Molecular Pathogenesis and Therapeutic Targets in Epithelial Ovarian Cancer

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Abstract   Ovarian cancer, the most aggressive gynecologic cancer, is the foremost cause of death from gynecologic malignancies in the developed world. Two primary reasons explain its aggressive behavior: most patients present with advanced disease at diagnosis, and die of recurrences from disease that has become resistant to conventional chemotherapies. In this paper on epithelial ovarian cancer (EOC), we will review molecular alterations associated with the few precursor lesions identified to date, followed by the more commonly recognized processes of de novo carcinogenesis, metastasis, and the development of chemoresistance. We will propose a unifying model of ovarian epithelial tumorigenesis that takes into account various hypotheses. We will also review novel approaches to overcome the major problem of chemoresistance in ovarian cancer. Finally, we will discuss advances and new challenges in the development of mouse model systems to investigate EOC precursor lesions, progression, metastasis, and chemoresistance. J. Cell. Biochem. 102: 1117–1129, 2007.   © 2007 Wiley-Liss, Inc.

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Epithelial cancer of the ovary is the most lethal gynecologic malignancy in the United States, with approximately 22,000 new cases and 16,000 deaths occurring annually [Jemal et al., 2007]. Due to the relative lack of specific signs and symptoms of this disease and the lack of effective screening programs, epithelial ovarian cancer (EOC) is diagnosed at advanced stages in most patients, contributing to low overall cure rates. Furthermore, after primary surgical resection and subsequent platinum-taxane based chemotherapy, to which most patients respond initially, the majority of patients eventually recur with chemoresistant disease and die of metastatic disease. For all stages, the 5-year survival rate is 45%, but in patients with advanced disease, it is approximately 30% [Aletti et al., 2007]. Here, we review two major challenges in ovarian cancer care, specifically what is known (and still unknown) about the process of carcinogenesis and the development of chemoresistance. In particular, we focus our attention on recent advances in the development of new therapeutic approaches, and new models for better understanding the nature of this disease.

ORIGINS, PRECURSOR LESIONS, AND DE NOVO CARCINOGENESIS

EOC constitutes 90% of ovarian malignancies and is classified into distinct histologic categories consisting of serous, mucinous, endometrioid, clear cell, transitional, mixed, and undifferentiated subtypes [Bell, 2005]. Although Mullerian metaplasia of ovarian surface epithelium and its inclusion glands is generally considered as the origin of high-grade EOC [Bell, 2005], accumulating evidence suggests

that the epithelial lining of the fallopian tube may provide an alternative site of origin for high-grade serous EOC in BRCA mutation-positive women [Dubeau, 1999; Crum et al., 2007; Lee et al., 2007]. EOC can arise as a result of de novo carcinogenesis, tubal carcinoma implants, or, less commonly, progression from precursor lesions as a result of genetic instability and K-ras, Braf, β-catenin, or PTEN mutations [Shih Ie and Kurman, 2004; Bell, 2005; Fukumoto and Nakayama, 2006]. Some investigators propose that there are two distinct pathways of tumorigenesis for low versus high-grade carcinomas. Low-grade neoplasms arise in a stepwise manner from precursors such as cystadenomas, borderline tumors, or endometriosis. The more common high-grade neoplasms appear to arise de novo without definable precursor lesions [Shih Ie and Kurman, 2004; Bell, 2005] (Fig. 1). Loss of p53 function is thought to be an early molecular event associated with de novo carcinogenesis of high-grade serous, endometrioid, and clear cell carcinoma; BRCA dysfunction is also considered to be an early event associated with de novo carcinogenesis of high-grade serous EOC [Bell, 2005; Fukumoto and Nakayama, 2006]. Borderline tumors frequently contain wild-type p53 and may serve as precursor lesions for low-grade and mucinous EOC [Shih Ie and Kurman, 2004; Bell, 2005; Christie and Oehler, 2006; Fukumoto and Nakayama, 2006]. Low-grade EOC arising from borderline tumors frequently maintain wild-type p53 phenotype while acquiring unique genetic alterations [Shih Ie and Kurman, 2004; Bell, 2005; Christie and Oehler, 2006; Fukumoto and Nakayama, 2006]. For example, serous borderline tumors frequently contain BRAF or K-ras mutations [Singer et al., 2003], LOH on chromosome Xq [Cheng et al., 1996], and microsatellite instability [Haas et al., 1999]. Mucinous borderline tumors contain K-ras mutations [Scambia et al., 1997]; endometrioid and clear cell borderline tumors arising in endometriosis contain β-catenin and PTEN mutations and microsatellite instability [Fukumoto and Nakayama, 2006] (Fig. 1). These genetic alterations are believed to be early events in the tumorigenesis of the respective tumor histotypes [Fukumoto and Nakayama, 2006]. In vitro and in vivo models of ovarian cancer also support the role of these genes in tumorigenesis [Boyd, 2005]. Heritable mutations in DNA mismatch repair genes, such as hMLH1 and hMSH2, can also be considered as precursor genetic events in Lynch syndrome-associated ovarian cancer [Lu and Broaddus, 2005]. Although several models of ovarian cancer have been established by targeted alteration of some of these pathways [Boyd, 2005], a comprehensive approach to analyze collectively the contributions of these genes in tumorigenesis and histologic differentiation of ovarian cancer is still needed. For example, although Lynch syndrome-associated ovarian carcinomas display diverse histologic phenotypes, no ovarian-specific mouse model is yet available to investigate early genetic changes associated with histologic differentiation in Lynch-associated ovarian carcinomas. Moreover, although expression of particular Hox genes is associated with specific ovarian histology [Cheng et al., 2005], no mouse model is yet available to investigate whether targeted expression of specific Hox genes determines histology of ovarian carcinoma. Future studies involving targeted alterations of these genes in ovarian surface epithelium and fallopian tube epithelium are essential for determining the origins of ovarian cancer and defining the contributions of these genes to tumorigenesis and histologic differentiation of ovarian carcinomas.

PROGRESSION

Recent transcriptomic analysis of serous carcinoma indicates that several oncogenic pathways, such as β-catenin, Ras, and Src, are deregulated in serous carcinoma, and that deregulation of these oncogenic pathways is associated with poor prognosis in patients with incomplete response to platinum-based chemotherapy [Dressman et al., 2007]. These results therefore suggest that aberrant activation of oncogenic pathways likely represent additional genetic alterations associated with progression in high-grade serous tumors that frequently contain p53 and BRCA dysfunction as early genetic events.

Current models of progression for low-grade carcinomas of serous and mucinous type suggest that these tumors may arise from borderline tumors of low malignant potential [Shih Ie and Kurman, 2004; Bell, 2005; Christie and Oehler, 2006; Fukumoto and Nakayama, 2006]. Endometrioid and clear cell cancers arising from a background of endometriosis can also arise from a borderline tumor. As indicated in
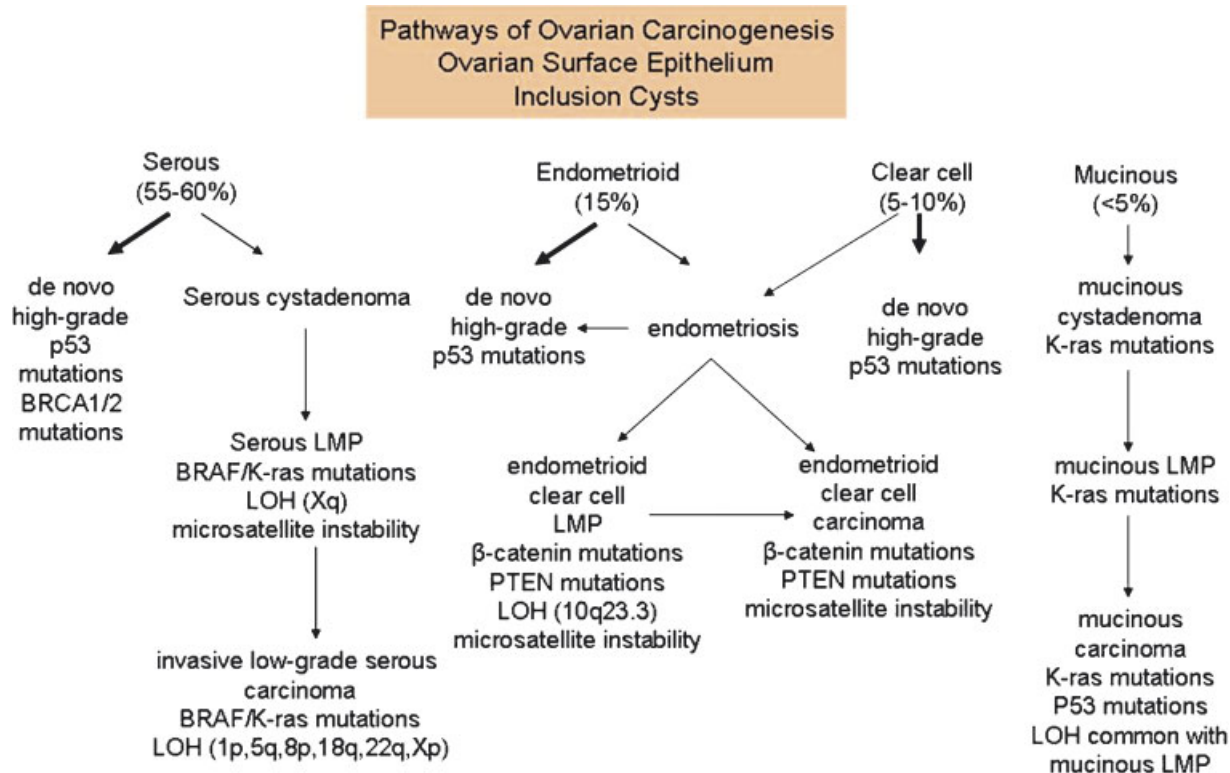
Fig. 1. Molecular features and progression pathways in EOC. The less common low-grade EOC are proposed to arise from step-wise progression of precursors, such as borderline tumors to carcinoma. High-grade EOC is proposed to arise from de novo carcinogenesis with as yet unidentified precursor lesions. *Proportions of all EOC; less common histotypes not included. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 1, these carcinomas have identifiable precursor lesions and early genetic alterations. However, additional genetic alterations, required for progression from borderline tumors to frank carcinoma, are not fully understood. Since biospecimens can be readily collected from these tumors, a thorough genetic analysis should be performed to investigate the step-wise development of these low grade EOCs. In addition to single-nucleotide polymorphism and microsatellite marker analyses to establish the clonal origin of these carcinomas, targeted mutational analyses that focus on kinome, cell cycle genes, transcription factors, known oncogenes, and tumor suppressors should be investigated to identify the potential role of these genes in the progression of EOC. Toward this goal, the identification of novel mutational targets in ovarian cancer that will be generated from the Ovarian Cancer Genome Project should facilitate the analysis of the clonal origin and evolution of ovarian tumors.

METASTASIS

From the standpoint of tumor characteristics that correlate with ultimate patient mortality, the ability to metastasize has obvious significance [Liotta and Kohn, 2005]. Historically, tumor metastases have been explained by the clonal selection of a metastatic phenotype among a heterogeneous population of tumor cells. However, some suggested that genes that only specify metastasis would not be selected for during early tumor development since these genes would not necessarily promote tumor growth [Bernards and Weinberg, 2002]. Therefore, it was suggested that genes that promote metastasis should also provide a growth advantage and thus may be selected for early in carcinogenesis [Bernards and Weinberg, 2002]. Consistent with this hypothesis, genome-wide gene expression analysis between early (I–II) and late stage (III–IV) ovarian cancers showed little differences in gene expression [Shridhar

et al., 2001]. Moreover, transcriptomic analysis and comparative genomic hybridization analysis between primary carcinomas and their respective metastases indicated similar gene-expression profiles and chromosomal alterations [Bayani et al., 2002; Hibbs et al., 2004; Israeli et al., 2004]. Nonetheless, recent reports indicate that a number of genes are differentially expressed in primary ovarian tumors compared to metastatic omental tumors [Lancaster et al., 2006; Bignotti et al., 2007]. For example, Bignotti et al. reported that stromal-derived factors and metastasis predictive genes, such as plasminogen activator, metalloproteinase, collagen, vascular endothelial growth factors (VEGFs), endothelin, fibroblast growth factor, thrombospondins, integrins, and chemokines are overexpressed in metastases compared to primary tumors. Moreover, it is also possible that differential expression of a small set of genes in metastases compared to primary sites may be the result of tumor–stromal interaction in new tumor niches. Mouse models of ovarian cancer with tissue-specific genetic manipulation should provide valuable insights into the role of metastatic genes in ovarian carcinoma progression.

RESISTANCE TO CHEMOTHERAPY

Ovarian cancer is considered one of the more chemosensitive solid tumors as 80% of patients respond to initial chemotherapy [Berkenblit and Cannistra, 2005; Aletti et al., 2007]. Unfortunately, despite initial responses, most of these tumors later relapse and will eventually become resistant to chemotherapy. Thus, chemoresistance can be viewed as the final step in tumor progression and is mainly responsible for the majority of ovarian cancer-related deaths. Resistance to chemotherapy in ovarian cancer can be classified into two groups: de novo resistance and acquired resistance [Agarwal and Kaye, 2003]. Approximately, 20% of advanced-stage ovarian tumors do not respond to chemotherapy initially, and are considered to have de novo resistance. Those tumors that respond initially and later recur are considered to acquire resistance as a result of the selection of drug-resistant clones during treatment with chemotherapy. Although several studies have been performed to identify gene signatures associated with resistance to chemotherapy [Hartmann et al., 2005; Jazaeri et al., 2005; Spentzos et al., 2005; Bild et al., 2006], no

consistent profile has emerged, suggesting that some of the genes in these signatures may be secondary phenomena rather than the primary processes associated with chemoresistance. Therefore, two of the most relevant avenues of translational research are the identification of patients who most likely will not benefit from conventional approaches, and development of new therapeutic approaches that will allow a biologically based, personalized therapy.

NEW THERAPEUTIC APPROACHES

Technologic advances that allow us to examine the molecular machinery driving cancer cells have helped to identify numerous mediators within ovarian cancer cells that can be targeted with new molecular strategies. The promise that new therapeutics may offer to these patients can be considered a real alternative (or adjunctive) strategy especially if outcome can be related to the "molecular signature". Several new therapeutic approaches and associated clinical studies and outcomes are discussed below (see Table I).

ATP Binding Cassette (ABC) Transporter Inhibitors

One possible target to enhance chemosensitivity is to prevent drug efflux in cancer cells by blocking ATP binding cassette (ABC) transporters [Szakacs et al., 2006]. Several transporters have been identified in this class of molecules, but only Pgp (ABCB1), and to a lesser extent MRP1 and ABCG2 inhibitors have been evaluated in clinical trials. PSC-833, an inhibitor of ABCB1, has been tested in association with carboplatin and taxol in a phase III clinical trial, but there was no improvement in survival [Joly, 2002]. A better definition of the inclusion criteria for patients with overexpression of ABC genes may be needed to determine the potential benefit of these targeted therapies.

Glutathione Inhibitors

Inactivation of cisplatin by sulfur-containing molecules is one of the mechanisms involved in platinum resistance. Ethacrynic acid (inhibitor of GST-mediated conjugation of glutathione to cisplatin) and Buthionine sulfoximine (inhibitor of γ-glutamyl cysteine synthetase in the synthesis of glutathione), have been tested in

TABLE I. New Therapeutics in Ovarian Cancer

New therapeutics	Target	Trial (phase)	Outcome	References
ABC transporters				
PSC-833 + Carbo + Taxol vs. Carbo + Taxol	ABCB1	III	No benefit DFS (762 patients)	Joly [2002]
Glutathione inhibitors				
Ethacrynic acid + Thiotepa	GST-mediated conjugation	I	RR: N.E. (five patients)	O'Dwyer et al. [1991]
Buthionine sulfoximine + Melphalan	γ-Glutamyl cysteine synthetase	I	One PR (20 patients)	O'Dwyer et al. [1996]
Anti-angiogenic molecules				
Bevacizumab	VEGF	II	RR 21% (62 patients)	Burger et al. [2005]
Thalidomide		II	One PR (10 patients)	Abramson et al. [2002]
Carboxyamidotriazole		II	One PR (36 patients)	Hussain et al. [2003]
Apoptotic pathway enhancers				
TRM1	TRAIL-R1 agonist	I	RR: N.E.	Tolcher et al. [2004]
Growth factor receptors blockers				
Trastuzamab	HER-2/erbB-2	II	RR: 7.3% (41 patients)	Bookman et al. [2003]
CI-1033	Erbb	II	RR: 0% (105 patients)	Campos et al. [2005]
Gefitinib	EGFR, erbB1	II		Schilder et al. [2005]
Erlotinib HC1	EGFR, erbB1	II		Gordon et al. [2005]
Cell-cycle inhibitors				
Seliciclib (CYC202; R-roscovitine)	Cdk 1, 2, 7, 9	I	0 PR (21 patients)	Benson et al. [2007]
Flavopiridol	Cdk	I		Gries et al. [2003]
17AAG	HSP90	I		Haluska et al. [2004]
PS341	Proteasome	I		Aghajanian et al. [2002]
Signal transduction pathway inhibitors				
LY294002	PI3K	Pre-clin		Hidalgo and Rowinsky [2000]
RAD 001	mTOR	I		Hidalgo and Rowinsky [2000]
CCI 779	mTOR	I		Hidalgo and Rowinsky [2000]
BAY 43-9006	Raf-1 kinase	I	Two PR (11 patients)	Strumberg et al. [2003]
ISIS 5132	c-raf kinase	II	RR: 0% (22 patients)	Oza et al. [2003]
R115777	Farnesyltransferase	I		Johnston [2001]
SCH66336	Farnesyltransferase	I		Johnston [2001]
SU 6656	Src	Pre-clin		

DFS, disease free survival; RR, response rate; PR, partial response; N.E., not evaluated.

phase I clinical trials, but their efficacy in phase II and III clinical trials has not been evaluated yet [O'Dwyer et al., 1991, 1996].

Anti-Angiogenic Molecules

VEGF is the most important driving factor behind angiogenesis in ovarian cancer. Different agents have been developed to inhibit VEGF or its receptors (VEGFR and VEGFR2) [Ferrara, 2005]. Bevacizumab (Avastin) is a recombinant humanized monoclonal antibody against VEGF, which has shown activity in ovarian cancer. Specifically, bevacizumab was given as a single agent in a phase II study (women with recurrent ovarian or primary peritoneal cancer) in GOG170. The response rate was 21% (13 of 62 women enrolled), and 40% of patients had a progression free survival of 6 months or more [Burger et al., 2005]. Another study showed a 28% response rate of bevacizumab in combination with low-dose metronomic oral cyclophosphamide in patients with recurrent ovarian/peritoneal cancer [Garcia et al., 2005]. One very serious side effect seen with bevacizumab is bowel perforation. Wright et al. performed a retrospective analysis of ovarian cancer patients treated with bevacizumab and reported that 8 of 158 (5%) patients treated with bevacizumab developed bowel perforation [Wright et al., 2006]. An ongoing phase III trial from GOG is comparing standard carboplatin and paclitaxel with either placebo or bevacizumab in patients with suboptimal stage III and stage IV disease. Thalidomide [Abramson et al., 2002] and carboxyamidotriazole [Hussain et al., 2003] have been tested in phase II trials, with less encouraging results as compared to bevacizumab.

Apoptotic Pathway Enhancers

Activation of the extrinsic apoptotic pathway through death-receptor signaling has also been explored and shown to be implicated in the response to various chemotherapy agents. The ability of TRAIL to target specifically tumor cells led to the introduction of an agonistic

monoclonal antibody to TRAIL-R1, TRM1 in phase I clinical trials [Tolcher et al., 2004].

Growth Factor Receptor Blockers

Inhibitors of the erbB family of receptor tyrosine kinases have been studied in ovarian cancer. This receptor family and their ligands promote tumorogenesis through a variety of stimulatory and cell survival pathways. Overexpression of EGFR has been shown to be associated with platinum resistance and consequent poor prognosis in ovarian cancer. Trastuzumab (Herceptin), a humanized monoclonal antibody against HER-2/erbB-2, has been shown to have a limited response rate (7.3%) in a phase II trial including patients with recurrent ovarian or primary peritoneal carcinoma with HER-2 overexpression [Bookman et al., 2003]. Small molecule inhibitors (Gefitinib, Erlotinib HC1) of the epidermal growth factor receptor (EGFR, erbB1) have shown minimal activity in phase II clinical trials [Gordon et al., 2005; Schilder et al., 2005]. Lapatinib, a potent dual inhibitor of EGFR and HER-2/erbB2, is currently being evaluated in several clinical trials in ovarian cancer. The underlying hypothesis is that a dual inhibitor of these important pathways could have significant therapeutic advantages over single receptor inhibitors. However, in a recent phase II study, no objective responses were observed in a cohort of 105 patients with platinum resistant disease using the small molecule pan-Erbb inhibitor CI-1033 [Campos et al., 2005].

Cell-Cycle Inhibitors

Cyclin dependent kinase (cdk) and mitotic kinesin KSP/Eg5, are potential targets for alternative treatments in ovarian cancer. Seliciclib (CYC202; R-roscovitine) is a selective, orally bioavailable inhibitor of cyclin-dependent kinases 1, 2, 7, and 9 recently studied in a phase I clinical trial. No objective responses were noted in the 21 patients enrolled, but disease stabilization was observed in eight patients [Benson et al., 2007]. Flavopiridol, an inhibitor of cdks, causes cell cycle arrest or apoptosis based on the relation of the transcription factor E2F1 and RB. 17AAG is an inhibitor of heat shock protein 90 (HSP90), a chaperone protein required for the function of several protein kinases. Inhibition of HSP90 leads to degradation of oncogenic proteins, including Raf-1 and mutant p53, causing cell-cycle arrest. PS-341 is a small-molecule proteosome inhibitor that prevents depletion of proteins important in cell cycle, apoptosis, and drug resistance (p53, c-myc, NF-κB family members). All these molecules have been recently evaluated in either pre-clinical or phase I clinical trials [Adams, 2002; Aghajanian et al., 2002; Haluska et al., 2004; Bible et al., 2005].

Small Molecule Inhibitors of Signal Transduction Pathways

PKC and PKA, PI3K/Akt, Ras/Raf/MAPK, and Src pathways can be activated following ligand−cell receptor interaction. This activation leads to proliferation, cell growth, and ultimately survival signals. Since several members of these oncogenic pathways have been shown to be expressed in different cancers, they naturally represent a potential target for novel treatments. To target the PI3K/Akt pathway, LY294002, a PI3K inhibitor showed efficacy in vitro, but was not utilized in clinical trials because of its poor pharmacological profile. Rapamycin analogs RAD 001 and CCI 779 inhibit mTOR, that lies downstream of PI3K, and are currently being studied in phase I clinical trials [Hidalgo and Rowinsky, 2000].

Targeting the Ras/Raf/MAPK pathway, BAY 43-9006, a specific Raf-1 kinase inhibitor, has been studied in phase I trials, with limited efficacy reported (only 2 out of 11 patients showed partial response) [Strumberg et al., 2003]. ISIS 5132 is a 20-base phosphorothioate DNA oligonucleotide against human c-raf kinase, a downstream effector of ras oncogene function. A phase II trial of ISIS 5132 in 22 patients with recurrent ovarian cancer failed to show any benefit with this drug for these patients [Oza et al., 2003].

Farnesylation of Ras is necessary for its activation and is catalyzed by the enzymes farnesyltransferases. R115777 and SCH66336, two small-molecules that inhibit farnesyltransferase are currently been evaluated in clinical trials [Johnston, 2001].

Activation of Src has been shown to be capable of stimulating the Ras-MAP kinase pathway by phosphorylation of Shc by Src itself. Furthermore, the activation of the Ras-MAP kinase pathway by G protein-coupled receptors seems to be correlated to Src activation and subsequent Shc phosphorylation. A small molecule inhibitor of Src (SU6656) has been characterized and identified [Blake et al., 2000]. SU 6656

has been shown to be effective in pre-clinical studies in ovarian cancer cell lines. Interestingly, the efficacy of this molecule is correlated with Src expression, suggesting that pre-screening of oncogenic pathway expression in tumors might guide further clinical trials [Dressman et al., 2007].

Oncolytic Viral Therapy

Therapeutic oncolytic viruses have unique mechanisms of action that should be effective in apoptosis-resistant cancer cells. For example, wild-type measles virus was recognized to be oncolytic when natural infection led to regression of established cancers in humans [Liu et al., 2007]. The virus exerts its cytopathic effect by binding to cells that express one of its receptors, gaining entry to the cell and then co-opting it to transcribe the viral fusion antigen that is then expressed on the surface of the cell. This in turn mediates cell:cell fusion with neighboring cells until a large syncytia develops, containing 50–100 cells that are no longer viable. Ovarian cancers express CD46, one of the receptors for measles virus. CD46 is a complement regulatory protein that allows cells to evade complement fixation. We at Mayo are nearing the completion of a phase I trial of the attenuated measles virus (Edmonston strain used for vaccinations), given intraperitoneally to women with recurrent ovarian or peritoneal cancer [Peng et al., 2002]. The agent has been very well tolerated and there is early evidence of disease stabilization in several patients.

MOUSE MODELS OF HUMAN EOC

Over the past 5 years a few genetically engineered mouse models of ovarian cancer have been generated. From these models it is widely accepted that both inactivation and activation of specific pathways can lead to ovarian cancer development of different histologies and phenotypes [Garson et al., 2005]. These models have also been extremely useful to determine the functional contributions of individual pathways that contribute to the development of ovarian cancer and more importantly to test the molecular mechanisms associated with resistance to targeted therapy.

Xenograft Models

Earlier models included xenotransplanted cancer cells with defined genetic alterations and in vitro transformed mouse ovarian epithelial cells [Maines-Bandiera et al., 1992]. The biological differences between human and rodent cells make extrapolating results from mice to humans difficult. Therefore, a more reliable model that truly reflects human ovarian cancer is to genetically engineer nonmalignant human ovarian epithelial cells to become tumorigenic. While there is still a controversy as to whether the ovarian surface epithelial cells are the primary origin of ovarian cancer cells [Crum et al., 2007], several experimental models that manipulated these cells in vitro provided additional support for this concept. Auersperg et al. were the first group to introduce Kirsten murine sarcoma virus into rat OSE cells and show that subcutaneous (s.c.) or intraperitoneal (i.p.) injection of these cells in immuno-compromised mice resulted in endometrioid tumors [Adams and Auersperg, 1981]. Subsequent studies that manipulated these cells to express SV40 T antigen [Leung et al., 2001] and or E6 and E7 genes [Gregoire et al., 2001] immortalized these cells. In contrast to SV40 T antigen immortalized cells, the E6 and E7 immortalized OSE cells resulted in spontaneous progression from a benign to an invasive phenotype [Gregoire et al., 2001]. However, introduction of oncogenic HRAS or KRAS into SV40 T/t antigen immortalized OSE cells allowed them to form s.c tumors in immuno-compromised mice [Liu et al., 2004]. While these xenograft models were informative to test individual oncogenes whose activation leads to tumor formation in vivo, they did not reflect changes that potentially occurred very early or the initiating events in tumorigenesis.

Development of Syngeneic Ovarian Cancer Model

Tumor development from xenografted cells is achieved only in immuno-compromised mice. This was overcome in the very first mouse model of ovarian cancer generated by Roby et al. who used mouse ovarian epithelial cells (MOSE cells) from virgin mice to generate transformed cells by repeated passage in culture [Roby et al., 2000]. These ID8 clonal lines formed tumors both in syngeneic and nude mice models. Taking advantage of the spontaneous transformation of mouse ovarian surface epithelial cells in culture, Roberts et al. [2005] characterized distinct transitional stages of ovarian cancer as the cells progressed from a premalignant

nontumorigenic phenotype to a more aggressive malignant cancer. These MOSE specific models identified cellular and molecular changes associated with early and late stages of ovarian cancer in an immunocompetent environment.

Genetically Engineered Mouse Models

While the above described models utilized in vitro transformation of the MOSE cells followed by implantation into syngenic mice, an ideal model to investigate genetic alterations associated with ovarian cancer would be to generate a mouse model where tumors arise directly from the OSE cells. The mouse ovary offers a distinct advantage to introduce specific gene alterations since these genes can be readily delivered by intrabursal injection of adenoviruses [Flesken-Nikitin et al., 2003]. Using Cre recombinase mediated recombination, a target gene with loxP sites flanking the gene can either be activated or inactivated. Using this approach, Flesken-Nikitin et al. [2003] inactivated two key tumor suppressor genes, *p53* and *Rb* in OSE cells of the mouse. In these models, recombinant adenovirus expressing Cre is injected under the ovarian bursa of double transgenic mice bearing floxed copies of *p53* and *Rb*. Inactivation of both genes resulted in a high prevalence (33 of 34 mice) of ovarian tumors in these mice, with 24% of mice also having abdominal ascites, while inactivation of either *p53* or *Rb* resulted in much lesser incidence of ovarian carcinogenesis. With a similar approach, Dinulescu et al. initially generated mice expressing a mutated *K-ras* gene that developed benign endometrioid lesions involving the OSE [Dinulescu et al., 2005]. In the second phase of this elegant experiment, the mice in which K-ras could be activated were crossed with mice where *Pten* could be inactivated using Cre-mediated recombination. These mice expressed activated Ras and lacked *Pten*. These mice developed invasive endometrioid carcinoma of the ovary within 7–12 weeks post injection. In a more recent report, the group lead by Vanderhyden [Clark-Knowles et al., 2007], using mice with conditional expression of *Brca1*, inactivated *Brca1* in the mouse OSE with intrabursal injection of recombinant Adv-Cre and reported that these mice developed more preneoplastic changes, such as hyperplasia, epithelial invaginations, and inclusion cysts (frequently seen in older women) compared to control ovaries. In a

different approach, Orsulic et al. [Orsulic, 2002] used the RCAS retroviral vector to introduce oncogenes into the OSE cells from transgenic mice carrying the RCAS receptor TVA. Tumorigenicity in both syngenic and immunocompromised mice revealed that p53 deficiency in combination with two oncogenes (*c-Myc*, *K-RAS*, or *AKT*) were required for tumor formation [Orsulic, 2002]. The first transgenic model of EOC in which 5′ regulatory sequences of the mouse *MISIIR* gene driving the expression of SV40 T antigen in the reproductive tract including the OSE was developed by Connolly et al. [2003]. The tumors developed by these mice histologically were poorly differentiated with occasional cysts and papillary structures present at the surface of the ovary.

Advantages of the Genetically Engineered Mouse Models

The generation of genetically engineered mouse models has opened new avenues of research to answer fundamental questions pertaining to targeted therapy. For example, these models will enable testing the efficacy of small molecule inhibitors (MEK inhibitor PD98059, mTOR inhibitor rapamycin, and/or AKT inhibitors) of pathways activated by oncogenes such as AKT, K-RAS, or c-Myc. Mabuchi et al. [2007] used the MISIIR-Tag transgenic mouse model to test the effect of RAD001 (Everolimus), an mTOR inhibitor and showed that RAD001 markedly delayed tumor onset and progression. In an elegant study, Xing et al. [Xing and Orsulic, 2005a,b] generated several mouse cell lines with defined genetic alterations based on their original model. They demonstrated that the mTOR pathway was an excellent target for the treatment of cells that depend on Akt signaling but was ineffective in cells that utilized alternative pathways for proliferation, independent of Akt signaling. These results have major implications for the development of pathway-targeted therapy. Based on the results from the above model, we can now test the efficacy of interrupting multiple pathways simultaneously as a means to treat ovarian cancers that exhibit resistance to single agent therapy.

Limitations and Challenges of the Genetically Engineered Mouse Models

The relevance of each model to human ovarian cancer is limited by the prevalence of

these genetic alterations in ovarian cancer. Moreover, due to the specific alterations of genetically defined targets in some mouse models, the models lack genetic heterogeneity, and do not reproduce the full spectrum of human EOC tumors. For example, Dinulescu et al. exploited K-*ras* and *Pten* alterations to produce endometrioid type ovarian cancer [Dinulescu et al., 2005]. Although *Pten* is frequently mutated or deleted in endometrioid type ovarian cancer, it is not commonly altered in other histologic subtypes of ovarian cancer [Dinulescu et al., 2005]. Therefore, this mouse model is useful for studying the carcinogenesis pathway that leads to this particular histology of ovarian cancer. On the other hand, the mouse models generated by Orsulic et al., Flesken-Nikitin et al., and Connolly et al. [Orsulic et al., 2002; Connolly et al., 2003; Flesken-Nikitin et al., 2003] appear to be more relevant for the biology of de novo arising higher grade EOCs. A limitation of the Flesken-Niktin and Connolly models is that they yield poorly differentiated tumors that do not show step-wise progression with histologic differentiation, and therefore do not permit the investigation of EOC progression. Future challenges include the generation of mouse models that accurately reflect human EOC in terms of histologic differentiation and provide insight into step-wise progression. These models should not be limited to the ovarian surface epithelium but should also target the fallopian epithelium. In addition, the role of Hox genes in histologic differentiation should be investigated in these mouse models. Finally, targeted genetic alterations in the stem cell population within the ovary or fallopian tube should be investigated to define the role of these cells in EOC tumorigenesis.
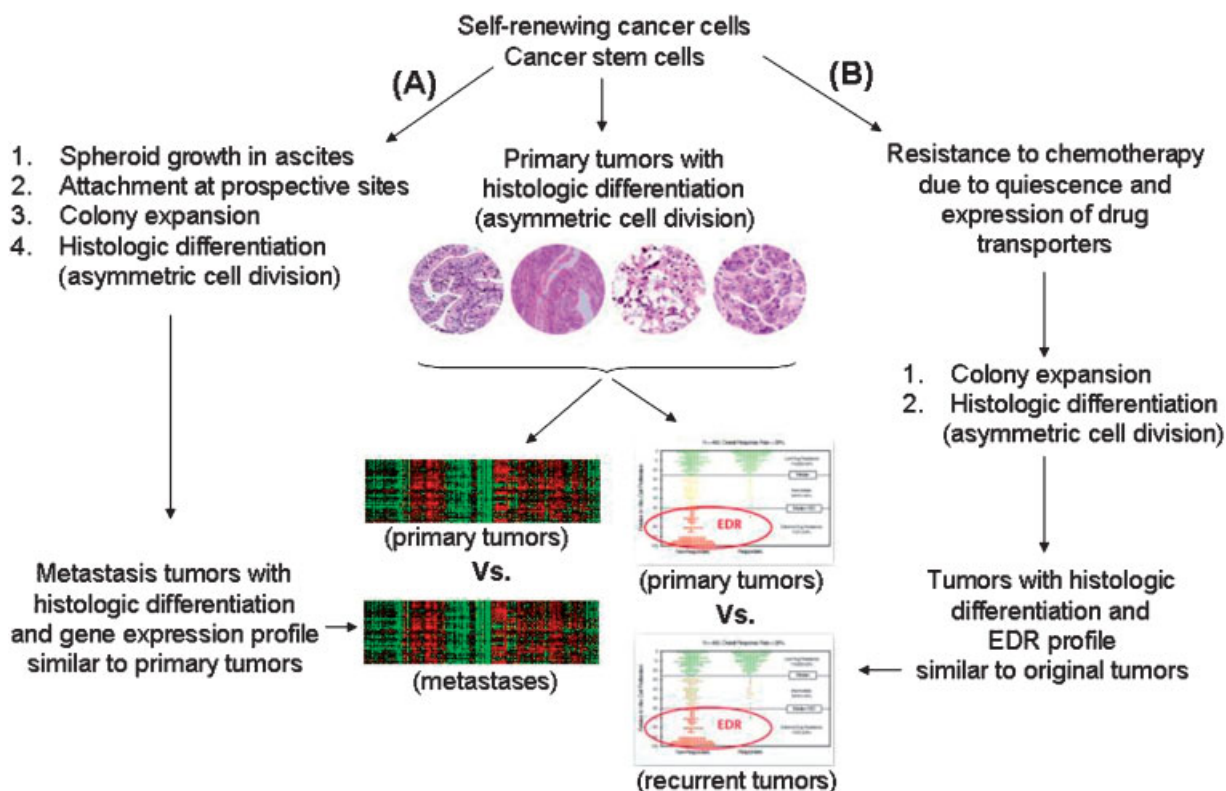
CANCER STEM CELL MODEL

Cancer stem cells are defined as self-renewing cancer cells with potential to reconstitute the wide spectrum of heterogeneous tumor populations. Recent studies have indicated the presence of an ovarian cancer Hoechst-low side-population of self-renewing cells with differentiation potential and high tumorigenicity [Bapat et al., 2005; Szotek et al., 2006]. Due to their stem cell-like characteristics with ability to differentiate into tumors with different histologies, these putative cancer stem cells provide an attractive model to reflect the various histologies observed in ovarian carcinomas (Fig. 2). They also provide a model of cancer metastasis in which these cells are able to colonize, expand, and differentiate into heterogeneous tumor phenotypes similar to primary tumors. In such a model, both the primary tumors and metastases would display similar genetic and expression profiles because both populations are supposedly derived from the same lineage of cancer stem cells (Fig. 2). Consistent with this model, transcriptomic and comparative genomic hybridization studies of primary tumors and metastases show conservation of expression and genetic alterations between two populations [Bayani et al., 2002; Hibbs et al., 2004; Israeli et al., 2004]. Finally, owing to their quiescence and the expression of drug transporters, these stem cells are generally considered to be resistant to chemotherapy and therefore may provide a model of chemoresistance. In such a model, cancer stem cells that are innately resistant to chemotherapy will persist after chemotherapy and repopulate a heterogeneous tumor with phenotype similar to the original primary tumors. Since these repopulated tumor cells were not directly selected by chemotherapy, those cells that were originally chemosensitive would remain sensitive to chemotherapy (Fig. 2). Consistent with this model, Tewari et al. reported the conservation of in vitro extreme drug resistance profile between primary tumors and recurrent tumors [Tewari et al., 2005]. This model would also explain why those patients that responded to initial rounds of platinum-based chemotherapy, with more than 12 months of remission, can usually be successfully re-treated with platinum-based therapy. Therefore, cancer stem cells could potentially provide a unifying model of ovarian cancer that reflects histologic diversity, metastasis, and resistance to chemotherapy. Better understanding of the biology of these self-renewing cancer cells, and their role in ovarian carcinogenesis, may be possible with new mouse models.

CONCLUSIONS

Two vital areas in ovarian cancer research are the identification of early events in ovarian carcinogenesis that could yield markers for early detection and the development of tools to overcome chemoresistant disease. The identification of early precursor lesions and genetic

Fig. 2. Cancer stem cell model. Cancer stem cells are defined as self-renewing cells with potential of histologic differentiation. **A**: This model posits that due to their self-renewing and clonogenic potential, these cells, when disseminated, may be able to colonize and expand to produce distant metastases. Because both primary and metastases arise from cancer stem cells, they are expected to produce similar genetic alterations and gene expression profiles. **B**: Similarly, cancer stem cells, owing to their quiescence and expression of drug transporters, may be resistant to chemotherapy, allowing them to persist and expand after chemotherapy. Because both primary and recurrent tumors arise from these cancer stem cells, they are expected to possess similar drug resistant profiles.

alterations in these lesions should facilitate the generation of mouse models that accurately reflect human EOC that could in turn promote the discovery of markers for early detection. The identification of stem cells in ovary and fallopian tube epithelium, and targeted genetic alterations in these cells, should also provide a model to test the role of these cells in tumorigenesis of EOC and in the development of chemoresistance. These new tools are essential to investigate the cellular and molecular changes associated with the initiation of ovarian cancer and to provide models to test targeted therapeutic agents to combat this deadly malignancy.

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