Ovarian cancer is composed of a heterogeneous group of tumors that are derived from the surface epithelium of the ovary or from surface inclusions. They are classified into serous, mucinous, endometrioid, clear cell, and Brenner (transitional) types corresponding to the different types of epithelia in the organs of the female reproductive tract (1–3). Each histologic subcategory is further divided into three groups: benign, intermediate (borderline tumor), and malignant, reflecting their clinical behavior (1). In developed countries, serous tumors account for about 60% of all ovarian epithelial tumors; therefore, serous borderline tumors (SBT) are the major focus of this review.

The modern history of SBTs begins in the 1950s and 1960s when investigators from the United States and Europe recognized that there was a subset of serous carcinomas that had a significantly better prognosis even when they presented as advanced stage tumors and were inadequately treated (4). The International Federation of Gynecology and Obstetrics in 1971 and subsequently the WHO in 1973 therefore created a separate category for these tumors, designating them “borderline” or “low malignant potential” (5). In addition, because women with advanced-stage borderline tumors had a very good prognosis, extraovarian lesions were classified as “implants” rather than metastases. The biggest challenge in the management of women with these tumors is to identify the subset that will behave in a malignant fashion and to develop effective treatment for them. This challenge can only be addressed if the pathogenesis of these tumors is elucidated.

Molecular Studies of Ovarian Borderline Tumors

Ovarian serous carcinoma has been traditionally graded as well differentiated, moderately differentiated, and poorly differentiated, implying that serous carcinoma begins as a well-differentiated neoplasm that progresses over time to a poorly differentiated tumor. The recent molecular studies summarized below, however, have challenged this view and offered an alternative “dualistic” model of serous carcinogenesis.

Molecular genetic findings. Molecular genetic studies aimed at delineating the pathogenesis of SBTs have highlighted the importance of the KRAS signaling pathway. Activating mutations in KRAS and one of its downstream mediators, BRAF, have been identified in a variety of human cancers, and mutations in either KRAS or BRAF result in constitutive activation of the RAS/RAF/mitogen-activated protein kinase (MAPK) kinase (MEK)/MAPK signaling pathway (6). KRAS mutations were first reported in SBT by Mok et al. (7). Recent studies have verified the original finding and further showed that mutations in KRAS and BRAF characterize SBTs and low-grade serous carcinomas (8–11). Mutations in either codons 12 and 13 of KRAS or codon 599 of BRAF occur in two thirds of SBT and low-grade serous carcinomas (8). In contrast, none of the 112 high-grade serous carcinomas (usual type of serous carcinoma) that we have analyzed contained KRAS or BRAF mutations. These findings provide compelling evidence indicating that KRAS and BRAF mutations are predominantly confined to low-grade serous ovarian carcinomas and their putative precursor, SBTs. The frequent mutations in KRAS and BRAF in SBTs in the progression of low-grade ovarian serous carcinoma tumor are analogous to melanoma and colorectal carcinoma in which mutations in RAS (KRAS in colorectal
tumor and NRAS in melanoma) and BRAF genes also occur in their preinvasive stages, nevus and adenomatous polyp, respectively (12–15). Thus, it is likely that mutations in RAS and RAF genes are involved in early tumorigenesis, but the mutations are not sufficient for a malignant transformation. Interestingly, none of these three types of tumor that were analyzed showed mutations in both RAS and BRAF and lends further support to the view that RAS and BRAF mutations have an equivalent effect on tumorigenesis. In view of the absence of KRAS and BRAF mutations in high-grade serous carcinoma, it would seem that the development of high-grade serous carcinoma involves a pathway not related to the mutations in the RAS/RAF/MEK/MAPK signaling pathway. This conclusion is supported by the finding of p53 mutations in >50% of high-grade ovarian serous carcinomas and the rare finding of mutant p53 in SBTs and low-grade serous carcinomas (16, 17).

Two recent studies have employed mutational analysis to characterize the early molecular genetic events in the development of SBTs. In one study, 30 consecutive serous cystadenomas, the putative precursor of SBTs, were analyzed for KRAS and BRAF mutations and none were found (18). A subsequent study that analyzed eight serous cystadenomas containing small SBTs compared the mutational status of KRAS and BRAF in the SBT and the adjacent epithelium from the cystadenoma (19). It was found that four SBTs contained activating KRAS mutations at codon 12 and three SBTs contained mutant BRAF at codon 599. One case contained wild-type KRAS and BRAF. Moreover, the mutation detected in the SBT component of the tumor was identical to the mutation in the cystadenoma epithelium adjacent to the SBTs in 86% of informative cases. Unlike SBTs, cystadenoma epithelium lacks cytologic atypia. These findings have several important implications in thus far as the pathogenesis of SBT is concerned. First, they suggest that mutations of KRAS and BRAF are early events associated with tumor initiation as occurs in melanoma (14) and colorectal carcinoma (20). Moreover, it would seem that they precede the development of a SBT indicating that some cystadenomas are precursors of SBTs. Second, the frequency of mutations in KRAS and BRAF in cystadenomas associated with SBTs was significantly higher than those without SBTs. This finding together with the fact that SBTs are relatively uncommon compared with cystadenomas (1, 21–24) suggests that only a small proportion of serous cystadenomas are neoplastic with the potential to progress to SBTs.

The biological effects and clinical implication of KRAS and BRAF mutations in serous borderline tumor. The biological effects of mutations in KRAS and BRAF in the development of low-grade carcinoma are likely mediated by the constitutive activation of MAPK, the downstream target of the KRAS/BRAF/MEK/MAPK (extracellular signal-regulated kinase) signaling pathway (25). This is supported by the observation that activating mutations in these genes are oncogenic in experimental cell culture systems (12, 26, 27) probably through a constitutive activation of MAPK which in turn regulate many downstream targets that are important for tumor development (28, 29). It should be noted that activated MAPK or extracellular signal-regulated kinase can also be observed in conventional high-grade serous carcinomas probably through an epigenetic mechanism other than activating mutations of KRAS and BRAF (25, 30), because mutations in both genes are rarely found in high-grade serous carcinomas.

What is the biological significance of activation in the MAPK signaling pathway in SBT and low-grade serous carcinoma? Pohl et al. applied long serial analysis of gene expression to identify genes that are regulated by activated MAPK in low-grade serous carcinoma cells that harbor a BRAF mutation (31). The transcriptome of these cells was compared with that of CI-1040-treated cells, a compound that selectively inhibits MEK, the upstream regulator of MAPK (28). The most striking changes after MEK inhibition were down-regulation of cyclin D1, COBRA1, and transglutaminase-2 and up-regulation of tumor necrosis factor-related apoptosis-inducing ligand, thrombospondin-1, optineurin, and palladin. Among all the differentially expressed genes, cyclin D1 showed the greatest alteration of gene expression. Cyclin D1 plays an important role in the cell cycle transition from G1 to S phase through its association with cyclin-dependent kinases 4 and 6 (32, 33). In ovarian tumors, overexpression of cyclin D1 is associated with low-grade tumors (34–36), a finding consistent with our view that cyclin D1 is a downstream target of active MAPK, which is constitutively expressed in most low-grade ovarian tumors because of frequent activating mutations in KRAS and BRAF. Future experiments are necessary to determine whether mutations of KRAS or BRAF are sufficient to initiate the development of SBTs or whether additional genetic “hits” are required in tumorigenesis.

Because CI-1040 can inhibit the KRAS/BRAF/MEK/MAPK pathway, it is likely that this compound and other emerging MEK inhibitors may be an effective therapeutic agent for patients with SBTs and low-grade serous carcinomas. Pohl et al. have recently shown that CI-1040-treated ovarian serous tumors harboring either KRAS or BRAF mutations showed marked growth suppression (G1 cell cycle arrest) compared with tumors containing wild-type KRAS and BRAF in vitro (31). Normal cells including ovarian surface epithelial cultures and ovarian stromal cells did not show significant growth inhibition by CI-1040 treatment. In addition, CI-1040-induced apoptosis occurred more frequently in tumor cells with either KRAS or BRAF mutations than in those with wild-type sequences. These findings indicate that an activated MAPK pathway is critical in tumor growth and survival of ovarian tumors with KRAS or BRAF mutations and suggest that the CI-1040-induced phenotypes depend on the mutational status of KRAS and BRAF in ovarian tumors. Because SBTs and low-grade serous carcinomas have a high frequency of mutations in KRAS and BRAF, it will be important to determine if treatment with CI-1040 can prolong disease-free interval and overall survival in patients with advanced-stage SBTs.

Genetic instability in serous borderline tumors. Microsatellite instability and changes in DNA copy number (or chromosomal instability) reflect the genetic instability in tumor cells (37). Microsatellite instability has been studied in SBTs using 69 microsatellite markers (38). Similar to high-grade serous carcinoma in which frequency of microsatellite instability is rare (39), SBTs did not show evidence for microsatellite instability in 19 SBTs studied. In contrast, SBTs showed DNA copy number changes as evidenced by chromosomal and allelic imbalance based on comparative genomic hybridization and digital PCR analysis, respectively. Two independent studies using comparative genomic hybridization have shown that the level of chromosomal imbalance in SBTs and low-grade serous carcinomas is similar to each other and is
which precedesthe development of SBT.

progression (i.e., the cystadenoma stage), are referred to SBT. Mutationsof KRAS genes in SBTs and BRAF occur at a very early stage of progression (i.e., the cystadenoma stage), which precedes the development of SBT.

Relationship of Serous Borderline Tumor to Ovarian Serous Carcinoma in Tumor Progression

In the past, the role of SBTs in the progression of ovarian serous carcinoma was not clear (11, 44–47). Until recently, molecular genetic studies together with clinicopathologic observations suggested that SBTs were unrelated to serous carcinoma (11, 46, 48, 49). Recent molecular genetic and morphologic studies, however, suggest there are two main pathways of tumorigenesis that correspond to the development of low-grade and high-grade serous carcinoma (50–55). This has led to the proposal of a new model of serous carcinogenesis, which reconciles the lack of association of SBTs with serous carcinoma on the one hand and the occasional malignant behavior of SBTs on the other (54).

In one pathway, invasive low-grade serous carcinoma develops from a noninvasive (i.e., in situ tumor) traditionally termed SBT (56), mimicking the adenoma-carcinoma sequence in colorectal carcinoma in which carcinoma evolves through a continuum of histologically recognizable precursor lesions (ref. 57; Fig. 1). Detailed histopathologic analysis shows that SBTs consist of two noninvasive tumors at different stages of tumor progression, a benign tumor that has been termed “atypical proliferative serous tumor” and an intraepithelial (in situ) carcinoma termed “low-grade serous carcinoma” (noninvasive micropapillary serous carcinoma), which is the immediate precursor of invasive low-grade serous carcinoma (9, 54). Atypical proliferative serous tumor and intraepithelial low-grade serous carcinoma can be thought of as analogous to dysplasia and carcinoma in situ of the cervix. That is to say, the atypical proliferative serous tumor is a benign proliferative lesion that can progress to intraepithelial low-grade serous carcinoma, which is the immediate precursor of invasive low-grade serous cancer. Recurrence of SBT as high-grade serous carcinoma has been reported (58). Ortiz et al. have compared mutational profiles of p53 and KRAS genes in SBTs and the subsequent serous carcinomas from the same patients and found that SBTs were molecular genetically different from the serous carcinomas (46). Although these serous carcinomas were considered as grade 1 in that study,
it is not clear what they classified as grade 1 corresponds to what we classify as low-grade serous carcinomas or high-grade serous carcinoma.

**Peritoneal Implants Associated with Serous Borderline Tumor**

SBTs are frequently associated with peritoneal implants that are extravarian lesions. This terminology, “implants,” was used because it was not clear to early investigators what these lesions were. They may be benign implants from the SBT, analogous to implants of endometriosis, or they may be benign reactive lesions that develop independently on peritoneal surfaces (i.e., mesothelial hyperplasia; ref. 59). Alternatively, they are indeed metastases from the ovarian tumor or an independent primary peritoneal carcinoma. In the late 1970s and 1980s, investigators, thinking that the implants may be prognostic indicators, subdivided them into “noninvasive” and “invasive” types based on their microscopic appearance (60). Although initially there was considerable debate regarding the prognostic significance of this distinction, a recent review of the literature showed that the presence of invasive as opposed to noninvasive implants was the most important prognostic indicator (61). It is generally accepted that mortality in patients with SBTs is limited to those with extraovarian disease, but there is considerable controversy about how they should be treated, particularly because the distinction between noninvasive and invasive implants can be difficult.

Only a few molecular analyses have been done on peritoneal implants and have been limited to noninvasive implants. The results are conflicting. Investigators assessing allelic imbalance and the mutational status of KRAS have shown identical findings in SBTs and the synchronous implants of the same patient, supporting the implantation theory (42, 62, 63). On the other hand, clonality assays using X chromosome inactivation have shown different inactivation patterns in the SBTs and peritoneal lesions, providing evidence that some implants arise independently (64, 65). Elucidating the molecular features of the invasive as well as different types of noninvasive implants will clarify their nature and will have a major effect on the management of patients with advanced stage tumors.

**Molecular Alterations in Nonserous Borderline Tumors**

Unlike SBTs, mucinous, endometrioid, and clear cell borderline tumors are often associated with their corresponding carcinomas. Accordingly, these nonserous borderline tumors have been thought to possibly represent an intermediate stage in the stepwise progression to carcinoma (Table 1). In mucinous carcinoma for example, morphologic transitions from cystadenoma to a borderline tumor and to intraepithelial carcinoma and invasive carcinoma have been recognized for some time. In addition, an increasing frequency of KRAS mutations at codons 12 and 13 has been described in cystadenomas, borderline tumors, and mucinous carcinomas, respectively (7, 11, 66–68) and using microdissection, the same KRAS mutation has been detected in mucinous carcinoma and in the adjacent mucinous cystadenoma and borderline tumor (7). Other than KRAS mutations, molecular genetic changes, including microsatellite instability, have rarely been reported in mucinous borderline tumors (38). Similarly, morphologic data showing a frequent association of endometriosis with endometrioid adenofibromas and endometrioid borderline tumors and their topographical distribution, adjacent to invasive well-differentiated endometrioid carcinoma, suggest a stepwise progression in the development of endometrioid carcinoma. These tumors are characterized by frequent mutation in β-catenin (69, 70), and to a lesser extent, mutation in PTEN (71). Moreover, similar molecular genetic alterations, including loss of heterozygosity at 10q23 and mutations in PTEN, have been reported in different stages of tumor progression in the same specimen (71–75). The molecular genetic and histopathologic findings suggest a precursor role of endometrioid borderline tumors in the development of ovarian endometrioid carcinoma. A recent report using an engineered mouse model shows that KRAS and PTEN mutations play an important role in the development of endometriosis and endometrioid carcinoma of the ovary (76). In that study, expression of oncogenic KRAS or conditional PTEN deletion within the ovarian surface epithelium gave rise to preneoplastic ovarian lesions with an endometrioid glandular morphology. Furthermore, the combination of the two mutations in the ovary led to the induction of invasive and

**Table 1. Ovarian borderline tumors and associated molecular genetic changes in tumor progression**

<table>
<thead>
<tr>
<th>Type of ovarian borderline tumor</th>
<th>Major molecular genetic alterations</th>
<th>Precursor lesions and invasive carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>BRAF and KRAS mutations (~ 67%)</td>
<td>Precursor: serous cystadenoma or adenofibroma; Progression to: invasive low-grade serous carcinoma</td>
</tr>
<tr>
<td>Mucinous</td>
<td>KRAS mutations (~ 60%)</td>
<td>Precursor: mucinous cystadenoma; Progression to: intraepithelial carcinoma then to invasive mucinous carcinoma</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>LOH or mutations in PTEN (20%), β-catenin gene mutations (~ 50%), microsatellite instability (13–50%)</td>
<td>Precursor: endometriosis, endometrioid adenofibroma; Progression to: intraepithelial carcinoma then to invasive endometrioid carcinoma</td>
</tr>
<tr>
<td>Clear cell</td>
<td>KRAS mutations (5–16%), microsatellite instability (~ 13%)</td>
<td>Precursor: endometriosis, clear cell adenofibroma; Progression to: intraepithelial carcinoma then to invasive clear cell carcinoma</td>
</tr>
<tr>
<td>Brenner (transitional type)</td>
<td>Not yet identified</td>
<td>Precursor: Brenner tumor; Progression to: malignant Brenner (transitional cell) carcinoma</td>
</tr>
</tbody>
</table>
widely metastatic endometrioid ovarian carcinomas. The ovarian cancer model provides cogent molecular evidence of the role of KRAS and PTEN in the development of endometrioid carcinoma. Like endometrioid tumors, clear cell borderline tumors are also frequently associated with endometriosis, clear cell adenofibromas, and clear cell carcinoma, but molecular evidence for the stepwise progression is lacking, because molecular markers specific to clear cell neoplasms have only recently been identified (77, 78). These findings also provide further evidence of the close relationship of endometrioid and clear cell carcinoma and point to a common precursor lesion for these two neoplasms.

**Future Directions**

Despite recent advances, there are several challenges unique to borderline tumors that must be overcome to better understand the behavior and pathogenesis of SBTs and develop new management strategies. Several examples are discussed below.

**Etiologic role of KRAS and BRAF mutations in initiating serous borderline tumor.** Although the RAS signaling pathway has been extensively studied in SBTs, it is not known whether mutations of KRAS and BRAF alone are sufficient to induce SBTs in vivo. Murine models must be established to express the mutant forms of KRAS and BRAF to determine if expression of mutant KRAS and BRAF induces SBT-like lesions in mice. Furthermore, analysis of SBT genomes is critical for the identification of potential molecular genetic alterations that play a key role in tumorigenesis. New technologies, including digital karyotyping, array comparative genomic hybridization, and high-throughput mutational analysis, are now available to address these issues (79). The molecular genetic studies of SBTs will not only provide the essential data for future identification of new oncogenes and tumor suppressors in these tumors but will also provide molecular markers to refine the diagnostic and prognostic criteria of primary tumors and implants.

One of the challenges in studying SBTs is how to isolate and enrich tumor cells, because in SBTs, unlike most solid tumors, the tumor cells are arranged as a single layer and overlie abundant stroma. Therefore, the tumor represents only a small fraction of the pooled sample. Laser capture microdissection is a powerful technique for isolating tumor cells from SBTs; however, it is labor intensive and difficult to obtain sufficient DNA and RNA for assays. Several techniques may be useful to overcome these difficulties. For example, tumor cells can be isolated from fresh SBTs and short-term cultured (31). Genomic DNA can be then purified, and cell biology assays done on the cultured cells. In addition, genomic DNA and RNA can be directly purified from the fresh tissues using in situ lysis by applying a mild extraction buffer directly onto the surface of fresh SBTs (80).

**Molecular definition of peritoneal implants.** It is important to determine whether molecular genetic features may be superior to morphologic features as prognostic markers. For example, pathologists acknowledge that the distinction of noninvasive and invasive implants at times can be very difficult. Molecular genetic studies including mutation analysis and allelic imbalance will determine whether different kinds of implants have distinct molecular genetic profiles and whether these profiles can predict outcome. Correlation of the molecular genetic findings with the morphology and behavior of implants may disclose previously unrecognized morphologic features that may assist the surgical pathologist in making the correct diagnosis.

**Target-based therapy for serous borderline tumor at advanced stages.** Novel therapeutic targets can be identified to improve the management of SBT patients with advanced-stage tumors (i.e., with metastatic low-grade carcinoma). Although SBTs occur in women of all ages, most present in women of reproductive age. In perimenopausal and menopausal women, hysterectomy and bilateral salpingo-oophorectomy are generally the treatments of choice, but in reproductive age women, issues of fertility and sterilization must be weighed against treatment for what is regarded as cancer, albeit a low-grade one. Because SBTs and low-grade serous carcinoma are indolent tumors, they do not respond to conventional cytotoxic chemotherapy; therefore, there is considerable debate regarding the best therapy for women with advanced-stage disease. Although many women are followed with no treatment, there are no reliable tumor markers that can be used to monitor the disease process. Recent molecular genetic studies may provide clues for the development of novel target-based therapy. For example, in most SBTs and low-grade serous carcinomas, there is constitutive activation of the MAPK signaling pathway due to frequent mutations in the KRAS and BRAF genes, the upstream regulators of MAPK. Accordingly, it will be important to test whether CI-1040 and other MEK inhibitors can prolong disease-free interval and overall survival in patients with advanced-stage SBTs.

**Conclusions**

Recent molecular studies, including analyses of mutational status, DNA copy number changes, and gene expression profiles, have shed new light on the pathogenesis of SBTs and provide a model for studying the development of ovarian serous carcinoma. In this model, serous carcinoma is subdivided into low-grade and high-grade types that have distinct pathways of tumorigenesis (54). Low-grade serous carcinomas develop in a slow stepwise fashion from SBTs and intraepithelial carcinoma, whereas the majority of high-grade serous carcinomas develop rapidly, presumably from inclusion cysts or ovarian surface epithelium. In the dualistic model, the SBT is a distinct entity that represents the putative precursor of invasive low-grade serous carcinoma and is unrelated to high-grade (usual type) serous carcinoma. This model is the first step in an attempt to elucidate the molecular pathogenesis of ovarian carcinoma but should not be construed as implying that other pathways of tumorigenesis do not exist. For example, we have observed high-grade serous carcinomas associated with a SBT or a low-grade carcinoma on rare occasions. In these cases, both low-grade and high-grade components shared the same mutations, suggesting that high-grade serous carcinomas may occasionally develop from a preexisting SBT or low-grade serous carcinoma.

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1. R. Dehari, unpublished data.
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Molecular Pathogenesis of Ovarian Borderline Tumors: New Insights and Old Challenges

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