Diseases of the Vulva

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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 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.

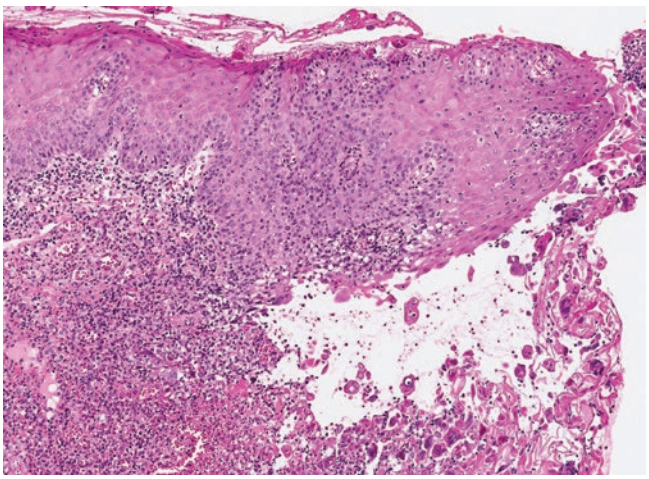

Fig. 1.1 Herpetic ulcer of vulva (low magnification)

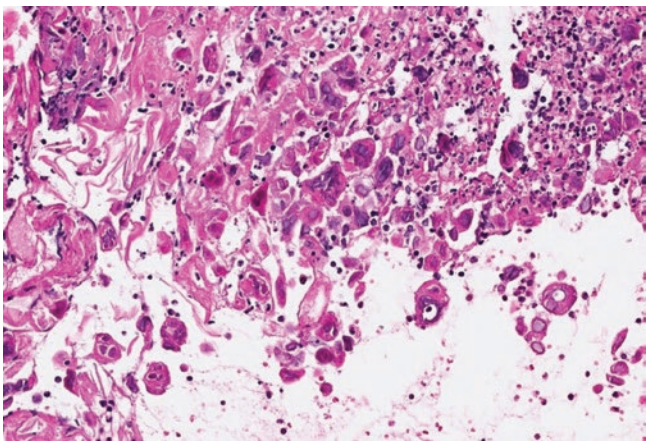

Fig. 1.2 Herpetic ulcer of vulva (high magnification). Note the multinucleation and ground-glass appearance of the nuclei

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

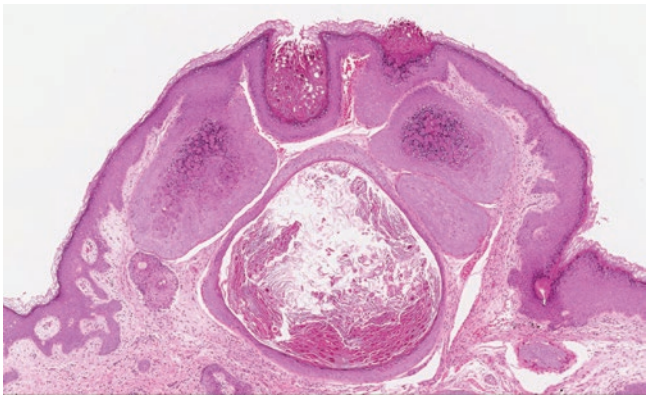

Fig. 1.3 Molluscum contagiosum, typical histological features

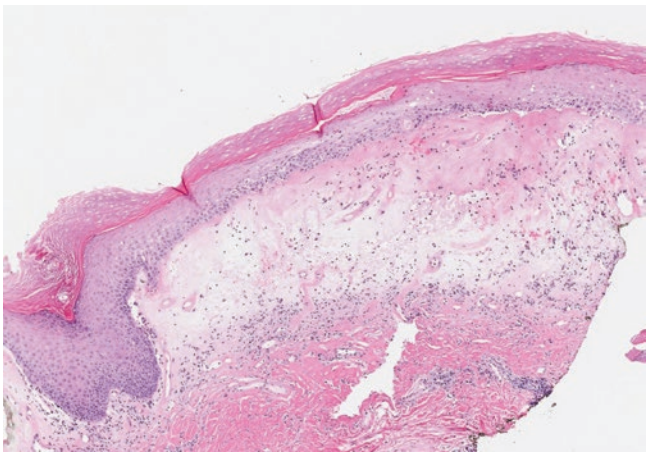

Fig. 1.4 Well-developed lichen sclerosus, thin epidermis with extensive subepithelial homogeneous hyalinized zone

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-

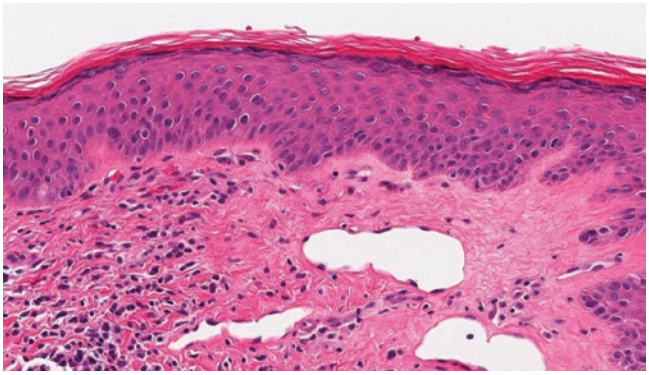

Fig. 1.5 Early lichen sclerosus with thickened basement membrane, entrapped lymphocytes, and early papillary dermal sclerosis

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.

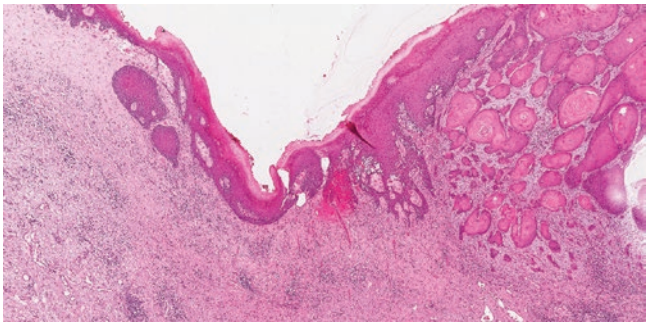

Fig. 1.6 Invasive keratinizing squamous cell carcinoma (right side) and associated lichen sclerosis (left side)

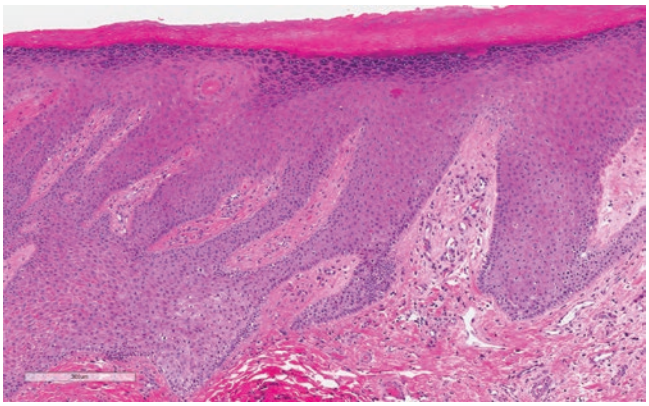

Fig. 1.7 Lichen simplex chronicus

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 nonpathogenic 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

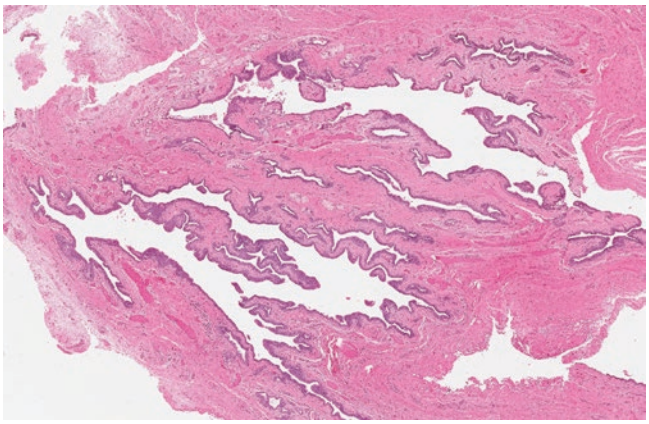

Fig. 1.8 Bartholin duct cyst

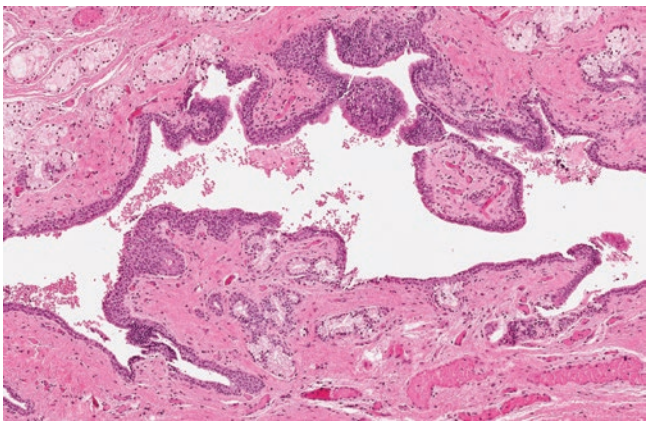

Fig. 1.9 Bartholin duct cyst, note the adjacent normal Bartholin's gland acini

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

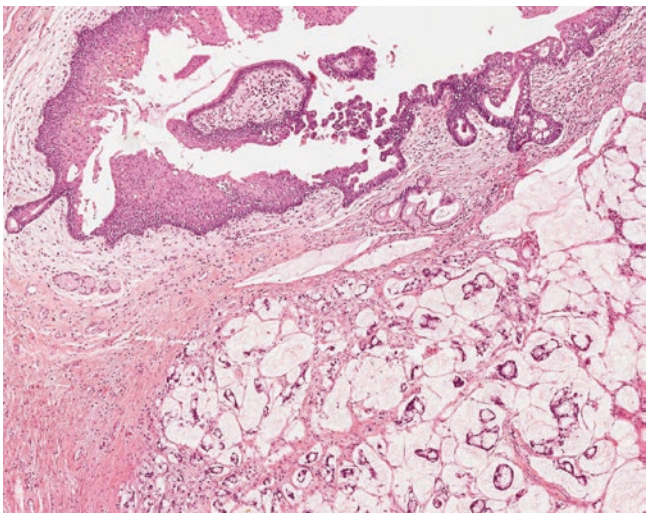

Fig. 1.10 Mucinous adenocarcinoma of the Bartholin gland

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

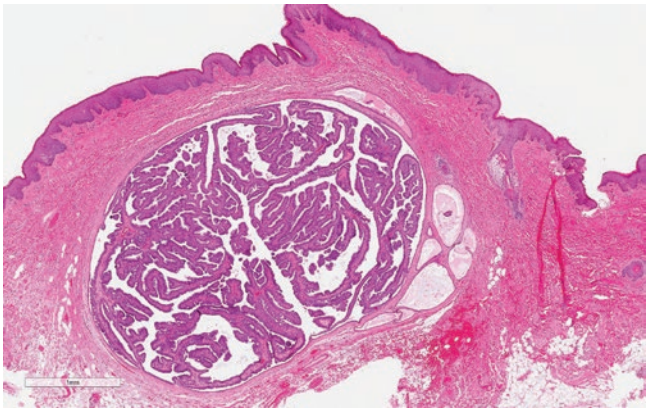

Fig. 1.11 Hidradenoma papilliferum. Note circumscribed complex proliferation with papillary and glandular architectures

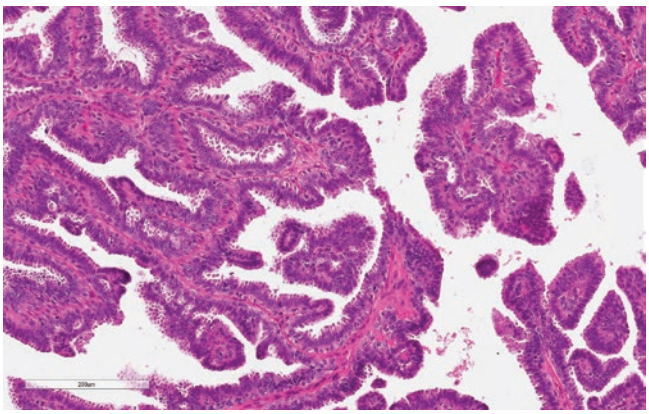

Fig. 1.12 Hidradenoma papilliferum. High-power view shows myoepithelial cells and bland columnar epithelial cells

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].

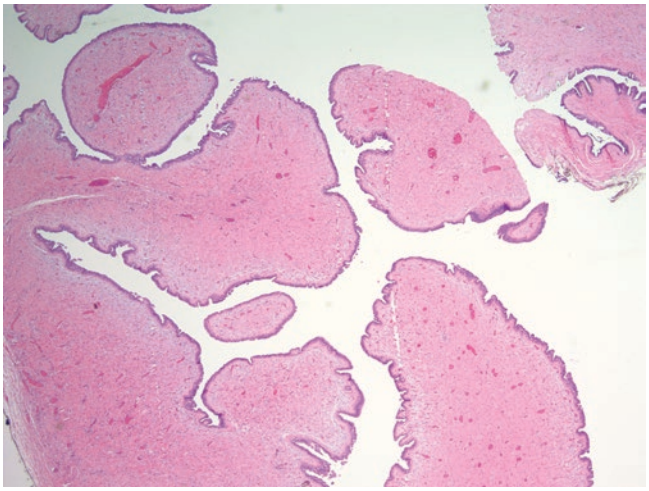

Fig. 1.13 Benign mammary-type phyllodes tumor in the vulva

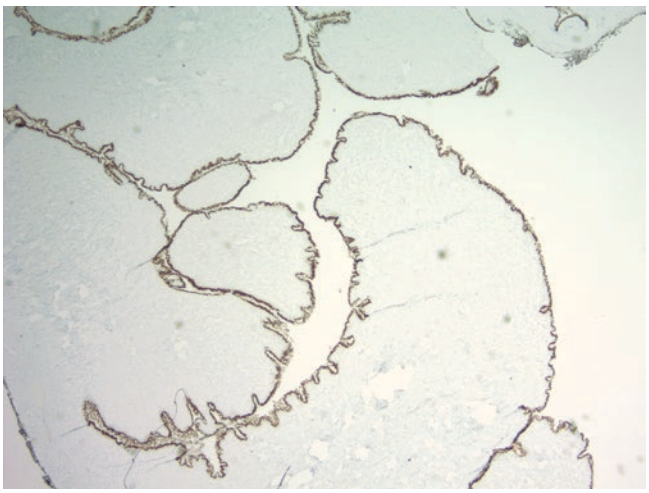

Fig. 1.14 Benign mammary-type phyllodes tumor in the vulva. GATA3 is positive

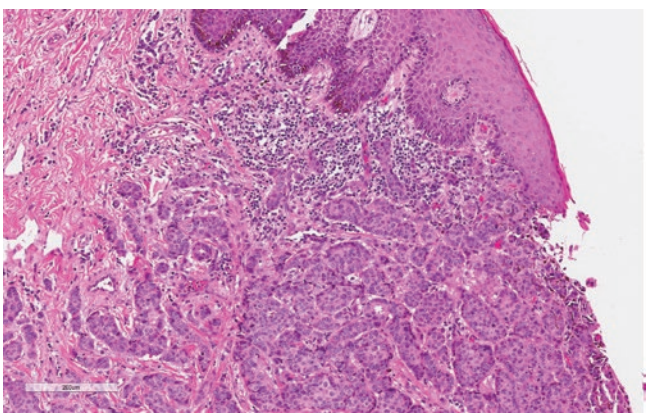

Fig. 1.15 Invasive mammary carcinoma in the vulva

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

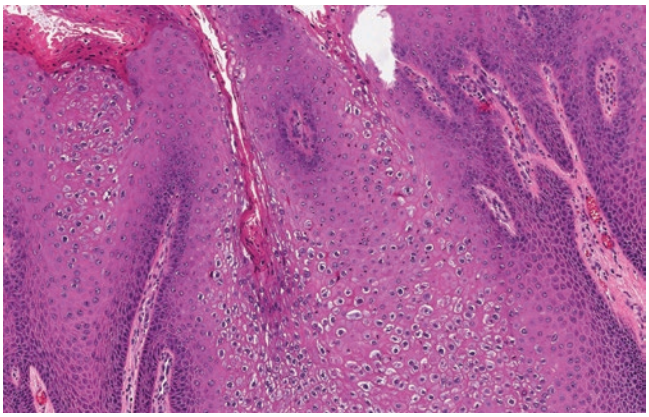

Fig. 1.17 Condyloma acuminatum. Note the koilocytotic changes

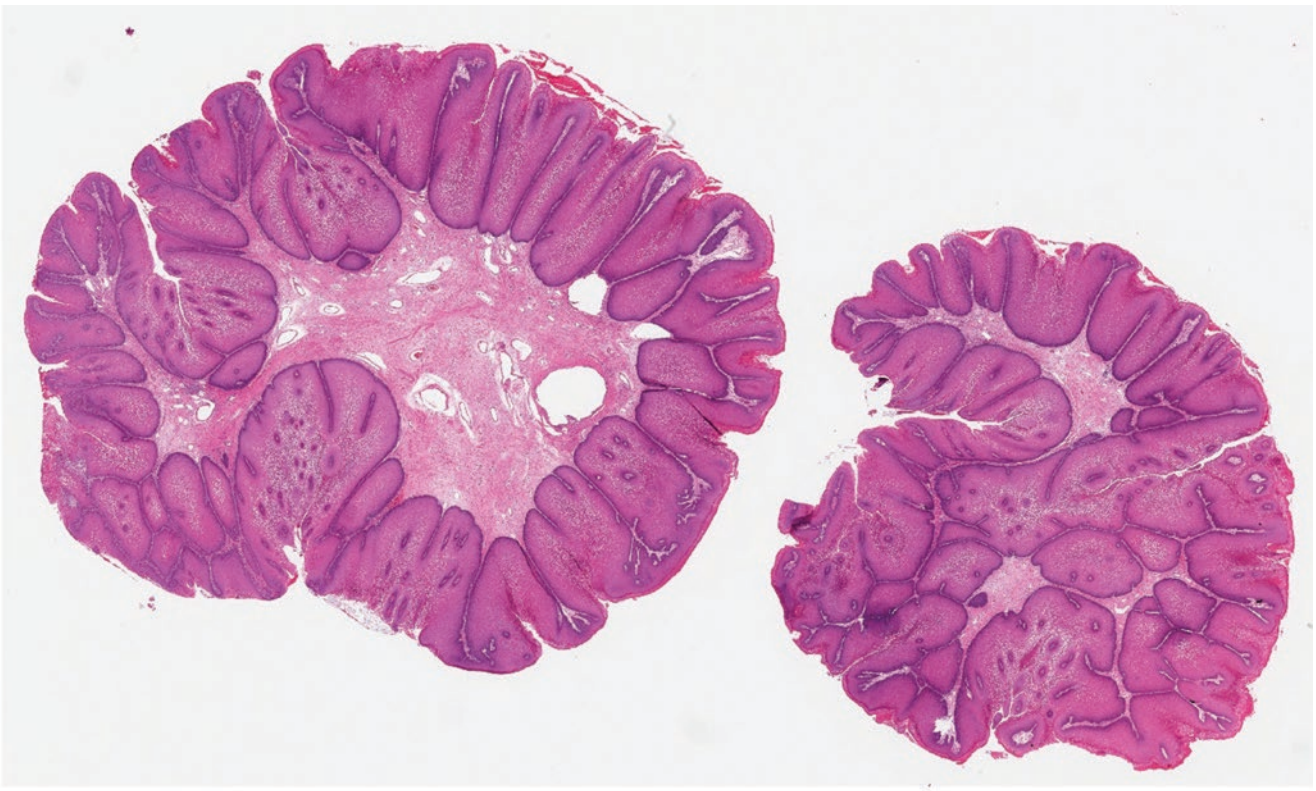

Fig. 1.16 Condyloma acuminatum. Low-power view shows typical papillary architecture

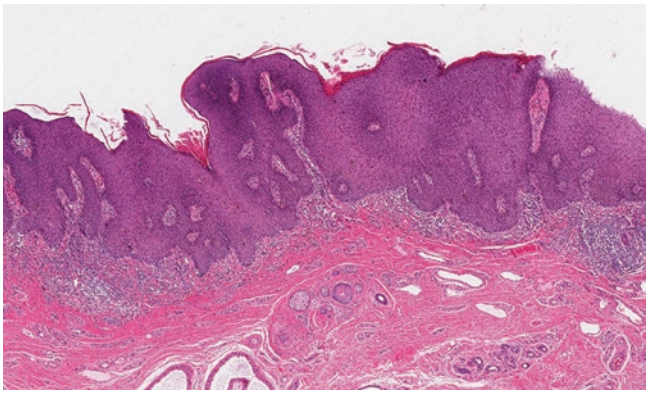

Fig. 1.18 Condyloma acuminatum without the koilocytotic changes

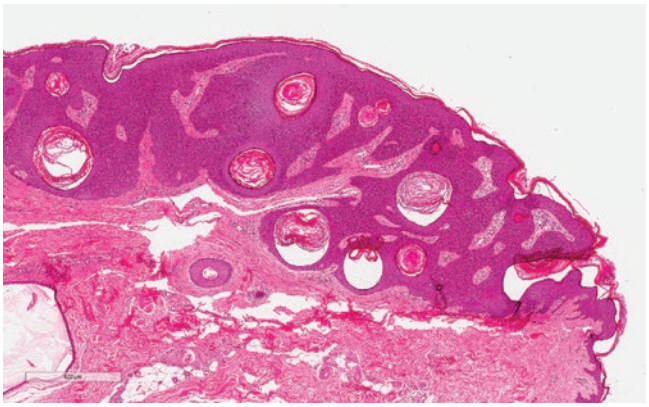

Fig. 1.20 Seborrheic keratosis

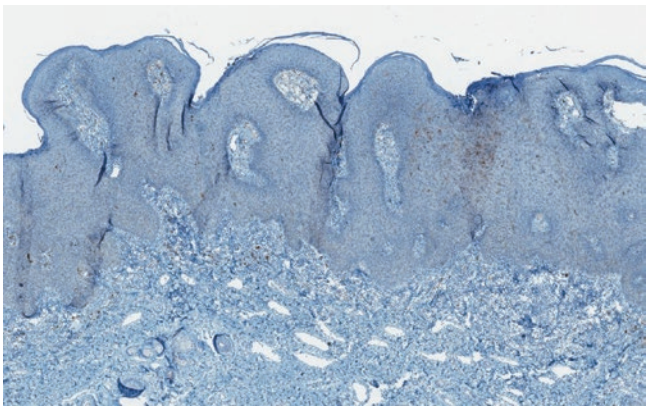

Fig. 1.19 Immunostain for p16 is weak and focal in condyloma acuminatum with features concerning for high-grade squamous intraepithelial lesion (uVIN)

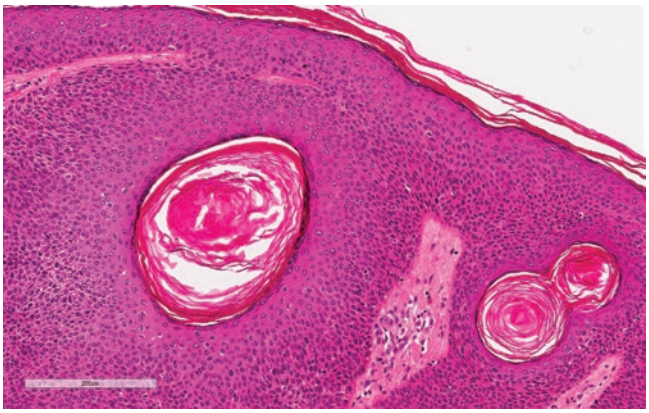

Fig. 1.21 Seborrheic keratosis. Note the keratin filled horn cyst and absence of cytological atypia

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

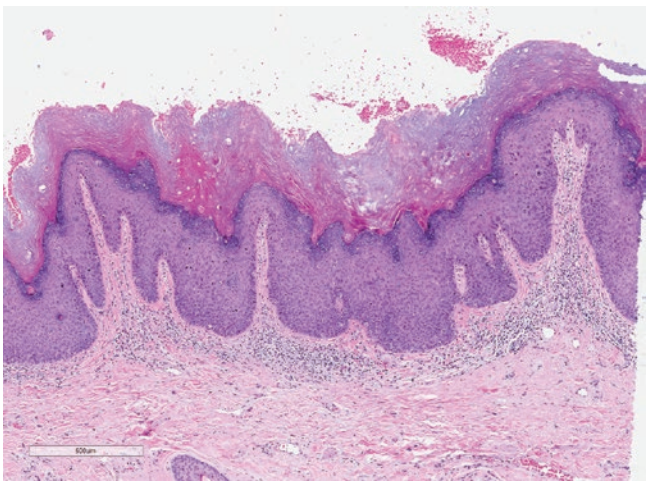

Fig. 1.22 HPV-associated HSIL. The surface is hyperkeratotic

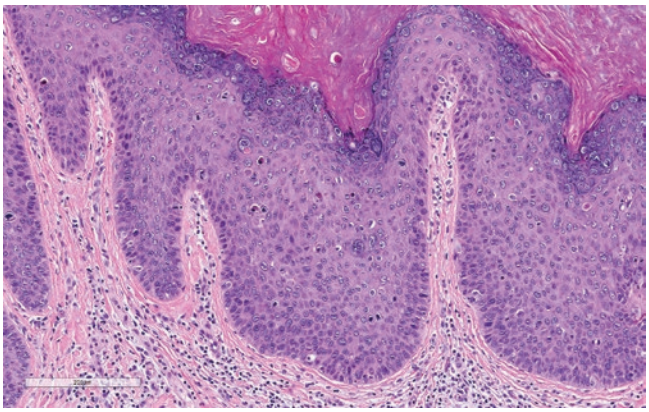

Fig. 1.23 HPV-associated HSIL. Note full-thickness atypia

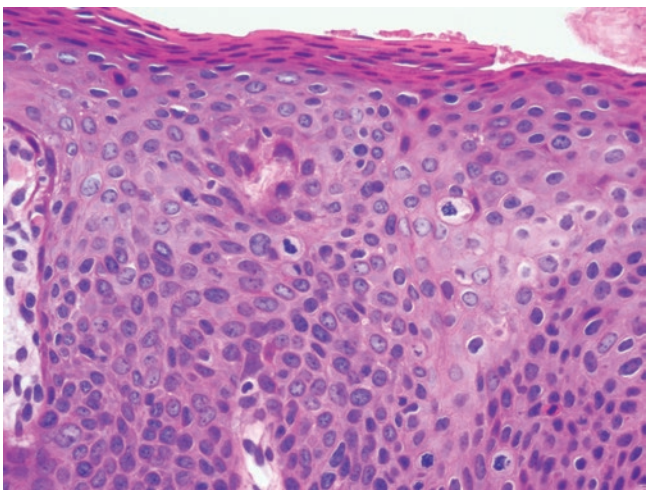

Fig. 1.24 HPV-associated HSIL, frequent mitotic figures

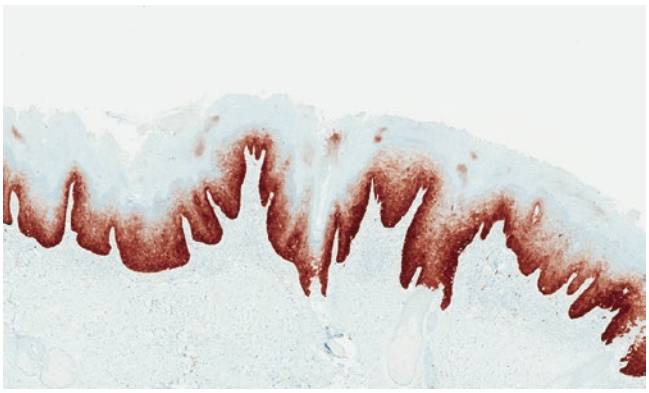

Fig. 1.25 HPV-associated HSIL, immunostain for p16 shows block-like pattern

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

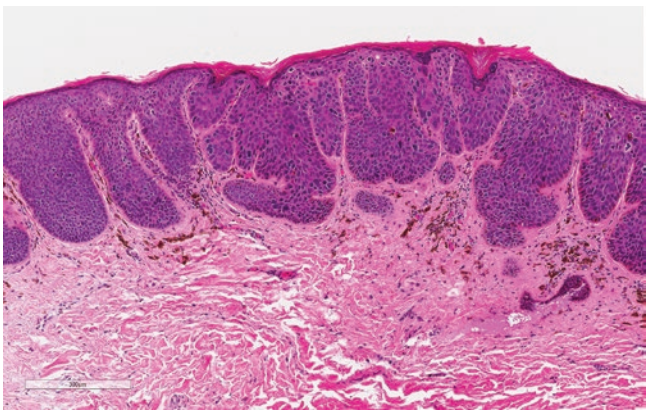

Fig. 1.26 HPV-associated HSIL with basaloid features

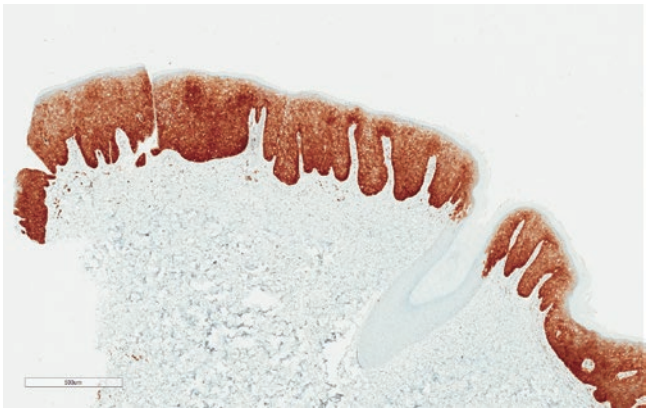

Fig. 1.27 HPV-associated HSIL with basaloid features, immunostain for p16

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 acuminatum, 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

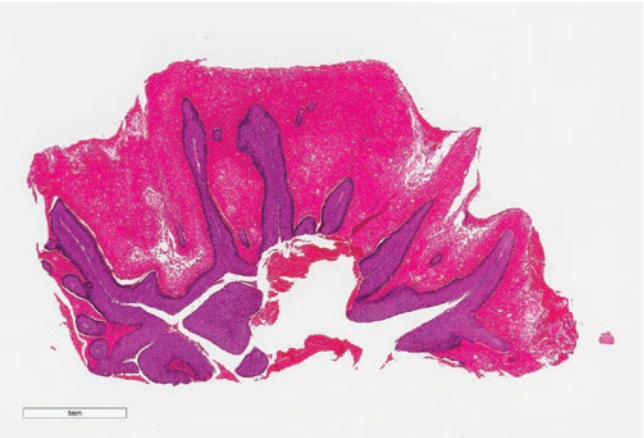

Fig. 1.28 HPV-associated HSIL, warty type. Low power shows spiking surface with hyperkeratosis and parakeratosis

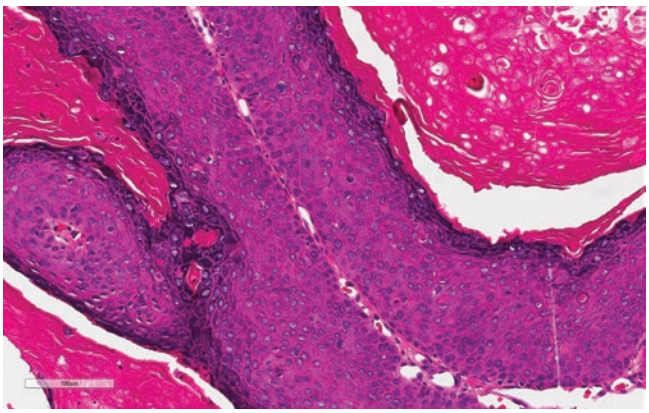

Fig. 1.29 HPV-associated HSIL, warty type. High power shows full-thickness atypia

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

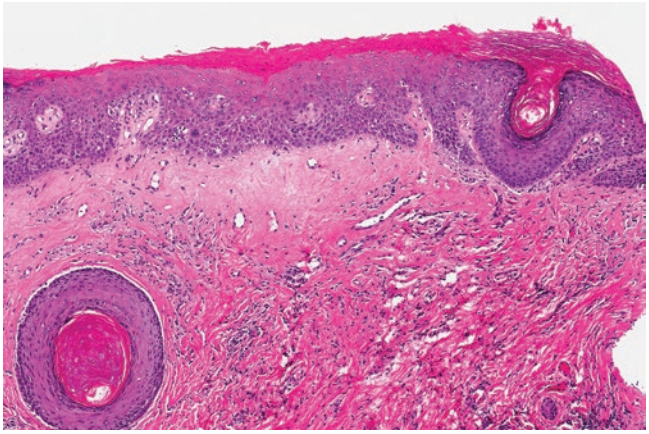

Fig. 1.30 HPV-independent VIN, low-power view with uninvolved spumous epithelium at the right edge

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.

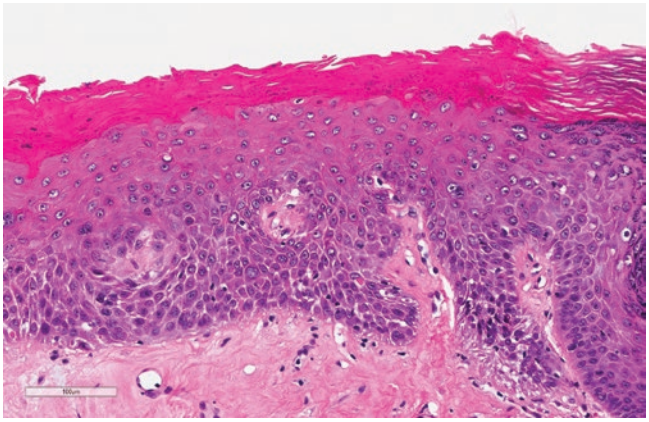

Fig. 1.31 HPV-independent VIN, high-power view with atypia of the basal cells

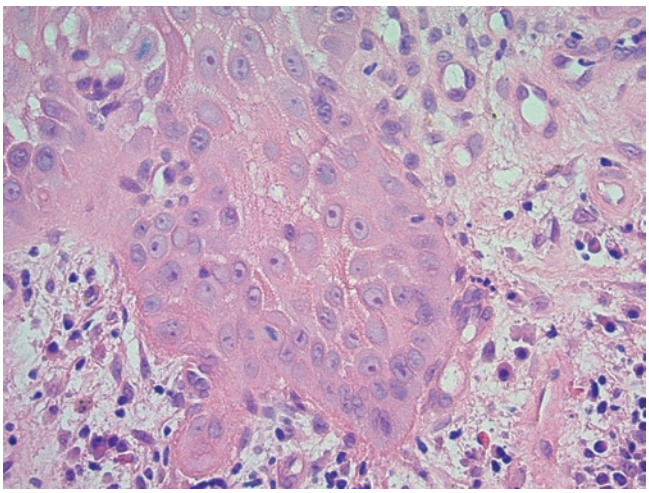

Fig. 1.32 HPV-independent VIN, note the prominent intercellular bridges and nuclear atypia

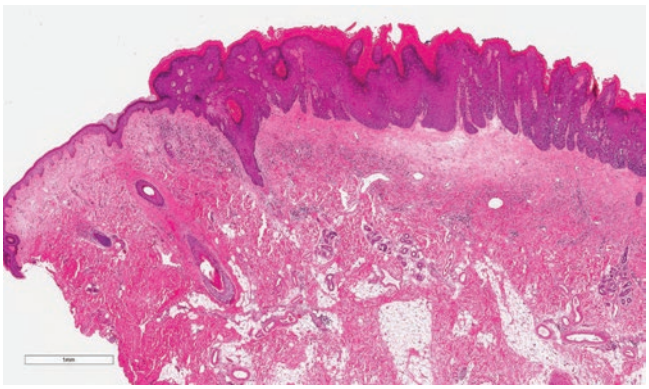

Fig. 1.33 HPV-independent VIN in a background of LS

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

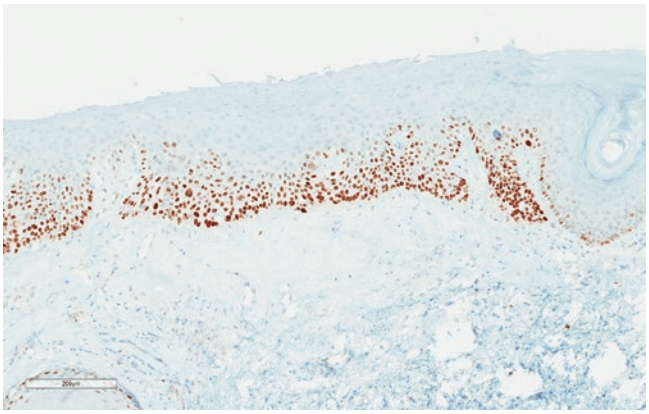

Fig. 1.34 Immunostain of p53 in HPV-independent VIN (missense mutation)

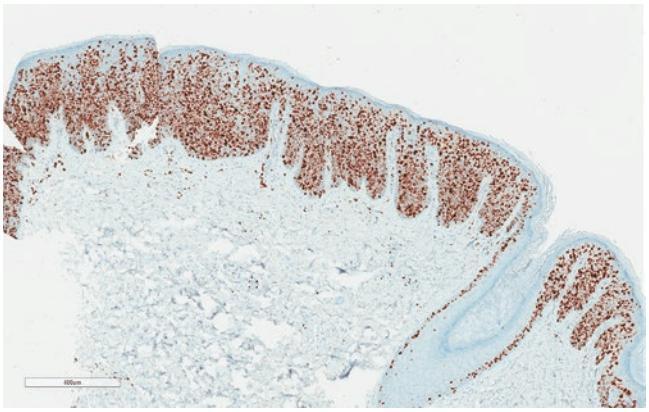

Fig. 1.35 Immunostain of Ki-67 in high-grade squamous intraepithelial lesion

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 epithelium (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.

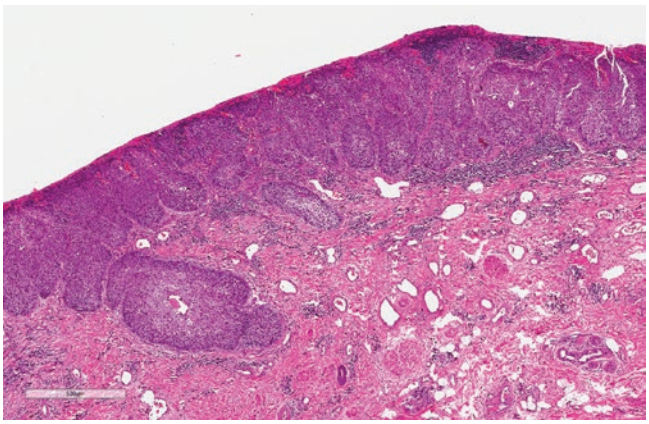

Fig. 1.36 Differentiated VIN with basaloid morphology simulating uVIN

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.

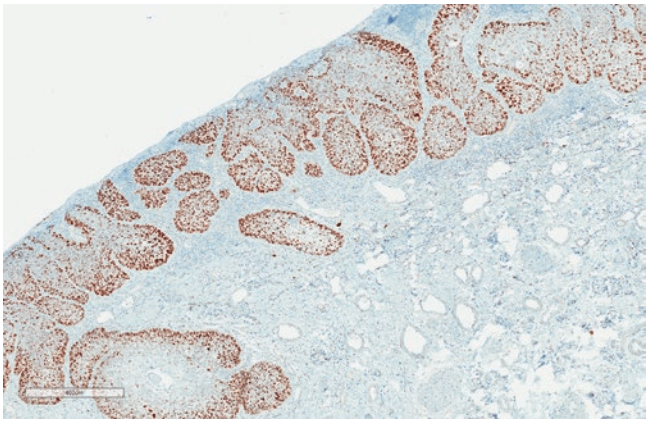

Fig. 1.37 Differentiated VIN with basaloid morphology, aberrant p53 immunostain

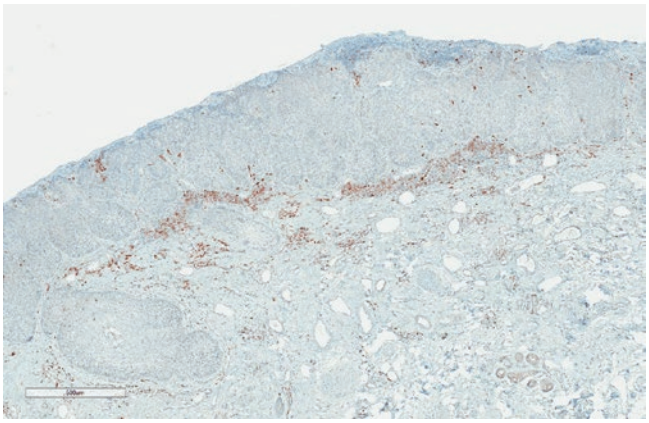

Fig. 1.38 Differentiated VIN with basaloid morphology, negative p16 immunostain

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

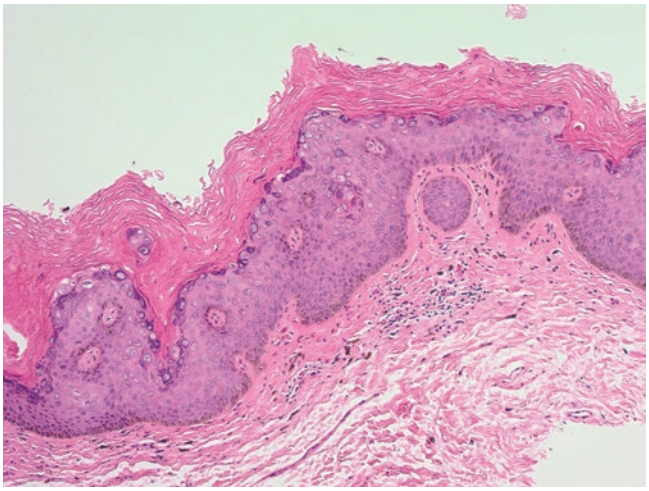

Fig. 1.39 HPV-related HSIL (uVIN2)

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

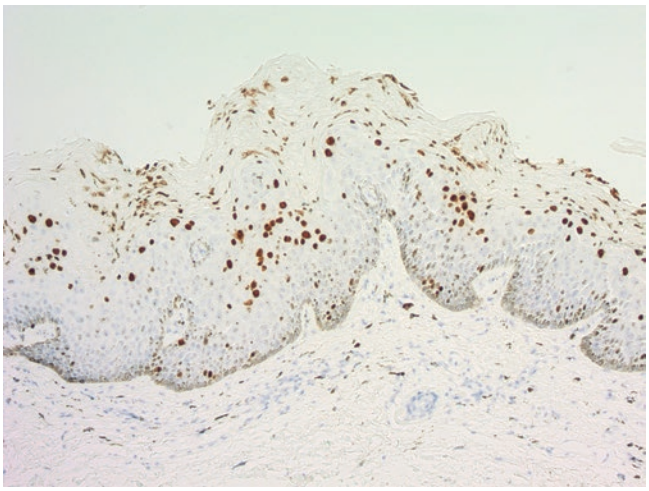

Fig. 1.40 Positive RNA ISH for high-risk HPV in HPV-related HSIL (uVIN2)

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 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.

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

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

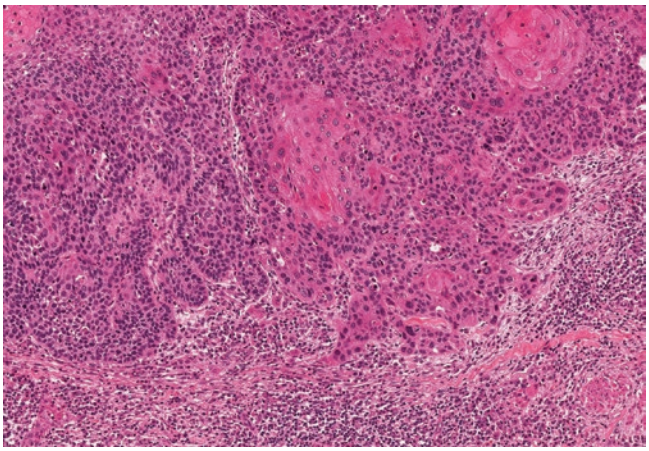

Fig. 1.41 Squamous cell carcinoma with superficial invasion. Note the disrupted basement membrane

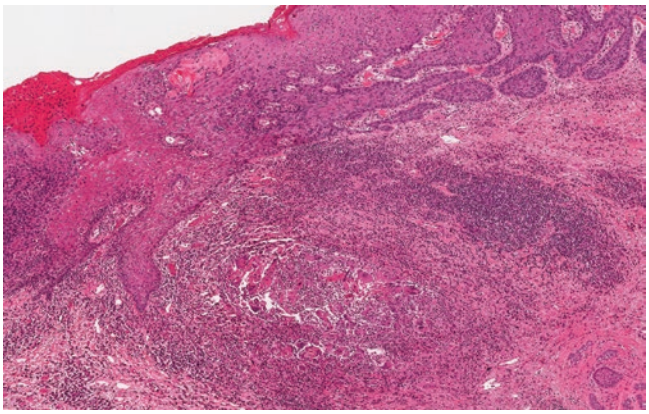

Fig. 1.42 Squamous cell carcinoma with superficial invasion and reactive stromal changes

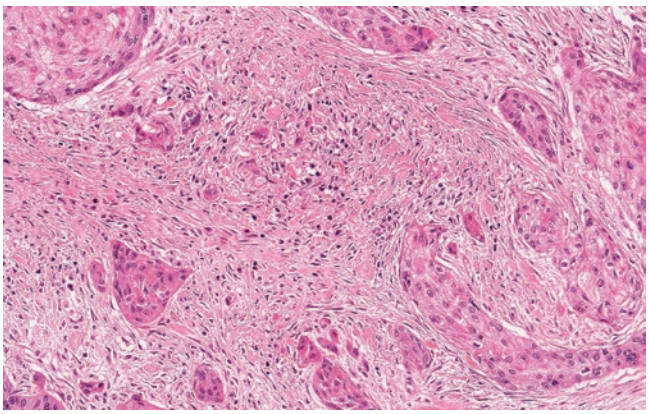

Fig. 1.43 Invasive squamous cell carcinoma, individual tumor cells

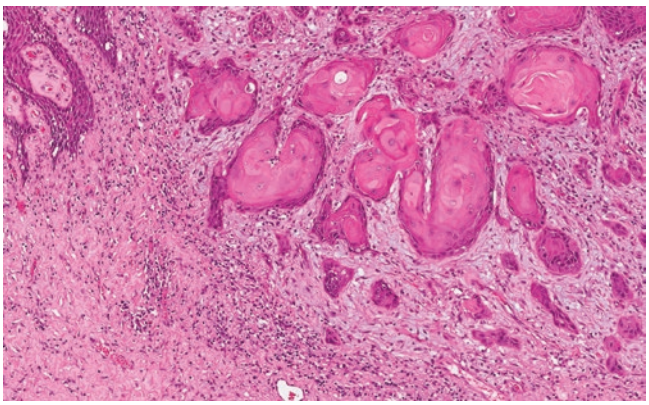

Fig. 1.44 Invasive squamous cell carcinoma. Note the paradoxical maturation

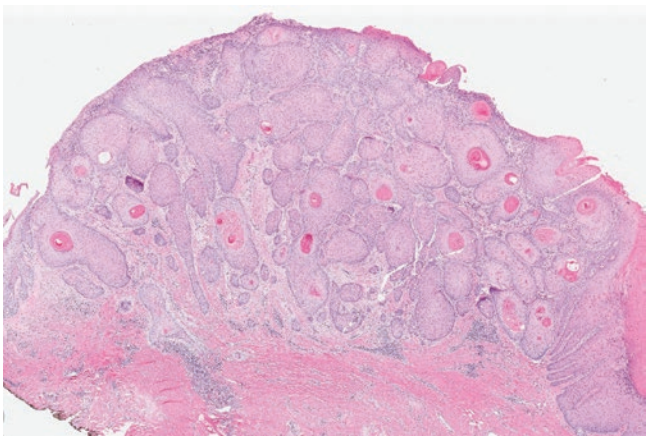

Fig. 1.45 Invasive keratinizing squamous cell carcinoma

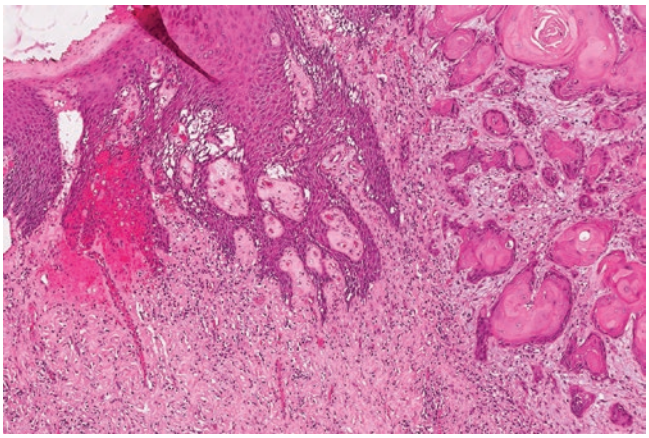

Fig. 1.46 Invasive keratinizing squamous cell carcinoma and associated lichen sclerosis

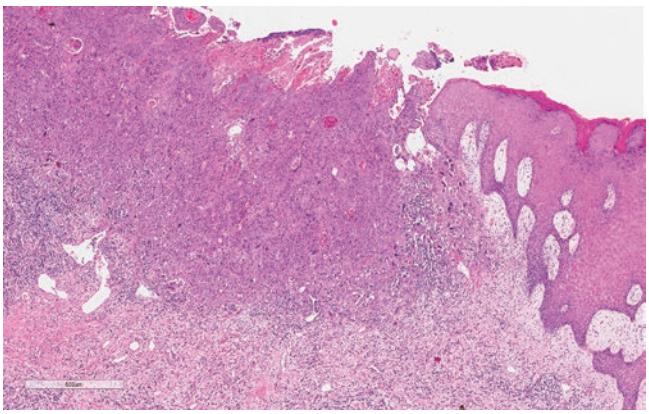

Fig. 1.47 Poorly differentiated vulvar SCC with surface ulceration

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 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-

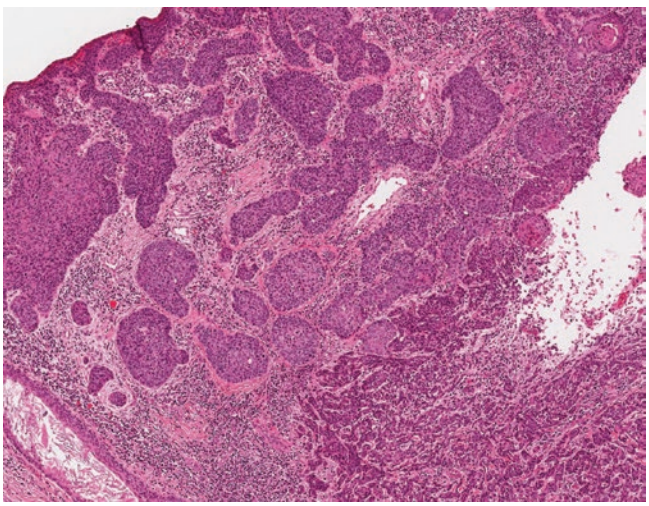

Fig. 1.48 Invasive squamous cell carcinoma, basaloid type, low-power view

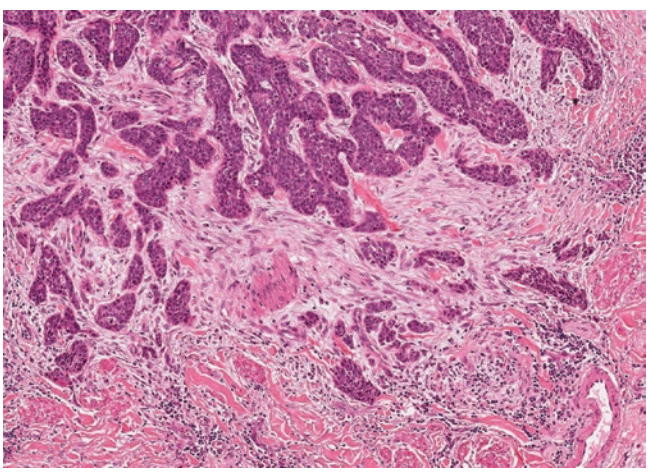

Fig. 1.49 Invasive squamous cell carcinoma, basaloid type with desmoplastic stroma

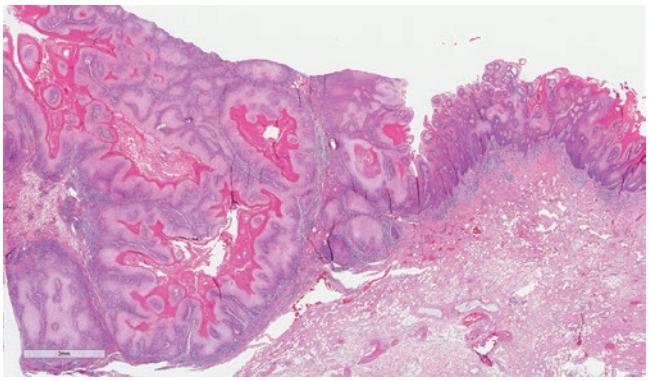

Fig. 1.50 Invasive squamous cell carcinoma, verrucous type

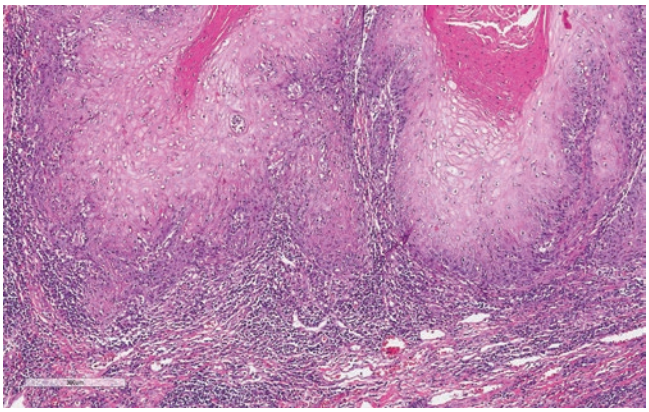

Fig. 1.51 Invasive squamous cell carcinoma, verrucous type. Note the pushing broad front, and minimal cytological atypia

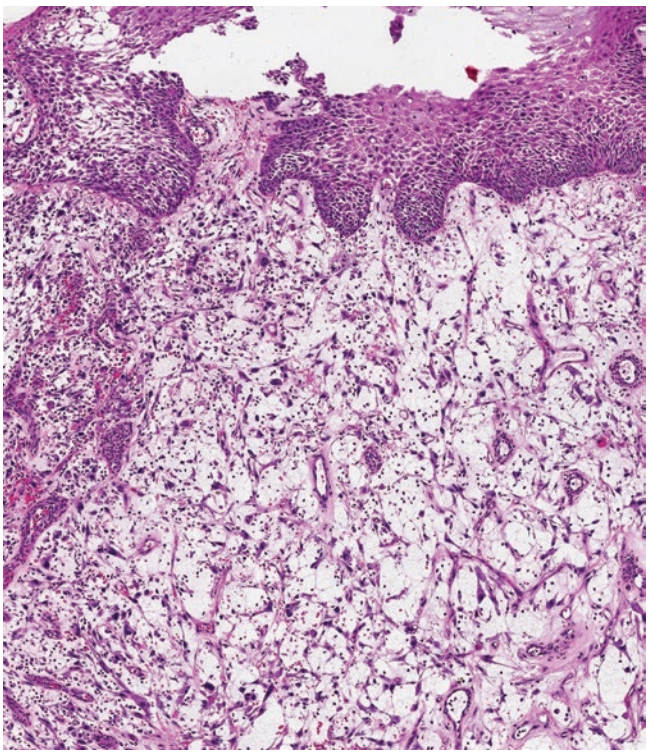

Fig. 1.52 Invasive squamous cell carcinoma with sarcomatoid features

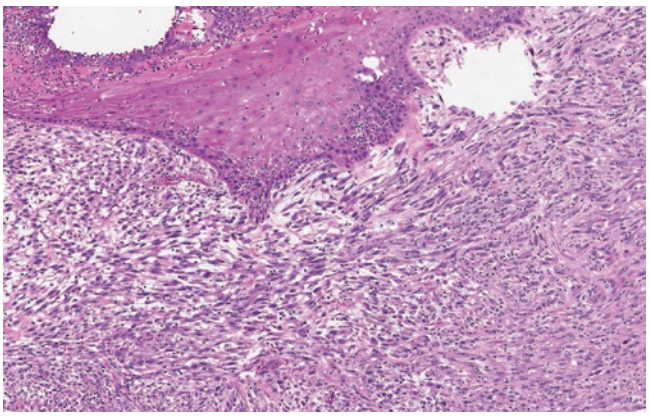

Fig. 1.53 Invasive squamous cell carcinoma with spindle cell features

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.

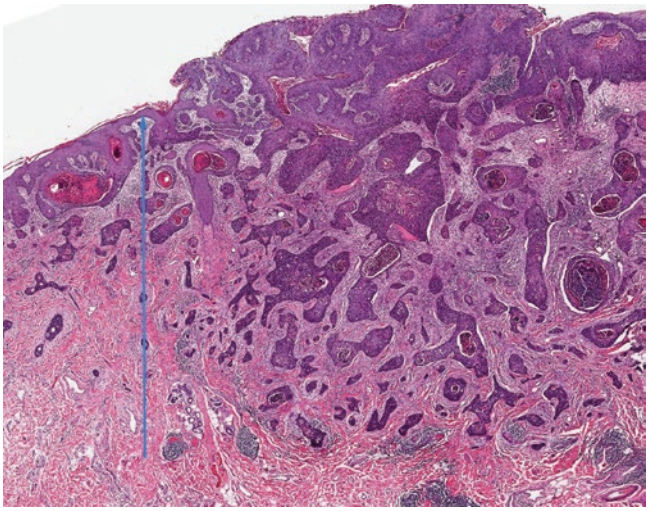

Fig. 1.54 Depth of stromal invasion of spumous cell carcinoma (blue line)

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

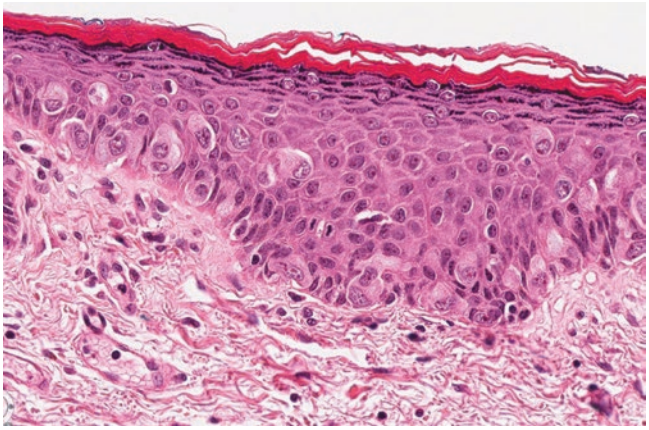

Fig. 1.55 Primary extramammary Paget disease. Typical pagetoid involvement of the basal layer of the epidermis

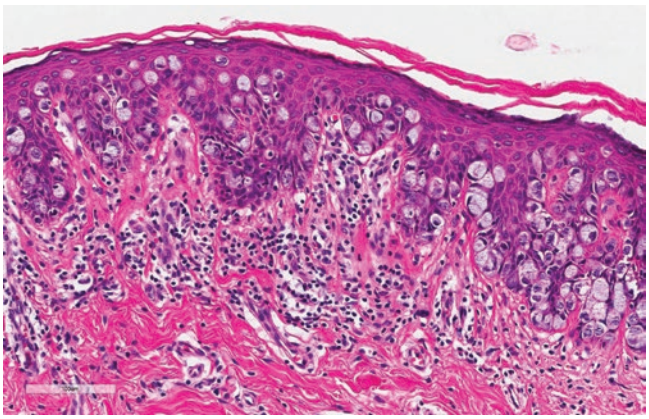

Fig. 1.56 Primary extramammary Paget disease. Signet ring-type tumor cells with abundant intracytoplasmic mucin

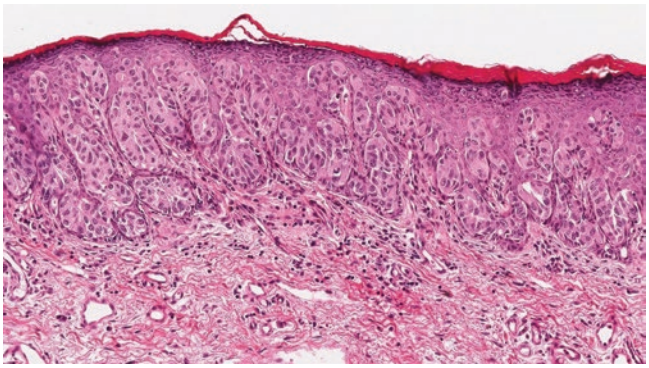

Fig. 1.57 Primary extramammary Paget disease. Note the nests of tumor cells and full-thickness involvement

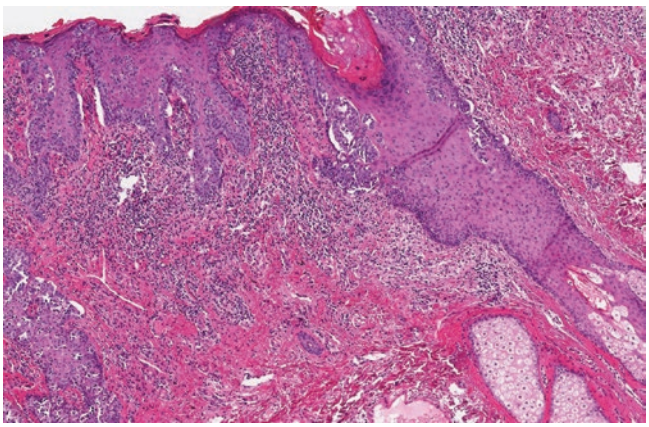

Fig. 1.58 Primary extramammary Paget disease with involvement of adnexal structure

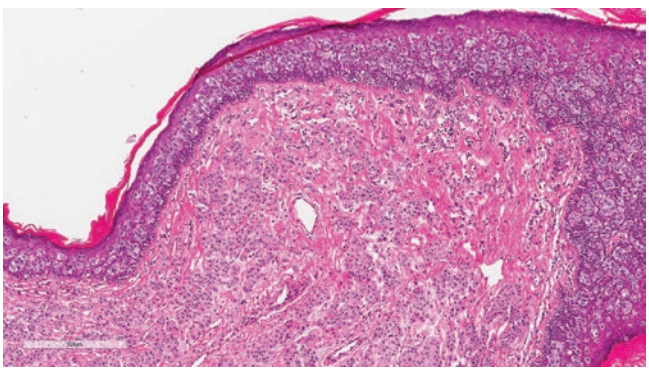

Fig. 1.59 Invasive extramammary Paget disease

may be difficult to discern, especially in a small biopsy specimen. When numerous, the Paget cells may involve much of the epidermis, giving a thickened and disarrayed appearance and mimicking VIN. When mainly located at the basal layer, Paget disease may also be confused with melanoma in situ. In problematic cases, immunohistochemical stains are useful to facilitate the diagnosis.

18. How Does Immunohistochemical Study Help Differentiating Primary Paget Disease from Secondary Paget Disease and Other Vulvar Neoplasia?

Immunohistochemistry helps distinguish primary EMPD from secondary EMPD and distinguishes EMPD from other vulvar neoplasia such as melanoma and VIN. Paget cells are typically diffusely and strongly positive for CK7, an excellent marker for intraepidermal and invasive EMPD (Fig. 1.60). The positivity of CK7 facilitates the distinction of EMPD from hyperplastic and dysplastic squamous cells; the latter are usually negative for CK7. However, normal Toker cells and Merkel cells are positive for CK7 and must be distinguished morphologically. Paget cells are also usually positive for CEA, CAM 5.2, GCDFP-15, MUC1, androgen receptor, and mucin stain (Fig. 1.61). Her-2 positivity has been found in Paget cells in 40–60% of cases, while estrogen receptor and progesterone receptor are usually negative [83, 84].

To distinguish primary from secondary EMPD, a panel of antibodies should be applied, including CK7, CK20, CEA, GCDFP-15, CDX2, SATB2, and mucin stain. Primary vulvar Paget cells are usually positive for CK7, GCDFP-15, and mucin stain, but negative for CK20. Secondary EMPD cells usually are negative for GCDFP-15. Secondary EMPD derived from colorectal carcinoma is usually positive for CK20, CDX2, and SATB2 but negative for CK7. Secondary EMPD derived from underlying urothelial carcinoma is usually positive for CK7, CK20, GATA3, and p63. However, it is important to point out that GATA3 alone has no value for differentiating between primary and secondary vulvar Paget disease derived from the urological tract. GATA3 can also be expressed in most primary EMPD cells [84].

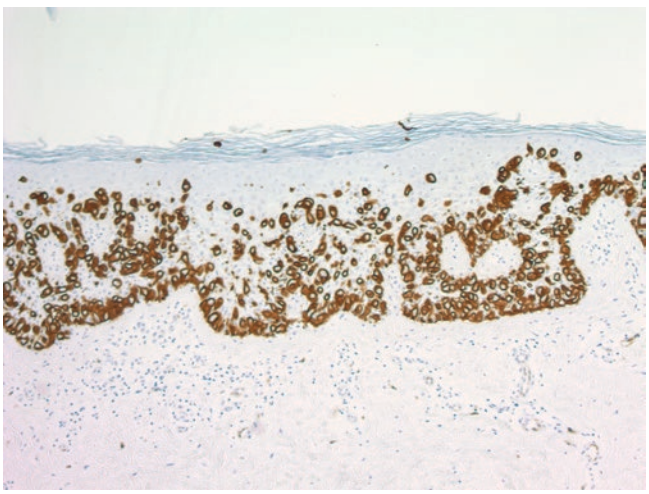

Fig. 1.60 Primary extramammary Paget disease. The neoplastic cells are positive for CK7

In contrast to Paget cells, melanoma cells lack mucin and usually form a continuous proliferation sitting directly on the basement membrane. The cytoplasmic pigment is often seen in neoplastic melanocytes. However, since cytoplasmic pigment can occasionally be seen in Paget cells, it is not a reliable morphological feature for melanoma. Because S-100 protein is sometimes expressed in Paget cells, other more specific melanocytic markers such as MART-1/Melan-A, HMB45, and SOX10 are recommended.

Overexpression of p16 is a hallmark in HPV-associated high-grade squamous intraepithelial lesion (uVIN). However, p16 immunoreactivity with variable intensity has been observed in cases of intraepithelial and invasive EMPD [82]. Similarly, GATA3 also expressed in most cases of primary EMPD and HPV-associated high-grade squamous intraepithelial lesion (uVIN) [85]. Therefore, p16 and GATA3 immunohistochemistry have no role in separating Paget disease from HPV-related high-grade squamous intraepithelial lesions (Table 1.2).

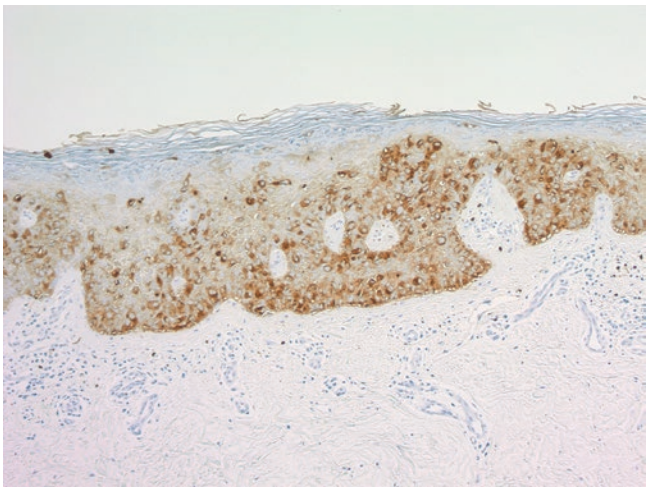

Fig. 1.61 Primary extramammary Paget disease. The neoplastic cells are positive for CEA

19. What Are the Histological Features of the Vulvar Fibroepithelial Polyp and Fibroepithelial Polyp with Stromal Atypia?

Fibroepithelial polyps, clinically often referred to as skin tag, are benign, polypoid, or pedunculated lesions that arise from the subepithelial stroma of the vulva, vagina, and perineum. The pathogenesis of fibroepithelial polyp is not clearly understood. Fibroepithelial polyps most commonly occur in reproductive age women, often during pregnancy, and may regress after delivery [86]. They may also occur in postmenopausal women on hormonal replacement therapy. So it might represent a reactive hyperplastic process to hormone rather than a true neoplasm. Most polyps are less than 5 cm, although rare giant fibroepithelial polyps associated with congenital lymphedema have been reported [87]. Fibroepithelial polyps are typically solitary, although multiple polyps may be seen.

Histologically, fibroepithelial polyps are stromal growth typically covered by squamous epithelium, which may exhibit varying degrees of hyperplasia (Fig. 1.62). Epidermal squamous epithelium is usually mild to moderate hyperplastic with no evidence of koilocytosis, a feature separating fibroepithelial polyp from condyloma. The stroma can be variably cellular but is usually fibrous to edematous and sparsely cellular. Stromal cells are usually bland. Sometimes stellate stromal cells with or without multinucleated stromal cells can be seen near the superficial stroma or adjacent to vessels (Fig. 1.63). The vessels are usually thin-walled, sometimes thick-walled vessels can be seen centrally within the polyp. The pathological diagnosis of a typical fibroepithelial polyp is usually straightforward, with no need for an ancillary study. However, occasionally the squamous hyperplasia and hyperkeratosis are prominent with morphological features overlapping with condyloma or warty type of VIN. In such a situation, HPV in situ hybridization will be very helpful. Immunostaining for p16 is useful in polypoid

Table 1.2 Commonly used markers for distinguishing primary vulvar EMPD from secondary EMPD, HSIL/SCC in situ and melanoma in situ

Markers	Primary EMPD	Secondary EMPD from TCC	Secondary EMPD from CRC	HSIL/SCC in situ	Melanoma in situ
CK7	+	+	–	–	–
GCDFP15	+	–	–	–	–
CK20	–	+	+	–	–
CDX2	–	–	+	–	–
Uroplakin III	–	+	–	–	–
GATA3	–/+	+	–	–/+	–
P63	–	+	–	+	–
HMWCK	–	–	–	+	–
Melan A	–	–	–	–	+
HMB45	–	–	–	–	+

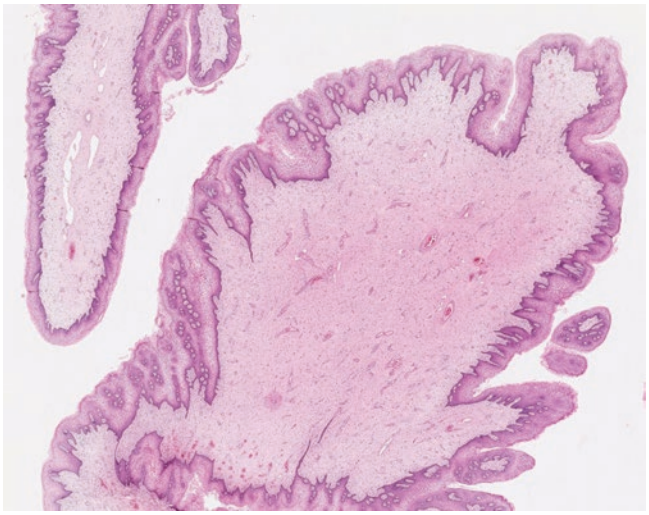

Fig. 1.62 Fibroepithelial polyp

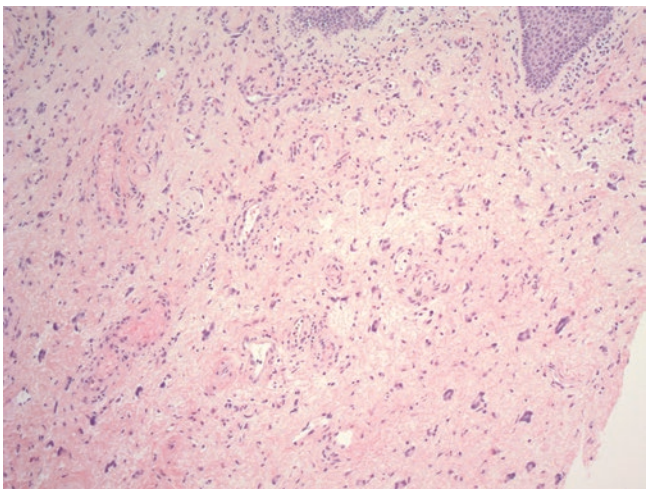

Fig. 1.63 Fibroepithelial polyp. Note the stellate and multinucleated stromal cells

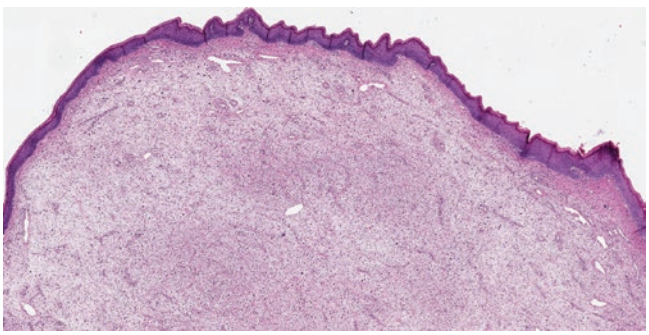

Fig. 1.64 Fibroepithelial polyp with markedly increased stromal cellularity, low-power view

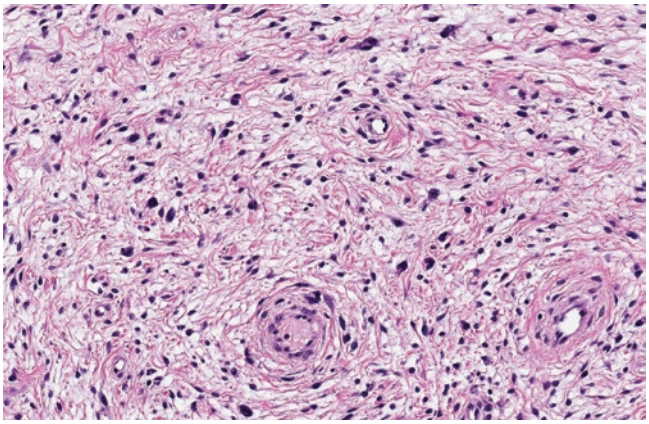

Fig. 1.65 Fibroepithelial polyp with nuclear pleomorphism with cytological atypia

lesions only if HPV-associated high-grade squamous dysplasia is suspected.

Occasionally, the stroma of a fibroepithelial polyp exhibits worrisome histological features, such as markedly increased stromal cellularity, nuclear pleomorphism with cytological atypia, and increased mitotic activity (Figs. 1.64 and 1.65). These worrisome histological features are particularly, but not invariably, present in polyps that occur during pregnancy [88]. The terms such as atypical fibroepithelial polyp, cellular pseudosarcomatous fibroepithelial stromal polyp, and pseudosarcoma botryoides have been used to describe the fibroepithelial polyps with these worrisome histological features [88, 89]. Most of these atypical features are seen in bigger polyps secondary to irritative processes. Given no evidence of aggressive or malignant behavior observed in these atypical polyps, authors recommend using the terminology of "fibroepithelial polyp with atypical stromal cells or with atypical features" to avoid unnecessary overtreatment. Local excision is almost always curative in most patients with vulvovaginal or peritoneal fibroepithelial polyps.

20. What Are the Features of Deep (Aggressive) Angiomyxoma, and What Are Differential Diagnoses?

Deep angiomyxoma, also referred to as aggressive angiomyxoma, is a deeply located, locally infiltrative mesenchymal tumor of the vulva and perineum in the women of reproductive age. The etiology remains unknown. The typical clinical presentation is a painless mass in the soft tissue of the vulvovaginal region, pelvis, and peritoneum. Tumors

vary in size but are often large (>10 cm) and poorly circumscribed. The tumors are often much larger and deeper than the initial clinical impression on pelvic examination. Radiological imaging is necessary to assess the extent of the tumor before surgery. Because the tumor has potential for local recurrence in approximately 30–40% of cases, if incompletely excised, wide local excision with 1 cm margins is considered optimal and adequate treatment [90–94]. It is important to distinguish deep angiomyxoma from its mimics because of the great clinical impact.

Grossly the typical finding is an ill-defined, solid tumor with a myxoid, edematous, or sometimes gelatinous appearance. Characteristic histological features include uniformly hypocellular loose myxoid stroma composed of small, bland, spindled or stellate cells, numerous medium to large-sized vessels with thin to thick and often hyalinized walls haphazardly scattered throughout the tumor, perivascular condensation of delicate collagen fibers, and bundles of brightly eosinophilic smooth muscle cells near the vessels (Figs. 1.66, 1.67, and 1.68). Stromal mast cells and extravasated erythrocytes are commonly seen. Rare multinucleated cells may be present. Mitotic figures and nuclear atypia are absent or rare. At the tumor periphery, fat, skeletal muscle, and nerves may be entrapped because of the infiltrative border of the tumors. When recur, the stroma usually becomes more fibrous and even hyalinized, making it difficult to distinguish recurrent tumor from adjacent non-neoplastic connective tissue.

To distinguish deep angiomyxoma from other tumors with a myxoid stroma, characteristic histological appearance is most helpful. The differential diagnosis mainly includes cutaneous myxoma, cellular angiofibroma, and angiomyofibroblastoma. Cutaneous myxoma typically presents as a superficially located, well-demarcated mass usually less than 5.0 cm with lobulated growth pattern, delicate thin-walled capillaries, and scattered inflammatory cells, particularly

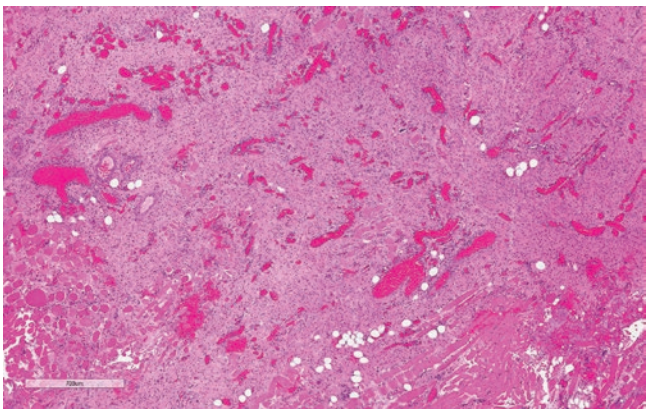

Fig. 1.66 Deep angiomyxoma. The tumor infiltrates adipose tissue and skeletal muscle

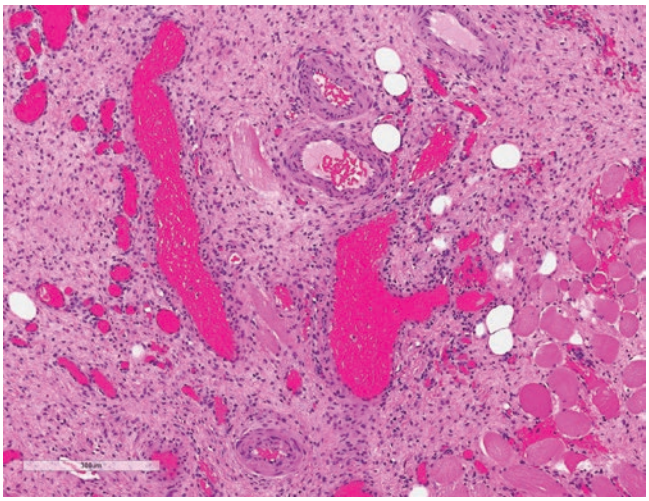

Fig. 1.67 Deep angiomyxoma. Note the admixed thin- and thick-walled vessels of varying sizes

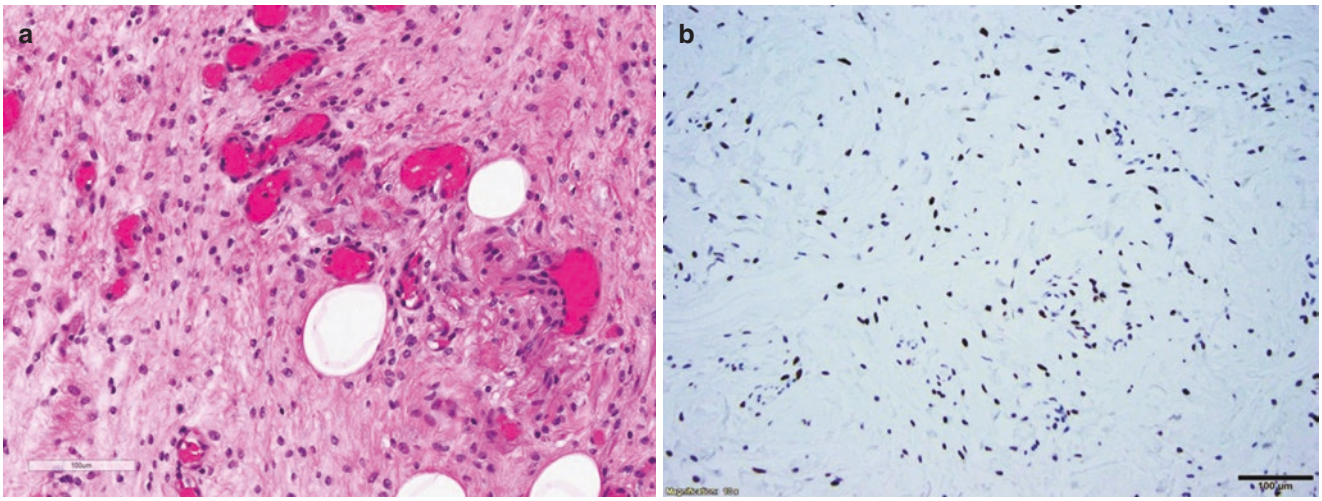

Fig. 1.68 Deep angiomyxoma. (**a**) Note small, bland, loosely arranged tumor cells and the perivascular delicate collagen fibers. (**b**) Nuclear expression for HMGA2

polymorphonuclear leukocytes. Deep angiomyxomas differ from cutaneous myxoma by their deep location, relatively large size, infiltrative borders, medium-sized thick-walled vessels, and immunoreactivity for desmin, ER, and PR. Compared to angiomyofibroblastoma, deep angiomyxoma has infiltrative margin, typically less prominent vascular component with larger thicker walled vessels and uniformly less cellular. Cellular angiofibroma is usually smaller in size and relatively well-circumscribed, lacking prominent myxoid appearance, much more cellular with small to mid-sized vessels, and immunohistochemically diffusely reactive to CD34.

Immunohistochemistry with a panel of antibodies can be applied, mainly for differential diagnosis from its mimics rather than confirming the diagnosis because there are no specific immunohistochemical markers for deep angiomyxoma. The tumor cells are typically positive for vimentin, desmin, and actin, particularly in the myoid bundles. In almost all cases, positive nuclear stains for estrogen receptor (ER) and progesterone receptor (PR) are present. Positive CD34 may be observed but usually focal, not as diffuse and strong as seen in cellular angiofibroma. Some studies found that structural rearrangements of 12q15 with the involvement of HMGA2 in approximately one-third of the cases of deep angiomyxoma [94, 95]. The immunohistochemical study also shows that nuclear expression for HMGA2 is found in 50% of aggressive angiomyxomas. However, HMGA2 gene rearrangements do not always correlate with nuclear HMGA2 protein expression. In some cases, there is HMGA2 rearrangement but no protein expression and vice versa [96]. Therefore, the immunohistochemical reactivity of HMGA2 may be useful in confirming the morphological impression of aggressive angiomyxoma, but further studies are necessary to fully assess its diagnostic utility in terms of sensitivity and specificity.

21. What Are the Histological Features and Immunohistochemical Profiles of Cellular Angiofibroma?

Cellular angiofibroma is a rare benign mesenchymal tumor that predominantly occurs in the vulva or perineum of middle-aged women. Clinically it often presents as a painless, not so deeply located, relatively small (<3 cm), well-circumscribed subcutaneous mass [97, 98]. It is easier to be completely excised and behaves in a benign fashion with no recurrent potential [99]. Malignant transformation to sarcomatous proliferation is extremely rare [100, 101].

Gross examination often reveals a superficially located, white, or yellowish, well-demarcated mass with a firm, rubbery consistency. Microscopically, cellular angiofibroma is relatively well-circumscribed but not encapsulated, although focal infiltration into surrounding soft tissue may be present. These tumors are characteristically moderately cellular and composed of bland uniform spindled cells with ovoid nuclei and scant palely eosinophilic cytoplasm arranged in short intersecting fascicles (Fig. 1.69). Another characteristic feature is numerous small- to medium-sized blood vessels, often with a thick and hyalinized wall and admixed wispy collagen bundles (Fig. 1.70). A minor component of adipose tissue is commonly present within the tumor or at the periphery. Mitotic activity is variable, usually sparse. Inflammatory cells and mast cells are often present. Necrosis and nuclear pleomorphism are typically absent. Atypia occasionally may be present, most commonly in the form of scattered hyperchromatic multinucleated cells. Rarely, an abrupt transition to a discrete sarcomatous component can occur, which may exhibit features of the atypical lipomatous tumor, pleomorphic liposarcoma, or pleomorphic sarcoma, not otherwise specified [101] (Fig. 1.71). Although morphologically worrisome, the clinical follow-up has not found an association with malignant behavior. Therefore, the biological signifi-

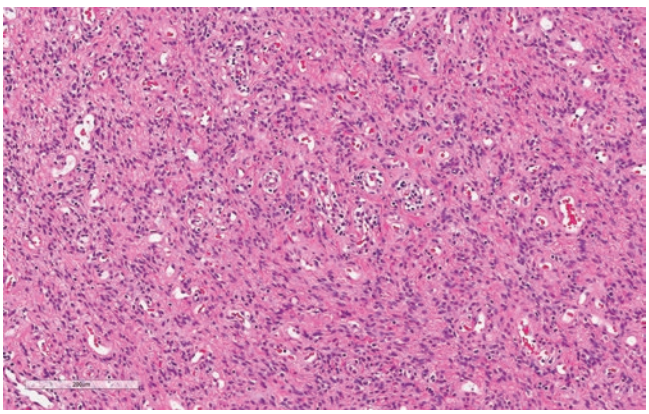

Fig. 1.69 Cellular angiofibroma. The bland uniform spindled cells are arranged in short intersecting fascicles

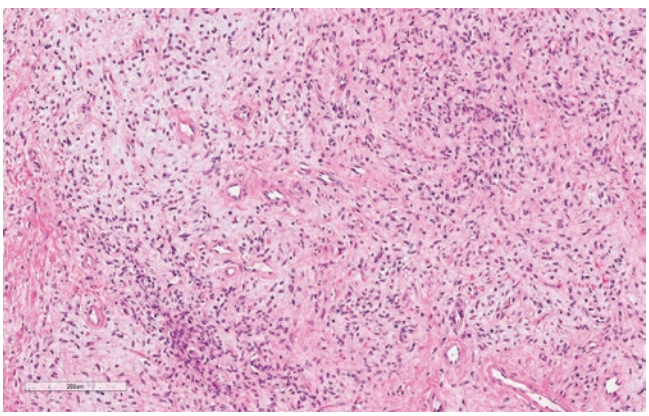

Fig. 1.70 Cellular angiofibroma. Note the small- to medium-sized blood vessels with a thick and hyalinized wall

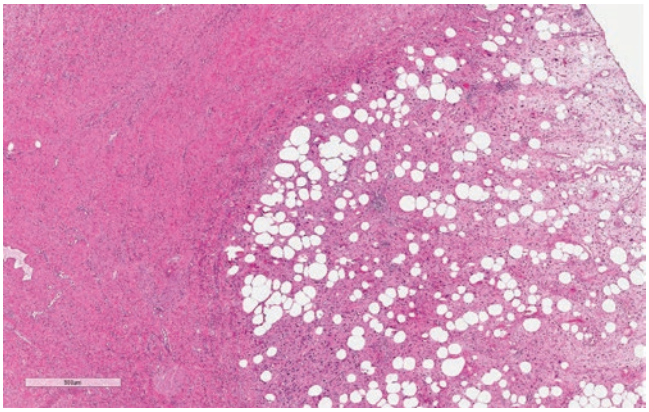

Fig. 1.71 Cellular angiofibroma with an abrupt transition to a discrete sarcomatous component

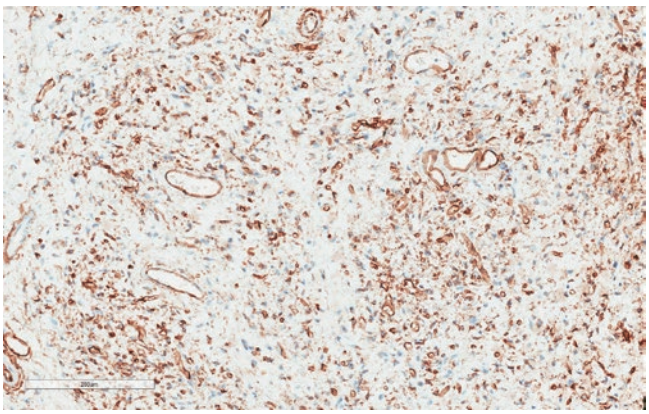

Fig. 1.72 Cellular angiofibroma. The spindle cells are positive for CD34 immunostaining

cance of atypia and sarcomatous transformation remains unclear.

Immunohistochemically, the spindle cells of cellular angiofibroma are diffusely reactive for CD34 in many cases and less commonly reactive for smooth muscle actin and desmin (Fig. 1.72). Immunoreactivities with ER and PR are seen in half of the cases [97, 98]. Neoplastic spindle cells are negative for keratin, epithelial membrane antigen (EMA), STAT6, and S-100 protein. Overexpression of p16 may occur in the atypical and sarcomatous cells in contrast to the absence of staining in the typical tumor [101].

Histopathologically, cellular angiofibroma shares some morphological features with extramammary myofibroblastoma and spindle cell lipoma. Also, all three tumors are immunohistochemically reactive to CD34. Recent studies reveal that genetic alterations at the 13q14 region harboring the FOX1A1 gene have been identified in all three tumors [102, 103]. Both extramammary myofibroblastoma and cellular angiofibroma are well-circumscribed tumors composed of short intersecting fascicles of bland, CD34-positive spindle cells within a collagenous stroma. However, the vascular component of extramammary myofibroblastoma is typically less prominent, although it can show a similar degree of hyalinization. Spindle cell lipoma is rare in the vulva; it contains CD34-positive spindle cells similar to those of cellular angiofibroma. However, they typically contain a prominent adipocytic component and inconspicuous thin-walled vessels.

A solitary fibrous tumor (SFT) is rare in the female genital tract but has overlapping features with cellular angiofibroma, including the presence of fat and CD34 positivity spindle cells. However, SFTs usually have more variable cellularity, dense hyaline collagen bundles, areas of hyalinization, and hemangiopericytoma-like vessels. Immunoreactivity to STAT6 in SFT but lack of it in cellular angiofibroma assists in differential diagnoses. When a smooth muscle neoplasm comes into the differential diagnosis, diffuse immunopositivity for one or more of the smooth muscle markers desmin, SMA, and h-caldesmon is supportive of a smooth muscle neoplasm [104].

22. What Are the Histopathological Features and Differential Diagnoses of Angiomyofibroblastoma?

Angiomyofibroblastoma is an unusual benign mesenchymal tumor, occurring primarily in the vulvovaginal area of women in productive age. It usually grows slowly and clinically, often mistaken as a Bartholin's cyst. Most tumors are less than 5 cm and can be large up to 12 cm in size. Clinically angiomyofibroblastoma rarely recurs if completely excised.

Grossly it is a well-circumscribed vulvar mass. The characteristic histological findings are a well-demarked tumor with abundant small to medium-sized thin-walled blood vessels and alternating hypocellular edematous and hypercellular areas (Fig. 1.73). Spindled or oval stromal cells aggregate around the vessels (Fig. 1.74). The tumor cells are plumped bland, may have an epithelioid or a plasmacytoid appearance (Fig. 1.75). Mitotic figures were absent or very sparse. Thin-walled blood vessels in the tumor usually are lack of prominent hyalinization [105].

The immunohistochemical study is nonspecific but can be applied to rule out other vulvar mesenchymal tumors. The stromal cells are strongly and diffusely reactive for vimentin and desmin in most cases, only focal for smooth muscle actin and CD34. Hormone receptors ER and PR are consistently positive in tumor cells.

Angiomyofibroblastoma can be distinguished from deep angiomyxoma by its circumscribed borders, perivascular condensation of plump stromal cells, numerous non-hyalinized blood vessels, and alternating hypocellular and hypercellular areas.

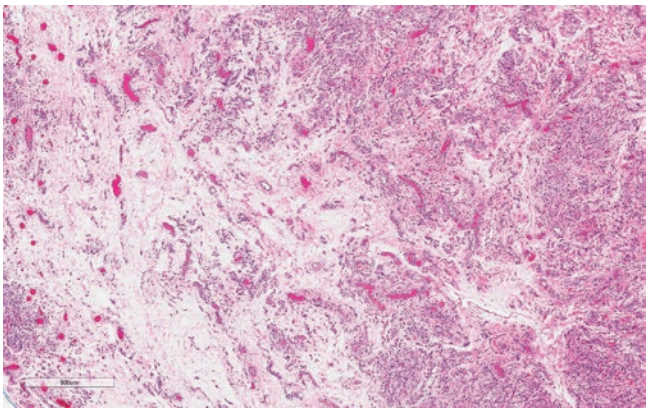

Fig. 1.73 Angiomyofibroblastoma. Alternating hypercellular and hypocellular zones

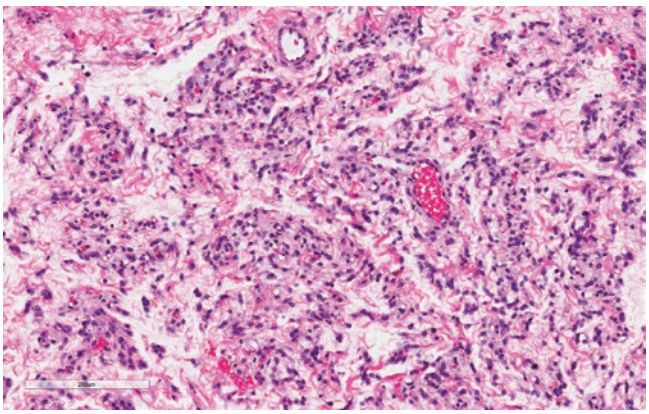

Fig. 1.74 Angiomyofibroblastoma, perivascular aggregate of bland spindled stromal cells

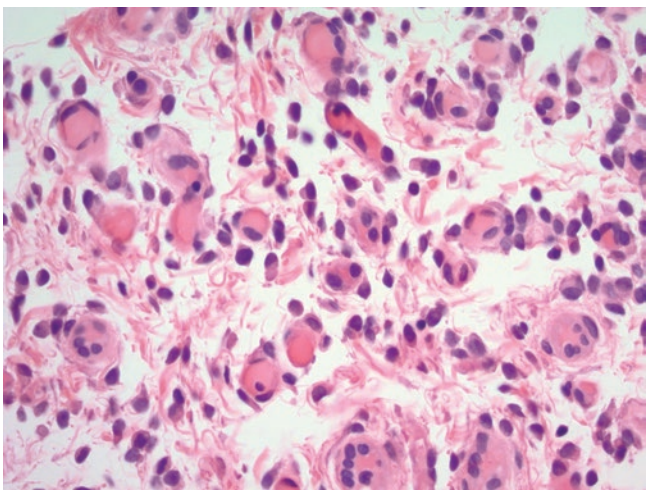

Fig. 1.75 Angiomyofibroblastoma, the stromal cells have an epithelioid or a plasmacytoid appearance

Angiomyofibroblastoma and cellular angiofibroma also have overlapping clinical and histological features. Clinically both tumor s are superficially located and most commonly seen in women of productive age. Morphologically both tumors are composed of bland spindled cells with a prominent vascular component. Some histological features may be helpful in distinguishing these two lesions. Cellular angiofibroma usually has numerous small to medium-sized blood vessels with a thickened and often hyalinized wall, while angiomyofibroblastoma usually has delicate, small, thin-walled vessels. Also, cellular angiofibroma has rather uniform cellularity with short intersecting fascicles of bland spindle cells. In contrast, angiomyofibroblastoma has variable hypocellular and hypercellular zones, and the tumor cells tend to be perivascular with a more plump appearance. Moreover, although not specific, a desmin+/CD34− immunophenotype favors angiomyofibroblastoma, while diffuse CD34+ in the spindle cells supports the diagnosis of cellular angiofibroma.

23. What Do We Need to Know About Smooth Muscle Tumors in the Vulva?

Unlike those of the uterus, smooth muscle tumors are uncommon in the distal female genitalia. Smooth muscle tumors of the vulva occur over a wide age range but most common in the fourth and fifth decades [106, 107]. Clinical they usually present as a painless, slow-growing, and well-circumscribed mass. Tumors may be of varying size but are often less than 5 cm. Histologically there are three principle patterns: spindled, myxoid, and epithelioid (Fig. 1.76). While spindled type is the most common type, a myxoid and/or hyalinized stroma is disproportionately common in the vulva [107]. Similar to uterine counterparts, vulvar leiomyomas usually exhibit the characteristic gross appearance and microscopic features of benign smooth muscle tumors of the myometrium. Vulvar leiomyomatosis is extremely rare. It has recently been linked to being part of esophageal-vulvar leiomyomatosis, which maybe familial and is thought to associate with an X-linked Alport syndrome [108, 109]. This rare condition is characterized by multiple ill-defined nodular smooth muscle proliferation in the vulva. Patients may have synchronous or metachronous leiomyomatosis of the esophagus, which can be seen in several generations in the same family. Currently, there is no evidence of vulvar leiomyomatosis linked to hereditary leiomyomatosis and renal cell carcinoma syndrome.

Leiomyosarcoma is rare in the vulva but does represent the most common subtype of sarcoma at this site. Vulvar

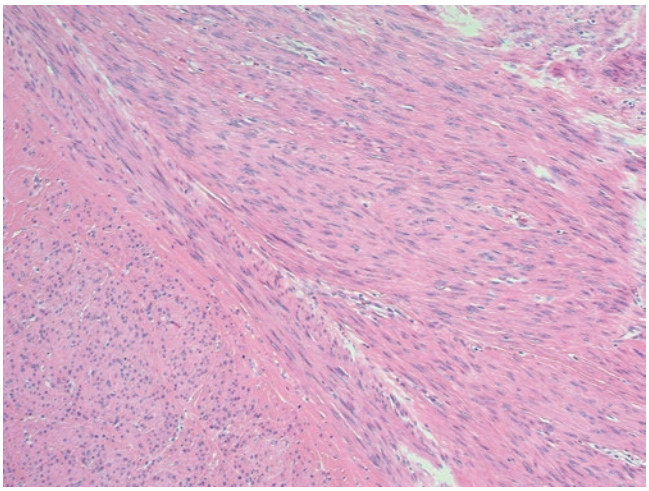

Fig. 1.76 Leiomyoma of the vulva with spindled morphology

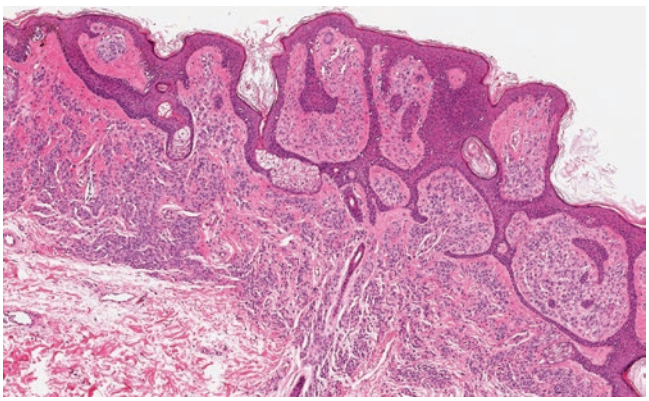

Fig. 1.77 Intradermal nevus of the vulva

leiomyosarcoma typically presents as a rapidly growing tumor in postmenopausal women [110]. Histologically, most vulvar leiomyosarcoma has a similar morphological appearance to their uterine counterparts. In regards to the criteria in classification of vulvar smooth muscle tumors and to predict recurrent or metastatic potential, a recent study with a large series of cases indicated that it could achieve high sensitivity and specificity in classifying vulvar smooth muscle tumors using WHO criteria for uterine smooth muscle tumors [110]. Furthermore, it suggested that circumscription or peripheral infiltration did not seem to be a reliable indicator of malignant potential.

The differential diagnosis can be wide and include not only site-specific vulvar mesenchymal tumors discussed in this chapter but also other entities such as spindle variant of malignant melanoma and dermatofibrosarcoma protuberans. The characteristic histological features and immunoreactivity with multiple smooth muscle markers assist in establishing a diagnosis.

24. What Are the Clinical and Histological Features of Benign Melanocytic Lesions in the Vulva?

Pigmented vulvar lesions are present in approximately 10% of women in the US [111, 112]. They represent a broad spectrum of different entities, including benign and malignant melanocytic proliferation and non-melanocytic proliferations. Overall vulvar melanocytic nevi represent roughly 23% of clinically pigmented vulvar lesions [111, 112]. Most vulvar nevi are intradermal nevi or compound, but other uncommon variants, such as congenital, dysplastic, blue, and Spitz nevi, have been reported (Fig. 1.77). A subgroup of nevi in the genital region demonstrates different but often worrisome histological features from those seen in other anatomic locations.

Atypical genital nevi (AGN) AGN is referred as atypical melanocytic nevi of the genital type. It fits within the category of "nevi of specific sites" and maybe seen anywhere along the anatomic milk line, including the breast, axilla, periumbilical region, and groin [113–115]. Despite the worrisome histological features, AGN has a benign clinical course; no recurrence or metastasis has been reported after complete excision [113, 116, 117]. Therefore, recognition of this group of melanocytic lesions is important to avoid over diagnosis and subsequent treatment.

AGN usually occurs in younger women of reproductive age, representing 5% of vulvar nevi. These patients may have a personal or family history of dysplastic nevi or malignant melanoma [118]. Atypical genital nevi are often detected clinically during routine gynecological examinations, pregnancy, or clinical surveillance because of a personal or family history of dysplastic nevi or melanoma. Most vulvar nevi are located on the labia majora or labia minora, and less commonly, the clitoral hood. The majority of AGN have some atypical clinical features, as they are often hyperpigmented and larger on average than typical nevi, sometimes with irregular borders [113, 118].

AGN has histological features overlapping with those of dysplastic nevi and malignant melanoma. The distinct histological feature of AGN is their prominent, enlarged nests of naval cells with variation in size and shape at the dermoepidermal junction. Variable pigmentations are often present [118]. AGN may have histological features raising concern for melanoma, such as moderate to severe cytological atypia, pagetoid spread, and adnexal involvement (Fig. 1.78). The melanocytes are usually larger than those seen in common nevi showing enlarged nuclei, prominent nuclei, abundant cytoplasm, and fine pigments. Lentiginous growth and Pagetoid spread are typically focal. Melanocytes frequently

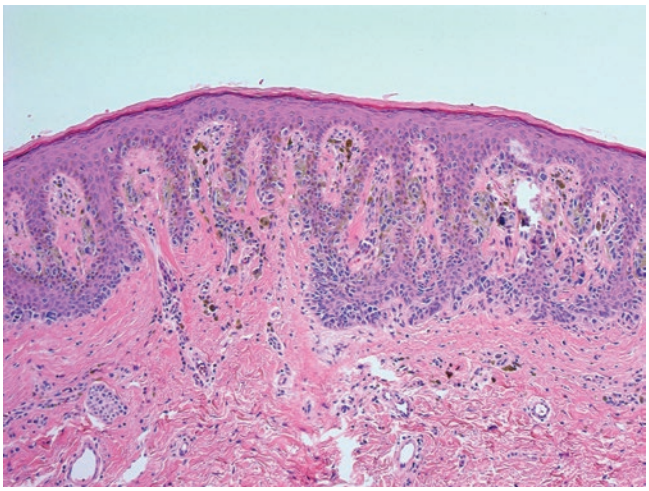

Fig. 1.78 Atypical melanocytic nevi of the genital type, moderate cytological atypia, and pagetoid spread

show skin adnexal involvement, either as single cells or as nests. No or rare mitotic activity or necrosis is present. The maturation of melanocytes is present in the dermal nests. AGN is not considered precursors of dysplastic nevi or melanomas. Complete excision is the treatment of choice if clinically indicated. The main differential diagnoses include dysplastic nevus and vulvar melanoma.

Dysplastic nevi are rare and have no predilection for the genital area, but they are well documented in the vulva of women of all ages. It has an increased risk of developing malignant melanoma. Dysplastic nevi show significant overlap with AGN in epidemiology and clinical presentation. Histologically, dysplastic nevi are characterized by a more prominent lentiginous growth pattern with elongation and bridging of the rete ridges. Melanocyte atypia is scattered and random, rather than relatively uniform, mild to moderate atypia of most AGN. Intracytoplasmic melanin pigment is fine and evenly distributed, compared with the coarse pigmentation in AGN. Dysplastic nevi also show a more prominent host reaction, with the characteristic concentric, eosinophilic fibroplasia, and lamellar fibroplasia in contrast to broad zones of superficial dermal fibrosis in AGN [113, 118]. However, there is a significant morphological overlap between AGN and dysplastic nevi, and the reliable distinction is not always possible.

Take-Home Message It is more important to distinguish AGN from vulvar melanoma from a clinical standpoint. Atypical genital nevus is largely a disease of premenopausal women with a peak age in the 20s and 30s. In contrast, vulvar melanoma is exceedingly rare in this age group and is primarily a disease of elderly adults with a peak incidence in the sixth and seventh decade. Clinically, vulvar melanoma tends to present as a large and often ulcerated tumor with irregular borders. Histologically, melanoma tends to show a more asymmetric growth with poorly delineated borders, more prominent and irregular Pagetoid spread, greater cytological atypia, and lacks dermal maturation seen in AGN. Also, the dermal mitotic activity can be identified in most melanoma, whereas it is rarely found in AGN. The invasive component lacks maturation with depth and is characterized by expansile growth. Also, atypical mitoses, apoptosis, and tumor necrosis may be identified in melanoma [119].

25. What Are the Clinical and Histopathological Features of Vulvar Malignant Melanoma?

Vulvar melanoma is a rare and aggressive malignancy that accounts for 2.4–10% of all vulvar malignancies [120–122]. Approximately 3–7% of all melanomas in women occur in the vulvar region. The malignant vulvar melanomas often occur de novo. The malignant transformation from a preexisting nevus in the vulva is much less common than in extra-vulvar melanomas. There are important differences in anatomic consideration, surgical approach, and biological behavior between vulvar and non-vulvar melanomas [123–125]. The overall survival rate in vulvar melanoma is much worse compared with the survival rate in other cutaneous melanoma across all stages [126–129].

Vulvar melanoma is most commonly seen in Caucasian women in their sixth or seventh decade. The usual locations are the labia majora, less commonly the clitoris, and the labia minora; only 12% arise in hair-bearing areas [130]. Multifocal tumors occur in approximately one-third of cases. Vulvar melanoma is commonly present as an asymmetrical pigmented macule, papule, or mass, often with an irregular border and often larger than 7 mm. Pruritus, bleeding, or a changing in size are also common presentations [131, 132]. Ulceration is present in half of the cases of vulvar melanoma [126]. Up to one-third of the vulvar melanomas are not pigmented [131, 132].

If a vulvar melanoma is suspected, a full-thickness punch biopsy specimen is warranted to ensure adequate depth for staging purposes [132]. Histologically, the tumor consists of marked atypical melanocytes arranged in confluent nests and sheets containing varying amounts of melanin within the epidermis and dermis. Almost half of the vulvar melanomas are of the superficial spreading type, followed by nodular and lentiginous, and spindle cell types [126]. In the intraepidermal component, the tumor cells usually have enlarged nuclei with prominent nucleoli and abundant eosinophilic cytoplasm. The invasive component may be spindle cell type, epithelioid type, or mixed spindle–epithelioid type with a myxoid or desmoplastic stromal response (Fig. 1.79). Cytoplasmic melanin can vary from copious to absent.

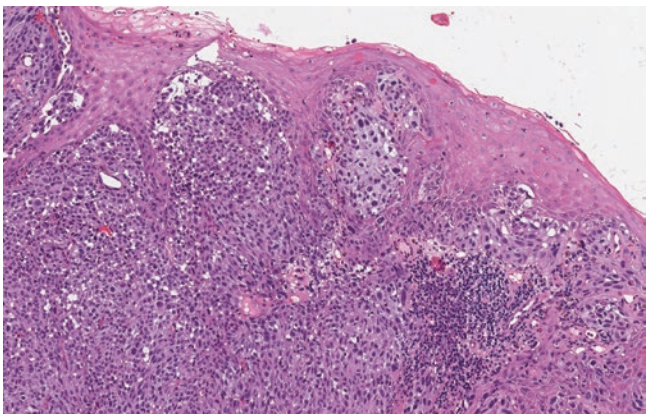

Fig. 1.79 Malignant melanoma of the vulva

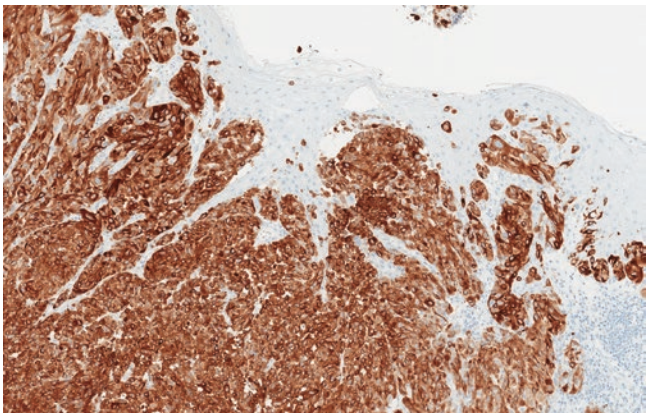

Fig. 1.80 Malignant melanoma of the vulva. The neoplastic cells are positive for Melan A

Mitotic figures are usually abundant. The studies of the correlation of histological type with survival show inconsistent results; some studies found the superficial spreading type is generally associated with a better prognosis [126, 132]. Differential diagnoses of malignant melanoma include atypical genital nevi and dysplastic nevi, Paget disease, squamous cell carcinoma, including carcinoma in situ/pagetoid VIN. The tumor cells of both Paget disease and malignant melanoma can contain melanin pigment, and thus its presence does not aid in the differential diagnosis. For distinction of poorly differentiated squamous cell carcinoma or adenocarcinoma from the invasive component of melanoma or sarcoma from spindle cell melanoma, malignant junctional component, cytoplasmic melanin pigment, and positivity for melanocytic markers, such as Melan A, Sox10, HMB-45, or MART1, facilitate the diagnosis (Fig. 1.80). As in other sites, the diagnosis of melanoma should always be considered in the differential diagnosis when dealing with a poorly differentiated malignant vulvar tumor.

For vulvar malignant melanoma thicker than 1 mm, the examination of lymph nodes is generally recommended [123, 133]. Once diagnosed, the AJCC staging system should be used for vulvar melanoma instead of the FIGO system used for squamous cell carcinoma. Breslow thickness, ulceration, and lymph node involvement are important negative prognostic indicators [126, 128, 129]. Most studies in vulvar melanoma propose a minimum cut-off value for high-risk melanomas of 1.5 mm tumor thickness [128, 129, 134]. Lymph node status is also prognostic for recurrence. Recent studies showed that the mitotic rate was an independent predictor of survival [126, 135, 136].

Due to late diagnosis, the prognosis of vulvar melanoma is poor, with estimated 5-year survival ranging from 37% to 50%. Surgery remains the primary treatment modality for all resectable melanomas. A surgical margin of 0.5–1.0 cm for melanoma in situ, 1 cm for invasive melanoma with a Breslow thickness ≤1 mm, 1–2 cm for a Breslow thickness of 1.01–2 mm, and 2 cm for a Breslow thickness of ≥2.01 mm is generally recommended [137, 138].

The medical treatment of melanoma recently has changed with FDA and EMA approval of CTLA-4, PD-1, BRAF, and MEK inhibitors [139–142]. Immunotherapy with monotherapy or combination therapy with one or more of the following drugs, ipilimumab, pembrolizumab, nivolumab, vemurafenib, dabrafenib, and trametinib, may be considered in women with advanced/metastatic melanoma. Due to the relatively high number of KIT mutations in vulvovaginal melanoma, tyrosine kinase inhibitors may be a treatment option in the future [143–145].

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