Precancerous Lesions of the Gynecologic Tract

Diagnostic and Molecular Genetic Pathology

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Preface

Many malignancies have a morphologically recognizable precursor lesion, a fact that at least theoretically offers the opportunity to intercept the malignancy before its development or to diagnose and treat it at an early stage. The cervix represents an enduring model for using precancerous lesion-centered screening and management programs to reduce the mortality and morbidity associated with a cancer. In the larger female genital tract, precancerous and putative precancerous lesions abound, and the past several years has seen the description of new lesions as well as an evolution in our diagnostic approach to, and understanding of, the long-existing ones. In this book, we aim to produce a comprehensive overview of precancerous lesions of the gynecologic tract, authored by an international group of authors well versed in the various areas. The chapters are arranged in broad, organ-based subsections that should facilitate their review. Contributors were encouraged to discuss lesions that are well established as precancerous lesions, such as the squamous intraepithelial neoplasms of the lower genital tract as precursors of squamous cell carcinomas at these sites, as well as the more newly reported putative precursors, such as atypical lobular endocervical glandular hyperplasia as a precursor for cervical adenocarcinomas exhibiting gastric differentiation. In each chapter, emphasis is placed on diagnostic pathology as well as on those aspects of their molecular pathology that may illuminate the pathogenesis of each lesion described. There is a separate chapter on the cytopathology of precancerous lesions in the cervix, and there are two chapters on the clinical management of precancerous lesions in the gynecologic tract. It is my hope that this text will be a valuable resource to gynecologic pathologists, residents, students, and other interested medical practitioners on the current state of knowledge on precancerous lesions of the gynecologic tract.

San Diego, CA — Oluwole Fadare, MD

Acknowledgments

The successful completion and publication of this text is a testament to the hard work of the contributors, who have all taken the time to produce well-illustrated and comprehensive treatises that would hopefully enhance the reader's understanding of the various precancerous lesions that are covered. I would like to express my thanks to trainees, mentors, and collaborators, all of who continue to inspire new questions about gynecologic pathology and enhance my understanding of old ones. Finally, my never-ending appreciation goes to Abby Fadare, for everything.

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Part I

Ovary, Fallopian Tube and Peritoneum

Precursors of High-Grade Serous Carcinoma

1

Patricia A. Shaw, Blaise Clarke,
and Sophia H.L. George

Abbreviations

BRCA1	Breast cancer 1, early onset
BRCA2	Breast cancer 2, early onset
Ca125	Cancer antigen 125
FTE	Fallopian tube epithelium
HGSC	High-grade serous carcinoma
LGSC	Low-grade serous carcinoma
MEP	Mucosal epithelial proliferation
OSE	Ovarian surface epithelium
PTEN	Phosphatase and tensin homolog
Rb1	Retinoblastoma 1
RRSO	Risk-reducing salpingo-oophorectomy
SCOUT	Secretory cell outgrowth
SEE-FIM	Sectioning and extensively examining the fimbriated end
STIC	Serous tubal intraepithelial carcinoma
STIL	Serous tubal intraepithelial lesion
TP53	Tumor protein p53

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Introduction

High-grade serous carcinoma (HGSC) is the most common and deadliest of epithelial ovarian cancers, accounting for about 70 % of all ovarian carcinomas and approximately 90 % of advanced stage II/IV ovarian cancers. It is typically diagnosed in perimenopausal and postmenopausal women, presenting with advanced stage disease. HGSC is not associated with signs or symptoms of early disease, and there is no effective screening test that influences long-term outcome. Advances in primary cytoreductive surgery, improved delivery of platinum-based first-line chemotherapy, and development of innovative targeted therapies have increased the progression-free and overall survivals, but despite these advances, most patients eventually recur and die of their disease, with a 60 % mortality at 5 years [1].

A consistent impediment to reducing mortality in HGSC has been the inability to diagnose HGSC at an early and potentially curable time in the disease course. Large trials using both transvaginal ultrasound and the serum marker CA125 failed to demonstrate an impact on mortality when using both modalities for ovarian cancer screening [2]. While new targeted therapies offer promise in the improved management of HGSC, it is possible that there will not be a major impact on HGSC mortality without more effective preventative and

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Table 1.1 Summary of reported protein expression and genomic alterations demonstrated in the p53 signature, STIL, and STIC lesions in comparison to HGSC

Alterations	P53 signature	Serous tubal intraepithelial lesion (STIL)	Serous tubal intraepithelial carcinoma (STIC)	High-grade serous carcinoma
P53	Mutated (LOH)	Mutated	Mutated (LOH)	Mutated (LOH)
BRCA1/BRCA2			Mutated/LOH	Mutated/LOH/ hypermethylated
Genes (FISH)			CCNE1 Amp hTERT Amp	CCNE1 Amp hTERT Amp
Protein expression	$Pax8^+$, $Bcl2^+$, $Pax2^-$, $\gamma H2AX^+$	Up—CCNE1, p16, stathmin	Up—CCNE1, Rsf1, FASN, p16, stathmin, HMGA2, increase in $CD68^+$ cells Down—LKB1, FoxoA3, Rb1	Up—CCNE1, p16, stathmin, Rsf1, FASN Down—LKB1, FoxoA3, Rb1
Chromosomal copy number alterations		Deleted—1p31.1, 1q21.1, 6p21.3, 8p11.2, 11q12.3, 12p13.3, 12q24.3, 15q11.2 Amplified—3q26.1, 4q13.2, 14q32.2, 20q13.2	Deleted—2p14, 2q31.1, 3q22.3, 3q26.3, 6p21.3, 6p11.2, 11q12.2-13.3, 18q12.1, 19p13.1, 20p13-p11.2, 22q13.3 Amplified—4p16.3, 8q13.1-q24.3, 10q26.3, 11q15.5-15.4, 12a12-13.1, 12q24.33, 12q24.4, 16q13.3 18 19q13.2-13.43	Extensive genomic rearrangements (TCGA 2011)

screening modalities. Consequently, there is a need to understand the molecular and genetic events which precede clinically evident HGSC. Until relatively recently, this was a serious challenge, in large part because there was no known histological precursor of HGSC. More knowledge about the classification and the natural history of the HGSC precursors, now recognized to exist in the fallopian tube, and their rate of progression to invasive carcinoma, is required before more effective early detection strategies can be developed [3, 4].

Historical Perspective

Classification of Serous Carcinoma

Until recently, the most common type of (surface) epithelial ovarian cancer was classified as serous carcinoma (also known previously as papillary serous carcinoma, papillary serous cystadenocarcinoma) and graded using various three-tiered systems which were subjective and predicated loosely on architectural patterns and/or nuclear pleomorphism; the Silverberg–Shimizu grading system did apply objective criteria based on scoring of architecture, nuclear pleomorphism, and mitotic count. Despite the lack of consistency of grading, tumor grade was considered to be a prognostic factor in serous carcinoma.

Unlike endometrioid and mucinous carcinoma histotypes, most cases of serous carcinoma were not believed to arise in association with serous borderline tumors. In 1996, Kurman and his collaborators described a subset of serous borderline tumors that were at increased risk of recurrence and death [5]. The term *noninvasive micropapillary carcinoma* was proposed to separate these tumors from the usual type of serous borderline tumor and to link it to the invasive counterpart, a cytologically low-grade carcinoma which we now recognize as low-grade serous carcinoma (LGSC). Subsequent molecular pathology studies identified an increased frequency of *K-ras* mutations in LGSC and its precursors, but not in the more conventional serous carcinoma, which we now diagnose as high-grade serous carcinoma [6]. In contrast, mutations of the tumor suppressor

TP53 were identified in most HGSC, now known to be present in 98 % of HGSC, but were not seen in LGSC [7]. At about the same time, Malpica and Silva described a two-tier grading system based on cytological atypia and mitotic count which stratified ovarian carcinoma into two prognostic groups. They also noted the association of the low-grade tumors with borderline tumors [8]. In light of these findings, Kurman proposed that serous carcinoma be divided into two distinct entities with two different pathways of tumorigenesis. This was the first step in elucidating the pathogenesis of HGSC.

The dualistic model of ovarian cancer, developed by Kurman et al., proposed that Type I cancers, including endometrioid, mucinous, clear cell, and low-grade serous carcinomas, have a stepwise progression from benign to borderline and to carcinomas, are slow growing, are diagnosed at an early stage, and have a relatively indolent course. In contrast, the Type II tumors, of which high-grade serous carcinoma is the most frequent example, are aggressive, rapidly growing, and present at an advanced stage of spread, with poorly understood precursor lesions [9]. Morphologically, high-grade serous carcinoma has variable architecture, including the classic papillary pattern, often with bridging and fusion of papillae resulting in slit-like spaces, solid, pseudo-endometrioid glandular pattern, and transitional-like pattern. Multiple patterns may exist in any one tumor. All HGSC have high-grade nuclei and a high mitotic rate, usually with more than 25 mitoses per 10 high power fields. Molecularly, unifying features of HGSC include mutation of the tumor suppressor gene *TP53*, chromosome instability, and a high proliferation rate, frequently associated with alterations of the retinoblastoma pathway [7, 10, 11].

HGSC Cell of Origin

HGSC has no known morphologic precursor lesion in the ovary, and therefore, little was known of the early molecular/genetic events of serous carcinogenesis, a major obstacle in identifying markers of predisposition or of early stage ovarian cancer. Epidemiological studies of ovarian cancer risk factors, such as infertility, and protective factors, such as increased parity and oral contraceptive use, suggested that an increased number of lifetime ovulations may play a role in the development of ovarian carcinoma [12–15]. The importance of reproductive risk factors seemed to support the traditional theory of ovarian cancer origin, the incessant ovulation theory, first proposed by Fathalla in 1971 [16]. In the absence of native epithelium in the normal ovary, the ovarian surface epithelium (OSE) was the favored ovarian cancer cell of origin. OSEs are a modified mesothelial layer, express calretinin, are PAX8 negative, and are non-ciliated, with few immune cells associated with the monolayer [17]. It was suggested that with each ovulatory event, OSE damage and subsequent repair eventually led to acquisition of genetic abnormalities leading to carcinogenesis. This theory required that OSE undergo metaplasia to an epithelial cell type prior to the events of malignant transformation, often within ovarian cortical epithelial inclusion cysts [18]. In further support of the OSE cell of origin, there were reports that (1) atypia (reported as dysplasia or ovarian intraepithelial neoplasia) was present in surface epithelium adjacent to early stage ovarian carcinoma, (2) an increased number of cortical inclusion cysts were present in nonmalignant ovaries contralateral to ovarian cancer (histotype not specified), and (3) OSE adjacent to ovarian cancer had a higher incidence of metaplastic and hyperplastic changes [19–22]. For the most part, however, common histological changes associated with cancer precursors, such as cytological atypia, cellular stratification, and mitotic activity, have not been identified or of present are extremely rare in the ovary, indicating that if OSE was indeed the cell of origin of ovarian serous carcinoma, any precursor lesion must be very transient or the molecular progression of the disease is not associated with a morphological counterpart. Most of the publications describing early cancers or non-invasive cancer precursors within the ovary were reported prior to the routine histological examination of the fallopian tube. It is possible that the few images published purporting to represent

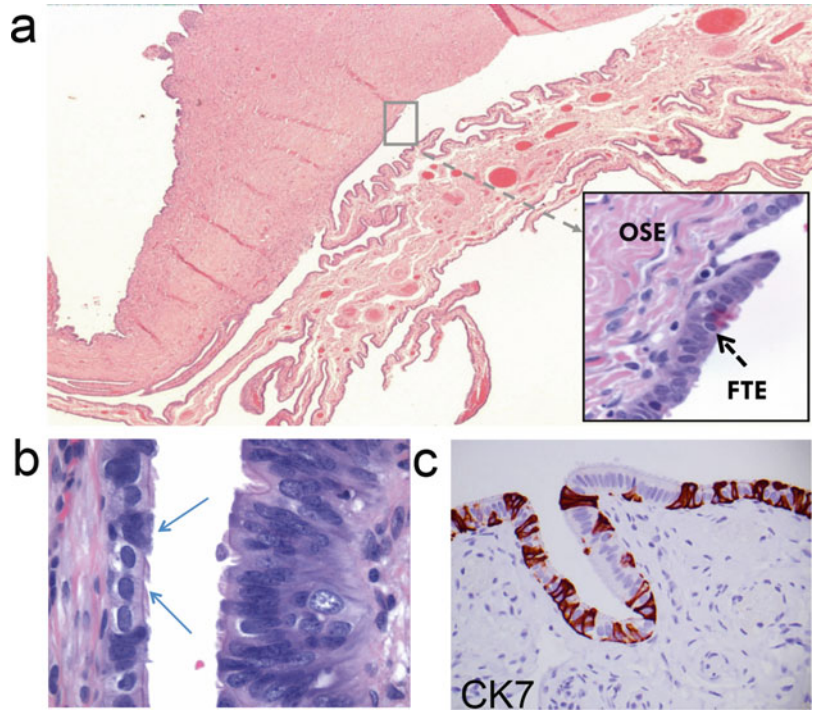

Fig. 1.1 Normal fallopian tube. (**a**) Single plica of the tubal fimbria is attached to the ovarian surface. The picture insert shows a higher magnification with a transition from tubal-type epithelium (FTE) to ovarian surface epithelium (OSE). (**b**) Normal tubal mucosa with varied stratification and a mixture of ciliated and secretory cells. (**c**) CK7 immunohistochemistry highlights the distribution of secretory cells (CK7 positive) and ciliated cells (CK7 negative)

dysplasia and early intraepithelial carcinoma within cortical inclusions actually represent cancerization of cortical cysts [23].

Convincing evidence in support of the fallopian tube epithelium (FTE) as the cell of origin emerged as pathologists began to examine the ovaries and fallopian tubes after risk-reducing surgery in women at high genetic risk of ovarian cancer. The two candidates for cell of origin, FTE and OSE, share common embryological origin, as well as close anatomic proximity. The Mullerian duct, which forms the fallopian tube, uterus, and endocervix, is formed by invagination of the embryonic coelomic epithelium, which also gives rise to mesothelium, including the OSE. The tube, lined by the hormonally sensitive FTE, has functions in ovum pickup and transport, facilitation of fertilization, and support of preimplantation embryo development in the first days after fertilization. It is divided into the interstitial portion (within the uterus), the isthmus, the ampulla, and the fimbria. Of particular note, and an important candidate for the source of serous carcinoma, is the FTE covering the fimbria, which has fingerlike projections in close contact to the OSE and ovarian surface and directly exposed to the peritoneal cavity (Fig. 1.1). It is of interest to note that genetic mouse models deleting BRCA1, Rb1, and *TP53* genes from the OSE resulted in leiomyosarcomas [24] not high-grade serous carcinoma, and in contrast, targeted deletion of *BRCA1*, *TP53*, and *PTEN* in fallopian tube epithelia resulted in high-grade serous carcinoma with phenotypic and genomic alterations congruous with human HGSC [25].

BRCA and HGSC

A major advance in our understanding of HGSC tumorigenesis was the discovery in the mid-1990s that more than 90 % of hereditary ovarian cancers were associated with inherited germline mutations of *BRCA1* and *BRCA2*. BRCA1/BRCA2 proteins have multiple functions including a critical role in the repair of double-stranded

DNA breaks through homologous recombination. Loss of either BRCA1 or BRCA2 protein leads to deficient double-stranded DNA repair, which in turn increases the risk of chromosomal rearrangements. The lifetime risk of developing ovarian cancer is 40–60 % for *BRCA1* mutation carriers and 10–20 % for *BRCA2*. Epidemiological studies indicated a predominance of HGSC in hereditary ovarian cancer, with a lack of mucinous carcinomas and borderline tumors [26, 27]. Blinded histopathological review using current definitions of histological classification demonstrates that the BRCA-deficient cancers are exclusively HGSC type [28–30].

The efficacy of risk-reducing salpingo-oophorectomy in women at high risk based on family history or germline mutations of *BRCA1* or *BRCA2* in preventing HGSC has been demonstrated, reducing the lifetime risk of HGSC to 5 % and decreasing mortality from all causes by 77 % [31, 32]. Because women with *BRCA* mutations are at the highest risk of developing HGSC, one might expect that if morphologic precursors of HGSC exist within the ovary, they would be detected in prophylactic oophorectomy specimens. However, a preliminary non-blinded report of histological features differentiating "cancer-prone" ovaries from ovaries at lower risk was not validated by other authors in more carefully controlled studies, nor was a reproducible histologic cancer precursor identified in the ovaries from high-risk patients [20, 33–38].

The addition of carcinoma of the fallopian tube to the list of BRCA1-/BRCA2-associated malignancies led to more comprehensive examination of the fallopian tube in prophylactic specimens. Support for the role of FTE in HGSC tumorigenesis was soon discovered, by the discovery of occult carcinomas and descriptions of putative cancer precursors in the fallopian tube epithelium [39–46].

Historically, precursor lesions of FTCa were not well defined, and the terms mucosal epithelial proliferation (MEP), hyperplasia, and dysplasia have been applied variably in the literature to histological lesions of the FT epithelium (FTE). Mild hyperplasia of the FTE was reported to be a frequent finding and considered to be a normal, non-pathologic finding, but epithelial proliferation with nuclear atypia, which was described in moderate and severe MEP of FTE, was abnormal [47].

The finding of occult cancers and cancer precursors in the fallopian tubes of women at genetic high risk of serous carcinoma indicated an important and previously unsuspected role of the tubal epithelium (FTE) in *BRCA* mutation-associated serous carcinogenesis. These findings suggested an etiology of hereditary serous carcinoma other than that of malignant transformation of OSE cells. Early spread from a small clinically undetected carcinoma of the tubal fimbria, which is in direct contact with the peritoneal cavity and with the ovarian surface, would explain the lack of early detection by current technologies and the formation of ovarian masses, because the ovary is a fertile soil for growth of metastatic carcinomas from multiple sites. It would also explain the frequent peritoneal spread early in the disease course and many cases of presumed primary peritoneal carcinoma. While the observations leading to this hypothesis pertain to carriers of germline mutations, it seemed possible they would also apply to the more common sporadic serous carcinomas, which share molecular alterations with hereditary epithelial ovarian cancer, including loss of function of BRCA proteins [10, 48, 49].

High-Grade Serous Carcinoma, Occult

The rare finding of clinically incidental serous carcinoma was first reported in 1965 and was described further in a larger series by Bell and Scully in 1994[50]. It was noted in these early reports of "early de novo carcinoma" that despite the small size of the lesions, present within the ovarian cortex or on the ovarian surface and measuring up to 7 mm, an adverse outcome including recurrence and death was possible. The increase in prophylactic surgery in mutation carriers and the more comprehensive histological examination of salpingectomy specimens in recent years has led to the discovery of an increasing number of low-volume cancers at an early stage.

By definition, occult carcinoma is not detected preoperatively, and transvaginal ultrasound and serum CA125 are frequently reported as normal

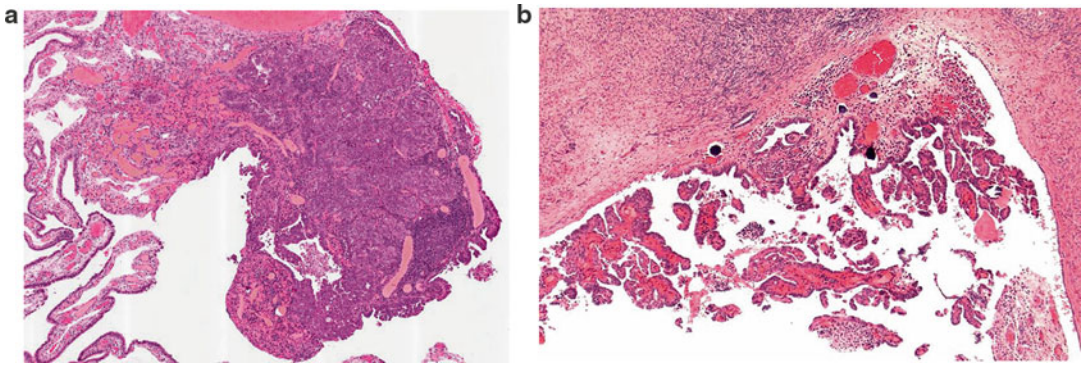

Fig. 1.2 Occult invasive carcinoma. (**a**) High-grade serous carcinoma in the tubal fimbria, measuring 1.6 mm, with adjacent serous tubal intraepithelial carcinoma. (**b**) Surface deposits of carcinoma on the ipsilateral ovary. The patient was a 44-year-old BRCA1 mutation carrier. Reprinted with permission [45]

within the year prior to risk-reducing salpingo-oophorectomy (RRSO). The first reports of occult carcinoma in *BRCA* mutation carriers indicated a range of 2.3–10.4 % incidence of occult carcinoma at the time of RRSO, and there was a surprisingly high incidence of carcinomas involving the distal end, fimbria, of the fallopian tubes [41, 45, 51, 52]. Ovarian involvement was also detected, but, in at least some of the cases, if the tube was carefully examined, the ovarian involvement was metastatic from the fallopian tube (Fig. 1.2). The frequency is higher in reports when examination of the fallopian tubes and ovaries is performed by meticulous fine sectioning and by consistent review limited to gynecologic pathologists at a single institution and lower in multi-institution studies without centralized pathology review. The incidence also varies with age of the patient at the time of RRSO and is more frequent with documented germline mutations of BRCA1/BRCA2 and more frequent with *BRCA1* than *BRCA2* mutations; it varies with the type of mutation (known deleterious, etc.) and, according to some studies, on whether the patient has a prior history of breast cancer [53, 54].

Macroscopic

In the majority of cases, careful macroscopic examination will be unremarkable, but if visible, small pale nodules may be detected involving the fimbria, distal fallopian tube, or ovary, usually the ovarian surface. Most cases involve the fallopian tube or the fallopian tube and ovary, with a minority of cases involving only the ovary. Even though these tumors are not detected clinically, the stage of the carcinoma ranges from stage 1A to 3C [32, 51, 55, 56].

Microscopic

Occult carcinomas in RRSO specimens are usually HGSC [45, 54, 55]. Like clinically evident HGSC, architectural features vary, but they often have a mixed solid/papillary architecture. Because many of the carcinomas involve the distal end of the fallopian tube, there may also be deposits of tumor on the ovarian surface (Fig. 1.2).

Outcome

The carcinomas involving the fimbria, which are in close contact with the ovarian surface and are directly exposed to the peritoneal cavity, may be associated with microscopic spread to the ipsilateral ovary and the peritoneal cavity and have a significant risk of recurrence, reported to be as high as 43 %, despite the small size of the primary tumor [56, 57]. It is possible that undetected occult tubal carcinoma account

for a significant proportion of cases diagnosed as primary peritoneal carcinoma in women with germline mutations. There have been few studies documenting the follow-up of patients presenting with occult, low-volume disease, but it appears that even with early stage, low-volume disease, and with adjuvant chemotherapy, there is still a significant risk of recurrence [57]. Peritoneal spread may be present with a distal tubal carcinoma of only a few millimeters maximum dimension [45].

Molecular Pathology

HGSC is a genetically unstable tumor, characterized by a varied histomorphology unified by marked pleomorphism, a high mitotic rate, and biomarker expression reflective of the most common molecular alterations. The latter includes the near-ubiquitous presence of a mutation in the tumor suppressor p53 (*TP53*), resulting in either overaccumulation of p53 protein by immunohistochemistry (missense—60 % of analyzed cases) or complete loss of protein expression (frameshift/splicing junctions/nonsense—39 % of analyzed cases). Mutations of p53 are already present in early stage HGSC, and mutant *TP53* is likely an essential driver mutation required for the early pathogenesis of HGSC. HGSC demonstrates widespread intratumoral heterogeneity in mutation, copy number, and expression profiles, indicating complex and highly individual evolutionary routes in HGSC progression. The only somatic mutation present within all samples, i.e., common in multiple tumor sites and in multiple tumor patients, was *TP53* mutation; *TP53* mutation, the most stable genomic feature of HGSC, appears to be the common route to malignant transformation [58].

Recently, Hunter and colleagues reported no difference in the level of genomic aberration observed in early low-volume occult tubal carcinomas compared with high-grade serous carcinomas, suggesting that, at least in BRCA1/BRCA2 mutation carriers, genomic instability is an early event in HGSC carcinogenesis [59].

Serous Tubal Intraepithelial Carcinoma

Intraepithelial lesions with morphological features of malignancy but no evidence of stromal invasion, now known as serous tubal intraepithelial carcinoma (STIC), are detected in any one of the three clinical settings: (1) HGSC of presumed ovarian, tubal, peritoneal origins, (2) in prophylactic salpingectomy specimens from BRCA1/BRCA2 mutation carriers, and (3) rarely as an incidental finding in routine surgical specimens. These lesions have been recognized and reported in the past and were thought by some to represent evidence of multicentric tumorigenesis in Müllerian-type epithelium [60]. Other terms used in the literature include dysplasia, atypical mucosal epithelial proliferation, and carcinoma in situ [39, 47, 60].

Serous tubal intraepithelial carcinoma is defined as a localized lesion characterized by morphological atypia, abnormal p53 expression (reflecting the presence of a p53 mutation), and increased proliferation rate [18, 61]. Tubal intraepithelial carcinoma has been seen in association with HGSC for many years, but this finding was interpreted as evidence of a "field effect" of tumorigenesis—the secondary Müllerian system [60]. Careful examination of the fallopian tubes prophylactically resected from BRCA1/BRCA2 mutation carriers led to the description of STIC in the absence of invasive disease, an important finding which, along with the description of "dysplasia" in the RRSO specimens, supported the concept that HGSC, at least in BRCA mutation carriers, had its origin in the fallopian tube. Subsequently, STIC was reported in up to 61 % of tubes from patients with clinically evident HGSC, supporting the currently favored theory that STIC is the immediate precursor of HGSC in both hereditary and sporadic forms of HGSC [62–64].

Microscopic

Many STIC lesions are small, making the diagnosis sometimes challenging. The reproducibility of the diagnosis using morphological criteria

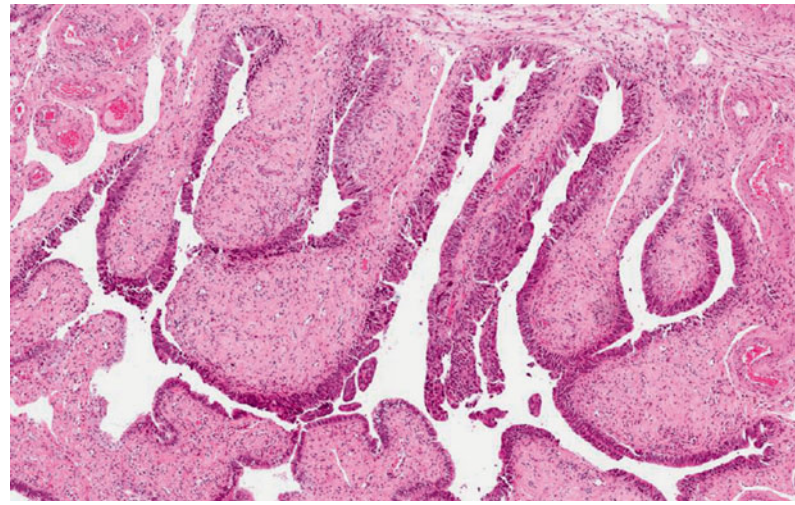

Fig. 1.3 Serous tubal intraepithelial lesion (STIC). A large lesion involving multiple plicae and demonstrating cellular stratification and detachment in addition to atypia and increased proliferation

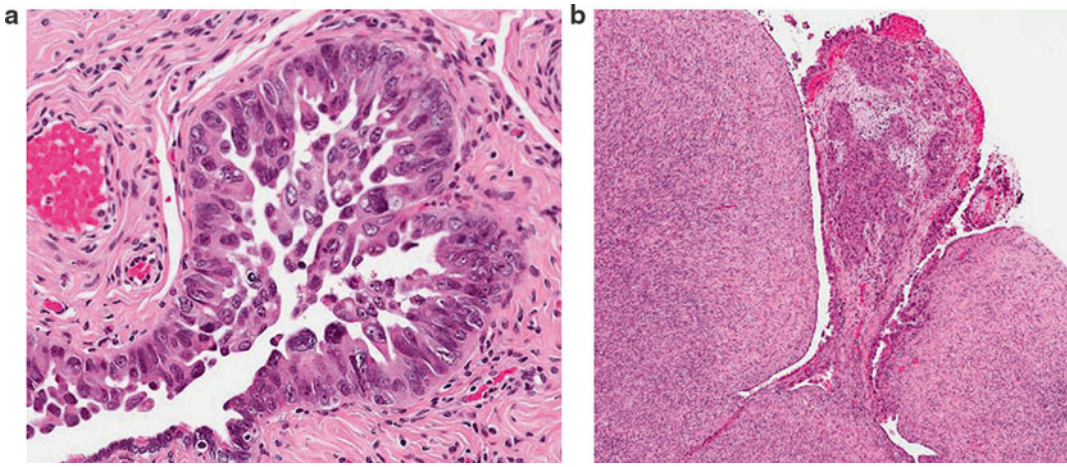

Fig. 1.4 (**a**) Serous tubal intraepithelial lesion (STIC). This lesion has prominent tufting, with cell detachment. (**b**) A microscopic focus of high-grade serous carcinoma on the ipsilateral ovarian surface

alone is only moderate among experienced gynecological pathologists [65, 66]. Like HGSC, STIC has a variable histological appearance, and the morphological spectrum of changes is wide. STIC lesions have varying degrees of stratification, and some STIC has exfoliation of cells, sometimes with a growth pattern reminiscent of the slit-like spaces, epithelial "fractures" seen so commonly in the invasive counterpart (Fig. 1.3). Detachment of malignant-appearing cells may be associated with superficial implants of the ipsilateral ovary (Fig. 1.4).

Using current definitions which include the use of immunohistochemistry to improve diagnostic reproducibility, all cases of STIC have:

1. Morphological atypia, which includes not necessarily all but a combination of the following features: nuclear enlargement, hyperchromasia, irregularly distributed chromatin, nucleolar prominence, loss of polarity, apoptosis, epithelial tufting, and mitotic activity
2. Abnormal p53 expression by immunohistochemistry: diffuse intense nuclear positivity,

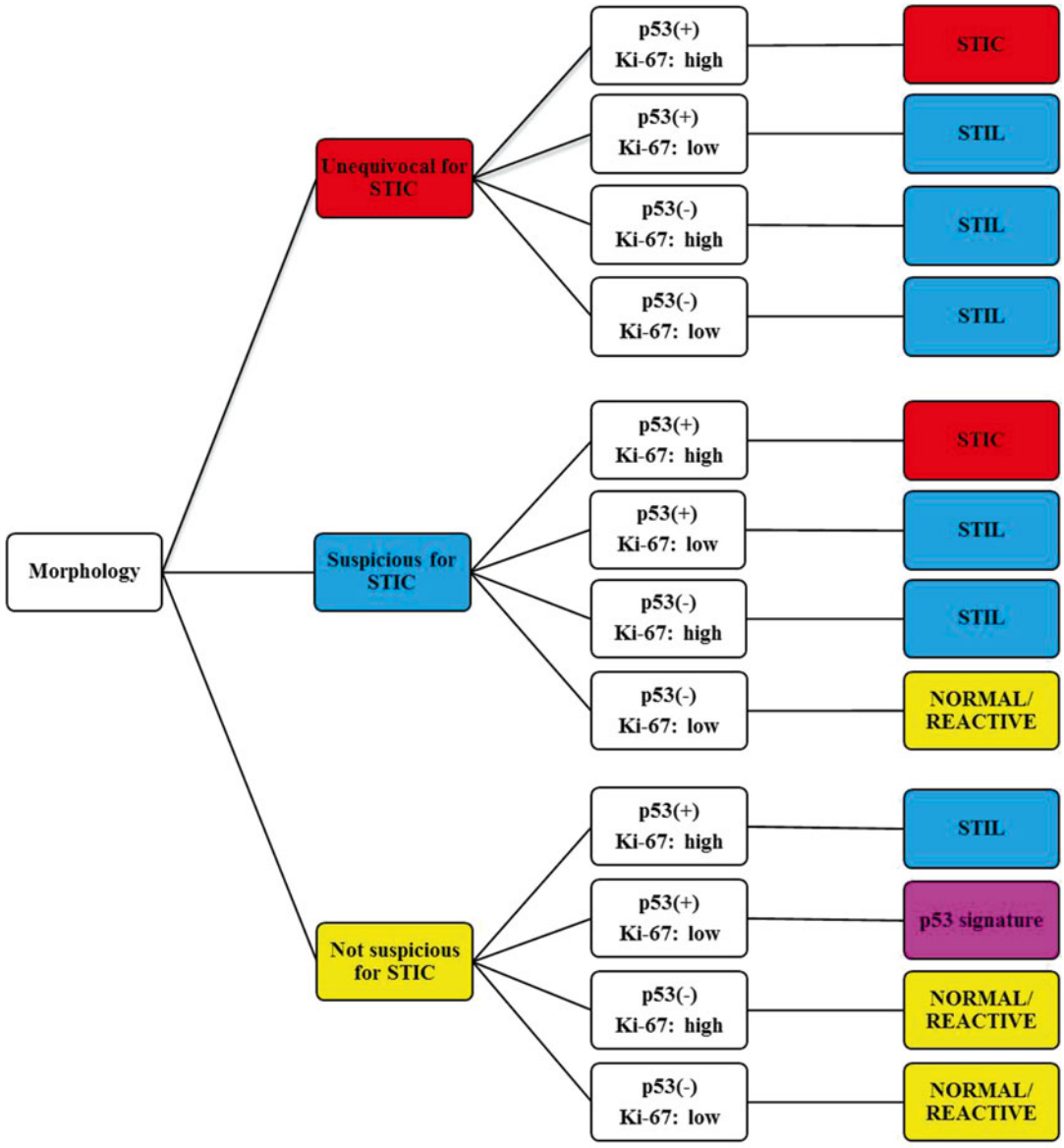

Fig. 1.5 Algorithm for the diagnosis of tubal intraepithelial lesions, using morphology and immunohistochemistry. Ki67 expression is considered high if positive in at least 10 % of lesion cells. P53 is considered positive with either a diffusely positive pattern or a null pattern of expression. Negative p53 in this chart reflects normal, wild-type expression. Reprinted with permission [61]

or negativity in all lesional nuclei, the "null" pattern of expression

3. Increased proliferation, with at least 10 % of tumor nuclei expressing Ki67 by immunohistochemistry

A diagnostic algorithm has been developed to improve the reproducibility of the diagnosis of tubal precursor lesions, independent of the pathologist's level of experience (Fig. 1.5) [61, 66]. In brief, suspected tubal lesions are first assessed by

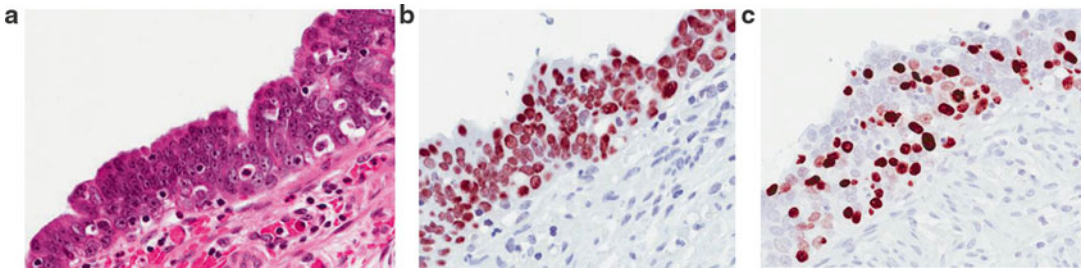

Fig. 1.6 Serous tubal intraepithelial lesion (STIC). (**a**) H&E. (**b**) P53 with diffuse nuclear overexpression. (**c**) Ki67

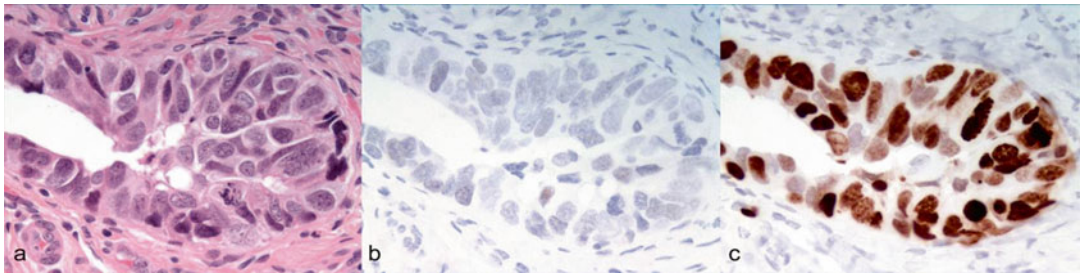

Fig. 1.7 Serous tubal intraepithelial lesion (STIC). (**a**) H&E. (**b**) P53 (null pattern of expression). (**c**) Ki67 [82]

morphological criteria and categorized as: (1) not suspicious for STIC, (2) suspicious for STIC, or (3) unequivocal for STIC. Immunostains help to further categorize the lesion.

A diagnosis of STIC requires that a lesion is assessed:

1. To be suspicious for STIC or unequivocal for STIC
2. To have an abnormal p53 staining pattern, either intense nuclear positivity in greater than 75 % of the lesional cells or 0 % labeling (null pattern)
3. To have increased proliferation as indicated by greater than 10 % of the lesional cells showing positive Ki67 staining

Lesions not meeting all of these criteria are not diagnosed as STIC, but may be diagnosed as serous tubal intraepithelial lesion (STIL), or p53 signature, or normal/reactive (Figs. 1.6 and 1.7).

An additional biomarker which has not yet been widely validated is laminin γ1, which has been proposed as an alternate biomarker of potential use in those STICs which have no p53 staining [67]. This marker may be useful in the diagnosis of STICs with a null pattern of p53 expression.

Molecular Pathology

STICs found in association with HGSC have been shown to have matching mutations of *TP53* in 93 % of cases, supporting the clonal relationship between STIC and HGSC and providing further evidence that the STIC precedes HGSC [68]. The pattern of p53 expression by immunohistochemistry is highly concordant with the type of p53 mutation present [68]. Diffuse intense staining in STIC corresponds with missense mutations (overaccumulation of abnormal protein), and complete loss of staining corresponds to null mutations (due to splice, frameshift, and nonsense mutations). Weak, patchy, isolated cell positivity corresponds to wild-type *TP53*. Complete loss of staining is usually easily interpreted with wild-type positivity in the background uninvolved tubal epithelium.

Abnormal expression of p53 is present in close to 100 % of STICs, as in HGSC. Other translational changes, present in HGSC with varying frequencies, have also been detected by immunohistochemistry with similar levels of expression between STIC and synchronous HGSC: p16 overexpression (CDKN2A), loss of Retinoblastoma protein (Rb) [11], upregulation of the PI3K pathway (stathmin) [69], loss of FOXO3a [70], loss of Pax2 [70, 71], and loss of LKB1 [72]. Similarly, upregulation of oncogene products cyclin E, Rsf-1, and fatty acid synthase (FASN) is present in both STIC and HGSC [73]. In addition, shortened telomeres, important in early carcinogenesis, have been documented in STIC [74].

FISH studies have identified genomic aneuploidy in STIC lesions associated with HGSC, in chromosomes 1, 8, 11, and 17 [75]. Other studies have identified additional genomic similarities between STIC and HGSC, including overexpression of cyclin E [73] and amplification of human telomerase (hTERT) [74]. Additional genomic studies are ongoing, which will clarify the nature of the earliest genomic alterations further.

A concerted effort to molecularly annotate HGSC by the TCGA resulted in a seminal paper of a comprehensive catalog of the major genomic alterations within HGSC, including transcription, translation, and genomic rearrangements [10]. How early these changes occur in the disease course could be probed more comprehensively, and investigations are ongoing. However, STICs are often small and are diagnosed in formalin-fixed paraffin-embedded tissues, leading to technical challenges in the characterization of the genomic alterations which immediately precede HGSC. While much is still to be learned about the transcriptional, mutation, and genomic changes in STIC, it appears that STIC and invasive HGSC share many aberrations, indicating that although STIC is a noninvasive lesion, the cells have the propensity for metastasis without the requirement of invasion into adjacent stroma prior to peritoneal spread.

Outcome

The morphological features of STIC and the molecular associations reported to date suggest that STIC is a malignant lesion, and it has been recommended that STIC be staged as serous carcinoma, Stage 1A [76]. The clinical outcome of patients with STIC, in the absence of positive peritoneal washings or other diseases, is not yet well understood and cannot be predicted for an individual patient. There is at least one published report of a patient with a STIC but no evidence of invasive disease recurring with advanced-stage disease [57], although one small series indicates a favorable outcome [77]. Nevertheless, the accurate diagnosis of STIC clearly has significant clinical implications, although current information does not yet provide clear direction on how patients with a STIC diagnosis should be managed. Because STICs share molecular and genetic alterations with HGSC and at least 15 % of HGSCs are associated with germline mutations of *BRCA1* or *BRCA2*, patients with an incidental diagnosis of STIC may also have a higher risk of carrying a deleterious mutation, and therefore, referral to a genetic counselor is likely indicated.

Some patients may be offered adjuvant treatment including chemotherapy, so it is important to not overcall this diagnosis.

Controversy

There is considerable variation in the reported incidence of STIC. This is due to a number of factors, but in large part to study design. Most importantly, study populations vary widely; some are observational, resulting in an overestimate of STIC frequency. Only a few are inclusive of sequential cases with documented germline mutations of *BRCA1* and *BRCA2* [45, 55, 77]. The frequency of STIC lesions increases with age and is lower with oral contraceptive use [78], and this is not controlled for in publications to date. STIC is also seen with a lower frequency in women with a strong family history but with

negative germline testing. Finally, and importantly, the histological criteria used to detect the precursor lesions vary from study to study. The inclusion of immunohistochemistry for p53 and Ki67 in the diagnostic algorithm significantly improves the reproducibility of the diagnosis, and the studies reporting a lower frequency have not necessarily followed this approach [54, 61, 66, 79–82]. Taking these factors into consideration, the estimate for STIC frequency in BRCA1 mutation carriers is between 5 and 10 % and is likely somewhat lower in BRCA2 mutation carriers. It should also be noted that the incidence of STIC in women at no known genetic risk is not zero [54, 82, 83]. This is an important factor and should be kept in mind when processing salpingectomy specimens, particularly as the clinical relevance of a STIC diagnosis is still uncertain.

The recognition, description, and molecular/genetic analyses of STICs are extremely important in furthering our understanding of how HGSC begins and will influence future preventative and early detection strategies. Care must be taken however in the interpretation of tubal lesions in the setting of HGSC resection specimens. It is possible that lesions which are consistent with STIC may in fact be peritoneal/mucosal spread from HGSC tumor. While considered to be rare, mucosal implants on the tubal fimbria from non-gynecological cancers do occur, and it is possible that some cases of apparent STIC may represent spread, not origin, from an ovarian tumor [84].

The traditional recommendations for assigning a site of origin to pelvic HGSC may no longer be relevant. A recent proposal uses the presence or absence of STIC in determining the site of origin [76]. While this proposal has not yet been widely adopted, it is reasonable that the fimbriated ends of the tubes of all cases of HGSC be examined in toto following a SEE-FIM-like protocol and that assignment of the site of origin of HGSC be made taking involvement of the tubal epithelium into consideration. Primary peritoneal carcinoma should only be diagnosed if the fallopian tube has been examined in toto and found to be negative for STIC and carcinoma and if the size of ovarian cortical involvement is limited to less than 5 × 5 mm. Recognition of STIC and the fimbrial epithelium in HGSC surgical resection specimens will vary, based on a number of factors, and in some circumstances the site of origin will be undesignated, but should then be considered to be tubal/ovarian, distinguishing those cases from an endometrial origin. Currently, the clinical management of HGSC is independent of the pathologist's designation of site of origin and will be increasingly based on genetic alterations rather than designated site of origin. Recognition of STIC is important, but the presence or absence of STIC in a clinical case of HGSC does not necessarily prove the site of origin.

P53 Signature

The term p53 signature was proposed by Crum and colleagues to describe a morphologically indistinct lesion which can be detected only with the use of immunohistochemistry. The p53 signature is defined as a focus of benign-appearing non-ciliated tubal epithelium with nuclear overexpression of p53 but no increased proliferation compared to the background tubal epithelium (Ki67 < 10 %). Overexpression of p53 should be seen in a minimum of 12 consecutive cells [46, 85]. P53 signatures are frequent in the fallopian tubes of women at both low and high genetic risk of HGSC, with an incidence of 11–46 % of resected tubes from women with or without germline mutations and with and without HGSC [46, 49, 82]. Because they are seen with a relatively high frequency in premenopausal women with no known genetic predisposition to HGSC and because, in at least one study, p53 signature is not associated with ovarian cancer risk factors, this lesion may be considered to be a latent cancer precursor [78, 86].

Microscopic

P53 signatures cannot be distinguished by routine H&E examination alone. Once detected by immunohistochemistry, p53 signatures may in retrospect appear to be distinct from the background tubal epithelium. The cells are non-ciliated and have a secretory cell phenotype, and this fact

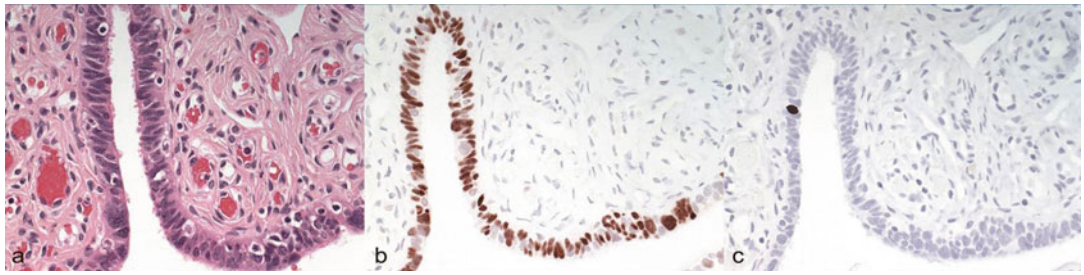

Fig. 1.8 P53 signature. (**a**) H&E. (**b**) P53. (**c**) Ki67 [82]

indicates they represent a type of secretory cell outgrowth (SCOUT), and it is this feature that makes the lesion appear to be distinct. Some lesions may have occasional residual ciliated cells. The cells may have minimal atypia, but by definition there are no diagnostic features of malignancy (Fig. 1.8).

P53 signatures typically involve the tubal fimbria and distal end of the tube. They may be multifocal and bilateral and are seen more frequently in tubes with malignant changes (STIC) [46, 82]. Other immunohistochemistry stains of secretory tubal cells are also expressed in the p53 signature, including PAX8, HMFG2, and CK7.

Molecular Pathology

As might be expected with the intense positive nuclear staining seen in p53 signatures, at least some of these lesions are associated with mutations of the p53 gene [46]. P53 signatures also upregulate phosphorylated, γH2AX, a biomarker reflective of concomitant DNA damage (double-stranded breaks) [46]. The co-localization of p53 signatures with γH2AX suggests that the p53 signature is caused by DNA damage and that the coexistence of p53 mutations (present in at least some p53 signatures) and unrepaired double-stranded DNA breaks may coexist prior to malignant transformation.

There is some evidence that altered cell cycle checkpoints may be present in p53 signatures, particularly in those lesions associated with *BRCA1* germline mutations. Norquist et al. reported that expression of the cell cycle inhibitor p27 within the p53 signature in *BRCA1* mutation group was significantly lower than in *BRCA2* mutation carriers or in the control group, whereas no difference in p21 expression was seen [49]. Strictly speaking, p53 signatures by definition do not have an increased proliferation, but in this study some p53 signatures did have increased Ki67 expression. In the same study, the authors demonstrated that in *BRCA1* mutation carriers, the wild-type allele remains intact, indicating that both loss of normal function of p53 and loss of the p27-regulated G0-S cell cycle checkpoint precede *BRCA1* loss of heterozygosity.

Li-Fraumeni syndrome is a rare familial disorder defined by the inheritance of a germline p53 mutation. Fallopian tubes resected from women with this syndrome have a dramatically increased frequency of p53 signatures, with as many as 20 signatures identified per section [87]. Patients with this syndrome have an increased lifetime risk of breast, brain, soft tissue, and blood cancers, but they do not have an increased risk for high-grade serous carcinoma.

To summarize, the p53 signature is a morphologically benign lesion but immunohistochemically distinct lesion, commonly seen in women at both low and high genetic risk of HGSC. Furthermore, women with Li-Fraumeni syndrome are not at increased risk for HGSC despite having numerous p53 signatures in the distal end of the fallopian tube. These observations indicate that additional genotoxic event(s) must occur prior to malignant transformation. The p53 signature is thought to be one of if not the earliest recognizable precursor lesions of high-grade serous carcinoma, because of the ubiquity and prevalence of the mutations in *TP53*. The signature itself is a

benign focus of epithelial cells with no or subtle changes in nuclear atypia, polarity, and an expansion of secretory cells [86, 88, 89]. The cells in the p53 signature have limited proliferative capacity and although loss of normal p53 function is necessary for a diagnosis of p53 signature, it is not sufficient to promote carcinogenesis; at least one more genotoxic event is required for malignant transformation.

Currently, p53 signatures are not reported clinically and are considered to be of research interest only.

Serous Tubal Intraepithelial Lesion

Diagnostic criteria for STIC and for p53 signature incorporate morphology and biomarker interpretation. When using these criteria, there are tubal lesions which demonstrate more features of atypia and/or proliferation than would be expected in a p53 signature, but do not fulfill the criteria required for a reproducible diagnosis of STIC. This group of lesions is not yet well characterized, and the clinical relevance of these lesions is poorly understood. Other terms have been applied to this group, including atypical hyperplasia, proliferative p53 signature, tubal intraepithelial lesion in transition (TILT), and tubal atypia, but we prefer the designation serous tubal intraepithelial lesion (STIL).

Microscopic

STILs vary from having mild to marked atypia, may or may not have abnormal nuclear p53 expression, and often have some increased proliferation based on Ki67 expression when compared to the background tubal epithelium. Because these lesions are not well understood, it is recommended that the STIC diagnostic algorithm be followed. A STIL diagnosis is most commonly made with the following combination of findings:

1. Morphology unequivocal for STIC, abnormal p53 expression, Ki67 less than 10 %
2. Morphology suspicious for STIC, abnormal p53 expression, Ki67 less than 10 %

These features indicate that significant alterations have occurred, but that the criteria for a diagnosis of intraepithelial carcinoma are not fulfilled (Figs. 1.9 and 1.10).

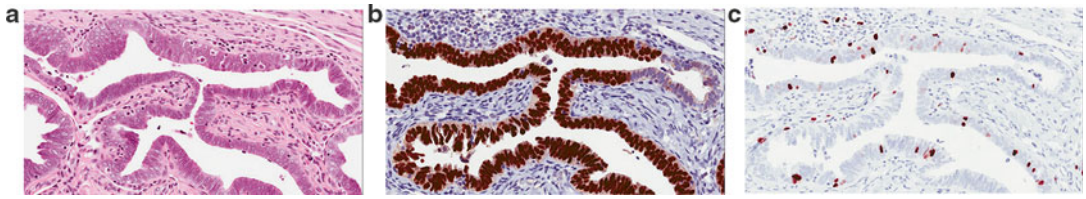

Fig. 1.9 Serous tubal intraepithelial lesion (STIL). (**a**) H&E. (**b**) P53. (**c**) Ki67. Abnormal morphology at least suspicious for STIC, diffuse p53 expression, with increased Ki67 expression that is less than 10 % of the lesion cells

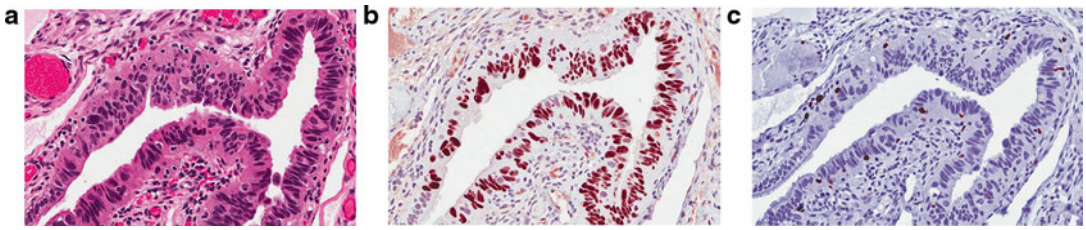

Fig. 1.10 Serous tubal intraepithelial lesion (STIL). (**a**) H&E. (**b**) P53. (**c**) Ki67. Abnormal morphology at least suspicious for STIC, diffuse p53 expression, but no increased Ki67 expression

Using the diagnostic algorithm, it is also possible, though much less likely, to see:

3. Morphology unequivocal for STIC, normal p53 expression, Ki67 greater than 10 %
4. Morphology unequivocal for STIC, normal p53 expression, Ki67 less than 10 %
5. Morphology suspicious for STIC, normal p53 expression, Ki67 less than 10 %

Controversy

It seems likely, because of the widespread variation of morphological features and biomarker expression in this group, that some of these lesions are in fact benign or reactive changes, not cancer precursors, and others, particularly those STILs with abnormal p53 expression, are cancer precursors with variable transcriptomic/genomic alterations resulting in variable histological phenotypes. Because of the uncertainty of both diagnostic reproducibility and clinical relevance, some authors have recommended that a diagnosis of STIL (or proliferative p53 signature) not be used in clinical practice. An alternative to this, which we currently practice, is that a lesion with significant atypia and abnormal p53 expression and increased proliferation, but the proliferation is less than the 10 % cutoff, is diagnosed as STIL. The diagnosis is accompanied by a comment indicating that an atypical lesion of uncertain clinical relevance is present and that there is no diagnostic evidence of intraepithelial carcinoma.

Secretory Cell Outgrowths

The p53 signature is the best characterized HGSC benign cancer precursor. One group has reported another entity that they consider to be a benign cancer precursor, with some similarities to the p53 signature [90, 91]. Secretory cell outgrowths (SCOUTs) are frequent in the fallopian tube and, unlike p53 signatures, are seen throughout the fallopian tube mucosa, not just in the anatomically high-risk distal end. They are linear outgrowths of secretory cells, which stand out from the normal tubal mucosa mix of ciliated and non-ciliated cells. They have no atypia and no increased proliferation. They may appear to have some crowding, being more prominent in the mucosa. Like p53 signatures, they are seen in women of both low and high genetic risk of HGSC, but they are seen more frequently in cases of pelvic HGSC, suggesting that they may also be a benign latent cancer precursor. P53 signatures may be SCOUTs with additional molecular/genetic alterations.

Currently, SCOUTs are not reported clinically and are of research interest only.

Processing of Salpingectomy Specimens

Risk-reducing salpingo-oophorectomy will be increasingly adopted as a preventative strategy in women at high genetic risk, in both academic and community practice settings. Given the reluctance of premenopausal women to undergo surgical menopause, an alternative approach, salpingectomy with ovarian retention, has been proposed as an interim strategy under investigation [92–94]. It is also likely that opportunistic salpingectomy with ovarian retention will be increasingly considered in low-risk women undergoing hysterectomy or tubal ligation [95]. It is therefore important pathologists standardize the processing of resected tubal specimens.

The Association of Directors of Anatomic and Surgical Pathology recommended a two-tier approach to gross examination of the fallopian tube, depending on the level of suspicion for an occult invasive or intraepithelial carcinoma [43, 96]:

1. All salpingectomy specimens are fixed for at least 4 h with care in the handling of the fimbriated ends, protecting the integrity of the fimbrial mucosa.
2. All salpingectomy specimens are sectioned at a maximum of 2–3 mm intervals, with the exception of the fimbriated end.

(a) Benign conditions (minimum[1]):
- Three sections are submitted representing isthmus, ampulla, and infundibulum/fimbria. A single H&E section is prepared from each block.

(b) Risk-reducing salpingo-oophorectomy (SEE-FIM protocol):
- The distal 2 cm of the fimbriated end is transected.
- The fimbria is sectioned parallel to the long axis and may be further sectioned longitudinally.
- The remainder of the tube is sectioned at 2–3 mm cross sections.
- All sections of the fallopian tube are submitted in toto.
- Similarly, the ovaries are fixed for a minimum of 4 h prior to sectioning, serially sectioned perpendicular to the long axis at 2–3 mm intervals, and submitted in toto.
- A single H&E section plus several unstained sections *or* one H&E plus p53 plus Ki67 should be taken from the infundibulum/fimbria blocks. A single H&E section from the other tubal and ovary blocks is sufficient.

(c) HGSC debulking surgery (SEE-FIM protocol or SEE-FIM protocol)

The aim of the SEE-FIM protocol is to maximize the surface area of the fimbria for histological examination. It is our practice to longitudinally section the fimbria as described in the protocol, in all salpingectomy specimens. To ensure optimal fixation times for interpretation of subsequent immunohistochemistry, all salpingectomy specimens are fixed for 24–48 h. It is our practice for all RRSO specimens to prepare one H&E section plus p53 and Ki67 sections in infundibulum/fimbria blocks. An alternative is to include unstained sections from the same ribbon strip as the H&E section. It has been our experience that detection of small STIC lesions may be missed on H&E examination alone, but it would be unlikely to miss a STIC with the addition of p53 and Ki67 immunohistochemistry. It has also been our experience that assessment of suspicious lesions may be compromised if additional sections are not included in the initial sectioning of the tissue blocks. Finally, in most circumstances, multistep deeper level sectioning is not necessary, but may be performed if peritoneal washings are positive for malignant cells and no lesion is detected in initial tube/ovary sections [97].

[1] Because there is a low risk of STIC and occult carcinoma in women considered to be at low risk of HGSC, it has been recommended that the tubal fimbriae be examined following a SEE-FIM-like protocol, and this is our current practice [83].

Summary

Classification and understanding of high-grade serous carcinoma precursors continues to evolve, but it is clear that the fallopian tube plays an important role in ovarian carcinogenesis. The careful processing of salpingectomy specimens and a uniform diagnostic approach incorporating morphology and immunohistochemistry are needed to optimize the diagnosis of early, low-volume high-grade serous carcinoma and its immediate precursor, serous tubal intraepithelial carcinoma.

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2 Precursors of Low-Grade Serous Adenocarcinoma of the Ovary: Pathology and Molecular Pathways

Kate Lawrenson and Paulette Mhawech-Fauceglia

Introduction

Epithelial ovarian cancer (EOC) is the leading cause of death due to gynecologic malignancy in women in the United States, with 22,240 new cases and 14,030 women estimated to have died of ovarian cancer in 2013 [1]. The majority of EOCs are of serous histology, and it is now widely accepted that ovarian serous carcinomas fall into two distinct categories: high grade and low grade. High-grade serous ovarian carcinomas (HGSCs) are the most common subtype of EOC, whereas low-grade serous carcinomas (LGSCs) are less common and represent approximately 3 % of all ovarian surface epithelial carcinomas. The two types are distinct in terms of pathogenesis, molecular pathways, treatment response, and patient prognosis. HGSCs are classified as Type II carcinomas in the Shih and Kurman dualistic model of ovarian cancer development [2]. Type II carcinomas exhibit distinct genetic hallmarks including high levels of genetic instability and *TP53* mutations. HGSCs are de novo carcinomas and it is thought that a large proportion originate from the fallopian tube fimbriae [3, 4]. LGSCs are Type I tumors, which are more genetically stable and frequently harbor alterations in the mitogen-activated protein kinase (MAPK) signaling pathway. Unlike HGSC, they follow a stepwise progression from inclusion cyst to serous cystadenoma, serous borderline tumor, serous borderline tumor with micropapillary pattern, and finally to invasive low-grade serous carcinoma. However, the pathogenesis of this subtype is not fully understood and the cellular origins are a recent topic of debate. In this chapter we will be discuss the histology, grading, pathogenesis, and molecular characteristics of low-grade serous carcinomas.

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Histology

Serous borderline tumors (SBTs)/serous tumor of low malignant potential (LMP) represents 25–30 % of non-benign serous tumors and occurs in women 30–50 years of age. In the majority of cases they are unilateral and usually present at an early stage (stage I) [5]. The WHO defines SBT as an "ovarian tumor of low malignant potential exhibiting an atypical epithelial proliferation of serous type cells greater than that seen in its benign counterpart but without destructive stromal invasion" [6].

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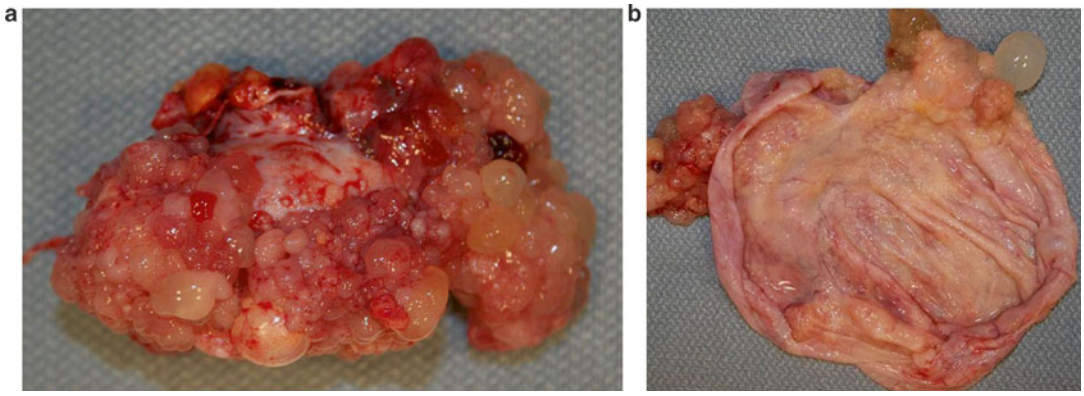


Fig. 2.1 (**a**) Ovarian cyst with surface involvement by friable papillary excrescences. They appear to cover most of the ovarian cyst surface. (**b**) The inner lining of the same ovarian cystic mass is mainly smooth. However, there are areas showing irregular friable vegetating masses

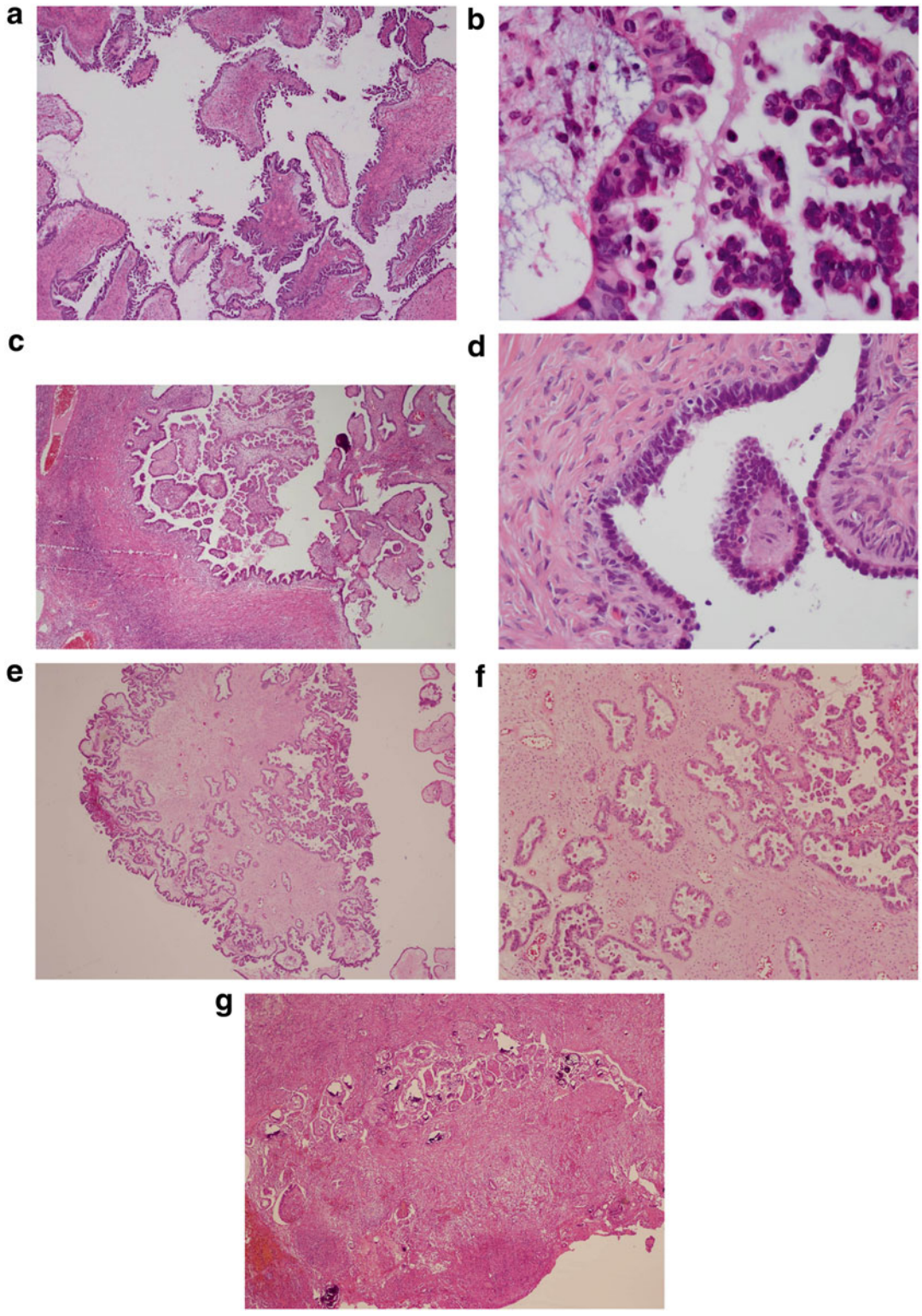
Grossly, the mass is usually partially cystic and partially solid. Polypoid excrescences are present on the outer surface of the ovary or within the cyst lumen (Fig. 2.1a, b). The papillary structures are yellow in color, soft, and friable. SBT can be readily differentiated from the hard, stocky, white excrescences that are usually characteristic of serous cystadenofibroma. SBTs can be subgrouped into tumors with typical and tumors with micropapillary patterns.

Typical SBT

Typical SBT makes up the majority of SBT or (90 %). A diagnosis of SBT/LMP is based on three main characteristics: (1) epithelial stratification and cellular budding where the tumor cells become detached from the papillae and appear to float in the cystic lumen with no fibrovascular core. (2) The tumor cells have mild to moderate cytologic atypia. (3) There is lack of stromal invasion. Microscopically, the papillae are lined by stratified cuboidal to columnar epithelial cells. These papillae show branching and complex structure. The epithelial cells have high nuclear-to-cytoplasmic ratio (N/C), and the nuclei are hyperchromatic with prominent nucleoli. Mitotic figures are frequently present (Fig. 2.2a–c). While the histologic criteria may suggest that a diagnosis of serous LMP is straightforward, sometimes the diagnosis of serous LMP can be challenging as these diagnoses are subject to numerous pitfalls, including the following:

Serous LMP May Have Variants Some SBTs present with intracystic mucin and can mimic mucinous adenocarcinoma. The key to make the

Fig. 2.2 (**a**) Cut section of these vegetating masses reveals papillary structure with fibrovascular stalks. These structures are lined by stratified cuboidal to columnar epithelial cells. (**b**) There is stratification of tumor cells which they start getting detached and float in the lumen. These cells exhibit moderate cellular atypia with high nuclear/cytoplasmic ratio. (**c**) The main characteristic feature of ovarian serous borderline tumor is the absence of ovarian stromal invasion. (**d**) Cut surface of benign serous cystadenoma. The cyst is lined by cuboidal epithelium. There are few areas where the cells appear to be stratified. However, due to the lack of cytologic atypia, this mass is considered as benign and this pseudo-stratification is due to tangential section. (**e**) Lower magnification showed tumor cells that seem to infiltrate fibrous stroma. (**f**) Higher magnification, these cells seemed to invade the stalk of the papillae and not the ovarian stroma which can be a major pitfall. (**g**) Microscopic features of autoimplantations are very similar to the features of desmoplastic noninvasive implant. They are defined by clusters of tumor cells in a background of extensive hemorrhage, fibrosis, and acute chronic inflammation. Frequent psammoma bodies are seen. These autoimplantations are usually seen on the surface of the ovary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diagnosis is that the mucin is intracystic, not intracytoplasmic, as usually seen in mucinous tumors. The second variant is that the tumor can have a cribriform pattern and can mimic endometrioid tumor. While these variants do not carry any significance on prognosis, they can create a diagnostic challenge for pathologists.

Tangential Cut Caution should be practiced when one sees what appears to be epithelial proliferation without cytologic atypia, because tangential sectioning of the lining of a benign serous cystadenoma can give the impression of proliferation of the epithelial lining (Fig. 2.2d).

Stromal Invasion By definition, SBT lacks stromal invasion. This is a major criterion to differentiate SBT from serous adenocarcinoma. Therefore, invasion of the stalk of the papillae should not be considered as ovarian stromal invasion as illustrated in Fig. 2.2e, f.

Autoimplantation Another pitfall is the failure to differentiate between stromal invasion and autoimplantation, which is the invagination of the tumor on itself creating the illusion of a stromal invasion, as shown in Fig. 2.2g. Grossly, serous LMP tumors exist as well-demarcated plaques on the surface of the ovary. It is essential to mention that autoimplantations are localized superficially on the surface of the ovary and are morphologically similar to desmoplastic noninvasive implants with disorganized groups of tumor cells embedded in dense stroma with hemorrhage, chronic inflammation, mesothelial proliferation, and massive necrosis.

Micropapillary SBT

Micropapillary SBT (MSBT) accounts for 5–10 % of all SBTs. The significance of this subtype is debated among pathologists. Some authors have found a close association between MSBT and invasive implants and urged to call this entity as "micropapillary serous carcinoma" [7, 8]. Others preferentially use the term MSBT, avoiding the use of the term of "carcinoma," to minimize the possibility of over-treating patients [7, 8]. The general agreement on the significance of micropapillary architecture in SBTs is that there is a significant increase in incidence of invasive peritoneal implants [9]. Molecular studies show that MSBT has a similar gene expression profile as low-grade serous carcinoma and distinct from typical SBT [10]. MSBT is the only surface epithelial stromal tumor with a well-defined adenoma-carcinoma sequence, where LGSC is thought to arise in a stepwise fashion from a benign cystadenoma through BST to an invasive low-grade serous carcinoma [11]. Microscopically, MSBTs show highly complex micropapillary growth in a filigree pattern, growing in a nonhierarchical fashion from stalk which has been aptly described as a "Medusa head"-like appearance. Micropapillae are at least five times as long as they are wide [12] (Fig. 2.3a–c). Micropapillary foci should occupy an area of at least 5 mm, since micropapillary foci of less than 5 mm have no bearing on clinical outcome [12].

SBT with Microinvasion

Microinvasion is defined as single cells or few clusters of cells similar to those seen in the overlying SBT that infiltrate the stroma. One or more foci may be present but none should exceed 10 mm^2 or not exceeding 3 mm or 5 mm. SBT with microinvasion appears to have no significance on disease outcome, with 10-year survival rate of 86 % [12].

Peritoneal Implants

Peritoneal implants are classified into epithelial invasive and noninvasive implants and desmoplastic noninvasive implants. Implants are a heterogeneous group of lesions and various types may coexist; therefore, multiple biopsies of numerous foci of suspicious lesions at the time of surgery and extensive tumor sampling by the pathologist are essential for the accurate evaluation of peritoneal implants. Differentiating invasive and noninvasive implants can be challenging, but given the increased probability of tumor

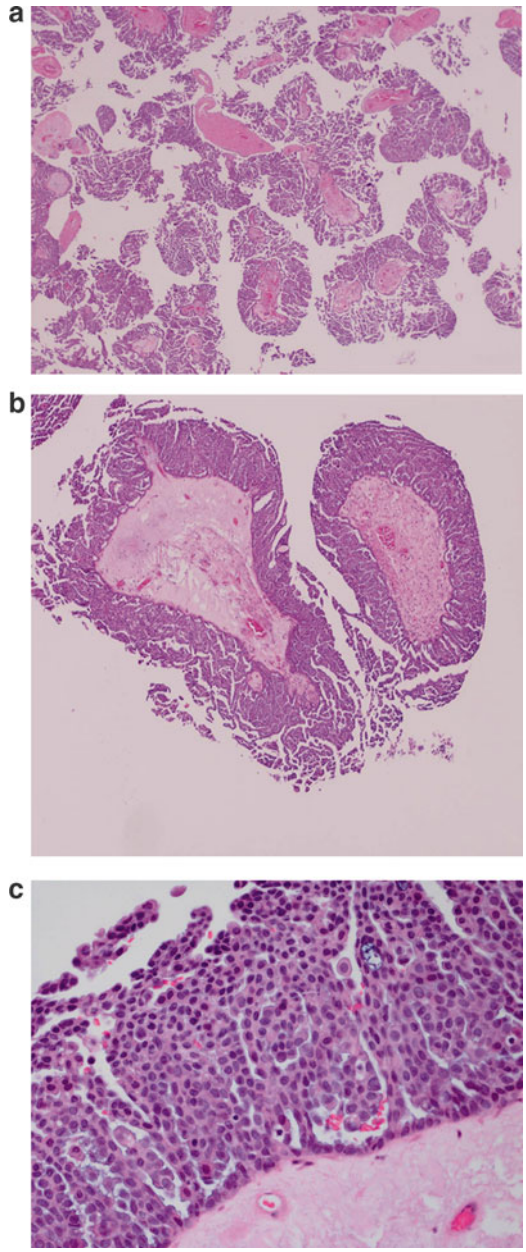

Fig. 2.3 (**a**) There is highly complex micropapillary growth in a filigree pattern, growing in a nonhierarchical fashion from stalk. These are described as a "Medusa head"-like appearance. (**b**) Micropapillae should be at least five times as long as they are wide. (**c**) Cytologically, tumor cells are somewhat bland looking exhibiting mild atypia and very infrequent mitotic figures

recurrence for invasive implants, accurate diagnoses have a significant impact on patient prognosis and clinical management of the case.

Epithelial noninvasive implants are characterized by the presence of papillae within cystic spaces exhibiting mild cytologic atypia. There are frequent psammoma bodies and no stromal reaction or destruction with mild degree of inflammatory cells (Fig. 2.4a, b). SBTs with noninvasive implants are considered indolent, with 5-year survival rates of 95 % and recurrence rates are typically low, ranging from 8 % to 32 % [13].

Epithelial invasive implants are characterized by haphazardly distributed glands and clusters of branching papillae infiltrating the adipose tissue and stroma. The epithelial cells have moderate to marked cytologic atypia. Psammoma bodies are sparsely distributed throughout the tumor, and the associated stroma is composed of dense fibrous tissue with mild degree of inflammation (Fig. 2.4c). Patients with SBT with invasive implants have higher chances of developing low-grade carcinomas many years after initial diagnosis [14].

Desmoplastic noninvasive implants are defined by clusters of irregular glands tumor cells exhibiting mild cytologic atypia. Frequent psammoma bodies are seen (Fig. 2.4d, e). There is no stromal reaction; on the contrary, the stroma is loose and may have granulation tissue-like features with neutrophilic infiltrates and hemorrhage.

Ovarian Grading Systems and Low-Grade Ovarian Serous Carcinoma

Before we discuss the molecular characteristics of low-grade ovarian serous carcinoma (LGSC), it is worth discussing the grading system for epithelial ovarian cancer. There are at least five grading systems that are in use by pathologists worldwide. The most commonly used around the world are from the International Federation of Gynecology Oncology (FIGO) and the World Health Organization (WHO). The FIGO grading system [15] is based on the ratio of glandular or papillary pattern to solid growth of the tumor: grade 1 tumors when <5 % is solid growth, grade II when 5–50 % is solid growth, and grade 3

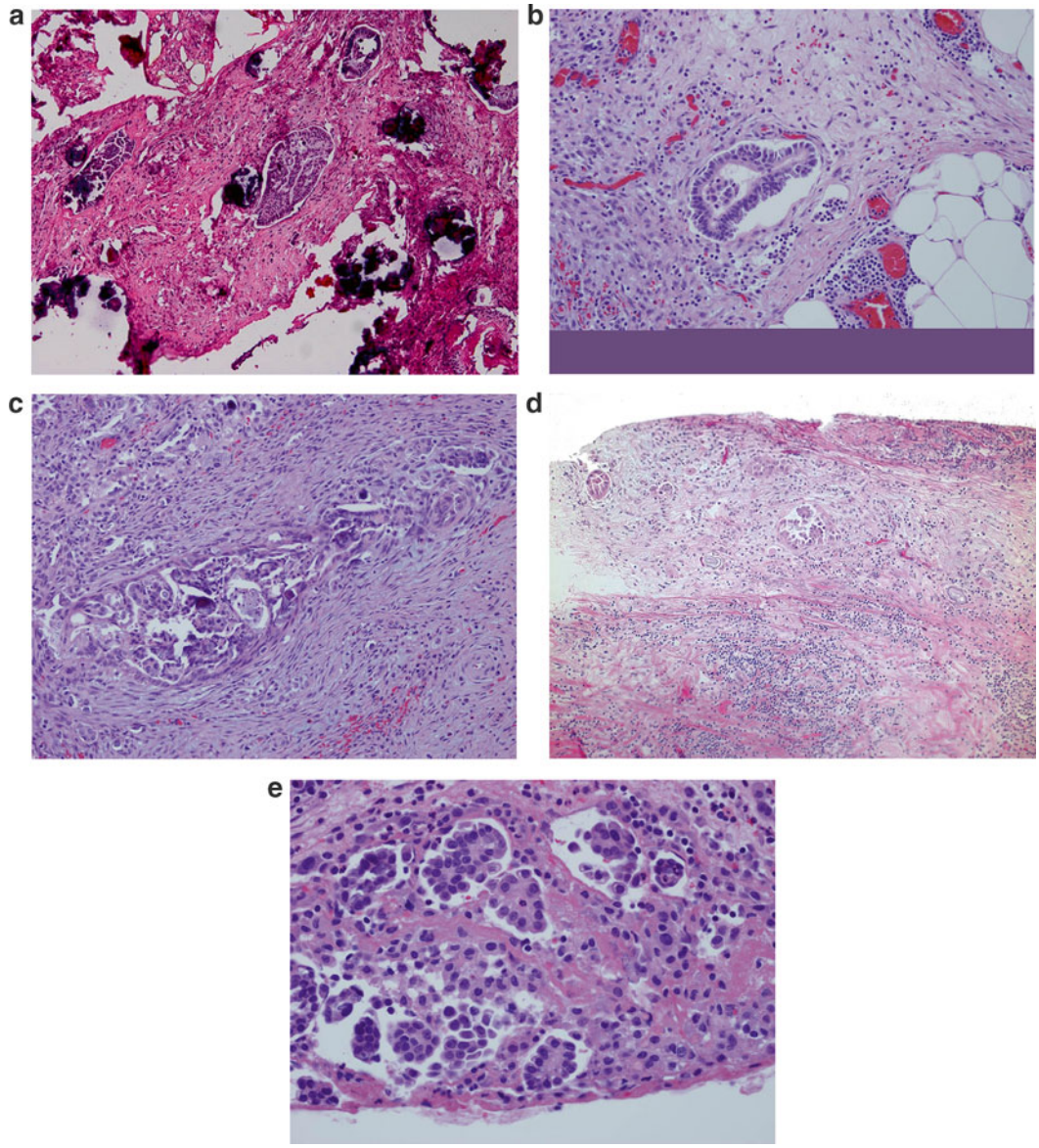

Fig. 2.4 (**a**) Noninvasive implants are characterized by cluster of tumor cells embedded in a fibrous tissue with no desmoplastic reaction. Psammoma bodies are frequently present. (**b**) Empty spaces are seen surrounding these clusters. (**c**) Invasive implants are characterized by complex papillae structures that seemed to infiltrate the stroma. There is extensive desmoplastic reaction with proliferation of fibrous tissue and chronic inflammation. Psammoma bodies are usually infrequent. (**d**) Desmoplastic noninvasive implants are defined by clusters of papillae usually seen on the surface. These papillae are seen in a background of fibrotic stroma with extensive chronic inflammation. (**e**) Closer magnification shows very bland-looking cells surrounded by empty spaces

tumors when >50 % is solid growth. The WHO system is more subjective, as it depends on the impression of the pathologist assessing the tumor architecture and cytologic features. It is considered an intuitive method where there are no actual objective criteria for grading. The other system used commonly in the United States is the Gynecologic Oncology Group (GOG) grading system [16]. Basically, the GOG system borrows the grading system from cancer occurring in other sites, depending on the histologic type; for example, the FIGO system for grading endometrial cancer

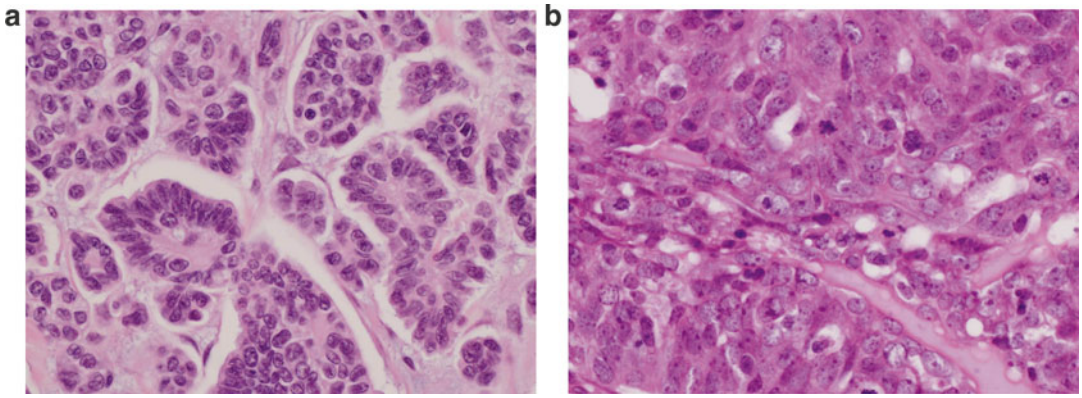

Fig. 2.5 (**a**) Low-grade serous carcinoma shows tumor cells with mild atypia and very few mitotic. (**b**) High-grade serous carcinoma is defined by tumor cells with moderate to severe atypia and high mitotic rate

will be used when the tumor is endometrioid type, and when the tumor is transitional cell type, the same grading system as for transitional cell carcinoma of the bladder is used. Clear cell carcinoma is not graded at all.

Of particular importance for basic and clinical research is the lack of reproducibility of the three grading systems and the frequent disparities between diagnoses by different pathologists using the same grading system [17, 18]. As a result, the significance of tumor grade to prognosis varies in the literature. It is clear that classification of EOC histological subtype and grading based on molecular markers would significantly improve reproducibility of diagnoses and enable more accurate clinical studies to be performed.

An additional grading system that is commonly used is Silverberg's grading system [19]. Silverberg and his colleagues tried to create a grading system using the Nottingham grading system of the breast, which is based on architecture, cytologic atypia, and mitotic counts. Each is given a number and then they added to a score. As the criteria for this system are very defined and very objective, it is not surprising that this system shows a high degree of reproducibility among pathologists. In addition, using this grading system, tumor grade was shown to be a predictive factor for survival, with lower tumor grade associated with a more favorable outcome [20]. Lastly, the MD Anderson two-tier grading system grades each tumor as low grade or high grade [21]. Low-grade tumors are defined as tumors with mild atypia and a low frequency of mitotic figures (<12 mitoses/10 high-power fields), whereas high-grade tumors are tumors with moderate to severe atypia and high mitotic rates (Fig. 2.5a, b). This final grading system is only applied to serous carcinoma and again shows good intra-observer reproducibility [22]. Moreover, the two-tier system reveals prognostic associations that are consistent with those seen when using the Silverberg's grading system [22].

Accurate grading of serous carcinomas as low-grade or high-grade is crucial for multiple reasons: (1) LGSCs and HGSCs are associated with markedly different prognoses, (2) LGSCs are usually cisplatinum resistant and so may often not receive standard chemotherapy, and (3) LGSCs may benefit from novel therapeutics designed to interrupt signaling pathways activated in this tumor type specifically.

Cellular Origins

Ovarian LGSCs are relatively rare tumors, which makes investigating the origins challenging. LGSCs can arise de novo but others clearly evolve in a stepwise manner beginning with a benign serous cystadenoma which progresses to a serous borderline tumor (SBT) which then develops into an invasive LGSC, as described above [23]. Not all borderline tumors will develop

into invasive cancer but the proportion that do tend to have invasive implants upon presentation. LGSCs are a distinct entity to high-grade serous counterparts and are associated with distinct somatic alterations, clinical characteristics, and epidemiological risk factors. Although some case reports identified low-grade and high-grade components within the same tumor, this appears to be a rare occurrence and the distinct somatic profiles of LGSC and HGSC most strongly support the hypothesis that the two entities are different diseases and LGSC is not a precursor of HGSC [23]. The majority of HGSCs appear to originate from secretory cells in the fimbrial portion of the fallopian tube [4, 24–26]. Although recent pathological evidence has suggested a fallopian origin for at least a subset of LGSCs, classically it has been thought that LGSCs originate from ovarian surface epithelial cells (OSECs). A third model for LGSC origins is the endometrial model. Each of these three cell-of-origin models is discussed in more detail below.

Ovarian Epithelial Cells

Historically it was thought that the majority of LGSCs arise from ovarian epithelial cells, a layer of simple, cuboidal, mesothelial-type epithelial cells covering the surface of the ovary. OSEC-type cells can also line simple cysts within the ovarian cortex, termed cortical inclusion cysts (CICs). CICs arise from invaginations of the ovarian surface that occur following ovulation. Invaginations that fuse at the top create OSEC cysts, where OSECs are in close proximity to the mitogenic environment of the ovarian stroma. Interestingly, there is a relationship between body mass index (BMI) and number of CICs [27]. BMI is associated with borderline and low-grade serous cancer risk, but not HGSC risk [28], consistent with an ovarian origin for the former histological subgroup, but not for the latter.

In this model the microenvironment of the ovarian stroma plays a key role in the early genesis of LGSC by promoting Müllerian differentiation of OSECs. Evidence shows OSECs exhibit marked phenotypic plasticity, which some argue enables the cells to differentiate into the histologically diverse subtypes of EOC during cancer development [29]. However, theories supporting OSECs as cells of origin for serous ovarian cancer have recently come under scrutiny and have been heavily criticized. The lack of expression of EOC markers in OSECs, the divergent embryological origins of OSECs and Müllerian-type epithelium, and the scant evidence of early-OSEC-derived neoplastic lesions have all been used to question the validity of the OSEC as a precursor cell for serous EOCs.

Fallopian Epithelial Cells

Recent pathological evidence, as well as data from in vitro and in vivo models, has demonstrated that a significant proportion of high-grade serous ovarian cancers (HGSCs) originate from secretory epithelial cells located in the epithelium of the fallopian tube fimbriae [4, 24–26, 30]. This has led researchers to look more closely into whether LGSCs could also have a tubal origin. A key observation is the morphological similarity of LGSCs to the fallopian tube: LGSCs can contain both secretory and ciliated epithelia that closely resemble the morphology and immunohistochemical staining profile of normal tubal epithelium. The ratio of ciliated to secretory cells in fallopian-type inclusion cysts and serous cystadenomas is similar, with an increase in the proportion of secretory cells in borderline tumors progressing to a near absence of ciliated cells in LGSC [31]. Extensive sectioning and examination of fallopian tubes from patients with LGSC has identified regions of papillary tubal hyperplasia occurring more commonly in women with atypical proliferative serous tumors than in unaffected women [32]. Moreover, chronic salpingitis has been identified in association with ovarian serous borderline tumors, and secretory cell outgrowths (considered to be a precursor lesion) are more common in fallopian tubes from women with serous borderline tumors compared to controls [33]. Finally, mutational analyses have identified identical mutations in the *KRAS* proto-oncogene in serous borderline tumors and endosalpingiosis,

suggesting co-occurrence of the two represent different stages of the disease continuum [34].

So how do tubal epithelial cells become relocated to the ovary? This process is not fully understood, but it is known that two types of CIC exist within the ovary—PAX8 negative, calretinin positive cysts, thought to be derived from OSECs, and PAX8 positive, calretinin negative cysts, proposed to be tubal in origin [31]. However, it is worth noting that this conclusion is based on the assumption that PAX8 is never expressed by OSECs, which in our own unpublished data we find to be incorrect (in a large series of 27 normal ovaries, nearly half express PAX8). Moreover, detailed examinations of ovaries find transitions of OSEC-type to cuboidal (tubal)-type epithelial cells within the same cyst, suggesting OSECs can undergo a metaplasia and acquire tubal characteristics [35]. Nonetheless, a benign process termed endosalpingiosis does bring tubal epithelium into the ovary, which is a likely source of tubal type epithelium within CICs.

Endometriosis Epithelial Cells

An alternative hypothesis is that LGSCs may arise from other types of Müllerian epithelial cells, particularly from endometrial epithelial cells ectopically located to the ovary via the common process of retrograde menstruation. In around 10 % of women, the endometrial epithelial cells engraft and form functional glands within the ovary and at other sites, a condition termed endometriosis. While there is currently little pathological or experimental evidence to support this theory, epidemiological studies find that endometriosis is associated with an increased risk of LGSC (with an odds ratio of 2.11, 95 % confidence interval 1.39–3.20) [36], and it is clear that this association warrants furtherinvestigation.

The Microenvironment of the Ovary

While the cellular origins of LGSC are not yet clear, one unifying theme in the above three models is the vital role played by the specific microenvironment of the ovary, as it appears that cystic structures within the ovary are hotspots for neoplastic transformation. Markers of oncogenic stress are upregulated in CICs relative to the surface epithelium [37], likely due to the effects of mitogenic molecules such as estrogen or the genotoxic and pro-inflammatory effects of follicular fluid [38]. Elucidating the pathways involved in stromal-epithelial cross talk during the developmentof LGSC will likely be essential for our understanding of the earliest stages of these tumors.

Somatic Genetic Characteristics of LGSC

In contrast to high-grade serous ovarian cancers, which nearly always contain *TP53* mutations [39] and which display widespread copy number aberrations and chromosomal rearrangements, *TP53* mutations are rare in LGSCs, and LGSCs typically do not contain significant amounts of chromosomal disruption. LGSCs are characterized by mutations in *KRAS*, *BRAF*, and *ERBB2* (Table 2.1). Collectively, *KRAS* and *BRAF* muta-

Table 2.1 Mutations commonly found in low-grade serous ovarian carcinoma

Pathway	Gene	Frequency of alteration (%)	Reference	Common mutations	Effect on pathway
MAPK	*KRAS*	18–30	[40, 42]	G12V, G12D	Activating
MAPK	*NRAS*	9[a]	[43]	Q61R, Q61K	Activating
MAPK	*BRAF*	35–48	[40, 42, 44]	V600E	Activating
MAPK	*ERBB2*	6	[44]	c.2325dupTACGTGATGGCT, c.2322dupGCATACGTGATG, c.2324dupATACGTGATGGC	Activating

[a]Of all invasive cases with adjacent borderline malignancies

tions are found in about two-thirds of all LGSCs and in 61 % of serous borderline tumors, in a mutually exclusive fashion [40]. Mutations in these genes result in constitutive activation of the mitogen-activated protein kinase (MAPK) pathway. *KRAS* is a GTPase that transduces extracellular mitogenic signals into the cell, via the MAPK and also phosphoinositide 3-kinase (PI3K) pathways. In ovarian LGSCs *KRAS* is commonly mutated at codon 12, which renders the protein constitutively active in the absence of upstream mitogenic signals. Matching *KRAS* mutations can be detected in ovarian serous borderline tumors that recur as LGSC, strongly suggesting that LGSC develops from SBTs harboring activating *KRAS* mutations [41]. In LGSCs, *BRAF* is commonly mutated at position 600, where a valine to glutamate substitution renders the kinase constitutively active in the absence of activating stimuli. *BRAF* mutations are associated with better patient prognoses than *KRAS* mutations, because the most aggressive and recurrent LGSCs tend not to harbor *BRAF* alterations [42]. *BRAF* mutations are also associated with early tumor stage, which may suggest that *BRAF* alterations are early events in the genesis of ovarian LGSC. *RAS* molecules, such as *KRAS*, are major upstream regulators of *BRAF*, which is thought to explain the mutual exclusive manner in which *KRAS* and *BRAF* mutations are found in LGSC [42] and other solid tumors. Activation of the MAPK pathway can also occur via activation of *ERBB2* or *NRAS* also occur, although these alterations occur at a lower frequency than perturbations in *KRAS* or *BRAF* [43, 44]. Other key molecular alterations in BST/LGSC include p16(INK4A) [45] and maintained expression of p21(WAF) [46, 47], which could relate to the lower proliferative indices of these tumors relative to high-grade counterparts.

Conclusion

While the origins of high-grade serous ovarian cancer have been hotly debated, the cellular origins of low-grade serous ovarian cancer have been somewhat overlooked. It is, however, not yet clear whether LGSCs arise from ovarian or fallopian epithelial cells or from Müllerian-type epithelial cells within the uterus. The Cancer Genome Atlas project has generated a comprehensive catalogue of the somatic alterations in HGSC, profiling copy number alterations, mutations, as well as the transciptome and methylome and yielding novel candidate therapeutic targets [39]. However LGSCs were not included in this project, and similar analyses of somatic genetic alterations that occur early during the development of SBTs and LGSCs remain somewhat lacking. Although it is likely that activation of the MAPK pathway is an early event, more detailed analyses of the somatic events that lead to the genesis of LGSC will likely reveal novel opportunities for early detection and therapeutics.

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Precancerous Lesions of Ovarian Clear Cell and Endometrioid Carcinomas

3

Andres A. Roma

Precancers are lesions that precede the development of invasive cancers [1]. In other words, a precancer is one lesion that if left unchecked would eventually develop into a cancerous lesion. Ovarian clear cell and endometrioid cancers have a strong epidemiological link with endometriosis. Endometriosis has been the lesion most often associated and/or preceding ovarian clear cell carcinoma (in 50–90 % of cases) and endometrioid carcinomas (in up to 40 % of cases) [2, 3]. However, ovarian clear cell carcinomas have also been associated with clear cell adenofibromatous lesions [3]. Recently, there has been an attempt to explain most ovarian cancers with an origin outside the ovary, in particular the fallopian tube, as opposed to classic literature that reported the ovarian surface epithelium as the direct source of ovarian carcinomas [4, 5]. In this chapter, the author will attempt a comprehensive review of the precancerous lesions of ovarian clear cell and endometrioid carcinomas.

If a man will begin with certainties, he shall end in doubts; but if he will be content to begin with doubts he shall end in certainties.
Sir Francis Bacon. The Advancement of Learning (1605)

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Endometriosis

Nowadays, based on clinical, histopathological, and genetic evidence that will be reviewed herein, endometriosis is identified as the most likely precursor lesion for endometrioid or clear cell ovarian lesions. While not all patients with endometriosis will eventually develop malignancy, patients with endometriosis have a higher risk of developing the aforementioned carcinomas. Data from 13 ovarian cancer case–control studies, part of the Ovarian Cancer Association Consortium, were pooled in a review study, and logistic regression analysis was undertaken to assess the association between self-reported endometriosis and the risk of ovarian cancer [6]. Self-reported endometriosis was associated with a significantly increased risk of clear cell (odds ratio 3.05, 95 % CI 2.43–3.84, $p<0.0001$), low-grade serous (odds ratio 2.11, 1.39–3.20, $p<0.0001$), and endometrioid invasive ovarian cancer (odds ratio 2.04, 1.67–2.48, $p<0.0001$). No association was noted between endometriosis and risk of mucinous (odds ratio 1.02, 0.69–1.50, $p=0.93$) or high-grade serous invasive ovarian cancer (odds ratio 1.13, 0.97–1.32, $p=0.13$) or borderline tumors of either subtype (serous odds ratio 1.20, 0.95–1.52, $p=0.12$; mucinous odds ratio 1.12, 0.84–1.48, $p=0.45$).

From a practical standpoint, recognizing and diagnosing endometriosis is important for the

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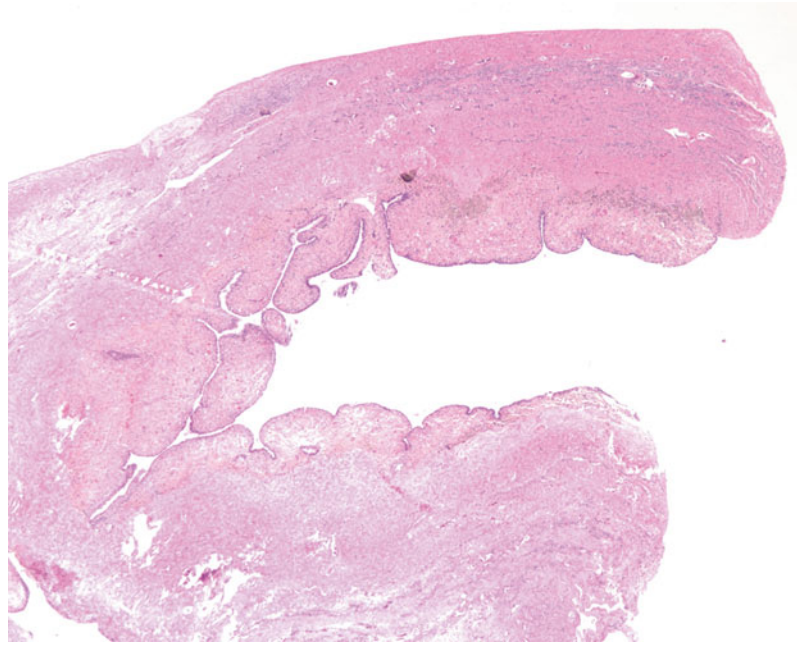

Fig. 3.1 Low power examination of ovarian endometriotic cyst lined by epithelium and underlying stroma with remote hemorrhage represented by pigment-laden macrophages. H&E 20×

obvious reason that it can help pathologists relate it with synchronous or metachronous lesions in the patient and provide an origin and accurate typing of the subsequent lesion(s) that might otherwise be problematic. The gold standard to diagnose endometriosis is still the microscopic examination of the hematoxylin and eosin (H&E) stained sections (Figs. 3.1, 3.2, 3.3, 3.4, and 3.5). Its diagnosis is usually straightforward and based on the presence of endometriotic-type epithelium, cellular endometrial-like stroma and recent or remote hemorrhage. It is important to note that most pathologists require two of these three components to establish the diagnosis (Fig. 3.6). An exception that the author has rarely encountered in clinical practice is the presence of only stroma with pseudo-decidualized changes due to previous hormonal treatment in patients with known or suspected endometriosis (Fig. 3.7).

While the endometrial-type cysts or glands in endometriosis have an epithelium that resembles the endometrial epithelium, the same metaplastic changes that occur in the endometrium can be seen in endometriotic lesions obscuring or making the diagnosis more challenging (Fig. 3.8). Similarly, the stroma might be overlooked or misinterpreted as cellular ovarian stroma and may be very subtle and or barely perceptible and discontinuous to the periglandular zone, while many other lesions can demonstrate areas of hemorrhage (Figs. 3.9, 3.10, and 3.11).

History of Endometriosis

Most of our knowledge of endometriosis can be traced back to Dr. John Albertson Sampson's publications. However, the first description of ovarian endometriosis is credited to William Wood Russell, who presented a paper to the Johns Hopkins Medical Society in 1899 entitled "Aberrant portions of the Müllerian duct found in the ovary" [7]. Pick later reported that Rokitansky's *cystosarcoma adenoides ovarii uterinum*, described by the latter in his textbook of pathologic anatomy published in 1861, could represent ovarian endometriosis [7].

Endometriosis was the primary focus of research of Dr. Sampson from 1921 and continued until the end of his career [7–9]. These contributions

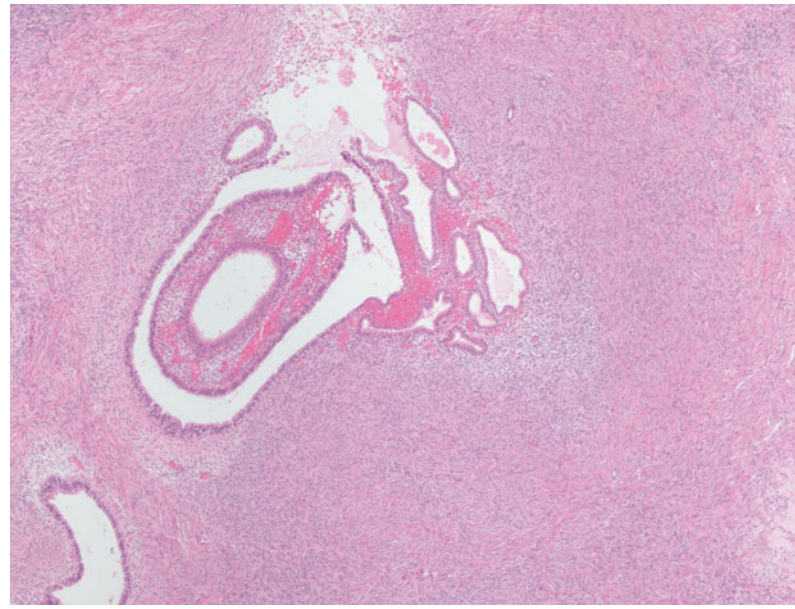

Fig. 3.2 Ovarian stroma with endometrial-type glands associated with endometrial-type stroma and recent hemorrhage. H&E 400×

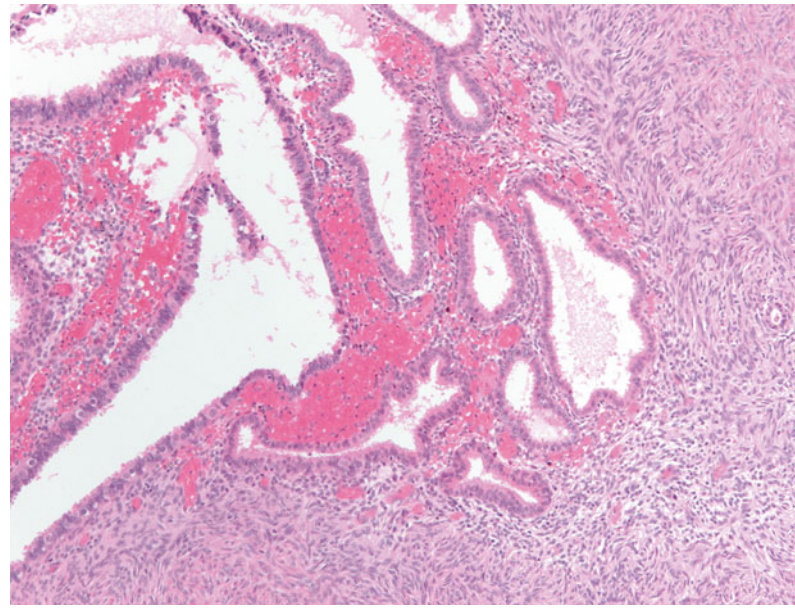

Fig. 3.3 High-power examination of Fig. 3.2 to better demonstrate endometrial-type glands and stroma devoid of atypia. H&E 200×

earned him the appellation "Father of Endometriosis." Not only were these articles of significant scientific importance, but they were also written solely by him, but their number (a total of 18, 14 published in the 1920s), their length (mean 35 pages), and their numerous gross and microscopic illustrations would be distinctly unusual today. Most of the work was conducted by himself in his own laboratory and with his own technician, independent of the Pathology Department of Albany Hospital, where he worked [7]. In 1936, Cattell and Swinton reviewed the

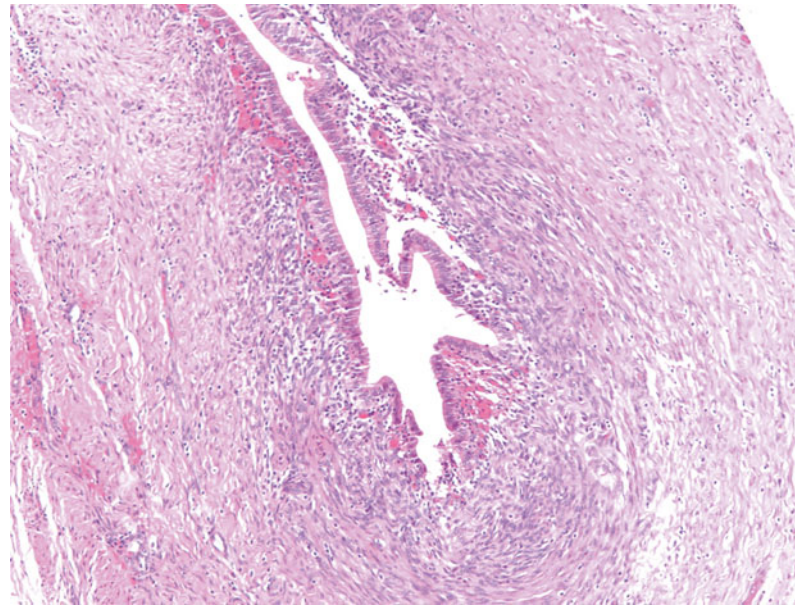

Fig. 3.4 Example of endometriosis better demonstrating cellular endometrial-type stroma. H&E 200×

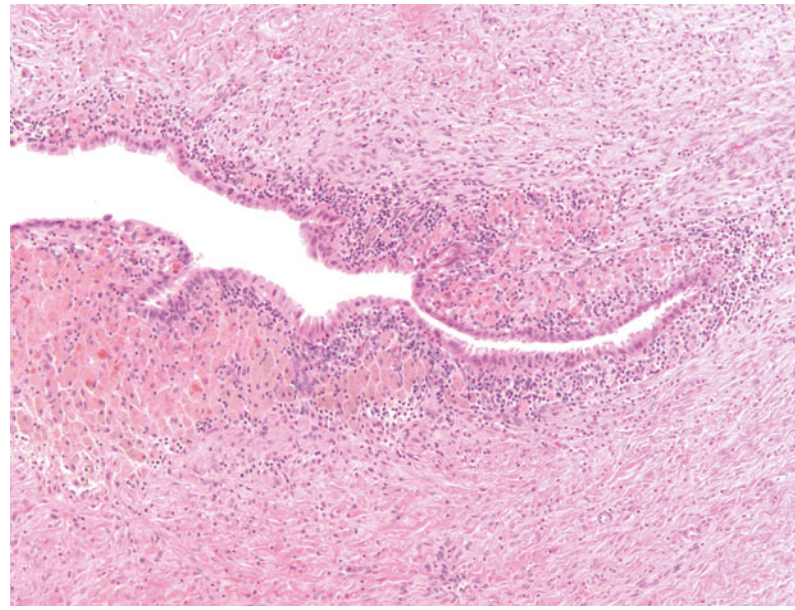

Fig. 3.5 Ovarian endometriotic cyst with abundant pigment-laden macrophages. H&E 200×

literature prior to 1921, estimating that fewer than 20 reports of what would be interpreted today as endometriosis were published worldwide to that date [7].

The first report of extraovarian endometriosis is credited to Rokitansky in 1860, referring extraovarian endometriotic lesions as "adenomyomas" because of the frequent admixture of endometrial tissue with benign smooth muscle. That report was followed by similar reports by von Recklinghausen between 1893 and 1896. Originally, endometriosis was considered a congenital lesion of either Wolffian or Müllerian origin. This theory was subsequently supplanted by the coelomic or serosal metaplasia theory (attributed to Iwanoff). In his 1898 article, Iwanoff proposed that ectopic

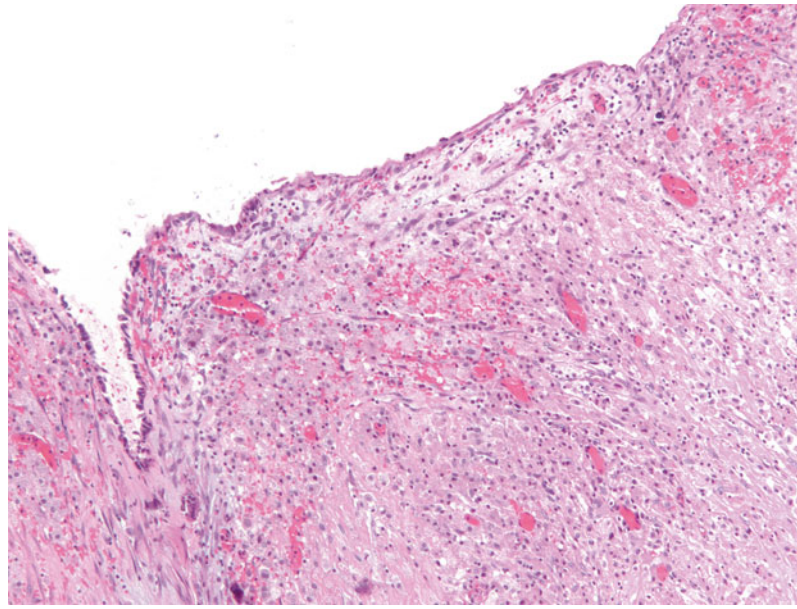

Fig. 3.6 Adjacent area to Fig. 3.5 that is a more challenging example of endometriosis with abundant histiocytes. H&E 200×

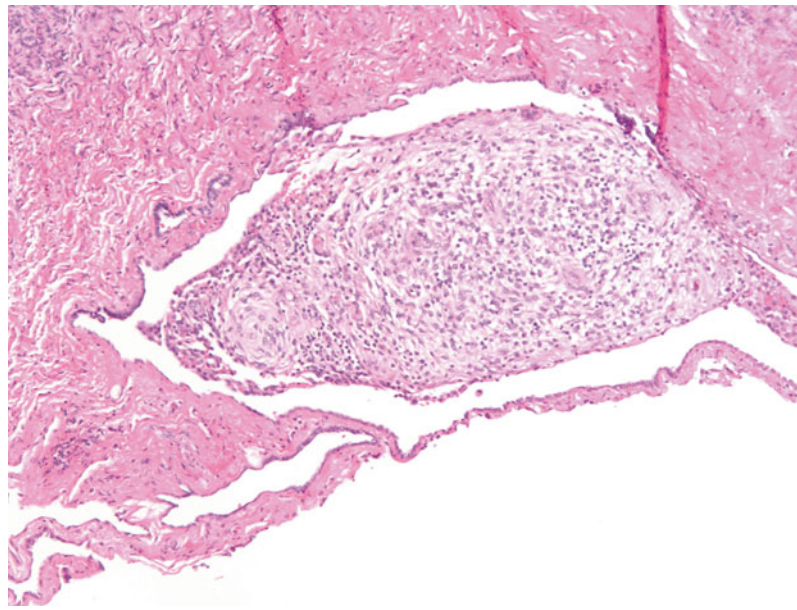

Fig. 3.7 Ovarian surface adhesions with clusters of stromal only cells with decidualized changes in patient with known endometriosis receiving hormonal treatment. H&E 200×

endometrial tissue is a result of metaplasia of the peritoneum. Robert Meyer believed that peritoneal inflammation stimulated the metaplastic transformation of the mesothelium to endometrial-like tissue [7]. A more detailed description of Sampson's papers and the history of endometriosis can be found in the excellent publications by Dr. Philip Clement [7–9].

Origin of Endometriosis

Much on this topic has been published and debated, but it is still controversial. In Sampson's first paper, he postulated the idea that endometriosis could be due to two possibilities. The endometrium during normal menstrual period might take a backward direction and flow into

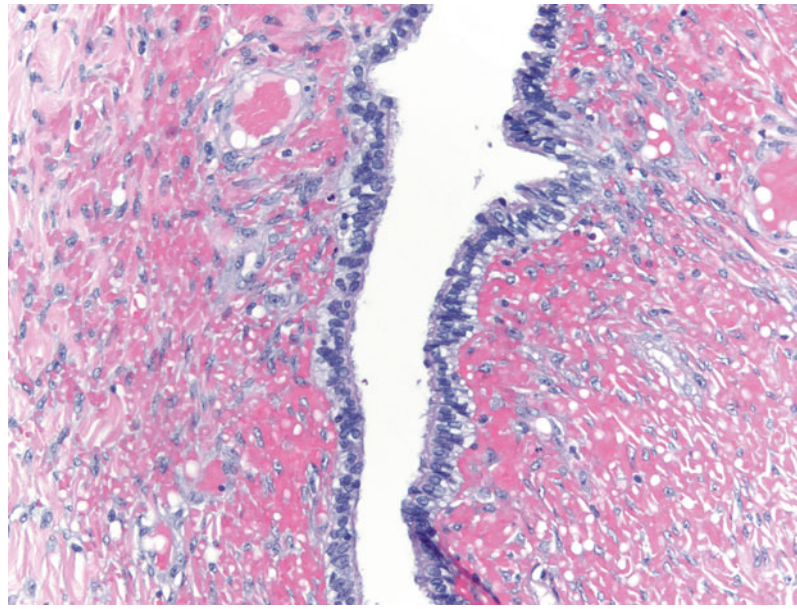

Fig. 3.8 Area of endometriosis with ciliated metaplasia as well as clear cell change on the *right*, in an otherwise classic example of endometriosis. H&E 200×

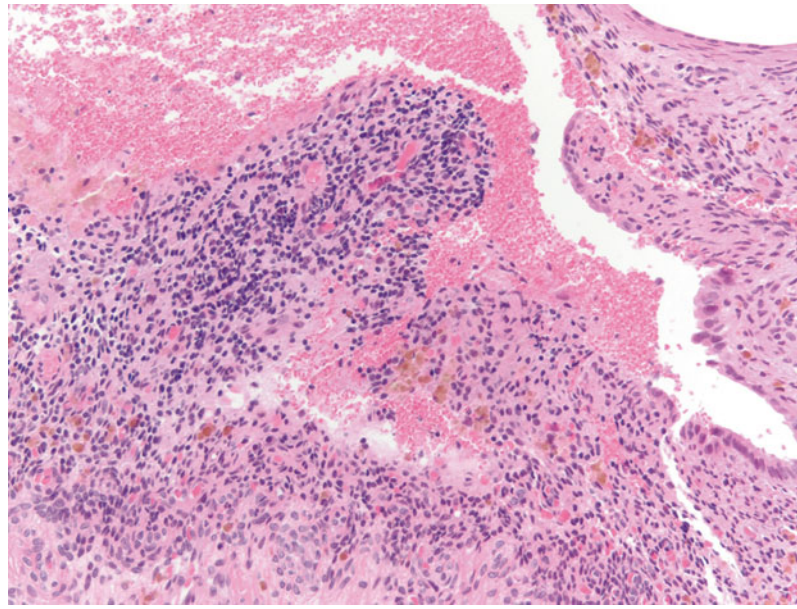

Fig. 3.9 Stroma predominant endometriosis with focal endometrial-type epithelium on the *right*. Also note pigment-laden macrophages consistent with remote hemorrhage. H&E 200×

the peritoneal cavity and attach to any organ, a concept that later would develop into the implantation theory (abnormal menstruation with a backward flow through the tube). Sampson postulated that "menstrual blood might at times escape into the pelvis, carrying with it some of the epithelium lining the cyst cavity; this epithelium may become implanted in the cul-de-sac or other portions of the pelvis and there give rise to other foci of endometrial tissue" (Fig. 3.12). Another possibility was that an ovarian endometrioma may rupture, spreading its endometrial-type lining into the peritoneum, indicating that at the time, endometrioma or ovarian "hematoma" was

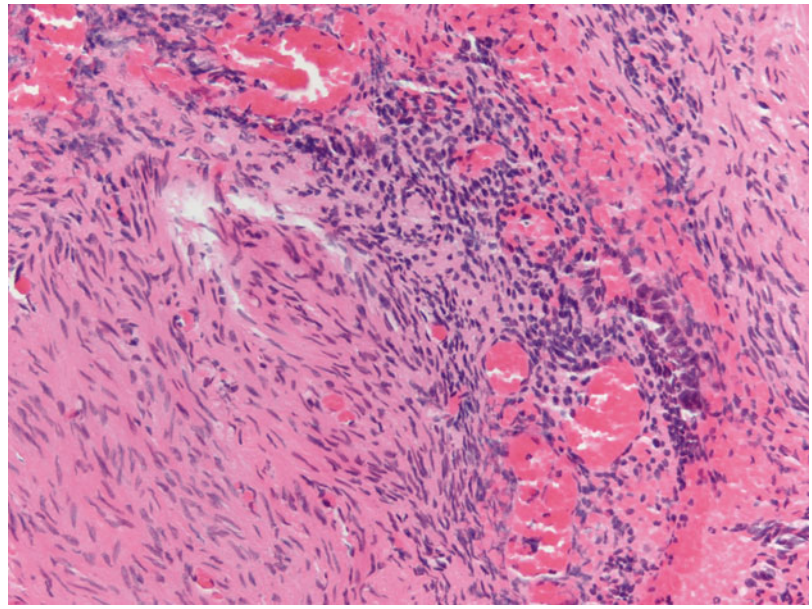

Fig. 3.10 Example of stroma predominant endometriosis with focal endometrial-type epithelium on the *right*. Note recent hemorrhagic foci. H&E 200×

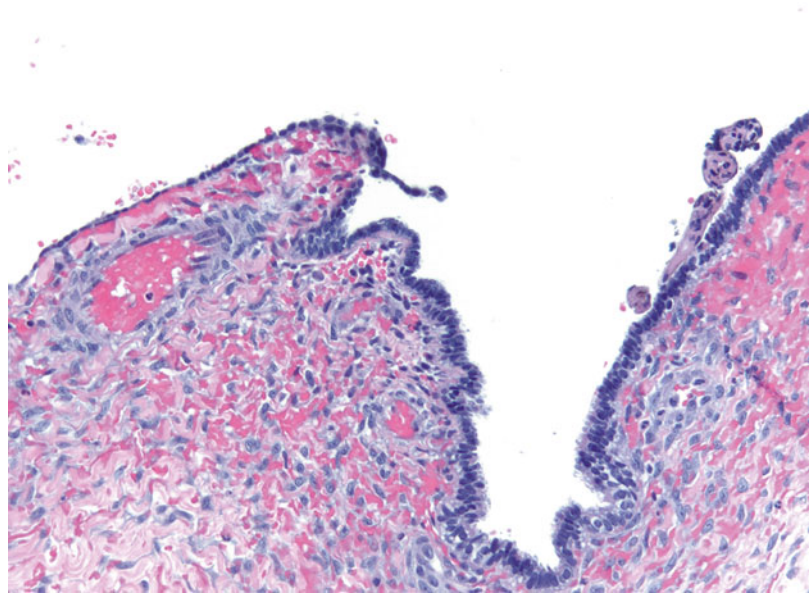

Fig. 3.11 Same case as Fig. 3.8 showing predominantly ciliated epithelium, not supportive of endometriosis. Also note lack of endometrial-type stroma. Other areas had classic features of endometriosis. H&E 200×

believed as an independent lesion and not associating that ovarian endometriosis might also be related to transtubal spread of endometrial tissue [7, 10, 11]. Evidence for the theory proposed by Sampson included finding the endometriotic lesions during the menstrual life of women, the presence of a retroflexed uterus favoring a backward flow of menstrual blood, patent tubes indicating that transtubal spread was possible, and the occurrence of endometriosis in the most susceptible areas of the ovary for this occurrence [7] (Fig. 3.13). Additional observations that supported his theory were reported in subsequent publications including "…blood may be observed

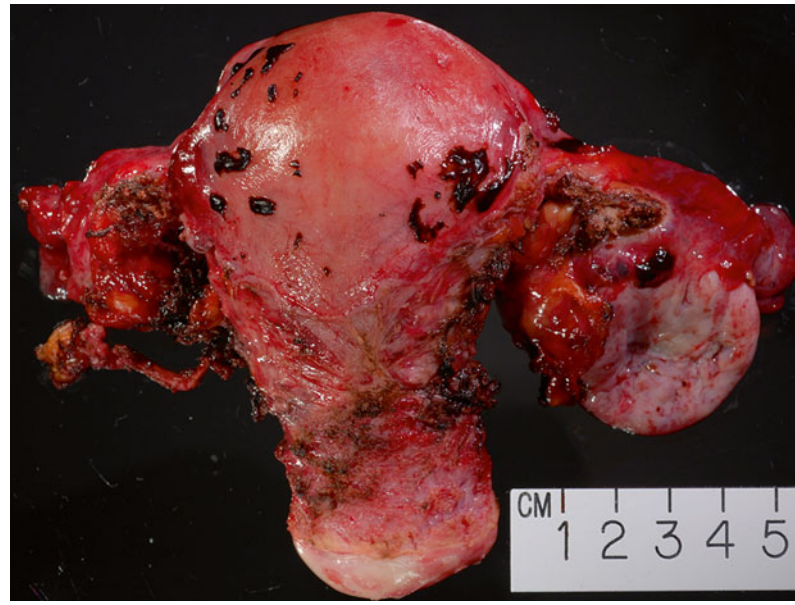

Fig. 3.12 Gross example of endometriosis involving uterine serosa as well as ovarian surface on the *right*. The enlarged ovary was in part replaced by endometriotic cyst

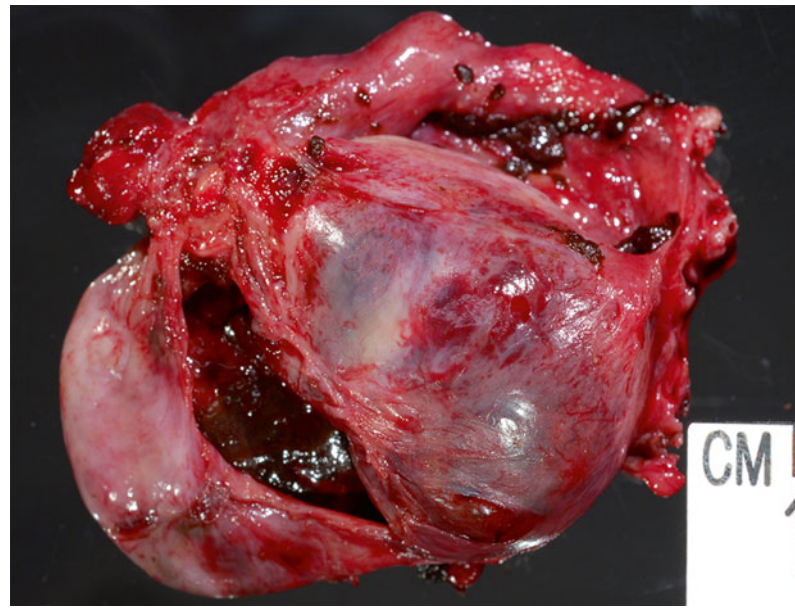

Fig. 3.13 Enlarged ovary replaced by cystic lesion containing chocolate-type content grossly consistent with endometriosis

escaping through the lumen of the fimbriated ends of the tubes of women operated upon during menstruation…" and finding endometrial tissue within tubal lumina in removed fallopian tubes [7] (Figs. 3.14 and 3.15). In 1926, he was able to quote in support of this belief the experimental work of Jacobson who successfully "transplanted" endometrial tissue into the peritoneum of rabbits [12]. In a paper published in 1927, Sampson reported a case of embolic endometriosis in the venous circulation. He described a patient with myomatous uteri who was menstruating at the time of surgery. Injecting the uterine cavity with water and melted gelating, he noted how the menstruating blood was "escaping from the severed uterine and ovarian veins" [13, 14].

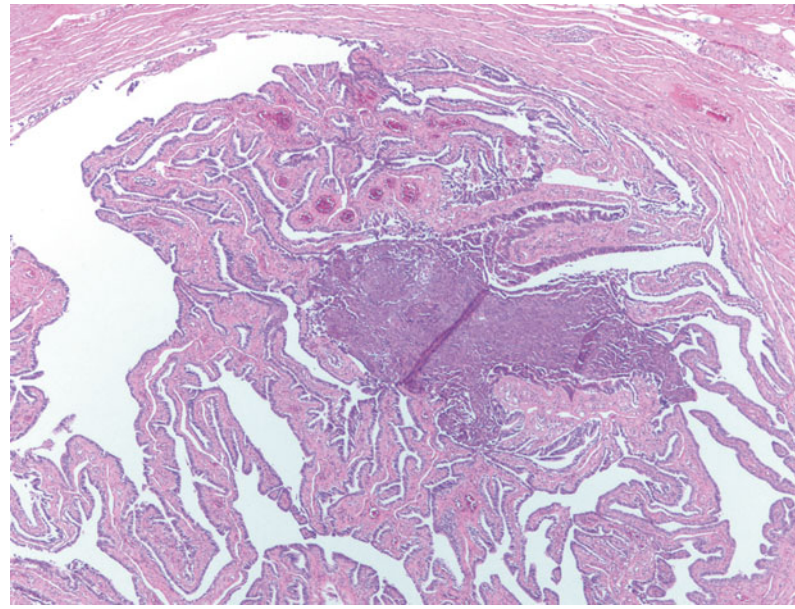

Fig. 3.14 Endometrial-type tissue involving the lumen of an otherwise normal fallopian tube, suggesting that endometrial tissue can travel through the tube. H&E 100×

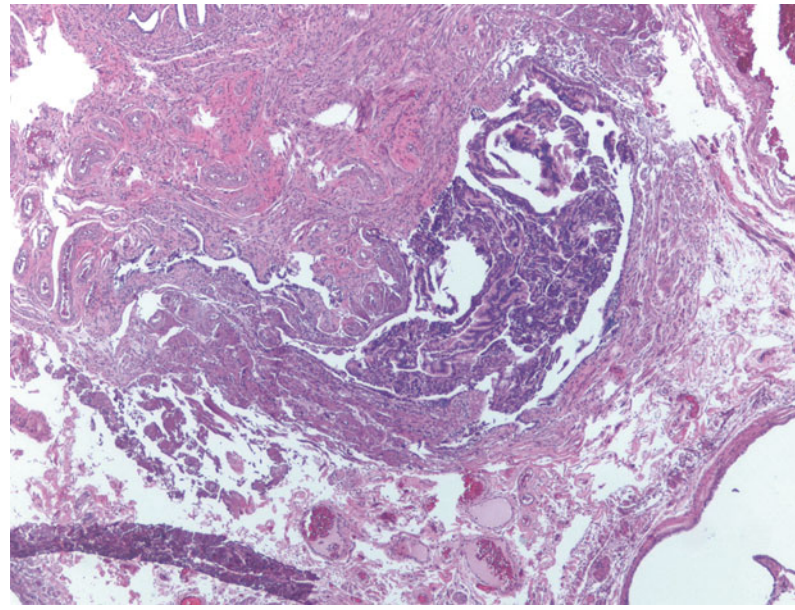

Fig. 3.15 Patient had multiple foci of endometriosis involving ovaries, uterine serosa, and peritoneum as well as endometrial-type tissue involving the fallopian tube lumen. H&E 100×

He suggested that "bits of uterine mucosa, occasionally, might escape into the venous circulation during menstruation" adding another possibility to the pathways of endometriosis. It was also Sampson who introduced the term endometriosis: "The nomenclature of misplaced endometrial or Müllerian lesions is a difficult one to decide upon….A variety of lesions is produced by misplaced endometrial or Müllerian tissue, and it is difficult to classify all of them as true tumors…. The term endometriosis is more descriptive than mullerianosis and is correct in the majority of instances because we believe that the uterine mucosa is the chief source of these lesions" [7].

However, Sampson's work provoked a revival of the metaplasia theory proposed by Meyer [12]. Criticisms on the retrograde menstruation were based on the belief that sloughed endometrial

tissue had become anoxic and nonviable and accordingly was unable to grow at another location [10, 13]. Novak, reexamining the histology of hundreds of fallopian tubes, found particles of uterine tissue lying free in the lumen of the tube in only seven instances. In none had the patient been menstruating. In at least five of them, the endometrial tissue was so large that it was not possible for them to have entered through the tiny tubes orifice, and as endometrial tissue was also found in a number of the adjacent ovaries, Novak believed that the fragments were traveling down the tube and not up [13, 15]. He challenged the retrograde menstrual theory to explain such a common condition as pelvic endometriosis. The proponents of the metaplasia theory pointed out that all the genital epithelia were derived from the coelomic epithelium and so are related to the peritoneum, indicating that an irritant can induce the peritoneum to transform itself into endometrial tissue, since the endometrium and endosalpinx may be looked on as modified peritoneum [13, 15].

There is still debate today about the origin of endometriosis, and it is possible that there is more than one cause of endometriosis, with the dominant causative factors varying depending on the location. There are three clinically distinct forms of endometriosis, including endometriotic implants on the surface of the pelvic peritoneum and ovaries (peritoneal endometriosis), ovarian cysts lined by endometrioid mucosa (endometriomas), and complex solid masses comprised of endometriotic tissue within adipose and fibromuscular tissue, residing between the rectum and the vagina (rectovaginal endometriotic nodule) [16]. Their causes could be the same or different in each site. What is known since the early twentieth century is that endometriosis is related to uninterrupted ovulatory cycles [16]. It was felt that the increase in the endometriotic rate in the 1930s was due to delayed marriages, the lack of early child bearing, probably associated with the economic difficulties of the great depression era [15]. Meigs agreed with these concepts and recommended young couples to have children early and practice contraception after (not before) they have children: “couples should be taught how to have children, not to avoid them” [15].

Based on the lack of uniform opinion (and definitive research models proving or disproving these theories) regarding the origin of endometriosis in the ovary (or others sites), different models are currently being proposed. Proponents of the coelomic metaplasia theory support that the peritoneal–mesothelial covering of the adult ovary retains the ability to differentiate into serous, endometrioid, and mucinous epithelia, acquiring a Müllerian epithelium [16, 17]. Recently a different theory has been proposed: direct implantation of tubal epithelium into the ovary to form an inclusion cyst, which in turn might be the site of origin of ovarian serous carcinoma is an alternative theory to that of metaplasia from the surface epithelium (mesothelium) [4] (Figs. 3.16 and 3.17). Implantation of fallopian tube epithelium from the fimbria at the time of ovulation when the surface epithelium is disrupted can explain the derivation of low-grade and high-grade serous carcinomas [4]. Also, entrapment of exfoliated endometrial epithelium, most likely in areas of prior ovulation can be incorporated into the ovarian tissue, developing into endometriotic cysts [4, 5].

Irrespective of the cause, it is unclear what determines the fact that some, but not most, women develops endometriosis, since most women have backflow menstruation into the peritoneal cavity, but endometriosis occurs in only 5–10 % [18]. Two mechanisms could potentially explain the preferential implantation of endometrial tissue onto the peritoneal surface in some patients: molecular defects (activation of oncogenic pathways) and/or immunologic abnormalities (failure of the immune system to clear implants from the peritoneal surface) [18–26].

Metaplastic Changes and Atypical Morphologic Features of Endometriosis

Before molecular and/or genetic studies were widely available, morphologic features were identified to try to predict or detect patients with endometriosis at risk of developing malignancy. The first lesions described were atypical endome-

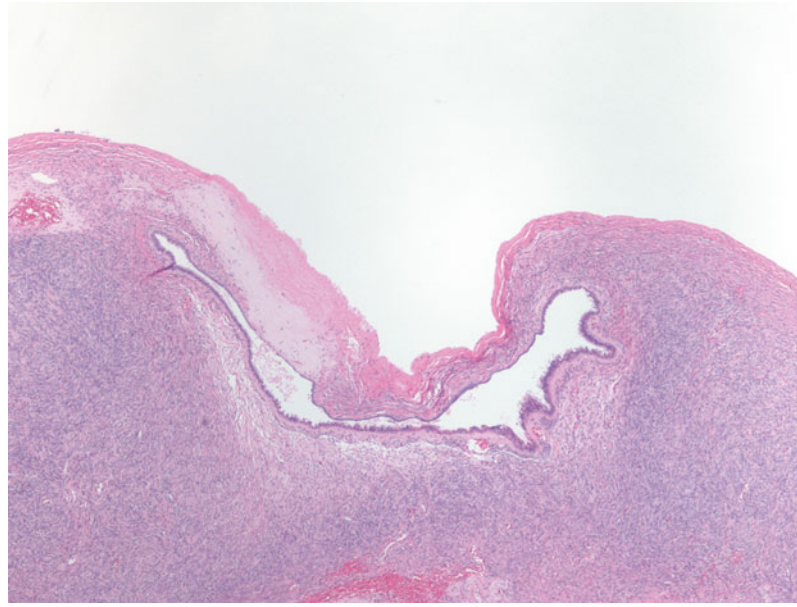

Fig. 3.16 Epithelial-lined cyst adjacent to ovarian surface with area of hyalinized stroma. Possible are implantations of fallopian tube epithelium after ovulation. H&E 40×

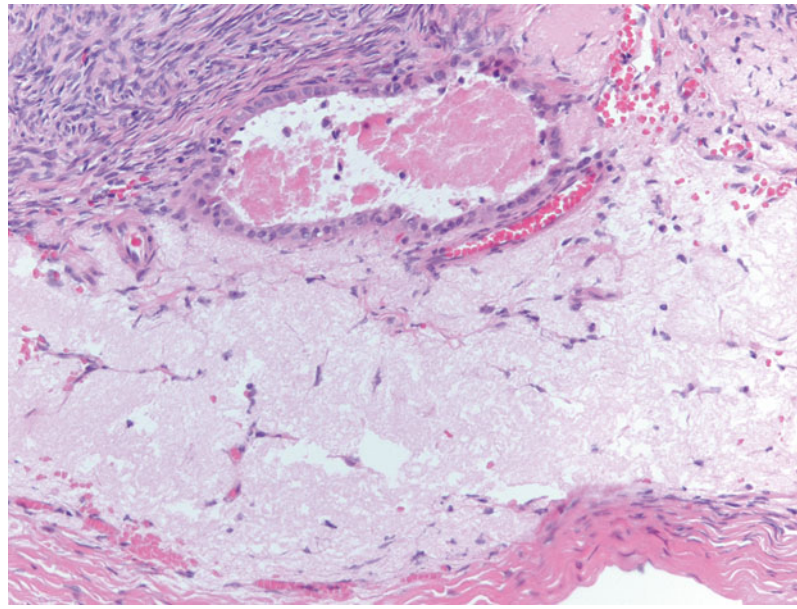

Fig. 3.17 High-power examination of epithelial-lined cyst adjacent to ovarian surface with area of hyalinized stroma. H&E 200×

triosis and hyperplasia within an ovarian endometriotic cyst [27–44]. In some cases, both terms were used interchangeably; more specifically, cases of hyperplasia within ovarian endometriosis were designated atypical endometriosis, similar to cases of ovarian endometriosis harboring only cytologic atypia. Atypical endometriosis is usually characterized by a focal or multifocal finding of an eosinophilic epithelium, though in some cases, focal clear cytoplasm could be seen, in addition to cells with irregular nuclei of variable size, enlarged or with bizarre changes, and/or hyperchromatic with smudged chromatin and/or prominent nucleoli (Figs. 3.18, 3.19, 3.20, 3.21, and 3.22). The atypical cells are lined in a simple epithelium, but the cysts may be focally

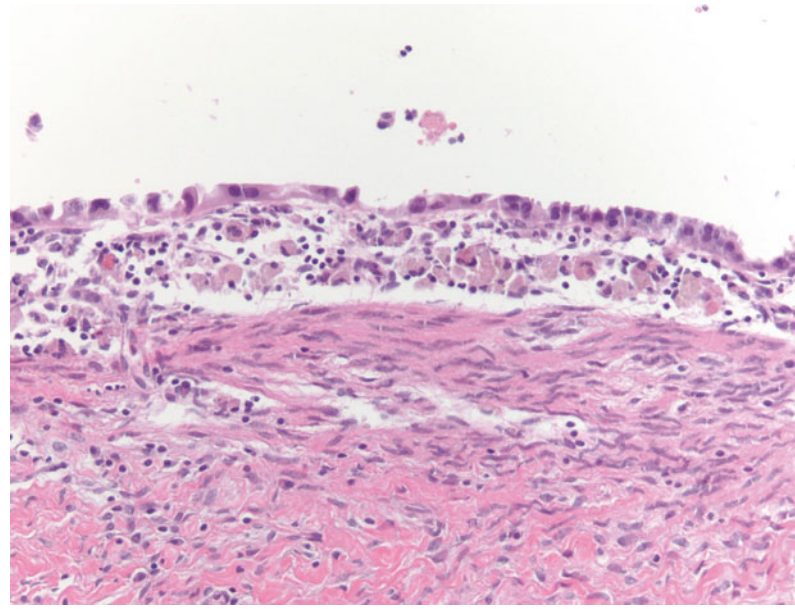

Fig. 3.18 Area of endometriotic cyst showing epithelial lining with larger nuclei, pleomorphism, and high nuclear to cytoplasmic ratio, consistent with atypical endometriosis. H&E 200×

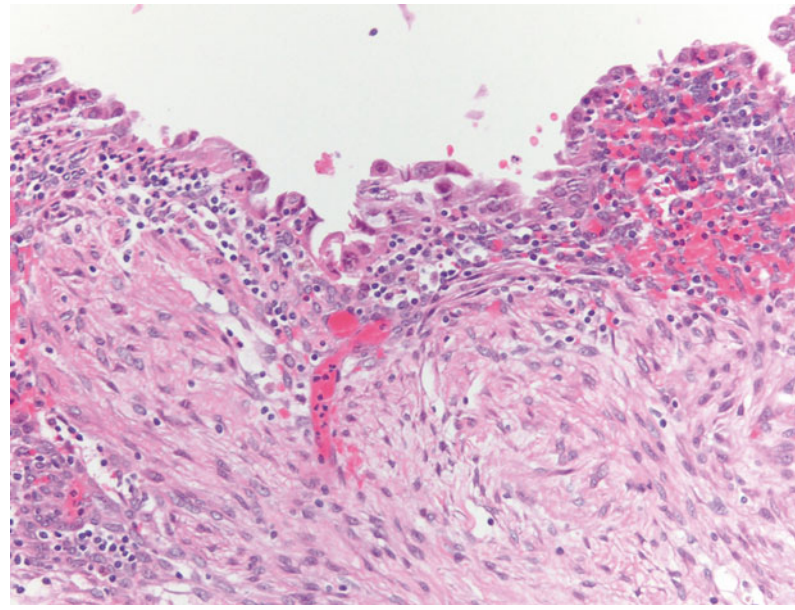

Fig. 3.19 Another example of atypical endometriosis. In this case, there is abundant inflammation, and the changes could be reactive in nature. H&E 200×

stratified as small papillary structures [45]. Inflammatory cells can accompany this epithelium. It is often difficult to determine the true nature of these changes, which may range from being entirely reactive changes (and accordingly benign) to, at the opposite end of the spectrum, being compatible with a neoplasm and mimicking or suggesting a clear cell carcinoma (Figs. 3.22, 3.23, 3.24, and 3.25). Frequently the epithelium is discontinuous, leaving the underlying stroma with hemosiderin-laden macrophages, pseudoxanthoma cells, fibrinous material, and/or

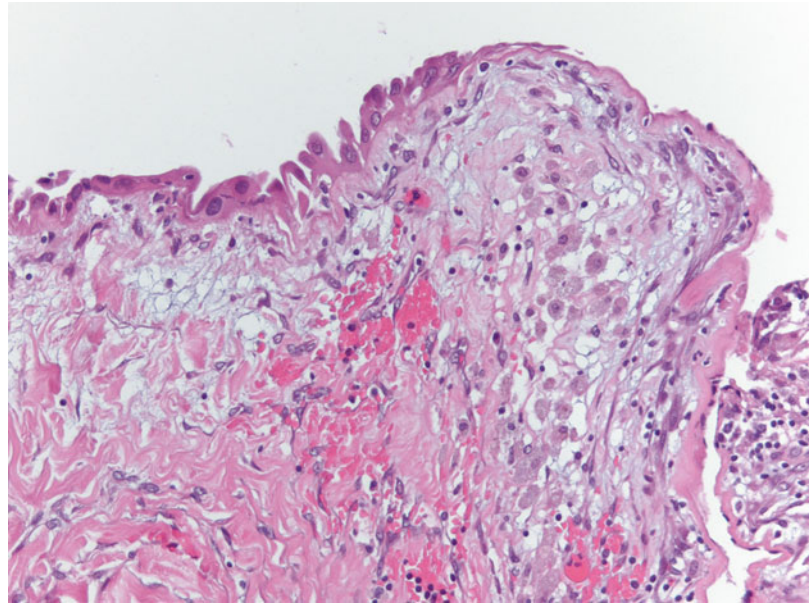

Fig. 3.20 Different areas of atypical endometriosis; same case as Fig. 3.19. H&E 200×

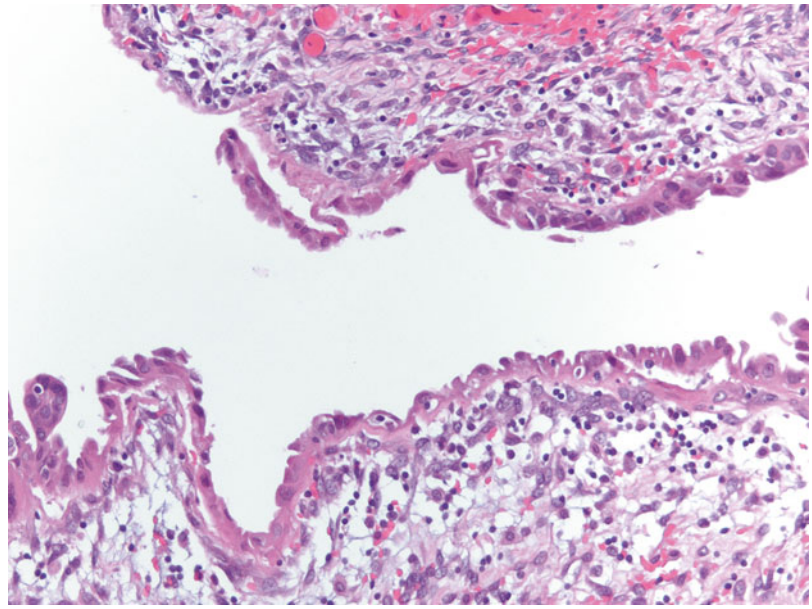

Fig. 3.21 Different areas of atypical endometriosis; same case as Fig. 3.19. H&E 200×

loose fibrous tissue with hemorrhage. Epithelial stratification and/or papillae can be seen [27]. Cases of hyperplasia within endometrioma show similar features to that seen in the endometrium, including crowded, simple, or complex glands with or without cytologic atypia (Figs. 3.26, 3.27, 3.28, and 3.29).

The rate of atypical endometriosis is very low, in the range of 1.7–3.6 % as reported by Fukunaga et al. and Czernobilsky and Morris [28, 29]. The rate of malignant transformation in atypical endometriosis was 25 % in Fukunaga's study (one of four patients with atypical endometriosis developed carcinoma after 2.5 years of follow-up).

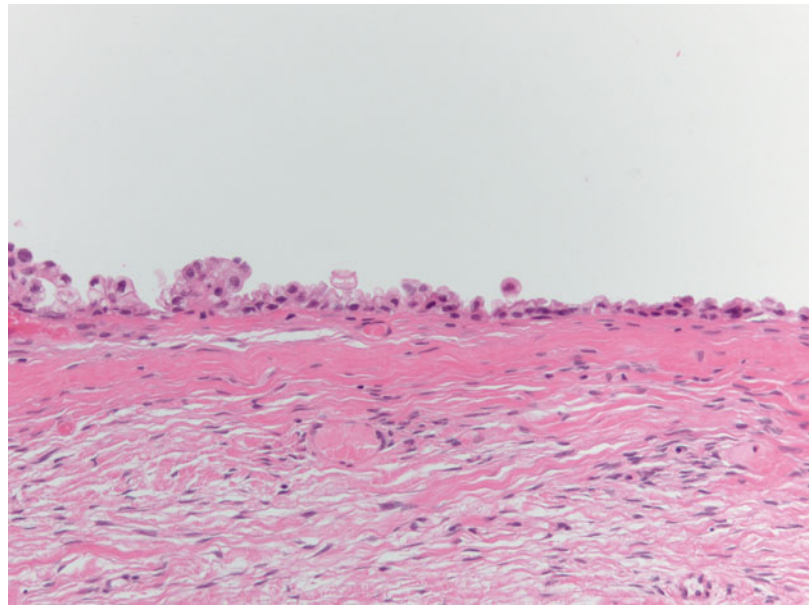

Fig. 3.22 Atypical endometriosis. In this case, the cytoplasm shows clear cell change and incipient papillary formations, raising the possibility of progression to clear cell carcinoma. H&E 100×

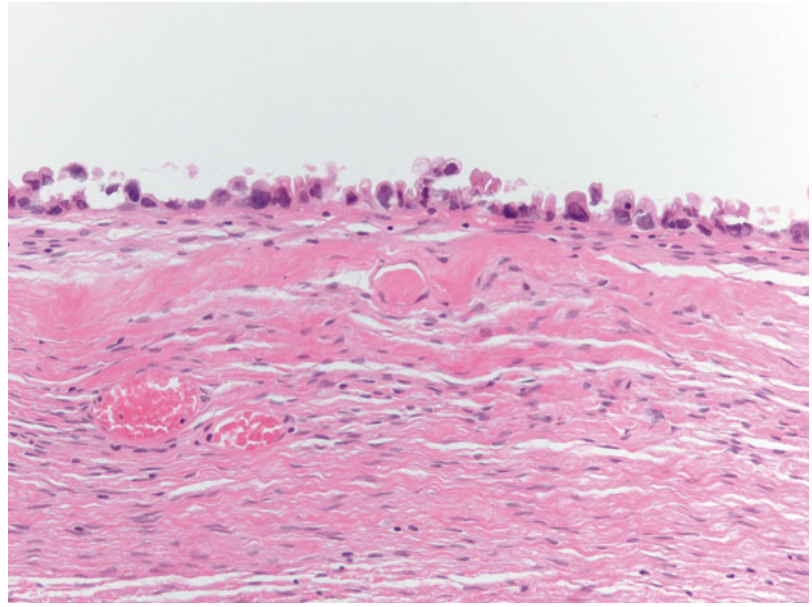

Fig. 3.23 High-power examination showing clear cell change in the cytoplasm. Other areas showed classic solid and glandular features of clear cell carcinoma. H&E 200×

While in Seidman's study, only 1 of 20 patients with complex atypical hyperplasia or "early carcinoma" developed clinically evident endometrioid adenocarcinoma after 8.6 years of follow-up [28, 33]. A recent report described the case of a patient that started with ovarian endometriosis and progressed to atypical ovarian endometriosis and ovarian endometrioid carcinoma 10 years after the original diagnosis [46].

Two things are worth emphasizing at this point: first, the importance of thorough histologic sampling of the tissue when significant atypia or hyperplasia is identified in endometriosis (submitting the entire lesion is recommended) and

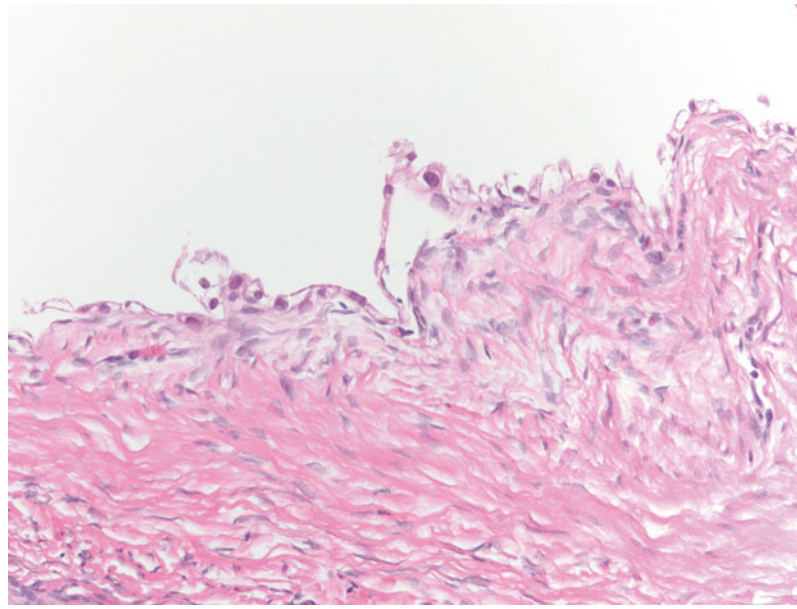

Fig. 3.24 Examples of atypical clear cells lining an endometriotic cyst suggesting incipient clear cell carcinoma (or carcinoma in situ). H&E 200×

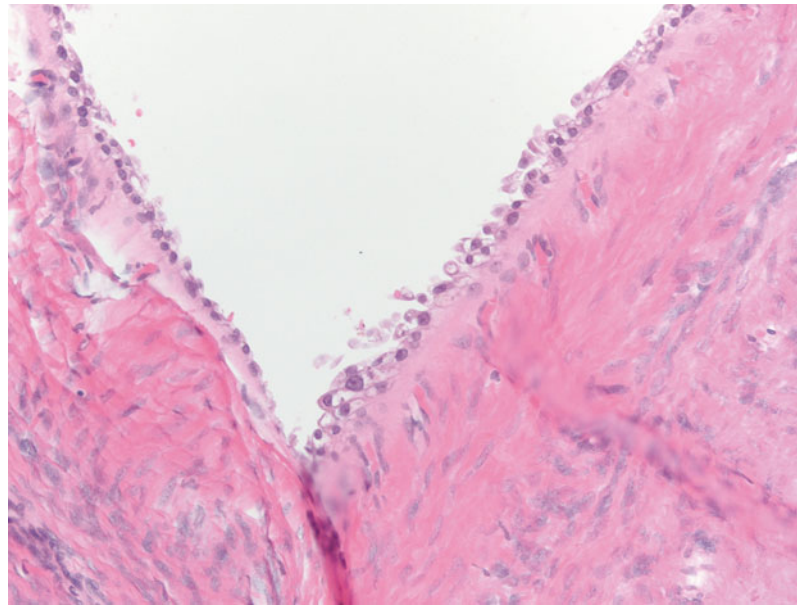

Fig. 3.25 Examples of atypical clear cells lining an endometriotic cyst suggesting incipient clear cell carcinoma (or carcinoma in situ). H&E 200×

second, the metaplastic changes that can occur in endometriosis and mimic neoplastic changes.

Similarly to the uterine endometrium, the epithelial lining of ovarian endometriosis can undergo metaplastic, hyperplastic, and atypical changes and even malignant transformation [29, 32, 44]. Metaplasia is a replacement of the endometrial epithelium with an epithelium that is normally encountered in another organ of derivation [32, 44]. Metaplastic changes can create difficulty

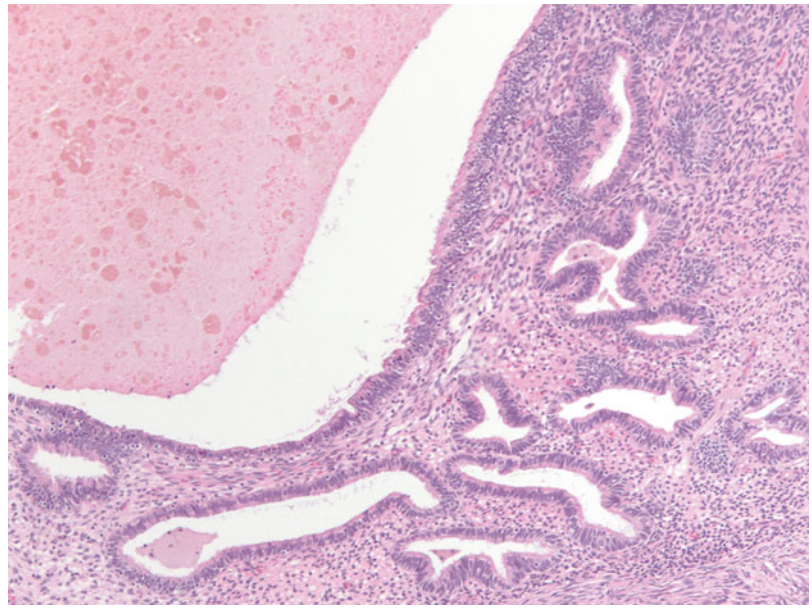

Fig. 3.26 Clustered endometrial glands in endometriotic cyst, consistent with hyperplasia within ovarian endometriosis. H&E 200×

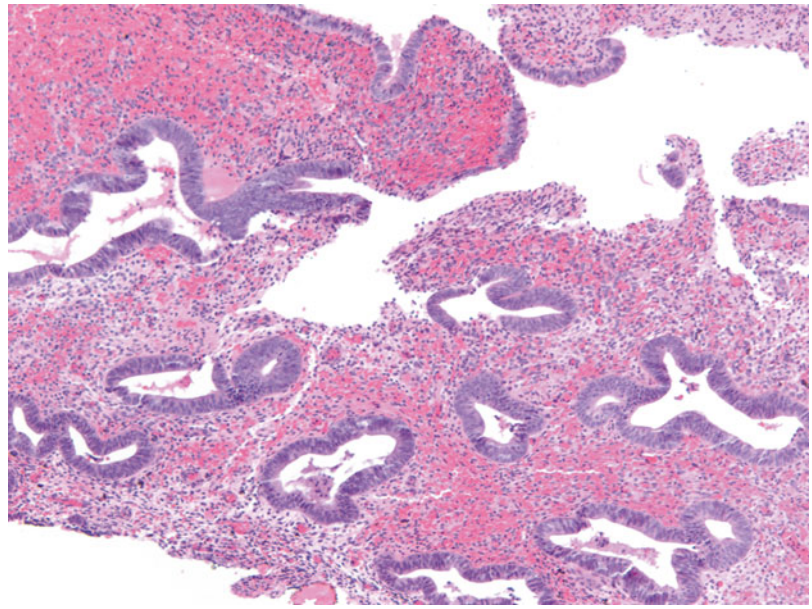

Fig. 3.27 Detached fragments of hyperplastic endometrial glands within a large endometriotic cyst. H&E 100×

diagnosing the underlying endometriosis or suggesting a different diagnosis (Figs. 3.30 and 3.31). Most common changes include ciliated change, exemplifying the close relationship of tubal-type epithelium, eosinophilic, hobnail or clear cell, squamous, and mucinous metaplasia. In a study of 388 ovarian endometriosis cases, the most common type of metaplasia was eosinophilic or oncocytic (5.6 %) followed by mucinous metaplasia (5.3 %), while the other types of metaplasia accounted for less than 2 % each [32]. The majority of metaplasias in ovarian endometriosis are observed in cases not associated with malignant epithelial tumor or atypia and should not be

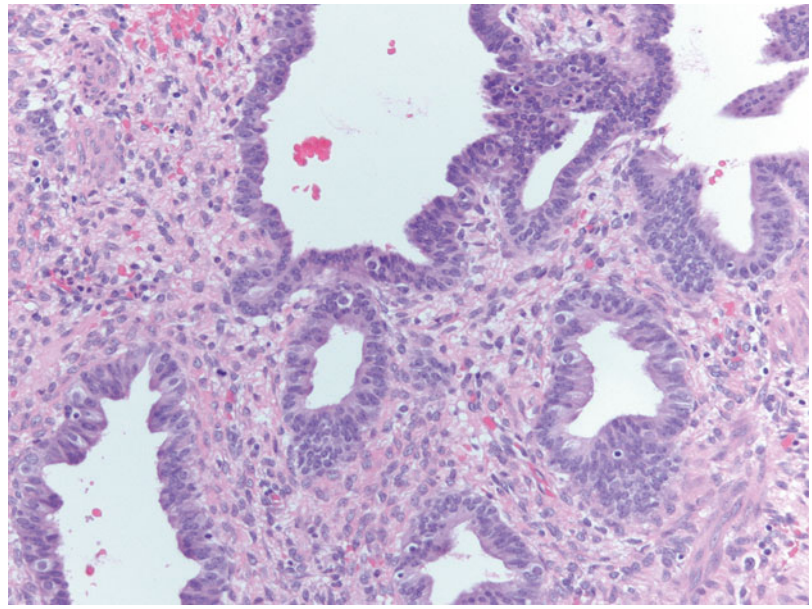

Fig. 3.28 High-power examination showing cytologic atypia consisting of focal enlarged round nuclei and prominent nucleoli. H&E 200×

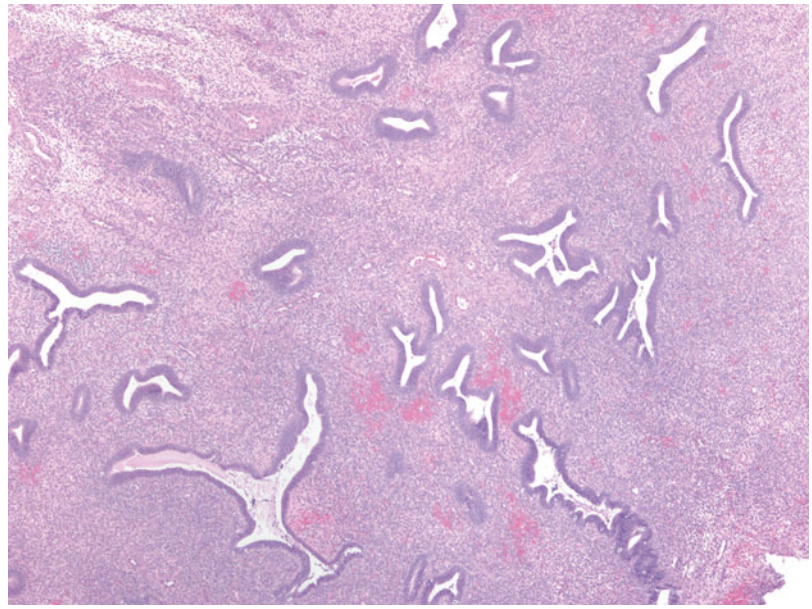

Fig. 3.29 Low-power examination of polypoid area within an endometriotic cyst, consistent with polypoid endometriosis, differential diagnosis of hyperplasia. H&E 40×

interpreted as neoplastic features [44]. However, cases of mucinous borderline tumors, endocervical type, also associated with endometriosis usually harbor areas of endometriosis with mucinous metaplasia adjacent to the tumor [44].

Occasionally, endometriotic glands in women with an intrauterine or ectopic pregnancy exhibit overt secretory changes that may include Arias-Stella reaction [47] (Figs. 3.32, 3.33, 3.34, and 3.35). The changes include nuclear enlargement and hyperchromasia and/or optically clear nuclei with prominent nucleoli and cytoplasmic vacuolization or clearing due to accumulation of intracellular glycogen. Arias-Stella changes in glands

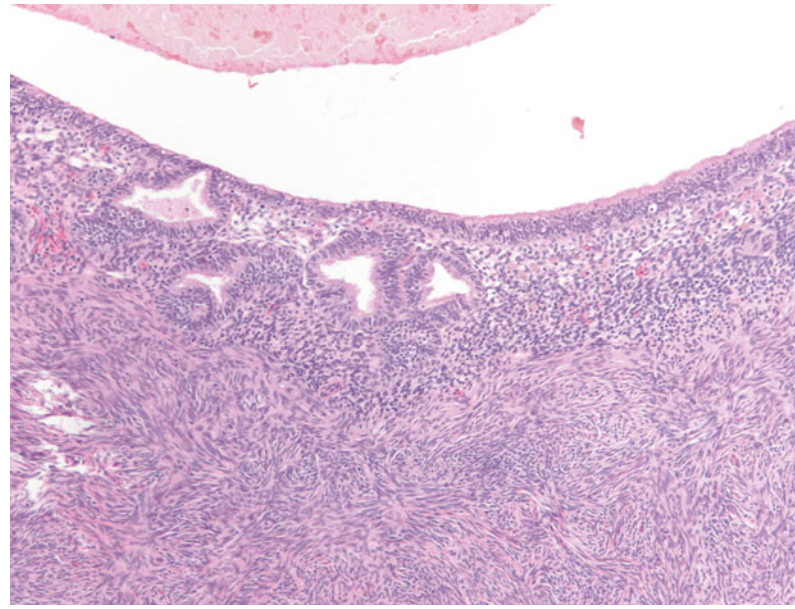

Fig. 3.30 Low- and high-power examinations of an endometriotic cyst. Note the lining with cilia, indicative of tubal metaplasia and the cellular endometrial-type stroma and occasional pigment-laden macrophages. H&E 100×

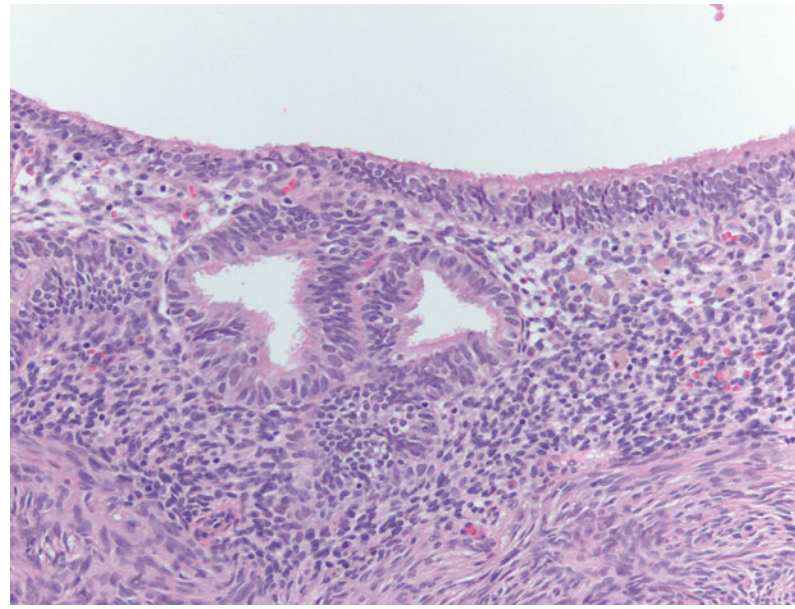

Fig. 3.31 Low- and high-power examinations of an endometriotic cyst. Note the lining with cilia, indicative of tubal metaplasia and the cellular endometrial-type stroma and occasional pigment-laden macrophages. H&E 200×

containing nuclei showing markedly pleomorphic features (monstrous cell type) can in occasion be seen. These changes mimic atypical endometriosis or clear cell carcinoma. The history of pregnancy, or changes seen in a young and fertile patient, might alert to the possibility of the presence of benign features. If the lesion presents in postmenopausal patients or if the differential diagnosis is still problematic, immunostains for KI-67 and Estrogen receptors (ER) can distinguish Arias-Stella reaction from clear cell carcinoma and other types of high-grade carcinomas [48]. In one study that comparatively assessed the expression of these markers in uterine carcinomas

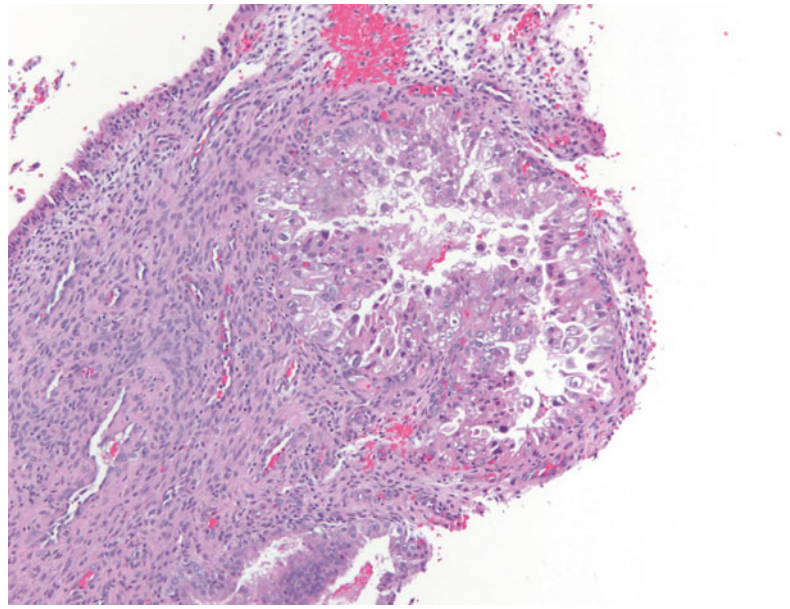

Fig. 3.32 Low power examination of Arias-Stella change within endometriotic cyst raising the possibility of clear cell carcinoma. Patient was 37 weeks pregnant, and the ovarian cyst was resected during cesarean section. H&E 100×

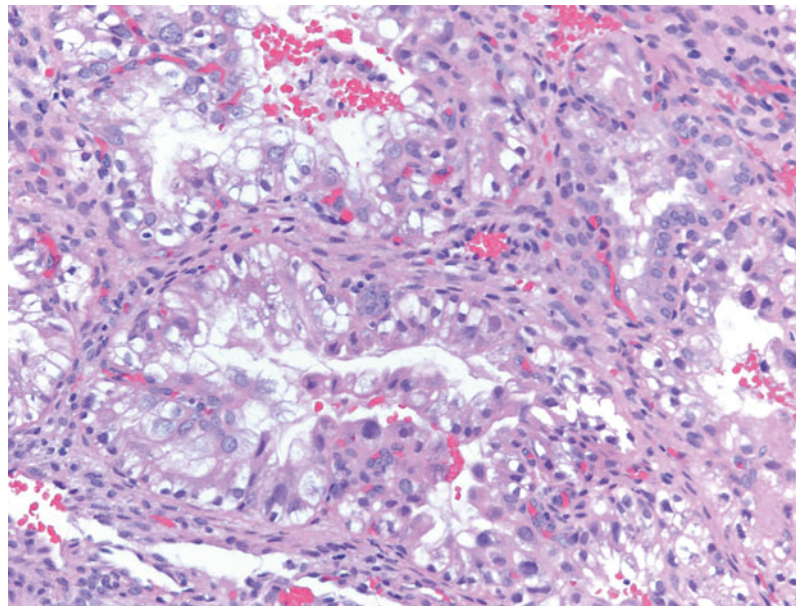

Fig. 3.33 High power examination of Arias-Stella change within endometriotic cyst raising the possibility of clear cell carcinoma. Patient was 37 weeks pregnant, and the ovarian cyst was resected during cesarean section. H&E 200×

and uterine Arias-Stella reaction [48], the former had a low KI-67 proliferation index, while clear cell carcinoma expressed KI-67 broadly with increased intensity (Fig. 3.31). ER was negative in all tested clear cell carcinoma cases, while Arias-Stella reaction was positive in about 50 % of the cases.

Molecular Features of Ovarian Endometriosis and Associated Carcinomas

Molecular distinctions between endometrium and ovarian endometriosis have been detected including increase production of estrogen,

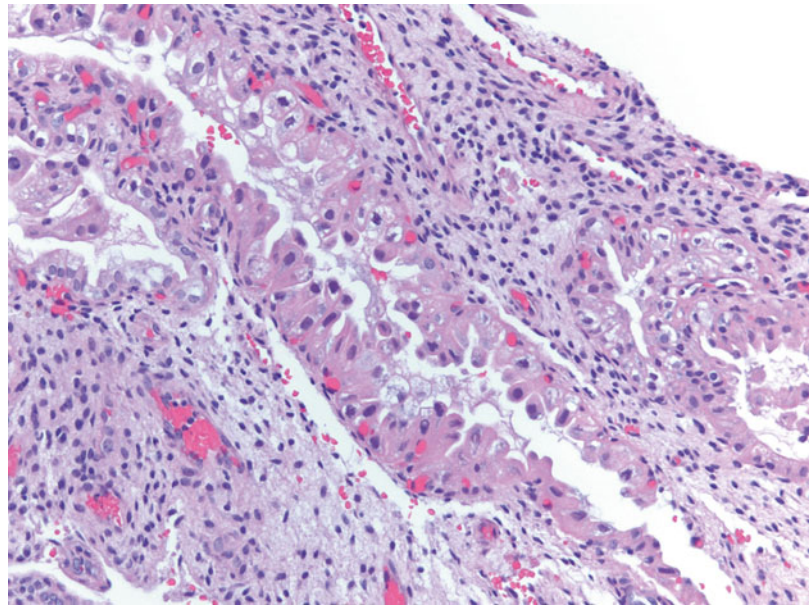

Fig. 3.34 Arias-Stella change within endometriotic cyst raising the possibility of clear cell carcinoma. Patient was 37 weeks pregnant, and the ovarian cyst was resected during cesarean section. H&E 200×

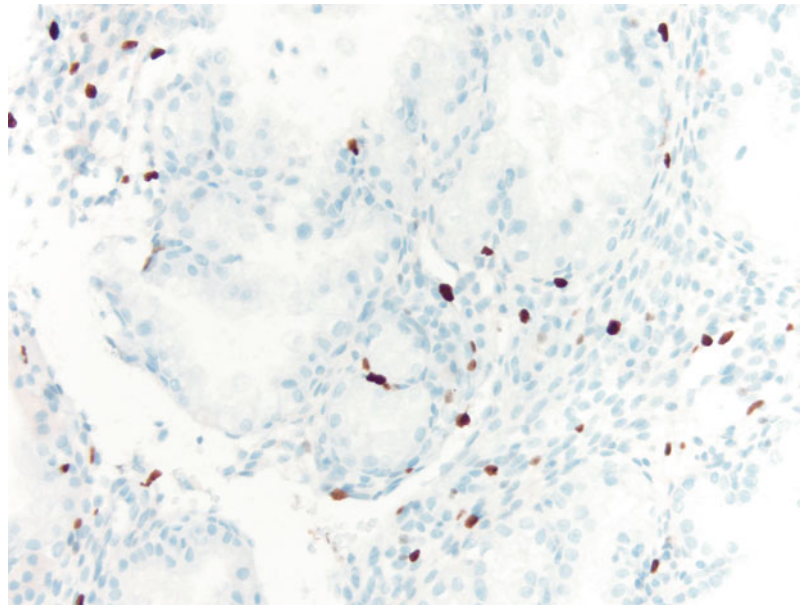

Fig. 3.35 Immunostain for KI-67 in case of Arias-Stella change showing only focally positive nuclei, while clear cell carcinoma demonstrates a more diffuse staining pattern. 200×

prostaglandins, metalloproteinases, inflammatory cytokines, and chemokines in endometriotic tissues [20–24, 49–54]. Increased levels of acute inflammatory cytokines including several interleukins were proposed to help with implantation of endometrial tissue fragments onto ovarian and peritoneal surfaces. In addition, hormones, such as estrogen, play an important role in endometriosis, in part regulated by promoter methylation of the estrogen receptor β gene [18, 55].

Ovarian endometriosis bears genetic instability or damages caused by iron-dependent oxidative stress. DNA damage and loss of heterozygosity (LOH) caused by oxidative stress are critical factors in the carcinogenic process with down-regulation of some tumor suppressor genes and

overexpression of specific candidate oncogenes implicated in tumorigenesis [56]. Different molecular changes are responsible in development of ovarian clear cell carcinoma and endometrioid carcinoma from endometriosis.

In 1996, British investigators examined for the first time DNA from endometriosis to analyze if these samples harbored alterations that could also be seen in ovarian endometrioid carcinoma [57]. While the cases of endometriosis did not harbor alterations in *TP53* and *KRAS* genes, allelic losses in tumor suppressor genes located on chromosome 9p (18 %), 11q (18 %), and 22q (15 %) were identified. Eleven (28 %) of the 40 cases demonstrated LOH at one or more of these loci. In a latter study, Sato et al. evaluated the tumor suppressor gene *PTEN/MMAC1*, located on chromosome arm 10q (10q23.3) [58]. LOH at this site occurred in 56 % of endometriotic cysts, similar to that seen in 42 % ovarian endometrioid carcinomas and 27 % clear cell carcinomas. Somatic mutations in phosphatase and tensin homolog (*PTEN*) were identified in 20 % ovarian endometrioid carcinomas, 8 % ovarian clear cell carcinomas, and 20 % endometriotic cysts.

Other studies have found LOH within ovarian endometriosis (including atypical endometriosis) at candidate ovarian tumor suppressor gene loci and LOH events or other genetic alterations common to the endometriosis and synchronous ovarian carcinomas, including *TP53* [41, 42, 59]. Recent whole genome or targeted sequencing studies have identified frequent mutations of *PTEN* (14–20 %), *CTNNB1* (16–53.3 %), and *KRAS* most commonly associated with ovarian endometrioid cancer [60–63].

Based on the different morphology, it is understandable that clear cell carcinoma harbors different molecular changes, although a few commonalities exist. Kuo KT et al. demonstrated ovarian clear cell carcinomas with mutations of *PIK3CA* (33 %), *TP53* (15 %), *KRAS* (7 %), *PTEN* (5 %), *CTNNB1* (3 %), and *BRAF* (1 %) genes [64]. Sequence analysis of *PIK3CA* in 28 clear cell carcinomas and clear cell carcinoma cell lines showed a mutation frequency of 46 %. Samples with *PIK3CA* mutations showed intense phosphorylated AKT immunoreactivity. These findings demonstrate that ovarian clear cell carcinomas have a high frequency of activating *PIK3CA* mutations [64].

Additional studies evaluated *PIK3CA* mutation in clear cell carcinomas. Yamamoto et al. detected somatic mutations of the *PIK3CA* gene in 10/23 (43 %) ovarian clear cell carcinomas, and in all cases the type of mutation was H1047R in the kinase domain [65]. The identical H1047R mutation was also detected in the coexisting endometriotic epithelium, adjacent to the clear cell carcinoma, in nine of ten (90 %) cases. Moreover, in six of the nine lesions, the H1047R mutation was identified even in the endometrioses lacking cytologic atypia supporting evidence of endometriosis as a precursor lesion. In a subsequent study, these investigators increased the sample size to 88 informative cases, and *PIK3CA* gene mutations were identified in 39 % of cases [66]. Findings also associated with the mutation included cystic tumor, the presence of adjacent endometriosis, prominent papillary architecture of tumor growth, the presence of hyalinized and mucoid stroma, and the absence of clear cell adenofibroma components [66].

Protein markers specific for ovarian clear cell carcinoma associated with endometriosis have also been evaluated. Hepatocyte nuclear factor-1β (HNF-1β), a transcription factor shown to be significantly upregulated in ovarian clear cell carcinoma, is rarely expressed in ovarian non-clear cell carcinoma specimens [67], while endometrial non-clear cell carcinomas have varied staining [68]. Similarly, Kato et al. identified expression of HNF-1β in 9 of 17 clear cell tumors associated with endometriosis, including five cases of reactive endometriotic epithelium and four cases of atypical endometriosis [68]. In the same study, 16 of 40 cases of endometriosis not associated with a primary clear cell ovarian carcinoma also displayed HNF-1β expression. However, the expression of HNF-1β was almost exclusively detected in the epithelium showing inflammatory atypia [69].

Lastly, molecular/genetic changes have been identified in endometriotic lesions adjacent to malignancy, linking endometriosis, clear cell carcinoma, and endometrioid carcinoma. Wiegand et al. identified mutations of the tumor suppressor gene *ARID1A* that are common to endometrioid

and clear cell ovarian carcinomas [70]. In their study, mutations in AT-rich interactive domain-containing protein 1A (ARID1A) were seen in 55 of 119 ovarian clear cell carcinomas (46 %), 10 of 33 endometrioid carcinomas (30 %), and none of the 76 high-grade serous ovarian carcinomas [70]. A total of 17 samples (12 of ovarian clear cell carcinoma and 5 of endometrioid carcinoma) each had two validated ARID1A mutations. In addition, immunohistochemical expression loss of BAF250a protein correlated strongly with the ovarian clear cell carcinoma and endometrioid carcinoma subtypes and the presence of *ARID1A* mutations, implicating *ARID1A* as a tumor suppressor gene frequently disrupted in ovarian clear cell and endometrioid carcinomas. Two patients with ovarian clear cell carcinomas carrying *ARID1A* mutations had contiguous atypical endometriosis. Both cases demonstrated *ARID1A* mutations and loss expression of BAF250a protein in the clear cell carcinoma and the contiguous, atypical endometriosis, but not in distant areas of endometriosis [70]. HNF-1β was expressed in the ovarian clear cell carcinoma but not in the contiguous atypical or distant endometriosis.

In an immunohistochemical evaluation of ARID1A (BAF250a) protein expression in 90 clear cell carcinoma cases, Yamamoto et al. reported the intensity of immunoreactivity for BAF250a as negative, weakly positive, and strongly positive in 44 %, 22 %, and 33 % of tumors, respectively. Compared to tumors immunoreactive for BAF250a, BAF250a-negative tumors were significantly associated with the presence of adjacent endometriosis and more frequently harbored *PIK3CA* mutations (P=0.013) [66].

The same group analyzed *PIK3CA* mutation and ARID1A immunoreactivity in ovarian clear cell carcinomas associated with endometriosis and clear cell adenofibroma (another precursor lesion of clear cell carcinoma described later) [71]. ARID1A immunoreactivity was deficient in 17 (61 %) of the 28 endometriosis-associated carcinomas and 6 (43 %) of the 14 adenofibroma-associated carcinomas. Among the precursor lesions adjacent to the 23 ARID1A-deficient carcinomas, 86 % of the classic endometriosis (12 of 14) and 100 % of the atypical endometriosis (14 of 14), benign (3 of 3), and borderline (6 of 6) clear cell adenofibroma components were ARID1A deficient. In contrast, in the 19 patients with ARID1A-intact carcinomas, all of the adjacent precursor lesions retained ARID1A expression regardless of their types and presence/absence of atypia. Analysis of 22 solitary endometrioses and 10 endometrioses distant from ARID1A-deficient carcinomas showed that all of these lesions diffusely expressed ARID1A. Among the 42 clear cell carcinomas, somatic mutations of *PIK3CA* were detected in 17 (40 %) tumors. The majority (71 %) of these were ARID1A-deficient carcinomas. These results suggest that loss of ARID1A protein expression occurs as a very early event in ovarian clear cell carcinoma development, similar to the pattern of *PIK3CA* mutation, and frequently coexists with *PIK3CA* mutations [71]. This indicates that the loss of BAF250a protein expression is suggestive for the presence of ARID1A mutations and represents a useful marker of malignant transformation of endometriosis [62].

Clear Cell Adenofibroma

Besides endometriosis, clear cell adenofibroma is another precursor lesion that has been associated with clear cell carcinoma. Schiller and colleagues described a variant, of what is now known clear cell carcinoma, with an adenofibromatous pattern among cases referred to as "parvilocular cystoma" [72, 73]. The same tumor was later named "mesonephroma" or mesonephric tumor, but based on its association with endometriosis, including DES-exposed carcinoma in vaginal adenosis (endometriosis), it is currently named clear cell carcinoma and is widely considered to be of Müllerian origin [74–76].

Features of Clear Cell Adenofibroma

The tumor is composed of glands separated by abundant fibromatous stroma. The glands are typically small to medium size, mostly round, uniform, and occasionally filled with eosino-

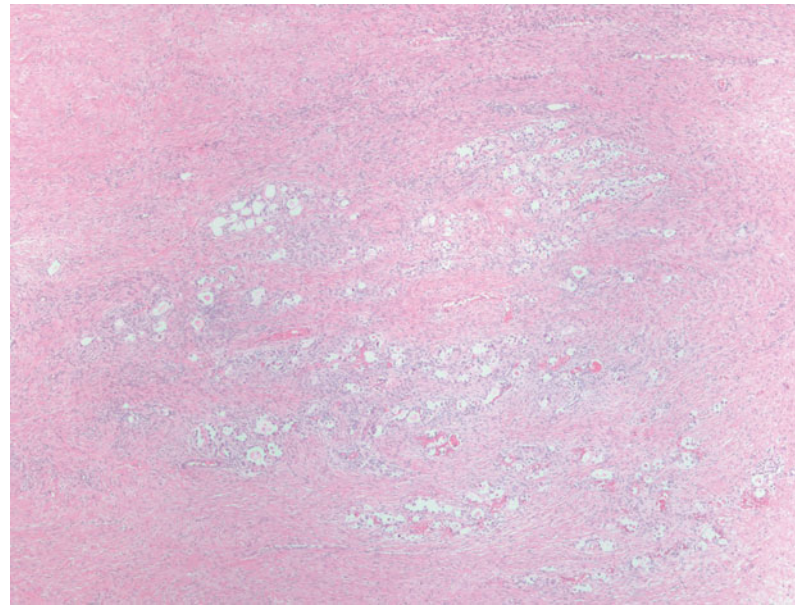

Fig. 3.36 Clear cell adenofibroma composed of fibromatous stroma and glands lined by cells with clear cytoplasm and hobnail features

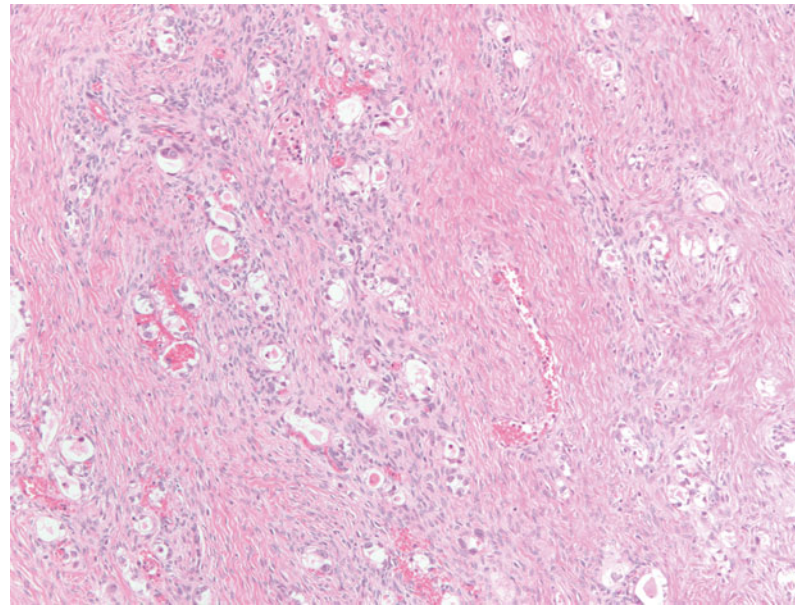

Fig. 3.37 High-power examination of clear cell adenofibroma. H&E 200×

philic secretions. They are lined by one or two layers of flat to low-cuboidal cells with scant to moderate pale or clear cytoplasm. The nuclei are usually small, uniform, and oval to round. The nuclei exhibit either no or mild atypia, but notable nuclear atypia is not present. Mitotic figures are usually not identified. When tumors have similar features but exhibit greater cytologic atypia or mitotic figures but fall short of a diagnosis of clear cell carcinoma, they are usually diagnosed as borderline clear cell tumors. Other features in the latter tumors include complex glandular pattern, crowded glands, and epithelial stratification [76–80] (Figs. 3.36, 3.37, 3.38, 3.39, 3.40, 3.41, 3.42, 3.43, 3.44, 3.45, 3.46, 3.47, and 3.48).

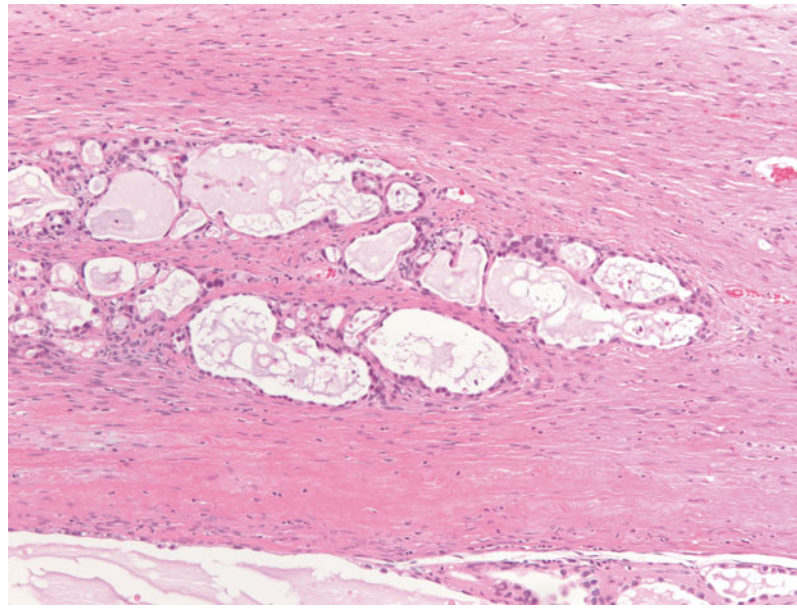

Fig. 3.38 Clear cell tumor with focal nuclear atypia raising the possibility of a borderline tumor. H&E 100×

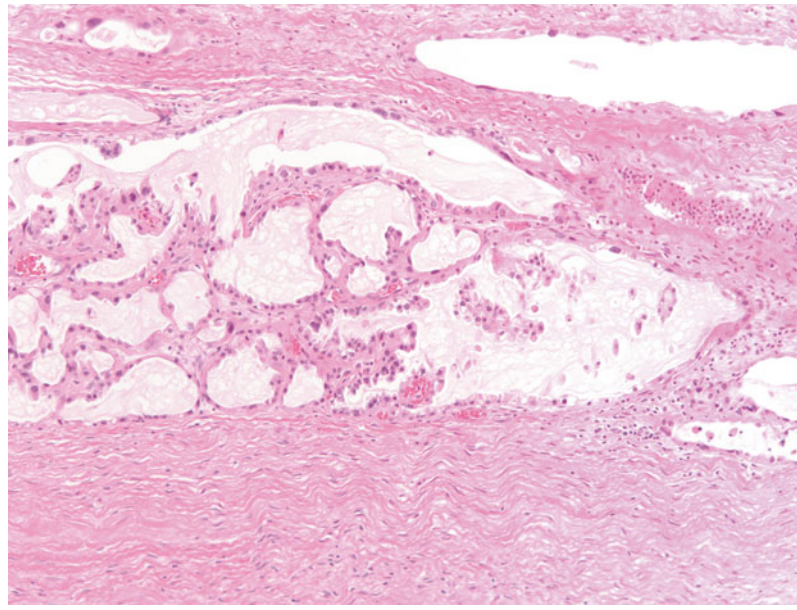

Fig. 3.39 Clear cell tumor with focal nuclear atypia raising the possibility of a borderline tumor. H&E 100×

Evidence for Clear Cell Adenofibroma as Precursor Lesion of Clear Cell Carcinoma

In 1985, Bell and Scully described several tumors with clear cell features, including three tumors with clear cell features that showed no significant epithelial atypia and were classified as benign. Twelve tumors contained glands or small solid nests composed of epithelial cells with nuclear characteristics of low-grade malignancy without invasion of the stroma and were designated as borderline tumors. Three predominantly borderline tumors with focal microinvasion of the stromal

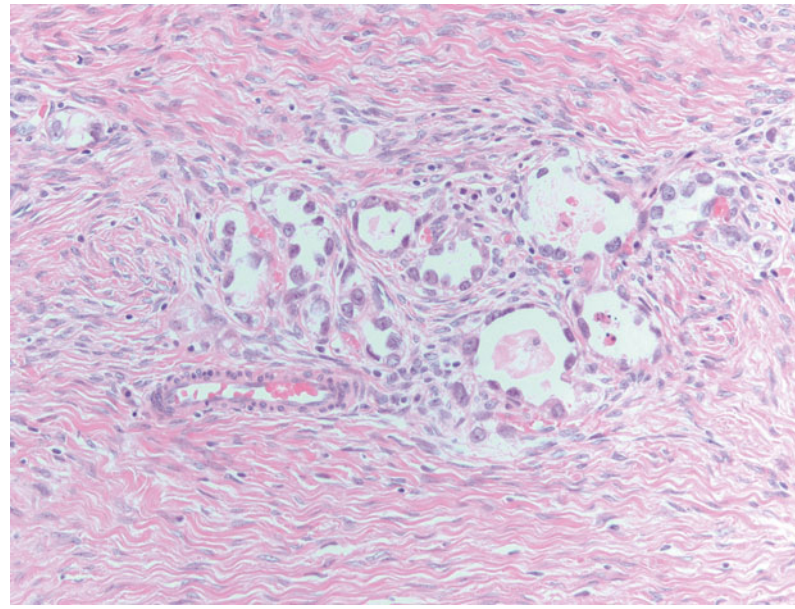

Fig. 3.40 High-power examination highlighting nuclear atypia and hobnail changes. H&E 200×

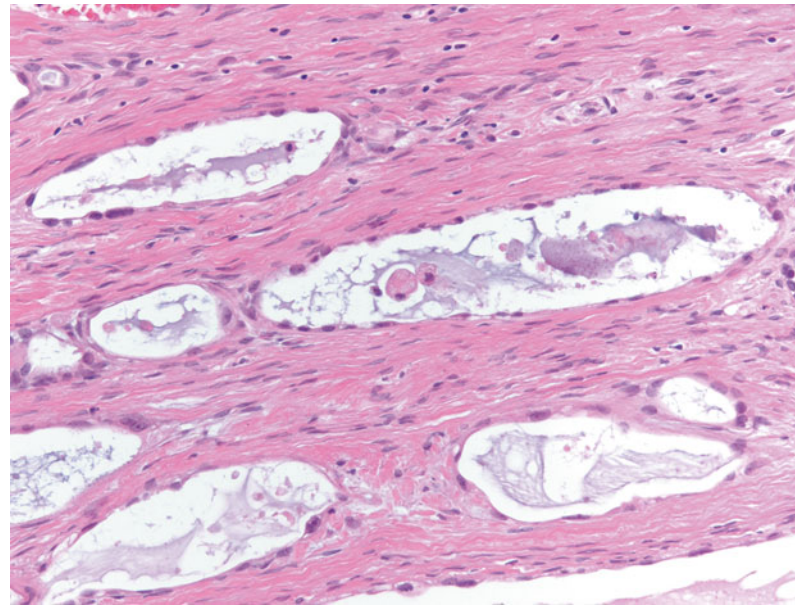

Fig. 3.41 High-power examination highlighting nuclear atypia and hobnail changes. H&E 200×

component were also described [77]. These and other reports of small clear cell carcinomas or microinvasive tumors arising in a fibromatous background suggested an adenofibroma–carcinoma sequence [3, 78–80].

However, recently, two pathways for the development of clear cell carcinoma have been proposed including the endometriosis–clear cell carcinoma relation explained in the previous section and the adenofibroma–carcinoma sequence. [3, 80–82]. Different clinicopathologic and molecular findings between endometriosis-associated clear cell carcinoma and those tumors containing an adenofibromatous background have been reported, proposing that each may have a different pathogenesis [3, 80–82]. However, some

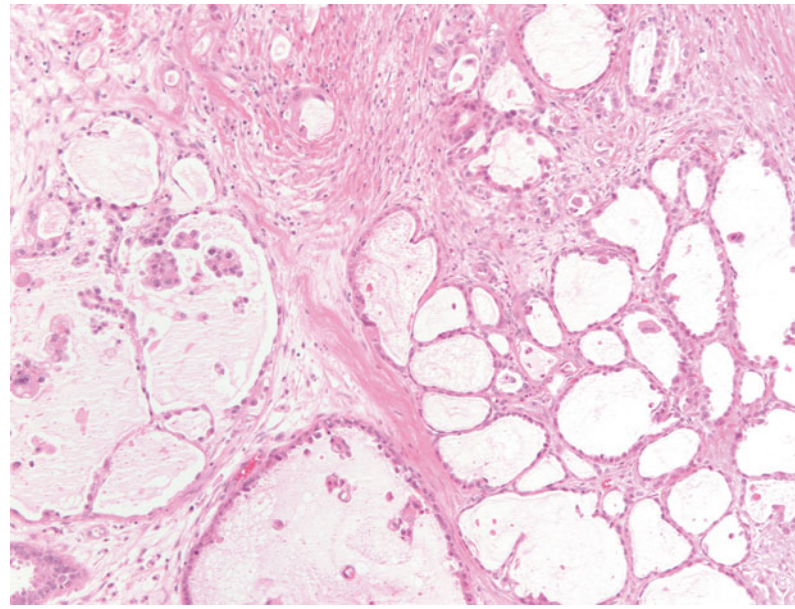

Fig. 3.42 Different patterns of clear cell carcinoma. Predominantly glandular. H&E 100×

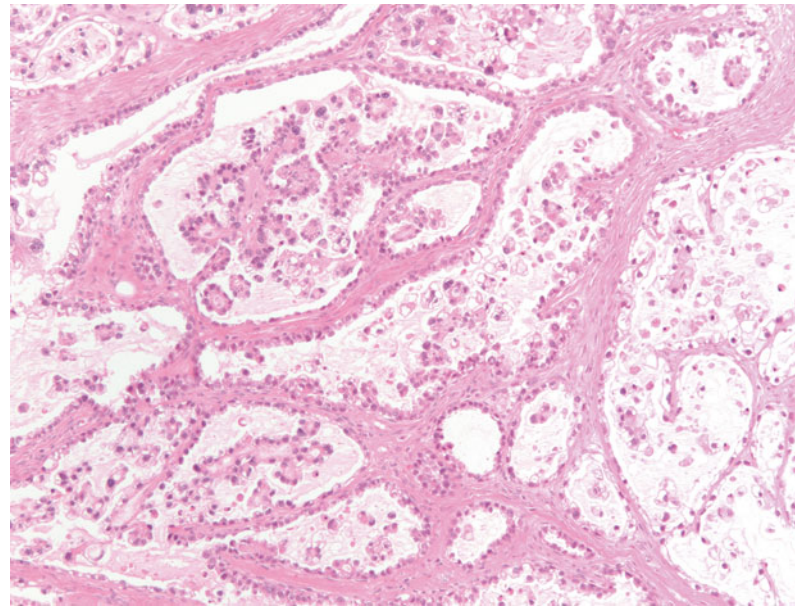

Fig. 3.43 Different patterns of clear cell carcinoma. Glandular and focal papillary patterns in clear cell carcinoma. H&E 100×

adenofibromatous tumors have been associated with endometriosis making unclear if these are really two different pathways.

Yamamoto et al. studied 14 clear cell carcinomas associated with a fibromatous background that included clear cell adenofibroma and borderline tumor [80]. For all informative loci, the frequency of LOH in clear cell carcinoma was 49 % (54/110 loci) and was significantly higher than those in the components of clear cell adenofibroma (22 %, 20/92 loci) and borderline tumor (30 %, 25/83 loci). The concordance

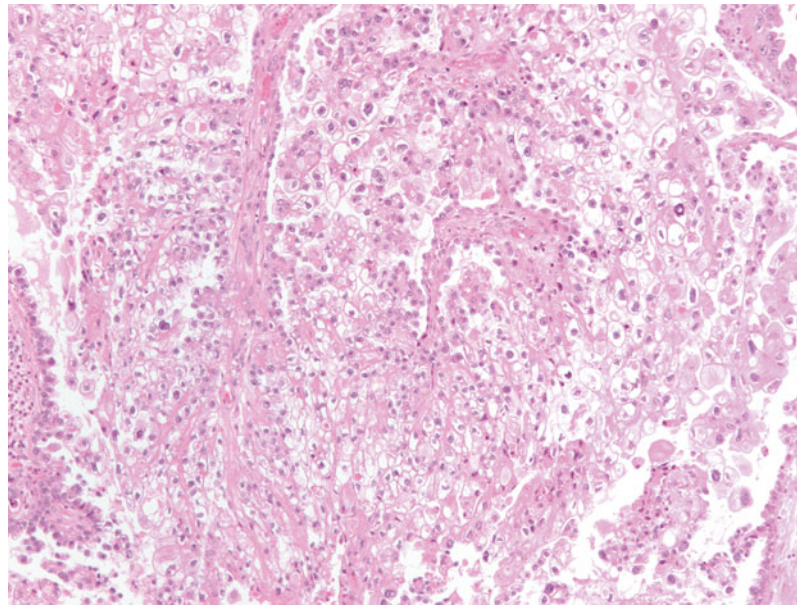

Fig. 3.44 Different patterns of clear cell carcinoma. Glandular and focal papillary patterns in clear cell carcinoma. H&E 100×

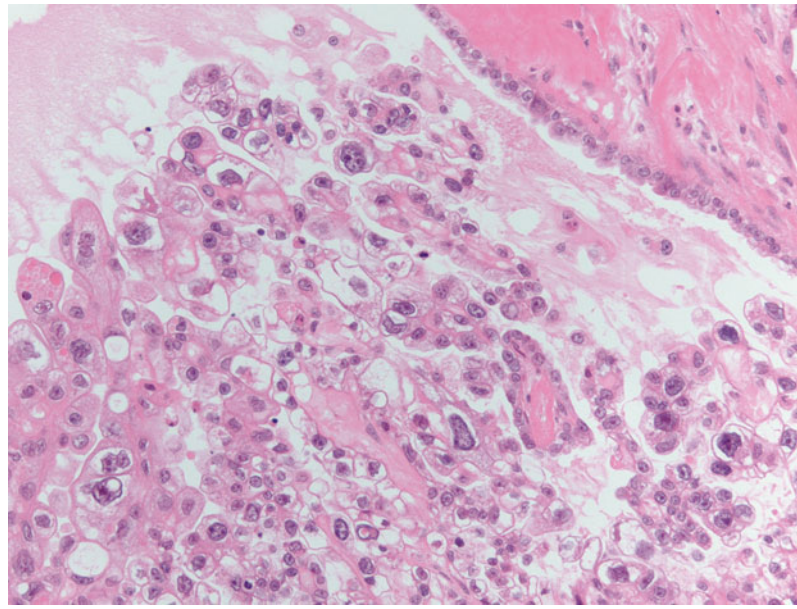

Fig. 3.45 High-power examination of papillary pattern of clear cell carcinoma with significant nuclear atypia. H&E 200×

rate in allelic patterns at all informative loci was 74 % between adenofibroma and carcinoma, 81 % between borderline tumor and carcinoma, and 95 % between adenofibroma and borderline tumor. An identical LOH pattern, involving the same alleles, was identified between adenofibroma and carcinoma in 13 (93 %) of the cases, very unlikely to have occurred by chance. Among the markers examined, LOH on 5q, 10q, and 22q was frequent in both adenofibroma and carcinoma, whereas LOH on 1p and 13q was rare in adenofibroma but frequent in carcinoma.

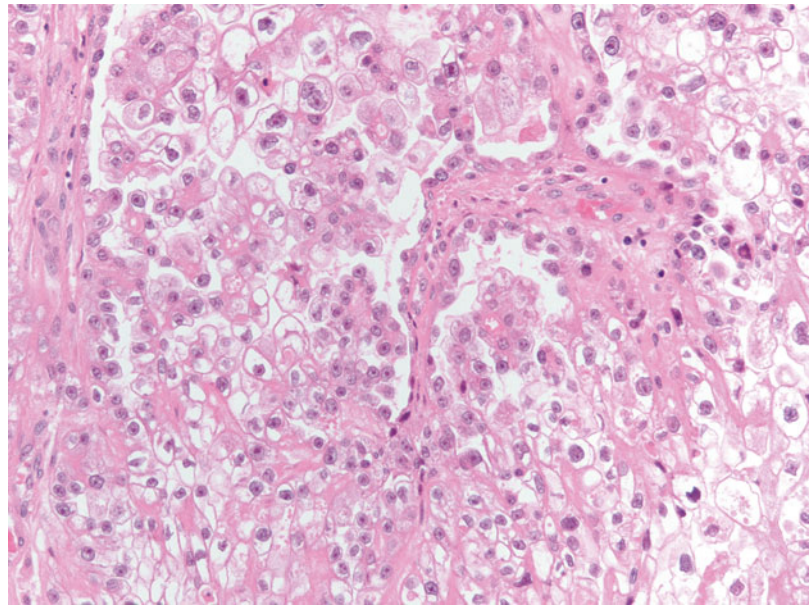

Fig. 3.46 High-power examination of papillary pattern of clear cell carcinoma with significant nuclear atypia. H&E 200×

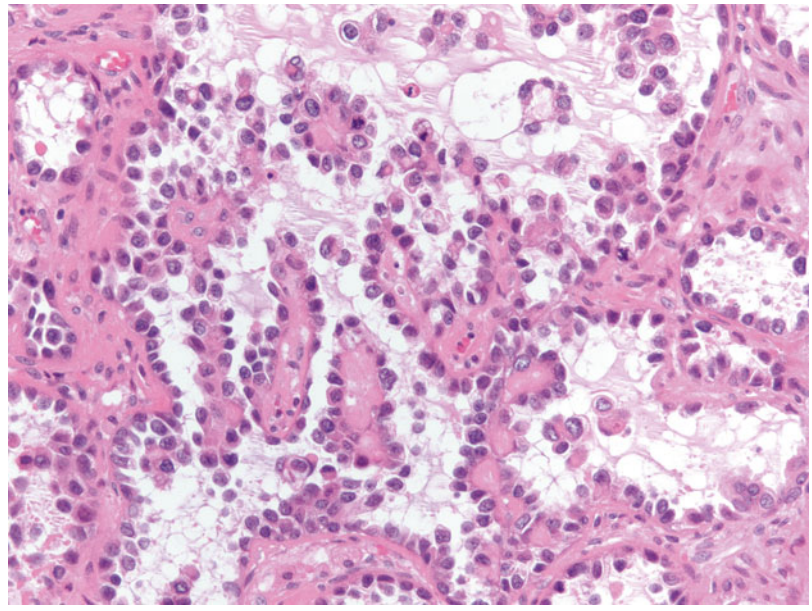

Fig. 3.47 Papillary pattern of clear cell carcinoma with hyalinized stroma and hobnail epithelium. H&E 100×

These findings suggested that clear cell adenofibroma can be a clonal precursor of ovarian clear cell carcinoma [80].

The same investigators compared LOH in clear cell carcinomas with a fibromatous background (14 cases) and those associated with endometriosis (20 cases) [81]. For all informative loci, the frequency of LOH was not statistically different between the two carcinoma groups: 38 % (66/172 loci) in the endometriosis-associated carcinomas and 35 % (40/113 loci) in the clear cell carcinomas associated with a fibromatous background. However, LOH differences were detected at several loci; the frequencies of

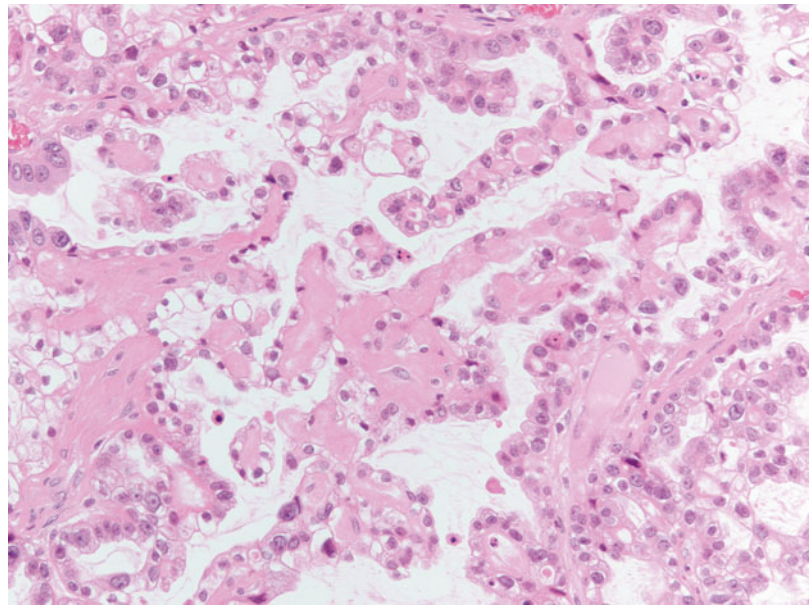

Fig. 3.48 Papillary pattern of clear cell carcinoma with hyalinized stroma and hobnail epithelium. H&E 100×

LOH at chromosomes 3p, 5q, and 11q were significantly higher in the endometriosis-associated carcinomas than in the clear cell-associated carcinomas, further supporting the presence of two distinct carcinogenic pathways to ovarian clear cell adenocarcinoma [81].

Subsequent morphologic studies compared clear cell carcinomas that were predominantly cystic and associated with endometriosis and those that had a fibromatous background including adenofibroma or borderline tumor [3, 82]. Veras et al. analyzed 122 clear cell carcinoma cases [3]. Cystic clear cell carcinoma was more frequently diagnosed as stage I compared with adenofibromatous clear cell carcinoma (75 % vs. 44 %). Conversely, adenofibromatous clear cell carcinomas were diagnosed more often in advanced stages (stages II–IV) compared with cystic clear cell carcinomas (56 % vs. 18 %). Clear cell carcinomas with the cystic and adenofibromatous background were associated with endometriosis and atypical endometriosis. However, endometriosis was found in 91 % of ztriosis was seen in 62 % of cystic clear cell carcinomas. Endometriosis was found in 44 % of clear cell carcinomas with adenofibromatous background, and atypical endometriosis was seen in 11 % of these cases. Cystic clear cell carcinomas were predominantly papillary (47 % of cases), whereas none of the adenofibromatous carcinomas displayed a predominantly papillary pattern. A more favorable outcome was observed for cystic clear cell carcinoma compared with adenofibromatous clear cell carcinoma (2-year and 5-year survival for the cystic clear cell carcinomas was 82 % and 77 % and was 62 % and 37 % for adenofibromatous clear cell carcinomas) [3].

Zhao et al. reviewed 472 clear cell neoplasms including 427 carcinomas [82]. One third of carcinomas had an adenofibromatous background. Similarly to the study by Veras et al., endometriosis was found in all types of tumors, but it was more frequent in carcinomas with cystic background (endometriotic cysts). Tumors associated with a cystic background occurred in younger patients; these tumors more commonly had a mixed carcinoma component of non-clear cell type, had more papillary architecture, were more frequently oxyphilic, and were more frequently associated with atypical endometriosis. The authors finally speculated that some clear cell carcinomas are derived from epithelial atypia arising in an endometriotic cyst that later evolves

into clear cell carcinoma, while in other carcinomas, non-cystic endometriosis induces a fibromatous reaction resulting in the formation of adenofibroma, which then develops into borderline tumor and subsequently clear cell carcinoma. The absence of endometriosis or adenofibromatous components in some clear cell carcinomas may be due to overgrowth and obliteration by the invasive carcinoma [82].

Based on all the presented data, two distinctive pathways occur in ovarian clear cell carcinomas. Clear cell carcinoma, predominantly cystic tumors but probably solid tumors as well, arises from endometriosis and atypical endometriosis. Clear cell carcinoma, predominantly solid tumors, arises from clear cell adenofibromas or borderline tumors. It is still uncertain whether these benign/borderline clear cell lesions also are developed from endometriosis.

Endometrioid Adenofibroma

Similarly to clear cell carcinoma, endometrioid adenofibroma and borderline tumor have been proposed as possible precursor lesions of ovarian endometrioid adenocarcinoma; however, these tumors have been less frequently studied than their clear cell counterpart.

Features of Endometrioid Adenofibroma

Kao et al. reported the first series of 12 endometrioid adenofibromas, in a paper titled "Unusual cystadenofibromas" that also included mucinous and clear cell adenofibromas [83]. Two years later, Roth et al. reported 10 additional cases and classified endometrioid adenofibromas as benign, proliferating, and those associated with adenocarcinoma (low malignant potential) [84].

Grossly, these lesions have variable size, from small to very large, over 15 cm lesions. They have either smooth fibroma-like external surface or show a cauliflower-like appearance with papillary excrescences, usually thick of fibrotic, as opposed to the more edematous ones seen in serous borderline tumors. Cut surface is firm and densely fibrous with multiple cysts. If the cysts are microscopic, the term "adenofibroma" is the preferred one, while if the lesion is predominantly cystic, "cystadenofibroma" should be the preferred diagnosis.

Microscopic examination reveals that the tumors consist of well-spaced endometrial-type glands set in fibrous stroma. The glands vary from tubular to dilated and often had pseudostratified columnar lining. Nuclei are regular and uniform. Mitoses are not apparent. Squamous metaplasia can be present. The stroma usually appears less cellular and more collagenized.

If there is a greater degree of epithelial proliferation, more complicated architectural pattern with occasional intraglandular papillary infoldings, epithelial atypia, and mitosis, the designation "endometrioid borderline tumor" or atypical proliferative tumor is justified [85]. Areas of glandular crowding are usually seen resembling those seen in endometrial hyperplasia. Occasionally, it is difficult to determine when a lesion is complex and atypical enough to reach a diagnosis of well-differentiated endometrioid adenocarcinoma. Criteria that are applicable in the uterus, with all associated difficulties in their application, are used, including a combination of complex or confluent glandular or papillary architecture with gland fusion, stromal invasion, and significant atypia (Figs. 3.49, 3.50, 3.51, 3.52, 3.53, 3.54, 3.55, 3.56, 3.57, 3.58, and 3.59).

Evidence for Endometrioid Adenofibroma as Precursor Lesion of Endometrioid Carcinoma

After describing endometriosis, Sampson reported cases of ovarian carcinoma that he believed arose from endometriosis [86, 87]. To qualify, these cases should be seen in an endometriotic background and could not be demonstrated as invading from elsewhere. After that study, investigators reported ovarian carcinomas that morphologically resembled endometrial carcinomas, some seen in an endometriotic background, while others were accompanied by similar tumors

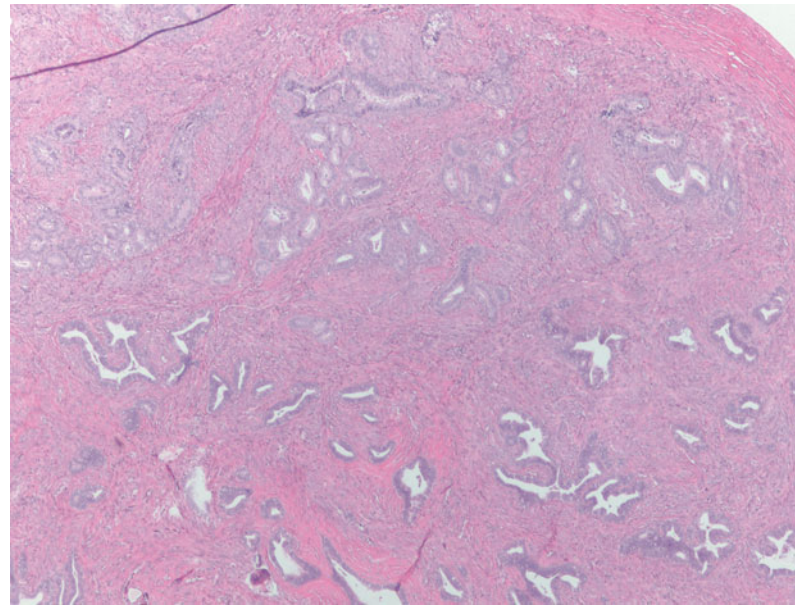

Fig. 3.49 Endometrioid adenofibroma with focal clustered glands diagnosed as borderline tumor. H&E 40×

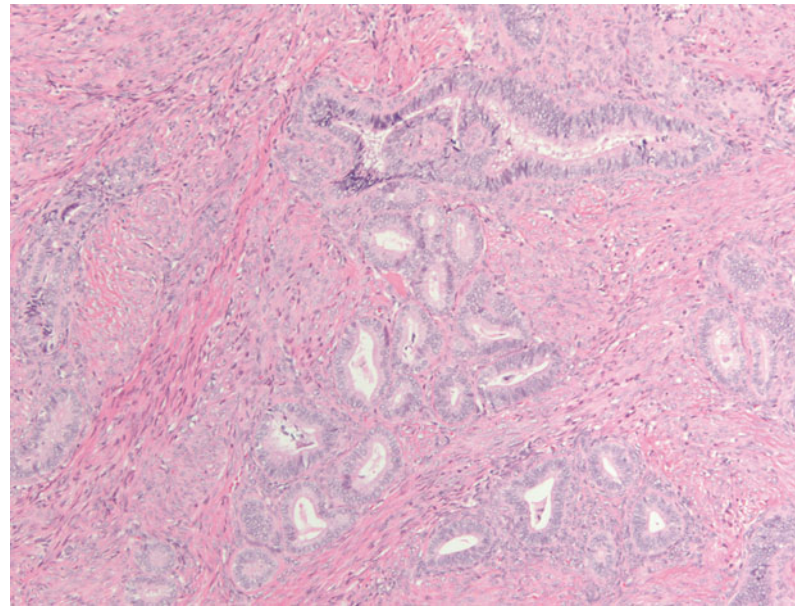

Fig. 3.50 High power of Fig. 3.49, showing glandular cluster with minimal atypia, not sufficient for a diagnosis of carcinoma. H&E 100×

in the uterus [87]. To unify terminology, the Cancer Committee of the International Federation of Gynecologic and Obstetrics appointed a group of experts in the field who agreed on the term endometrioid carcinoma to designate primary ovarian carcinomas morphologically resembling carcinomas arising in the uterus [87].

Scully and colleagues described ovarian endometrioid carcinomas, those associated with endometriosis, and those where endometriosis was not evident. In addition, they also described three occurrences of benign endometrioid tumors, one of them composed of epithelial glands of endometrial-type, foci of

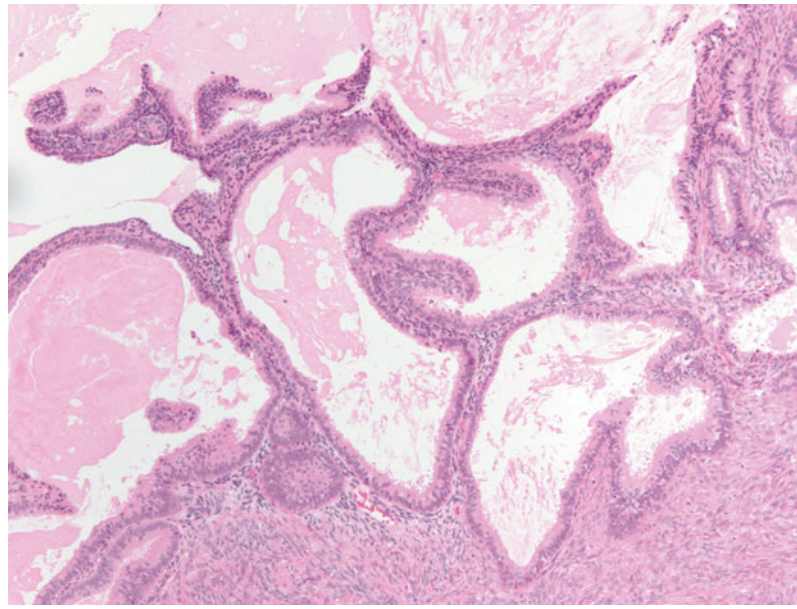

Fig. 3.51 Endometrioid tumor with crowded glands but lacking significant atypia. The background showed endometrioid adenofibroma, and this areas qualified as borderline tumor. H&E 100×

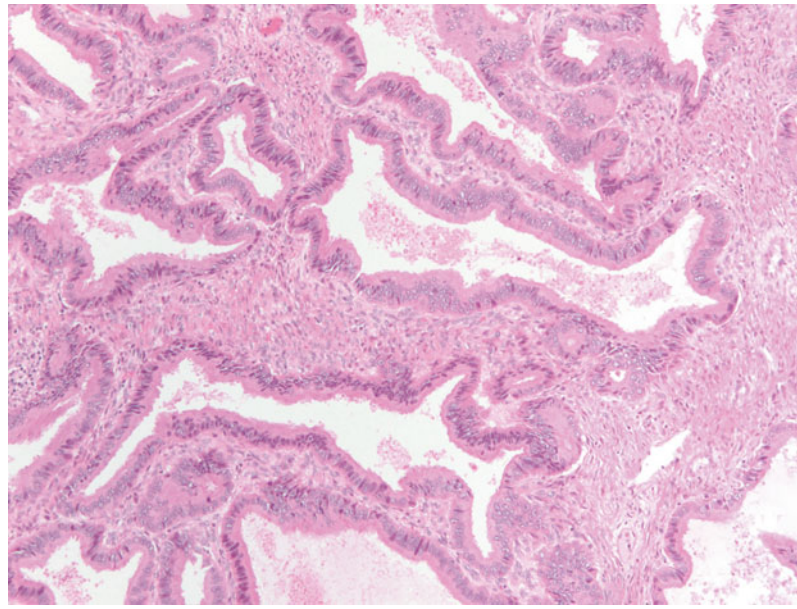

Fig. 3.52 Contralateral ovary as above figure with crowded and irregular glands. Note nuclear atypia consistent with well-differentiated endometrioid adenocarcinoma. H&E 100×

squamous differentiation, and fibrotic background: endometrioid adenofibroma (adenoacanthofibroma). Previously, in 1949, Hughesdon reported an adenofibroma with areas of endometriosis [88]. This study prompted inclusion of endometrioid adenofibroma into the 1973 World Health Organization (WHO) as a separate entity [89]. In the following decades, several studies reported tumors that were described as endometrioid adenofibroma, some of which were associated with endometriosis and others combined with adenocarcinoma [82, 83, 90–93].

In summary, endometrioid adenofibromas was first recognized in the background of ovar-

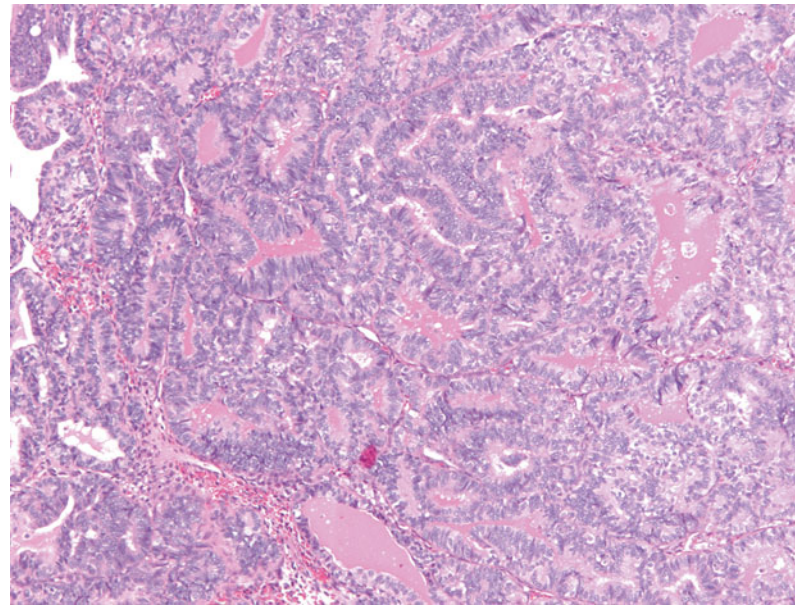

Fig. 3.53 Different patterns of endometrioid adenocarcinoma with glandular and papillary/villoglandular features. H&E 100×

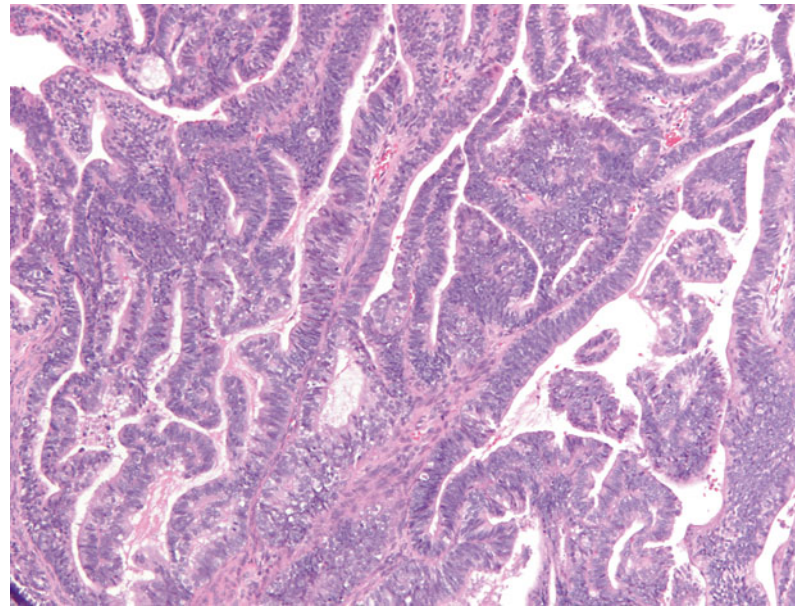

Fig. 3.54 Different patterns of endometrioid adenocarcinoma with glandular and papillary/villoglandular features. H&E 100×

ian endometrial-like carcinomas with fibromatous stroma. Some authors classified them as benign endometrioid adenofibroma, proliferative or associated with carcinoma, suggesting a link or adenofibroma–carcinoma sequence. Endometrioid adenofibroma was also included as a benign counterpart of endometrioid carcinoma in the 1973 WHO classification [87]. In addition, some of these tumors occurred in a background of endometriosis in the same or contralateral ovary or in another pelvic site, linking all these lesions with endometrioid carcinoma.

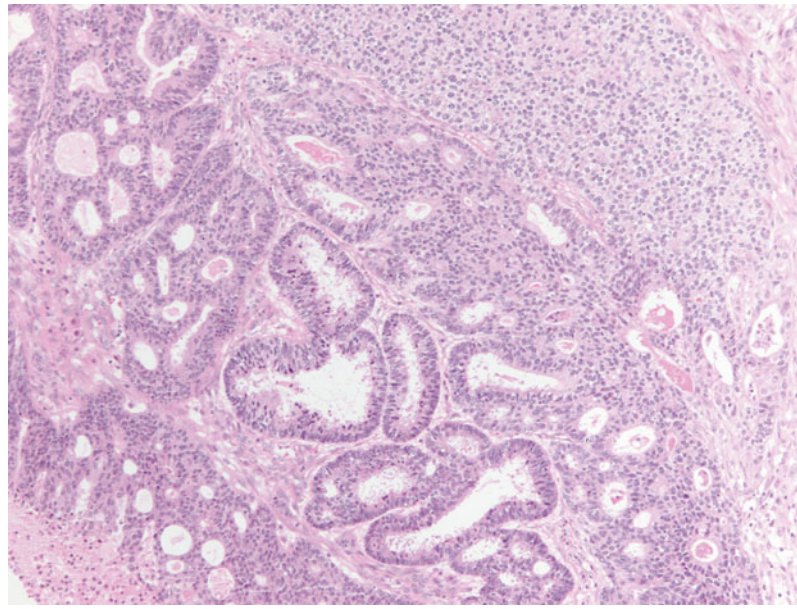

Fig. 3.55 Endometrioid adenocarcinoma with glandular and associated solid component consistent with high-grade endometrioid carcinoma. H&E 100×

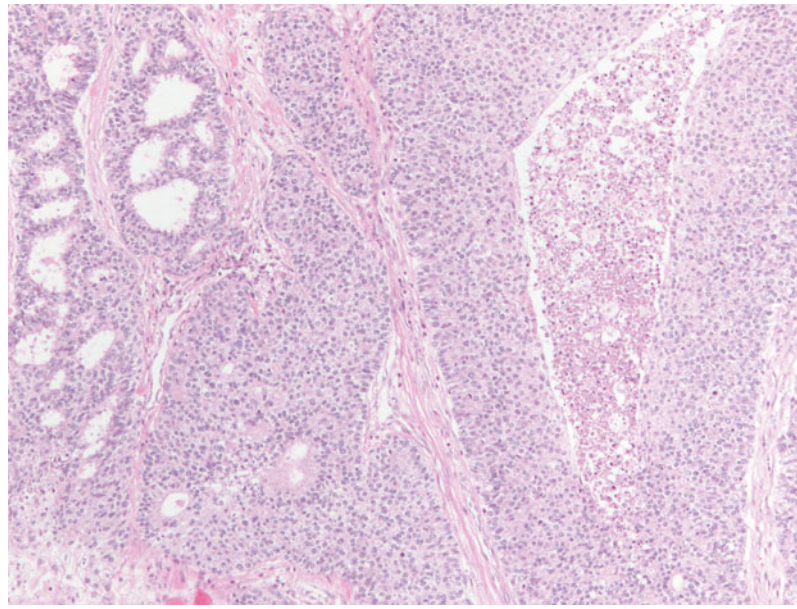

Fig. 3.56 Endometrioid adenocarcinoma with glandular and associated solid component consistent with high-grade endometrioid carcinoma. Note central necrosis. H&E 100×

Fallopian Tube Origin of Ovarian Endometrioid Carcinomas?

In the past decade, an attempt to determine the origin of the ovarian surface epithelial tumors has provided a new potential site of origin: the fallopian tube epithelium [4, 5, 94, 95]. The origin of ovarian epithelial tumors from the surface epithelium had been questioned for some time [96]. In addition, as previously mentioned, while Sampson's retrograde menstruation theory (transtubal spread of endometrial tissue) is widely accepted, it is still controversial, leaving room for other possible precursors of endometrioid and/or clear cell carcinoma.

Recently, some studies were published linking tubal epithelium and ovarian carcinomas with endometrioid features [97–99]. The Zheng group

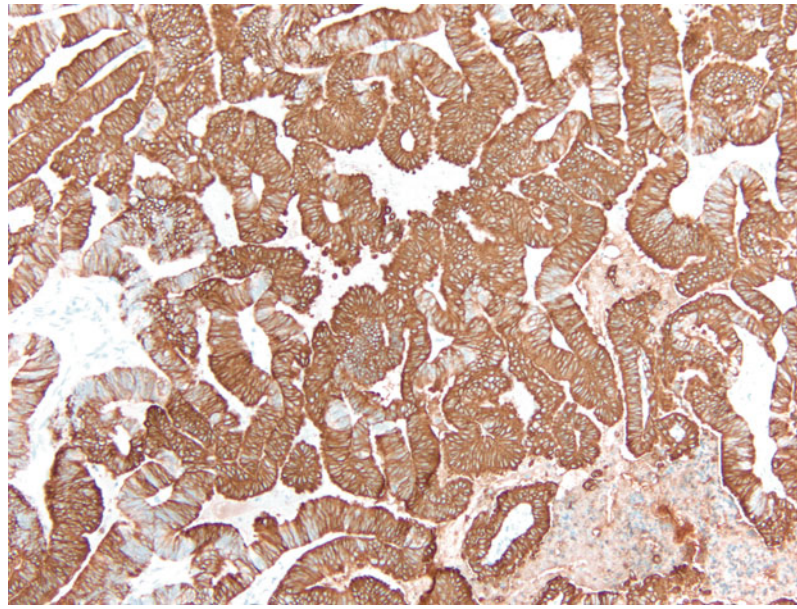

Fig. 3.57 Diffuse cytoplasmic immunostain for cytokeratin 7 to differentiate from metastatic carcinoma from colonic origin. 100×

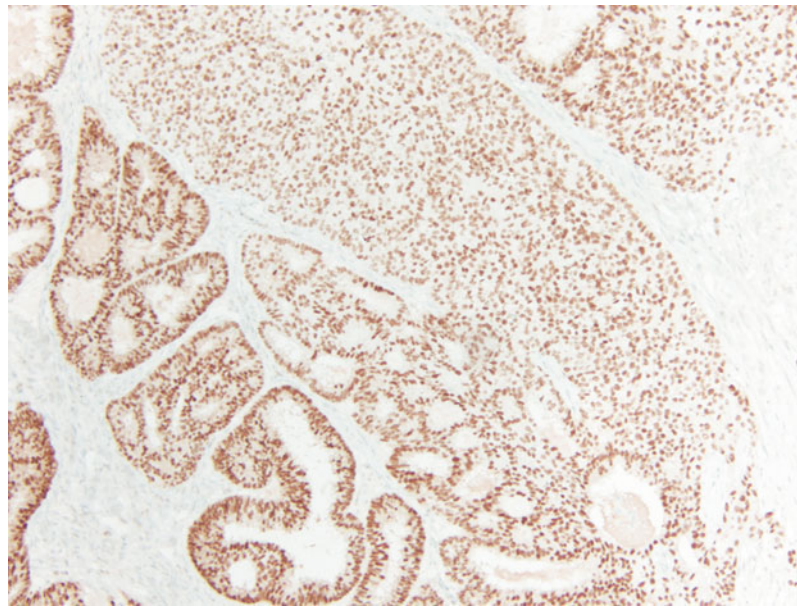

Fig. 3.58 Diffuse nuclear immunostain for PAX8 that supports a Müllerian origin and differentiates from metastatic carcinoma from colonic origin. 100×

recently reported a novel study that raised the possibility that the fallopian tube is a contributor to ovarian endometriosis [100]. The basic rationale is that tubal mucosa is known to be able to form endometrial-like tissue at the morphologic level. Tubal epithelia may then shed viable cells onto the ovarian surface possibly leading to endosalpingiosis or ovarian epithelial inclusion cysts, a common finding within the ovary in 30 % of the cases [100]. Previously, the authors reported that the ovarian epithelial inclusions could be transformed into ovarian endometriosis through a probable metaplastic process [17]. The authors studied the differentially expressed genes *FMO3*

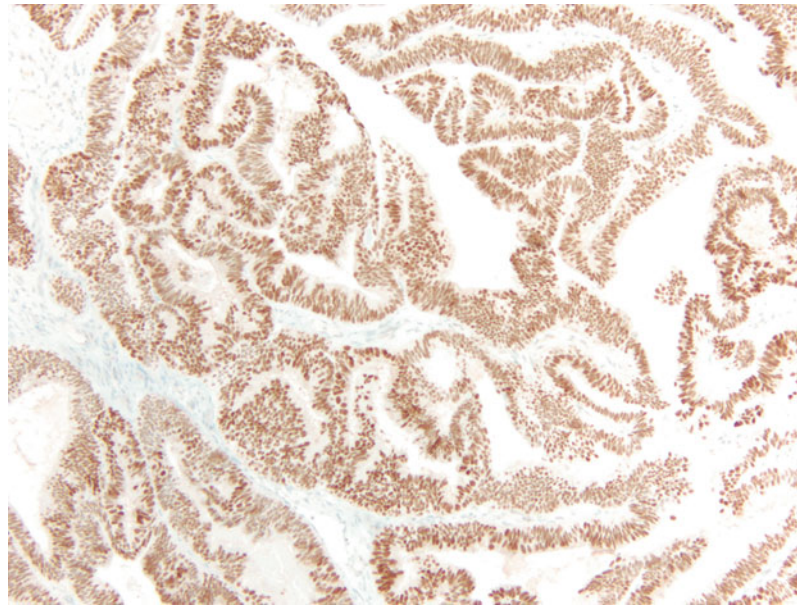

Fig. 3.59 Diffuse nuclear immunostain for PAX8 that supports a Müllerian origin and differentiates from metastatic carcinoma from colonic origin. 100×

and *DMBT1*. *FMO3* is highly expressed in the tubal epithelia while low in the endometrium. In contrast, *DMBT1* is high in the endometrium but low in the fallopian tube. In 32 ovarian endometriosis cases analyzed by real-time PCR, 18 (56 %) showed a high level of *FMO3* expression and a low level of *DMBT1* expression. However, 14 (44 %) ovarian endometriosis cases showed a reversed expression pattern with these two markers. Results were similarly seen utilizing western blot and immunohistochemistry. The findings suggest that approximately 60 % of the ovarian endometriosis cases studied may be derived from the fallopian tube, whereas about 40 % of the cases may be of endometrial origin [96]. This data is preliminary and has yet to be reported from other laboratories.

Van der Horst et al. reported on a mouse model that mutations (activation of Wnt/b-catenin) in the distal oviduct resulted in precursor lesions that developed into ovarian tumors, resembling human endometrioid ovarian cancer [97]. In the study, adenomatous polyposis coli (APC) knockout mice were used to study the activation of Wnt/b-catenin signaling in Müllerian duct-derived organs. Using nuclear b-catenin staining, Wnt/b-catenin signaling activation was confirmed in the entire epithelium of the adult Müllerian duct (fallopian tube, including fimbrial epithelium and endometrium) but was absent in ovarian surface epithelium. In addition, 62.5 % of mice developed tumors in the distal and fimbrial part of the tube. In the ovaries, mainly at young age, in 16.3 % of mice, epithelial inclusion cysts were noted, which developed further into endometrioid ovarian tumors, resembling human endometrioid ovarian cancer.

Lastly, Tanwar et al. similarly reported that APC-deleted mice with β-catenin expression led to the development of epithelial inclusion cysts [98]. However, in this study, the epithelium originating from the ovarian cysts was the ovarian surface epithelium and not the tubal epithelium. High-grade ovarian lesions composed of tightly packed villoglandular histology were observed in older APC-deleted mice. PTEN expression was elevated in the early lesions but lost after progression to more advanced tumors. Knockdown of APC or expression of a gain-of-function of β-catenin similarly induced human ovarian surface epithelial cells to develop into tumors with endometrioid histology. Expression of HOXA10

was induced in both the advanced APC-deleted murine tumors and in the tumor xenografts of human ovarian surface epithelium with knocked down APC. These results suggested that reduced APC activity is sufficient to induce formation of epithelial inclusion cysts and support ovarian inclusion cyst development and that induced HOXA10 expression and loss of PTEN are key mechanisms driving endometrioid differentiation. Additional studies are required, but these studies add a new possibility for the development of ovarian endometrioid carcinomas.

Summary

In this chapter, the author reviewed the most common precursor lesions of endometrioid and clear cell carcinomas of the ovary, including new molecular advances providing insights into their molecular pathogenesis and origins.

The issue of precursor lesions causes one to reflect on the puzzling discrepancy between the two gonads, ovary and testis, in their incidences of the different tumor types. The testis primarily harbors germ cell tumors and less commonly sex cord tumors, while epithelial tumors are extraordinarily rare. The testis is also lined by epithelium, a modified mesothelium. The testis, however, lacks the company of the fallopian tube. The ovary, which is also covered by a modified mesothelium, primarily harbors epithelial tumors. Although Sampson, close to a century ago, suggested that the endometrial-like tissue and carcinomas seen in the ovary were related with extraovarian tissue (endometrial tissue), a theory suggesting metaplasia of the ovarian surface epithelium was for most part of the century accepted as the most likely explanation. The same coelomic metaplasia theory led most investigators to believe that epithelial tumors within the ovary were originating in the only epithelium identified in the ovary (surface epithelium). The fallopian tube was ignored for the most part. It probably did not help that the most common sections submitted of the tube were the infundibular or ampullary regions, instead of the fimbria.

At present, the pendulum appears to have swung to the opposite side, and an attempt to explain all epithelial ovarian lesions as arising for the tubal epithelium is increasingly favored by investigators. Time and additional research will hopefully uncover the real truth. What is clear, for now, is that at least a subset of clear cell and endometrioid adenocarcinomas evolves from endometriosis.

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Clinical Management of Selected Precancerous Lesions of the Uterus, Fallopian Tube, and Ovary

4

Xiaomang B. Stickles

Introduction

Precancerous lesions of the upper female genital tract are frequently diagnosed only after a definitive surgical procedure has been performed. The exception is precancerous lesions of the endometrium, which are usually diagnosed on either office endometrial sampling via pipelle biopsy, or at the time of dilation and curettage. This chapter will review current clinical management and the existing data that supports these recommendations. For those lesions that are typically diagnosed post hoc, subsequent treatment is also reviewed.

Precancerous Lesions of the Uterus

Endometrial Hyperplasia

Simple endometrial hyperplasia is associated with a relatively low risk of progression to carcinoma. Therefore, nonsurgical treatment is usually chosen. Progesterone has been the cornerstone of nonsurgical treatment since the 1960s. This could be administered either systemically via oral or intramuscular route or locally via an intrauterine device. Traditionally, endometrial hyperplasia with atypia has been an indication for hysterectomy due to the high association with concurrent and subsequent endometrioid adenocarcinoma. An alarming increase in the rate of endometrial hyperplasia diagnosed during reproductive years has been seen due to the obesity epidemic in the United States and developed world. This has lead to a spike in interest in nonsurgical management for these precancerous histologies with higher risk of progression to cancer.

Various types of agents and routes of delivery have been examined. The response rate varies in the literature and is as high as 90 %. On average, the response rate is about 70 %. Most early series from the 1980s and 1990s consisted of less than ten patients. The larger studies to date are summarized in Table 4.1. Based on existing literature, it appears that most lesions that would regress with progesterone treatment will do so within the first 2 years, and the vast majority of these regress within the first year. There is not a clear correlation between progesterone dose and chance of regression. Few older studies were designed with this question in mind. A recent study by Marra et al. did not show a statistically significant high rate of regression with higher doses, though authors acknowledge that the study

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Table 4.1 Endometrial hyperplasia and carcinoma treated with progesterone

Author	# Complex atypical hyperplasia	# Endometrial carcinoma	Agents used	Response rate (%)	Median follow-up months (range)
Ferenczy (1989)	20	0	MPA	50	5.5 years (2–7 years)
Randall (1997)	17	12	MA, MPA	86	41 (9–79)
Jobo (2001)	20	0	MPA	75	66 (8–281)
Kaku (2001)	10	29	MPA	80	31.5 (10–133)
Minaguchi (2007)	12	19	MPA	91	40.7 (2–109)
Ushijima (2007)	17	28	MPA	67	47.9 (25–73)
Wheeler (2007)	18	26	Oral progestin, LNG-IUD	52	11 (not stated)
Wildemeersch (2007)	8	0	LNG-IUD	87	32 (14–90)
Yu (2009)	17	8	MPA	82	34.6 (7–114)
Signorelli (2009)	10	11	Natural progesterone	57	98 (35–176)
Gunderson (2014)	17	29	MA, LNG-IUD, MPA, misc	65	35 (2–162)
Marra (2014)	89	0	Natural progesterone (3 doses)	89	Not stated
Simpson (2014)	19	25	MPA, MA	55	39 (5–128)
Kudesia (2014)	13	10	Oral prog, LNG-IUD, oral prog+levo IUD	61	13 (3–74)
Total	287	197		71	

was limited by its small sample size (60 with simple hyperplasia, 72 with complex hyperplasia) [1]. However, this study sample is robust compared to most of the prior published series.

Consensus on conservative management of complex hyperplasia with atypia consists of serial endometrial sampling every 3–6 months for up to 2 years. Conception should be attempted once the hyperplasia has regressed. Often, these patients require assisted reproductive technologies as the risk factors for their endometrial pathology are also common causes of infertility, namely, components of metabolic syndrome such as obesity, PCOS, and insulin resistance. It is therefore crucial that patients receive evaluation and treatment planning with an infertility specialist in parallel with the treatment for their endometrial hyperplasia.

Reproductive outcomes in women with endometrial hyperplasia and low-grade endometrial carcinoma treated conservatively are mixed and not particularly encouraging. In a small series of 12 women with endometrial carcinoma treated with high-dose medroxyprogesterone acetate (MPA 400–600 mg/day), 70 % of patients attempting to conceive did, and 50 % had full-term deliveries [2]. However, the majority of patients recurred during follow-up and half went on to have subsequent hysterectomy. Yu and colleagues reported four pregnancies among 25 patients treated conservatively with MPA 100–500 mg/day [3]. A total of 14 patients attempted conception, and 13 required assisted reproductive techniques. All four pregnancies were in patients with initial diagnosis of atypical hyperplasia, and three term deliveries were reported with the fourth patient being lost to follow-up.

Nonetheless, medical management is never definitive, and hysterectomy should be performed upon completion of childbearing. For those patients who have completed childbearing, hysterectomy is the standard treatment. In patients

undergoing definitive surgical treatment, frozen section should be obtained to evaluate for the possibility of a carcinoma. In younger premenopausal women, the question of ovarian preservation vs. complete staging with bilateral salpingo-oophorectomy is raised. The incidence of ovarian metastasis from an endometrial cancer in a patient with a preop diagnosis of complex hyperplasia is exceedingly low. It is likely in the range of 1–3 % [4]. A multi-institutional review from the 1990s to 2000s revealed an alarming 25 % rate of synchronous ovarian malignancies in young women with a preoperative diagnosis of endometrial cancer [5]. However, 50 % of patients in this series had at least one first- or second-degree relative with malignancy, and 35 % had a first-degree relative with malignancy. This raises the question of whether this cohort accurately represents the risk of ovarian metastasis or synchronous malignancy in the typical population of young women with endometrial hyperplasia as a result of obesity and metabolic syndrome. Another series including 37 patients with endometrial cancer under the age of 45 found an 11 % rate of synchronous ovarian malignancy [6]. A Korean Gynecologic Oncology Group study specifically evaluated ovarian preservation (both intentional and incidental) at the time of hysterectomy with a diagnosis of endometrial malignancy on final pathology [7]. Mean age was 38 years. 175 patients had preservation of at least one ovary, and 31 of whom had a preoperative diagnosis of endometrial hyperplasia. Follow-up was robust with a median of 55 months. Two of the seven recurrences had adnexal metastasis (1.1 %). Both had high risk for recurrence, including one with endometrioid histology who rejected adjuvant treatment and the other with serous histology. Another series reported 13 patients with preservation of at least one ovary at the time of surgery for known endometrial malignancy [8]. There was not a statistically significant difference in overall survival in patients with and without ovarian preservation. However, for stage I patients, there was an improved disease-free survival in women without ovarian preservation, but no improvement in overall survival. Extrapolating from limited data, women with preoperative diagnosis of endometrial hyperplasia should have a lower incidence of ovarian metastasis or synchronous malignancy compared to those with a known endometrial carcinoma. It would be reasonable to retain at least one ovary in young premenopausal women undergoing hysterectomy for endometrial hyperplasia.

Serous Endometrial Intraepithelial Carcinoma

Serous endometrial intraepithelial carcinoma (EIC) is an early form of conventional endometrial serous carcinoma (uterine papillary serous carcinoma) that displays noninvasive patterns of growth in the endometrium but which paradoxically retains the ability for extrauterine spread, presumably through the fallopian tube lumens [9–13]. Accordingly, although EIC is generally conceptualized as an early step in the evolution of endometrial serous carcinoma, it is a *fully* malignant *precursor* lesion, rather than *potentially* malignant *precancerous* lesion. EIC and conventional endometrial serous carcinoma are largely identical at the molecular and immunophenotypic levels, and as previously noted, both can metastasize and are thus fully malignant [9–13]. Furthermore, there is no difference in patient outcomes between patients with advanced stage EIC and patients with advanced stage conventional serous carcinomas [11]. Therefore, clinical management for EIC is identical to the management for patients with conventional serous carcinomas [9]. There is a significant body of evidence that the lesion *endometrial glandular dysplasia* is the true precancer for endometrial serous carcinoma [14–16]. However, at present, there is insufficient data to definitively recommend management approaches when one encounters this diagnosis in a sampling specimen.

Serous lesions of the uterus are treated surgically, unless the patient's comorbidities prohibit surgery. Surgical staging consists of hysterectomy, bilateral salpingo-oophorectomy, pelvic and para-aortic lymphadenectomy, and omentectomy. Though serous lesions of the uterus are

frequently compared to serous ovarian carcinomas, unlike serous ovarian carcinomas, minimally invasive surgery is acceptable for serous lesions of the uterus. Parenthetically, in cases of endometrioid adenocarcinoma, there is some evidence that the use of a uterine manipulator in minimally invasive surgery leads to an increase in the incidence of positive cytology. A series from Korea as well as another from Memorial Sloan Kettering both showed a small increase in positive cytology [17, 18]. However, another series of 42 patients from the University of Vermont compared to cytology obtained before and after placement of uterine manipulators. None of the 42 patients had positive cytology either before or after placement [19]. The clinical significance of this in endometrioid histology is questionable. However, there is the concern among gynecologic oncologists that positive cytology for serous lesions of the uterus may be more significant. Some gynecologic oncologists advocate laparoscopic occlusion of the fallopian tubes prior to placement of the uterine manipulator at the time of surgery. There is insufficient data to support or refute this practice. However, laparoscopic tubal occlusion adds minimal operative time and risk to the overall procedure and should be considered in cases of known serous histology.

Serous Tubal Intraepithelial Carcinoma

Serous Tubal Intraepithelial Carcinoma, or STIC, is usually diagnosed as an incidental finding at the time of risk-reducing bilateral salpingo-oophorectomy. There is no current consensus on the management of incidentally diagnosed STIC. Whether or not surgical staging is warranted has been examined by a few retrospective series. Surgical staging for tubal carcinoma is the same as for ovarian carcinoma: bilateral salpingo-oophorectomy, total hysterectomy, omentectomy, peritoneal biopsies, and pelvic and para-aortic lymphadenectomy. Some controversies exist in the use of minimally invasive techniques in staging. Proponents point to the rapid recovery and similar lymph node count achieved through minimally invasive staging. Opponents argue that the mesentery and bowel cannot be adequately assessed via laparoscopy. The details are beyond the scope of this chapter.

A series by Olivier et al. showed three occult tubal carcinomas and two occult ovarian carcinomas in 58 patients with BRCA1 mutations [20]. All five patients underwent subsequent staging, two patients were upstaged, and both developed recurrent disease. The single patient who remained stage 1A was disease-free at 46 months of follow-up. A series by Wethington et al. of 593 risk-reducing surgeries, mostly in BRCA-mutated patients, found an incidence of 2 % (12 cases) [21]. Of these 12 cases, seven went on to have some subsequent surgical intervention. Only one patient had positive cytology. None had any omental, lymph node, or peritoneal involvement on biopsy. None of the patients received any chemotherapy, and no recurrences were noted at follow-up (median 28 months, range 16–44 months). The author concluded that given the low yield of finding metastatic disease, staging is not recommended for incidentally diagnosed STIC.

Another series by Powell et al. of BRCA 1- and BRCA 2-mutated patients followed 17 patients with high-grade noninvasive neoplasia of the fallopian tube, including one patient that also had an occult ovarian noninvasive lesion [22]. All but two had a recorded preop CA-125 level, and all were in the normal range. Of these 17 patients, 2 had positive cytology, and 10 went on to have some additional staging surgery. Four patients received chemotherapy with carboplatin and paclitaxel. Two of these four patients had positive cytology and had undergone additional staging surgery. The other two had negative cytology and did not undergo additional surgery. Only one patient recurred 43 months after risk-reducing surgery and was disease-free at 16 months following completion of debulking followed by chemotherapy. All 17 patients were alive at last follow-up.

Gilks et al. examined the incidental finding of STIC in 21 patients without BRCA mutation (though one patient had Li-Fraumeni syndrome) [23]. Six patients were reported to have under-

Table 4.2 Incidentally diagnosed STIC

Author	# Patients with STIC	# Patients surgically staged	# Upstaged	Follow-up months (range)	# With recurrent cancer
Olivier (2004)	5/58 (8.6 %)	5 (100 %)	2 (40 %)	12	2 (40 %)
Wethington (2013)	12/593 (2 %)	7 (58 %)	0	28 (16–44)	0
Powell (2013)	17/407 (4 %)	10 (59 %)	0	80 (40–150)	1 (5.9 %)
Gilks (2015)	21[a]	6 (29 %)	0	Not given	1 (4.8 %)
Mean/Total	55 (4.9 %)	28 (51 %)	7.1 %		4 (7.3 %)

[a]Series of STICs only

gone staging, and two were upstaged. One recurrence was seen in a patient who remained stage IA after full staging and received no adjuvant chemotherapy. Clinical outcome for the remaining 15 patients was not reported. Table 4.2 provides a summary of above case series.

Based on limited retrospective data, it seems reasonable to offer staging surgery, particularly in the presence of positive cytology, for patients with incidentally diagnosed STIC. Empiric chemotherapy without additional staging surgery or evidence of spread beyond the tube or ovary is not warranted. Undoubtedly, the treatment of incidentally diagnosed STIC will continue to evolve as the body of evidence increases.

Ovarian Atypical Endometriosis

The incidence of ovarian atypical endometriosis with coexisting carcinoma was discussed in an earlier chapter. Though literature supports ovarian atypical endometriosis as a true premalignant entity, the temporal relationship between development of ovarian atypical endometriosis and invasive carcinoma is scantly reported. In a handful of case reports and series, it ranges from 10 months to 5 years [24–26]. The proportion of patients with ovarian atypical endometriosis without concurrent malignancy that will go on to develop an invasive carcinoma is poorly reported. In a single series that included four patients with ovarian atypical endometriosis who were followed for mean of 2.5 years (range 1.3–3.5 years), only one patient went on to develop and endometrioid carcinoma in the abdominal wall, which occurred 18 months after initial diagnosis [27]. Another study examining differential nuclear organizing region silver staining (AgNOR) included ten patients with ovarian atypical endometriosis. Two patients subsequently developed invasive clear cell carcinoma, at 10 months and 3 years after ovarian atypical endometriosis diagnosis [25]. The patient with short interval died soon after diagnosis. The other was disease-free at last follow-up 27 months after debulking surgery. Due to the rarity of the situation, no conclusive recommendation could be made for or against definitive surgical intervention. For the older patient who has completed childbearing, one should consider bilateral salpingo-oophorectomy with total hysterectomy as definitive and preventative treatment. For the younger patient in whom fertility preservation is an important consideration, long-term serial imaging of remaining ovary(ies) should be done. The role of CA-125 has not been explored. In case reports, CA-125 was either not done or normal even at the time of cancer diagnosis [26]. It is unlikely to be of significant benefit and should not be used as a surveillance tool.

Miscellaneous Ovarian Precursors

Endometrioid borderline tumors are the third most common type of borderline tumors, after serous and mucinous. The largest series ever reported included 31 patients [28]. All but three patients had unilateral tumors. Limited clinical information and outcomes were reported. Seventeen patients underwent bilateral salpingo-oophorectomy and hysterectomy. The remainder had bilateral salpingo-oophorectomy, unilateral

Table 4.3 Endometrioid borderline tumors

Author	# Patients	# Undergone hysterectomy	# With endometrial pathology	Follow-up	# Recurrence
Bell (1985)	20	12	7	6.3 years (1–13 years)	0[a]
Snyder (1988)	31	19 (1 prior)	12	3.8 years (0.8–11.2 years)	0
Bell (2000)	31	17	3	48 months	0
Roth (2003)	30	18	7	Not stated	0
Uzan (2012)	16	5	1	24 months (12–132 months)	1
Total	128	71	30		1 (0.8 %)

[a]One patient had a second primary endometrioid adenosquamous carcinoma of contralateral ovary 2 years after diagnosis

salpingo-oophorectomy, or cystectomy. Sixteen patients had surgical staging and all were noted to be stage I. Two patients were known to have received chemotherapy, though clinical follow-up data was available in only 11 patients. During a mean duration of 48 months, no recurrences were noted. The most recent series by Uzan et al. reported 16 patients, six of who had some sort of staging procedure. One patient received adjuvant chemotherapy, another received vaginal brachytherapy for a synchronous endometrial malignancy. This series contains the only recurrence ever reported in the English literature. She did not undergo staging at the time of diagnosis; experienced two recurrences, treated with surgery and chemotherapy; and was without evidence of disease at 72 months of follow-up [29]. Table 4.3 summarizes the clinical characteristics of patients in the larger series [28–32]. Compared to other histologic subtypes of borderline tumor, endometrioid borderline tumors of the ovary have a higher rate of synchronous endometrial pathology. Therefore, hysterectomy should be part of definitive surgical management.

Clear cell borderline tumors arising out of adenofibromas of the ovary are very rare, comprising less than 1 % of borderline ovarian tumors. The majority of patients are older than 50 years, and all reported cases containing information on clinical follow-up were stage I. The largest series ever reported that offered descriptions of clinical course included 12 patients [33]. In this series, all but two patients underwent bilateral salpingo-oophorectomy. No patient received adjuvant treatment. Unfortunately, four were lost to follow-up. After a median follow-up of 28 months (range 2–129 months), no recurrences were noted. To date, there is only one reported case of recurrent disease, and another of possible but unconfirmed recurrence. The series of 11 patients reported by Bell et al. included one patient who received radiation for a pelvic recurrence, followed by surgical resection for a second pelvic recurrence. She died of unrelated cause. Another patient had a lung nodule noted on surveillance imaging. However, no pathologic diagnosis or treatment was ever done due to her advanced age [30]. The largest series of 41 cases did not provide any prognostic or clinical follow-up data [34]. Multiple other case reports all reported patients without evidence of recurrence at last follow-up [35–38]. Most patients had bilateral salpingo-oophorectomy and hysterectomy, with the diagnosis made after the fact. The vast majority of patient had unilateral tumors. Given no recurrences were noted in the uterus, it stands to reason that bilateral salpingo-oophorectomy is sufficient treatment. There were two patients with endometrial hyperplasia and one with endometrial polyps in the series by Bell et al. [30]. Whether the endometrial pathology has any correlation with ovarian pathology is unknown. Nonetheless, hysterectomy at the time of bilateral salpingo-oophorectomy is probably prudent as the absolute morbidity it adds to the procedure is relatively low. However, if the diagnosis of clear cell adenofibroma was made after bilateral salpingo-oophorectomy, then a repeat surgery for the purpose of hysterectomy is likely not warranted. No definitive recommendations

can be made regarding adjuvant therapy given lack of data evaluating its use, but observation after surgery seems like the most reasonable course of action based on existing literature.

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Part II

Uterine Corpus

5 Putative Precursor Lesions of Gestational Trophoblastic Neoplasia

Natalia Buza and Pei Hui

Introduction

Gestational trophoblastic neoplasms (GTN) include three distinct malignant tumors—choriocarcinoma, epithelioid trophoblastic tumor (ETT), and placental site trophoblastic tumor (PSTT)—arising from different types of trophoblast. Gestational choriocarcinoma—the most aggressive form of GTN—is composed of neoplastic villous intermediate trophoblast, syncytiotrophoblast, and cytotrophoblast, whereas ETT and PSTT originate from chorion laeve-type and implantation site-type intermediate trophoblast, respectively.

Gestational choriocarcinoma nowadays is preceded by complete hydatidiform mole (CHM) in approximately 25 % of cases, while majority of tumors (~50 %) develop following a term pregnancy, non-molar abortion (~22–23 %), and less commonly ectopic pregnancy (2–3 %) [1]. CHM is associated with a 2–3 % risk of progression into choriocarcinoma, while partial hydatidiform moles (PHMs) carry minimal risk (less than 0.5 %) for choriocarcinoma [2–4]. Some studies also suggest that the prognosis of CHM may be affected by its genotype; heterozygous complete moles have been reported to have a higher risk of malignant transformation, compared with the homozygous ones [5–7]. While the traditional histological diagnostic criteria of choriocarcinoma include absence of chorionic villi, rare cases of in situ or intraplacental choriocarcinoma have been reported [8–15].

Unlike gestational choriocarcinoma, tumors of intermediate trophoblast origin—ETT and PSTT—do not have well-characterized precursor lesions. Placental site nodules—benign proliferations of chorionic-type intermediate trophoblast—show some morphologic similarities to ETT, but lack significant cytological atypia and mitotic activity. Rare cases of atypical placental site nodule—with intermediate features between ETT and benign placental site nodule—have also been described and have been proposed as possible precursor lesion to ETT [16, 17]. Exaggerated proliferation of implantation site intermediate trophoblast (exaggerated placental site) may mimic PSTT and may be considered as its benign counterpart, but thus far, definite pathogenetic link or an intermediate lesion between the two entities has not been established.

Hydatidiform Moles

Hydatidiform moles are nonneoplastic proliferations of the villous trophoblast with a potential for aggressive clinical behavior, in the form of

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persistent gestational trophoblastic disease (GTD) or less commonly choriocarcinoma. The two forms of molar gestations—complete and partial hydatidiform moles—share some features on the clinical, histological, and genetic level: both are abnormal gestations incompatible with fetal survival, and they have hydropic changes and trophoblastic proliferation in the chorionic villi and demonstrate paternal dominance in their genomes. However, distinction and precise classification of the two entities are crucial due to the marked difference in their risk of subsequent aggressive behavior or overt malignant transformation.

Genetic Basis of Molar Gestations

Complete hydatidiform moles (CHMs) are characterized by a paternal-only genome, most commonly with a diploid, homozygous (monospermic), 46XX genotype [18]. Approximately 10–20 % of CHMs show a heterozygous (dispermic) 46XX or 46XY genotype, and less common tetraploid cases have also been reported [19–21]. Another rare subset of complete moles has sparked significant interest in recent years, presenting as recurrent CHM with strong familial tendency [22]. These cases represent a rare exception to the androgenetic-only genome of CHM, as they are biparental (monoandric monogynic), with homozygous or compound heterozygous mutations of the NLRP7 gene on chromosome 19q13.4 or the KHDC3L gene on chromosome 6q13, disrupting the normal genetic imprinting pattern [23–27].

Partial hydatidiform moles (PHMs) typically have a triploid—diandric monogynic—genome, resulting from two sperms fertilizing a haploid ovum (dispermic, heterozygous PHM) in over 90 % of cases and less commonly arise from a single fertilizing sperm followed by duplication of the paternal chromosome set (monospermic, homozygous PHM) [28, 29]. Rare cases of tetraploid PHM with three haploid paternal chromosome sets have also been reported [30, 31]. While early studies also raised the possibility of diploid PHM [32, 33], more recent data suggest that they probably do not exist [34].

Clinical Presentation

The clinical presentation of hydatidiform moles has changed significantly over the past few decades as a result of availability and advances in diagnostic ultrasound technology and highly sensitive serum human chorionic gonadotropin (hCG) detection. The classic clinical symptomatology of CHM—vaginal bleeding during second trimester, excessive uterine size, hyperemesis, and toxemia—is less common; nowadays, most patients present with missed abortion (absence of fetal heart beat on ultrasound) and vaginal bleeding during the first trimester of pregnancy [35–37]. Patients with PHM are usually diagnosed in the late first—or early second—trimester as a missed or incomplete abortion. Vaginal bleeding is also common, and an ultrasound may show focal cystic changes of the placenta, not uncommonly with fetal development [38].

Gross and Microscopic Features

Complete Hydatidiform Mole

The evacuation specimen in well-developed CHM is usually voluminous with grossly apparent hydropic change in the chorionic villi, resembling a "bunch of grapes" in appearance. Early cases of CHM, however, will have a smaller specimen volume and may not show grossly identifiable villous hydrops.

The histologic hallmarks of well-developed CHM include marked, diffuse villous enlargement and edema with cistern formation and diffuse, circumferential trophoblastic hyperplasia (Fig. 5.1). The villous contours are typically smooth and round, but surface invaginations resulting in trophoblastic pseudo-inclusions are also common. Cytological atypia is nearly always present in villous and implantation site trophoblast, usually accompanied by brisk mitotic activity. The villous stroma is usually hypocellular due to the marked edema and is devoid of any vessels or fetal red blood cells. On the other hand, in very early complete moles (VECMs)—evacuated before 12 weeks of gestation—the trophoblastic proliferation and hydropic

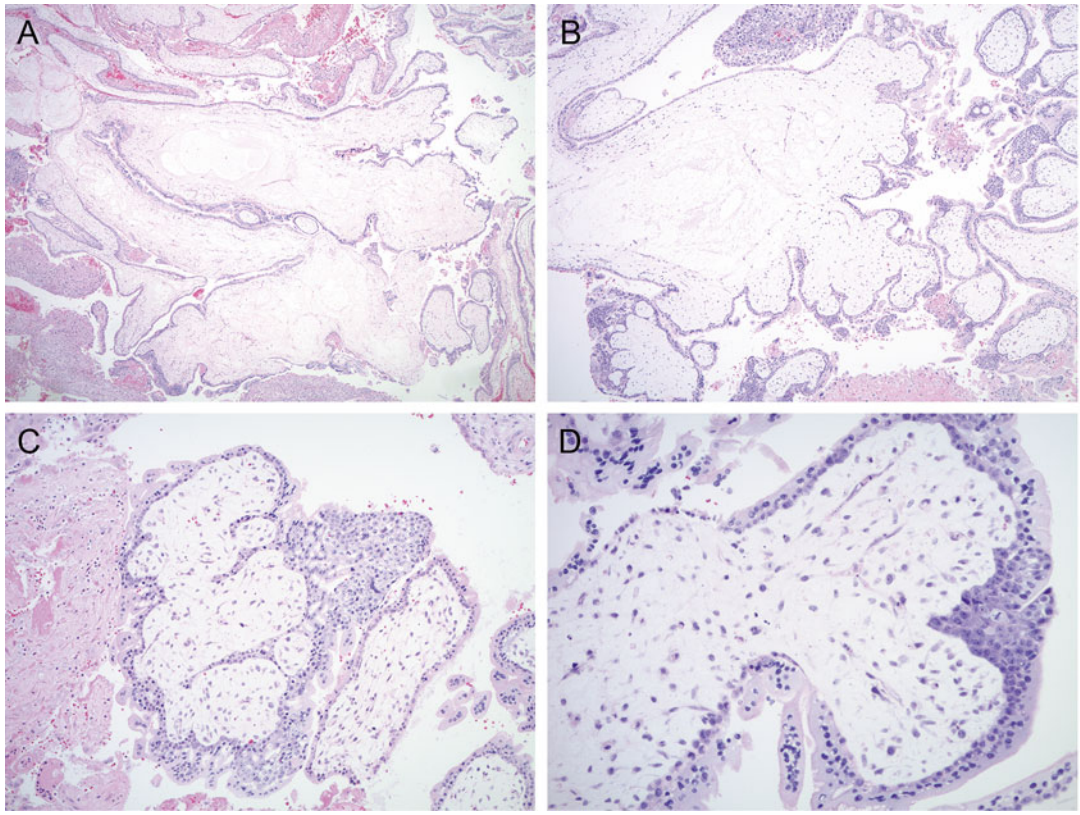

Fig. 5.1 Microscopic features of complete hydatidiform mole (CHM). (**a**, **b**) Well-developed CHM with large hydropic villi and central cistern formation. Circumferential trophoblast hyperplasia is also present. (**c**, **d**) Early CHM with polypoid, "cauliflower-like" villi; hypercellular, myxoid villous stroma with karyorrhexis; and circumferential trophoblast hyperplasia

change are not fully developed yet, and instead, the villous stroma appears hypercellular and myxoid with stellate fibroblasts and prominent karyorrhectic debris. Rarely primitive fetal vessels and even nucleated red blood cells may be seen in VECM [32, 33, 39, 40]. Unlike in well-developed CHM, the villous size is usually within the normal range, and the villi are polypoid and "cauliflower" shaped with less frequent trophoblastic pseudo-inclusions. Fetal parts or other non-villous extraembryonic structures (e.g., yolk sac) are not present in CHM.

Partial Hydatidiform Mole

The specimen volume in partial moles is typically less than that of CHM, and grossly visible hydropic change is rare, especially during the first and early second trimester. Fetal development may be seen, usually with mild to moderate symmetrical intrauterine growth restriction and characteristic malformations (e.g., syndactyly) [41].

Histologically, there are usually two populations of chorionic villi in PHM—large, hydropic villi (ranging between 1 and 6 mm in size) in the background of small or normal appearing ones. The villous contour is irregular, scalloped with surface invaginations, and round to oval trophoblastic pseudo-inclusions. Trophoblastic hyperplasia is typically mild to moderate and focal, without significant cytological atypia. Cistern formation is not uncommon. Fetal vessels with nucleated red blood cells are often seen (Fig. 5.2).

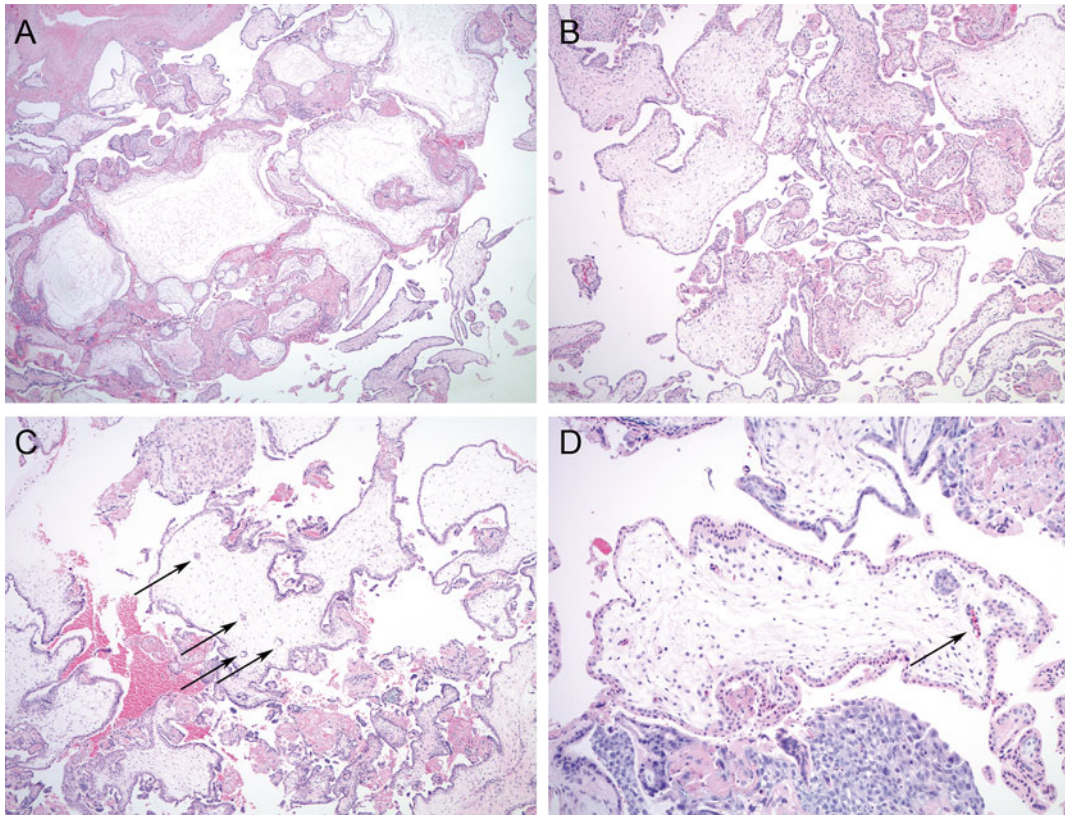

Fig. 5.2 Microscopic features of partial hydatidiform mole (PHM). (**a**, **b**) Two villous populations: large hydropic villi with occasional cistern formation in the background of smaller villi. (**c**) Irregular villous contours and trophoblastic pseudo-inclusions (*arrows*). (**d**) Fetal vessels with nucleated red blood cells are commonly seen (*arrow*)

Ancillary Studies

The diagnosis of hydatidiform moles is often challenging based on clinical and morphological features alone. The microscopic changes are not entirely specific and show significant overlap between complete and partial mole, especially when evacuated at an early gestational age. In addition, non-molar gestations with or without identifiable genetic abnormalities can also mimic hydatidiform moles at the morphologic level. Ancillary studies are often necessary to differentiate between complete and partial hydatidiform moles and to rule out non-molar mimics.

Ploidy Analysis

Determination of the number of complete haploid sets of chromosomes can separate diploid gestations from triploid, tetraploid, or other aneuploid ones. However, it does not provide information about the parental origin of chromosome sets; thus, a diploid CHM cannot be separated from diploid non-molar hydropic abortion based on DNA ploidy. In addition, it is unable to differentiate between triploid—diandric monogynic—partial moles and non-molar digynic monoandric triploidy, which constitute at least one third of all triploid gestations and are not associated with increased risk of GTN [29, 42, 43].

Conventional karyotyping has been used for several decades to assess ploidy and is very helpful in identifying chromosomal trisomy syndromes, which often mimic PHM morphologically. However, it requires fresh tissue and is time and labor intensive, limiting its utility in routine practice. Ploidy analysis can also be performed on formalin-fixed paraffin-embedded material by flow cytometry, although it may be prone to technical problems and interpretation errors, leading to potential misclassification of ploidy [44–47]. Another technique—polymorphic deletion probe (PDP) fluorescent in situ hybridization (FISH)—has been recently reported for chromosomal enumeration in suspected molar gestations, but similar to the other methods, it suffers from both technical limitations and interpretation problems [48].

P57 Immunohistochemistry

Various immunohistochemical markers, including cell cycle proteins (E2F-1, CDK2, cyclin E, p27, p57) and proliferation markers (proliferation cell nuclear antigen [PCNA], Ki-67), have been explored for their diagnostic utility in hydatidiform moles [49–51]. However, only p57 immunostain has been found useful in routine diagnostic pathology practice. P57 gene is located on chromosome 11p15.5 and encodes a cyclin-dependent kinase inhibitor protein [52, 53]. Since the gene is paternally imprinted—preferentially expressed from the maternal allele—maternal genetic material is necessary for a normal p57 protein expression pattern: strong nuclear staining in cytotrophoblasts, intermediate trophoblasts, intervillous trophoblast islands, and villous stromal cells and absent staining in syncytiotrophoblasts [54]. Normal placentas, non-molar hydropic abortions, chromosomal trisomies, digynic triploid cases, and partial moles all show normal p57 staining patterns, due to the presence of maternal genetic material in their genomes. Complete moles, on the other hand, lack p57 immunoreactivity in cytotrophoblast and villous stromal cells, but retain p57 expression in intervillous intermediate trophoblasts and villous endothelial cells (Fig. 5.3). Maternal decidua always shows positive nuclear staining, serving as an internal positive control.

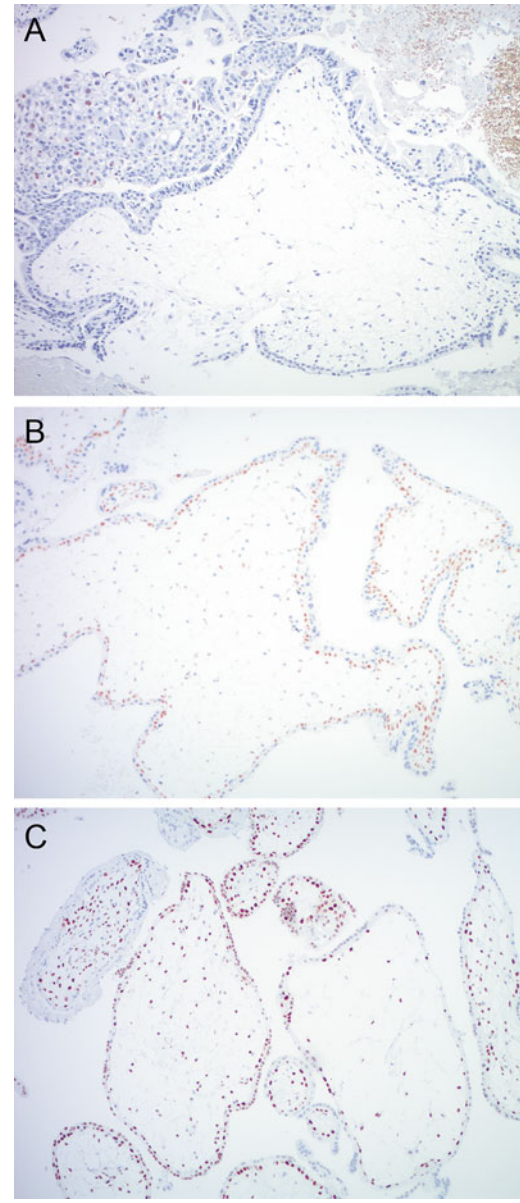

Fig. 5.3 P57 immunohistochemistry. (**a**) CHM with absent p57 staining in villous stroma and cytotrophoblast. Intervillous intermediate trophoblast shows positive nuclear staining (*upper left corner*). PHM (**b**) and non-molar hydropic abortion (**c**) show normal p57 immunostaining pattern

P57 immunohistochemistry is a useful adjunct test in the diagnostic workup of hydatidiform moles, as it can separate CHM from PHM and from other non-molar hydropic gestations. However, partial moles and their non-molar

mimics contain maternal genetic material and thus show normal p57 staining pattern and cannot be separated from each other based on p57 immunohistochemistry. Additionally, some complete moles may show focal p57 staining, due to incomplete imprinting, twin gestation (admixture of CHM and normal villi), or rare androgenetic/biparental mosaic/chimeric gestation [55, 56]. Another rare potential pitfall is CHM with retention of maternal chromosome 11, resulting in normal p57 expression [57, 58], and rare p57-negative PHM due to loss of maternal chromosome 11 [59]. The p57 immunohistochemical pattern of biparental complete moles is identical to androgenetic CHM as a result of mutations of the NLRP7 or KHDC3L genes and disruption of the normal imprinting pattern [60].

Short Tandem Repeat Genotyping

Short tandem repeats (STR) are repetitive, genetically stable DNA sequences of 2–7 nucleotides, which are highly prevalent in the noncoding regions of the genome [61]. STR polymorphism—difference in the number of repeats at each STR locus among different members of a species—can be analyzed and used to distinguish between individuals. STR genotyping is widely used for identity testing in forensics and more recently has also become part of the routine diagnostic workup for molar gestations at some large academic centers [21, 62, 63].

One of the advantages of STR genotyping compared to other ancillary molecular techniques is that it can be performed on formalin-fixed paraffin-embedded tissue samples, following dissection of pure maternal and fetal tissues from unstained sections. Numerous commercial kits, e.g., PowerPlex® 16 System (Promega) are available for DNA extraction and PCR amplification. Comparison of the allelic profiles between maternal and fetal (chorionic villous) tissues at 15 STR loci provides information about the parental genetic contribution to the villous tissue and the relative proportions of maternal and paternal genetic material. Complete moles show paternal-only alleles—either in a homozygous or heterozygous pattern—in at least two informative STR loci (Fig. 5.4). PHM can be diagnosed in the presence of two unique paternal alleles in addition to one maternal allele in at least two loci (dispermic or heterozygous PHM) or one paternal allele in duplicate quantity and one maternal allele at every STR locus (monospermic or homozygous PHM) (Fig. 5.5). Digynic triploidy can be reliably distinguished from PHM using STR genotyping by the presence of one paternal and two maternal alleles. A biallelic profile with balanced maternal and paternal contributions indicates a non-molar abortion (Fig. 5.6). Chromosomal trisomies may also be identified by genotyping as a single allelic gain, although not all chromosomes are represented among the 15 STR loci (Fig. 5.7) [64]. Rare potential pitfalls of genotyping interpretation also exist and require close morphologic correlation and p57 immunostaining in some cases to avoid misclassification. For example, biparental CHM shows a biparental allelic profile on genotyping; however, the histological features and p57 expression pattern are diagnostic and are indistinguishable from those of a diandric complete mole [23, 25]. A case of egg donor pregnancy has also been reported with morphologic features suspicious for PHM and genotyping results mimicking a complete mole, due to lack of maternal alleles in the villous tissue [65]. In addition, twin pregnancy with coexisting CHM and normal fetus and cases with mosaicism/chimerism could also interfere with the interpretation of genotyping data [66, 67].

Differential Diagnosis

Spontaneous non-molar hydropic abortions may show significant villous enlargement and edema, occasionally even with cistern formation, mimicking a complete or partial mole on the morphologic level. However, the villous contour is round or oval without invaginations, and trophoblastic hyperplasia is absent or mild and polarized (not circumferential) (Fig. 5.8). They show normal p57 expression pattern and a balanced biallelic profile on genotyping.

The morphologic changes of an early non-molar gestation and a very early complete mole

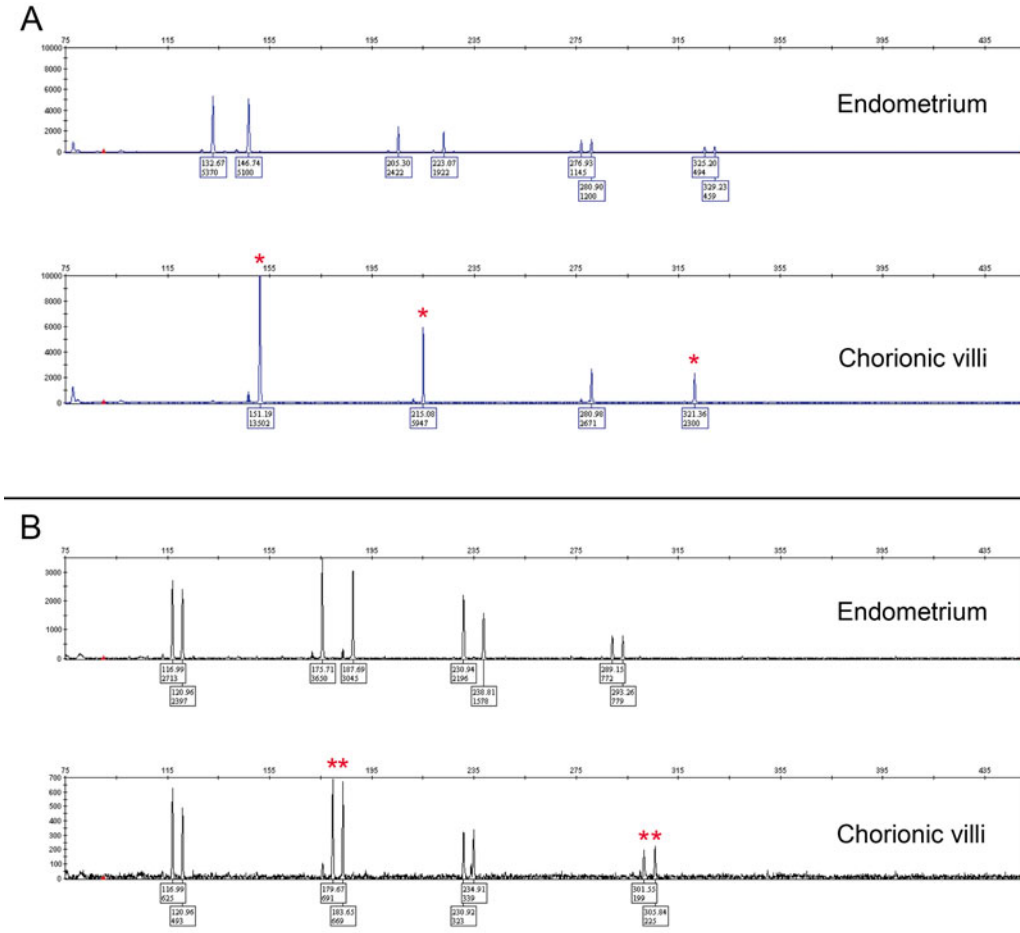

Fig. 5.4 STR genotyping of complete hydatidiform moles (CHM). (**a**) In homozygous (monospermic) CHM, the chorionic villi show a unique paternal allele (*asterisk*) in duplicate quantity and absence of maternal alleles in at least two informative loci. (**b**) In heterozygous (dispermic) CHM, there are two unique paternal alleles (*asterisk*) and absence of maternal alleles in at least two informative loci

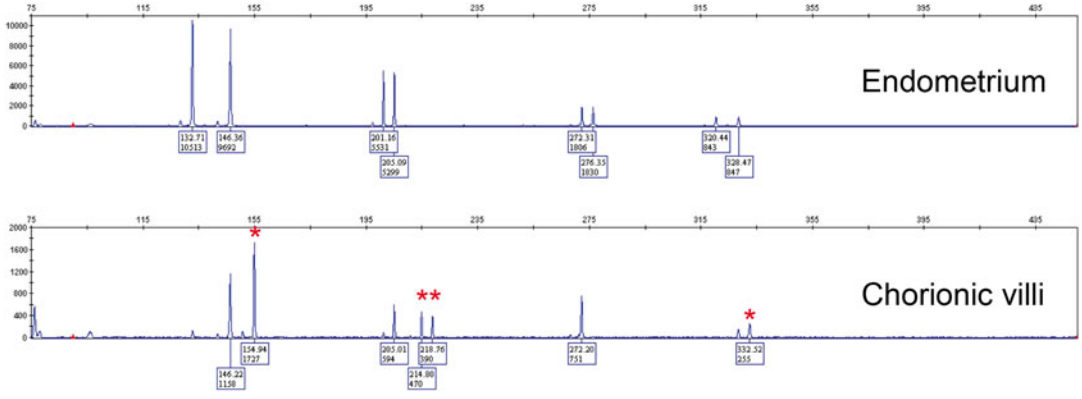

Fig. 5.5 STR genotyping of partial hydatidiform mole (PHM). Dispermic PHM shows a triploid pattern with two unique paternal alleles (*double asterisk*) or one paternal allele (*asterisk*) in duplicate quantity in addition to one maternal allele

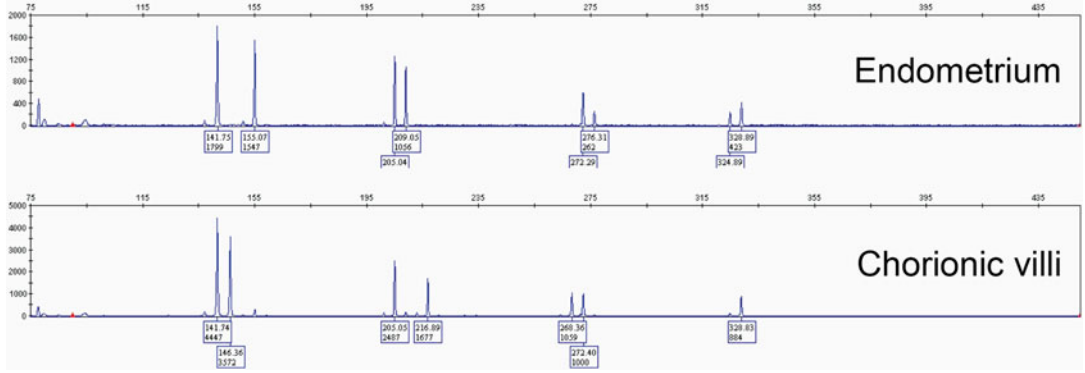

Fig. 5.6 Non-molar hydropic abortion showing a balanced biparental allelic pattern on genotyping

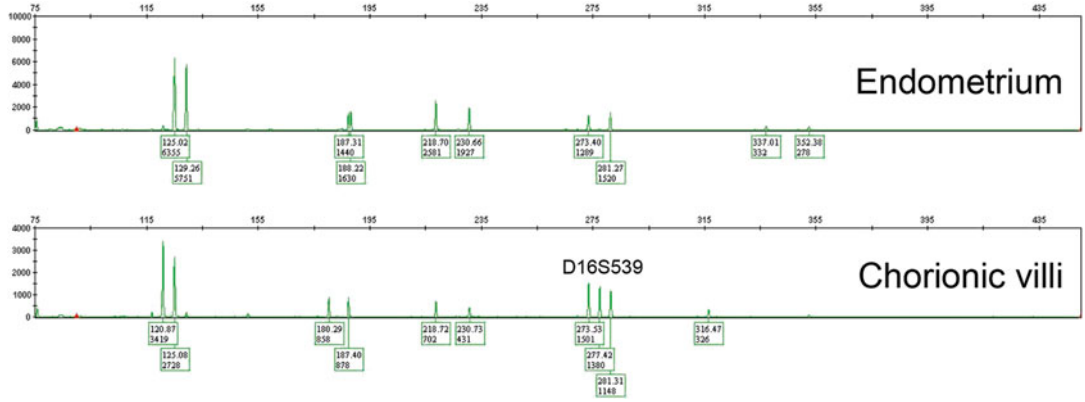

Fig. 5.7 Trisomy 16. Three alleles identified at locus D16S539, other loci show normal biparental allelic pattern

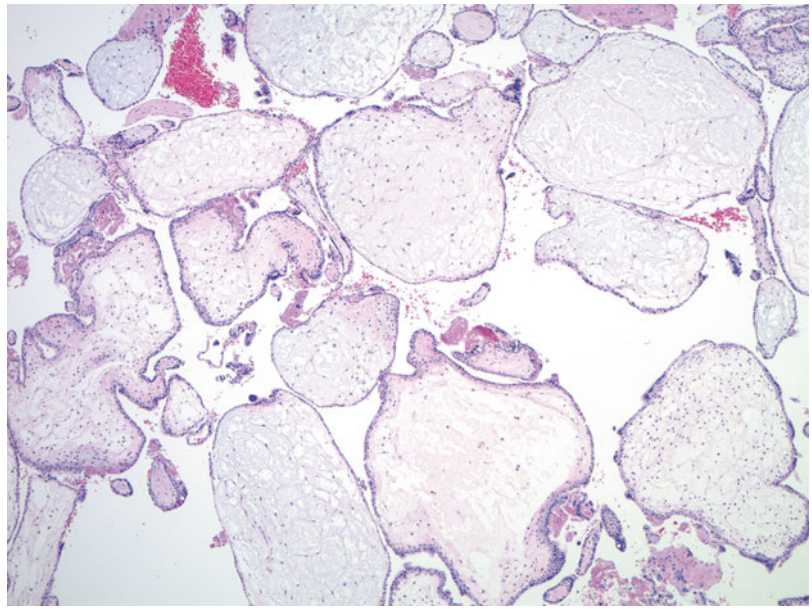

Fig. 5.8 Non-molar hydropic abortion. The villous shape is *round* or *oval*; no trophoblastic pseudo-inclusions or significant trophoblast hyperplasia is seen

(VECM) may overlap in the form of villous size and villous stromal cellularity. Trophoblastic proliferation—even if it is only mild or moderate—is circumferential or random in VECM, in contrast to the polarized trophoblastic proliferation of early normal pregnancy. Presence of fetal parts and well-formed villous stromal vessels with nucleated red blood cells essentially rules out a complete mole. P57 immunohistochemistry and genotyping can be used to resolve difficult cases.

Chromosomal trisomies (especially trisomies 7, 8, 13, 15, 16, 18, 21, and 22) often have hydropic, irregularly shaped chorionic villi with frequent trophoblastic pseudo-inclusions, morphologically simulating PHM (Fig. 5.9). Trophoblastic hyperplasia may also be present, more commonly in trisomies involving chromosomes 7, 15, 21, and 22 [68]. However, when compared with trisomy syndromes and non-molar hydropic abortions, the combination of cistern formation and maximum villous size of ≥2.5 mm has been shown to have a 90 % positive predictive value for partial mole [64]. P57 immunohistochemistry does not help distinguishing PHM from trisomies, as they both show normal expression pattern due to the presence of maternal genetic material. Genotyping can identify diandric triploidy—diagnostic of partial mole—and separate it from common trisomy syndromes showing a single allelic gain.

Digynic monoandric triploidy—comprising approximately one third of all triploid gestations—may also present a diagnostic challenge morphologically and also on ploidy analysis. Similar to PHM, it may show villous contour irregularity with trophoblastic pseudo-inclusions, mild hydropic change, and syncytiotrophoblast sprouts (Fig. 5.10) [69]. However, unlike PHM, digynic triploidy is not associated with increased risk of persistent GTD or GTN. Molecular genotyping can be used to determine the parental origin of the haploid chromosome sets in the triploid genome and precisely separate the two entities.

Placental mesenchymal dysplasia is a late gestational age mimic of PHM, presenting with stem and terminal villous hydrops, rare cistern formation, aneurysmal stem vessels, and peripheral stem villous chorioangiomatoid change, usually in late second trimester [43, 70]. Trophoblastic hyperplasia and trophoblastic pseudo-inclusions are typically absent [70]. Fetal abnormalities are common, in the form of intrauterine growth restriction or Beckwith-Wiedemann syndrome

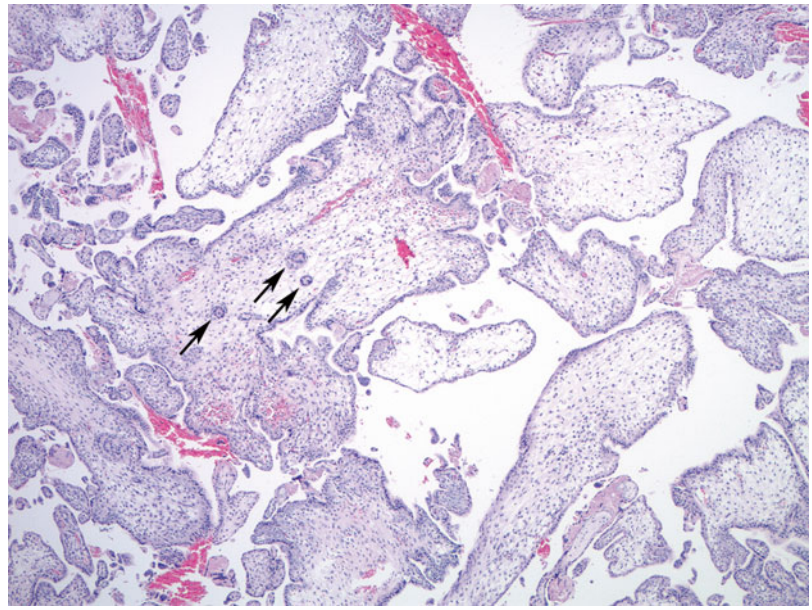

Fig. 5.9 Trisomy 13 with villous hydrops and irregular villous contours, resulting in trophoblastic pseudo-inclusions (*arrows*)

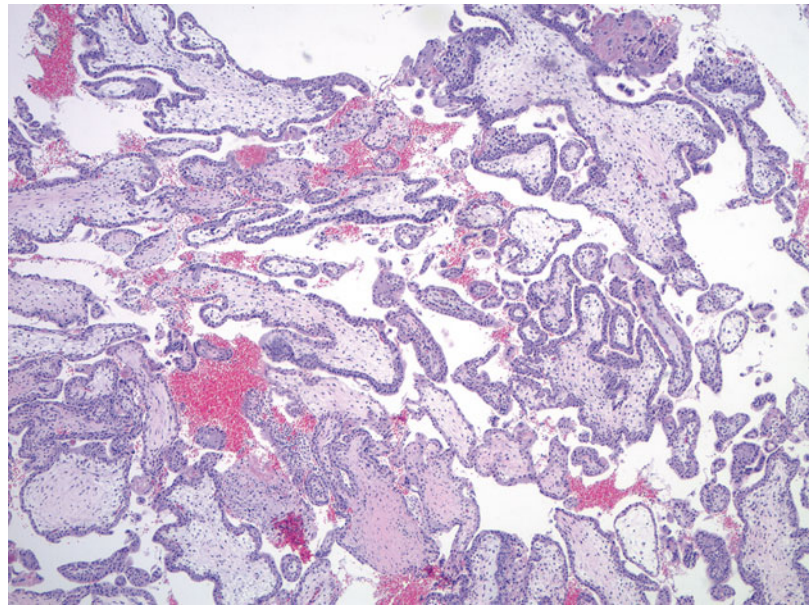

Fig. 5.10 Digynic triploidy showing mild villous hydrops and scalloped villous surface, mimicking PHM

[71]. Genotyping shows a balanced biparental allelic pattern, allowing clear separation from a partial molar gestation.

Proposed Diagnostic Algorithm of Hydatidiform Moles

Integration of ancillary techniques into the routine diagnostic workup of molar gestations is necessary to avoid diagnostic misclassification based solely on morphologic findings. Two algorithmic approaches have been recently proposed to combine morphologic evaluation with p57 immunohistochemistry and DNA genotyping [55, 72, 73]. According to one approach, all cases with morphologic suspicion for either complete or partial hydatidiform mole are subjected to p57 immunohistochemistry first, and cases with absent staining are diagnosed as CHM [55, 73]. Cases with equivocal staining pattern or morphological features are further analyzed by STR genotyping. Another algorithm advocates genotyping on all cases with morphologic features of complete or partial mole, to obtain a definitive diagnosis based on the parental genetic contribution to the villous tissue [72]. Rare cases with a biallelic genotyping pattern and strong morphologic suspicion for CHM are also evaluated by p57 immunohistochemistry as a second step, to rule out a biparental CHM.

The two algorithmic approaches may also be combined: cases with histologic features of CHM can be subjected to p57 immunostaining, and the lack of reactivity would confirm the diagnosis of complete mole. However, cases showing characteristics of PHM microscopically are evaluated by genotyping (without p57 immunostaining) [74].

Invasive and Metastatic Hydatidiform Mole

Invasive mole is characterized by myometrial and/or vascular invasion by molar villi and is seen in approximately 10–15 % of CHM and up to 5 % of PHM cases [75–79]. The molar villi may rarely spread to the broad ligament, vagina, and vulva and even metastasize to distant sites, such as lungs [80, 81]. Histologically, invasive CHM has diffuse villous hydrops and trophoblastic hyperplasia, which occasionally may be exuberant and shows marked cytological atypia, resembling choriocarcinoma in iso-

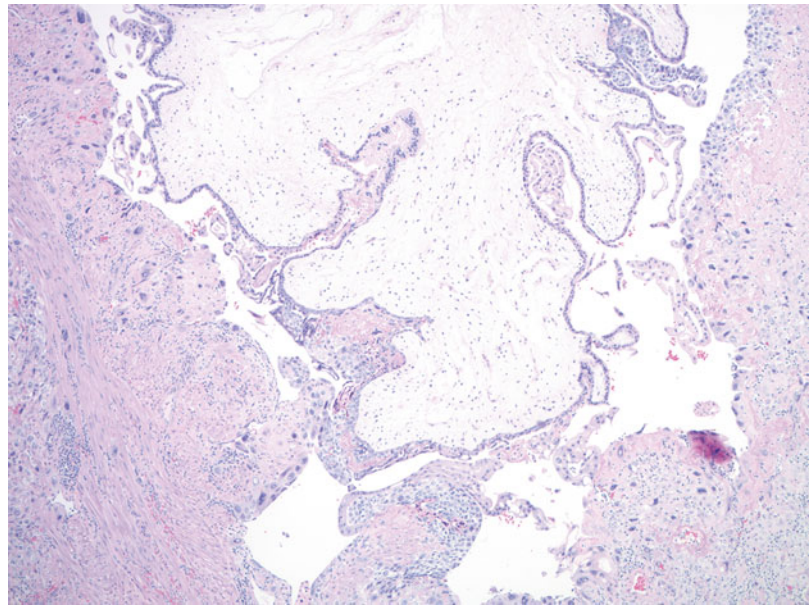

Fig. 5.11 Invasive complete mole. Large hydropic molar villi seen invading into the myometrium in a hysterectomy specimen

lation (Fig. 5.11). Such lesions may be considered as "emerging" or "in situ" choriocarcinoma (see next section). Precise distinction between these entities is typically not crucial for clinical management purposes, as they are all encompassed under the term persistent GTD and are often treated based on clinical parameters alone [82].

In Situ or Intraplacental Choriocarcinoma

Histological diagnostic criteria of choriocarcinoma traditionally include bilamellar growth pattern with mononuclear trophoblast rimmed by a layer of multinucleated syncytiotrophoblast, severe cytological atypia, high mitotic activity, and absence of chorionic villi. However, it has been proposed more recently that an intermediate or precursor lesion also exists: "emerging" or "in situ" choriocarcinoma in the presence of non-molar or molar villi with exuberant trophoblastic hyperplasia and marked cytological atypia (Fig. 5.12) [13, 83, 84]. It should also be noted that very early gestations may have foci of sheet-like trophoblastic proliferation with high mitotic activity mimicking an "in situ" choriocarcinoma, although significant cytologic atypia is absent.

Rare cases of intraplacental choriocarcinoma have also been documented in a full-term placenta [8–12, 14, 15, 85]. The initial presentation of some of these patients was metastatic choriocarcinoma, and the intraplacental primary lesion was only discovered after careful reexamination of the placenta. As these lesions are often small—measuring less than 1 cm—they may be missed on gross examination. Hence, some investigators recommend sectioning of the placenta at 5 mm intervals and sampling of any hemorrhagic mass lesions [10].

Atypical Placental Site Nodule

Placental site nodule (PSN) or plaque—a benign, reactive proliferation of chorion laeve-type intermediate trophoblast—is most commonly an incidental finding in endometrial curettings, although patients may present with irregular uterine bleeding [86–89]. PSN is typically small, ranging from 4 to 10 mm in size [89]. Microscopically, it is characterized by haphazardly arranged mononuclear or less often multinucleated trophoblast in a hyalinized matrix, showing variable cellularity, often with zonation (Fig. 5.13). Mild nuclear atypia and nuclear pseudo-inclusions are not

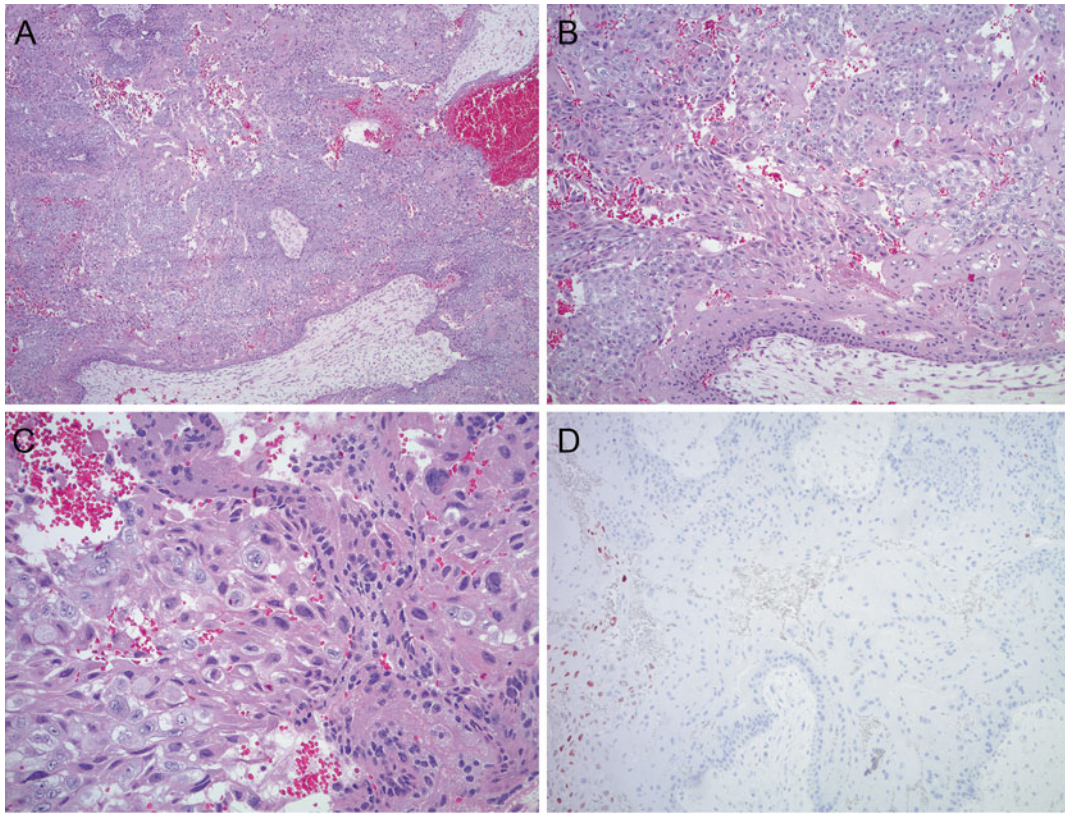

Fig. 5.12 (**a**–**c**) "Incipient choriocarcinoma": exuberant biphasic, atypical trophoblastic proliferation in the presence of complete molar villi. (**d**) P57 immunostain is negative in villous stroma and cytotrophoblast, confirming complete hydatidiform mole. Positive internal control in intermediate trophoblast (*left side* of image)

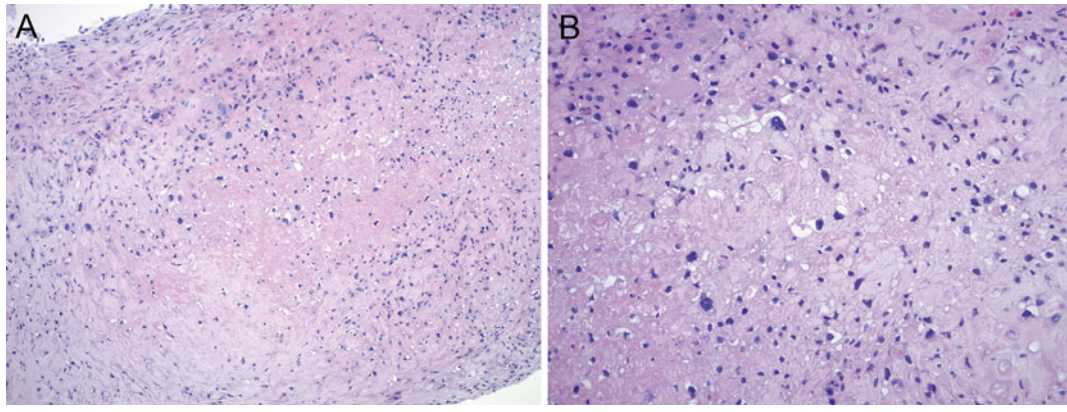

Fig. 5.13 Placental site nodule (PSN). (**a**) Small fragment of PSN in a cervical curettage specimen with relatively low cellularity and central hyalinization. (**b**) Mild nuclear atypia and multinucleation are not uncommon

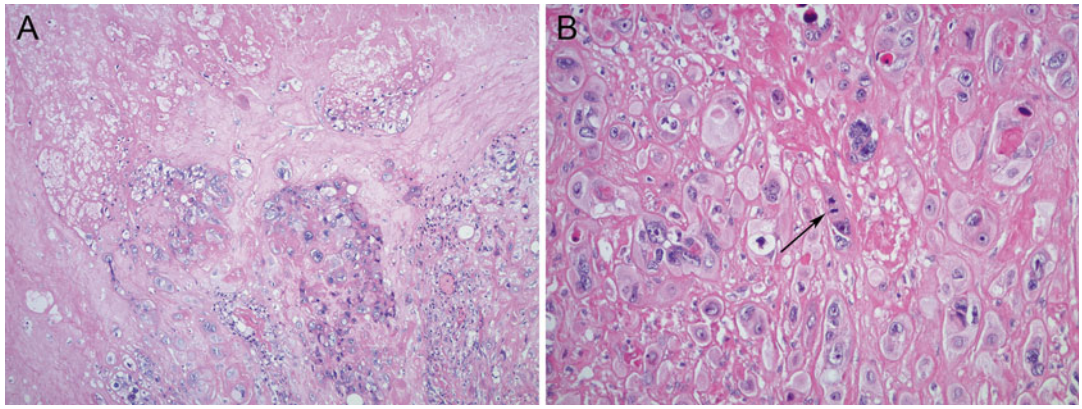

Fig. 5.14 Epithelioid trophoblastic tumor (ETT) shows increased cellularity, necrosis (**a**), and moderate to marked nuclear atypia with mitotic figures (*arrow*, **b**)

uncommon; however, mitotic figures are rare or absent. Immunohistochemical stains for cytokeratins (CAM 5.2, AE1/AE3, 34βE12), EMA, p63, human placental lactogen (hPL), and hCG show variable positivity [89]. The proliferative index by Ki-67 immunostain is less than 8 %, and cyclin E immunostaining is weak or absent [87].

Epithelioid trophoblastic tumor (ETT) on the other hand is a malignant neoplasm composed of chorion laeve-type intermediate trophoblast, usually forming a larger mass lesion (0.5–4 cm), often in the cervix or lower uterine segment [90, 91]. Unlike in PSN, moderate nuclear atypia and increased mitotic activity—typically ranging from 1 to 10/10 high power field (HPF)—are seen (Fig. 5.14). Rare cases with even higher mitotic count—48/10 HPF—have also been reported [91]. Central areas of eosinophilic, hyalinized material, and necrotic debris are also characteristic. Ki-67 immunostain shows >10 % positivity, and cyclin E immunostain is strongly, diffusely positive.

Atypical PSN (APSN) shows intermediate morphologic features between PSN and ETT—larger size, increased cellularity, moderate nuclear atypia, presence of mitotic figures, and Ki-67 proliferation index between 8 and 10 % [92]—and has been proposed as a precursor lesion to ETT [16, 93] (Fig. 5.15). Shih and Kurman reported intimate association between PSN and ETT within the same specimen in 2 of 14 ETT cases [90]. Transformation of PSN into ETT and/or PSTT has also been described in two recent case reports with both lesions present in the same specimen (curettage or hysterectomy) in close proximity, with a microscopic atypical transitional area in between [17, 94]. In a series of 42 PSNs, four cases showed atypical microscopic features, but no recurrences or GTN was seen on follow-up [87]. Most recently, 21 atypical PSNs—the largest series to date—have been reported, 3 of which (14 %) were associated with malignant GTD: one patient had concurrent atypical PSN and PSTT, one patient developed PSTT after 16 months, and one patient was diagnosed with ETT 6 months after the initial diagnosis [95]. Based on these data, it has been suggested that patients with APSN should be evaluated by imaging studies to rule out an underlying mass lesion and would require clinical follow-up due to the approximately 10–15 % risk of malignant GTD. Serum hCG measurement has also been recommended, although it appears to be unreliable for early detection of malignant transformation in this setting [95].

Atypical Exaggerated Placental Site

Exaggerated placental site (EPS) is a reactive proliferation of intermediate trophoblast at the implantation site in a concurrent—or recent—normal, ectopic, or molar pregnancy. The trophoblastic cells are large, pleomorphic with abundant

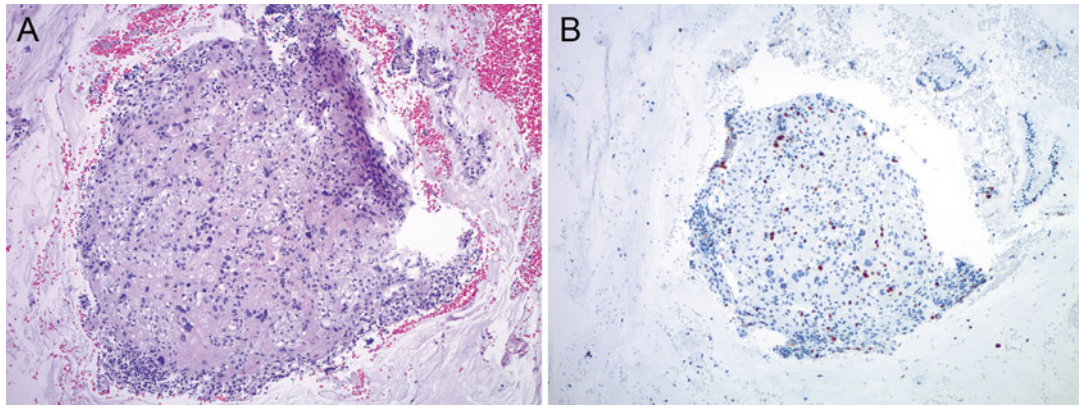

Fig. 5.15 Atypical placental site nodule (APSN). Small fragment (~2 mm) of APSN in a curettage specimen with high cellularity and at least moderate nuclear atypia (**a**). Ki-67 immunostain shows a slightly increased proliferation index (**b**)

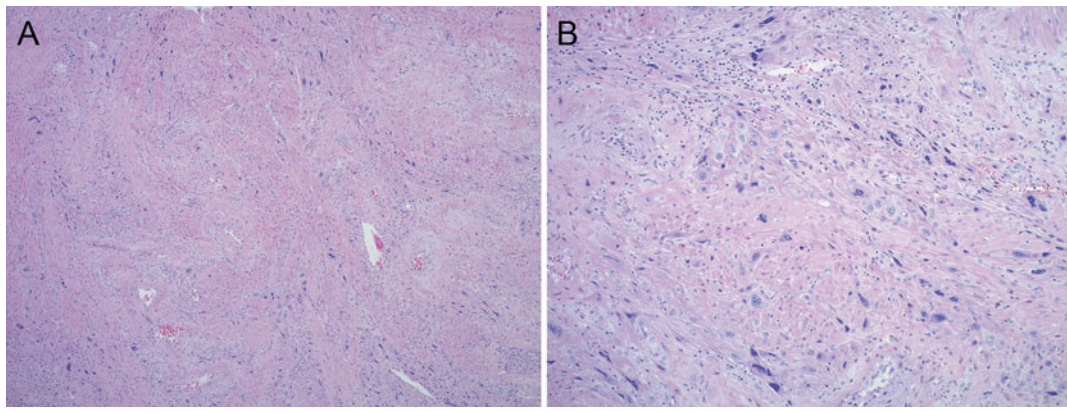

Fig. 5.16 Exaggerated placental site (EPS). Large, pleomorphic intermediate trophoblasts infiltrate the underlying myometrium dissecting between individual smooth muscle fibers (**a**). Multinucleated cells are evenly distributed (**b**)

eosinophilic cytoplasm, and infiltrate the underlying myometrium dissecting between individual smooth muscle fibers. Most lesional cells are mononuclear, but variable number of multinucleated trophoblasts are also present which are evenly distributed throughout the lesion (Fig. 5.16). Mitotic figures are absent, and the Ki-67 proliferation index is low (less than 1 %). Chorionic villi are often seen adjacent to EPS, which likely represents the upper end of the morphological spectrum of normal implantation site.

The neoplastic counterpart of implantation site intermediate trophoblast proliferation—with capacity for locally aggressive behavior and rarely for distant spread—is placental site trophoblastic tumor (PSTT) [96]. PSTT typically forms a mass lesion ranging between 1 and 10 cm, with infiltrative borders, splitting between the myometrial fibers. The tumor cells are large, predominantly mononuclear, with moderate to marked nuclear atypia, and often with prominent nucleoli (Fig. 5.17). Multinucleated cells may also be seen, but unlike in EPS, they are irregularly distributed throughout the tumor. Mitotic figures may be present, ranging from 0 to 22/10 HPF, falling between 2 and 4/10 HPF in most tumors. Necrosis is not uncommon. The tumor cells are positive for cytokeratin AE1/AE3, hPL,

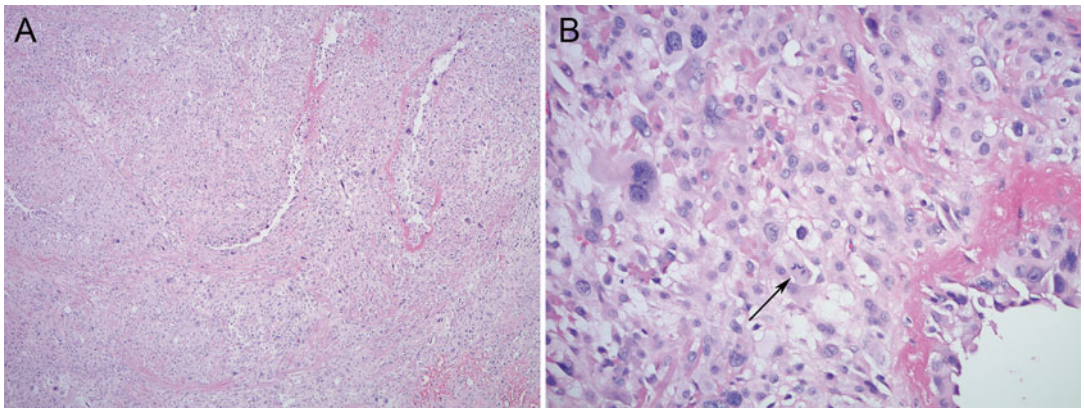

Fig. 5.17 Placental site trophoblastic tumor (PSTT). Infiltrative, hypercellular tumor composed of atypical, predominantly mononuclear intermediate trophoblast. The tumor cells invade and replace vessel walls—recapitulating a feature of normal implantation site (**a**). Mitotic figures may be present (*arrow*, **b**)

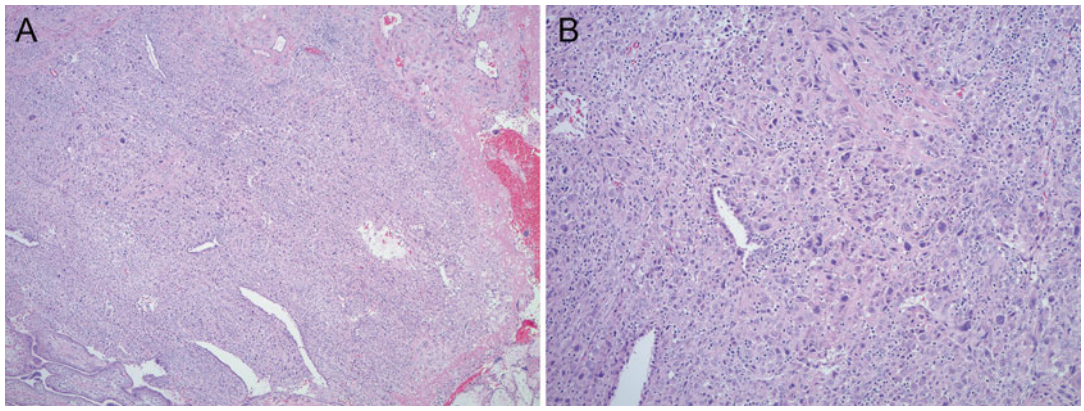

Fig. 5.18 "Atypical" exaggerated placental site. Large sheets of intermediate trophoblast at the implantation site with increased cellularity in an evacuation specimen. Chorionic villi are present—in the *left* and *right lower corners* of the image (**a**). There is at least moderate nuclear atypia, and multinucleated trophoblasts are rare (**b**)

and Mel-CAM immunostains and negative for p63 [92, 97, 98]. The Ki-67 proliferation index usually falls between 10 and 30 % [92].

The genetic link between EPS and PSTT has been questioned by a recent study showing that while development of PSTT requires a paternal X chromosome, all PSTTs had XX genome, and only 45 % of EPS cases demonstrated the same [99]. Nonetheless, rare cases show intermediate morphologic features between EPS and PSTT and may be interpreted as atypical EPS (Fig. 5.18) [84]. However, currently there are no data available on the biology and clinical significance of such lesions.

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Putative Precursors of Uterine Sarcomas

6

Qing Zhang and Jian-Jun Wei

Abbreviations

ALM	Atypical leiomyoma
CGH	Comparative genomic hybridization
EMA	Epithelial membrane antigen
EMT	Epithelial to mesenchymal transition
ESN	Endometrial stromal nodule
ESS	Endometrial stromal sarcoma
FIGO	International Federation of Gynecology and Obstetrics
FISH	Fluorescence in situ hybridization
HDCA8	Histone deacetylase 8
HGESS	High-grade endometrial stromal sarcoma
LGESS	Low-grade endometrial stromal sarcoma
LMS	Leiomyosarcoma
MMMT	Malignant mixed Mullerian tumor
SMA	Smooth muscle actin
STUMP	Smooth muscle tumors of uncertain malignant potential
UAS	Uterine adenosarcoma
UCS	Uterine carcinosarcoma
ULM	Uterine leiomyoma
USMT	Uterine smooth muscle tumors
UUS	Uterine Undifferentiated Sarcoma
WHO	World Health Organization

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Introduction

Uterine sarcomas are rare tumors that account for approximately 1 % of female genital tract malignancies, 3–7 % of uterine cancers [1], and an estimated 7 % of all soft tissue sarcomas [2]. Based on the 2014 World Health Organization (WHO) classification, uterine malignancies with a sarcomatous component were classified into carcinosarcomas (~5 % of all uterine malignancies), leiomyosarcomas (~1–2 %), endometrial stromal sarcomas (~1 %), undifferentiated sarcomas (<1 %), and adenosarcomas (<1 %) (Fig. 6.1) [3]. Previously, uterine sarcomas were staged similar to endometrial carcinomas. In 2009, a new International Federation of Gynecology and Obstetrics (FIGO) classification and staging system was designed specifically for uterine sarcomas to reflect their distinctive biologic behavior from epithelial malignancies (Table 6.1) [4].

For past 20 years, molecular studies provided many new findings of the different genomic and

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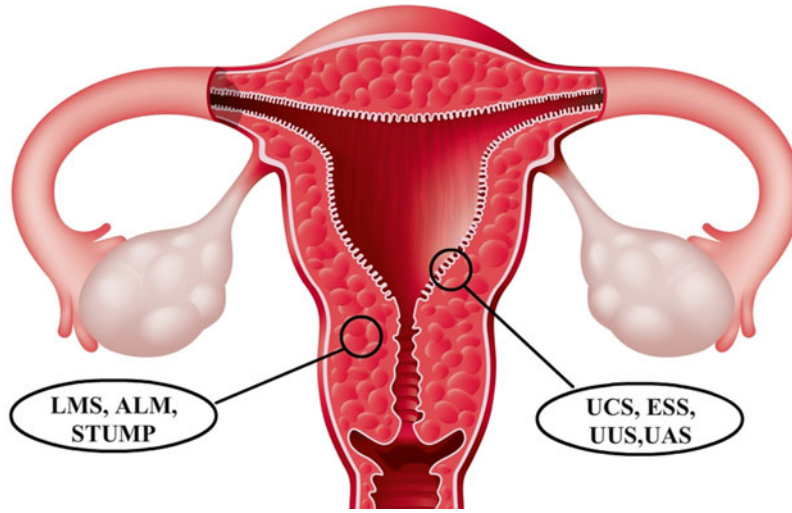

Fig. 6.1 Cartoon diagram highlights the anatomic site for tumor origin of uterine sarcoma

genetic alterations in uterine sarcomas. These findings help us to better understand the complexity and relationship between normal and tumor cell types. However, the primary causes of uterine sarcoma remain largely unknown. In this chapter, we review the current literature on the clinical and pathological presentation and focus on the common and early molecular alterations in each of the uterine sarcoma types. In addition, we will provide some insights into the molecular biology, potential diagnostic biomarkers, and the tumorigenesis of uterine sarcomas, as well as their possible origin.

Uterine Carcinosarcoma

Clinical Features

Uterine carcinosarcoma (UCS), also referred to as "malignant mixed Müllerian tumor (MMMT)," accounts for almost half of all uterine sarcomas and 5 % of malignant uterine tumors [5]. Although the majority of UCSs appear at advanced ages (~50–70 years, with a median age of 65 years), a small number has been reported in patients under 40 years old. The symptoms are similar to endometrial carcinomas, with vaginal bleeding, pelvic mass, and lower abdominal pain. The incidence is increased in patients with an increased exposure to estrogen and pelvic radiation. As reported, patients treated with tamoxifen are eight times more likely to have UCS, and up to 30 % of patients with UCSs have a history of pelvic irradiation [6]. Patients with UCS usually present with extrauterine spread (41 % in stages III and IV) [7]. The serum level of CA125 is also elevated in most cases especially in extrauterine disease and deep myometrial invasion. The 5-year disease-specific survival is 47 % for stage I, 35 % for stage II, 22 % for stage III, and 10 % for stage IV [7].

Pathological Features

Grossly, UCSs are typically large, bulky, polypoid masses, filling the uterine cavity and prolapsing through the cervical canal with deep infiltration into the myometrium (Fig. 6.2). The cut surface often shows areas of hemorrhage, necrosis, and cystic change and frequently extends beyond the uterus.

Microscopically, UCSs are biphasic tumors, which include epithelial and sarcomatous components. The epithelial component is usually high grade (Fig. 6.2). The most common epithelial component is serous (two-thirds of cases) followed by endometrioid with other epithelial components including clear cell, squamous, and undifferentiated carcinoma. Most cases (72 %) consist of a single type of epithelial component, and the remaining (28 %) show 2–3 mixed epithelia. For the sarcomatous component, 80 % of

Table 6.1 FIGO staging for uterine sarcomas (2009) [3]

Stage	Surgical-pathologic findings
Leiomyosarcomas and endometrial stromal sarcomas	
I	Tumor limited to the uterus
IA	Tumor Less than or equal to 5 cm
IB	Tumor More than 5 cm
II	Tumor extends beyond the uterus, within the pelvis
IIA	Tumor involves adnexa
IIB	Tumor involves other pelvic tissues
III	Tumor invades abdominal tissues (not just protruding into the abdomen)
IIIA	One site
IIIB	More than one site
IIIC	Metastasis to pelvic and/or para-aortic lymph nodes
IV	
IVA	Tumor invades the bladder and/or rectum
IVB	Distant metastasis (excluding adnexa, pelvic, and abdominal tissues)
Carcinosarcomas	
I	Tumor confined to the corpus uteri
IA	Tumor limited to endometrium or invades less than half myometrial invasion
IB	Tumor invades one-half or more of the myometrium
II	Tumor invades stromal connective tissue of the cervix but does not extend beyond the uterus
IIIA	Tumor involves serosa and/or adnexa (direct extension or metastasis)
IIIB	Vaginal involvement (direct extension or metastasis) or parametrial involvement
IIIC	Metastases to pelvic and/or para-aortic lymph nodes
IV	The bladder and/or bowel mucosa and/or distant metastases
IVA	Tumor invades bladder mucosa and/or bowel (bullous edema is not sufficient to classify a tumor as T4)
IVB	Distant metastasis (includes metastasis to inguinal lymph nodes, intraperitoneal disease, or the lung, liver, or bone. It excludes metastasis to para-aortic lymph nodes, vagina, pelvic serosa, or adnexa)
Adenosarcomas	
I	Tumor limited to the uterus
IA	Tumor limited to the endometrium/ endocervix
IB	Tumor invades less than half the myometrium
IC	Tumor invades one-half or more of the myometrium
II	Tumor extends beyond the uterus, within the pelvis
IIA	Tumor involves adnexa
IIB	Tumor involves other pelvic tissues
III	Tumor involves abdominal tissues
IIIA	One site
IIIB	More than one site
IIIC	Metastasis to regional lymph nodes
IV	
IVA	Tumor invades bladder or rectal mucosa
IVB	Distant metastasis

the cases show high-grade sarcoma. The most common histological type is undifferentiated with areas of leiomyosarcoma, rhabdomyosarcoma, chondrosarcoma, osteosarcoma, and liposarcoma. The majority (67 %) reveal only one type of sarcoma, while the rest show multiple histologic differentiations [8, 9].

Immunohistochemistry and Genetic Aberrations

Due to the biphasic nature, the immunophenotypes are slightly different. For example, the epithelial component shows diffuse immunoreactivity for epithelial membrane antigen (EMA) and cytokeratin, whereas the sarcomatous component can be positive for cytokeratin, but is usually patchy or focal. In contrast, sarcoma is positive for vimentin and other mesenchymal markers depending on the differentiation, such as desmin and caldesmon (smooth muscle differentiation), myogenin, and MyoD1 (rhabdoid differentiation). Diffuse immunoreactivity for P53 was firstly reported in 30 % (5/17) of UCSs, and there was no disparity between the epithelial and sarcomatous components [10]. Recent studies have confirmed that P53 is commonly overexpressed in UCSs with a positive rate ranging from 30 % to 80 %. Overall, 70–80 % of cases have a concordant immunostaining pattern for P53 in carcinoma and sarcoma. The MDM2 oncogene encodes a protein that binds to and inactivates the p53 gene product. MDM2 overexpression is found in 17 (4/23)–26 % (11/43)

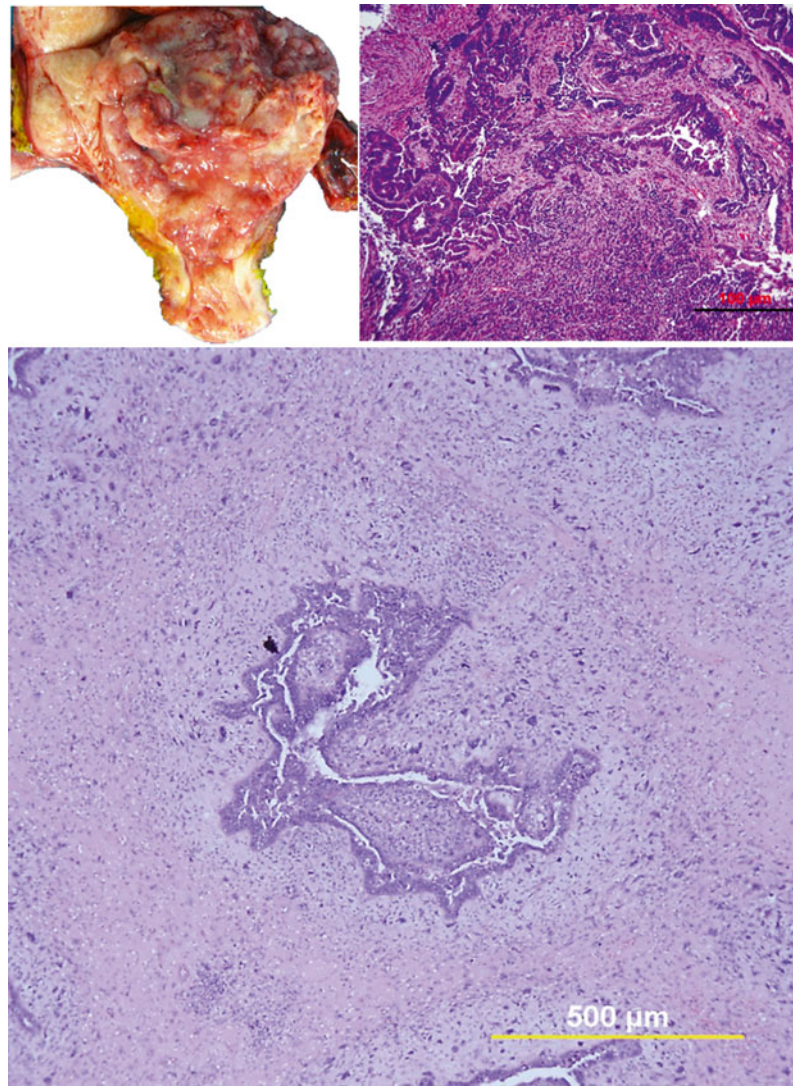

Fig. 6.2 Uterine carcinosarcoma. (**a**) Photomacrograph illustrates typical gross appearance of polypoid growth pattern of carcinosarcoma. (**b**) and (**c**) photomicrographs show biphasic tumor sections containing malignant epithelial component (high-grade serous carcinoma) and mesenchymal component (undifferentiated sarcoma)

of UCSs [11, 12]. P16 overexpression is very common in UCSs, and it was reported to be as high as 96.7 % and 86.7 % of carcinoma and sarcoma, respectively [12–15]. Other gene mutations and dysregulation in UCS include but are not limited to TGF-β, Rb, HER-2, VEGF, ERβ, CATs, β-catenin, COX-2, and PTEN [16–22]. Notably, ERα, PR, IGF1R, and CD10 are often downregulated or completely lost in sarcoma [23]. A study examined the expression of 39 epithelial to mesenchymal transition (EMT)-related genes. Acquired markers of EMT were found to be upregulated, and attenuated markers of EMT were downregulated in UCSs. High expression of phospho-SMAD2/3 (p-SMAD2/3) indicated that TGF-β seems to play a major role in UCS. In the same study, chromosomal gains at 19q13, which includes the TGFB1 locus, were also identified by chromosomal Gene Set Enrichment Analysis (GSEA)and comparative genomic hybridization (CGH) microarrays in UCS [24]. In addition, upregulation of the Akt/β-catenin and Rb pathway may be essential for the establishment and maintenance of phenotypic characteristics of UCSs through the regulation of E-cadherin mediated by the transactivation of the Slug gene [25].

Global genomic analysis by comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) in a series of 30 carcinosarcomas revealed that chromosomal gains (85 %) are more common than losses (30 %) in UCSs. Chromosomal amplification is frequently observed on chromosome 8q (42 %) and 20q (70 %). In these regions, c-myc (8q24.12) and ZNF217 (20q13.2) amplification can be identified in 78 % and 87 % of UCSs. Amplification of ZNF217 was mostly seen in both tumor components, whereas amplification of c-myc was observed less often in the sarcomatous than in the carcinomatous component [26]. These two genes correlate to distant metastases and poor prognosis [26, 27].

Theories of Tumor Origin

There are several hypotheses for the cell origin of UCS, including (1) monoclonal stem cell combination (origin from a common cell origin), (2) tumor collision (origin from two distinct malignant cell populations), and (3) composition (origin from metaplastic transformation from one neoplastic cell type) (Fig. 6.3). An early study illustrated a conversion of carcinomatous cells to sarcomatous cells using four clonal cell lines from ovarian carcinosarcoma [28]. An in vitro experiment on the EMTOKA cell line (a human UCS cell line) found that the same intermediate filament (IF), HER-2, P53 expression, and the ultrastructure characteristics can be found in EMTOKA and its clones [29]. These two studies support the monoclonal theory that the epithelial component and sarcomatous component may be derived from a common stem cell. A recent study examined the panel of immunoreactivity for MLH1, MSH2, MSH6, PTEN, P53, β-catenin, and cyclin D1 in 40 UCSs. They found that expression patterns of P53, MSH2, and MSH6 corresponded between the epithelial and mesenchymal components [30]. Furthermore, the two components also harbored similar chromosomal aberrations [26]. The presence of similar immunohistochemistry and genetic aberration patterns in both histological components in most UCSs are evident in favor of the monoclonal theory for either stem cell origin or composition theory due to the metaplastic transformation, whereas presence of different types of epithelial or sarcomatous components within one tumor can be better explained by the stem cell theory. It seems that some observations in favor of the biclonal origin theory will require the examination of additional cases and studies [31–33].

Occasionally, collision tumors can be seen in clinical cases. For example, Jin et al. found that one of the 15 UCSs was probable collision tumors [33]. PAX8 is a useful immunohistochemical marker for these epithelial neoplasms of gynecologic origin. A study shows that the epithelial component strongly expressed PAX8 in 97 % of (36/37) the tumors, but only 27 % (10/37) mesen-

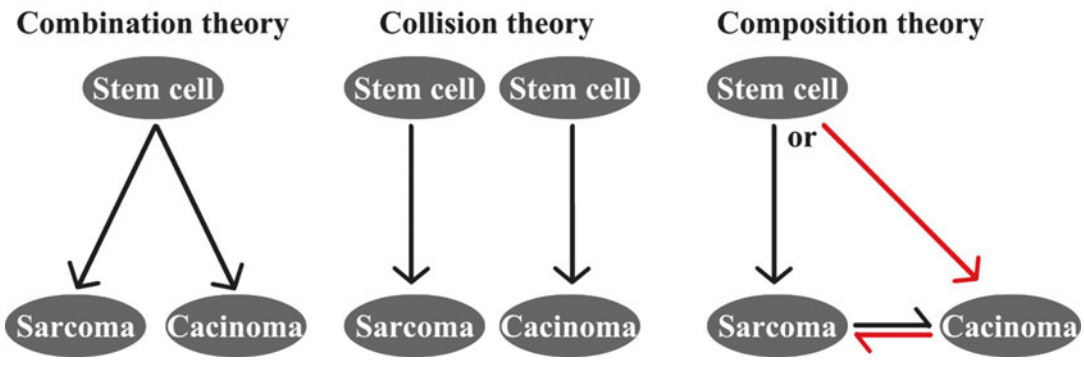

Fig. 6.3 Proposed mechanisms for tumorigenesis of uterine carcinosarcomas

chymal component showed PAX8 expression in 27 cases (10/37) with variable expression [34].

Unfortunately, the precursor lesion for UCS remains unknown, and most cases are diagnosed in their invasive form.

Leiomyosarcoma

Clinical Features

Leiomyosarcoma (LMS) is the second most common type of uterine sarcoma, accounting for ~1–2 % of uterine malignancies [35]. According to the Surveillance, Epidemiology, and End Results (SEER) database, the incidence of uterine sarcomas from 1979 to 2001 was 0.36 per 100,000 women/year and may be increasing among women in the United States [2]. Most tumors were stage I (68 %), whereas stages II, III, and IV tumors represented 3 %, 7 %, and 33 % of cases respectively [36]. This disease is prevalent in postmenopausal women with the mean age of 60 years old. Signs and symptoms include abnormal vaginal bleeding (56 %), palpable pelvic mass (54 %), and pelvic pain (22 %). Preoperative distinction between benign and malignant smooth muscle tumors remains a challenge in the diagnosis. In hysterectomy for benign uterine smooth muscle tumors, 1/800 cases turns out to be leiomyosarcoma. A small proportion of tumors are identified as malignancies either through manifestations of their rupture (hemoperitoneum) and/or extrauterine extension or metastases. Interestingly, the development and progression of LMS seem not to be associated with hormones. Rather, histories of hereditary retinoblastoma or prior pelvic radiation are considered as the main risk factors.

Pathological Features

Grossly, LMSs are mainly a solitary uterine mass. They are usually large with a mean diameter of 10 cm (only 25 % are <5 cm). The cut surface is typically soft, yellow or tan, fleshy, necrotic, and hemorrhagic with irregular and infiltrating borders (Fig. 6.4).

Most LMSs are spindle cell and hypercellular. Tumor cells show moderate to severe nuclear atypia and a high mitotic rate [generally exceeding 10 mitoses per 10 high-power fields (HPFs)]. Tumor necrosis (coagulated necrosis) can be seen in most cases. Epithelioid and myxoid LMSs, however, are rare variants with mild to moderate nuclear atypia, and the mitotic rate is often <5/10 HPF [37]. In clinical practice, the diagnosis of LMS can be sometime problematic due to a wide

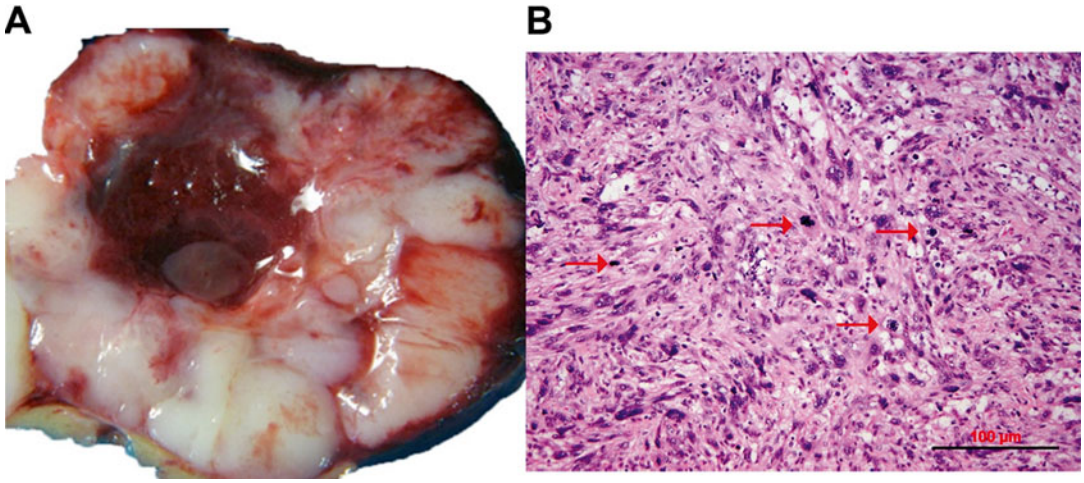

Fig. 6.4 Uterine leiomyosarcoma. (**a**) Gross appearance of leiomyosarcoma, characterized by tan and fleshy cut surface with hemorrhage and true tumor necrosis. (**b**) Histology section shows hypercellular spindle cell proliferation, prominent cytologic atypia, and brisk of mitoses

Table 6.2 Benign andborderline uterine smooth muscle tumors [38]

Leiomyoma variants with mimic malignancy
Atypical leiomyoma (leiomyoma with bizarre nuclei)
Smooth muscle tumors of uncertain malignant potential (STUMP)
Cellular leiomyoma
Mitotically active leiomyoma
Myxoid leiomyoma
Epithelioid leiomyoma
Leiomyoma with massive lymphoid infiltration
Smooth muscle tumors with unusual growth patterns
Disseminated peritoneal leiomyomatosis
Benign metastasizing leiomyoma
Intravascular leiomyomatosis
Lymphangioleiomyomatosis

spectrum of histologic features shared with other uterine smooth muscle tumors (USMTs) as listed in Table 6.2. In a review of 356 cases with an original diagnosis of LMSs, only 72.7 % cases (259/356) could be confirmed as malignant tumors, whereas 27.3 % (97/356) were reclassified as benign or leiomyoma variants [35].

Immunohistochemistry and Genetic Aberrations

LMSs usually express smooth muscle markers such as smooth muscle actin (SMA), desmin, h-caldesmon, and histone deacetylase 8 (HDCA8). Only ~20–30 % cases express ER and PR [39–42]. A significantly higher level of cell proliferation (illustrated by a high Ki-67 index) is present in the majority of LMSs [43–47]. Recently, P16 has been discovered to be a new biomarker for LMS identified through global gene profile analyses [48]. Emerging data showed that ~71–100 % of LMSs are strongly and diffusely immunoreactive for P16, and therefore, it may be a useful adjunct immunomarker for distinguishing between benign and malignant uterine smooth muscle tumors [40, 43, 45, 49–56]. Additionally, PTEN, FASCIN, pAKT, pS6RP, P4EBP1, and β-catenin positive can be seen in most LMS [39, 57–60]. Mutation and overexpression of P53 have also been described in a significant number of LMSs (~20–43 %) but not in ULMs [46, 61–66] (Fig. 6.5). None of these markers, however, are absolutely discriminatory in the differential diagnosis between LMS and its potential mimics.

Based on gene profile analysis, many cell cycle genes, oncogenes, and transcription factors are dysregulated and may be related to LMS tumorigenesis (Table 6.3) [48, 67–71]. In addition, LMSs present complex numerical and structural chromosomal aberrations, including frequent losses of 10q (where PTEN harbored), 12q, and 13q. A gain of 17p and losses of 2p and 16q are occasionally observed [72–77].

Theories of Origin

The pathogenesis of uterine LMS is poorly understood. It is generally believed that LMSs arise de novo [78]. Lately, this theory is being challenged as recent studies show that some uterine LMSs may be related to preexisting ULMs [79–81]. For example, one study shows that there are benign-appearing tumor components, defined as "leiomyoma-like" (Fig. 6.6), within LMS. These leiomyoma-like areas have fewer distinctive genomic alterations compared to their truly malignant counterpart. Furthermore, a high-density and chip-based analysis by CGH illustrates some different but partially shared genetic alterations between "leiomyoma-like" and fully malignant areas within one tumor mass. The findings suggest that some uterine LMS may arise from a preexisting benign uterine smooth muscle tumor [82].

A mouse model utilizing a deletion of PTEN alleles results in widespread smooth muscle cell hyperplasia and a high rate of abdominal LMSs with a very rapid onset and elevated incidence (~80 %) of LMS. Apparently, the AKT-mTOR pathway plays a critical role for smooth muscle cell transformation and LMS genesis [83].

Atypical leiomyoma (ALM), as defined by Stanford researchers [84], was considered to be an intermediate-grade uterine smooth muscle tumor (USMT). As of now, ALM is a clinically benign disease, characterized by an absence in its

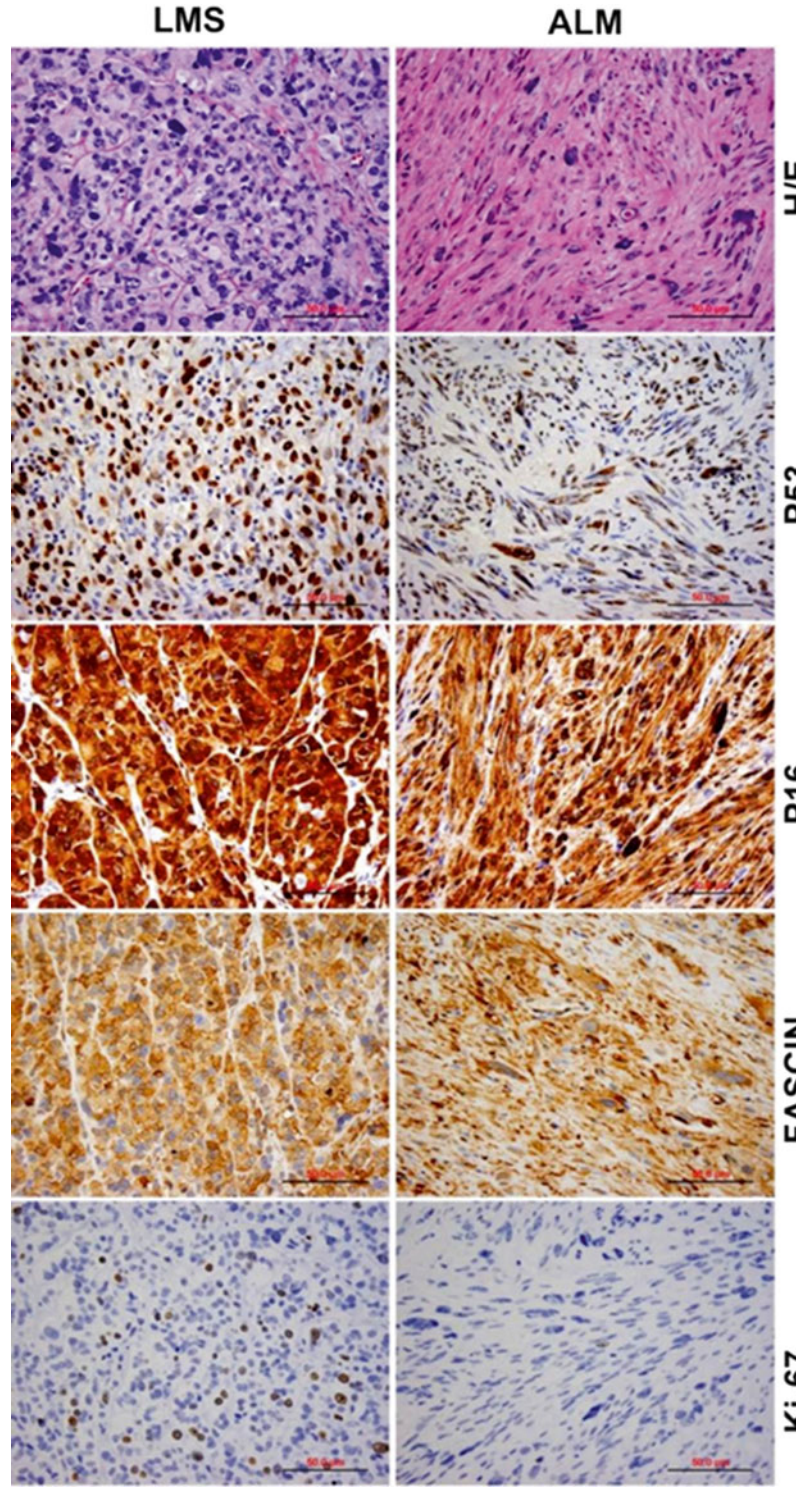

Fig. 6.5 Side-by-side comparison of leiomyosarcoma (LMS, *left*) and atypical leiomyoma (ALM, *right*) by histology and immunohistochemistry

Table 6.3 Up- and downregulated genes in uterine LMS [48, 67–71]

Function	Genes
Upregulated	
Cell cycle regulation	CDKN2A, CDK4, CDKN2A, CCND1, CCND3, CKS2, FOXM1, PTTG1, PRC-1, UBE2C, COPS3, MDM2
Cell homeostasis	GRIA2, NPTX2, CRABP2, POPDC2, ST5, TOP2A
Cell structure	ACTC1, DIAPH3, DCX, COL5A2, COPS3, THBS2, PLP1
Signal transduction	MAP3K8, PIK3R1, IL17B, TSPAN31, SPP1
Growth factors	IGF1, IGFBP5, TGFB3
Transcription factors	E2F1, RB1, GLI1
Proteinases	MMP9, CAPN6
The actin cytoskeleton	CALD1, SLMAP, DMD, ACTG2, CASQ2, CFL2, MYLK, LPP
Organ development	ADD3, ANTXR, FLJ39632, TCF4, FBN1, SNAI2, SPRY1, XPOT, FSTL1
Downregulated	
Metabolism	ALDH1A1, ALDH1B1
Cell cycle and structure	CDKN1A, DPT, KRT19, CNN1
Cell homeostasis	TNXB
Oncogenes	Mutations in KIT, MED12, IRF1
Signal transduction	MAP3K5, RNASE4

Table 6.4 Pathological criteria for atypical leiomyoma and smooth muscle tumors of uncertain malignant potential (STUMP) [38, 84, 86]

	Standard
ALM	Cytological atypia: in 4×, focal, multifocal, or diffuse moderate to severe cytological atypia
	Mitosis figure: <5 MF/10 HPF
	Necrosis: Absent
STUMP	Tumor cell necrosis in a typical leiomyoma
	Necrosis of uncertain type with ≥10 MF/10 HPFs or marked diffuse atypia
	Marked diffuse or focal atypia with borderline mitotic counts
	Necrosis difficult to classify Problematic finding such as epithelioid or myxoid change is present

contribution to fatalities in patients [85]. Due to its unusual presentation of histologic features, these tumors have been known as: atypical leiomyoma, symplastic leiomyoma, and leiomyoma with bizarre nuclei (Fig. 6.7). The tumor origin and histogenesis of ALM remain largely unknown. Recently, several studies attempted to reexamine and evaluate the nature of ALM using the tumors' clinical, histology, and immunohistochemistry aspects [56, 86–88]. Despite the benign clinical course of ALM, the overlap of several histological features and immunoprofiles were found between ALM and LMS. The histogenesis and its relationship to LMS draw great attention. To address this issue, comparison of the molecular and gene expression patterns in these two types of tumors was investigated recently. It was found that ALM shared or was closely related to LMS in many gene mutations including *P53*, *MED12*, and *PTEN* [46, 60–66, 72–77, 86, 89–101] (Fig. 6.8). The findings of shared genetic and molecular alterations as well as the histologic features between ALM and LMS suggest a close relation for the histogenesis of these two different diseases (Fig. 6.9) [86]. In fact, the early changes of ALM and tumor progression of LMS may be far more complex than we expected, and the findings of the shared genetic changes between ALM and LMS may either truly reflect the stepwise tumor progression or require some as of yet unidentified molecular changes which occur only in LMS but not ALM. Additional studies are needed to further characterize the nature of ALM.

Uterine smooth muscle tumors of uncertain malignant potential (STUMP) show unequivocal histological features (i.e., coagulative tumor cell necrosis, nuclear atypia, and mitotic activity), but do not meet all diagnostic criteria for LMS, ULM, or its variants [3]. STUMP is a heterogeneous group tumors which cannot be classified as definitively benign or malignant; the nature of histopathology may be benign, intermediate, or malignant (Table 6.4) [38, 84]. Therefore, STUMP is an entity for diagnosis of exclusion. The mean age at diagnosis was 45 years, which is younger than LMS and similar to leiomyoma variants [5, 86]. The incidence of STUMP is also

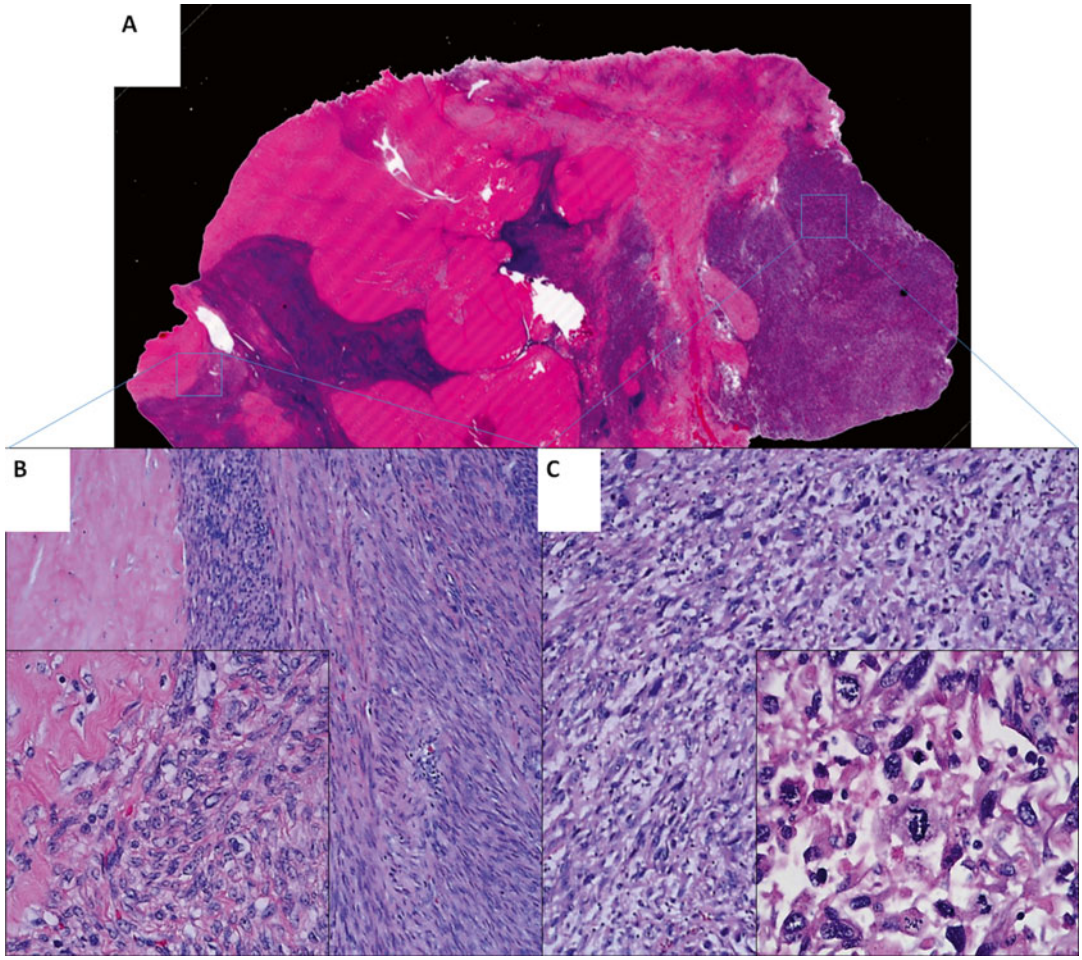

Fig. 6.6 A large leiomyosarcoma with leiomyoma-like area. A full mounted tissue section (**a**) with benign leiomyoma-like area, showing spindle cell tumor with minimal cytologic atypia, low mitotic count, and hyalinized change (**b**) and area of fully malignant area, characterized by hypercellular spindle cell proliferation with high-grade cytologic atypia and frequent mitoses/atypical mitosis (**c**)

unknown. The recurrence rate in patients with uterine STUMP was 6.7 % in comparison to LMS at 66.7 % recurrence [102–104]. Consequently, STUMP is considered also as an intermediate or early malignant tumor type [86, 102–104]. The relationship between STUMP and LMS has not yet been fully established.

Differential diagnosis of STUMP, ALM, and LMS can be challenging, and several markers such as P53, P16, FASCIN, Ki-67, ER, and PR can be potentially used for diagnosis. For example, *P53* mutations are slightly lower in STUMP [23 % (7/30)] and ALM [18 % (12/112)] than in LMS (20–43 %) [46, 61–66] (Fig. 6.8). P16 is an important marker for LMS where diffuse immunoreactivity for P16 is present in 79 % (260/329) of LMS, 56 % (67/119) of STUMP, and 32 % (13/41) of ALM, respectively (Fig. 6.8) [40, 43, 45, 49–56]. FASCIN expression is also similar between STUMP [50 % (2/4)] and LMS [79 % (31/39)] [57, 58].

Recent identification of *MED12* mutations in ULMs can be a useful marker in the differential diagnosis. Nearly 70 % of ULMs harbor *MED12* mutations, but the mutation rate is very low in STUMP, ALM, and ULM (less than 15 %, Fig. 6.8) [60, 86, 89–101].

Based on the pattern of the molecular alterations, some STUMP may represent early or precursor lesions of LMS (Fig. 6.9).

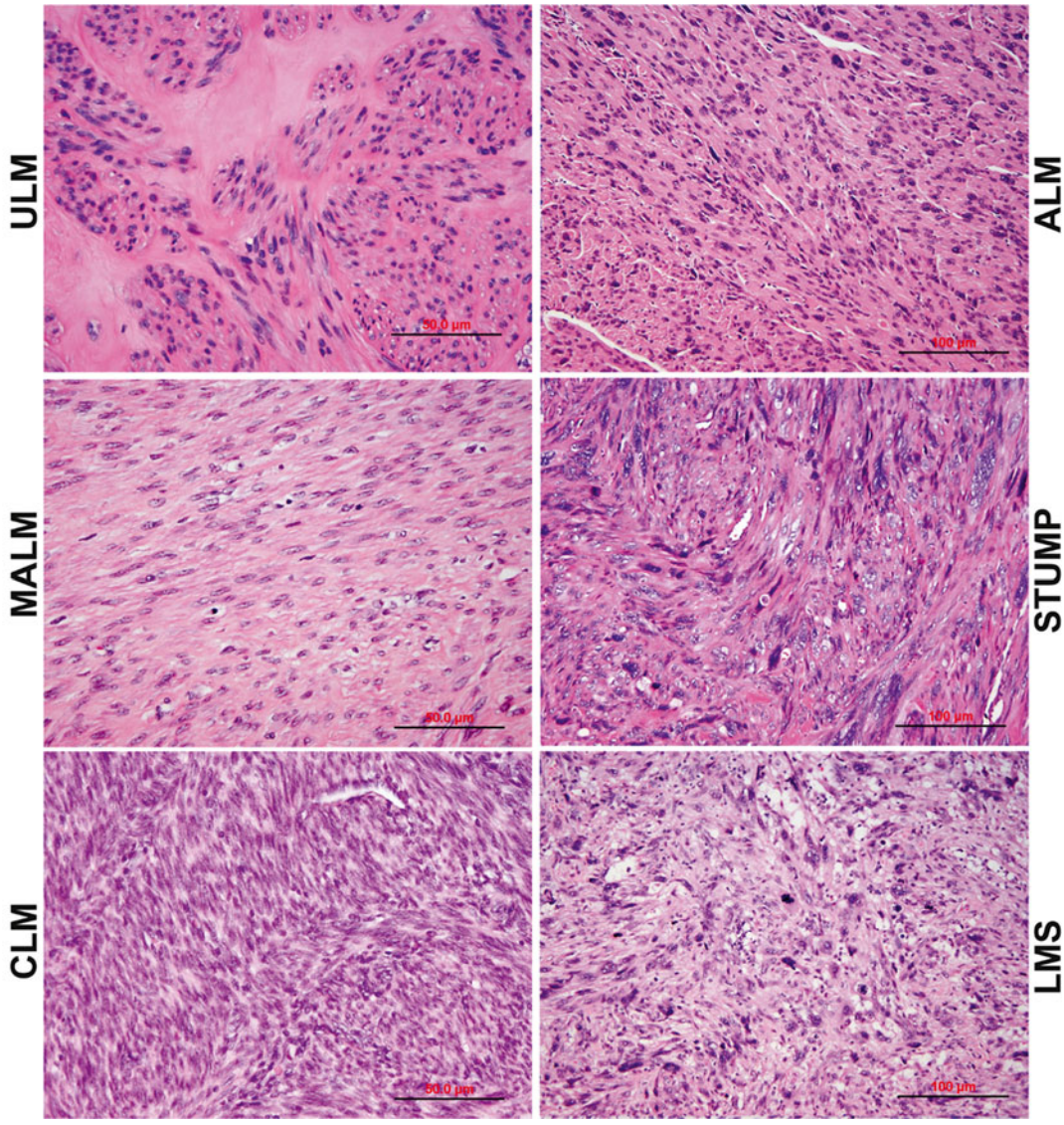

Fig. 6.7 Histologic variants of uterine smooth muscle tumors (USMTs). Photomicrographs illustrate the histologic examples of six USMT variants, including usual type leiomyoma (ULM), cellular leiomyomas (CLM), mitotically active leiomyomas (MALM), atypical leiomyomas (ALMs), uterine smooth muscle tumors of uncertain malignant potential (STUMP), and leiomyosarcomas (LMSs)

Endometrial Stromal Sarcomas

Clinical Features

Endometrial stromal sarcoma (ESS) accounts for approximately ~10–15 % of all uterine sarcomas and occurs over a wide age range with a mean age of 50 years old [35, 105]. Patients commonly present with abnormal uterine bleeding, pelvic pain, pelvic mass, and dysmenorrhea but as many as 25 % of them are asymptomatic [106]. Occasionally, metastasis may be the initial presentation. The cause of the disease is unknown but may be related to obesity, diabetes, ovarian polycystic disease, estrogen, tamoxifen therapy, and pelvic radiation [107, 108]. Most cases (60 %) present with FIGO stage I disease, and the remainder are in stages II–IV [105].

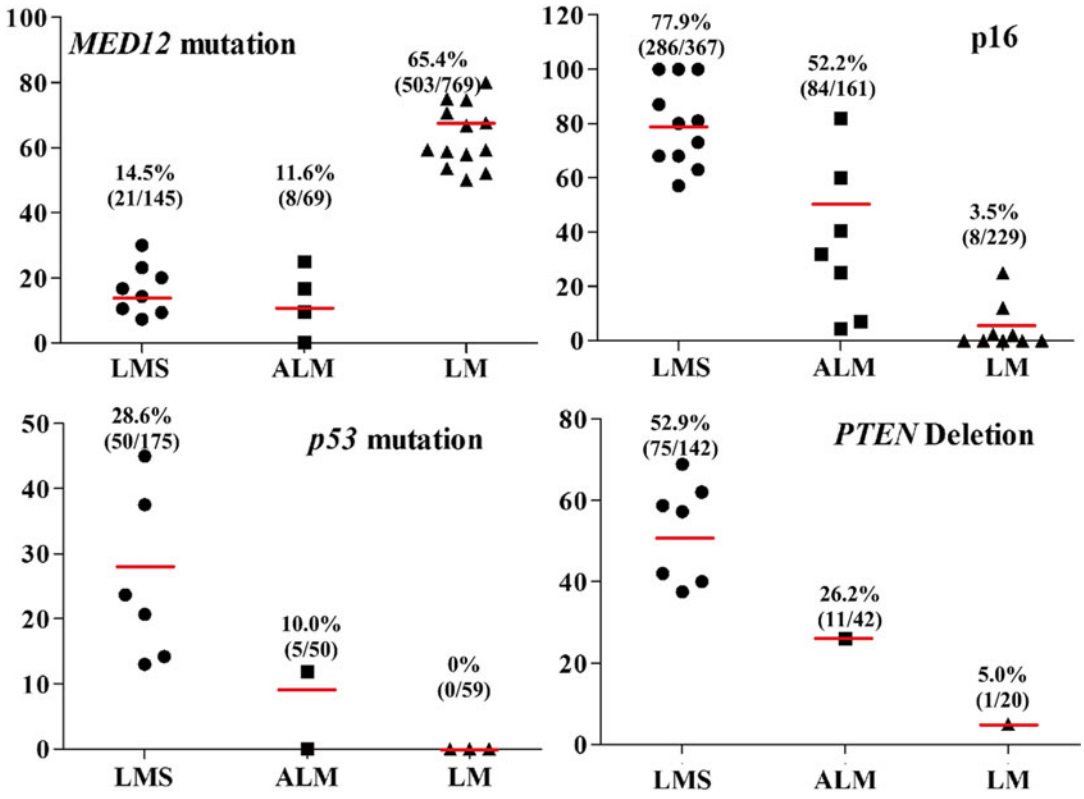

Fig. 6.8 Dot plot analyses summarized available published data for the gene mutations and expression pattern among leiomyoma (LM, *triangles*), atypical leiomyoma (ALM, *squares*), and leiomyosarcoma (LMS, *round*) [40, 43, 45, 46, 49–56, 60–66, 72–77, 86, 89–101]. The average rates of mutations for each category are listed above

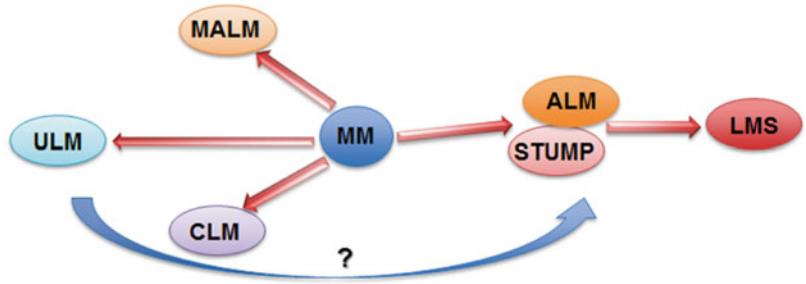

Fig. 6.9 Proposed relationship of tumorigenesis in different USMT subtypes [86]

Pathological Features

According to 2014 WHO classification, endometrial stromal tumors (ESTs) can be divided into three subtypes: endometrial stromal nodule (ESN), low-grade (LGESS)/high-grade (HGESS) endometrial stromal sarcoma, and undifferentiated uterine sarcoma (UUS) on the basis of cytologic atypia, differentiation, mitotic count, and immunoprofile (Fig. 6.10) [3]. ESN is a benign endometrial stromal tumor that has a well-circumscribed margin. For ESS, the tumor size ranges from 5 to 10 cm, and the cut surface is usually yellow to tan with areas of hemorrhage and possible necrosis [105, 106]. Tumors grow polypoid or intramural masses. The common growth pattern is wormlike plugs of tumor that fill and distend to the myometrial veins (intravascular) with frequent extension

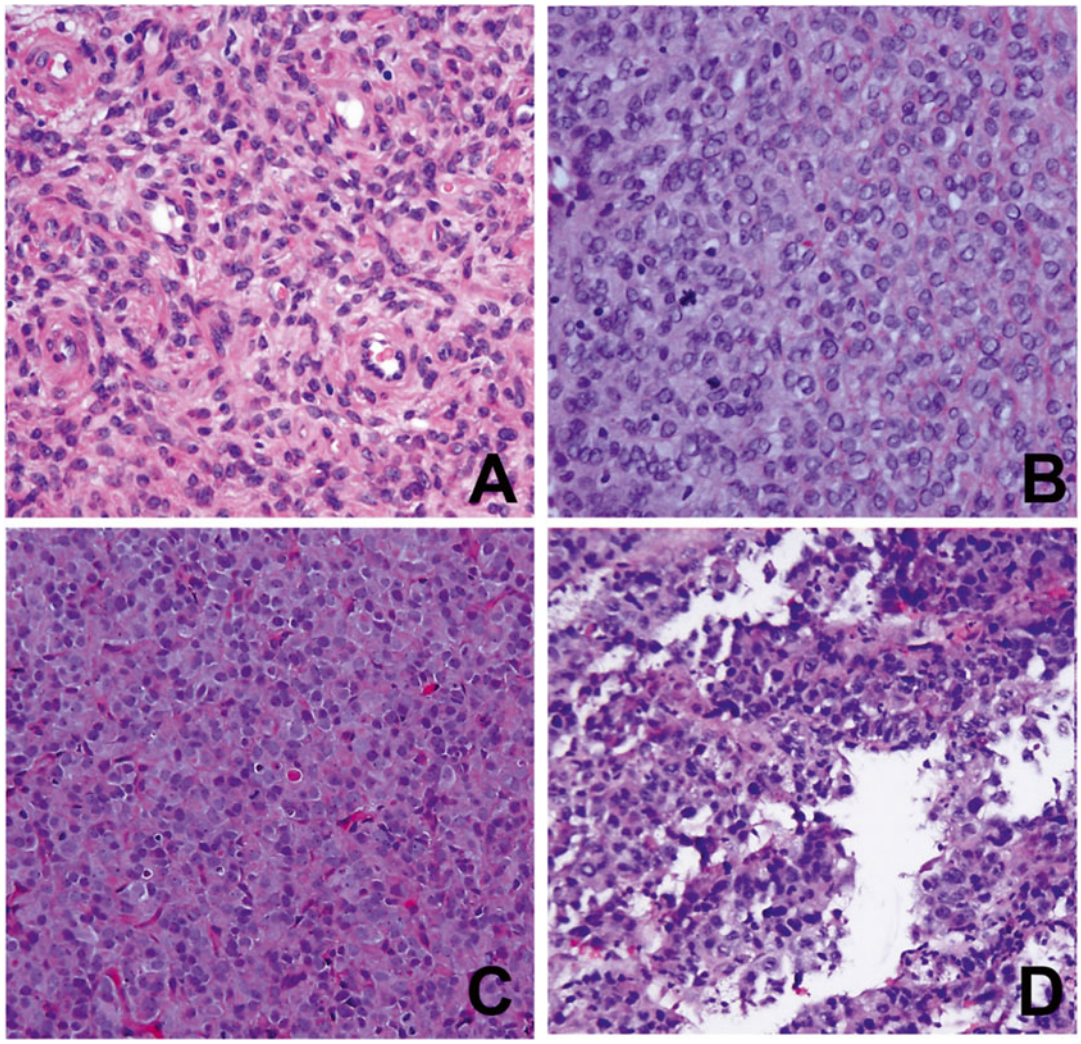

Fig. 6.10 Histology and cytology ofuterine stromal sarcoma. (**a**) Low-grade endometrial stromal sarcoma (LGESS). (**b**) High-grade endometrial stromal sarcoma (HGESS). (**c**) Uterine undifferentiated sarcoma of uniform type (USS). (**d**) Uterine undifferentiated sarcoma of pleomorphic type

to parametrical veins and lymphatics. LGESS shows minimal to no cytological atypia and low mitotic activity (usually <5/10 HPF). The mitotic activity of HGESS is typically >10/10 HPF and is typically very striking [109].

Immunohistochemistry and Genetic Aberrations

CD10, a membrane glycoprotein that functions as a cell surface enzyme, is a feature marker for ESS and is diffusely positive in almost all ESS. ER (only α-isoform), PR, vimentin, α-SMA, and keratin are also immunoreactive in ESS [110–112]. Nuclear β-catenin and WT-1 can be positive in ESS [113–115], whereas desmin, h-caldesmin, and HDCA8 are generally negative [112, 116]. C-Kit can be positive, but C-Kit mutations are not observed [117]. In those ESSs with smooth muscle differentiation or sex cord-like differentiation, the heterologous elements are usually reactive for smooth muscle markers, CD10, inhibin, calretinin, melan-A, CD99, and WT-1 [112, 118]. Interferon-induced transmembrane protein 1 (IFITM1) recently has been found

as a sensitive and specific marker for endometrial stromal differentiation across the spectrum from proliferative endometrium to metastatic stromal sarcoma. IFITM1 has been reported to be highly sensitive and specific in the distinction between endometrial stromal tumors and uterine smooth muscle tumors (72.7 % and 86.7 %, respectively) [119].

ESS is a genetically heterogeneous group of neoplasms harboring distinct cytogenetic abnormalities. An unusual derivative chromosome generated by the insertion of chromosome 19 into chromosome 10 near centromere [ins(10;19)(p11;p13q13)] was first reported in an ESS case in 1988 [120]. The characteristic cytogenetic abnormalities in ESSs are nonrandom chromosome translocations. Nearly 80 % of LGESSs harbor a specific chromosomal translocation t(7;17)(p21;q15), resulting in fusion genes of JAZF1 and JJAZ1(SUZ12) [121–123]. Other rearrangements include t(6;7)(p21;p15), t(6;10;10)(p21;q22;p11), t(1;6)(p34;p21), and t(X;17)(p11.2;q21.33) which lead to PHF1-JAZF1, EPC1-PHF1, MEAF6-PHF1, and MBTD1-CXorf67 rearrangements [123–126]. Nearly 60 % of HGESSs harbor specific translocation at t(10;17)(q22;p13) which gives rise to the YWHAE-NUTM2A/NUTM2B fusion protein [127]. A recent study showed that diffusely (≥70 %) moderate to strong nuclear immunoreactivity for cyclin D1, which is a target gene of β-catenin, is seen in HGESSs with YWHAE-NUTM2A/NUTM2B fusion but is negative in LGESSs with JAZF1-JJAZ1 fusion [128]. Moderate to strong membranous/cytoplasmic C-Kit staining can be seen in all YWHAE-NUTM2A/NUTM2B positive tumors (12/12); however, no hotspot mutations of C-Kit are observed [129].

Theories of Origin and Precursor Lesion

It is believed that ESS originated from endometrial stromal cells. This is consistent with their similar immunoprofile to endometrial stromal cells. The abnormal expression of nuclear β-catenin indicated that the Wnt signaling pathway may play an important role in the pathogenesis of ESSs. ESN is a clinical benign endometrial stromal tumor, but the immunohistochemistry profile for endometrial stromal nodule is almost identical to LGESS. ESNs are typically immunoreactive for vimentin, ER, PR, and CD10 [110–112]. Kurihara S et al. reported that 37.5 % (3/8) of ESNs showed nuclear β-catenin expression and 25 % (2/8) were positive for cyclin D1. Meanwhile, promoter hypermethylation and subsequent suppression of secreted frizzled-related proteins (SRFPs) could also be found in 37.5 % (3/8) of ESNs compared with 58.6 % (17/29) of ESSs [130]. JAZF1-JJAZ1 fusion was found in 60–100 % of ESN [131, 132], but the rearrangements of YWHAE-NUTM2A/NUTM2B, PHF1-JAZF1, EPC1-PHF1, and MEAF6-PHF1 were not found in ESNs. The relationship between ESN and LGESS has not been established, and it deserves further investigation.

Uterine Undifferentiated Sarcomas

Clinical Features

Uterine undifferentiated sarcomas are poorly differentiated sarcomas arising in the endometrium or myometrium, lacking any resemblance to proliferative-phase endometrial stroma, have high-grade cytological features and no specific type of differentiation (Fig. 6.10) [3]. Because of the low incidence and few published series, the knowledge of UUSs is limited. They may account for approximately 3–6 % uterine sarcomas [133, 134]. The median age of diagnosis ranges from 42 to 75 years old. The most common symptoms are postmenopausal vaginal bleeding, abdominal pain, and other signs secondary to extrauterine spread [135]. Approximately 70 % of patients are diagnosed with stages III to IV according to the FIGO classification, and preferential metastatic locations include the peritoneum, lungs, intra-abdominal lymph nodes, and bone [38, 136].

Pathological Features

Grossly, UUSs are often polypoid masses (usually >10 cm), with a fleshy, gray to white cut surface and prominent areas of hemorrhage and necrosis. On microscopic examination, the common histology is characterized by high and pleomorphic nuclear atypia, high mitotic activity, and prominent tumor necrosis and lacks apparently smooth muscle or endometrial stromal differentiation (Fig. 6.10). It is important to note that the distinction between undifferentiated/dedifferentiated endometrial carcinoma and UUS with nuclear uniformity can be difficult, particularly in biopsy samples. According to a recent study, the undifferentiated sarcomas can be further defined as uniform and pleomorphic types. Uniform type shows spindle cells or round cells with permeative myometrial involvement and lymphovascular invasion. Pleomorphic type shows high-grade cytological atypia with marked nuclear pleomorphism, brisk of mitotic activity (almost always exceeding 10 MF/10 HPF and sometimes approaching 50 MF/10 HPF), and destructive infiltration of the myometrium.

Immunohistochemistry and Genetic Aberrations

Typically,the UUSs show no immunoreaction for ER and PR, but weak ER and PR positivity can be observed in the uniform type. The pleomorphic type frequently shows P53 overexpression. CD10 can be positive in some undifferentiated sarcomas, similar to LMS, rhabdomyosarcoma, and UCS. Focal immunoreactivity for SMA, desmin, EMA, and keratin positivity can be seen [135].

The genetic alterations in UUS are generally unknown as genetic, and molecular analysis for the UUSs is scant. Available data by sequencing analysis showed that missense TP53 mutations [135] can be observed in rare cases of UUS. Cytogenetic analysis of UUSs showed a complex karyotype, with many structural and numerical chromosomal aberrations [137]. The frequent genomic alterations include gains on 2q, 4q, 6q, 7p, 9q, 20q and losses on 3q, 10p, 14q [122].

Theories of Origin

The tumor origin of the UUS remains unknown.

Uterine Adenosarcomas

Clinical Features

Uterine adenosarcoma (UAS) makes up 5 % of uterine sarcomas and mainly occurs in postmenopausal women but may also be diagnosed in adolescents and young adults [35]. In a recent study, the majority of the patients were diagnosed between the age of 40 and 65 years with 38 % older than 65 and 10 % younger than 40 [138]. The most common symptom of UASs is vaginal bleeding, but some patients present with pelvic pain, vaginal discharge, or symptoms related to uterine enlargement. Patients with previous pelvic radiotherapy and long-term unopposed estrogen therapy, in particular tamoxifen therapy, are at high risk.

Pathological Features

UAS is a rare Müllerian adenosarcoma, mixed with a benign epithelial and mesenchymal components which resembles low-grade endometrial stromal sarcoma (Fig. 6.11). About a quarter of this tumor contains a high-grade sarcoma with tendency of sarcomatous overgrowth [3]. Macroscopically, UAS is typically polypoid and fills most of or the entire uterine cavity and sometimes may protrude through the cervical OS. UASs commonly arise from the endometrium or adenomyosis but rarely from the endocervix. The tumor size ranges from 1 to 17 cm (with a mean of 6.5 cm) [3, 139]. The cut surface may show variably sized cysts containing watery or mucoid fluid or clefts. Tumors with hemorrhage and necrosis can be seen. Adenosarcomas with sarcomatous overgrowth usually show myometrial invasion.

Microscopically, the epithelial component is usually benign endometrial epithelia with and without mucinous or squamous differentiation.

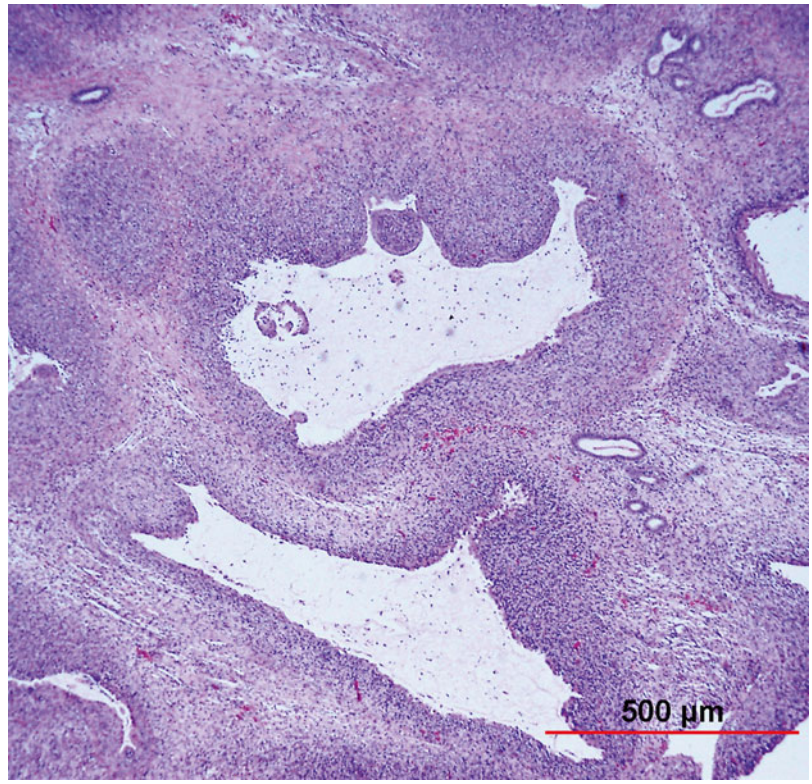

Fig. 6.11 Photomicrograph of a biphasic tumor (adenosarcoma) contains benign and cystic dilated endometrial glands surrounded by hypercellular and malignant endometrial stromal cell proliferation

The mesenchymal component is malignant, resembling LGESS. Heterologous mesenchymal elements (rhabdomyosarcoma, chondrosarcoma, liposarcoma, and sex cord stromal) can be found in ~10–15 % of cases. At low power, the glands are cystic, and the stroma concentrates around them forming periglandular cuffs [139]. The stromal component shows variable mitotic activity (usually >4 MF/HPF). But if the characteristic leaflike architecture is present with periglandular cuffing, the diagnosis can be made even in the absence of mitotic figures. Adenosarcoma with sarcomatous overgrowth can be seen in approximately 10 % of UAS. It often shows greater nuclear pleomorphism and mitotic activity with myometrial and vascular invasion.

Immunohistochemistry and Genetic Aberrations

The immunophenotype of UAS without sarcomatous overgrowth resembles that of ESS. CD10, WT1, ER, PR, and certain SMAs are often positive [140–143]. In contrast, UAS with sarcomatous overgrowth had a strong immunoreactivity for WT-1, Ki-67, and P53, but CD10, ER, and PR were often negative [140, 141]. 90 % (18/20) of UASs are immunoreactive for CD10, but only 63 % (5/8) of UAS with sarcomatous overgrowth are CD10 positive [143]. Ki-67 can be used to distinguish UASs from other benign tumors, such as endometrial polyps and atypical polypoid adenomyomas. Ki-67-positive nuclei are higher in the periglandular zone (~20 %) than the adjacent stroma (<5 %) [140]. The genetic and epigenetic aberrations in UAS remain largely unknown. A recent mouse model showed that HMGA1a transgenic mice developed aggressive uterine tumors resembling UAS. This study suggested that HMGA1a may play an important role in UAS development [144].

Theories of Origin

The molecular mechanisms and tumor origin of sarcomagenesis in UAS remain unknown.

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Precancerous Lesions of Endometrioid Adenocarcinoma

7

Susanne K. Jeffus and Charles M. Quick

Introduction

The WHO's recent adaptation of a two-tiered classification scheme based on endometrioid intraepithelial neoplasia (EIN/atypical hyperplasia (AH) and non-atypical hyperplasia) reflects the evolution of our understanding of endometrial precancers (WHO 2014) [1]. EIN/AH, as a diagnostic category, represents the histologic manifestation of underlying molecular aberrations that can reliably separate endometrial precancer from estrogen-driven hyperplasia. In this new diagnostic schema, a diagnosis of EIN/AH carries the connotation that the patient has an endometrial precancer and is at a significant risk of developing an adenocarcinoma. Conversely, patients that are exposed to excess estrogen are prone to develop hyperplasia, designated non-atypical hyperplasia by WHO 2014. This does not, however, represent an uncoupling of non-atypical and atypical hyperplasia, as hormonally driven non-atypical hyperplasia represents the backdrop for genetic aberrations that allow for endometrial precancers such as EIN/AH to develop.

Changes in Diagnostic Systems: WHO 1994–2014

For two decades the WHO 1994 classification system has provided a relatively consistent method for classification of endometrial lesions. This system allowed for assignment of patients by their risk of developing, or having, carcinoma. The four basic diagnostic categories included in the WHO 1994 schema included hyperplasias (simple and complex hyperplasia without atypia) and atypical hyperplasias (simple and complex atypical hyperplasia).

The "typical hyperplasias" were associated with a proliferative response to excess estrogenic stimulation. In these cases, the entire endometrial compartment would show the signs (increased gland-to-stroma ratio) of excess estrogen. This glandular proliferation was subdivided into "simple" and "complex" based on the proportion of glands relative to stroma and the complexity of the glands in question. Presence of complicated architecture and epithelial proliferation (tufting or budding) necessitated the designation of complex hyperplasia. Within the WHO 2014 system, the essence of the "typical

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hyperplasia" remains unchanged. The WHO 2014 category of "hyperplasia without atypia" consists of cases that would be deemed simple or complex non-atypical hyperplasia in the WHO 1994 system. Hyperplasia without atypia may range from mildly crowded glands to complex, crowded glandular proliferations, as long as the glands in question do not appear cytologically altered compared to the patients background endometrium (termed cytologic demarcation, discussed later in this chapter).

Atypical hyperplasias in the WHO 1994 system consisted of crowded proliferations of glands with atypical cytologic features (Table 7.1). The identification of these features, even if focal, was sufficient to warrant a diagnosis of hyperplasia with atypia. At the time, the combination of the cytologic and architectural features into one classification system represented a great leap forward in the methodology used to classify proliferative endometrial lesions; however, the application of the features of cytologic atypia proved difficult as evidenced by numerous publications [2–5]. Application of these cytologic features in the WHO 2014 system continues to be difficult; however, comparison of possible foci of EIN/AH with the patient's background endometrium (if available) can provide much needed help. The recognition that EIN/AH represents a precancerous population of glands coupled with a much simpler to wield two-tiered system has led to much better reproducibility among pathologists [6, 7]. With the formal adaptation of this two-tiered system, pathologists should benefit from easier-to-apply criteria, and patients should benefit from more precise diagnoses.

Reclassification of hyperplastic endometria from the WHO 1994 system to the EIN system (which aligns with the current WHO 2014 classification) has been studied [7]. Reclassification of WHO 1994 cases using the four-tiered system of hyperplasia with and without atypia using strict EIN (WHO 2014) criteria demonstrated that the majority of cases considered complex atypical hyperplasia comprise the EIN/AH group. Interestingly, 44 % of cases of complex, non-atypical and 4 % of cases of simple, non-atypical hyperplasia were reclassified as EIN/AH [7]. This comes as little surprise as many pathologists have had difficulty with vexing cases of non-atypical hyperplasia that are worrisome, but not diagnostic of atypical hyperplasia. The utilization of cytologic demarcation in the EIN/AH system will help to alleviate this issue.

Table 7.1 Histologic features of cytologic atypia as defined by The WHO

Features of cytologic atypia
1. Loss of nuclear polarity
2. Irregular nuclear shape
3. Irregular nuclear contours
4. Vesicular chromatin
5. Hyperchromatic chromatin
6. Prominent nucleoli

Background: Morphometry to Subjective Practice

In the late 1970s, advances in computer hardware and software allowed for computerized morphometric analysis of pathologic samples. These techniques were applied to endometrial samples in the hopes of identifying more objective methods for evaluation of precancerous lesions [8–11]. As these methods were refined and correlated with outcome, specific architectural [12] and cytologic [13, 14] features were identified that could predict patients predisposed to carcinoma. Combinations of measurable architectural and cytologic features as well as the volume percentage of stroma present in the samples were measured and used to calculate a D-score (Table 7.2) [15]. Cases that were subjected to computerized morphometry were reliably classified into probable precancer (EIN), unknown, and probable

Table 7.2 Measurements taken to determine D-score

Components of D-score
1. Volume percent stroma
2. Standard deviation of the shortest nuclear axis
3. Gland outer surface density

benign categories with relative success; however, the requirements for resources and technical expertise to carry out this methodology were great, limiting its adaptation outside of a few large reference centers. Through the efforts in developing computerized morphometry, evaluation of volume percent stroma as a keystone of precancer identification was realized, and this determination became one of the key histologic features utilized when making a diagnosis of EIN/AH.

Another key component of the WHO 2014 EIN/AH classification is cytologic demarcation. This premise differs from the identification of cytologic atypia in that it is a comparison of clonal vs. non-clonal glands within the same specimen. Initially this association was described in patients with endometrial carcinoma. It was noted that adjacent to the carcinoma there were monoclonal populations of endometrial glands which were histologically different from the patient's native endometrium [16]. These precancerous glands formed the histologic basis for the EIN/AH schema which is in place today. Volume percent stroma and cytologic demarcation from these early studies were combined with size cutoffs and exclusion of mimics to create the EIN classification system, which as a result, represents a histologic descriptor of underlying genetic mutations [17].

EIN/AH and Cancer Risk

Studies of patients with a diagnosis of EIN/AH have shown that the WHO 2014 system is more effective at predicting patients that will progress to, or concurrently have, endometrial carcinoma [18]. A patient with a new diagnosis of EIN/AH will suffer from occult, concurrent carcinoma (defined as a cancer diagnosis in 1 year) in one-third of cases [19]. Patients that do not develop adenocarcinoma in the first year have been found to carry a 45-fold increase in risk of progression to low-grade endometrioid carcinoma compared to those with non-atypical hyperplasia [20]. While these quoted figures are striking, the exact percentage of patients that might progress to adenocarcinoma is impossible to determine, as many patients are definitively treated with hysterectomy at the time of diagnosis of EIN/AH; however, these are comparable with those reported for the previous WHO 1994 classification [21, 22].

Endometrial Carcinoma Is Derived from EIN/AH

Central to the theory of precancerous lesions is the idea that carcinomas can be directly linked to their putative precursors. Progressive microsatellite changes in precancerous and cancerous endometrium have demonstrated a direct link between nonfamilial, precancerous endometrium, and adjacent endometrial carcinomas [23]. Subsequently, microsatellite allotype mapping has shown that endometrial precancers (histologically EIN/AH) develop into endometrial adenocarcinoma, and that additional mutations can lead to intratumoral genetic heterogeneity in various regions of the tumor that could lead to tumor progression [24]. These elegant studies support the idea that tumor progression occurs through physical extension of clonal lesions, i.e., endometrial precancers.

EIN/AH Differs from Normal Endometrium

Normal endometrial glands represent a polyclonal population of tissue from which monoclonal precursor lesions arise. Indeed, EIN/AH represents a novel population of monoclonal glands that are genetically and histologically distinct from the background native endometrium [16, 25]. As previously mentioned, subsets of these lesions have microsatellite instability which has been mapped to show direct continuity between histologically identifiable precancers and their adjacent tumors [23]. While these mutations set EIN/AH apart from normal endometrium, they do not convey the ability to invade or metastasize, which also sets them apart from endometrial adenocarcinoma.

EIN/AH Shares Genetic and Phenotypic Features with Endometrial Carcinoma

Cells undergoing transformation from benign to precancerous harbor genetic mutations that confer growth advantages and set them apart from normal background endometrium. These same mutations also link these cells to the subsequent adenocarcinomas. Studies have repeatedly demonstrated that EIN/AH and adenocarcinoma contains similar, nonrandom X-chromosome inactivation as well as specific, conserved microsatellite mutations in lesions within individuals [23–27]. Further supporting evidence has been described involving single-gene mutations that are known to be associated with endometrioid endometrial adenocarcinoma. Studies have identified inactivation of *PTEN* [28–30], *PAX2* [31], mutations in *K-RAS* [32–34], and hypermethylation of *hMLH1* [35] in precancerous endometrial lesions. In fact, *PTEN* and *PAX2* mutations are such common events in EIN/AH that immunohistochemical staining has been suggested as a diagnostic adjunct in difficult cases (described later).

EIN/AH Can Be Diagnosed

The synthesis of the years of scientific exploration into the roots of endometrial carcinogenesis has led to the development of the benign hyperplasia/EIN sequence [36]. This sequence outlines specific histologic features that can reliably be identified by routine light microscopy. Furthermore, these features can reliably identify glandular proliferations as precancerous. The acceptance of this diagnostic schema has culminated in its adoption by the WHO as the formal classification system for endometrial precancers [1].

Endometrial Hyperplasia without Atypia

Endometrial hyperplasia without atypia (benign hyperplasia) is most commonly seen in perimenopausal women with symptoms of abnormal uterine bleeding, but can occur in any woman with a systemic and unopposed excess of estrogen. The etiologic differential diagnosis for elevated estrogen not counterbalanced by progesterone is broad and includes chronic anovulation, hormone replacement, or estrogen-secreting ovarian tumors. Depending on the duration of unopposed estrogen on the endometrium, architectural changes range from a predominantly normal proliferative pattern with occasional cystically dilated glands (disordered proliferative endometrium) to progressive gland crowding with gland branching and dilation (the so-called benign hyperplasia sequence). According to the 2014 WHO Classification of Tumors of the Female Reproductive Organs, the new diagnostic term "endometrial hyperplasia without atypia" encompasses the 1994 WHO classification terms of "simple hyperplasia without atypia" and "complex hyperplasia without atypia" [1, 36, 37].

The earliest histologic changes of unopposed estrogen are collectively referred as "disordered proliferative endometrium." Most importantly, the endometrium shows a normal gland-to-stroma ratio. Isolated dilated glands are interspersed in a background of a mitotically active proliferative pattern endometrium (Fig. 7.1). Additionally, tubal metaplasia may accompany these changes. In contrast to the preserved normal gland-to-stroma ratio in a disordered proliferative pattern, continuous unopposed estrogen results in a global increase in the gland-to-stroma ratio (hyperplasia). Best appreciated on low-power examination, the hyperplastic endometrium shows a "regularly irregular" pattern; normal proliferative glands are admixed with more crowded branching or dilated glands (Fig. 7.2). Of note, the cytologic features of the glands are unchanged from field to field. Nuclei are "pencil shaped" and demonstrate an orderly columnar arrangement with respect to the basement membrane (Fig. 7.3). Mitotic figures are present, but not overly abundant nor atypical. Stromal hemorrhage and breakdown as well as reparative epithelial changes are not uncommon [1, 17, 37]. A monoclonal precancerous lesion or frank carcinoma is notably absent. Prolonged and

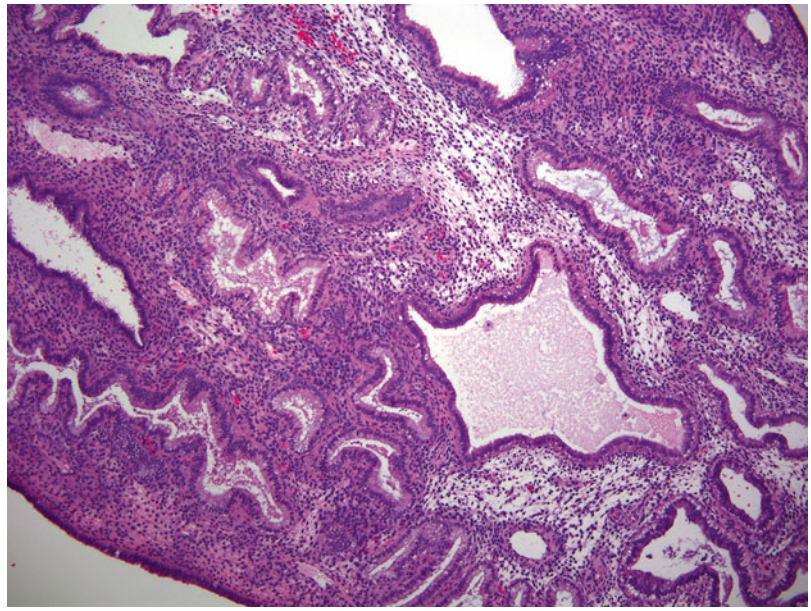

Fig. 7.1 Disordered proliferative endometrium displaying an admixture of normal and cystically dilated glands

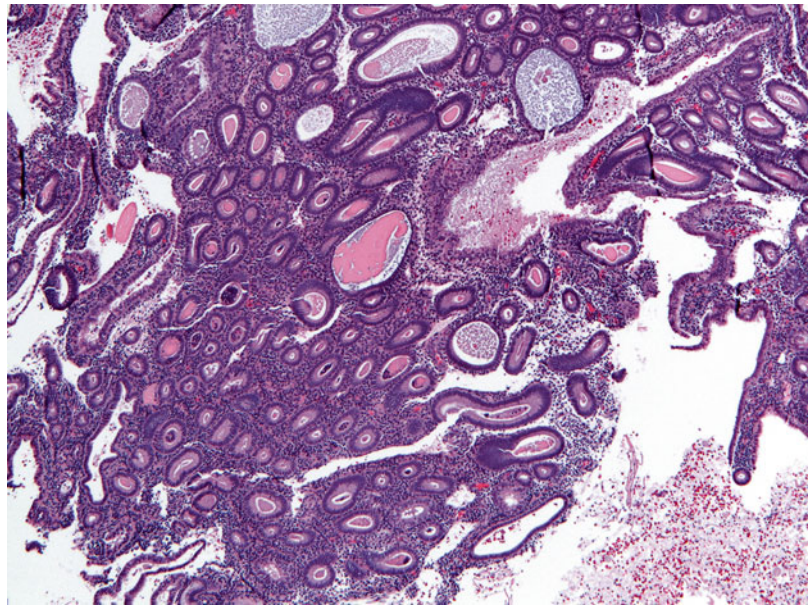

Fig. 7.2 Hyperplasia without atypia (low power) comprised of a population of slightly crowded glands and intervening stroma. Note the presence of a few microcysts, indicative of a chronic anovulatory state

unopposed estrogen exposure carries a two- to tenfold risk of endometrial carcinoma [38–40]. In a long-term follow-up study by Kurman et al., progression to carcinoma was seen in 1 % (simple hyperplasia) and 3 % (complex hyperplasia) of "untreated" patients with endometrial hyperplasia without atypia [41]. Treatment of endometrial hyperplasia without atypia centers on hormonal therapy or elimination of the cause of estrogen excess (e.g., weight loss in case of obesity).

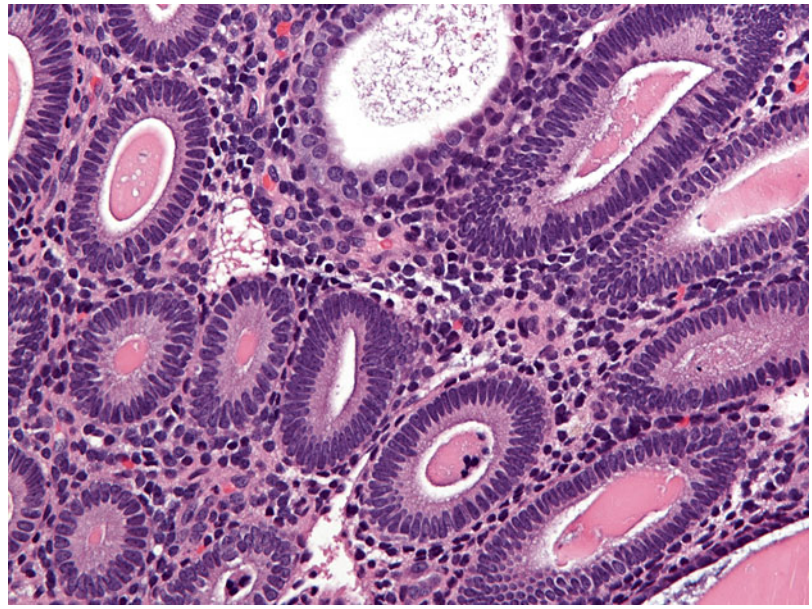

Fig. 7.3 Hyperplasia without atypia (high power) demonstrating crowded glands with bland nuclei

Endometrial Intraepithelial Neoplasia/Atypical Hyperplasia

Endometrioid intraepithelial neoplasia/atypical hyperplasia (EIN/AH) has recently been adapted by the 2014 WHO Classification of Tumors of the Female Reproductive Organs as the standard terminology for precancerous endometrial lesions, essentially eliminating the usage of the 1994 WHO terms of simple atypical hyperplasia and complex atypical hyperplasia [1, 37]. This paradigm shift occurred due to evidence that (1) the 1994 WHO classification scheme suboptimally risk stratified patients according to the biology of the disease and (2) diagnoses relied heavily on assessment of cytologic atypia which was found to be poorly reproducible, even among experts [3, 15, 18, 36, 42–44]. While histologic overlap exists, there is no direct diagnostic correlation between the 1994 WHO classification and the 2014 EIN/AH scheme (e.g., the terms complex atypical hyperplasia and EIN should not be used interchangeably). EIN/AH represents a distinct monoclonal precancerous lesion to endometrioid (type I) endometrial carcinoma (Table 7.3).

The diagnostic criteria for EIN/AH are fourfold and include architectural gland crowding (VPS < 55 %), cytologic demarcation of the lesional focus from background endometrium, size of more than 1 mm (one-half of a 10× field is a helpful guide), and exclusion of benign mimics and carcinoma (Table 7.4). All of these criteria must be met for a diagnosis of EIN/AH (Fig. 7.4). Gland crowding, whether focal or diffuse, is best assessed at low-power magnification, and as stated, the proportion of endometrial glands should be greater than 1:1. While crowded, glands are still separated by stroma, a crucial distinguishing characteristic between EIN/AH and endometrioid carcinoma. Lesional glands appear tubular or dilated and branching. As one moves from the epicenter of the focus of crowded glands, the clonal gland population will become slightly less crowded, and interspersed normal glands may be identified (Fig. 7.5). Of course, this pattern of glandular crowding is predicated on having a relatively intact fragment of tissue to examine.

One of the central tenants of EIN/AH is cytologic demarcation. Lesional glands must display

Table 7.3 Summary of clinical and diagnostic parameters of hyperplasia without atypia and EIN/AH

2014 WHO	1994 WHO	Origin	Histology	Genetics	Treatment	Risk
Hyperplasia without atypia	Simple hyperplasia without atypia Complex hyperplasia without atypia	Excess estrogen (anovulatory cycles, polycystic ovarian syndrome, obesity, estrogen-producing tumor)	"Regularly irregular" pattern Diffuse endometrial involvement Crowded proliferative glands with branching and dilation; no cytologic demarcation	Polyclonal	Hormonal therapy	Two- to tenfold risk of developing endometrial carcinoma in the future
EIN/AH	Simple hyperplasia with atypia Complex hyperplasia with atypia	Endometrial Precancer	Focal or diffuse endometrial involvement Gland crowding (VPS < 55 %) Cytologic demarcation Size: >1 mm Exclusion of benign mimic and carcinoma	Monoclonal	Hormonal therapy or surgery (hysterectomy)	33–41 % risk of concurrent endometrial carcinoma 45-fold increased risk to develop endometrial carcinoma within 1 year

altered cytology (difference in nuclear and/or cytoplasmic features) compared to the background endometrium (Fig. 7.6). The cytologic features of EIN/AH vary widely and are predominantly dependent on the hormonal environment. For example, nuclei may be cigar shaped, pseudostratified, or rounded with prominent nucleoli. The cytoplasm may demonstrate typical endometrioid morphology or show altered differentiation (e.g., secretory, mucinous, eosinophilic) (Fig. 7.7). As such, the criteria for classical atypia (rounded nuclei, vesicular chromatin, prominent nucleoli, anisonucleosis, loss of polarity) are not a requirement; rather, it is the comparison to the background endometrium that establishes whether the criterion for cytologic demarcation is met.

At minimum, the requirement for size of EIN/AH is 1 mm measured in a single dimension. Of note, multiple foci are not additive. This 1 mm cutoff is historically based in the morphometric studies used to evaluate volume percent stroma in precancerous lesions. Adhering to a minimum size of 1 mm prevents overcalling of small areas of compressed glands as well as other potentially subdiagnostic or artifactual lesions. Lesions that are smaller than 1 mm, yet felt to be cytologically altered and crowded, are best classified as "focal gland crowding" (discussed later).

Before rendering a diagnosis of EIN/AH, benign mimics and carcinoma must be excluded. Examples of the former include but are not limited to artificial gland crowding, telescoping of glands, hyperplasia without atypia, endometrial polyps, endometrium with reparative change or tubal metaplasia, and fragments of normal lower uterine segment. When glandular growth shows a back-to-back cribriforming pattern without intervening stroma, villoglandular, mazelike, or solid growth, the diagnosis of endometrioid adenocarcinoma is warranted (Fig. 7.8). Because EIN/AH is the immediate precursor to endometrioid adenocarcinoma, both are commonly present in an endometrial sample. If uncertainty about the presence of carcinoma prevails, a diagnosis of "at least atypical hyperplasia/endometrioid intraepithelial neoplasia" is appropriate with a comment

Table 7.4 EIN/AH diagnostic criteria

Diagnostic criteria for EIN/AH (all must be met for the diagnosis)
Gland crowding (VPS < 55 %)
Cytologic demarcation from background endometrium
Size: at least 1 mm
Exclusion of benign mimics and carcinoma

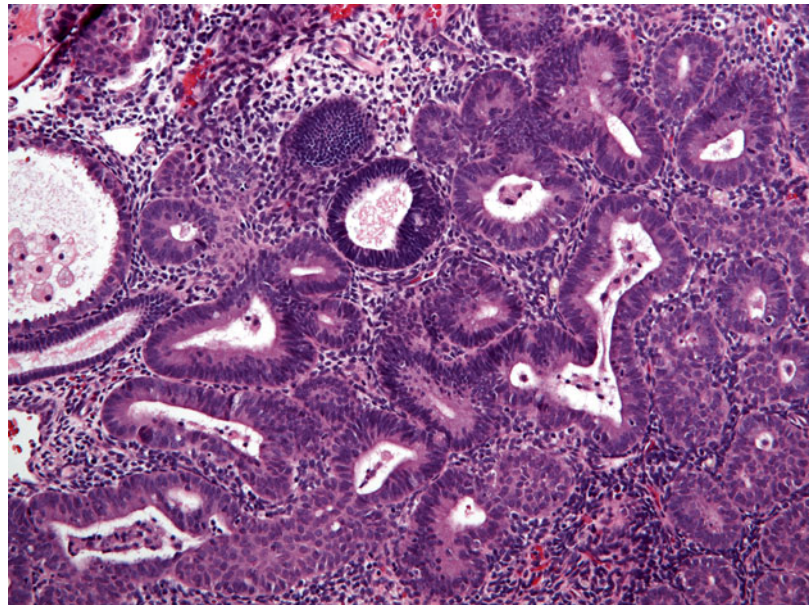

Fig. 7.4 EIN/AH denoted by the presence of crowded glands that differ from the background endometrium. In this figure the EIN/AH is lighter in staining intensity than the background endometrial glands (*center*), but does not display generally acceptable atypical nuclear features

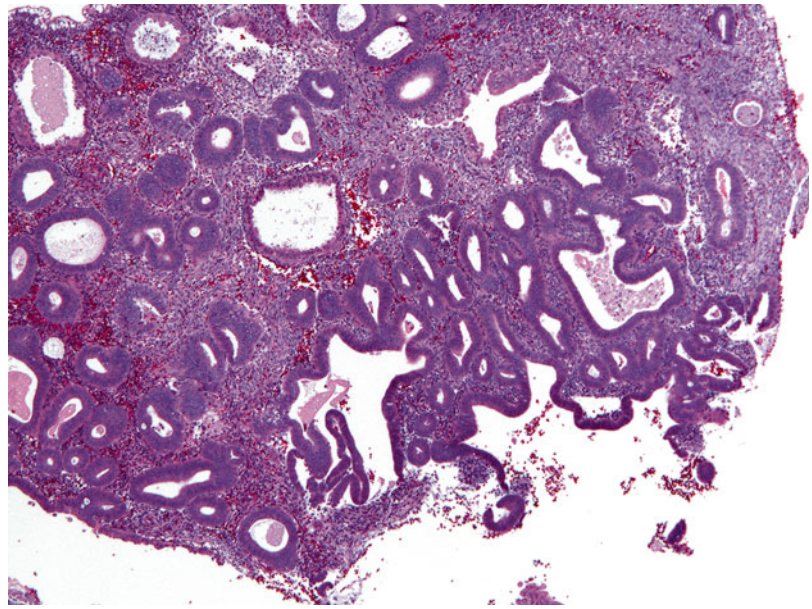

Fig. 7.5 A low-power view of gland crowding in which anovulatory glands compose slightly more than half of the tissue in the demonstrated field

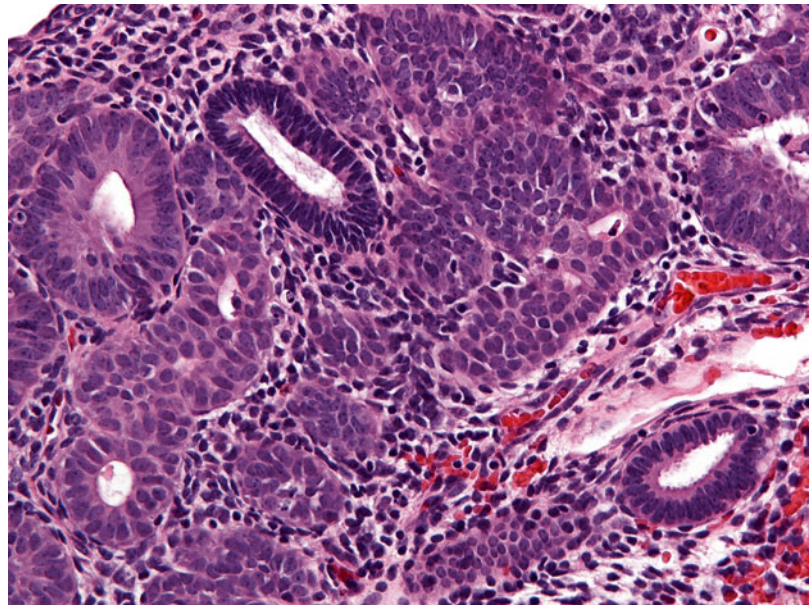

Fig. 7.6 Cytologic demarcation can be easily identified in this image. Normal glands stain much darker and are surrounded by crowded glands with altered cytology; here, the cells have more rounded nuclei with nucleoli

that the histopathologic features in the sample fall short of a definitive diagnosis of endometrioid adenocarcinoma. Therapeutic options for EIN/AH consist of hormonal therapy and surgery (hysterectomy). The decision about which treatment option is pursued rests with the clinician and is influenced by various patient-related factors such as age, desire for future fertility, and comorbidities.

Complicating Factors in the Diagnosis of EIN/AH

Sampling Artifacts and Specimen Fragmentation

Commonly observed in biopsy/curettage specimens, gland crowding due to artificial gland compression or telescoping of glands can

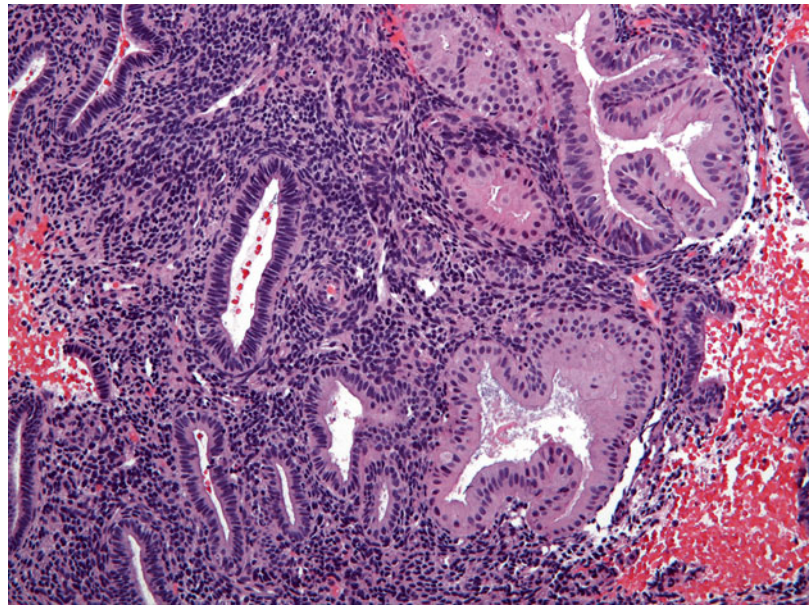

Fig. 7.7 In this example of cytologic demarcation, the EIN/AH is composed of enlarged cells with ample eosinophilic cytoplasm. Note that the nuclei are not significantly "atypical"

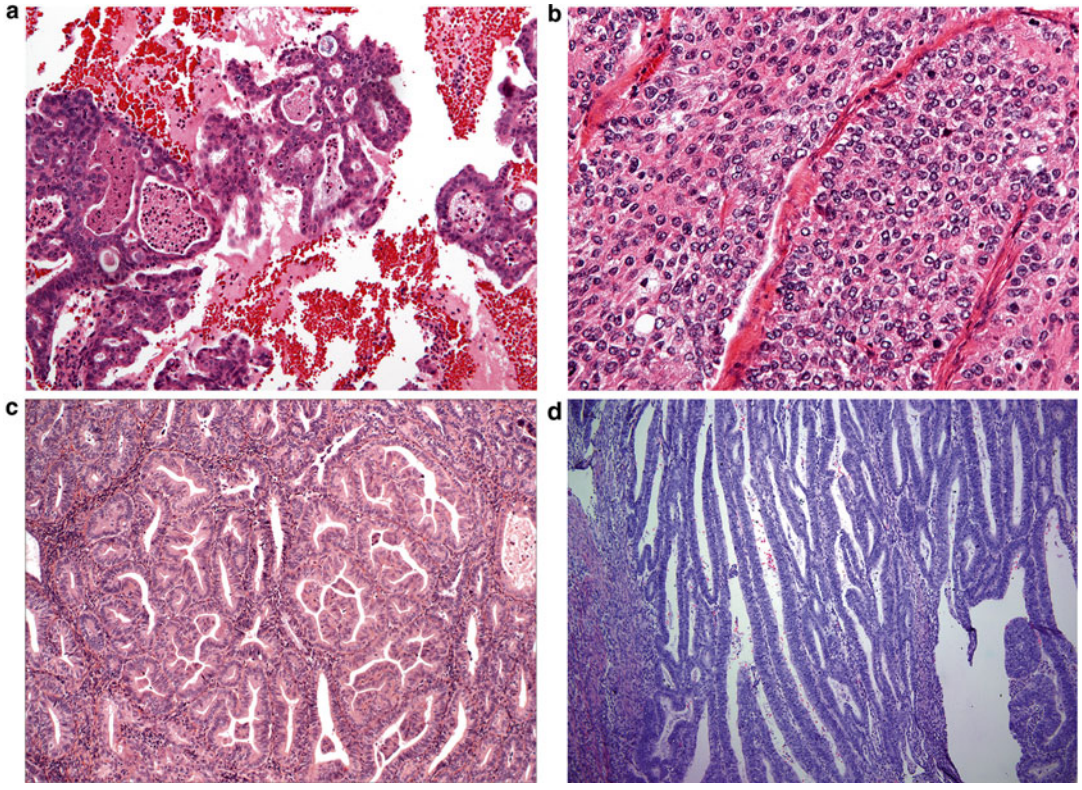

Fig. 7.8 Exclusion of endometrial adenocarcinoma is key in diagnosing EIN/AH. Histologic features indicative of carcinoma include (**a**) cribriforming, (**b**) non-morular solid growth, (**c**) papillary or mazelike glandular configurations, and (**d**) villoglandular architecture composed of thin papillary cores

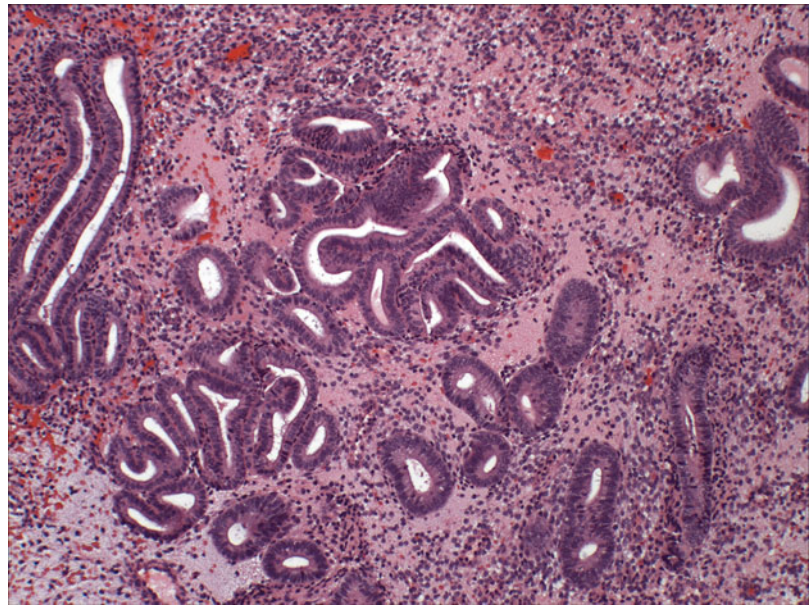

Fig. 7.9 Telescoping artifact may mimic glandular crowding. Note the absence of cellular stroma adjacent to the artifactually compressed glands

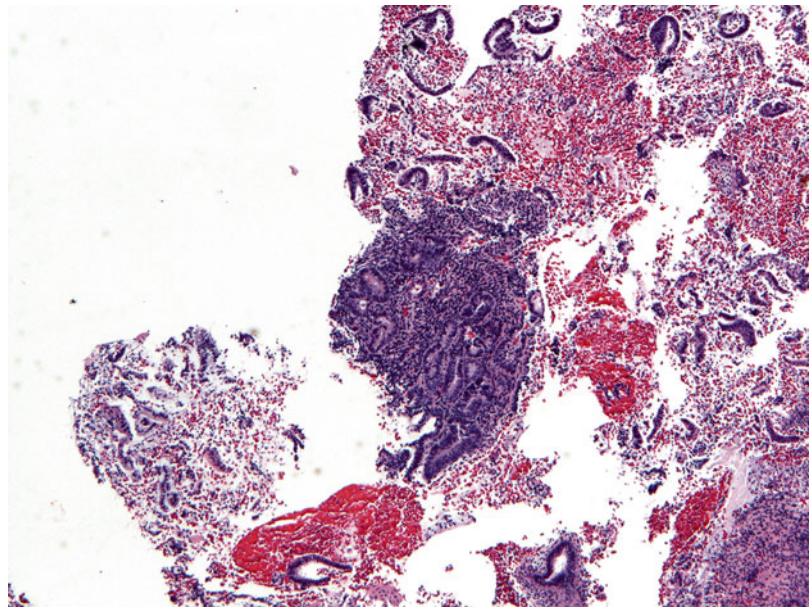

Fig. 7.10 This small fragment of tissue contains a cluster of crowded glands that is suspicious for EIN/AH

occur; however, this is usually a focal finding, and the absence of cytologic demarcation is a helpful clue (Fig. 7.9). Fragmentation of samples represents a particular challenge to the pathologist. The EIN/AH criteria should be rigidly applied to prevent overdiagnosis and retain diagnostic specificity. The diagnosis of EIN/AH should only be rendered in intact fragments that meet all diagnostic criteria and contain intact intervening stroma. Even if extensively fragmented, the size criterion of 1 mm must be met in a single intact fragment; separate lesional foci should not be added to meet the size threshold. If a fragmented biopsy demonstrates features suspicious for EIN/AH (Fig. 7.10), obtain additional levels. If still no

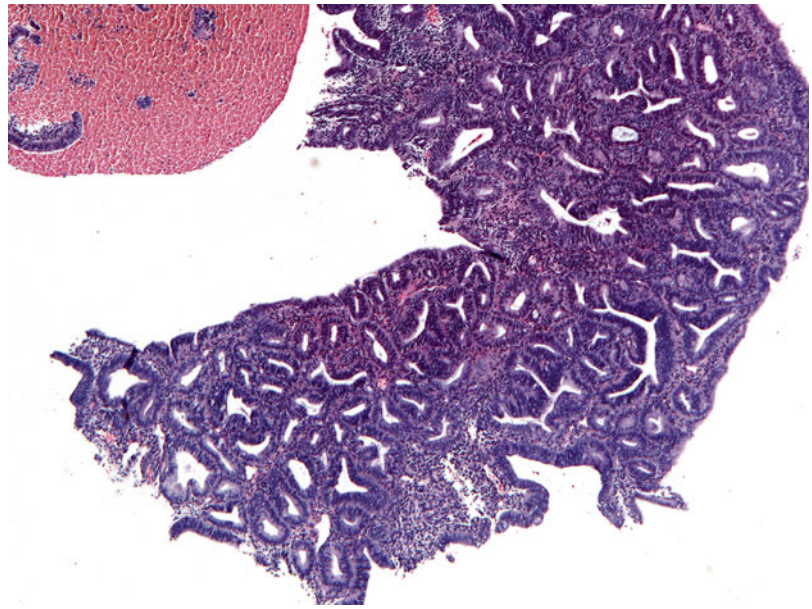

Fig. 7.11 Occasional samples will lack apparent normal background glands to evaluate for cytologic demarcation (so-called over-run EIN/AH)

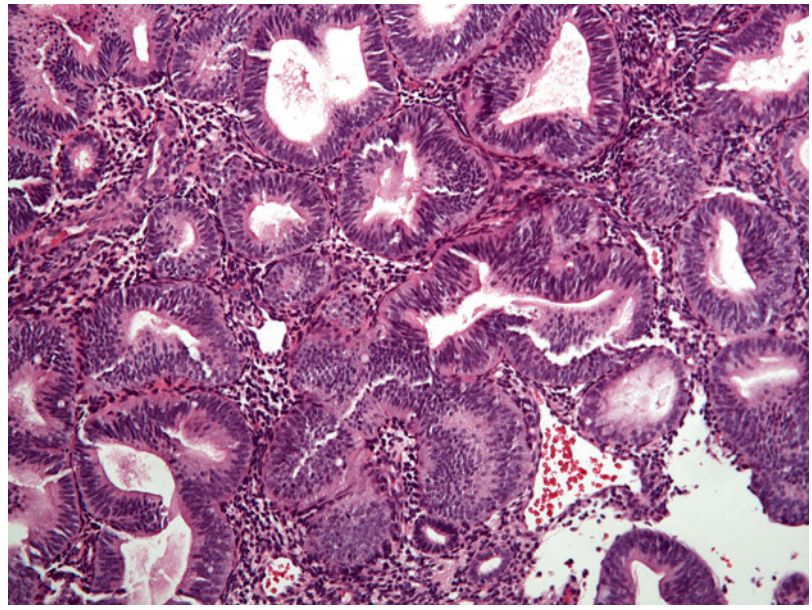

Fig. 7.12 In cases of over-run EIN/AH, a diligent search for normal background glands will often demonstrate rare normal glands (*bottom center*)

resolution is achieved, a descriptive diagnosis with a comment advising re-biopsy in 3 months is recommended.

So-Called Over-Run EIN/AH

A discrete focus of gland crowding with appropriate cytologic demarcation and size leads to a straightforward diagnosis of EIN/AH. However, in one-fifth of endometrial samples, EIN/AH encompasses the entire specimen, so-called over-run EIN/AH (Fig. 7.11). On low-power examination, the differential diagnosis includes endometrial hyperplasia without atypia. Hence, a careful search for cytologic demarcation becomes critical. Native endometrial glands in over-run EIN/AH are often interspersed between lesional glands or are present at the periphery (Fig. 7.12). If no background endometrium is identified, the

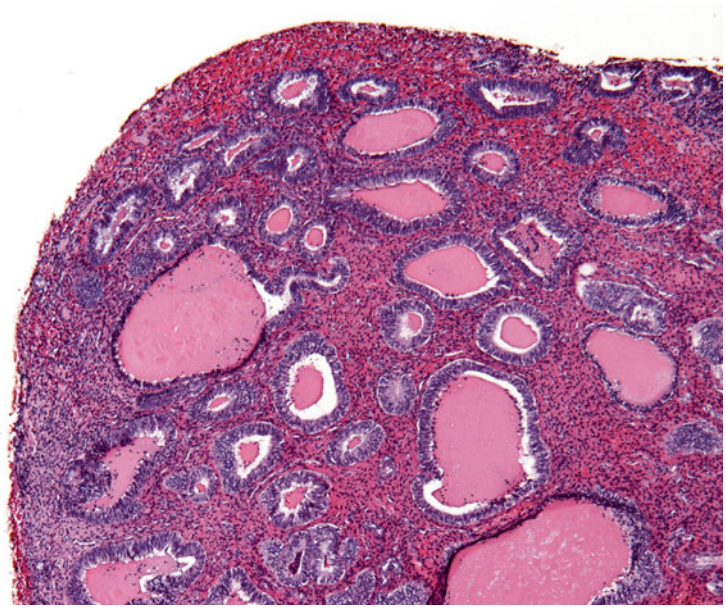

Fig. 7.13 A classic example of an endometrial polyp composed of oddly shaped, scattered glands and fibrous stroma

pathologist must decide whether the architectural complexity and cytologic atypia are in keeping with a diagnosis of EIN/AH and cannot be explained by a benign process. Immunohistochemistry (see below) such as staining with PAX2 may be of value in this setting.

Polyps

Fragments of benign endometrial polyps can be confused with EIN/AH because glands may be crowded, haphazardly arranged, dilated and branched, and may display altered cytology. The fibrous stroma and thick-walled vessels are key features for the correct diagnosis (Fig. 7.13). One-fifth of EIN/AH arise in an endometrial polyp. Postmenopausal women are more likely to have polyps containing EIN/AH compared to premenopausal women [45]. Assessment for features of EIN/AH within a polyp can be challenging due to the inherently variable distribution and variable cytology of glands. All criteria for the diagnosis of EIN/AH must be met; of note, evaluation of cytologic demarcation is performed by comparing the cytology of the crowded glands to the background glands *within* the polyp rather than to the native endometrium outside of thepolyp (Fig. 7.14) [45].

EIN/AH with Altered Differentiation

The most commonly encountered metaplasias include mucinous, tubal, squamous, and papillary syncytial metaplasia. Less frequently encountered are eosinophilic (oxyphilic), hobnail, and secretory metaplasia. Because these metaplasias can be seen in benign, precancerous, and frankly malignant endometrial glands, the alternative designation "altered differentiation" has been proposed. Altered differentiation is causally related to the hormonal environment or specific mutations (e.g., beta-catenin in formation of squamous morules) [46–48]. In a study by Carlson et al., squamous morular and tubal differentiation were the most commonly encountered states of altered differentiation in EIN/AH; squamous and mucinous metaplasia showed the highest observer reproducibility ($K > 0.64$) [45].

Squamous metaplasia either occurs focally on the endometrial surface in the form of mature, nonkeratinizing, and well-differentiated squamous cells, as an exaggerated diffuse response referred to as ichthyosis uteri, or as morular metaplasia. The latter are well-circumscribed, round (mulberry-like) aggregates of ovoid and spindle cells originating from their glandular counterparts and often obliterating the gland

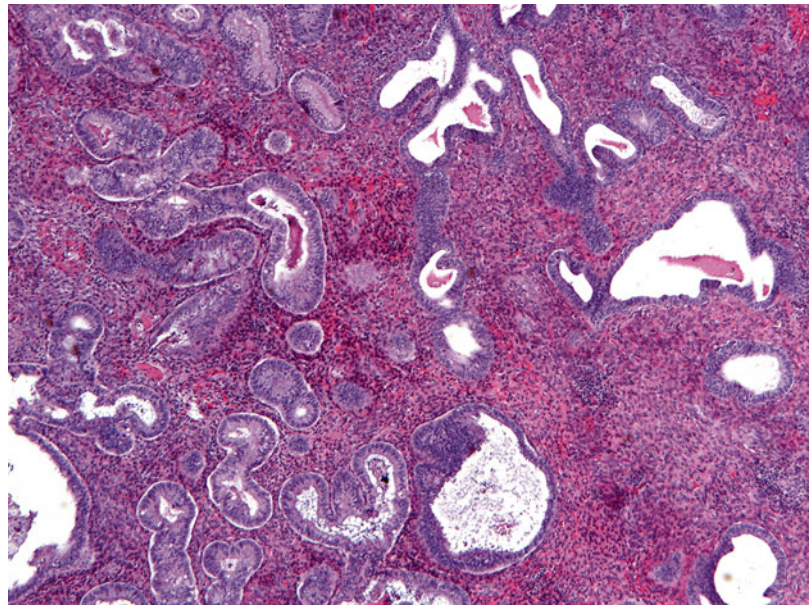

Fig. 7.14 Diagnosis of EIN/AH within a polyp may be difficult. Cytologic demarcation between gland populations within the polyp is required. In this example the crowded glands on the *left half* have slightly different cytoplasmic characteristics than the benign glands on the *right*

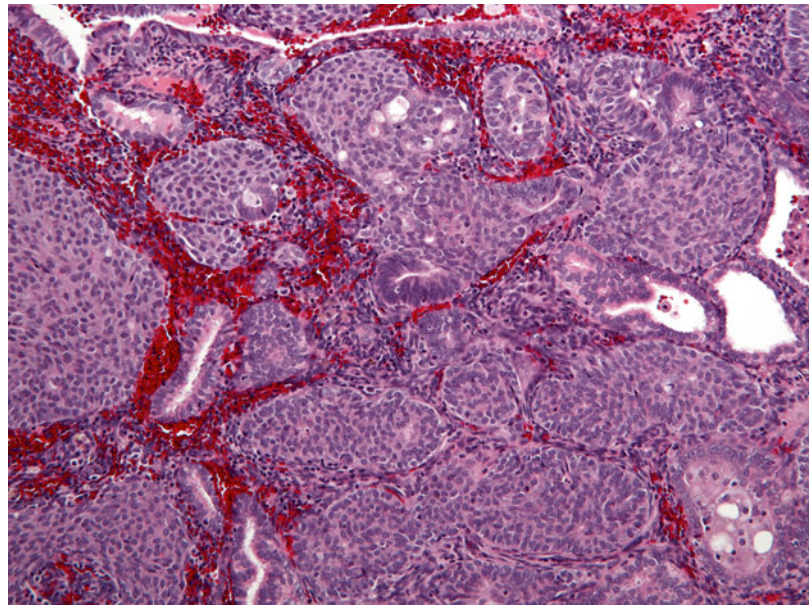

Fig. 7.15 The presence of squamous morules may obscure glandular architecture. The presence of focal nuclear swirling, streaming, or partially involved glands may be a helpful clue

lumen (Fig. 7.15). Central necrosis and clear cell change within morules can occur. They are hormone receptor (ER, PR) negative, functionally inert (low Ki-67) elements that should be excluded during assessment for gland crowding in EIN/AH or when grading endometrioid adenocarcinoma. Because morular metaplasia is associated with EIN/AH and endometrioid adenocarcinoma, when seen in isolation, their presence should trigger a comment with a recommendation for close clinical surveillance [49]. The most common molecular alteration in morular metaplasia is a mutation in the *CTNNB1* gene (β-catenin) [50]. Aside from nuclear and cytoplasmic beta-catenin expression by immunohistochemistry, morules also express membranous

CD10 and nuclear CDX2 [50]. Squamous morules can mimic granulomas, solid, or spindle cell differentiation in an endometrioid adenocarcinoma, or a smooth muscle neoplasm. Immunohistochemical stains can hence be valuable in defining the disease process.

Mucinous metaplasia varies in architectural complexity and cytologic appearance (endocervical or intestinal type). Nucci et al. subdivided mucinous metaplasias into types A, B, and C based on architectural complexity [51]. Type A mucinous metaplasia is composed of a monolayer of cells with no significant atypia or complexity. Type B mucinous metaplasia may contain mild complexity with pseudo-papillae. Type C mucinous metaplasia consists of mucinous epithelium with complex cribriforming, microglandular, or villous architecture and has a high (75 %) association with adenocarcinoma (Fig. 7.16). For all practical purposes, patients with type C mucinous metaplasia should be treated similarly to patients with non-mucinous EIN. Cases with type A and type B mucinous metaplasia have a low risk of progression and may be followed by additional sampling.

Tubal metaplasia is common and an effect of unopposed estrogen levels. It is characterized by cilia, clear round cells and mimics the histologic appearance of the fallopian tube. While frequently encountered in benign endometria, EIN and adenocarcinoma can show prominent tubal differentiation. When seen in conjunction with scattered, cystically dilated glands (disordered proliferative endometrium), it can be a sign of chronic anovulation.

Papillary syncytial metaplasia is now recognized as a reactive/reparative process. This form of "metaplasia" is commonly encountered in samples with stromal breakdown, hemorrhage, and fibrin thrombi (Fig. 7.17). The epithelium contains neutrophils and can show mild to moderate nuclear atypia. Its diagnostic separation from (papillary) serous carcinoma is imperative. Postmenopausal status, atrophic background, strong, diffuse p53 positivity, and a high Ki-67 index would favor high-grade serous carcinoma. Hobnail change is a type of papillary syncytial metaplasia. It is characterized by cells with clear to eosinophilic cytoplasm and nuclei that bulge into the luminal space (Fig. 7.18). Its reactive etiology needs to be distinguished from Arias-Stella effect, clear cell carcinoma, and radiation change. Key features favoring malignancy include complex architecture, nuclear pleomorphism, and high mitotic index.

To the novice, normal secretory endometrium can be a diagnostic pitfall. Glands are crowded and show extensive architectural complexity. However, the torturous glands, while crowded, show an orderly arrangement and demonstrate cytologic uniformity. Sub- or supranuclear vacuoles or luminal secretions may be present. The diagnosis of EIN/AH with secretory differentiation is uncommon and challenging, particularly when arising within a secretory background. Parra-Herran et al. showed that EIN/AH with secretory differentiation is most commonly seen in premenopausal women (average age of 45), is predominantly associated with circulating progestin (endogenous or exogenous), and has a greater tendency to regress compared to traditional EIN/AH [52]. Glands in EIN/AH with secretory change are larger, more complex, and demonstrate a haphazard arrangement compared to normal secretory endometrium. Defining cytologic features includes vacuolated cytoplasm, an apical ruffled border, nuclear rounding, nuclear overlap, and vesicular chromatin [52, 53]. These cytologic changes are relatively bland; therefore, identification of cytologic demarcation is crucial (Fig. 7.19). EIN/AH arising in a secretory background can also lack luminal complexity and resemble proliferative type glands allowing its distinction from the secretory nonneoplastic background (Fig. 7.20). This suggests that some precancerous lesions arising in a secretory background are relatively unaffected by hormonal influences [52, 53].

Hormonal Therapy

One of the treatment options for EIN/AH is hormonal therapy (progestin). This alternative to surgical intervention is offered to patients who

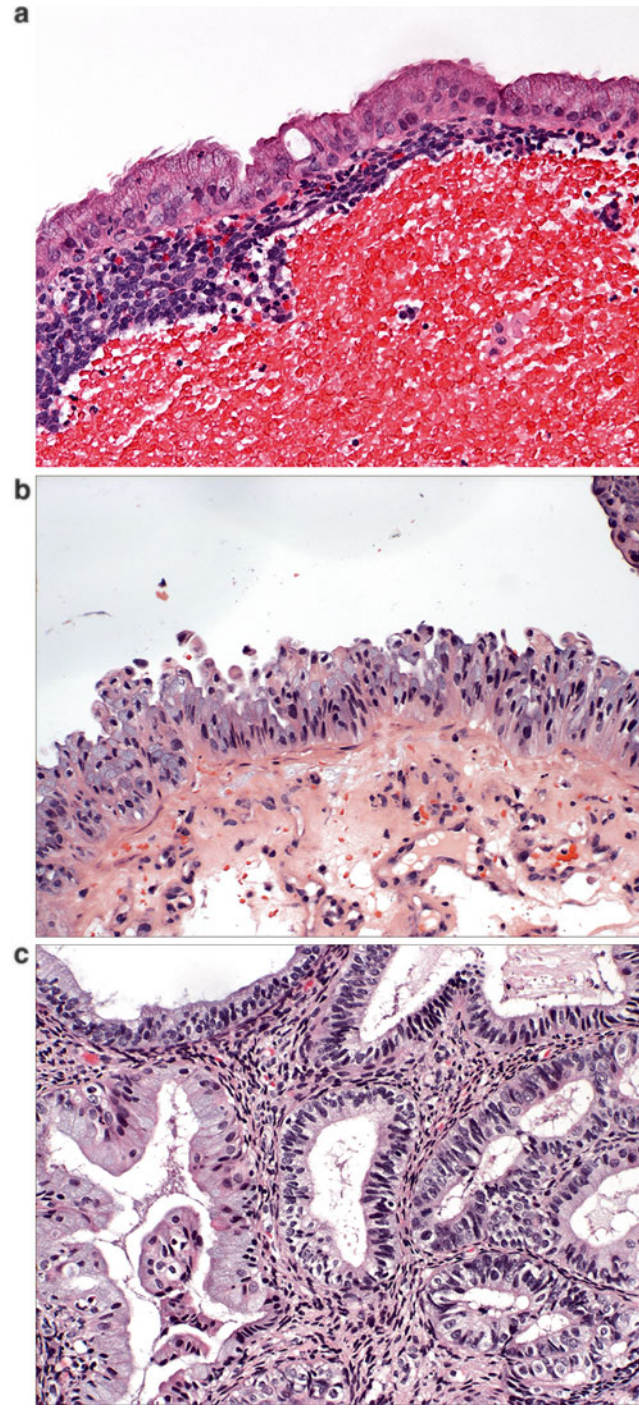

Fig. 7.16 Mucinous metaplasia may confound the evaluation for EIN/AH. Mucinous metaplasia may be (**a**) simple (type A), (**b**) stratified and bland (type B), or (**c**) complex (type C). Type C mucinous metaplasia is traditionally treated with hysterectomy

desire fertility, or are poor surgical candidates. Hormonal therapy remains to be standardized. Common treatment approaches are continuous progestin administration (e.g., intrauterine device) or discontinuous therapy that allows for a withdrawal bleed. A withdrawal bleed potentially

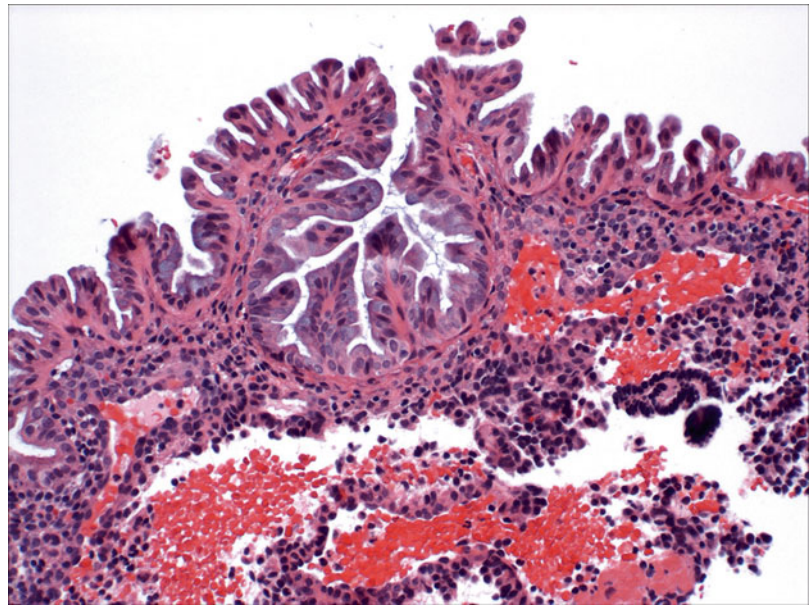

Fig. 7.17 Endometrial epithelial repair may present as papillary syncytial metaplasia. The superficial location, degenerative nuclear features, and presence of breakdown can be helpful in separating this from a malignant process

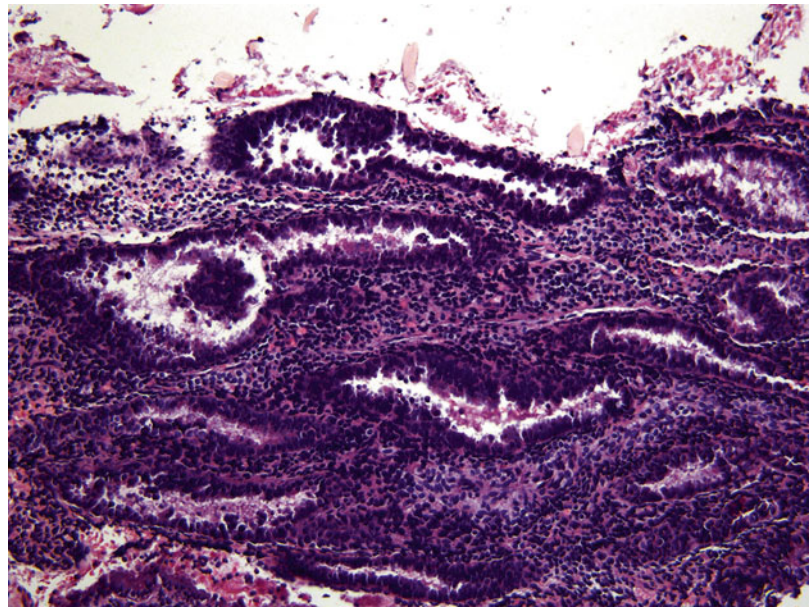

Fig. 7.18 Irritation of the endometrial epithelium may lead to hobnail metaplasia (exfoliation artifact) in which the epithelial cells shed into the lumen and may assume a hobnailed architecture. This should not be confused with clear cell carcinoma

results in shedding of the neoplastic tissue. Surveillance biopsy after a withdrawal bleed is usually performed to ascertain treatment effect [54].

Exogenous hormone administration causes pseudo-decidualization of the stroma. The stromal compartment as a whole expands; stromal cells appear plump with abundant eosinophilic cytoplasm (Fig. 7.21). Due to the stromal expansion, lesional glands (EIN/AH or adenocarcinoma) become less crowded. In addition, malignant glands can become more cytologically bland and contain cytoplasmic metaplasias, particularly eosinophilic and mucinous types. In a complete treatment response, the glandular compartment involutes and becomes essentially atrophic in appearance (small nuclei,

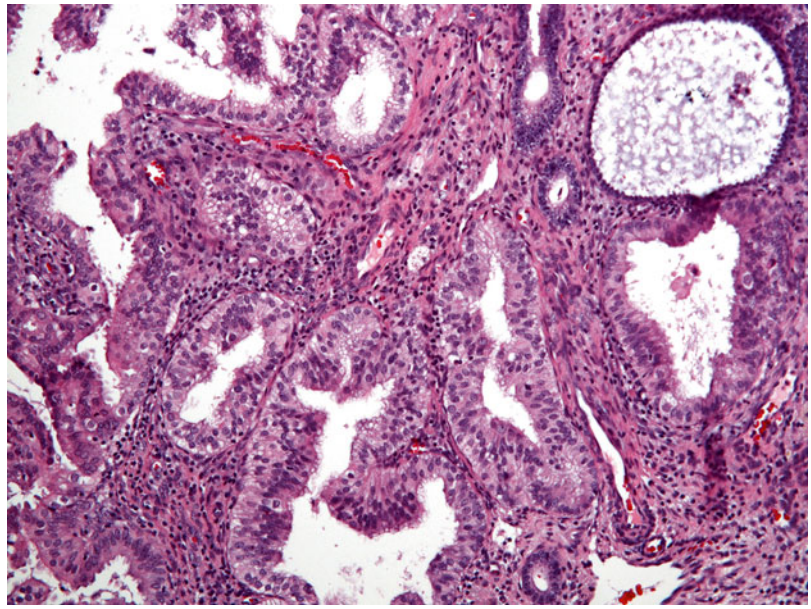

Fig. 7.19 Secretory EIN/AH often displays secretory changes within the glands as well as bland cytology. Normal endometrial glands are present in the *upper right* aspect

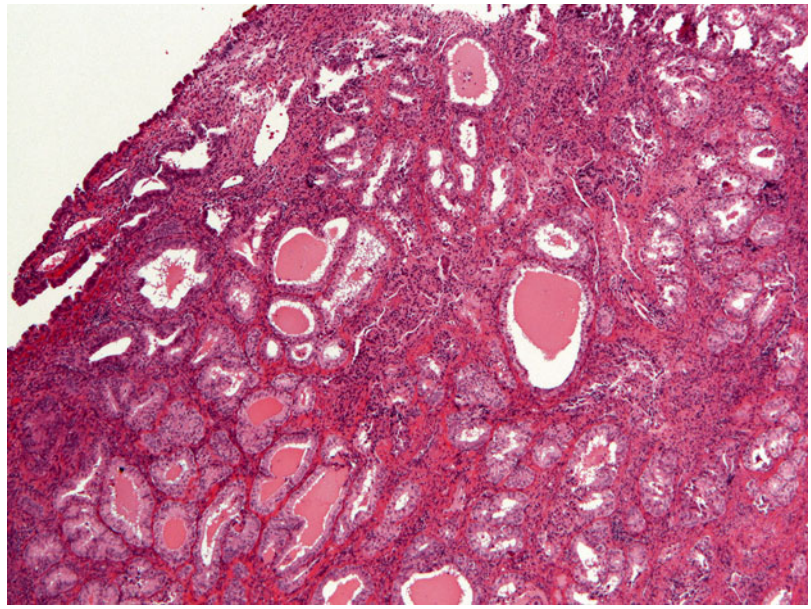

Fig. 7.20 Low power of secretory EIN/AH will often demonstrate a more orderly glandular proliferation when compared to the background secretory glands, which may resemble proliferative endometrium. Dense eosinophilic secretions are often present

lack of mitotic activity) (Fig. 7.22). If squamous morules are part of the (pre-) cancerous process, hormonal therapy will cause involution of the glandular components but not the hormone-receptor negative squamous elements. However, not all precancerous or cancerous endometria show a complete response to progestin creating diagnostic difficulty. Therefore, it is best practice for the pathologist to compare the surveillance biopsy to the patient's previous endometrial sample(s) and describe in a comment which morphologic changes are seen. If persistence of a precancer or cancer is identified, in our practice, a diagnosis of "residual endometrial

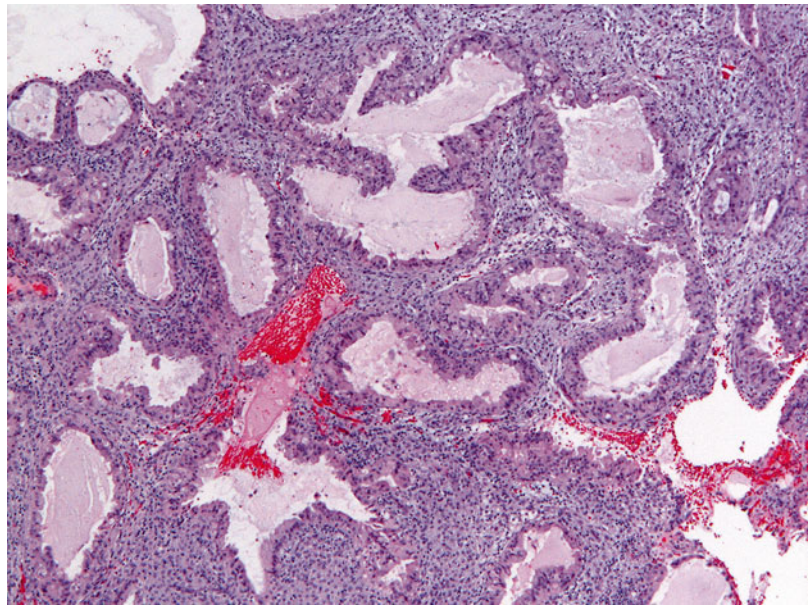

Fig. 7.21 Progestin-treatedEIN/AH often displays a decrease in the amount of crowding. Additionally, the involved glands often show a striking eosinophilic or mucinous change

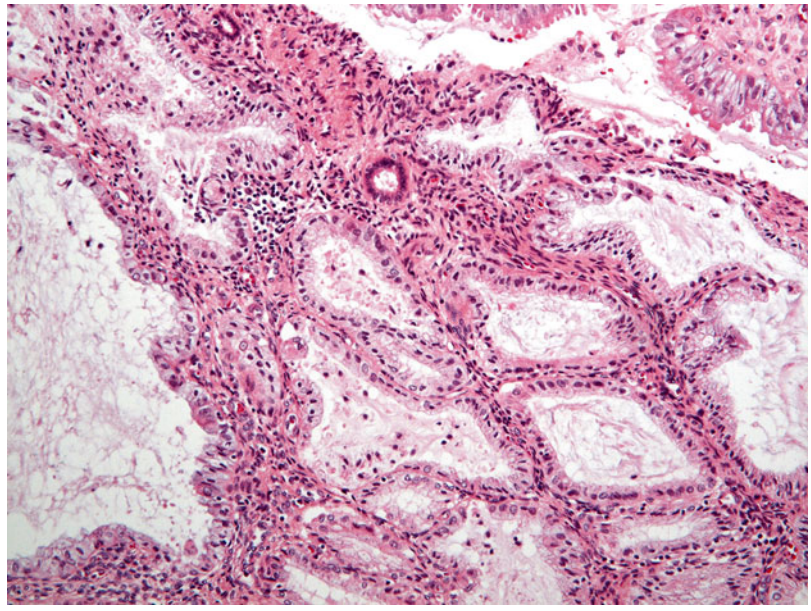

Fig. 7.22 Eosinophilic and mucinous changes in EIN/AH treated with progestins. Note the presence of a single uninvolved, benign gland

intraepithelial neoplasia with progestin effect (see comment)" is rendered. Relaying detailed information on resolution, persistence or even progression of the original lesion is crucial to determine if definitive surgical intervention is required [48, 55].

Subdiagnostic Lesions

Occasionally, the pathologist will encounter endometrial lesions which fulfill some but not all criteria for the diagnosis of EIN/AH. For example, a common dilemma is the presence of a focus

of crowded glands with cytologic demarcation which fails to meet the minimum size requirement. This may represent an under-sampled EIN/AH which is better seen after obtaining additional levels. If deeper levels do not resolve the diagnostic dilemma, it is our practice to designate subdiagnostic lesions as "focus of gland crowding with altered cytology" with a comment that not all the criteria for EIN/AH are met in this sample and that re-biopsy in 3–6 months is recommended. Huang et al. studied the clinical outcome of such ambiguous foci, which represented 0.3 % of their institutional reports from 2001 to 2009 [56]. Twenty-three percent (33 out of 143) of cases designated as "gland crowding" had a subsequent neoplastic process (EIN/AH or carcinoma). The authors of this study reemphasized that these rare subdiagnostic lesions do not represent pre-EIN/AH and are not part of the EIN/AH reporting scheme. Instead, these diagnostically challenging lesions should be reported descriptively with emphasis on clinical follow-up and re-sampling within 1 year.

Value of Immunohistochemistry in the Diagnosis of EIN/AH

Application of the EIN/AH scheme can be challenging in the setting of obscured or sparse non-lesional background endometrium, highly fragmented samples, altered differentiation, or a secretory background. Therefore, biomarkers may be of diagnostic value in select circumstances to aid identification of precancerous lesions with a high risk of progression to carcinoma. In addition, biomarkers have the potential value of training the eye of a pathologist who is first learning the EIN/AH system. Two such biomarkers, PTEN and PAX2, have been previously investigated in endometrial precancers.

PTEN

PTEN is a tumor suppressor gene regulating cell proliferation in the endometrium. PTEN is expressed in normal endometrium (nuclear and cytoplasmic staining). Its inactivation is seen in up to 49 % of histologically normal endometrial glands, in 44–63 % of EIN (Fig. 7.23) and 68–83 % of endometrial carcinoma [27, 30, 31]. Some of the histologically normal-appearing glands with sporadic inactivation of PTEN are shed during menstruation. It is the persistence of these glands over time that puts the endometrium at risk for clonal expansion and additional genetic alterations. PTEN undoubtedly plays a strong role in the progressive development of EIN/AH and endometrioid adenocarcinoma; however, the frequent PTEN inactivation in "normal" endometrium limits its potential as a useful biomarker. In addition, the technical and interpretative challenges of thePTEN antibody (nuclear and cytoplasmic staining) have further prevented its application.

PAX2

The PAX2 gene product is a nuclear transcription factor; it plays an important role in the embryologic development of the mullerian and urogenital system. PAX2 is expressed in normal endometrium. Monte et al. showed loss of PAX2 in 36 %, 71 %, and 77 % of normal, precancerous, and cancerous endometrium, respectively [31]. Simultaneous loss of PTEN and PAX2 may in fact represent a key causal event in the evolution of clonal precursor lesions of the endometrium [31]. In contrast to PTEN, the PAX2 biomarker is technically robust and shows strong nuclear expression in normal endometrial glands. Loss of nuclear staining can be used to assist in the identification of clonal endometrial precursor lesions.

Few studies have explored the utility of PAX2 in the diagnosis of precancerous lesions of the endometrium [31, 53, 57–59]. Quick et al. utilizing the EIN scheme found PAX2 a helpful and technically robust biomarker. PAX2 nicely delineated EIN lesions due to its loss of nuclear staining in lesional glands compared to the strong nuclear staining in non-lesional background endometrium (Fig. 7.24). PAX2 proved valuable when background glands were difficult to iden-

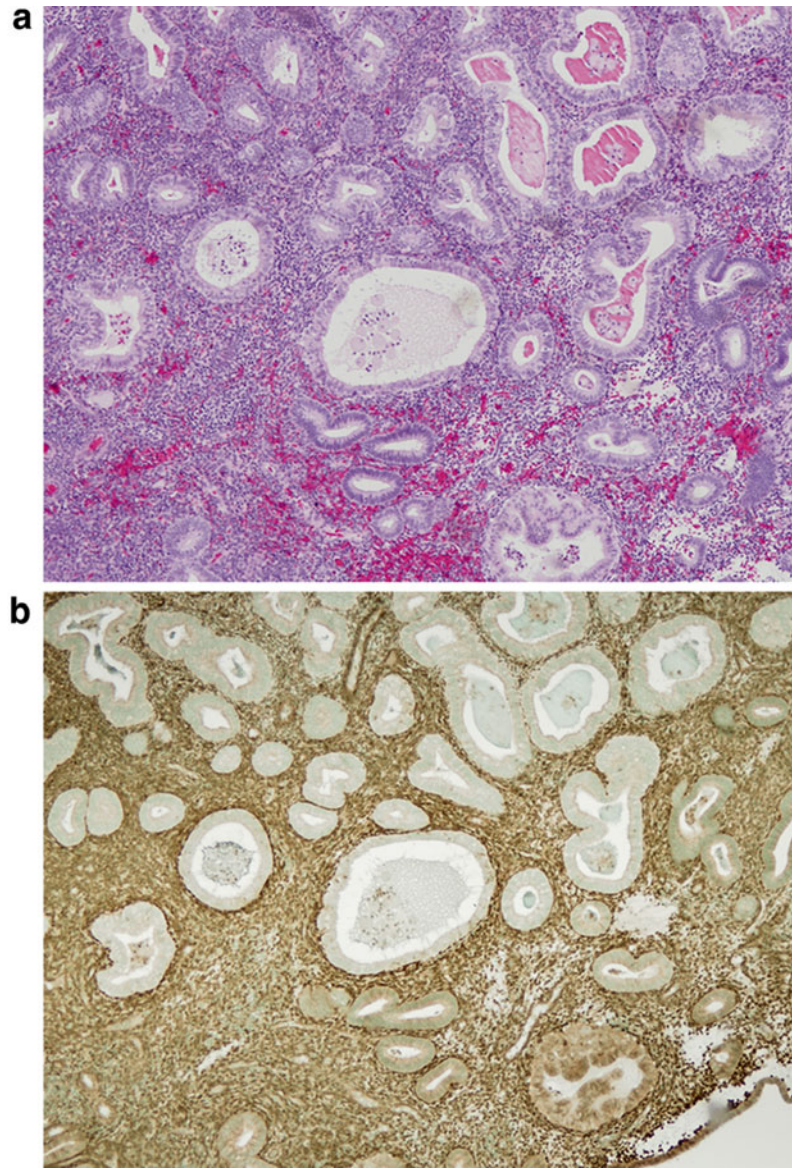

Fig. 7.23 Cytoplasmic PTEN loss in EIN/AH may be a helpful immunohistochemical adjunct (Photo courtesy of Dr. George L. Mutter)

tify (so-called over-run EIN) and in biopsies with a background of secretory endometrium. The authors concluded that PAX2 is a helpful adjunct immunohistochemical stain, particularly for pathologists who are learning to apply the EIN scheme for the first time [57]. Allison et al. classified endometrial precursor lesions using the 1994 WHO system; the authors scored PAX2 loss as complete (0 % cells staining), partial (1–75 % cells staining), and minimal to no loss (76–100 % cells staining) [58]. PAX2 loss correlated with the increasing severity of the hyperplasia but was less helpful in distinguishing hyperplasia with atypia from hyperplasia without atypia. In a recent publication by Joiner et al., the utility and expression of PAX2 were studied in a side-by-side comparison using both the 1994 WHO and EIN endometrial precancer classification systems in a single study [59]. In contrast to Allison et al., the authors classified PAX2 expression as normal (retention of nuclear staining compared to background endometrium) or altered

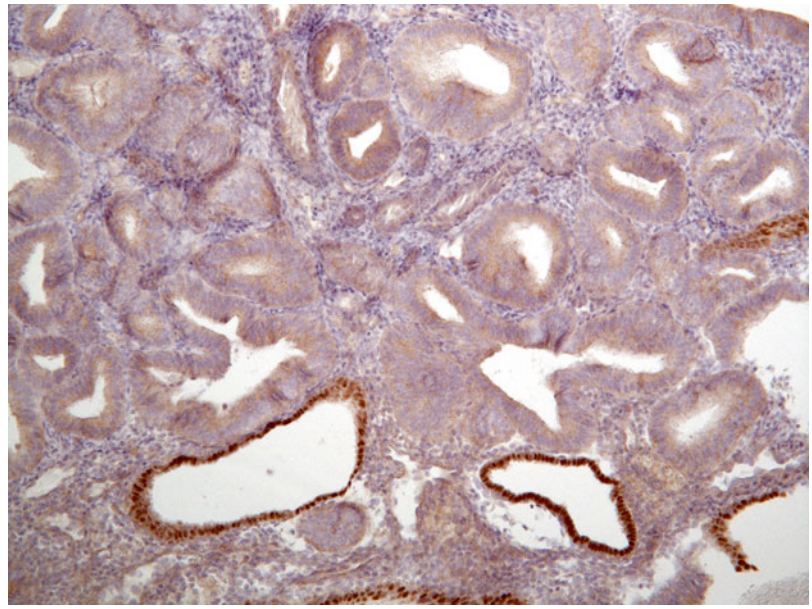

Fig. 7.24 Nuclear PAX2 loss in EIN/AH may be helpful in difficult cases

[complete loss (null), decreased, or increased staining compared to background endometrium]. The most frequent alteration in the study (86.3 %) was complete loss of nuclear staining of PAX2. In addition, PAX2 alterations were seen in most cases of EIN (33/36, 92 %) compared to benign hyperplasia (2/13, 15 %) and showed overall a better correlation with EIN than with the 1994 WHO classification.

Caution should be utilized regarding the routine use of PAX2 as one-third of normal endometrial glands can demonstrate sporadic inactivation of PAX2. However, the literature has shown that PAX2, when carefully applied to select cases, can be a valuable adjunct stain and training tool when the features of EIN/atypical hyperplasia are a diagnostic consideration.

Workflow/Approach to Endometrial Sampling

Most endometrial biopsies and curettages are performed for abnormal uterine bleeding. The surgical pathologist must correctly identify which patient's symptoms are related to an underlying precancer or cancer compared to those who are bleeding for other reasons. The "other" category is diverse and includes diagnostic entities such as atrophy, submucosal leiomyoma, endometrial polyp, or estrogen-driven changes such as disordered proliferative endometrium and hyperplasia without atypia. A systematic approach to the endometrial sample at hand is most useful. We recognize that the 1994 WHO classification system has been widely adapted and applied over the last 10 years. Within this scheme, the approach to an endometrial sample consisted of first assessing for gland-to-stroma ratio. If increased, the extent of architectural crowding and complexity determined the designation of simple or complex hyperplasia. The next and most crucial step was high-power examination of lesional glands to evaluate for the presence or absence of cytologic atypia.

With the adaptation of the EIN/AH scheme, pathologists must consciously retrain their minds to a new diagnostic approach. The first step remains the low-power assessment for an increase in the gland-to-stroma ratio (for practical purposes, greater than one). The next step is to identify if cytologic demarcation is present: the pathologist must search for the patient's native endometrium and compare its cytologic features (nuclear and/or cytoplasmic) to the cytologic appearance of the crowded glands. So-called

atypia as previously defined by the 1994 WHO scheme is not a necessity. Rather, it is simply the difference in cytologic appearance between the crowded glands and native endometrium that fulfills the requirement for demarcation. In conjunction with the minimum size criterion of 1 mm and exclusion of benign mimics and carcinoma, the diagnosis of EIN/AH is subsequently established. Immunohistochemical staining with PAX2 may be of value in select cases. Online training tools are available at www.endometrium.org [60].

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Precancerous Lesions of Endometrial Serous Carcinomas

8

Oluwole Fadare and Wenxin Zheng

Introduction

Although endometrial serous carcinomas (ESCs) only represent approximately 10 % of endometrial carcinomas [1], they are estimated to account for 39 % of endometrial cancer-associated mortality [2]. 33–52.6 % of ESC cases are found to be confined to the uterus after comprehensive surgical staging [3–5], and 16–25 % of all cases are confined to the uterine corpus without myometrial invasion [6–8]. It is now well recognized that tumors in the latter group may show extrauterine extension [9–12], hence the recommendations that all patients with ESC be surgically staged in a comprehensive manner [5, 13–15].

The overall survival for ESC patients with true stage I disease after comprehensive staging is relatively favorable and ranges from 83 to 100 % [3, 9, 12]. In contrast, patients with advanced stage disease have a 5-year survival of less than 40 % [3, 8], which has been attributed to an admixture of chemoresistance, metastatic manifestations at distant sites, and/or other factors [13]. These observations highlight the fact that improvements in the survival of ESC patients are likely to be most efficacious by identifying tumors at their earliest possible stage of development and instituting the most appropriate management once so identified. Serous carcinomas in the female genital tract, in general, display an intrinsic capacity for metastases once they are morphologically recognizable as a carcinoma, irrespective of their size or growth patterns at their primary site. Therefore, the ultimate task that eliminates the possibility of metastases is to identify the precancerous lesion and ablate or remove it [13]. The spectrum of putative precancerous and precursor lesions for ESC that has been proffered during the last quarter century is embodied in the model of ESC development that was recently proposed [16, 17]. In this model, serous carcinomas originally start as p53 signatures [18–20], followed by the true *pre*cancer (endometrial glandular dysplasia) [21–26], the architecturally intraepithelial precursor lesion (serous endometrial intraepithelial carcinoma) [27–32], and the well-developed conventional neoplasm [16, 17]. In this section, the authors comprehensively appraise the various components of the model [1–78].

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Serous Endometrial Intraepithelial Carcinoma

Historical Evolution and Nomenclature

ESC was formally described as a distinct clinicopathologic entity in 1980 [33]. However, the first description of a related intraepithelial lesion was possibly by Lee and Belinson in a 1990 report [27]. In their series of 227 consecutive operable clinical stage I endometrial carcinomas, the authors noted that 25 % of recurrences were unassociated with myometrial or lymphovascular invasion and that recurrence in these "noninvasive cases was strongly associated with ESC, even when present only focally or manifested by typical cytological features in the absence of well-formed papillae" [27]. Sherman first applied the term "intraepithelial carcinoma" to describe a lesion that was associated with 89 % of their cases of ESC. This lesion was defined by surface endometrium or glands composed "of cytologically malignant cells closely resembling the (associated) invasive serous carcinoma" [28]. Morphologically similar lesions were subsequently described as endometrial intraepithelial carcinoma [30], endometrial carcinoma in situ [29], or uterine surface carcinoma [31]. The term "serous EIC" was introduced in the 2003 classification of the World Health Organization, in recognition of the invasive tumor the lesion definitionally resembles [34]. The aforementioned reports all described EIC as displaying the same basic clinicopathologic profile: (1) a strong association with ESC (or mixed endometrial carcinomas with a serous component) that accordingly resulted in them been seen in the same age groups, (2) an apparently intraepithelial or surface growth pattern, and (3) foci that may be present adjacent to the tumor, isolated from the tumor, and/or diffusely present. Wheeler et al. [11] introduced the term "minimal serous carcinoma" to describe lesions that include serous EIC or superficial, non-myoinvasive ESC less than 1 cm. The authors noted that the pathologic distinction of serous EIC from superficial, non-myoinvasive ESC (i.e., ESC with only endometrial stromal invasion as inferred from glandular crowding or glandular confluence) is difficult and lacks clinical significance [11, 36].

Morphology and Differential Diagnosis

Changes of serous EIC are typically seen in glands or surface epithelium and are frequently multifocal. The glands are usually separate from other similar glands and generally do not display an abundance of crowding or confluence. By definition, serous EIC must be lined by epithelial cells whose features render them morphologically indistinguishable from the conventionally invasive serous carcinoma if viewed at high magnification. Thus, the cells may variably display severe pleomorphism, hobnail nuclei, hyperchromasia with coarse chromatin pattern, nucleolomegaly, intraglandular budding, eosinophilic cytoplasm, and abundant mitotic figures, including "atypical" forms (Figs. 8.1, 8.2, 8.3, and 8.4). A given case may display the aforementioned in a "glandular" pattern, a "flat" pattern in the overlying epithelium, or both (Figs. 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, and 8.8). Lesions may show abrupt transition to background endometrium or display transitional areas to EmGD, which then transition to background endometrium (Figs. 8.5 and 8.6). Single glands may also show either or both interfaces. Serous EIC has a distinct association with endometrial polyps, especially in the postmenopausal population [65–67]. In one study of 40 minimal serous carcinomas, 21 cases (52.5 %) were associated with an endometrial polyp [12].

The histologic differential diagnosis includes papillary syncytial metaplasia (PSM) with degenerative atypia [56–58], radiation-associated atypia, or metastases. The overlap between PSM and serous EIC is related to potentially significant atypia and increased expression of p16 and p53, concomitant with decreased expression of hormone receptors in the former [56–58] (Figs. 8.9 and 8.10). However, unlike serous EIC, PSM lacks mitotic figures, has a low proliferative

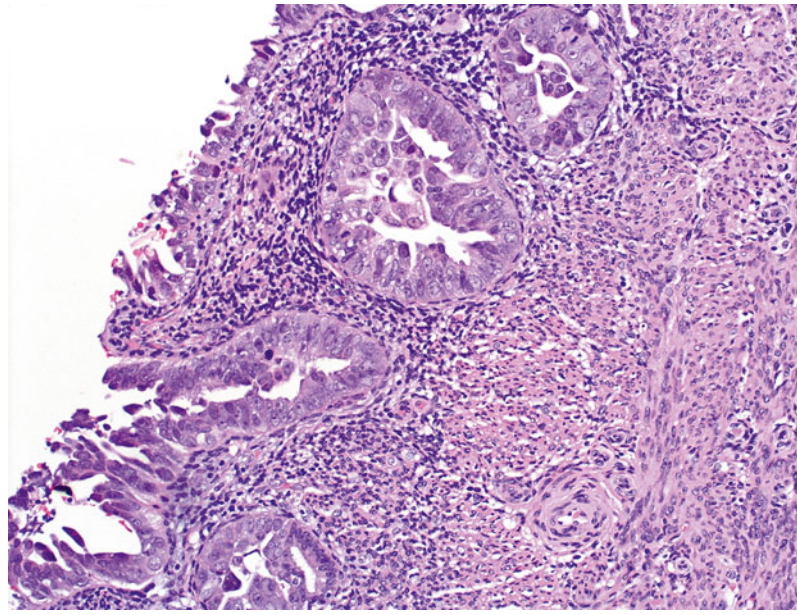

Fig. 8.1 Serous EIC in a glandular pattern and flat pattern

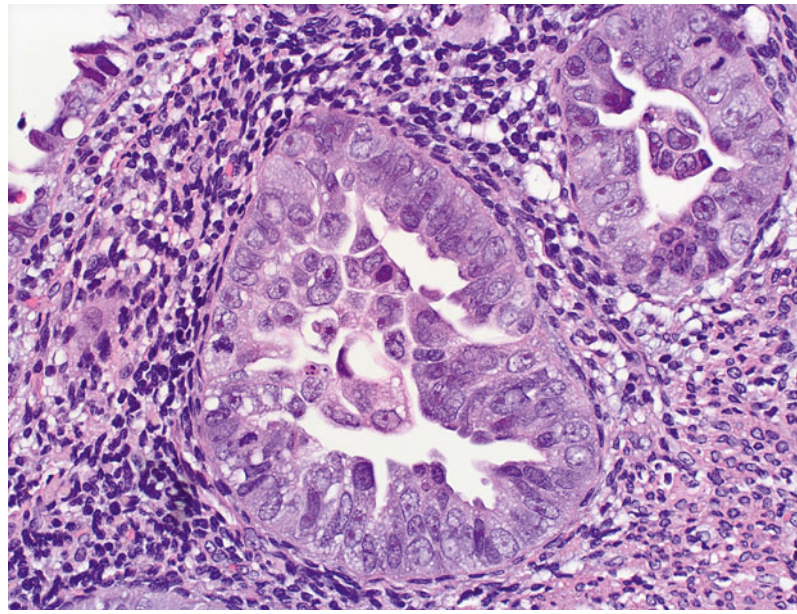

Fig. 8.2 Serous EIC, glandular pattern: cytologic features – a given case may variably show severe pleomorphism, hobnail nuclei, hyperchromasia with coarse chromatin pattern, nucleolomegaly, intraglandular budding, eosinophilic cytoplasm, and abundant mitotic figures

index, lacks expression of HMGA2, and has a wild-type pattern of p53 staining [56–58]. Radiation-associated atypia is usually more diffuse, lacks mitotic figures and has a low proliferative index, lacks a serous carcinoma-like immunophenotype, and does not display the intraglandular budding that is typical of serous EIC (Figs. 8.11 and 8.12). Endocervical adenocarcinoma in situ may also present as an intraepithelial lesion in an endometrial polyp or in background endometrium [63]. As detailed below, adnexal serous carcinomas may also present as minute or focal lesions in endometrial samples [64].

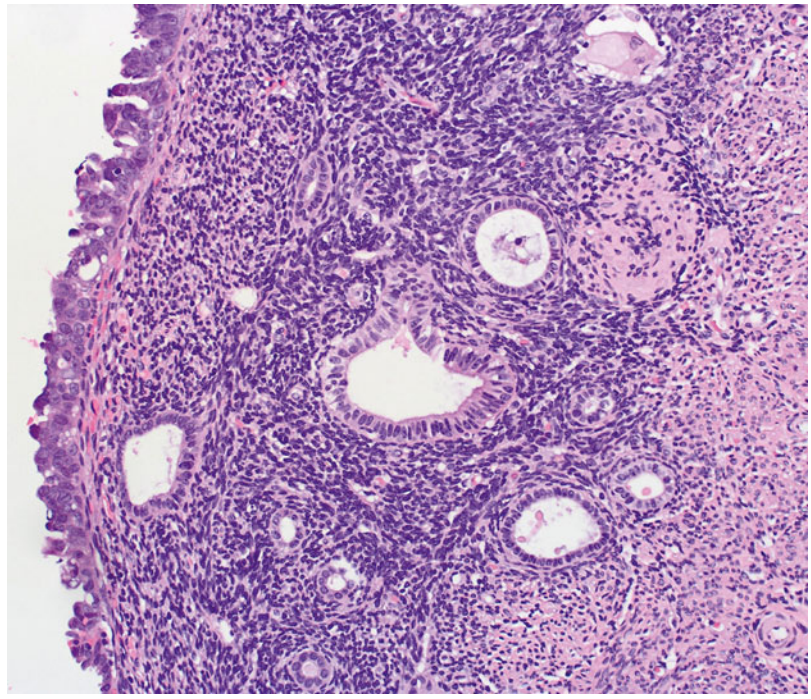

Fig. 8.3 Serous EIC in a flat pattern, left field

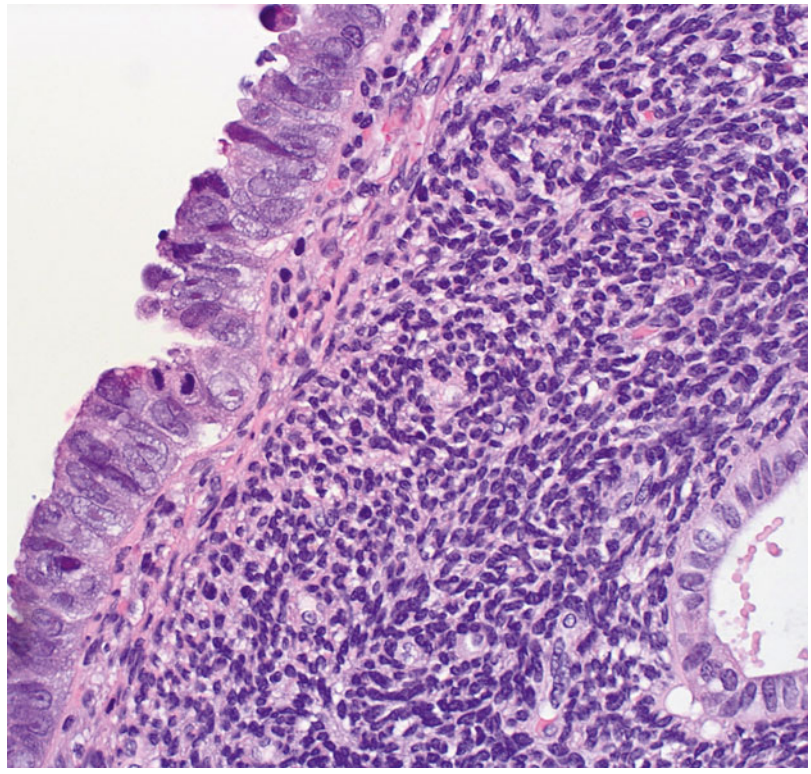

Fig. 8.4 Serous EIC in a flat pattern, cytologic features

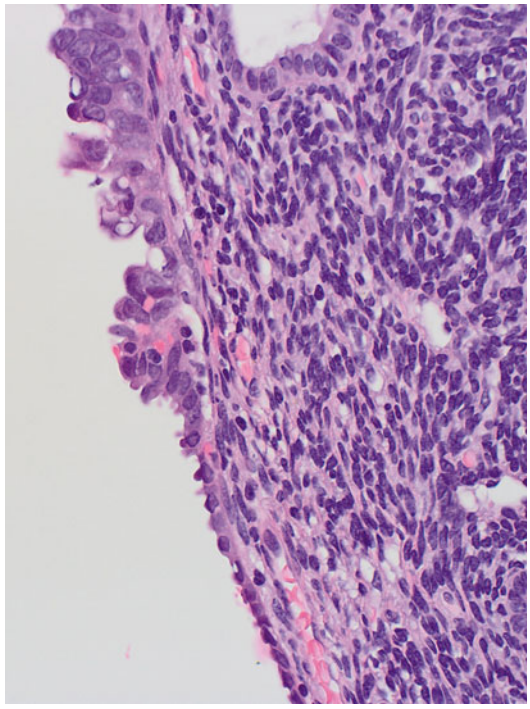

Fig. 8.5 Serous EIC (*upper field*) showing transition to EmGD (*midfield*) and to resting endometrium (*lower field*)

Serous EIC, Extrauterine Extension, and Pelvic Serous Carcinogenesis

The notion that non-myoinvasive or superficially invasive ESC may potentially show extrauterine extension had long been recognized, even from some of the earliest descriptions of ESC (1). Serous EIC has been identified in 58–89 % of ESC [28, 35]. Although EIC is rarely identified without concomitant ESC, analyses of small groups of patients have conclusively established that EIC may be associated with peritoneal carcinomatosis in the absence of any other serous neoplasm in the uterus [36, 37]. The frequency with which this occurs is not entirely clear. However, for the larger group of lesions defined as minimal or only very superficially invasive, up to two-thirds of cases may be associated with extrauterine extension [16]. Morphologically, the extrauterine deposits display the same features as the endometrial lesions [38] and are usually less than 2 cm each [39]. Similarly, both intrauterine and extrauterine lesions have similar immuno-

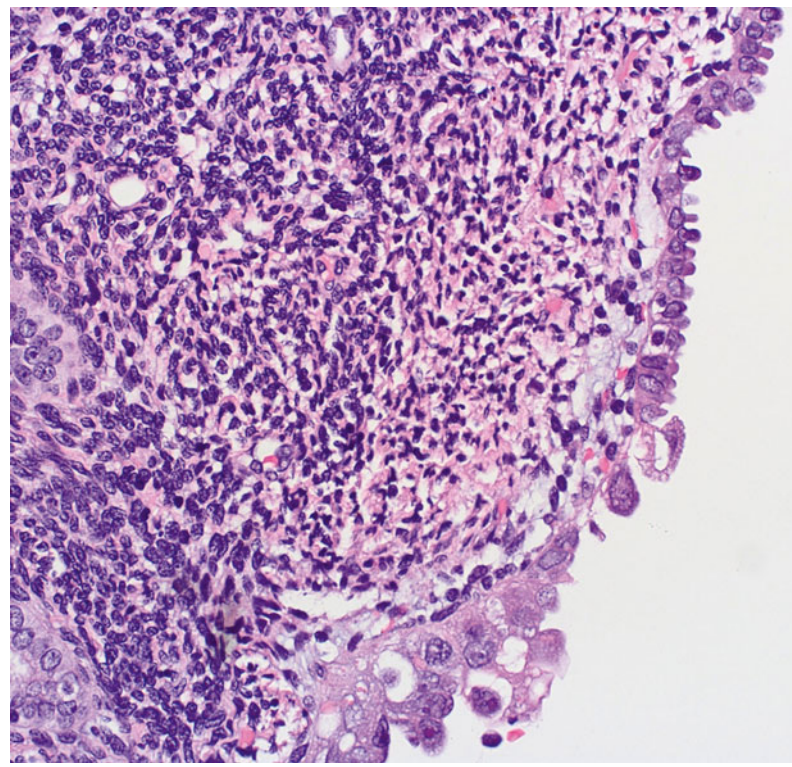

Fig. 8.6 Serous EIC (*lower field*) showing abrupt transition to resting endometrium (*upper field*)

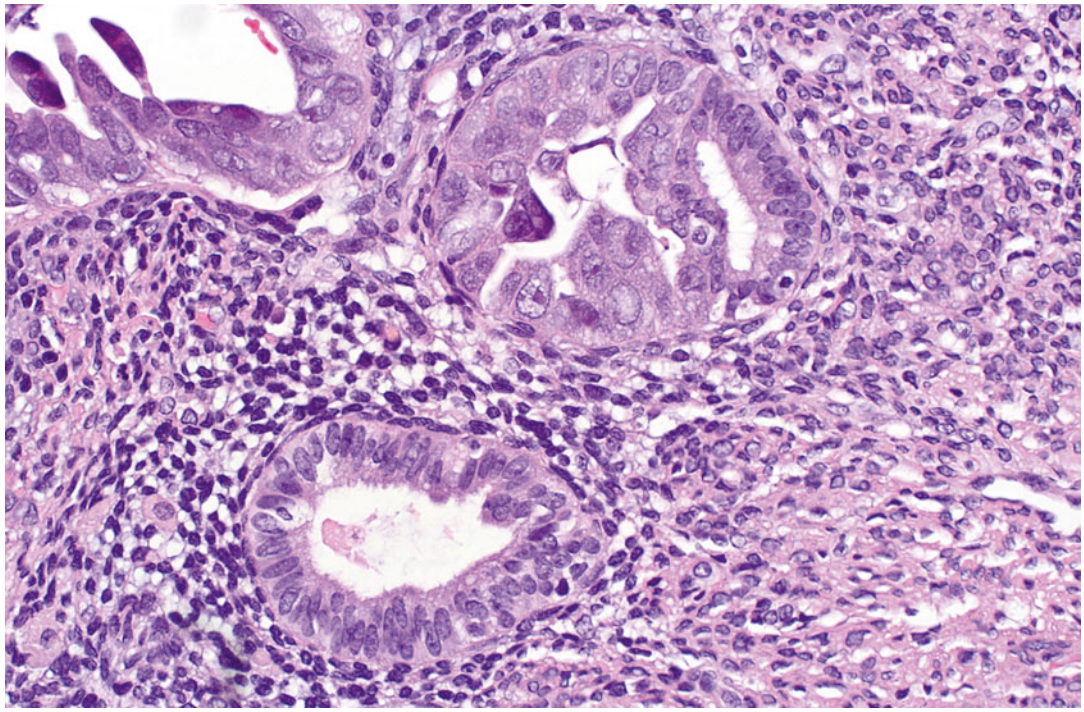

Fig. 8.7 Serous EIC showing abrupt transition to nonneoplastic cells within a single gland (*upper field*)

phenotypes regarding estrogen receptor, progestin receptor, p53, WT1, and Ki-67 expression [38]. At the molecular level, both uterine and extrauterine lesions within a given patient have been shown to display identical *TP53* mutations [39, 40]. However, the interrelationships between these lesions may be more complicated.

A very small subset of serous carcinomas in the endometrium may actually represent either drop metastases from the adnexa that manifest in the endometrium with an EIC-like intraepithelial growth pattern. Many of such lesions are associated with extrauterine serous carcinomas that are independent primaries as evidenced by the concomitant presence of corresponding precancerous lesions at the extrauterine site. It is known that some cases of EIC may be associated with serous tubal intraepithelial carcinoma (STIC) in the fallopian tube. In one study of 22 consecutive cases of serous carcinomas involving the endometrium [41], the fallopian tubes were submitted according to the SEE-FIM protocol [42]. 50 % of cases showed adnexal involvement, including five wherein a STIC was identifiable. In all five cases, the endometrial lesions tended to be noninvasive or only minimally invasive of the myometrium, and identical *TP53* mutations were shared by both tubal and endometrial lesions in a subset of cases [41]. In another study 8 % of ESC showed STIC when the fallopian tube was processed using the SEE-FIM protocol [35]. Extensive sectioning of the endometrium in patients with adnexal serous carcinomas has identified serous EIC in about 15 % of cases [43]. In our own recent analysis of samples from 21 patients with serous EIC, cellular lineage relationships between intrauterine and extrauterine lesions were assessed by *TP53* mutation analysis. Based on the patterns of concordant or discordant mutation for this gene, we concluded that the extrauterine disease associated with serous EIC may be from the endometrium (47.6 % of cases), adnexa (23.8 %), or both (28.6 %) [39]. All of the aforementioned findings, in total, suggest either

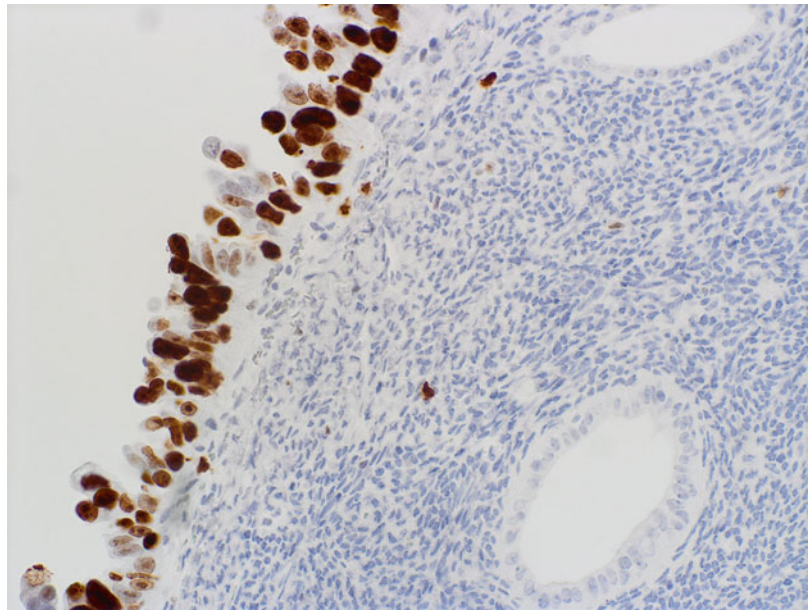

Fig. 8.8 Serous EIC showing aberrant pattern of p53 expression

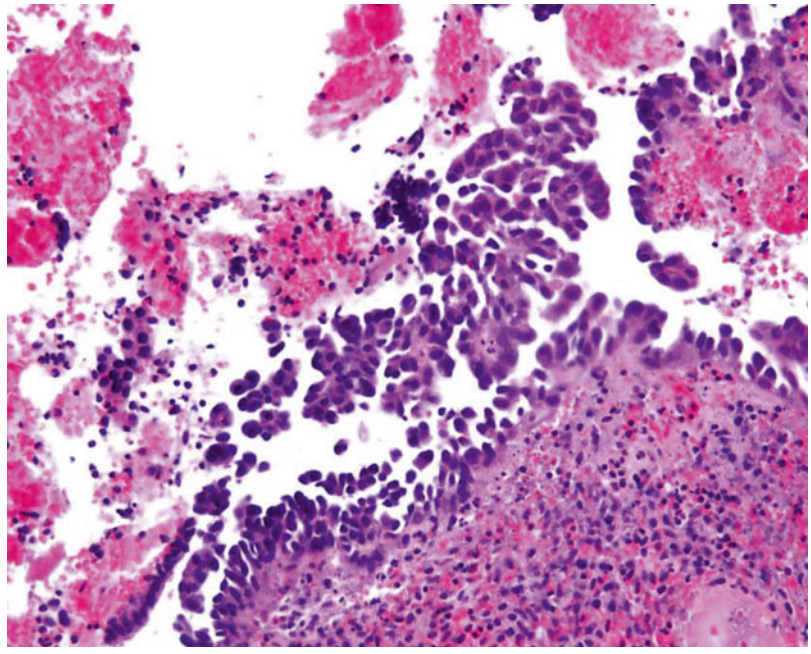

Fig. 8.9 Papillary syncytial metaplasia, a lesion in the differential diagnosis with serous EIC

that some cases of ESC (EIC or otherwise) are of extrauterine origin, that the primary lesion accordingly contributes to the extrauterine disease burden, or that both the intrauterine and extrauterine lesions are independent, being simply reflective of an increased propensity of serous carcinogenesis that is generalized in the upper genital tract.

Serous EIC as a Precursor Lesion

The conclusion by Ambros et al. in 1995 that serous EIC "is the likely precursor" for uterine tumors displaying serous differentiation was based on their intraepithelial growth pattern as well as their statistically significant, nearly exclusive association with carcinomas with a

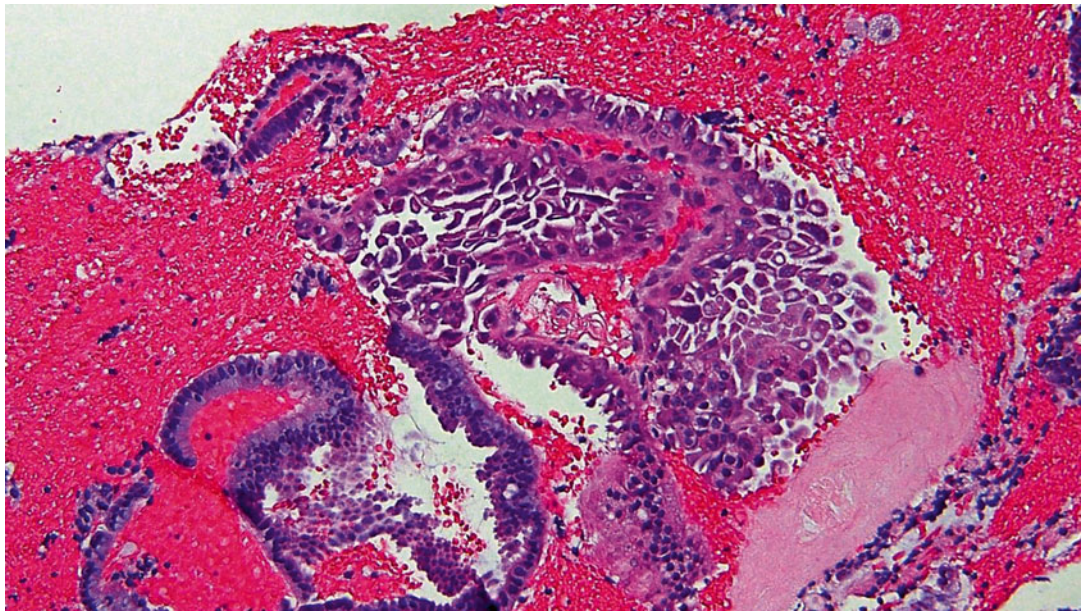

Fig. 8.10 Papillary syncytial metaplasia, a lesion in the differential diagnosis with serous EIC

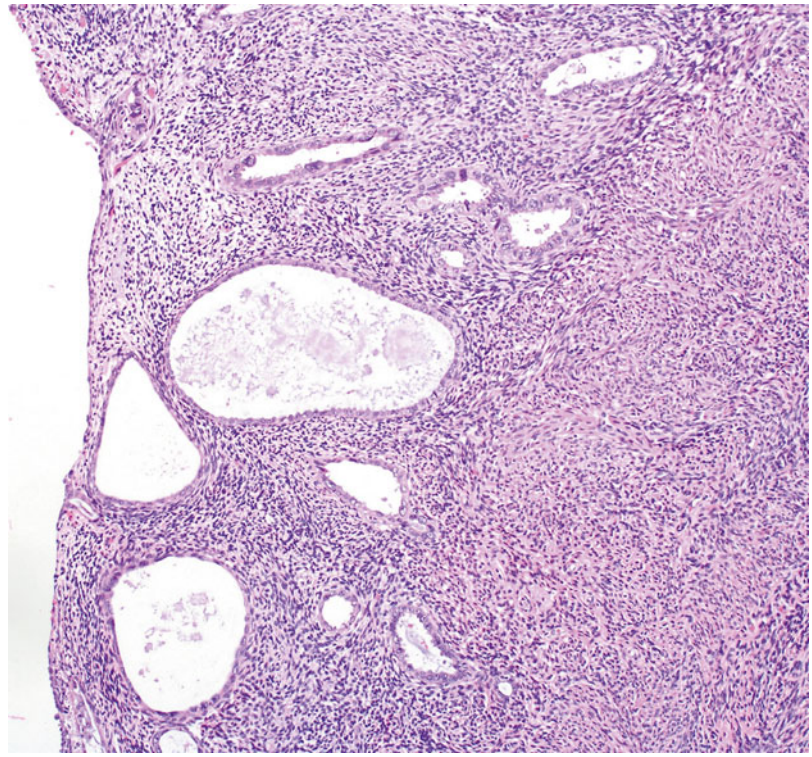

Fig. 8.11 Radiation-associated glandular atypia in the endometrium, a finding that is in the differential diagnosis with serous EIC

serous component [30]. Subsequently published studies have shown that serous EIC and ESC display broadly similar phenotypic patterns, including a comparable frequency of expression of proteins such as p53, p16, IMP3, HMGA2, Nrf2, hormone receptors, Cyclin E1, and HER2/

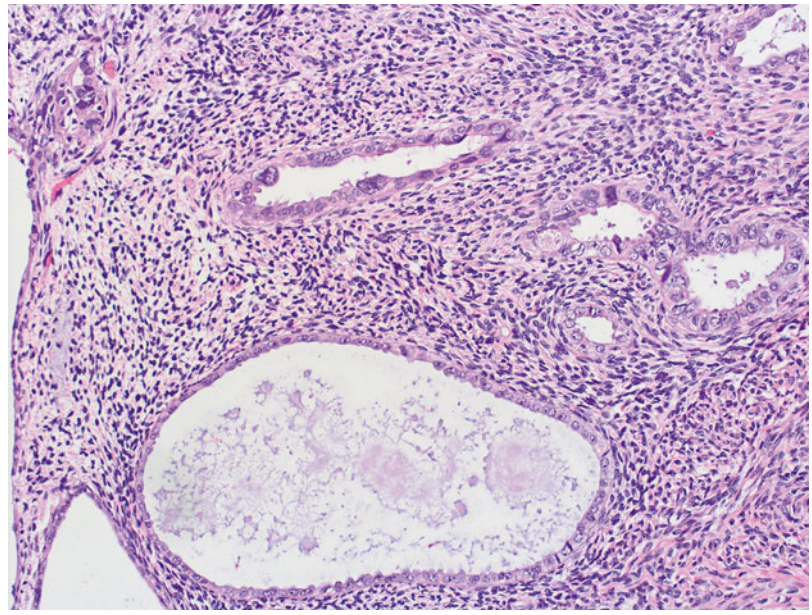

Fig. 8.12 Radiation-associated glandular atypia in the endometrium: Higher magnification of Figure 8.11

neu [44–51]. This is not unexpected, since serous EIC is definitionally composed of cells that are identical to ESC.

At the molecular level, serous EIC and ESC have been shown to be very similar and to share a broad molecular profile. Kuhn et al. [52] performed a comparative mutational analysis in samples from nine patients with serous EIC and ESC pairs. All nine pairs showed concordant *PIK3CA*, *PPP2R1A*, and *TP53* mutational profile, whereas eight of the nine pairs had concordant *FBXW7* mutation status between these two components. The single discordant pair contained a *FBXW7* p.Asp440Asn mutation that was absent in serous EIC but present in the associated ESC [52]. *FBXW7* mutation may be associated with Cyclin E1 overexpression at the protein level, as can *CCNE1* amplification, the underlying gene for the Cyclin E1 protein. It has subsequently been shown in another paired analysis that 45 % of ESC and 41 % of serous EIC showed *CCNE1* amplification as assessed by fluorescent in situ hybridization and that there was generally a high concordance in *CCNE1* copy numbers between the pairs [53]. However, there is evidence that although serous EIC and ESC are similar, they are not identical, and this may lend credence to the postulation that serous EIC precedes ESC. The frequency of *TP53* mutations is generally higher in ESC than in serous EIC (72–78 % in serous EIC vs. 90–96 % in ESC) [24, 54]. Loss of the wild-type p53 allele, as inferred from loss of heterozygosity at chromosome 17p, has been observed in 100 % of ESC and in 43 % of serous EIC [54]. Occasional cases of serous EIC and their associated ESC display discordant *TP53* mutations (7 % of cases in our analysis [24]). Additionally, as previously indicated, *FBXW7* mutations in the same patient are also occasionally discordant between these lesions. Also supporting the concept that serous EIC precedes ESC is the morphologic observation of their growth pattern and size. It is logical that a small, highly proliferative lesion with an intraepithelial growth pattern will eventually become a large lesion with an invasive growth pattern. Accordingly, serous EIC is placed before ESC in our model and is its likely precursor [16, 17]. However, this placement of serous EIC is merely a conceptual component of the framework by which endometrial serous carcinogenesis can be studied and understood. For practical and clinical purposes, this distinction is irrelevant, since the evidence indicates that serous EIC is already a cancer with significant potential for metastasis [36, 37], rather than a true precancer that is associated with an increased risk of cancer but which is in and of

itself not malignant [55]. Serous EIC, when identified alone, is therefore best conceptualized as ESC at an early pathologic phase and/or with an unusual intraepithelial growth pattern, and patients with serous EIC should be managed in the same manner as their counterparts with the conventionally invasive neoplasm [13, 32].

Endometrial Glandular Dysplasia

Studies published during the past decade have established endometrial glandular dysplasia (EmGD) as the most likely precancerous lesion for ESC [21–26, 59]. EmGD was originally described in 2004 [21], following works predicated on the hypothesis that there is a morphologically identifiable lesion that precedes serous EIC and which is precancerous. In the seminal reports that described the entity, the endometrium adjacent to a cohort of serous EIC, ESC, and endometrioid carcinoma cases was evaluated in detail. Atypical glandular lesions were identified 53 % of the ESC cases and 1.7 % of the endometrioid carcinomas [21]. These lesions were then studied and form the basis of the EmGD entity. The average age of the patients with an EmGD lesion was 65 years (range 57–79 years) [21].

Morphology, Immunophenotype, and Differential Diagnosis

EmGD are microscopic lesions, and it is rare for any single focus to exceed 2 mm. However, we have occasionally encountered them extensively involving an endometrial polyp. By definition, EmGD shows a level of atypicality that renders them distinct from the background endometrium, but which is not as severe as is characteristic of serous EIC (Figs. 8.13, 8.14, 8.15, 8.16, 8.17, 8.18, and 8.19). This atypicality is manifested in

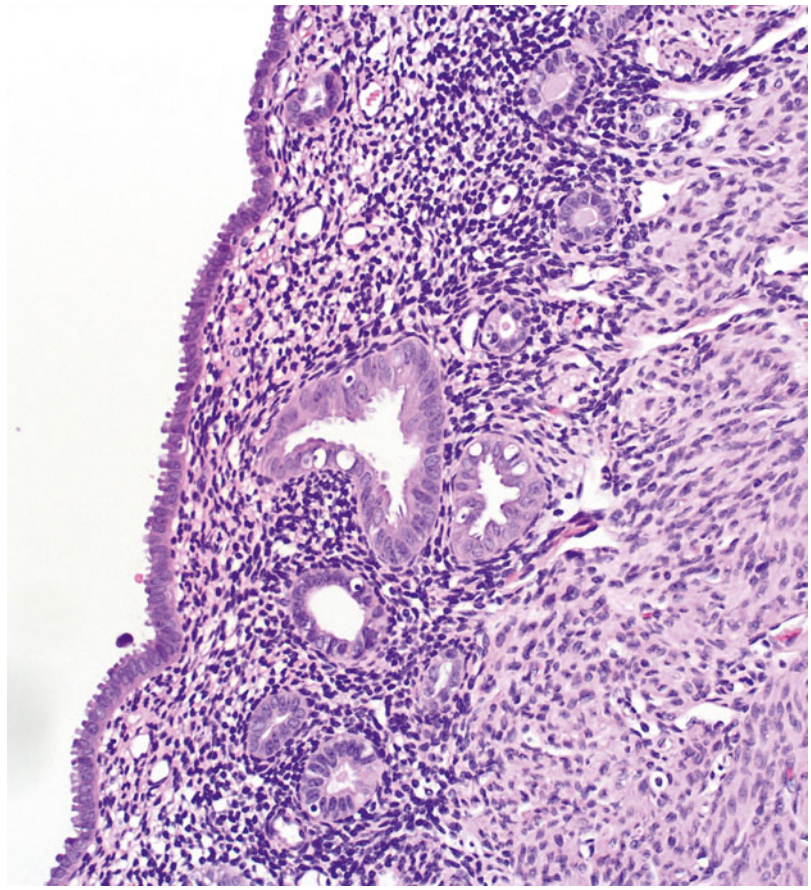

Fig. 8.13 A focus of EmGD, represented here as a pair of glands (center field) showing notably increased atypia above the background and overlying epithelia

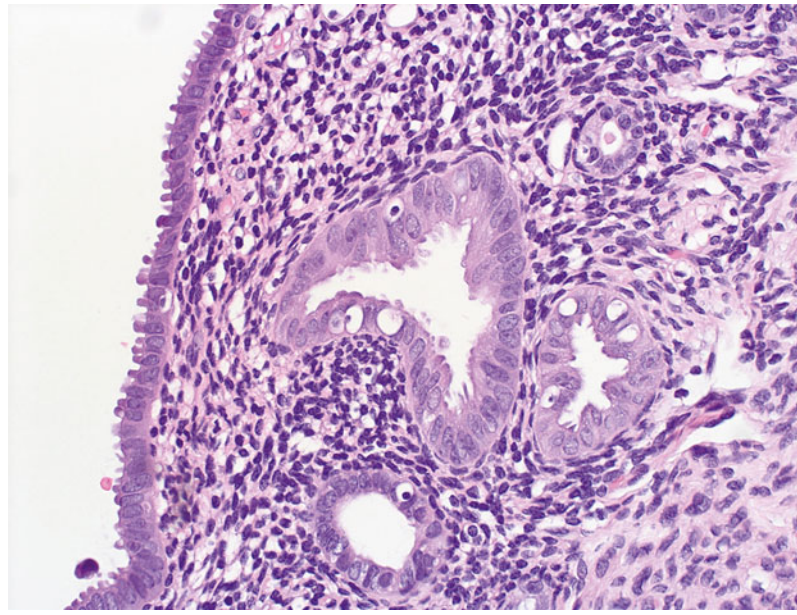

Fig. 8.14 EmGD: higher magnification of glands shown in Fig 8.13

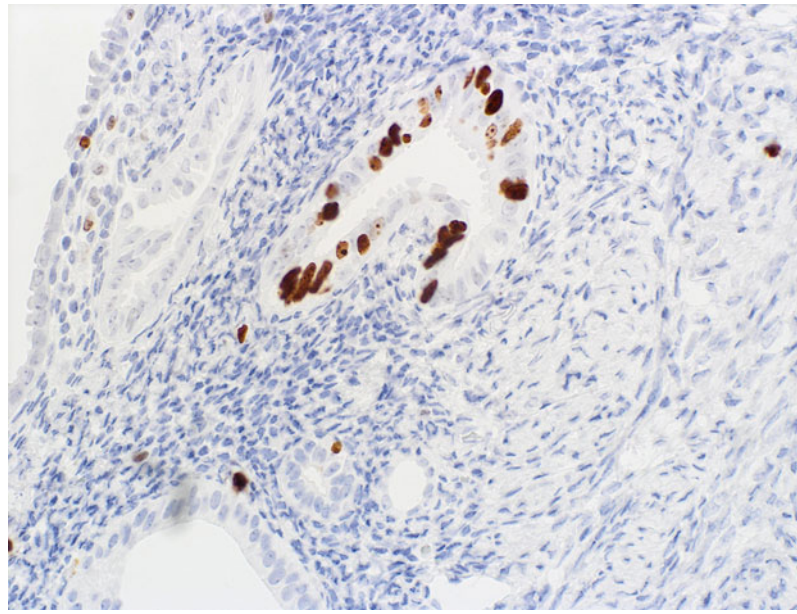

Fig. 8.15 A focus of EmGD showing increased proliferative activity relative to background epithelia, as assessed with MIB1 immunohistochemistry

oval to round nuclei that are typically two- to threefold enlarged as compared with the background resting endometrium (in which about 80 % of them are seen [21]). This is as compared with serous EIC cells, which are usually 4–5 times larger. EmGD may be in the form of single or clustered glands or in overlying flat epithelium. EmGD is frequently multifocal. Nucleoli may be seen but are not conspicuous. The chromatin pattern is variable. Mitotic figures and apoptotic bodies are not readily apparent [21]. Transitional areas between EmGD and serous EIC are frequently observed [21].

Most cases of EmGD are recognizable by paying careful attention to morphologic attributes. However, given that EmGD definitionally occu-

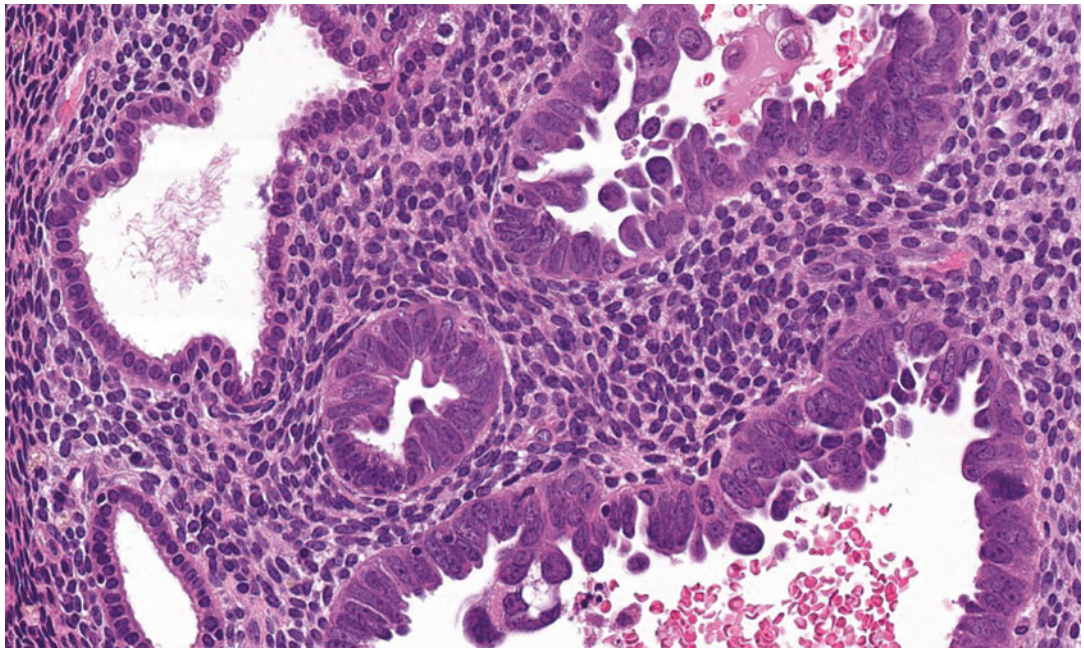

Fig. 8.16 EmGD (*middle gland*) compared with background endometrial glands (*left field*) and serous EIC (*right field*)

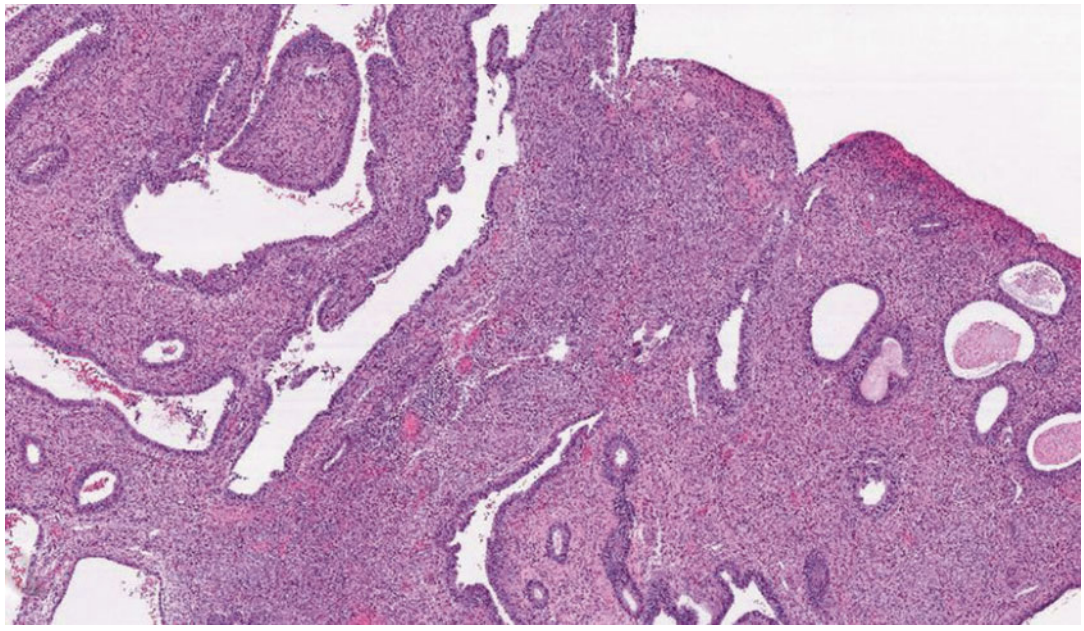

Fig. 8.17 A rare case of EmGD diffusely involving an endometrial polyp

pies a nebulous "intermediate" point between well-recognizable morphologies (resting endometrium and serous EIC), diagnostically problematic cases may be encountered. In these cases, which are most relevant in a biopsy or curettage, the differential considerations include a benign gland with reactive or metaplastic changes and EmGD. Immunohistochemical studies may be

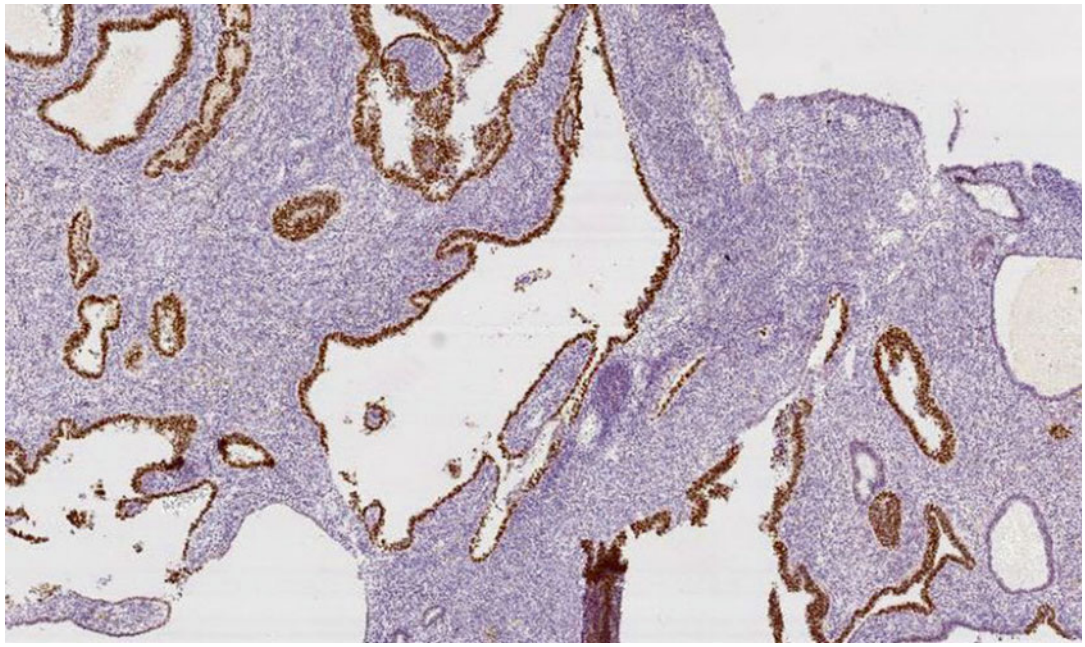

Fig. 8.18 A rare case of diffuse EmGD involving an endometrial polyp. This case showed diffuse expression of the p53 protein

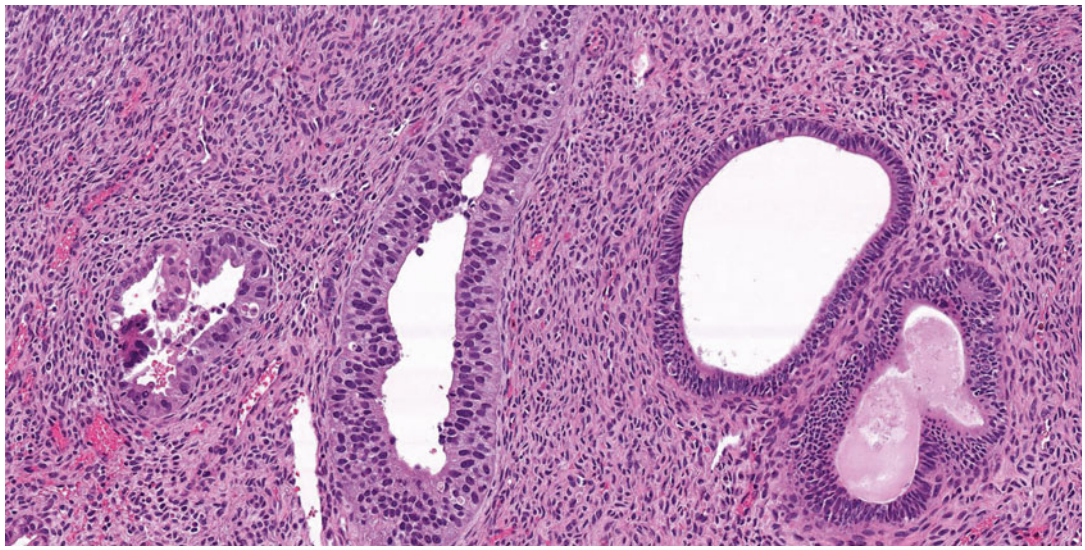

Fig. 8.19 Same case pictured in Figs. 8.17 and 8.18. Note cytologic differences between p53-expressing (*left field*) and p53 wild-type (*right field*) glands

useful in these scenarios. IMP3 may highlight these foci relative to background endometrium, but IMP3 is relatively insensitive for this purpose [48]. Their MIB1 proliferative index is typically distinct from the background [21] (Figs. 8.13, 8.14, and 8.15). Thus, comparison with the

background endometrium is important in assessing these foci, and if the background endometrium is not resting, MIB1 is less useful. p16 may be useful in delineating glands that are biologically abnormal but whose morphologic features are equivocal, since EmGD most frequently expresses p16 diffusely, whereas background glands show a "mosaic" pattern (Figs. 8.20 and 8.21). However, p16 must be utilized in conjunction with other markers, so that tubal metaplasias,

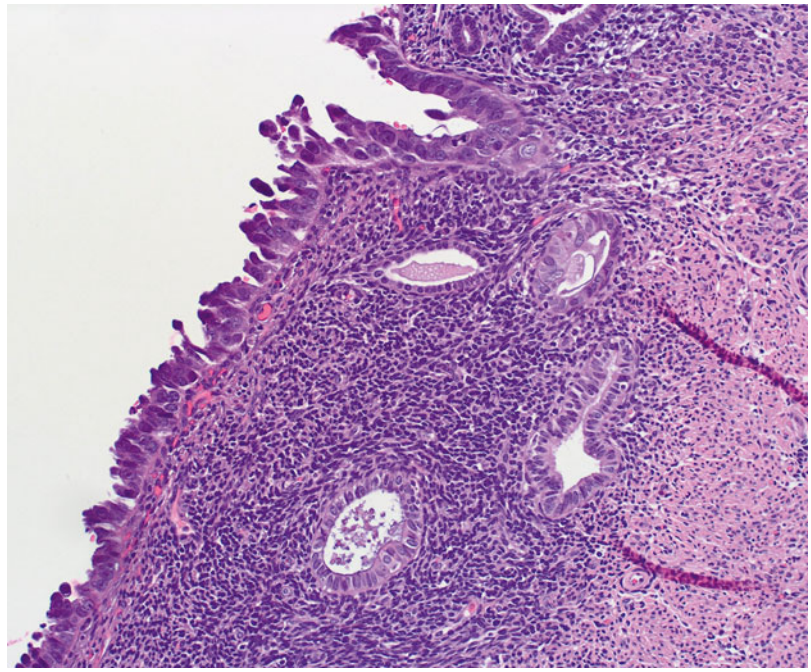

Fig. 8.20 Underlying the serous EIC (left field) are 2 subepithelial glands with mild atypia, which raises the diagnostic question of tubal metaplasia with degenerative nuclear changes versus EmGD

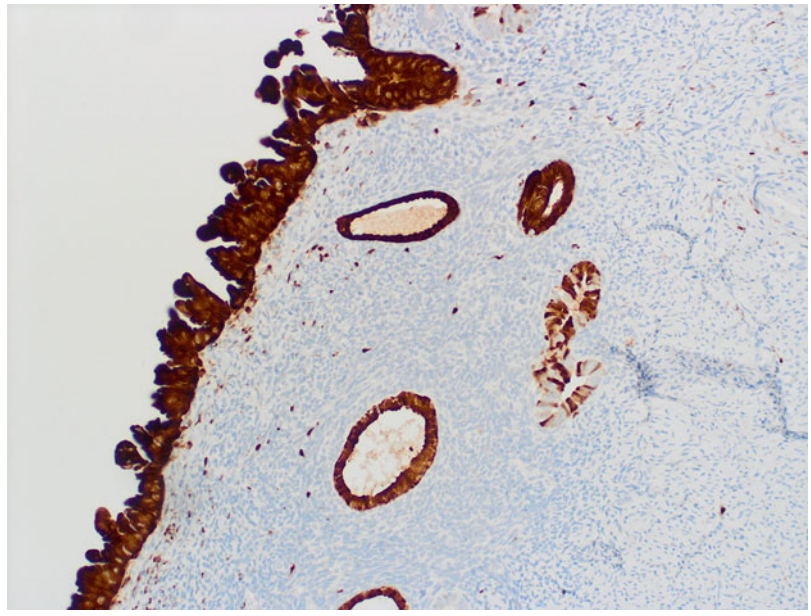

Fig. 8.21 Same case shown in figure 8.20, highlighting the limited utility of p16 immunohistochemistry in distinguishing tubal metaplasia with reactive changes from EmGD. p16 was diffusely expressed in the serous EIC, in some of subepithelial glands with tubal metaplasia, and in a "mosaic" pattern in the single subepithelial gland without tubal metaplasia (right field). p16 should always be used in conjunction with other markers in this context. In this case, the metaplastic glands showed no increase in proliferation as assessed by MIB1

which may be cytologically atypical and are usually p16 positive, are not mischaracterized as EmGD. In our original studies, p53 immunohistochemistry was also useful, since most of our EmGD cases had p53 staining scores that were intermediate between serous EIC and resting endometrium [21]. Based on current understanding of p53 staining patterns, only diffuse staining is acceptable as being indicative of an aberrant *TP53* status. Distinct and significant p53 staining in a putative EmGD (i.e., morphologically atypical) gland relative to the background is consistent with EmGD. Problems associated with the use of p53 include the fact that only 43 % of EmGD lesions display a *TP53* mutation [24] and the fact that a subset of *TP53*-mutated cases lack an intact protein and accordingly are p53 negative by immunohistochemistry. In general, EmGD foci also display reduction (relative to uninvolved glands) or loss of expression of the estrogen and progesterone receptors.

EmGD as a Precancer

In 2004, the US National Cancer Institute sponsored a consensus conference on precancers, and participants defined five criteria that putative precancers must meet: (1) there is a method by which the precancer can be diagnosed; (2) it differs from the background normal tissue from which it originates; (3) the precancer shares some but not all molecular and phenotypic attributes with the cancer into which it develops; (4) there is evidence that when the precancer develops into cancer, the cancer arises from cells within the precancer; and (5) there is some evidence that the precancer is associated with an increased risk of cancer [55].

The available evidence suggests that EmGD is the most likely precancerous lesion to ESC. As indicated in the sections on morphology and immunohistochemistry, there are modalities by which EmGD can be diagnosed, the lesions are definitionally distinct from the background endometrium and most frequently have a phenotype that is distinct from it as well. As such they fulfill criteria 1 and 2. Since *TP53* mutations appear to be one of the earliest molecular events in serous carcinogenesis, they offer a valuable framework for studying their putative precancers. Linkage analyses of the *TP53* gene provide supportive evidence that EmGD is a precancer. The load of *TP53* mutations tends to progressively increase from EmGD to serous EIC and ESC. In our study, the rates of *TP53* mutations in resting endometrium, EmGD, serous EIC, and ESC were 0 %, 43 %, 72 %, and 96 % [24]. The differences between EmGD and serous EIC or ESC were statistically significant [24]. Loss of heterozygosity at the chromosomal region for *TP53* and 1p is observed in 31 % and 18 % of EmGD, respectively, but in 78 % and 47 % of cancerous areas within the same uteri [22]. When EmGD, serous EIC, and ESC are present in the same uterus, the specific *TP53* mutations within these lesions are generally highly concordant [24]. Similar findings have been found by loss of heterozygosity analyses using microsatellite polymorphic DNA markers [22] or by HUMARA-based analyses [16]. These studies, especially the concordance of *TP53* mutations between EmGD and ESC/serous EIC within the same uteri, provide compelling linkage evidence that the cancers in these uteri originated from the EmGD, and the differences in mutational load indicate that EmGD displays some but not all the properties of the well-developed malignancy. Then, the latter can also be intuited from morphologic differences and frequency of expression of markers such as IMP3 (which are significantly more frequently expressed in the cancers) [48]. In mice, deletion of the *TP53* gene homologue Trp53 in an endometrium-specific fashion resulted in the development of a variety of type II endometrial malignancies. Analysis of the background, non-neoplastic endometrium in these mice identified a series of lesions whose features were diagnostic of EmGD and serous EIC [60]. Interestingly, in Trp53 knockout mice, the protein KPNA (karyopherin alpha 2), a mediator of nucleocytoplasmic transport [61], is expressed in ESC but at comparably low levels in EmGD or serous EIC and not at all in normal endometrium [62]. This not only reinforces the aforementioned point that differences exist between serous EIC and ESC and

between EmGD and serous EIC and ESC but also that EmGD is distinct from the background endometrium.

The fact that only half of EmGD cases display *TP53* mutations indicates that although this is an early molecular alteration, either (1) the half without *TP53* mutations display cytologic atypia but are actually not neoplastic or (2) these lesions possess an undefined molecular alteration other than *TP53* that renders them neoplastic and cytologically atypical and only acquire *TP53* as a primary "driver" molecular event to malignant transformation later in their evolution, since *TP53* is the most common molecular alteration in ESC [52]. It is also worthwhile to note that a different pathway of evolution may be operational for a subset of cases, since not all cases of ESC display *TP53* mutation, and a variety of authors using a variety of different analytic approaches have consistently demonstrated that anywhere from 4 to 18 % of cases lack this mutation [24, 52, 54].

The final hallmark of a precancer is that there is some evidence that the precancer is associated with an increased risk of cancer [55]. The risk for malignancy posed by an isolated diagnosis of EmGD in an endometrial sampling has not been systematically evaluated in long-term prospective studies. It is important to note that such studies are difficult to construct even for the significantly more common precancers for Type I endometrial carcinomas, since it would likely entail a patient "observation only" approach after diagnosis. Additionally, the risk to life posed by failing to prevent the evolution of a lesion to ESC is likely to be significantly higher than to endometrioid carcinoma. The aforementioned EmGD-associated risk is presumed to be elevated based on a single retrospective study of endometrial biopsies and curettages that preceded the diagnoses of ESC in a large cohort [23]. We studied the endometrial samples that preceded (3 months or earlier) the hysterectomies for 250 ESC cases and a control group of 258 benign cases. 27 biopsy specimens from the ESC group and 29 samples from benign control group were ultimately assessed in detail. A total of ten EmGD cases were identified from both groups, 90 % of which were from the ESC group. The period between the EmGD-bearing biopsy and the diagnosis of ESC or serous EIC ranged from 16 to 98 months (average 33 months) [23].

All of the above findings justify the characterization of EmGD as a precancerous lesion that precedes serous EIC.

p53 Signatures

A p53 signature is a morphologically unremarkable segment of epithelial cells displaying moderate to strong immunoreactivity for the p53 protein as assessed by immunohistochemistry (Fig. 8.22). These foci were identified by immunohistochemical analyses of the background endometrium in carcinomas and have been proposed as the first step in endometrial serous carcinogenesis in our model [16, 17]. They are multifocal in at least 89 % of cases [18]. Zhang et al. identified p53 signatures in the endometrium associated with 39.1 % of ESC, 37 % of serous EIC, and 3.3 % of endometrioid carcinomas and in 1.7 % of benign endometrial samples [18]. Jarboe et al. identified p53 signatures in 70 % associated with 70 % of serous EIC and in 4 % of 137 endometrial polyps [19]; other authors have reported less well-defined increases in p53 expression in endometrial polyps [68, 69, 73]. Koi et al. [70] identified p53 signatures in 11 % of the endometrial tissues of 82 women with a variety of benign diseases. Nguyen et al. identified p53 signatures in the background endometrium associated with 24 % of ESC, 0 % of CCC, and 20 % of carcinosarcomas [20]. In the latter study, of the eight non-endometrioid tumors with p53 signatures, 88 % were associated with serous EIC [20]. The p53 signatures are occasionally observed in direct continuity with serous EIC [19]. These signatures display a low proliferative index (4–5 %) [19, 20] and express estrogen receptor-alpha [20, 70]. In our analysis of microdissected samples of p53 signatures, 42 % of cases showed at least one *TP53* gene mutation [18]. A high degree of concordance was identified between the EmGD, serous EIC, and ESC lesions within a given uterus, as at least one identical *TP53* gene mutation

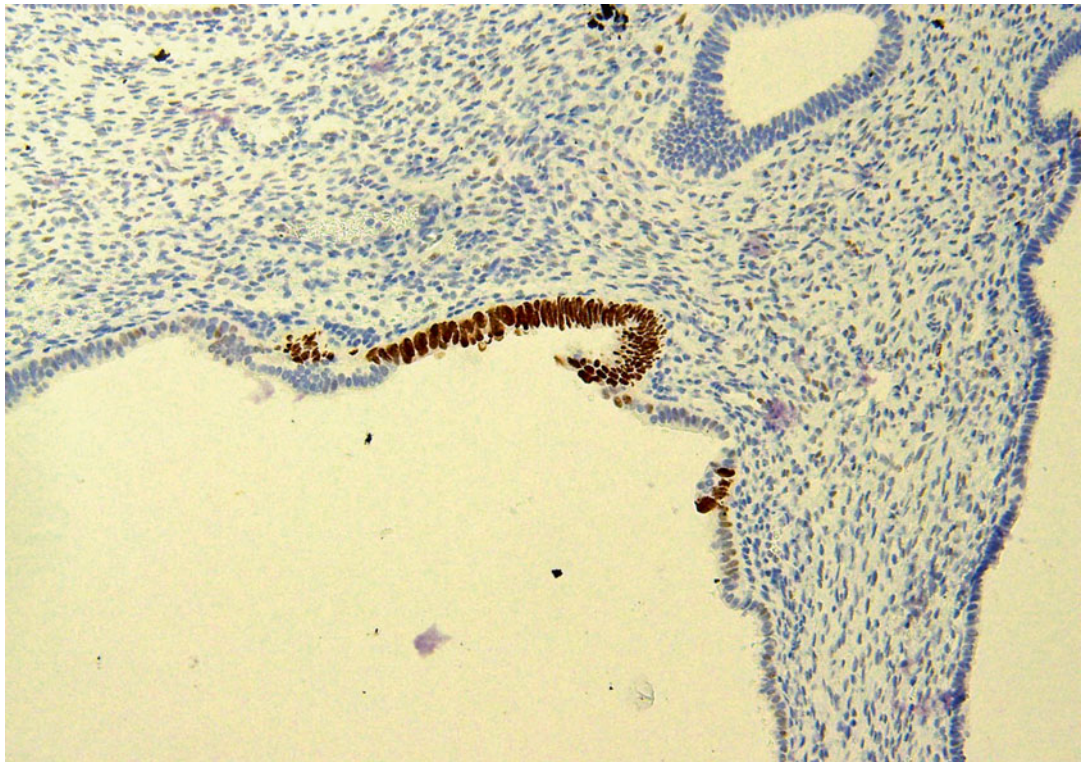

Fig. 8.22 A p53 signature in an endometrial polyp

was found in all three lesions in 50 % of cases [18]. Similarly, Jarboe et al. [19] found identical *TP53* mutations in paired p53 signatures and serous carcinomas in two (67 %) of three cases analyzed.

The aforementioned studies provide evidence that links the signatures to EmGD and to serous EIC and ESC. However, the complicated role that p53 may play in endometrial pathophysiology suggests that p53 signatures are unlikely to represent obligate precursors. P53, as well as other proteins and genes that are integral to its function (the so-called p53 network [71]), can be activated from their usual "inactive" state by a variety of stimuli, including DNA damage, ultraviolet light, and oncogenes [71, 72]. All eventuate in stabilization of the p53 protein, its resultant increase in affected cells, and ultimately, demonstrability of expression by immunohistochemistry [71–73]. Since p53 signatures associated with carcinomas show evidence of DNA damage as assessed by γH2AX immunohistochemistry [19], DNA damage is likely one of the primary initiating factors in at least this subset of cases. A generalized model has been proposed wherein DNA damage activates kinases (such as ATM, Chk1, and Chk2), which in turn phosphorylate the p53 protein and block its interactions with its negative regulator (MDM2), leading to stabilization of the p53 protein [71]. In many organs, p53 plays a major tumor suppressor role since its activation can elicit both apoptotic death and cell cycle arrest [73]. However, in a constantly cycling organ with a high turnover rate, such as the premenstrual endometrium, its physiologic roles are less well defined. p53 expression in the late proliferative phase endometrium is significantly higher than in the late secretory phase endometrium [74]. Whether this "physiologic" p53 accumulation is due to an effect of estrogen causing transcriptional upregulation or an accumulation of replication errors due to high cell turnover is unclear. In support of the possibility of some interaction between p53 and ER-alpha are in vitro

studies of breast cancer, wherein cells transfected with the wild-type p53 result in an eightfold increase in transcription from the ER promoter [77]. While it is unlikely that p53 regulates ER expression in the endometrium in a similar fashion, it does suggest a baseline potential for interaction between these two factors. Parenthetically, there is an increased expression of p53 mRNA in the eutopic endometrium of women with endometriosis as compared with controls [75], and in general, there is an increased endometrial expression of anti-apoptotic proteins and decreased expression of pro-apoptotic proteins in women with endometriosis compared with controls [76].

The significance of p53 expression in nonneoplastic endometrium must thus be assessed within the context of the aforementioned potential for a robust role for p53 in endometrial pathophysiology. Nonetheless, the data indicates that:

1. Relative to the other histotypes and normal endometrium, p53 signatures have a strong association with serous EIC and ESC [18], but may also be associated with carcinosarcomas [20].
2. p53 signatures associated with ESC are associated with *TP53* mutations in 42 % of cases [18], suggesting that *TP53* mutation occurs prior to malignant transformation, at least as recognizable morphologically.
3. p53 signatures associated with ESC show DNA damage, as compared with p53 signatures associated with benign polyps [19]; the co-expression of p53 and γH2AX suggests that the p53 signature is caused by DNA damage; the lack of DNA damage in p53 signatures associated with endometrial polyps and unassociated with ESC suggests that p53 became stabilized through other mechanisms in this subset.
4. Congruent *TP53* mutations have been identified in p53 signatures when they associated with EmGD, serous EIC, and ESC in the same uterus, in at least 50 % of cases [18]; occasional p53 signatures display a *TP53* mutation that is different from the *TP53* mutation seen in the ESC within the same uterus [19], which supports the concept that additional mutations can be acquired during the course of progression or that multiple p53 signatures, each with different *TP53* mutations, may occur within the same uterus, with only a subset progressing to EmGD or serous EIC or ESC. A similar pattern of p53 signature multicentricity with variable mutational patterns is well described in the fallopian tube [78].

These findings form the basis for our model, whereby p53 signatures transform to EmGD. [16, 17]. The precise composite of additional molecular events that must occur to facilitate this transition is currently unclear. p53 signatures, by definition, are not morphologically identifiable, and we generally do not report p53 signatures in clinical specimens.

Summary

The current model of endometrial serous carcinogenesis calls for p53 signatures to originate (predominantly but not necessarily) from resting endometrium and to then transform to EmGD , serous EIC, and ESC in progressive order [16, 17]. Large aspects of this model were based on the study of the *TP53* gene and expression of its associated protein within the constituent lesions of the model. This model would not explain all cases of ESC and assumes that additional molecular events are progressively being acquired during progression. At least 42 % of p53 signatures associated with ESC will display *TP53* mutations; it is unclear whether the 58 % of cases that are devoid of the mutation undergo senescence or apoptosis or whether they progress to EmGD by acquiring other molecular events (including, potentially, other *TP53* mutations). Similarly, only 43 % of EmGD displays *TP53* mutations, and what ultimately becomes of the *TP53* wild-type subset is also unclear. To some extent, it is not entirely clear what percentage of even the *TP53*-mutated cases of p53 signatures or EmGD undergo persistence, senescence, or apoptosis. Both appear to require additional molecular

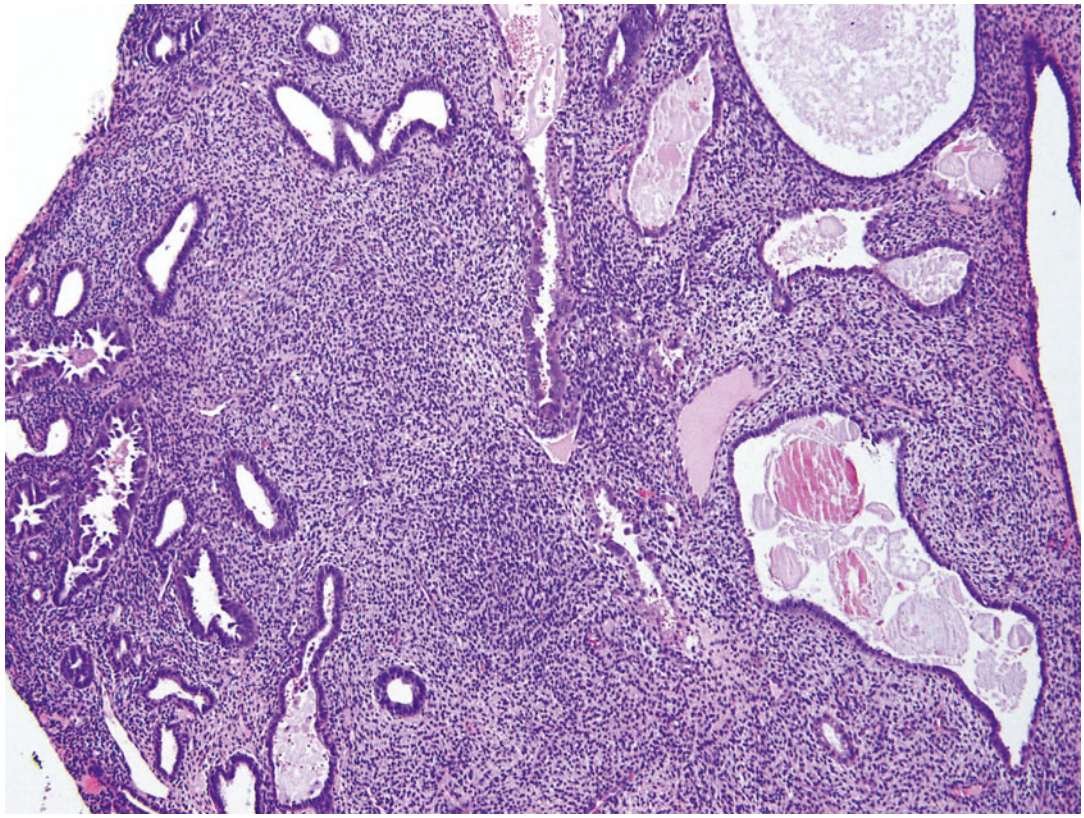

Fig. 8.23 Serous EIC arising from an endometrial polyp. Careful inspection of this image clearly establishes that there is a level of cytologic atypicality that is intermediate between serous EIC glands and the background endometrial glands. Studies of these glands formed the basis for delineating the clinicopathologic profile of the lesion EmGD

events for malignant transformation. What is clear is that a subset persists and progresses to serous EIC, as evidenced by concordant *TP53* mutations between p53 signatures, EmGD, and serous EIC lesions within the same uterus. It is certainly possible that in addition to the *TP53-dependent* pathways outlined above, there is an entire separate *TP53*-independent pathway by which clonal populations are selected from the endometrium, and that *TP53* mutations occur as a later event in this pathway. Multiple *TP53* mutations are identifiable at the p53 signature and EmGD phases, and it is likely that only small proportion actually progresses. Our model makes room for these possibilities. The currently available evidence, however, indicates that at least a subset of ESC evolves through a p53 signature → EmGD → serous EIC → ESC pathway. Since serous EIC and ESC are already cancers and p53 signatures lack any clinical significance as a diagnostic statement, the best chance for reducing the incidence and mortality for patients with ESC is to diagnose its precancerous lesion, which all available evidence indicates is EmGD (Fig. 8.23). This will allow the full clinicopathologic profile of the lesion to be defined, including their natural history, and, ultimately, prevent the development of ESC in a subset of patients.

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Part III

Uterine Cervix, Vulva and Vagina

Vulvar Intraepithelial Neoplasia

9

Demaretta S. Rush and Edward J. Wilkinson

Historic Perspective and Terminology

Over the nearly 100 years that intraepithelial lesions of the vulva have been recognized and described, a bewildering variety of names have been applied to them. In the last 10 years, there have been several proposed revisions to the terminology for these lesions, and as yet there is no universal agreement as to whether all of these revisions are warranted and which system of nomenclature is to be preferred, with the result that we are now seeing several systems being used simultaneously. The lack of consistency in the nomenclature over time, and even between contemporary publications, can make it difficult to interpret past literature and to follow new developments. This is an unfortunate, but perhaps unavoidable consequence of the long-standing commitment of those who study and treat these diseases to maintaining a terminology that accurately reflects the biology of the disease and the clinical significance of the diagnosis in the face of progressive evolution in the understanding of these lesions. In this chapter, we have elected to use the International Society for Study of Vulvovaginal Disease (ISSVD) 2004 terminology, but we present below a brief description of all past and current nomenclature in historical context, which we hope will help the reader to translate between them as necessary when encountering both historic and current literature on the subject. The evolution of terminology for intraepithelial lesions of the vulva is further summarized in Table 9.1.

The earliest description of intraepithelial neoplasia of the vulva was published in France in 1922 [1]. As these lesions were increasingly recognized, they came to be known as "Bowen disease" due to their similarity to lesions described in the nonvulvar skin first described by Bowen in 1912 [2]. Similar terms such as "Bowen dermatosis" [3] were also used in reference to vulvar intraepithelial disease in the earliest pathologic literature. By the 1950s, terms such as "intraepithelial carcinoma" [4]) and "carcinoma in situ" [5], thought to better represent the biology of the lesions, were added to the repertoire. The first recognition that there seemed to be more than one type of intraepithelial neoplasia of the vulva came in 1961, with the publication of two seminal papers by Abell and Gosling [6, 7] in which they described both a more common "Bowen's type" and a less common "simplex" type of intraepithelial carcinoma. By the 1970s, all of

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Table 9.1 The evolution of terminology for vulvar squamous lesions 1976–2014

ISSVD 1976	ISSVD 1986	WHO 1994	ISSVD 2004	LAST[a] 2012	WHO 2014
Hyperplastic dystrophy with atypia, mild	Vulvar intraepithelial neoplasia (VIN) I	Mild dysplasia (VIN 1)	–	Low-grade squamous intraepithelial lesion (LSIL)	LSIL
Hyperplastic dystrophy with atypia, moderate	Vulvar intraepithelial neoplasia (VIN) II	Moderate dysplasia (VIN 2)	VIN, usual type (uVIN)	High-grade squamous intraepithelial lesion (HSIL)	HSIL
Hyperplastic dystrophy with atypia, severe	Vulvar intraepithelial neoplasia (VIN) III *or* VIN III, differentiated type	Severe dysplasia (VIN 3)	VIN, usual type (uVIN) *or* VIN, differentiated type (dVIN)		HSIL *or* differentiated-type VIN
Squamous carcinoma in situ (CIS)		CIS *or* CIS, differentiated type			

[a]LAST = Lower Anogenital Squamous Terminology Standardization Project. This terminology does not address lesions of the differentiated type

these terms, in addition to a variety of other clinically derived terms such as "erythroplasia of Queyrat," "leukoplakia," "leukoplakic vulvitis," and "bowenoid papulosis," were all being used to describe intraepithelial lesions of the vulva. It was, at the same time, becoming increasingly clear that not all of these lesions exhibited the same biologic behavior.

By this time, it was also becoming evident that intraepithelial lesions of the vulva, as of the cervix, consisted of both full-thickness lesions with severe nuclear atypia and high proliferation and of lesser degrees of intraepithelial changes. In 1976, the ISSVD published the first of several reports on the nomenclature of vulvar diseases [8], with the intent to establish a more uniform and clinically relevant terminology. The report introduced the term "squamous carcinoma in situ" to refer to the most severe lesions with severe, full-thickness cellular abnormalities and classified less severe abnormalities under the heading of "hyperplastic dystrophy with atypia," which were subdivided into mild, moderate, and severe categories.

Around the same time the ISSVD made this first attempt to standardize the diagnostic terminology of vulvar lesions, major advances were beginning in the understanding of cervical disease which would come to impact the understanding of vulvar disease. In the cervix, squamous lesions had long been categorized as either dysplasias, which were not considered malignant, or carcinoma in situ, which was malignant by definition. But during the 1960s, it became increasingly evident that carcinoma in situ was not, in fact, a distinct histopathologic and biologic entity. In 1973, Richart introduced a new terminology for cervical intraepithelial disease which eliminated the term. The precursors of cervical carcinoma, he argued, were better understood as a single disease process evolving through a spectrum of morphologic changes that he termed cervical intraepithelial neoplasia (CIN) [9]. In 1982, Crum and Richart introduced the term "vulvar intraepithelial neoplasia" (VIN) for description of vulvar squamous lesions, reflecting the growing understanding that these lesions were pathogenetically similar to those of the cervix [10, 11]. The term was accepted by the ISSVD in 1986 [12] when it recommended that that term be used for all squamous intraepithelial lesions of the vulva, with lesions subcategorized into three tiers of severity, designated VIN I, VIN II, and VIN III. To incorporate those relatively uncommon vulvar intraepithelial lesions previously referred to predominantly as the "simplex" type, a subcategory termed VIN III, differentiated type, was included. This differentiated VIN lesion did not have a counterpart in the cervix.

For the next 18 years, aside from a revision in 1989 recommending the replacement of the roman numerals with Arabic ones [13], the ISSVD nomenclature remained stable; however, competing systems of nomenclature were introduced. In 1994, the World Health Organization (WHO), in the second edition of "Histologic Typing of Female Genital Tract Tumors" [14], addressing the issue of inconsistent terminology for vulvar lesions, accepted VIN 1–3 as an acceptable alternative for three of the four tiers in its preferred terminology of dysplasia/carcinoma in situ for intraepithelial neoplasia of the vulva. In this system, lesions were categorized as mild dysplasia (VIN 1), moderate dysplasia (VIN 2), severe dysplasia (VIN 3), and carcinoma in situ, the latter of which included a subset of "carcinoma in situ, simplex type." This publication also introduced the overarching term "squamous intraepithelial lesion" into the mix as a general term to cover all of the described subcategories with the exception of "carcinoma in situ, simplex type."

Up to this point, the evolution of nomenclature for intraepithelial neoplasia closely mirrored that of the uterine cervix. The majority of intraepithelial lesions of the vulva showed a morphology similar to those of the cervix, and especially as it became clear that lesions in both sites were strongly associated with oncogenic human papillomavirus (HPV), the carcinogenicity of which was becoming increasingly well understood, it was tempting to try to fit vulvar lesions into that new paradigm.

While there were certainly many important similarities between intraepithelial neoplasias of the vulva and cervix, significant differences pre-

vented consideration of the two as completely analogous. To begin with, there was the mysterious entity of the "differentiated" or "simplex" lesions, which did not have a morphologic counterpart in the cervix and showed little association with HPV, suggesting an alternative etiology must exist for them. There was also the unexplained difference in the low-grade lesions of these different sites, with low-risk HPV-type-related exophytic condylomata appearing commonly on the vulva but rarely in the cervix, while flat low-grade squamous intraepithelial neoplasias were common on the cervix but rare on the vulva. By far the most significant and troubling issue, however, was that as research into the association of HPV with cervical and vulvar disease progressed, the association in vulvar lesions proved considerably weaker than that in the cervix. While almost all CIN 2 and 3 lesions, as well as associated cervical squamous carcinomas and even a significant portion of CIN 1, consistently demonstrated the presence of high-risk HPV viral subtypes [15–17], the findings in vulvar lesions were less consistent. Studies reported a wide range, from 53 % to 92 % [18–21], of VIN 2 and 3 lesions to contain high-risk HPV viral DNA, but the average HPV positivity rate was considerably lower than in the cervix. What was even more troublesome was that significantly fewer vulvar squamous cell carcinomas than VIN cases were HPV positive, with studies finding anywhere from 0 to 48 % [18, 21–26] and averaging on the lower side of this range.

The perplexing failure of oncogenic HPV to explain vulvar intraepithelial squamous lesions as well as it did for cervical intraepithelial lesions led some investigators to look more closely at vulvar cancers and their associated precursors. In so doing, it became evident that there was, in fact, a significant difference in morphology between HPV-positive and HPV-negative tumors, as there was for their associated intraepithelial lesions. HPV-negative tumors tended to be well-differentiated, keratinizing squamous cell carcinomas [19, 22, 23] and to be associated with adjacent non-neoplastic vulvar dermatoses like squamous hyperplasia or lichen sclerosus [27, 28], or the differentiated type of VIN as an associated intraepithelial component [28]. HPV-positive tumors tended to show a warty or basaloid pattern and to have associated VIN 3 with similar morphology in a large percentage of cases [18, 19, 22, 23, 25, 29]. Epidemiologic data also showed significant differences between women with HPV-positive tumors and those with HPV-negative ones, with the former presenting about 20 years earlier [22, 23, 29] and more often also affected by other HPV-related disease of the cervix, vagina, or vulva [20, 27, 30–32]. The evidence was building that squamous carcinoma of the vulva was less homogeneous than that of the cervix. Taken together, the morphologic, virologic, and epidemiologic data suggested that, unlike in the uterine cervix, there was not just one pathway to squamous carcinoma of the vulva, but two, with two distinct intraepithelial precursor lesions, only one of which was related to HPV [23, 25, 33], as summarized in Table 9.2.

By this time, advances in the understanding of HPV pathogenesis in the cervix had led to further change in how cervical squamous intraepithelial lesions were viewed. The biologic evidence supported the existence of only two, rather than three, types of lesions. Thus began a shift, first in the reporting of cervical cytology specimens and subsequently in reporting tissue biopsies, to the classification of lesions into either "low-grade" (CIN 1) or "high-grade" (CIN 2–3) categories. This shift was justified not only on the grounds that it better reflected the biologic evidence, but also that it allowed for more reproducible diagnosis [34, 35] and was more practical, given that most clinicians already treated CIN 2 and CIN 3 lesions the same way.

The evolving understanding of how HPV influenced the development of cervical cancer, and that there were both HPV-dependent and non-HPV-dependent pathways to vulvar carcinoma , led to yet another revision in terminology for vulvar disease. In 2003, the WHO revised its terminology to favor the use of VIN over the synonyms of dysplasia and carcinoma in situ, defining both warty and basaloid types associated with HPV, which were further classified into VIN 1, 2, and 3, and maintaining the previous terminology of "carcinoma in situ, simplex type" for lesions with a

Table 9.2 The two pathways to squamous carcinoma of the vulva

	HPV-related squamous carcinoma of the vulva	Non-HPV-related squamous carcinoma of the vulva
Patient age	Younger (40s–50s)	Older (60s–70s)
Associated diagnoses	Other HPV-related lesions of the lower anogenital tract (condyloma, cervical, vaginal and/or anal intraepithelial neoplasias)	Non-neoplastic dermatoses (lichen sclerosus, squamous hyperplasia, etc.)
Associated in situ lesion	uVIN	dVIN
Tumor morphology	Warty and/or basaloid	Well-differentiated keratinizing

differentiated morphology [36]. In 2004, the ISSVD undertook more drastic revisions, abandoning the category of VIN 1 altogether and combining VIN 2 and 3 into one category of simply "VIN" [37, 38]. In this system, VIN is subclassified into two categories, the usual type, abbreviated "uVIN," and the differentiated type, abbreviated "dVIN," and uVIN is further subclassifiable as warty, basaloid, or warty/basaloid if desired. The decision to abolish the category of VIN 1 has been controversial, but the justifications given were that the diagnosis is rare without an accompanying higher-grade lesion [39], that it is poorly reproducible [34, 40], and that, unlike in the cervix, evidence is lacking that it may progress to a higher-grade lesion [37, 41]. The same arguments which had been used to support the combination of CIN 2 and 3 into one category of "high-grade" lesions were found to be applicable to VIN 2 and VIN 3 [34, 39], at least the HPV-related types, and ultimately to all HPV-related lesions of the lower anogenital tract. It is for this reason that a recent proposal has been made to standardize the nomenclature for all HPV-related lesions of the lower anogenital tract ; the Lower Anogenital Squamous Terminology Standardization Project (LAST) proposes that a two-tier system of LSIL and HSIL be used to refer to all HPV-related squamous intraepithelial lesions of the vulva, vagina, cervix, anus, perianal area, and penis [42], rather than the organ-specific intraepithelial neoplasia nomenclatures, with the option to maintain that specific nomenclature in parentheses; thus, lesions formerly categorized as VIN 2 or 3 or uVIN in ISSVD terminology would be designated HSIL (VIN 2–3). This system pertains only to HPV-related lesions, so it makes no recommendation as to terminology for differentiated lesions of the vulva. Most recently, the WHO has adopted identical terminology, retaining the term "differentiated-type VIN" for those lesions not related to HPV [43].

As is evident from the terminology used and from their earliest descriptions, intraepithelial lesions have always been recognized as preinvasive lesions. This understanding was based initially on morphologic grounds, and later supported by the observation that these lesions were observed adjacent to invasive disease in 7–25 % of cases examined [4, 7, 44], and further supported by the observation that when followed over time a significant proportion of patients with these lesions developed squamous carcinoma [6, 44]. These observations continued to be made over the ensuing decades, and the strength of the association of VIN with carcinoma has even increased over time, as more attention has been paid to vulvar disease and interventions have been initiated earlier in the disease process, with later studies reporting the presence of intraepithelial disease adjacent to vulvar carcinoma in 20–100 % of cases [18, 27, 45–54]. This strong association remains compelling evidence that VIN is a precancerous lesion. But association does not prove causation, as the association of squamous hyperplasia, lichen sclerosis, and other non-neoplastic vulvar dermatoses with vulvar carcinoma has been noted to be as strong, if not stronger than that of VIN. It has not been until relatively recently that the underlying biology of VIN has begun to come to light, and there is a tremendous amount still to discover, but these investigations have continued to provide additional support to the long-held understanding of VIN as a precancerous lesion.

Vulvar Intraepithelial Neoplasia, Usual Type

Epidemiology

The vast majority of in situ squamous lesions of the vulva, comprising 82.3–98 % of reported series, are uVIN [28, 51, 55, 56], and the increasing incidence of VIN since the 1970s [16, 57–63] as well as a decreasing age of diagnosis [29, 60, 64], has been largely attributed to the role of HPV in the pathogenesis of this disease. The factors known to increase the risk of developing uVIN are principally related to the risk of contracting HPV or to the presence of established infection in the lower anogenital tract. Thus, early age of first intercourse; a history of multiple sex partners, of genital warts, and of cervical intraepithelial neoplasia; and immunosuppression are all associated with increased risk. The incidence has been shown to increase in women up to the fifth decade and then to decline gradually [16, 57].

The average age of patients with uVIN in recent studies ranges from 38 to 53 years [31, 32, 41, 47, 51, 58, 65–69]. Like those with cervical HPV-related disease, the majority of patients are smokers [70–72], and anywhere from 25 % to 75 % [20, 31, 32, 52, 58, 67, 71, 72] have been reported to have HPV-associated lesions elsewhere in the lower anogenital tract, depending on how many sites were examined and how disease was defined. Prior, synchronous or subsequent squamous cell carcinoma of the vulva is found in approximately one quarter of patients [41, 51, 73].

Morphology

Grossly, uVIN may appear as a white, erythematous, or pigmented plaque, papule, or polypoid area on the affected epithelium, dependent on the histomorphology of the particular lesion. Multiple synchronous lesions are reported to be present in 36–77.5 % [32, 67, 69, 74], a finding which is more common in younger women.

The precancerous nature of uVIN has never been doubted, largely because the morphology is so abnormal. Microscopically, two distinct and strikingly abnormal histologic patterns of uVIN have been described. They are not, however, mutually exclusive. Mixed forms are common and may be diagnosed as such, and, as there is no clinical difference between them, it is acceptable to abstain from designating either specific type as long as the distinction from dVIN is clear. It is also not uncommon for uVIN of either warty or basaloid type to be seen adjacent to or admixed with condyloma acuminatum, particularly in immunosuppressed patients [75]. As the clinical behavior will be dominated by the uVIN in these cases, it is imperative that the uVIN be recognized and diagnosed correctly.

The warty type of uVIN is so named because of the spiky or undulating surface which lends them an exophytic gross appearance similar to condyloma. In this type of uVIN, there is usually striking acanthosis and hyperkeratosis, often accompanied by hypergranulosis and parakeratosis (Fig. 9.1). The thickened epithelium is disorganized and cellular maturation is markedly decreased, although the most superficial layers retain some degree of maturation and typically show at least focal koilocytic change (Fig. 9.2). Extreme cellular pleomorphism is characteristic, often with numerous multinucleated, apoptotic, and dyskeratotic forms (Fig. 9.3). There is usually moderate to abundant eosinophilic cytoplasm in the more superficial layers, and intercellular bridges and cell borders are well defined (Fig. 9.4). Nuclear atypia is pronounced, with irregular nuclear membranes and coarsely granular, hyperchromatic chromatin (Fig. 9.5). Mitotic activity is readily apparent throughout all layers of the epithelium, including abnormal mitotic figures (Fig. 9.6).

The basaloid type of uVIN has a relatively flat surface, and these lesions present grossly not as exophytic lesions but as macules or plaques. Like warty uVIN, the epithelium is thickened, and hyperkeratosis, parakeratosis, and koilocytosis may be present, but all of these features are seen to a lesser degree than in uVIN (Fig. 9.7). The lack of maturation in basaloid uVIN, however, is

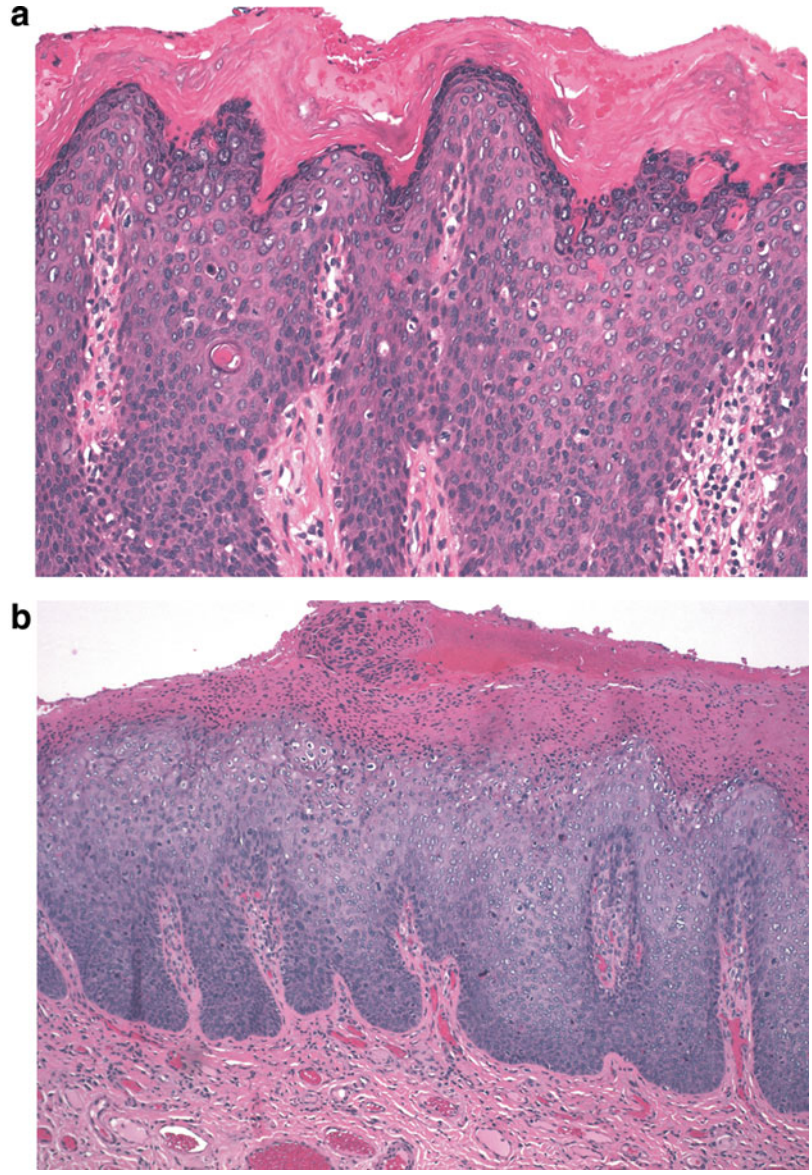

Fig. 9.1 (**a**) This case of uVIN, warty type, shows a thickened epithelium (acanthosis) with an undulating surface, hypergranulosis, and pronounced hyperkeratosis. (**b**) In this uVIN, the acanthotic epithelium is surfaced with a thick layer of parakeratosis

much more striking, and there is very little, if any, maturation even at the surface. Rather than the extreme pleomorphism seen in warty UVIN, in basaloid uVIN the cell population is quite uniform, comprised of small, immature cells with enlarged hyperchromatic nuclei and scant cytoplasm, with poorly defined cellular borders (Fig. 9.8). Mitotic activity is not significantly different from that seen in the warty type.

In both types of uVIN, the rete pegs are often widened and deepened while the dermal papillae are narrowed and extend more superficially than normal (Fig. 9.9). Both types of uVIN may contain intracellular and extracellular pigment, sometimes quite prominent (Fig. 9.10), which should not be taken as evidence of melanocytic differentiation. Both also commonly involve skin appendages (Fig. 9.11), which may be misconstrued as invasion, particularly in a superficial biopsy. At the same time that it is important to avoid making this mistake, it should be kept in mind that it is not uncommon for a lesion consid-

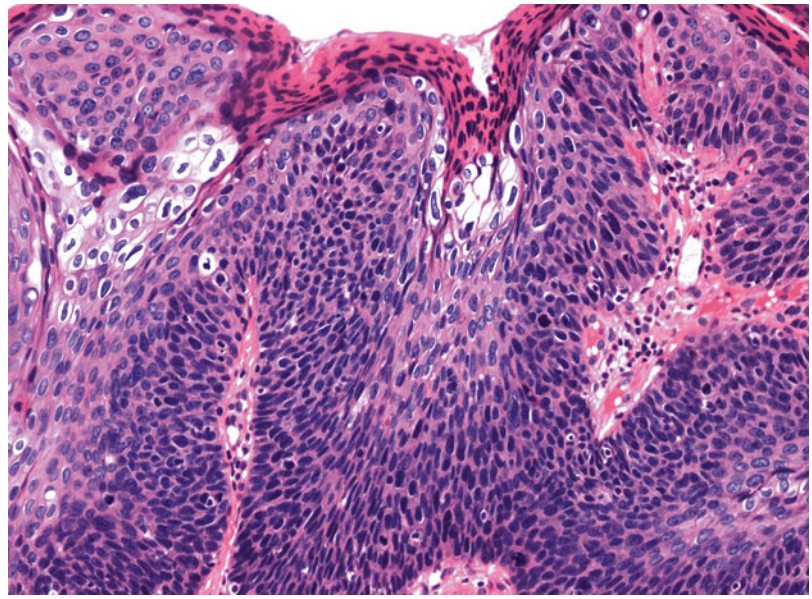

Fig. 9.2 Focal koilocytic change in the warty type of uVIN. Surface parakeratosis is also present

ered to be uVIN to be discovered to have focal invasion upon pathologic evaluation of the excised specimen. Such occult cancers, usually with only minimal invasion, have been reported in up to 22 % of cases [32, 67, 72, 74, 76–78], a finding which not only bolsters the concept of uVIN as a precancerous lesion but also demands careful evaluation of all excisions.

Rare variants of VIN have been described with pagetoid or mucinous morphology. These are usually seen in association with concurrent invasive carcinoma and with associated intraepithelial squamous cell abnormalities with the more typical appearance of uVIN. Lesions with a pagetoid morphology, in which clusters and nests of atypical squamous cells are found scattered within an otherwise normal epithelium rather than replacing the full thickness, have been confirmed to express p16 and half have been found to have integrated HPV, consistent with the observed relationship to uVIN [79, 80]. Immunohistochemistry has provided further evidence that these cells represent an aberrant morphology of uVIN rather than Paget disease as they do not stain with mucin or CEA, a finding which may be useful in difficult cases [79, 80]. Another VIN variant in which cells with mucinous cytoplasm are admixed throughout the epithelium of an otherwise typical uVIN has been termed "VIN with mucinous differentiation" [81]. This variant has also been shown to be positive for p16 and to contain HPV 16, in keeping with the observed association with uVIN.

Immunophenotype

As p16 is overexpressed as a result of aberrant cell cycle activation by viral oncoproteins, its expression is extremely sensitive and specific for the presence of virus (124). Consequently, uVIN stains strongly for p16 in 95–100 % of cases [50, 65, 82–84], as do their associated carcinomas [85]. Even HPV-negative lesions with the histologic appearance of uVIN have been found to express p16 in the majority of cases [85], suggesting these cases represent failure of detection rather than true absence of the virus. The pattern of staining is either nuclear or nuclear and cytoplasmic, involving a continuous area or "block" of epithelium from the basal layer through at least the bottom third of the epithelial thickness (Fig. 9.12). A similar pattern may be seen with proExC, another marker of active HPV infection [86]. Ki-67/MIB1, also a marker of cell cycle activation, is likewise strongly expressed in

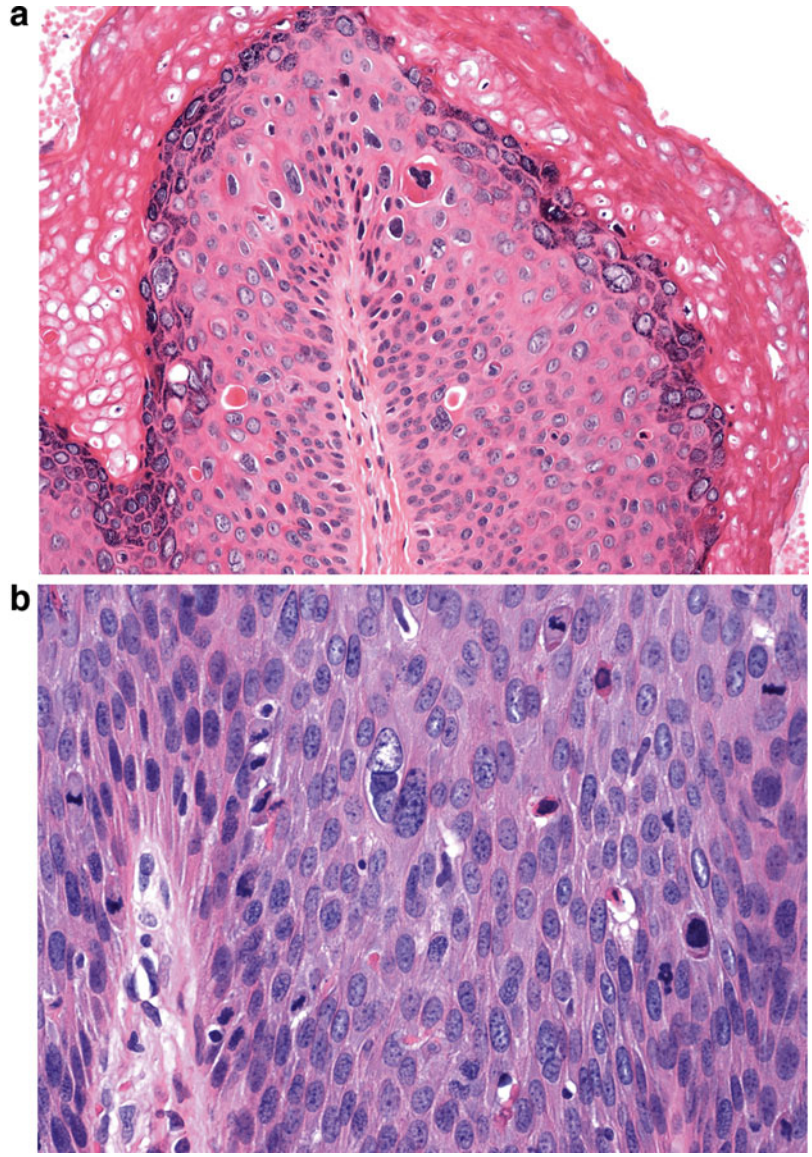

Fig. 9.3 (**a**) Extreme cellular pleomorphism, as seen here, is common in uVIN, warty type. Multinucleated cells, dyskeratotic cells, and apoptotic bodies are also present, along with surface hypergranulosis and hyperkeratosis. (**b**) Two large cells in the center of this field, one of which is multinucleated exemplify the cellular pleomorphism of uVIN, warty type. Again, small apoptotic bodies are also present. Several mitotic figures are also present in this field

uVIN, showing nuclear positivity in the majority, if not all, cells throughout the full thickness of the epithelium (Fig. 9.13). Studies of p53 expression in uVIN have been somewhat contradictory, with some studies reporting varying levels of overexpression [50, 87, 88] and some reporting no expression at all [65], but p53 does not appear to play a significant role in HPV-associated tumors, which show very low p53 expression [50, 87], and it does not appear to be useful marker in uVIN.

Molecular Findings

That tumors of basaloid and warty morphology have been shown to have adjacent uVIN in 53.8–100 % of cases [23, 29, 50, 56, 89, 90] has been taken as strong evidence that uVIN gives rise to these tumors. More significantly, over the past two decades or so there has been an increasing understanding of the molecular alterations underlying the profoundly altered epithelial morphology of uVIN, which has further clarified its

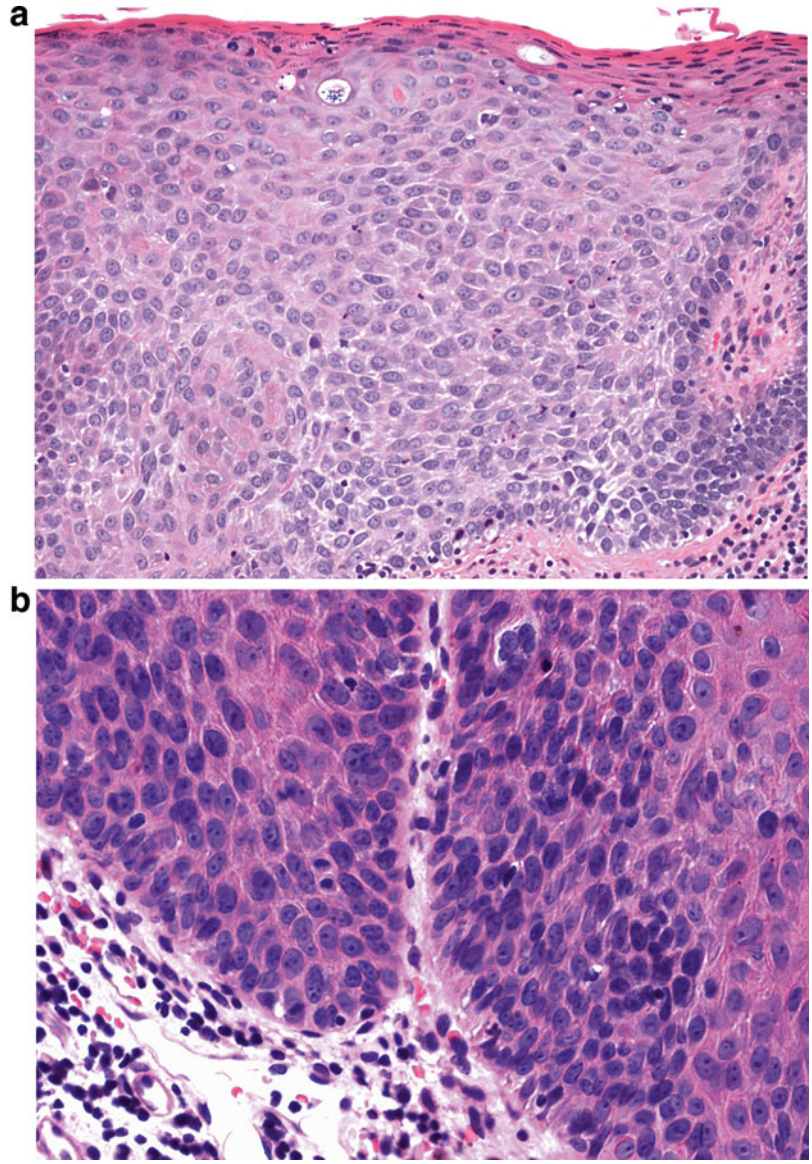

Fig. 9.4 (**a**) Prominent intercellular bridges account for the linear white spaces between many of the cells in this case of uVIN, emphasizing well-defined cell borders. The cells here show an appreciable amount of eosinophilic cytoplasm. (**b**) Higher power shows the well-defined cell borders and hairlike extensions of intracellular bridges between the cells

relationship to invasive disease. The relationship of uVIN and vulvar cancer to HPV infection, with high-risk viral types identified in 57–97.1 % of uVIN [47, 56, 65, 68, 70, 71, 91–97] and from 69.4 % to 90 % [50, 52, 84, 89, 93] of basaloid/warty carcinoma, has provided some of the strongest evidence that uVIN is truly a precancerous entity. Over the last three decades, it has become firmly established that HPV is a causative agent of a variety of cancers, ultimately leading to official classification as a carcinogen by the WHO in 2005 [98]. The mechanisms by which the virus acts to induce neoplasia through the actions of viral oncoproteins E6 and E7, which have been shown to interfere with the normal cell cycle in such a way as to promote genetic instability and the continued proliferation of genetically altered cells [83, 98, 99], have become quite well understood, primarily through work done on cervical cancer and its precursors.

The natural history of HPV infection of the vulva remains less well understood than in the

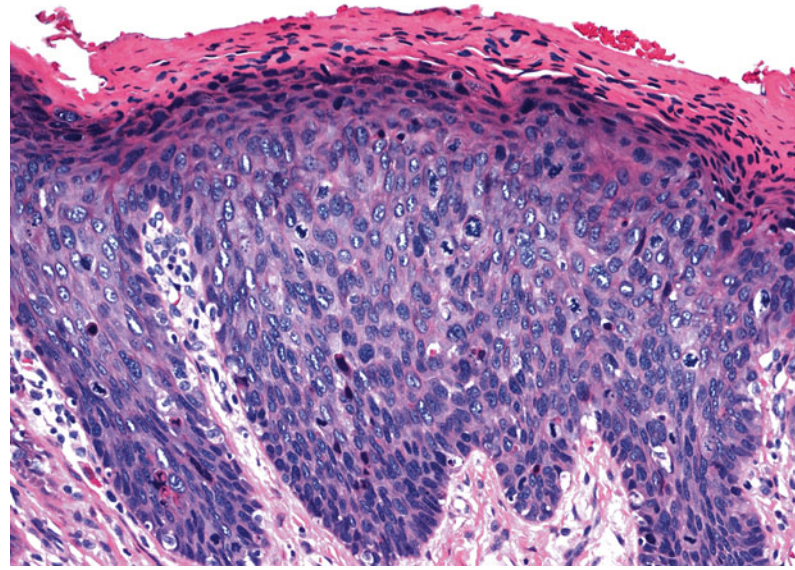

Fig. 9.5 The nuclear features of uVIN, warty type, are evident here even at a relatively low power. In addition to the nuclear pleomorphism, there is coarsely granular, hyperchromatic chromatin with areas of nuclear clearing

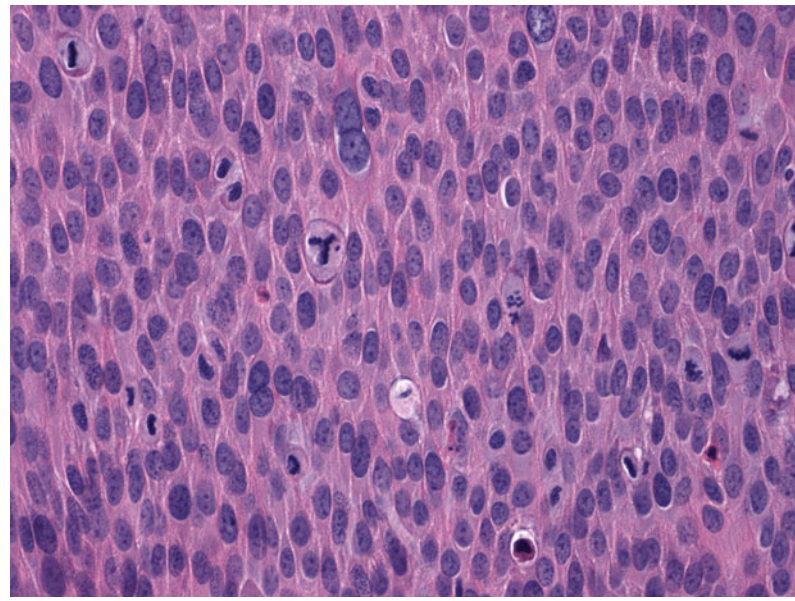

Fig. 9.6 Numerous mitotic figures, including an abnormal, tripolar figure in the central part of the field

cervix. This is in part due to the fact that cervical disease is more common and that the asymptomatic cervix is routinely screened for the presence of HPV, while the vulva is not. This has provided an abundance of cervical material for study, allowing investigators to elucidate the course of HPV-induced disease in this site in great detail, while data involving vulvar lesions is relatively scant. The few studies that have looked for HPV on the vulva of asymptomatic patients have shown that nearly three quarters of women with HPV detected in the cervix will also have the virus detected on the vulva [70, 100]. In one prospective study of HPV-naïve women, of 1196 incident high-risk HPV infections of the vulva, only 11 uVIN developed [91] demonstrating that

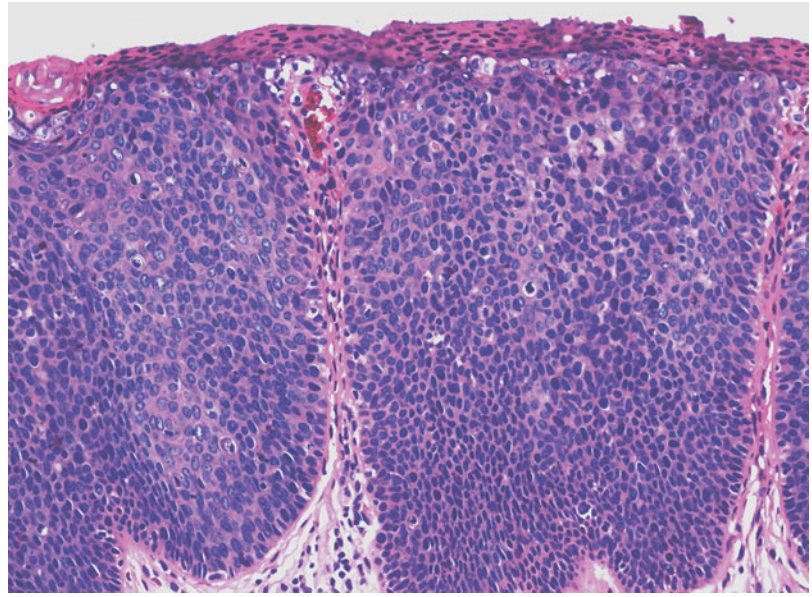

Fig. 9.7 Basaloid uVIN. The epithelium is acanthotic with a thin layer of surface parakeratosis, but the nuclei are more uniform and there is less cytoplasm than is typical of warty uVIN

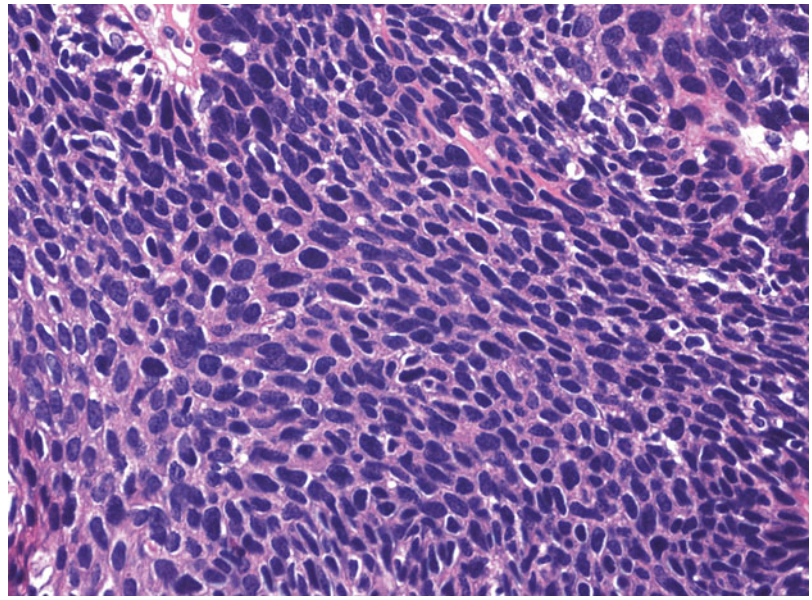

Fig. 9.8 On high power, the cells of basaloid uVIN are small, with scant cytoplasm and relatively indistinct cell borders (compare to warty uVIN in Fig. 9.4). The nuclei are hyperchromatic and enlarged, but much less pleomorphic than in warty uVIN (compare to Fig. 9.3)

the vast majority of patients will clear the infection without ever developing VIN. For those few patients who do, the mean time from incident HPV infection to VIN was found to be 18.5 months [91]. The highest rates of detection of high-risk virus, not surprisingly, are seen in patients with current uVIN lesions, and in most cases, the detection is in multiple sites (27). The rate of virus detection drops by about half in patients with a history of treated VIN [70] consistent with the finding that half of vulvar infections have been found to persist after treatment [91].

The distribution of HPV subtypes is different in uVIN than in high-grade squamous intraepithelial lesions of the cervix. In both sites, HPV 16 is the most common subtype, but in the cervix, the second most common subtype is HPV 18, while in the vulva HPV 18 is only rarely

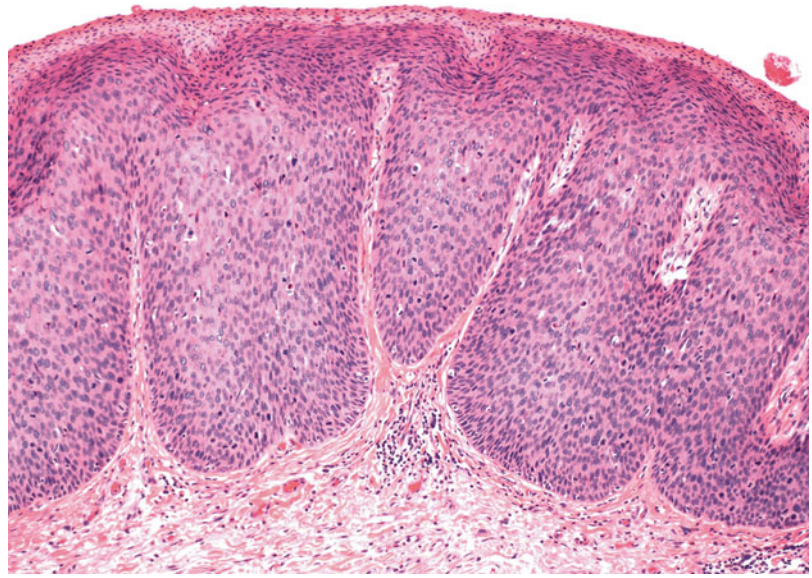

Fig. 9.9 Wide, deep rete pegs with narrow dermal papillae extending far superficially, a characteristic architecture seen in both types of uVIN

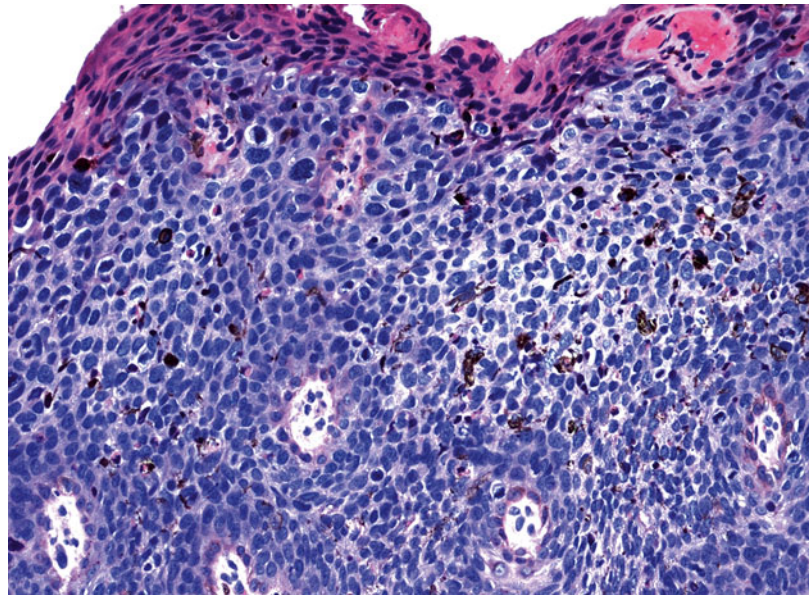

Fig. 9.10 Clusters of coarse pigment granules are scattered throughout the epithelium in this case of warty uVIN, which would have given the lesion a brown appearance grossly

detected and the second most common subtype is HPV 33 [20, 52, 56, 89, 91, 94, 96, 101–105]. Because many studies, following the lead established in cervical research, have limited their analysis to HPV types 16 band 18, this may account for some of the discrepancies between the rates of HPV positivity in the cervix compared to the vulva. Interestingly, there is evidence that it is a single infection that is responsible for the multiple lesions seen in patients with multifocal and multicentric disease. In the majority of cases analyzed, the same viral subtype was identified in both the vulvar and cervical lesions [52], or in multiple vulvar lesions of the same patient [106].

This integration of the viral genome is considered a critical step in viral carcinogenesis. Although it is not required for progression of disease, viral integration results in increased expression of viral oncogenes E6 and E7, and it makes sense that it would increase the likelihood of progression. Studies in vulvar disease support the significance of viral integration in the pathogenesis of squamous cell carcinoma and also support the idea that the disease evolves through a precursor VIN lesion. In one case of HPV 16-related carcinoma with adjacent uVIN, the virus was detected in both integrated and episomal form in both the uVIN and the tumor, but the integrated form was predominant in the tumor [97]. A later study on a single patient with multifocal uVIN and an associated carcinoma was shown to have only episomal HPV 16 in two loci of uVIN, but had integrated virus in two other uVIN sites and in the tumor [107]. In a larger study, integrated HPV was found in 24 of 25 uVIN adjacent to squamous cell carcinoma [104]. In another, viral integration was found in 38 % of HPV 16- or HPV 18-positive cases of uVIN, all of which were multifocal and multicentric and one of which progressed to invasive carcinoma [106]. In addition, this study found the same viral-type transcript pattern in all specimens from the same patient with multiple foci of disease in 83.3 % of cases, suggesting the multiple lesions are monoclonal. One study detected the same viral integration site in vulvar lesions as in previously diagnosed

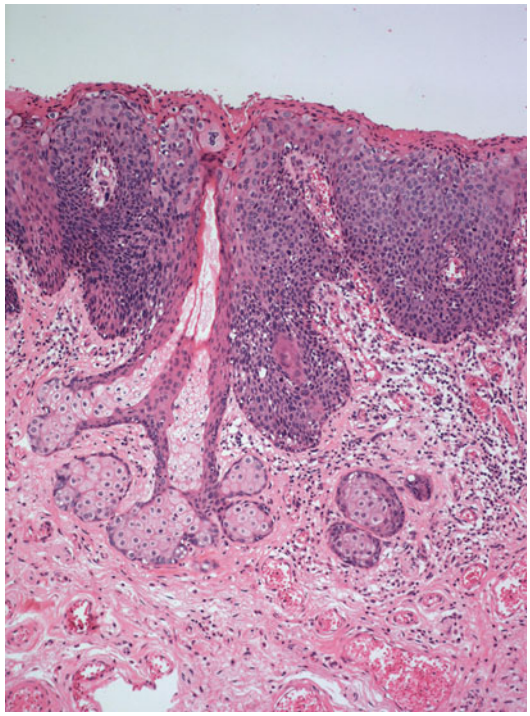

Fig. 9.11 VIN extending down a sebaceous gland. Note how much deeper the lesion penetrates along this gland than does the adjacent lesion

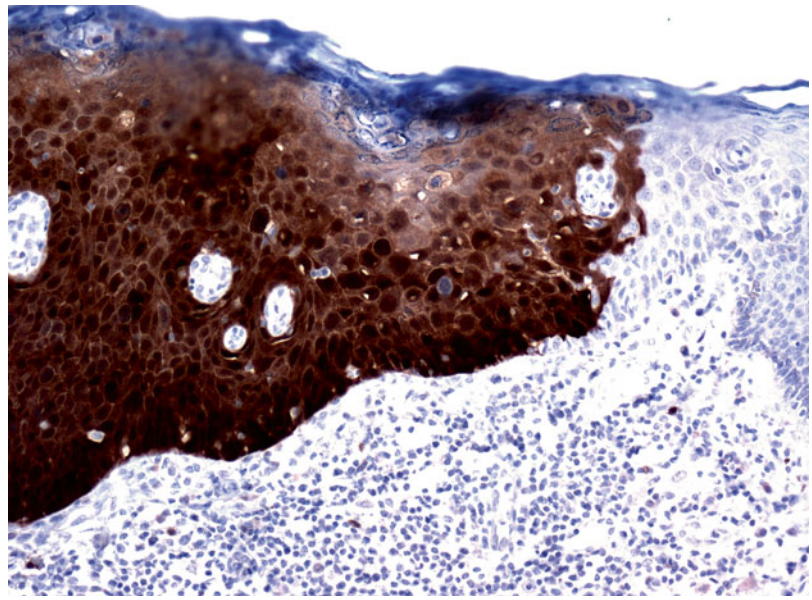

Fig. 9.12 The pattern of p16 staining in uVIN. There is intense nuclear and cytoplasmic staining through the entire epithelial thickness in this lesion. Note the clear distinction for the adjacent normal epithelium, which is p16 negative

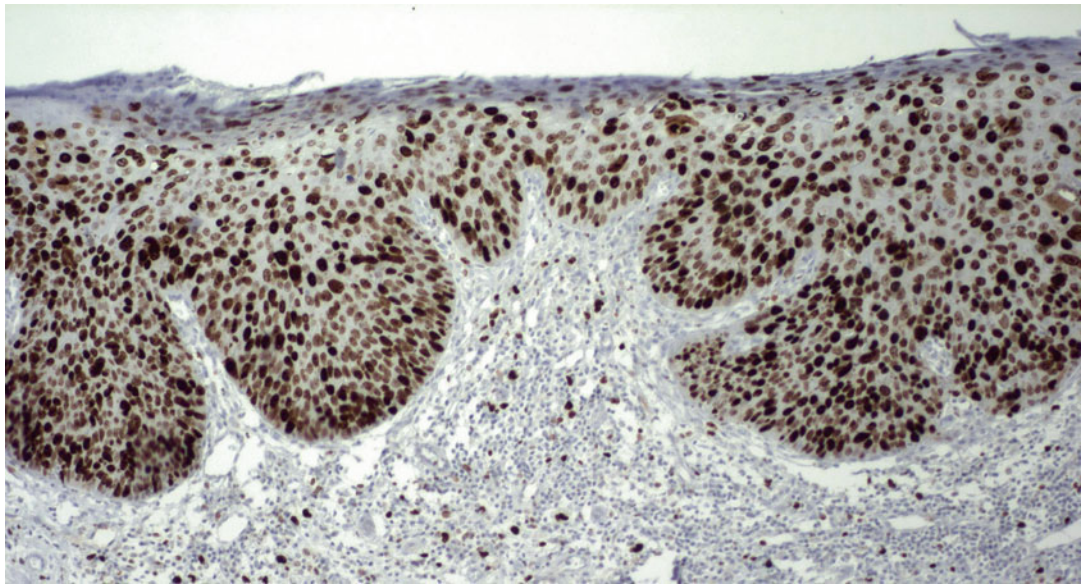

Fig. 9.13 The majority of the cells in uVIN show strong nuclear positivity for Ki/67/MIB-1 through all layers of the epithelium

cervical lesions of the same patient, raising the possibility that a single clone might be responsible for disease in multiple sites [108].

Other genetic similarities have been found between uVIN and vulvar squamous cell carcinoma to support the evolution of the former to the latter. Clonal evolution of VIN lesions was first hypothesized in 1981, when studies by Fu and Wilkinson showed all of the cases of VIN they analyzed were aneuploid [109, 110]. Further studies confirmed a monoclonal origin in the majority of cases [97, 111, 112], and comparison of in situ lesions and the associated invasive tumors has shown the same clone in both lesions [112]. Allelic imbalance has been demonstrated in the majority of uVIN [113] and to be higher in VIN associated with cancer than in VIN alone, suggesting that increasing genetic instability drives the progression to invasive disease [114]. Further analysis has shown that the tumors associated with uVIN, as well as the uVIN in different sites, when multifocal lesions are present, may not be completely identical [112, 114], suggesting continued clonal evolution within the invasive tumor. Some specific genetic alterations have been described in uVIN, including loss on chromosome 17 and gains in the long arm of chromosome 3 [63, 65, 115]. The latter alteration, reported in up to 50 % of uVIN, is of particular interest as the same alteration is also commonly seen in squamous cell carcinomas of both vulva and cervix [65, 115, 116].

Patient Outcome

Recurrence of uVIN is common and has been reported in 28.7–72.5 % of cases [20, 31, 32, 66, 71, 72, 117–119]. Most recurrences have been reported within 4 years [117, 118], but patients remain at risk for life and close life-long follow-up is imperative. Multifocal infection sites are likely responsible for at least part of the recurrence risk [70]. Recurrences consist of both treatment failures, where the lesion recurs in the same location treated previously and of new "field effect" lesions related to the progression of HPV infection at another site. Only one study has considered the two separately, finding an equal number of both, but that recurrence at the site of previous treatment appeared in a median time of 2.4 years as opposed to a median time of

13.5 years in the "field effect" lesions [64]. Most studies have found recurrence to be more likely if lesions are multifocal [31, 32, 69, 71, 77, 117, 119], but some have failed to find a correlation between recurrence and multifocality [67, 120]. Similarly, most studies have found recurrence to be more common if the initial excision margins are positive [31, 64, 66, 67, 77] and if the patient continues to smoke [19, 66, 72, 117], although one study has challenged both of these findings [120]. Immunosuppression [117], cryosurgical treatment [67], and larger lesion size [66] have all been associated with increased risk of recurrence. Whether surgical excision or laser treatment has a higher risk of recurrent disease remains controversial, with studies showing conflicting results [117, 118]. Not surprisingly, given its viral etiology, the immune microenvironment in uVIN has recently been shown to differ from normal controls [121] and the specific alteration of increased CD14-positive macrophages to correlate with recurrence and progression of disease [122].

Subsequent invasive squamous carcinoma of the vulva is reported in treated patients in 2.3–16 % of reported cases, with most studies finding progression to occur in less than 7 % of patients and in less than 8 years [20, 31, 32, 58, 64–67, 69, 71, 117, 118, 123]. Progression has been reported to occur more frequently and rapidly in patients who are immunosuppressed [71, 117], in multifocal disease [117], in women who continue to smoke [117], and in patients of advanced age [58] although not all studies have confirmed these findings [66]. The median time to progression of a uVIN lesion to invasive squamous cell carcinoma has been reported from 41.4 to 109 months [58, 66, 67, 117], and cases have been reported up to 18 years following initial treatment [123]. One study reported two peaks in progression, the first at 2–3 years and the second at 7–8 years post treatment [117]. For obvious reasons, fewer studies have been able to report on the progression rate in untreated disease, but a small number of patients who have refused initial treatments have been followed and most have been found, ultimately to progress to invasive disease within 1–8 years [64, 67, 123].

A small subset of patients with the histologic findings of uVIN will regress with no further treatment. This phenomenon was first reported as "reversible vulvar atypia" by Friedrich in 1972 [124]. The term "Bowenoid papulosis" was coined to describe the condition in 1979, a term that describes a subset of disease which is distinct clinically but not morphologically, and therefore no longer accepted as a pathologic diagnosis but remains in clinical use. The patients in this fortunate group are usually young and often pregnant, with multiple pigmented papular lesions [64, 67, 125]. In recent case series, they have comprised 1.2 % [67] and 12 % [64] of patients with VIN, and the median time to regression was reported as 9.5 months [64].

Vulvar Intraepithelial Neoplasia, Differentiated Type

Epidemiology

Although the increasing incidence in VIN seen since the 1970s is predominantly due to uVIN in young women, as discussed above, the diagnosis of dVIN has also been seen with increasing frequency in recent years [58]. Nonetheless, it remains a minority of VIN, comprising only 2–18 % of cases [28, 41, 51, 55]. Unlike uVIN, however, the increasing frequency of diagnosis of dVIN is most likely due to increased recognition of the disease, rather than any true increase in its incidence. The reasons for the relative rarity of the diagnosis of dVIN as compared to uVIN are as yet incompletely understood. It may truly be an uncommon occurrence, although it seems likely that both misdiagnosis as benign reactive disease and its rapid progression to invasive disease contribute to its underrecognition an underreporting. Over the last decade and a half, as it has become clear that there are two different pathways to squamous carcinoma of the vulva, the awareness and acceptance of the diagnosis of dVIN appear to have increased, and it is likely to be diagnosed with greater frequency in the future.

The mean age of patients at initial diagnosis of dVIN ranges from 66.8 to 73 years in recent

series [51, 58, 65, 90, 126]. Only 3 % of patients have been reported to have multicentric disease [58], and approximately one half have multifocal disease [127]. There appears to be a close association between dVIN and vulvar dermatoses, particularly lichen sclerosus and squamous hyperplasia. Associated lichen sclerosus has been identified in dVIN in 33.3–56 % of cases [90, 127] and associated squamous hyperplasia in 83.3 % [127]. In fact, it has long been a question whether these entities, particularly lichen sclerosus, might be considered precursors to squamous carcinoma as well. Many studies have reported the strong association of vulvar dermatoses with cancer, reporting associated lichen sclerosus in up to 71 % of vulvar squamous carcinomas [23, 46, 48–50, 52, 128] and associated squamous hyperplasia or lichen simplex chronicus in up to 83 % [22, 27, 33, 46, 49, 50, 128]. Patients with lichen sclerosus of the vulva, though not of the nonvulvar skin, have long been known to be at increased risk of developing vulvar cancer. The risk is generally estimated at about 4–5 % [129], but one prospective study of women with symptomatic lichen sclerosus found it to be much higher, reporting the eventual development of vulvar cancer in 21 % of patients followed [128]. It appears that lichen sclerosus and squamous hyperplasia of the vulva are also closely related to one another, and several studies have shown that vulvar lichen sclerosus associated with cancer is, in fact, thickened and hyperplastic [106, 128, 130] and that this is what differentiates it from "ordinary" non-vulvar lichen sclerosus which does not progress to cancer.

Looking more closely at the association between the vulvar dermatoses, dVIN, and squamous cell carcinoma, however, it seems that the association of the dermatoses with cancer is due, in large part, to the strong association with dVIN, and it is the dVIN, in turn, which is strongly associated with carcinoma. Supporting this are the findings that in cases of lichen sclerosus without associated carcinoma, dVIN is seen only rarely, but in cases of lichen sclerosus with associated cancer, dVIN is relatively common [128, 131]. It has been found that when lichen sclerosus is not associated with concurrent cancer, uVIN is a more likely associated finding than dVIN [132]. It is also reported that in cases of carcinoma associated with lichen sclerosus, only a minority of tumors have lichen sclerosus alone, while most have both dVIN and lichen sclerosus [47, 51, 129], and in one lichen sclerosus patient followed with successive biopsies for 11 years, areas of dVIN were eventually reported prior to the ultimate development of invasive carcinoma [133], seeming to confirm that dVIN is the more direct link to malignancy. This study also reported the median time to the development of vulvar squamous cell carcinoma in patients with lichen sclerosus as nearly four times as long as that for dVIN [133]. Given the current evidence, it appears that the association of vulvar dermatoses with cancer is due to a long and gradual accumulation of changes with progression through a dVIN stage, which may go unrecognized. Recently, squamous carcinomas of the vulva arising in association with lichen planus of the vulva have been reported, all of which were HPV negative and the majority of which had adjacent dVIN, suggesting lichen planus-associated carcinoma may also arise through dVIN as a preinvasive intermediary [134].

Morphology

While there has never been any question as the precancerous nature of uVIN, the nature of dVIN has long been a controversial topic, largely because unlike uVIN, the morphology of dVIN is much less strikingly abnormal and shows significant overlap, both grossly and microscopically, with the vulvar dermatoses which often accompany it. Grossly, dVIN lesions may present as focal gray white areas with a roughened surface, as ill-defined raised white plaques, or as discrete elevated nodules, ranging in size from 0.5 to 3.5 cm [127]. The lesions may be deceptively subtle and difficult to distinguish from associated lichen sclerosus or other dermatosis. Similarly, on microscopic examination, the histologic changes can be difficult to recognize and distinguish from associated dermatoses. The diagnosis is notoriously confusing and controversial, and

there are still authors who do not accept its existence, considering it rather as a variant of squamous hyperplasia or lichen sclerosus with atypia [23]. Because the atypical cells are confined to the basal and parabasal layers of the epithelium in dVIN, some have even designated it as a low-grade lesion [135]. It is almost certain that a significant number of cases previously reported in the literature as various vulvar "dystrophies" were really dVIN, as was recently suggested by a retrospective study of lichen sclerosus, in which the authors contended that only 30 % of cases diagnosed as lichen sclerosus which progressed to cancer, as opposed to 94 % of cases which did not progress, were really lichen sclerosus, reclassifying 41 % of cases as dVIN [133]. The tremendous difficulty in making the diagnosis was again illustrated in another recent study, in which it was shown that even with focused education only a small increase in interobserver agreement could be achieved [136]. Some of the resistance to the recognition of dVIN as a distinct pathologic diagnosis stems from the lack of any premalignant lesion of similar appearance in either the squamous mucosa of the vagina and cervix or in the nongenital skin. Yet similar lesions are described in oral cavity and larynx, which also show aggressive behavior, strong propensity to develop invasion [137, 138], suggesting an analogous disease process does indeed exist in squamous mucosa elsewhere in the body.

The main reason the diagnosis of dVIN is so difficult is that the diagnostic changes are present mainly in the base of the lesion, while the superficial layers of the epithelium are essentially normal and do not stand out from the surrounding tissue, very unlike uVIN, where there is such striking lack of maturation and nuclear atypia in the entire epithelium. One of the hallmarks of dVIN, and the reason it is so termed, is its increased proportion of more differentiated and mature cells, so that instead of displaying abnormally immature cells in the upper layers, as in uVIN, the epithelium in dVIN shows abnormally mature cells in the lower layers of the epithelium. Cells in the basal and parabasal layers develop premature keratinization, developing abundant, brightly eosinophilic cytoplasm and prominent intracellular bridges, as are normally seen in the superficial epithelium (Fig. 9.14). These cells may form whorled aggregates towards the base of the epithelium, often with accompanying keratin pearl formation (Fig. 9.15). Nuclear atypia is also a feature of these cells and is manifest by hyperchromatic, irregular, and variably sized nuclei with coarse nuclear chromatin and prominent macronucleoli. A second type of atypical cell is present in the basal layers, typically surrounding this abnormally mature cells, consisting of smaller cells with less abundant and eosinophilic cytoplasm but similar nuclear features, and the most basal cells may show striking nuclear atypia with little eosinophilic cytoplasm (Fig. 9.16). Architectural changes may also be seen in the lower layers of the epithelium, where the rete pegs are typically elongated, often distinctly narrowed, and show branching and frequently anastomosing patterns (Fig. 9.17). Towards the surface of dVIN, however, the only significant change may be parakeratosis, and there is little to distinguish these upper layers from those of normal or reactive epithelium.

In addition to the subtlety of the cellular changes and their restriction to the basal and parabasal location, another reason dVIN is more difficult to discern than uVIN is that the abnormal proliferation is much less readily apparent. Although the basal layer may be noticeably expanded in dVIN, mitotic activity is much less pronounced than in uVIN (Fig. 9.18). Atypical mitotic figures may occur and are an extremely helpful feature if they do, but they are usually few, if any. The epithelium of dVIN is often markedly acanthotic, but it can also be normal in thickness or even atrophic appearing, masking its proliferative nature.

Only one unusual variant of dVIN has been described, consisting of cases with basaloid morphology indistinguishable from basaloid uVIN, but lacking expression of p16, and associated with HPV-negative keratinizing squamous cell carcinoma [139].

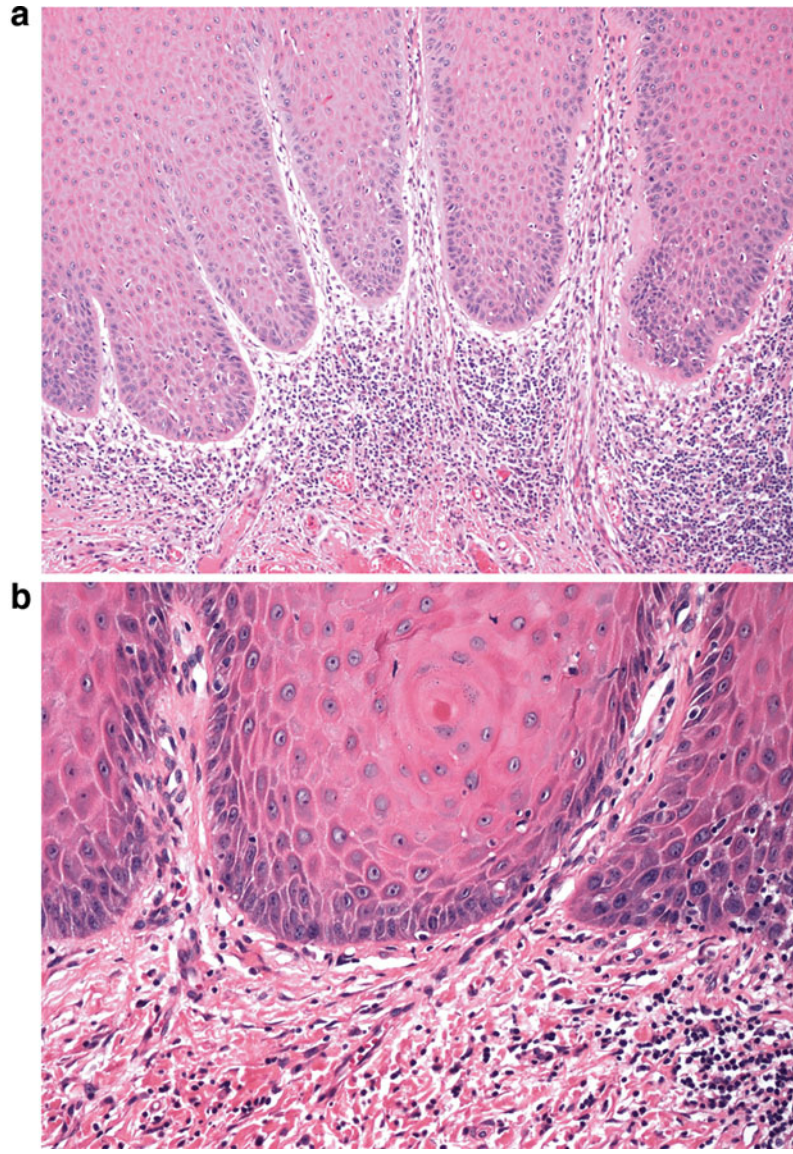

Fig. 9.14 (**a**) In dVIN, even the parabasal cells show abundant, brightly eosinophilic cytoplasm typical of the surface of keratinizing squamous epithelium. Only a thin layer of basal cells remains relatively immature appearing. (**b**) Higher power shows the prominent intercellular bridges between the cells in dVIN

Immunophenotype

Immunohistochemical studies consistently demonstrate absence of staining for p16 in dVIN (Fig. 9.19) [65, 82, 84, 85, 126] and only basal and parabasal staining with ProexC [86] providing further evidence that it is not HPV related and is etiologically distinct from uVIN. Ki-67/MIB 1 stains only a thin layer of basal and parabasal cells in dVIN and never shows staining above the lower third of the epithelium, consistent with the localization of the abnormal cells (Fig. 9.20) [126, 140]. MIB-1 staining shows a distinctly different pattern from dVIN in squamous hyperplasia, where it will extend throughout the entire thickness of the epithelium [140], but the staining pattern in lichen sclerosus is very similar to dVIN, staining only the basal and some parabasal epithelium. On the whole, there is little evidence of reliable, diagnostically useful, qualitative or quantitative differences in staining between the vulvar dermatoses and

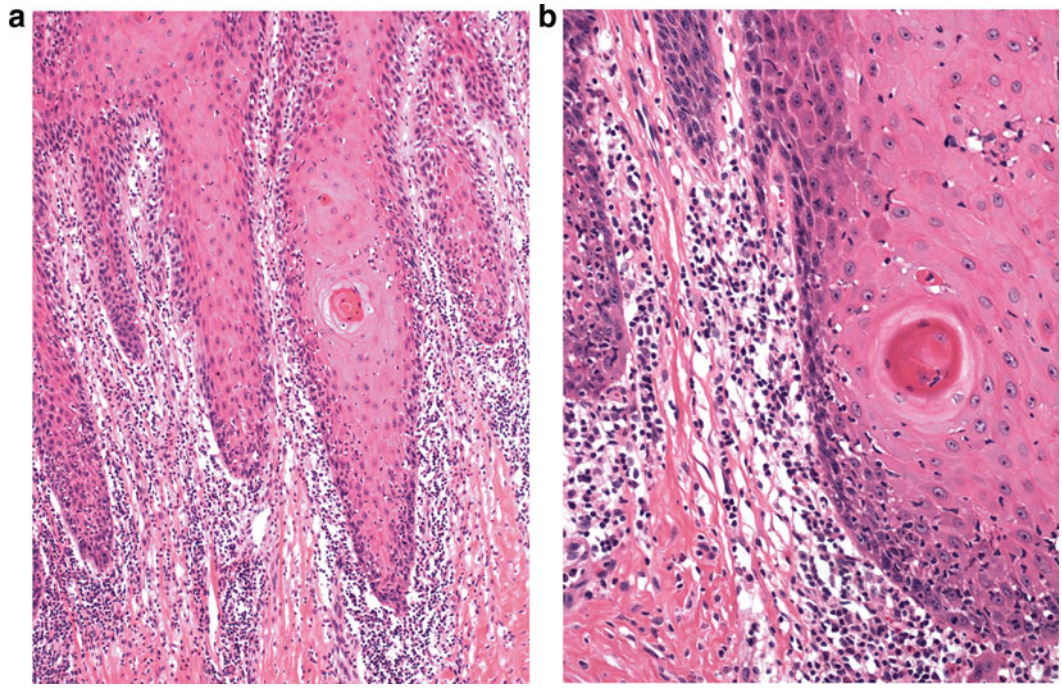

Fig. 9.15 (**a**) A keratin pearl is seen about halfway down a deep rete ridge in this case of dVIN. There is extensive associated chronic inflammation in this case, which is not uncommon. (**b**) Another keratin pearl in dVIN, on higher power, shown closer to the base of the epithelium

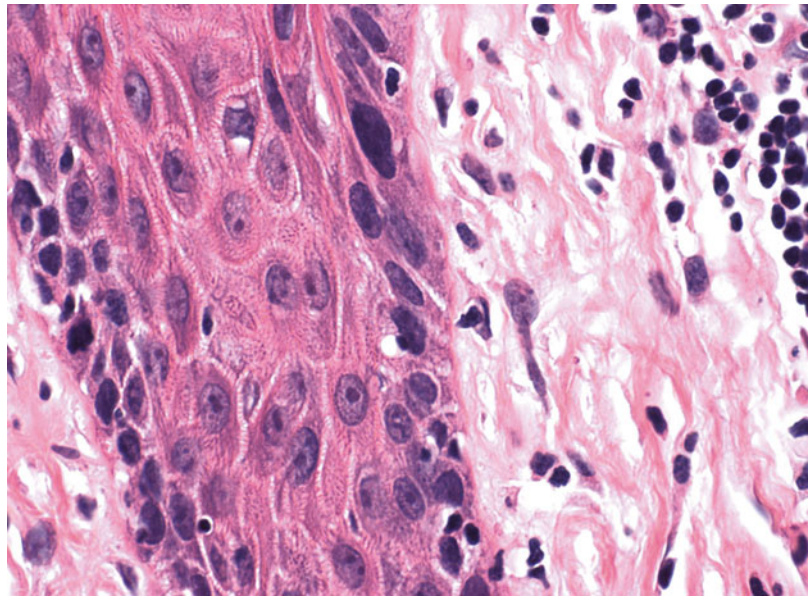

Fig. 9.16 High-power magnification of dVIN showing the characteristic nuclear features. The cells at the base of the epithelium, with very little cytoplasm, show markedly hyperchromatic and pleomorphic nuclei, while the cells farther from the base with more cytoplasm show more uniform nuclear shape with readily apparent prominent macronucleoli

dVIN, which limits the use of immunohistochemistry in the differential diagnosis. In many studies, however, immunohistochemical findings do appear to support the idea of evolution of vulvar dermatoses to dVIN. The MIB-1 labeling index has been reported to increase in cases

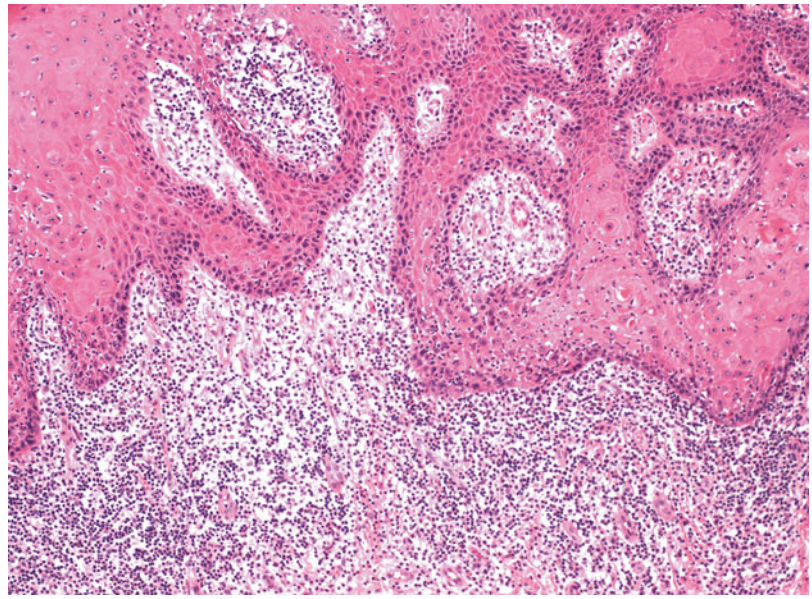

Fig. 9.17 The pattern of anastomosing rete ridges seen here is a very helpful feature in identification of dVIN

with lichen sclerosus that progress to cancer [141, 142] and to be higher still in dVIN [139]. No clear cutoff is yet established, however, making these differences of little use for diagnostic purposes. One recent study reported the use of cytokeratin 17 as a useful marker for dVIN [143], which may prove to be of value with further study.

Much has been made of the overexpression of p53 in dVIN, which has been reported in many studies in the majority of dVIN [71, 85, 87, 88, 90, 126, 127, 144]. The pattern of p53 staining is described as showing a high labeling index in the basal layer, producing continuous or near-continuous linear staining, with some suprabasilar cells staining as well (Fig. 9.21). In practice, this is less useful than one would wish as a marker for dVIN, as, again, lichen sclerosus and squamous hyperplasia have been shown to show a similar pattern, especially when associated with adjacent squamous cell carcinoma [85, 87, 131, 142, 145]. The labeling index is reportedly higher in dVIN than other conditions [90, 127, 146], but like MIB-1 staining, this is of little practical use in the differential diagnosis of difficult lesions.

Molecular Findings

HPV has been reported in only 0–13.2 % of dVIN and associated keratinizing vulvar squamous carcinomas [41, 52, 65, 83, 89, 93, 96, 127, 144], indicating little if any role for HPV in its pathogenesis or progression. Interestingly, in one study in which 10.5 % of dVIN associated with carcinoma were found to be HPV positive, only one case was found to be p16 positive and none had integrated HPV [104], suggesting that even in the minority of cases in which HPV is detected, it is rarely of biologic and pathogenetic significance.

What does appear to play a role in dVIN and the related HPV-negative cancers, as would be suggested by the immunohistochemical findings discussed above, is p53 mutation. Such mutations have been reported in the majority of cell lines from vulvar squamous cell carcinoma [147] and in up to 76.5 % of clinical cases [24, 148–150], and they are much more common in HPV-negative tumors. The majority of associated HPV-negative cancers have been found to overexpress p53 [50, 88], in contradistinction to HPV-positive tumors [50, 87, 151], and the fact that mutations in p53 have been identified in up to

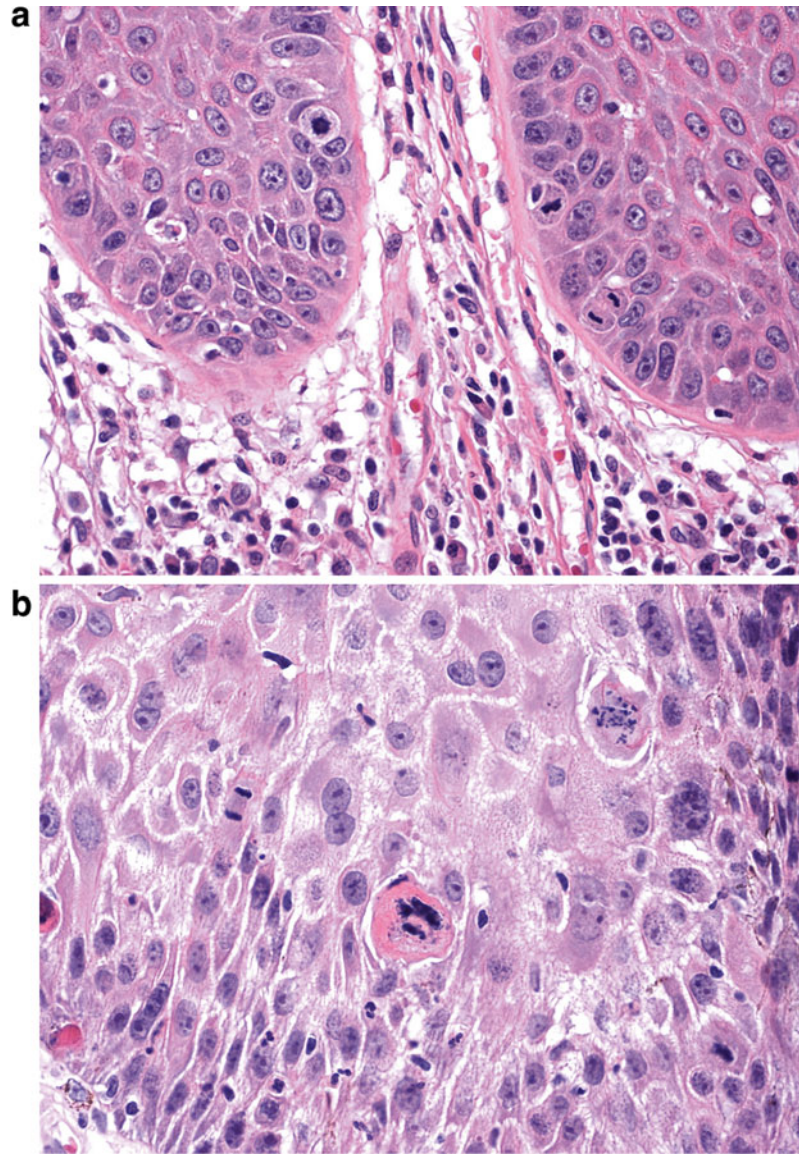

Fig. 9.18 (**a**) Several mitotic figures are shown here in the basal layer of dVIN. (**b**) The abnormal nuclear material in the large dyskeratotic cell in the center of the field is degenerative change, but towards the *upper right hand corner* and abnormal "dispersed" mitotic figure is evident in the parabasal layer of this case of dVIN. Some pigment is also visible in the parabasal layers of this case

60 % of dVIN [150] appears to support that dVIN is the progenitor of these tumors. The fact that p53 mutations have also been reported in a smaller proportion of lichen sclerosus and squamous hyperplasia [149] suggests that a proportion of these lesions do indeed progress to dVIN and that p53 mutation is an early event in the development of HPV-negative tumors [148]. In half of cancers with p53 mutations, adjacent dVIN was found to have an identical mutation [24, 150], but to date no adjacent dermatosis has been reported with mutation identical to the tumor, supporting that dVIN is the more direct precursor. VIN associated with squamous cell carcinoma has been found to be more likely to have aberrant p53 than VIN occurring alone [151], suggesting a crucial role for these mutations in the progression of disease.

Other genetic abnormalities are identified in dVIN as well. In one study, half of dVIN were found to be polyploid and the other half aneuploid [110], though in another only 38 % were

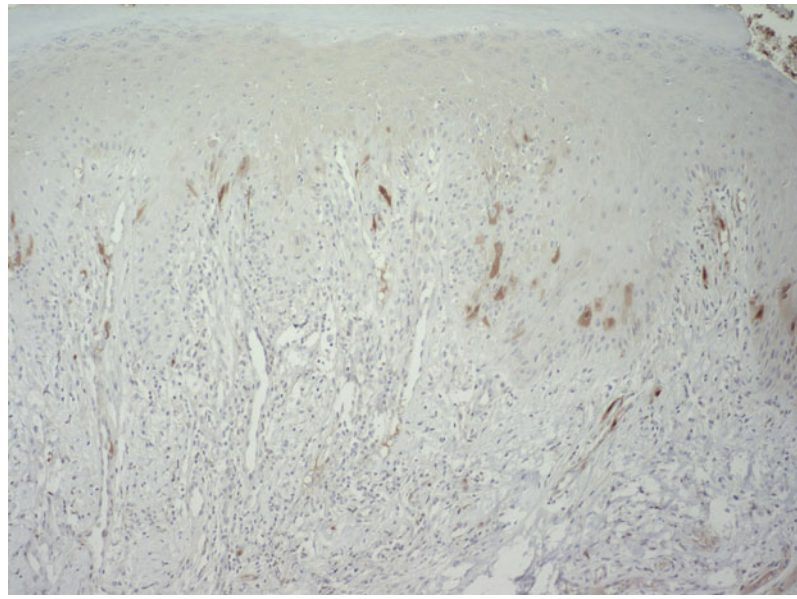

Fig. 9.19 Although there is focal cytoplasmic reactivity for p16 in this case of dVIN, the strong positive "block" indicative of HPV infection, as seen in uVIN, is absent in dVIN (compare to Fig. 9.12)

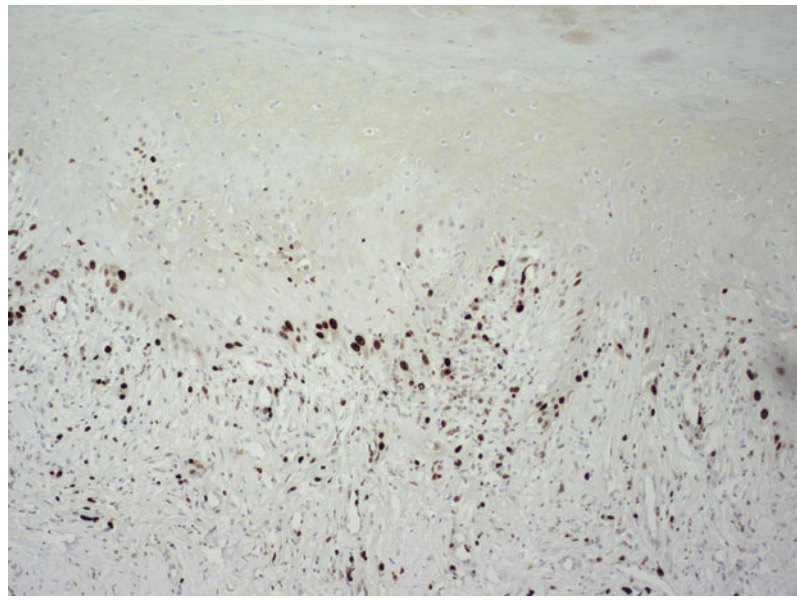

Fig. 9.20 Ki-67/MIB-1 stains only basal cells and some parabasal cells in this case of dVIN (compare to Fig. 9.13)

aneuploid or tetraploid [146]. Allelic imbalance has been reported in 53 % of dVIN [113], and in one case, it was shown to be similar to that in the associated squamous carcinoma [152]. Lichen sclerosus and squamous hyperplasia have shown a similar frequency of allelic imbalance [113, 152], and some cases have also been reported to be monoclonal [97, 111] suggesting some genetic changes may begin to accrue in the dermatoses as they evolve towards dVIN. Microsatellite instability has also been reported in dVIN, as well as in some cases of lichen sclerosus and squamous hyperplasia, but not in HPV-positive uVIN [113], a finding which both demonstrates molecular evidence of a close relationship between the dermatoses and dVIN and supports that dVIN is

Fig. 9.21 The pattern of p53 staining in dVIN. Nearly all of the basal cells, and some scattered suprabasal cells, show nuclear positivity

pathogenetically distinct from uVIN. While HPV-associated tumors and their precursors have been shown to have loss in chromosome 17, HPV-negative tumors and their precursors had gains instead [63], further evidence that vulvar carcinoma develops along two distinct pathways. Gains of 3q26, however, as found in the majority of vulvar squamous carcinomas [65, 115] and half of uVIN, were also identified in all cases of dVIN examined [65], suggesting this genetic abnormality may represent an area in carcinogenesis where both the HPV-dependent and HPV-independent pathways converge.

Patient Outcome

Due to the difficulty, and sometimes reluctance, in making the diagnosis, there is a relative paucity of information regarding the natural history of dVIN. It is clear that the association of dVIN with carcinoma is much stronger than that of uVIN; up to 85.7 % of patients with dVIN have been found to have previous, concurrent, or subsequent vulvar carcinoma [41, 51, 73, 127], and up to 80.8 % of vulvar carcinomas have been reported to have adjacent dVIN [47, 90, 104, 126]. The understanding that dVIN has a greater propensity to progress to invasive disease is based largely on observations that it is rarely encountered without an associated carcinoma. In one study, only one of six cases evaluated was still in situ [126], and in another only 26.7 % were still in situ [51]. Progression is reported in 25–32.8 % [58, 127, 133] of treated cases and has been reported to be more likely if the margins of resection are positive [153]. Progression has been reported to occur anywhere from 5 to 55 months [58, 127, 133, 153], with a median time of 22.8–28 months [58, 133], generally much shorter than is reported for uVIN. The proximity of the median age for dVIN to the median age of vulvar carcinoma has been taken as further evidence of the rapid progression of dVIN to carcinoma [41, 58].

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Vaginal Intraepithelial Neoplasia 10

Philip P.C. Ip and Ka Yu Tse

Vaginal Intraepithelial Neoplasia

Historic Evolution

Vaginal cancer was first described by Cruveilhier in the nineteenth century, but that of premalignant squamous intraepithelial lesion was not reported until 1933 by Hummer [1, 2]. The use of the term vaginal intraepithelial neoplasia, VaIN, has become popularized ever since the 1980s [3–6].

Epidemiology

The true incidence of VaIN is unknown, but it is estimated to be 0.2–0.3 per 100,000 women in the United States. VaIN is uncommon and only 1 % as frequently diagnosed as cervical intraepithelial neoplasia (CIN). This is likely related to the fewer studies on VaIN than the cervical and vulval counterparts [7, 8]. The global incidence is also difficult to determine, partly because of the difference in HPVgenotype distributions. Increase in VaIN detection has been related to the clinical practice of cytology screening and colposcopy in patients with abnormal cervical cytology [9, 10]. With the introduction of HPV prophylactic vaccines, the VaIN incidence is expected to decline [11]. It should be noted, however, 40 % of VaIN II/III is not associated with infection by HPV 16 and 18, the genotypes currently covered by the commercially available vaccines, and the benefit for preventing HPV-related lesions may not be as great as for the cervix [12]. The nanovalent vaccine, recently approved by the US Food and Drug Administration (FDA), is expected to cover additional high-risk genotypes and may protect against VaIN more effectively.

VaIN tends to occur in an older age group [6, 13–15]. The mean age of diagnosis for VaIN III is 53 years, which is 10 years older than that for CIN III [2, 16–19]. There seems to be no difference in age between those diagnosed with VaIN I, II, or III [16]. The maturation of the vaginal epithelium and its thickness are dependent on hormonal status. The higher incidence of VaIN observed in older individuals (postmenopausal) or in those who had iatrogenic menopause after bilateral salpingo-oophorectomy or external pelvic irradiation may in part be explained by the presence of thin, atrophic, and easily traumatized vaginal epithelium (see under pathogenesis below).

More than two-thirds of women with VaIN had a history of hysterectomy for benign, premalignant

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(especially CIN), or malignant lesions [20–22]. The VaIN is usually found in the vaginal vault occurring as a persistent or recurrent lesion or may represent a new lesion [6, 20, 23–25]. New lesions may be a result of reinfection or reactivationof latent HPV [26, 27].

Pathogenesis

Almost all VaINs are associated with infection of both low- and high-risk genotypes of HPV. HPV DNA is detected in the dysplastic cells in at least 80 % of cases [28]. It has been noted that HPV 16 and 18 were frequently found in 60 % of VaIN II/III and 41 % of VaIN I [12, 29]. The overall genotype patterns in VaIN are slightly different to those found in the cervical counterpart (Table 10.1) [28].

Unlike the cervix, the vagina (and vulva) lacks the squamocolumnar junction where HPV preferentially infects. This in part accounts for the lower frequency of VaIN compared with the cervical counterpart. HPV gains entry into the vaginal squamous epithelium through micro-openings, such as those secondary to microtrauma or erosion, and accesses and preferentially binds to the epithelial basement membrane before binding to the cell surface receptors and infecting basal cells [30, 31]. The microtrauma also induces wound healing and activates cell division in cells that are infected with HPV [32, 33]. At the same time, the virus undergoes DNA replication in these basal cells, but viral gene expression and protein production do not usually occur until the keratinocytes are differentiated and situated nearer to the surface of the epithelial layers in the stratum spinosum and granulosum. The duration of this infectious process is variable and takes at least 3 weeks. Subsequent integration of DNA into host genome is vital for neoplastic progression [34, 35]. It should be pointed out that even though HPV infection is necessary for neoplastic transformation, the majority of infection will resolve with time as it is usually cleared by the host's immune system, specifically cell-mediated immune response. It is estimated that 70 % of the infection will resolve spontaneously within 1 year and about 90 % will resolve within 2 years [36–38]. Only persistent infection would result in an increased risk of deregulation of viral gene expression and neoplastic progression [39–41]. It is thought that the persistence is in part a result of suppression of the host innate and adaptive immune responses [40, 42, 43].

Table 10.1 Low- and high-risk HPV genotype distribution in LSIL and HSIL of the vagina and cervix (modified from Srodon et al. [28])

	Vagina		Cervix	
	[a]Low-risk HPV	[a]High-risk HPV	[a]Low-risk HPV	[a]High-risk HPV
LSIL	81	18, 51	6, 11	18
	42, 43, 54, 62, 67	56, 66		16, 66
		16, 39, 52, 58, 67		35, 51
				31, 33, 45, 68
				26, 39, 52, 53, 58, 59
HSIL	CP6108	16	None	16
		58		31, 35
		31		18
		35, 51, 52, 66		58
				52, 56, 59, 66, MM1, MM3

[a]The order of appearance of each genotype indicates their relative frequency found in the study of Srodon et al. [28]

Other risk factors for VaIN are similar to those for CIN and contribute to an increased risk of HPV infection in the vagina. These include concurrent HPV infection of other lower genital sites, smoking (carcinogenic effect of tobacco), use of oral contraceptives, immunosuppression (including human immunodeficiency virus infection), and external pelvic radiotherapy for other malignancies [6, 16, 44–50]. Women who were exposed to in utero diethylstilbestrol (DES) have vaginal adenosis more frequently [51–53]. This results in greater number of squamocolumnar junctions resembling those in the cervical transformation zone which then becomes the targets

for HPV infection and subsequent development of VaIN, squamous cell carcinoma, or adenocarcinoma [54–57].

Nomenclature

Traditionally, VaIN was classified using a three-tier system of VaIN I, II, or III according to the degree of dysplasia, similar to those described for the cervix. In agreement with the Bethesda System for Cytopathology and the 2013 LAST project recommendations, the WHO 2014 working group agreed to replace this traditional three-tier histologic classification of squamous intraepithelial neoplasia (i.e., VaIN I, II, and III) into a two-tier system of low- and high-grade squamous intraepithelial lesions (i.e., SIL, as LSIL or HSIL, respectively) [4, 58, 59]. The rationale for this change is that the two-tier system is more histologically reproducible than the three-tier system and can divide patients into two managerial or biological subgroups. Lesions regarded as condyloma and VaIN I are morphological manifestation of transient HPV infection and will be classified as LSILs. HSILs are a result of persistent infection by high-risk HPV, are precancerous, and will replace the VaIN II/III category.

Gross Features

Patients with either LSIL (condyloma and VaIN I) or HSIL (VaIN II/III) in the vagina are asymptomatic, and the lesions are usually grossly invisible. The usual presentation is an abnormal vaginal smear result. They may also be detected by colposcopy incidentally during investigation for abnormal cervical cytology. The lesions are represented by a white or pink discolored area with or without a raised plaque with irregular granular surface. They are usually more visible after application of acetic acid or Lugol's iodine solution under colposcopy (Figs. 10.1 and 10.2). The upper third of the vagina is more frequently involved. About 50 % of lesions are multifocal, and in 75 %, there is a preceding or concurrent SIL or carcinoma in the cervix or vulva [6, 20, 24, 25, 60–63].

Microscopic Features

SIL is characterized by various degrees of epithelial dysplasia but without invasion through the underlying basement membrane. The cells in SIL show nuclear atypia, partial loss of or disordered squamous cell maturation, dyskeratosis, and

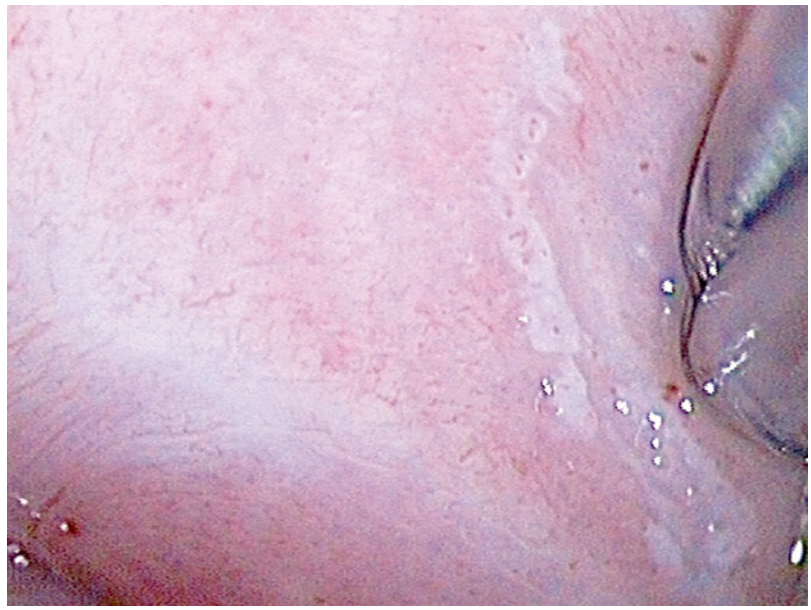

Fig. 10.1 VaIN I (LSIL) after application of 5 % acetic acid under colposcopy. Faint acetowhite areas in lateral vaginal wall with fine punctation and fine mosaicism (*right*)

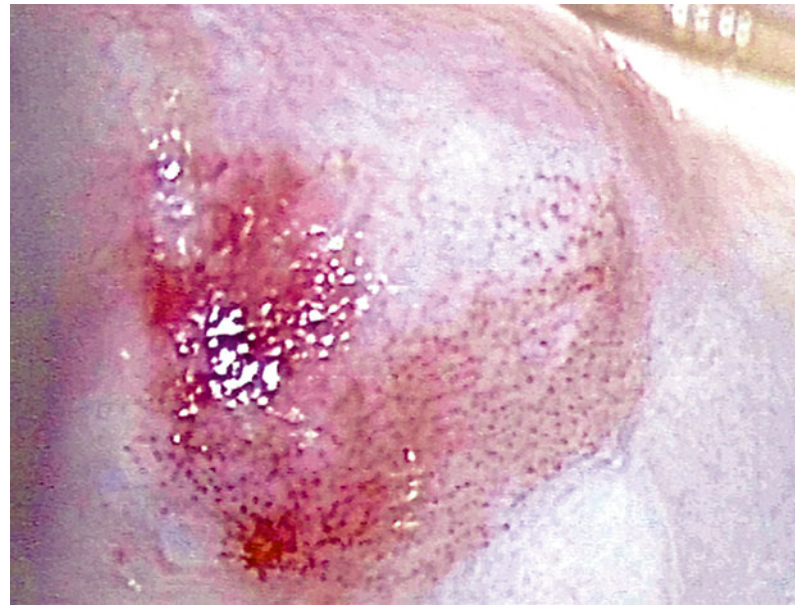

Fig. 10.2 VaIN II/III (HSIL)after application of 5 % acetic acid under colposcopy. Dense acetowhite area with coarse punctation (1–2 o'clock)

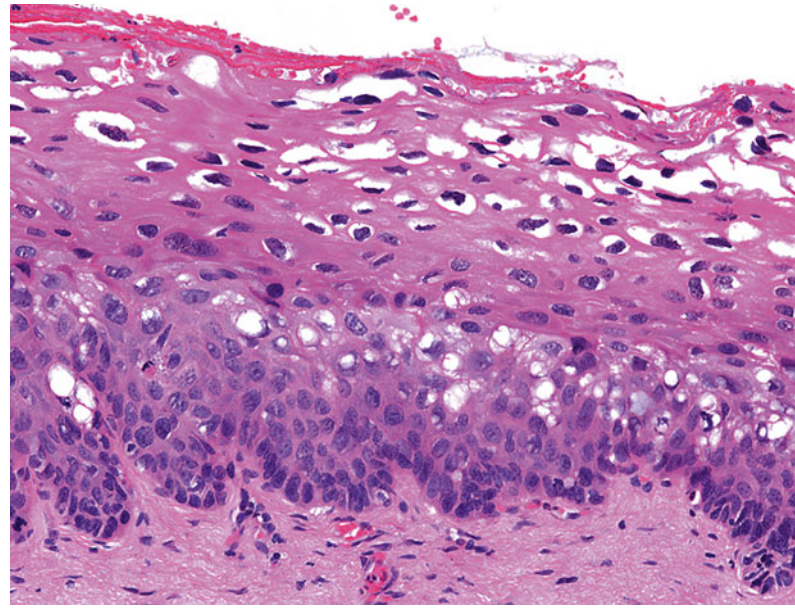

Fig. 10.3 VaIN I and condyloma (LSIL). Dysplastic epithelium involving the lower one-third of the epithelium. Typical koilocytes are striking in the upper, mature layers of the epithelium. H&E ×40

increase in mitotic activity, including the presence of atypical mitoses. LSIL (condyloma and VaIN I) is diagnosed when the dysplastic epithelium involves the lower one-third of the epithelial layer (Fig. 10.3); koilocytic atypia is defined by nuclear enlargement with or without binucleation, nuclear membrane irregularity, hyperchromasia, and perinuclear halo. These HPV cytopathic effects are normally found in mature squamous cells (Fig. 10.4). HSIL (VaIN II/III) is diagnosed when the involvement of the epithelial thickness exceeds that of LSIL (Figs. 10.5, 10.6, 10.7, and 10.8).

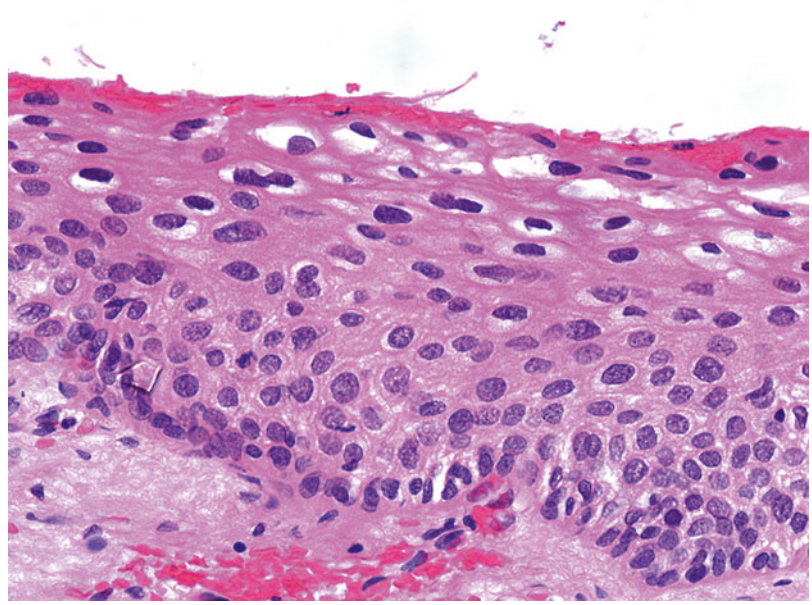

Fig. 10.4 Condyloma (LSIL). Mild epithelial dysplasia may be difficult to appreciate in thin and atrophic epithelium. There is minimal atypia involving the basal and parabasal cells, but koilocytes are more readily identified in the upper layers. H&E ×40

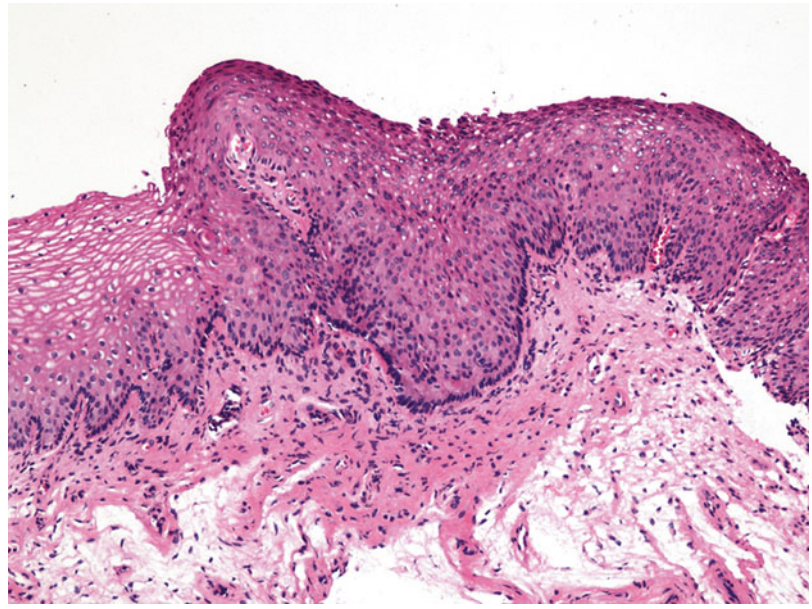

Fig. 10.5 VaIN II (HSIL). Focus of HSIL is usually well demarcated from the adjacent nondysplastic epithelium and can be appreciable under medium magnification. H&E ×20

Immunohistochemistry

p16

The LAST recommendation has determined that p16 immunostain is useful in routine pathologic diagnosis of SIL [64]. p16 is a cyclin-dependent kinase inhibitor encoded by the tumor suppressor gene, *CDKN2A*. It arrests cell cycle by inactivation of the cdk4-cyclin D and cdk6-cyclin D complex, which controls the transition to S-phase by phosphorylation of Rb. Binding of HPV E7 to pRB essentially leads to its inactivation and causes an inverse rise in p16 protein [65]. This rise in p16 indirectly indicates the presence of high-risk HPV infection and can be detected by immunohistochemistry, especially when the staining is

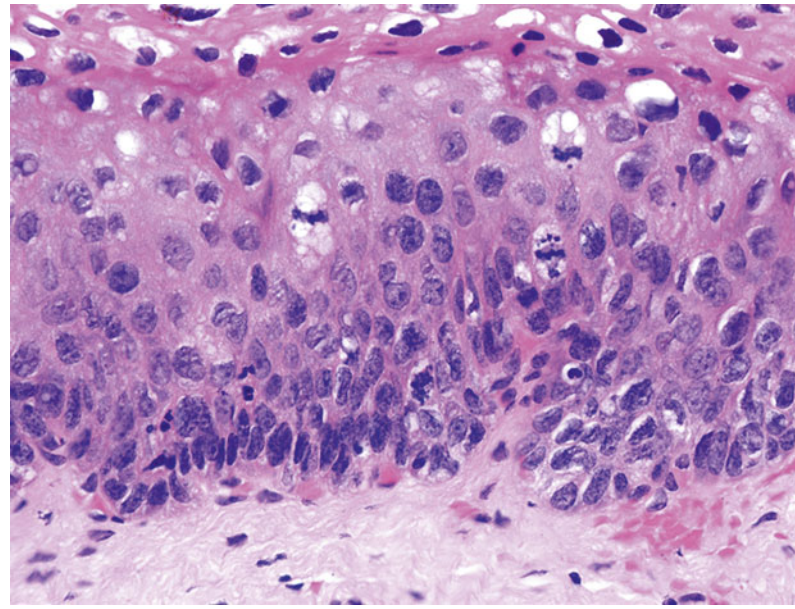

Fig. 10.6 VaIN II (HSIL). Dysplastic epithelium with mitotic figures involving the lower two-thirds of the epithelium. Limited maturation is preserved nearer to the surface. H&E ×40

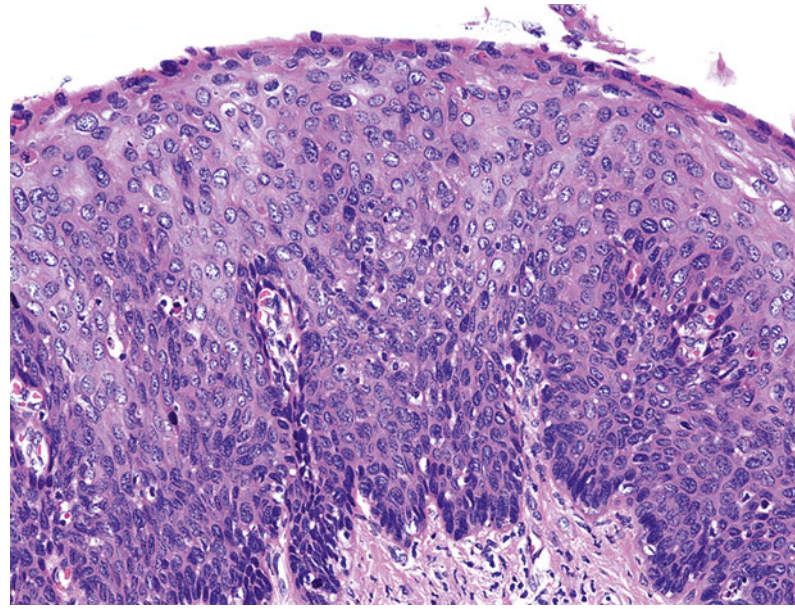

Fig. 10.7 VaIN III (HSIL). Full-thickness epithelial dysplasia. H&E ×20

diffuse and strong [66–74]. This immunostain has been found to be useful in distinguishing HSIL (VaIN II/III) from benign mimics (see below) but not from LSIL (condyloma and VaIN I) as the latter have been shown to express p16 in up to 50 % of cases [66, 72–74]. It should also be noted that the frequency of p16 positivity in vaginal SIL is considerably less than that in the vulval and cervical counterparts (62 % of vaginal versus 85 % of vulval and 90 % of cervical HSILs) [75]. The LAST working group recommends the judicious use of p16 stain and that it should only be used to confirm a morphological impression of HSIL (VaIN II/III) but not used merely to distinguish between LSIL (condyloma and VaIN I) and HSIL (VaIN II/III) (Fig. 10.9) [4].

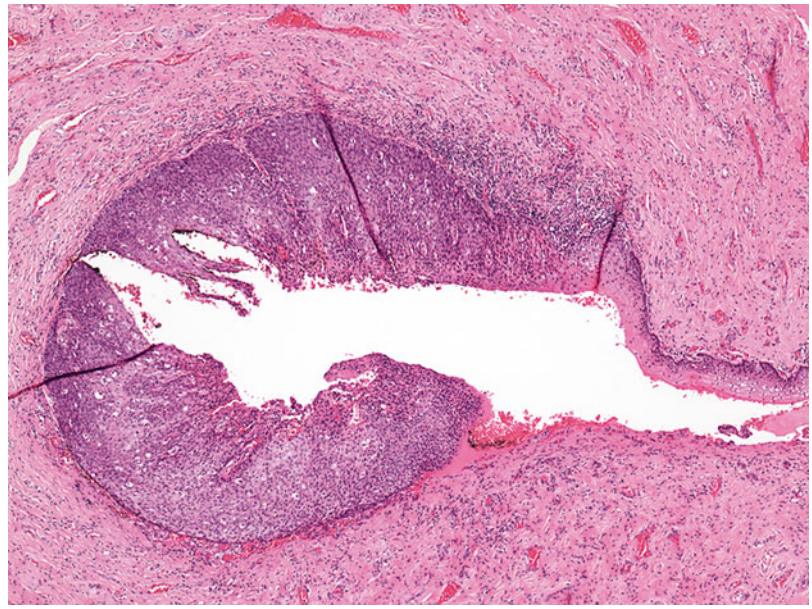

Fig. 10.8 A typical case of VaIN III (HSIL) involving the vaginal vault. The dysplastic focus (*left upper* and *lower*) shows sudden transition to normal (*top right*) and ulcerated mucosa (*lower right*). H&E ×10

Ki-67

The MIB1 proliferative index may be estimated by using the immunohistochemical stain ki-67. This is a nonhistone protein expressed in the cell nucleus in all phases of the cell cycle except for G0 and early G1 [71]. In normal, non-inflamed, nonneoplastic squamous or metaplastic epithelium, the staining is limited to the parabasal cells. In LSIL and HSIL, the staining extends to the intermediate squamous cells and all layers of the epithelium, respectively (Fig. 10.10) [69, 72, 76, 77]. It is a very sensitive but nonspecific marker as reactive inflammatory lesions may also result in similar staining patterns as SILs [69]. The concurrent use of p16 does not seem to improve the accuracy of identifying and grading SIL [72].

ProEx C

ProEx C is an antibody that targets the expression of topoisomerase II-alpha and minichromosome maintenance protein-2, where these two genes are shown to be overexpressed in cervical cancers [71, 78]. The expression of ProEx C indicates an underlying aberrant S-phase induction [79]. In normal nonneoplastic epithelium, ProEx C staining is confined to the basal cells. In 90–92 % of HSIL, the staining extends to both the lower and upper half of the epithelium. Staining in LSIL is more variable, and metaplastic epithelium may also show suprabasal cell staining. Like p16, ProEx C is proposed to be useful in distinguishing between HSIL and reactive lesions but not between HSIL and LSIL [78, 79].

Differential Diagnoses

The lack of epithelial cell maturation in transitional cell metaplasia may potentially be confused with SIL. The cells in the former may have mild to moderate nuclear atypia and may have perinuclear halos. Unlike SIL, however, cell nuclei in transitional cell metaplasia are often spindle with tapered ends and have longitudinal nuclear grooves. The nuclei of the parabasal cells are usually oriented vertically to the basement membrane while those nearer to the surface are horizontal. Mitotic figures are usually rare [80]. As noted earlier, the vaginal mucosa is particularly thin and atrophic in postmenopausal women and is susceptible to trauma and erosion. Reactive atypia in these cases may be difficult to distinguish from true SILs. However, mitotic activity is uncommon in atrophy. Administration of topical

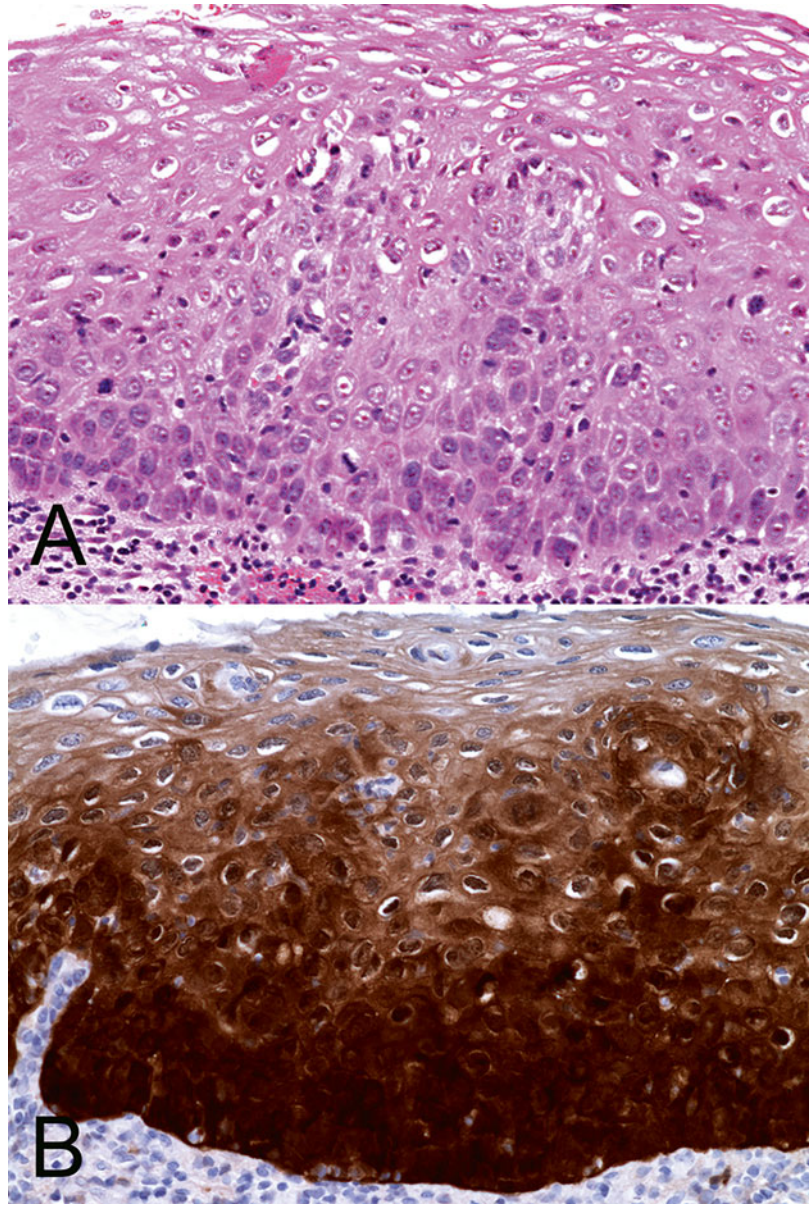

Fig. 10.9 (**a**) Recommended use of p16 immunostain to confirm a morphological impression of VaIN II (HSIL). H&E ×40. (**b**) There is almost full-thickness epithelial staining with p16. The cells are stained diffusely and intensely

estrogen cream in the vagina would induce epithelial maturation, and truly dysplastic lesions will then be apparent in cytology or biopsy. In those cases without coexisting active inflammation, the use of ki-67 and p16 immunostains may facilitate the differential diagnosis of SIL from reactive atypia, postirradiation atypia, atrophy, transitional cell metaplasia, and immature squamous metaplasia within adenosis [75, 77]. In nondysplastic conditions, the p16 stain is either negative or may show patchy staining, while the ki-67 index is usually low. Extramammary Paget's disease is characterized by the presence of intraepithelial large cells with abundant cytoplasm and vesicular nuclei. They usually involve the basal epithelial layers but sometimes can be extensive, making it difficult to distinguish from SIL, particularly in small biopsies. Paget's cells usually contain cytoplasmic mucin and are immunoreactive for CK7 and GCDFP-15.

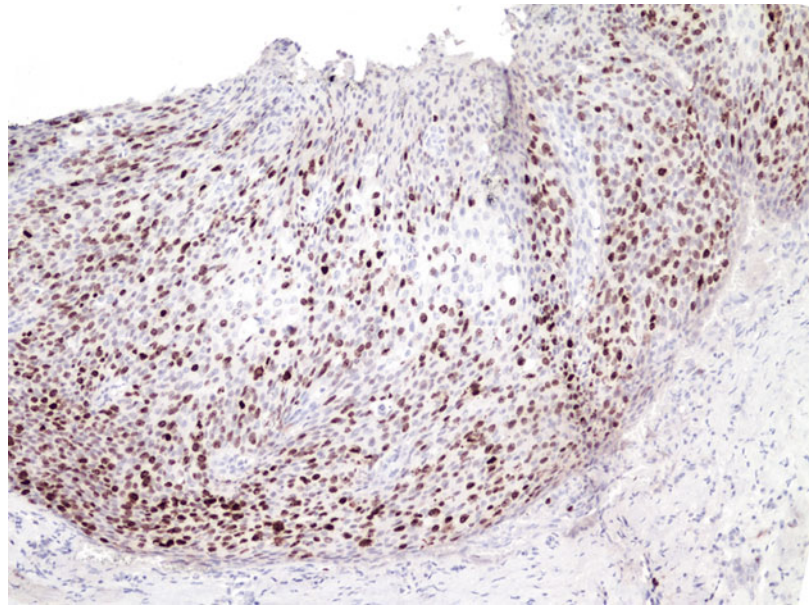

Fig. 10.10 The MIB1 proliferative index (ki-67) is elevated with positive staining in nuclei reaching the upper layers of the epithelium

Molecular Properties

The infected basal and parabasal epithelial cells initially remain morphologically normal because HPV gene expression is inhibited to a maintenance level only. Productive gene expression is tightly regulated until the cells have begun terminal squamous differentiation. At this juncture, the appearance of nuclear atypia such as enlargement and hyperchromasia is related to the rise in E6 and E7 viral proteins. In high-risk HPV genotypes E6 and E7, viral proteins cause cell cycle disruption. E6 binds to p53 protein and inhibits apoptosis, while E7 binds to pRb protein and activates the transcription factor E2F, thereby causing DNA replication [81–84]. These events cause keratinocytes to lose terminal differentiation, and histologically, the affected cells resume an immature appearance and manifest as HSIL. As a result of the suppression of the host innate and adaptive immune responses and for other unknown reasons, some high-risk HPVs have evolved the ability to become persistent for many years. The deregulation of E6 and E7 genes in persistent infection leads to an increase in cell proliferation of the basal/parabasal cells, i.e., those that are still capable of cell division, which is directly responsible for genomic instability, accumulation of genetic errors, and viral integration into the host genome [34, 85–88].

Infection caused by the low-risk HPV genotypes usually results in LSILs, which are clinically flat and inconspicuous lesions and will regress spontaneously with time. The production of viral DNA mainly occurs in cells that can no longer divide, i.e., intermediate squamous cells [89]. In low-risk HPV, the E6 and E7 viral proteins, respectively, do not bind p53 and pRb proteins with as high affinity as those in high-risk genotypes, and the precise mechanisms of their contribution to neoplastic transformation are unclear. Nonetheless, the role of wound healing response in causing the proliferation of infected basal/parabasal cells harboring the virus is thought to play an important part [90–92]. LSILs are DNA stable lesions, and the enlarged nuclei are either euploid or polypoid. The nuclear atypia is accompanied by cell maturation and acquisition of cytoplasm. Koilocytic change is related to an increase in cytokeratin binding protein E4 [93].

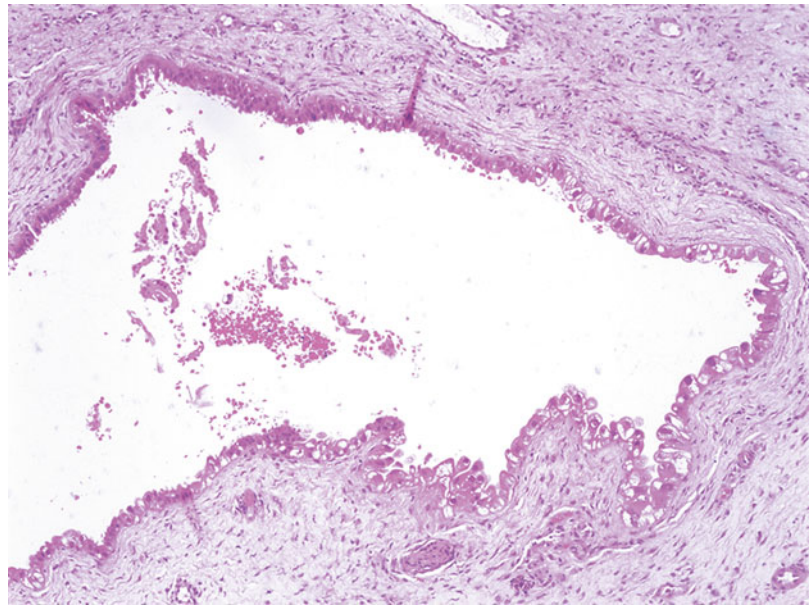

Fig. 10.11 Vaginal adenosis in a case associated with a coexisting clear cell carcinoma. A "premalignant" gland showing dysplasia and clear cell change (*right lower*). H&E ×20

Patient Outcome

The natural history of vaginal SIL is less well studied. In general, LSIL that is associated with low-risk HPV infection usually resolves spontaneously around 2 years as the virus is cleared [36–38]. Some HSIL related to high-risk HPV is more likely to develop into persistent infection and subsequent progression into carcinoma. In several studies, 3–8 % of SIL progressed into squamous cell carcinoma in spite of treatment and close surveillance [15, 61, 94–96]. Local recurrence is more likely with larger lesions, inadequate excision, and involvement of margins [13]. Recurrence may also be related to the fact that these lesions tend to be multifocal and clinically invisible, and complete excision is difficult to achieve [19, 97]. The risk of recurrence is not related to the grade of VaIN (SIL) though VaIN III may recur at a much shorter interval from the time of its first treatment [16, 95]. Smoking may lead to recurrence and progression into carcinoma [98]. There are no proven histologic biomarkers that could predict which cases of SIL are more likely to progress to carcinomas. Nonetheless, follow-up with high-risk HPV molecular testing will identify patients with persistent infection who are more likely to have progressive disease [99–102].

Premalignant Glandular Lesions of the Vagina

Vaginal mucosa is normally devoid of glands and primary adenocarcinomas are rare. Adenocarcinoma in situ of the vagina has not been a well-defined entity, and criteria of diagnosis have not been established. Lesions considered as premalignant in this context have been described when they are found contiguous to the invasive tumors and usually show various degrees of epithelial dysplasia. These include adenosis, endometriosis, mesonephric remnants, urethral diverticulum, Skene's gland, and endocervicosis [57, 103–112]. Some cases develop as a result of incomplete excision of cervical adenocarcinoma in situ [113].

Vaginal adenosis has been implicated as a precursor lesion to adenocarcinoma. The majority of clear cell carcinoma of the vagina described in the past was found in association with adenosis in the setting of in utero DES (Fig. 10.11) [51–53]. With discontinued usage of DES, the incidence of these tumors is decreasing. Apart from this association, vaginal adenosis may also arise de novo, secondary to tamoxifen, CO_2 laser

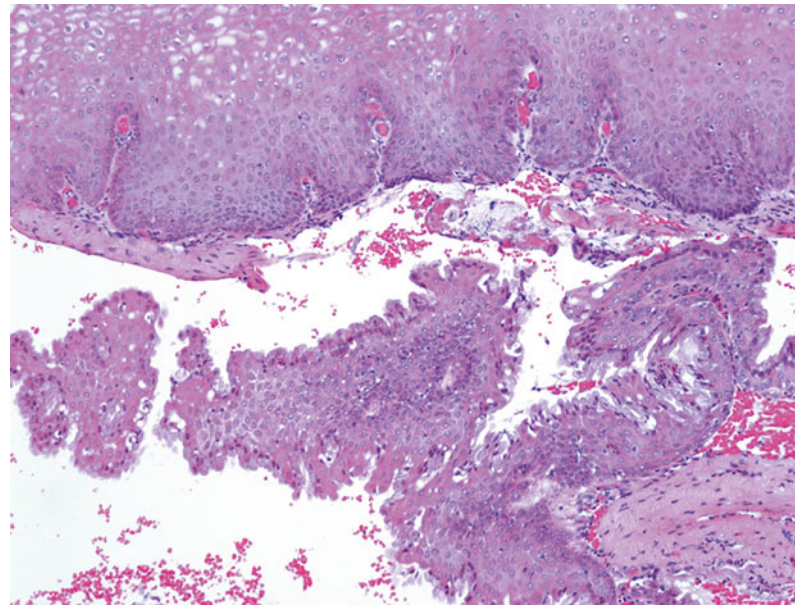

Fig. 10.12 Vaginal adenosis. The glandular epithelium is commonly found beneath the surface mucosa. H&E ×20

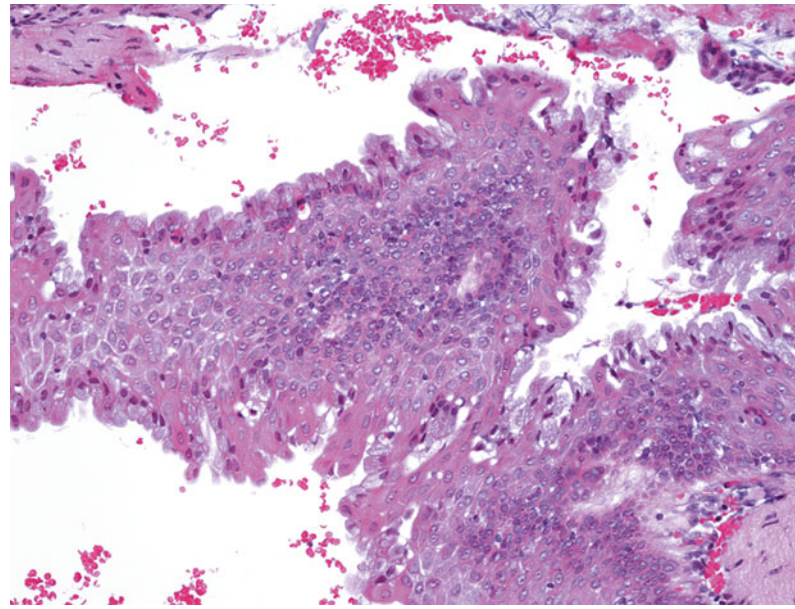

Fig. 10.13 Vaginal adenosis. The mucinous epithelium often undergoes squamous metaplasia (*left*). H&E ×40

vaporization, topical 5-fluorouracil treatment for vaginal condyloma/SIL, and Stevens-Johnson syndrome [56, 114–117]. Grossly, vaginal adenosis is granular and red. Most are found involving the upper third. In those related to DES, there may be other coexisting congenital abnormalities such as transverse ridges of the vagina [54]. Microscopically, the adenosis consists of glands, either replacing the surface squamous epithelium or being found in the superficial vaginal stroma. The glands are lined by endocervical-type mucinous epithelium, but tuboendometrioid-type epithelium may also be found. Squamous metaplasia of these epithelia is common(Figs. 10.12, 10.13, and 10.14). As noted earlier, the squamocolumnar junctions in these may become the targets for HPV infection and hence develop into premalignant and malig-

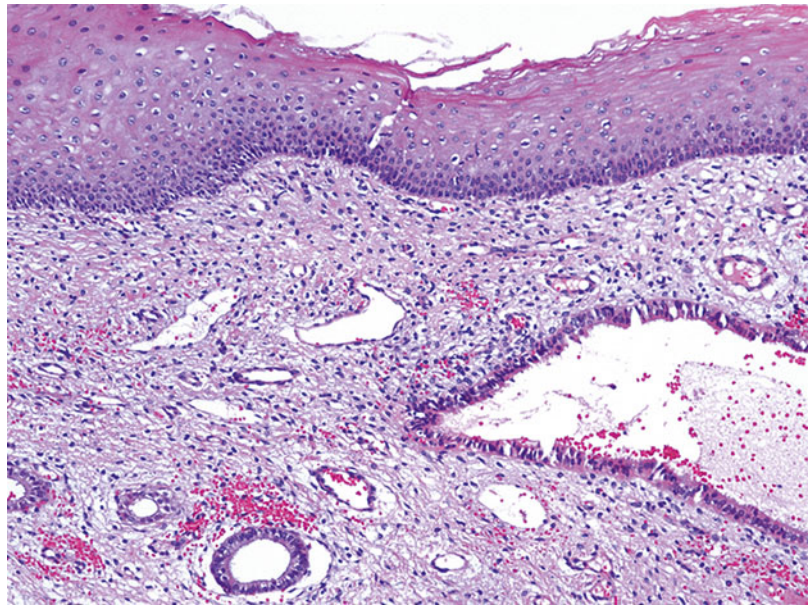

Fig. 10.14 Vaginal adenosis. The glandular epithelium may be of ciliated tuboendometrioid type. H&E ×20

nant lesions [54–57]. Intestinal metaplasia is rare and seldom gives rise to an intestinal-type adenocarcinoma [105, 118, 119].

About two-thirds of primary endometrioid carcinoma of the vagina is secondary to a preexisting endometriosis. In these, the latter shows transition into the invasive tumor, supporting the view that this is indeed the premalignant lesion [57, 103]. Endometriosis of the vagina is rare. Some are secondary to trauma induced by previous hysterectomy, and these are usually of the superficial type. Others may be an extension of pelvic endometriosis involving pouch of Douglas or rectovaginal septum, and these are often found deep in the vaginal stroma. The etiologies for malignancy arising from endometriosis include unopposed estrogen and tamoxifen treatment [120–123]. Histologically, vaginal endometriosis is identical to those found elsewhere, but in cases in which there is an established adenocarcinoma, the glandular epithelium usually shows dysplasia resembling atypical hyperplasia of the endometrium.

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Cytology of Cervical Precancerous Lesions

11

Zaibo Li and Chengquan Zhao

Introduction

Cervical cytology screening (Papanicolaou [Pap] test) has markedly reduced mortality from cervical cancer, especially squamous cell carcinoma, which comprises the majority of cervical cancers (up to 90 %) [1, 2]. This cervical cancer mortality reduction is not only due to an increase in the detection of early stage invasive cervical cancer which has a 5-year survival rate of 92 % but also the detection and treatment of precancerous lesions [1, 2]. In the USA, more than 50 % of women with invasive cervical cancer either have never had cervical cytology tests or have not been screened periodically [3, 4].

The Pap test was invented by and named after Dr. Papanicolaou to detect potentially precancerous/cancerous cells sampled from transformation zone, the junction of the ectocervix and endocervix [5]. Conventional Pap test involves plating cervical samples obtained by brush and spatula on a microscope slide and then preserving samples with fixative solution. In liquid-based cytology, samples from the brush and spatula are transferred into a liquid fixative solution, and then cells are trapped onto a filter and plated onto a glass slide in a monolayer.

Pap test results are reported according to the Bethesda System (TBS), which was first introduced in 1988 and revised in 1991 and 2001 [6–8]. The newly revised TBS will be published in the earlier months of 2015. The intent of TBS is to distinguish between abnormalities that are unlikely to progress to cancer and those that are more likely to indicate a precancerous or cancerous lesion and to standardize and improve the clinical usefulness of Pap test reports.

This chapter will introduce epithelial abnormalities of cervical cytology based on TBS 2014 Classification and include the following categories: atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells-cannot exclude HSIL (ASC-H), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), squamous cell carcinoma, atypical glandular cells-not otherwise specified (AGC-NOS), atypical glandular cells-endocervical (AGC-EC) , atypical glandular cells-endometrial (AGC-EM), endocervical Adenocarcinoma in situ (AIS), adenocarcinoma (endocervical, endometrial, not otherwise specified), and other malignant neoplasms.

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Squamous Cell Abnormality

Atypical squamous cells
- Atypical squamous cells of undetermined significance (ASC-US)
- Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H)

Squamous intraepithelial lesions
- Low-grade squamous intraepithelial lesions (LSIL)
- High-grade squamous intraepithelial lesions (HSIL)

Squamous cell carcinoma

Glandular Cell Abnormality

Atypical glandular cells (AGC)
- Atypical glandular cells-NOS (AGC-NOS)
- Atypical endocervical cells (AGC-EC)
- Atypical endometrial cells (AGC-EM)

Cervical adenocarcinoma in situ (AIS)
Endocervical adenocarcinoma
Endometrial adenocarcinoma
Other malignant neoplasms

Squamous Abnormality

Atypical squamous cells (ASC) are defined as squamous cells with equivocal (uncertain diagnostic) findings that are suggestive for squamous intraepithelial lesions (SIL), which are qualitatively or quantitatively insufficient for a definitive interpretation. ASC is not a single biologic entity but rather includes changes that may be the results of nonneoplastic conditions (inflammation, atrophy, air-drying, etc.) or neoplasia (cervical intraepithelial neoplasia or carcinoma). It is recommended that ASC rate should be kept under 5 % for all Pap smears in a laboratory, and the ASC/SIL ratio should not exceed 3:1. All ASC interpretation should be categorized as either ASC of undetermined significance (ASC-US) or ASC-cannot exclude a high-grade squamous intraepithelial lesion (ASC-H).

ASC-US

Definition

ASC-US represents squamous cells with findings that are either suggestive of low-grade SIL (LSIL) or SIL of indeterminate grade and is recommended to comprise more than 90 % of all ASC interpretations in a laboratory.

Morphology

The area of the nucleus in a normal intermediate squamous cell is approximately 35 μm^2, which is used as a comparison for nuclei of atypical squamous cells. ASC-US cells may show some of the following features (Fig. 11.1):

1. Nuclei are about 2½–3 times the area of the nucleus of a normal intermediate squamous cell.
2. Slight increase in nuclear to cytoplasmic ratio (N/C).
3. Nuclear membrane is smooth to slightly irregular.
4. Nuclear hyperchromasia is absent to light.
5. Chromatin is finely granular and evenly distributed.
6. Nucleoli/chromocenters are inconspicuous or absent.
7. Cells present singly and in sheets.
8. Atypical parakeratosis may present as small orangeophilic cells with moderate cellular pleomorphism, slight increase in nuclear size, and vesicular or pyknotic chromatin.

Fig. 11.1 (continued) nuclear enlargement. (**d**) ASC-US versus LSIL. Squamous cells with multinucleation, hyperchromasia, nuclear enlargement, and possible perinuclear halo. (**e**) Atypical parakeratosis. Cluster of squamous cells with irregular hyperchromatic nuclei and dense orangeophilic cytoplasm. (**f**) Single squamous cell with irregular hyperchromatic nucleus and possible perinuclear halo. (**g**) ASC-US versus LSIL. Single squamous cell with hyperchromatic nucleus and perinuclear halo. (**h**) Atypical repair. Single and cluster of cells with features suggestive of repair, but also enlarged nuclei, increased N/C ratio, irregular chromatin distribution, and conspicuous nucleoli

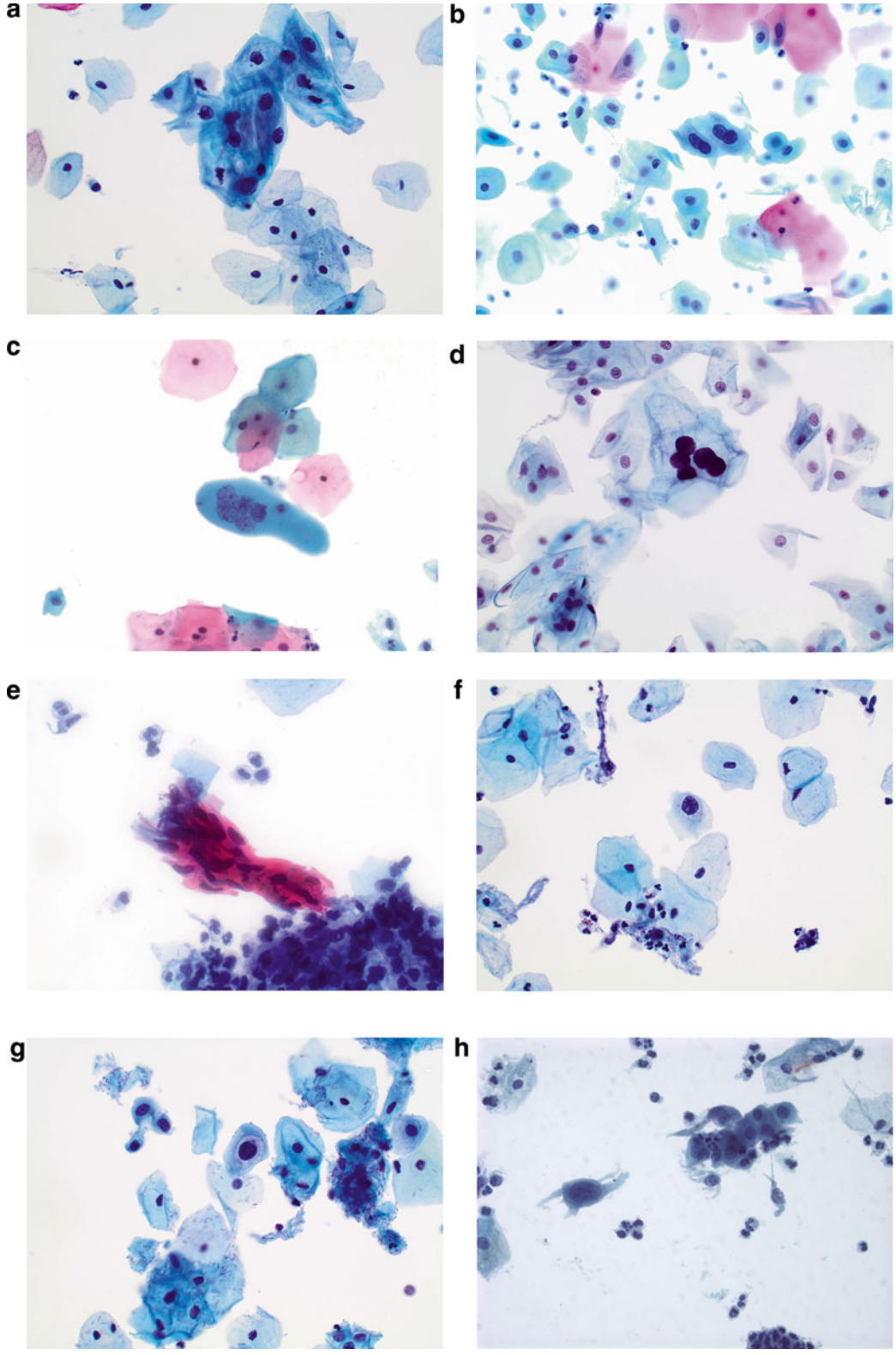


Fig. 11.1 ASC-US (liquid-based preparation, LBP). (**a**) Mature squamous cells with nuclear enlargement, hyperchromasia, but even chromatin distribution. (**b**) ASC-US versus LSIL. Squamous cells with binucleation and nuclear enlargement: these are the only cells found on this Pap smear. (**c**) Squamous cells with multinucleation and

9. Suggestive HPV cytopathic effect and/or binucleation may be present, but fall short of LSIL.

ASC-US includes the following patterns:

1. Mature intermediate squamous cells with changes that resemble koilocytes, but lack all of the features of typical koilocytes (e.g., definite hyperchromasia, sharp perinuclear halos, or binucleation).
2. Atypical parakeratosis with dense orangeophilic cytoplasm, minimal nuclear irregularity, and mild nuclear atypia.
3. Atypical repair cells with features of repair and presence of marked nuclear size variation, prominent nucleoli, and irregular chromatin distribution.
4. Atypia of atrophy with nuclear enlargement, hyperchromasia, or irregular nuclear contours.
5. ASC-US may refer to cells with atypical nuclear features due to air-drying artifact in conventional smears or distorted cells at the rim of liquid-based cytology specimens.

Differential Diagnosis

Reactive Changes The nucleus in cells with reactive changes may increase up to two times the area of the nucleus of a normal intermediate squamous cell with hypochromasia, fine chromatin, and smooth nuclear contour. The N/C ratio is low, and the cytoplasm is degenerative. Nucleoli may be seen (Fig. 11.2).

LSIL The nucleus in LSIL cells is usually more than three times the area of the nucleus of a normal intermediate squamous cell with irregular nuclear shape and contour, hyperchromasia, binucleation/multinucleation, and coarse chromatin. Nucleoli are usually inconspicuous. Typical koilocytes have sharply defined perinuclear halos.

Follow-Up Results In a screened US population, approximately 50 % of ASC-US cases are positive for high-risk human papillomavirus (hr-HPV). Approximately 10–20 % of women with ASC-US were proven to have CIN2–3 on biopsy. Approximately 30–50 % of women with ASC-US were proven to have CIN1 on biopsy [9].

Management The 2012 updated consensus management guidelines recommend that high-risk HPV testing is the preferred method for women ages 25 years or older, although repeat cytology at 1 year is acceptable. Colposcopy is not an option for women with ASC-US in the 2012 updated guideline. For women ages 21–24 years with ASC-US, repeat cytology at 1 year is the preferred method, although high-risk HPV testing is acceptable.

ASC-H

Definition

ASC-H represents squamous cells with findings that are suggestive of high-grade SIL (HSIL) and accounts for less than 10 % of ASC cases.

Morphology(Fig. 11.3)

Small cell pattern (atypical immature squamous metaplasia): immature (small) squamous cells with high N/C ratio but mild-moderate nuclear atypia

1. Small fragments or individual cells
2. Nuclei size is 1½–2½ times larger than normal intermediate cell nuclei
3. Smooth to slightly irregular nuclear membrane
4. Increased N/C ratio, similar to that of HSIL
5. Finely granular and evenly distributed chromatin
6. Inconspicuous or absent nucleoli

Hyperchromatic crowded group (HCG) pattern:

1. Groups of crowded cells with sharp linear edges
2. Polygonal cells containing nuclei with loss of polarity or difficultly visualized nuclei and dense cytoplasm

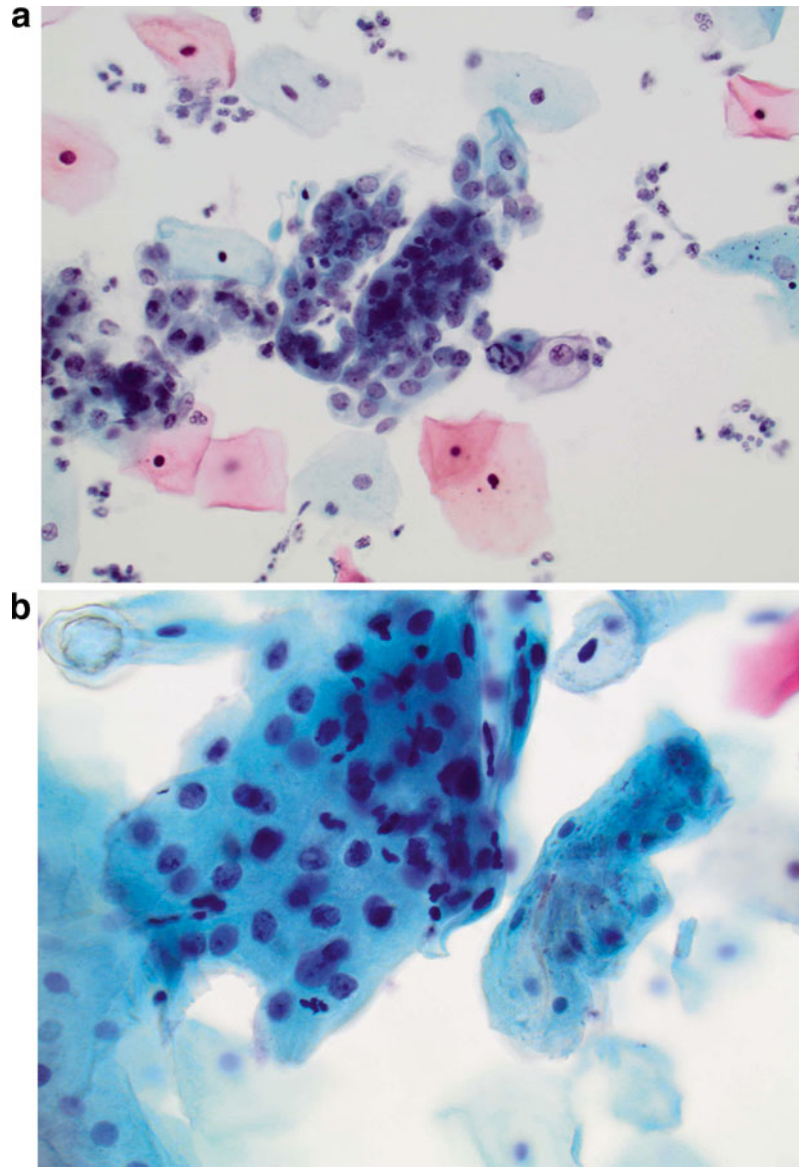


Fig. 11.2 Reactive endocervical cells(liquid-based preparation, LBP). (**a**) Endocervical cells with enlarged nuclei, fine chromatin, regular nuclear contour, and prominent nucleoli arrange in a glandular pattern. (**b**) Reactive squamous cells in the presence of inflammation. Clusters of squamous cells with enlarged nuclei, regular nuclear contour, and prominent nucleoli

Differential Diagnosis

1. **Squamous metaplasia:** mostly in sheets and cobblestone-like clusters or individual cells with homogenous dense cytoplasm, uniform round to oval nuclei, smooth nuclear membranes, and fine chromatin (Fig. 11.4).
2. **Atrophy:** sheets/single parabasal cells with abundant cytoplasm, uniform round to oval nuclei, smooth nuclear membranes, and fine chromatin. Cell borders are usually distinct.
3. **Repair:** flat sheets with maintained polarity (streaming) containing cells with minimal nuclear enlargement (1½–2 times enlargement), smooth nuclear membrane, mild hyperchromasia, finely granular and evenly distributed chromatin, prominent single or multiple nucleoli, dense cytoplasm with

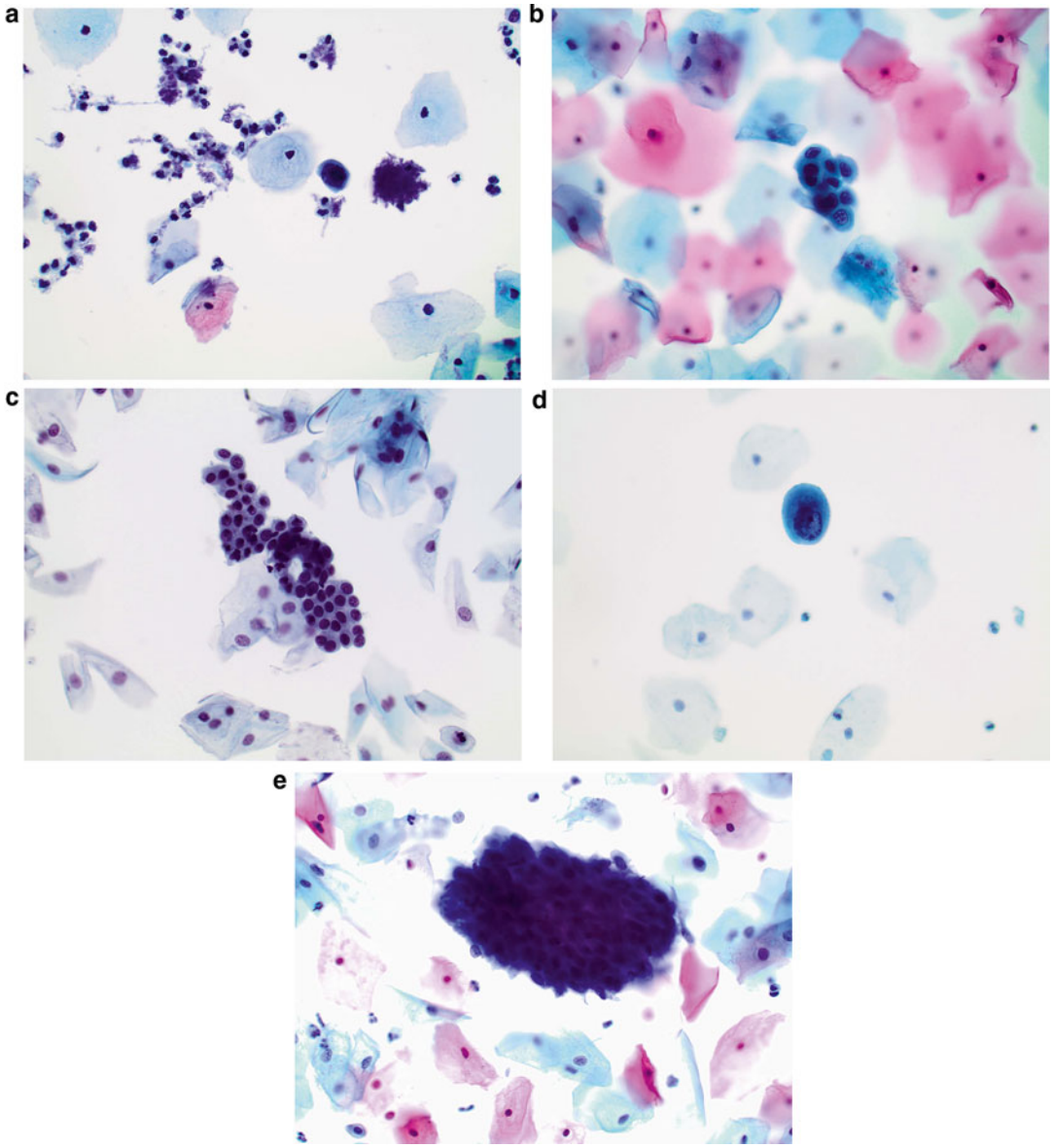


Fig. 11.3 ASC-H (liquid-based preparation, LBP). (**a**) Single small cell with scant cytoplasm, high N/C ratio, and a dark hyperchromatic nucleus which shows round nuclear contour and irregular chromatin distribution. (**b**) A loose cluster of cells with metaplastic cytoplasmic change, high N/C ratio, hyperchromatic nuclei, variation in size of nuclei, and minimal irregular nuclear contour. (**c**) A relatively large cluster of cells with thin rim of cytoplasm, high N/C ratio, and round hyperchromatic nuclei. (**d**) Single small cell with metaplastic cytoplasmic change, high N/C ratio, and a dark hyperchromatic irregular nucleus and irregular chromatin distribution. (**e**) A hyperchromatic group of cells with dark nuclei and nuclei overlapping, which are difficult to be ascertain

polychromasia, or vacuolization in a background of inflammation (Fig. 11.5).

4. **Radiation:** markedly enlarged cells with no significant increase of N/C ratio, bizarre cell shapes, variable nuclear size, binucleation/multinucleation, smudging chromatin, prominent single/multiple nucleoli, and cytoplasmic vacuolization.
5. **Histiocytes:** streaming single cells with round, ovoid, and bean-shaped nuclei, foamy cytoplasm, a low N/C ratio, and fine cytoplasmic vacuoles.

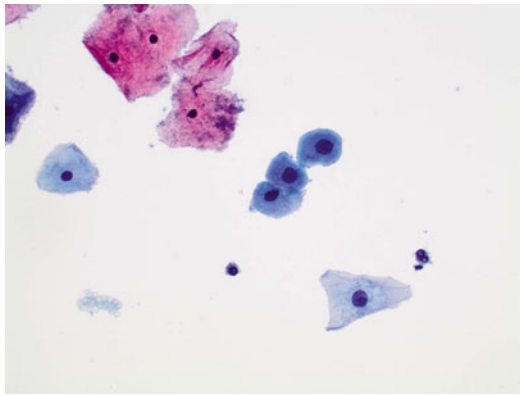


Fig. 11.4 Squamous metaplasia (liquid-based preparation, LBP). Individual cells with homogenous dense cytoplasm, uniform round to oval nuclei, smooth nuclear membranes, and fine chromatin

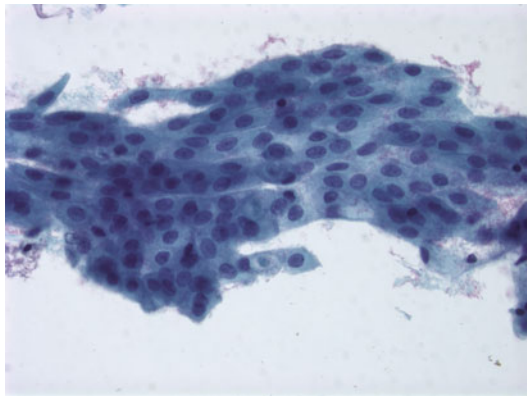


Fig. 11.5 Repair (liquid-based preparation, LBP). Flat sheets with maintained polarity containing cells with minimal nuclear enlargement, smooth nuclear membrane, mild hyperchromasia, finely granular and evenly distributed chromatin, prominent nucleoli, and dense cytoplasm

6. **HSIL:** cells with conspicuous nuclear atypia, a greater N/C ratio (nuclear size > 2× the size of normal intermediate cells), more pronounced irregular nuclear contours, and marked coarse chromatin.
7. **Endometrial cells:** small cells with bean-shaped nuclei and smudgy chromatin in 3D clusters (Fig. 11.6).

Follow-Up Results

Approximately 30–50 % of ASC-H cases prove to have CIN2 or CIN3 on follow-up biopsy, and the incidence is higher in younger patients [10].

Management

The 2012 updated consensus management guidelines recommend women with ASC-H cytology to undergo colposcopy regardless of HPV results. Reflex HPV testing is not recommended. Recent studies and CAP survey results indicate that HPV-positive rate is about 50–60 % in women with ASC-H Pap test.

Follow-up of patients with HPV-negative ASC-H results yielded very low rates of detectible CIN2/3 (1.6 %) and no diagnoses of cervical cancer. Triage of study patients with HPV-negative ASC-H results to routine HPV and cytology cotesting at 1 year was a safe follow-up option [11].

LSIL

Definition

LSIL represents squamous cells with mild dysplasia or koilocytosis caused by both low-risk and high-risk HPV infections. The majority of LSIL represent a transient HPV infection which usually regresses within 1–2 years. Less than 2 % of LSIL will progress to invasive cervical carcinoma if untreated.

Koilocytosis represents HPV-associated cytopathic effect in mature intermediate or superficial cells.

Morphology (Fig. 11.7)

1. Singly or sheets of mature/superficial squamous cells with distinct cytoplasmic borders.
2. Slightly increased N/C ratio.
3. Nuclear size >3 times the size of a normal intermediate nucleus.
4. Variable nuclear size and shape.
5. Smooth to slightly irregular nuclear membranes.
6. Variable hyperchromasia.
7. Binucleation or multinucleation may be seen.
8. Slightly coarse but evenly distributed chromatin.
9. Koilocytosis: squamous cells with well-defined perinuclear cavitation and a dense peripheral rim of cytoplasm together with nuclear abnormalities.

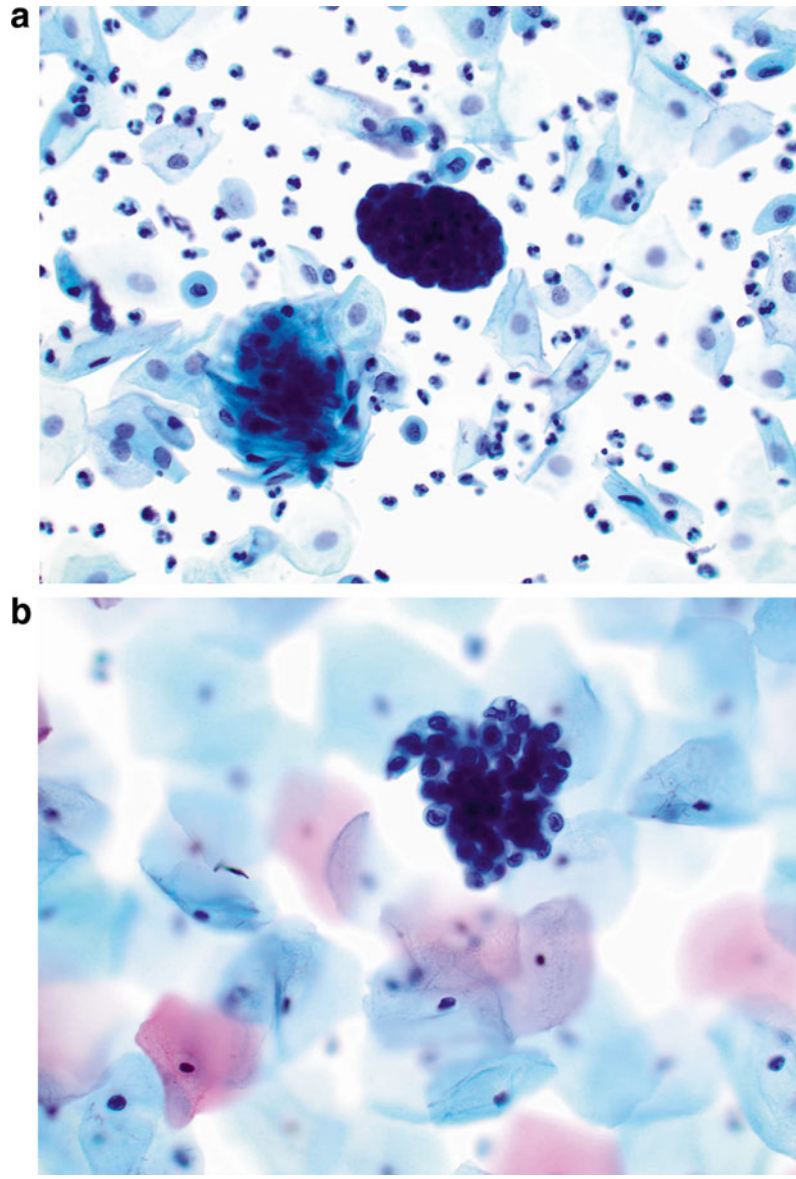


Fig. 11.6 Endometrial cells (liquid-based preparation, LBP). (**a**) A tight cluster of small cells in 3D structure. (**b**) Small cells with bean-shaped nuclei

10. Other LSIL cells include macrocytes, kite cells with cytoplasmic tails, polka dot cells with cytoplasmic globules, and balloon cells with clear cytoplasm.

Differential Diagnosis

1. **Reactive squamous cells with nonspecific halos**. Nonspecific perinuclear halos usually show small clearings without nuclear atypia and can be caused by trichomoniasis infection, other inflammatory changes, or artifact during slide preparation (Figs. 11.8 and 11.9).
2. **Reactive endocervical cells** can show enlarged nuclei and hyperchromasia which may mimic LSIL cells. However, reactive endocervical cells usually show regular nuclear contour and prominent nucleoli (Fig. 11.10).
3. **Parakeratosis or atypical parakeratosis (ASC-US)**. The cells with parakeratosis are much smaller than koilocytes and do not display atypia. The cells with atypical parakera-

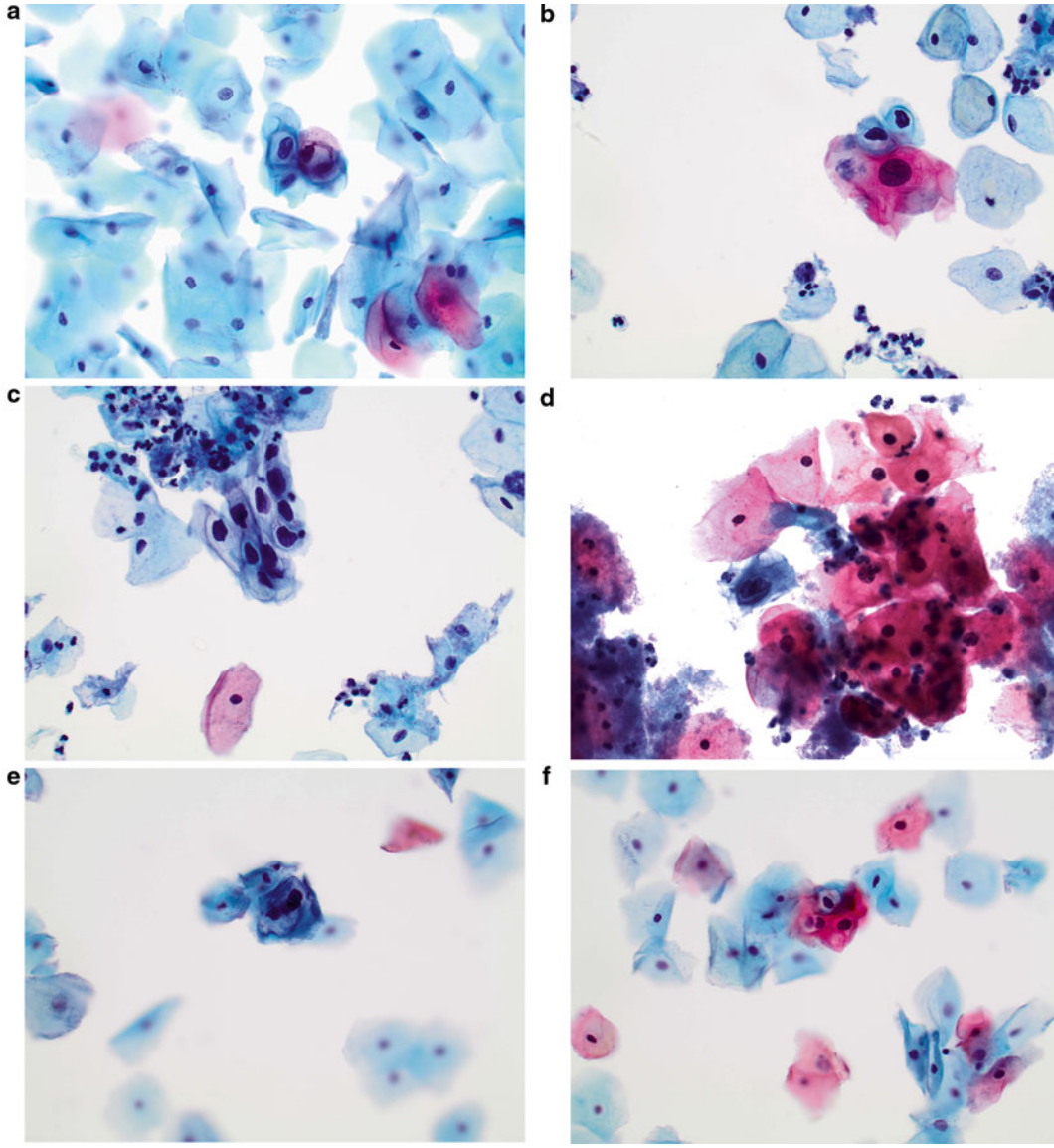
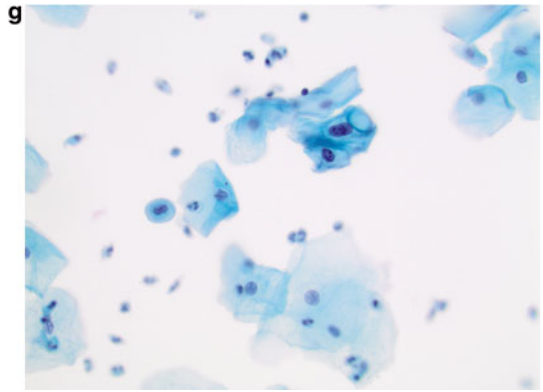


Fig. 11.7 LSIL (liquid-based preparation, LBP). (**a**) Squamous cells with slightly increased N/C ratio, slightly coarse chromatin, irregular and hyperchromatic nuclei, well-defined perinuclear cavitation, and a dense peripheral rim of cytoplasm. (**b**) Single squamous cells with slightly enlarged, irregular, and hyperchromatic nuclei, well-defined perinuclear cavitation, and a dense peripheral rim of cytoplasm. (**c**) A cluster of squamous cells with enlarged, highly irregular, and hyperchromatic nuclei, relatively abundant dense cytoplasm, slightly increased N/C ratio, and well-defined perinuclear cavitation. (**d**) Single squamous cells with binucleation, well-defined perinuclear cavitation, and relatively abundant dense cytoplasm. (**e**) Single squamous cells with multinucleation and well-defined folded perinuclear cavitation. (**f**) Squamous cells with small, but highly hyperchromatic, nuclei and well-defined perinuclear cavitation. (**g**) LSIL versus ASC-US. Cells with slightly enlarged, slightly hyperchromatic nuclei and binucleation. (**h**) SIL, grade cannot be determined. Atypical squamous cells with irregular hyperchromatic nuclei, moderate amount of cytoplasm, and slightly increase N/C ratio. Based on the nuclear features, these cells are interpreted as SIL, but not gradable due to moderate amount of cytoplasm and absence of perinuclear halo



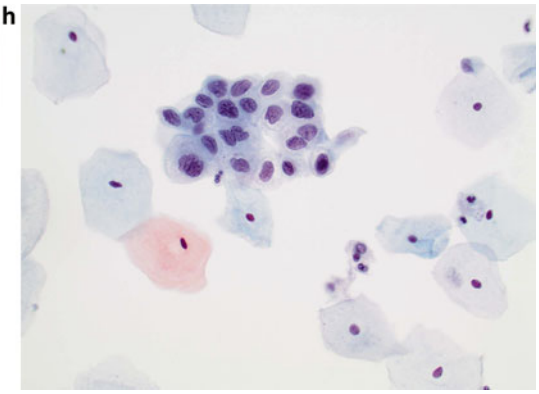


Fig. 11.7 (continued)

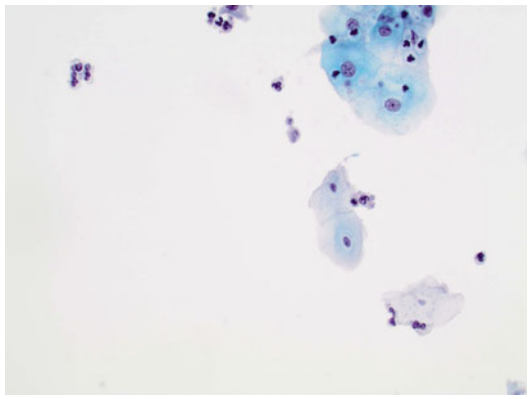


Fig. 11.8 Reactive squamous cells in the presence of trichomonas vaginalis. Clusters of squamous cells with enlarged nuclei, regular nuclear contour, and prominent nucleoli on the *upper right corner*. Trichomonas vaginalis can be seen in the *center* of the image (liquid-based preparation, LBP)

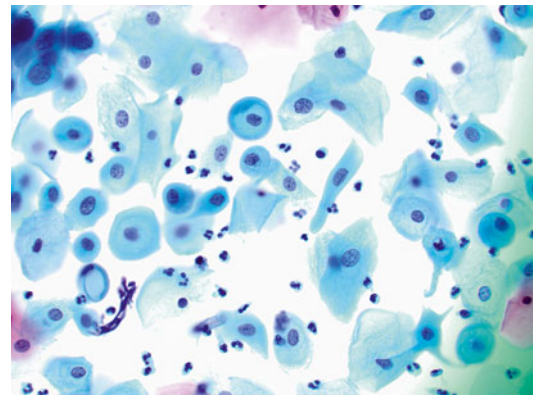


Fig. 11.9 Nonspecific clear perinuclear halos can be seen in glycogenated intermediate cells during pregnancy or other conditions. These cells lack typical nuclear features of LSIL (liquid-based preparation, LBP)

tosis show nuclear atypia but not enough for a diagnosis of LSIL cells (Fig. 11.1).

4. **Postpartum changes.**Navicular cells found in pregnant women can show empty vacuoles which may mimic perinuclear halos. However, nuclei are round to oval and uniform with fine and evenly distributed chromatin.

Follow-Up Results

Approximately 15–25 % of LSIL cases prove to have histologic CIN2 or CIN3 during follow-up biopsies [12].

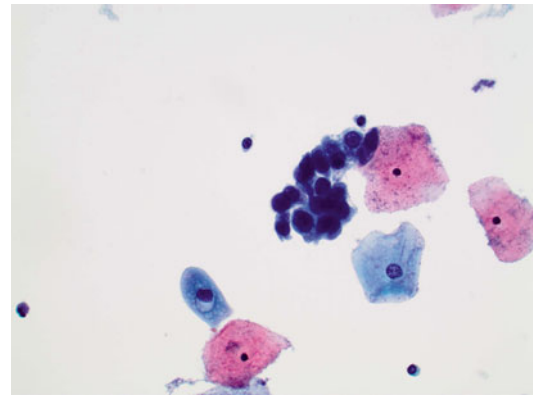


Fig. 11.10 Reactive endocervical cells with enlarged nuclei and hyperchromasia, but regular nuclear contour. A nonspecific clear perinuclear halo is also seen in a reactive squamous cell (liquid-based preparation, LBP)

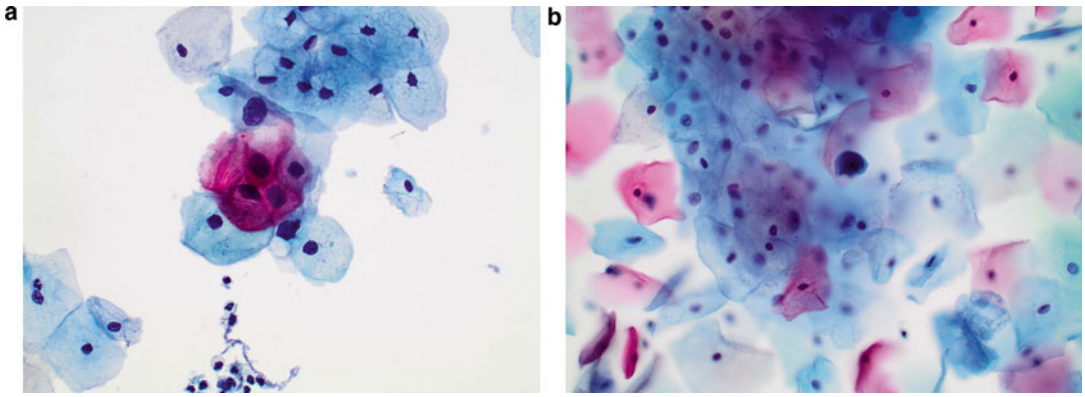


Fig. 11.11 LSIL-H (liquid-based preparation, LBP). LSIL cells (**a**) were found on this Pap smear together with ASC-H cell (**b**)

Management

The 2012 updated consensus management guidelines recommend women with LSIL cytology and no HPV test or a positive HPV test to undergo colposcopy. If contesting shows HPV-negative LSIL, a repeat co-testing at 1 year is preferred, but colposcopy is acceptable. For women with LSIL who are aged 21–24 years, follow-up with cytology at 12-month intervals is recommended. For pregnant women with LSIL, colposcopy is preferred. HPV-positive rate is about 70–80 % in women with LSIL Pap test. HPV prevalence declines in older age groups. CIN 2/3 follow-up rate was very low in women 50 years and older with HPV-negative LSIL. HPV testing can be an option for old women in the guideline. In recent years, more women with LSIL Pap test had HPV testing result due to the increase of co-testing for women 30 years and older. Some research findings support recent recommendations for repeat co-testing after 1 year as an appropriate option for patients with HPV-negative low-grade squamous intraepithelial lesion (LSIL) [13].

LSIL-H (LSIL-Cannot Exclude HSIL)

LSIL-H represents cases with LSIL that also contain a few cells that are suspicious for, but not diagnostic of, HSIL (Fig. 11.11). Studies have found that these women have a significantly higher likelihood of a high-grade intraepithelial lesion on biopsy than women with LSIL only. The HPV-positive rate is also higher in women with LSIL-H than that in women with LSIL only. LSIL-H is a unique category of cytologic abnormality associated with distinctive HPV and CIN 2/3+ diagnostic rates. LSIL-H is not included in the 2001 TBS category, but has been used by some laboratories [14].

HSIL

Definition

HSIL represents squamous cells with moderate dysplasia, severe dysplasia, and carcinoma in situ. Most HSILs are caused by persistent high-risk HPV infection and hence have increased progressing potential. Approximately 1.4 % of HSIL cases will progress to invasive cervical squamous cell carcinoma.

Morphology (Fig. 11.12)

1. HSIL cells often occur singly, in small aggregates, or syncytial-like hyperchromatic groups with irregularity of polarity.
2. HSIL cells are typically smaller than LSIL cells, but vary from small basal-type to larger LSIL-like cells.
3. Markedly increased N/C ratio.
4. Irregular nuclear contours, hyperchromasia, and chromatin clumping or granularity.
5. Inconspicuous nucleoli.

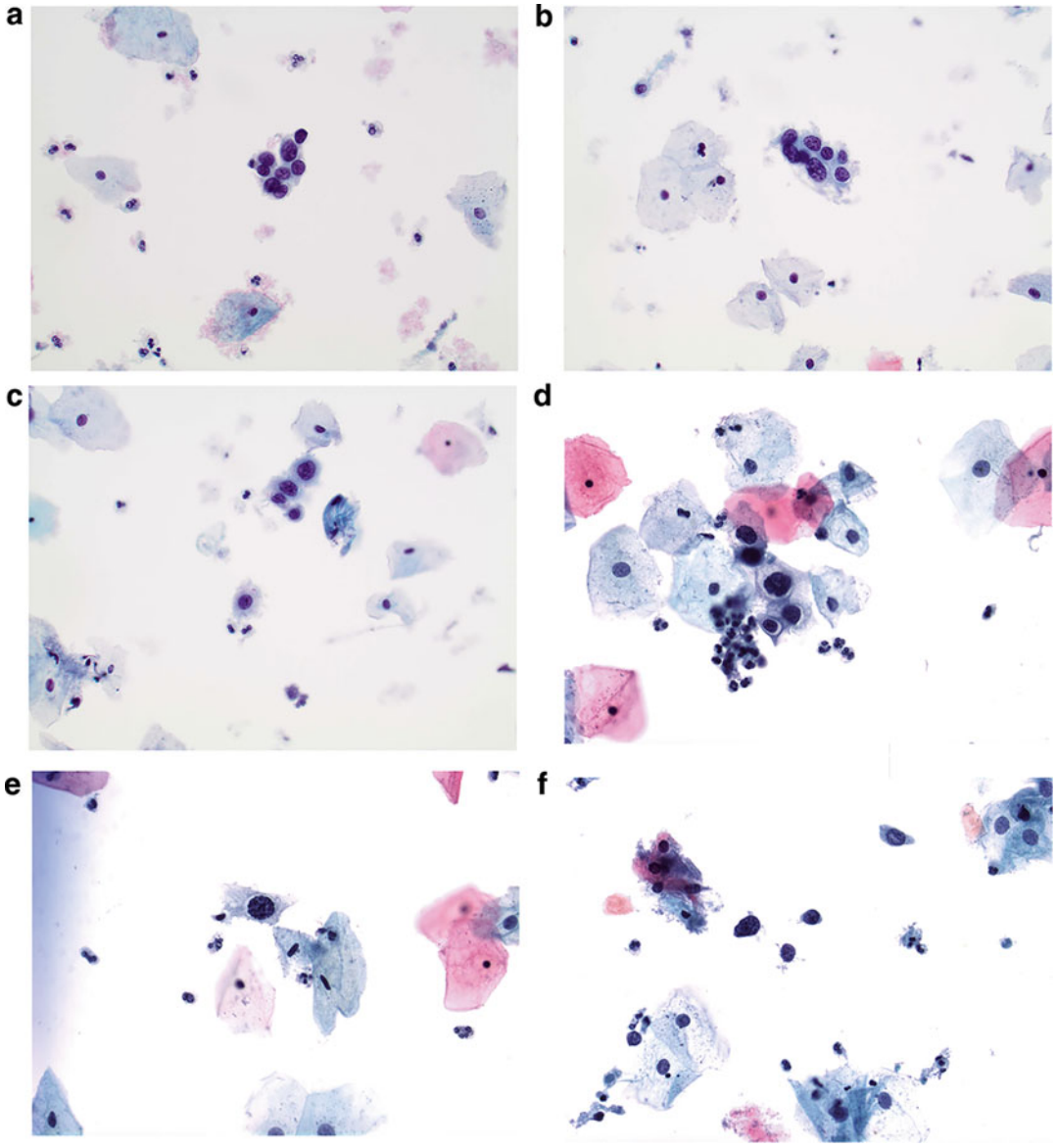


Fig. 11.12 HSIL (liquid-based preparation, LBP). (**a**) Small aggregate of small cells with markedly increased N/C ratio, irregular nuclear contours, hyperchromasia and chromatin clumping or granularity, and inconspicuous nucleoli. (**b**) Small aggregate of small cells with variation in size of nuclei, markedly increased N/C ratio, hyperchromasia, and chromatin clumping. (**c**) Single small cells with variation in size of nuclei, markedly increased N/C ratio, hyperchromasia, and chromatin clumping. (**d**) Cells can be large, but also contain enlarged hyperchromatic nuclei with irregular nuclear contour. The markedly increased N/C ratio is maintained. (**e**) Single HSIL cell with very coarse chromatin and high N/C ratio. (**f**) Single HSIL cells with variation in size, high N/C ratio, and coarse chromatin. (**g**) A cluster of hyperchromatic group cells with high N/C ratio, irregular nuclear contour, and coarse chromatin. (**h**) A small group of HSIL cells with very small nuclei, but markedly high N/C ratio. (**i**) Large HSIL cells with hyperchromatic nuclei, irregular nuclear contour, and markedly increased N/C ratio. (**j**) HSIL cells with involvement of endocervical glands. (**k**) HSIL cells involving endocervical glands can contain vacuoles

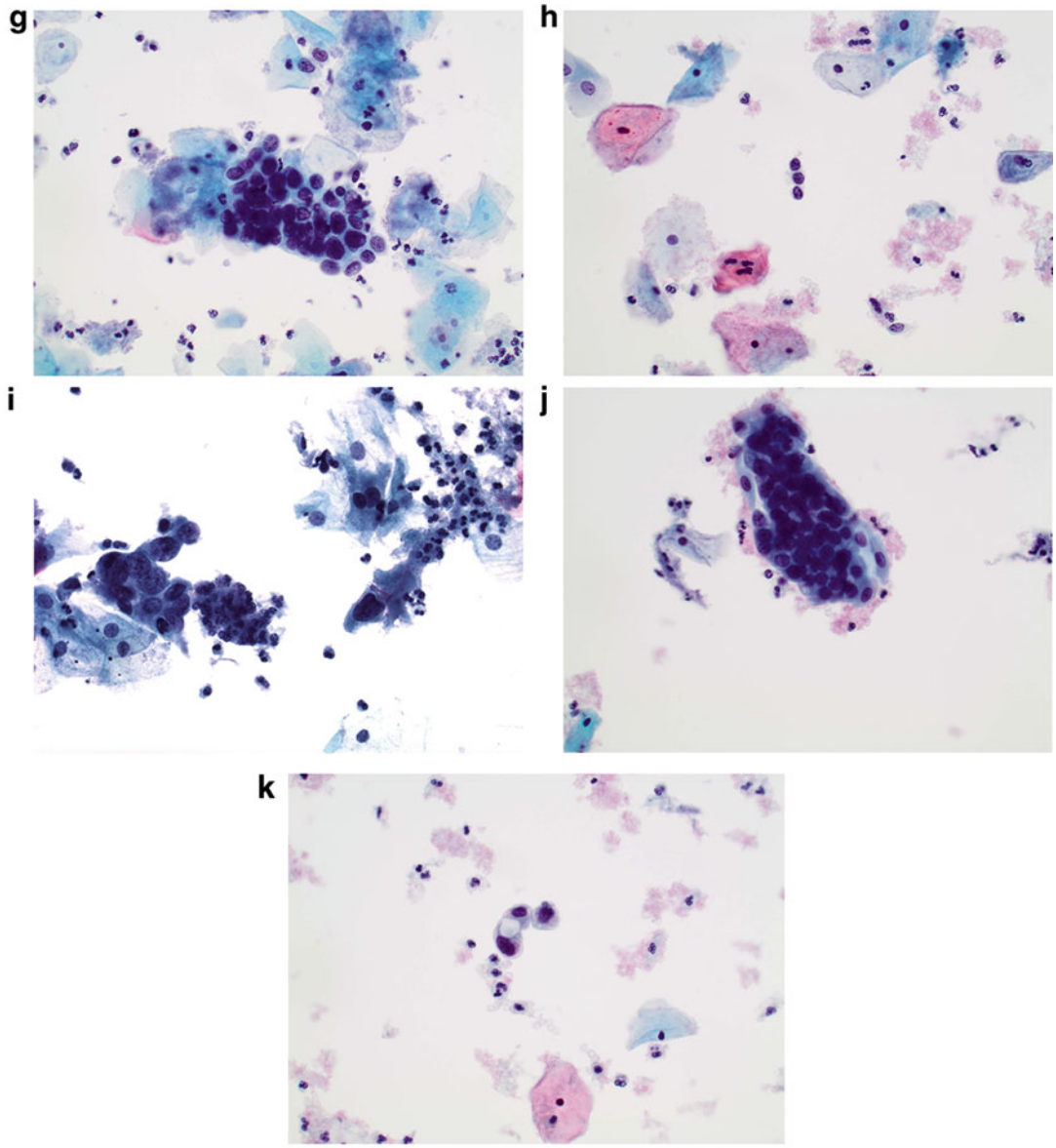


Fig. 11.12 (continued)

Differential Diagnosis

1. **LUS:** Cells are in cohesive tissue fragments with uniform round/oval and well-ordered nuclei, evenly distributed chromatin, and mildly increased N/C ratio. The nucleoli may be present.
2. **Herpes:** Cells infected with HSV can be multinucleated or mononuclear cells with nuclei exhibiting ground glass appearance due to intranuclear viral particles and enhancement of the nuclear envelop due to margination of chromatin. Intranuclear inclusions and nuclear molding may be present (Fig. 11.13).

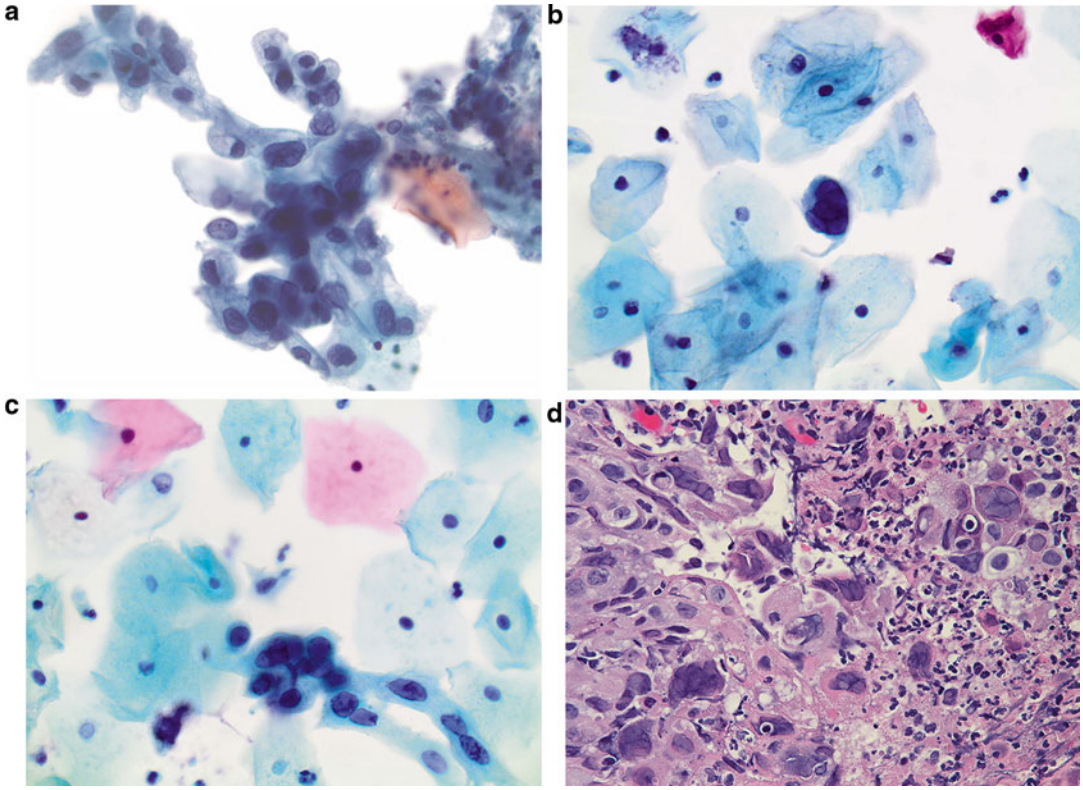


Fig. 11.13 Herpes. (**a–c**) Liquid-based preparation. (**d**) H&E stain of biopsy specimen. Mononuclear or multinucleated cells with nuclei exhibiting ground glass appearance, margination of chromatin, and nuclear molding

3. **Adenocarcinoma in situ (AIS) cells** show atypical nuclei but more glandular differentiation such as nuclear feathering and rosettes.
4. **Squamous cell carcinoma cells** often show prominent nucleoli and background necrosis.
5. **Follicular cervicitis** shows small lymphocytes with less hyperchromasia.

Follow-Up Results

Approximately 60 % of HSIL cases prove to have CIN 2+ lesions at colposcopy. Five-year cervical cancer risk is 8 % among women 30 years of age, and older and the risks are modified by HPV test results [15].

Management

The 2012 updated consensus management guidelines recommend that women with HSIL cytology should undergo immediate loop electrosurgical excision or colposcopy is acceptable, except in special populations. Triage using either reflex HPV testing or repeat cytology alone is unacceptable.

Squamous Cell Carcinoma

Definition

Squamous cell carcinoma represents malignant cells with squamous differentiation. Cervical squamous cell carcinoma usually affects women between the ages of 40 and 55 years, about 10 years later than intraepithelial lesions.

Morphology

Keratinizing Squamous Cell Carcinoma (Fig. 11.14)

1. Individual or small clusters with marked variation in cellular size and shape.
2. Caudate and spindle cells can be present with dense orangeophilic cytoplasm, and

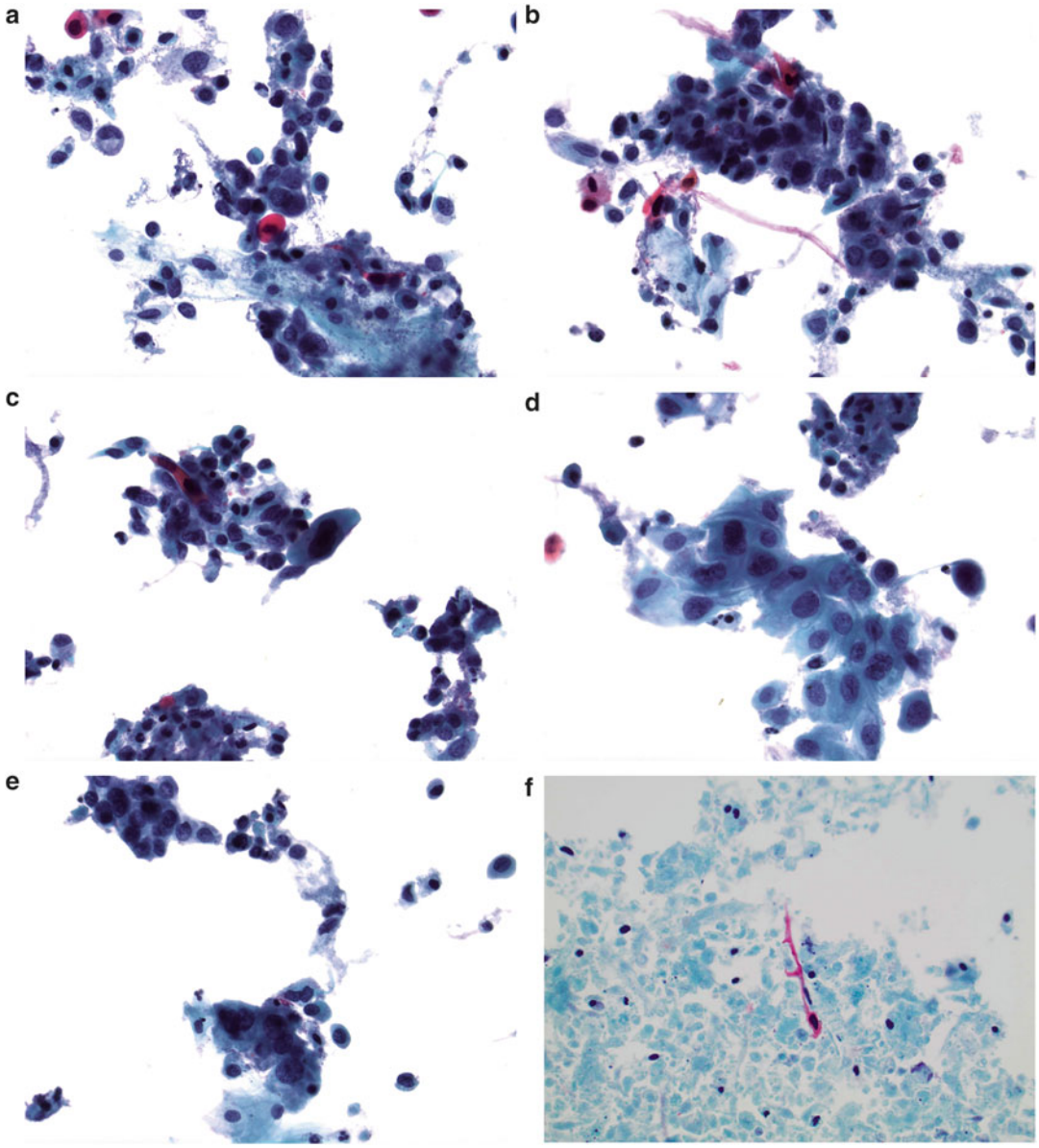


Fig. 11.14 Keratinizing squamous cell carcinoma (liquid-based preparation, LBP). (**a**) Individual or small clusters of squamous cells with marked variation in cellular size and shape, keratotic changes including hyperkeratosis, or pleomorphic parakeratosis with dense orangeophilic cytoplasm. Nuclei with variable size and shape, irregular membrane, hyperchromasia, and coarsely granular and irregularly distributed chromatin. (**b**) Clusters of squamous cells with marked variation in cellular/nuclear size and shape, keratotic changes, hyperchromasia, and coarse chromatin. (**c**) Very large bi-/multinucleated cells can be seen. (**d**) Cytoplasm is usually dense. (**e**) Tumor diathesis (clinging diathesis in LBP: necrotic material at the periphery of the cell groups in liquid-based preparation) can be seen. (**f**) Tadpole cell with ample orangeophilic cytoplasm

cells often have ample cytoplasm forming unusual cell shapes such as "tadpole" or "fiber" cells.
3. Keratotic changes including hyperkeratosis or pleomorphic parakeratosis.
4. Nuclei with variable size and shape, irregular membrane, hyperchromasia, coarsely granular and irregularly distributed chromatin with parachromatin clearing, and conspicuous nucleoli but less common than nonkeratinizing squamous cell carcinoma.
5. Background of tumor diathesis (clinging diathesis: necrotic material at the periphery of the cell groups in liquid-based preparation).

Nonkeratinizing Squamous Cell Carcinoma (Fig. 11.15)
1. Single or syncytial sheets of cells with poorly defined cell borders
2. Enlarged nuclei with prominent irregular nuclear membrane, coarse and unevenly distributed chromatin with distinct parachromatin clearing, and prominent nucleoli
3. Cyanophilic, vacuolated, or dense cytoplasm
4. Background of tumor diathesis

Differential Diagnosis

1. **Repair.** Reparative cells can be large and hyperchromatic with prominent nucleoli but tend to be arranged in flat cohesive sheets with less haphazardly arranged cells and contain less nuclear atypia.
2. **LSIL.** LSIL cells especially koilocytes can mimic keratinizing squamous cell carcinoma cells with a lower N/C ratio but tend to have less nuclear atypia without background of tumor diathesis.
3. **HSIL.** HSIL cells with significant nuclear atypia can mimic nonkeratinizing squamous cell carcinoma but rarely have prominent nucleoli, bizarre cell shapes (tadpole and fiber cells), and a tumor diathesis.
4. **Benign endometrial cells.** Endometrial cells with blood can mimic small cell nonkeratinizing SCC, but they tend to have uniform bean-shaped nuclei without prominent nucleoli. Clinical history of menstrual period may be helpful.
5. **Adenocarcinoma.** Cervical adenocarcinoma cells tend to have 3D arrangement with glandular formation with delicate, granular, vacuolated cytoplasm, more prominent nucleoli, and less background tumor diathesis.

Atypical Glandular Cells

Definition

Atypical glandular cells represent glandular cells with morphologic changes which fall short of an interpretation of adenocarcinoma either quantitatively or qualitatively. It can be divided into AGC-NOS (not otherwise specified), AGC-EC (endocervical), and AGC-EM (endometrial). AGC denotes an increased level of risk for neoplasia and not a specific precursor lesion.

Morphology (Fig. 11.16)

AGC-EC represents endocervical cells with morphologic changes which fall short of an interpretation of endocervical adenocarcinoma in situ or invasive adenocarcinoma either quantitatively or qualitatively.

1. Sheets or strips of endocervical glandular cells with ill-defined cell borders, nuclear crowding/overlapping
2. Increased N/C ratio, but more cytoplasm than atypical endometrial cells
3. Enlarged nuclei with hyperchromasia, moderately coarse chromatin, and occasional mitosis
4. Rare cell groups with rosetting or feathering

AGC-EM represents endometrial cells with morphologic changes which fall short of an interpretation of endometrial adenocarcinoma either quantitatively or qualitatively.

1. Small groups of cells (usually 5–10 cells per group) with ill-defined cell borders, scant cytoplasm, or occasional vacuoles.
2. Nuclei are slightly enlarged compared to normal endometrial cells with mild hyperchromasia and occasional small nucleoli.

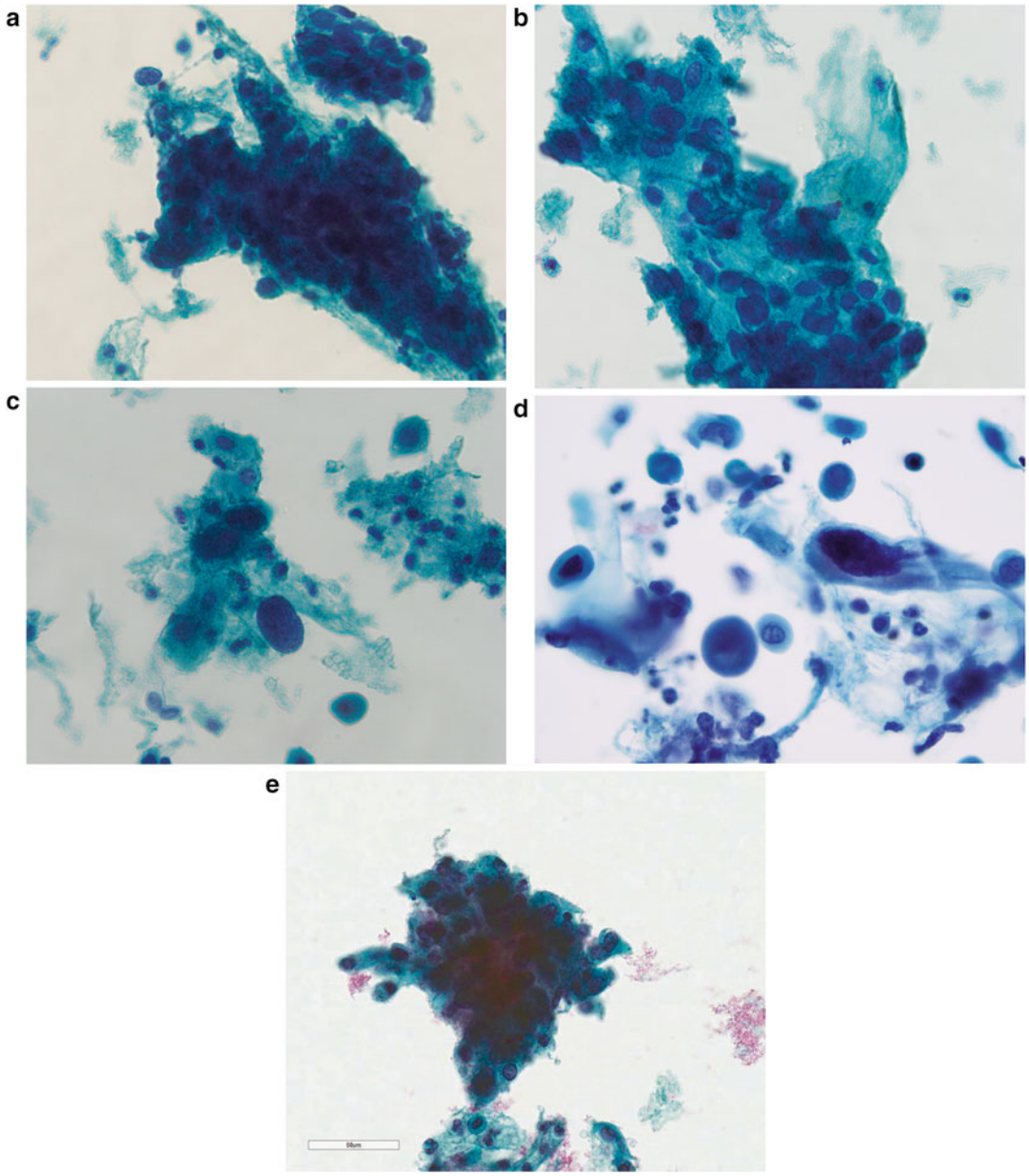


Fig. 11.15 Nonkeratinizing squamous cell carcinoma (liquid-based preparation, LBP). (**a**) Syncytial sheets of cells with poorly defined cell borders, dense cytoplasm, enlarged nuclei, prominent irregular nuclear membrane, and coarse chromatin. (**b**) Poorly differentiated squamous cell carcinoma cells with very large nuclei and prominent nucleoli. (**c**) Single poorly differentiated squamous cell carcinoma cells with very large nuclei, scant cytoplasm, and coarse chromatin. (**d**) Single poorly differentiated squamous cell carcinoma cell with markedly irregular and large nucleus. (**e**) Tumor diathesis (clinging diathesis in LBP: necrotic material at the periphery of the cell groups in liquid-based preparation) can be seen

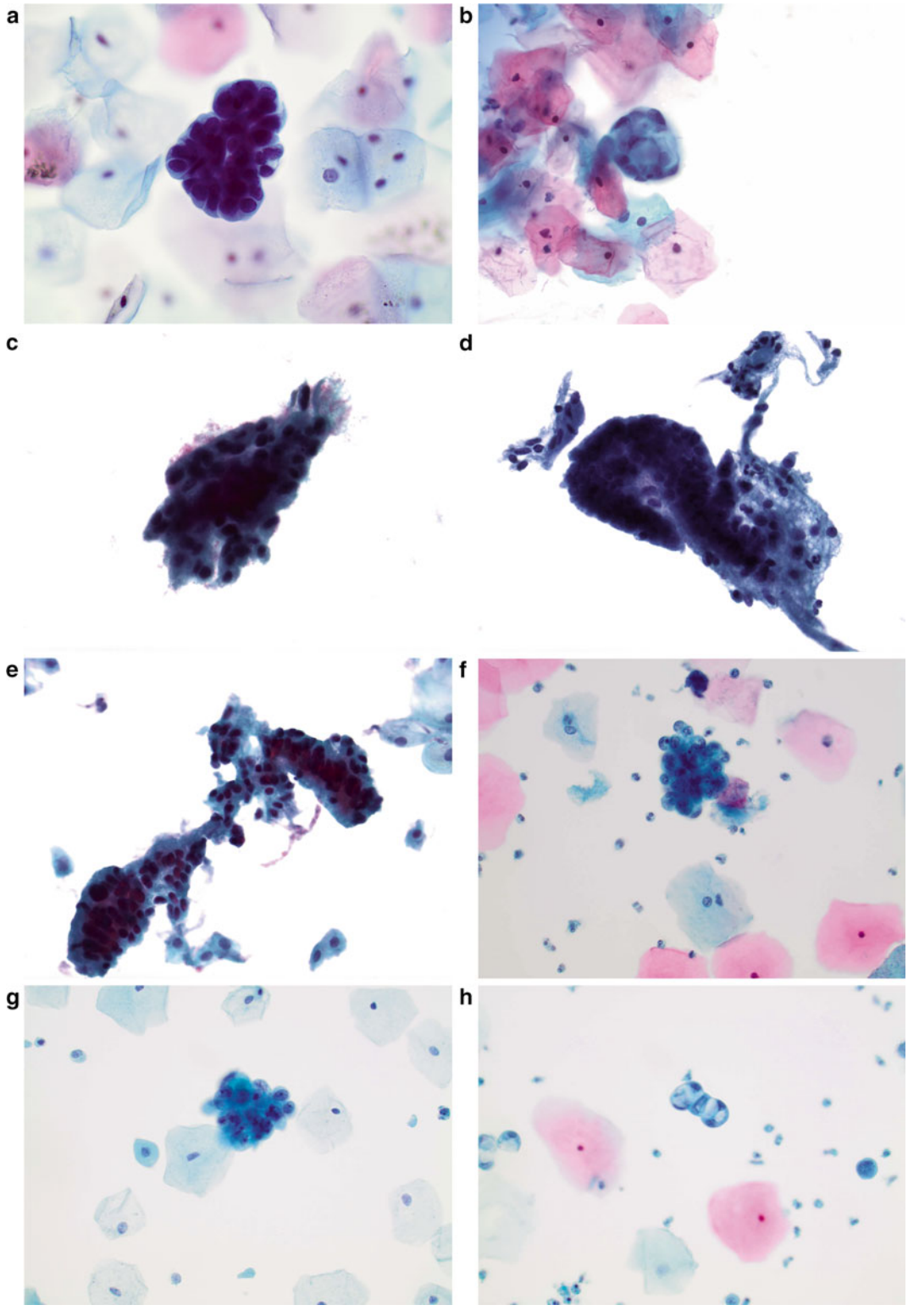


Fig. 11.16 Atypical glandular cells (liquid-based preparation, LBP). (**a**) A group of hyperchromatic cells in a glandular architecture with enlarged nuclei. (**b**) Sheets endocervical glandular cells with ill-defined cell borders, nuclear crowding/overlapping, increased N/C ratio, and vacuoles. (**c**) Sheets endocervical glandular cells with ill-defined cell borders, nuclear crowding/overlapping, enlarged nuclei with hyperchromasia, and slightly coarse chromatin.

Differential Diagnosis

1. **Normal endocervical cells.** These cells are in honeycomb/palisading arrangements with delicate cytoplasm, small round uniform nuclei, smooth nuclear membranes, and vesicular and evenly distributed chromatin. Nucleoli may be seen in reactive endocervical cells which may have slightly enlarged nuclei with minimal hyperchromasia.
2. **Normal endometrial cells.** These cells are present in tight three-dimensional cell clusters or loose cell group arrangements with small round/oval/bean-shaped nuclei, fine and evenly distributed chromatin, and minimal/no nuclear atypia.
3. **Tubal metaplasia.** It represents columnar cells with cilia and/or terminal bars. These cells are in small groups or pseudostratified/crowded groups with round/oval nuclei which may be enlarged, pleomorphic, and hyperchromatic.

Follow-Up Results

AGC can be associated with benign lesions including polyps and metaplasia, but also with neoplasias including adenocarcinomas of the endometrium, cervix, ovary, fallopian tube, and other sites. Follow-up results showed that approximately 20 % of women with AGC prove to have CIN2+, 3 % prove to have adenocarcinoma in situ, and 5 % prove to have invasive adenocarcinoma [16, 17].

Although the cancer risk is lower in women younger than 35 years of age with AGC, the risk of CIN 2+ is higher, and intensive assessment is warranted at all ages. In the KPNC cohort, CIN 3+ was found in 9 % of women aged 30 years and older with AGC cytology, with cancer in 3 %.

Management

The 2012 updated consensus management guidelines recommend women with all subcategories of AGC except atypical endometrial cells to have colposcopy with endocervical sampling regardless of HPV result. In women 35 years of age and older or women younger than 35 years but at increased risk for endometrial neoplasia with all subcategories of AGC, endometrial sampling is recommended in conjunction with colposcopy and endocervical sampling. For women with atypical endometrial cells, initial evaluation with endometrial and endocervical sampling is preferred, but colposcopy is acceptable either at the initial evaluation or deferred.

Adenocarcinoma In Situ

Definition

Endocervical AIS is an in situ tumor without invasion, and it occurs 10–15 years earlier than invasive adenocarcinoma. Since AIS is also associated with HPV infection, it does coexist with CINs in approximately 20–50 % of patients. A diagnosis of AIS on a Pap test does not exclude invasive adenocarcinoma, and a histological examination is necessary for a definitive diagnosis.

Morphology (Fig. 11.17)

1. Cells in groups and strips with rosette formation, feathering, crowding, and/or stratification with indistinct cell borders
2. Finely vacuolated cytoplasm
3. Increased N/C ratio

Fig. 11.16 (continued) (**d**) Strips of endocervical glandular cells with enlarged hyperchromatic nuclei, coarse chromatin, and occasional prominent nucleoli. (**e**) Strips of endocervical glandular cells with enlarged hyperchromatic nuclei and variation in nuclear size and shape. (**f**) Small cluster of cells with ill-defined cell borders, scant cytoplasm, enlarged nuclei, and occasional small nucleoli. (**g**) Rare mitosis can be present. (**h**) Occasional vacuoles can be present

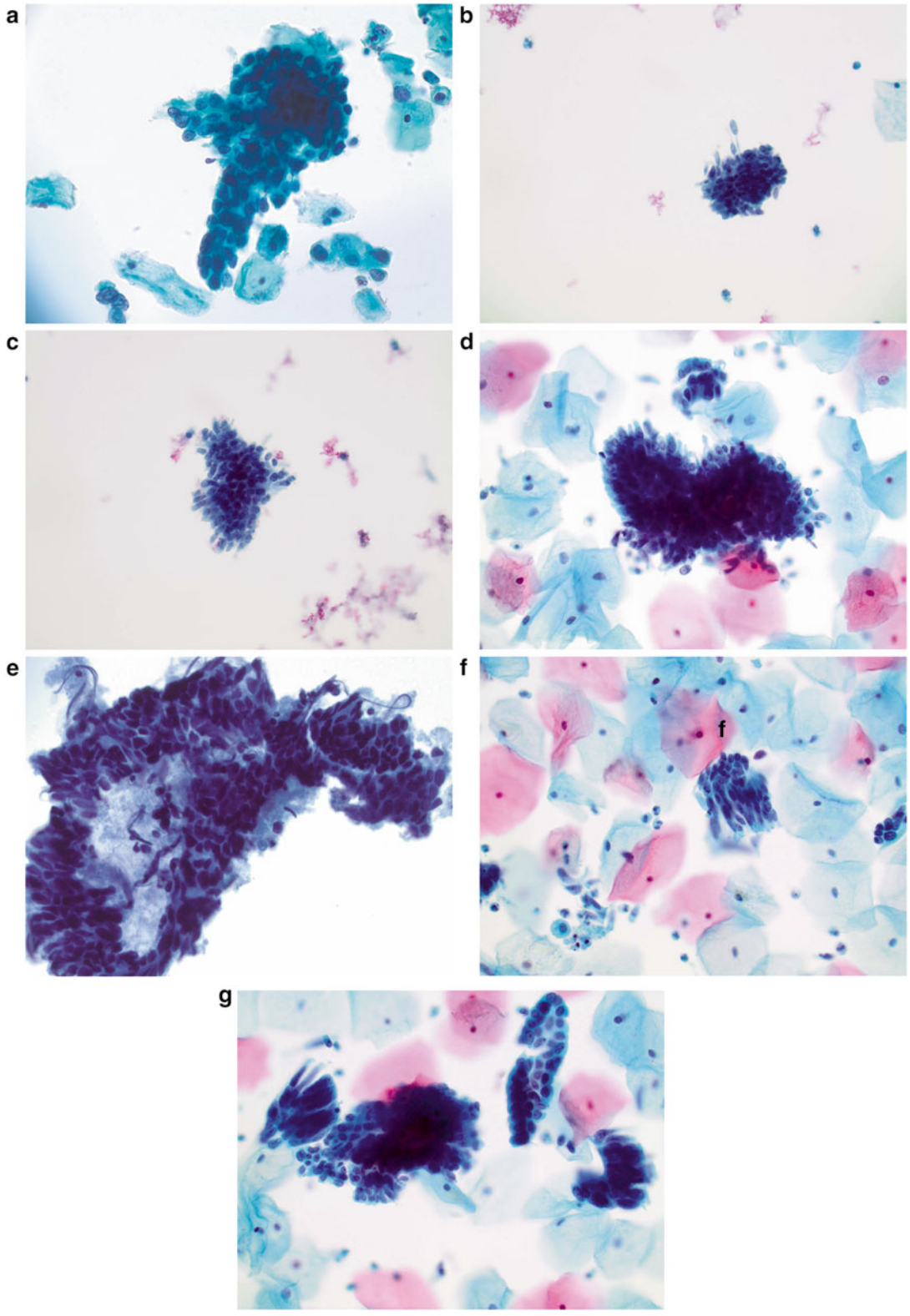


Fig. 11.17 Adenocarcinoma in situ (AIS) (liquid-based preparation, LBP). (**a**) Strip of endocervical cells with indistinct cell borders, nuclear crowding and stratification, enlarged hyperchromatic nuclei, and inconspicuous nucleoli. (**b**) Small cluster of glandular cells with elongated nuclei, relatively smooth nuclear membrane,

4. Elongated nuclei with smooth to markedly irregular nuclear membrane, hyperchromasia, evenly distributed fine to coarsely granular chromatin, inconspicuous nucleoli, and variable mitosis

Differential Diagnosis

1. **Normal/reactive endocervical cells.** These cells are in honeycomb/palisading arrangements with delicate cytoplasm, small round uniform nuclei, smooth nuclear membranes, and vesicular and evenly distributed chromatin. Nucleoli may be seen in reactive endocervical cells.
2. **Normal endometrial cells.** These cells are usually in tight three-dimensional cell clusters or loose cell group arrangements with small round/oval/bean-shaped nuclei, fine and evenly distributed chromatin, and minimal/no nuclear atypia. Feathering, rosettes, and mitoses are not seen.
3. **Tubal metaplasia.** These cells are columnar cells with cilia and/or terminal bars in small groups or pseudostratified/crowded groups with round/oval nuclei which may be enlarged, pleomorphic, and hyperchromatic.
4. **HSIL.** HSIL cells can form hyperchromatic crowded groups and have mitoses as AIS cells. However, features of glandular differentiation and feathering are not seen in HSIL cells.
5. **Invasive adenocarcinoma.** The finding of prominent nucleoli and tumor diathesis favors a diagnosis of invasive adenocarcinoma.

Follow-Up Results

Besides adenocarcinoma, CINs were also found in approximately 20–50 % of women with AIS cells on Pap smear.

Management

The 2012 updated consensus management guidelines recommend women with all subcategories of AIS to have colposcopy with endocervical sampling regardless of HPV result. In women 35 years of age and older or women younger than 35 years but at increased risk for endometrial neoplasia with AIS, endometrial sampling is recommended in conjunction with colposcopy and endocervical sampling.

Adenocarcinoma

Adenocarcinoma cells detected on Pap tests can be from endocervix, endometrium, or extrauterine in origin (vagina, ovaries, fallopian tubes, and metastasis) (Table 11.1).

Table 11.1 Comparison between endocervical and endometrial adenocarcinomas

Cellular feature	Endocervical adenocarcinoma	Endometrial adenocarcinoma
Architecture	Strips, sheets, and 3D clusters	Individual and 3D clusters
Cell size	Larger	Smaller
Cell shape	Columnar, less round	Round to oval/cuboidal
Cytoplasm	Abundant, granular, and eosinophilic, distinct cell border	Scant, vacuolated, polymorphonuclear engulfment, indistinct cell border
Nuclei	Enlarged, thickened irregular nuclear membrane	Rounded up, smaller
Chromatin	Coarse chromatin	Fine to clumped chromatin
Nucleoli	Prominent macronucleoli, multiple	Small inconspicuous
Diathesis	Clinging dirty tumor diathesis	Watery tumor diathesis

Fig. 11.17 (continued) hyperchromasia, evenly distributed chromatin, nuclear stratification, and feathering. (**c**) Small cluster of glandular cells with nuclear crowding, stratification, and feathering. (**d**) Large cluster of hyperchromatic group of cells with elongated nuclei, nuclear overlapping, stratification, and occasional small nucleoli. (**e**) Large cluster of hyperchromatic group of cells with enlarged elongated nuclei and slightly coarse chromatin. (**f**) Mitosis can be present. (**g**) Occasional small prominent nucleoli can be present

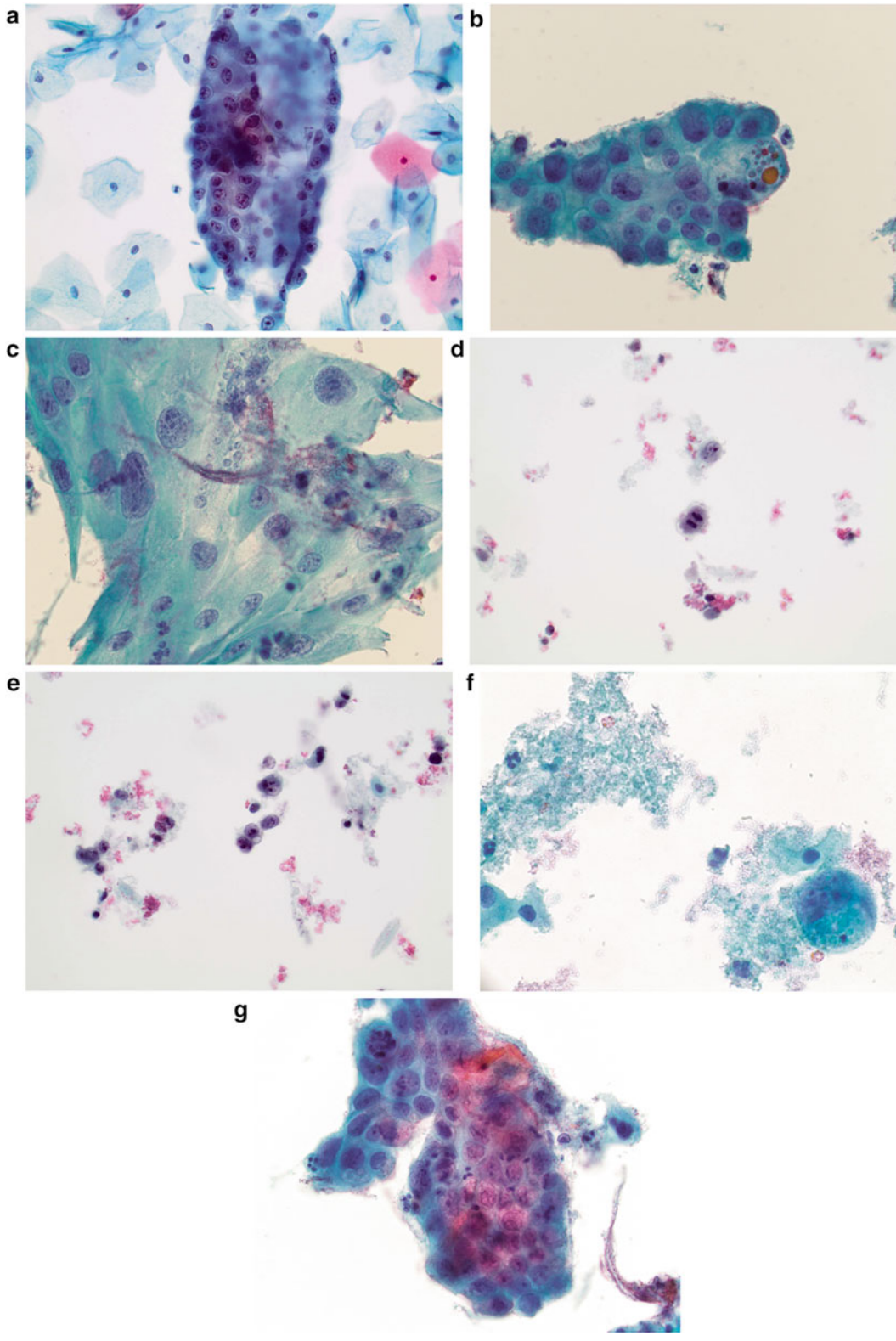


Fig. 11.18 Endocervical adenocarcinoma(liquid-based preparation, LBP). (**a**) Endocervical adenocarcinoma cells with markedly enlarged nuclei, pleomorphic shape, irregular coarse chromatin, and prominent macronucleoli. (**b**) Endocervical adenocarcinoma cells with markedly enlarged nuclei, variation in size and shape, irregular

Morphology

Endocervical Adenocarcinoma (Fig. 11.18)

1. Strips, sheets, or 3D clusters of glandular cells with distinct cell borders and abundant eosinophilic cytoplasm
2. Enlarged nuclei with thickened and undulating irregular nuclear membrane, coarsely granular, hyperchromatic chromatin, and prominent nucleoli (may be multiple and irregular)
3. Necrotic tumor diathesis and/or blood

Endometrial Adenocarcinoma (Fig. 11.19)

1. Single or well-preserved three-dimensional groups of variably sized glandular cells with anisonucleosis and nuclear overlapping/crowding
2. Vacuolated cytoplasm with frequent large vacuoles and polymorphonuclear engulfment
3. Round up nuclei with thickened nuclear membrane, fine to clumped chromatin, and small nucleoli
4. Watery tumor diathesis

Differential Diagnosis

1. **Normal/reactive endocervical cells.** These cells are in honeycomb/palisading arrangements with delicate cytoplasm, small round uniform nuclei, smooth nuclear membranes, and vesicular and evenly distributed chromatin. Nucleoli may be seen in reactive endocervical cells.
2. **Normal endometrial cells.** These cells are usually small round/oval/bean-shaped nuclei, fine and evenly distributed chromatin, and minimal/no nuclear atypia.
3. **Tubal metaplasia.** These cells are columnar cells with cilia and/or terminal bars in small groups or pseudostratified/crowded groups with round/oval nuclei.
4. **AIS.** AIS cells can have feathering, but with less atypia and inconspicuous nucleoli. The finding of tumor diathesis favors invasive adenocarcinoma.

Normal Endometrial Cells

The risk of endometrial lesions is very low in young women with normal endometrial cells in Pap test. The age to report the normal endometrial cells has been changed from 40 years and above to 45 years and above in the updated 2014 TBS.

Others

Other malignant neoplasms which can occur in Pap tests include some rare variants of cervical adenocarcinoma [villoglandular adenocarcinoma, minimal deviation adenocarcinoma (adenoma malignum), intestinal-type adenocarcinoma, endometrioid cervical adenocarcinoma, clear cell carcinoma, serous carcinoma, mesonephric adenocarcinoma, adenosquamous cell carcinoma, glassy cell carcinoma, adenoid cystic carcinoma and adenoid basal cell carcinoma] and other types of malignant neoplasm [small cell and large cell neuroendocrine carcinomas, extramammary Paget's

Fig. 11.18 (continued) coarse chromatin, and prominent nucleoli. (**c**) Endocervical adenocarcinoma cells with markedly enlarged nuclei, variation in size and shape, irregular coarse chromatin, prominent macronucleoli, and plenty cytoplasm. (**d**) Mitosis can be easily seen. (**e**) Occasional vacuoles can be seen. (**f**) Necrotic tumor diathesis and pleomorphic tumor cell. (**g**) Tighter cluster of poorly differentiated endocervical adenocarcinoma cells with less cytoplasm, enlarged pleomorphic nuclei, and prominent nucleoli and tumor diathesis

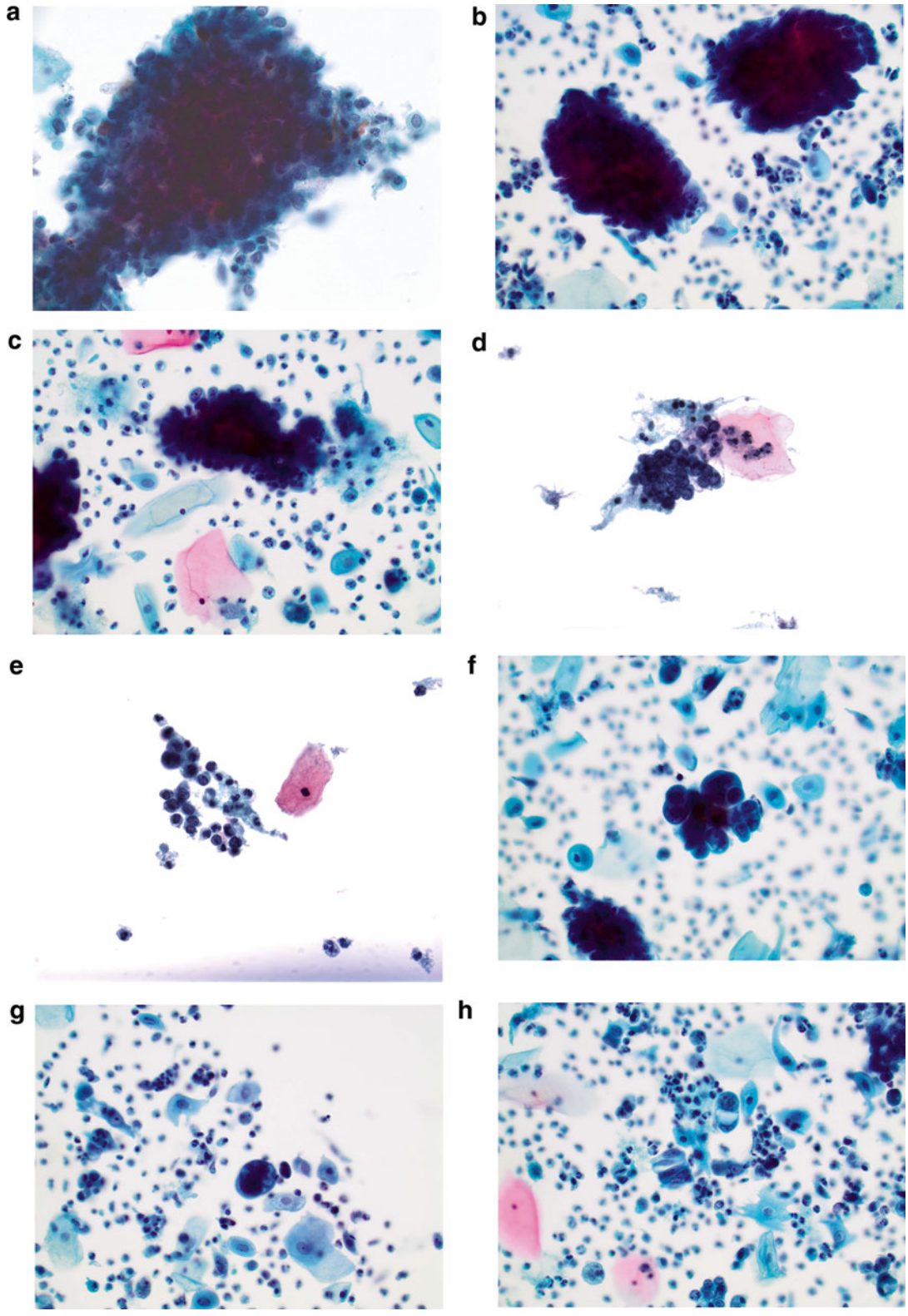
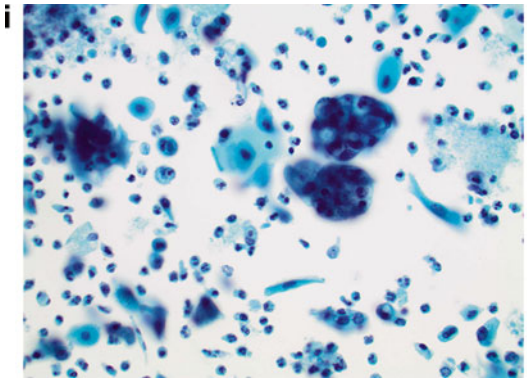


Fig. 11.19 Endometrial adenocarcinoma(liquid-based preparation, LBP). (**a**) Three-dimensional group of variably sized glandular cells with anisonucleosis and nuclear overlapping/crowding, round nuclei, thickened nuclear membrane, fine to clumped chromatin, and small nucleoli. (**b**) Three-dimensional groups of glandular cells with



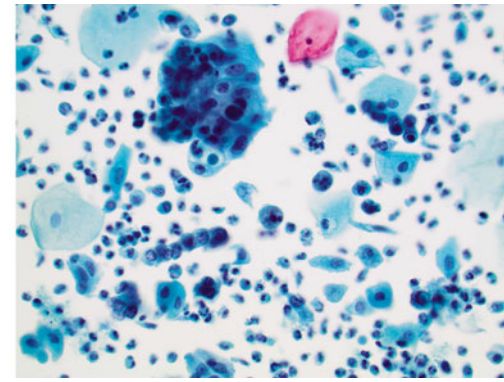


Fig. 11.19 (continued) nuclear overlapping and crowding. (**c**) 3D group with papillary projection. (**d**) Cluster of well-differentiated endometrial endometrioid adenocarcinoma cells with slightly enlarged nuclei, scant cytoplasm, nuclear overlapping, and mild coarse chromatin. (**e**) Cluster of well-differentiated endometrial endometrioid adenocarcinoma cells with variation in size, scant cytoplasm, and mild coarse chromatin. (**f**) Small group of moderately differentiated endometrial endometrioid adenocarcinoma cells with enlarged nuclei, scant cytoplasm, high N/C ration, and coarse chromatin. (**g**) Single poorly differentiated endometrial adenocarcinoma cells with markedly enlarged nuclei, irregular nuclear contour, and markedly coarse chromatin. (**h**) Large vacuoles can be frequent and push nuclei to the periphery. (**i**) A small cluster of endometrial adenocarcinoma cells with prominent cytoplasmic vacuoles. (**s**) Small nucleoli and polymorphonuclear engulfment can be seen

disease, metastatic carcinoma (ovarian, colorectal, breast, and other sites), malignant melanoma, lymphoma, myeloid sarcoma, malignant mixed Müllerian tumor, rhabdomyosarcoma, leiomyosarcoma, Ewing sarcoma, and gestational trophoblastic diseases]. These entities are very rare and are not detailed here.

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Precursors of Cervical Adenocarcinomas

12

Yoshiki Mikami and Atsumi Kojima

Revised Classification and Emerging Concepts of Cervical Adenocarcinomas

The recently revised World Health Organization (WHO) classification, which was launched in 2014, subdivides invasive adenocarcinoma into endocervical adenocarcinoma of usual type (Fig. 12.1), mucinous carcinoma, villoglandular carcinoma, endometrioid carcinoma, clear cell carcinoma, mesonephric carcinoma, and adenocarcinoma admixed with neuroendocrine carcinoma (Table 12.1) [1]. This revision represents a significant paradigm shift, including the separation of true mucinous carcinoma; the employment of the term "usual type," replacing a vague diagnostic term "mucinous adenocarcinoma of endocervical type"; and the concept of HPV-related and HPV-unrelated adenocarcinomas.

Mucinous carcinoma is defined as a tumor composed of cells with abundant intracytoplasmic mucin and, in the previous version of the WHO classification (2003), included endocervical type, intestinal type, signet-ring cell type, minimal deviation type (MDA, "adenoma malignum") (Fig. 12.2), and villoglandular type [2]. In the mucinous category, endocervical type, defined as adenocarcinoma composed of cells resembling normal endocervical cells, was the most common, accounting for approximately 90 % of all cervical adenocarcinomas in the WHO 2003 scheme. However, in reality, the most common adenocarcinomas encountered by pathologists are very frequently mucin deficient or have only a tiny amount of intracytoplasmic mucin demonstrated by mucin staining (Fig. 12.1), and adenocarcinomas without any specific direction of differentiation or characteristic morphology, including serous, clear cell, endometrioid, or mesonephric carcinomas, have been designated as endocervical-type mucinous adenocarcinoma. This means that this subtype is a "waste basket" category and thus the term "usual type" has been proposed and incorporated into the revised WHO classification (2014) [1, 3]. In this context, combined with the observation that true mucinous carcinomas show aggressive behavior [4, 5], mucinous carcinoma was separated from the usual type to include gastric type (Fig. 12.3), intestinal type, and signet-ring cell type [1]. Gastric type mucinous carcinoma (GAS) is an emerging subtype of mucinous carcinoma, which shows aggressive clinical behavior and spectrum of morphological differentiation, encompassing MDA as its extremely well-differentiated variant [1, 6].

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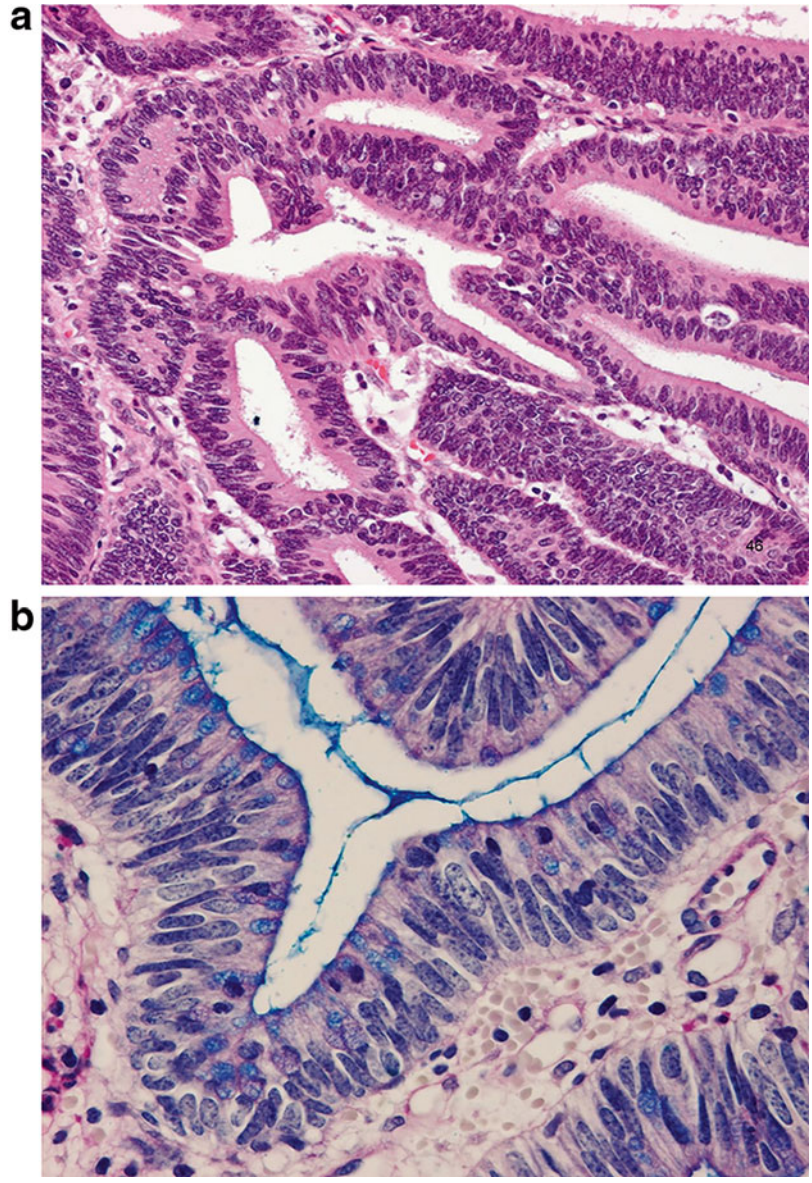

Fig. 12.1 Usual-type adenocarcinoma of the cervix, composed of tall columnar cells with significant nuclear enlargement, elongation, stratification, and hyperchromasia. The cytoplasm is rather dark, and intracytoplasmic vacuoles are absent or obscured (**a**). PAS-Alcian blue double staining showing the absence or paucity of intracytoplasmic mucin (**b**)

The detection rate of high-risk HPV in adenocarcinoma cases varies from approximately 62 % to up to 97 % [7–11], reflecting a variety of factors including differences in patient population, age distribution, socioeconomic status, and geography. From the view of the implication of high-risk HPV, cervical adenocarcinomas can be divided into two categories (Table 12.2), and precancerous glandular lesions may be best discussed with regard to their relationship with high-risk HPV and differently for each morphologic subtype. The representative HPV-related tumor includes usual type and intestinal type carcinomas, whereas HPV-negative tumors include GAS, serous carcinoma, endometrioid carcinoma, clear cell carcinoma, and mesonephric carcinoma with some exceptions [9–13]. There are some studies demonstrating the detection of HPV in cases of serous and endometrioid carcinomas [9–14]; however, this may be mostly a consequence of different criteria employed for the individual studies. Usual type endocervical adenocarcinomas occasionally show micropapillary growth patterns, imparting a close resemblance to serous carcinoma. In other

Table 12.1 World Health Organization classification of glandular lesions of the uterine cervix (4th ed., 2014) [1]

Glandular tumor and precursors
Adenocarcinoma in situ
Adenocarcinoma
Endocervical adenocarcinoma, usual type
Mucinous carcinoma, NOS
Gastric type
Intestinal type
Signet-ring cell type
Villoglandular carcinoma
Endometrioid carcinoma
Clear cell carcinoma
Serous carcinoma
Mesonephric carcinoma
Adenocarcinoma admixed with neuroendocrine carcinoma
Benign glandular tumors and tumor-like lesions
Endocervical polyp
Mullerian papilloma
Nabothian cyst
Tunnel clusters
Microglandular hyperplasia
Lobular endocervical glandular hyperplasia
Diffuse laminar endocervical glandular hyperplasia
Mesonephric remnants and hyperplasia
Arias-Stella reaction
EndocervicosisEndometriosis
Endometriosis
Tuboendometrioid metaplasia
Ectopic prostate tissue

words, a subset of serous carcinoma of the cervix is a morphologic variation or a consequence of high-grade transformation of usual type, which are HPV-related. On the other hand, a significant number of usual types might have been regarded as endometrioid carcinoma because of a paucity of intracytoplasmic mucin, resulting in its higher incidence in some studies (up to 30 %) [3, 15]. Using strict criteria, endometrioid carcinoma is a rare neoplasm, accounting for less than 10 % of all cervical adenocarcinomas [3], and most tumors diagnosed as such are considered HPV-negative.

Clear cell carcinoma shows features similar to ovarian and endometrial clear cell carcinoma. Moreover, it is well known to be associated with in utero exposure to diethylstilbestrol (DES), a synthesized estrogen [16], and recent studies have shown that it is mostly not associated with HPV infection [12, 14, 17]. Notably, Ueno et al. demonstrated an increased EGFR or HER2 expression or activation of AKT or mTOR in all clear cell carcinomas in their series, which suggests that tyrosine kinase inhibitors targeting the AKT-mTOR pathway are potential treatment regimens for this particular tumor [17].

Mesonephric carcinoma, characterized by columnar or cuboidal neoplastic cells arranged in a glandular pattern containing diastase-resistant Periodic acid-Schiff (PAS)-positive proteinaceous eosinophilic secretions, with occasional papillary or reticular patterns, is considered to be derived from mesonephric remnant and is typically located deep in the cervix away from the transformation zone, which is the preferential site of HPV-related adenocarcinoma development.

The concept of GAS has been proposed based on the observation of clinicopathologic features of MDA. MDA was demonstrated to show a gastrointestinal phenotype by immunohistochemistry using HIK1083 and anti-MUC6 antibodies, both of which recognize pyloric gland mucin of the stomach [18]. Although their phenotypic characteristics were considered to be specific for MDA, one of the authors (YM) revealed that a subset of so-called endocervical-type mucinous adenocarcinomas can be positive for these two markers [19]. Because this tumor shares some characteristics, including the morphology [6] and absence of HPV detection [12, 14, 20], with MDA (although it does not match completely with prototypical MDA because of less differentiation), these tumors were separated as a distinct entity (GAS) [1, 6]. Morphologically, GAS is characterized by abundant clear or pale eosinophilic cytoplasm and distinct cell borders, and it shows aggressive clinical behavior [6].

General Aspects of Cervical Adenocarcinoma Precursors

Based on the Surveillance, Epidemiology, and End Results (SEER) Program data, Wang et al. demonstrated that adenocarcinoma accounts for

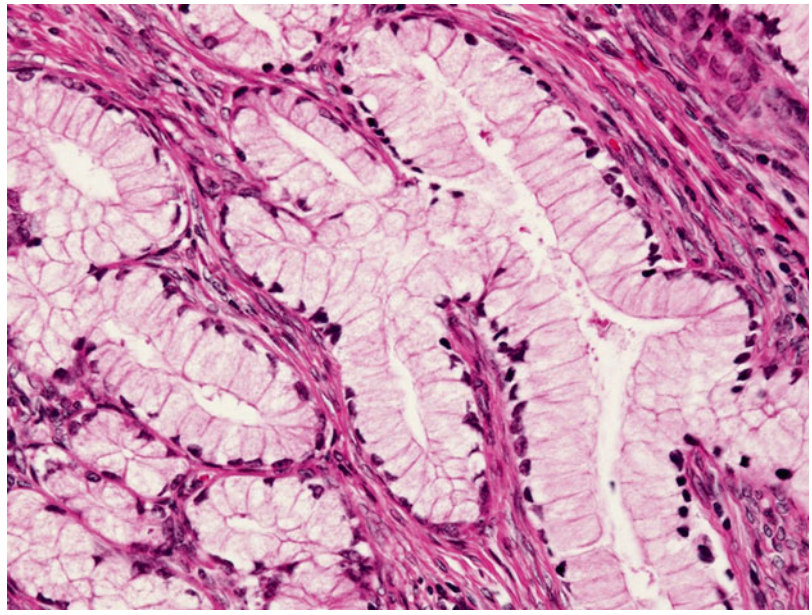

Fig. 12.2 Minimal deviation adenocarcinoma ("adenoma malignum"), which is true mucinous carcinoma, is characterized by well-formed glands composed of cells with abundant intracytoplasmic mucin and basally located nuclei with bland nuclear morphology

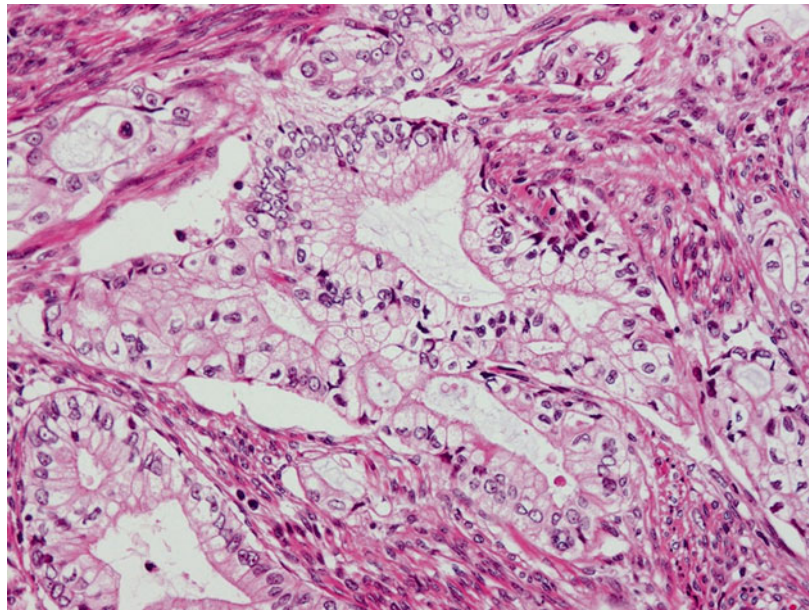

Fig. 12.3 Gastric type mucinous carcinoma, characterized by abundant clear or pale eosinophilic cytoplasm and distinct cell borders. Some cytologic features are shared by minimal deviation adenocarcinoma (MDA), but architectural abnormalities and distinct nuclear anaplasia are straightforward to indicate their malignant nature in contrast to MDA

approximately 20 % of all cervical carcinomas, but the cervical intraepithelial neoplasia 3 (CIN 3) to adenocarcinoma in situ (AIS) ratio is 34, suggesting a low incidence of AIS as a precursor of invasive carcinoma compared with CIN 3 [21]. In addition, they revealed that AIS to adenocarcinoma and CIN 3 to squamous cell carcinoma (SCC) ratios are 0.8 and 6.69, respectively [21]. The low incidence of AIS and higher incidence of its invasive counterpart may be explained by four possibilities:

1. Cervical adenocarcinomas are not derived from AIS.

Table 12.2 Division of cervical adenocarcinoma based on HPV implication

High-risk HPV-positive adenocarcinoma	High-risk HPV-negative adenocarcinoma
• Usual type (previously called endocervical type mucinous adenocarcinoma) • Intestinal type mucinous carcinoma • Serous carcinoma[a] • Endometrioid carcinoma[a]	• Gastric type mucinous carcinoma (including minimal deviation adenocarcinoma) • Mesonephric carcinoma • Serous carcinoma • Endometrioid carcinoma

[a]Results are conflicting in the literature, possibly due to a function of different diagnostic criteria; by using the strict criteria, it appears that these two types are mostly HPV-unrelated neoplasms

2. AIS rapidly progresses to invasive carcinoma with a very short interval, escaping the screening program.
3. AIS cannot be detected by conventional sampling because of its topographic characteristics, i.e., the location beneath the surface epithelium in the cervical canal.
4. Limitations of the current diagnostic criteria for interpreting Pap smears, particularly in cases of AIS with minimal cytologic atypia.

Regarding common AIS types, in a series of early invasive adenocarcinomas, AIS was identified in up to 85 % of cases [22], and the time-to-progression to its invasive counterpart, ranging from 2 to 8 years, appears not significantly different from high-grade squamous intraepithelial lesion (HSIL) (CIN 2/3) [22–24], and therefore the latter two might be main sources of the low detection rate of AIS [25]. In the literature, however, there are cases of small invasive carcinoma without AIS, suggesting a rapid progression [22]. Information on the natural history of AIS is still limited, and thus, in cases of uncommon subtype of AIS, a variety and combination of factors may be considered.

AIS and Its Variations

AIS is defined as an intraepithelial lesion containing malignant-appearing glandular epithelium that carries a significant risk of developing into invasive adenocarcinoma if not treated [1]. By definition, desmoplastic stromal reaction indicating destructive invasion is absent in cases of AIS, although intraglandular tufting or cribriform growth may be seen. In the literature, Friedell and McKay first described AIS in 1953 [26], and thereafter the lesion has been widely accepted as a precancerous lesion. The rationale indicating the precancerous nature of AIS includes:

1. AIS frequently coexists with invasive adenocarcinoma [22, 26].
2. AIS and invasive adenocarcinoma have common cytologic features [26].
3. The AIS patient age is lower than in those with invasive adenocarcinoma by 10–15 years [21, 27].
4. High-risk HPV, particularly types 16 and 18, is commonly detected in AIS [28–30].
5. The expression pattern of biomarkers including $p16^{INK4a}$ and CD44 in cases of AIS is similar to those of invasive adenocarcinoma [31–35].

Clinically, AIS is commonly asymptomatic and is mostly detected by screening. Patient age varies from 18 to 75 with a mean age of 35 in one large study [27]. The incidence varies, e.g., 1 in 25,000–475,000 women, depending on race, geography, and socioeconomic status [36, 37].

In addition to the common usual type (endocervical type) as described by Friedell et al. (Figs. 12.4 and 12.5) [26, 38], there are a variety of morphologic subtypes of AIS, including intestinal type [38] (Fig. 12.6), endometrioid type (Fig. 12.7) [38], clear cell type (Fig. 12.8) [39], serous type (Fig. 12.9), and tubal or ciliated type (Fig. 12.10) [40]. Gastric type of AIS (Fig. 12.11) has also been described [19]. The subclassification of AIS does not appear to have any impact on patient management, but pathologists are encouraged to be aware of the morphologic spectrum of AIS of the uterine cervix to avoid misinterpretation and in order to understand the histogenesis of cervical adenocarcinoma.

Microscopically, the usual type of AIS is typically located in the transformation zone with

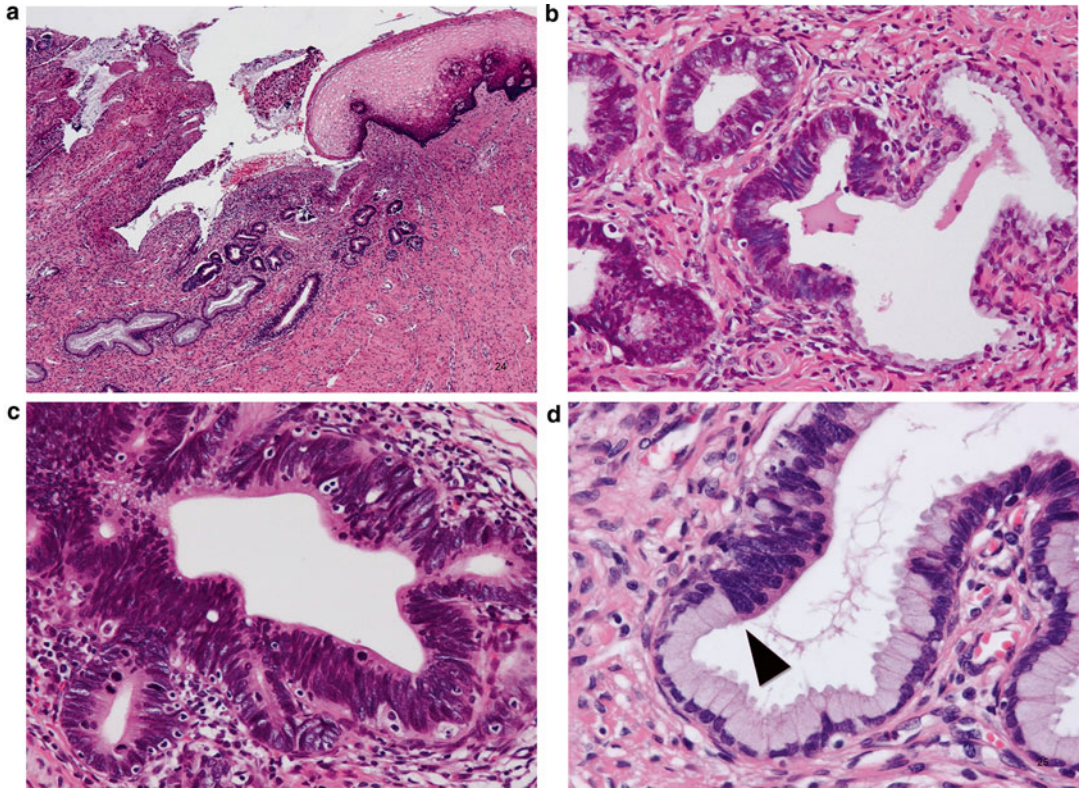

Fig. 12.4 Adenocarcinoma in situ (AIS), usual type. The lesion is typically found in the transformation zone where (**a**) preexisting endocervical glands are replaced by columnar cells with malignant-appearing cells (**b**). These tall columnar cells show moderate to marked nuclear enlargement, nuclear stratification, and heterogeneity in nuclear size and hyperchromasia, with occasional mitotic figures (**c**), and sometimes show a distinct border adjacent to preexisting endocervical epithelium (**d**), which is not a feature of reactive atypia

some exceptions. On low power view, it can be recognized by deeply stained columnar epithelium (Fig. 12.4a, b) because of moderate to marked nuclear enlargement, overlapping, and hyperchromatism (Fig. 12.4c), in contrast to the basally located small nuclei of normal glandular epithelium. Atypical columnar cells replace preexisting mucus-secreting columnar cells, and the contour of the endocervical glands is preserved. The atypical cells commonly extend from the superficial to the deeper portion of the glands and frequently show a clear front with preexisting nonneoplastic glandular epithelium (Fig. 12.4d). It should be kept in mind that AIS involving nonspecific endocervical glandular hyperplasia may show gland crowding, making early invasion a diagnostic concern. Therefore, the distribution and architecture of the background nonneoplastic glands should be considered, as well as the architectural complexity of atypical glands, desmoplastic stromal reaction, and inflammatory reaction. Although there is heterogeneity with respect to nuclear size and shape, the nucleolus is usually absent or small, and vesicular nuclei with conspicuous nucleoli and significant nuclear pleomorphism are rather indicative of invasive adenocarcinoma. The amount of intracytoplasmic mucin is also lower. Mitotic figures and apoptosis and the existence of the front formation are diagnostically important [41], and, in the absence of these features, pathologists should be careful and consider AIS mimics including tubal metaplasia as differential diagnoses. The characteristic front formation indicates that AIS arises de novo

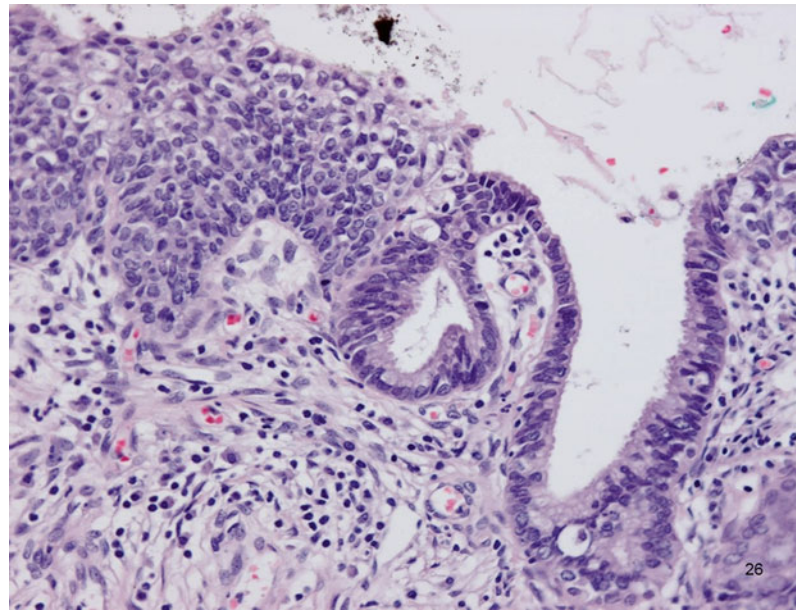

Fig. 12.5 Adenocarcinoma in situ (AIS) associated with high-grade squamous intraepithelial lesions (HSIL) (CIN 3) identified in the transformation zone, suggesting a common implication of high-risk HPV. This should be distinguished from adenosquamous carcinoma in situ or stratified mucin-producing intraepithelial lesion (SMILE)

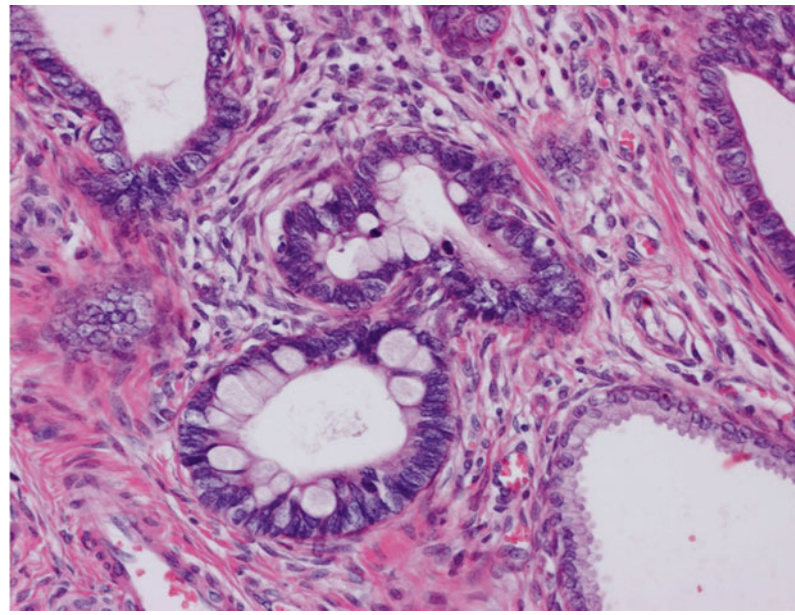

Fig. 12.6 Adenocarcinoma in situ (AIS), intestinal type, composed of a mixture of columnar cells and goblet cells

without a precursor lesion, which is biologically and morphologically intermediate between normal epithelium and AIS [42]. Approximately two-thirds of cases are incidentally found on biopsy or conization for HSIL (Fig. 12.5) [38].

The intestinal type AIS is characterized by an admixture of goblet cells (Fig. 12.6). Neuroendocrine cells that show immunoreactivity for chromogranin can be also identified, but Paneth-like cells are rarely seen. McCluggage et al. demonstrated CDX2 positivity in cases of intestinal type AIS, indicating the true enteric differentiation of this particular lesion. Nuclear abnormalities may be minimal, and therefore pathologists are encouraged to perform a diligent search when encountering a lesion interpreted as intestinal

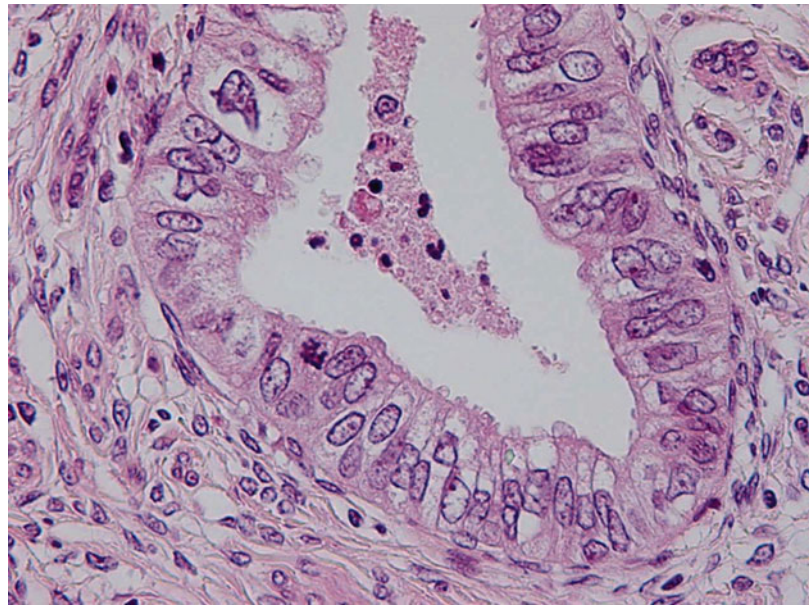

Fig. 12.7 Adenocarcinoma in situ (AIS), endometrioid type, characterized by mucin-deficient columnar cells with cilia. Nuclear morphology, including enlarged vesicular nuclei and nucleoli, nuclear stratification, and mitotic figures indicate the malignant nature of the lesion

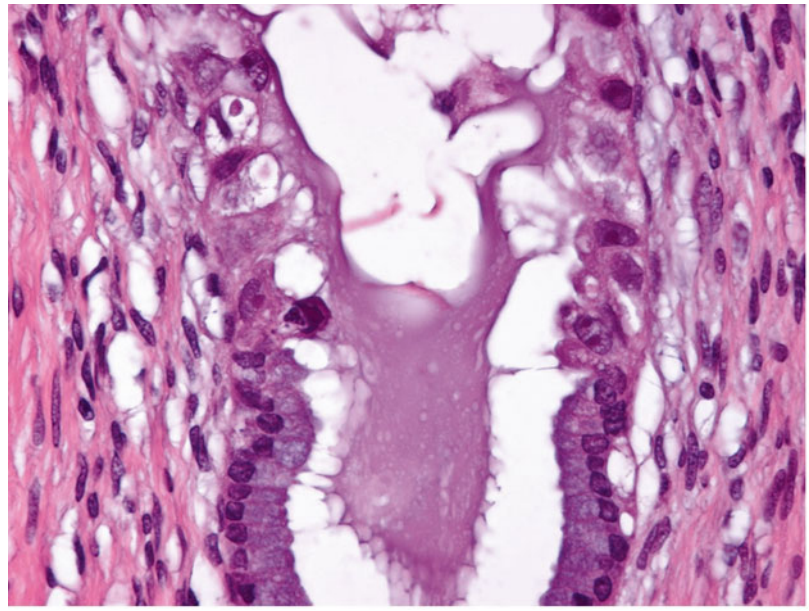

Fig. 12.8 Adenocarcinoma in situ (AIS), clear cell type. Atypical cells with abundant clear cytoplasm, showing hobnail appearance, lining the preexisting endocervical glands

metaplasia since it may represent a neoplastic condition [43].

Endometrioid type AIS is composed of cells with almost no intracytoplasmic mucin (Fig. 12.7). This type, like the tubal type, may be associated with tuboendometrioid metaplasia [40], and thus the associated invasive adenocarcinoma is located in the higher portion of the endocervical canal away from the transformation zone [22].

Interestingly, Jaworski et al. examined AIS subtypes, and in their series, they found that endometrioid type more frequently coexists with usual type and/or intestinal type and is only occasionally found alone and that intestinal type is

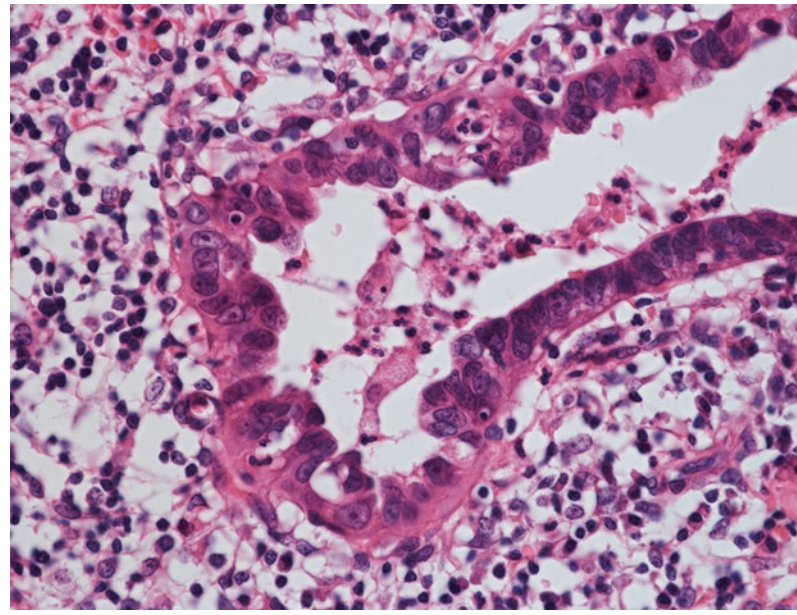

Fig. 12.9 Adenocarcinoma in situ (AIS), serous type. Replacement of preexisting normal endocervical cells replaced by cells with significant nuclear pleomorphism, showing heterogeneity in nuclear size and shape

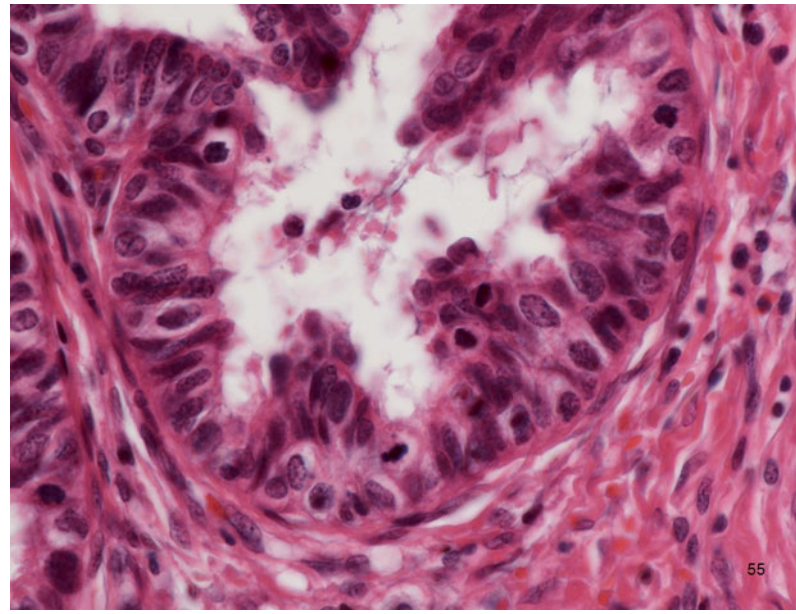

Fig. 12.10 Adenocarcinoma in situ (AIS), tubal type. Atypical columnar cells with cilia and intermingling of peg cell-like cells

always in association with usual type and/or endometrioid type [38]. These results suggest possibilities that intestinal type AIS is derived from usual-type AIS and that the endometrioid type may be classified into two groups: HPV-negative de novo type and HPV-positive usual type-related; the latter might represent mucin-deficient usual type AIS.

Clear cell type AIS shows atypical cells with abundant clear or pale eosinophilic cytoplasm and hobnail appearance (Fig. 12.8). It imparts a close resemblance to the Arias-Stella reaction and thus can be a diagnostic pitfall, although, from a practical point of view, it may be better to think of a benign diagnosis in a pregnant woman.

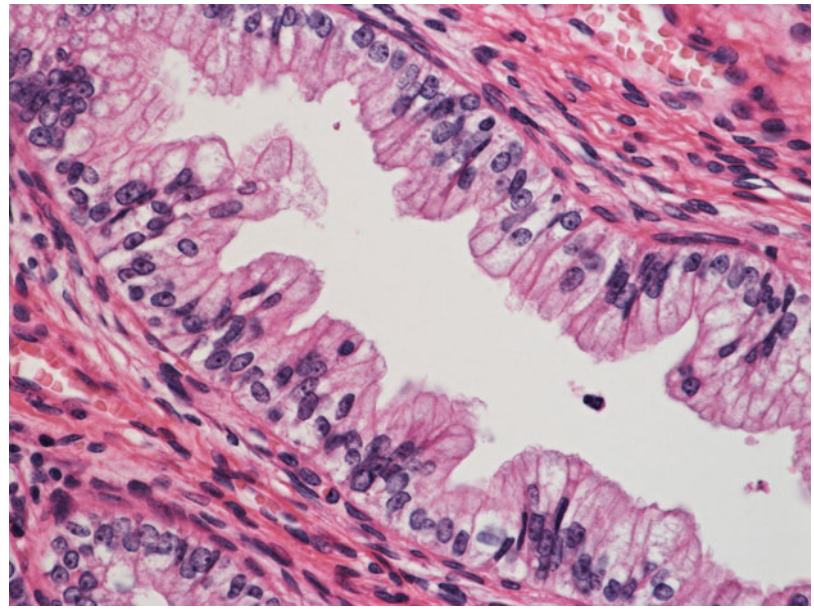

Fig. 12.11 Adenocarcinoma in situ (AIS), gastric type, characterized by lining cells with abundant clear or pale eosinophilic cells with distinct cell borders; the nuclear morphology is rather unremarkable and thus is a diagnostic concern

Gastric type AIS is characterized by abundant clear or pale eosinophilic cytoplasm and enlarged vesicular nuclei with conspicuous nucleoli (Fig. 12.11). Lobular endocervical glandular hyperplasia (LEGH) with intraepithelial carcinoma (atypical LEGH) is distinguished from this condition by its characteristic lobular architecture, but both of these conditions might be GAS precursors [19]. It should be kept in mind that the nuclear abnormalities in cases of gastric type AIS may be very unremarkable and thus can be overlooked without familiarity of its cytologic features. Immunohistochemically, this type is positive for HIK1083 and MUC6, which are representative gastric markers [19]. Occasionally, it can be positive for $p16^{INK4a}$ [19], suggesting an implication of high-risk HPV, but this remains undetermined.

A unique precancerous lesion is described as a stratified mucin-producing intraepithelial lesion (SMILE). It is characterized by stratification of epithelium with cells containing intracytoplasmic mucin vacuoles (Fig. 12.12) [44], and Boyle et al. found that it is identified on 0.6 % of cervical specimen [45] (Fig. 12.12). SMILE, also called mucoepidermoid carcinoma in situ [46] or adenosquamous carcinoma in situ [47] by some authors in the literature, is commonly associated with AIS, invasive adenocarcinoma, or adenosquamous carcinoma, as well as HSIL, and is currently considered a variant of AIS [44, 45]. It may be best regarded as clonal expansion of the reserve cell population located in the transformation zone, which is capable of divergent differentiation [45]. However, Lee et al. demonstrated that both HSIL and AIS occur in association with adenosquamous carcinoma, suggesting that intraepithelial lesions showing bimorphic differentiation are also a precursor of this tumor [22].

A panel of markers contribute to the correct diagnosis of AIS (Fig. 12.13). In cases of usual type AIS, $p16^{INK4a}$, which is induced by the integration of high-risk HPV DNA resulting in loss of function of Rb/E2F controlling promoter of the $p16^{INK4a}$ gene, is diffusely positive [31, 32]. Conversely, normal endocervical glands and endocervical glandular hyperplasia are negative. Tubal metaplasia and occasional cases of LEGH are also positive, but staining intensity is weak. Estrogen receptor (ER) expression is lost or decreased in AIS, whereas it is positive in normal endocervical glands [33]. Nonspecific endocervi-

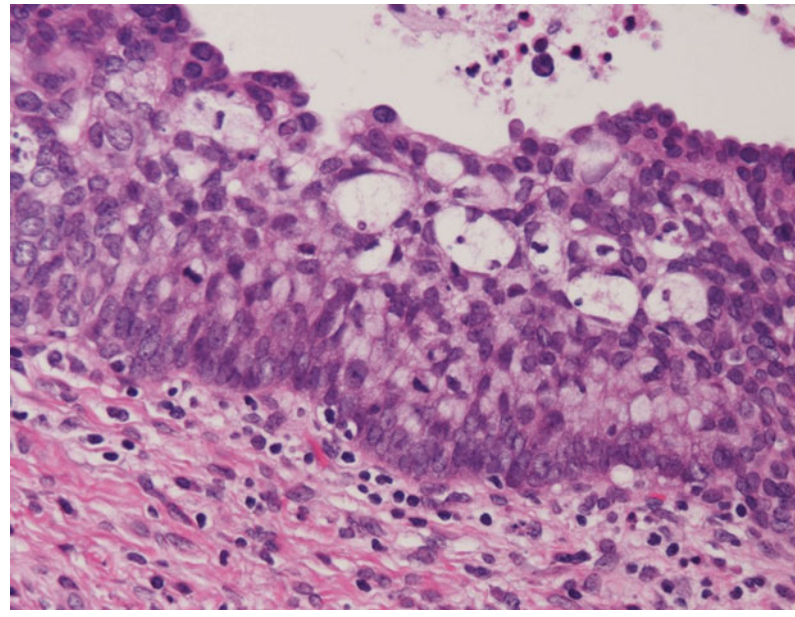

Fig. 12.12 Stratified mucin-producing intraepithelial lesion (SMILE). Intraepithelial proliferation of stratified cells with abundant cytoplasm and mucin vacuoles

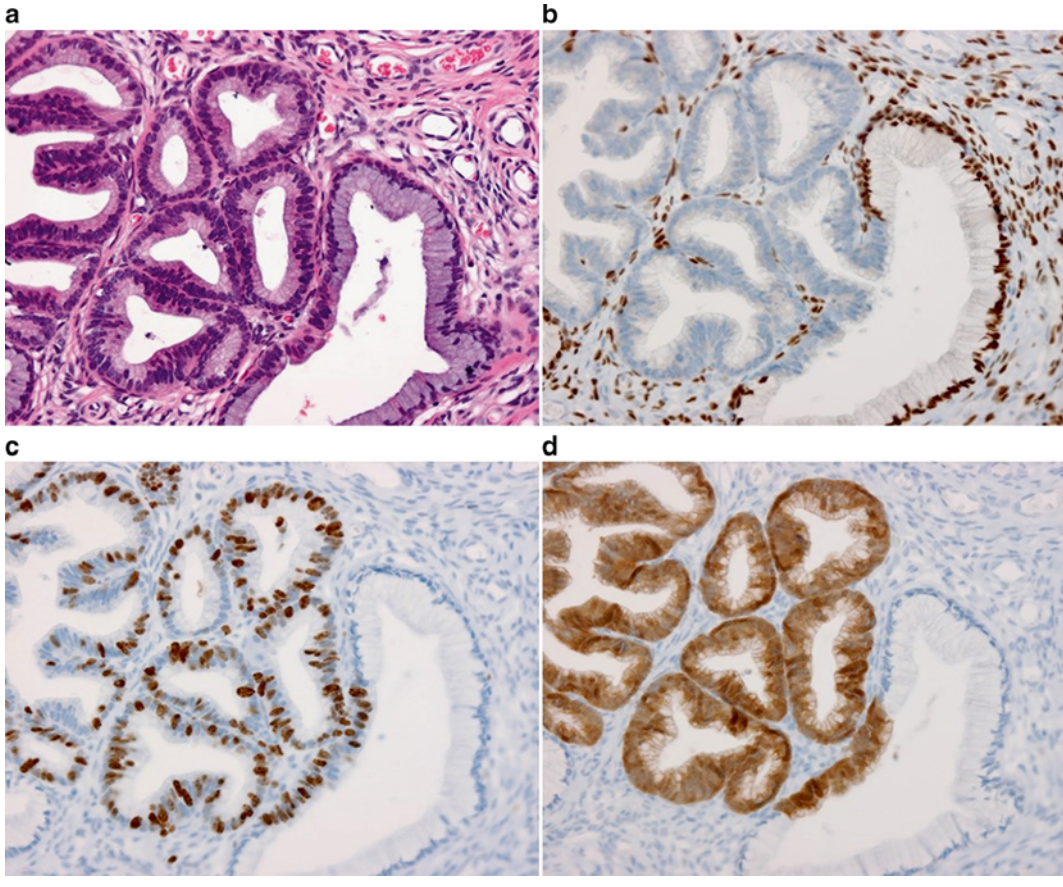

Fig. 12.13 Immunohistochemical phenotype of adenocarcinoma in situ (AIS), usual type. AIS (**a**, left side, HE) typically shows negative staining for estrogen receptor (ER) (**b**), Ki-67 labeling index exceeding 50 % (**c**), and diffuse and strong immunoreactivity for $p16^{INK4a}$ (**d**). Conversely, the adjacent normal endocervical glandular epithelium (right side) shows a nuclear staining for ER, almost no Ki-67-positive cells, and a negative staining for $p16^{INK4a}$

cal glandular hyperplasia and tubal metaplasia are also positive for ER, but LEGH is typically negative. The Ki-67 labeling index commonly exceeds 25 % in cases of AIS and can reach up to 40 % [43, 48, 49]. Although $p16^{INK4a}$ immunohistochemistry is diagnostic, the combination of $p16^{INK4a}$, ER, and Ki-67 is recommended in challenging cases rather than using a single marker. In addition, it should be emphasized that usual and intestinal types can be identified by this panel, but unusual types, including clear cell type, gastric type, and serous type, may need different diagnostic considerations.

Controversies on the Term Glandular Dysplasia

The term glandular dysplasia has been used to describe precancerous lesions of adenocarcinoma [50, 51] and was employed in the previous version of WHO classification (2003) [2], which defines it as an atypical glandular lesion characterized by significant nuclear abnormalities that are more striking than those in glandular atypia but fall short of the criteria for AIS. However, there has been no sufficient evidence supporting the existence of glandular dysplasia as precancerous lesions, in contrast to squamous intraepithelial lesions (SIL). In fact, AIS is commonly present adjacent to preexisting normal glandular epithelium showing front formation without an intervening atypical epithelium intermediate between AIS and normal epithelium. Some studies demonstrated that the average age of patients with glandular dysplasia is higher than or not significantly different from those with AIS [52]. In addition, the detection rate of high-risk HPV is not high in this particular lesion [43]. More importantly, the biologic significance of glandular dysplasia, i.e., the risk for developing invasive adenocarcinoma, remains undetermined. Based on these reasons, glandular dysplasia as a diagnostic entity has been questioned by gynecologic pathologists and oncologists [42]. Some examples of glandular dysplasia might be benign conditions, including reactive changes and tubal metaplasia [43]. However, it appears realistic that the lesion recognized as glandular dysplasia associated with adenocarcinoma, HSIL, or squamous cell carcinoma is considered to represent AIS with low-grade nuclear abnormalities because of the detection of high-risk HPV DNA and increased Ki-67 labeling index [43]. In other words, the condition designated as "glandular dysplasia" includes both benign and malignant conditions. In reality, small foci of AIS might be diagnosed as glandular dysplasia by some pathologists.

In the United Kingdom, the term cervical glandular intraepithelial neoplasia (CGIN) is commonly used [53, 54], which is divided into low grade (LG-CGIN) and high grade (HG-CGIN), and each is considered to correspond to glandular dysplasia and AIS, respectively, in the WHO 2003 terminology. However, in routine practice, the diagnostic category of LG-CGIN is strictly used to denote a neoplastic condition, based on morphology with the aid of immunohistochemistry using the aforementioned panel including $p16^{INK4a}$, and should be managed by gynecologists in a manner similar to AIS. In other words, LG-CGIN represents morphologically low-grade AIS (Fig. 12.14) [55]. Therefore, pathologists are encouraged to avoid the use of the term glandular dysplasia and to inform clinicians of the recommended optimal management when making a diagnosis of LG-CGIN or low-grade AIS. Otherwise, pathologists may mislead clinicians, and the lesion might be managed in the same way as low-grade squamous intraepithelial lesions (LSIL), resulting in unnecessary follow-up. The natural history of LG-CGIN and the relationship between LG-CGIN and HG-CGIN remain undetermined. Since it is rare to see coexistence of both conditions in the same lesion, a transition from LG-CGIN to HG-CGIN appears unlikely, and each may be a precursor of low-grade and high-grade adenocarcinoma, respectively, although this issue should be scrutinized by further studies.

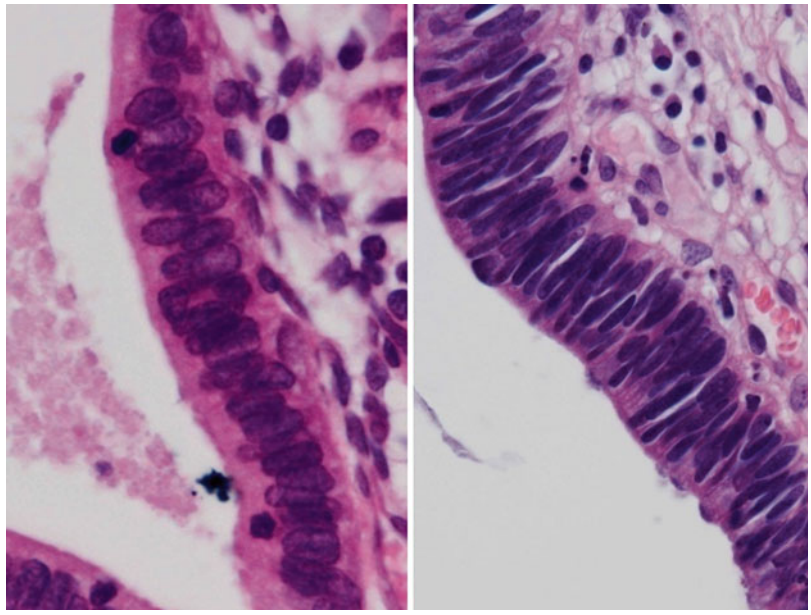

Fig. 12.14 Comparison of low- and high-grade atypia in adenocarcinoma in situ (AIS). A subset of AIS shows bland nuclear morphology (a), compared with a prototypical AIS showing significant nuclear hyperchromasia, enlargement, and overlapping (b). The latter lesion, which was confirmed to be negative for estrogen receptor (ER) and positive for $p16^{INK4a}$ and shows an increase of Ki-67 labeling index exceeding 50 %, is thus now considered to be low-grade AIS or CGIN and might have been interpreted as glandular dysplasia in the past

Atypical Lobular Endocervical Glandular Hyperplasia (Atypical LEGH) /Pyloric Gland Metaplasia

Endocervical glandular hyperplasiais divided into three types, including nonspecific type, diffuse laminar type [56], and lobular type (LEGH) [57]. Among these, LEGH is characterized by proliferation of small glands arranged in a lobular fashion surrounding dilated glands and represents pyloric gland metaplasia (Fig. 12.15); thus, it shows a gastric phenotype as shown by positivity of HIK1083 and anti-MUC6, two representative gastric markers [58, 59]. LEGH per se is considered a benign condition [57–59], but there is some evidence supporting the neoplastic nature of this condition. For instance, significant cytologic abnormalities or architectural abnormalities [19], which are immunohistochemically positive for p53 and show increased Ki-67 labeling index [60], may be identified limited to LEGH without destructive stromal invasion (Fig. 12.16), and adenocarcinoma and LEGH with or without atypia may coexist [19, 61]. In addition, Kawauchi et al. demonstrated abnormalities in DNA copy number similar to MDA in cases of atypical LEGH [62]. In addition, LEGH shows negative or only weak staining for $p16^{INK4a}$, and high-risk HPV is not detected in this condition. Therefore, there seems to be an HPV-independent pathway linking a subset of LEGH and adenocarcinoma, which can be gastric type or prototypical MDA. Recently, Matsubara et al. demonstrated the mutation of *GNAS*, *KRAS*, or *LKB1* genes [63], which are mutually exclusive, in LEGH cases, thus implicating these genetic abnormalities in this pathway and illustrating the precancerous nature of LEGH. However, it should be kept in mind that LEGH is not an uncommon condition, and only a subset of LEGH showing cytologic or architectural abnormalities is considered to be at risk for developing invasive carcinoma. LEGH with or without atypia can also occur in patients with

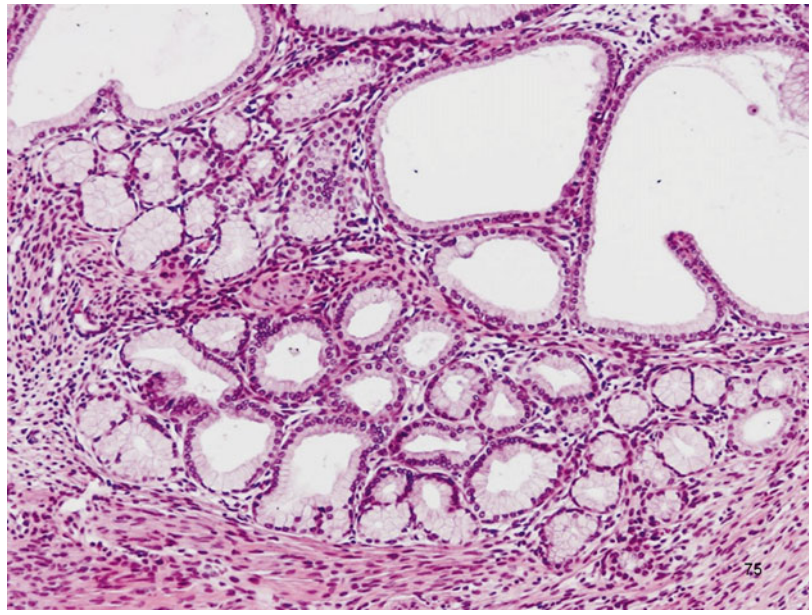

Fig. 12.15 Lobular endocervical glandular hyperplasia (LEGH), characterized by proliferation of small glands in a lobular fashion, surrounding dilated glands, imparting a resemblance of pyloric gland of the stomach

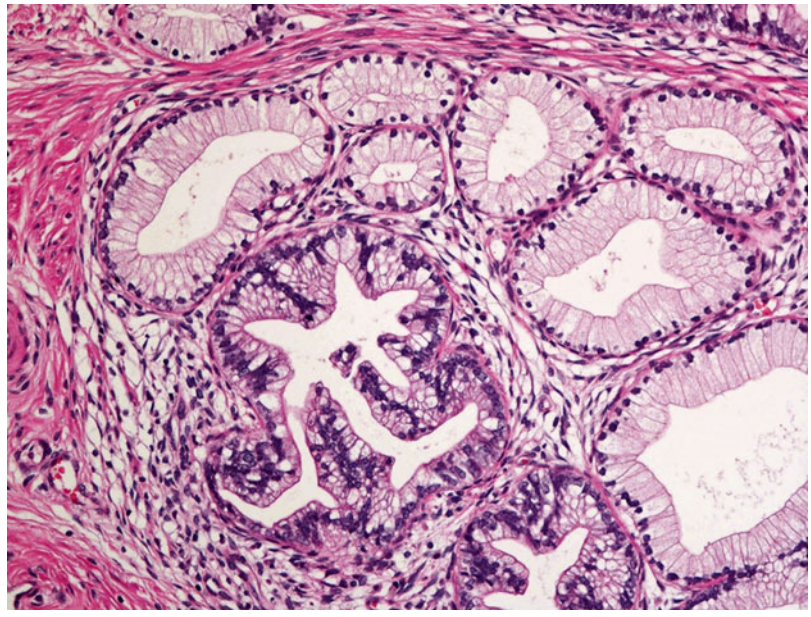

Fig. 12.16 Atypical lobular endocervical glandular hyperplasia (LEGH)/pyloric gland metaplasia (PGM), showing significant cytologic abnormalities with its characteristic architecture preserved

Peutz-Jeghers syndrome (PJS), which is well known to be associated with MDA [19, 61, 64–68]. Only rarely is the papillary proliferation of mucinous cells lining the fibrovascular stroma without destructive stromal invasion identified in a patient with PJS (Fig. 12.17), which may be considered to be a preinvasive state of MDA ("adenoma malignum in situ") [67].

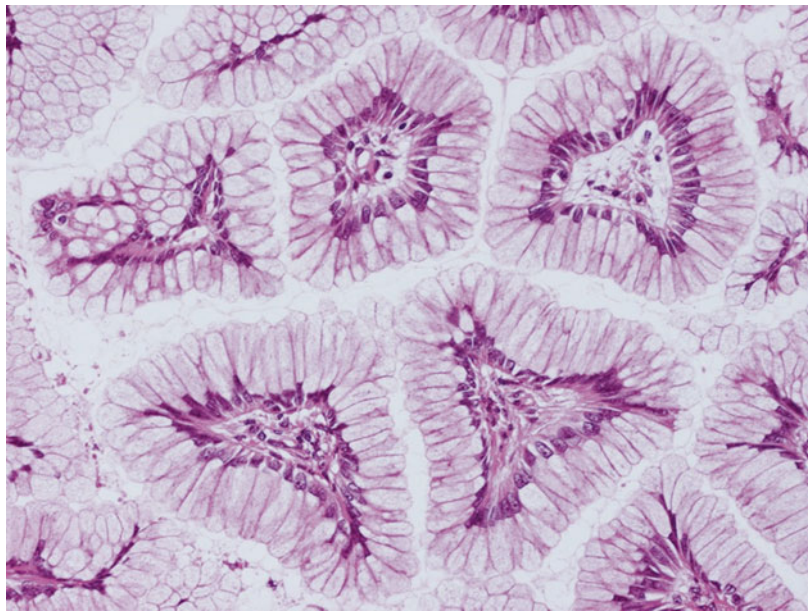

Fig. 12.17 Papillary proliferation of columnar cells with abundant intracytoplasmic mucin, identified in case of Peutz-Jeghers syndrome with lobular endocervical glandular hyperplasia (LEGH). Destructive stromal invasion was not identified. This lesion might be challenging on biopsy specimens, because, in the current classification of endocervical glandular lesions, it does not fit with any neoplastic or pseudoneoplastic lesions. Considering the association with atypical LEGH and Peutz-Jeghers syndrome, this condition is likely precancerous, and some authors may call this a preinvasive state of minimal deviation adenocarcinoma (MDA)

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Precancerous Lesions of Squamous Cell Carcinoma of the Cervix: Squamous Dysplasia

13

Lynn Hirschowitz and C. Simon Herrington

Historical Evolution and Terminology

Over the past century, the terminology used to describe precancerous lesions of the cervix has been a source of confusion to pathologists and clinicians alike. However, over recent decades, advances in our understanding of the pathogenesis and behavior of these lesions [1] have refined the histopathological classification of precancerous cervical lesions. The current histopathological classification of cervical intraepithelial lesions is now reproducible, closely reflects their biological relationship to HPV infection, and reliably informs clinical decision-making.

The earliest recorded description of cervical dysplasia is attributed to Sir John Williams [2] in the late 1800s. In 1900, Cullen recognized the spatial relationship of noninvasive epithelial lesions to adjacent invasive squamous neoplasms of the cervix [3]. The recognition of preneoplastic cervical lesions aligned closely with the cytological identification (and classification) of cellular abnormalities in the Pap smear. The Pap smear was developed in the first half of the 1900s by Dr George Papanicolaou, based at Cornell University Medical College, in the Department of Anatomy [4–6]. During the mid-1900s, the cytological abnormalities in Pap smears were shown to correlate with histological changes in cervical squamous epithelium, and broadly similar systems were adopted for classification and grading of the cytological and histological abnormalities [7, 8]. The development of a universal classification and grading system was subsequently hampered by confusing terminology (including terms such as "anaplasia" and "basal cell hyperplasia"), varied definitions of "cervical dysplasia," and (as we gained better understanding of the natural history of preneoplastic lesions) the introduction of different histological and cytological terminologies in different parts of the world.

There was lack of clarity when the term "dysplasia" was originally used in the mid-1900s. The term "dysplasia" simply describes an abnormality of growth or development, not a process involved in neoplasia. As Buckley et al. [9] stated, "it is not clear whether those who wish to retain this term consider that a cell in dysplastic cervical epithelium is an abnormal non-neoplastic cell, a cell which has undergone malignant change or one which, in some ill undefined manner, occupies a hypothetical middle ground between neoplastic and nonneoplastic cells."

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Notwithstanding the strict meaning of the word, the International Committee on Histological Definitions proposed the following consensus definition of dysplasia [10]: "all other disturbances of differentiation of the squamous epithelium of lesser degree than carcinoma *in situ*." The definition was later felt to be broad and imprecise [11, 12], and the WHO proposed that "dysplasia is a lesion in which part of the thickness of the epithelium is replaced by cells showing varying degrees of atypia" [13]. This was considered more acceptable, although the grading of "dysplastic" lesions into mild, moderate, or severe dysplasia and the lack of established criteria for this grading system were originally viewed with some skepticism.

Introduction of the term "carcinoma in situ" caused further confusion because of the implication that there was a biological distinction between this entity and dysplasia [14, 15]. The distinction of carcinoma in situ from severe dysplasia was arbitrary and not reproducible, and there was no evidence that severe dysplasia had any less potential for progressing to invasive carcinoma than did carcinoma in situ [16–18]. It was becoming increasingly clear from studies of histomorphology and follow-up of the natural history of cervical dysplasia that "dysplasia" and carcinoma in situ were only stages in the evolution of a single process, and the term "cervical intraepithelial neoplasia" was adopted to encompass "dysplasia" of all grades including carcinoma in situ [9, 19–23].

Cervical intraepithelial neoplasia (CIN) was applied as an umbrella term for neoplasia confined to the epithelium and not invading the stroma. CIN was subdivided into grades according to the relative thickness of epithelium occupied by neoplastic cells of basaloid type. Cases of CIN3 were shown to be more likely than those of CIN1 and CIN2 to progress to invasive carcinoma. The time taken for CIN1 lesions to progress to carcinoma was much longer, and patients with CIN1 could be kept under surveillance. The risk of progression was lower for CIN2 than CIN3. The CIN terminology redefined "dysplasia" as a neoplastic lesion, eschewed the concept of a two-stage process in the evolution of cervical intraepithelial neoplasia, and underscored the biological and clinical unity of dysplasia and carcinoma in situ. Pathologists were no longer expected to differentiate between severe dysplasia and carcinoma in situ, and grading of an intraepithelial lesion was of prognostic and therapeutic significance for individual patients.

Studies of the biology of human papillomavirus (HPV) and cervical oncogenesis during the 1980s indicated that squamous carcinoma and its precursor lesions were almost always caused by infection with specific high-risk (HR) types of HPV [24–28]. It also became clear that there was considerable subjectivity in differentiating between CIN2 and CIN3, and in the USA, the need was recognized for a more biologically and therapeutically relevant and histologically reproducible two-tier system of high- and low-grade CIN [29–31], mirroring the system for grading cervical cytological abnormalities.

The Bethesda system for grading cervical cytological abnormalities was introduced in the USA in 1988, revised in 1991 [32] and again in 2001 [33]. The Bethesda system is a highly reproducible, two-tier grading system of low- and high-grade squamous intraepithelial lesions (LSIL and HSIL). A similar two-tier system was proposed for histology but not supported by professional organizations and not initially adopted. However, in 2001 and 2006, the American Society for Colposcopy and Cervical Pathology (ASCCP) Consensus Guidelines for clinical management of histological abnormalities in the cervix adopted two-tier terminology (HSIL and LSIL), although a three-tier system was retained for the management of adolescents and young women [34]. More recently, a two-tier system was proposed for grading and classifying all HPV-associated lesions of the lower anogenital tract [35, 36]. A two-tier system improves diagnostic reproducibility, better reflects the known biology of HPV-associated lesions, and does not have a deleterious effect on patient management as compared with a three-tier system [37]. The new terminology was adopted at the most recent College of American Pathologists (CAP)-ASCCP Lower Anogenital Squamous Terminology (LAST) consensus conference [38–40].

A three-tier histological grading system (CIN1, CIN2, and CIN3) is still followed in the

UK [41]. The original rationale was that this permitted direct correlation with the three-tier cytological grading of dyskaryosis in use at the time and ensured continuity in the recording, transfer, and storage of coded data to existing local, regional, and national databases. However, the three-tier grading system is of little value in guiding patient management. Patient management is based on a two-tier grading system, of low- (CIN1) and high-grade (CIN2 and CIN3) abnormality. A three-tier cytological grading system of mild, moderate, and severe squamous dyskaryosis was in use in the UK until 2012, when a two-tier system ("low grade" and "high grade") was adopted for squamous dyskaryosis [42]. The histological grading system will need to be reviewed now that a two-tier system has been adopted for cervical cytology reporting in the UK.

Epidemiology

Cervical carcinoma is the fourth most common cancer in women worldwide; over half a million new cases were diagnosed in 2012 [43]. It is now established that virtually all (if not all) cervical carcinomas are caused by human papillomavirus (HPV) infection [44]. The human papillomaviruses (HPVs) are found across mammalian species, are well conserved, and infect humans globally. They infect squamous cells throughout the body, and infection has been implicated most importantly in the development of cervical cancer and its precursor squamous intraepithelial lesions (also termed cervical intraepithelial neoplasia—see above). HPV infection is also associated with benign lesions, namely, viral warts.

Genital HPV infection is very common, with a cumulative 3-year incidence in one study of over 40 % in young sexually active women [45]. Almost 200 HPV types are currently recognized [46]. These are divided into five genera, alpha, beta, gamma, mu, and nu, of which the alpha papillomaviruses are relevant to mucosal infection and the development of genital neoplasia. Alpha papillomaviruses are further divided into high- and low-risk types, based on their association with neoplastic disease. The concept of low- and high-risk HPV infection was based on the relative risk associations of these HPV types with intraepithelial and invasive disease of the uterine cervix. At one time, an intermediate risk group was also defined on the basis that it was associated more with intraepithelial than invasive disease [47], but as further data have accumulated, this group has been largely subsumed into the high-risk category. Twelve HPV types, namely, HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, are considered to be high-risk types, with a further group, including HPV 68 and 73, showing some association with invasive disease [48].

Most HPV infections, including those with high-risk HPVs, are transient [45]. Cervical neoplasia can therefore be thought of as an uncommon complication of a common infection. The factors that underlie progression are poorly understood, although persistent infection is clearly important [49].

Current concepts of HPV-associated disease recognize two forms of infection: productive infection, which leads to a low-grade lesion, and transforming infection, which leads to a high-grade lesion. The normal, productive life cycle is associated with coordinated viral gene expression and production of infectious viral particles. In transforming infection, productive infection fails, with loss of coordinated expression of viral genes and, in particular, upregulation of early gene expression, which leads to abnormalities of cell cycle checkpoint control and accumulation of genetic abnormalities. From a pathological point of view, low-grade squamous intraepithelial lesions (LSILs) represent productive infections and high-grade squamous intraepithelial lesions (HSILs) and generally represent transforming infections [50].

Productive HPV infection produces a characteristic koilocytic morphology. This indicates vegetative viral replication and is seen typically in viral warts and LSILs, in which the viral life cycle proceeds relatively normally. In a small subset of HPV infections, infection persists and leads to the development of high-grade SILs and invasive carcinomas. In these cases, the viral DNA is often integrated into the host genome, and normal regulation of E6 and E7 expression is lost through disruption of the E1/E2 gene region (discussed below). In such situations, the uncon-

trolled expression of E6 and E7 in cycling squamous epithelial cells leads to expansion of the basal cell compartment, associated with accumulation of genomic abnormalities, leading to cytological abnormality. Infection of cervical glandular epithelium also occurs but is less common. It is also nonproductive, as viral replication requires squamous differentiation. Some HPV types, particularly HPV 18, are particularly associated with this process, resulting in the development of CGIN/adenocarcinoma in situ (AIS) and adenocarcinoma [51].

Molecular Biology of HPV Infection

HPVs are double-stranded DNA viruses and comprise an approximately 8 kb circular genome enclosed within an icosahedral capsid composed of viral L1 and L2 capsid proteins [52]. Viral replication and production of viral particles requires squamous differentiation, which underlies the tight association between HPV infection and squamous epithelial surfaces. The genomes of all HPVs are similar, containing genes that code for "early" (E1, E2, E4, E5, E6, E7) and "late" (L1, L2) proteins. These two blocks of genes are separated (between L and E) by a noncoding region [or long control region (LCR)], which contains glucocorticoid response elements and the origin of DNA replication [53]. The HPV genes encode only one protein with enzyme activity (E1, a helicase), and therefore, HPVs must subvert the host DNA replication and protein synthesis machinery in order to replicate. This involves interference with cell cycle control, inducing DNA replication in postmitotic differentiated squamous cells, and inhibition of apoptotic pathways, preventing cell death in response to infection.

The Functions of the HPV Proteins

The E1 protein acts as a DNA helicase [54] and cooperates with the E2 protein to control viral DNA replication [55]. The E2 protein facilitates the transport of viral DNA to the host nucleus and, once within the nucleus, promotes binding to host DNA. It also binds to the long control region of the viral genome, repressing the expression of E6 and E7. E2 also has strong proapoptotic effects. The N-terminal of the protein has been shown to be responsible for this effect and induces the caspase cascade directly by activating caspase 8. E2 itself is then cleaved as part of the cascade, functioning as both an inducer and target of the cascade's activity [56].

The E1^E4 protein is formed by fusion of E4 with part of the E1 protein and is the most abundant viral gene product [57]. It has potent proapoptotic potential [58], and although unclear, its role appears related to genome amplification and virus release. The E5 protein is also thought to be involved in genome amplification, and it plays a role in koilocyte formation [59]. L1 and L2 are the major and minor capsid proteins, respectively, which form the capsid coat of the virus [60].

The E6 and E7 genes encode the main transforming proteins. When expressed inappropriately in cycling cells, these drive immortalization and neoplastic transformation. The E6 protein is expressed early in viral infection and interacts with a large of number of cellular proteins, many of which are cell cycle regulators. The best known, and most significant, effect of E6 expression is the abrogation of function of the key tumor suppressor protein p53 by direct binding and consequent degradation [61, 62]. Loss of p53 function leads to failure of the checkpoint between the gap 1 (G1) and synthesis (S) phases of the cell cycle. Cells can therefore accumulate DNA damage without undergoing cell cycle arrest or apoptosis [63].

HPVs also evade cellular apoptotic processes in order to replicate without inducing cell death. p53 is proapoptotic, and therefore, its degradation by E6 inhibits apoptosis. Additionally, E6 can prevent apoptosis by mechanisms that do not involve binding to p53 [64]. Conversely, under some circumstances, E6 has proapoptotic effects, which in the normal viral life cycle may relate to virion release.

The E6 protein also activates telomerase reverse transcriptase (hTERT), inhibiting the normal shortening of telomeres that occurs when cells divide and extending cell lifespan [65].

The predominant effect of E7 protein expression is abrogation of the function of the retino-

blastoma (pRb) protein. E7 binds to non-phosphorylated pRb, releasing E2F transcription factors and hence driving progression into S phase. E7 also blocks the effects of the cyclin-dependent kinase inhibitors (CDKIs) p21 and p27 [66, 67], and as pRb inhibits expression of p16, E7 expression also leads to the overexpression of p16. This overexpressed p16 is functionally silent, however, as cells do not require cyclin-dependent kinase (CDK) 4/6, which is the target of p16 inhibition, for cell progression. Thus, E7 effectively substitutes for the phosphorylation of pRb by CDK4, and p16 overexpression has no effect. In clinical practice, this increase in p16 expression is being used increasingly as a surrogate marker for E7-expressing high-risk HPV infection [68, 69], and guidelines for its use in this context have been published [40].

E7 also promotes chromosomal instability by inducing abnormal numbers of centrosomes. E6 shares a similar function, acting in synergy to promote further abnormalities in host chromosome and DNA structure [70].

E7 also has antiapoptotic effects, reducing activation of pro-caspase-8, which is an initiator of the caspase cascade [71]. In experimental models, E7-immortalized cells appear almost completely resistant to cell death via the extrinsic pathway. However, inhibition of nuclear export of proteins using leptomycin B induces widespread apoptosis of keratinocytes overexpressing HPV 16 E7 [72].

High-Risk Versus Low-Risk HPV Type

Both high-risk and low-risk HPV types can, and indeed need to, induce productive infection of squamous epithelium. However, why some HPV types can lead to neoplastic transformation, and others do so only rarely, is not entirely clear.

One difference between high- and low-risk HPVs is the propensity of the former to integrate into the host genome rather than remaining in an extrachromosomal (episomal) form [73, 74]. HPV integration occurs at widely distributed sites in the human genome, but the point at which the circular viral genome breaks to allow integration is consistently within the E1/E2 region, downstream of the E6 and E7 genes and leaving the relationship between the LCR and E6/E7 intact. As one role of the E2 protein is to repress the expression of E6 and E7 through binding to E2 binding sites in the LCR, the failure of E2 expression leads to enhanced expression of E6 and E7 [75]. In addition, as E1 and E2 are required for viral DNA replication, loss or disruption of this region leads to failure of viral replication. Moreover, the proapoptotic effect of E2 is lost.

A further difference between high- and low-risk HPV types lies in the functions of the E6 and E7 proteins. For example, high-risk HPV E6 proteins degrade p53 more effectively than low-risk HPV E6 proteins [76], and high-risk HPV E7 proteins bind pRb with greater affinity than low-risk HPV E7 proteins [77].

Overall, low-risk HPVs, whose genomes remain as episomes and do not integrate into host chromosomes, alter cell cycle control to drive viral replication in postmitotic differentiated squamous epithelial cells, primarily through the action of E7. Viral genes and proteins are expressed in a coordinated way, culminating in the production of viral particles. High-risk HPVs can also do this, but, when coordinated expression of HPV genes is disrupted, for example, by HPV integration, inappropriate high-level expression of particularly E6 and E7 occurs in dividing cells. This induces genetic instability, activation of telomerase, inhibition of apoptosis, and many other changes, resulting eventually in neoplastic transformation. It should be stressed, however, that this represents an uncommon outcome of HPV infection, the probability of which increases over time. This may explain the association between persistent HPV infection and increased risk of neoplastic transformation.

Morphology

Terminology

As noted in section "Historical Evolution and Terminology," a three-tier grading system is still used in the UK for the histological reporting of squamous intraepithelial neoplasia [41], whereas a

two-tier classification is used in the USA. A two-tier classification is also recommended by the WHO [50]. The WHO lists the following synonyms for HSIL: CIN2, CIN3, moderate and severe squamous dysplasia, and squamous carcinoma in situ (CIS). For LSIL, the following synonyms are listed: CIN1, mild squamous dysplasia, flat condyloma, koilocytotic atypia, and koilocytosis.

The reason for including HPV-associated lesions in LSIL is that it is difficult to differentiate morphologically between pure HPV-associated lesions such as flat condyloma and CIN1. LSIL therefore reflects the morphological manifestations of an active, differentiation-dependent, transient HPV infection on squamous cells and incorporates lesions that show HPV effect (koilocytotic atypia) with or without cytonuclear changes of mild dysplasia or CIN1 [38–40, 48, 78]. This terminology is further justified on the basis of the poor histological reproducibility of the spectrum of intraepithelial lesions in this diagnostic category and the lack of biological markers that meaningfully separate the lesions.

HSIL represents lesions that are classified as precancerous and which, if left untreated, carry a significant risk of progressing to cancer. CIN2 and CIN3 are included in the definition, and the inclusion of carcinoma in situ takes account of the fact that there are no reproducible histological criteria to allow its distinction from CIN3. There is no biomarker that defines the intermediate category of CIN2, and the variability in histological interpretation of high-grade lesions justifies the amalgamation of CIN2 and CIN3 into a single category [78, 79]. In general, there are no significant differences in management of HSIL (CIN2) and HSIL (CIN3) although some clinicians argue that in young patients a diagnosis of HSIL (CIN2) allows for the possibility of lesion regression and potentially spares young women complications related to child-bearing that might result from excision of cervical tissue [37, 80].

HPV Infection

It is important to note that LSIL and HSIL may coexist in the cervix, not only in a single area or cervical quadrant but also in other separate areas or quadrants, and colposcopy may not reliably distinguish LSIL from HSIL [81]. Furthermore, in 20–40 % squamous intraepithelial lesions, multiple HPV genotypes can be identified; conversely, when multiple lesions are present, they may result from infection by the same or different HPV subtypes [82]. Quint et al. [82] also demonstrated that almost every HPV type found in CIN by laser capture microdissection was associated with a biologically separate area of CIN, although there was often abutting ("colliding") of virologically discrete foci to produce what appeared morphologically to be a single CIN lesion. Quint et al. [82] proposed the concept of "one virus, one lesion" (using virology rather than morphology to define a lesion).

Histomorphology does not allow the prediction of HPV subtype, although according to the 2014 WHO fascicle, there are data to suggest that HPV 16 and 18 may produce more rapidly growing, larger lesions. The difficulty in subcategorizing LSILs of similar biological potential into flat condylomas, CIN1, and koilocytosis without atypia supports the recommendations of the LAST project to unify the nomenclature of these lesions.

Criteria for Grading of Squamous Intraepithelial Lesions

Despite the fact that the histological features of squamous dysplasia are well described, there is considerable interobserver variability in the grading of squamous intraepithelial lesions, particularly low-grade lesions [83–85].

The defining histological features of squamous intraepithelial lesions are nuclear abnormalities, alterations in epithelial maturation, and presence of mitotic activity.

Nuclear Abnormalities

Nuclei are of variable size and usually enlarged. They are irregular in shape and hyperchromatic with irregular chromatin clumping and may have a wrinkled nuclear membrane. The nuclear/cytoplasmic ratio is increased and the polarity of the nuclei may be altered, giving a disorganized arrangement. The term "nuclear atypia"

encompasses this range of nuclear changes. Note the following:

- In contrast to reactive atypia, where nucleolation may be prominent, nucleoli are inconspicuous in preinvasive lesions.
- Nuclear atypia is often random and variable in CIN, whereas reactive nuclear atypia tends to be homogeneous in distribution.
- Neoplastic nuclear atypia correlates closely with alterations in epithelial maturation.

Alterations in Epithelial Maturation

In the cervix, the term "maturation" is used synonymously with epithelial differentiation. As squamous epithelial cells mature, the nuclear/cytoplasmic ratio decreases toward the epithelial surface, so that the cytoplasm predominates in superficial cells and the nuclei are small or absent.

The extent of maturation relative to the thickness of the epithelium is one of the features used to grade squamous intraepithelial lesions. There is no or little epithelial maturation in severe dysplasia. Instead, immature and atypical squamous cells extend through the full thickness of the epithelium, so that cells with a high nuclear/cytoplasmic ratio are present at the epithelial surface. In contrast, LSIL/low-grade squamous intraepithelial lesions show maturation in the upper two-thirds of the epithelium, and a low nuclear/cytoplasmic ratio is present in squamous cells near the surface. Mild nuclear atypia involving the full thickness of the epithelium is a characteristic of CIN1.

Mitotic Activity [86, 87]

Normal cervical squamous epithelium shows minimal mitotic activity. When present, it is confined to the parabasal layers. Most, but not all, cases of squamous intraepithelial neoplasia show increased mitotic activity. Mitoses may be present at all levels of the epithelium, including in the superficial third in CIN3/HSIL. Atypical mitotic figures (which reflect aneuploidy) are sometimes seen (Fig. 13.1).

Criteria for the Diagnosis of LSIL/CIN1/Mild Dysplasia including HPV-Associated Changes [88, 89]

Not all of the abnormalities that are typical of low-grade squamous intraepithelial neoplasia are found in every case, although nuclear atypia is

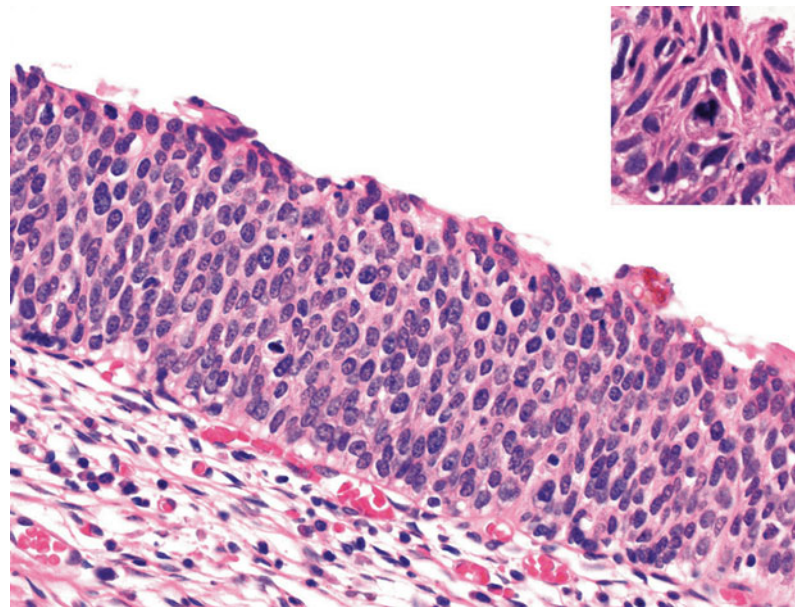

Fig. 13.1 HSIL/CIN3 and atypical mitotic figure. Nuclear atypia extends through the full epithelial thickness without any cytoplasmic maturation in the superficial epithelial layers. Note the nuclear size and nuclear/cytoplasmic ratio and the intraepithelial mitotic activity. Most cases of squamous intraepithelial neoplasia show increased mitotic activity, including atypical forms (inset)

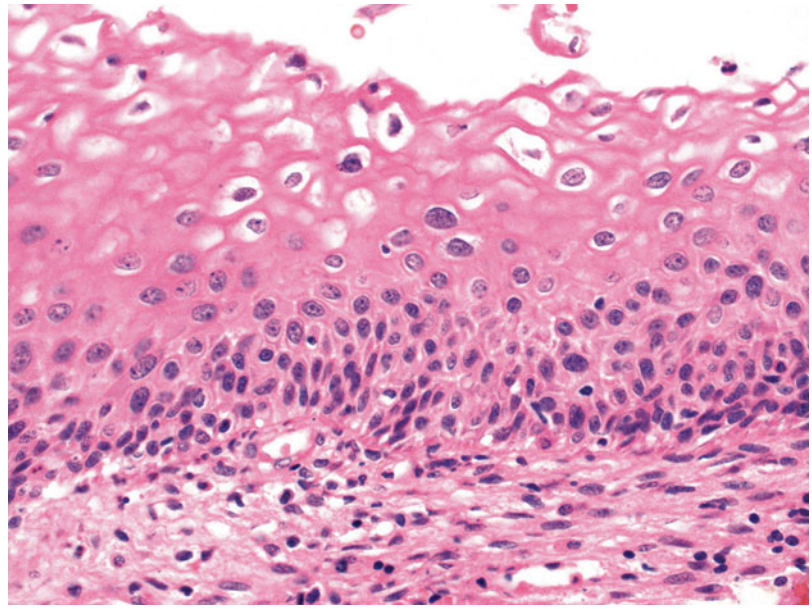

Fig. 13.2 LSIL/CIN1. Some degree of nuclear abnormality extends through the full epithelial thickness, but atypical nuclei are most prominent in the basal third of the epithelium, in the basal and parabasal cells. Koilocytosis (HPV effect) is present in the squamous cells in the upper third of the epithelium. The koilocytes have a well-defined nuclear halo with a sharp or "hard" edge and tend to have a smaller crenated nucleus—sometimes described as "raisinoid"

essential for the diagnosis (Fig. 13.2). Some degree of nuclear abnormality extends through the full thickness of the squamous epithelium, but atypical nuclei are most prominent in the basal third, in proliferating basal and parabasal cells. Cytoplasmic maturation is present in the upper two-thirds of the epithelium although the nuclear/cytoplasmic ratio is increased. Mild nuclear hyperchromasia is usual, and sometimes irregularities are visible in the nuclear membrane. A well-defined nuclear halo with a sharp or "hard" edge may be present in squamous cells in the upper third of the epithelial thickness; if this feature is associated with the nuclear abnormalities described above, the abnormality is designated HPV effect, koilocytosis or koilocytotic atypia [90]. Usually there is acanthosis, papillomatosis, hyperkeratosis, or parakeratosis at the epithelial surface, and individual cell keratinization may be present [91]. Normal mitotic figures may be increased. Atypical mitoses are uncommon and if present support a diagnosis of LSIL/CIN1. LSIL may be identified in immature and atrophic squamous epithelium and is often seen in association with HPV infection, as too is multinucleation.

LSIL must be distinguished from HSIL/CIN2/CIN3 and benign mimics of LSIL, including a range of inflammatory processes and infections. Immunohistochemistry (see section "Histogenesis and Immunophenotype" below) may be helpful.

Criteria for the Diagnosis of HSIL (CIN2 and CIN3)

There is poor interobserver variability for the diagnosis of CIN2 [79] and in follow-up excision specimens, over a half of patients who had CIN2 biopsies are found to have CIN3 [92]. The intermediate state of CIN2 is felt to be a "mix of biological CIN1 and CIN3" [38].

In both HSIL (CIN2) (Fig. 13.3) and HSIL (CIN3) (Fig. 13.1), nuclear atypia extends through the full thickness of the epithelium and is more marked than in LSIL/CIN1. There is increased nuclear size and nuclear/cytoplasmic ratio and irregularity of the nuclear outline. Cytoplasmic maturation is present in the upper third of the epithelium in HSIL (CIN2) but usually absent or confined only to the superficial epithelial layers in HSIL (CIN3). Mitotic figures (including abnormal forms) are increased and found at all levels of the epithelium.

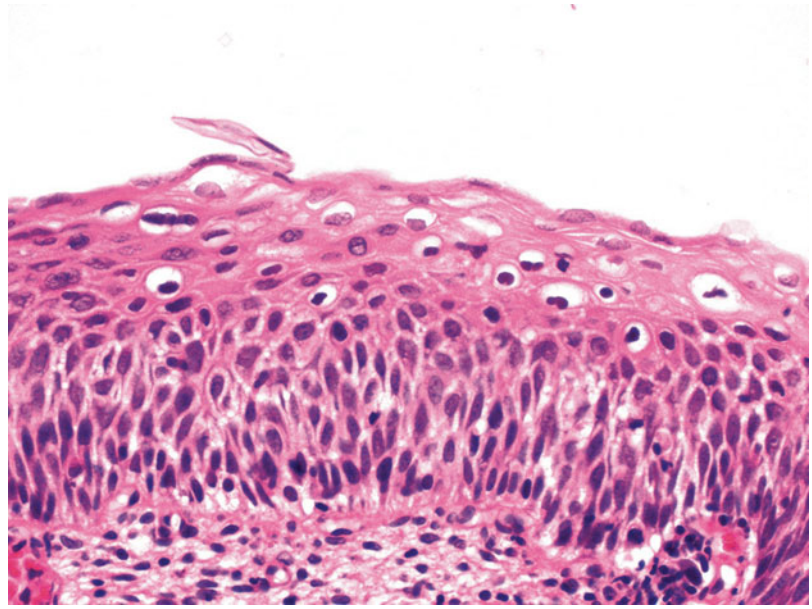

Fig. 13.3 HSIL/CIN2. Nuclear atypia extends through the full thickness of the epithelium but is most prominent in the lower half to two-thirds. There is some cytoplasmic maturation in the upper third of the epithelium, where koilocytes are also seen

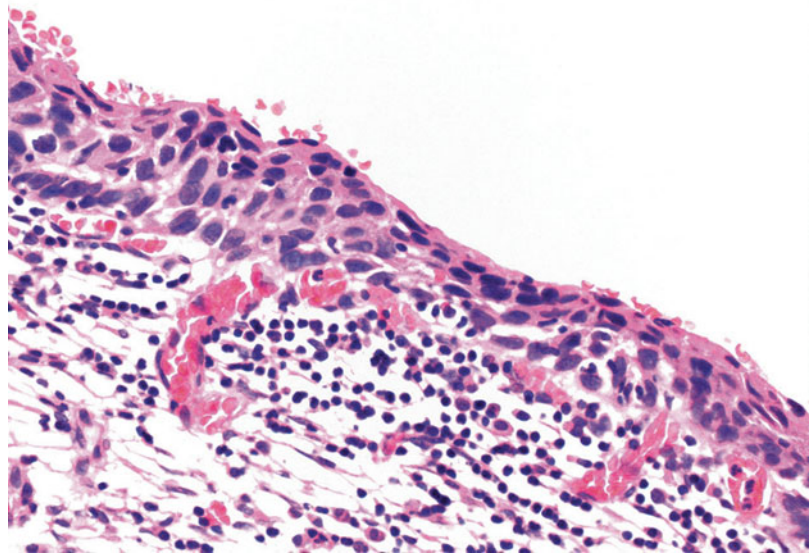

Fig. 13.4 Thin HSIL. This high-grade squamous intraepithelial lesion is <10 cells thick and shows full thickness nuclear atypia with an increase in nuclear size and nuclear/cytoplasmic ratio

Nucleolation is not usually a feature of high-grade dysplasia and if present should prompt the exclusion of an under-sampled squamous carcinoma, reparatory changes, or severe inflammatory atypia. p16 expression is not present in reparative or inflammatory atypia [38].

The 2014 WHO fascicle [50] describes three variants of HSIL: thin HSIL, keratinizing HSIL, and papillary squamous carcinoma in situ. Thin HSIL (Fig. 13.4) represents a high-grade squamous intraepithelial lesion that is <10 cells thick and may be differentiated from immature metaplasia and cervical atrophy by immunohistochemistry. Keratinizing HSIL (Fig. 13.5) is usually found on the ectocervix and resembles HPV-associated HSILs at cutaneous sites such as

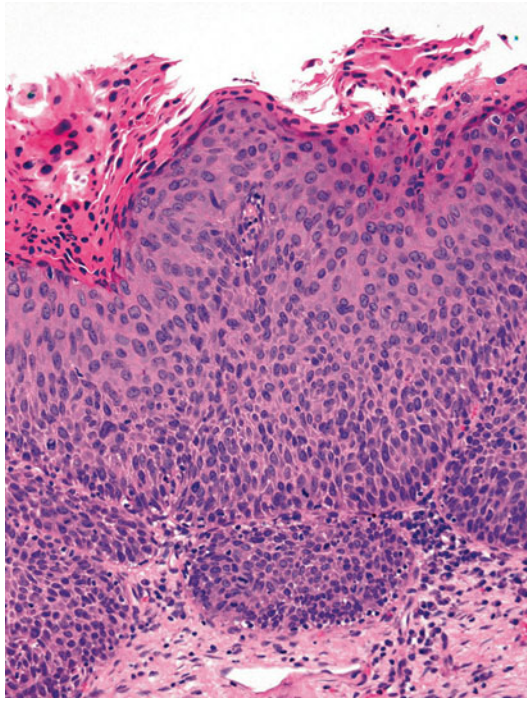

Fig. 13.5 Keratinizing HSIL. This variant of HSIL has a thick layer of keratinization on the surface and markedly pleomorphic and severely atypical cells throughout the squamous epithelium

the vulva. Keratinizing HSIL has a thick layer of keratinization on the surface and markedly pleomorphic and severely atypical, dyskeratotic cells within the squamous epithelium. Papillary squamous carcinoma in situ should be diagnosed only if the lesion has been completely excised and the absence of stromal invasion confirmed. This is a papillary lesion with a squamotransitional morphology: the papillae are fine and are overlain by severely dysplastic epithelium, which resembles neoplastic urothelium [93, 94].

Histogenesis and Immunophenotype

Histogenesis

Both LSILs and HSILs develop as a result of HPV infection. Over 80 % of LSILs result from infection by high-risk (HR) subtypes of HPV [95, 96]. The remainder—true LSILs—result from infection by low-risk (LR) HPV types. Over 90 % of HSILs are caused by infection with HR HPV [97]. Traditionally, squamous intraepithelial lesions (SILs) were thought to progress in a stepwise fashion from LSIL to HSIL. However, this is controversial. Although some studies have shown that HSIL may arise de novo without progression from LSIL [98] and that specific HPV subtypes are associated with individual SILs [48, 82], variations in tissue sampling and histological interpretation of biopsy material [99] have clouded the issue.

Human papillomaviruses (HPVs) typically infect basal cells in the stratified squamous epithelium of the cervical transformation zone. It is likely that HPV infection of the basal layer is acquired through mild abrasion or "microtrauma" of the squamous epithelium [100]. The basal cells are sometimes referred to as reserve cells or stem cells because they and the contiguous parabasal cells are the only cervical epithelial cells capable of cell division and are therefore responsible for maintenance and regeneration of the squamous epithelium. They have the potential to differentiate along one or more epithelial lines: squamous, glandular, or neuroendocrine. When basal cells are committed to squamous differentiation, they mature throughout the epithelial thickness in an orderly fashion. This maturation takes place at both morphological and molecular levels. If morphologically normal basal cells become infected with HPV, the infection is typically nonproductive with only maintenance levels of gene expression (see above). There is normally close regulation of productive HPV gene expression, and this takes place only in cells that have begun squamous maturation and lost their proliferative capacity [101–104]. Early virus proteins are expressed in the parabasal zone, and as squamous differentiation proceeds, there is viral DNA synthesis and induction of all viral genes, with production and assembly of virions in the squamous cells just beneath the epithelial surface. Morphologically, such lesions manifest as LSILs and are characterized by koilocytic atypia, nuclear enlargement, and hyperchromasia. These features are the result of viral proteins that affect

DNA synthesis and the structure of intermediate filaments in the host cell cytoplasm. However, the absence of koilocytes in cytology samples and the absence of koilocytic atypia in histological specimens should not be interpreted as an absence of HPV gene expression.

In HSILs which are usually associated with HR HPV infection, coordination is lost between cellular differentiation and viral early gene expression, and the viral oncogenes E6 and E7 are inappropriately expressed in a population of immature-looking squamous cells that retain the capacity to divide and initiate unregulated cell proliferation [48, 105]. With the continued proliferation of this population of cells, the normal epithelium is overrun by epithelial cells that show disorderly squamous maturation and have a basal cell-like morphology, i.e., the epithelium shows the morphological features of HSIL (CIN2 and 3).

Immunophenotype

Immunohistochemistry is helpful to confirm a diagnosis of CIN—the morphological changes of LSIL/CIN1 may be subtle in thin metaplastic epithelium, and distinguishing HSILs from benign mimics such as immature squamous metaplasia, transitional metaplasia, and atrophy may be problematic. Reactive/inflammatory changes, morphological changes associated with repair and regeneration, and tangential sectioning can also mimic both HSIL and LSIL. Finally, immunohistochemistry may be of value in identifying CIN at resection margins of loop biopsies where there is significant electrothermal/cautery artifact.

The immunohistochemical markers most commonly used to confirm a diagnosis of CIN are those that corroborate HPV infection (p16 and ProEx C) and confirm proliferation in the atypical cells (MIB-1) [106, 107].

The p16 gene, *CDKN2A*, (previously *P16INK4A*) [108] is a tumor suppressor gene that encodes a cyclin-dependent kinase inhibitor, a protein involved in cell cycle regulation. p16 acts with the retinoblastoma protein, another tumor suppressor, in a negative feedback loop to control cell proliferation. When HR HPV DNA integrates into the host cell genome, the viral oncoprotein E7 binds to the retinoblastoma protein and inactivates it, with resultant loss of negative feedback, and p16 overexpression (see above). p16 is therefore a surrogate marker of HR HPV infection. Significant p16 expression manifests as diffuse, strong, cytoplasmic, and nuclear immunopositivity ("block" immunopositivity) in squamous lesions associated with HR HPV infection, and the extent of labeling correlates with the grade of the histological abnormality [109]. In one study, over 99 % of cases with histologically confirmed HSIL (CIN 3) were p16-positive [110]. According to recommendations of the LAST project [38], the labeling should be present in the basal layer and extend upward to involve at least one-third of the epithelium (Fig. 13.6). The "one-third" condition has been suggested to add specificity but is arbitrary. Full-thickness labeling or extension into the upper third or half of the epithelium is not required to call a specimen positive.

Interpretation of p16 immunolabeling can be problematic as the intensity and distribution of the staining may be variable, particularly when associated with LR HPV infection and a range of lesions in the cervix that may mimic CIN. Focal or patchy nuclear staining is regarded as nonspecific and may be present in reactive conditions as well as LSIL and immature metaplasia (Fig. 13.7). p16 may help in the diagnosis of CIN2 but is not recommended if the differential diagnosis on H&E morphology is between LSIL (CIN1) and normal epithelium, because LSIL (CIN1) can be p16-negative; only 30 % of cases of CIN1 are p16-positive [110–113]. p16 immunohistochemistry is recommended when assessing biopsy specimens that appear morphologically low grade but associated with high-grade antecedent cytology, when there is a high risk of missed high-grade disease.

It is important that the immunohistochemistry is interpreted in an adequate biopsy, in which a precancerous lesion is morphologically under consideration. Tiny, disrupted epithelial fragments, detached single epithelial cells, and other material of a suboptimal nature may lead to mis-

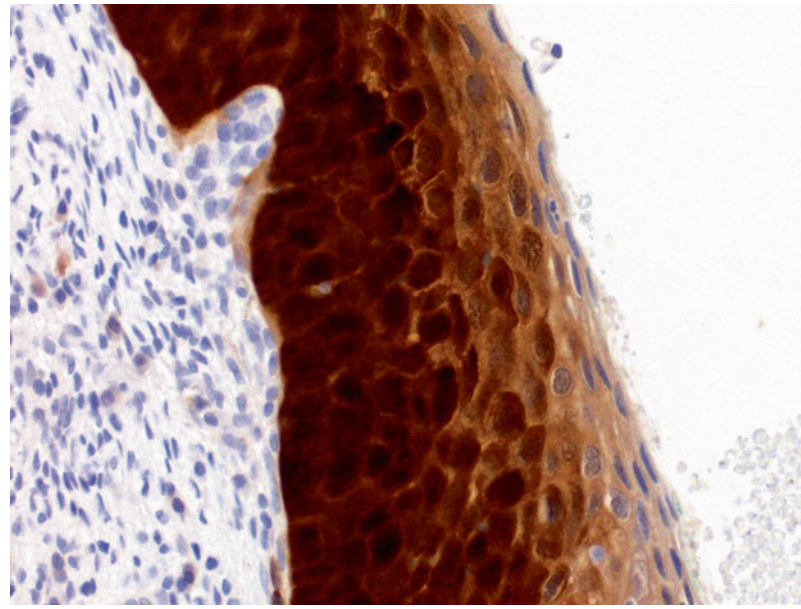

Fig. 13.6 Immunolabeling of p16 in HSIL. The labeling is both nuclear and cytoplasmic and extends from the basal layer upward through most of the thickness of the epithelium. According to the recommendations of the LAST project, this pattern of "block" staining should be present in the basal epithelium and involve at least one-third of the epithelial thickness

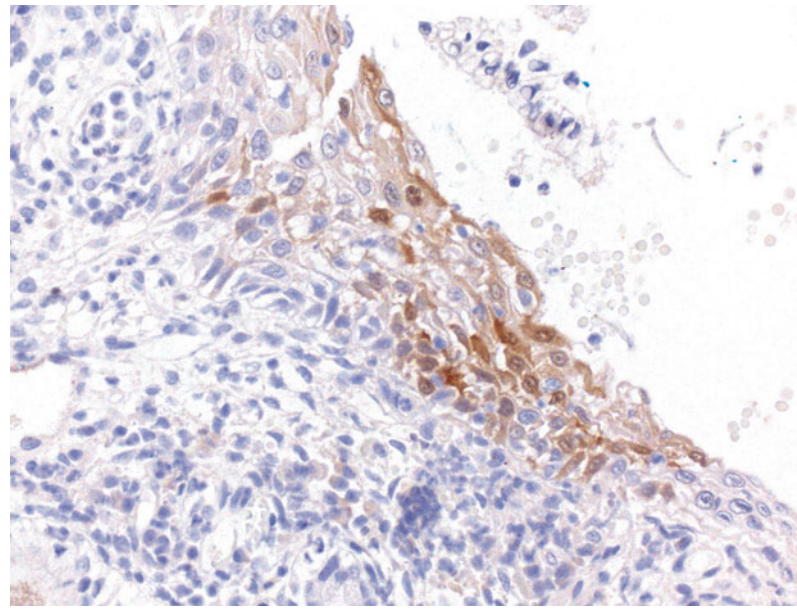

Fig. 13.7 Immunolabeling p16 in LSIL. This example illustrates focal or patchy nuclear and cytoplasmic p16 in LSIL, but the labeling pattern is regarded as nonspecific and may also be present in reactive conditions as well as immature metaplasia

interpretation of p16 immunohistochemistry. The LAST study concluded that p16 is the most reliable biomarker of the activation of E6/E7-driven cell proliferation in the context of HPV infection, and its use is therefore recommended in the assessment of lower anogenital tract SILs [38].

ProEx C, a cocktail of antibodies against topoisomerase II-alpha and minichromosome maintenance two proteins, is overexpressed in CIN and cervical carcinoma [114, 115]. Only nuclear positivity in the upper two-thirds of the epithelium is considered significant. Several studies have confirmed that ProEx C is comparable to p16 and MIB-1 in identifying HSIL in formalin-fixed tissue and in its differentiation from benign mimics [116–118]. However, a lit-

erature review carried out as part of the LAST project [38] found insufficient data to recommend ProEx C (or MIB-1) for use, alone or in combination, in the diagnosis of CIN. The LAST report acknowledged that some pathologists or institutions might choose to use these other markers as an adjunct, within a broader panel or in selected cases when p16 labeling is equivocal. Since both ProEx C and MIB-1 show very specific nuclear staining, the immunohistochemistry may be easier to interpret.

MIB-1 is a monoclonal antibody against Ki-67, a nuclear cell proliferation-associated antigen that is expressed in all active parts of the cell cycle (G1, S, G2/M) [119–121]. Expression of MIB-1 is usually confined to basal and parabasal cells. Detection of this antigen in the middle and upper thirds of the squamous epithelium is therefore significant. HSIL (CIN2 and CIN3) usually shows widespread nuclear MIB-1-positivity in all layers of the epithelium [122]. In LSIL (CIN1), staining is not widespread and only small clusters of immunopositive squamous cells may be present in the upper two-thirds of the epithelium [123]. Tangential sectioning and intraepithelial lymphocytes associated with inflammatory changes may give the impression that MIB-1-immunopositive cells are in the upper two-thirds of the squamous epithelium, and pathologists should be mindful of this diagnostic pitfall.

Patient Outcomes

LSIL

In histologically confirmed LSIL, regression occurs within 12 months on average [50]. The risk of progression correlates with HPV type (HPV 16 infection is associated with a high risk of progression), immunosuppression, smoking, and older age. The biopsy procedure itself affects the natural history of LSIL—regression occurs in up to one-third of cases, and this reduces the predictive value of biomarkers [92]. Even after colposcopy and a histological diagnosis of LSIL, there is 10 % chance that HSIL has been missed by the biopsy [124–126]. In some studies, the risk of progression was found to be increased when LSIL was associated with p16 expression [127, 128]. However, to date, no combination of biomarkers has been shown reliably to predict whether a LSIL lesion will regress, persist, or progress.

HSIL

Testing for HPV DNA 12 months after treatment is the best predictor of recurrent or residual disease [129]. The size of the lesion and the presence of margin involvement are also predictive of recurrence. There are no significant differences in outcome based on the modality of treatment (loop electrosurgical excision, conization, laser ablation, or cryotherapy). To date, no biomarkers have proven reliable in predicting which HSILs are most likely to progress.

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Clinical Management of Selected Precancerous Lesions in the Lower Genital Tract

14

Hironori Tashiro and Hidetaka Katabuchi

Clinical Management of the Cervical Precancerous Lesions

Precancerous Lesions of the Uterine Cervix in This Chapter

1. High-grade squamous intraepithelial lesion (HSIL). Synonyms: cervical intraepithelial neoplasia grade 2 (CIN2), grade 3 (CIN3)
2. Adenocarcinoma in situ (AIS). Synonyms: high-grade cervical glandular intraepithelial neoplasia (HG-CGIN)

Clinical Management

Cervical cytology screening programs have been associated with a reduction in cervical carcinoma incidence and mortality. As human papillomavirus (HPV) infections have been shown to increase the risk of cervical cancer, prevention programs incorporating HPV vaccination, in addition to screening and diagnostic programs, have become the standard of care in developed countries, including the United States and Canada. Methods for triaging cytological and histological cervical abnormalities detected by these programs have recently undergone rapid development. As only a small percentage of cervical precancerous lesions progress to invasive carcinoma, programs have recently focused on identifying patients at highest risk of disease progression. Despite the number of cervical cancer screening programs worldwide, the clinical management of precancerous lesions (HSIL and AIS) detected by these programs is largely consistent between developed countries.

Management of Patients with Cervical High-Grade Squamous Intraepithelial Lesions

Cervical carcinoma affects a higher proportion of younger women compared to most other types of carcinoma. Routine cytologic screening is the most frequently used method of determining the presence of cervical intraepithelial neoplasia (CIN). Screening aims to identify women with CIN in order to reduce the risk of developing invasive carcinoma [1]. Since 1968, the clinical management of CIN has involved the categorization of lesions according to a three-tier stratification system: CIN1, CIN2, and CIN3. However, in 2013,

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the Lower Anogenital Squamous Terminology (LAST) project, conceived and sponsored by the American Society for Colposcopy and Cervical Pathology (ASCCP) and the College of American Pathologists (CAP), advocated a two-tier system with CIN1 termed as low-grade squamous intraepithelial lesions (LSIL) and CIN2 and CIN3 together termed as high-grade squamous intraepithelial lesions (HSILs). The two-tier system is more biologically relevant and histologically reproducible than the previous three-tier system. Subsequently, the two-tier system has been adopted by the newly released 2014 WHO classification [2]. It is, henceforth, anticipated that the new two-tier system will be adopted for the clinical management of CIN.

Colposcopy and appropriately directed biopsy are frequently utilized in the management of patients with cervical HSIL due to their utility in ruling out invasive squamous cell carcinoma. Osler reported a meta-analysis of clinical follow-up studies of biopsy-confirmed CIN published between the 1950s and 1990s. When stratified into three grades of severity, the composite data indicated approximate likelihoods of regression, persistence, progression, and progression to invasion for CIN1 of 60 %, 30 %, 10 %, and 1 %, respectively. The corresponding approximations for CIN2 were 40 %, 40 %, 20 %, and 5 %, respectively. For CIN3, the likelihood of regression was 33 % and the likelihood of progressing to invasion was greater than 12 %. Thus, higher-grade lesions are more likely to persist and less likely to regress [3]. Considering the natural history of progression of squamous intraepithelial lesions (SIL) to carcinoma, HSIL (CIN2, 3) can be regarded as true precancerous lesions requiring clinical management and treatment, whereas LSIL (CIN1) do not benefit from immediate surgery and should be managed with observation alone [4, 5]. Clinical management of SIL depends on the patient's age, likelihood of reliable follow-up, and the histologic severity of the lesion. The identification of women at highest risk of cervical carcinoma depends predominantly on HSIL severity [5]. Regarding severity, clinicians often request that pathologists do everything possible to identify cases of HSIL that can be safely followed up to allow the possibility of HSIL regression, thereby sparing young women of childbearing age of complications potentially arising from cervical excision [6–9]. Excisional treatment of HSIL in young women can often be postponed in cases not associated with high-risk HPV types 16 and 18 on a case-by-case basis. Attempts have been made to improve HSIL evaluation through the use of biomarkers, such as p16INK4a immunohistochemistry. However, no biomarkers have yet proven clinically reliable for distinguishing cases of HSIL requiring treatment from cases that can be safely followed up. Currently, one important principle in the management of cervical HSIL is the avoidance of overtreatment. Techniques for definitive treatment of HSIL include excision with shallow conization and performance of the loop electrosurgical excision procedure (LEEP). Other ablative techniques include laser vaporization and cryotherapy [10]. Efforts to achieve effective management, limit complications, and preserve reproductive function have led to increased use of local treatment for HSIL. Cure rates have been shown to be similar for excisional and ablative techniques. Theoretically, excisional techniques have advantages over ablative methods as they allow comprehensive histological assessment of excised tissue and the entire transformation zone with accurate measurement of excision margins [11]. However, clinicians should inform young women wishing to conceive that excisional treatment may be associated with adverse pregnancy outcomes [6–9].

Management of Patients with Cervical Glandular Precancerous Lesions: Adenocarcinoma In Situ

A relative and absolute increase in the incidence of adenocarcinoma of the uterine cervix has occurred in the United States during the past four decades [12, 13]. In younger patients with invasive adenocarcinoma, a small increase in prevalence has been observed, mainly in patients aged 30 years or less [14]. An increased prevalence in younger women has been also found for adenocarcinoma in situ (AIS) [15, 16]. Adenocarcinoma represents approximately 15 %–20 % of all cervical carcinoma [17]. In 1991–1995, the overall

incidence of squamous carcinoma in situ of the cervix in white women in the United States was 41.4 per 100,000, whereas the incidence of AIS was only 1.25 per 100,000 [16, 18]. Thus, AIS represents a much smaller proportion of cervical precancerous lesions compared to squamous lesions. These findings may indicate a shorter interval for progression of AIS to invasive cancer or that many cases of AIS are missed. This fact represents a substantial issue in the clinical management of AIS. Moreover, there is increasing recognition of a spectrum of benign, premalignant, and malignant cervical glandular lesions exhibiting gastric differentiation. Lobular endocervical glandular hyperplasia (LEGH) lesions, including so-called atypical LEGH, may represent precursor lesions for non-HPV-related cervical adenocarcinomas exhibiting gastric differentiation, including minimal deviation adenocarcinoma and cervical gastric-type adenocarcinoma [19, 20]. Deep knife biopsy is necessary for the histological assessment of LEGH following imaging studies, such as echography or magnetic resonance imaging, as cervical smear, colposcopy, and punch biopsy are unable to detect LEGH lesions. Thus, the natural history of glandular precancerous lesions in not as well understood as that of squamous precancerous lesions, and therefore, their management remains controversial, with currently utilized treatments ranging from LEEP and conization to hysterectomy [21–26]. Conservative treatments, except for ablative treatments, should be considered in young women on a case-by-case basis in order to preserve the uterus [27–32].

Management of Patients with Specific Conditions

Studies evaluating the clinical management of cervical cancer during pregnancy are limited. The cervix is highly vascularized during pregnancy; thus, excisional treatments, such as LEEP or conization, should preferably be avoided during pregnancy. Moreover, clinicians are more reluctant to perform excisional procedures during pregnancy due to the risk of cervical inflammation leading to chorioamnionitis resulting in abortion or preterm labor. As treatment for cervical HSIL can be safely postponed until the postpartum period [33], the ability to distinguish HSIL from invasive carcinoma has been a major step forward in the management of these patients [34]. Biopsies of the most colposcopically abnormal areas are recommended to minimize bleeding. When distinguishing precancerous lesions from invasive carcinoma proves challenging with colposcopy and usual biopsy in pregnant women, diagnostic excisional technique should be considered to rule out invasive carcinoma until the second trimester [35].

In postmenopausal women, accurate diagnosis and adequate outcomes have been reported with the use of short-term local estrogen replacement therapy (ERT) and a short period of cytological and colposcopic follow-up. A single course of local ERT can help distinguish true precancerous changes: ERT often results in cervical ectropion, allowing the entire squamocolumnar junction to be visualized [36].

Clinical Treatments (Fig. 14.1)

Conization

In the United States, conization of the uterine cervix is primarily performed as a diagnostic tool and secondarily as a therapeutic option in patients that are young and desire future fertility [11]. However, conization is used for definitive treatment in other countries. Cold-knife conization has been the standard treatment for precancerous lesions of the cervix for many years. Large conization is an effective option for lesion removal but carries an increased risk of complications.

In cervical HSIL, shallow conization capturing all abnormal tissue is preferred to large conization in patients desiring future fertility (Figs. 14.2a–c and 14.3). Colposcopy allows accurate assessment of lesion size prior to conization. In shallow conization, an incision is made in the mucous membrane of the ectocervix at a location and depth that is certain to include all abnormal lesions. Morphometric data indicate the vast majority of HSIL are less than 5 mm

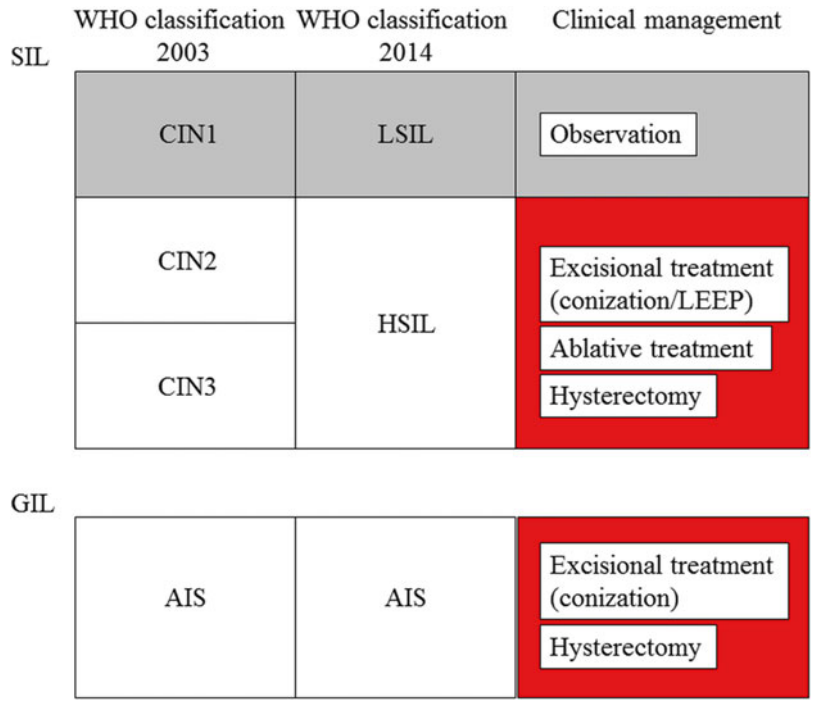

Fig. 14.1 Overview of clinical management for precancerous cervical lesions. *WHO* World Health Organization, *SIL* squamous intraepithelial lesion, *GIL* glandular intraepithelial lesion, *CIN1* cervical intraepithelial neoplasia grade 1, *CIN2* cervical intraepithelial neoplasia grade 2, *CIN3* cervical intraepithelial neoplasia grade 3, *LSIL* low-grade squamous intraepithelial lesion, *HSIL* high-grade squamous intraepithelial lesion, *AIS* adenocarcinoma in situ

deep, suggesting treatments extending to a depth of around 1 cm are adequate in women with satisfactory colposcopy [37]. Although therapeutic conization should preferably be avoided in pregnant patients with HSIL, diagnostic shallow conization of the index lesion is recommended in pregnant women when microinvasive carcinoma is suspected from cervical smear, colposcopy, and/or biopsy findings [35] (Fig. 14.2d–f).

The demonstration of residual disease in subsequent hysterectomy specimens has been a major concern with the use of regular conization for cervical AIS. Residual AIS has been reported in up to half of patients with uninvolved margins of conization [38] and in up to 80 % of patients with involved margins [39]. Therefore, cylindrical and deep conization running parallel to the endocervical canal and including the transformation zone and deep glands is recommended as an alternative excisional option in the vast majority of AIS cases [15, 31] (Fig. 14.2g–i). Since cylindrical and deep conization confers a much higher risk of postconization cervical stenosis, techniques are required to prevent stenosis. The use of retained nylon threads tied to an intrauterine device reportedly prevents cervical stenosis [40]. Pathologic assessment of the length and depth of AIS involvement should be performed in order to ensure adequate removal of AIS lesions [41], and postconization endocervical curettage (ECC) which is performed above the conization bed after the excision would provide valuable prognostic information regarding the risk of residual AIS [42]. In addition, strong consideration should be given to high-risk HPV testing in conjunction with cervical cytology or ECC in light of recent data supporting its value in prediction of recurrent AIS [43–45].

Regarding reduction of operative hemorrhage during cornization, the most frequently used techniques are lateral suturing of the descending branches of the cervical arteries and direct injection of a vasopressor into the cervical stroma. The Sturmdorf method involving suturing of resultant raw surface flaps of cervical epithelium, is unnecessary in the majority of cases. Hemorrhage may occur as a result of incomplete division of the vessels, or it may be associated with secondary infection of the defect site. Treatment is usually conservative,

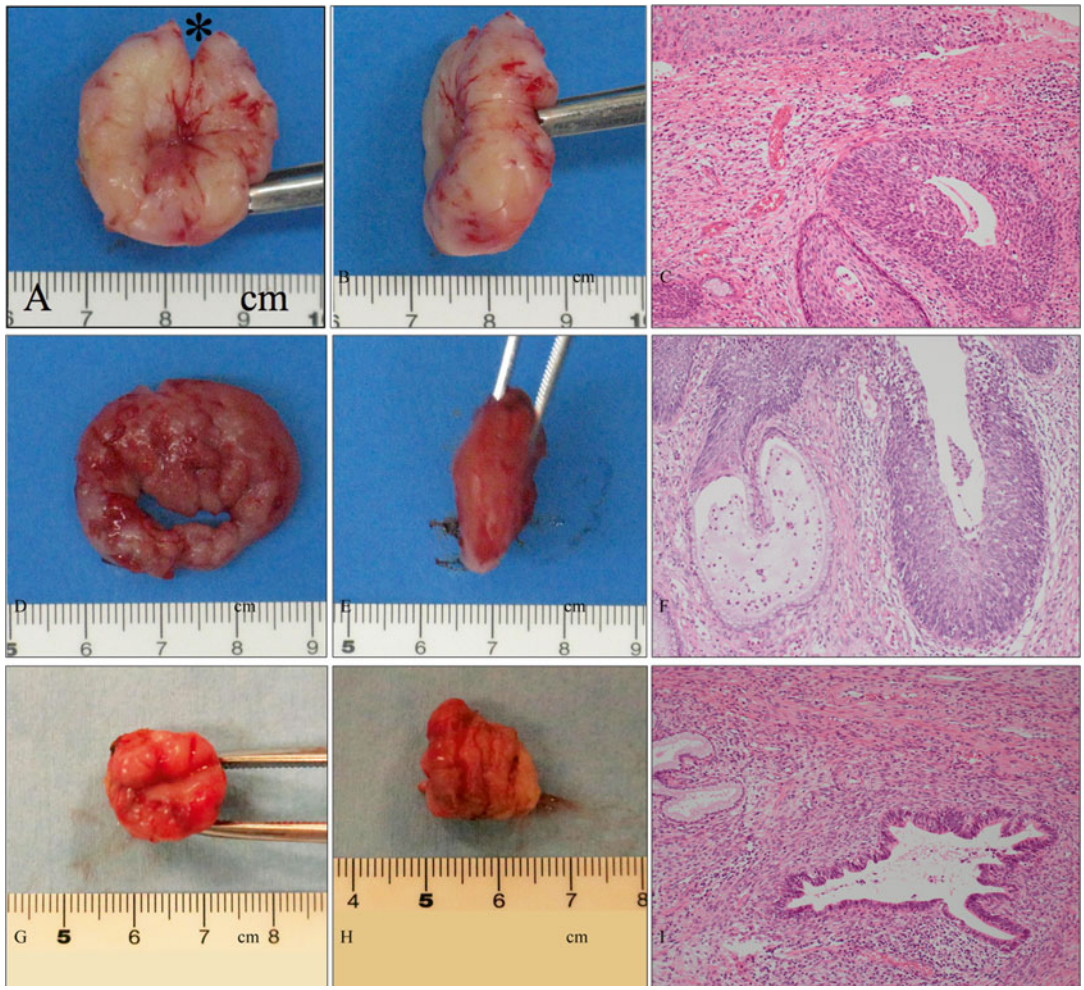

Fig. 14.2 Various shapes of conization. (**A**–**C**) Gross photography of a cervical specimen (**A**, front view; **B**, side view) obtained from therapeutic shallow conization involving a small incision (*) orientated at 12 o'clock and microphotography of the specimen (**C**) demonstrating HSIL. The patient was a 28-year-old woman, gravidity 1, parity 1, who wished for another baby. The patient became pregnant 3 months after this conization and delivered a healthy baby at 38 weeks of pregnancy. (**D**–**F**) Gross photography of a cervical specimen (**D,** front view; **E**, side view) obtained from diagnostic shallow conization during pregnancy and microphotography of the specimen (**F**) demonstrating HSIL. The patient was a 28 year-old woman, gravida 3, parity 2, at 21 weeks of pregnancy. Preoperatively, cervical smear and punch biopsies indicated HSIL with a suggestion of stromal invasion. Finally, HSIL without invasion (**F**) was confirmed by the diagnostic shallow conization, and the patient delivered a healthy baby at 39 weeks of pregnancy. (**G**–**I**) Gross photography of a cervical specimen (**G**, front view; **H**, side view) obtained from therapeutic cylindrical conization for AIS and microphotography of the specimen (**I**) demonstrated AIS at a localized area of the endocervix. The patient was a 34-year-old woman, gravidity 2, parity 2, who wished to preserve her fertility. Histological analysis and cervical cytological examination following conization indicated no residual cervical AIS (**C**, **F**, **I**: hematoxylin-eosin staining, magnification ×10 for **C**, **F**, **I**)

although occasionally surgical management may be required. Significant cervical stenosis and infertility are rare complications dependent on the amount of endocervix removed. Conization is associated with adverse obstetric outcomes, including preterm delivery and perinatal mortality, with excision depth posited as a risk factor [37].

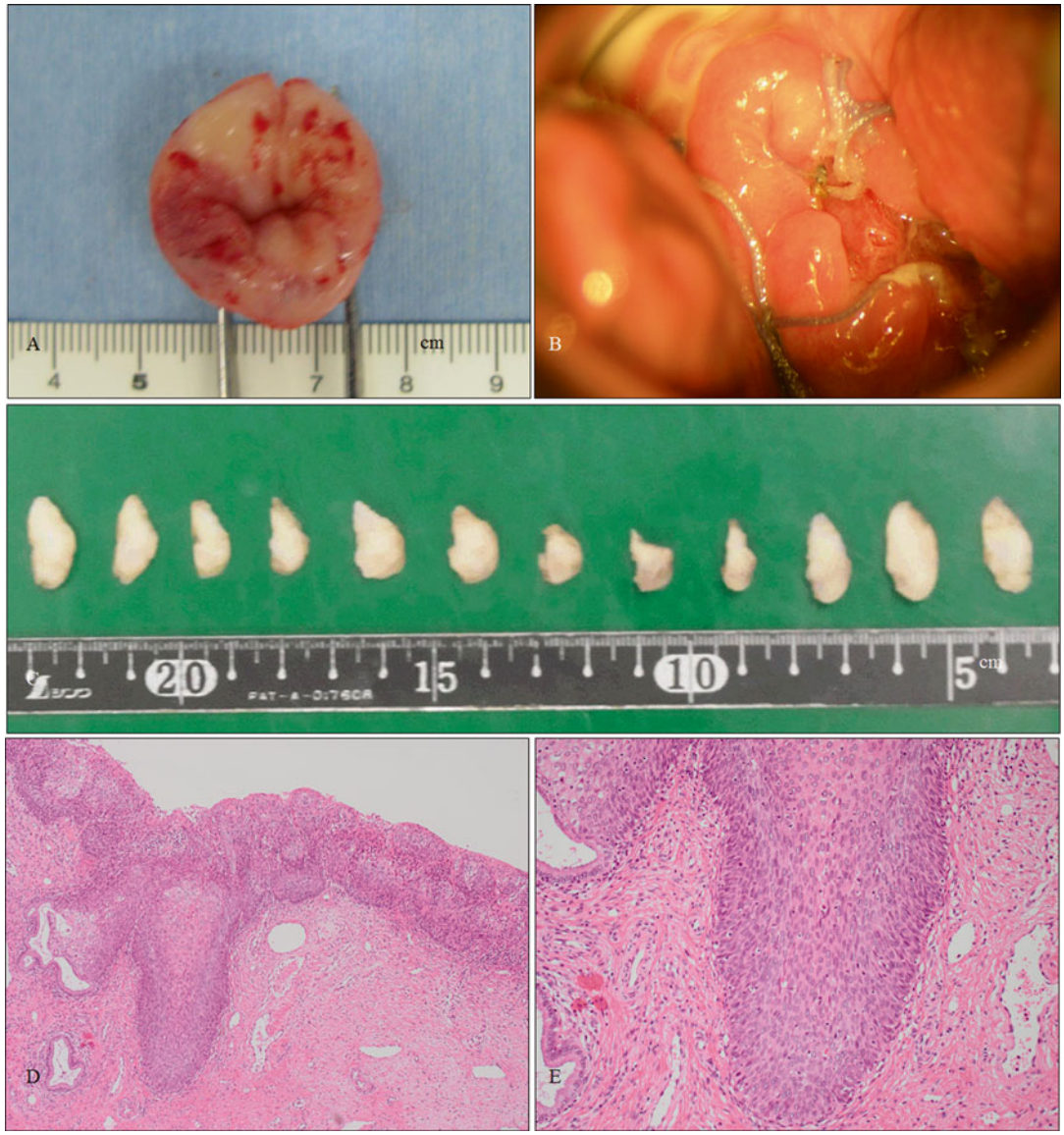

Fig. 14.3 Shallow conization in a case of cervical HSIL. (**A**, **B**) Gross photography of a specimen obtained from conization (**A**) and the cervix of the patient 1 month after conization (**B**). The patient was a 25-year-old woman, nulligravida, who had a 2-year history of cervical and vulvar HSIL referred to our hospital. The vulvar lesion is shown in Fig. 14.8. (**C**) Gross photography of the 12 divided specimens following formalin fixation for pathological analysis. (**D**, **E**) Microphotographs of cervical specimens demonstrating HSIL (**D**, **E**: hematoxylin and eosin staining, magnification ×4 for **D** and ×10 for **E**)

Carbon dioxide (CO_2) lasers, "light amplification by stimulated emission of radiation," may be utilized as an alternative to cold-knife conization to reduce the complication risk. Several studies have shown that rates of primary and secondary hemorrhage, and residual disease, are comparable between laser and cold-knife conizations and that compared with cold-knife conization, laser conization is associated with a statistically significant decrease in the risk of cervical stenosis and inadequate colposcopy at follow-up [1]. The major issue with laser conization is the difficulty in evaluating excised lesions and margins due to laser-induced coagulation [46]. Recently, coniza-

tion using an ultrasonic scalpel has been shown to be a reliable method avoiding thermal artifacts associated with laser conization [47, 48].

Loop Electrosurgical Excision Procedure

LEEP is currently one of the most frequently used techniques for the effective eradication of cervical HSIL [49, 50]. An appropriately sized loop is chosen to encompass the entire lesion for removal in one block. The depth of the excised tissue varies; however, a depth of 6–7 mm is conventionally used. This technique provides adequate tissue for histopathological assessment. Large studies have demonstrated early invasive lesions not recognized by colposcopy may be identified with LEEP [51]. Thermal artifact is the most substantial issue with LEEP in general practice with approximately 10 % of specimens reported as unreadable and 30–50 % having significant coagulation artifact [46, 51]. The main side effect of LEEP is secondary hemorrhage, similar to conization. LEEP may cause cervical stenosis, occurring in approximately 6 % of cases. Previous loop excision and the volume of excised specimens have been shown to be independent predictors of stenosis [52]. Duggan et al. observed no difference in recurrence rates between LEEP and conization [53]. A prospective study found lower rates of preterm premature membrane rupture, preterm delivery, and low birth weight (<2500 g) with LEEP than with cold-knife conization and no difference in mean birth weight, cesarean delivery, labor induction, or neonatal intensive care unit admission [54].

Ablative Methods

Ablative techniques, including laser vaporization and cryotherapy, are second-choice treatments for cervical HSIL. CO_2 laser vaporization is widely accepted as one of the most effective ablative treatments for cervical HSIL, and there is no evidence of differing outcomes between laser vaporization and excisional LEEP [55, 56] (Fig. 14.4). Cryosurgery, in use

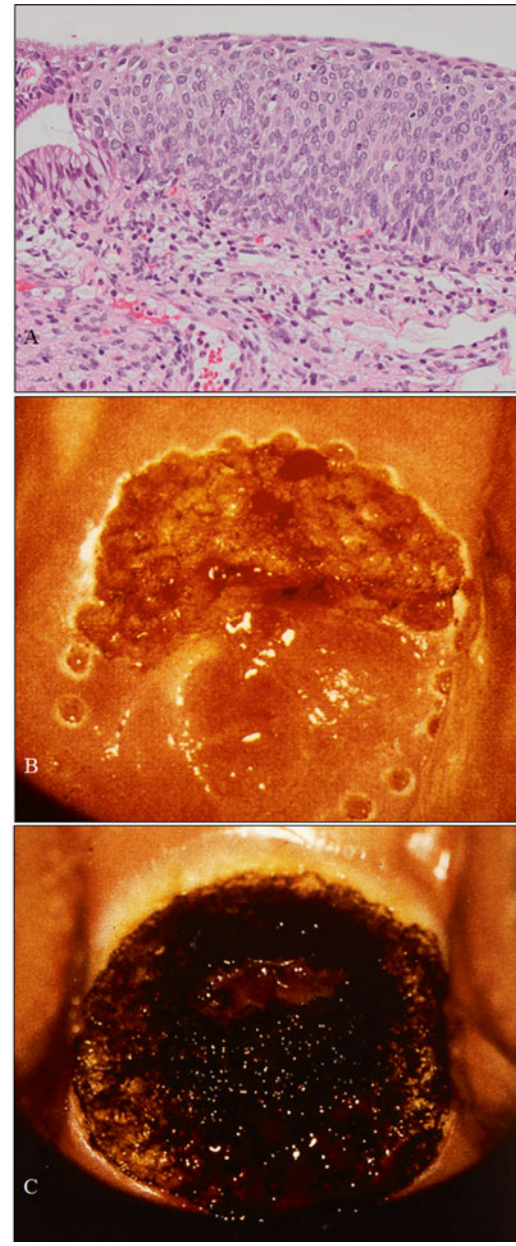

Fig. 14.4 A case with cervical HSIL treated by CO_2 laser evaporation. (**A**) Microphotography of a biopsy sample demonstrating HSIL consistent with cytological and colposcopical findings. The patient was a 28-year-old woman, nulligravida, who hoped for a baby in the near future. (**B**) Margin of a planned area for vaporization marked using a laser beam. (**C**) Vaporization of the entire transformation zone including the HSIL using a CO_2 laser beam (**A**: hematoxylin-eosin staining, magnification ×10)

for the past four decades, is relatively simple and can be performed effectively in developing countries [57]. However, it is associated with a higher rate of subsequent morbidity, including invasive carcinoma, than laser vaporization and excisional methods [58, 59]. Ablative techniques preclude histological assessment as they destroy the epithelium of the transformation zone (Fig. 14.4). Prior to the use of ablative therapies, histological diagnosis by colposcopically directed biopsy should be undertaken in order to exclude invasive carcinoma [60]. The entire transformation zone and the lesion should be fully visible with colposcopy, and there should be concordance among all cytological, colposcopic, and histological findings. Ablative methods are appropriate in women with lower severity ectocervical HSIL and contraindicated by HSIL extending into the endocervical canal, AIS, and clinical suspicion of invasive carcinoma. Despite these reservations, laser vaporization provides control over the depth of destruction and hemostasis and improves healing with minimal damage to adjacent tissues. Laser vaporization also has utility in treating multiple HSILs in the lower genital tract, including the cervix, vagina, and vulva [11].

Hysterectomy

Hysterectomy is indicated for cervical HSIL, particularly in postmenopausal patients or patients accepting of permanent sterilization. However, HSIL is not considered an appropriate sole indictor for hysterectomy as multiple alternative therapies are currently available. Conversely, hysterectomy has been proposed as the gold-standard treatment of AIS [39, 41]. Currently, there is a lack of consensus regarding the safety of conization alone for the treatment of AIS. In particular, hysterectomy should be considered when the residual AIS is identified following conization.

In patients requiring hysterectomy for definitive treatment of HSIL or AIS, vaginal hysterectomy or minimally invasive techniques such as a laparoscopic hysterectomy are appropriate in developed countries.

Clinical Management of the Vaginal Precancerous Lesion

Precancerous Lesion of the Vagina in This Chapter

1. High-grade squamous intraepithelial lesion (HSIL). Synonyms: vaginal intraepithelial neoplasia grade 2 (VaIN2), grade 3 (VaIN3)

Clinical Management

Management of Patients with High-Grade Squamous Intraepithelial Lesions

Diagnoses of primary vaginal carcinoma must satisfy the International Federation of Gynecology and Obstetrics (FIGO) criteria, including sparing of the uterine cervix. According to these criteria, vaginal carcinoma is rare and affects older women aged greater than 60 years. The posterior wall of the upper third of the vagina is the most frequently involved site [61]. Coexistence of vaginal intraepithelial neoplasia (VAIN) and vaginal carcinoma is rare, comprising 0.4 % of all intraepithelial neoplasia affecting the lower female genital tract. VAIN has been shown to be associated with high-risk human papillomavirus (HPV) infection, with cervical intraepithelial neoplasia (CIN) seen in up to 43 % of women [62–65]. VAIN reportedly occurs in the presence of multiple HPV-associated lesions affecting the lower genital anogenital tract. The natural history of VAIN is thought to be largely similar to that of CIN with high-grade VAIN considered a precancerous lesion [62, 66]. Although a three-tier system stratifying vaginal intraepithelial neoplasia into VAIN1, VAIN2, and VAIN3 categories has been previously widely used in clinical management, a two-tier system categorizing vaginal intraepithelial neoplasia as either LSIL or HSIL has recently been proposed and adopted in the newly released 2014 WHO classification [67]. It is, henceforth, anticipated that the new two-tier system will be adopted in the clinical management of VAIN.

Vaginal SILs are commonly asymptomatic. Cytologic screening is the conventional method of identifying the presence of vaginal SIL. The vagina should be carefully inspected by colposcopy for obvious abnormalities, with particular attention paid to the upper vagina. Vaginal HSIL commonly involves the upper third of the vagina or the vaginal vault following hysterectomy and is frequently multifocal involving other regions of the lower genital tract, termed "lower genital tract neoplastic syndrome" (Fig. 14.5). Colposcopic examination and directed biopsy are the mainstays of accurate vaginal HSIL diagnosis. In typical colposcopic findings, vaginal HSILs are ovoid and slightly raised, exhibiting a thickened acetowhite epithelium and a raised external border. Lesions with a papillary surface or abnormal vascular patterns, such as punctuation or mosaic, should be examined by multiple biopsies to rule out invasive carcinoma [61] (Fig. 14.5). Colposcopy is often a poorer predictor of histological findings in vaginal SIL than in cervical SIL. Even in cases where colposcopy reveals mild and atrophic changes, histologic findings occasionally demonstrate more severe changes (Fig. 14.6).

Evidence regarding the optimal clinical management of HSIL is lacking. Empirical practices include surgical excision or ablation of suspicious areas [68, 69]. Conservative surveillance in selected populations has been reported previously; however, the efficacy and safety of these approaches is not well known [65]. Renavelu et al. suggested the following limitations frequently

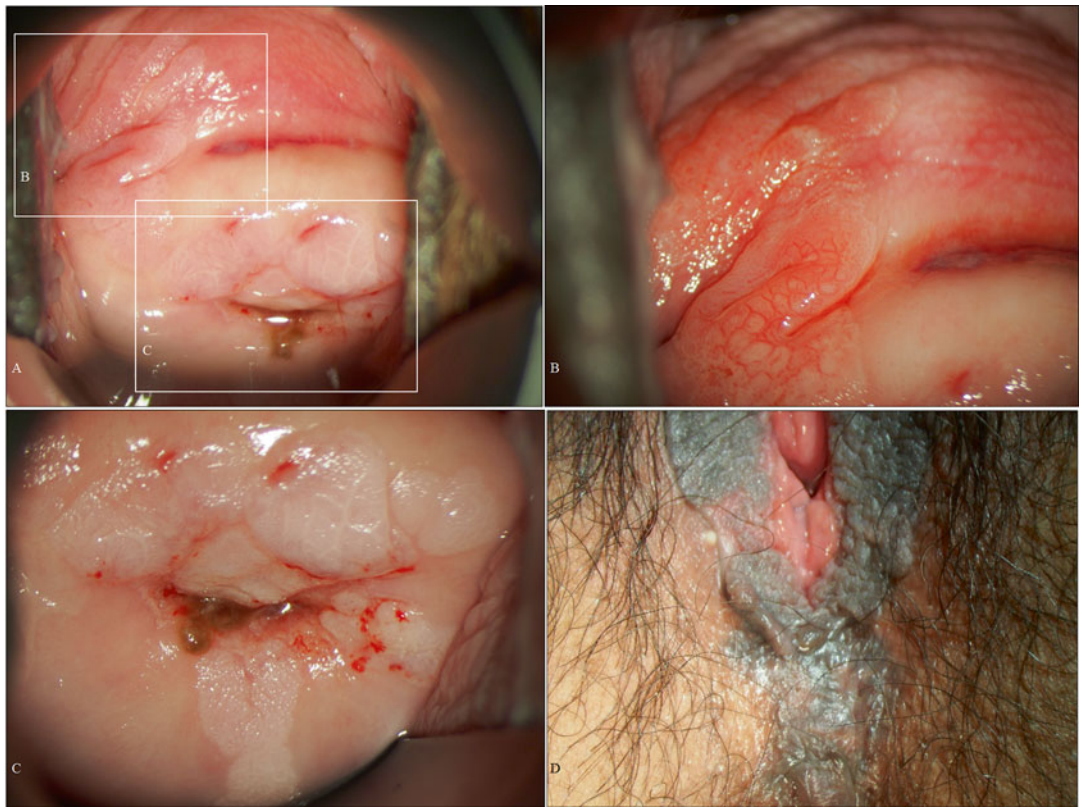

Fig. 14.5 A case of vaginal HSIL accompanied by other multifocal lesions of the lower genital tract. (**A**–**D**) Colposcopic and gross findings of lower genital neoplastic syndrome. The patient was a 43-year-old woman, nulligravida, who had been receiving immunosuppressive therapy for myasthenia gravis. Multiple lesions involving the upper third of the vagina (**A**, **B**), other uterine cervical sites (**C**), the vulva (**D**), the perineum, and the anus. (**E**–**J**) Microphotography of the biopsies demonstrating cervical HSIL (**E**, **F**), vaginal HSIL (**G**, **H**), and vulvar HSIL (**I**, **J**) (**E**–**J**: hematoxylin-eosin staining, magnification ×10 for **E**, **G**, **I** and ×20 for **F**, **H**, **J**)

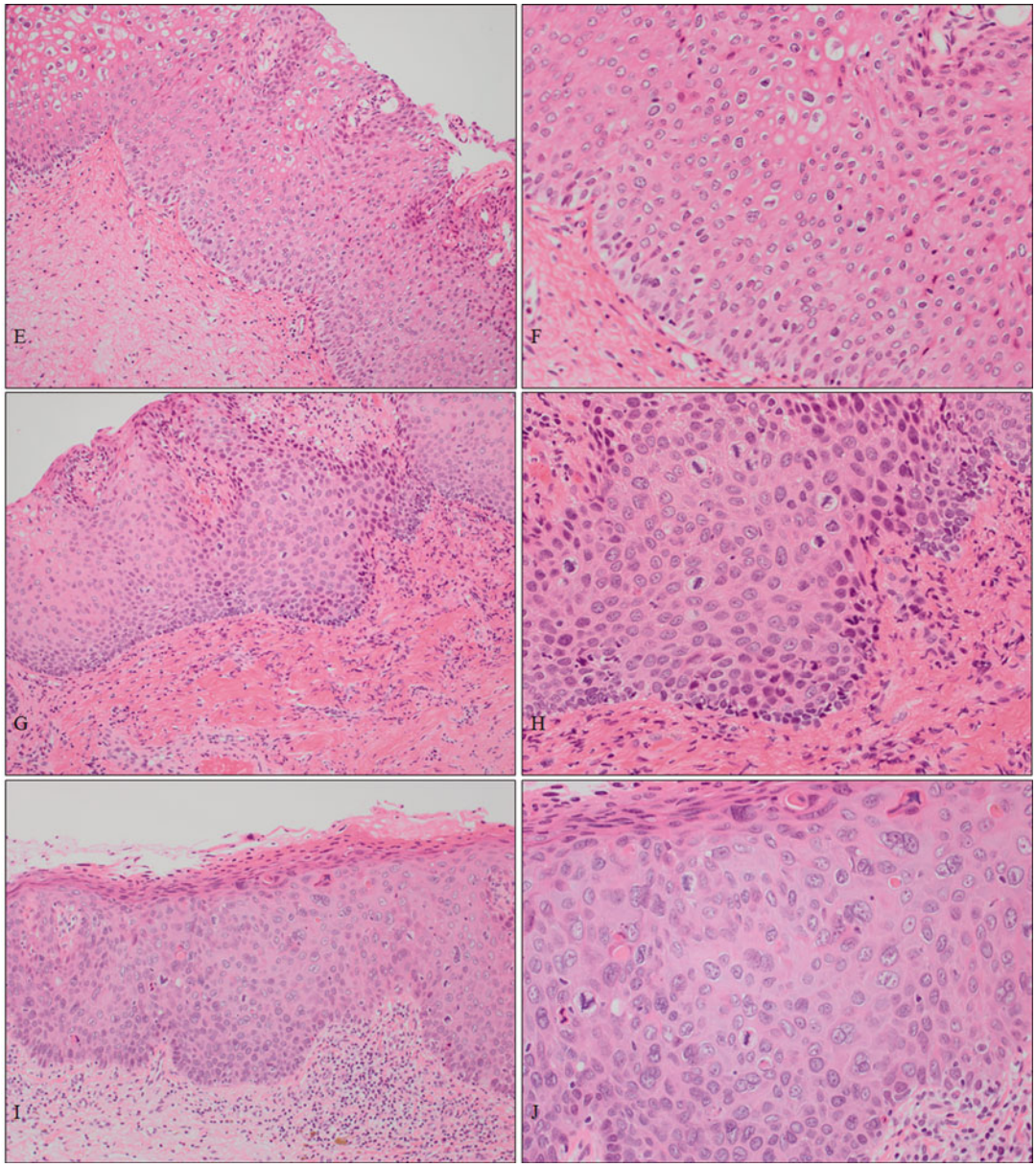

Fig. 14.5 (continued)

apply to HSIL studies: (a) clear definitions of "remission" and "recurrence" are lacking; (b) the majority of case series are smaller; (c) those of comparable size date back 10 years; (d) previous papers report a mixed series of LSIL and HSIL; and of particular note, (e) the role of abnormal cytology as a preclinical indicator of disease recurrence has not previously been established among women posttreatment [65].

Clinical Treatments (Fig. 14.7)

There is no general consensus regarding the optimal treatment for vaginal HSIL. However, HSIL can be successfully treated by either excision or laser vaporization with reported success rates ranging from 69 % to 79 % for both treatments [66, 70, 71]. The major advantage of laser vaporization therapy is the ability to precisely control

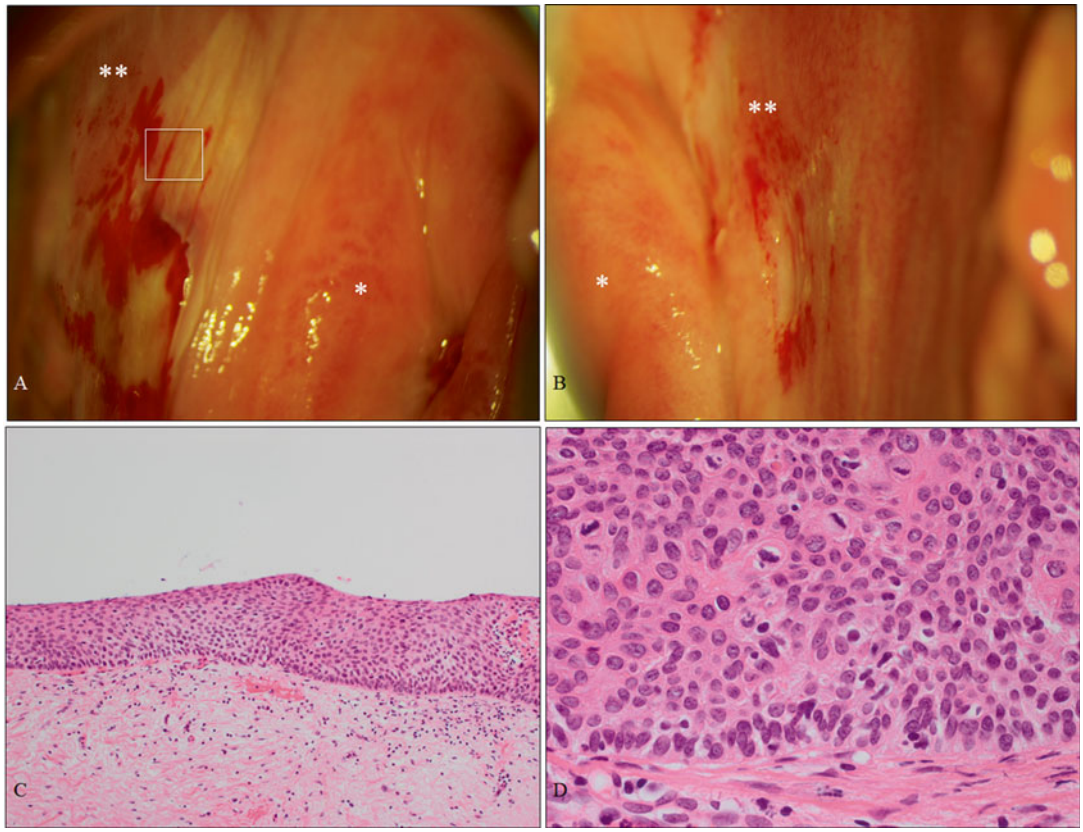

Fig. 14.6 A case of vaginal HSIL alone affecting the vaginal fornix. (**A**, **B**) Colposcopic findings at the right fornix (**A**) and left fornix (**B**) revealing mild acetowhite lesions. The patient was a 61-year-old woman without cervical or vulvar lesions and no previous history of HPV-related disease. (**C**, **D**) Microphotography demonstrating HSIL (**C**, **D**: hematoxylin-eosin staining, magnification ×4 for **C** and ×40 for **D**) . * uterine cervix; ** vaginal fornix

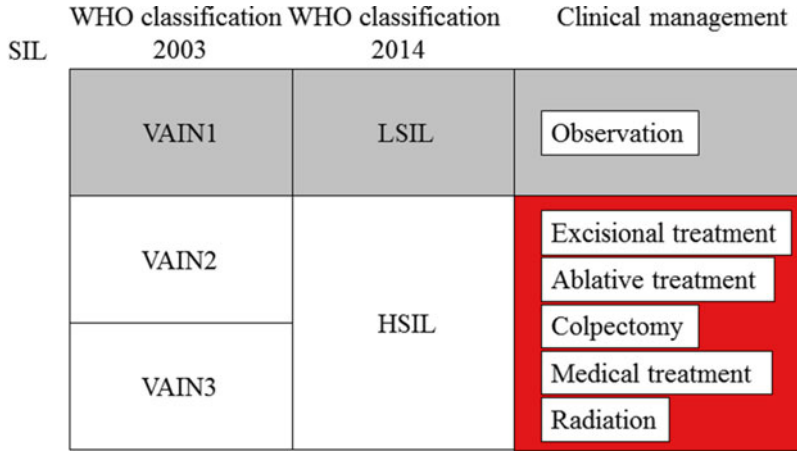

Fig. 14.7 Overview of clinical management for vaginal precancerous lesions. *WHO* World Health Organization, *SIL* squamous intraepithelial lesion, *VAIN1* vaginal intraepithelial neoplasia grade 1, *VAIN2* vaginal intraepithelial neoplasia grade 2, *VAIN3* vaginal intraepithelial neoplasia grade 3, *LSIL* low-grade squamous intraepithelial lesion, *HSIL* high-grade squamous intraepithelial lesion

the depth and width of destruction through direct vision provided by colposcopy [72]. Although cancer progression rates for vaginal HSIL appears to be less than those of cervical HSIL, studies of patients with long-term follow-up after excision or laser vaporization reported subsequent vaginal carcinoma development in approximately 5 % of HSIL patients [61, 66, 71, 73].

Excisional Methods

Local excision is the main therapeutic approach for vaginal HSIL. Ideally, local excision of a small and isolated area is performed under local anesthesia. Excision of large or multiple areas can be performed under general anesthesia. When lesions involve the upper area or vaginal vault following hysterectomy, upper colpectomy may be appropriate. Alternatively, total colpectomy followed by vaginal restoration with a split-thickness skin graft may be considered. However, major surgery carries a risk of bladder and/or bowel fistula formation or shortening and narrowing of the vagina. Therefore, aggressive surgical procedures are considered inappropriate for the treatment of HSIL.

Ablative Methods

CO_2 laser vaporization is an efficient treatment of histologically proven lesions with the absence of invasive carcinoma confirmed cytologically and colposcopically that may performed under local or general anesthesia [71]. Prior to vaporization, the lesion is injected with mixture of saline and anesthetic or saline alone that acts as a necessary protective buffer that prevents penetration to deeper tissues. Vaginal HSIL can be effectively treated by CO_2 laser vaporization to a depth of 1.5 mm, sufficient for the destruction of epithelium containing vaginal SIL without damaging surrounding structures [71, 72]. Cavitational ultrasonic surgical aspiration (CUSA) may be effective and safe for the treatment of VAIN. CUSA allows selective removal of diseased tissue with minimal damage to surrounding healthy tissue [74, 75].

Other Treatments

Topical application of 5-fluorouracil (5-FU) cream has been advocated by a varying proportion of investigators over the last three decades and was most frequently employed in the 1980s [61]. Vaginal burning with ulceration and vaginal bleeding, occasionally requiring surgery, are common complications of topical 5-FU administration. Combination therapy consisting of laser vaporization and 5-FU treatment may be preferred for the treatment of multifocal lesions, particularly in post-hysterectomy cases with deep vaginal angles [76]. Different treatment schedules and dose levels have been investigated to identify regimens that maintain efficacy while decreasing adverse effects. Some investigators have advocated surface irradiation as brachytherapy using an intravaginal applicator [77–79]. However, brachytherapy is associated with risk of recurrence and marked vaginal stenosis, increasing the difficulty of subsequent therapies [61]. A recent cohort study reported high rates of regression and cure of vaginal HSIL in patients treated with intravaginal estrogen alone or in combination with other treatment modalities. This treatment may represent a viable alternative therapy [80].

Clinical Management of the Vulvar Precancerous Lesions

Precancerous Lesions of the Vulva in This Chapter

1. High-grade squamous intraepithelial lesion (HSIL). Synonyms: vulvar intraepithelial neoplasia grade 2 (VIN2) and grade 3 (VIN3), usual-type vulvar intraepithelial neoplasia (uVIN)
2. Differentiated-type vulvar intraepithelial neoplasia (dVIN). Synonyms: carcinoma in situ of simplex type

Clinical Management

Management of Patients with Vulvar High-Grade Squamous Intraepithelial Lesion

The incidence of vulvar intraepithelial neoplasia (VIN) is increasing in women under 50 years of age [81]. The rising prevalence of human papillomavirus infection (HPV) has led to a continuously

increasing incidence of VIN and vulvar carcinoma [82–84]. In 1986, the International Society for the Study of Vulvar Disease (ISSVD) introduced the term, VIN, to designate precursors of vulvar squamous cell carcinoma. Although VIN was initially considered a single category, strong evidence has subsequently accumulated over the last two decades indicating the existence of at least two distinct clinicopathologic subtypes, one associated with high-risk HPV infection and a second independent of HPV infection. In 2004, the ISSVD proposed a classification system reflecting these two subtypes: HPV-associated usual VIN (uVIN) and HPV-independent differentiated VIN (dVIN). Following this proposal, dVIN is considered a clinical distinct entity from uVIN. The natural history of uVIN is thought to be similar to that of cervical intraepithelial neoplasia (CIN) with high-grade uVIN considered a precancerous lesion [85]. The three-tier classification system has previously been used in the clinical management of VIN, but there has been renewed interest in the use of the two-tiered squamous epithelial lesion stratification system describing low-grade SIL (LSIL) and high-grade SIL (HSIL), nomenclature that more accurately reflects the similar natural history of uVIN to other lower anogenital tract HPV-associated intraepithelial lesions. The two-tiered squamous epithelial lesion stratification system for uVIN and use of the term dVIN were recently adopted in the newly released 2014 WHO classifications [86].

Vulvar HSIL has a diverse clinical presentation. Nonspecific symptoms, including pruritus, irritation, and pain, are observed in approximately 60 % of cases. In younger patients, symptoms are often preceded or accompanied by condylomas. Since patients commonly present asymptotically or with nonspecific symptoms, accurate vulvar inspection during routine gynecologic examination is important. Vulvar lesions may be red, white, or pigmented in color and either flat or raised and may coexist with erosions or ulcers [85, 87, 88]. These findings should prompt further vulvar examination using a magnification instrument such as a colposcope. The use of acetic acid is not recommended as it is nonspecific for vulvar HSIL [89]. Biopsy of the most suspicious part of the lesion should be performed under local anesthesia to confirm diagnosis. The diagnosis of vulvar HSIL is made from the clinical appearance and subsequent biopsy findings. Unifocal lesions are most commonly observed around the vaginal introitus. Perianal skin is involved in 10–15 % of the patients with vulvar HSIL [89].

The considerable temporal difference of 20–30 years between the peak incidence of vulvar HSIL and invasive vulvar carcinoma suggests that there may not always be a causal link between these two conditions [90, 91]. The risk of progression to invasive carcinoma in vulvar HSIL is considered to be low [88, 92, 93]. Further, vulvar HSIL is commonly multifocal, whereas invasive vulvar carcinoma is most frequently unifocal. The behavior of vulvar HSIL is not apparently comparable to that of cervical HSIL. Spontaneously regressing vulvar HSIL, so-called bowenoid papulosis [94], characterized by small, multifocal, papular, and hyperpigmented lesions affecting the labia majora and/or perianal skin is known to occur in younger women (Fig. 14.8). The high rate of regression has been reported in patients under 30 years and has been shown to be particularly common in pregnant women [92, 95].

Management of Patients with Differentiated-Type Vulvar Intraepithelial Neoplasia

Despite the fact that dVIN was first described in the 1960s by Abell as a highly differentiated form of vulvar carcinoma in situ, until more recently, the pathological entity had not gained wide attention because its existence had clinically been questioned [96, 97]. Recent knowledge has revealed that dVIN is often observed in areas of lichen sclerosus in older women [97, 98]. It is estimated that dVIN accounts for a small proportion with up to 5 % of all VIN lesions compared with uVIN [99, 100]. White or red lesions in areas of hyperkeratosis, ulceration, and the presence of a rough and irregular surface are all suspicious for dVIN (Fig. 14.9). Patients are often symptomatic with a long history of lichen sclerosus of vulvar itching and/or burning, and dVIN diagnosis might be frequently missed. Due to the highly malignant potential of

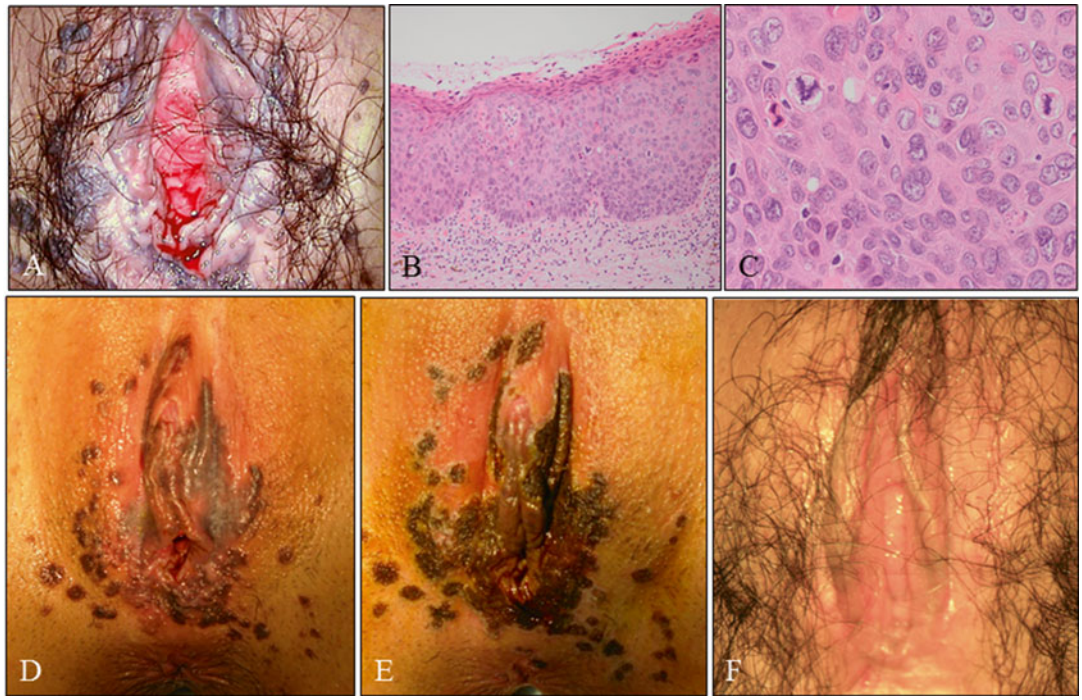

Fig. 14.8 Case of a young woman with vulvar HSIL, the so-called bowenoid papulosis. (**A**) Gross vulvar findings demonstrating multiple lesions. This is the same patient as shown in Fig. 14.3. The patient was a 25-year-old woman, nulligravida, with a 2-year history of cervical (Fig. 14.3) and vulvar HSIL referred to our hospital. (**B**, **C**) Microscopy of lesions demonstrating vulvar HSIL. (**D**, **E**) Pre- (**D**) and post- (**E**) laser therapeutic findings demonstrating pigmented and papular lesions ablated by laser vaporization. (**F**) Gross findings 2 months following laser vaporization and imiquimod therapy (**B**, **C**: hematoxylin-eosin staining, magnification ×10 for **B** and ×40 for **C**)

dVIN, non-healing ulcers and/or newly developed white hyperkeratotic lesions in patients with lichen sclerosus should be periodically biopsied or excised without delay to obtain a representative histopathological diagnosis [91]. It should be emphasized that the recognition of dVIN in patients with lichen sclerosus can be extremely challenging owing to associated ulceration and fissuring [85].

Clinical Treatments (Fig. 14.10)

Clinical treatment is indicated for vulvar HSIL and dVIN but not LSIL. There is a low risk of progression from HSIL to invasive carcinoma [91], and there have been many more case reports of spontaneous regression of vulvar HSIL in young women compared to cervical HSIL [95] [92]. Surgical treatment in young women is associated with a risk of psychosexual sequelae [101]. Careful observation or conservative treatment should be considered in young patients with vulvar HSIL confirmed by accurate and repeated biopsy. However, cases of vulvar HSIL where invasion cannot be ruled out should be treated by surgical excision (Fig. 14.11). Surgical treatment should be performed in older women due to the highly malignant potential of dVIN. Choice of therapy depends on the extent of disease, the location of the lesions, and, importantly, the desires of the patient.

Wide Local Excision

An important advantage of surgical local excision is that it allows complete histologic assessment and the early identification of invasive lesions [102]. Most localized lesions can be managed effectively by local excision with end-to-end suture of the skin defect. Wide local excision has utility in the treatment of large individual lesions and multifocal lesions (Fig. 14.11). Although primary closure

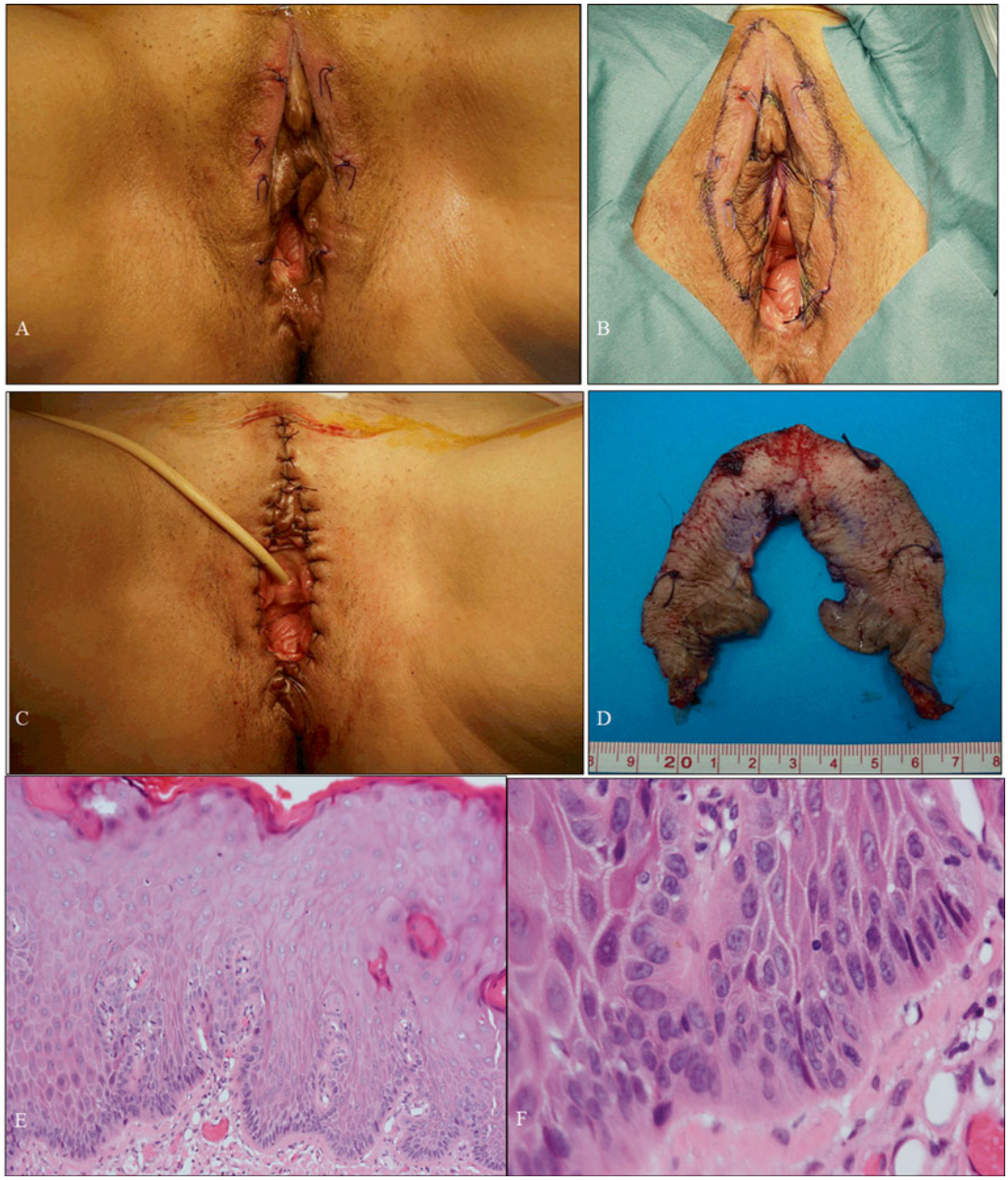

Fig. 14.9 A case of differentiated VIN (dVIN). (**A**–**C**) Gross photography of the vulva at first presentation at our hospital (**A**), preoperatively (**B**), and postoperatively (**C**). The patient was a 60-year-old woman, gravidity 1, parity 1, with leukoplakia. Her complaint was severe itch sensation of her vulva. Vulvar biopsies indicated VIN, and high-risk HPV test of the vulvar skin was negative. (**D**) Macroscopic image of specimen obtained from simple vulvectomy preserving clitoris. (**E**, **F**) Microscopic findings demonstrating dVIN (**E**, **F**: hematoxylin-eosin staining, magnification ×4 for **E** and ×40 for **F**)

of the surgical defects is usually obtained due to the elasticity of the vulvar skin, a use of split-thickness skin grafts taken from the buttocks or the medial aspect of the thigh is sometimes required for the repair. Rates of recurrence have been reported to be almost threefold higher in cases where margins were positive for residual HSIL [103]. It has been demonstrated that HPV

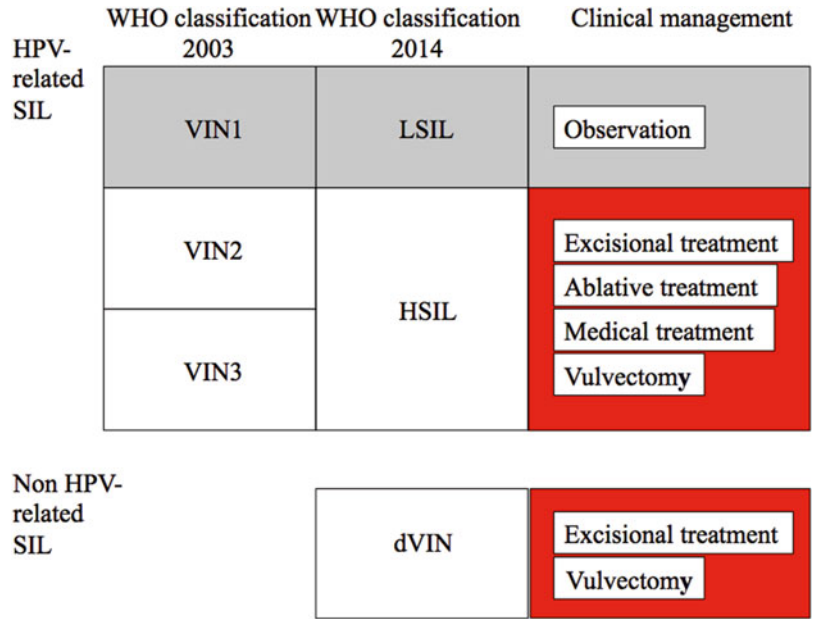

Fig. 14.10 Overview of clinical management for vulvar precancerous lesions. *WHO* World Health Organization, *SIL* squamous intraepithelial lesion, *VIN1* vulvar intraepithelial neoplasia grade 1, *VIN2* vulvar intraepithelial neoplasia grade 2, *VIN3* vulvar intraepithelial neoplasia grade 3, *LSIL* low-grade squamous intraepithelial lesion, *HSIL* high-grade squamous intraepithelial lesion

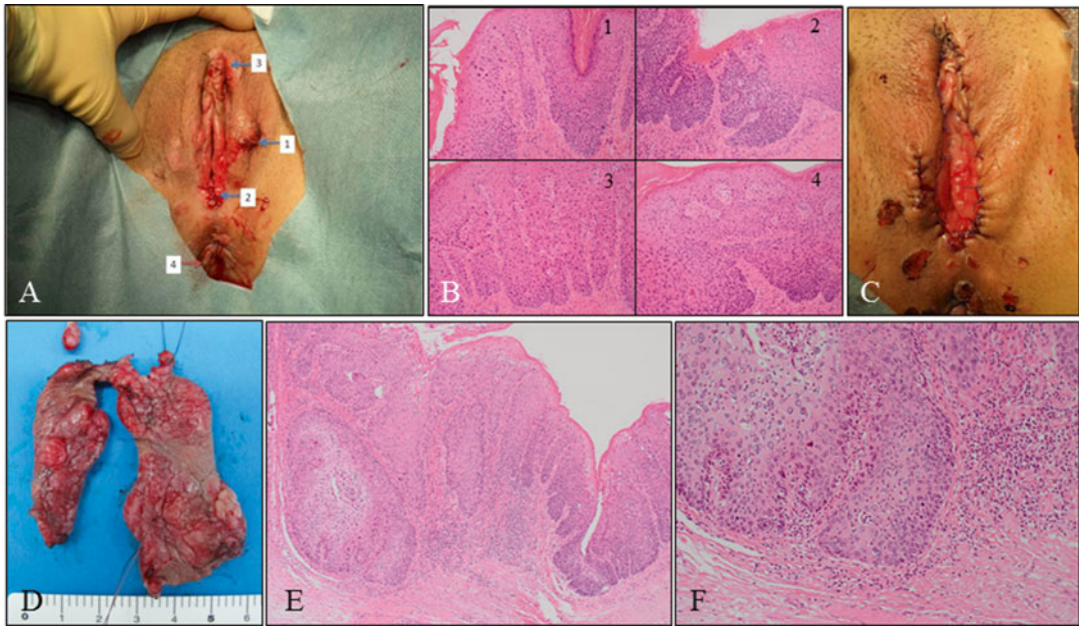

Fig. 14.11 Vulvar HSIL lesions with clinically suspected invasion. (**A**) Macroscopic findings of a vulvar lesion demonstrating four sites of biopsies (1–4). (**B**) A panel of microphotography of the biopsies (**B**: 1–4) indicating vulvar HSIL. The patient was a 39-year-old woman, nulligravida, with myelodysplastic syndrome with leukopenia. The patient had coexisting cervical HSIL. (**C**) Macroscopic findings following wide local excision and laser therapy. (**D**) Specimen obtained from wide local excision containing vulvar lesions clinically suspicious for invasion. (**E**, **F**) Histology indicating vulvar lesions were HSIL with invasive regions. The patient underwent radical vulvectomy with inguinal lymphadenectomy (**B**, **E**, **F**: hematoxylin-eosin staining, magnification ×4 for **D** and ×10 for **B**1–4, **F**)

infection after the wide local excision, as assessed by HPV test, is associated with an increased risk of recurrence [104].

Simple Vulvectomy

Unrecognized invasive carcinoma has been noted in up to 20 % of vulvectomy specimens in patients undergoing biopsies in patients with vulvar precancerous lesions [105, 106]. Therefore, simple vulvectomy is useful in patients with HSIL when there is clinical suspicion of occult invasion, or in patients with dVIN with highly malignant potential (Fig. 14.9). However, simple vulvectomy is associated with a high incidence of postoperative psychosexual problems. More conservative treatment should be considered in younger women with HSIL. Skinning vulvectomy with skin grafting, which was first described in 1968 and then resumed in 1986, has been developed for the removal of vulvar skin at risk of malignant transformation and replacement with ectopic epidermis from a donor site [107, 108]. Skinning vulvectomy with skin grafting preserves vulvar subcutaneous tissue providing an optimal cosmetic and functional result [109]. Reported recurrence rates following skinning vulvectomy range between 12 % and 30 %. Failure is primarily due to positive excision margins [110, 111].

Ablative Therapy

Ablative therapy may be used as an alternative to the excision of vulvar lesions principally with the use of laser vaporization [112, 113]. The disadvantage of ablative therapy is the potential for necrotic vulvar ulceration and slow wound healing. CO_2 laser vaporization in the management of HSIL has become the treatment of choice for many patients, particularly those with multifocal disease (Fig. 14.8e). Laser vaporization requires accurate histological evaluation using mapping biopsies to rule out invasion prior to laser vaporization. Repeated laser vaporization treatments are occasionally required due to a reported recurrence rate of 5–40 % [114, 115]. Recurrence occurs as a result of ineffective treatment depth or lateral extension. Benedet et al. evaluated 165 women with vulvar SIL. Of the 122 patients with vulvar HSIL (VIN3), the mean thickness of the epithelium was 0.52 mm (range, 0.1–1.9 mm). In patients with hair follicles involved with vulvar SIL, the mean depth of involvement was 1.9 mm (range, 1–3.4 mm). Only 19 patients had appendiceal involvement. Age did not appear to affect the thickness of involved epithelium. Satisfactory therapeutic and cosmetic results may be obtained with laser vaporization of hairy and glabrous skin to depths of 2 mm and 1 mm, respectively [116]. Pain, which may be severe, is the major disadvantage of laser therapy. Small portions of the vulva may be treated on an outpatient basis; however, general anesthesia is required for the treatment of large areas of the vulva.

Medical Therapy

Medical therapy for HSIL and dVIN has the potential to preserve vulvar appearance and function [117]. Similar to ablative treatments, medical therapy does not provide histological specimens meaning occult invasion may be missed. In the 1980s, 5-FU was used as a topical chemotherapeutic agent for VIN. Failure and recurrence rates for 5-FU were high with low levels of patient compliance. Pain and burning frequently limit the duration of 5-FU treatment [118]. A number of alternative therapeutic options are now available.

Imiquimod

Imiquimod and imidazoquinoline amine are classified as immune response modifiers. Imiquimod 5 % cream is widely used for the treatment of genital warts with proven efficacy in terms of lesion clearance and lower recurrence rates compared with conventional surgical treatments [119, 120]. HPV-related lesions are associated with decreased expression of proinflammatory Th1 cytokines, tumor necrosis factor (TNF)-α, and interferon (INF)-γ. Imiquimod is a topical immune response modifier that induces the secretion of proinflammatory Th1 cytokines. Imiquimod acts by activating macrophages via binding to cell surface receptors, such as Toll-like receptor (TLR)-7. These receptors function as the primary sensors of the innate immune system for the recognition of microbial pathogens.

Imiquimod is a potent TLR-7 agonist and thereby induces the synthesis and release of endogenous proinflammatory cytokines. In addition, imiquimod stimulates natural killer cell activity and induces the proliferation and differentiation of B-lymphocytes [121]. Imiquimod is considered a candidate therapeutic option for HPV-related HSIL, particularly the so-called bowenoid papulosis, in young patients (Fig. 14.8). Meta-analysis of randomized controlled trials has revealed that women treated with topical imiquimod have a better response than women receiving a placebo. Three of the four trials included assessed the effectiveness of topical imiquimod versus placebo in women with HSIL; the other examined low- versus high-dose indole-3-carbinol in a similar population of women. Meta-analysis of these three trials found the proportion of women who responded to treatment at 5–6 months was much higher in the group who received topical imiquimod than in the group who received placebo (relative risk (RR) = 11.95, 95 % confidence interval (CI) 3.21–44.51). A single trial reported similar results at 12 months (RR = 9.10, 95 % CI 2.38–34.77). Only one trial reported common adverse events in the imiquimod group. One trial found no significant differences in quality of life or body image between imiquimod and placebo groups [81]. The majority of patients reported local burning sensations, swelling, and pain. Imiquimod appears to be effective in reducing the prevalence of recurrent HPV and could therefore be used a complementary treatment to surgery to prevent recurrence rather than as an isolated treatment for severe vulvar intraepithelial neoplasia with the potential for progression to carcinoma [122].

Topical Photodynamic Therapy

Photodynamic therapy (PDT) utilizes the interaction between a tumor-localizing photo sensitizer, 5-aminolaevulinic acid (ALA), and light of an appropriate wavelength to bring about molecular oxygen-induced cell death [123]. A small number of studies have assessed the efficacy of PDT in treatment of vulvar HSIL. Unifocal and small lesions often responds well to PDT; however, these lesions can be easily removed surgically. Multifocal and/or pigmented lesions have been shown to be less likely to respond to PDT [115, 124]. Failure to respond to PDT is associated with detectable HPV levels [115, 125]. PDT appears to have equal efficacy to that reported for laser vaporization and local excision [115, 126]. PDT has been shown to not induce ulcers or scar formation with decreased healing times compared with laser vaporization [115]. Larger randomized control studies are required to confirm the effectiveness of PDT in HSIL.

Other Candidate Medical Treatments

Cidofovir, an acyclic nucleoside analogue, has potent antiviral activity against a broad range of DNA viruses, including HPV [127]. The drug was developed for the treatment of acquired immune deficiency syndrome (AIDS) [128]. The effect of cidofovir in HPV-related disease is thought to be mediated by the induction of apoptosis in HPV-infected cells. A study describing the use of cidofovir in usual VIN has been reported [129]. Twelve patients with usual VIN, 10 of which completed follow-up, were treated with cidofovir 1 %. Four patients showed complete resolution of all symptoms and visible lesions, and three patients had partial responses. One patient had invasive disease diagnosed in a residual lesion following a partial response to treatment. Local side effects were limited to ulceration of the affected mucosa with no effect on surrounding normal tissue observed. More studies are required to evaluate the role of cidofovir in the treatment of vulvar HSIL.

Therapeutic HPV vaccines have been designed to generate cell-mediated immunity against HPV-infected cells expressing the early viral proteins E6 and E7 which act as oncogenes and tumor-specific antigens. The principle of action of HPV vaccination is the induction of cellular immunity against usual VIN lesions, eliciting specific immunity against HPV E6 and E7 proteins [130, 131]. A clinical study of vaccination with synthetic long peptides representing the entire length of the two HPV-16 oncoproteins, E6 and E7, demonstrated treatment efficacy in vulvar HSIL over a period of 12–24 months. Clinical responses

in HPV-16-positive women have been demonstrated following administration of a synthetic long-peptide vaccine against the HPV-16 oncoproteins E6 and E7 [132]. Clinical trials of both PDT and therapeutic HPV vaccination have demonstrated an association between clinical responses and tumor-infiltrating lymphocytes [125, 133]. Another clinical trial using a combination of imiquimod and therapeutic HPV vaccination found imiquimod followed by vaccination achieved histological clearance of lesions at 52 weeks in almost 60 % of a heavily pretreated cohort of women with vulvar HSIL [134].

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