 21,000 new cases in 2008 according to the American Cancer Society and National Cancer Institute. This makes it the most lethal malignancy of the female genital tract. If ovarian cancer is found at an early stage, the 5-year survival rate is 92%. However, less than 20% of ovarian cancer is diagnosed at an early stage which decreases the 5-year survival rate to 15-45% (Nossov et al., 2008). The most common form of OCA is epithelial, which can be further divided into 4 main pathologies: serous (70%), endometriod (20%), mucinous (5-10%), and clear cell (5-10%) (Neesham, 2007). Other forms of OCA arise from the germ cell or stromal cell layer.

Risk factors for ovarian cancer

Several risk factors increase the possibility for ovarian cancer. The most well known is hereditary mutations in *BRCA1* or *BRCA2*, which accounts for 10% of cases (Carvalho and Carvalho, 2008; Landen et al., 2008). Mutations in *BRCA1* in a first degree relative increase OCA risk by 40-50%, while mutation in *BRCA2* increase risk by 20-30% (Soegaard et al., 2008). Other risk factors include mismatch repair mechanism mutations, polycystic ovarian syndrome (PCOS) (Landen et al., 2008), infertility (Cetin et al., 2008), smoking (Modugno et al., 2002; Rossing et al., 2008), talcum powder use, ovarian cysts, endometriosis, and hyperthyroidism (Ness et al., 2000). Both BRCA and nibrin (NBN) proteins function in double-strand break repair, cell cycle control, meiosis, and telomere formation. In one case study, the OCA patient was found to have heterogous mutations in both *BRCA* and *NBN* (Porhanova et al., 2008). Heterozygous *BRCA* results in hereditary breast cancer, while *NBN* heterozygosity results in Nihmengen Breakage Syndrome (NBS). NBS has been implicated in increased cancer risk as well as

other severe yet non-cancer related syndromes such as growth and mental retardation (Huang et al., 2008). A considerable portion of the literature suggests that NBS increases the risk for B-cell non-Hodgkin's lymphoma (Huang et al., 2008) and melanoma (Debniak et al., 2003), but has not been associated with either hereditary or sporadic prostate cancer (Hebbring et al., 2006) despite claims that NBS can be characterized by gonadal failure (Gladkowska-Dura et al., 2008). One group suggested that NBS may contribute to the formation of rare ovarian carcinomas (Plisiecka-Halasa et al., 2002), but further evidence is needed to confirm that finding. PCOS was reported to increase OCA risk 2-fold (Schildkraut et al., 1996; Cetin et al., 2008). It was suggested that infertility drugs and treatments alter the hormonal environment, thereby acting as co-factors in cancer formation. It was also proposed that infertility in itself could a risk factor most likely due to endometriosis and ovulatory abnormalities. However, infertility could be a symptom of OCA rather than a cause (Venn et al., 1999). Women with a history of endometriosis greater than 10 years are at particularly increased risk, although it has not been linked to the cause of cancer (Cetin et al., 2008).

More recently inflammation has been proposed to be a causative agent. Viral and bacterial agents produce chronic inflammation, which can lead to cancer in the GI tract, liver, and other cells mediated by oxidative stress producing reactive oxygen species (ROS), which reduce cellular adhesion by altering the MMP:TIMP ratio to promote metastasis, and reactive nitrogen species (RNS) forming adducts, which induce DNA and protein damage (Hold and El-Omar, 2008). Pelvic inflammatory disease has also been associated with OCA. Inflammation is known to promote increased cellular division, DNA excision and repair, oxidative stress, and a high concentration of cytokines and prostaglandins (Ness et al., 2000).

Factors that decrease the risk for OCA include multiple pregnancies, lactation lasting longer than 24 months, and oral contraceptive use (Ness et al., 2000; Landen et al., 2008), suggesting that decreased ovulation and cyst formation are protective against cancer formation. The first pregnancy is known to decrease the risk by 50% with a moderate decrease with subsequent pregnancies. Tubal ligation and hysterectomy also reduces the risk, especially in combination. Age at menarch, age at menopause, and menstrual pattern are not associated with risk (Ness et al., 2000).

Detection strategies

Current detection strategies include ultrasound and blood analysis for the tumor marker CA-125 (Neesham, 2007). However, both techniques have their drawbacks. The ovarian surface is dynamic in premenopausal women, so cancer could be mistaken for functional cysts. The CA-125 blood test is a measure of epithelial antigen protein expressed on the coelomic epithelium, which includes the ovarian epithelium (Neesham, 2007). However, the test lacks sensitivity, detecting only 50% of stage 1 disease, and has a high false positive rate (Lowe et al., 2008; Nossov et al., 2008) that can result from a variety of situations including gynecological origin such as endometriosis, fibroids, hemorrhagic ovarian cysts, menstruation, acute pelvic inflammatory disease, and first trimester pregnancy. Endometrial, pancreas, bladder, breast, liver, and lung cancer can also cause a false positive as well as inflammatory conditions, renal disease, and cirrhosis (Neesham, 2007). Blood analysis for CA-125 is only appropriate for detection of epithelial OCA (90% of OCA). More

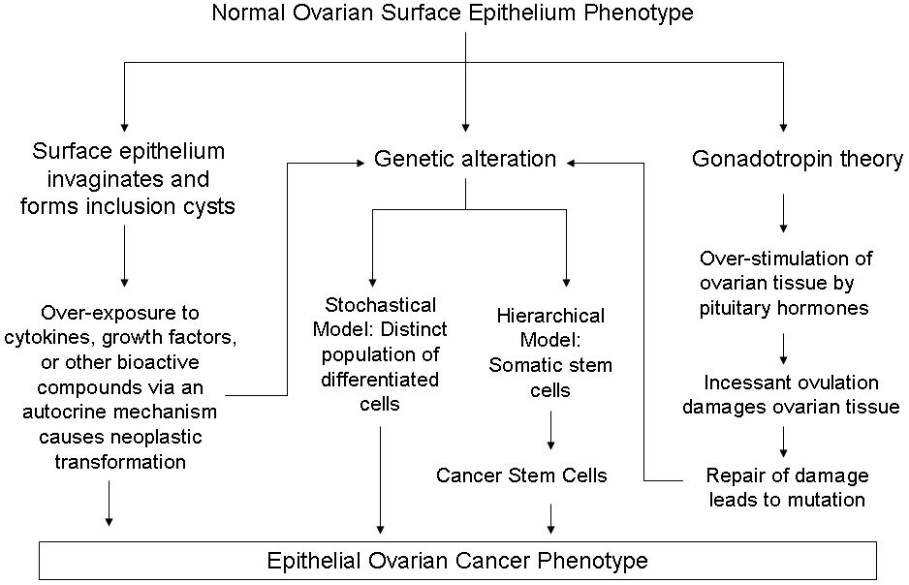

Fig. 1. Comprehensive diagram of the theories of the etiology of epithelial ovarian cancer.

recently mesothelin, an epithelial biomarker, and HE4 were proposed as possible candidates for early detection to complement the CA-125 serum test to help improve the sensitivity (Hellstrom and Hellstrom, 2008; Lowe et al., 2008; Nossov et al., 2008).

Prognostic markers

OCA can be classified into two broad categories: high-grade and low-grade. High-grade tumors are characterized by rapid growth and relative sensitive to chemotherapy without the presence of a definite precursor lesion. Low-grade tumors are classified by less rapid cell growth, relatively insensitivity to chemotherapy, and shared molecular characteristics similar to low-malignancy potential neoplasms. Molecular and pathway analysis indicates that the two grades utilized different signaling pathways that share p53 as a common branch point. Low- grade tumors have intact p53 while high-grade tumors have dysfunctional p53 (Landen et al., 2008).

Prognostic factors are characterized by tumor grade, disease stage, and vascular invasion. Tomić et al. (2003) correlated p53 overexpression with vascular invasion. Nm23 and Her2 correlated with vascular invasion, high grade, and advanced disease stage. Univariant analysis for advanced clinical stage, high grade, vascular invasion, and positive staining for p53, nm23, and Her2 proteins indicated overall shorter survival. However, using Cox multivariant analysis, only the stage correlated to survival. Cox proportional hazard regression multivariant analysis of early stage showed that only vascular invasion correlated to survival, while nothing correlated to an advanced disease stage.

Treatment

Treatment usually involves cytoreductive surgery followed by a chemotherapy regimen of platinum-based chemotherapy such as carboplatin-taxane (Rocconi et al., 2008). Agents used with cytoreductive surgery tend to induce expression of Cyclooxygenase-2 (COX-2). COX-2 mRNA and protein overexpression has been found in ovarian serous adenocarcinomas and were associated with altered expression of p53 and SMAD4 and increased expression of Her-2 (Erkinheimo et al., 2004). When COX-1 and COX-2 mRNA and protein expression were analyzed from patients who had not undergone cytoreductive treatment, COX-1 expression was elevated and compartmentalized to the epithelium, whereas normal ovarian epithelium did not express COX-1 or -2 mRNA at a detectable level (Gupta et al., 2003).

Microtubule-interfering agents (MIA) have been used to treat Her-2 positive breast cancer; however, it was found that MIA increase prostaglandin E2 (PGE2) synthesis via increasing COX-2 mRNA levels (Subbaramaiah et al., 2000). This makes COX-2 an attractive target for prevention and treatment. While epidemiological evidence from case-controlled studies suggests that non-steroidal anti-inflammatory drugs (NSAIDs) reduce the risk of breast cancer, cohort studies disagree. A population-based study from Long Island found an inverse relationship between regular aspirin use (defined as 1 or more tablets per week for 1 year) and breast cancer risk in hormone-receptor positive tumors. However, the Women's Health Study found no correlation between low-dose of aspirin (100 mg every other day) and reduction of risk for breast or OCA. The non-COX-specific NSAID indomethacin inhibits ovulation in a dose-dependent manner, implying that it could decrease the risk of OCA (Crew and Neugut, 2006). Treatment with indomethacin, SC-560 (COX-1 specific inhibitor), or celecoxib (COX-2 specific

Table 1. Factors that increase or desrease the risk for ofrmation of ovarian cancer.

Risk factors	Factors that decrease risk
Genetic mutations	Multiple pregnancies
BRCA 1/2	Lactation>24 months
Nibrin protein	Oral contrceptive use
Polycystic ovarian syndrome (PCOS)	Tubal ligation
Infertility	Hysterectomy
Ovarian cysts	
Endometriosis	
Hyperthyroidism	
Inflammation	
Personal habits	
Smoking	
Talcum powder use	

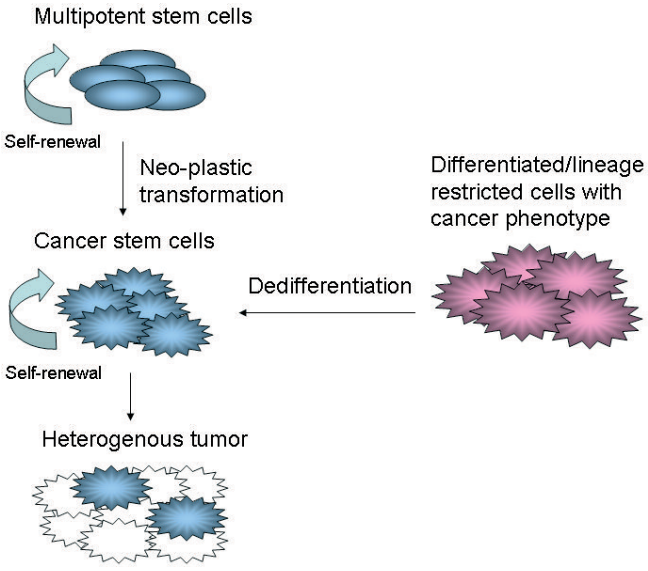

Fig. 2. Hierarchial model of tumorigenesis and formation of cancer stem cells.

inhibitor) only reduced cell numbers at a concentration of 50-100 times greater than what can be achieved *in vivo* (Gupta et al., 2003).

While the initial response rate to first-line platinum-based chemotherapy is 65-80%, most ovarian carcinomas relapse, contributing to a low 5-year survival rate (Fehrmann et al., 2007).

Etiology

The etiology of epithelial OCA remains mostly unknown. Several theories have been proposed as the mechanism for carcinogenesis. One such theory indicates that the ovarian surface epithelium (OSE) can undergo invagination and form inclusion cysts. These inclusion cysts can then undergo neoplastic transformation either through access to stromal-derived growth factors, cytokines, and bioactive compounds, which are normally restricted by the tunica albuginea, or from neoplastic progression in crypts promoted by an autocrine mechanism from OSE-derived cytokines and hormones that accumulate to bio-active levels (Auersperg et al., 2001). Another theory is the "gonadotropin theory" (Bose, 2008). The gonadotropin theory suggests that pituitary gonadotropins, i.e., luteinizing hormone (LH) and follicle stimulating hormone (FSH), overstimulate the ovarian tissue causing excessive ovulation. Androgens, estrogens, progesterones, and insulin-like growth factor-I (IGF-I) may also contribute to cancer pathogenesis (Lukanova and Kaaks, 2005) as well as a genetic component. Currently, about 10% of OCA cases are hereditary (Carvalho and Carvalho, 2008; Landen et al., 2008). Of these, 90% are due to *BRCA1* or *BRCA2* mutations (Landen et al., 2008). Other contributing genes could be alterations of p53 expression and overexpression of Her2/*neu*, PIK3A, AKT, and c-myc (Tammela and Odunsi, 2004). $P27^{kip1}$ has been proposed as an oncogene because of its ability to expand stem cells even though it mainly functions as a cyclin-dependent kinase inhibitor (CKI) to regulate the cell cycle. Using a knock-in version of P27 that was unable to bind to the Cdk:cyclin complex ($P27^{ck-}$) produced spontaneous tumor formation in multiple tissues, including ovarian hyperplasia (Besson et al., 2007). The other reigning models for tumorigenesis include the stochastical model, where a distinct population of cells acquires somatic mutations leading to metastasis, and the hierarchical model, where the tumor is initiated by cancer stem cells (CSCs) (Odoux et al., 2008).

Cancer stem cells (CSCs)

Cancer stem cells (CSCs) are described as "multipotent cells capable of forming heterogeneous tumors in immunodeficient mice at high efficiency" (Polyak and Hahn 2006). Five defining criteria has been established to verify the existence of CSCs: 1) self-renewal, 2) restriction to a small percentage of the tumor population, 3) ability to reproduce the heterogenous tumor phenotype, 4) multipotent differentiation into non-tumorigenic cells, and 5) expression of distinctive cells markers (Clarke et al., 2006; Dalerba et al., 2007; Olempska et al., 2007; Zhang et al., 2008). Based on this criteria, CSCs have also been referred to as cancer-initiating cells (Zhang et al., 2008) or tumor-initiating cells (Clarke et al., 2006). CSCs are thought to be somatic stem cells that have undergone mutations transforming them into a cancer phenotype (Dontu et al., 2004). Evidence has suggested the presence of ovarian somatic stem cells, although their source differs (Bukovsky et al., 2004; Johnson et al., 2005). Others have suggested that CSCs are formed from lineage-restricted or differentiated cells with a cancer phenotype that dedifferentiate into CSCs (Srivastava and Nalbantoglu, 2008), a phenomenon that has been seen in cell culture with the epithelio-mesenchymal transition (EMT) (Bukovsky et al., 2004). CSCs were first identified in acute myeloid leukemia (Lapidot et al., 1994) and have since been identified in many types of solid tumors including ovarian (Bapat et al., 2005; Zhang et al., 2008), breast (Al-Hajj et al., 2003), prostate (Collins et al., 2005), liver (Chiba et al., 2006; Suetsugu et al., 2006), brain (Singh et al., 2003), lung (Dome et al., 2006), melanoma (Grichnik et al., 2006), colon (O'Brien et al., 2007; Odoux et al., 2008), and pancreas (Olempska et al., 2007). Moreover, expression of CD133 (a neural stem cell marker) has been found in the aforementioned systems and is thought to correlate with CSC (Baba et al., 2009). While CD133 has been used to sort for hematopoietic stem cells, Ferrandina et al. (2008) used flow cytometry to isolate $CD133^+$ cells from primary ovarian carcinomas. The $CD133^+$ cells represented less than 1% of the total population, which is one of the defining characteristics of CSCs. The $CD133^+$ cells were able to form colonies and had a higher proliferation potential than $CD133^-$ cells. Upon comparing the percentage of $CD133^+$ in 8 normal ovaries, 5 benign ovarian tumors, 16 primary ovarian carcinomas, and 25 omental metastases, Ferrandina et al. (2008) found that the ovarian carcinomas has a significantly higher percentage than all other groups.

Baba et al. (2009) showed that $CD133^{+/+}$ has also been found in the OCA cell lines OVCAR-8, while $CD133^{+/-}$ has been found in the OCA cell lines A2780, PEO1, OVCA432, OVCAR-2, and OV90. A single $CD133^-$ cell from A2780 or PEO1 produced more $CD133^-$ cells. In contrast, a single $CD133^+$ cell from the same cell lines was able to undergo asymmetric division and produce $CD133^+$ and $CD133^-$ cells. Further investigation by immunohistochemical analysis revealed that 10 of 32 advanced stage primary serous epithelial ovarian carcinomas contained $CD133^+$ cells at the luminal edges of glandular structures. Since a characteristic of CSCs is to form xenograft tumors, $CD133^+$ and $CD133^-$ cells isolated from A2780 were injected into nude mice. $CD133^+$ cells showed heightened aggressiveness with a larger tumor size and shorter latency. Immunohistochemical analysis of the xenograft tumors showed that the tumors derived from

$CD133^+$ cells contained both positive and negative staining suggesting that the original cells underwent asymmetric division. Tumors derived from $CD133^-$ cells showed no to extremely low staining for CD133. $CD133^+$ cells from cell lines showed increased resistance to cisplatin than CD133- progeny.

Several other groups found $CD44^+$ in human epithelial OCA and breast tumor cells (Al-Hajj et al., 2003; Dontu et al., 2004; Bapat et al., 2005; Bourguignon et al., 2008; Zhang et al., 2008). Bapat et al. (2005) isolated clones from the ascite samples of a malignant grade IV serous adenocarcinoma. Nineteen of sixty-five clones were immortalized, which showed variations in morphology and growth rates. Ten representative clones were selected and showed an up-regulation CD44, as well as c-met, epidermal growth factor receptor, E-cadherin (9 of 10), Snail (2 of 10), and Slug. Snail and Slug are known mediators of the EMT (Kurrey et al., 2005). Two of the immortalized clones, designated A2 and A4, were selected for the remaining experiments. Bapat et al. (2005) showed that the two clones had continued mutagenesis, evidenced by an increased proliferation rate and ability to form organized spheroids. These clones also showed the stem cell markers Nestin, Oct4, and Nanog, which was reduced upon tumor formation in nude mice.

Bourguignon et al. (2008) used the breast cancer cell line MCF-7 and OCA cell line SK-OV-3.ipl (established from ascites that developed in a nude mice given an intraperitoneal injection of SK-OV-3 cells) to show that hyaluronan-CD44 interaction recruits Nanog into the complex. Nanog is functionally coupled to Stat-3 to increase transcription of Stat-3 specific target genes encoding proliferation-related proteins in a hyaluronan-dependent manner in both MCF-7 and SK-OV-3.ipl, leading to rapid cell growth. Furthermore, Bourguignon et al. (2008) showed that *MDR1* expression was significantly enhanced with both cell lines with a CD44/Nanog/Stat-3 dependent manner. The increased *MDR1* expression led to lower IC_{50} value for doxorubicin and paclitaxel and reduced the ability of these agents to cause cell death in both MCF-7 and SK-OV-3.ipl.

Zhang et al. (2008) sought to characterized CSCs derived primary stage III serous adenocarcinomas. Tumor cells were isolated in an anchorage-independent manner, which clustered into spheres. Single cells from the spheroids were cultured under differentiating condition. The spheroids were able to adhere and differentiate into an epithelial morphology, forming symmetric colonies. Furthermore, the spheroids expressed *Oct-4, nestin, Nanog, SCF, Notch-1,* and *Bmi-1* analyzed by reverse transcription-PCR, suggesting an undifferentiated phenotype. Expression of ABCG2, a membrane efflux transporter, was up-regulated in spheroids compared to bulk tumor or differentiated cells. The spheroids were grown under stem cell-selective or differentiating conditions to examine if the spheroids cells possessed a CSC chemoresistance phenotype. Using cisplatin and paclitaxel, the IC_{50} values were increased under stem cell-selective conditions and also showed increase cell survival to both agents. When the spheroids were injected into nude mice, they were able to reconstitute the tumor, as hence were designated as CSCs. $CD44^+CD177^+$ cells were highly tumorigenic and formed heterogenous tumors reconstituting the original tumor phenotype compared to CD44-$CD117^-$. As few as 100 $CD44^+CD177^+$ cells resulted in tumor formation with a shorter latency.

Alvero et al. (2009) isolated $CD44^+$ cells from malignant ovarian ascites samples obtained from patients with stage III/IV ovarian cancer. $CD44^+$ cells were able to form spheroids and maintained this phenotype over 20 passages, suggesting that they have the ability to self-renew. Furthermore, $CD44^+$ cells were xenografted into nude mice to determine their tumorigenicity. Purified $CD44^+$ cells formed tumors that were 10% $CD44^+$ and 90% $CD44^-$, which was representative of the original tumor phenotype. A differential global gene expression profile of $CD44^+$ cells and $CD44^-$ cells showed that there was difference in genes belonging to families associated with control of cell death and apoptosis, signal transduction, transcription regulation, and control of cell differentiation. One of these genes was myeloid differentiation factor 88 (MyD88), which was up-regulated 10-fold in $CD44^+$ cells. MyD88 is a component of the toll like receptor 4 (TLR4) pathway and led to NF-κB activation causing an increase in IL-6, IL-8, MCP-1, and GROα levels. When $CD44^+$ cells treated with paclitaxel, a known ligand of TLR4, there was an increase in NF-κB production. $CD44^-$ cells underwent apoptosis in response to palitaxel treatment. The MyD88 pathway was shown to play an important role in chemotherapy resistance in $CD44^+$ cells and recapitulation of the original tumor.

Side Population (SP)

The side population (SP) was first found in hematopoietic stem cells by their ability to efflux the lipophilic dye Hoechst 3342; subsequently, they were shown to protect recipients from lethal irradiation at low cell doses (Goodell et al., 1996). SP cells have since been found in cell lines established from solid tumors, including neuroblastoma (JF, SK-N-SH, IMR32, LAN-1, and LAN-3) and epithelial OCA (SK-OV-3 and PA-1) (Hirschmann-Jax et al., 2004), and in a variety of tissues and tumor types (Patrawala et al., 2005; Moserle et al., 2008). SP cells are characterized by their expression of highly selective transporters ABCG2 (Zhou et al., 2001) and multidrug resistance-associated transporter p-glycoprotein (MDR1) (Hirschmann-Jax et al., 2004) and the other ABC transports ABCB1, ABCC1, and ABCC2 (Patrawala et al., 2005). These transporters give them the ability to efflux lipophilic substrates such as the dye Hoechst 33342 and many types of chemotherapy agents such as doxorubicin and imatinib (Jones et al., 2004), leading to chemotherapy resistance and tumor redevelopment (Hombach-Klonisch et al., 2008; Rocconi et al., 2008). SP cells were shown to be

sensitive to reserpine (Patrawala et al., 2005) and to the ABCB1 inhibitor verapamil (Goodell et al., 1996; Srivastava and Nalbantoglu, 2008), which reverses their phenotype (Moserle et al., 2008).

Patrawala et al. (2005) analyzed the SP various cancer cell lines including prostate, breast, colon, bladder, glioma, melanoma, cervix, and ovary. Even though the SP is rare (~0.01-5%), the authors were able to develop a system that would reliably detect a SP of ~0.01%. SP cells displayed an increased tumorigenicity compared to non-SP cells, forming tumors with as few as 100 SP cells with ~60% efficiency, whereas 200,000 non-SP did not. Purified SP cells were passaged *in vivo*, resulting in an increase in the population of SP cells similar to the first-generation tumor, suggesting that SP cells give rise to non-SP cells. SP cells also expressed *Notch* and ß-catenin in higher levels, suggesting that SP cells have stem-like features. Dysregulation of Wnt/ß-catenin, Jagged/Notch, and Hedgehog signal pathways have been implicated in carcinogenesis (Perryman and Sylvester, 2006).

Szotek et al. (2006) sorted SP and non-SP cells from human OCA cell line MOVCAR 7 and 4306 by ability to efflux Hoechst 33342 and flow cytometry. Upon culturing equal numbers of the cell, SP cells from both cell lines formed colonies, survived numerous passages, and produced both SP and non-SP cells. Non viable cells were gated out using propidium iodine to minimize the confounding cytotoxic effect of Hoechst 33342. Cell cycle analysis revealed that a majority of SP cells to be in G_1, whereas non-SP cells had a higher percentage of cells in S phase. By injecting purified SP (82.6% purity) and non-SP (92.3%) cells from MOVCAR 7 in to the dorsal fat pad of nude mice, SP cells showed a potential to initiate earlier tumor growth at lower numbers.

Moserle et al. (2008) analyzed SP and non-SP cells from cell lines established from patients diagnosed with stage IIIb to IV epithelial OCA and xenografted into nude mice (designated PDOVCA#1-6) for their proliferation capacity, cell cycle status, apoptosis level, and tumorigenicity. The resulting tumors were then sorted based on their ability to efflux Hoechst 33342 to separate the SP cells and non-SP cells to 98±1% and 90±3% purity respectively. Compared to non-SP cells, SP cells had a higher proliferation, reduced apoptosis rate (not due to Hoechst 33342), and increased tumorigenicity based on growth time and number of injected cells. Cell cycle analysis revealed that there were more non-SP cells in S and G2/M phase. The higher proliferation and reduced apoptosis of SP cells translated into a higher tumorigenicity, displaying both a greater efficiency and increased rate. The purified SP cells also generated tumors that contained both SP and non-SP cells similar to those measured in tumor resulting from unsorted cells. Furthermore, IFN-α treatment correlated to the SP cells and reduced their proliferation through up-regulation of the transcriptome. However, it did not modify the expression of ABCG2 and MDR1 transporters.

Conclusions

While the etiology of cancer has remained elusive, several theories about tumorigenesis have been proposed. Perhaps the most intriguing is the theory that cancer can be initiated from CSCs. Not only do CSCs have the ability to reconstitute the original tumor phenotype, they seem to do so more rapidly and with a lower cell count number than their related progenitors. The CSCs also account for chemotherapy resistance, particularly paclitaxel and doxorubicin, two of the current chemotherapy agents used clinically, through expression of the highly selective transporters ABCG2 and MDR1. Clearly, the need to be able to identify and target CSCs remains necessary for successful treatment of OCA. Evidence suggests that OCA CSCs can be identified as $CD44^+CD117^+CD133^+$. While CSCs and the SP have been defined differently, there are some similarities between the two populations. Both populations seem to contribute to chemotherapy resistance via the transporters ABCG2 and MDR1 have tumorigenic properties, and stem cell-like characteristics. However, further experiments are required to examine if the SP can more stringently match the definition of CSCs (Moserle et al., 2008).

A molecular analysis of CSC and the SP could provide valuable insight into gene dysregulation and signaling pathways that may trigger tumorigenesis, as shown with MyD88 being involved with chemotherapy resistance. CSCs and/or the SP could show differences between high- and low-grade tumors as indicated by their sensitivity to chemotherapy as one of their differentiating characteristics.

To verify the gonadotropin hypothesis, experiments are needed to show that exposure to gonadotropins can induce ovarian epithelium transformation into a cancer phenotype.

Acknowledgements. This work was supported by a NIH/National Cancer Institute R01 grant (CA124630). Authors are thankful to Mr. Andrew March for his editorial corrections and comments.

References

Al-Hajj M., Wicha M.S., Benito-Hernandez A., Morrison S.J. and Clarke M.F. (2003). Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad .Sci USA 100, 3983-3988.

Alvero A.B., Chen R., Fu H.H., Montagna M., Schwartz P.E., Rutherford T., Silasi D.A., Steffensen K.D., Waldstrom M., Visintin I. and 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., Wong A., Choi K., Kang S. and Leung P. (2001). Ovarian surface epithelium: Biology, endocrinology, and pathology. Endocr. Rev. 22, 255-288.

Baba T., Convery P.A., Matsumura N., Whitaker R.S., Kondoh E., Perry T., Huang Z., Bentley R.C., Mori S., Fujii S., Marks J.R., Berchuck A. and Murphy S.K. (2009). Epigenetic regulation of CD133 and

tumorigenicity of CD133+ ovarian cancer cells. Oncogene 28, 209-218.

Bapat S., Mali A., Koppikar C. and Kurrey N. (2005). Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Res. 65, 3025-3029.

Besson A., Hwang H., Cicero S., Donovan S., Gurian-West M., Johnson D., Clurman B., Dyer M. and Roberts J. (2007). Discovery of an oncogenic activity in p27kip1 that causes stem cell expansion and a multiple tumor phenotype. Genes Dev. 21, 1731-1746.

Bose C. (2008). Follicule stimulating hormone receptor in ovarian surface epithelium and epithelial ovarian cancer. Oncol. Res. 17, 231-238.

Bourguignon L., Peyrollier K., Xia W. and 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.

Bukovsky A., Caudle M., Svetlikova M. and Upadhyaya N. (2004). Origin of germ cells and formation of new primary follicles in adult human ovaries. Reprod. Biol. Endocrinol. 2, 20.

Carvalho J. and Carvalho F. (2008). Is Chlamydia-infected tubal fimbria the origin of ovarian cancer? Med. Hypoth. 71, 690-693.

Cetin I., Cozzia V. and Antonazzo P. (2008). Infertility as a cancer risk factor – a review. Placenta 29, 169-177.

Chiba T., Kita K., Zheng Y.W., Yokosuka O., Saisho H., Iwama A., Nakauchi H. and Taniguchi H. (2006). Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. Hepatology 44, 240-251.

Clarke M.F., Dick J.E., Dirks P.B., Eaves C.J., Jamieson C.H., Jones D.L., Visvader J., Weissman I.L. and Wahl G. M. (2006). Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res. 66, 9339-9344.

Collins A.T., Berry P.A., Hyde C., Stower M.J. and Maitland N.J. (2005). Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res. 65, 10946-10951.

Crew K. and Neugut A. (2006). Aspirin and NSAIDs: effects in breast and ovarian cancers. Curr. Opin. Obstet. Gynecol. 18, 71-75.

Dalerba P., Cho R.W. and Clarke M.F. (2007). Cancer stem cells: models and concepts. Annu. Rev. Med. 58, 267-284.

Debniak T., Górski B., Cybulski C., Jakubowska A., Kurzawski G., Lener M., Mierzejewski M., Masojç B., Medrek K., Kladny J., Zaluga E., Maleszka R., Chosia M. and Lubi*ƒ*ski J. (2003). Germline 657del5 mutation in the NBS1 gene in patients with malignant melanoma of the skin. Melanoma Res. 13, 365-370.

Dome B., Timar J., Dobos J., Meszaros L., Raso E., Paku S., Kenessey I., Ostoros G., Magyar M., Ladanyi A., Bogos K. and Tovari J. (2006). Identification and clinical significance of circulating endothelial progenitor cells in human non-small cell lung cancer. Cancer Res. 66, 7341-7347.

Dontu G., El-Ashry D. and Wicha M. (2004). Breast cancer, stem/progenitor cells and the estrogen receptor. Trends Endocrinol. Metab. 15, 193-197.

Erkinheimo T., Lassus H., Finne P., van Rees B., Leminen A., Ylikorkala O., Haglund C., Butzow R. and Ristimäki A. (2004). Elevated cyclooxygenase-2 expression is associated with altered expression of p53 and SMAD4, amplification of HER-2/neu, and poor outcome in serous ovarian carcinoma. Clin. Cancer Res. 10, 538-545.

Fehrmann R., Li X., van der Zee A., de Jong S., te Meerman G., de Vries E. and Crijns A. (2007). Profiling studies in ovarian cancer: a review. Oncologist 12, 960-966.

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

Gladkowska-Dura M., Dzierzanowska-Fangrat K., Dura W., van Krieken J., Chrzanowska K., van Dongen J. and Langerak A. (2008). Unique morphological spectrum of lymphomas in Nijmegen breakage syndrome (NBS) patients with high frequency of consecutive lymphoma formation. J. Pathol. 216, 337-344.

Goodell M.A., Brose K., Paradis G., Conner A.S. and Mulligan R.C. (1996). Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J. Exp. Med. 183, 1797-1806.

Grichnik J.M., Burch J.A., Schulteis R.D., Shan S., Liu J., Darrow T.L., Vervaert C.E. and Seigler H.F. (2006). Melanoma, a tumor based on a mutant stem cell? J. Invest. Dermatol. 126, 142-153.

Gupta R., Tejada L., Tong B., Das S., Morrow J., Dey S. and DuBois R. (2003). Cyclooxygenase-1 is overexpressed and promotes angiogenic growth factor production in ovarian cancer. Cancer Res. 63, 906–911.

Hebbring S., Fredriksson H., White K., Maier C., Ewing C., McDonnell S., Jacobsen S., Cerhan J., Schaid D., Ikonen T., Autio V., Tammela T., Herkommer K., Paiss T., Vogel W., Gielzak M., Sauvageot J., Schleutker J., Cooney K., Isaacs W. and Thibodeau S. (2006). Role of the Nijmegen breakage syndrome 1 gene in familial and sporadic prostate cancer. Cancer Epidemiol. Biomarkers Prev. 15, 935-938.

Hellstrom I. and Hellstrom K. (2008). SMRP and HE4 as biomarkers for ovarian carcinoma when used alone and in combination with CA125 and/or each other. Adv. Exp. Med. Biol. 622, 15-21.

Hirschmann-Jax C., Foster A., Wulf G., Nuchtern J., Jax T., Gobel U., Goodell M. and Brenner M. (2004). A distinct "side population" of cells with high drug efflux capacity in human tumor cells. Proc. Natl. Acad. Sci. USA 101, 14228–14233.

Hold G. and El-Omar M. (2008). Genetic aspects of inflammation and cancer. Biochem. J. 410, 225–235.

Hombach-Klonisch S., Paranjothy T., Wiechec E., Pocar P., Mustafa T., Seifert A., Zahl C., Gerlach K., Biermann K., Steger K., Hoang-Vu C., Schulze-Osthoff K. and Los M. (2008). Cancer stem cells as targets for cancer therapy: select cancers as examples. Arch. Immun. Ther. Exp. 56, 165-180.

Huang J., Grotzer M.A., Watanabe T., Hewer E., Pietsch T., Rutkowski S. and Ohgaki H. (2008). Mutations in the Nijmegen breakage syndrome gene in medulloblastomas. Clin. Cancer Res. 14, 4053-4058.

Johnson J., Bagley J., Skaznik-Wikiel M., Lee H., Adams G., Niikura Y., Tschudy K., Tilly J., Cortes M., Forkert R., Spitzer T., Iacomini J., Scadden D. and Tilly J. (2005). Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. Cell 122, 303-315.

Jones R., Matsui W. and Smith B. (2004). Cancer stem cells: are we missing the target? J. Natl. Cancer Inst. 96, 583–585.

Kurrey N.K., Amit K. and Bapat S.A. (2005). Snail and Slug are major determinants of ovarian cancer invasiveness at the transcription level. Gynecol. Oncol. 97, 155-165.

Landen C. Jr, Birrer M. and Sood A. (2008). Early events in the pathogenesis of epithelial ovarian cancer. J. Clin. Oncol. 26, 995-1005.

Lapidot T., Sirard C., Vormoor J., Murdoch B., Hoang T., Caceres-Cortes J., Minden M., Paterson B., Caligiuri M. and Dick J. (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 367, 645-648.

Lowe K., Shah C., Wallace E., Anderson G., Paley P., McIntosh M., Andersen M., Scholler N., Bergan L., Thorpe J., Urban N. and Drescher C. (2008). Effects of personal characteristics of serum CA125, mesothelin, and HE4 levels in healthy postmenopausal women at high-risk for ovarian cancer. Cancer Epidemiol. Biomarkers Prev. 17, 5480-2487.

Lukanova A. and Kaaks R. (2005). Endogenous hormones and ovarian cancer: Epidemiology and current hypotheses. Cancer Epidemiol. Biomarkers Prev. 14, 98-107.

Modugno F., Ness R. and Cottreau C. (2002). Cigarette smoking and the risk of mucinous and non-mucinous epithelial ovarian cancer. Epidemiology 13, 467-471.

Moserle L., Indraccolo S., Ghisi M., Frasson C., Fortunato E., Canevari S., Miotti S., Tosello V., Zamarchi R., Corradin A., Minuzzo S., Rossi E., Basso G. and Amadori A. (2008). The side population of ovarian cancer cell is a primary target of INF-α antitumor effects. Cancer Res. 68, 5658-5668.

Neesham D. (2007). Ovarian cancer screening. Austr. Fam. Physician 36, 126-128.

Ness R., Grisso J., Cottreau C., Klapper J., Vergona R., Wheeler J., Morgan M. and Schlesselman J. (2000). Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer. Epidemiology 11, 111-117.

Nossov V., Amneus M., Su F., Lang J., Janco J., Reddy S. and Farias-Eisner R. (2008). The early detection of ovarian cancer: from traditional methods to proteomics. Can we really do better than serum CA-125? Am. J. Obstret. Gynecol. 199, 215-223.

O'Brien C.A., Pollett A., Gallinger S. and Dick J.E. (2007). A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 445, 106-110.

Odoux C., Fohrer H., Hoppo T., Guzik L., Stolz D., Lewis D., Gollin S., Gamblin T., Geller D. and Lagasse E. (2008). A stochastic model for cancer stem cell origin in metastatic colon cancer. Cancer Res. 68, 6932-6941.

Olempska M., Eisenach P., Ammerpohl O., Ungefroren H., Fandrich F. and Kalthoff H. (2007). Detection of tumor stem cell markers in pancreatic carcinoma cell lines. Hepatobiliary Pancreat. Dis. Int. 6, 92-97.

Patrawala L., Calhoun T., Schneider-Broussard R., Zhou J., Claypool K. and Tang D. (2005). Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2$^+$ and ABCG2$^-$ cancer cells are similarly tumorigenic. Cancer Res. 65, 6207-6219.

Perryman S. and Sylvester K. (2006). Repair and regeneration: opportunities for carcinogenesis from tissue stem cells. J. Cell Mol. Med. 10, 292–308.

Plisiecka-Halasa J., Dansonka-Mieszkowska A., Rembiszewska A., Bidzinski M., Steffen J. and Kupryjanczyk J. (2002). Nijmegen breakage syndrome gene (NBS1) alterations and its protein (nibrin) expression in human ovarian tumours. Ann. Hum. Genet 66, 353-359.

Polyak K. and Hahn W.C. (2006). Roots and stems: stem cells in cancer. Nat. Med. 12, 296-300.

Porhanova N., Sokolenko A., Sherina N., Ponomariova D., Tkachenko N., Matsko D. and Imyanitov E. (2008). Ovarian cancer patient with germline mutations in both BRCA1 and NBN genes. Cancer Genet. Cytogenet. 186, 122-124.

Rocconi R., Matthews K.S., Kemper M.K., Hoskins K.E. and Barnes M.N. (2008). Chemotherapy-related myelosuppression as a marker of survival in epithelial ovarian cancer patients. Gynecol. Oncol. 108, 336-341.

Rossing M., Cushing-Haugen K., Wicklund K. and Weiss N. (2008). Cigarette smoking and risk of epithelial ovarian cancer. Cancer Causes Control 19, 413-420.

Schildkraut J., Schwingl P., Bastos E., Evanoff A. and Hughes C. (1996). Epithelial ovarian cancer risk among women with polycystic ovary syndrome. Obstet. Gynecol. 88, 554-559.

Singh S.K., Clarke I.D., Terasaki M., Bonn V.E., Hawkins C., Squire J. and Dirks P.B. (2003). Identification of a cancer stem cell in human brain tumors. Cancer Res. 63, 5821-5828.

Soegaard M., Kjaer S., Cox M., Wozniak E., Høgdall E., Høgdall C., Blaakaer J., Jacobs I., Gayther S. and Ramus S. (2008). BRCA1 and BRCA2 mutation prevalence and clinical characteristics of a population-based series of ovarian cancer cases from Denmark. Clin. Cancer Res. 14, 3761-3767.

Srivastava V.K. and Nalbantoglu J. (2008). Flow cytometric characterization of the DAOY medulloblastoma cell line for the cancer stem-like phenotype. Cytometry A 73, 940-948.

Subbaramaiah K., Hart J., Norton L. and Dannenberg A. (2000). Microtubule-interfering agents stimulate the transcription of cyclooxygenase-2. Evidence for involvement of ERK1/2 AND p38 mitogen-activated protein kinase pathways. J. Biol. Chem. 275, 14838-14845.

Suetsugu A., Nagaki M., Aoki H., Motohashi T., Kunisada T. and Moriwaki H. (2006). Characterization of CD133$^+$ hepatocellular carcinoma cells as cancer stem/progenitor cells. Biochem. Biophys. Res. Commun. 351, 820-824.

Szotek P., Pieretti-Vanmarcke R., Masiakos P., Dinulescu D., Connolly D., Foster R., Dombkowski D., Preffer F., Maclaughlin D. and Donahoe P. (2006). Ovarian cancer side population defines cells with stem cell-like characteristics and Müllerian-inhibiting substance responsiveness. Proc. Natl. Acad. Sci. USA 103, 11154-11159.

Tammela J. and Odunsi K. (2004). Gene expression and prognostic significance in ovarian cancer. Minerva Ginecol. 56, 495-502.

Tomić S., Ilić Forko J., Babić D., Sundov D., Kuret S. and Andelinović S. (2003). c-erbB-2, p53, and nm23 proteins as prognostic factors in patients with epithelial ovarian carcinoma. Croat. Med. J. 44, 429-434.

Venn A., Watson L., Bruinsma F., Giles G. and Healy D. (1999). Risk of cancer after use of infertility drugs with *in-vitro* fertilization. Lancet 354, 1586-1590.

Zhang S., Balch C., Chan M., Lai H., Matei D., Schilder J., Yan P., Huang T.-M. and Nephew K. (2008). Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Res. 68, 4311-4320.

Zhou S., Schuetz J.D., Bunting K.D., Colapietro A.M., Sampath J., Morris J.J., Lagutina I., Grosveld G.C., Osawa M., Nakauchi H. and Sorrentino B.P. (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.

Accepted June 24, 2009