Endometriosis-associated ovarian neoplasia

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Summary

This article reviews the most relevant pathological and molecular features of ovarian tumours that are associated with endometriosis. Endometriosis is a common condition, affecting 5–15% of all women, and it has been estimated that 0.5–1% of cases are complicated by neoplasia. The most common malignant tumours in this setting are endometrioid adenocarcinoma and clear cell adenocarcinoma, each accounting for approximately 10% of ovarian carcinomas in Western countries. A minority of cases are associated with Lynch syndrome. These carcinomas are often confined to the ovaries at presentation in which case they have relatively favourable outcomes. However, high-stage tumours, particularly clear cell carcinomas, generally have a poor prognosis and this partly reflects relative resistance to current treatment. Histological diagnosis is straightforward in the majority of cases but some variants, for example endometrioid carcinomas with sex cord-like appearances or oxyphil cells, may create diagnostic difficulty. Similarly, clear cell carcinomas can show a range of architectural and cytological patterns that overlap with other tumours, both primary and metastatic, involving the ovaries. Endometriosis-associated borderline tumours are less common, and they often show mixed patterns of differentiation (seromucinous tumours). Atypical endometriosis may represent an intermediate step in neoplastic progression and some of these lesions demonstrate immunohistological and molecular alterations similar to those observed in endometriosis-related tumours. ARID1A mutations are relatively common in all of these tumours, but each has additional characteristic molecular alterations which are likely to be of increasing clinical relevance as targeted therapies are developed. Less is known of the pathogenesis of rarer endometriosis-associated ovarian tumours including endometrioid stromal sarcoma, mesodermal (Müllerian) adenosarcoma, and carcinosarcoma. This article also briefly reviews the issue of synchronous endometrioid carcinomas of the endometrium and the ovary, including the most recent developments on pathogenesis.

Key words: Endometriosis; tumour; endometrioid; clear cell; molecular; immunohistochemistry.

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INTRODUCTION

Neoplasia is a rare but significant complication of endometriosis, occurring in approximately 0.5–1% of cases.1 Endometriosis-associated neoplasms (EANs) usually occur in the ovaries, often arise in younger patients, and encompass a range of tumours most of which are clinically malignant.2 However, many of these tumours are confined to the ovaries at the time of diagnosis (stage I) and the overall prognosis is favourable. The most common EANs are endometrioid ovarian carcinoma (EOC) and clear cell carcinoma (CCC), each accounting for approximately 10% of all ovarian adenocarcinomas in Western countries.3 Borderline endometrioid and clear cell tumours are also associated with endometriosis but occur much less commonly. The third major category of EANs are those epithelial neoplasms which demonstrate Müllerian mucinous (endocervical-like) or mixed differentiation, also known as Müllerian mucinous/mixed epithelial (MM/ME) or seromucinous tumours.2 EANs demonstrate distinctive and partly overlapping molecular alterations which may lead to the development of specific targeted therapies for high-stage and recurrent tumours. Synchronous endometrioid neoplasms involving the ovary and endometrium as well as extraterine endometrial (endometrioid) stromal neoplasms, mesodermal (Müllerian) adenosarcomas and carcinosarcomas may also be associated with endometriosis. Although not discussed further here, it should be noted that these tumours also rarely arise in extraovarian sites such as the pelvic peritoneum or bowel wall, often in association with endometriosis.

ENDOMETRIOSIS

Endometriosis affects 5–15% of women in the reproductive age range and is defined by the presence of endometrial-like tissue (epithelial and/or stromal elements) outside the uterine corpus.4–6 The most commonly affected sites are the peritoneum and the pelvic organs, particularly the ovaries. While the pathogenesis of endometriosis remains disputed, and is probably multifactorial, the likeliest mechanism in most cases is transtubal dissemination of endometrial tissue into the peritoneal cavity with subsequent implantation and growth in susceptible women.7 In support of this mechanism is the finding that the eutopic endometrium of patients with endometriosis shows functional alterations such as altered cell cycle regulation and an increased capacity to implant and induce angiogenesis.8,9 It is noteworthy that some endometriotic lesions, particularly those involving the ovary,
appear to be monoclonal, a feature usually considered a hallmark of neoplasia. Moreover, a proportion of endometriotic lesions demonstrate immunophenotypic and/or molecular alterations that also characterise EANs, and these are found more commonly when endometriosis is associated with an ovarian tumour. However, it has been shown recently that deep infiltrating endometriosis (typically extra-ovarian), which is rarely associated with the development of EANs, also demonstrates cancer-associated somatic mutations in a significant proportion of cases.

The morphological features of endometriosis are well-documented and most cases present no diagnostic difficulty. However, histological variants such as those in which the epithelial and/or stromal components demonstrate metaplastic or reactive changes may be more challenging, and endometriotic lesions that mainly or entirely comprise stromal elements are probably under-recognised. As with the eutopic endometrium, the appearances of endometriosis can be significantly influenced by treatment including hormonal therapy. One variant, polypoid endometriosis, is worthy of specific comment since this may be misinterpreted clinically and histologically as a true neoplasm. Some such cases reflect microanatomical location of endometriosis close to a mucosal surface or cyst lining since this facilitates a polypoid growth but other cases more closely resemble polyps developing in the endometrium.

A further important variant is atypical endometriosis. This term has been applied to two processes, both of which occur most frequently in the ovary. The first corresponds to architecturally complex and cytologically atypical proliferative lesions that resemble atypical hyperplasia/intraepithelial neoplasia arising in the endometrium. These lesions sometimes coexist with EOC, further emphasising a neoplastic continuum analogous to that observed in the endometrium. Second, and more common, are alterations in the lining epithelium of endometriotic cysts characterised by varying degrees of cellular stratification and disorganisation, inflammation and cytological atypia, often accompanied by ‘metaplastic’ alterations (ciliated, eosinophilic, hobnail, squamous and/or clear cell) (Fig. 2). It is often less clear whether such changes are reactive or degenerative in nature, or whether they represent a step in the neoplastic progression of endometriosis towards an EAN. While most lesions are clinically benign, possibly representing analogous changes to those sometimes observed in the endometrium, and there may be anatomical continuity between atypical endometriosis and an ovarian neoplasm, usually EOC or CCC (Fig. 3). Some EANs include cystic elements where the lining epithelium is cytologically malignant and in such cases it can be difficult to determine whether this represents cystic change within an overtly malignant tumour or ‘in situ’ carcinoma developing within an endometriotic cyst. From a pathogenetic perspective, it has been proposed that the combination of an inflammatory milieu, hyper-oestrogenic state and high iron levels may potentiate carcinogenesis within endometriotic cysts.

ENDOMETRIOSIS-ASSOCIATED OVARIAN NEOPLASIA

Endometrioid adenocarcinoma

EOC arises most commonly in the perimenopausal or post-menopausal age group, in the fifth and sixth decades of life, with a mean age of 56 years. It has been suggested that a high proportion of EOCs arise from endometriotic cysts, since ipsilateral ovarian and pelvic endometriosis is seen in up to 42% of these patients. EOC is associated in 15–20% of cases with endometrioid carcinoma of the endometrium. The pathogenesis of this association and the possibility that some ovarian tumours represent metastasis of the uterine neoplasms is discussed below. Patients with tumours associated with endometriosis are 5–10 years younger on average than those not associated with endometriosis. EOC may be asymptomatic, or present as a pelvic mass, with or without pain, but presentation is typically non-specific. Serum CA 125 is elevated in over 75% of patients, and some have endocrine-related symptoms/signs secondary to steroid hormone production by peritumoural or intratumoural luteinised ovarian stroma. Like other EANs, most EOCs are low-stage at the time of diagnosis, being confined to the ovary and adjacent pelvic structures. Approximately 20% of tumours are bilateral.

Grossly, EOC are typically large with mean size of 15–20 cm. The external surface is usually smooth while the cut surface usually shows friable solid soft masses associated with haemorrhage (Fig. 4). Cystic areas may be seen, and may be filled with mucoid material. Remnants of an endometriotic cyst may be identified at the periphery of the mass. Occasionally, the tumour presents as a mural nodule in the
setting of an endometrioid cyst. Predominantly solid, adenofibromatous tumours may also occur.

Microscopically, EOC closely resembles its endometrial counterpart and may show all the morphological variants seen in the uterus. It encompasses a spectrum of neoplasms with variable degrees of glandular differentiation, although the majority are grade 1 or 2 tumours demonstrating a predominant glandular architectural pattern. This feature is more frequently seen in tumours associated with endometriosis. In well-differentiated EOC the glands are typically small but some can be large and cystically dilated, and may be round to oval or show irregular, angulated profiles. The neoplastic glands are typically lined by stratified, eosinophilic epithelial cells with well-defined luminal borders, and often these show smooth, rounded contours (Fig. 5). Marked architectural complexity with fusion of the glands and cribriforming are also seen, and there may be focal or confluent necrosis with intraluminal necrotic debris. Some neoplasms demonstrate a microglandular pattern. Mitoses typically range up to 5 per 10 high-power fields.

Squamous differentiation is frequent and a useful clue to endometrioid differentiation but, as in the endometrium, the terms adenoacanthoma or adenosquamous carcinoma are not recommended (Fig. 5). The squamous component can have variable histological appearances, but low-grade EOC typically shows rounded intraluminal aggregates of cytologically bland squamous cells lacking keratinisation ('morules'). Occasionally, the squamous elements may show abortive differentiation with spindle cell morphology often merging imperceptibly with more obvious squamous or glandular elements. Low-grade EOCs with squamous differentiation can show an adenofibromatous growth, with glands embedded in a prominent fibromatous stroma. Keratin production can be present and this may be associated with a histiocytic and giant cell reaction. Such foreign body granulomatous reactions can also occur in the peritoneum, but in the absence of viable tumour cells these do not affect staging or prognosis. The rare high-grade EOCs can show areas of frankly malignant squamous differentiation that may be admixed or sharply separated from the glandular elements. The squamous tumour nests may show central necrosis with cellular debris and pyknotic nuclei.

The presence of necrosis in EOC may raise the differential diagnosis of metastatic colorectal carcinoma, particularly
since the latter can show a pseudo-endometrioid appearance. However, necrosis in colorectal carcinoma is typically more extensive and often demonstrates a characteristic intraluminal pattern (often referred to as ‘dirty necrosis’), frequent disposition of glands in a ring at the edge of the necrotic material (garland pattern), and the presence of focal segmental necrosis of the glandular neoplastic elements.29 The presence of squamous differentiation, an adenofibromatous background, or endometriosis, all of which favour EOC, may be helpful in this differential diagnosis.

Secretory change, characterised by the presence of subnuclear or supranuclear cytoplasmic vacuoles in the tumour cells, is focally present in a proportion of low-grade EOCs. This finding should not be misinterpreted as CCC which has distinctive architectural features as discussed below. It is important to note that EOC may coexist with CCC, since both components may arise from pre-existing endometriosis. A villous papillary pattern may also be seen, and typically the papillae demonstrate connective tissue cores. There may be mucin production, and the neoplastic gland lumens may contain extracellular mucin or sometimes colloid-like material. Additional microscopic features that may be seen in EOC include oxyphil, glycogen-rich, ciliated, or balloon-like cells.30 Transitional and adenoid cystic-like patterns may also be seen raising the possibility of an extra-ovarian primary,31 but as with other histological variants the diagnosis can usually be confirmed based upon the clinical and immunohistochemical findings, and the presence of more characteristic EOC areas on thorough tumour sampling. EOCs may also exhibit a prominent spindle-cell pattern.32

The stroma in EOC usually has a non-specific appearance but there may be a desmoplastic reaction in widely infiltrative tumours. EANs, including EOCs, may show condensation of ovarian stromal cells, often with luteinisation, at the tumour periphery or admixed with the neoplastic component.33 This is typically seen in low-grade tumours and may be associated with production of steroid hormones which, on occasion, may be associated with clinical endocrine manifestations. The elaboration of steroid hormones by stromal cells could

Fig. 3 (A) Intracystic low-grade endometrioid adenocarcinoma associated with atypical endometriosis. The cyst lining shows varying degrees of cellular stratification. (B) Higher magnification of cyst lining showing transition from normal epithelium (upper right) to multilayered atypical epithelium with areas of squamous differentiation (left).

Fig. 4 Gross appearance of an ovarian endometrioid carcinoma. (A) The capsular surface is smooth. (B) The cut surface shows a tumour mass adjacent to an endometriotic cyst.
also contribute towards tumour growth in hormone receptor positive cases.34

As with endometrioid adenocarcinoma of the endometrium, grading of EOC is based upon the proportion of solid (non-glandular) tumour, excluding areas of squamous differentiation; grade 1 tumours have <5% solid elements, grade 2 tumours show 5–50% solid growth, and grade 3 tumours show >50% solid architecture. High-grade EOC have a predominant poorly differentiated appearance with marked pleomorphism and high mitotic index. These tumours should be distinguished from high-grade serous carcinoma (HGSC) (see below). In the past, a significant proportion of HGSCs were misdiagnosed as high-grade endometrioid or mixed serous-endometrioid carcinomas because of the presence of a ‘glandular’ growth. However, numerous pathological and molecular studies have demonstrated that the vast majority of these tumours are in fact HGSCs.35,36 Interestingly, a pseudo-endometrioid pattern in HGSC is one of the histological features that characterises tumours with germline or somatic BRCA-1 mutations.37

Occasionally, a solid growth or microglandular pattern with small rosette-like glands resembling Call–Exner bodies and monotonous cells including the presence of nuclear grooves in EOC may suggest the diagnosis of adult granulosa cell tumour.38–40 In other cases, a trabecular or tubular pattern may simulate a Sertoli cell tumour. If the stroma shows condensation or luteinisation, this differential diagnosis may be challenging. Helpful features include the finding of mucin within the microglands of EOC as well as focal squamous differentiation, adenofibromatous background, and the presence of ovarian endometriosis or obvious endometrioid elements. Moreover, EOC generally show more overtly atypical cytological features. Age, presence of hormonal symptoms, bilaterality and pattern of metastatic spread are all important features in this differential diagnosis.

It is also important to remember that EOC may be associated with a somatic germ cell component, typically in the form of a yolk sac tumour (YST).41,42 The latter may exhibit a typical reticular-microcystic pattern with or without Shiller–Duval bodies, but also enteric differentiation with the glands showing subnuclear vacuolation.

Different patterns of invasion (expansile and destructive) have been reported in EOC.43 Expansile invasion is characterised by a confluent glandular growth, and has been associated with good prognosis in some series. Destructive invasion is characterised by obvious stromal invasion with abnormal glands and small nests of tumour cells infiltrating the stroma, inducing a marked desmoplastic reaction with inflammatory response.

**Immunohistochemistry**

Common immunohistochemical and molecular alterations in EANs are summarised in Table 1. In contrast to serous carcinoma, EOC is usually negative for WT1, only focally (patchily) positive for p16, and usually diffusely positive for progesterone receptor (PR) and vimentin, particularly in low-grade tumours.44,45 PAX8 staining is typically positive and EOC frequently shows nuclear β-catenin expression. Immunostaining for p53 protein usually shows a wild-type pattern but high-grade EOC may show mutation pattern staining.46 Alpha-inhibin, steroidogenic factor-1, and FOXL-2 may be useful in the differential diagnosis with sex cord-stromal tumours.47–49 An immunohistochemical panel including PR, vimentin, cytokeratin (CK)20, CK7, CDX2, and SATB-2 generally will differentiate EOC from metastatic colorectal tumours.50

An immunohistochemical panel including PR, vimentin, cytokeratin (CK)20, CK7, CDX2, and SATB-2 generally will differentiate EOC from metastatic colorectal carcinoma. If the pathological and immunohistochemical features are not typical, gene-expression microarray analysis can be helpful.50 Immunohistochemistry for germ cell tumour markers including SALL4, CDX2, villin and glypican 3 can
be used to confirm the presence of a somatic YST component.51

Molecular findings

Molecular alterations in EOC are generally similar to those of the uterine counterpart, although with some variation in the expression profile.52–58 The main molecular alterations are: microsatellite instability (MI), and mutations in PTEN, KRAS, PIK3CA, ARID1A, and CTNNB1 (β-catenin) genes. MI has been demonstrated in 12–20% of EOC.55 Patients with tumours from Lynch syndrome/hereditary non-polyposis colon cancer kindreds have an inherited germline mutation in the DNA mismatch repair (MMR) protein genes MLH-1, MSH-2, MSH-6 or PMS-2 (‘first hit’), but EOC develops only after the deletion or mutation of the second corresponding allele (‘second hit’). In sporadic tumours, MLH-1 inactivation by promoter hypermethylation is the main cause of MMR protein deficiency.

The tumour suppressor gene PTEN, located on chromosome 10q23.3, is frequently abnormal in EOC with somatic mutations occurring in 20% cases. In agreement with Knudson’s two-hit proposal, loss of heterozygosity (LOH) at 10q23 frequently coexists with somatic PTEN mutations, and in combination this leads to activation of the PI3K/AKT pathway which plays a key role in the regulation of cellular homeostasis. Activated AKT modulates the expression of several genes involved in cell cycle progression and in the suppression of apoptosis. Mutations in PIK3CA may also contribute to the alteration of the PI3K/AKT signalling pathway in EOC. PI3K is a heterodimeric enzyme consisting of a catalytic subunit (p110) and a regulatory subunit (p85), and the PIK3CA gene, located on chromosome 3p26.32, codes for the p110 catalytic subunit of PI3K.

CTNNB1, the gene encoding β-catenin, maps to 3p21. β-catenin appears to be important in the functional activities of both APC and E-cadherin. β-catenin is a component of the E-cadherin-catenin complex which is critical in cellular differentiation and the maintenance of normal tissue architecture. β-catenin is also important in signal transduction, with increased cytoplasmic and nuclear levels inducing transcriptional activation through the LEP/Tcf pathway. The APC protein down-regulates β-catenin by cooperating with glycogen synthase kinase 3β (GSK-3β), inducing phosphorylation of the serine-threonine residues coded in exon 3 of CTNNB1, and its degradation through the ubiquitin-proteasome pathway. Mutations in exon 3 of CTNNB1 occur in 38–50% of EOC and result in stabilisation of the β-catenin protein, its cytoplasmic and nuclear accumulation, and subsequent participation in signal transduction and transcriptional activation through the formation of complexes with DNA binding proteins. Mutations in ARID1A, and loss of expression of the corresponding protein BAF250a, occur in approximately 30% of EOC but these are more frequent in CCC and are discussed further below.56–58

Clear cell adenocarcinoma

This malignant epithelial neoplasm accounts for approximately 10% of ovarian carcinomas in Western countries but it is relatively common in Japan where it represents up to 25%
of ovarian carcinomas.57,58 As with most ovarian tumours, CCC usually presents with symptoms of a pelvic mass including pain, obstruction or abdominal swelling, and often there is history of previous or concurrent endometriosis. Occasional patients present with complications related to hypercalcaemia or thromboembolism.59,60 The majority of tumours are confined to the ovary at the time of diagnosis, and although traditionally considered a high-grade malignancy, patients with stage IA CCC have a relatively favourable outcome with 80–90% 5-year survival.61,62 However, the prognosis is guarded when there is positive peritoneal cytology, ovarian capsule rupture or capsular tumour involvement (stage IC). Patients with high-stage CCC generally have very poor outcomes, even worse than similar stage HGSC, and this may partly reflect tumour resistance to standard platinum-based chemotherapy.63,64 However, patients with MMR protein-deficient and/or Lynch syndrome-associated high-stage CCC may have unexpectedly long survival,65 and this may reflect tumour immunogenicity with the potential for immunomodulatory therapy in such cases.66

Most CCC are unilateral, typically 10–15 cm in diameter, and they often exhibit both solid and cystic components in variable proportion, the latter sometimes comprising a background adenofibroma (Fig. 6). In cystic tumours, the carcinoma may present as solitary or multifocal intraluminal papillary or nodular areas within an otherwise smooth cyst lining. As with EOC, the tumour may also form a distinct mass in continuity with an endometriotic cyst, and endometriosis may be identified in the contralateral ovary or in other pelvic/peritoneal sites.67

Microscopically, CCC characteristically shows tubulocystic, solid or papillary growth patterns and these are often admixed in individual cases (Fig. 7).68 Stromal hyalinisation is often present, particularly within papillary areas, and this can be a useful pointer to the diagnosis in cytological preparations and in intra-operative assessment.69,70 Nuclei are typically hyperchromatic and sometimes show apical distribution imparting a ‘hobnail’ appearance. Cytoplasmic clearing is present at least focally in most cases but cytoplasmic eosinophilia is not uncommon. Occasional cases demonstrate intracytoplasmic eosinophilic inclusions, psammoma bodies, colloid-like luminal secretions or signet ring-like cells with eccentric nuclei and intracytoplasmic vacuoles (Fig. 7). Mitotic activity is variable in CCC but is often less prominent than in other high-grade carcinomas. Cellular stratification and marked nuclear pleomorphism are not typical of CCC and should raise the possibility of an alternate diagnosis such as HGSC with clear cell change.67

Although the histological diagnosis of CCC is usually straightforward, the range of architectural and cytological patterns described above potentially overlap with many other tumours, both primary and metastatic, involving the ovaries and occasionally this creates diagnostic difficulty, particularly in small biopsy specimens or during intra-operative assessment. For example, CCCs with a papillary pattern may be mistaken for borderline serous tumours, the presence of signet ring-like cells may raise concern for metastatic carcinoma, and cases associated with a prominent lymphoplasmacytic infiltrate can mimic dysgerminoma (Fig. 8).71,72 Interestingly, prominent immune infiltrates and myxo-hyaline stromal change in CCCs appear to be mutually exclusive, and the former has been associated with high-stage tumour presentation.71 The presence of ‘abrupt’ zonal tumour necrosis, with no associated stromal reaction, is also characteristic of CCC and sometimes this can be a useful diagnostic pointer on frozen section examination (Fig. 8).72

It is stressed that cytoplasmic clearing is not present in all CCCs and that this is a non-specific finding since it can be seen in other primary ovarian neoplasms as well as many tumours metastatic to the ovary.74,75 A useful supportive clue is the presence of endometriosis (including atypical endometriosis) which has been recorded in 20–40% of CCCs in...
better outcomes. These tumours generally present in gestational tissues and other non-tumour sites. It is currently controversial whether the histological pattern of CCC has prognostic significance, and in most centres these tumours are considered high-grade by definition and are not further sub-classified. However, some studies have suggested that CCCs with an adenofibromatous component have better outcomes. These tumours generally present in older women, are less often associated with endometriosis, less frequently show papillary morphology and have a lower incidence of ARID1A mutations, together suggesting that they may be distinct biologically. Conversely, a multi-centre study of 122 CCCs by Veras et al. found that CCCs associated with an adenofibroma had a poorer prognosis and this was related to more frequent high-stage presentation. Immunochemistry

As noted above, many types of tumour can show clear cell appearances and immunohistochemistry can be useful when the diagnosis of CCC is uncertain. In practice, the selected antibody panel should be tailored according to the particular histological differential diagnosis but in the first instance the demonstration of a PAX8 and CK7 positive/CK20 negative immunoprofile is useful general support of a primary gynaecological neoplasm. However, none of these markers is specific for CCC and it should be noted that unlike most other EANs, CCCs are usually negative, or only focally positive, for hormone receptors (Fig. 9). In the context of a confirmed primary gynaecological neoplasm, expression of hepatocyte nuclear factor 1β (HNF1β), napsin A and z-methylacyl CoA racemase (AMACR) is supportive of CCC (Fig. 9). However, HNF1β can be expressed by non-neoplastic and endometriotic epithelium, as well as some endometrioid, serous and metastatic carcinomas, while AMACR, although relatively specific, has only moderate sensitivity for CCC. At present, napsin A appears the most sensitive and specific marker for CCC but staining is often focal and this could be problematic in small biopsy or cytology samples.

Immunohistochemistry is useful in distinguishing tubo-ovarian HGSC with clear cell change (usually diffusely WT1 positive and mutation-pattern p53 staining, Fig. 9) from CCC which shows the opposite staining pattern. As discussed below, approximately 50% of CCCs show loss of ARID1A/BAF250a expression (Fig. 9). Occasionally, YST/primitive endodermal tumour enters the differential diagnosis of CCC, particularly in younger women. While a SALL4/ARID1A/BAF250a immunoprofile would favour YST, it should be noted that a minority of CCCs express AFP and glypicin 3 while YST may be positive for CK7. In this context, high serum AFP levels, the presence of additional germ cell tumour components and lack of associated endometriosis would favour YST. MMR protein immunodeficiency would also support a diagnosis of CCC over other potential diagnostic mimics but as discussed below only a minority of tumours demonstrate this finding.

Molecular findings

The SWI/SNF complex comprises multiple subunits which have critical roles in DNA repair and in the control of cellular proliferation, differentiation and motility. Most subunits have functional tumour suppressor activity and therefore loss of function promotes carcinogenesis. SWI/SNF complex deficiencies occur in approximately 20% of all human cancers, and in the context of gynaecological neoplasia, mutational inactivation of the subunit ARID1A has been demonstrated in approximately half of all ovarian CCC. ARID1A mutations can be demonstrated indirectly with loss of BAF250a protein expression although it should be noted that mutational status and immunohistochemistry do not correlate exactly. Mutations in ARID1A are not specific to CCC since they also occur in approximately 30% of endometrioid and ovarian endometrioid adenocarcinomas, but in the context of an ovarian neoplasm, ARID1A mutation (or loss of BAF250a expression) supports the diagnosis of an EAN. Loss of BAF250a expression has also been demonstrated in some typical and atypical endometriotic lesions adjacent to ARID1A-deficient CCC and EOC, but not in anatomically distant endometriotic lesions, suggesting that inactivation is an early step in tumourigenesis. PIK3CA mutations occur in 30–40% of ovarian CCCs and they commonly coexist with ARID1A mutations. However, AKT2 gene amplifications have also been described in 37% and 14% of tumours, respectively, and therefore SWI-SNF complex and Met/P1K3CA/AKT pathway dysregulation may act...
synergistically to promote carcinogenesis in a significant subset of CCC.109 Experimentally, genetic modifications of Arid1a and Pik3ca lead to the development of a murine ovarian tumour that resembles CCC.55 This tumour model is also characterised by activation of the interleukin-6/STAT3 signalling pathway, a feature of human CCC. PIK3CA mutations are more common in endometriosis-associated, cystic and papillary CCCs, and in tumours showing a myxohyaline stroma, whereas an inverse correlation with an associated adenofibromatous tumour component is noted.10² ARID1A-deficiency is also less common in CCC associated with an adenofibroma, further suggesting a correlation between mutation status and tumour morphology.39,10¹ Some reports have suggested that PIK3CA mutation or over-expression may be associated with a favourable prognosis in CCC,10² but this has not been found in all studies.103 PTEN alterations are less common in CCC than in EOC but mutation and/or loss of heterozygosity have been reported in 6–20% of tumours.11² Somatic mutations in the telomerase reverse transcriptase (TERT) promotor were reported in 37/233 (15.9%) ovarian CCC, and these mutations were generally mutually exclusive with PIK3CA mutation and loss of ARID1A expression.11³ Thus, TERT alterations may have a significant pathogenetic role in a subset of tumours.

As noted above, it is now well recognised that ovarian neoplasia occurs more commonly in patients who have Lynch syndrome due to germline mutations in genes encoding DNA MMR proteins. Such tumours account for approximately 1–2% of all ovarian carcinomas and most are EOCs or CCCs.11³ Conversely, however, most studies have found that <10% of ovarian CCCs demonstrate MI or MMR protein immunodeciency.6¹,65,11⁷,11⁸ Nevertheless, reflex immunohistochemistry or MI testing has been advocated in all ovarian EOC and CCC to identify those potentially arising in Lynch syndrome.12⁰ It is noteworthy that patients with MMR protein-deficient/Lynch syndrome-associated high-stage ovarian CCCs may have an unexpectedly favourable clinical outcome but this finding needs to be confirmed in larger studies.12³

Müllerian mucinous/mixed epithelial (seromucinous) tumours

Ovarian tumours exhibiting predominant Müllerian mucinous (endocervical-like) or mixed Müllerian differentiation have undergone a series of nomenclature changes but perhaps they are now best categorised as MM/ME tumours.4,1²¹,1²⁴ They may also be referred to as seromucinous
tumours as in the current WHO classification. The relative proportion of cells exhibiting mucinous or other types of differentiation (endometrioid, squamous, serous/ciliated, clear cell, hobnail, eosinophilic, or indeterminate) differs in individual tumours, giving rise to a range of histological appearances, and occasionally squamous differentiation predominates. These tumours share an association with endometriosis (50% of cases), often show bilateral ovarian involvement (20–30% of cases), and may demonstrate ARID1A mutations. Therefore, current evidence suggests that MM/ME tumours represent a single entity with a morphological spectrum that varies according to the range and relative proportion of the cellular differentiation patterns.

MM/ME neoplasms are typically cystic, often with thick collagenous and/or muscularised walls, and most are classified as borderline based upon the presence of cytological atypia, which is usually mild, and a papillary architecture that grossly and microscopically mimics that of borderline serous tumours (Fig. 10); micropapillary epithelial proliferation may also be present. Stromal microinvasion is seen in 10–20% of tumours and non-invasive peritoneal implants may be seen rarely but these findings do not adversely affect prognosis. As with other EANs, concurrent endometriosis may show atypical features, often with mucinous differentiation (Fig. 10). Malignant MM/ME tumours (seromucinous carcinomas) are relatively rare and they are often associated with borderline tumours, suggesting a ‘type I’ pattern of ovarian tumour progression analogous to that of mucinous and low-grade serous neoplasms. While most of these neoplasms have a favourable prognosis, occasional high-stage MM/ME carcinomas have been associated with a fatal outcome. A recent study showed poor inter-observer agreement in the diagnosis of malignant MM/ME tumours (seromucinous carcinomas) even amongst specialist gynaecological pathologists, and immunohistological and molecular studies suggested that most of these tumours demonstrated an endometrioid or, less commonly, a low-grade serous carcinoma phenotype. Therefore, it remains to be seen whether malignant MM/ME tumours truly represent a distinct entity or whether they might be better categorised as other tumour subtypes.

The distinction between borderline MM/ME tumours and the more common primary (gastro-) intestinal borderline mucinous tumours (IBMT) is important. Features favouring a MM/ME neoplasm include bilaterality, a spectrum of Mullerian epithelial phenotypes, association with endometriosis, characteristic stromal neutrophil infiltrate, and lack of intestinal differentiation (absence of goblet, Paneth and neuroendocrine cells). Immunohistochemical and molecular findings also differ as discussed below.

Immunohistochemistry

ME/MM neoplasms generally exhibit a CK7, PAX8, ER, PR and vimentin positive immunophenotype (Fig. 10), negative or only focal WT1 and CEA staining, and typically lack expression of gastrointestinal markers such as CK20 or CDX2. Basal cells, similar to the subcolumnar reserve cells of normal endocervical mucosa, may be present and are highlighted by p63, 34BE12 and CK17 immunostaining. In contrast, primary IBMT typically co-express CK7 and CK20, express PAX8 inconsistently, and otherwise show an intestinal phenotype (ER, PR and vimentin negative, CDX2 positive).

Molecular findings

An important feature linking borderline MM/ME tumours with other EANs is ARID1A mutation and/or loss of BAF250a expression which occur in approximately one-third of cases, similar to the incidence in EOC. KRAS

![Fig. 10](A) Borderline mixed Mullerian/mixed epithelial tumour. (A) Low magnification demonstrating intracystic papillary architecture. (B) The papillae are lined by cells showing variable endometrioid, mucinous or eosinophilic appearances. (C) Adjacent atypical endometriosis shows focal mucinous differentiation. (D) The tumour cells including micropapillary areas strongly express ER.

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mutations may also be important drivers in this tumour subgroup since they were recorded in 11/16 (69%) borderline tumours in one series,138 and in 6/9 (67%) and 21/30 (70%) carcinomas in two further reports.133,145 In the study by Rambau et al.,133 PIK3CA, PTEN and ARID1A mutations were also detected in 37%, 19% and 16% of cases, respectively, and 30% of tumours showed concurrent KRAS and PIK3CA mutations.

**Ovarian endometrioid stromal sarcoma, mesodermal adenosarcoma, and carcinosarcoma**

These relatively rare tumours arise much more frequently in the uterus and therefore a primary endometrial neoplasm has to be excluded before assuming an ovarian origin. However, primary ovarian endometrioid stromal sarcomas (ESSs), mesodermal (Müllerian) adenosarcomas (MAs), and carcinosarcomas/malignant mixed Müllerian tumours are well documented, and they may be associated with (and presumed to arise from) ovarian endometriosis.136,157 Low-grade extra-uterine ESSs can demonstrate similar cytogenetic alterations to their uterine counterparts including JAZF1/SUZ12 rearrangements suggesting a common pathogenesis,148–150 and an ovarian high-grade ESS with a YWHAE/NUTM2B rearrangement has also been described recently.151

Primary ovarian MAs show similar histological appearances to the corresponding uterine neoplasms including the finding of sex cord-like elements and high-grade sarcomatous overgrowth.152–154 A greater proportion of ovarian cases present at high-stage, possibly reflecting the relative ease with which such tumours can spread to the peritoneum, and the overall prognosis is less favourable. Interestingly, while MAs have traditionally been regarded as mixed epithelial-stromal tumours, a recent study of endometrial tumours suggests that these are fundamentally mesenchymal neoplasms since only the stromal cells show molecular alterations and there is no clonal relationship with the epithelial component.155 There are limited data on the molecular pathogenesis of MAs, and these are based upon primary uterine tumours. However, PIK3CA/AKT/PTEN pathway alterations have been demonstrated in 72% of neoplasms,156 potentially representing a therapeutic target in high-stage or metastatic cases,157 while approximately 20–25% show amplifications of MDM2, CDK4, HMGA2 and/or TERT.158–161 Mutations in FGFR2, KMT2C and DICER1 were also each identified in 2/19 (11%) uterine MAs in one study,136 the latter finding is of particular interest since a recent case report documented an endometrial MA arising in a patient with a germline DICER1 mutation.158 In this regard it should be noted that the histological distinction of MA and embryonal rhabdomyosarcoma, a tumour well-recognised in the DICER1 syndrome, can be difficult, but most embryonal rhabdomyosarcomas in patients with DICER1 syndrome arise in the cervix rather than the uterine corpus.159

Primary carcinosarcomas of the ovary and fallopian tube, like their uterine counterparts, are clinically aggressive malignancies often presenting at high stage.160,161 Most tumours show serous epithelial differentiation and occasionally these arise in patients with germline BRCA mutations or are associated with serous tubal intraepithelial carcinoma (STIC), suggesting a close pathogenetic relationship with the more common tubo-ovarian HGSC.162–164 However, some of the neoplasms are associated with endometriosis, and/or demonstrate endometrioid rather than serous differentiation, and such tumours may be considered EANs. Immunohistochemical and molecular data, mainly derived from studies on uterine tumours, generally support a clonal relationship between the epithelial and mesenchymal components and it is now accepted that these tumours are fundamentally carcinomas with stromal differentiation possibly representing a form of epithelial-mesenchymal transition.165–167 In addition to TP53 mutations, 50–60% of cases demonstrate P13K pathway alterations, and occasional cases show additional endometrioid-type molecular changes including KRAS and PTEN mutations.168

**Synchronous endometrioid carcinomas of the endometrium and ovary**

Up to 20% of EOCs coexist with an endometrioid carcinoma of the endometrium and a similar association occurs less commonly with ovarian CCC (Fig. 11). The favourable outcome in cases in which the tumour is restricted to both organs has suggested that these neoplasms are independent, and probably arise as a neoplastic field effect in different areas of the Müllerian tract.162,169

A number of clinicopathological features has traditionally been used to distinguish whether synchronous ovarian and endometrial neoplasms represent two independent tumours, or a primary endometrioid carcinoma of the endometrium with metastasis to the ovary/ovaries. Features favouring two independent primaries include: (1) low histological grade, (2) different microscopic appearances, and (3) lack of myometrial invasion, lympho-vascular space invasion, or tubal involvement of the uterine tumour. Conversely, features favouring metastasis are: (1) high histological grade, (2) similar microscopic appearances, (3) discrepant tumour sizes, (4) bilateral ovarian involvement, (5) superficial and/or invasive pattern of tumour growth in the ovary, and (6) presence of extensive myometrial, vascular and/or tubal invasion. Moreover, the presence of ovarian endometriosis or of endometrial hyperplasia is supportive of ovarian and endometrial origin, respectively.

Molecular techniques (single gene or high throughput approaches) may be used in difficult cases. Similarity of molecular features is interpreted as suggestive of an identical tumour in both locations, while the presence of different
molecular profiles supports independent origin. Next generation sequencing has been applied to a small number of tumours, and has shown that endometrial and ovarian tumours are clonal in the majority of cases. This possibly surprising result appears contradictory to the generally indolent behaviour of these tumours, and it has been suggested that this may relate to the pathway of spread. According to this hypothesis, spread of an endometrial carcinoma to the ovary by vascular invasion results in a conventional metastatic tumour with a generally poor prognosis. However, transtubal dissemination without vascular invasion can result in ‘drop-like metastasis’ in the ovary that behaves clinically more like an independent low-stage neoplasm. Further studies are required to confirm this hypothesis.

CONCLUSION

EANs represent a heterogenous group of tumours and while most are malignant they have a relatively favourable prognosis when confined to the ovary. Borderline EANs occur less commonly and many show mixed patterns of Müllerian differentiation. Atypical endometriosis can be identified in a significant proportion of tumours and may represent an intermediate step in neoplastic progression. EANs commonly show mutations in the SWI/SNF complex component ARID1A (BAF250a) but the most common malignant neoplasms, EOC and CCC, show additional distinctive genetic signatures, and these are likely to become increasingly important in the era of personalised and targeted tumour therapies.

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