

## Review

# Current concepts in ovarian epithelial tumorigenesis: correlation between morphological and molecular data

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**Summary.** Ovarian carcinoma is the most lethal gynaecological malignancy, most tumours being advanced at presentation. However, little is known about precursor lesions and the cell of origin of epithelial ovarian malignancy. In this review, the proposed cell of origin is discussed as well as recent molecular data relating to ovarian cancers of different morphological types. It is stressed that ovarian carcinoma is a heterogeneous group of neoplasms with several different morphological types, each with their own underlying molecular genetic events. Recent data suggest that mucinous ovarian cancers and a small subset of serous cancers (low grade ovarian serous carcinoma) develop through a well-defined adenoma-carcinoma sequence while the much more common high grade ovarian serous carcinoma develops *de novo* from the ovarian surface epithelium or the epithelium of cortical inclusion cysts. The realisation that various morphological types of epithelial ovarian cancer are associated with different molecular genetic events is a major advance in the study of ovarian cancer. It can be anticipated that this will lead to the development of specific therapeutic agents of value against a specific tumour type.

**Key words:** Ovary, Pathogenesis, Morphological types, Molecular genetics

## Introduction

Despite representing only 4% of cancers in women in the USA, ovarian carcinoma is the most lethal gynaecological malignancy (Gershenson et al., 1996; Shih and Kurman, 2004). Most cases are advanced at presentation and the overall five-year survival rate is only 20-30% (Wang, 2002; Brewer et al., 2003). Early diagnosis at a stage when cure might be expected is uncommon. A further challenge is that few experimental

models of this malignancy exist (Auersperg et al., 2002; Brewer et al., 2003). Nelly Auersperg has commented that "in spite of the clinical importance of epithelial ovarian cancer, the initiating events and early stages in ovarian epithelial carcinogenesis are among the least understood of all major human malignancies" (Auersperg et al., 2002).

Most ovarian malignancies are epithelial in type. However, ovarian carcinoma is a complex and heterogeneous group of neoplasms rather than a single disease entity (Nicosia et al., 2003). There are several morphological types, chiefly serous, mucinous, endometrioid, clear cell, transitional and undifferentiated. Additionally, mixed tumours not uncommonly occur. Limited understanding of early events in ovarian tumorigenesis is compounded by an incomplete comprehension and recognition of putative precursor lesions. Moreover, the nature of the cell of origin is a matter of some debate (Dubeau, 1999; Feeley and Wells, 2001). Nevertheless, there have been some significant recent advances in our understanding of the pathogenesis of ovarian epithelial malignancy and it is likely that the demonstration of molecular differences between tumour types will be a significant advance (Schwartz et al., 2002). It is beyond the remit of this review to provide a comprehensive *précis* of the clinical pathology of the various types of ovarian carcinoma. Rather, some pertinent issues will be addressed in relation to the morphology and molecular pathology of the different subtypes. Recent data, largely based on molecular genetic observations, have resulted in a suggested model for classification of carcinomas into types which are clinically relevant (Shih and Kurman, 2004).

## The morphological spectrum of ovarian epithelial malignancy

Ovarian carcinomas exhibit a diverse spectrum of morphology with several different tumour cell types, as stated previously. Recent molecular studies have revealed marked genetic heterogeneity in these tumours

(Fox and Singh, 2002) and it is becoming increasingly apparent that different genetic alterations are characteristic of the various morphological types. Previous understanding of ovarian tumour biology was compromised by early studies which did not discriminate between tumour types, making it difficult to correlate molecular alterations with a specific tumour morphology. In future studies this should be rectified by molecular analysis of each tumour type in isolation as a specific and well-defined morphological entity. Recent insights have been generated by such an approach (Mandai et al., 1998; Obata et al., 1998; Garrett et al., 2001; Staebler et al., 2002; Gilks, 2004), as discussed in detail later.

Ovarian carcinomas (the term carcinoma being restricted to epithelial malignancies and not including malignant tumours of germ cell or sex cord-stromal origin) are among the most morphologically variable tumours of any organ (Auersperg et al., 2002). They may resemble the müllerian epithelium of the fallopian tube (serous tumours), endocervix (mucinous tumours) or endometrium (endometrioid tumours). Clear cell, transitional, undifferentiated and mixed carcinomas also occur, as well as a variety of other rare types. Although the concept of a cell of origin is still controversial, compelling evidence points towards ovarian surface epithelium (OSE), a modified form of peritoneal mesothelium (Auersperg et al., 2001, 2002; Feeley and Wells, 2001; Bao et al., 2002; Brewer et al., 2003; Cvetkovic, 2003). OSE possesses a capacity for divergent differentiation along the müllerian pathways mentioned above in view of its embryological derivation from the coelomic epithelium near the nephrogenital ridge, a precursor of normal müllerian epithelia in the female (Auersperg et al., 2001). This capacity for divergent differentiation is also reflected in the occasional finding of foci of ciliated epithelium or, more rarely, mucinous epithelium lining the surface of the ovary. Although widely accepted as being of müllerian origin, it seems possible that transitional cell tumours may arise from OSE by an analogous process of wolffian rather than müllerian differentiation since the wolffian ducts share a common origin in the coelomic epithelium of the nephrogenital ridge (Fox and Singh, 2002).

Each histological type of ovarian epithelial neoplasia encompasses a spectrum of tumours comprising benign, borderline and malignant. The relationship of benign, borderline and malignant tumours within each morphological subtype is an area of debate (Shih and Kurman, 2004) and is discussed further in the sections on serous and mucinous tumours. A prime challenge has been the identification of molecular markers linking benign, borderline and malignant tumours to each other as a means of proving a continuum of neoplastic progression from benign through borderline to malignant (Campbell et al., 2002), similar to the adenoma-carcinoma sequence described in colorectal neoplasia (Fearon and Vogelstein, 1990).

With regard to the prevalence of morphological

types of primary ovarian epithelial malignancy, serous carcinomas account for the majority of cases followed by endometrioid and clear cell carcinomas. It is important to note that the ovary is a common site of metastatic carcinoma. A recent population-based study has shown that mucinous carcinomas account for only 3% of primary ovarian epithelial malignancies (Seidman et al., 2004). This is in contrast to the older literature where mucinous carcinomas were reported to be much more prevalent and undoubtedly reflects the fact that primary ovarian mucinous carcinomas were over-diagnosed in the past due to misinterpretation of metastatic mucin-secreting adenocarcinomas from sites such as the colorectum, appendix, pancreas, biliary tree and endocervix (Seidman et al., 2003). Additionally, virtually all cases of pseudomyxoma peritonei are now known to be of gastrointestinal rather than ovarian origin (Ronnett et al., 1995). Furthermore, many neoplasms which would have previously been designated as ovarian mucinous carcinomas on the basis of nuclear stratification and atypia but without overt stromal invasion are now considered to represent mucinous borderline tumours with intraepithelial or intraglandular carcinoma (Riopel et al., 1999). The advent of differential cytokeratin immunohistochemistry, together with other immunohistochemical markers, has helped to classify many mucinous carcinomas in the ovary as metastatic. It is now widely accepted that bona fide primary ovarian mucinous carcinomas are rare and are typically low stage at presentation (FIGO Stage I/II). With an advanced stage ovarian mucinous carcinoma, a metastasis should always be suspected. In contrast, most serous tumours present at an advanced stage (FIGO Stage III/IV) (Seidman et al., 2004). The prevalence of primary transitional carcinoma of the ovary varies widely between different studies, undoubtedly reflecting the fact that different criteria are used in various institutions to make this diagnosis (Eichhorn and Young, 2004; Seidman et al., 2004).

As can be seen from the foregoing discussion, ovarian carcinoma represents a complex heterogeneous group of neoplasms of different morphological types. This is reflected by a variety of underlying molecular genetic alterations, some of which are relatively type-specific. This infers that specific genetic changes are markers of tumour type and indicates that differences in prognosis and response to treatment are likely between these different types (Gilks, 2004). Conversely, it is true that tumours of identical histological type may possess different genetic alterations, helping to account for the variable prognosis and response to chemotherapy often seen within a specific morphological category (Bast, 2003). In the following sections, specific genetic events associated with the different morphological types of ovarian cancer are discussed. First, the likely cell of origin of epithelial ovarian cancer will be discussed in some detail followed by a consideration of likely initiating events in the development of ovarian surface epithelial neoplasia.

### Cell of origin of ovarian epithelial neoplasia

The recognition of some similar genetic abnormalities in different morphological types of ovarian carcinoma suggests that there may be a commonality of early genetic alteration with divergence during further development of invasive malignancy (Hauptmann et al., 2002). Difficulties lie in the identification and study of precursor lesions (Feeley and Wells, 2001).

#### *Ovarian surface epithelium (OSE)*

In 1872, Wells (cited in Bao et al., 2002) suggested that ovarian cancers originate from the 'germinal' epithelium covering the ovarian surface. However, OSE remained neglected experimentally until the development of OSE culture systems in the 1980s (Adams and Auersperg, 1981; Auersperg et al., 1984). Recent years have witnessed a significant increase in the ability to study OSE but a detailed understanding of its role in the development of ovarian malignancy has not yet been achieved. The study of OSE has been hindered by its fragile nature as a monolayer which is commonly abraded and lost at oophorectomy unless handled with due care.

OSE is derived from the coelomic epithelium, a mesodermally derived epithelium (Auersperg et al., 2001). Although sharing a common embryological derivation, OSE (in essence a specialised form of mesothelium) exhibits a different phenotype compared with non-ovarian peritoneal mesothelium, possibly due to the influence of local hormonal factors related to ovarian steroidogenesis (Brewer et al., 2003). This is exemplified by expression of the cell surface glycoprotein CA125 which is present in müllerian epithelia and mesothelia but not in normal OSE (Kabawat et al., 1983). Since CA125 expression is regarded as a marker of differentiation, it has been suggested that OSE is less differentiated and therefore less committed to a mature mesothelial phenotype than the remainder of the pelvic peritoneum (Auersperg et al., 2001). As illustrated by its polydirectional müllerian differentiation in normal development, embryonic coelomic epithelium may progress along different phenotypic pathways. It is possible that neoplastic transformation of OSE may lead to recapitulation of müllerian pathways, resulting in the range of epithelial phenotypes recognised by pathologists. It is also apparent that, under certain circumstances, the peritoneal mesothelium can give rise to tumours of müllerian type without ovarian involvement.

In addition to the embryological rationale outlined above, early evidence linking ovarian carcinoma to OSE was derived from histopathological observations in the rarely-detected small early-stage carcinomas in contralateral 'normal' ovaries in women with unilateral ovarian carcinoma (Deligdisch et al., 1995) and in prophylactic oophorectomies in women with a strong

family history of ovarian cancer. Such 'cancer-prone' or 'cancer-associated' ovaries have been reported to more frequently exhibit surface epithelial pseudostratification, papillomatosis, deep cortical epithelial invaginations, increased numbers of epithelial inclusion cysts and increased stromal activity compared to normal controls (Salazar et al., 1996). Such changes have been suggested to represent a 'pre-neoplastic' phenotype (Bingham et al., 2001), particularly in women harbouring *BRCA1* and *BRCA2* mutations (Brewer et al., 2003) but this is by no means proven and these changes have not been validated in several more recent blinded studies (Deligdisch et al., 1999; Werness et al., 1999), although one group identified subtle changes in such ovaries using morphometric methods (Deligdisch et al., 1999). In this study it was found that surface epithelial nuclei were larger with more heterogeneously dense chromatin in *BRCA1* mutant specimens compared to controls (Deligdisch et al., 1999). Cell culture studies using OSE from women with and without family histories of, or predisposition to, ovarian carcinoma are difficult to interpret. An enhanced epithelial phenotype has been reported in OSE derived from 'cancer-prone' females (Auersperg et al., 1995). However, a similar comparison of OSE phenotypes showed no significant differences (Piek et al., 2004). The spectrum of putative precursor lesions has been reviewed comprehensively by Feeley and Wells (Feeley and Wells, 2001) who cite observations supporting an origin of ovarian carcinomas from OSE such as surface atypia adjacent to invasive carcinoma and atypia in epithelial inclusion cysts (Godwin et al., 1993; Mittal et al., 1993). This atypia has been termed 'ovarian intraepithelial neoplasia' (Plaxe et al., 1990).

The central concept in the model of OSE as the precursor of ovarian carcinoma is a process of 'differentiating up' from an 'uncommitted' dual epithelial-mesothelial phenotype to an overtly epithelial phenotype (Auersperg et al., 2001; Feeley and Wells, 2001), associated with induction of E-cadherin expression (Sundfeldt et al., 1997). Normal OSE does not express E-cadherin but rather maintains cell-cell adhesion via N-cadherin, a characteristic of mesothelial cells (Auersperg et al., 1997, 2001). E-cadherin expression in non-neoplastic OSE varies with both location and morphology. Normal OSE has been shown to be negative for E-cadherin whereas the epithelium of cortical invaginations and inclusion cysts, which commonly exhibits müllerian metaplasia with a ciliated phenotype, is often strikingly positive (Sundfeldt et al., 1997).

The intricate physiology of normal OSE is beyond the scope of this review. Briefly, OSE actively transports materials to and from the peritoneal cavity and serves as a diffusion barrier, separating the underlying ovarian stroma from the peritoneal cavity. OSE is hormonally responsive and expresses oestrogen and progesterone receptors (Bao et al., 2002). OSE cells also possess lysosome-like inclusions and produce proteolytic

enzymes which have been hypothesised to play a role in follicular rupture and stromal remodelling (Auersperg et al., 2001). A role in extracellular matrix synthesis and a myofibroblast-like contractile function have also been suggested as additional components of ovulatory repair (Auersperg et al., 1994; Kruk et al., 1994). Possible subversion of such functions by neoplastic transformation may result in enhancement of an invasive capability.

#### *Epithelial inclusion cysts*

In 1971, Fathalla proposed that the repeated rupture or wounding of the ovarian surface at ovulation is followed by rapid proliferation of OSE, providing a fertile ground for mutational events (Fathalla, 1971). This hypothesis also provided a mechanism for the formation of epithelial cortical inclusion cysts. Nulliparity, early menarche and late menopause may therefore increase ovarian cancer risk by maximising the total number of ovulation/repair episodes during a lifetime. Although pregnancy or hormonal contraception were initially thought to diminish the risk of malignancy by simply decreasing the number of ovulation/repair episodes, studies using progestins in macaques provided evidence for induction of apoptosis in OSE cells (Rodríguez et al., 1998). It is possible that progestins promote apoptosis in genetically abnormal OSE cells as a protective mechanism, thus guarding against malignant transformation.

Epithelial inclusion cysts are widely regarded as likely precursors of ovarian carcinomas, especially of serous type. Inclusion cysts may be a site of origin of ovarian intraepithelial neoplasia, a useful concept which allows consideration of ovarian cancer in the same manner as other epithelial cancers with an identifiable precursor lesion (Brewer et al., 2003). Inclusion cysts are traditionally regarded as sequelae of ovulation whereby proliferating foci of OSE migrate into the underlying stroma and become incorporated into a healing follicle after rupture (Auersperg et al., 2001). However, evidence to the contrary has been presented by Scully who has proposed that inclusion cyst formation is not solely related to repair since downgrowth of OSE into ruptured follicles is rarely observed histologically (Scully, 1995). Moreover, inclusion cysts are more numerous in multiparous than in nulliparous women (Scully, 1995). Patients with polycystic ovarian syndrome, characterised by infrequent or absent ovulation, are reported to have five times more inclusion cysts than normal controls (Resta et al., 1993). Possible alternative mechanisms for inclusion cyst formation include para-ovarian inflammatory adhesions with entrapment of pockets of OSE and an active remodelling process involving dynamic interaction of proliferating stroma and OSE (Scully, 1995). An association has been drawn between inclusion cysts and deep cortical epithelial invaginations (Tresserra et al., 1998). Disputes regarding aetiology notwithstanding, it seems likely that

inclusion cysts are more prone to undergo malignant transformation than epithelium on the surface of the ovary (Scully, 1995). Although rarely identified, early carcinomas tend to be found in the cortical parenchyma where inclusion cysts commonly reside rather than on the surface (Feeley and Wells, 2001). Similarly, müllerian metaplasia is much more common in inclusion cysts than in surface epithelium (Resta et al., 1993). Immunoreactivity for tumour markers such as CA125 and CA19-9 has been shown to be increased in inclusion cysts compared to OSE (Blaustein et al., 1982). Increased p53 immunoreactivity has also been demonstrated in the epithelium of inclusion cysts, especially those showing müllerian metaplasia and cytological atypia (Hutson et al., 1995).

It is possible that incorporation of OSE into the stromal substance of the ovary represents a novel milieu in comparison to the microenvironment of the ovarian surface and its normal physiological relationship with the peritoneal cavity. Entrapment of OSE within ovarian stroma may thus disrupt the normal epithelial-stromal relationship (Brewer et al., 2003). Growth factors and cytokines which would otherwise diffuse into the peritoneal cavity may become concentrated within inclusion cysts (Feeley and Wells, 2001).

In spite of the evidence presented above, some difficulties exist with the surface epithelial hypothesis of ovarian carcinogenesis. A primary criticism is that the ovary does not normally contain cells with a müllerian phenotype (Dubeau, 1999), although this is rebutted by the capacity of the OSE for multidirectional differentiation as described previously (Auersperg et al., 2001). Some scepticism that OSE (and the epithelium of inclusion cysts) represents the cell of origin of ovarian carcinomas is based on the rarity of well-described precursor lesions, unlike the situation in other epithelial organs where it is commonplace to identify precursor lesions (Dubeau, 1999). The “secondary müllerian system” has been put forward as an alternative origin for ovarian cancer. This proposal is based on the capacity of the pelvic and abdominal peritoneum to differentiate into müllerian epithelia; accordingly, many small foci of müllerian-like epithelium may be found in the para-ovarian and paratubal regions, omentum, pelvic peritoneum and pelvic lymph nodes (Lauchlan, 1994). This model finds some favour by providing a rational explanation for müllerian-type tumour development outside the ovary, primary peritoneal serous carcinoma being well described (Taus et al., 1997; Halperin et al., 2001). Nevertheless, the biological basis for OSE and cortical inclusion cysts as the precursor of the majority of ovarian carcinomas is better established and more widely accepted by pathologists and scientists.

#### **Pathways of ovarian epithelial tumorigenesis**

Heterogeneity of tumour types and underlying genetic alterations is a major challenge to understanding ovarian carcinoma. A common controversy is whether or

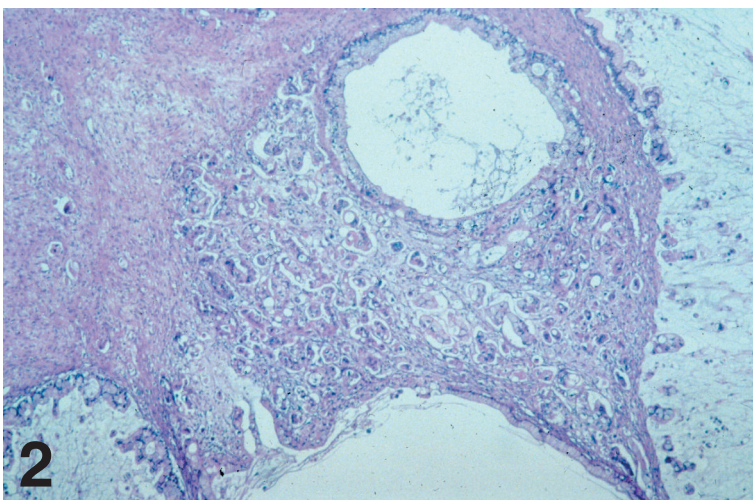
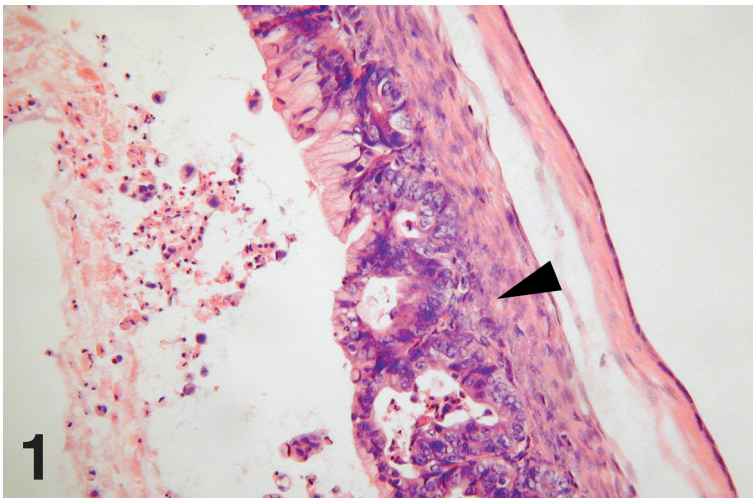
not a continuum of stepwise progression exists from benign through borderline to malignant tumours, similar to the well-defined adenoma-carcinoma sequence in the colorectum, or whether benign, borderline and malignant tumours of various morphological types represent separate biological entities and as such arise *de novo* (Scully, 1995; Cvetkovic, 2003). There is a danger of attempting to follow the paradigm of colorectal carcinoma as a sequence of neoplastic progression where well-defined morphological stages of tumour progression are mirrored by specific genetic alterations (Fearon and Vogelstein, 1990). Indeed, the relative rarity of small early-stage carcinomas and of identification of well-defined precursor lesions has led many to suggest that most ovarian carcinomas arise *de novo* rather than through a neoplastic continuum (Campbell et al., 2002).

As emphasised, there is a clear need to distinguish between the various morphological tumour types in terms of molecular alterations if different (or common) pathways are to be elucidated. In this regard, significant

advances have been made in recent years. In the following sections, genetic alterations in serous, mucinous and endometrioid neoplasia will be considered, these being the most common morphological types and those in which genetic events are best documented. As stated previously, primary ovarian mucinous carcinomas are less common than clear cell carcinomas, but benign and borderline mucinous tumours are much more common.

### *Mucinous neoplasia*

In contrast to serous neoplasms, it is not uncommon in mucinous tumours to see areas of benign, borderline and malignant neoplasm side by side (Figs. 1, 2). This underscores the necessity to adequately sample mucinous ovarian tumours since the intratumoral heterogeneity may be marked. Based on morphological observations, and supported by molecular data (discussed in the following paragraphs), it is currently



**Fig. 1.** Ovarian mucinous tumour with area of borderline neoplasm and adjacent area showing marked nuclear atypia (arrow) amounting to intraepithelial carcinoma. x 200

**Fig. 2.** Ovarian mucinous tumour showing abrupt transition between borderline and invasive areas. x 100

thought that there is a continuum of mucinous neoplasia from benign to borderline to borderline with intraepithelial or intraglandular carcinoma to microinvasive carcinoma and ultimately to overtly invasive mucinous carcinoma (Hart, 2005).

Mucinous ovarian tumours commonly exhibit mutations in the *KRAS* proto-oncogene on chromosome 12 (Cuatrecasas et al., 1997; Mandai et al., 1998; Garrett et al., 2001; Takeshima et al., 2001; Gemignani et al., 2003). The protein product, ras, serves to transduce growth signals from cell surface tyrosine kinase receptors via the ras-raf-MEK-ERK-MAPK signalling pathway with consequential effects on gene transcription (Peyssonnaud and Eychene, 2001). Point mutations at codons 12, 13 or 61 constitutively activate the GTPase domain and contribute to neoplastic transformation. Codon 12 is the site of most K-ras mutations (Bos, 1989).

A number of studies have undertaken mutational analysis of *KRAS* in benign, borderline and malignant ovarian mucinous tumours and in these different epithelial components in the same neoplasm. One group analysed 20 mucinous tumours using a stringent microdissection-based approach to separate contiguous benign, borderline and malignant areas within carcinomas (Garrett et al., 2001). *KRAS* mutations were demonstrated in 50% of mucinous borderline tumours and carcinomas. Furthermore, identical mutations were demonstrated in benign, borderline and malignant foci within the same tumour. This observation supports a progression through a neoplastic continuum and suggests that *KRAS* mutation is an early event in the evolution of ovarian mucinous neoplasms. A caveat of such an interpretation is that benign-appearing areas within a mucinous carcinoma may represent well-differentiated malignant elements rather than a pre-existing benign mucinous cystadenoma. Laser microdissection-based mutational analyses also report homogeneous

distribution of *KRAS* mutations in the various types of contiguous epithelium within mucinous carcinomas (Mandai et al., 1998; Takeshima et al., 2001).

Mutational analyses have also been undertaken of *KRAS* in conjunction with its major effector *BRAF* (a serine-threonine kinase). Interestingly, *KRAS* and *BRAF* mutations tend to be mutually exclusive (discussed in the section on serous tumours) suggesting that they are functionally equivalent in their tumorigenic effects (Gemignani et al., 2003). Despite *KRAS* mutations being found in 50% of mucinous tumours examined, *BRAF* mutations have not been demonstrated. The prevailing theme in such analyses of mucinous ovarian tumours is that the relative frequency of *KRAS* mutations increases from benign through borderline to malignant tumours (Cuatrecasas et al., 1997). This correlates with well-documented morphological observations of a neoplastic continuum in ovarian mucinous neoplasia (Puls et al., 1992; Russell and McCluggage, 2004).

### Serous neoplasia

There are undoubtedly more complex pathways of serous ovarian tumorigenesis than is the case with mucinous neoplasms. Although a morphological continuum from benign to borderline to malignant, similar to that seen in mucinous neoplasia (discussed earlier), is an attractive model of tumorigenesis, it is relatively rare to identify benign or borderline elements in serous carcinomas (Puls et al., 1992). However, a dualistic model of ovarian serous carcinogenesis has been proposed recently which is now gaining widespread acceptance (Fig. 3). In this dualistic pathway the much more common high grade ovarian serous carcinoma (OSC) (Fig. 4) develops directly from OSE or the epithelium of cortical inclusion cysts through an as yet poorly described and understood precursor lesion (Singer et al., 2002). The much less common low grade

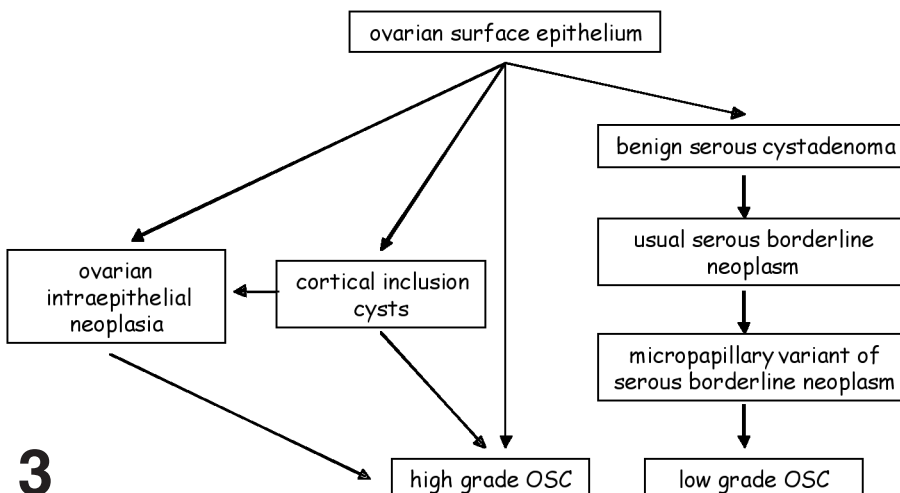


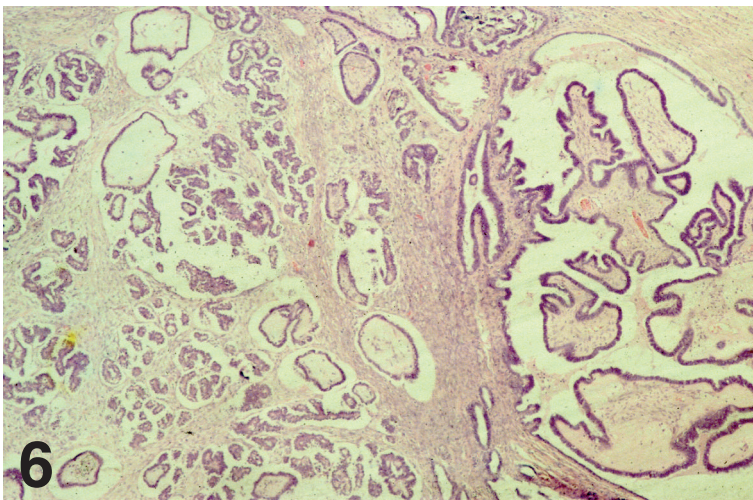
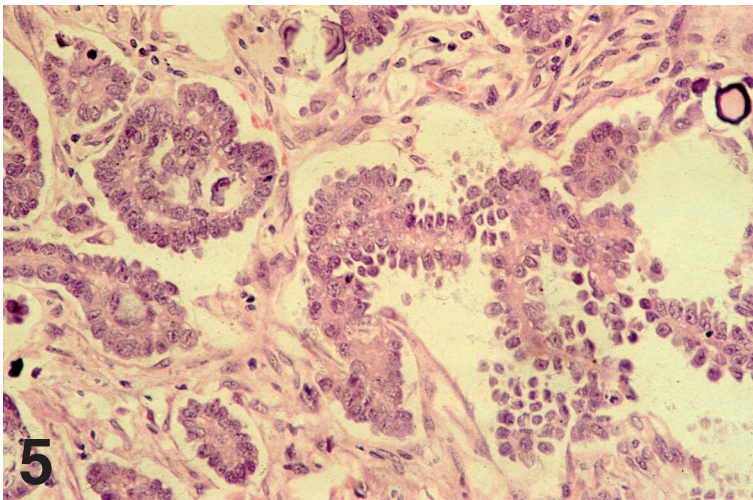
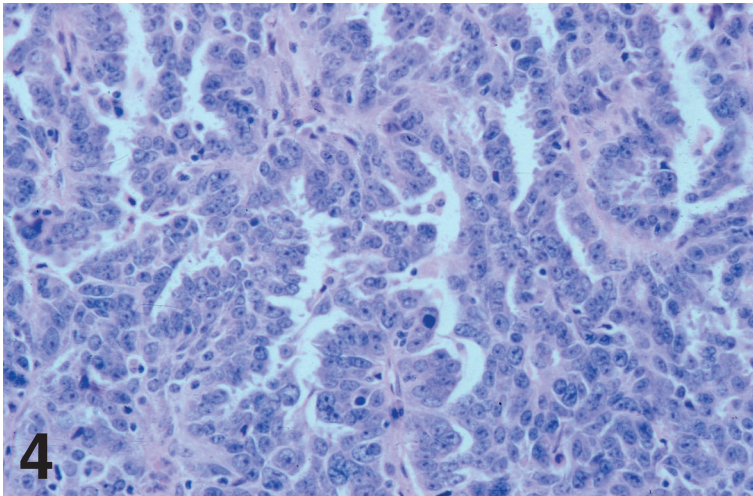
Fig. 3. A proposed dualistic scheme for the development of ovarian serous carcinoma.

OSC (Fig. 5) develops through an adenoma-carcinoma sequence. In this pathway there is a continuum from benign serous cystadenoma to a usual type of borderline

serous cystadenoma to a micropapillary variant of serous borderline tumour to an invasive low grade OSC (Fig. 6). Morphological evidence for this dualistic pathway is

derived from the observation that it is extremely uncommon to see benign and borderline elements associated with high grade OSC, in contrast to the situation with low grade OSC where an admixture of elements is commonly identified (although these low grade neoplasms are rare). These pathways have different underlying genetic events which are discussed below. In addition, immunohistochemical differences have been demonstrated between low grade and high grade OSC with increased expression of p53, MIB1 and bcl-2 in high grade compared to low grade carcinomas (O'Neill et al., 2005).

Mutations of *TP53* have been shown to be the most frequent and consistent molecular alteration in OSC (Schuyer et al., 1999; Wen et al., 1999; Fallows et al., 2001) and recently these have been demonstrated to be common in high grade OSC, in contrast to low grade OSC where these mutations are rarely identified. The rarity of identifiable precursor lesions and associated benign or borderline elements in high grade OSC has led to the hypothesis that these neoplasms acquire *TP53* mutations relatively early in their natural history and arise de novo from OSE or the epithelium of cortical inclusion cysts (Feeley and Wells, 2001; Shih and Kurman, 2004). This is supported by the observation of enhanced p53 immunoreactivity in inclusion cysts adjacent to high grade OSC, discussed previously (Hutson et al., 1995). In contrast, mutations in *TP53* are rare in low grade OSC. In these tumours, mutations in *KRAS* and *BRAF* are commonly identified. *KRAS* mutations are the most frequent genetic alteration in serous borderline tumours and low grade OSC (Haas et al., 1999; Diebold et al., 2003), reported in a third of borderline tumours (Singer et al., 2002) and a third of low grade OSC (Singer et al., 2003). The frequency of *BRAF* mutations at codon 599 seems to mirror that of *KRAS* closely. Mutation of *BRAF* has been demonstrated in 28% of serous borderline tumours and 30% of low grade OSC but not in high grade OSC (Singer et



**Fig. 4.** Typical high grade ovarian serous carcinoma. x 200

**Fig. 5.** Typical low grade ovarian serous carcinoma with a micropapillary pattern. x 200

**Fig. 6.** Transition between borderline serous cystadenoma and invasive low grade serous carcinoma. x 100

al., 2003). Thus, if considered together, *KRAS* or *BRAF* mutations were found in 65% of low grade OSC and 61% of serous borderline tumours but were not present in high grade OSC. Interestingly, *KRAS* and *BRAF* mutations are not found in the same tumours (discussed previously), suggesting that they are mutually exclusive. The similar frequency of mutations in *KRAS* and *BRAF* in serous borderline tumours and low grade OSC and the identification of similar mutations in microdissected areas of benign serous cystadenoma adjacent to serous borderline neoplasms suggests that mutations in *KRAS* and *BRAF* are early events in the evolution of low grade OSC.

Patterns of chromosomal imbalance in serous tumours also fail to support a clear relationship between benign, borderline and malignant neoplasms. It is important to note that many such studies are likely to include mainly cases of high grade OSC since these are much more common than low grade neoplasms. Karyotypic analysis has revealed an increased frequency of numerical chromosomal aberrations in serous carcinomas relative to benign and borderline tumours (Evans et al., 1999). Allelic imbalance of various chromosomal loci has been shown to be increased in invasive carcinoma relative to borderline tumours, more so in serous than in mucinous neoplasms (Evans and Herrington, 2001). It seems likely that the acquisition of chromosomal abnormalities is an early event in the development of high grade OSC. Comparative genomic hybridisation analyses provide further evidence of different chromosomal aberrations in ovarian serous neoplasia. High grade OSC has been shown to possess at least twice the number of chromosomal imbalances as low grade OSC (Hauptmann et al., 2002). Serous borderline tumours and high grade OSC exhibit different patterns of chromosomal imbalance, suggesting that most invasive carcinomas (high grade) do not arise from borderline tumours. Taken together, such chromosomal studies suggest that most serous carcinomas (high-grade) are characterised by chromosomal instability and are thus substantially different compared to low grade OSC and serous borderline tumours. It should be noted that the majority of familial ovarian carcinomas arising in a background of *BRCA1* or *BRCA2* mutation-mediated genetic instability are high grade OSC (Lakhani et al., 2004).

It can be summarised that, using a variety of investigative techniques, recent studies have concluded that serous borderline tumours (and low grade OSC) and high grade OSC are unrelated biologically (Ortiz et al., 2001; Singer et al., 2002). The dualistic model described thus provides a conceptual framework within which to begin to understand the relationship between serous borderline tumours and invasive carcinoma (Russell and McCluggage, 2004).

Before leaving the subject of OSC, some mention needs to be made of micropapillary serous carcinoma (MPSC), an entity about which there has been much debate in the literature. MPSC has been divided into

non-invasive and invasive forms by one prominent group of investigators headed by Robert J Kurman (Burks et al., 1996; Seidman and Kurman, 1996). Invasive MPSC is synonymous with low grade OSC, although this may also exhibit other architectural patterns (Shih and Kurman, 2004). There is a paucity of studies but invasive MPSC is thought to pursue a relatively indolent course compared to high grade OSC with a much lower proliferative index, a poor response to chemotherapy and a five-year survival rate in the region of 55% (Burks et al., 1996). High grade OSC, by comparison, is a highly aggressive neoplasm and exhibits rapid progression with an overall five-year survival rate of approximately 30% (Smith Sehdev et al., 2003), although there is often a good initial response to platinum-based chemotherapy. The entity described as non-invasive MPSC by Kurman and colleagues is a non-invasive tumour which, in contrast to the usual serous borderline tumour, is characterised by a micropapillary, cribriform or solid architecture with the papillae required to be five times as long as they are wide. Such an area must be at least 5mm in dimension. These non-invasive micropapillary tumours are more commonly bilateral and exophytic than the usual serous borderline tumour and are more likely to be associated with invasive peritoneal implants. Kurman and colleagues prefer to consider non-invasive MPSC as a form of low grade carcinoma and refer to usual serous borderline tumours as 'atypical proliferative serous tumours' to emphasise their usual benign behaviour. However, this view is not universally accepted, especially since non-invasive MPSC does not behave in a more aggressive manner in the absence of invasive peritoneal implants (Eichhorn et al., 1999; Prat and De Nictolis, 2002). Thus, although a micropapillary pattern, as defined above, in a serous borderline tumour may be a marker of an increased likelihood of invasive peritoneal implants, in the absence of such implants the behaviour is no different than that of a usual serous borderline tumour. In the recent World Health Organisation classification, although the concept of non-invasive MPSC is discussed, this tumour is retained in the borderline category (Tavassoli and Devilee, 2003).

A pragmatic approach taken by many pathologists is that micropapillary serous borderline tumours are an intermediate step between a "typical" serous borderline tumour and a low grade OSC (Russell and McCluggage, 2004). The similar behaviour to usual serous borderline tumours in the absence of invasive peritoneal implants is the reason for the reluctance of most authorities to consider micropapillary serous borderline tumour a variant of serous carcinoma (Eichhorn et al., 1999; Prat and De Nictolis, 2002). There is, in addition, a danger of creating further subdivisions which may result in confusion amongst pathologists and oncologists. The designation of a non-invasive tumour as a low grade OSC may result in the administration of chemotherapy which is probably inappropriate in these cases. Although the concept of non-invasive MPSC as an entity clinically

distinct from usual serous borderline tumour is far from robust, allelic imbalance of chromosome 5q has been reported to be more frequent in non-invasive micropapillary neoplasms compared with usual serous borderline tumours (Singer et al., 2002). Similarly, allelic imbalance of chromosome 1p is more common in low grade OSC than in non-invasive tumours with a micropapillary pattern (Singer et al., 2002).

Thus, there is now accumulating evidence for a dualistic pathway of ovarian serous carcinogenesis. As further evidence of the genetic disparity between high grade OSC and serous borderline tumours, *c-erbB2* amplification and over-expression has been identified in up to 67% of carcinomas but not in borderline tumours (Ross et al., 1999).

### *Endometrioid neoplasia*

Endometrioid carcinoma, the second most common form of ovarian carcinoma, is associated with endometriosis in up to 39% of cases (Sainz et al., 1996), suggesting an origin from ectopic endometrium rather than the ovarian surface epithelium. However, an alternative possibility is that ovarian endometriosis represents a specific form of müllerian metaplasia and this provides a similar potential origin for endometrioid carcinoma as epithelial inclusion cysts may do for high grade OSC. The figure quoted for the proportion of ovarian endometrioid carcinomas which arise in endometriosis is likely to be an underestimate since it is possible that other cases of endometrioid carcinoma arise in endometriosis but this is overgrown and obliterated by the neoplasm. It is interesting that there is an even stronger association between ovarian clear cell carcinoma and endometriosis (Stern et al., 2001). Genetic events underlying the development of ovarian clear cell carcinoma are not discussed in this review since few studies have investigated this tumour type.

A point of some importance is the difficulty amongst histopathologists in reproducible and accurate classification of many high grade ovarian carcinomas. There is considerable morphological overlap between high grade serous and endometrioid carcinoma and interobserver reproducibility is poor. This has implications for molecular studies aimed at dichotomising different tumour types based on patterns of gene expression since neoplasms may be incorrectly classified pathologically and thus incorrectly assigned to a specific category. It is interesting that high grade serous and endometrioid carcinomas have not been reliably distinguished in terms of gene expression profiling (Schwartz et al., 2002; Gilks, 2004). Thus, much of our present understanding of genetic events in endometrioid carcinoma of the ovary relates to low grade endometrioid carcinoma.

Ovarian endometrioid carcinoma has been shown to share similar molecular alterations to uterine endometrioid carcinoma (Matias-Guiu et al., 2001; Lax, 2004), including mutation of the  $\beta$ -catenin and K-ras

genes but with a lower rate of microsatellite instability and mutation or deletion of the PI3K phosphatase PTEN (Obata et al., 1998; Catusus et al., 2004; Dinulescu et al., 2005). Abnormalities of other members of the PI3K pathway have been reported, including PI3KCA which has been shown to be mutated or amplified in 30% of ovarian carcinomas in general and 45% of malignancies of endometrioid or clear cell type (Campbell et al., 2004). Although the genetic alterations in ovarian endometrioid carcinomas are similar to those seen in uterine endometrioid carcinomas, there are differences in the frequencies of different events between ovarian and uterine endometrioid carcinoma.

The association of ovarian endometrioid carcinoma (and clear cell carcinoma) with endometriosis raises many interesting questions about the biology of endometriosis which have been addressed in other reviews (Wells, 2004). Evidence supporting endometriosis as a precursor of some morphological types of ovarian carcinoma has been accruing in recent years, one example being the identification of 'atypical endometriosis' adjacent to endometrioid carcinoma (Seidman, 1996; Fukunaga et al., 1997; Fukunaga and Ushigome, 1998). Studies of clonality and loss of heterozygosity also support a pre-neoplastic role for endometriosis in some cases. However, a mechanism for the association of endometriosis with endometrioid carcinoma is not well established. A mouse model of neoplastic transformation of peritoneal endometriosis has been reported using K-ras transformation in conjunction with PTEN deletion (Dinulescu et al., 2005). In this intriguing study, expression of an oncogenic K-ras allele or deletion of PTEN in ovarian surface epithelium led to atypical endometrioid epithelial proliferations. A combination of both genetic interventions resulted in invasive endometrioid carcinoma with widespread metastatic disease. Such models provide further evidence of a role for K-ras and PTEN in ovarian endometrioid carcinoma.

### **Conclusion**

It is evident that the importance of phenotypic/genotypic relationships, previously neglected, is now being addressed in the field of ovarian epithelial malignancy. It is interesting to note that current therapeutic strategies for ovarian carcinoma are not based on morphological subtype. To this end the demonstration of a variety of pathways of ovarian carcinogenesis may have clinical impact by grouping pathogenetically unrelated (and related) tumours into defined therapeutic categories. It is also evident that our understanding of ovarian carcinoma is hampered by a lack of recognition of precursor lesions, especially with regard to high grade OSC, the most common type of ovarian epithelial malignancy. Future studies addressing underlying molecular alterations in ovarian carcinomas should include rigorous histopathological analysis to classify tumours into well defined morphological groups. New

morphological and molecular classifications are critically dependent upon careful correlation with the clinical aspects of the various neoplastic types in order to ascertain if there are significant prognostic differences and responses to therapy.

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