REVIEW

The changing landscape of gynaecological cancer diagnosis: implications for histopathological practice in the 21st century

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The era of molecular medicine has led to dramatically improved understanding of the genetic events that give rise to different types of cancers. In the case of gynaecological malignancies, this has resulted in distinct shifts in how these tumours are diagnosed in routine surgical pathology practice, with an increased emphasis on accurate subtype diagnosis. This has happened across all sites in the gynaecological tract and for most cell types, but in ways that are site-specific and may appear to be subtle, as in most instances the diagnostic terminology has not changed. For example, the diagnosis of clear cell carcinoma of the ovary is still in use, but the diagnostic criteria and clinical implications are different in 2017 from what they were in 2000. As a result, there can be a failure to appreciate how important these changes are and the resulting necessity of incorporating them into our daily practice. In this review we will describe changes in diagnostic surgical pathology occasioned by improved understanding of molecular events during pathogenesis, for cancers of ovary/tube, endometrium, cervix and vulva, and highlight how current practice differs from that of only a few years ago.

Keywords: cancer, endometrial carcinoma, gynaecological classification, histopathology, molecular genetics, ovarian carcinoma, prognosis

Introduction

In the 19th century, extending into the early 20th century, there was great debate about whether microscopy was of value in the diagnosis of cancer, as in most cases a diagnosis could be made based on macroscopic examination.1 The debate ended in favour of histopathological examination being able to contribute meaningfully, a result borne out over subsequent decades, with microscopic examination providing many important insights; for example, the submacroscopic pathology of precursor lesions that forms the basis of cervical screening. The classification of cancer evolved from being based only on anatomical location to being subclassified further based on cell type. Thus, ovarian carcinoma was subdivided further based on the histopathological features of the cells and their resemblance to normal Müllarian cell lineages, such as serous or endometrioid. This was formalized in the 1975 World Health Organization (WHO) classification of tumours of the female genital tract.2

Further progress was possible in refining these subtypes based on routine histopathological assessment, but a fundamental limitation hindered progress; this was the lack of a gold standard to inform further
refinement of the subclassification and improve reproducibility of diagnosis. This can be illustrated by the example of serous versus endometrioid carcinoma of the ovary. There were large variations in practice, resulting in the irreproducible diagnosis of endometrioid, serous and mixed endometrioid/serous carcinoma of the ovary, but no way to determine which was the correct practice and move to more uniform diagnostic practice. In the absence of a reference standard, histopathological diagnoses cannot be assessed statistically by calculation of sensitivity, specificity and negative and positive predictive value; instead we are left measuring reproducibility, performed typically through calculation of kappa statistics. The diagnosis of carcinoma involving the ovary, for example, was highly reproducible, but subtype diagnosis, and even distinction between primary and secondary ovarian involvement, was irreproducible through the 1990s.

Molecular medicine started, arguably, with the development of robust techniques for immunostaining. This included improved reagents (murine monoclonal antibodies, with higher-affinity rabbit monoclonal antibodies appearing in recent years) and heat-induced antigen retrieval, improving the staining of formalin-fixed paraffin-embedded tissues dramatically. More recently, nucleic acid technologies have emerged and the high-throughput techniques, especially mRNA expression profiling and next-generation sequencing, have been applied to large numbers of human tumour samples. These latter two techniques work best with fresh or fresh frozen rather than formalin-fixed samples, but there has been progress recently in their application to formalin-fixed tumour tissue. Molecular-based subcategories of common cancers associated with prognosis, response to therapy or underlying hereditary cancer susceptibility syndromes were identified, such as basal-like breast carcinoma, which was recognized only through gene expression profiling. These molecular techniques were heralded as a replacement for microscopy in tumour diagnosis; predictions of the demise of histopathology have proved to be exaggerated but there is no denying the impact of molecular techniques on diagnosis, as will be discussed below.

The emergence of targeted therapy for cancer has been the biggest impetus for molecular-based subclassification of cancer. Adjuvant human epidermal growth factor receptor 2 (HER2)-targeted therapy emerged as the standard of care for breast cancers with overexpression/amplification of HER2 in 2005, at which point all breast cancers had to be tested at diagnosis. Another example is the demonstration of targeted therapy against mutant epidermal growth factor receptor (EGFR) in pulmonary adenocarcinoma. The cost and morbidity of the targeted treatments placed a premium on accurate testing of predictive markers and there is now a consensus that 90–95% sensitivity and specificity (at a minimum) should be the goal. It is interesting that the expectations for diagnostic classification far exceed the expectations around response rate when targeted therapies are given, which can be disappointingly low, and there remains much work to be conducted to improve the ability to predict those patients who will or will not respond to the therapy. None the less, the bar for reproducible subclassification of cancers has been raised permanently, and histopathological diagnosis of cancer subtypes in the 21st century should meet the same high standards that have been established for oestrogen receptor (ER) and HER2 testing in breast cancer, if these histopathological features are to be used to guide treatment: this should be our expectation and aspiration, otherwise histological subclassification serves no purpose. The progression from morphological subclassification, which often resulted in poorly reproducible categories with imprecise diagnostic criteria, gave way to distinct categories with supporting molecular markers, i.e. classification based on a combination of histology, immunohistochemistry (IHC) and molecular markers, as shown in Figure 1, with further progress possible to purely molecular categories.

The influence of molecular discoveries on diagnosis played out differently for each site in the female reproductive tract. Our aim is to highlight these differences and emphasize current standards of practice, and how we are now able to deliver highly reproducible subclassification of gynaecological malignancies that reflects the underlying molecular pathology accurately. Because the subclassification differs from site to site, this review will be in four sections: ovary/tube, uterus, cervix and vulva.
Ovary and fallopian tube

Carcinoma of the ovary, considered formerly to be a single disease with a common origin from the ovarian surface epithelium and considerable morphological variability, is now recognized to be five distinct ‘histotypes’: high-grade serous (HGSC), clear cell (CCC), endometrioid (EC), low-grade serous (LGSC) and mucinous (MC) carcinoma. These differ with respect to precursor lesions, patterns of spread, biomarker expression, survival and association with hereditary cancer syndromes. They also have very distinct underlying molecular abnormalities, and the ovarian carcinoma histotype diagnosis reflects those potentially targetable molecular changes. These differences between histotypes are summarized in Table 1,

The molecular underpinnings for the shift in diagnostic criteria started with WT1 immunostaining that demonstrated how most so-called high-grade endometrioid and transitional cell carcinomas were indistinguishable from HGSC (WT1-positive) but distinct from low-grade EC (WT1-negative). Another major breakthrough was recognition, on both morphological and molecular grounds, that HGSC and LGSC were readily separable, with the former showing abnormal p53 immunostaining in 95% of cases, which the latter almost never do. A final major shift was with the demonstration that fewer than 1% of ovarian carcinomas are truly of mixed type, i.e. two morphologically and immunophenotypically/molecularly distinct components, and most mixed carcinomas are admixtures of histotypes that are associated with endometriosis, i.e. EC, CCC, de-differentiated and seromucinous carcinoma, as well as seromucinous and, occasionally, serous borderline tumour. The histotype designations changed only subtly from the WHO 2003 classification, and it would be easy to underestimate the degree of change in practice. A recent study, comparing histotype diagnoses made in 2003 to those made by the same pathologist in 2014, however, shows the dramatic shift clearly: only 56% of cases had the same histotype diagnosis based on review using 2014 criteria, compared to the diagnosis from 2003. This does not indicate simple lack of reproducibility in diagnosis, as an independent review of the cases in 2014 by a second pathologist resulted in 98% agreement with the first pathologist, even though they practise in different countries, have different educational backgrounds and have not worked together previously. This highlights the very high degree of reproducibility of ovarian carcinoma histotype diagnosis when current diagnostic criteria are used, meeting the high expectations that we should have for any clinically actionable classification system.

The histotypes of ovarian carcinoma form the basis for assessment of risk of hereditary cancer syndromes; patients diagnosed with HGSC have an approximately 20% likelihood of an inherited BRCA1 or BRCA2 mutation, while those with EC or CCC have a risk of Lynch syndrome identical to that of patients presenting with endometrioid or colorectal carcinoma. To not screen patients presenting with HGSC for germline BRCA1 and BRCA2 mutations denies their female relatives, who may be mutation carriers, the possibility of highly effective risk-reducing surgery. It is also not rational to offer reflex mismatch repair (MMR) testing for colorectal and endometrial carcinomas without including ovarian EC and CCC in the reflex testing.

Accurate histotype diagnosis underpins targeted treatment. In this regard, using histotype to guide screening for hereditary breast and ovarian cancer syndrome should be distinguished from the current push by industry sponsors who manufacture Poly [ADP ribose] polymerase (PARP) inhibitors to perform reflex testing for somatic BRCA1 and BRCA2 mutations on all HGSC. This is being conducted to identify those patients eligible to receive palliative treatment with PARP inhibitors, even though BRCA mutation status is an imperfect predictive test; some patients without mutations respond to treatment while patients with mutations may not respond. It is an interesting commentary that universal BRCA mutation testing is more likely to enter practice to guide palliative treatment rather than to identify candidates for life-saving risk reducing surgery, based on a well-funded push by the pharmaceutical industry. Histotype-specific treatment in non-HGSC is in evolution, but with evidence that CCC respond to radiotherapy (while HGSC do not) there has been a move towards offering adjuvant chemoradiation in some centres for this histotype. For the chemoresistant LGSC, trials of alternative therapies are under way with up-front cytoreductive surgery being the cornerstone of treatment, as they are unlikely to respond to neoadjuvant chemotherapy.

Molecular diagnostics have led to improved diagnosis of adult granulosa cell tumour (aGCT) and small-cell carcinoma of hypercalcaemic type (SCCHT). Both were recognized based solely upon morphology. In the case of aGCT, a pathognomonic mutation (FOXL2 134W mutation) was identified through whole-genome sequencing of four cases. Characterization of cases drawn largely from the consultation files of Dr
Robert Scully showed that the mutation was present in 94% of aGCT, but not in other tumour types.\textsuperscript{40,41} We have shown subsequently that approximately 20% of cases diagnosed very consistently as aGCT in other centres are not aGCT, based on absence of the mutation and other clinical and pathological features, including patient outcome.\textsuperscript{42} In retrospect, if FOXL2 mutation testing had been applied to most archival series of aGCT rather than the very accurately diagnosed cases from the Scully consultation files, it would have been possible to conclude that presence of the mutation was a favourable prognostic feature, found in approximately 80% of cases of ‘aGCT’, had the histopathological diagnosis been held to be the ‘gold standard’. Instead, we now accept FOXL2 mutation as being pathognomonic of aGCT, and cases with atypical features should be subject to mutational analysis.\textsuperscript{42,43} This will make more accurate diagnosis of aGCT possible by all pathologists, to a level that in the past was attained only by very skilled and experienced experts.

The story of SCCHT is similar, in that the original cases were identified primarily in the consultation files of Dr Scully, based solely upon morphological

| Table 1. Features of the five major histological types of ovarian carcinoma\textsuperscript{19-24} |
|---------------------------------|-------|-------|-------|-------|-------|
| Mean age                        | HGSC  | CCC   | EC    | LGSC  | MC    |
| Frequency among stages III/IV OC* | 87.7% | 4.5%  | 2.5%  | 5.3%  | 1.2%  |
| Frequency among stages I/II OC*  | 35.5% | 26.2% | 26.6% | 1.9%  | 7.5%  |
| >10-year survival\textsuperscript{23} | 24.4\% | 58.7% | 59.7% | 24.4\% | 87%\textsuperscript{24} |
| 5-year survival (stage III only) | 40%   | 23%   | 66%   | 71%   | NA    |
| Origin                          | Fallopian tube | Endometriosis | Endometriosis | Fallopian tube | Germ cell, transitional epithelium |
| Molecular abnormalities         | Genomic instability; TP53 mutation; homologous recombination DNA damage repair defects; CCNE1, NOTCH3 activation; Rb, NF1 inactivation | Wnt-catenin activation; ARID1A-chromatin remodelling complex inactivation; PI3K activation; PTEN inactivation, MMR abnormalities | ERBB2/KRAS/BRAF/MEK pathway activation | ERBB2/KRAS/BRAF/MEK pathway activation |
| Inherited syndromes             | HBOC  | LS    | LS    | ?     | ?     |
| Sensitivity to platinum-based chemotherapy | Sensitive | Relatively resistant | Sensitive | Relatively resistant | Relatively resistant |
| Adjuvant/Targeted therapies licenced or in trial | PARP; immune checkpoint inhibitors | Radiotherapy; Sunitinib | mTOR inhibitors | Selumetinib (MEK1/2 inhibitors) | Trastuzumab |

HGSC, High-grade serous carcinoma; CCC, Clear cell carcinoma; EC, Endometrioid carcinoma; LGSC, Low-grade serous carcinoma; MC, Mucinous carcinoma; ARID1A, AT-rich interaction domain1A; BRAF, B-Raf proto-oncogene, serine/threonine kinase; CCNE1, Cyclin E1; ERBB2, Oestrogen-related receptor b2; HBOC, Hereditary breast and ovarian cancer; KRAS, Kirsten rat sarcoma viral oncogene homologue; LS, Lynch syndrome; MEK, Mitogen-activated protein (MAP) extracellular signal-related kinase (ERK) kinase; MMR, DNA mismatch repair; NA, Not available; NF1, Nuclear factor 1; PI3K, Phosphatidylinositol 3-kinase; PTEN, Phosphatase and tensin homologue; Rb, Retinoblastoma protein.

\*Cancers categorized as ‘other’ (other cancer) in Reference 20 are excluded from these rows.

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features.\textsuperscript{44} These features are quite variable, however, as exemplified by the large-cell variant of SCCHT, and accurate diagnosis is problematic given that most pathologists would see, at most, one case during their career. With recognition of the frequent mutation of SMARCA4 and concomitant loss of SMARCA4/BRG1 and SMARCA2/BRM protein expression,\textsuperscript{45,46} we now have specific and sensitive molecular markers that will allow accurate diagnosis (Figure 2). This is important, given that many cases have a hereditary basis.\textsuperscript{47,48}

**Uterus**

Endometrial carcinomas have been subclassified based on cell type, just as was performed for ovarian carcinoma, but molecular studies have made it clear that tumours of apparently the same histotype arising at different primary sites are not the same disease. This is most striking in the case of high-grade serous carcinoma of tubo-ovarian and uterine origin. The former commonly have BRCA but not PPP2R1A mutations, while uterine serous carcinomas (USC) have a very low frequency of BRCA mutations but frequently have PPP2R1A mutations.\textsuperscript{49,50} There are differences between endometrioid carcinomas of ovary and endometrium but they are more subtle, such that in an individual case it is not possible to determine the primary site based on molecular analysis.\textsuperscript{51} That carcinomas of ovary and endometrium can be indistinguishable morphologically does not indicate identity.

Endometrial carcinomas were divided into Types 1 and 2 tumours in the early 1980s, with the former associated with unopposed oestrogenic stimulation of the endometrium.\textsuperscript{52} The morphological correlates of Types 1 and 2 were shown subsequently to be low-grade endometrioid endometrial carcinomas (EEC) and high-grade USC, respectively.\textsuperscript{53} This classification never entered diagnostic practice, as there were too many cases that defied classification as either Types 1 or 2. With respect to histotype diagnosis, endometrial carcinoma has been plagued by poor reproducibility\textsuperscript{54–57} and the recognition that significant numbers of cases show ‘ambiguous morphology’,\textsuperscript{58} a situation that has not improved during the past 15 years, in contrast to the dramatic improvements in the reproducibility of ovarian carcinoma histotype diagnosis.

The genomic analysis of endometrial carcinomas performed through The Cancer Genome Atlas (TCGA) resulted in a four-part genomic classification of endometrial carcinoma: (i) ultramutated/polymerase ε (POLE) (characterized by mutations in the exonuclease domain (EDM) of DNA polymerase epsilon, POLE); (ii) hypermutated/MSI (characterized by microsatellite instability, MSI); (iii) low copy-number abnormalities; and (iv) high copy-number abnormalities,\textsuperscript{59} a classification with direct prognostic significance.\textsuperscript{60} Recent

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Figure 2. A, Small-cell carcinoma of the ovary hypercalcaemic type. B, SMARCA4/BRG1 loss in small-cell carcinoma of hypercalcaemic type (SCCHT). C, SMARCA2/BRM loss in SCCHT (Figure 2A–C courtesy of Dr Anthony Karnezis, Vancouver General Hospital).
studies incorporating molecular classification in high-grade endometrial carcinomas have demonstrated that the greatest lack of agreement in histotype diagnosis lies within the ultramutated/POLE, hypermutated/MSI and copy-number high/serous-like categories. The first group, ultramutated/POLE, accounting for 8–10% of cases, has been characterized recently as showing endometrioid morphology with high-grade or ambiguous features (Figure 3) but very favourable clinical outcome. The second category, hypermutated/MSI, has been better characterized over many years; features described as Lynch syndrome-related are ambiguous morphology and combinations of histotypes (including the presence of an undifferentiated component), isthmic location and lymphocytic infiltrates. MSI-related tumours demonstrate superior responses to adjuvant therapy and are potential candidates for immunomodulatory therapies.

It has become clear, therefore, that the only way to make endometrial carcinoma diagnosis reproducible and clinically actionable is to incorporate strategies that reflect the molecular subtype. The TCGA molecular classification system required very costly analysis of unfixed tumour samples, and this led to a search for surrogate markers of the TCGA molecular groups that could be used in routine clinical practice, i.e. inexpensive and applicable to formalin-fixed paraffin-embedded tissue. Sequencing for mutations in the POLE EDM is a surrogate for the ultramutated group, while either microsatellite instability testing or immunostaining for MMR proteins can serve as a surrogate for the hypermutated group, and are equivalent in terms of performance. TP53 mutational analysis or abnormal p53 immunostaining can serve as surrogate markers for the presence of high somatic copy-number abnormalities (CN-high), with the latter being less expensive and used more readily in practice. Thus iterative application of MMR IHC, POLE EDM sequencing and p53 IHC can serve to assign cases to one of the four major molecular subgroups. Implicit within such a move would be the rejection of purely morphologically defined entities such as the variants of EEC. While it is possible to recognize a range of appearances within a tumour histotype, incorporating these into a classification system serves no clinical purpose as they are poorly reproducible, present a bewildering array of terms that generate confusion among our clinical colleagues and have no prognostic or therapeutic relevance. It is not clear at present whether the full molecular classifier should be applied to all EC. For example, p53 IHC may not be needed for grade 1 EEC.

De-differentiated/undifferentiated carcinomas of the endometrium (UEC) were first described by Silva in 2006. Although distinctive, there remained challenges in distinguishing between solid pattern EEC or USC and UEC; molecular markers have emerged more recently. UEC have a distinct molecular profile, characterized by frequent MMR protein expression abnormalities (53–70% of cases), frequent loss of expression of one of the SWItch-sucrose non-fermentable (SWI/SNF) proteins and mutation of the corresponding gene, and wild-type p53 expression. This is a work in

Figure 3. Polymerase ε (POLE)-mutant endometrial carcinoma. A, These cases may show endometrioid architecture. B, Irregular luminal outline suggesting serous differentiation. C, Tumour cells show high nuclear grade. D, Bizarre tumour nuclei may be seen.
progress, but it seems highly likely that UEC will emerge as a distinct morphological/molecular entity.

The current WHO classification of endometrial sarcomas is a good illustration of a therapeutically relevant histological classification based on molecular insights.\(^{19}\) Endometrial stromal sarcomas (ESS) are defined as mesenchymal tumours composed of cells resembling those of non-neoplastic proliferative-phase endometrial stroma. These tumours characteristically have a rich arteriolar vasculature and demonstrate characteristic ‘finger-like’ myopermeative and intravascular growth. Poorly differentiated endometrial sarcomas comprise a distinct category and are composed of larger cells with nuclear hyperchromasia and pleomorphism, seen easily at low power. Following the original studies by Norris and Taylor in the 1960s,\(^{78}\) ESS were classified into low- and high-grade based on mitotic activity of \(<\) or \(\geq10/10\) high-power fields, with 100% versus approximately 50% 5-year disease-specific survival in the two groups.\(^{79,80}\) In 1990, Chang et al.\(^{81}\) published the largest series of ESS to date in which they showed that separation of these tumours on the basis of mitotic index alone was not prognostically relevant. This was reflected in the 2003 WHO classification of endometrial sarcomas into two categories: ESS, low-grade and undifferentiated endometrial sarcoma (UES), the latter showing marked cytological atypia and mitotic activity; in this classification there was no separation of a high-grade ESS category based on mitotic activity or otherwise.\(^{39}\) In retrospect, and in the light of recent molecular findings, it has become clear that using a combination of cytological atypia and mitotic activity, rather than either of these features alone, three distinct entities can be recognized.\(^{82}\)

The 2014 WHO classification places malignant endometrial stromal and related tumours into three categories: low-grade ESS (LGESS), high-grade ESS (HGES), and undifferentiated uterine sarcoma (UES, previously UES). These diagnoses carry different prognostic and therapeutic implications; LGESS are indolent, with a low risk of recurrence, which may occur several years following initial surgery, and have potential for responding to hormonal therapies, while UES are aggressive with no effective therapies identified currently; HGESS occupies an intermediate position, presenting typically at advanced stage, with frequent recurrences occurring within a few years after first surgery, and potentially responsive to adjuvant chemo- and radiotherapy.\(^{82}\)

Both LGESS and HGESS are karyotypically simple translocation-associated malignancies, in contradiction to the karyotypically complex UES. LGESS most commonly harbours a t(7,17)(p16;q21) translocation, resulting in a JAZF1–SUZ12 fusion gene encoding an abnormal oncoprotein that causes transcriptional dysregulation. Other translocations with different fusion products also occur, resulting in analogous effects on transcriptional regulation in endometrial stromal progenitor cells. The newly defined HGESS is characterized by a t(10,17)(q22;p13) translocation resulting in a YWHAE–NUT fusion gene,\(^{83}\) encoding an oncoprotein of the 14–3–3 family of ubiquitously expressed proteins which regulate a variety of cellular functions associated with cancer development and progression.\(^{84}\) The YWHAE–NUT HGESS is characterized by myoinfiltrative growth including vascular permeation resembling that in LGESS. The constituent cells, however, are higher grade, i.e. showing larger nuclei with high mitotic activity and irregular nuclear contours (Figure 4A). Depending on the amount of cytoplasm, these may appear as round blue cell tumours or more epithelioid. In approximately half of these cases, the tumour contains morphologically low-grade areas (Figure 4B), closely intermingled or clearly demarcated from the high-grade areas. Thus defined, YWHAE–NUT HGESS includes cases classified previously as low-grade ESS but showing nuclear atypia and high mitotic activity either focally or exclusively, as well as cases classified previously as UES but showing relatively uniform nuclei. In addition to morphology and cytogenetics, diagnosis is aided by IHC: unlike LGESS, HGESS or HGESS areas in a tumour with mixed morphological features lack significant ER, PR and CD10 expression, and are characterized by diffuse strong nuclear expression of cyclin D1 (Figure 4C).\(^{85}\) In cytogenetic terms UUS is characterized by a complex karyotype with widespread structural and numerical chromosomal aberrations. Morphologically, this is a diagnosis of exclusion after other specific uterine sarcomas, carcinosarcoma with a predominant mesenchymal component, undifferentiated carcinoma and other mimics have been ruled out.

**Cervix**

A purely morphology-based histological classification of endocervical adenocarcinomas has been carried forward in the 2014 WHO classification system. This illustrates a system with overlapping morphological features and imprecise cut-points between categories, lacking in reproducibility and prognostic significance and unrelated to underlying molecular abnormalities.
or aetiology. Such a system can be worse than no subclassification as it is meaningless, and hence likely to be ignored. There is no significant difference between human papilloma virus (HPV)-related ‘endocervical adenocarcinoma, usual type’, ‘endometrioid’ (‘an adenocarcinoma arising in the cervix that has endometrioid morphological features’) and ‘mucinous adenocarcinoma, NOS’ (not otherwise specified) (‘a mucinous adenocarcinoma that cannot be classified as any of the specific types of adenocarcinoma’); such terms are not used consistently by pathologists, the criteria for their application are highly subjective, and they are ignored by clinicians when planning patient management as being irrelevant. They are simply part of the morphological spectrum of HPV-associated cervical adenocarcinomas, with different patterns co-existing frequently in a single case (Figure 5); such descriptive subclassifications are pseudo-diagnoses, having the appearance of legitimate diagnoses but being essentially meaningless. They add nothing to our diagnostic reports and have served as a distraction, delaying recognition of the important subset of HPV-independent cervical adenocarcinomas, so that we have only an incomplete understanding of their natural history, range of morphological features and relationship to underlying genetic syndromes such as Peutz–Jegher’s syndrome. It is these latter cases of HPV-independent p16 IHC-negative (or only focally positive) cervical adenocarcinomas that will become more prominent as vaccination for HPV becomes more widespread, but our current classification system has served to obscure these uncommon tumours. As a group these HPV-independent tumours are reported to be more aggressive, suggesting that these should be singled out for more radical management at the time of diagnosis, but lack of accurate subclassification of cervical adenocarcinomas as HPV-associated or HPV-independent has hampered systematic prospective investigation. The problem for cervical squamous cell carcinoma (SCC) is less acute, as HPV-independent cervical SCC appears rare, although its subclassification is also rife with meaningless morphological categories. Just as for variants of EEC, it is time to drop meaningless morphological subclassifications of HPV-associated cervical carcinomas.

**Vulva**

There are two pathways of vulvar squamous cell carcinogenesis, and the *in-situ* and invasive diseases associated with each pathway show distinctive but overlapping and therefore not pathognomonic features, first described by Abell more than 50 years ago. With the discovery of the aetiological role of
HPV in squamous neoplasia, the pathways could be identified as being HPV-associated and HPV-independent: tumours arising from these pathways differ with respect to aetiology, risk factors, precursor lesions, age at diagnosis, natural history, morphology and response to treatment (Table 2).97–99 Routine histopathological examination can identify cases correctly as HPV-associated [basaloid or warty invasive carcinoma, high-grade squamous intraepithelial lesion (HSIL)] or HPV-independent [well-differentiated keratinizing invasive carcinoma, differentiated vulvar intraepithelial neoplasia (dVIN)] in 81% of cases.100 While this is reasonably accurate, it is not accurate enough if this designation is to be used to guide treatment.97 In order to be clinically actionable this distinction has to be more robust, and p16 immunostaining has emerged as a good surrogate for nucleic acid-based testing.100,101 Polymerase chain reaction (PCR) for HPV DNA is sensitive but not specific, while in-situ hybridization (ISH) is specific but not as sensitive; both techniques are also more expensive and have a longer turnaround time than an immunostain. p16 immunostaining, looking for ‘block’ positivity with moderate to strong staining of the nucleus and cytoplasm of all cells in the lower-most third of the epithelium as an indicator of oncogenic HPV infection, has excellent performance characteristics (100% sensitive, 98–99% specific)100,102 and is implemented easily in practice. There are very occasional lesions (1% of cases) where there is strong p16 positivity but no evidence of HPV.100 These cases can be resolved by application of HPV ISH or other molecular techniques when the clinical scenario and morphological features suggest HPV-independent disease, but there is strong p16 immunoreactivity.

There has been a step towards classifying vulvar squamous neoplasia routinely as HPV-independent versus HPV-associated, as the precursor lesions are diagnosed differently based on WHO/Lower Anogenital Squamous Terminology (LAST),19,103 with the designation HSIL (VIN2/3) for HPV-associated and dVIN for at least a subset of HPV-independent in-situ lesions, and p16 serves to distinguish between these possibilities. A problem with the existing classification
of VIN is that not all in-situ lesions of HPV-independent squamous cell carcinoma show a high degree of cellular differentiation, as suggested by the designation dVIN. There are two possible pragmatic solutions; such in-situ lesions can be diagnosed either as dVIN (noting in the microscopic description that there is moderate or greater nuclear atypia) or the designation 'HPV-independent high-grade VIN' could be adopted.

With regard to invasive squamous cell carcinoma (SCC) of the vulva, it is now time to designate this routinely as HPV-independent or HPV-associated; the practice in gynaecological pathology lags behind that of head and neck pathologists, where p16 immunostaining is carried out routinely to allow accurate subclassification of oropharyngeal SCCs. Recent studies demonstrate that, like oropharyngeal HPV-associated SCC, HPV-associated vulvar squamous cell carcinoma (VSCC) shows better prognosis with lower recurrence rates and disease-specific mortality.

It could be argued that HPV status does not influence treatment of vulval neoplasia, so why do it; this is circular (and defeatist) reasoning. The only certain way to keep HPV status from guiding patient management for vulvar carcinoma, given that it is of prognostic significance and is used at another body site in planning treatment, is to continue not to determine and report the HPV status routinely.

Conclusions

It is becoming apparent that histological classification systems for cancers in the 21st century must have two attributes: first, they must reflect tumour biology and behaviour, and secondly they must be highly reproducible in routine practice. Purely morphological categorization of tumours is only justifiable if this reflects tumour biology accurately, as in the case of ovarian carcinomas. Histotype diagnosis, when it provides only arbitrary, irreproducible and clinically irrelevant distinctions between categories (e.g. HPV-associated endocervical adenocarcinoma classification), must give way to systems that reflect behaviour accurately or predict response to a particular therapy. That this has not occurred naturally in gynaecological cancers begs the question of why, and a wide range of reasons is encountered for not adopting the practices recommended above.

A common reason is that subclassification is not requested by clinicians or does not affect patient management (e.g. ovarian carcinoma is a single disease/treated the same way). Histopathologists must not forget that we are consultant physicians, and we determine how best to process and further analyse samples submitted to our laboratories; if we believe that further testing is indicated, it is our responsibility to carry this out on behalf of our patients. It is the discoveries made by pathologists that demonstrated cervical precancerous changes and the origins of HGSC, each of which has resulted in development of life-saving cancer prevention strategies. Rather than waiting until we are asked by our clinical colleagues, we should do what we are responsible for in the light of new knowledge. By contrast, if we do not change our practice, we could be held responsible in the future for withholding information and hampering progress which influences patient management and outcomes materially.

An important barrier is the expense of adopting a wider panel of IHC or a nucleic acid-based test in routine practice. This can be offset by ensuring that there is no wastage; given the frequency with which CK7 and CK20 are carried out, to no effect, on
omental biopsies from patients who clinically have HGSC [adnexal mass(es), ascites, omental involvement], it should be possible to save enough within the IHC laboratory budget to cover the costs of more relevant testing. As another example, the panels of immunostains performed frequently to distinguish USC from EEC would be better replaced by MMR and p53 immunostaining.

It has been argued that we should have patient consent for MMR testing. This issue has been debated for years, and is now resolved. It is now agreed widely that MMR immunostaining is a screening test and not a definitive genetic test (which is carried out only with patient consent); it has the same implications in a pathology report as describing an endometrial carcinoma of the lower uterine segment with a prominent lymphoid infiltrate as being possibly Lynch syndrome-related, something we would be remiss in not reporting. Furthermore, MMR immunostaining now has additional diagnostic significance for identification of MSI/hypermethylated endometrial carcinoma, another impetus for universal testing. In a similar way it may be felt that reporting HPV status on vulvar cancer specimens could stigmatize patients. In an era where we are moving to primary HPV testing for cervical screening and identify head and neck cancers routinely as being HPV-associated or -independent, in our opinion this issue no longer applies.

In summary, we have highlighted molecular-based advances that have enabled reproducible subclassification of common malignancies of the female reproductive tract. The subclassification schemes vary based on site, from almost purely morphologically based for ovarian carcinomas to morphological, with biomarker use in selected cases (cervix and vulva), to a purely biomarker-based classifier for endometrial carcinoma. All can be implemented in routine practice, which will continue to evolve as new entities are discovered and new techniques and algorithms validated. For the present it is crucial to recognize both the strengths but also the limitations of a purely morphological diagnosis and not to hesitate to use ancillary techniques where morphology is known to be insufficient. A fundamental driver of these changes is the development of treatments individualized to the patient to maximize survival and quality of life after a devastating diagnosis of cancer. While every member of the team has to keep up with the changing landscape and allow a responsive evolution of their practice, the pathological diagnosis is central to these changes, and more than ever before the focus is upon the histopathologist.

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