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Practical Gynecologic Pathology

Frequently Asked Questions

Springer

Practical Anatomic Pathology

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This Book Series is designed to provide a comprehensive, practical and state-of-the-art review and update of the major issues and challenges specific to each subspecialty field of surgical pathology in a question and answer (Q&A) format. Making an accurate diagnosis especially from a limited sample can be quite challenging, yet crucial to patient care. This Book Series, using the most current and evidence-based resources 1) focuses on frequently asked questions in surgical pathology in day-to-day practice; 2) provides quick, accurate, terse, and useful answers to many practical questions encountered in daily practice; 3) emphasizes the importance of a triple test (clinical, radiologic, and histologic correlation); 4) delineates how to appropriately utilize immunohistochemistry, in situ hybridization and molecular tests; and 5) minimizes any potential diagnostic pitfalls in surgical pathology. These books also include highly practical presentations of typical case scenarios seen in an anatomic pathology laboratory. These are in the form of case presentations with step-by-step expert analysis. Sample cases include common but challenging situations, such as evaluation of well-differentiated malignant tumors vs. benign/reactive lesions; distinction of two benign entities; sub-classification of a malignant tumor; identification of newly described tumor and non-tumor entities; workup of a tumor of unknown origin; and implementation of best practice in immunohistochemistry and molecular testing in a difficult case. The Q&A format is well accepted, especially by junior pathologists, for several reasons: 1) this is the most practical and effective way to deliver information to a new generation of pathologists accustomed to using the Internet as a resource and, therefore, comfortable and familiar with a Q&A learning environment; 2) it's impossible to memorialize and digest massive amounts of new information about new entities, new and revised classifications, molecular pathology, diagnostic IHC, and the therapeutic implications of each entity by reading large textbooks; 3) sub-specialization is a very popular practice model highly demanded by many clinicians; and 4) time is very precious for a practicing pathologist because of increasing workloads in recent years following U.S. health care reforms. This Book Series meets all of the above expectations. These books are written by established and recognized experts in their specialty fields and provide a unique and valuable resource in the field of surgical pathology, both for those currently in training and for those already in clinical practice at various skill levels. It does not seek to duplicate or completely replace other large standard textbooks; rather, it is a new, comprehensive yet concise and practical resource on these timely and critical topics.

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Preface

While textbooks of gynecological pathology at various comprehensive levels are ubiquitous, readers are often seeking specific target information, particularly of recent update, that may be buried in lengthy text and time consuming to extract from such systemic textbooks. In order to provide a quick review or summary of major diagnostic challenges in gynecologic pathology, this volume embraces a Q/A format by providing the desired diagnostic knowledge. Our specific emphasis is to combine the diagnostic approach as it relates to morphology and the relevant ancillary tests which impact on clinical outcome or management. We intend to provide the concise, accurate answers for a variety of common, yet challenging diagnostic scenarios that are relevant to and frequently encountered in daily practice. Each topic has been addressed by subspecialty gynecological pathologists in the field, making this a valuable handbook for a broad spectrum of readers, particularly pathology trainees, junior pathologists, and general practicing pathologists.

As a result of the complex pathologic findings and molecular alterations, many gynecologic neoplasia and their related precursor lesions are frequently challenging, especially for less common entities. A short but condensed summary for some of the difficult, prevailing, and emerging topics dominates the content of this book. Covering female genital organs in 16 chapters, the book walks readers through current topics, controversies, and diagnostic dilemmas for common gynecologic diseases with histological criteria and key ancillary study tools, richly illustrated with histological figures and tables. To reflex the current advances in diagnostic gynecological pathology, the topics are closely correlated with the updated disease classifications and diagnostic criteria, particularly those recognized by international gynecological pathology societies. To name a few, cervical cancers are discussed using HPV-based classification of cervical cancers endorsed by the International Endocervical Adenocarcinoma Criteria and Classification (IEACC) in Chaps. 3 and 4. Chapter 5 covers major features and considerations of various endometrial adenocarcinomas as well as how newly developed molecular tests influence prognosis and therapeutic strategy, recently advocated by the International Society of Gynecological Pathologists (ISGyP) Endometrial Carcinoma Project. Chapter 6 highlights the major pitfalls for diagnosis of many histologic variants of uterine smooth muscle tumors and other mesenchymal neoplasms. Many unique genetic mutations or alterations associated with leiomyoma variants and endometrial stromal tumors are discussed. Recent advances in genomic and specific gene mutations that are linked to gynecologic neoplasms or diseases are discussed in many chapters including but not limiting to Chaps. 6, 7, 14, and 16. Chapter 7 presents an algorithm for the differential diagnosis of gestational trophoblastic diseases using a combination of histology and molecular approach to help guide readers towards accurate diagnosis. Chapters 9 and 10 discuss the histology and clinical correlation of ovarian epithelial and germ cell tumors based on the International Germ Cell Consensus Classification (IGCCC) and the new WHO classification, both of which serve as valuable resources for practicing pathologists at either subspecialty or general practice setting. Practical discussions of many newly established immunohistochemical and molecular markers are provided in facilitating the differential diagnosis for many challenging entities or tumor types in Chaps. 15 and 16.

At routine sign-out microscope with pathology trainees or at an educational slide seminar, the teaching most frequently takes the format of providing a specific answer to a specific

question raised in real time in the presence of a particular specimen at hand. "Getting to the point" is perhaps the best approach for knowledge retention. Therefore, facilitation of an effective reading by providing a short concise summary for some of the most difficult, prevailing, and emerging concepts in gynecologic pathology is what we have hoped to achieve in this book.

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Diseases of the Vulva

1

Gloria Zhang, Yun Zhao, and Bin Yang

Abbreviations

AGN	Atypical genital nevi
AJCC	The American Joint Committee on Cancer
CD34	Cluster of designation 34
CDC	Centers for disease control and prevention
CEA	Carcinoembryonic antigen
CIN	Cervix intraepithelial neoplasia
CK	Cytokeratin
CMV	Cytomegalovirus
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
DNA	Deoxyribonucleic acid
dVIN	Differentiated vulvar intraepithelial neoplasia
EMA	European Medicines Agency
EMPD	Extramammary Paget disease
ER	Estrogen receptor
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FIGO	The International Federation of Gynecology and Obstetrics
GCDFP15	Gross cystic disease fluid protein 15
HMB45	Human Melanoma Black 45
HMGA2	High mobility group protein A2
HPV	Human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
HSV	Herpes simplex virus
ISH	In situ hybridization
ISSVD	International Society for the Study of Vulvovaginal Disease
LAST	Lower Anogenital Squamous Terminology
LP	Lichen planus
LS	Lichen sclerosus
LSC	Lichen simplex chronicus
LSIL	Low-grade squamous intraepithelial lesion
MART-1	Melanoma antigen recognized by T-cells-1
NOS	Not otherwise specified
PD-1	Programmed cell death protein 1
PR	Progesterone receptor
Rb	Retinoblastoma
RNA	Ribonucleic acid
SATB2	Special AT-rich sequence-binding protein 2
SCC	Squamous cell carcinoma
SFT	Solitary fibrous tumor
SMA	Smooth muscle actin
SOX10	SRY-related HMG-box 10
uVIN	Usual-type vulvar intraepithelial neoplasia
VaIN	Vagina intraepithelial neoplasia
VIN	Vulvar intraepithelial neoplasia

1. What Are the Clinical and Histological Features of the Common Viral Infections in the Vulva?

Human Papillomavirus Infection

According to the CDC, human papillomavirus (HPV) infection is the most common sexually transmitted infection in the United States. Most sexually active persons become infected with HPV at some point in their lives. The infections in most cases are transient, but when HPV infection persists, it can cause health problems like genital warts, dysplastic lesions, and cancer. There are about 120 types of HPV that affect different parts of the body, at least 40 of which can affect the

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genitals area [1]. Based on their oncogenic potential, different subtypes of HPV are classified as low-risk types, such as HPV 6 and HPV 11, and high-risk types, such as HPV 16 and HPV 18. Genital warts are by far the most common manifestation, and almost all genital warts are caused by low-risk HPV. In contrast, high-risk HPV, most commonly HPV 16 and HPV 18, are linked to vulvar intraepithelial neoplasia (VIN) and squamous cell carcinoma [2]. However, the HPV oncogenic risk on the vulvar skin and mucosa seems less than that on the cervix. The clinical and histological features of genital warts and squamous dysplasia are discussed in detail in the next parts.

Genital Herpes Simplex Virus Infection Genital herpes simplex virus (HSV) infection is a significant global public health problem because there is a dramatic upsurge in genital HSV infections documented from seroprevalence studies. The perinatal transmission of HSV can also lead to fetal morbidity and mortality. Most genital herpes is associated with HSV type 2, with a recent increase being noted for HSV type 1. The virus replicates at the infection site, then travels to the dorsal root ganglia through a retrograde axonal flow. It remains in a latent phase with reactivation occurring spontaneously or following stimuli such as fever, stress, ultraviolet radiation, or immunosuppression. The clinical manifestations of primary genital HSV infection are highly variable. The initial presentation can be severe with vulva pain, dysuria, fever, tender inguinal lymphadenopathy, and headache. In some patients, however, the infection can be mild, subclinical, or asymptomatic. The classic lesions often appear 3–7 days after exposure. There are vesicles arranged in clusters on an erythematous base that evolve to pustules and erosions [3, 4]. These lesions are excruciatingly painful. The perineum, perianal skin, cervix, vagina, and urinary tract are often synchronously involved. Accompanying regional lymphadenopathy may last for more than a week. The lesions usually persist for 2–6 weeks and heal without scarring unless there is a secondary infection. Genital HSV infection is more severe and protracted in immunosuppressed individuals. There are no apparent differences in the clinical presentation between HSV-1 and HSV-2. The diagnosis is usually apparent clinically and can be confirmed through serologic tests, tissue culture, direct immunofluorescence, or molecular techniques. In cytological smears or biopsy from the base or edges of a newly formed vesicle or ulcer, the infected keratinocytes can be identified. The characteristic cytological features are the homogeneous ground-glass appearance of the nuclei. The cells can be multinucleated with molding, eosinophilic intranuclear inclusions, and dense eosinophilic cytoplasm (Figs. 1.1 and 1.2). Biopsies often show an intraepidermal blister resulting from infected cell swelling and losing attachment (ballooning degeneration) with inflammation. Follicles are more often involved in recurrent
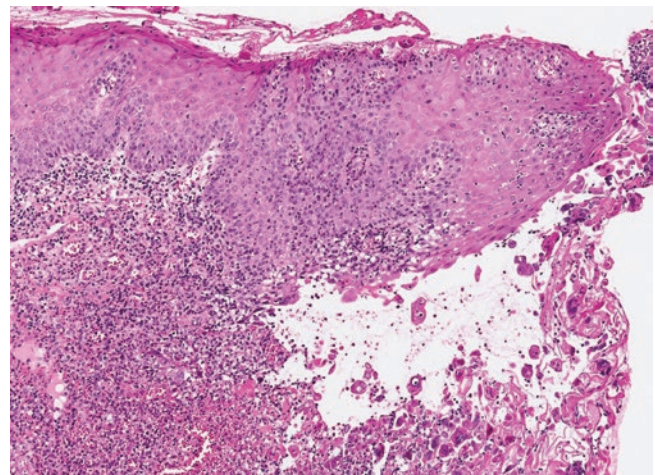

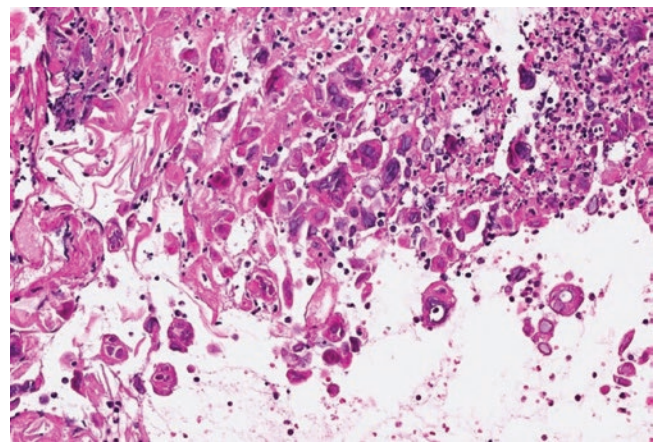**Fig. 1.1** Herpetic ulcer of vulva (low magnification)

Fig. 1.2 Herpetic ulcer of vulva (high magnification). Note the multinucleation and ground-glass appearance of the nuclei

lesions. Sometimes it is difficult to appreciate when a few infected cells admixed with many inflammatory cells. Occasionally the infected cells with dark nuclei, in the absence of blister, may mimic squamous dysplasia. In those cases, an immunohistochemical study using anti-HSV can help distinguish these lesions.

Molluscum Contagiosum Molluscum contagiosum is a poxvirus infection exhibiting single or multiple, 2–8 mm dome-shaped papules with a central umbilicated core of white material. This infection predominantly affects children and adolescents. Spontaneous regression commonly occurs. In adults, molluscum contagiosum occurs mainly as a sexually transmitted disease involving the vulvar and perianal regions. The histological features of molluscum contagiosum are pathognomonic [5]. At low magnification, there is endophytic growth of squamous epithelium arranged as lobules. Eosinophilic inclusion bodies fill the cytoplasm of infected
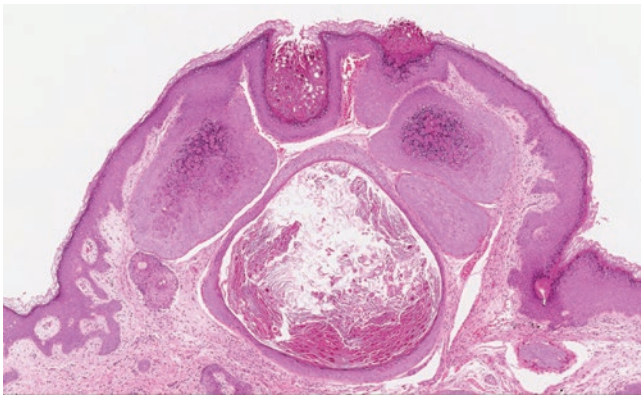
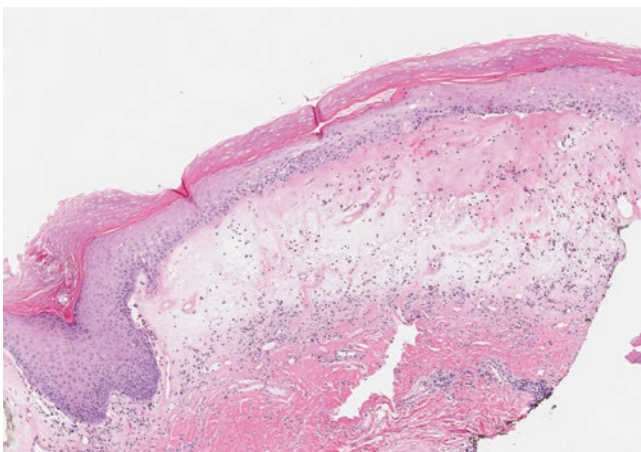**Fig. 1.3** Molluscum contagiosum, typical histological features

Fig. 1.4 Well-developed lichen sclerosus, thin epidermis with extensive subepithelial homogeneous hyalinized zone

cells above the basal layer (Fig. 1.3). The viral inclusion bodies become more basophilic as they enlarge. The inclusions can compress the nucleus of the infected keratinocytes to the periphery. In a fully evolved lesion, the epidermis may rupture due to the underlying viral proliferation pressure and produce the characteristic small white core.

Varicella zoster virus and cytomegalovirus (CMV) infections rarely occur in the vulva. They usually present with similar features to the lesions at other anatomic locations.

2. How to Correctly Interpret Lichenoid Vulvar Dermatoses?

Lichenoid vulvar dermatoses, most commonly lichen sclerosus and lichen planus, may have overlapping clinical and histopathological characteristics while each condition also has its unique features. An accurate diagnosis is essential for appropriate management because the nature and the prognosis of these conditions differ.

Lichen Sclerosus Vulvar lichen sclerosus (LS) is a chronic, progressive dermatologic condition in women of all ages with a peak incidence in postmenopausal women. The etiology of lichen sclerosus is mainly unknown. The clinical presentation of lichen sclerosus varies as the lesions progress. LS typically causes pruritus and presents as irregular, white, atrophic patches, which can involve any part of the vulva [6]. Most lesions are multiple. Bilateral, sometimes symmetrical, lesions occur in 80% of cases. The vulvar architecture remains intact in the early course of the disease. Over time, the affected skin becomes shiny and wrinkled with variable degrees of scarring, resulting in vulvar atrophy and deformity.

A vulvar biopsy is essential to confirm the diagnosis and exclude malignancy. The microscopic findings also vary considerably depending on the lesion's age, secondary changes related to pruritus-related scratching, excoriation, and treatment. The characteristic features of well-developed lesions include a thin epidermis with loss of the rete ridges, a subepithelial homogeneous zone that varies from edematous to hyalinized of variable thickness (Fig. 1.4). A band of lymphocytic infiltration beneath the homogeneous zone may or may not be present. Additional findings may include spongiosis, prominent thickening of the basement membrane, vacuolar alteration of basal keratinocytes, and sclerosis and/or ectasia of dermal vessels. Mitotic figures are usually rare or absent. Telangiectatic vessels and melanin incontinence correspond to focal red and brown appearance. The presence of eosinophils is present in up to half of the LS cases [7, 8]. Although the squamous epithelium is usually thinned, hyperkeratosis may occur in some LS cases due to superimposed lichen simplex chronicus. Histopathological changes in the early LS are subtle and can be diagnostically challenging. There is often a band of lymphocytic infiltration with minimal hyalinized sclerotic changes, a common feature shared by lichen planus and several other lichen dermatoses. Histological findings suggesting early LS include thickened basement membrane, which may be appreciable with the assistance of a PAS stain, lymphocyte entrapment by wiry dermal fibrosis, and dyskeratotic keratinocytes overlying columns of parakeratosis, and loss of papillary dermal elastin [7, 8] (Fig. 1.5).

An association between vulvar lichen sclerosus and squamous cell carcinoma has long been recognized. There is growing evidence that vulvar LS is a premalignant lesion. Pooled longitudinal studies indicate that about 4% of cases of longstanding LS progress to vulvar SCC [9]. A recent large study with histologically confirmed vulvar LS deter-
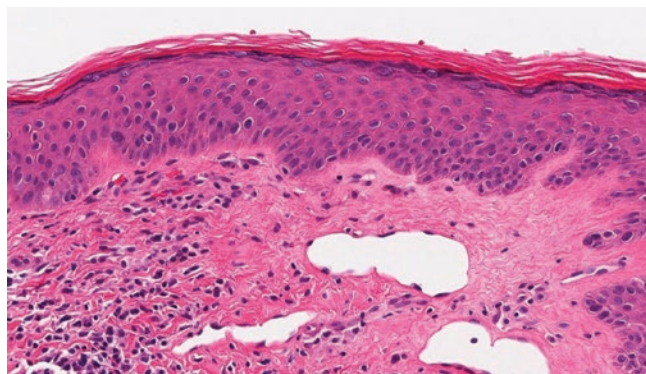
Fig. 1.5 Early lichen sclerosus with thickened basement membrane, entrapped lymphocytes, and early papillary dermal sclerosis

Fig. 1.6 Invasive keratinizing squamous cell carcinoma (right side) and associated lichen sclerosis (left side)

Fig. 1.7 Lichen simplex chronicus

mined that the 20-year incidence of vulvar SCC was 6.7% [10]. Up to 65% of cases of differentiated VIN and invasive keratinizing squamous cell carcinoma are associated with synchronous LS (Fig. 1.6). The presence of dyskeratosis and parakeratosis, hyperplasia, and/or basal cellular atypia should be noted as lichen sclerosus with these findings is more likely to progress to squamous cell carcinoma. Extragenital involvement is present in 20% of cases with no known association of subsequent dysplasia and malignancy.

Lichen Planus Lichen planus (LP) is a chronic, inflammatory, and immune-mediated disease of the skin and mucous membranes that occurs most commonly in women older than 40 years of age [6]. The characteristic skin lesions of lichen planus are thought to arise from a T-cell-mediated autoimmune response against the keratinocytes in the basal layer of the epidermis. The disorder is often associated with other autoimmune diseases. Whereas lichen sclerosus does not affect the vagina, lichen planus can manifest with concomitant vaginal involvement. Although the diagnosis of lichen planus can be made based upon the recognition of characteristic clinical manifestations, biopsy confirmation is usually recommended for vulvar lichen planus. Classic histological features of lichen planus include a band-like dermal infiltrate of lymphocytes at the dermal–epidermal junction, a wedge-shaped hypergranular layer, and "saw-toothed" rete ridges. Vacuolar change and apoptotic keratinocytes are present in the epidermal basal layer. As mentioned above, genital involvement by LP can sometimes be difficult to distinguish from early lichen sclerosus. Cytoid bodies, wedge-shaped hypergranulosis, extensive basal layer destruction, and pointed rete ridges favor lichen planus, but these findings are less common in the vulvar LP. For patients with hypertrophic lichen planus with epidermal squamous hyperplasia, a biopsy is usually performed to rule out squamous cell carcinoma or its precursor.
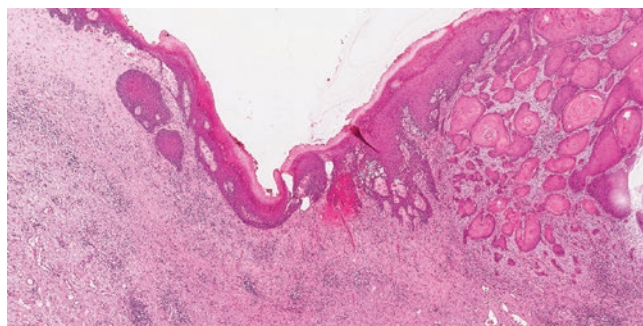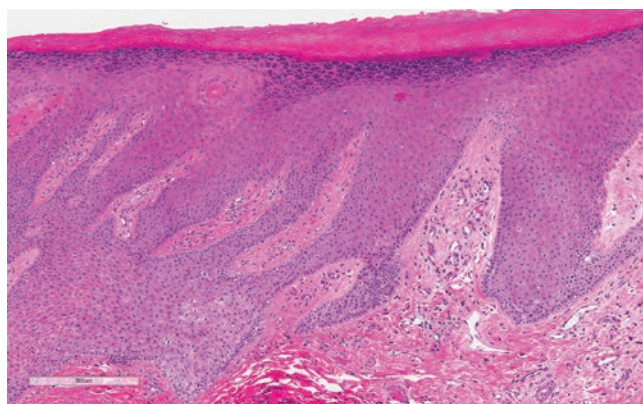
Lichen Simplex Chronicus Lichen simplex chronicus (LSC) is a common chronic inflammatory disorder that involves the vulva. It can be primary or secondary to or coexists with a wide variety of irritative and infectious conditions. A common clinical manifestation is intense vulvar pruritus, resulting in an itch-scratch cycle that can be uncontrollable. Physical examination usually reveals lichenified plaques with scale and accentuated cutaneous markings. The diagnosis of vulvar LSC is often based on patient history and physical examination; however, a skin biopsy can also aid in diagnosing equivocal cases. Histopathologically, vulvar LSC has psoriasiform hyperplasia, hypergranulosis, hyperkeratosis, and occasionally focal parakeratosis (Fig. 1.7). Notably, vulvar LSC often lacks the vertically oriented collagen fibers in the papillary dermis seen in extragenital LSC but more likely to have prominent fibroblasts [8].

Lichenoid Drug Eruption Lichenoid drug eruption has clinical and histological features similar to lichen planus. Lichenoid drug eruptions often have a prolonged latent period from introducing the drug to the cutaneous eruption. Some differentiating clinical factors suggestive of a lichenoid drug eruption include a more generalized distribution

with sparing of the classic sites of involvement of lichen planus, lack of Wickham's striae, and sparing of mucous membranes. Histopathologically, as lesions of lichen planus may have eosinophils, the presence of eosinophils alone cannot differentiate lesions of lichen planus and lichenoid drug eruptions. However, increased eosinophils and plasma cells may be a clue to a lichenoid drug eruption. Also, the dyskeratotic cells in lichen planus tend to be limited to the basilar layers, while the presence of increased numbers of dyskeratotic cells at all layers of the epidermis is more indicative of a drug-induced lesion [7].

3. How to Differentiate Deep-Seated Inflammatory Lesions in the Vulva, Such as Hidradenitis Supppurativa, Crohn's Disease, and Bartholin Cyst with an Abscess?

There are several conditions that can present as deep-seated ulcerative lesions in the vulvar.

Hidradenitis suppurativa is a chronic suppurative process of the apocrine gland-bearing skin that affects the axilla and vulva, particularly, often resulting in scarring and draining sinuses. Hidradenitis suppurativa is characterized by deep-seated, inflamed papules, and nodules that may occur anywhere in the anogenital area, most commonly in the inguinal and labiocrural folds, mons pubis, and perianal area [11]. The inflammatory papules and nodules are sometimes capped with white or yellow-white pustules. A very distinctive clinical feature is the presence of classic multiheaded or tombstone comedones ("blackheads"). The diagnosis of hidradenitis suppurativa is mainly made based on history and clinical findings, including clinically typical lesions, distribution in the typical locations, failure to respond within days to antibiotic therapy, and chronicity [11, 12]. Microscopic examination on a vulvar biopsy reveals acute and chronic inflammation, hyperplasia of the follicular epithelium, and apocrine glands dilated with keratinaceous material. The lesions of hidradenitis suppurativa are differentiated from furuncles and abscesses by bacterial culture. In hidradenitis suppurativa, cultures are usually negative or show only non-pathogenic organisms; however, the secondary infection does occur caused by a wide range of bacteria, including anaerobes. Bartholin gland abscesses typically are caused by *E. coli* or polymicrobial infection. Squamous cell carcinoma has been reported in longstanding cases of Hidradenitis suppurativa.

Crohn disease is a granulomatous inflammation, most commonly involving the small bowel, but occasionally the vulva and the perineum in adults and children. Genital Crohn disease is more frequent in children than in adults. Extraintestinal findings precede the gastrointestinal involvement in one-fourth of cases. The clinical appearance of vulvar Crohn disease is variable. When associated with a local colonic disease, perineal ulcers and large, edematous skin tags are characteristic. Sometimes fistulas extend from the affected bowel into the perineum and even vulvar skin or Bartholin glands, resulting in indurated, tender, inflamed areas that drain pus. The latter may mimic inflammation of Bartholin glands. The pathological changes include massive dermal and subcutaneous edema and markedly dilated lymphatics. Although non-caseating granulomas are diagnostic, in most cases, ill-formed aggregates of epithelioid histiocytes with variable numbers of lymphocytes are seen instead [13].

Hidradenitis suppurativa may be challenging to distinguish from Crohn disease. However, the latter usually does not affect axillae, is less painful, and usually has gastrointestinal manifestations. Fox–Fordyce disease (apocrine miliaria) sometimes coexists with hidradenitis suppurativa. Fox–Fordyce disease may present at puberty with pruritic papules of the axillae, vulva, and perianal regions.

Behçet disease is defined by the triad of recurrent oral ulcers, genital ulcers, and ocular inflammation. Behçet disease can cause deep painful ulcerations in the vulva that may lead to labial fenestration and gangrene. The pathogenesis is unknown. The histological features are usually nonspecific with mixed acute and chronic inflammation. Therefore, clinical and pathological correlation is essential for the diagnosis [14, 15].

4. What Are the Common Lesions in the Bartholin Glands?

Normal Bartholin glands consist of mucin-producing alveoli in the vulva draining eventually into a central duct. The ducts emerge onto the vestibule, one at each side of the vaginal orifice, in the groove between the hymenal ring and the labia minora. Blockage of the Bartholin glands' drainage ducts is a common etiology of a vulvar cystic mass, named Bartholin duct cyst, which may evolve into Bartholin duct abscess with superimposed infection [16]. Bartholin duct cyst often contains mucoid fluid. A Bartholin duct cyst is typically painless and slowly growing. Histologically the dilated ducts may be lined by squamous, transitional, mucinous, or flattened epithelium. Normal remnants of lobular mucus glands may be presented adjacent to the cyst. Acute and chronic inflammation can be seen when there is a superimposed secondary infection. A location consistent with Bartholin's origin and the presence of normal Bartholin's gland acini adjacent to the cyst facilitates distinction from cysts arising from minor vestibular glands [17] (Figs. 1.8 and 1.9).

Primary carcinoma of the Bartholin gland is rare, accounting for 0.1–5.0% of all vulvar malignancies and the risk factors are not well-established [18]. Benign tumors of the Bartholin gland are even rarer. Bartholin gland carcinoma is
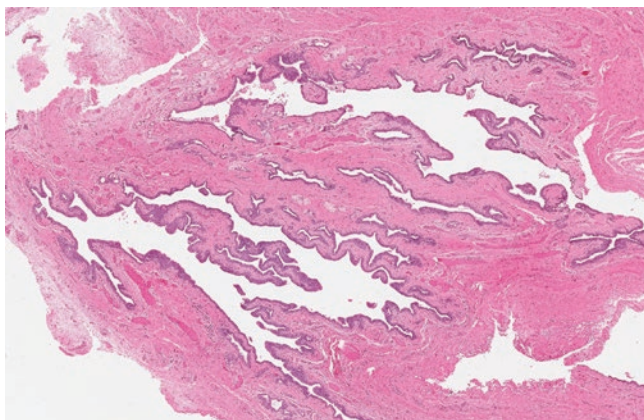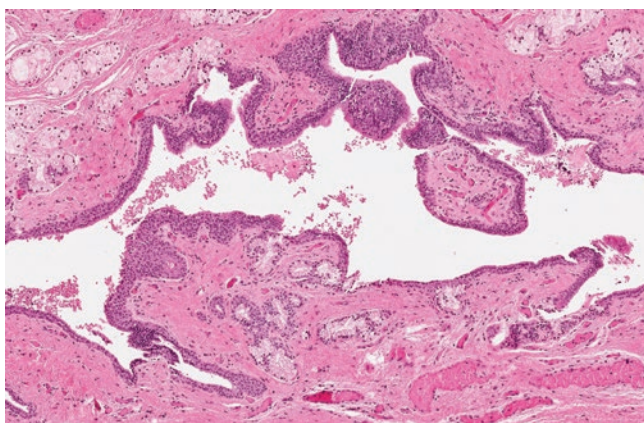
Fig. 1.8 Bartholin duct cyst

Fig. 1.9 Bartholin duct cyst, note the adjacent normal Bartholin's gland acini

Fig. 1.10 Mucinous adenocarcinoma of the Bartholin gland

Bartholin mass is required if any features suggestive of malignancy are present: solid component, cyst or abscess wall fixed to surrounding tissue, or mass unresponsive or worsening despite treatment.

5. What Are the Common Skin Adnexa and Mammary-Like Gland Tumors in the Vulva (Location and Histology)?

Despite the high concentration of skin appendages in the vulva, tumors derived from them are uncommon. In the vulvar region, hidradenoma papilliferum is the most common benign neoplasm, followed by syringoma and various types of cysts [22].

Hidradenoma papilliferum is a benign neoplasm that is thought to arise from mammary-like glands in the vulva. It occurs mainly in middle-aged women and usually presents as a 1–2 cm, solitary, round, firm, painless, or occasionally tender nodule. It is commonly seen on the labia majora and the outer lateral surfaces of the labia minora, less commonly the fourchette or clitoris or in the perineum. Although HPV has been identified in the lesion, the virus infection is not considered a causative factor. Histologically the tumor typically shows a circumscribed complex proliferation with papillary and glandular architectures (Fig. 1.11). The branching papillae have a fibrovascular core surrounded by an outer layer of small and often flattened myoepithelial cells and an inner layer of columnar or cuboidal epithelial cells with abundant pale eosinophilic cytoplasm (Fig. 1.12). It usually lacks cytological atypia and mitoses. Unusual features include sebaceous or squamous differentiation, foci resembling sclerosing adenosis, ductal adenoma, or sclerosing intraductal papilloma

typically deep, and the most common presentation is as a painless vulvar mass. The mass may be solid or cystic. Diagnosis is often delayed since findings on gross examination appear late in the course of the disease. On the other hand, a mass in the Bartholin complex is often misdiagnosed as an abscess or cyst. Fixation to underlying tissue is suspicious for malignancy. Squamous cell carcinomas are the most common malignant tumors arising from the Bartholin gland (about 40%), followed by adenocarcinoma NOS (25%) (Fig. 1.10) and adenoid cystic carcinoma (about 12%) [19, 20]. HPV has been identified in some Bartholin gland carcinomas of squamous and transitional type [21].

Take-Home Message The key to identifying a Bartholin mass is the anatomic location of the mass. Many vaginal and vulvar lesions can clinically mimic Bartholin gland disorders. The differential diagnoses of a vulvar mass should include cyst, leiomyoma, fibroma/fibroadenoma, hernia, accessory breast tissue, hidradenoma, endometriosis, lipoma, syringoma, hidradenitis suppurativa, and others. Biopsy of a
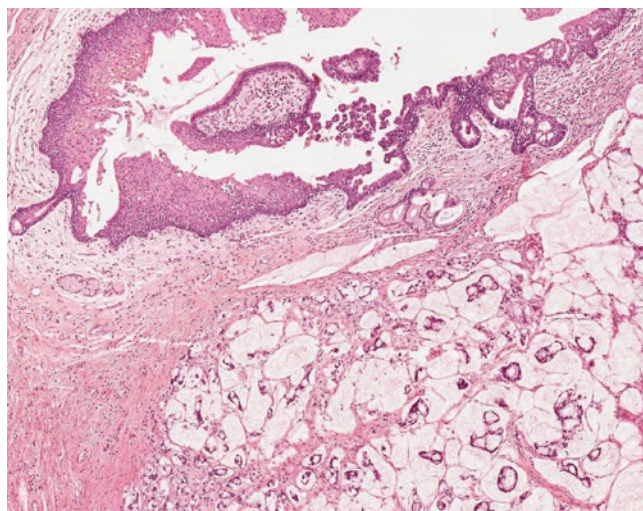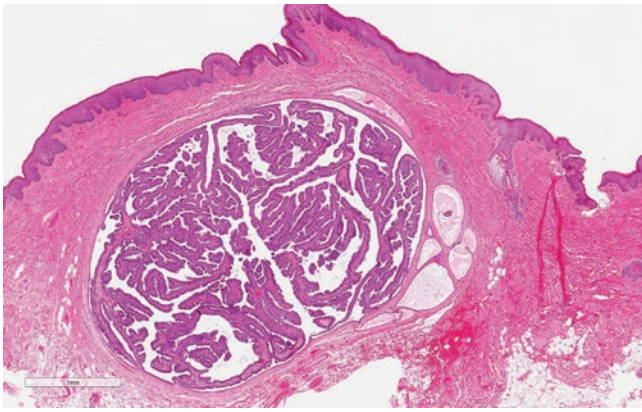
Fig. 1.11 Hidradenoma papilliferum. Note circumscribed complex proliferation with papillary and glandular architectures

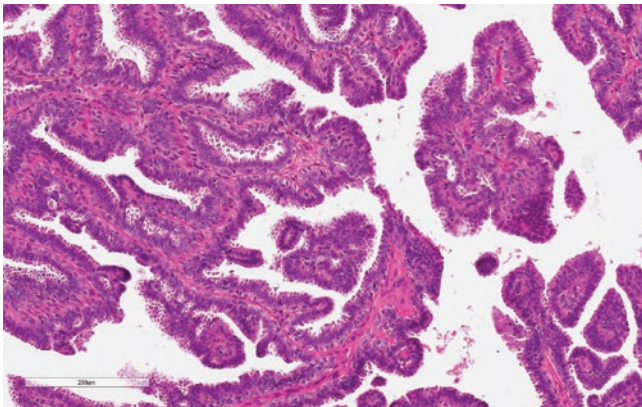**Fig. 1.13** Benign mammary-type phyllodes tumor in the vulva

Fig. 1.12 Hidradenoma papilliferum. High-power view shows myoepithelial cells and bland columnar epithelial cells

Fig. 1.14 Benign mammary-type phyllodes tumor in the vulva. GATA3 is positive

of the breast, numerous mitotic figures, inflammation, and calcification. Benign vulvar mammary-type tumors include hamartomas, fibroadenomas, benign phyllodes tumors, and intraductal papillomas (Figs. 1.13 and 1.14). Other benign apocrine vulvar tumors include apocrine cystadenoma, papillary apocrine fibroadenoma, apocrine tubular adenoma, and pigmented apocrine hamartoma.

A malignant mammary-like tumor can be seen but very rare. Mammary-like carcinoma is often large with frankly stromal invasion. It often has a smaller glandular architecture with cytological atypia such as enlarged nuclei and prominent nucleoli with readily seen mitosis (Fig. 1.15). Immunohistochemical studies with a panel of antibodies are useful to confirm the mammary-like carcinoma. Tumor cells are often positive for GATA3, ER, and PR. Rare cases of malignant transformation derived from hidradenoma papilliferum have been reported, including adenocarcinoma in situ, intraductal apocrine carcinoma, and adenosquamous carcinoma [23, 24].
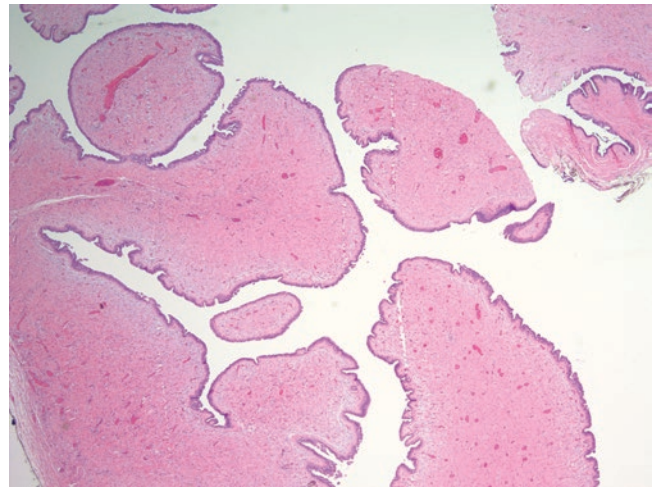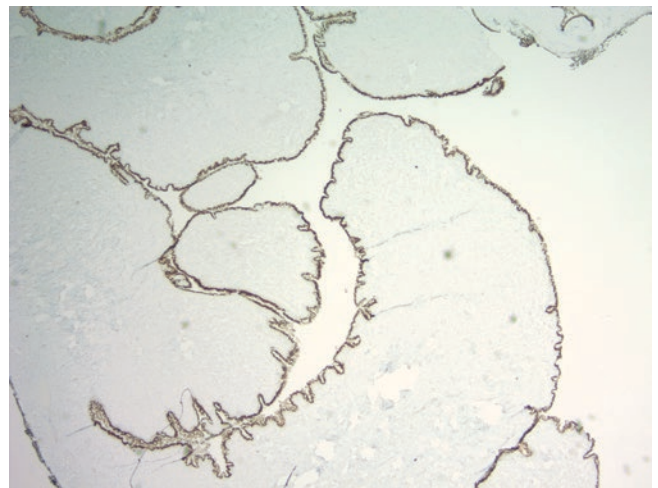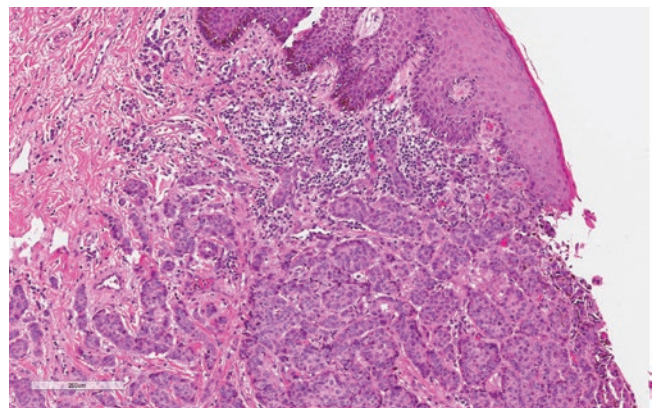

Fig. 1.15 Invasive mammary carcinoma in the vulva

6. What Are the Clinical and Pathological Features of Condyloma Acuminatum?

Condyloma acuminatum, also known as genital wart, is an exophytic papillary squamous lesion caused by low-risk type of HPV. HPV 6 and/or HPV 11 are detected in approximately 90% of genital warts, although co-infection with other low-risk or high-risk types of HPV is common [25–27]. Most condyloma acuminata occur in the moist areas of the labia minora and vaginal opening, but virtually all genital regions can be affected. Grossly condyloma acuminatum can be single or multiple, soft, and may appear as finger-like filiform, pearly dome-shaped, fungating, plaque-like, and usually non-pigmented [28, 29]. The size of condyloma acuminatum ranges from 1 mm to several centimeters.

Diagnosis of condyloma acuminata usually can be made clinically by physical examination. If the diagnosis is uncertain or atypical features are present, or the lesion is not responsive to the treatment, a biopsy is useful to confirm the diagnosis. Histological features of condyloma acuminatum include striking papillary architecture with fibrovascular cores, acanthotic squamous hyperplasia with hyperkeratosis, parakeratosis, hypergranulosis, and basal cell hyperplasia [30] (Fig. 1.16). Koilocytotic changes are typically present in the upper one-third layer of squamous epithelium with slightly enlarged, hyperchromatic nuclei and irregular, wrinkled nuclear membranes accompanied by perinuclear clearing (Fig. 1.17). The presence of koilocytotic changes facilitates the diagnosis of condyloma acuminatum, but it sometimes is focal and may not be seen in every case (Fig. 1.18). Mitotic activity is usually scattered and confined to the lower third of the epithelium. Some condyloma acuminatum needs to be distinguished from warty-type HPV-related high-grade squamous intraepithelial lesion, the latter usually has significant nuclear atypia and mitotic figures involving the full-thickness epithelium
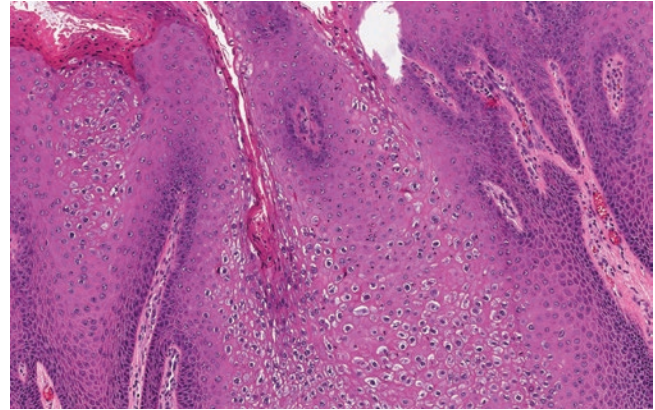

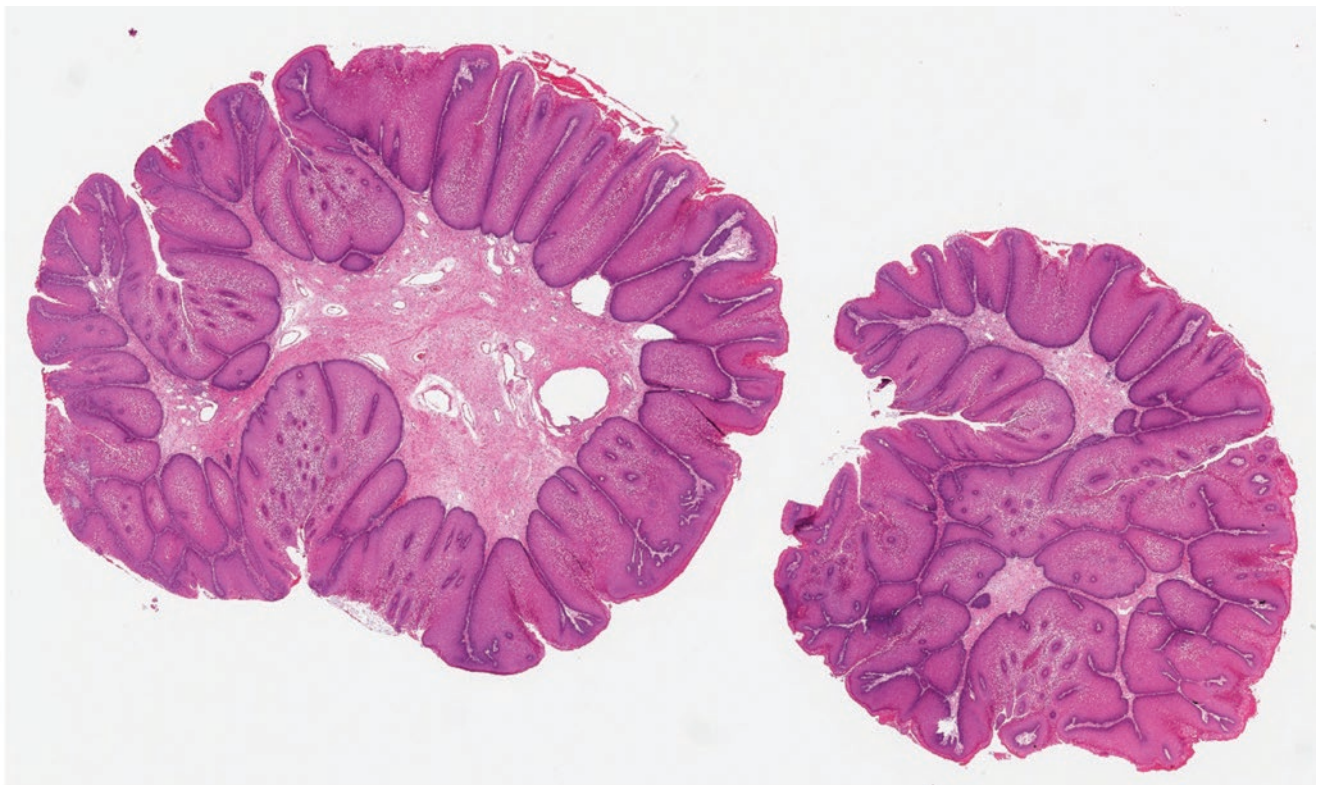**Fig. 1.17** Condyloma acuminatum. Note the koilocytotic changes

Fig. 1.16 Condyloma acuminatum. Low-power view shows typical papillary architecture
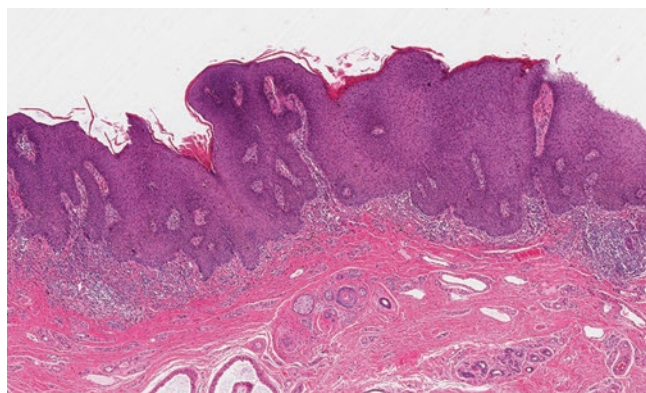
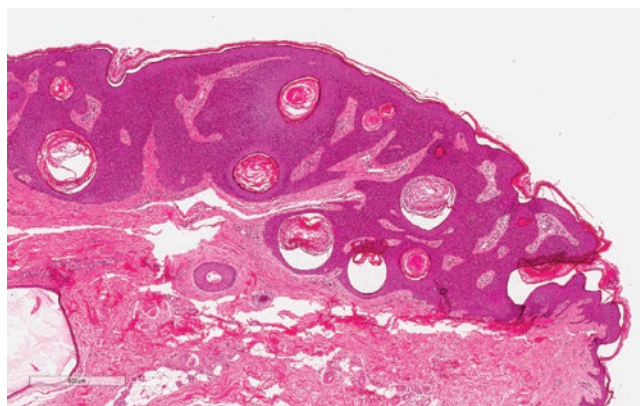**Fig. 1.18** Condyloma acuminatum without the koilocytotic changes
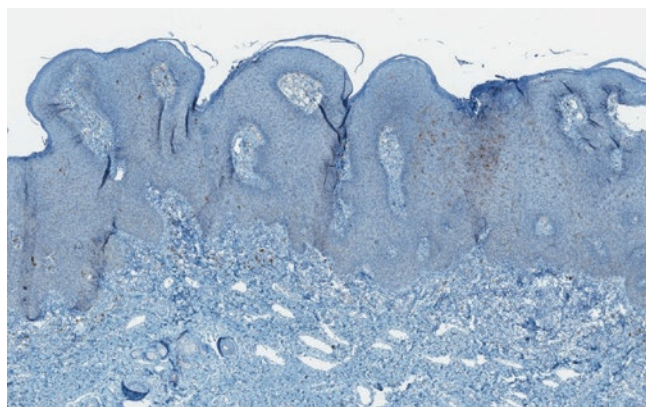

Fig. 1.20 Seborrheic keratosis

Fig. 1.19 Immunostain for p16 is weak and focal in condyloma acuminatum with features concerning for high-grade squamous intraepithelial lesion (uVIN)

Fig. 1.21 Seborrheic keratosis. Note the keratin filled horn cyst and absence of cytological atypia

and block-type p16 immunostaining pattern. Immunostaining for p16 is usually negative or focal and patchy in cases of condyloma (Fig. 1.19). Histologically some condyloma show features overlapping with seborrheic keratosis. Seborrheic keratosis may display variable papillomatosis, hyperkeratosis, and parakeratoses with no/minimal cytological atypia. Usually, pseudohorn cysts (intralesional cysts of loose keratin) are present and easily identified in seborrheic keratosis (Figs. 1.20 and 1.21). HPV positivity supports a diagnosis of condyloma acuminatum simulating seborrheic keratosis. In situ hybridization for HPV can be performed conveniently on paraffin-fixed tissue sections to detect low-risk HPV nucleic acid and establish the diagnosis of condyloma acuminatum.

Condyloma acuminatum is a benign lesion. After the initial appearance, the lesion may increase in number and size or regress spontaneously without treatment in up to 40% of cases [31]. Although treatment can eradicate the lesion, human papillomavirus infection may persist despite the resolution of visible warts and result in recurrence in 20–30% of patients overall [32, 33]. The risk of transformation from

condyloma acuminatum with a pure low-risk type of HPV infection to high-grade squamous dysplasia is vanishingly rare. Progression to VIN3 and invasive squamous cell carcinoma has been rarely reported in cases with a co-infection of high-risk HPV [34]. In the patients with known positive high-risk HPV tested in cervical PAP smears or patients with immunocompromised status, careful examination of each condyloma acuminatum for higher grade squamous dysplasia is required. If morphologically suspected, an immunohistochemical study with p16 helps identify high-grade squamous intraepithelial lesion. Condyloma acuminatum increases the risk of HIV transmission.

7. What Is the Difference Between Low-Grade Squamous Intraepithelial Lesion (VIN 1) and Condyloma?

Low-grade squamous intraepithelial lesion (LSIL) was proposed by the Lower Anogenital Squamous Terminology (LAST) Standardization project in 2012 and endorsed by the

2015 International Society for the Study of Vulvovaginal Disease (ISSVD). It is previously referred to as VIN1, mild squamous dysplasia, flat condyloma. There is general agreement that LSIL in the vulva is a poorly reproducible diagnosis with unclear behavior; the value or significance of the category is debatable [35, 36]. LSIL is commonly seen in the cervix but rare in the vulva. Based on the LAST study of the cervical squamous intraepithelial lesion, greater than 80% of cervical LSIL were caused by high-risk HPV, and approximately 10% of the patients eventually progressed into HSIL during follow-up. This is probably true in patients with vulvar LSIL, but there are limited studies on the vulva. In contrast, most vulvar condyloma acuminatum were caused by low-risk HPV; the risk of progression to high-grade dysplasia in an immunocompetent woman infected with only low-risk HPV is extremely rare to none. Unlike anal condyloma acuminatum, which often occurs in immunocompromised patients with a high frequency of co-infection of low-risk and high-risk of HPV types, vulvar condyloma usually occurs in immunocompetent women and with a much lower co-infection rate of low-risk and high-risk HPV types. Because of their different potentials of disease progression, it is essential to distinguish these two entities.

8. What Is the Difference in HPV Distribution in VIN1, CIN1, and VaIN1?

Although HPV infection is associated with low-grade squamous intraepithelial lesions in the cervix (CIN1), vagina (VaIN1), and the vulva (VIN1), the distributions of HPV subtypes are not the same. The distribution of low-risk types and high-risk types of HPV is 15% and 85% in CIN1, 25% and 75% in VaIN1, and 60% and 40% in VIN1 [37, 38]. In terms of disease distribution, condyloma acuminatum is much common in the vulva than in the cervix, whereas CIN1 is much more frequently seen in the cervix than VIN1 seen in the vulva. High-grade squamous intraepithelial lesion (VIN2-3) is much more frequently diagnosed pathologically than VIN1 in the vulva, whereas CIN1 is much more common than CIN2-3 in the cervix. The difference in disease distribution may reflect the difference in HPV subtype distribution and also may be explained by the physiological and pathogenic difference between the mucosa and the skin.

9. What Are the Etiology, Risk Factors, Clinical and Histological Features of HPV-Associated Squamous Intraepithelial Lesion?

The 2015 ISSVD terminology for vulvar squamous intraepithelial lesions proposed three terms [36]: low-grade squamous intraepithelial lesion (LSIL, flat condyloma, or HPV effect); high-grade squamous intraepithelial lesion (HSIL,

VIN usual type), and differentiated type (dVIN). 2020 WHO tumor classification [39] revises classifications based on the HPV status as squamous intraepithelial lesions, HPV-associated and vulvar intraepithelial neoplasia, HPV-independent (differentiated VIN). The former is further divided into low-grade squamous intraepithelial lesion (VIN1) and high-grade squamous intraepithelial lesion (VIN2 and 3, usual type).

HPV-related HSIL (uVIN) is the most commonly seen squamous lesion in the vulva and is associated with high-risk type HPV infection, especially HPV 16, which has been identified in >70% of cases [40]. Risk factors for HPV-related squamous intraepithelial lesions include early age of sexual intercourse and multiple sexual partners, cigarette smoking, herpes infection, and immunodeficiency or immunosuppression. HPV-related squamous intraepithelial lesions are often asymptomatic, although pruritus and/or pain may be noted. It usually presents with numbers of flat-topped papules, plaque-like, or verruciform lesions with variable color. Confluent or multifocal lesions exist in up to two-thirds of cases. In up to half the cases, synchronous or metachronous HPV-related intraepithelial or invasive squamous cell carcinoma are present in other sites, including the cervix, vagina, urethra, perineum, and anus [41].

Histologically, HPV-related HSIL (uVIN 2/3) can be flat or with spiking undulating surface with loss of squamous maturation; dysplastic squamous cells involves the lower two-thirds to the full thickness of the epithelium (Figs. 1.22, 1.23, 1.24, and 1.25). The dysplastic cells have a high nuclear/cytoplasm ratio with enlarged hyperchromatic nuclei, chromatin clumping, and nuclear pleomorphism. Increased mitotic activities are present beyond the basal layer with occasional abnormal mitosis. Dyskeratotic cells, apoptotic bodies, and poorly formed keratin pearls are frequently seen. Hair follicles and skin appendages are commonly involved. Based on the architecture and appearance of
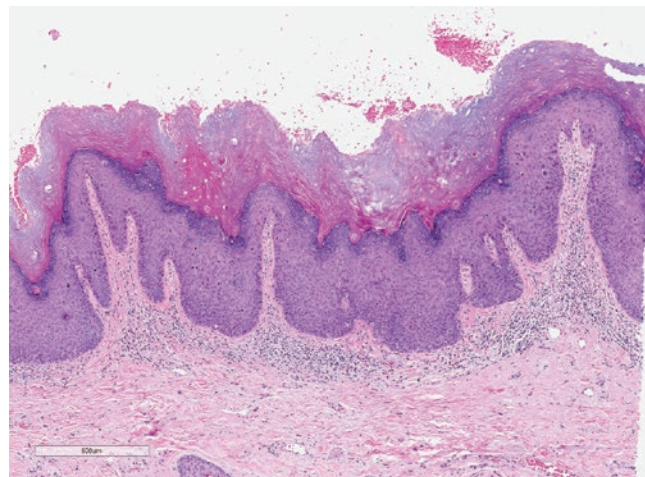

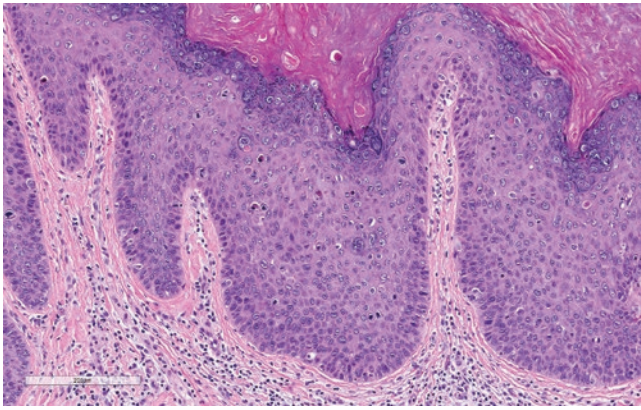**Fig. 1.22** HPV-associated HSIL. The surface is hyperkeratotic
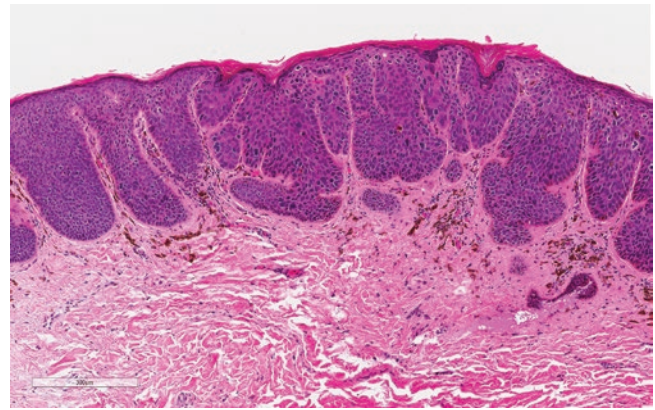
Fig. 1.23 HPV-associated HSIL. Note full-thickness atypia

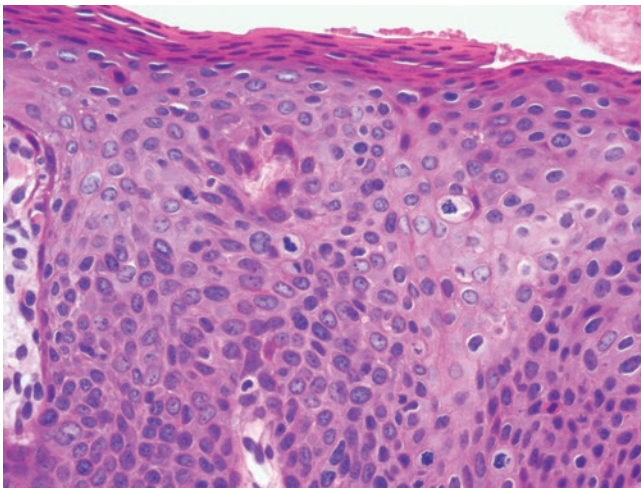**Fig. 1.26** HPV-associated HSIL with basaloid features

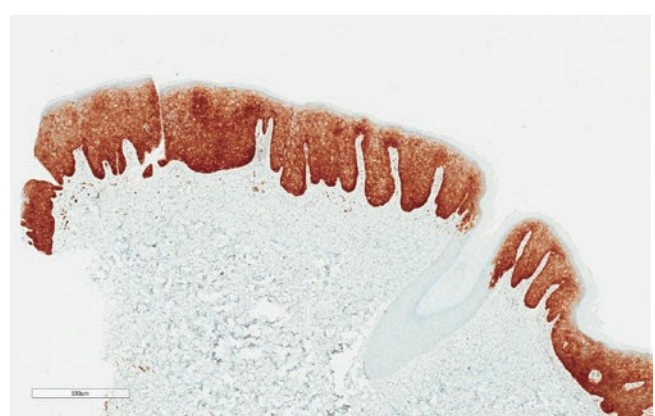**Fig. 1.24** HPV-associated HSIL, frequent mitotic figures

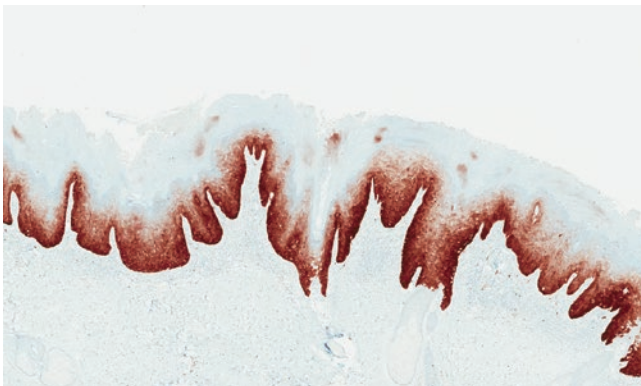**Fig. 1.27** HPV-associated HSIL with basaloid features, immunostain for p16

admixed in appearance. Warty uVIN can share some of the morphological features with condyloma acuminatum (Figs. 1.28 and 1.29). Dysplastic changes in the basal and parabasal layers of a warty or condylomatous lesion warrant a diagnosis of warty uVIN rather than condyloma accuminatum, which bears minimal cytological atypia. Pagetoid VIN is uncommon but important to recognize to avoid confusion with Paget's disease. Most uVIN is morphologically ready for rendering a diagnosis. When suspected, immunohistochemical stains shall be applied.

10. What Are Bowenoid Papulosis and Bowen Disease?

"Bowenoid papulosis" is a term clinically used to describe a relatively uncommon skin lesion of the genitalia of young. Most lesions are associated with high-risk HPV types, mainly HPV 16 but occasionally HPV 18 and other types. The vulva and the penis are more commonly involved than other sites. Bowenoid papulosis usually present as multiple
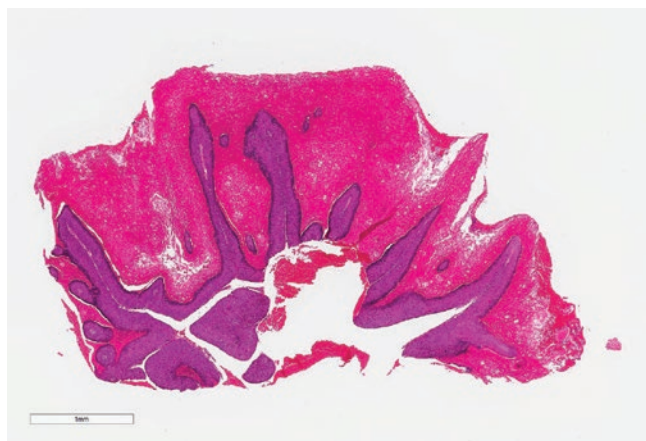

Fig. 1.25 HPV-associated HSIL, immunostain for p16 shows block-like pattern

the dysplastic squamous cells, some authors subdivide uVIN into flat basaloid and undulating warty variants [42] (Figs. 1.26 and 1.27). Such distinction has no known clinical significance; these variants are often synchronous and

Fig. 1.28 HPV-associated HSIL, warty type. Low power shows spiking surface with hyperkeratosis and parakeratosis

Fig. 1.30 HPV-independent VIN, low-power view with uninvolved spumous epithelium at the right edge
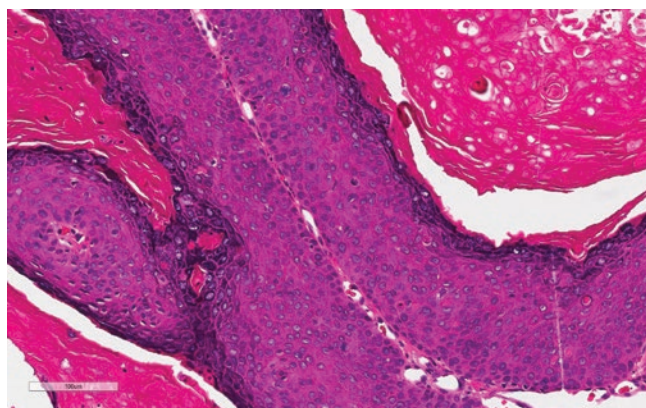

Fig. 1.29 HPV-associated HSIL, warty type. High power shows full-thickness atypia

small, firm, reddish-brown papules or plaques in the anogenital region. Classic lesions are sharply circumscribed and thus more easily treated by excision. It often regresses spontaneously, but 20% may recur after excision. Although clinically bowenoid papulosis resembles genital warts, histologically, it has a close resemblance to squamous cell carcinoma in situ. Bowen disease refers to squamous cell carcinoma in situ and is characterized by full-thickness atypia. However, due to a lack of distinct histological features, the use of this term is discouraged in the gynecological pathology, and the lesion is classified as vulvar intraepithelial neoplasia (VIN). Dermatologists still recognize Bowenoid papulosis as a distinct clinical variant.

11. What Are the Etiology, Risk Factors, Clinical and Histological Features of HPV-Independent Vulvar Intraepithelial Neoplasia (dVIN)?

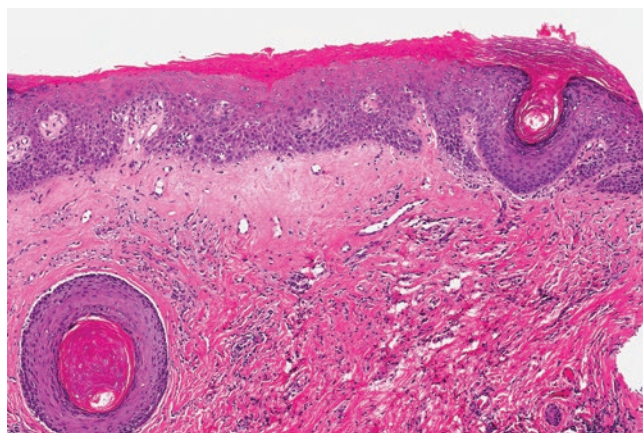Differentiated VIN (dVIN) is a high-grade squamous intraepithelial lesion and considered the putative precursor of non-HPV-related vulvar squamous cell cancer. Although this type of VIN comprises only less than 5% of all VIN lesions, it is probably the precursor of the most vulvar squamous cell carcinoma in postmenopausal women. The etiology for developing dVIN is still largely unknown. Chronic inflammatory vulvar diseases are considered as the main risk factors. Other possible risk factors include older age, vulvar irritation, and other oxidative and ischemic stress [43].

Clinically dVIN is usually seen in postmenopausal women with a mean age of 65 years. The lesion of dVIN is usually unifocal and unicentric and often associated with long-standing of lichen sclerosis or lichen planus. Differentiated VIN is found adjacent to vulvar squamous cell carcinomas in up to 80% of cases. Compared to uVIN, dVIN has a higher potential of malignant progression (33% in dVIN versus 5% in uVIN) to squamous cell carcinoma and over a shorter time frame [43, 44].

The histological features of dVIN are often subtle, making it challenging to distinguish dVIN from benign reactive squamous conditions. The involved squamous epithelium is usually flat with retained squamous maturation and normal thickness or slightly thickened or even with atrophic appearance. The characteristic morphological features include parakeratosis with loss of granular layer, atypical keratinocytes at the basal and parabasal layers, and prominent intercellular bridges or spongiosis [45, 46] (Figs. 1.30, 1.31, 1.32, and 1.33). The atypical cells are characterized with abundant eosinophilic cytoplasm, enlarged atypical vesicular nuclei with prominent nucleoli. Individual dyskeratotic cells with pink cytoplasmic keratin-like materials in the lower half near the basal portion of the epithelium and later forming small whorled keratin pearls (paradoxical maturation) can be seen. Scattered mitoses usually present in the basal layer but can extend to parabasal, even the intermediate levels of the epithelium. Unlike HPV-associated high-grade squamous intraepithelial lesions, skin appendages are usually not involved in dVIN. In more than 2/3 cases, dVIN is associated with lichen sclerosis. Therefore, dVIN should be carefully ruled out in any cases of lichen sclerosis in the vulvar biopsy.
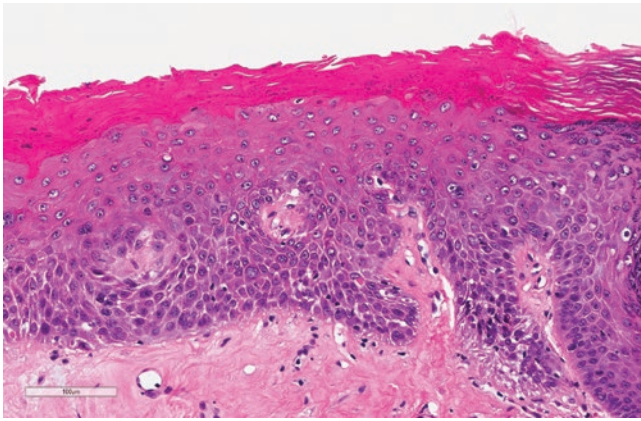
Fig. 1.31 HPV-independent VIN, high-power view with atypia of the basal cells
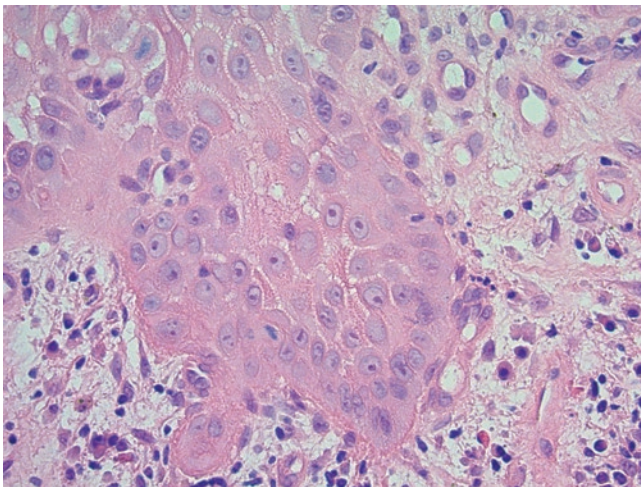

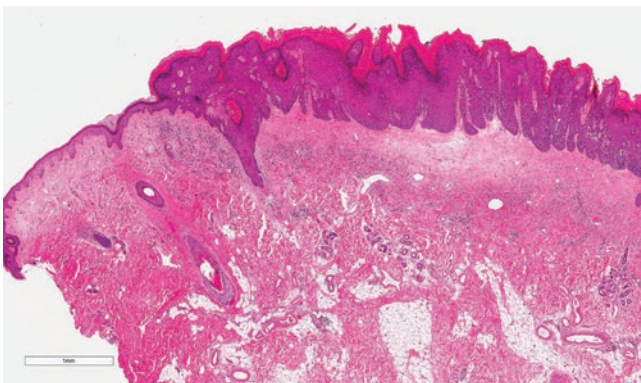**Fig. 1.32** HPV-independent VIN, note the prominent intercellular bridges and nuclear atypia

Fig. 1.33 HPV-independent VIN in a background of LS

12. What Is the Role of Immunohistochemistry in the Diagnosis and Classification of VIN and Vulvar SCC?

Although there are distinct morphological features between uVIN and dVIN in most cases and different associations with either HPV infection or lichen sclerosus, the distinction between these two entities is not always clear-cut due to overlapping histological and clinical features. Immunohistochemistry and occasionally HPV in situ hybridization can be helpful.

P16 P16 is a commonly used surrogate marker for oncogenic HPV infection. After viral integration into the host genome, inactivation of Rb by the viral E7 oncoprotein leads to overexpression of p16. There are three p16 immunostaining patterns: negative, focal/patchy, and diffuse block staining patterns [47–50]. The diffuse p16 staining pattern is defined as diffuse, strong, and continuous nuclear and cytoplasmic staining, also known as a "block-like" staining pattern (Figs. 1.26 and 1.28). This pattern should involve at least the lower half of the epithelium in the vulva (basal, parabasal, and most intermediate layers). The granular layer, surface hyperkeratosis, or parakeratosis are usually negative for p16. Diffuse p16 staining pattern is seen in HPV-associated high-grade squamous intraepithelial lesion (uVIN2-3). Focal/patchy p16 staining pattern is defined as scattered, non-continuous, mainly cytoplasmic and/or nuclear staining. Focal/patchy p16 staining pattern is usually seen in low-grade squamous intraepithelial dysplasia (VIN1) and condyloma acuminatum [51, 52]. Negative p16 staining pattern, no p16 immunoreactivities in the lesional cells, is usually seen in dVIN and other benign vulvar lesions not related to HPV infection.

TP53 Mutation of the TP53 gene is frequently seen in HPV-independent vulvar intraepithelial neoplasia/SCC [50, 53]. The p53 alterations can be detected by immunohistochemistry with an antibody reacting to both wild-type and mutant p53 proteins. Because wild–type p53 protein has a half-life as short as less than 20 min, p53 protein can be detected immunohistochemically only in a small subset of normal or benign tumor cells without harboring p53 mutation. In non-neoplastic vulvar squamous epithelium, p53 staining shows immunoreactivity in less than 10% of the nuclei in the basal epithelial layer with low staining intensity, representing the normal tissue amount of wild-type p53 protein [54]. In contrast, mutant p53 proteins have a prolonged half-life up to 20+ h; therefore, mutant p53 proteins can be detected immunohistochemically in most tumor cells harboring missense
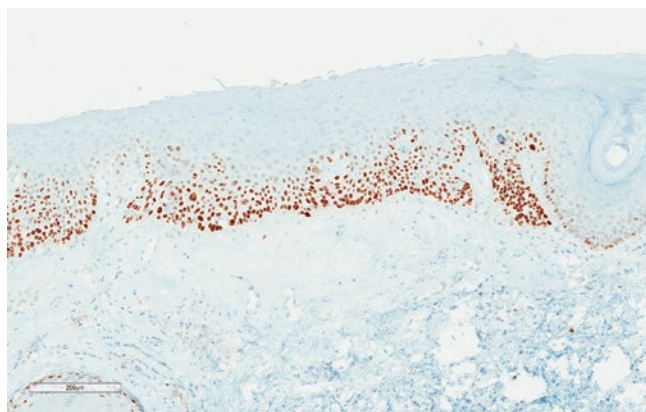
Fig. 1.34 Immunostain of p53 in HPV-independent VIN (missense mutation)

Fig. 1.35 Immunostain of Ki-67 in high-grade squamous intraepithelial lesion

Fig. 1.36 Differentiated VIN with basaloid morphology simulating uVIN

point mutations. Allelic deletion or nonsense mutations, resulting in a total lack of p53 protein production, were seen in a small percentage of vulvar dVIN/SCC. Two patterns of aberrant p53 immunostaining have been described corresponding to two different types of mutations: strong and diffuse pattern (>85% of tumor cells with strong nuclear staining) for missense mutations and completely negative pattern (null-pattern) for nonsense mutations. A totally negative p53 staining pattern is seen in 25–30% of dVIN and vulvar SCC not related to HPV infection [55].

P53 immunostaining pattern in dVIN is not as apparent as seen in invasive SCC. The typical p53 staining pattern seen in dVIN is increased nuclear p53 labeling in >90% of basal layer cells and extended to parabasal layers or intermediate layers. In most cases of dVIN, p53 staining is limited in the lower 1/3 or lower half of the neoplastic epithelium [50, 53, 56] (Fig. 1.34). In the dVIN cases with p53 nonsense mutation, a total lack of p53 immunostaining can be easily misinterpreted. Besides, p53 immunostain has its limitations. On the one hand, p53 overexpression can occur in longstanding LS and squamous hyperplasia; on the other hand, the normal epithelium can show very focal p53 staining, which could be confused with null-pattern [54, 57]. The staining pattern of p53 has to be interpreted with caution. A careful comparison with adjacent wild-type p53 staining pattern is helpful. In uVIN, the p53 staining pattern usually is similar to that of the adjacent normal epidermis.

Ki-67 Ki-67/MIB-1 may be a helpful marker to distinguish high-grade squamous dysplasia from squamous cell hyperplasia and normal epithelium. Both uVIN and dVIN show increased positive staining for Ki-67 in the basal and suprabasal layers, while the basal cell layer is characteristically negative for ki-67 in normal epithelium. The staining for Ki-67 in uVIN usually involves the full thickness of the epi-
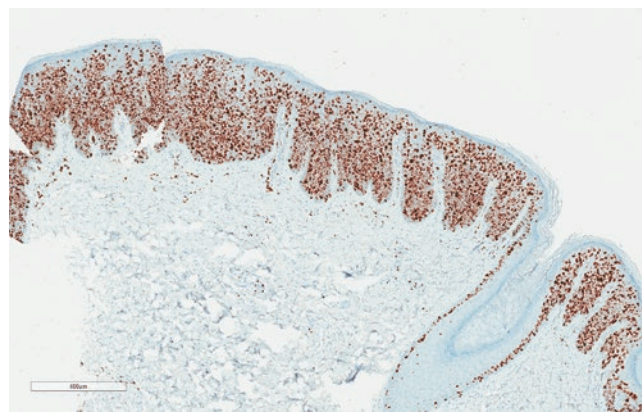
thelium (Fig. 1.35). In dVIN, Ki-67 staining is positive in the basal layer and a thin parabasal layer, which contrasts with the basal expression seen in LS [44, 58]. Other possible ancillary markers, including ProEx C, telomerase, β-catenin, osteopontin, sox2, and cyclin-D1, have been reported in a limited number of studies. Their diagnostic values need to be assessed with larger number of cases.
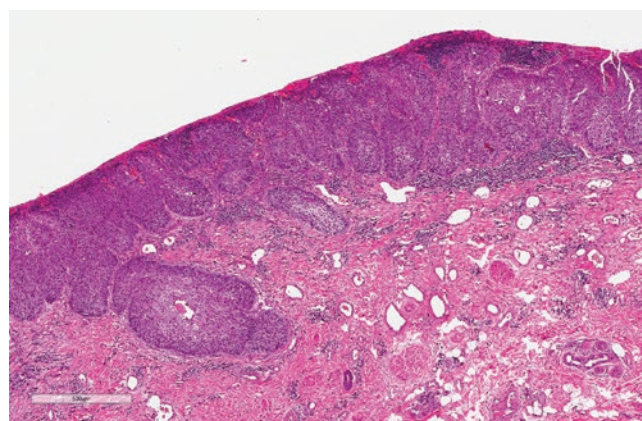
Take-Home Message Combined with clinical history and morphology, immunostain of p16 and p53 may be helpful to distinguish uVIN and dVIN. uVIN usually has a p16-positive/p53 wild-type/Ki-67 high immunoprofile, while dVIN usually has a p16-negative/p53 aberrant/Ki-67 high immunoprofile (Figs. 1.36, 1.37, and 1.38). However, the overlap of clinical features, morphological features, and immunoprofile exists. Cases of uVIN with aberrant p53 staining pattern and cases of LSIL with positive p16 staining have been reported. It is essential to keep this in mind to avoid misinterpretation.
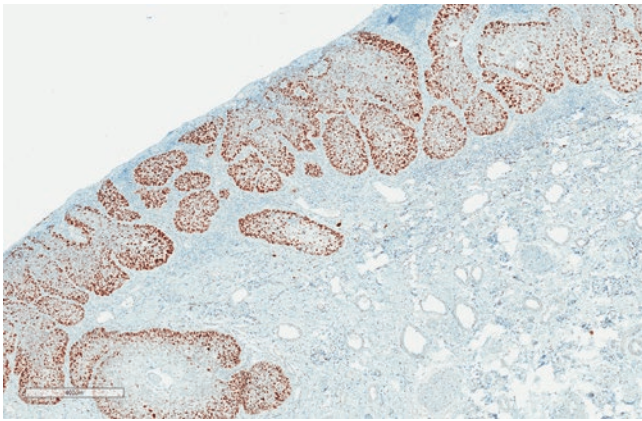
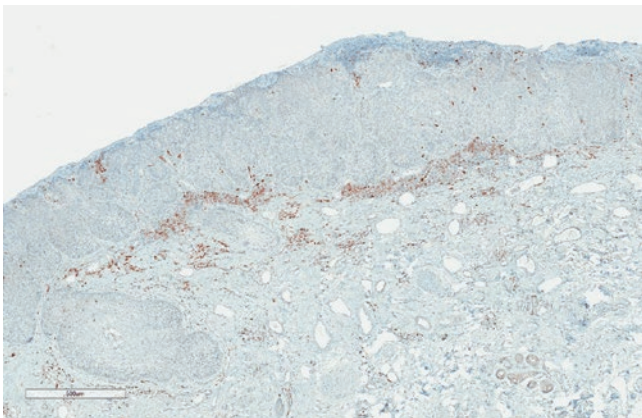**Fig. 1.37** Differentiated VIN with basaloid morphology, aberrant p53 immunostain

Fig. 1.39 HPV-related HSIL (uVIN2)

Fig. 1.38 Differentiated VIN with basaloid morphology, negative p16 immunostain

13. What Is the Sensitivity and Specificity of the HPV In Situ Hybridization Test? When Should We Apply the HPV In Situ Hybridization Test in the Practice?

The detection of high-risk HPV in tissue samples containing squamous cell carcinoma is important for their classification and prognosis. Immunohistochemistry for p16 is a commonly used surrogate marker for HR-HPV because of its high sensitivity, widespread availability, and low cost. However, its specificity is relatively low. So HPV-specific nucleic acid tests are often used in conjunction with immunohistochemistry for p16, particularly in cases with potential false-positive or borderline p16 immunohistochemical results, and cases may have non-HPV-related p16 overexpression [59–62].

In situ hybridization (ISH) is a viral nucleic acid-based molecular test that can directly detect HPV while preserving the morphology features of the lesions. A historic issue concerning the use of DNA ISH for viral detection has been its relatively low 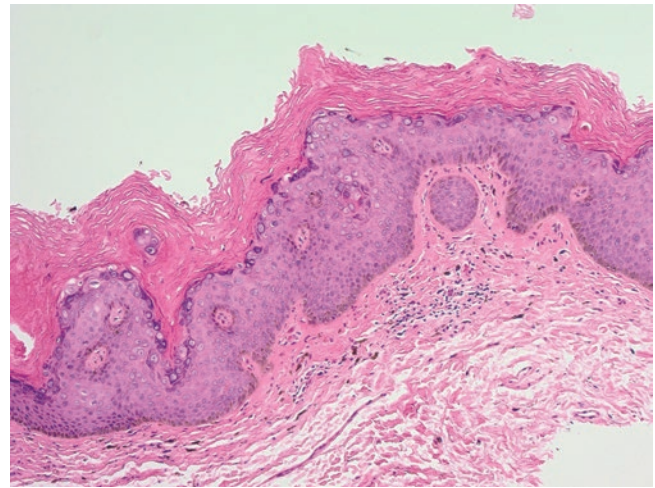sensitivity. Recent advances have improved signal-detecting methods with higher sensitivity. Detection of transcriptionally active HPV oncogenes E6/E7 is regarded as the gold standard for the presence of clinically relevant high-risk human papillomavirus. RNA ISH that detects E6/E7 mRNA has superior analytical sensitivity (≥97%) and specificity (93%) [59, 63]. The most likely reason for the superior performance of RNA ISH, compared to DNA ISH, is attributed to its abundance of the target mRNA, detection of many types of HPV in one reaction, and the technical aspects of signal amplification used in the RNA ISH tests [64]. Currently, commercially available RNA ISH tests on FFPE tissues can qualitatively detect viral mRNA in up to 28 HPV subtypes including low-risk cocktail (10 subtypes: 6, 11, 40, 43, 44, 54, 69, 70, 71, 74) and high-risk cocktail (18 subtypes: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) [63].

Take-Home Message HPV RNA ISH can be a useful ancillary tool in the following clinical scenarios: (1) vulvar papillary lesions suspicious for condyloma acuminatum; (2) differential diagnosis between warty VIN and condyloma acuminatum; (3) uVIN with ambiguous morphological features; (4) uVIN with equivocal p16 immunostaining pattern; (5) other situations need to know the HPV status or HPV low-risk versus high-risk types (Figs. 1.39 and 1.40). However, HPV ISH has no role in grading VIN since most VIN1 and almost all VIN2-3 result from an infection of high-risk type HPV.

14. What Are the Progression and Prognosis of HPV-Associated and HPV-Independent VIN/SCC?

Vulvar SCC accounts for about 4% of female reproductive tract cancer [65]. It is well accepted that there are two different pathways for the pathogenesis of vulvar squamous cell

Fig. 1.40 Positive RNA ISH for high-risk HPV in HPV-related HSIL (uVIN2)

carcinoma. However, morphological features overlap and cannot be used reliably to differentiate vulvar SCC derived from two different pathways [66, 67].

The HPV-associated VSCC usually occurs in women less than 50 years of age, and the incidence has increased from 2–5% to more than 30% of all vulvar SCC cases in the last decade [68]. More than 90% of all VIN cases are uVIN, but only 3–10% of them may progress to carcinoma, while high-risk HPV is found in less than 40% of vulvar SCC [69]. The associated invasive SCC is often non-keratinizing, basaloid, or warty in morphology and tends to be multifocal. It is usually diffusely and strongly positive for immunohistochemical stain for p16. Similar to head and neck squamous cell carcinoma, vulvar SCC associated with HPV has a better prognosis than the non-HPV-related SCC with overall improved survival and less recurrence [68, 70].

HPV-independent SCC is related to chronic inflammatory or autoimmune processes and involves differentiated VIN (dVIN). Differentiated VIN usually occurs in older women and only accounts for 2–16% of all VIN cases. Although dVIN is rare, it has a higher rate (up to 80%) to progress to invasive carcinoma. The associated invasive SCC is usually keratinizing and commonly associated with p53 mutations. The non-HPV-related vulvar SCC is a more aggressive clinically and prone to early metastasis and worse prognosis compared to the HPV-associated type.

Take-Home Message The distinction between uVIN and dVIN is important because dVIN has a greater risk of rapid transit to vulvar squamous cell carcinoma. Furthermore, dVIN-associated vulvar cancers have an increased risk of recurrence and higher mortality than those SCC arising from uVIN. A recent meta-analysis and review suggest that p53 and especially p16 expression status are of prognostic importance

Table 1.1 Summary of the features of HPV-associated and HPV-independent squamous cell carcinoma and their precursors

	HPV-associated squamous cell carcinoma	Non-HPV-associated squamous carcinoma
Prevalence	less common (approximately 1/3)	More common (approximately 2/3)
Age	Younger	Elder
Etiology	High-risk HPV infection (HPV 16 in >70% of the cases)	Unclear, often associated with lichen sclerosus
Pathogenesis	HPV viral oncoproteins E6 and E7 lead to degradation of p53 and RB1	Somatic p53 mutation in 80% of the cases
Risk factor	Cigarette smoking, compromised immune status	Vulvar dermatoses
Gross	Exophytic lesion, multifocality is common	Exophytic mass may be ulcerated
Histological features	Usually non-keratinizing, 1/3 may be keratinizing	Usually keratinizing with keratin pearl formation
Immunohistochemistry	P16 shows block staining, p53 shows wild-type pattern	Aberrant p53 staining pattern, p16 is typically negative
Associated vulvar intraepithelial neoplasia	uVIN	dVIN
Association with vulvar dystrophy	Rare	Common
Clinical outcome	Better outcome	Recurrence rate is higher, rapid progression is common

in women diagnosed with vulvar SCC. Table 1.1 summarizes the features of HPV associated and non-HPV-associated VIN and squamous cell carcinoma in the vulva.

15. What Are the Diagnostic Features for Stromal Invasion and Patterns of Invasive Squamous Cell Carcinoma?

Vulvar squamous cell carcinoma (SCC) is the most common histological type of vulvar cancer, comprising about 80% of vulvar malignancies [71, 72]. Gross features of vulvar carcinoma are highly variable. Women with vulvar SCC typically present with a unifocal vulvar plaque, ulcer, or mass (fleshy, nodular, or warty) on the labia majora; the labia minora, perineum, clitoris, and mons are less frequently involved. The tumor may be unrecognizable grossly, as in cases where

occult carcinoma is found in resections for a diagnosis of VIN. Lesions are multifocal in 5% of cases. A synchronous second malignancy, most commonly cervical neoplasia, is found in up to 22% of patients with a vulvar SCC [72].

Histological examination is necessary to confirm the diagnosis of invasive carcinoma and assess the depth of stromal invasion. Accurate diagnosis of superficial invasion can be challenging, especially on the biopsies. Tangential section and appendageal involvement may mimic stromal invasion. The appropriate orientation of the specimen helps illustrate the lesion and accurately measure the depth of invasion. Evidence of an irregular, angulated border/contour, individual tumor cells, paradoxical maturation, a disrupted basement membrane, and associated stromal reaction helps distinguish true invasion from VIN with the tangential cut (Figs. 1.41, 1.42, 1.43, and 1.44).

Vulvar SCC can have different morphological presentations, including keratinizing, warty, basaloid, and verrucous features. The majority of squamous cell carcinomas of the vulva are well-differentiated, keratinizing, and often arise on a background of lichen sclerosus with or without associated VIN (Figs. 1.45 and 1.46). In most cases, the surface is ulcerated (Fig. 1.47). The morphology of these vulvar SCC is identical to those occurring elsewhere on the skin, consisting
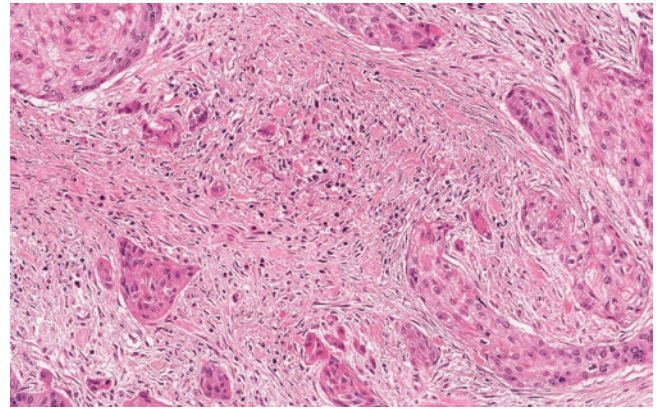

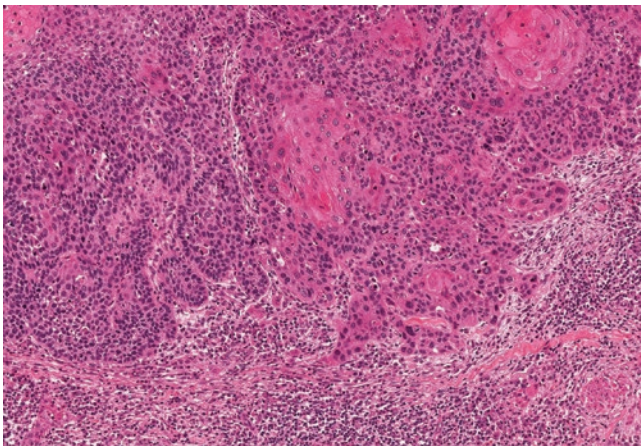**Fig. 1.43** Invasive squamous cell carcinoma, individual tumor cells

Fig. 1.41 Squamous cell carcinoma with superficial invasion. Note the disrupted basement membrane
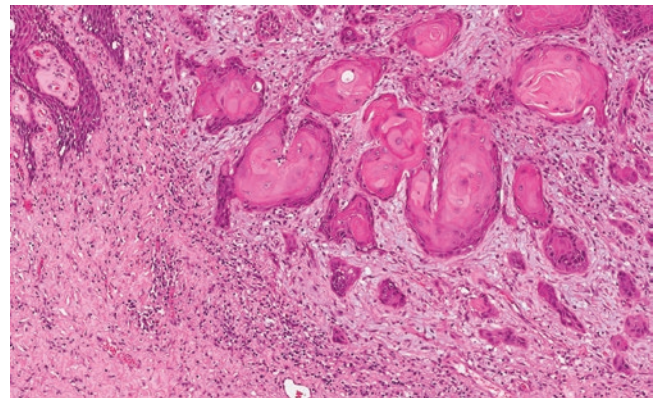

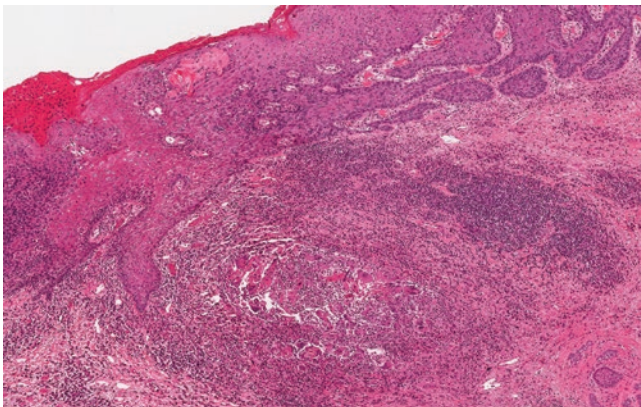**Fig. 1.44** Invasive squamous cell carcinoma. Note the paradoxical maturation

Fig. 1.42 Squamous cell carcinoma with superficial invasion and reactive stromal changes
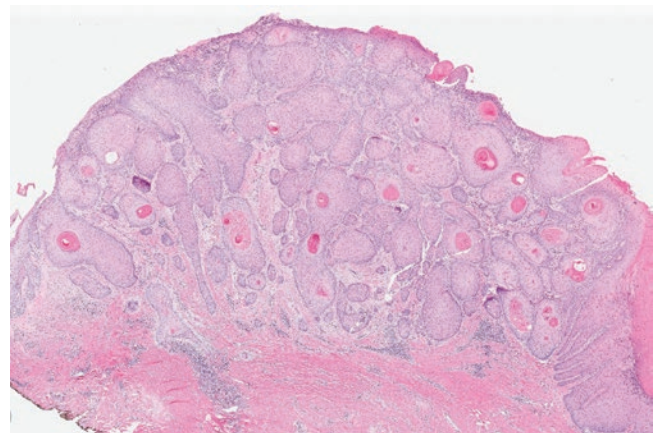

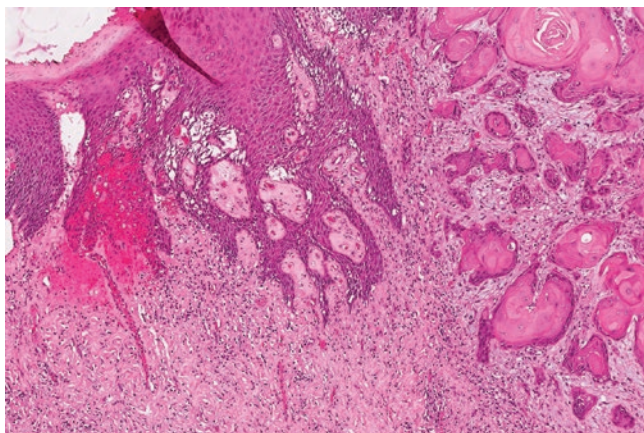**Fig. 1.45** Invasive keratinizing squamous cell carcinoma

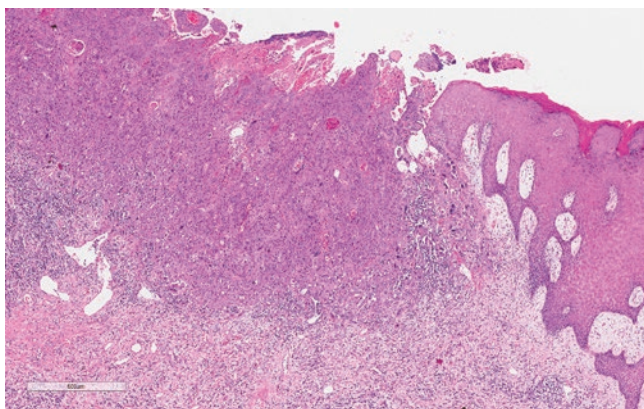**Fig. 1.46** Invasive keratinizing squamous cell carcinoma and associated lichen sclerosis

Fig. 1.47 Poorly differentiated vulvar SCC with surface ulceration

cytoplasm and oval nuclei containing evenly distributed coarsely granular chromatin (Figs. 1.48 and 1.49). Verrucous-type SCC is part of the spectrum of HPV-independent carcinomas and is characterized by verruciform growth pattern with pushing broad border of invasion. Verrucous SCC is well-differentiated with prominent hyper- and parakeratosis and variable keratinization. The tumor cells have abundant eosinophilic cytoplasm and minimal nuclear atypia (Figs. 1.50 and 1.51). Sarcomatoid changes and other uncommon findings may also present in vulvar squamous cell carcinoma (Figs. 1.52 and 1.53).

Take-Home Message Although there is an association of certain morphological patterns in vulvar SCC with either dVIN or uVIN, there are also tremendous overlapping mor-
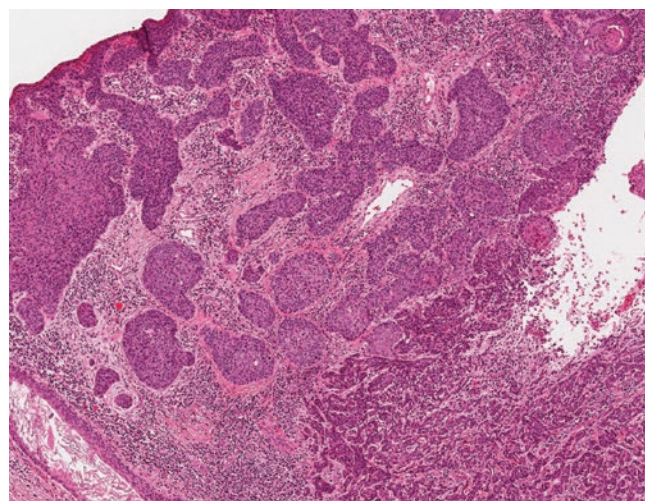

Fig. 1.48 Invasive squamous cell carcinoma, basaloid type, low-power view
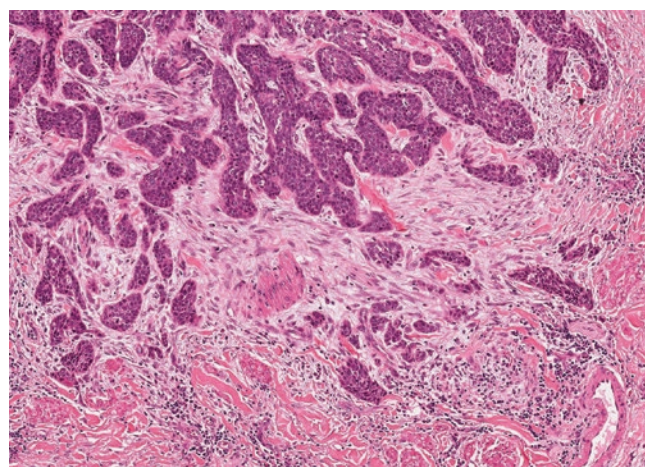
of irregularly shaped tongues and nests of squamous cells with abundant eosinophilic cytoplasm frequently showing whirling keratin pearls. The cells may invade in broad fronds or narrow finger-like projections with small clusters and single cells interspersed in irregular patterns. The nuclei show the same atypical features as those of dVIN. Stroma surrounding the invasive SCC often shows a desmoplastic response with chronic inflammation. Warty SCC and Basaloid SCC are significantly less common and are often associated with high-risk HPV infection and uVIN. About one-third of HPV-associated SCC also have keratinizing features. Warty SCC is distinguished by its exophytic papillary architecture. At the base of the lesion, irregularly shaped nests containing dyskeratotic cells and frequently keratin pearls are seen, similar to ordinary squamous cell carcinomas, but usually with a greater degree of nuclear pleomorphism and cytological atypia. Basaloid carcinoma consists of variably sized nests of basaloid squamous cells showing little to no maturation. The cells are relatively uniform, with scant

Fig. 1.49 Invasive squamous cell carcinoma, basaloid type with desmoplastic stroma
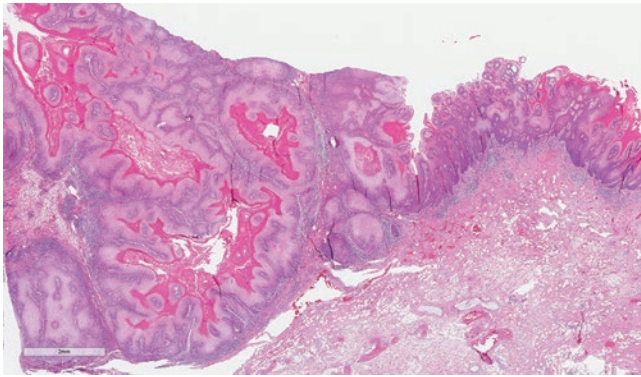
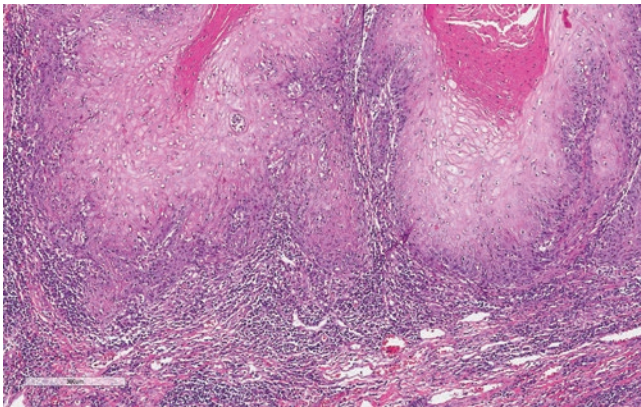**Fig. 1.50** Invasive squamous cell carcinoma, verrucous type

Fig. 1.51 Invasive squamous cell carcinoma, verrucous type. Note the pushing broad front, and minimal cytological atypia
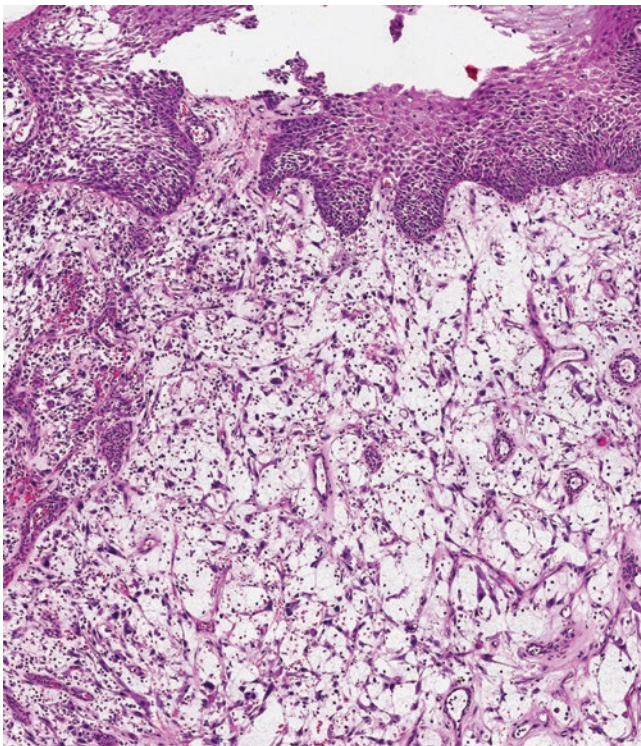

Fig. 1.52 Invasive squamous cell carcinoma with sarcomatoid features
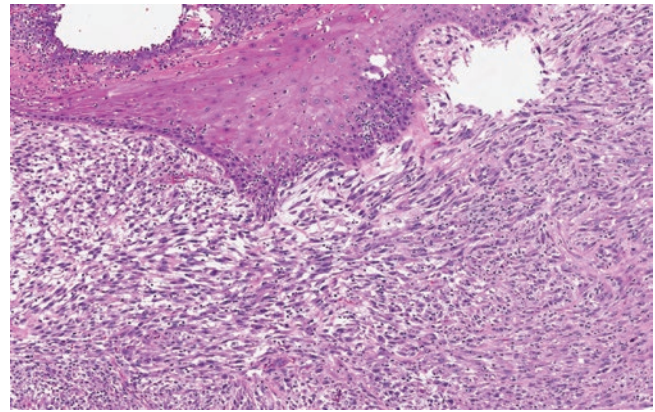

Fig. 1.53 Invasive squamous cell carcinoma with spindle cell features

phological features, especially in the late stage of vulvar SCC. Therefore, without the presence of adjacent VIN lesion or assistance of p53 and p16 immunohistochemistry, it is unreliable to define whether an invasive SCC is derived from dVIN or uVIN solely based on morphological features.

16. How to Measure the Depth of Invasion and Tumor Thickness in an Invasive SCC and their Value in Staging and Treatment?

The depth of invasion of squamous cell carcinoma is an important parameter for tumor staging and management, especially for small tumors. The depth of invasion is measured from the epithelial-stromal junction of the adjacent, most superficial dermal papilla to the deepest point of invasion (Fig. 1.54). The tumor thickness of an invasive squamous cell carcinoma is a separate measurement that is not used in staging. It is measured from the surface of the tumor or, if there is surface keratinization, from the bottom of the granular layer to the deepest point of invasion.

Based on the current AJCC and FIGO staging system, T1 lesions are tumors confined to the vulva with no extension to adjacent perineal structures. Based on the tumor size and, more importantly, the depth of invasion, T1 is further divided into T1a if the tumor is 2 cm or smaller and with stromal invasion no more than 1 mm, and T1b for tumor either greater than 2 cm or with stromal invasion greater than 1 mm. T1 patient will be managed with wide local excision. The importance of the depth of invasion in this group of patients is that it will decide whether they need an inguinal lymph node assessment [73, 74]. If clinically no palpable nodes on groin examination, for patients with stage T1a tumor, no lymphadenectomy is performed; for patients with stage T1b or higher tumor, inguinal lymphadenectomy is performed because of significantly increased risk of inguinofemoral lymph node metastases.
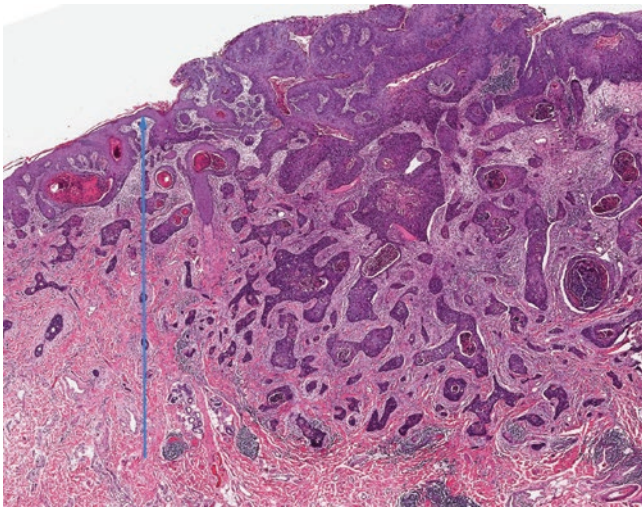
Fig. 1.54 Depth of stromal invasion of spumous cell carcinoma (blue line)

17. What Are the Clinical and Characteristic Histological Features of Extramammary Paget Disease?

Extramammary Paget disease (EMPD) is a rare malignancy accounting for about 1% of all vulvar cancers [75]. It most commonly involves the vulva in postmenopausal Caucasian women. The cell origin for primary EMPD is unclear and controversial. Different theories suggest EMPD may originate from either intraepidermal apocrine, or eccrine glands, or from pluripotent keratinocyte stem cells, or Toker cells of the epidermis [76, 77]. EMPD can be either a primary cutaneous carcinoma or a secondary carcinoma resulting from the epidermotropic spread or metastasis of an underlying internal malignancy. While mammary Paget's disease is almost always associated with underlying breast cancer, most primary EMPD is primary without underlying malignancy. Approximately 10–30% of EMPD cases represent secondary vulvar involvement by an underlying colorectal or urothelial cancer [76].

Vulvar EMPD usually presents as a less well-demarcated, red and thickened, eczematous plaques. Symptoms include longstanding tenderness, itching, irritation, and burning sensation. Early lesions are usually confined to the labia, but longstanding lesions may spread to the mons, clitoris, urethra, vagina, perianal area, and medial aspect of the thighs. It is usually multifocal, and the appearance is nonspecific, often confused with other vulvar rashes [78]. Vulvar biopsy should be performed in patients with suspicious lesions, including those with persistent pruritic eczematous lesions that fail to resolve with appropriate therapy.

Diagnosis is based upon characteristic histopathological features. The typical Paget cells are enlarged with abundant pale amphophilic cytoplasm, vesicular nuclei, and prominent nucleoli (Fig. 1.55). The pale cytoplasm of Paget cell is usually finely granular and contains intracytoplasmic mucin. Signet-ring cells with abundant cytoplasmic mucin occasionally are present (Fig. 1.56). Paget cells are typically distributed at the basal and suprabasal zone of the epidermis either singly or in nests but can spread into full epidermal thickness (Fig. 1.57). The pilosebaceous units and the hair follicles are involved in almost all cases (Fig. 1.58). The Paget cells appear not to be connected with the basement membrane and thus are different from melanoma in situ. There are no features of squamous differentiation, no visible intercellular bridges. Mitotic figures may be seen. Most Paget disease is confined to the epidermis; approximately 10–15% EMPD is dermal invasive [79–82] (Fig. 1.59). When Paget cells are few, they
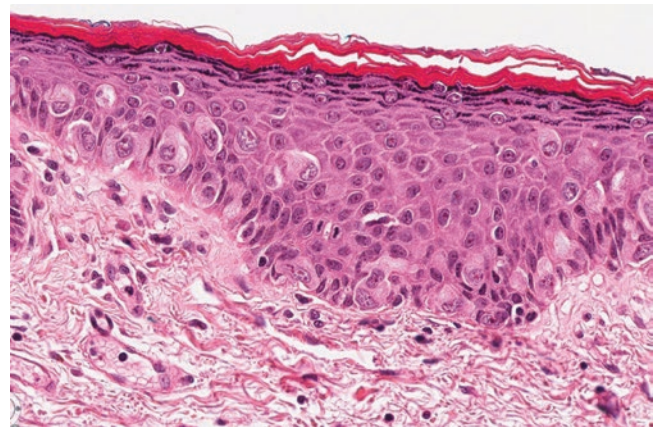

Fig. 1.55 Primary extramammary Paget disease. Typical pagetoid involvement of the basal layer of the epidermis
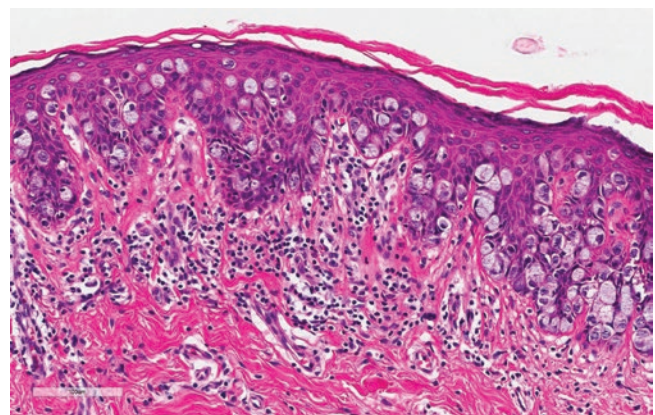

Fig. 1.56 Primary extramammary Paget disease. Signet ring-type tumor cells with abundant intracytoplasmic mucin