CHAPTER 1

EARLY DIAGNOSIS OF VULVAR CANCER

Peter Schlosshauer

INTRODUCTION

Surgery on vulvar malignancies has become more conservative over the past several decades. A radical vulvectomy with removal of bilateral inguinal lymph nodes en bloc, a mutilating procedure fraught with high complication rates still performed in the 1980s, has become a rarity in today’s practice. This may be partially due to the fact that tumors tend to be diagnosed at earlier stages, thanks to better patient education and improved healthcare. While a more conservative surgical approach is desirable for the patient, therapeutic efficacy should not be compromised. Because of the close proximity of important structures in the vulvar region, space is often very limited for the surgeon to achieve wide enough margins around the lesion. This chapter will specifically address the issues arising in the context of diagnosing and evaluating early stage vulvar malignancies and their precursor lesions. The current concepts of carcinogenic mechanisms of vulvar neoplasms are also reviewed.
Since a major part of the vulva is composed of skin, virtually any dermatologic disorder may be encountered on the vulva. For lesions unrelated to vulvar malignancies, including benign tumors and the manifestations of a generalized dermatologic or medical condition, the reader is referred to textbooks of gynecologic pathology, dermatopathology or dermatology.

NORMAL ANATOMY AND HISTOLOGY

The vulva is synonymous with the external female genitalia and includes the mons pubis anteriorly, the labia majora laterally, the labia minora medially, the clitoris at the anterior junction of the labia minora, the fourchette at the posterior junction of the labia minora, and the perineum extending posteriorly between the fourchette and the anus. The introitus (or vestibule) is the space limited by the labia minora laterally, the clitoris anteriorly, the fourchette posteriorly, and is separated from the vagina by the hymenal ring. Important structures related to the introitus are the vagina, the urethra, the paraurethral (Skene) glands and their ducts, and the major vestibular (Bartholin) glands, which are drained posterolaterally to the hymenal ring. In addition, there are minor vestibular glands around the external base of the hymenal ring. In the adult, the mons pubis, the labia majora and part of the perineum carry hair-bearing skin with adnexal structures, underlaid by ample adipose tissue.

While the clitoris and the lateral aspects of the labia minora are covered by keratinizing squamous epithelium, the introitus is lined by non-keratinizing squamous epithelium. Except for sebaceous glands, the labia minora do not carry any skin adnexal structures. The body of the clitoris consists of the richly vascularized corpora cavernosa and abundant nerve fibers. The mucinous Skene glands open into the introitus via a transitional-type epithelium lined duct. The Bartholin gland is composed of acini lined by columnar mucinous epithelium. Its duct typically displays mucinous epithelium proximally, transitional-type epithelium in its middle portion, and squamous epithelium close to the orifice. The lymphatic drainage of the vulva occurs generally to the ipsilateral femoral and inguinal lymph nodes.
However, lymph from the midline portions of the vulva may drain to both sides. In addition, there is a minor lymphatic drainage pathway from the clitoris via the urethral lymphatics to the interiliac, external iliac and obturator lymph nodes.

The rare occurrence of vulvar tumors that resemble lesions frequently found in the mammary gland has led to the theory that ectopic breast tissue may be encountered in the vulva as a result of incomplete regression of an embryologic “milking line”. Alternatively, such lesions may be derived from “mammary-like glands” that have eccrine and apocrine features and are a normal constituent of vulvar and perianal skin.

**CLINICAL IDENTIFICATION OF EARLY VULVAR NEOPLASMS**

The key to early diagnosis of a vulvar malignancy is of course clinical recognition of a suspicious lesion. Since it is not uncommon for patients to delay seeking medical attention, the first step to earlier diagnosis is improved patient education. Similar to breast self-examination, patients should be encouraged to self-examine the vulva regularly. The use of a mirror is helpful. Informed women will know to look for changes in skin color or thickness and the appearance of open sores or wart-like growths, and not to delay a visit to the gynecologist if a new lesion is noticed.

The understanding of the risk factors for developing vulvar neoplastic disease will increase the physician’s index of suspicion. These include a previous history of genital human papilloma virus (HPV) infection, cervical or vaginal intraepithelial neoplasia, lichen sclerosus, squamous hyperplasia, and chronic granulomatous diseases of the vulva. In recent years, immunosuppression due to HIV infection, status post organ transplantation, or chemotherapy has emerged as a major risk factor for intraepithelial or invasive neoplastic disease, especially in young women. Up to 50% of patients with vulvar neoplastic disease are asymptomatic. Also, symptoms may not be reported due to embarrassment or denial. Therefore, on routine annual gynecologic check-up visits, a careful anamnestic exploration always includes...
a question regarding changes or discomfort in the vulvar region. Common early symptoms include chronic vulvar pruritus or pain (“vulvodynia”), ulceration and bleeding, dysuria and “vaginal” discharge. Any of these complaints should alert the physician to the possibility of an underlying malignancy and prompt a thorough clinical examination, including the inspection and palpation of the vulva and inguinal lymph nodes. Biopsies should be taken liberally of any visible vulvar lesion. Symptomatic treatment or application of topical ointments for any condition without a defined working diagnosis is not recommended. The use of a colposcope and/or application of toluidine blue or 5% acetic acid (which on the vulva, as opposed to the cervix, may need to be applied for several minutes in order to visualize a dysplastic lesion!) may be helpful to identify a subtle lesion and choose the site for biopsy. Generally, it is preferable not to excise the entire lesion if no previous histopathologic diagnosis is available. It must be kept in mind that in the event of an invasive carcinoma, optimal results are achieved with an at least 1.0 cm disease free margin. Also, if there is stromal invasion of more than 1.0 mm in depth, inguinal lymph node dissection is recommended. Thus, an optimal treatment strategy should be developed based on biopsy results. Biopsies should be taken from areas that appear clinically most suspicious for invasion, avoiding necrotic areas. The biopsy should be deep enough and include underlying connective tissue to allow the evaluation of invasion, if present. The periphery of a lesion may show only in situ disease. Often, multiple biopsies are indicated, especially in multifocal disease.

**PROCESSING OF A SURGICAL SPECIMEN FOR PATHOLOGIC EVALUATION**

For optimal pathologic evaluation, a good collaboration between pathologist and surgeon is crucial. After obtaining a biopsy specimen, communication of pertinent clinical information to the pathologist is of utmost importance. While this is true for all surgical pathology, in the case of vulvar biopsies, it is essential for the pathologist to know whether the “lesion” appeared clinically as an exophytic papillary
structure, a pigmented spot, an ulcer, or something else. An accurately oriented specimen is necessary for correct identification of resection margins, in case they are involved by the pathologic process. Commonly, a stitch is attached to the specimen by the surgeon indicating a certain clockface position. For optimal preservation of orientation, and to prevent distortion during fixation, the resected specimen may be pinned onto cardboard before placed into 10% formalin. Additional information may be indicated on the cardboard, preferably with pencil, since other types of ink may be washed out by the formalin. The more clinical information is given to the pathologist, the more useful the rendered diagnosis will be.

Most surgical specimens resected from the vulva will fall into one of three categories:

1. An incisional biopsy represents lesional tissue removed without the intention to completely excise the lesion. These are typically small tissue fragments, a few millimeters in greatest dimension. When embedding the specimen into the paraffin block, care must be taken to orient the tissue such that a perpendicular section of the epidermis and underlying tissue will be seen on the slide. Incorrect embedding may not only compromise diagnostic accuracy, but can even destroy the tissue for further analysis. On incisional biopsies the pathologist is asked to give a mere diagnosis as to what kind of lesion is present (e.g. reactive versus neoplastic). If malignant, the report should also include a statement whether stromal invasion and/or lymphvascular involvement can be identified. Accurate evaluation of the size of the lesion and/or depth of invasion is not possible on incisional biopsies. The assessment of margins is less of an issue, since it is understood that the lesion was not completely excised. However, if the pathologist is not sure whether the specimen was meant to be an incisional or an excisional biopsy, it is prudent to ink the specimen and report on the status of the surgical resection margins anyway.

2. An excisional biopsy is taken with the intention to completely remove the lesion and achieve clear resection margins. These
specimens usually have the shape of an ellipse with a visible central lesion. Since the evaluation of margins is a required part of the pathologic evaluation, an excisional biopsy should always be inked. According to the surgeon’s indication for orientation, opposite resection margins can be inked in different colors (e.g. medial = black, lateral = red). For optimal assessment of the margins, it is recommended to cut off the tips of the elliptically shaped specimen, bivalve them longitudinally and submit them in separate cassettes. Then the remainder of the specimen is serially sectioned transversely and entirely submitted. Again, care must be taken to embed the sections “on edge” to allow optimal evaluation of the full thickness of the specimen.

3. After the diagnosis of a malignancy has been made, a radical procedure (wide resection, radical vulvectomy/hemivulvectomy) results in a larger specimen including inguinal lymph nodes. Correct orientation is still critical, but may be more easily recognizable from the specimen. Additional margins, e.g. vaginal and/or urethral margins, may need to be evaluated. Usually representative sections are sufficient to obtain the necessary pathologic information.

NON-NEOPLASTIC EPITHELIAL DISORDERS

Vulvar Dermatoses

Although the vulvar dermatoses lichen sclerosus (formerly known as lichen sclerosus et atrophicus) and squamous hyperplasia are not thought to be direct precursors of neoplastic lesions, they may be seen in association with vulvar carcinoma, especially with the human papillomavirus (HPV) unrelated keratinizing squamous cell carcinoma. Whether these associated dermatoses are due to similar stimuli that give rise to the cancer, or a reaction to the neoplastic process, is unclear. Regardless, the presence of these dermatoses should alert both clinician and pathologist to the possibility of an associated malignancy and prompt careful examination, close follow-up, and thorough pathological work-up of resected specimens. In addition, both
lesions may clinically mimic a neoplastic process and are therefore frequently biopsied. Lichen sclerosus and squamous cell hyperplasia may coexist (“mixed dystrophy”) and often present with vulvar pruritus. Visible lesions may be described as “leukoplakia” (literally “white plaque”), a term that is greatly appreciated by the pathologist as a piece of clinical information, but does not designate a specific histopathologic diagnosis.

Neither lichen sclerosus nor squamous hyperplasia are causally related to HPV infection.

**Lichen Sclerosus**

This disorder affects primarily postmenopausal women, but can be seen at any age, including children. Grossly, the epidermis is thinned, whitish and may show varying degrees of shrinkage leading to effacement of the labia minora, constriction of the introitus or clitoral phimosis (“kraurosis vulvae”). While it may affect the entire vulva including the perineum and perianal region, the vagina is not usually involved. Histologically, the fully developed lichen sclerosus is characterized by a thinned atrophic layer of viable squamous epithelium, marked hyper- and parakeratosis, flattening of the rete ridges at the dermoepidermal junction, a hyalinized hypocellular zone underneath the squamous epithelium, and a band-like chronic inflammatory infiltrate deep to the hypocellular zone (Fig. 1). In earlier stages, not all of these features may be present. Not infrequently, only the squamous epithelium will be obtained during the biopsy procedure, with no dermis available for evaluation. In such cases, the finding of a strip of hyperkeratotic squamous epithelium in which the layer of hyperkeratosis is thicker than the remaining viable epithelium is highly suggestive of the diagnosis of lichen sclerosus.

The etiology of lichen sclerosus remains unclear. Immunologic, genetic, hormonal, infectious and local reactive factors have been implicated. Vulvar lichen sclerosus is not associated with HPV infection, although HPV-positivity has been reported in a subset of penile lichen sclerosus cases.¹ There is an increased incidence of
p53 mutations in vulvar lichen sclerosus compared to the adjacent normal skin. Similar findings have been reported in the differentiated subtype of vulvar intraepithelial neoplasia (VIN), which is often associated with lichen sclerosus, suggesting that p53 mutations may be an early event in the carcinogenesis of non-HPV related vulvar squamous cell carcinoma. As stated above, lichen sclerosus is not considered a direct precursor of neoplastic disease, but histological evidence of lichen sclerosus is seen in approximately 61% of cases with invasive squamous cell carcinoma. Conversely, up to 21% of patients with symptomatic lichen sclerosus develop squamous cell carcinoma within a mean of four years. A recent study suggests that cases of lichen sclerosus that subsequently develop squamous cell carcinoma display a higher Mib-1 labeling index than those that do not develop carcinoma.

Fig. 1  Lichen sclerosus. The epidermis is atrophic and hyperkeratotic. Rete ridges are effaced. There is a hypocellular subepidermal layer and a sparse chronic inflammatory infiltrate underneath. Hematoxylin/cosin, original magnification 100×.
Squamous Hyperplasia/Lichen Simplex Chronicus

Formerly known as hyperplastic vulvar dystrophy, squamous hyperplasia is thought to be a non-specific reactive process due to a variety of irritants. Clinically, the involved area may appear whitish or reddened, excoriated due to scratching, but unlike lichen sclerosus, there is no shrinkage of the skin. Histologically, squamous hyperplasia shows somewhat opposite features as lichen sclerosus, including a thickening of the viable epithelium (acanthosis) and elongation of the rete ridges. There is a prominent granular cell layer and variable degrees of hyper-and parakeratosis. Intercellular bridges may be prominent due to edema (spongiosis). Of note, there is no significant cytologic atypia, and mitotic activity is confined to the basal cell layer (Fig. 2). If associated with a lymphocytic superficial dermal inflammatory

Fig. 2  Squamous hyperplasia/Lichen simplex chronicus. The epidermis is thickened and shows hyper- and parakeratosis. Rete ridges are elongated. There is a chronic inflammatory infiltrate within the superficial dermis. Hematoxylin/eosin, original magnification 100×.
infiltrate, it is also called lichen simplex chronicus (LSC). There is no dermal sclerosis. The differential diagnosis includes vulvar intraepithelial neoplasia (VIN), fungal infections, and other dermatoses like psoriasis or lichen planus, which need to be excluded in order to establish the diagnosis of squamous hyperplasia. Similar to lichen sclerosus, squamous hyperplasia is seen in association with over 50% of vulvar squamous cell carcinomas.

**Condylomata Acuminata**

Warts caused by human papillomavirus (HPV) types 6 and 11 are generally considered an infectious, sexually transmitted disease, and not a direct precursor lesion of neoplasia. However, epidemiologic analyses suggest that the long-term risk of developing invasive vulvar carcinoma is increased as much as 10-fold in patients hospitalized for anogenital condylomata acuminata.

More than 100 genetically distinct HPV types have been identified, most of which target a very specific epithelial region. On the vulva, HPV 6 and 11 are the most frequently encountered types, which give rise to sessile or papillary growths, known as condylomata acuminata. There are usually multiple lesions covering the vulvar skin to varying degrees. Moreover, they may be present in the vagina, cervix, urethra or perianal region. Most patients present in the third and fourth decade of life. Up to 50% of women with vulvar condylomata acuminata also have cervical HPV infections. The natural course of the disease is often very protracted, and it may take years until immunity is acquired and the warts are shed. For a more detailed discussion of the biology of HPV infections, the reader is referred to chapter 2, pp. 62–63.

Histologically, a condyloma acuminatum is characterized by a relatively thin fibrovascular core, surrounded by a thickened (acanthotic) epithelium. The pathognomonic criterion on routine light microscopy is the presence of koilocytes. These are squamous cells with an abnormal (hyperchromatic, enlarged, irregularly shaped) nucleus and a surrounding cytoplasmic clearing ("halo"), which are considered HPV-specific cytopathic changes. Multinucleation is frequent; however, an
occasional binucleated cell is non-specific, unless it displays the nuclear atypia required to classify it as a koilocyte. Likewise, a clear cytoplasm is not to be interpreted as a koilocytotic halo unless it is accompanied by an atypical nucleus. Koilocytotic changes tend to be more subtle on the vulva than they are on the cervix. Often, they are most obvious at the base of clefts between two adjacent papillary projections (Fig. 3). Mitotic figures are rare, not atypical, and confined to the basal or parabasal cell layers. Condylomata may display varying degrees of hyper-and parakeratosis, although the mere finding of parakeratosis is non-specific.

Rarely, vulvar condylomata acuminata may progress to VIN and invasive squamous cell carcinoma, especially if they are associated with high risk HPV types. The so-called “giant condyloma” (Buschke-Löwenstein) is now considered a well differentiated verrucous squamous cell carcinoma that is locally invasive, rarely if ever metastasizes and may be associated with HPV, including the “low-risk” type 6.

PRE-MALIGNANT SQUAMOUS EPITHELIAL LESIONS

In analogy to precursor lesions of the uterine cervix, intraepithelial premalignant squamous cell lesions of the vulva have been termed vulvar intraepithelial neoplasia (VIN). Over the past few decades, a dramatic increase in incidence of VIN has been observed, especially in women younger than 50 years of age. As a result the peak age for VIN has shifted from the seventh decade (in the 1970s) to the fifth decade. Up to 50% of patients with VIN have an associated cervical or vaginal neoplastic lesion. Clinically, VIN typically presents as single or multiple raised skin lesions that may involve any area of the vulva. In the perianal region, the term anal or perianal intraepithelial neoplasia is also used (AIN or PAIN, respectively). It may cause pruritus. The color may range from whitish over red, grey, to darkly pigmented. The term “bowenoid papulosis” refers to multiple pigmented papular lesions, while “erythroplasia of Queyrat” describes a reddish lesion involving the epithelium of the introitus. Both
Fig. 3 Condyloma acuminatum. (a) The lesion has a papillary architecture. The epithelium contains numerous koilocytes, especially at the base of clefts. Hematoxylin/eosin, original magnification 40×. (b) Higher magnification of an area containing koilocytes, which are characterized by an atypical nucleus surrounded by a clear cytoplasmic space (“halo”). Hematoxylin/eosin, original magnification 100×.
EARLY PATHOLOGIC DIAGNOSIS OF GYNECOLOGIC CANCER INCLUDING A CLINICIAN'S VIEW

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Two types of VIN are distinguished. The “usual” (also known as “classic”, “bowenoid” or “undifferentiated”) VIN comprises approximately 80% of all diagnosed VIN cases. It is HPV-associated (mostly types 16 and 18), occurs in a younger age group (third and fourth decades) and is often multifocal. These lesions are characterized by a lack of normal maturation as cells migrate from the basement membrane toward the surface. This is manifested by a persistently high nuclear/cytoplasmic ratio, cellular crowding, mitotic activity (including atypical mitotic figures) distant from the basement membrane, and the failure of the cell body to assume an orientation parallel to the skin surface. Acanthosis, hyper- and parakeratosis are also frequent features. Morphologically, basaloid, warty or mixed features are distinguished. Basaloid VIN is composed of relatively small cells with high nuclear/cytoplasmic ratio, hyperchromatic nuclei and coarse chromatin pattern (Fig. 4). Warty (condylomatous) VIN has larger cells with greater nuclear pleomorphism, clumped chromatin, frequent multinucleated cells and, especially towards the surface, koilocytotic changes (Fig. 5). Intermediate or mixed basaloid/warty lesions are frequent. Usual VIN is considered the precursor lesion of the HPV-associated basaloid and warty/condylomatous types of invasive vulvar squamous cell carcinoma.

The second VIN type is known as “differentiated” or “simplex” VIN. It is composed of cells that appear relatively mature with ample cytoplasm but enlarged atypical nuclei containing prominent nucleoli populating the basal and parabasal cell layers. An important diagnostic feature is the presence of dyskeratotic cells in the parabasal cell layer and keratin pearl formation within rete ridges (Fig. 6(a) and (b)). Differentiated VIN is typically unifocal, occurs in the postmenopausal age group, and is frequently seen in patients with a history of lichen sclerosus. On immunohistochemistry, p53 overexpression has been reported in the basal and parabasal layer of differentiated VIN, which may be useful to support the diagnosis (Fig. 6(c)). Ki-67 expression is confined to the basal cell layer in differentiated VIN (Fig. 6(d)). This is in contrast to normal vulvar epidermis, in which
Fig. 4  High grade VIN, basaloid type. The lesion is composed of cells with a high nuclear/cytoplasmic ratio. Numerous mitotic figures are present throughout the entire thickness of the epithelium. Hematoxylin/eosin, original magnification 100×.

Fig. 5  High grade VIN, warty (condylomatous) type. Cells have ample cytoplasm and koilocytotic features. Hematoxylin/eosin, original magnification 100×.
Fig. 6  Differentiated (simplex) VIN. (a) The epithelium is markedly thickened and appears relatively mature. Note keratin pearl formation in rete ridges. Hematoxylin/eosin, original magnification 40×. (b) High magnification shows nuclear atypia with prominent nucleoli, and dyskeratosis of individual cells. Hematoxylin/eosin, original magnification 400×.
Fig. 6 (Continued)  (c) Immunohistochemical stain for p53. Nuclear positivity is seen in basal and parabasal cells. Original magnification 100×. (d) Immunohistochemical stain for Ki-67 (MIB-1). Nuclear positivity is present in basal cells. Original magnification 100×.
the basal cell layer is typically negative and the parabasal cell layer positive for Ki-67. Increased expression of p53 and Ki-67 may herald transformation to invasive carcinoma. Differentiated VIN is HPV-negative and hence does not overexpress p16.

Differentiated VIN is thought to be the direct precursor of invasive HPV-unrelated keratinizing squamous cell carcinoma. Its risk of progression to invasive carcinoma is probably even higher than that for “usual” high grade VIN. Although the majority of vulvar carcinomas are HPV-unrelated, differentiated VIN is relatively rarely diagnosed, especially when not (yet) associated with an adjacent invasive carcinoma. Possible reasons for this phenomenon include that differentiated VIN may often remain unrecognized by the pathologist because of the more subtle morphologic changes, and that the progression time to invasive carcinoma may be rather short.

While differentiated VIN is not further graded, usual VIN was originally subdivided into mild (VIN 1, confined to lower third), moderate (VIN 2, involving lower and middle third), and severe (VIN 3, involving lower, middle and upper third of the epithelium) dysplasia. The term carcinoma in situ is applied to those lesions with no evidence of maturation at all throughout the full thickness of the epithelium. However, this is a purely academic distinction that has no clinical implications. For practical purposes, lesions classified as VIN 2 and 3 or carcinoma in situ are grouped together as high grade dysplasia.

Lesions that qualify for a diagnosis of VIN 1 are quite rare, and some authors even question the existence of such an entity. The International Society for the Study of Vulvar Disease (ISSVD) has recommended applying the term VIN to high grade (i.e. VIN 2 and 3, carcinoma in situ) lesions only without further grading, and discontinuing the use of the term VIN 1, the rationale being that “VIN 1” lesions and condylomata are manifestations of a viral infection rather than immediate precursors of invasive squamous cell carcinoma. However, a subset of vulvar “low grade” lesions does contain high risk HPV types, and these are morphologically indistinguishable from the low risk HPV-associated lesions. Discussion of the grading of
VIN, its appropriate terminology, and how this terminology reflects the biologic behavior of the lesions is ongoing.

High grade VIN can spontaneously regress, especially in young women. However, beyond 30 years of age, the potential to become invasive is great enough that surgical treatment is preferred. In contrast to the cervix, cytologic smears do not appear to be a practical tool to detect precursor lesions of vulvar carcinoma.

**INVASIVE CARCINOMA**

**Squamous Cell Carcinoma**

**Epidemiology, Etiology and Pathogenesis**

Squamous cell carcinoma of the vulva represents approximately 3%–5% of all female genital carcinomas. Eighty-six percent of all vulvar carcinomas are of squamous cell origin. A 20% increase in the incidence of invasive vulvar carcinoma has been observed in the USA over the past three decades. While invasive vulvar carcinoma is rare below the age of 30, the incidence continues to increase continuously thereafter without reaching a peak. Unlike VIN, no significant shift to a younger population has been observed in recent years. Not only is there this disparity between the incidence patterns of in situ and invasive vulvar carcinoma, but they also differ from the respective incidence patterns of cervical in situ and invasive carcinoma. These epidemiologic data suggest that a variety of as yet unidentified factors contribute to the progression from VIN to invasive vulvar carcinoma, and that these factors are different from those implicated in the majority of cervical carcinomas.

It is now well recognized that two types of vulvar squamous cell carcinoma can be distinguished based on epidemiology, pathogenesis and clinical presentation. The first group, comprising about one third of all vulvar squamous cell carcinomas, presents at a mean age of 55 years. Patients are often smokers. Tumors are positive for HPV DNA, mostly types 16 and 18. Histologically, these tumors are typically of the basaloid or warty types and often have associated “usual” VIN. The carcinogenetic mechanism is thought to be similar to that in
HPV-associated squamous cell carcinoma of the uterine cervix and involves integration of the viral DNA into the host cell genome, and inactivation of the p53 and retinoblastoma tumor suppressor proteins by the viral proteins E6 and E7, respectively (see chapter 2, pp. 62–65). Immunosuppressed patients are at increased risk to acquire HPV-related vulvar disease, including invasive squamous cell carcinoma. However, unlike cervical carcinoma, invasive vulvar carcinoma is not (yet) recognized as an AIDS-defining illness in HIV positive patients. The progression from in situ to invasive carcinoma appears to be associated with a reduction in the number of intraepithelial CD1a-positive antigen presenting dendritic cells in both HIV-positive and HIV-negative patients, further supporting a role of the immune system in the carcinogenic process. With the advent of HPV vaccines, a decrease in the incidence of these carcinomas is anticipated over the next decades.

Patients of the second group present at a mean age of 77 years. They tend to be non-smokers, and often have an associated vulvar dermatosis (lichen sclerosus or squamous hyperplasia). If there is VIN, it is of the differentiated (simplex) type. These tumors are HPV-negative and tend to be well differentiated and keratinizing. Mutations of the p53 tumor suppressor gene are thought to be an important event in the early pathogenesis of these HPV-unrelated tumors, since they are frequently found in lichen sclerosus, differentiated (simplex) VIN, and well differentiated keratinizing squamous cell carcinoma.

In addition, chronic granulomatous diseases of the vulva, especially granuloma inguinale, have been associated with an increased risk for developing vulvar carcinoma. Our knowledge about molecular genetic events in the pathogenesis of vulvar carcinoma is still very fragmentary. Apart from the alterations mentioned above, mutations in the PTEN tumor suppressor gene have been reported in both in situ and invasive squamous cell carcinoma of the vulva, suggesting a role in its early carcinogenesis. Other genes implicated in the development of vulvar carcinoma include p16ink4a, p15ink4b, the retinoblastoma (RB) tumor suppressor gene, and cyclin D1.
Histologic Subtypes

If not otherwise specified (NOS), vulvar squamous cell carcinomas are of the well to moderately differentiated, keratinizing type. Basaloid carcinoma is composed of relatively small cells with scant cytoplasm. It occurs in the younger patient population with a mean age of 54 years and is often associated with basaloid VIN. The majority of basaloid carcinomas are positive for HPV, mostly type 16. Basaloid carcinoma must be distinguished from basal cell carcinoma, which typically displays a palisading array of the outermost cells.

Similarly, warty (condylomatous) carcinoma presents at a mean age of 55 years, frequently shows associated warty VIN and is also associated with HPV, usually type 16. It is characterized by pronounced cytologic pleomorphism and koilocytotic changes. Of note, both basaloid and warty vulvar carcinomas may be associated with a synchronous cervical or vaginal squamous neoplasm.

Verrucous carcinoma is a well differentiated squamous cell carcinoma with a broad, “pushing” front of invasion that grows in a locally destructive fashion, but almost never metastasizes. Formerly known as “giant condyloma” of Buschke-Löwenstein, that designation is confusing and therefore no longer recommended. It has been found in association with lichen simplex chronicus and with vulvar acanthosis with “altered differentiation”, characterized by loss of the granular cell layer and multilayered parakeratosis,25 which may represent a precursor lesion. It is not clear whether verrucous carcinoma is causally related to HPV infection, but at least a subset of cases has been reported positive for various types, including the “low-risk” HPV type 6.8 Basal cell carcinoma, although generally very common elsewhere on the skin, occurs only rarely on the vulva. Its histopathologic hallmarks are the parallel alignment of the peripheral cells of the tumor nests (“palisading”), and the surrounding stromal retraction artifact. Basal cell carcinoma is locally aggressive, does not usually metastasize and has a good prognosis if completely excised.
Additional rare types of vulvar carcinoma include spindle cell, giant cell, adenoid squamous, adenoid basal, lymphoepithelioma-like, basosquamous and sebaceous cell carcinoma.

**Staging**

As is true for many other tumors, the single most important prognostic factor of vulvar carcinoma is its extent at the time of presentation, recorded as the tumor stage. Gynecologic oncologists tend to use the Fédération Internationale de Gynécologie et d’Obstétrique (FIGO) staging system, while the College of American Pathologists’ (CAP) guidelines for pathology reports on cancer cases require indication of the tumor stage in the Tumor-Node-Metastasis (TNM) format as published by the American Joint Committee on Cancer (AJCC). The TNM categories have been defined to correspond to the FIGO stages, but due to the different approaches some cases may fall into different stage groups depending on the system applied. Notably, the presence of any unilateral or bilateral regional lymph node metastasis will result in FIGO stage III or IVA, respectively, regardless of the size or depth of invasion of the primary tumor. FIGO stage IVB is defined by the presence of distant metastasis (Table 1).

Carcinomas with a depth of invasion of 1 mm or less (T1a, FIGO stage IA) are referred to as “superficially invasive” tumors. While the risk of metastatic disease in these cases is less than 5%, it sharply increases once the 1 mm threshold is passed. At 3 mm depth of invasion, regional lymph node metastases are seen in approximately 10% of cases. Depth of invasion is measured from the dermo-epidermal junction of the most superficial adjacent dermal papilla to the point of deepest invasion (Fig. 7). It is most accurately obtained by using a calibrated ocular under consideration of the magnification used. For practical purposes, if the diameter of the microscopic field at a specific magnification is known (e.g. at a magnification of 40× using a 4× objective and a 10× ocular, the diameter of the observed microscopic field is typically 5.0 mm), the depth of invasion can often be estimated with
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<th>FIGO stage</th>
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<td>Primary Tumor (T)</td>
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<td>Carcinoma <em>in situ</em>, high grade VIN</td>
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<td>T1</td>
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<td>Greatest tumor diameter 2 cm or less; confined to vulva or vulva and perineum</td>
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<td>T1a</td>
<td>IA</td>
<td>Stromal invasion 1 mm or less in depth, as measured from the dermoepidermal junction of the adjacent most superficial dermal papilla to the deepest point of invasion</td>
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<td>T1b</td>
<td>IB</td>
<td>Stromal invasion greater than 1 mm in depth</td>
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<td>T2</td>
<td>II</td>
<td>Greatest tumor diameter more than 2 cm; confined to vulva or vulva and perineum</td>
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<td>T3</td>
<td>III</td>
<td>Tumor of any size with contiguous spread to the lower urethra and/or vagina or anus</td>
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<td>T4</td>
<td>IVA</td>
<td>Tumor invades any of the following: upper urethra, bladder mucosa, rectal mucosa, or pelvic bone</td>
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<td>Distant metastasis cannot be assessed</td>
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<td>—</td>
<td>No distant metastasis</td>
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<tr>
<td>M1</td>
<td>IVB</td>
<td>Distant metastasis (including pelvic lymph node metastasis) present</td>
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</table>

Legend: Regional lymph nodes are the femoral and inguinal lymph nodes. The involvement of pelvic lymph nodes, including internal, external, common iliac and obturator lymph nodes, is considered distant metastasis. Vascular invasion has no impact on staging. The stage cannot be changed on the basis of disease progression, recurrence or response to radiation or chemotherapy prior to surgery. This classification applies to all subtypes of squamous cell carcinoma, Paget disease of the vulva, basal cell carcinoma and vulvar adenocarcinoma including Bartholin gland carcinoma. It does not apply to mucosal malignant melanoma.
sufficient accuracy, at least with respect to the 1 mm threshold. As opposed to carcinoma of the uterine cervix, the term “microinvasive carcinoma” is not clearly defined and should not be used to report on vulvar carcinoma. Instead, the pathology report should contain accurate measurements of the horizontal and deep tumor dimensions. The finding of vascular space involvement does not affect the staging, however, the pathology report should always contain a statement as to whether or not vascular space involvement was identified.

According to the FIGO report on the results of treatment, the five-year survival rates for vulvar carcinoma are 78.5%, 58.8%, 43.2% and 13.0% for FIGO stages I, II, III and IV, respectively. If inguinal lymph nodes are negative, the five-year survival rate is 80.7% as opposed to 62.9% with one positive lymph node, 30.4% with two and under 20% with more positive lymph nodes.28

Fig. 7 Measurement of depth of invasion. Pink = epidermis; tan = dermis; black line = intact basement membrane; red line = front of invasive carcinoma. The depth of invasion is measured from the dermoepidermal junction of the most superficial adjacent dermal papilla to the point of deepest invasion. (a) Tumor is extending directly from the epidermis. (b) An isolated invasive tumor cell nest underneath an intact basement membrane.
Since the pathologist depends on accurate clinical information with respect to laterality and location of lymph nodes or the presence of distant metastases that may not have resulted in a surgical specimen, it is prudent to report the tumor stage as pathologic (“pTNM”) stage with a disclaimer that this classification was determined based on the material and/or clinical information provided.

**Sentinel Lymph Nodes**

The clinical utility of selective extirpation of the sentinel lymph node in vulvar cancer is still under investigation. The idea is that the status of the sentinel lymph node, by definition the first lymph node to receive lymph fluid from the tumor region, is highly predictive for the presence of lymphatic metastases. Therefore, if the sentinel lymph node is negative, a complete inguinal lymph node dissection would not be necessary. However, it still remains to be determined whether the selective removal of the sentinel lymph node leads to a significant reduction of complications that are otherwise associated with a complete lymph node dissection (e.g. lymphedema), and how frequently groin recurrences will occur despite a negative sentinel lymph node, a scenario that carries a rather poor prognosis.

**Grading**

The AJCC suggests the following grading system: GX — grade cannot be assessed, G1 — well differentiated, G2 — moderately differentiated, G3 — poorly differentiated, and G4 — undifferentiated. However, the criteria to determine the grade if differentiation of vulvar squamous cell carcinoma are not defined. In fact, there is presently no generally agreed upon grading system that correlates well with clinical outcome and is applicable to the different histologic subtypes of vulvar carcinoma.

**Adenocarcinoma**

Primary vulvar adenocarcinomas are rare; the majority of cases fall under either Paget disease or Bartholin gland carcinomas.
Paget Disease

Vulvar Paget disease tends to occur in postmenopausal Caucasian women at a median age of 70 years. It usually presents as an erythematous, pruritic lesion that may have been present for years and even treated under various working hypotheses until finally the correct diagnosis is made based on a biopsy.

Histopathologically, Paget disease is characterized by relatively large malignant cells, occurring single or in clusters, located within the epidermis (Fig. 8) or extending into the dermis if it has become invasive. Depending upon the presumed cell of origin, a classification into three subtypes has been proposed:

Type 1: Primary vulvar Paget disease
(a) Intraepithelial, including surface epithelium and skin appendages.
(b) Invasive.

Fig. 8 Paget disease of the vulva. Large atypical cells are present within the epidermis, in clusters and individually. Hematoxylin/eosin, original magnification 100×.
(c) Presenting as a manifestation of an underlying primary vulvar invasive adenocarcinoma (e.g. of the Bartholin gland).

Type 2: Secondary Paget disease as a manifestation of an associated primary anal, colorectal or other non-cutaneous adenocarcinoma.

Type 3: Secondary Paget disease as a manifestation of an associated urothelial neoplasia, also known as pagetoid urothelial intraepithelial neoplasia (PUIN), or “pseudopaget disease”.

The histogenesis of vulvar Paget disease type 1a and 1b has long been a subject of debate. Among others, eccrine and apocrine glands, ectopic mammary glands, or pluripotential basal cells have been implicated. The recent discovery of Toker cells as a normal constituent of mammary-like glands in the vulvar region has added another potential cell of origin. Toker cells were first described in the breast as intraepithelial cells with clear cytoplasm that are found in approximately 10% of normal nipples. They are thought to be the cell of origin in rare cases of mammary Paget disease that are not associated with intraductal carcinoma. An analogous process has been proposed to occur in the vulva.29,30

While it is not usually possible to distinguish cells of the various Paget types on routine hematoxylin/eosin stained slides, the following adjunct studies may be helpful. Paget type 1a–c cells are positive for cytokeratin (CK) 7 and negative for CK20, much like epithelia of Müllerian origin. In addition, they tend to be positive for gross cystic disease fluid protein-15 (GCDFP-15) and carcinoembryonic antigen (CEA). Conversely, type 2 Paget cells are positive for CK20, negative for CK7, and negative for GCDFP-15. The caudal-related homeobox gene nuclear transcription factor (CDX-2) is expressed in intestinal cells of both the small and large bowel; limited reports on the expression of CDX-2 in the various subtypes of Paget disease suggest that it may be a specific and sensitive marker for type 2 Paget disease.31,32 Cells of Paget types 1 and 2 contain mucin and are positive on mucicarmine, Alcian blue and PAS (diastase-resistant) stains, whereas type 3 Paget cells (PUIN) are negative for mucin and negative for GCDFP-15. Similar to urothelial neoplasias, PUIN cells are positive for CK7 and uroplakin III, and may be positive for CK20.
Paget cells are HPV negative, unless they are a manifestation of an associated endocervical adenocarcinoma.

Immunohistochemical positivity for p53 and fatty acid synthase has been reported to be associated with invasive primary vulvar Paget disease (type 1(b)).\textsuperscript{33,34} Positivity was seen in Paget cells of both the intraepithelial and the invasive component. To distinguish Paget disease from malignant melanoma, which is its closest look alike, a marker for melanocytes (S-100 protein, HMB-45 or Melan-A) should be included in the panel of special stains (Table 2). Furthermore, the differential diagnosis includes pagetoid VIN (usually negative for CK7 and GCDFP-15) and mycosis fungoides (positive for lymphoid markers).

**Table 2.** Adjunct Studies in the Work-Up of Paget Disease and Its Differential Diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Paget disease</th>
<th>Pagetoid VIN</th>
<th>Malignant melanoma</th>
<th>Mycosis fungoides</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Type 1</td>
<td>Type 2</td>
<td>Type 3</td>
<td></td>
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<tr>
<td>Mucin markers</td>
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<tr>
<td>Mucicarmine</td>
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<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>PAS</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Alcian blue</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CK7</td>
<td>+</td>
<td>−*</td>
<td>+</td>
<td>−*</td>
</tr>
<tr>
<td>CK8</td>
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<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CK20</td>
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<td>+</td>
<td>+/*</td>
<td>−</td>
</tr>
<tr>
<td>Pan-CK</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−*</td>
</tr>
<tr>
<td>GCDFP-15</td>
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<td>−</td>
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</tr>
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<td>CDX-2</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Uroplakin III</td>
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<td>+/*</td>
<td>−</td>
</tr>
<tr>
<td>CEA</td>
<td>+</td>
<td>+</td>
<td>+/*</td>
<td>−</td>
</tr>
</tbody>
</table>

| Melanocytic markers |        |        |        |                  |                |
| S-100              | −      | −     | −      | −                 | +               |
| HMB-45             | −      | −     | −      | −                 | +               |
| Melan-A            | −      | −     | −      | −                 | +               |
| LCA                | −      | −     | −      | −                 | +               |

Legend: + positive; − negative; +/* most cases positive; −* usually negative, rarely positive; CK = cytokeratin; GCDFP = gross cystic disease fluid protein-15; CDX-2 = caudal-related homeobox-2; HMB-45 = human melanoma black-45; CEA = carcinoembryonal antigen; LCA = leukocyte common antigen.
Obviously, the correct diagnosis not only of vulvar Paget disease, but preferably of the subtype is of great clinical importance. Based on the results of histopathologic and adjunct studies, a thorough clinical work-up should be performed to rule out an associated carcinoma elsewhere. Of note, many patients with extramammary Paget disease also develop synchronous or metachronous breast carcinoma. Whether this is a true association, and whether there is a correlation with one of the subtypes of vulvar Paget disease, is unclear. Be that as it may, it is certainly warranted to include a breast examination in the work-up of patients with vulvar Paget disease. Similar to squamous cell carcinoma, the risk of lymph node metastases in invasive primary vulvar Paget disease (types 1b and 1c) increases sharply once the depth of invasion exceeds 1 mm.  

**Bartholin Gland Carcinoma**

Primary Bartholin gland carcinomas are rare. They occur at an average age of 50 years typically as a solid mass, deeply located in the soft tissue, while the overlying skin may be intact and unremarkable. Some cases are associated with vulvar Paget disease (type 1c). A variety of different histologic subtypes of Bartholin gland carcinomas have been described, including adenocarcinoma (40%); squamous cell carcinoma (40%); adenoid cystic carcinoma (15%); transitional cell carcinoma; and adenosquamous carcinoma. To establish the diagnosis of a Bartholin gland carcinoma, three criteria must be fulfilled: the tumor must arise at the site of a Bartholin gland, it must be histologically consistent with Bartholin gland origin, and metastasis from a distant primary tumor must be ruled out.

Squamous cell and adenocarcinomas of the Bartholin gland are usually positive for CEA, which may be helpful to support the diagnosis. A subset of primary Bartholin gland carcinomas, especially of squamous differentiation, is positive for high risk HPV types, which may contribute to its pathogenesis.
Skene Gland Carcinoma

Adenocarcinoma of the Skene glands is extremely rare. Its diagnosis can be supported by immunohistochemical positivity for PSA and prostate acid phosphatase, due to its phylogenetic analogy with the male prostate gland.

Malignant Melanoma

Malignant melanoma of the vulva occurs predominantly in white women and in the postmenopausal population. It is the second most frequent vulvar malignancy after squamous cell carcinoma and accounts for 6%–9% of all vulvar malignancies. Three histopathologic subtypes are distinguished: superficial spreading melanoma, nodular melanoma and mucosal/acral lentiginous melanoma. Tumor cells may display a wide morphologic spectrum, including epithelioid, dendritic and spindle cells (Fig. 9). Malignant melanoma may or may not produce melanin. Whenever a poorly differentiated malignant neoplasm is encountered on the vulva that is difficult to classify, melanoma needs to be ruled out.

The main differential diagnosis of superficial spreading melanoma is Paget disease. Cells of Paget disease tend to be larger and form clusters with occasional gland formation. VIN and squamous cell carcinoma may clinically mimic melanoma, especially if they are darkly pigmented.

Immunohistochemically, melanoma cells are positive for S-100 protein, HMB45 and Melan A, whereas Paget cells and squamous epithelial cells are negative for these markers. Conversely, Paget cells are positive for mucicarmine, PAS, CEA and/or cytokeratins, which are negative in melanoma.

For staging of vulvar melanoma, a modified Clark classification is applied, as proposed by the American College of Obstetricians and Gynecologists (ACOG) (Fig. 10):

Level I — Melanoma in situ.
Level II — Melanoma extends into superficial papillary dermis.
**Fig. 9** Malignant melanoma. Tumor cells are present within the epidermis and dermis. There is abundant melanin production. Hematoxylin/eosin, original magnification 100×.

**Fig. 10** Staging of malignant melanoma. In the Clark classification, levels are defined by the tissue layers involved. The Breslow system measures tumor thickness in mm from the lower border of the granular cell layer. Modified from http://www.med-ars.it. Illustration by Davide Brunelli, MD, with kind permission.
Level III — Melanoma fills and expands the papillary dermis.
Level IV — Melanoma extends into the reticular dermis.
Level V — Melanoma invades into fat or other subjacent tissues.

In addition, tumor thickness is correlated with prognosis. The Breslow system proposes measurement from the deep border of the granular layer to the deepest point of invasion. Lesions with a thickness of 0.75 mm or less have an excellent prognosis in that they virtually never metastasize or recur if completely excised.

Mesenchymal Tumors

Mesenchymal tumors of the vulva are generally rare, the most frequent entity being the benign leiomyoma. Not much is known about the pathogenesis and early stages of mesenchymal malignancies, and clinical management will be determined on a case by case basis. When faced with a spindle cell lesion of the vulva, the spindle cell variant of a squamous cell carcinoma should always be borne in mind, which can easily be confirmed due to its immunohistochemical positivity for cytokeratins. The following entities are somewhat specific to the vulva, or are frequently within the differential diagnosis of vulvar lesions.

Embryonal rhabdomyosarcoma typically is a tumor of infancy, although rare cases have been reported in young adults. It occurs more frequently in the vagina, and when both vulva and vagina are involved, it should be diagnosed as a primary vaginal tumor. The tumor presents as a fleshy, dark colored grape like mass that may ulcerate and bleed. Histologically, it is characterized by a proliferation of immature round to spindle shaped malignant cells. Cross striation may not be seen in all cases and is not required for the diagnosis. Cells are separated by a loose myxoid intercellular matrix. The most frequent subtype, sarcoma botryoides, is distinguished by the presence of a condensed layer of rhabdomyoblasts (cambium layer) underneath an epithelial surface.

Immunohistochemical markers for muscle differentiation (myoglobin, desmin, actin) as well as electron microscopy may be helpful
to support the diagnosis. Surgery or radiotherapy, in combination with chemotherapy, is very efficient, and disease-free survival rates exceed 70% at five years.36

Dermatofibrosarcoma protuberans (DFSP) is a locally aggressive tumor, usually occurring in adults at a median age of 54 years. The tumor arises in the dermis and is thought to be of fibrohistiocytic origin. Microscopically, it is composed of spindle cells arranged in a “storiform” pattern of radial whorls. The lesion is highly cellular, and entrapped adipocytes indicate infiltration of the underlying adipose tissue. Cytologically, cells are monomorphic and exhibit a moderate mitotic activity. The tumor tends to recur if not completely excised, but distant metastases are very rare. Tumor cells are positive for CD34, which is useful for confirmation of the diagnosis.

Aggressive angiomyxoma is typically seen in adult women younger than 40 years of age. It may arise in the vulva or in the pelvis, extending to the vulva. It presents as a subcutaneous mass, and if present in the posterior part of the labia majora, it may be clinically mistaken for a Bartholin gland cyst. Histologically, the tumor is composed of bland appearing spindle cells with low mitotic activity and abundant intercellular myxoid material, which gives the lesion a rather hypocellular appearance. A characteristic feature is the presence of clustered thick-walled muscular vessels (Fig. 11). Entrapped adipocytes or nerves reflect the locally invasive nature of the tumor. The spindle cells are positive for desmin, smooth muscle actin and muscle specific actin, but negative for S-100. CD34 is expressed in approximately 50% of cases. Aggressive angiomyxoma may recur locally, but distant metastases are extremely rare.37

Rearrangements of the high mobility group gene HMGA2, which encodes a non-histone chromatin associated protein that is thought to play a role during transcription, have been reported in about one-third of aggressive angiomyxomas.38 Similar alterations of the same gene are found in a variety of other benign mesenchymal tumors, including uterine leiomyomas, but not in angiomyofibroblastomas.

In the differential diagnosis, the most important entity is angiomyofibroblastoma. This is a well circumscribed benign tumor, composed of more epithelioid appearing cells with perivascular hypercellularity
and occasional multinucleation. It may have myxoid areas, but lacks thick walled vessels. Angiomyofibroblastoma tends to be positive for vimentin and desmin, but negative for actin. Myxoid malignant fibrous histiocytoma (myxofibrosarcoma) usually shows areas of higher cellularity displaying the typical “storiform” architecture. Marked cytologic atypia and mitotic activity including atypical mitotic figures are present at least focally. It may be immunoreactive for α1-antitrypsin and α1-antichymotrypsin. Myxoid neurofibroma diffusely expresses S-100. Myxoid leiomyoma lacks a prominent vascular pattern and is composed of myocytes with cigar-shaped nuclei that tend to be arranged in fascicles.

Also to be considered in the differential diagnosis of aggressive angiomyxoma is a peculiar tumor that may present as a unilateral labial swelling in prepubertal girls and has been variably termed “prepubertal vulval fibroma”, “childhood asymmetrical labium

Fig. 11  Aggressive angiomyxoma. The lesion is composed of bland appearing spindle shaped cells with abundant myxoid intercellular substance. Numerous vessels are present, some of them are thick walled. Hematoxylin/eosin, original magnification 40×.
majus enlargement (CALME)” or “prepubertal fibrous hyperplasia of the labium majus.”  

Although a benign tumor, it is discussed here within the differential diagnosis of malignant entities, with which it shares certain clinical and histologic features. It is rare in absolute case numbers and has only been recognized recently as a distinctive entity. However, it may comprise more than 20% of all pediatric vulvar soft tissue masses. It can be associated with peau d’orange changes of the overlying skin and may extend to pelvic structures on radiologic images. It is both macroscopically and microscopically poorly circumscribed, such that it may be difficult or impossible to completely resect it. Histologically, it is hypocellular and composed of cytologically bland spindle-shaped cells, growing without a particular architectural pattern, and surrounded by collagenous or edematous stroma (Fig. 12). The mitotic index is less than one mitotic figure per 10 HPF. Fat, nerves, vessels or skin adnexal structures may be entrapped in the lesion. Spindle cells are positive for CD34 in the majority of cases, but negative for muscle and neural markers. Despite its infiltrative growth pattern, the tumor appears to behave in a benign fashion, although recurrences have been observed after incomplete resection. Conservative surgery with an emphasis on cosmetically acceptable results is recommended. No metastatic disease has been reported. Clinically, the differential diagnosis includes lipoma, hemangioma and lymphangioma, furthermore embryonal rhabdomyosarcoma due to its similar age and site specificity. Histologically, the prepubertal fibrous lesion shares similarities with aggressive angiomyxoma and angiomyofibroblastoma. However, the latter two entities usually occur in adult women and are positive for desmin. In addition, angiomyofibroblastoma is well demarcated.

Although not specific to the vulva, a wide variety of other soft tissue tumors have been reported to occur there, including leiomyosarcoma; malignant fibrous histiocytoma; epithelioid sarcoma; malignant rhabdoid tumor; angiosarcoma; lymphangiosarcoma; angiomyoﬁbro sarcoma; hemangiopericytoma; Kaposi sarcoma; alveolar soft part sarcoma; malignant schwannoma; malignant granular cell tumor; liposarcoma; and eosinophilic granuloma. For more detailed information.
Fig. 12 Prepubertal vulval fibrous hyperplasia. (a) The lesion is composed of fibroblasts and collagen bundles. Entrapped adipocytes reflect the invasive nature of the lesion. Hematoxylin/eosin, original magnification 40×. (b) Higher magnification shows thick collagen bundles. Hematoxylin/eosin, original magnification 100×.
on these entities the reader is referred to textbooks of soft tissue pathology or review articles.

Other Malignant Tumors of the Vulva

Urethral carcinoma is rare and occurs mostly in elderly women. It tends to arise in the distal urethra or within diverticula and may present with bleeding and dysuria. Squamous cell carcinoma is the most frequent histologic type, followed by transitional cell carcinoma. The latter may be associated with pagetoid urothelial intraepithelial neoplasia (PUIN, type 3 Paget disease). Urethral adenocarcinomas have been subclassified into mucinous, clear cell or colloid types. For staging of urethral carcinoma, see Table 3.

Other malignancies that have been reported as primary vulvar tumors include yolk sac tumor (endodermal sinus tumor), malignant lymphoma, and Merkel cell tumor. Since these entities are extremely rare on the vulva, no experience has been accumulated as to their early detection. They display the same morphologic and immunophenotypic characteristics as in other sites. Once thought of in the differential diagnosis, they can be correctly identified by appropriate ancillary studies.

Accounting for about 8% of all vulvar neoplasms, metastatic tumors to the vulva are not infrequent. In the majority of cases, there is a primary malignancy of the genitourinary tract, squamous cell carcinoma of the uterine cervix being the most frequent. A metastatic tumor to the vulva always implies late stage disease, portends a poor prognosis and is generally treated palliatively.

Ancillary Studies

In the context of diagnosing early stage malignancies of the vulva and their precursor lesions, ancillary studies will be most useful in one of three scenarios: (1) Identification of HPV related lesions and differentiation from benign mimickers; (2) identification of very early and superficial stromal invasion; (3) the work-up of Paget disease, its subtypes and differential diagnosis. Additional applications are rather
Identification of HPV associated lesions

The most useful immunohistochemical markers to support the diagnosis of an HPV associated lesion are Ki-67 and p16\textsuperscript{Ink4a}. The Ki-67 antigen (recognized by the MIB-1 antibody) is expressed in the nuclei of proliferating cells during all phases of the cell cycle except G0. It is physiologically present in parabasal cells (while basal cells are usually negative!) of the vulvar squamous epithelium, which serve as a

<table>
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<tr>
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<th>Definition</th>
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<tr>
<td>TX</td>
<td>Primary tumor cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumor</td>
</tr>
<tr>
<td>Ta</td>
<td>Non-invasive papillary, polypoid or verrucous carcinoma</td>
</tr>
<tr>
<td>Tis</td>
<td>Carcinoma \textit{in situ}</td>
</tr>
<tr>
<td>T1</td>
<td>Tumor invades subepithelial connective tissue</td>
</tr>
<tr>
<td>T2</td>
<td>Tumor invades corpus spongiosum or prostate periurethral muscle</td>
</tr>
<tr>
<td>T3</td>
<td>Tumor invades anterior vagina or bladder neck</td>
</tr>
<tr>
<td>T4</td>
<td>Tumor invades other adjacent organs</td>
</tr>
<tr>
<td><strong>Regional Lymph Nodes (N)</strong></td>
<td></td>
</tr>
<tr>
<td>NX</td>
<td>Regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph node metastasis</td>
</tr>
<tr>
<td>N1</td>
<td>Metastasis in a single lymph node, 2 cm or less in diameter</td>
</tr>
<tr>
<td>N2</td>
<td>Metastasis in a single lymph node more than 2 cm in greatest dimension, or in multiple nodes</td>
</tr>
<tr>
<td><strong>Distant Metastasis (M)</strong></td>
<td></td>
</tr>
<tr>
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<td>Distant metastasis cannot be assessed</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastasis</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis present</td>
</tr>
</tbody>
</table>

Legend: Regional lymph nodes are the inguinal (superficial or deep), iliac (common, internal [hypogastric], obturator, external), presacral, sacral, and pelvic lymph nodes.
convenient internal control. Positivity for Ki-67 in the upper two thirds of the epithelium has been shown to correlate with HPV-infection.\(^42\) Note that although Ki-67 expression will be stronger and more diffuse in high grade lesions, positivity can be seen in the upper layers of the epithelium even in low grade lesions. Therefore, the Ki-67 reactivity can be used to differentiate HPV-associated lesions from non-HPV related changes, but is less helpful for the grading of VIN. Lymphocytes are frequently positive for Ki-67 and may lead to false positive interpretation, especially in heavily inflamed epithelia. Similarly, parabasal cells that appear to be located in higher epithelial layers due to tangential sectioning in suboptimally oriented biopsy specimens, or in the vicinity of stromal papillae extending towards the surface, should not be overinterpreted. Since koilocytotic changes on the vulva tend to be more subtle than on the uterine cervix, a Ki-67 stain can be very helpful to correctly identify a condyloma acuminatum and differentiate it from non-HPV related mimickers, e.g. fibroepithelial polyp, squamous papilloma or micropapillomatosis labialis. Dysplastic lesions of the vulva are usually characterized by mitotic activity beyond the parabasal cell layer, in which case a Ki-67 stain does not add much information. It is, however, very valuable when VIN is suspected in the absence of mitotic figures.

\(\text{P16, also known as inhibitor of cyclin dependent kinase 4a (INK4a), is a tumor suppressor protein that inhibits cell cycle progression at the G1-S interphase. It is regulated by the expression status of the retinoblastoma tumor suppressor protein. Because the E7 protein of high risk HPV types inactivates the retinoblastoma protein, p16 expression is upregulated and seen in nuclei and cytoplasm of infected cells. Thus, although p16 positivity is not specifically associated with an HPV infection, in the correct clinical setting, it is interpreted as a surrogate marker for the presence of high risk HPV types. P16 is strongly and diffusely positive in the basaloid and warty types of VIN (Fig. 13), but not in the HPV-unrelated differentiated (simplex) form of VIN, and not in verrucous carcinoma.}^43\) On the other hand, p16 is expressed in the subset of vulvar low-grade lesions, which is caused by high-risk HPV type infection. For unknown reasons, p16 positivity is also
43 Direct identification of HPV particles within the tissue is possible but less practical. The gold standard would be to extract DNA from the tissue and PCR-amplify HPV type-specific sequences. This, however, is too costly and labor intensive for routine purposes. *In situ* hybridization for specific HPV types or groups of HPV types can be done on tissue sections, but this method is fraught with low sensitivity.

**Identification of superficial stromal invasion**

The correct identification of early stromal invasion obviously has significant impact on patient prognosis and clinical management. While on most other sites of the skin it may be practical, in case of doubt, to
simply excise widely a questionable lesion, the vulva poses particular challenges: vulvar carcinoma has metastatic potential even if not deeply invasive; wide margins of excision may be difficult to obtain because of important adjacent structures, i.e. urethra or clitoris; and surgical treatment may include inguinal lymph node dissection. Therefore, the distinction between *in situ* and invasive disease is particularly critical on the vulva. In cases of questionable very superficial stromal invasion, the visualization of the basement membrane using antibodies against collagen type IV or laminin can be useful. While the basement membrane is interrupted in areas of recent or active invasion, some foci of clearly invasive or even metastatic tumor may be secondarily surrounded by basement membrane material. This is consistent with the theory that invasion happens in cycles of growth surges, in which the basement membrane is actively destroyed by tumor cells, followed by periods of quiescence, during which reformation of basement membrane material may occur, especially in well differentiated tumors. Hence, the finding of an interrupted basement membrane supports the diagnosis of an invasive process, whereas the presence of basement membrane material surrounding tumor cell nests does not rule out invasive disease. To highlight epithelial cells breaking through the basement membrane, double immunostaining for cytokeratin and basement membrane components has been proposed (Fig. 14). This may further enhance diagnostic accuracy, especially in cases where the dermoepidermal interface at the site of questionable invasion is obscured by a heavy inflammatory infiltrate.44

**Paget disease and its differential diagnosis**

The mucin containing cells of Paget disease types 1 and 2 can be demonstrated on mucicarmine, PAS (diastase resistant) and Alcian blue stains. In the majority of cases, one of these will be the single most important special stain to support the diagnosis of Paget disease. To rule out the possibility of a superficially spreading malignant melanoma, a melanocytic marker (S-100, HMB-45 or Melan-A) should be included in the panel. A lymphocytic marker (LCA) will help to identify mycosis fungoides.
To further classify the subtype of Paget disease, CK7 and CK20 are most useful: type 1 is typically positive for CK7 and in 85% of cases, negative for CK20, while the reverse is true for type 2. Positivity for CDX-2 appears to be specific for type 2 Paget disease, although experience with this intestinal marker is still limited. PUIN is positive for CK7, and frequently coexpresses CK20 and/or uroplakin III.

GCDFP-15 tends to be positive in type 1 Paget disease. This protein is also expressed in up to 50% of metastatic breast carcinomas, as well as in sweat gland and salivary gland carcinomas. Positivity is meaningful even if very focal and weak, which is often the case. CK8 (CAM5.2) is expressed in type 1 Paget cells, and similar to CK7, the surrounding squamous epithelium is negative for these markers. Along with mucin stains, CK7 and/or CK8 are useful tools not only to diagnose the disease but also to assess surgical resection margins or potential stromal invasion. Pagetoid VIN is positive for pan-cytokeratins.

Fig. 14  VIN with focus of early invasion. (a) Hematoxylin/eosin, original magnification 200×. (b) Double immunostain for cytokeratin (red) and collagen IV (dark brown), original magnification 200×. Arrowheads indicate areas of early invasion. Note defect in the continuity of the basement membrane. Reprinted with permission from Archives of Pathology & Laboratory Medicine. Copyright 2005. College of American Pathologists.
(AE1/AE3), but usually negative for CK7 and CK8, although rare CK7 positive cases have been reported. CEA positivity can be seen in all three types of Paget disease, including the majority of PUIN cases.

Immunohistochemical positivity for p53 or fatty acid synthase in intraepithelial Paget cells suggests that there may be an invasive component and should prompt careful examination of the specimen to rule out invasion. A summary of markers and their expression patterns that may be useful in the work-up of Paget disease and lesions in the differential diagnosis is provided in Table 2.

**Metastatic tumors**

Extensive discussion of metastatic tumors to the vulva is beyond the scope of this chapter. Most frequently, the primary tumor is located in the genitourinary tract. If ancillary studies are needed to determine the likely site of a primary tumor, it may be more cost effective to perform selected special stains in two or more successive rounds rather than a large battery of immunostains at once. For example, the first round may include few markers to determine the cell lineage (e.g. pan-CK for epithelial cells, vimentin for mesenchymal cells, S-100 as a melanocytic marker, and LCA as a lymphocytic marker), followed by a panel of markers for more specific differentiation analysis, depending on the results from the first round. Note that vimentin is not specific for mesenchymal cells, but is frequently coexpressed in endometrial endometrioid carcinoma, serous ovarian tumors, renal cell carcinoma (but not clear cell carcinoma of Müllerian origin!), and papillary thyroid carcinoma. Likewise, occasional melanocytic, mesenchymal (endometrial stroma) and lymphoid cells may be reactive for pan-CK.

Additional special stains that may be helpful in the diagnosis of early vulvar neoplasms include the following: the D2-40 antibody is directed against podoplanin, a membrane protein of lymphatic endothelium. It can be used to identify lymphvascular space invasion by various tumors and rule out tissue retraction artifact.

Overexpression of p53 has been reported in the basal and parabasal keratinocytes of differentiated (simplex) VIN, and may be
helpful to support that diagnosis. Of note, p53 positivity can also be seen in lichen sclerosus, in which case this is thought to be a reactive change rather than a marker of premalignancy.\textsuperscript{12,45}

REFERENCES


