Section 1

Biology of gynaecological cancers: our current understanding

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Chapter 1

Morphological sub-types of ovarian carcinoma: new developments and pathogenesis

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Introduction

In most developed countries, ovarian carcinoma is the second most common malignancy of the female genital tract, following endometrial carcinoma. Most cases present at an advanced stage and the overall prognosis is poor. Although clinically often considered as one disease, there is an increasing realisation that the various morphological sub-types of ovarian carcinoma are associated with distinct molecular alterations and have different natural history and prognosis.[1–3] Most studies lump the various morphological sub-types of ovarian carcinoma together, with the result that it is difficult to tease out the behaviour of the various tumour sub-types; with our current state of knowledge, this is not appropriate. Given these factors, and the realisation that some tumour sub-types, for example clear cell, mucinous and low-grade serous, do not respond well to traditional ovarian chemotherapeutic agents and that continuing trials are investigating the efficacy of various agents in some of these tumour sub-types, it is clear that accurate pathological typing of ovarian carcinomas will be critical in the future in directing therapy. To this end, it is recommended that central pathology review becomes mandatory in ovarian carcinoma (and other gynaecological tumours) trials when treatment is dependent on the morphological sub-type or some other pathological parameter. In the UK, this should be organised by the British Association of Gynaecological Pathologists (BAGP) representative on the National Cancer Research Institute (NCRI) Gynaecological Cancer Clinical Studies Group.

In this review, I cover the major morphological sub-types of ovarian carcinoma, including their pathogenesis, and discuss problematic areas in typing. Although sub-typing of most cases is possible on morphology alone, immunohistochemistry may assist in problematic cases. Before discussing the major sub-types of ovarian carcinoma, I cover some general issues regarding the relative frequencies of the various tumour types and tumour grading.

Major morphological sub-types of ovarian carcinoma and relative frequencies

The major morphological sub-types of ovarian carcinoma are serous, endometrioid, clear cell and mucinous.[4] Two relatively recent population-based studies[5,6] that included central pathology review have provided updated information regarding the relative frequencies of the major sub-types (Box 1.1). It can be seen that serous is the most common, followed by clear cell, endometrioid and mucinous in that order. This represents a change from many older studies where mucinous carcinoma was the second most common sub-type and accounted for approximately 12% of primary ovarian carcinomas,[7] a much higher frequency than in the two more recent studies; reasons for this apparent reduction are discussed later. These recent studies also indicate an increase and a decrease in the frequency of serous and of endometrioid carcinoma, respectively; this is probably a reflection of the fact that the distinction between a high-grade serous and an endometrioid carcinoma was previously poorly reproducible[8–12] and there is now a realisation that many neoplasms that were previously diagnosed as advanced stage, high-grade endometrioid carcinoma were, in fact, of serous type (discussed later). When divided into early stage (stage I–II) and late stage (stage III–IV), it can be seen that serous, clear-cell and endometrioid carcinomas are approximately equally represented in early stage whereas almost all advanced stage neoplasms are serous in type (Table 1.1). To put it another way, a high percentage of clear-cell, endometrioid and mucinous carcinomas are early stage and in fact these tumour types (especially endometrioid and mucinous) are usually confined to the ovary at diagnosis (stage I).

The traditional management of advanced stage ovarian carcinoma has for many years been surgical debulking followed by adjuvant chemotherapy. However, in some cases, upfront chemotherapy is administered, especially in women with miliary disease or widespread metastasis where optimal debulking is not considered feasible;

Box 1.1 Relative frequencies of sub-types of ovarian carcinoma based on two recent population-based studies[5,6]

- 68–71% serous
- 12–13% clear cell
- 9–11% endometrioid
- 3% mucinous
- 1% transitional
- 6% mixed

Table 1.1 Early-stage (I/II) versus late-stage (III/IV) distribution of sub-types of ovarian carcinoma based on two recent population-based studies[5,6]

Sub-type	Stage I/II	Stage III/IV
Serous	36%	88%
Clear cell	26%	5%
Endometrioid	27%	3%
Mucinous	8%	1%

this should then followed by surgery after three cycles, with a further three cycles of postoperative chemotherapy. The morphological features of ovarian carcinomas treated by chemotherapy often differ markedly from native tumours. Post-chemotherapy, many ovarian carcinomas have abundant clear or eosinophilic cytoplasm and the nuclear features are often bizarre.[13,14] There may be no residual tumour or it may be difficult to identify tumour cells owing to a pronounced chemotherapy effect with marked fibrosis, necrosis, inflammation, cholesterol cleft formation, haemosiderin deposition and dystrophic calcification. Unless there is no or minimal response, it can be very difficult to type an ovarian carcinoma following chemotherapy and there is a tendency to misdiagnose some as clear-cell carcinoma owing to the abundant clear cytoplasm.[13,14] If upfront chemotherapy is being administered, a pre-chemotherapy tissue biopsy (usually a percutaneous radiologically guided biopsy) should be considered the standard of care for definitive typing, rather than relying on cytology of the ascitic fluid in combination with serum CA125 levels and imaging. This is also the recommendation in the National Institute for Health and Clinical Excellence (NICE) guideline on ovarian cancer.[15] The procurement of a tissue biopsy also means that material is available for additional studies in the future; for example, targeted therapies are likely to be developed against specific proteins and if tissue is available this will be useful in assessing whether the target protein is present in the tumour cells. Tissue obtained at various stages in treatment may also be useful in assessing tumour progression and response to therapy.

Grading of ovarian carcinomas

Several grading systems for ovarian carcinoma are in use but pathological grading is often poorly performed.[16] Most of the grading systems are universal in that they can be applied to all the major morphological sub-types of ovarian carcinoma.[17,18] However, there is an increasing tendency to use different grading systems for different morphological sub-types of ovarian carcinoma and this practice has been recommended in the Royal College of Pathologists Cancer Datasets in the UK.[19] Grading of the various sub-types of ovarian carcinoma is discussed with each specific tumour type.

Serous carcinoma

The perceived relationship between benign, borderline and malignant ovarian serous neoplasms was controversial and a source of confusion for many years. It has always been tempting to speculate that a continuum of ovarian serous neoplasia exists, from benign to borderline to malignant. However, pathological evidence for this is lacking and, until recently, it was generally assumed that there was no firm relationship between borderline and malignant serous neoplasms, although occasionally the two were found to coexist. Recent studies have shed significant light on this and have convincingly demonstrated that there are two distinct types of ovarian serous carcinoma, low grade and high grade.[20–25] Although termed low-grade and high-grade serous carcinoma, it is important to emphasise that these are not two grades of the same neoplasm but rather two distinct tumour types with different underlying pathogenesis, molecular events, behaviour and prognosis. High-grade serous carcinoma is much more common than low grade. Low-grade serous carcinoma is thought to arise in many cases in a stepwise fashion from a benign serous cystadenoma through a serous borderline tumour to an invasive low-grade serous carcinoma. Thus, there is a well-defined adenoma–carcinoma

sequence. However, it is relatively uncommon to see areas of invasive low-grade serous carcinoma within a serous borderline tumour and, conversely, in many low-grade serous carcinomas a borderline component is not seen. It is thus not proven that all low-grade serous carcinomas arise from a pre-existing borderline tumour and it is possible that some or many do not. In contrast, high-grade serous carcinoma is not related to serous borderline tumour and was thought until recently to arise directly from the ovarian surface epithelium or the epithelium of cortical inclusion cysts with no well-defined precursor lesion. There is now emerging and quite compelling evidence (discussed in the next section) that many high-grade ovarian serous carcinomas actually originate from the epithelium of the distal fimbrial portion of the fallopian tube.[26–31] Instead of grading ovarian serous carcinoma using a three-tiered system (well, moderate, poor; grade 1, 2 or 3), there is now a growing tendency to classify these as either high grade or low grade; this is the recommendation in the Royal College of Pathologists Dataset on ovarian neoplasms[19] and the classification of a serous carcinoma as low grade or high grade has been shown to be highly reproducible among pathologists.[32] Almost all serous carcinomas that would have previously been classified as moderately or poorly differentiated represent high-grade neoplasms, while those that would have been classified as well differentiated may be either low grade or high grade using the current classification. Although two distinct tumour types, on rare occasions a low-grade serous carcinoma may transform into a high-grade carcinoma or a high-grade serous carcinoma may arise directly from a serous borderline tumour.[33] However, most low-grade serous carcinomas, when they recur, do so as low-grade neoplasms. Some use the term invasive micropapillary serous carcinoma as an alternative to low-grade serous carcinoma; I would not recommend this terminology since some low-grade serous carcinomas do not have a micropapillary architecture while, conversely, many high-grade serous carcinomas do have a micropapillary growth pattern. Figure 1.1 illustrates the postulated pathways of development of low-grade and high-grade serous carcinoma.

The underlying molecular events differ between low-grade and high-grade serous carcinoma. Low-grade serous carcinoma is associated with *KRAS* or *BRAF* mutation in approximately two-thirds of cases.[26–31] These mutations occur early in the evolution of low-grade serous carcinoma since they are also found in borderline and benign areas within the same neoplasm. *KRAS* and *BRAF* mutations appear to be mutually exclusive; in other words one, but not both, may be present in a

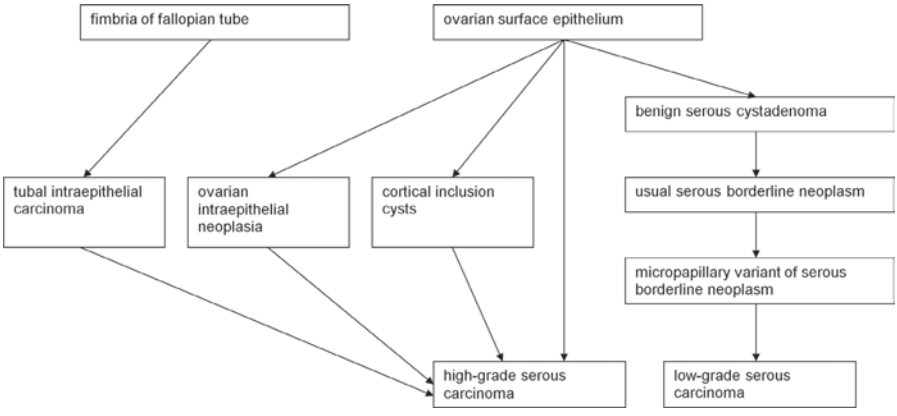

Figure 1.1 Postulated development of low-grade and high-grade ovarian serous carcinoma

particular neoplasm. Low-grade serous carcinoma is not associated with abnormalities of p53.[26–31] In contrast, high-grade serous carcinomas almost universally harbour p53 (*TP53*) mutations and this appears to occur early in neoplastic development;[26–31] these tumours are only rarely associated with *KRAS* or *BRAF* mutation. A study published in 2010 that used stringent techniques identified p53 dysfunction, nearly always representing mutation, in 97% of ovarian high-grade serous carcinomas.[34]

Evidence for the origin of high-grade pelvic serous carcinoma from the fallopian tube

There is convincing and accumulating evidence that many serous carcinomas of the ovary, fallopian tube and peritoneum currently classified as high grade (collectively referred to as high-grade pelvic serous carcinomas) are derived from the fimbria of the fallopian tube.[26–31,35] The initial evidence for this came from prophylactic salpingo-oophorectomy specimens from women with germline *BRCA1* or *BRCA2* mutation, who have a high risk of developing ovarian high-grade serous carcinoma. In initial studies, small high-grade ovarian serous carcinomas were occasionally identified. However, when pathologists began examining the fallopian tube in its entirety, it was found that there was more likely to be a small *in situ* or invasive high-grade serous carcinoma involving the fimbria of the fallopian tube; the *in situ* lesions are referred to as serous tubal intraepithelial carcinoma (STIC). This is relatively uncommon but is occasionally seen in prophylactic salpingo-oophorectomy specimens from women with *BRCA1* or *BRCA2* mutation. Further studies carefully examined the fallopian tubes in women with sporadic high-grade ovarian serous carcinoma and found similar lesions, usually STIC, involving the fimbria in a significant percentage of cases.[26,29] Furthermore, identical p53 mutations were demonstrated within the ovarian and tubal lesions. While this does not unequivocally prove that the origin is within the fallopian tube (this could represent a tumour within the ovary spreading along the fallopian tube), there is accumulating evidence that many high-grade pelvic serous carcinomas arise from the tubal fimbria. A p53 signature has also been identified in the fallopian tube.[26–31] This takes the form of small foci of intense p53 staining involving the secretory cells, most commonly of the fimbria, in the absence of morphological changes. p53 mutations have been demonstrated in some of these p53 signatures and these may represent the earliest stage of development of high-grade pelvic serous carcinoma. However, p53 signatures are extremely common in the fallopian tube even in women with benign disease and no hereditary predisposition to developing ovarian cancer and it is clear that only a small proportion will ever develop into a STIC.

It is probable that not all high-grade pelvic serous carcinomas are derived from the fallopian tube. A study published in 2010 that systematically examined the fallopian tubes in a consecutive series of ovarian carcinomas identified STIC in nearly 60% of high-grade ovarian serous carcinomas but not in other morphological sub-types of ovarian carcinoma.[29] It is possible that some high-grade serous carcinomas do arise from the ovarian surface epithelium or the epithelium of cortical inclusion cysts, the latter being lined by ciliated epithelium identical to that lining the fallopian tube. Another possibility in those cases in which no pre-malignant or malignant lesion is found in the fallopian tube is that tubal epithelium may exfoliate and become incorporated into the ovary and subsequently give rise to a high-grade serous carcinoma.

In summary, there is accumulating evidence that most high-grade pelvic serous carcinomas (high-grade serous carcinomas that are currently classified as ovarian, tubal or peritoneal in origin) arise from the fimbria of the fallopian tube. In most cases, the

malignant cells exfoliate from the fimbria into the pelvis and abdomen and result in the formation of an ovarian mass or masses with or without disease elsewhere in the pelvis and abdomen; this is conventionally referred to as ovarian high-grade serous carcinoma. In other cases, the malignancy remains localised to the fallopian tube, resulting in a fallopian tube high-grade serous carcinoma, or gives rise to extensive peritoneal disease in the absence of significant ovarian or tubal involvement; this is conventionally referred to as primary peritoneal high-grade serous carcinoma. It is likely that what are currently referred to as high-grade serous carcinomas of the ovary, fallopian tube and peritoneum are all different manifestations of the same disease and the designation high-grade pelvic serous carcinoma may be more appropriate. A consequence of these observations is that screening programmes for ovarian carcinoma may be relatively ineffective in downstaging high-grade serous carcinomas since these are most probably disseminated from the outset. However, it is possible that screening could be of value in picking up serous carcinomas when the burden of disease is lower. Future studies investigating the underlying molecular events in the development of high-grade pelvic serous carcinoma should concentrate on the distal fallopian tube.

Mucinous carcinoma

As mentioned above, primary ovarian mucinous carcinomas are relatively uncommon, with the two studies referred to earlier[5,6] suggesting that these account for only about 3% of primary ovarian carcinomas, a significantly lower percentage than in older studies. The reasons behind the apparent marked decline in primary ovarian mucinous carcinomas are well known. In older studies, it is likely that many presumed primary ovarian mucinous carcinomas, especially of advanced stage, were metastases from elsewhere. Advances in imaging, serum markers and preoperative workup have resulted in better recognition of metastatic ovarian neoplasms. Moreover, pathologists are now better at recognising the morphological features of metastatic mucinous carcinoma in the ovary,[1,36–42] including the well-known maturation phenomenon resulting in areas resembling benign and borderline mucinous cystadenoma. The use of differential cytokeratin staining and other immunohistochemical markers[43–50] has also improved the situation, although problems still exist. It is now clear that ovarian mucinous neoplasms associated with pseudomyxoma peritonei are almost always of appendiceal origin,[51–53] with the very rare exception of primary ovarian intestinal-type mucinous neoplasms arising in a dermoid cyst.[54] There has also been a redefinition of the criteria for diagnosis of a well-differentiated mucinous carcinoma with so-called expansile or non-destructive invasion and the distinction of this from a mucinous borderline tumour at the upper end of the spectrum with intraepithelial carcinoma;[1,36–38,55,56] this still represents a somewhat poorly reproducible area among pathologists and results in some variation in the reported prevalence of primary ovarian mucinous carcinomas between centres.

Most primary ovarian mucinous carcinomas are stage I and advanced stage neoplasms are extremely uncommon. In this scenario, a secondary neoplasm should always be strongly considered. One point I wish to make is that, although metastatic mucinous carcinomas in the ovary are still sometimes misdiagnosed as a primary ovarian mucinous carcinoma or even a mucinous borderline tumour owing to the pronounced maturation effect seen with some secondary mucinous carcinomas in the ovary, we have to some extent come full circle in that, in my opinion, there is now a tendency to overplay the possibility of a secondary neoplasm even when the pathological features are obviously those of a primary ovarian neoplasm. I feel

that, in a large majority of cases, the distinction between a primary and secondary mucinous carcinoma in the ovary can be achieved by careful pathological examination encompassing both the gross and microscopic findings and taking into account the distribution of the disease. It has been stated that, when a mucinous carcinoma is diagnosed in the ovary, further investigations such as colonoscopy and detailed imaging of the upper abdomen should be undertaken to exclude a primary neoplasm elsewhere. I feel this is unnecessary in most cases since, as discussed, basic pathological examination is usually sufficient to distinguish between a primary and secondary ovarian mucinous neoplasm.

Most primary ovarian mucinous carcinomas (and borderline tumours) are of the so-called intestinal (enteric or non-specific) type. A much more uncommon Müllerian type also exists.[57,58] Ovarian mucinous neoplasms of intestinal type comprise a spectrum or continuum from benign through borderline to malignant. In other words, intestinal-type ovarian mucinous carcinomas, like low-grade serous carcinomas, are thought to arise through a well-defined adenoma–carcinoma sequence from a benign cystadenoma through a borderline tumour to a mucinous carcinoma.[1,36–38] Similarly to low-grade serous carcinomas, ovarian mucinous tumours of intestinal type commonly exhibit *KRAS* mutations and identical mutations have been demonstrated in benign, borderline and malignant areas within the same neoplasm, suggesting that *KRAS* mutation is an early event in the evolution of these tumours.[59–61] Unlike the case with low-grade serous carcinomas, *BRAF* mutations are not a feature of ovarian mucinous neoplasms of intestinal type.

Invasion in primary ovarian mucinous carcinomas can be either expansile (non-destructive or confluent glandular) or infiltrative (destructive) in type;[1,36–38,55,56] the former is more common and is associated with a good prognosis. It may be difficult to diagnose a mucinous carcinoma with expansile invasion owing to the orderly growth pattern and absence of a stromal reaction and, as discussed, this is an area where there is significant inter-observer variability among pathologists. My criteria for the distinction between a borderline mucinous tumour with intraepithelial carcinoma and a carcinoma exhibiting expansile invasion are that the latter contains closely packed small- to intermediate-sized glands with a confluent back-to-back arrangement and no or minimal intervening stroma. A labyrinthine or cribriform growth pattern is also common and the epithelium should be cytologically malignant.[1]

Establishing a diagnosis of a mucinous carcinoma with infiltrative stromal invasion is straightforward but a secondary neoplasm should always be considered. It is stressed that primary ovarian mucinous tumours of intestinal type are commonly large and may be extremely heterogeneous within an individual neoplasm. It is not uncommon to see benign, borderline, borderline with intraepithelial carcinoma, and malignant areas side by side within the same neoplasm. Thorough pathological sampling is mandatory in such cases so that a small focus of invasion is not missed. Although there is no evidence base, the Royal College of Pathologists Ovarian Cancer Datasets recommend that primary ovarian mucinous carcinomas be graded in the same way as endometrioid carcinomas (see next section). Most primary ovarian mucinous carcinomas are well differentiated (grade 1), confined to the ovary and have a favourable prognosis. Advanced stage primary ovarian mucinous carcinomas have a very poor prognosis but are extremely uncommon.

As discussed above, most primary ovarian mucinous carcinomas (and borderline tumours) are of so-called intestinal type. The presence of goblet cells is not a prerequisite for an intestinal-type mucinous tumour and many of these exhibit gastric or pancreaticobiliary differentiation.[62] Intestinal-type ovarian mucinous neoplasms,

although typically positive with CK7, also commonly express, either focally or diffusely, enteric markers such as CK20, CDX2, CEA and CA19.9 and are negative with hormone receptors, CA125 and WT1.[63,64] CA19.9 especially is often diffusely positive and there may be elevation in the serum level of this marker.[65] Serum CA19.9 levels may be extremely high and are of no value in predicting preoperatively whether an ovarian mucinous neoplasm is benign, borderline or malignant.[65]

Endometrioid adenocarcinoma

Most, but not all, ovarian endometrioid adenocarcinomas are low grade and low stage (usually confined to the ovary). They often, although not always, arise from endometriosis (especially an endometriotic cyst) or a pre-existing borderline adenofibroma.[66,67] The reported prevalence of primary ovarian endometrioid adenocarcinoma is lower in recent than in older studies.[5,6] This is almost certainly due to the recognition that many neoplasms that would previously have been diagnosed as high-grade and high-stage endometrioid carcinomas are, in fact, serous in type. This is an area where previously there was poor reproducibility among pathologists and where WT1 staining may be useful (discussed later). With an endometrioid adenocarcinoma involving the ovary, there is not uncommonly a synchronous endometrioid proliferation, either pre-malignant or malignant, within the uterine corpus.[68]

In the Royal College of Pathologists Ovarian Cancer Dataset,[19] it is recommended that ovarian endometrioid adenocarcinomas be graded using the International Federation of Gynecology and Obstetrics (FIGO) system used to grade uterine endometrioid adenocarcinomas. Although less extensively studied, endometrioid adenocarcinomas of the ovary exhibit similar molecular events to those occurring in uterine endometrioid adenocarcinomas; these events include *PTEN*, β-catenin, *KRAS* and *PIK3CA* mutations and microsatellite instability.[69]

Clear-cell carcinoma

Clear-cell carcinomas have a characteristic morphological appearance typically consisting of an admixture of architectural arrangements including tubulocystic, glandular, solid and papillary. Hobnail cells and eosinophilic stromal hyalinisation are common. It is this admixture of architectural patterns that is more characteristic of clear-cell carcinoma than the presence of clear cells per se, cells with clear cytoplasm sometimes being a feature of both serous and endometrioid carcinomas. Most clear-cell carcinomas are diagnosed at early stage (stage I or II) and the majority arise in endometriosis. Careful pathological sampling, especially concentrating on cystic areas, will often reveal background endometriosis.

It is recommended that clear-cell carcinomas of the ovary be automatically graded as grade 3.[19] Since these neoplasms are often well differentiated architecturally, have a low cytological grade and are mitotically relatively inactive, formal grading may result in these being categorised as grade 1 or 2. The underlying molecular events in ovarian clear-cell carcinoma have not been extensively investigated[70] but a study published in 2010 identified *ARID1A* mutations in a significant percentage of cases.[71] It is generally assumed that clear-cell carcinomas are relatively resistant to the traditional chemotherapeutic agents used in the treatment of ovarian carcinoma and it is possible that this is because these neoplasms exhibit a low proliferation index.