Ovarian cancer is the fourth leading cause of cancer deaths among American women and the most lethal of the gynecologic malignancies. Although stage I ovarian cancers have a 5-year survival rate of over 90%, the majority of epithelial cancers are diagnosed at an advanced stage. A poorer prognosis with a 5-year survival rate of under 30% is associated with these advanced tumors. Improved early detection and population risk assessment require a better understanding of the molecular steps of carcinogenesis and the development of better animal models.

Here we review current theories of the histogenesis of ovarian cancer and correlate them with results from large-scale genomic and proteomic profiling studies. We describe how knowledge of tumor genetics and the application of investigational technologies are providing a detailed model of the early steps of carcinogenesis in ovarian cancer.

**PATHOGENESIS OF OVARIAN CANCER**

The cells covering the ovarian surface constitute a very small fraction (< 1%) of the total ovarian mass. Nonetheless, it is thought that most ovarian carcinomas arise de novo from invaginations of this ovarian surface epithelium (OSE) and underlying cortical inclusion cysts (CICs). This suggestion is formulated based on histologic findings that CICs are detected more frequently in ovaries contralateral to a stage I cancer and in ovaries removed prophylactically from women with a strong family history of ovarian cancer. CICs are likely to arise by two mechanisms: One is related to the ovulatory cycle. With the maturation of the follicle, the underlying OSE is subjected to apoptotic signals that ultimately aid in the rupture of the ovum through the ovarian surface. The resulting gap in the OSE has to be repaired. During this process, some OSE cells become entrapped within the cortex, giving rise to CICs. Aging likely accounts for a second mechanism of CIC generation. As the ovary ages it becomes atrophic and cerebriform on the surface. This process leads to invaginations of the OSE into the ovarian cortex and the formation of CICs. The signals that further modify the cyst epithelium and the triggers of malignant transformation are not known, but presumably result from the sequestration of the epithelium in the hormonally active ovarian stroma.

Whether CICs harbor neoplastic precursor lesions remains to be proven. A morphologic spectrum extending from normal epithelium to dysplasia, and invasive carcinoma has been observed within CICs of ovaries harboring stage I cancers and in ovaries removed prophylactically from women with a family history of carcinoma. Computer image analysis can distinguish differences in the nuclear chromatin among benign, borderline and malignant tumors. However, these results have not been correlated to molecular changes. Most significantly, metaplastic CICs have been found to carry p53 mutations, a finding common to serous carcinomas. Moreover, cancer-associated CICs contain p53 mutations. Further examination of rare early-stage tumors will undoubtedly aid in developing a consensus regarding the role of CICs in the pathogenesis of ovarian cancer.

An alternative hypothesis to the CIC model is that invasive carcinomas arise from so-called 'tumors of borderline malignancy'. Ovarian tumors are morphologically classified as benign, borderline or malignant. Tumors of borderline malignancy are a heterogeneous group defined by their lack of 'obvious invasion' of stroma and are characterized by a better prognosis than invasive carcinoma. Simplistically, it appears that these categories are sequentially related because (i) there is a 10–15 year time delay between the peak incidence of
benign ovarian tumors and carcinoma; (ii) there are similar epidemiologic risk factors for benign and borderline lesions and ovarian carcinoma; and (iii) there is an increased incidence of benign and borderline tumors in women with familial/genetic risk factors\textsuperscript{17}. However, only a minority of borderline lesions will precede an independent invasive carcinoma\textsuperscript{18}, and it is exceedingly rare to find histologic transition from borderline histology to invasive carcinoma in a single tumor\textsuperscript{19,20}.

In fact, serous type borderline lesions have been shown to be genetically distinct from their invasive counterpart. For example, serous borderline tumors\textsuperscript{9} typically contain different genetic changes than carcinomas. Serous borderline tumors show an absence of p53 mutations, loss of heterozygosity (LOH) on the long arm of the inactivated X chromosome and microsatellite instability. In contrast, invasive carcinomas have frequent p53 mutations and LOH on multiple somatic chromosomes (1p, 3p, 5q, 6q, 7p, 8p, 9q, 11p, 13q, 14q, 17q, 18q, 21q and 22q) and they lack microsatellite instability\textsuperscript{21-23}.

For mucinous-type tumors, there is a more intimate relationship between benign, borderline and invasive carcinoma than has been described for serous-type tumors. Mucinous-type tumors comprise less than 15% of ovarian tumors. Approximately 75% are benign, and the majority of the remainder are borderline. Primary invasive mucinous carcinoma is exceedingly rare and more often represents metastases from the appendix and gastrointestinal tract\textsuperscript{27} or overdiagnosis of borderline tumors. In many borderline mucinous tumors, the epithelium varies from atypical to adenomatous, a phenomenon referred to as ‘intraepithelial carcinoma’. True mucinous carcinomas with extensive stromal invasion are associated with such areas of intraepithelial carcinoma\textsuperscript{28}. Also, molecular studies have demonstrated a genetic link between benign borderline and invasive mucinous tumors\textsuperscript{29}.

Our current understanding suggests that the most common type of invasive ovarian carcinoma, the serous type, arises \textit{de novo} from OSE and CICs. In this subtype, there is little histologic and genetic relationship between benign, borderline and invasive tumors. Other, less common types exhibit more of a histologic and genetic continuum between borderline and invasive tumors.

**TUMOR HISTOLOGY: THE CELL OF ORIGIN**

The most common subtypes of ovarian epithelial tumors are histologically similar to other tumors arising in the female genital tract (fallopian tube, endometrium and endocervix). This similarity is consistent with the fact that, although continuous with the mesothelial lining of the peritoneal cavity, OSE shares a common embryologic origin with epithelia of Müllerian duct-derived tissues\textsuperscript{30,31}; they both arise from the coelomic epithelium in the area of the gonadal ridge. This common origin is evident in normal adult ovaries as the commonly observed histologic transformation of the OSE to a more columnar and ciliated cell type (Figure 2), a process referred to as Müllerian metaplasia.

Since carcinomas histologically resemble normal Müllerian-derived epithelium, such as that of the fallopian tube or endometrium, it is possible that metaplasia is an early step in neoplastic transformation. In the mature ovary, the surface cells have an uncommitted character with features of epithelial and mesenchymal-derived cells that express cyto keratin, laminin and collagen IV as well as vimentin, collagen I and III. In vitro (cell culture), OSE cells can shift between epithelial and mesenchymal morphology. For example, E-cadherin induces mesenchymal-to-epithelial transition in human OSE. The resulting surface epithelium expresses estrogen, progesterone and androgen receptors\textsuperscript{30,32}. In vivo, these stimuli for transformation are localized to the OSE by their intimate relationship to ovarian stroma and maturing follicles.

Thus, ovarian cancer is thought to arise via differentiation from mesenchymal to an epithelial (Müllerian) phenotype and a resulting sensitivity to hormonal and other triggers of transformation\textsuperscript{9,33,34}. An overactive or sustained response to one or several hormonal stimuli may leave the OSE vulnerable to transformation\textsuperscript{30,35}.

Other origins of ovarian carcinomas have been suggested. Whereas the majority are of the serous type arising from the OSE, it is well established that cancer can arise in foci of endometriosis. Ovarian cancers with endometrioid and clear-cell histologies are associated with endometriosis in 28% and 49% of cases, respectively\textsuperscript{36,37}. In addition, the risk factors for endometriosis and cancer are similar (early menarche, regular periods, short menstrual cycle and low parity). Moreover, tubal ligation is protective for endometrioid and clear-cell, but not serous cancers\textsuperscript{38}. Alternatively, some have postulated that all types of ovarian cancer arise from the embryologic remnants of the proximal Müllerian duct. These small tubular structures are often seen incidentally in the hilum of the ovary and throughout the adnexae. This would account for the morphologic similarity between ovarian and other Müllerian cancers.
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Figure 1 Model of ovarian carcinogenesis. Epithelial carcinomas are thought to arise from the surface epithelium or cortical inclusion cysts (CICs). CICs form either as a result of surface complexity that comes with aging or as part of the surface repair process following ovulation. These cysts, under the hormonal effects of the ovarian stroma, are more susceptible to malignant change.

Figure 2 Histology of carcinogenesis. (a) and (b) Cortical inclusion cysts (CICs) are a common incidental finding in all ovaries. Although they are typically lined by flat or a cuboidal cell type similar to that of the ovarian surface epithelium (c), they may undergo Müllerian metaplasia to a ciliated epithelium, resembling the lining of the fallopian tube. (d) Cancers arise from this metaplastic epithelium either in the ovarian surface or in CICs. (e) A high-power view of a papillary serous carcinoma.
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However, cytologic atypia in these remnants is rare and cancers predominantly occur in the ovarian cortex rather than in the hilum or paratubal areas.

THE ROLE OF OVULATION

The triggers for metaplastic and neoplastic transformation of the OSE remain unknown, but may be related to hormonal and structural changes during ovulation, pregnancy and lactation. It is a common belief that a woman’s risk of cancer increases with the number of ovulations. In fact, epidemiologic studies show that the risk of epithelial ovarian cancer is decreased by factors that suppress ovulation, including pregnancy, lactation and oral contraceptives. Further support for a causal role of ovulation comes from the observation that cancer of the OSE is rare in animal species that ovulate infrequently, whereas it is common in hens, which, like humans, are frequent ovulators. These observations support a model whereby CICs are increased by the number of ovulatory cycles, leading to an increase in transformation events.

Several causative mechanisms may explain the relationship between uninterrupted ovulation and the development of ovarian cancer. In one model, the surface epithelial cells overlying a maturing follicle undergo degradation by collagenases, plasminogen activators and lysosomal proteases during the process of follicular rupture. Epidermal growth factor stimulates OSE proliferation in cell culture and is thought to contribute to its post-ovulatory surface repair/proliferation. This alteration in growth factor exposure stimulates increased mitoses and possibly the mutational potential of the epithelial cells as they try to repair the wound left as a result of follicular rupture. Investigation along these lines has shown an association between detectable oxidative DNA damage with post-ovulatory remodeling. Cell culture studies of OSE from ovaries removed prophylactically from high-risk women also show that they retain an epithelial phenotype longer than control cells and are less responsive to wound healing signals related to ovulation.

NEW APPROACHES TO STUDY CARCINOGENESIS

Like other solid tumors, ovarian cancer is thought to result from an accumulation of genetic changes. Alterations in tumor suppressor genes and oncogenes have been shown to play an important role in ovarian carcinogenesis, with p53 and c-myc mutations commonly detected in serous carcinomas, k-ras mutations in mucinous carcinoma, and PTEN mutations in endometrioid tumors. Several technologies have been used for gene expression profiling of ovarian cancer with the goal of finding additional candidate genes that may either serve as a marker for early cancer detection or risk assessment, or that may become targets for specific chemotherapy.

Current methods for assessing global gene-expression profile alterations in different tissues include differential display, serial analysis of gene expression (SAGE), differential cDNA array, comparative hybridization of cDNA arrays, two-dimensional gel electrophoresis of cellular proteins and, more recently, proteomic serum patterns. The whole spectrum of approaches has been applied to ovarian cancer. As might be expected, each study has identified a unique set of genes whose expression is altered in the transition from benign OSE to carcinoma. Interestingly, like colonic cancers, the majority of upregulated genes identified are either surface antigens or secreted proteins. Moreover, limited comparison of the data from the various studies reveals that a subset of genes is shared among the various studies. These genes include HE4, Mucin 1, Ep-CAM, Mesothelin and CD9. These genes represent a promising list of candidate tumor markers. Further validation on human tissues and development of serum tests will determine their clinical utility.

Future study of these candidate genes will be facilitated by the recent production of a so-called ‘Ovachip’. This is a specialized cDNA array (gene chip) that analyzes expression of 516 genes chosen for their relevance to ovarian cancer. The choice of genes was based on more broad-based expression profiles using larger arrays. These focused studies may more easily demonstrate clusters of coordinately expressed genes, and molecular pathways involved in the development of cancer.

Although these studies have provided insight into the genetics of invasive carcinomas, they provide little understanding of the early steps in the transformation of the OSE to carcinoma. Defining this process will require development of appropriate animal models, and examination of ovaries from patients with early-stage cancer.

SERUM BIOMARKERS

Identification of markers that facilitate detection of early ovarian carcinoma will have great impact on the study of early ovarian carcinogenesis. Currently, cancer antigen 125 (CA-125) is the most widely used serum
biomarker for ovarian cancer. Serum concentrations of CA-125 are elevated (> 35 U/ml) in up to 80% of patients with advanced-stage disease and this marker is routinely used to follow response to treatment and disease progression. However, levels of CA-125 are highly correlated with tumor volume. Therefore, only 50% of patients with early-stage disease have elevated levels, thus limiting its use as a screening tool. Recently, transcriptional profiling of ovarian cancer cells using cDNA microarrays resulted in the observation that levels of prostatin (a serine protease) and osteopontin (a secreted bone morphogen) are markedly elevated in ovarian cancer cell lines compared to normal OSE. These have shown promise for use as serum screening tests. Other investigational serum markers, alone or in combination, have been reported to reflect disease course and relapse, but their lack of sensitivity or specificity limits their use as a cost-effective screening test.

An exciting new approach to cancer screening shifts the focus from monitoring single proteins to the analysis of thousands in a ‘proteomic spectrum’ using mass spectrometry. These technologies called matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) and surface-enhanced laser desorption and ionization time-of-flight (SELDI-TOF) separate low molecular weight serum proteins based on size and electrostatic charge, generating a complex map of proteins and their concentrations. When these maps are compared among patients with cancer, benign pelvic disease and a control group, patterns specific to cancer are identified. This is done without any knowledge of the nature of the specific proteins. Although not yet tested in a prospective trial, the technology was reported to have a positive predictive value among high-risk women of 94% compared to 35% for CA-125. This held true even in stage I disease. Identification of these protein targets in early-stage patients might provide insight into early pathways necessary for malignant transformation.

MODEL SYSTEMS

The genetic damage leading to cancer likely occurs in a stepwise process. In vitro models of pre-neoplastic and neoplastic ovarian epithelium have identified altered gene expression. For example, a comparison of cell cultures from normal OSE to a cancer cell model (immortalized cells overexpressing E-cadherin) showed decreased activity of cGMP-dependent protein kinases in tumor cells, whereas expression of MEK6, a regulator of the stress-sensitive p38-MAP kinase pathway, was increased. These data demonstrate that changes in several downstream signaling pathways correlate with progression to cancer. Such cell culture data is clinically relevant since increased phosphorylation of the targets of phosphatidylinositol-3-kinase (PI3K) occurs more frequently in OSE of BRCA1 mutation-carrying women than in the OSE of the general population. There is a therapeutic potential of PI3K inhibitors, which show a growth inhibitory effect on ovarian carcinoma.

A second approach to defining early neoplasia has been to study ovaries from women predisposed to carcinoma. Patients with mutations in the tumor suppressor genes BRCA1 and BRCA2 have predisposition to breast and ovarian carcinoma. Prophylactic oophorectomies detect occult early-stage carcinoma in 8–12%,5–66. The presence of histologic ‘pre-cancer’ lesions such as epithelial multilayering or tufting in these patients is controversial. However, molecular abnormalities including loss of heterozygosity (LOH) for BRCA1 and mutations of the p53 gene5 have been shown to occur prior to detectable cancer. Unfortunately, the relevance of BRCA1 mutations to sporadic cancers remains unclear since the rate of BRCA1 mutations in that group is very low. The estimated gene frequency in the general population is 0.0006, accounting for 5.7% of ovarian cancers in women below age 40, and 2.1% in women between 50 and 70 years of age.

Limited animal models of ovarian cancer exist. The ones most often used in the study of anticancer therapies are those produced by injecting human ovarian cancer cell lines into mouse peritoneum. Although these reflect the genetic makeup of the mature tumors, they do not provide a model of early carcinogenesis. More recently, a model was created by re-implanting mouse ovarian epithelial cells genetically altered in culture into the ovarian bursa (site of the ovaries) of adult mice. This model was created by re-implanting mouse ovarian epithelial cells genetically altered in culture into the ovarian bursa (site of the ovaries) of adult mice. This model better accounts for the effects of the local ovarian milieu. Importantly, the mouse tumors in this model arise from the OSE rather than the ovarian stroma and the histology of the tumors mimics that of papillary serous tumors in humans. This model is very exciting in that one can investigate the transforming role of specific candidate genes alone and in combination. However, ex vivo manipulation of the OSE precludes investigation of the cellular changes in early disease development, and the need for additional models remains.

CONCLUSIONS

Much remains to be learned before we fully understand the complexities of ovarian biology and the risks of
developing ovarian cancer. Improving methods of detection and treatment are of paramount importance. The foundations of a model of carcinogenesis are near. Genomic and proteomic approaches are providing candidate genes. Model systems in which to test them are sure to follow and change our approach to cancer screening and therapy.

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