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Origins and molecular pathology of ovarian cancer

Debra A Bell

Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Epithelial ovarian cancer comprises the majority of malignant ovarian tumors in adult women. These neoplasms are classified into distinct morphologic categories based on the appearance of the epithelium into tumors of serous, mucinous, endometrioid, clear cell, transitional, squamous, mixed and undifferentiated type. Current data indicate that each of these histologic subtypes is associated with distinct morphologic and molecular genetic alterations: high-grade serous and possibly endometrioid carcinomas most probably arise from surface epithelial inclusion glands with *TP53* mutations and dysfunction of *BRCA1* and/or *BRCA2*; low-grade serous carcinomas probably arise in a stepwise fashion in an adenoma–borderline tumor–carcinoma sequence from typical to micropapillary borderline tumors to low-grade invasive serous carcinoma via activation of the RAS–RAF signaling pathway secondary to mutations in *KRAS* and *BRAF*; mucinous carcinomas arise via an adenoma–borderline tumor–carcinoma sequence with mutations in *KRAS*; low-grade endometrioid carcinomas arise from endometriosis via mutations in *CTNNB1* (the gene encoding β-catenin) and *PTEN*. Although the morphologic data strongly support an origin of clear cell carcinoma from endometriosis, there is limited data on the genetic alterations in these uncommon tumors. Thus it is likely that most low-grade, relatively indolent ovarian carcinomas of serous, mucinous and endometrioid type arise from pre-existing cystadenomas or endometriosis whereas most high-grade serous carcinomas arise without an easily identifiable precursor lesion.
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Epithelial ovarian cancer comprises the majority of malignant ovarian tumors in adult women. These neoplasms are classified into distinct morphologic categories based on the appearance of the epithelium into tumors of serous, mucinous, endometrioid, clear cell, transitional, squamous, mixed and undifferentiated type.[1,2] Two types of ovarian surface epithelial lesions have been described as possible precursors of these carcinomas: lesions that appear *in situ* in surface epithelium or surface epithelial inclusion glands (SEIG); and pre-existing benign epithelial ovarian tumors and endometriosis. The morphologic and genetic data implicating each as a precursor lesion of the major histologic subtypes of ovarian carcinoma are discussed.

Correspondence: Dr DA Bell, MD, Department of Pathology, Warren 105, Massachusetts General Hospital, Fruit Street, Boston, MA 02114, USA.
E-mail: dbell@partners.org
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Surface epithelium and surface epithelial inclusion glands as precursor lesions

Morphologic Data

Two types of morphologic data are available regarding the malignant potential of ovarian surface epithelium and inclusion glands, alterations in these structures and studies on microscopic ovarian carcinoma.

Surface epithelium and surface epithelial inclusion glands

Despite the widespread acceptance of the origin of surface epithelial cancers from the ovarian surface epithelium and its inclusion glands, only rarely have putative precursor lesions been described at these sites. Two types of lesions have been suggested as possible precursors: proliferative–metaplastic changes and cytologic atypia. Ovaries have been examined for such lesions in four settings: (1) prophylactic oophorectomy specimens from patients with a strong family history of ovarian cancer or breast cancer or both, or with known *BRCA1* or *BRCA2* gene mutations; (2) uninvolved

ovaries contralateral to ovarian carcinomas or benign or borderline epithelial tumors or a combination of these tumors; (3) surface epithelium and epithelial inclusion glands adjacent to invasive ovarian carcinomas; (4) ovaries removed after positive or suspicious cul-de-sac aspirates performed to screen for ovarian cancer.

Unfortunately, most studies of the surface epithelium are limited by its fragility; it is usually denuded by allowing the surface to dry intraoperatively or by touching or rubbing it during removal or gross pathologic examination.[3,4] As a result, often all that remains for microscopic examination is residual intact epithelium either in crevasses below a gyriform surface or under the protection of fibrous adhesions.

Only rare examples of lesions showing significant epithelial atypia (severe dysplasia or carcinoma *in situ*) of the ovary have been reported.[5–11] These lesions are characterized by stratification of cells lining the ovarian surface or epithelial inclusion glands with loss of nuclear polarity and marked nuclear pleomorphism, hyperchromasia and chromatin clumping (Figure 1). In their study of cul-de-sac aspirates obtained for ovarian cancer screening, Graham *et al*[9] identified these changes adjacent to a microscopic serous carcinoma in one patient and in the surface epithelium of 20 additional patients with malignant cells in their aspirates.[7,8] Bell and Scully[6] noted severe cytologic atypia adjacent to or near incidentally detected microscopic invasive ovarian carcinomas in three of 14 women, and Gusberg and Deligdisch[5] noted such changes in two of three women whose ovaries had been removed prophylactically because of the finding of ovarian carcinoma in an identical twin. Mild-to-moderate atypia was noted in the third twin, and has also been reported in surface epithelium adjacent to grossly visible or microscopic ovarian carcinomas.[6,12–14] Ovarian carcinoma-*in situ* has been reported in only a single prophylactic oophorectomy specimen. This patient underwent a hysterectomy and bilateral salpingo-ooporectomy for persistent bleeding on Tamoxifen.[11] Sherman *et al*[4] identified mild atypia of the surface epithelium more frequently in the ovaries of women at increased risk for ovarian carcinoma (prophylactically removed ovaries from women with a strong family history of ovarian cancer or known *BRCA* mutations, or ovaries contralateral to ovarian cancer) than controls. Although this difference was statistically significant, the number of cases was small. Werness *et al*[15] were unable to identify a difference in the frequency of atypia in prophylactically removed ovaries and controls on light microscopic examination, but demonstrated a statistically significant increase in nuclear enlargement and chromatin heterogeneity in prophylactically removed ovaries morphometrically. Several other groups have failed to demonstrate epithelial atypia in the prophylactic oophorectomy specimens from women with a strong family history of ovarian cancer[16] or with known BRCA1 or 2 mutations.[17,18] Two groups of investigators have noted mild atypia of the surface epithelium in uninvolved ovaries contralateral to ovarian carcinomas or borderline tumors;[4,19] the latter group, however, failed to detect atypia in epithelial inclusion glands. Several other investigators were unable to demonstrate a statistically significant increase in atypia in ovaries contralateral to ovarian tumors over control ovaries.[20,21] Deligdisch *et al*[22] have noted 'ovarian dysplasia' as detected by a morphometric method utilizing discriminant analysis based on nuclear area and texture in both prophylactic oophorectomy specimens and in epithelium adjacent to carcinomas.

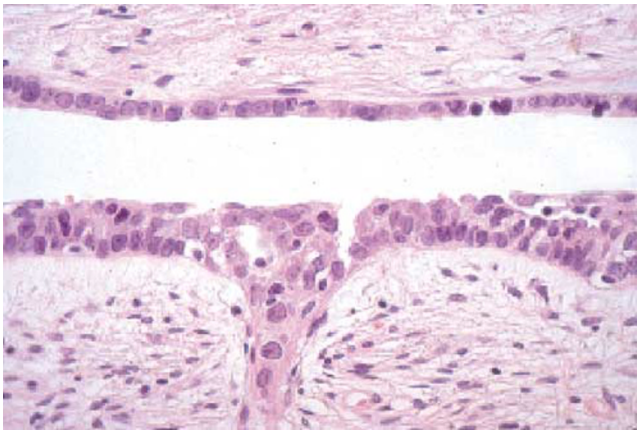

Figure 1 Ovarian carcinoma *in situ*. The epithelium is thickened by stratified cells showing loss of nuclear polarity and marked nuclear pleomorphism, hyperchromasia and chromatin clumping.

Although early studies had suggested that proliferative or metaplastic changes of the ovarian surface epithelium such as papillae, epithelial stratification and tufting, and metaplasia are precursors of ovarian carcinoma,[23] similar findings have been reported to be increased in frequency in only one more recent large series of prophylactic oophorectomy specimens from women with a strong family history of ovarian cancer.[16] The remainder of the recent studies[4,15,17,18,24] have failed to demonstrate any differences in the frequency of these histologic features between cases and controls. A few such studies have shown an increase in the number of inclusion cysts in prophylactic oophorectomy specimens[15] and in ovaries contralateral to ovarian carcinoma,[25] others have failed to confirm these findings.[16,19–21]

Microscopic surface-epithelial carcinomas

A very small number of microscopic or tiny gross carcinomas have been reported, usually as incidental findings at operations for other gynecologic disorders or, more recently, in prophylactic oophorectomy specimens from patients at high risk for the development of ovarian carcinoma.[6,8,9,16,22,26–28] The

largest study of such cases and the only one to include meaningful follow-up data was reported prior to widespread *BRCA* mutation testing and comprised 14 cases of ovarian carcinomas that had not been recognized preoperatively, intraoperatively or even on gross examination of the ovaries but were discovered only on microscopic examination.[6] The patients ranged in age from 27 to 65 (mean 50) years. Three women had a family history of ovarian cancer, six did not, and the family history was unknown or unreliable in the remaining cases. All of the tumors were incidental findings in patients operated on because of a gynecologic indication that did not include a suspicion of ovarian cancer. The tumors ranged in diameter from less than 1 to 7 mm. All of them were unilateral and four appeared to be multifocal. Surface involvement was found in five of the 13 cases in which this feature could be evaluated. The tumors had the typical microscopic features of larger clinically apparent tumors of the same cell type and were classified as serous in 10 cases, endometrioid in one, clear cell in one and undifferentiated in two. In all, 12 of the 14 tumors were grade 2 or 3 (Figure 2). Follow-up data of 2 or more years duration were available for 10 of the 14 patients. Five of the seven whose diagnoses had been made prospectively at the time of oophorectomy were alive without recurrence 2–12 years postoperatively. At the time of publication of the series one patient was alive with recurrent tumor and one had died of tumor. Subsequently, at least one patient originally categorized as alive without recurrence had died of tumor. Two of the three women whose ovarian tumors had been diagnosed for the first time on retrospective microscopic examination of previously removed ovaries after the development of peritoneal carcinomatosis 6, 7 and 10 years subsequently died and the third was alive with recurrent tumor.

Recent studies on prophylactic oophorectomy specimens from women with *BRCA 1* or *2* mutations have revealed surprisingly few cases of early ovarian carcinoma (three at the date of this review).[22,28–30] One of these patients died of tumor.[29]

Clearly, although the data are limited, the prognosis of patients with minute ovarian epithelial cancers is guarded. It is unclear, however, whether the subsequent peritoneal tumor in these women reflect late metastasis from the ovarian carcinoma or the development of a second primary tumor of the peritoneum. Further studies are necessary to answer this question.

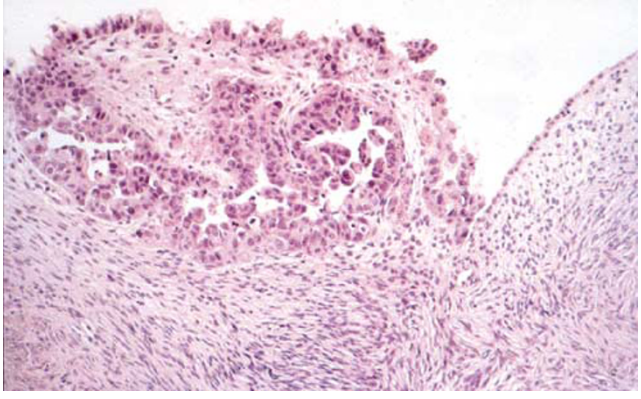

Figure 2 High-grade serous microcarcinoma. The tumor is present on the ovarian surface and in the superficial cortex.

Fallopian tube as the primary site of origin of epithelial ovarian carcinoma

Although very few cases of ovarian carcinoma-*in situ* or severe cytologic atypia or minute carcinomas have been identified in prophylactic salpingo-oophorectomy specimens, each such study that has included careful sampling of the fallopian tubes has reported examples of tubal carcinoma-*in situ* or severe atypia or small tubal carcinomas, especially in women with *BRCA1* mutations.[30–34] These findings have engendered speculation that 'ovarian' carcinomas in women with *BRCA1* mutations may originate from undetected exfoliated tubal carcinomas[30,33,35] and have led to the suggestion that ovarian and fallopian tube carcinomas in women with *BRCA* mutations should be termed 'female adnexal carcinoma'.[24,35]

Molecular Genetic Data

Surface epithelial inclusion glands

Only a small number of studies have examined the molecular genetic features of surface epithelium, its inclusion cysts or early carcinomas. Several small studies have examined the immunohistochemical expression of proliferation and differentiation-related proteins in nonatypical ovarian surface epithelium and SEIG in prophylactic oophorectomy specimens compared to controls.[15,18,24,36,37] Most of these studies failed to document differences in expression of p53, c-erbB2, and Ki67 in surface epithelium or SEIG. Additionally, Piek *et al*[24] also failed to note differences in expression of p21, p27, cyclin A, cyclin D, and estrogen receptor in surface epithelium or SEIG in prophylactic oophorectomy specimens *vs* controls. They did note increased expression of bcl-2 and progesterone receptor in SEIG epithelium in prophylactic oophorectomy specimens, however. In contrast, one study (36,37) found p53 overexpression in cortical clefts or inclusion cysts in 10 of 37 patients with *BRCA* mutations, and *TP53* mutations in three of these, two of which showed loss of heterozygosity (LOH) of wild-type *BRCA 1* or *2* alleles. Nnene *et al*[38] have noted that an increased level of morphologic abnormalities of ovarian surface epithelium and SEIG is associated with expression of p53 in normal ovaries, suggesting that abnormalities of p53 are an early event.

High-grade serous carcinomas
It has been well established that *TP53* mutations are frequent in both hereditary and sporadic high-grade serous carcinoma.[38–47] It has also been demonstrated that loss of *BRCA1* or *BRCA2* function by a variety of mechanisms is present in the majority of both sporadic and hereditary high-grade serous and endometrioid carcinomas.[48,49] Boyd and coworkers[36,37] and Mok and Bell (unpublished data) demonstrated *TP53* mutations in microcarcinomas and Werness *et al*[11] demonstrated loss of heterozygosity at *BRCA1* and *TP53* and overexpression of p53 in one microscopic ovarian surface carcinoma-*in situ*. A few studies have examined genetic abnormalities in surface epithelium or SEIG adjacent to coexisting conventional serous carcinoma. Hutson *et al*[10] demonstrated overexpression of p53 in SEIG associated with coexisting carcinoma. and Boyd and coworkers[36,37] demonstrated the same mutation in the carcinoma and the adjacent normal or atypical epithelium in a small number of cases. These data suggest that mutation of *TP53* is an early event in the development of ovarian carcinoma and is present prior to stromal invasion. Additionally, Shridhar *et al*[50] found that early (Stage I) compared to advanced (Stage III) carcinomas (that were mostly high-grade serous carcinomas) have similar patterns of gene expression.

Conclusions

These data demonstrating increasing atypia and common and accumulating genetic alterations in surface epithelial inclusion glands, ovarian 'carcinoma *in situ*', microcarcinomas and typical high-grade serous carcinoma suggest that high-grade serous and perhaps endometrioid ovarian adenocarcinomas arise from these structures. Additionally, the data from a small number of cases indicates that *TP53* mutation occurs in preinvasive epithelium with loss of *BRCA1* or *BRCA2* function in the majority of tumors, findings compatible with the high degree of genetic instability of these tumors. The suggestion that ovarian carcinomas are surface implants of occult fallopian tube carcinomas is deserving of further study.

Benign and borderline serous tumors as precursor lesions

Whether surface epithelial cancers arise in preexisting benign or borderline epithelial tumors of the same cell type and, if so, how often, has been specifically studied only recently. Benign ovarian neoplasms are almost always removed as soon as they are detected, and therefore their natural history is largely unknown. Evidence of a benign to malignant transformation is largely circumstantial and includes: (1) a generally observed older-age incidence of carcinomas than benign tumors of the same cell type; in that most investigators have reported progressively higher mean ages for benign, borderline, and invasive tumors in both the serous and mucinous categories, with average ages for the combined categories 44, 48 and 56 years, respectively;[51] (2) a reported five-fold increase in the frequency of benign epithelial tumors in first- and second-degree relatives of women with ovarian carcinoma;[52] (3) the presence of enlarging ovarian cysts on ultrasound prior to the development of carcinoma,[53] and (4) the frequent observation of various combinations of benign, borderline, and invasive neoplasia within the same specimen;[54,55]

Morphologic Data

Benign serous tumors
Several studies have shown a relatively low frequency of coexisting benign serous epithelium and serous carcinomas (the great majority of which were high grade) of 15–56%, and it is unusual to encounter a high-grade serous carcinoma as an incidental finding in a serous cystadenoma or borderline tumor, although such cases are rarely encountered.[54–56]

Serous borderline tumors
It is rare to see high-grade serous carcinoma arising in a serous borderline ovarian tumor of either typical or micropapillary type. It has been shown recently, however, that the majority of low-grade invasive serous ovarian carcinomas arise in association with serous borderline ovarian tumors of micropapillary type,[57] which in turn usually coexist with serous borderline tumors of the typical type (Figure 3a–d).[57–61]

Molecular Genetic Data

Benign serous tumors
Most studies have not detected p53 overexpression or *TP53* mutations (which are common in high-grade serous carcinomas) in serous cystadenomas.[62–65] However, a recent study utilizing microdissection[66] found *TP53* mutations in 6% (2/24) of benign serous tumors. Zheng *et al*[67] and Wolf *et al*[68] found that benign cysts in continuity with serous carcinomas have similar cytogenetic and mutational changes, suggesting that such cysts already have genetic abnormalities that predispose them to malignant transformation. An alternative interpretation is that such benign-appearing epithelium represents morphologic maturation of malignant epithelium and does not indicate a benign precursor lesion. Thomas *et al*[66] noted a surprisingly high frequency of LOH of 73% on at least one chromosome arm in benign serous tumors when 56 microsatellite markers on chromosomes 6, 7, 9, 11 and 17 (chromosomes that exhibit frequent LOH in typical ovarian carcinomas) were analyzed. The specific frequencies of LOH

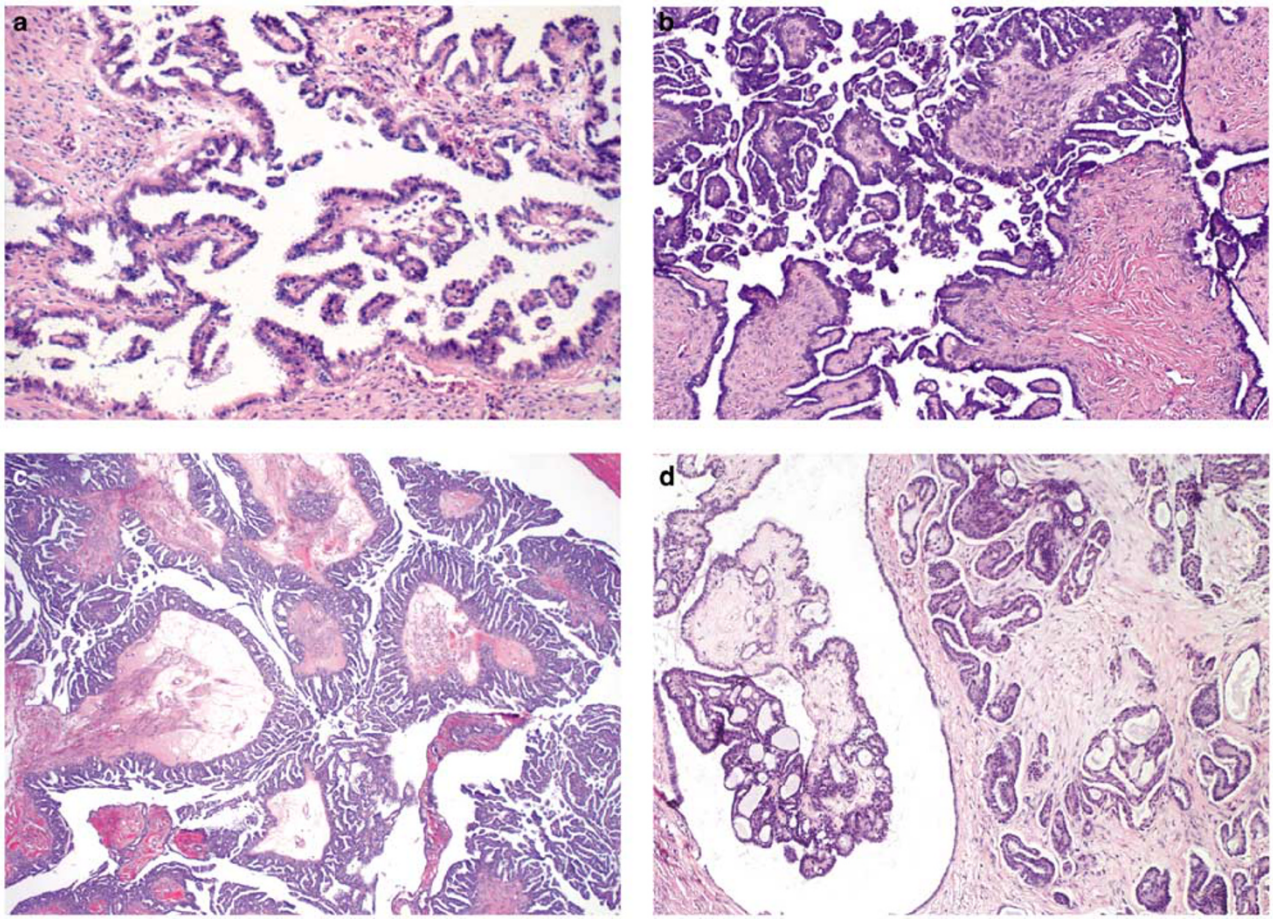

Figure 3 Probable progression of serous borderline tumor to invasive low-grade serous carcinoma. (**a**) Typical serous borderline tumor. (**b**) Typical serous borderline tumor (lower portion of field) coexisting with micropapillary serous borderline tumor. (**c**) Micropapillary serous borderline tumor. (**d**) Micropapillary serous borderline tumor with stromal invasion by low-grade serous carcinoma (right).

were 8, 23, 7, 10 and 7% for chromosomes 6, 7, 9, 11 and 17, respectively. In contrast, Tapper *et al*[69] found significant differences in gene expression between benign and malignant serous ovarian tumors.

Serous borderline tumors compared to high- and low-grade serous carcinoma

Most studies of serous neoplasms have shown different molecular changes in serous cystadenomas, borderline tumors and high-grade carcinomas, indicating that in most cases these tumor types develop via different genetic pathways.[42,70–74] *TP53* mutations and p53 immunohistochemical overexpression are uncommon in borderline serous tumors, whereas approximately 60% of high-grade serous carcinomas have detectable mutations or overexpression of *TP53*.[38–42,44,45] Serous borderline tumors have a higher rate of *KRAS* mutations (27–36%) than high-grade serous carcinomas (0–12%).[40,46,47,75–78] *BRAF* mutations, which are seen in 33–50% of serous borderline tumors of typical or micropapillary type, have not been identified in high-grade serous carcinoma.[74,76,78]

A few studies analyzing and comparing serous borderline tumors and low-grade serous carcinomas have been performed only recently. These studies have found a similar frequency of *KRAS* mutations in approximately one-third of typical or micropapillary serous borderline tumors and invasive low-grade serous carcinomas (invasive micropapillary serous carcinoma), and *BRAF* mutations in an additional similar proportion. Interestingly, *KRAS* and *BRAF* mutations are present only very rarely in the same neoplasm; thus, in aggregate these mutations are present in 60% of serous borderline ovarian tumors and 68% of low-grade serous carcinomas.[74,76,78] In contrast, *BRAF* mutations have not as yet been reported in high-grade conventional serous carcinoma and *KRAS* mutations have been reported only rarely (0–12%).[46,74,76,78]

Studies utilizing other techniques have also demonstrated substantial differences between borderline tumors and/or low-grade serous carcinomas and high-grade serous carcinomas. One study has

demonstrated that the allelic imbalance index gradually increases from typical to micropapillary serous borderline tumor to invasive low-grade serous carcinoma in contrast to the finding of high levels of allelic imbalance in even tiny high-grade ovarian serous carcinomas,[73] (Mok, Bell unpublished data). Comparative genomic hybridization (CGH) studies have also demonstrated differences between low- and high-grade serous carcinomas in that high-grade serous carcinomas showed significantly higher CNA frequencies than low-grade tumors.[79–81] Differences between low- and high-grade tumors were also demonstrated genetically. High-grade carcinomas showed under-representation of 11p and 13q, and over-representation of 8q and 7p, while 12p under-representation and 18p over-representation were present significantly more frequently in well and moderately differentiated tumors.[82]

These findings suggest that invasive low-grade serous carcinomas (or invasive micropapillary serous carcinomas) and high-grade serous carcinomas arise via different pathways, with low-grade carcinomas most probably arising in an adenoma–carcinoma sequence, with a progression from typical to micropapillary borderline tumors to invasive low-grade carcinoma via alteration of the RAS–RAF signaling pathway by mutations in either *KRAS* or *BRAF*.[73–79]

Conclusions

These data indicate that low- and high-grade serous carcinomas, in the great majority of cases, arise via different genetic pathways. Low-grade serous carcinomas most probably arise via an 'adenoma–borderline tumor-carcinoma' progression from typical to micropapillary serous borderline tumor to low-grade serous carcinoma via alteration of the RAS–RAF signaling pathway secondary to mutations in *KRAS* and *BRAF*. As noted in the previous section, high-grade serous carcinomas most probably arise from SEIG in the great majority of cases with *TP53* mutations and *BRCA1* or *BRCA2* dysfunction.

Benign and borderline mucinous tumors as precursor lesions

Morphologic Data

The studies of Scully *et al*[54] and Puls *et al*[55] showed coexisting benign mucinous epithelium in primary ovarian mucinous carcinomas in 74–90% of cases. Several recent detailed studies of invasive primary mucinous ovarian carcinomas of gastrointestional type showed coexisting mucinous borderline tumor in 67–69% of cases (Figure 4a, b).[83,84] The two studies describing endocervical-like mucinous carcinomas reported coexisting Mullerian mucinous or mixed cell type borderline tumors in 75–100% of cases.[85,86] These morphologic findings are highly suggestive that mucinous carcinomas arise from preexisting benign and borderline mucinous tumors.

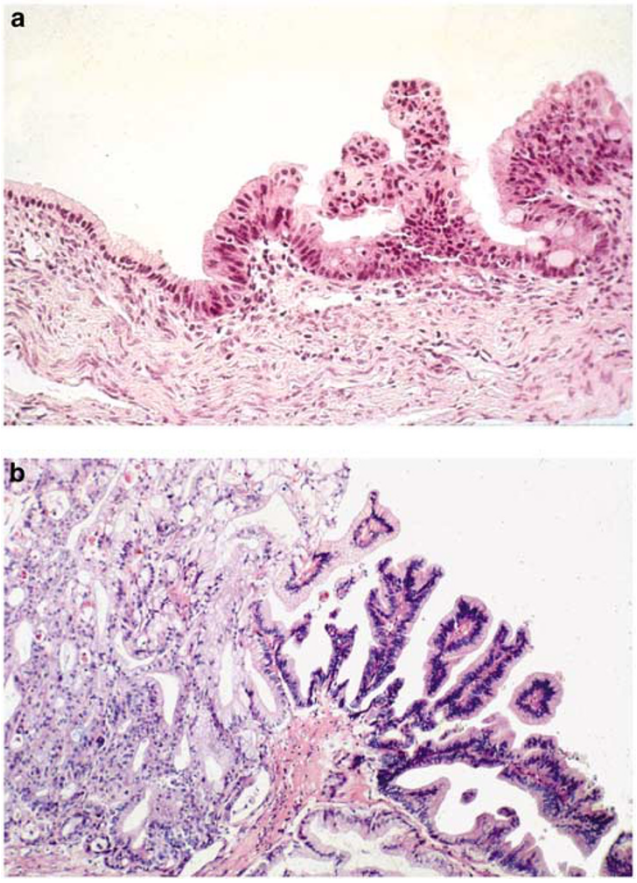

Figure 4 Probable progression of mucinous cystadenoma to mucinous carcinoma. (**a**) Mucinous cystadenoma (left) with a transition to mucinous borderline tumor (right). (**b**) Mucinous borderline tumor (right) in continuity with mucinous carcinoma (left).

Molecular Genetic Data

Greater similarities in mutation rates and overexpression of oncogenes and tumor suppressor genes have been reported in benign, borderline and malignant mucinous tumors than serous tumors. Most studies have shown a high and increasing frequency of *KRAS* mutations in mucinous cystadenomas, borderline tumors and carcinomas, ranging from 33 to 86%.[47,70,72,75,87,88] Several groups[75,87,88] have noted similar *KRAS* mutation patterns in benign, borderline and malignant mucinous areas within the same neoplasm, which suggests that *KRAS* mutation is an early event in mucinous tumorigenesis. It has also been noted that the rate of *TP53* mutations is lower in mucinous borderline tumors than mucinous carcinomas (13 *vs* 40%).[38,70,77] In contrast to serous borderline tumors,

mucinous borderline tumors have not shown *BRAF* mutations.[78,89] These data suggest that ovarian mucinous carcinomas arise via an adenoma–borderline tumor–carcinoma sequence with activation of the RAS–RAF signaling pathway by *KRAS* mutations.[78,89]

Conclusions

The majority of mucinous ovarian carcinomas arise via an adenoma–borderline tumor–carcinoma sequence with activation of the RAS–RAF signaling pathway secondary to *KRAS* mutations.

Endometriosis as a precursor lesion

Endometriosis, particularly cystic ovarian endometriosis, has been shown to be related to an increased risk of developing ovarian endometrioid and clear cell carcinoma in several epidemiologic studies.[90–94] Endometriosis-associated ovarian carcinomas are more frequently low-grade and low stage and have a more favorable prognosis than carcinomas not associated with endometriosis.[90] It has also been noted in one clinical study that 20% of endometrioid and 88% of clear cell carcinomas were preceeded by an ultrasonographically detected endometriotic cyst.[53]

Morphologic Data

Many types of neoplasms have been documented to arise in endometriosis morphologically, particularly endometrioid and clear cell carcinomas, and rarely, other types of surface epithelial carcinomas and even mesenchymal tumors such as endometrioid stromal sarcoma.[1,95–98] Endometrioid carcinoma has been reported to originate directly from endometriotic tissue in up to 24% of the cases in various series (Figure 5). Ipsilateral ovarian endometriosis has been detected in 11–31%, ovarian endometriosis of unspecified laterality in 9–20%, and pelvic endometriosis in 11–28% of cases of ovarian endometrioid carcinoma.[96,99–107] Well-differentiated or low-grade endometrioid ovarian carcinomas are associated with an especially high frequency of associated endometriosis (63%) or endometrioid adenofibromas (47%).[108]

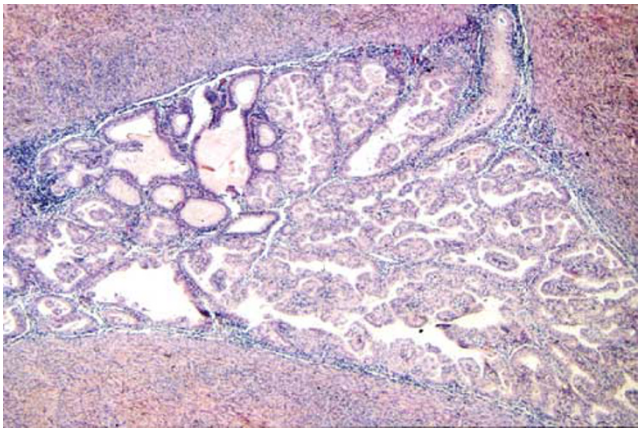

Figure 5 Endometrioid adenocarcinoma arising in endometriosis.

Russell[104] reported a 47% frequency of pelvic endometriosis in 30 cases of clear cell carcinoma of the ovary, and Aure *et al*[99] demonstrated a 24% frequency of ovarian endometriosis in 59 cases of the same type of tumor; both of those figures were surprisingly higher than the figures given by the same authors for the association of endometriosis with endometrioid carcinoma (28 and 9%, respectively). Although isolated examples of serous and mucinous carcinoma have been reported to originate in endometriosis, the association of these tumors in general with that disorder is not significant. Russell[104] found only a 2.5% frequency of pelvic endometriosis in 163 cases of serous carcinoma and a 6% frequency in 17 cases of mucinous carcinoma; and Aure *et al*[99] detected no ovarian endometriosis in 283 cases of serous carcinoma and only an 0.8% frequency, in 203 cases of mucinous carcinoma. Rutgers and Scully,[109] however, reported the finding of ovarian endometriosis in 30% of cases of mucinous borderline tumors of endocervical-like (Müllerian) type, which accounted for 15% of the mucinous borderline tumors in their series; in contrast, there was no significant association of ovarian endometriosis and the more common mucinous borderline tumor of intestinal type. Borderline tumors of mixed Müllerian epithelial cell types also have a frequent association with ovarian endometriosis.[110]

Hyperplasia in endometrosis as a precursor lesion

Because carcinoma of the endometrium often arises on a background of hyperplasia with cytologic and architectural atypicality, one might expect that a proportion of carcinomas arising in endometriosis would have similar precursors. Hyperplasia with varying degrees of architectural and cytologic atypia resembling that seen in the endometrium occurs in 2–7% of cases of ovarian endometriosis or ovarian endometriotic cysts without coexisting carcinoma and in 67–100% of endometriosis associated with coexisting carcinoma (Figure 6).[1,95,111–116] The prognostic significance of such changes is unclear because follow-up data have been limited to only a small number of women. Seidman[113] reported follow-up information on 13 women with complex atypical hyperplasia and seven women with 'early carcinoma', arising in ovarian endometriosis. All the women with complex atypical hyperplasia were followed for a mean of 8.5 years and survived without the development of carcinoma, however, most of the lesions were completely excised. Similarly, the four women with 'adenomatous hyperplasia' in ovarian endometriosis in the report

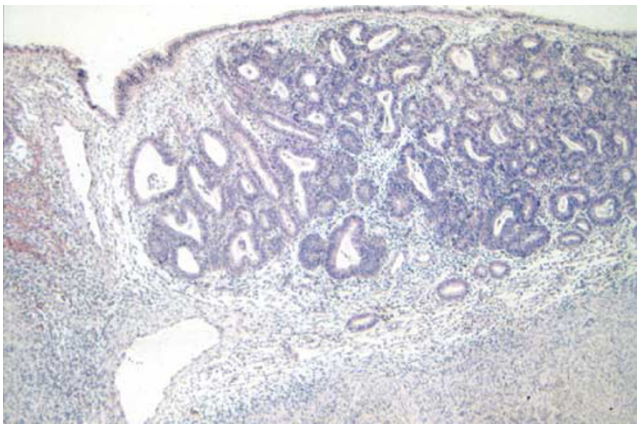

Figure 6 Atypical endometrial-like hyperplasia arising in an ovarian endometriotic cyst.

of Czernobilsky and Morris[111] were followed for 3–5 years without recurrence or the development of carcinoma, although 10 of their 11 patients with 'adenomatous hyperplasia' or severe atypia of cyst lining epithelium were treated by oophorectomy, presumably excising completely the atypical lesion. We have observed one case, however, in which the left ovary of a 55-year-old woman, which contained severely atypical endometriotic tissue, was dissected away from the pelvic wall; she received estrogen therapy for 3 years, and subsequently returned with an endometrioid adenocarcinoma that had arisen in the area where ovarian tissue had presumably been left behind. The outcome in this case is interesting in light of epidemiologic studies that have demonstrated a significant increase in the incidence of endometrioid carcinoma of the ovary in women on estrogen therapy[117,118] and in view of the number of reported cases of carcinoma developing in endometriosis in women receiving replacement therapy with estrogens unopposed by progesterone.[98]

Early carcinoma in endometriosis

Early carcinoma developing in an endometriotic cyst has also been specifically described in detail in only a few studies. Seidman[113] reported 11 examples of ovarian endometriosis containing foci that fulfilled the Kurman and Norris[119] criteria for endometrioid carcinoma of the endometrium. Seven women were followed for a mean of 8.6 years; in 'most' of the cases the lesions had been completely excised. Nevertheless, three of them had an adverse outcome. One had a poorly differentiated adenocarcinoma of the anterior vaginal wall 8.1 years later, one had an endocervical-like mucinous borderline tumor in the contralateral ovary 7 years later and the third patient was found to have 'early carcinoma' in foci of endometriosis in the contralateral ovary and the omentum 3 months after the initial diagnosis. Thus, although the prognosis of an individual completely excised example of early carcinoma arising in endometriosis is excellent, carcinoma may arise in residual foci of endometriosis at the same or other sites. For this reason, close follow-up of women with early carcinoma arising in endometriosis is warranted.

Atypical cyst lining cells as a precursor lesion

Two additional atypical lesions encountered in endometriosis—but rarely, if ever, in the endometrium—are characterized by cells showing varying degrees of atypia lining endometriotic cysts without endometrial glandular hyperplasia. In the first type, which comprises most of these cases, the lining epithelium is composed of a single layer of large polygonal cells with abundant eosinophilic cytoplasm and large hyperchromatic, often smudged nuclei. Acute inflammation is frequently present (Figure 7).[113,115,120,121] In the second less common type, large polygonal cells and hobnail cells are present with epithelial stratification and tufting. These cells may have eosinophilic, clear, or vacuolated cytoplasm and may contain intracytoplasmic mucin; their nuclei are usually markedly hyperchromatic and pleomorphic. This type of epithelium may also be associated with acute inflammation[111,113,114,120,122,123] and with mucinous endocervical-like (Mullerian) borderline tumors.[109,121] Seidman[113] obtained follow-up on 20 of 37 women with these two types of cyst lining atypias and found that the patients were all alive without the development of carcinoma after a mean of 8.6 years; 'most' of the lesions, however, were completely excised. Czernobilsky and Morris[111] reported that seven women with the second type of lining atypia were followed for 3–5 years without the development of carcinoma; again, most of the lesions were completely excised. Based on this data, Seidman[113] concluded that endometriotic cyst lining atypia was probably reactive or degenerative. In contrast, Moll *et al*[122] described a case in which a resected endometriotic cyst was lined by stratified, severely

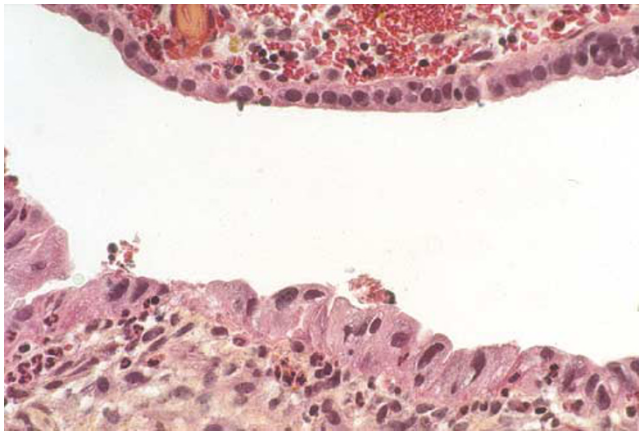

Figure 7 Endometriotic cyst lined by atypical cells with eosinophilic cytoplasm.

atypical hobnail cells; 3 years later, the patient had a clear cell carcinoma in the residual ovarian tissue; these authors suggested that such lining changes are preneoplastic. A single study of ploidy of atypical cyst lining cells has been performed. The investigators noted that all of the cases with 'mild' atypia and no stratification were diploid, but that three of six cases with 'severe' atypia, stratification and tufting, were aneuploid, suggesting a neoplastic potential.[120]

Molecular Genetic Data

Endometriosis

A small number of studies have analyzed genetic alterations in endometriosis unassociated with carcinomas.[124–131] They have shown that most benign-appearing ovarian endometriotic cysts are monoclonal.[124,125,129,130,131] Jiang *et al*[124,132] have shown loss of heterozygosity at several loci on chromosomes 9q, 11q and 22q in less than 20% of ovarian endometriotic cysts and Sato *et al*[126] found a higher frequency of LOH of 56% at 10q23.3. *PTEN* mutations have been noted in 21% of endometriotic cysts.[126] Several studies have failed to demonstrate either mutations or overexpression of p53[127,128,133] or *KRAS* mutations[128] in benign endometriotic cysts.

Endometrioid carcinoma

Low-grade ovarian endometrioid adenocarcinomas have been shown to have different genetic alterations than other histologic subtypes.[126–143] Mutations of *CTNNB1*, the gene that encodes β-catenin have been reported in 16–54% of cases.[134–140] Tumors associated with such mutations are more frequently low-grade and stage and have a favorable prognosis.[134,135,139] *PTEN*, which is frequently mutated in endometrioid carcinomas of the endometrium is mutated in approximately 20% of ovarian endometrioid carcinomas.[126,128] *TP53* mutations or overexpression or both have been reported in 42–63% of ovarian endometrioid adenocarcinomas.[127,128] Microsatellite instability has been reported in 12–19% of ovarian endometrioid adenocarcinomas.[140,141]

A small number of studies have examined ovarian endometrioid adenocarcinomas and adjacent synchronous endometriosis for common genetic events. Jiang *et al*[132] have demonstrated that endometriosis and adjacent endometrioid carcinoma share common genetic events such as loss of heterozygosity at the same loci involving the same allele, and have the same pattern of X chromosome inactivation (albeit in only two of two cases examined). Similarly, several groups of investigators have documented identical *PTEN* mutations and LOH patterns at 10q23 in endometriotic cysts or endometriosis coexisting with endometrioid carcinomas.[126,142] These findings are consistent with the morphologic observations of endometrioid carcinoma arising in endometriosis with mutations of *PTEN* and *CTNNB1*.

In contrast, high-grade or poorly differentiated endometrioid carcinomas have demonstrated similar genetic alterations to high-grade serous carcinoma.[49]

Clear cell carcinomas

Because of their rarity, much less data are available regarding the genetic alterations in clear cell adenocarcinomas. Recent molecular genetic studies have shown significantly lower *TP53* mutation rates and LOH rates at 17p13[143] in clear cell carcinomas than in other types of ovarian carcinomas and several studies have demonstrated that gene expression profiles may distinguish clear cell carcinoma from other histologic subtypes.[144,145] One study has shown identical LOH patterns at 10q23 and *PTEN* mutations in endometriotic cysts adjacent to clear cell carcinomas.[126] These findings suggest that some clear cell tumors arise from ovarian and pelvic endometriosis.

Conclusions

At least a subset of low-grade endometrioid adenocarcinomas arise from ovarian endometriosis with mutations of *CTNNB1* (β-catenin) and *PTEN*, probably progressing through endometrial-like hyperplasia as an intermediate step. High-grade endometrioid carcinomas probably arise via a different pathway involving *TP53* mutations.

Summary

The morphologic and molecular genetic data support different pathways of development for each of the major histologic types of ovarian carcinoma:

1. Low- and high-grade serous carcinoma most probably arise via different pathways, the former progressing along an adenoma–borderline tumor–carcinoma sequence involving mutations in *KRAS* and *BRAF* and the latter from alterations in surface epithelial inclusion glands involving mutations of *TP53* and dysfunction of *BRCA1* and/or *BRCA2*.
2. Mucinous carcinomas most probably arise via an adenoma–borderline tumor–carcinoma sequence with mutations of *KRAS*.
3. Endometrioid carcinomas arise from endometriosis with mutations of *CTNNB1* (the gene encoding β-catenin) and *PTEN.*
4. The genetic alterations in clear cell carcinoma are the least investigated but also support an origin from endometriosis.

The data also suggest that most lowgrade, relatively indolent ovarian carcinomas of serous, mucinous and endometrioid type arise from pre-existing

cystadenomas or endometriosis, whereas most high-grade serous carcinomas arise without an easily identifiable precursor lesion.

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