Pathology and Molecular Biology of Penile Cancer

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Learning Objectives
After reading this chapter you will be able to:

- Describe commonly encountered pre cursor penile lesions
- Discuss predisposing lesions related to penile cancer and their histopathological characteristics
- Outline the common histopathological subtypes of penile cancer
- Identify important histopathological features relevant to staging and grading of penile cancer along with their significance in disease outcomes
- List some significant molecular alterations and potential biomarkers used in penile cancer

Introduction

The rarity of penile cancer means that, in non-expert hands, there is a significant risk of misdiagnosis of both the subtype and staging. Accurate staging and grading of tumours are used to determine subsequent clinical management and patient follow up. Different subtypes of penile cancer have been defined, which appear to be associated with variable outcomes leading to treatment strategies [1].

There were significant changes to the 2016 World Health Organization (WHO) classification following the ISUP consultation on Penile Tumours, Boston, in March 2015 [2, 3]. Because of the rarity of penile tumours, this was not a consensus, but an

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expert-driven conference aimed at assisting pathologists who do not see these tumours on a regular basis [4]. The fifth edition of the WHO classification of urogenital tumours (WHO "Blue Book"), published in 2022, contains further significant revisions [5].

We attempt to discuss the key updates and pathological features that influence the management and need to be communicated clearly.

Precursor Lesions

Human papillomavirus (HPV)-associated penile intraepithelial neoplasia (PeIN) is a HPV-associated precursor lesion of invasive SCC, whereas differentiated PeIN is an HPV-independent precursor lesion of SCC. The most common HPV-associated PeIN subtypes are the basaloid (undifferentiated, a term that should be avoided) and warty [5] (Table 2.1). From the diagnostic point of view, most penile lesions can be correctly classified using Haematoxylin and eosin (H&E) stain, but immunohistochemical (IHC) or molecular analysis may be helpful in challenging cases.

Patients with HPV-positive tumours have a better prognosis than those with HPV-negative neoplasms, and therefore the identification of the virus in tumour tissue is becoming an important prognostic marker apart from the fact that immunohistochemical (IHC) or molecular analysis may be helpful in challenging cases [6]. The gold-standard test for HPV in tumour tissues is polymerase chain reaction (PCR) but the cost of the technique limits its availability in the majority of laboratories. However, in most cases, p16 has been shown to be an adequate surrogate marker for high-risk HPV [7]. Block-type p16 IHC is the most practical and reliable method to separate HPV associated from HPV-independent penile lesions [5]. Immunohistochemistry for p16 coupled with a proliferation marker Ki-67 is useful in the more challenging cases. Therefore, a panel comprising of p16, p53 and Ki-67 is adequate to correctly diagnose and differentiate benign lesions from pre-malignant lesions.

Immunohistochemistry for p16 is useful to separate differentiated PeIN (Figs. 2.1a–c) which is negative (Fig. 2.2a), whereas the majority of warty, warty-basaloid, and basaloid PeINs (Fig. 2.3a) show diffuse, strong and full thickness (en-bloc) reactivity in the atypical surface squamous epithelium for

Table 2.1 WHO Classification of PeIN

HPV independent	Differentiated
HPV-associated	Basaloid Warty Warty-basaloid
Others	Pleomorphic Spindle Clear cell Pagetoid

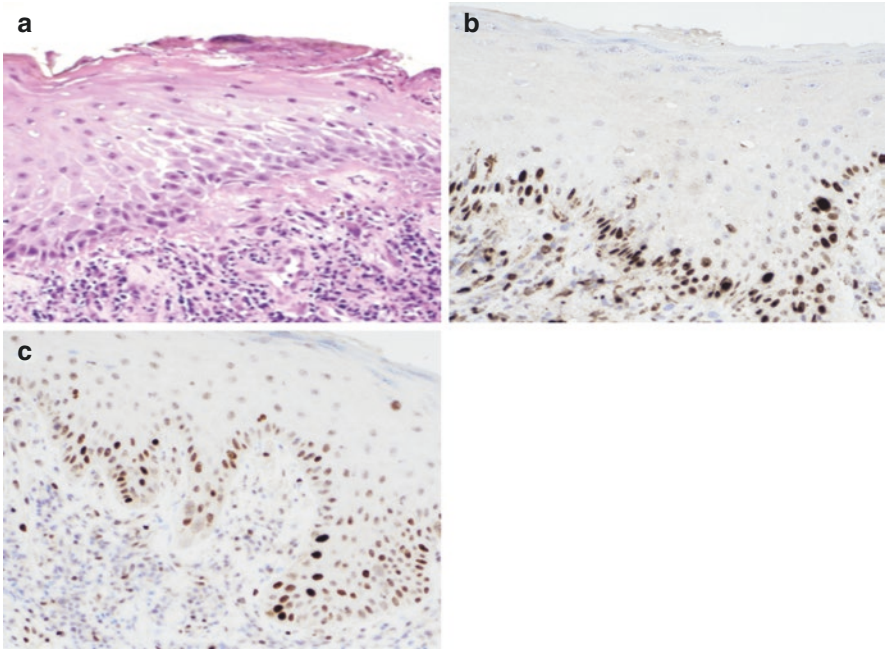

Fig. 2.1 (**a**) Haematoxylin and Eosin stain 20X magnification. Differentiated PeIN with surface maturation and marked atypia of the basal and parabasal keratinocytes. (**b**) MIB-1 stain 20X magnification. Overexpression of proliferation marker in the atypical basal and parabasal cells in differentiated PeIN. (**c**) Immunohistochemistry for p53 (20× magnification) can show 'wild type' staining in differentiated PeIN

p16 [8] (Fig. 2.3b). Differentiated PeIN tends to be associated more commonly with a chronic process such as lichen sclerosus [9]. Recognised different subtypes of differentiated PeIN are hyperplasia like, classic and pleomorphic (Figs. 2.2a–c). Ki-67 is helpful in differentiating the minimal basal atypia of reactive squamous hyperplasia. There is increased basal and parabasal expression with continuous staining in hyperplasia like differentiated PeIN in contrast to the scattered basal cells in benign reactive lesions (Figs. 2.2d and 2.4). Pleomorphic differentiated PeIN can be difficult to differentiate morphologically from undifferentiated PeIN but will be negative on staining for p16 (Fig. 2.2a) [10].

Where available, HPV genotyping can also help contribute to this diagnostic pathway. HPV 16 is the most common genotype and is detected in 71% of basaloid, 56% of warty- basaloid, and 20% of warty PeIN [11].

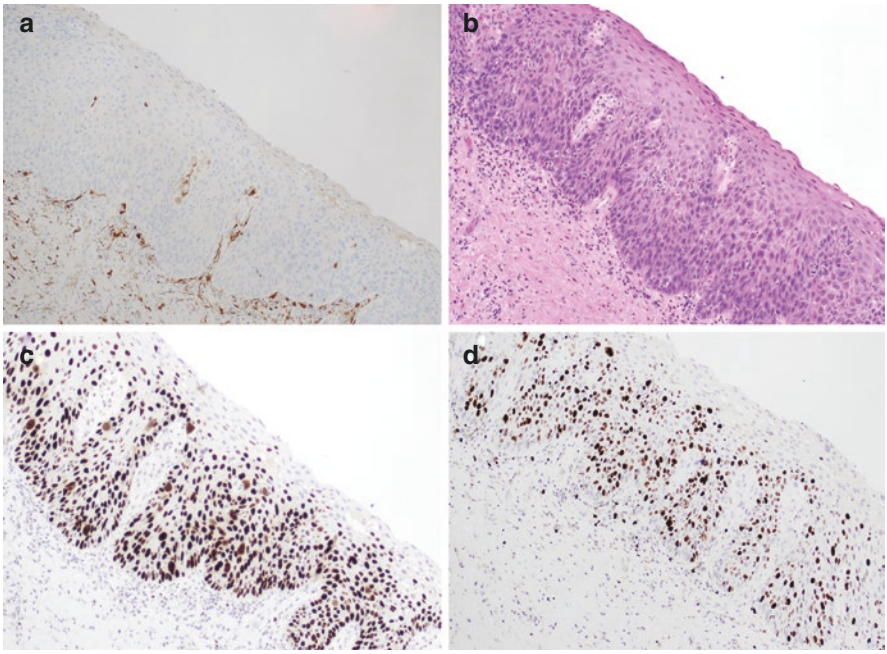

Fig. 2.2 (**a**) Immunohistochemistry for p16. Magnification 20X. No reactivity for p16 in differentiated PeIN. (**b**) Haematoxylin and Eosin stain 10X Magnification. Differentiated PeIN (pleomorphic variant): Marked pleomorphism of the basal and parabasal keratinocytes. There is surface maturation. (**c**) Immunohistochemistry for p53 10X. Differentiated PeIN: Mutant type staining with strong and diffuse overexpression of p53 in basal and parabasal layers. (**d**) Magnification 20X. Immunohistochemistry for MIB-1 overexpression in the atypical basal and parabasal cells of differentiated PeIN

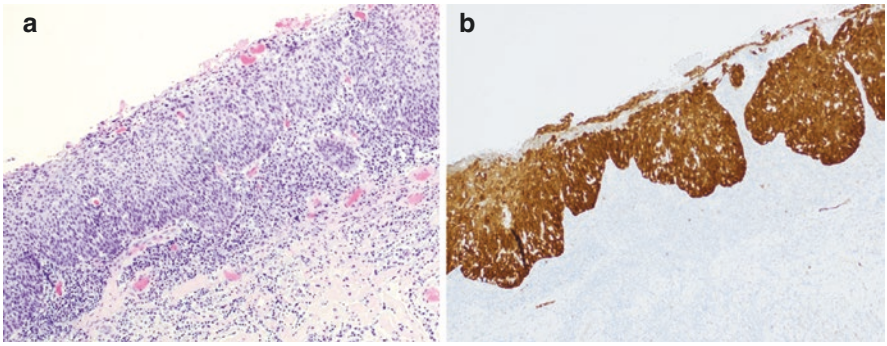

Fig. 2.3 (**a**) Haematoxylin and Eosin stain. Magnification 10X. Undifferentiated PeIN basaloid type. Full thickness atypia of the surface squamous epithelium with a monotonous population of basophilic cells. (**b**) Immunohistochemistry for p16 shows 'en-bloc' strong and diffuse reactivity in undifferentiated PeIN

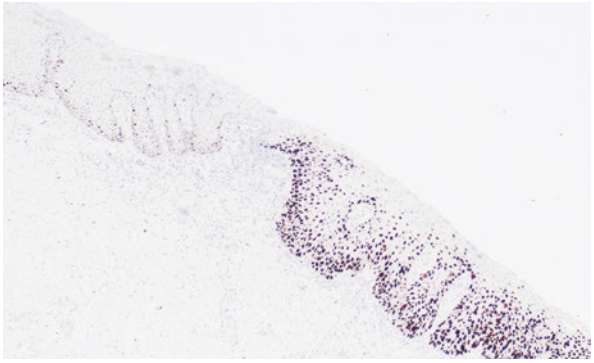

Fig. 2.4 Immunohistochemistry for p53. Magnification 4X. p53 overexpression (diffuse and continuous staining) in differentiated PeIN in contrast to the intermittent basal staining seen in the background surface squamous epithelium

Written reports should indicate the subtype and extent of PeIN and whether or not there is margin involvement.

In difficult cases with cytological abnormalities the category of 'atypia falling short of PeIN' with a recommendation for follow up may be used, to avoid over treatment.

Predisposing Conditions

Condylomata

Condylomata are benign, exophytic, wart-like lesions with koilocytosis and related to infection with low-risk HPV strains, such as HPV 6 and HPV 11 (Figs. 2.5a–c). However, some condylomata show cytologic atypia (Fig. 2.6) and contain high-risk HPV and may be seen in association with PeIN or invasive carcinoma with a variable morphologic spectrum [12]. Penile condyloma types include condyloma acuminatium (typical or atypical) and flat condyloma (typical or atypical).

Immunohistochemistry for p16 is recommended in atypical condylomas to detect the presence of high-risk HPV [13]. However, not all will show staining for p16 and in such cases further in situ hybridization (ISH) or PCR or other molecular techniques may be helpful. Lesions with high-risk HPV should be excised in their entirety and patients need to be more closely followed.

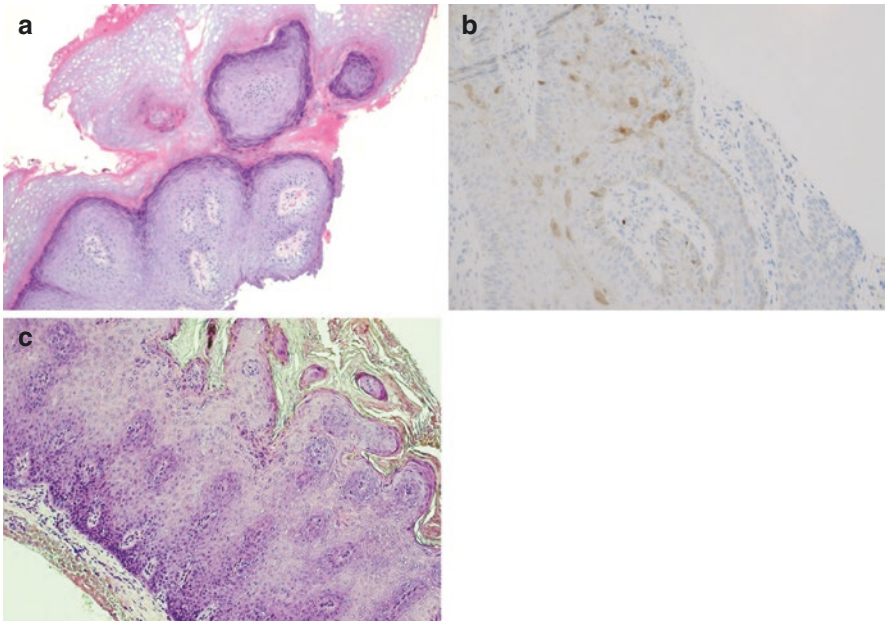

Fig. 2.5 (**a**) Hematocylin and Eosin 10X magnification. Typical Condyloma. Benign squamoproliferative lesion with hyperkeratosis, parakeratosis, acanthosis and papillomatosis of the surface squamous epithelium. (**b**) Immunohistochemistry for p16 shows focal/ scattered reactivity for p16 in the surface keratinocytes. (**c**) Haematoxylin and Eosin 10X. Typical condyloma

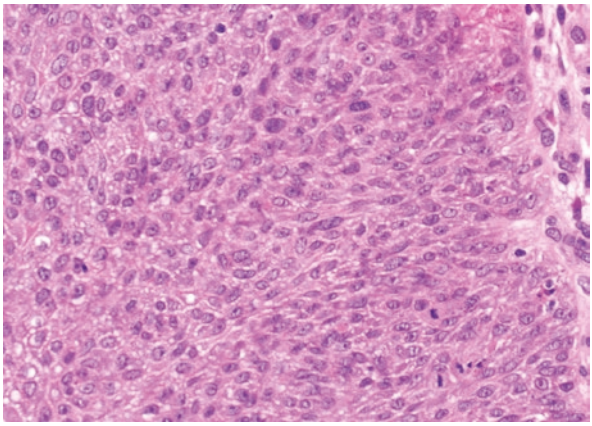

Fig. 2.6 Haemtoxylin and Eosin 40X. Atypical Condyloma. Mild to moderate pleomorphism with frequent mitotic figures

Lichen Sclerosus

The European Association of Urology guidelines identify lichen sclerosus (LSc) as a strong risk factor for penile squamous cell carcinoma but there are also studies to show that it is not associated with increased rates of adverse histopathological features [14, 15]. Differentiated PeINs not linked to HPV, affects elderly men, and is more commonly associated with lichen sclerosus [16, 17]. Histologically, early LSc

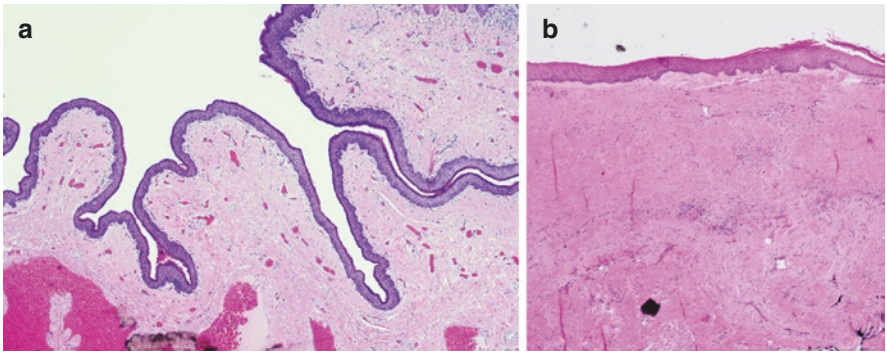

Fig. 2.7 (**a**) Haematoxylin and Eosin 4X. Lichen sclerosus. Attenuation and thinning of the surface squamous epithelium with subepithelial sclerosus and vascular ectasia. (**b**) Haematoxylin and Eosin 4X. Lichen sclerosus. Subepithelial sclerosus and a patchy lichenoid chronic inflammatory cell infiltrate

may show a mild or pronounced band- like CD8 and CD57 positive lymphocytic infiltrate often accompanied by a lymphocytic vasculitis, basal cell vacuolar degeneration and pigment incontinence. As the lesion progresses, subepidermal oedema and sclerosis develops with loss of dermal structures and vascular ectasia (Fig. 2.7a, b) [18]. The histopathology can show varying spectrum of changes depending upon the stage at sampling and it is therefore recommended to examine the specimen in its entirety as focal and early or 'burnt out' changes may be missed.

Penile Cancer

Most malignant tumours of the penis are squamous cell carcinomas (SCCs) originating in the inner mucosal lining of the glans, coronal sulcus, or foreskin. In the 2022 WHO Blue Book, scrotal tumour classification is mentioned separately for the first time [5].

Subtypes

Over 95% of penile cancers are squamous cell carcinomas, with rare instances of sarcomas, melanomas, or neuroendocrine carcinomas (NEC) (including large cell and small cell NEC) [19]. Other malignant lesions of the penis, all much less common than penile SCC, are melanocytic lesions, mesenchymal tumours, lymphomas and metastases. Penile metastases are frequently of prostatic or colorectal origin [14].

Several subtypes have been described and these have different prognostic profiles [20]. Most of these tumours may be diagnosed with H&E histology, but poorly differentiated neoplasms, can be further characterized by immunohistochemical (IHC) and molecular genetic analyses [2]. However, not all positive cases by PCR will be positive by p16 and vice versa, with about a 20% failure in the correlation [3, 21]. Nearly half of penile cancers are human papillomavirus (HPV)-related [22].

The 2016 WHO pathologic classification also adopts categorisation of subtypes of penile SCC as non–HPV-related and HPV-related cases [4, 12, 23]. The 2022 WHO classification followed this paradigm to subclassify tumours into HPV-associated and HPV-independent types (Table 2.2) [24]. It is recommended to report SCC as HPV associated or HPV independent in addition to the histologic diagnosis. If this is not possible, the designation SCC, NOS is acceptable.

A high prevalence of HPV infection is found in basaloid (76%) (Fig. 2.8), mixed warty-basaloid (82%) and warty penile (39%) SCCs while verrucous and papillary penile SCCs are HPV-negative [14].

Subtyping is required as verruciform carcinomas (papillary, warty, or verrucous carcinomas) tend to have better outcomes. Basaloid, acantholytic and sarcomatoid carcinomas are always high grade with a worse prognosis than the usual type of squamous carcinoma and therefore may more readily metastasise to distant sites such as the lung [19]. SCC of the usual type now includes pseudohyperplastic carcinomas and acantholytic/pseudoglandular carcinomas [5]. Verrucous carcinoma is a separate non-metastasising low-grade subtype including carcinoma cuniculatum as a pattern [25].

Up to 30% of tumours may show more than one pattern, all should be recorded in the report. Although not mandatory, reporting a percentage of the poorly differentiated subtypes in such cases is also helpful in guiding management.

Table 2.2 2022 World Health Organization classification of invasive epithelial tumours of the penis and scrotum

HPV associated	Basaloid squamous cell carcinoma Warty carcinoma Clear cell squamous cell carcinoma Lymphoepithelial carcinoma
HPV independent	Squamous cell carcinoma, usual type Verrucous carcinoma (including carcinoma cuniculatum) Papillary squamous cell carcinoma Sarcomatoid squamous cell carcinoma Squamous cell carcinoma, NOS

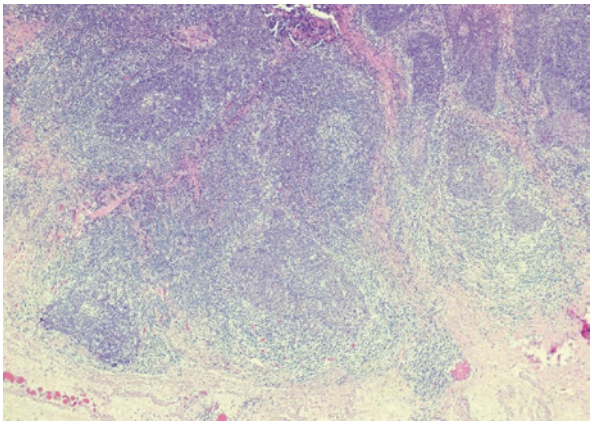

Fig. 2.8 Haematoxylin and Eosin 10X. Basaloid squamous cell carcinoma

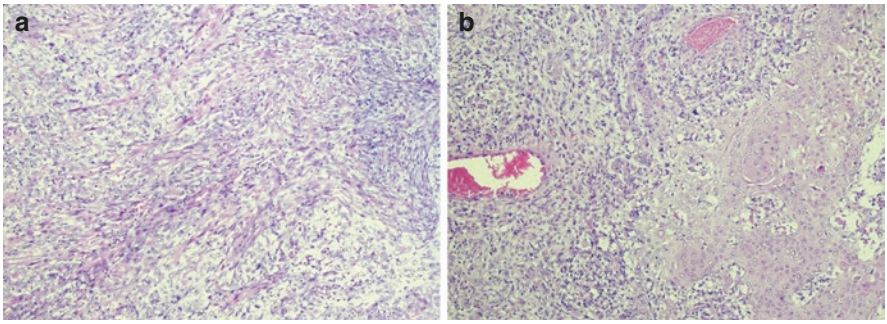

Fig. 2.9 (**a**) Haematoxylin and Eosin stain 10X. Sarcomatoid carcinoma composed of a prominent component of pleomorphic spindle cells in interlacing fascicles. (**b**) Haematoxylin and Eosin stain 10X magnification. An adjacent focus of admixed squamous cell carcinoma helps establish the diagnosis of sarcomatoid carcinoma

Tumour Grading

Differentiation between penile squamous cell carcinoma patients who can benefit from organ-sparing surgery and those at significant risk of lymph node metastasis is based on histopathological prognostic factors including histological grade subtype [26].

The 2016 WHO classification adopted the 3-tier grading method which defines well-, moderately-well and poorly differentiated carcinomas based on the degree of cytological atypia, keratinisation, intercellular bridges, and mitotic activity [4]. Sarcomatoid change is designated as grade 4 and is often in combination with other tumour types (Fig. 2.9a, b). The authors however advise caution in subtyping sarcomatoid variant if seen as focal/isolated cells or in foci adjacent to ulceration. Tumours are graded by the worst area even if this is the minor component.

Although, inconsistencies in concordance can result in under or over treatment of cases, it is important to highlight that recurrent tumours and re-excision specimens for residual tumours from different sites can show different grades.

Tumour Staging

The staging of penile cancer is covered in more detail in in Chap. 4. However, some pertinent issues with regards to pathology are important to consider here. The Royal College of Pathologists recommend the use of The American Joint Committee on Cancer tumour–node–metastasis (AJCC-TNM) staging system eighth edition for penile cancer as it considers factors widely used to predict patient prognoses, guide treatment, and evaluate treatment results. Differences between AJCC and Union Internationale Contre le Cancer eighth Edition

(UICC) staging systems are not minor and using the latter may lead to incorrect reporting of precancerous lesions, superficial tumours, and lymph node staging [27].

In the AJCC eighth edition, Ta disease indicates non-invasive localized squamous cell carcinoma, which allows for inclusion of historical variants other than verrucous carcinoma. This circumvents the confusion caused by UICC in which category pTa was specified as 'non-invasive verrucous carcinoma'. The Royal College of Pathologists guidelines state that this terminology should not be used, as it may falsely lead some pathologists to call verrucous carcinomas of the penis as non-invasive [27].

T1 is subcategorized into T1a and T1b based on the presence of lymphovascular invasion, perineural invasion and high-grade tumour/ poorly differentiated tumour. Urethral invasion is no longer a differentiator between T2 and T3 disease. T2 includes invasion of the corpus spongiosum and T3 involves invasion of the tunica albuginea and corpus cavernosum. The modification of the T2 and T3 stages are reflected in both UICC 8 and AJCC 8. For nodal staging, pN1 has been revised from a single lymph node metastasis to two unilateral inguinal lymph node metastases, while pN2 has been modified to three or more inguinal lymph node metastases [28]. The presence of extra nodal extension indicates an inguinal lymph node stage of pN3, no matter what the extent of the change [27].

Perineurial and Lymphovascular Invasion

Perineurial invasion has been added as an additional prognostic indicator in the AJCC eighth edition [27]. Vascular invasion should be recorded as it is a predictor of nodal metastases [29].

Excision Margins

The presence of microscopic involvement of surgical margins has implications for surgical outcome audit of pre-operative staging and/or surgical technique must be recorded by site and microscopic distance of the tumour from close margins in mm [19]. Providing the actual measurement of lateral extent of individual margins is of value to surgeons in reviewing their techniques. It additionally is part of the required dataset which is recommended to be reported in specimens from penile cancer as per the International Collaboration on Cancer Reporting (ICCR) [30] (Table 2.3).

Table 2.3 ICCR carcinoma of the penis dataset

Recommendation	Element name
Required	• Operative Procedure • Macroscopic Tumour Site • Macroscopic Maximum Tumour Dimensions • Histological Tumour Type • Histological Grade • Microscopic Maximum Tumour Dimensions • Extent of Invasion • Lymphovascular Invasion • Perineural Invasion • Margin Status • Lymph Node Status • Pathological Staging • Primary Tumour T Stage • Regional Lymph Node N Stage
Recommended	• Clinical Information • Tumour Focality • Block Identification Key • Associated Penile Intraepithelial Neoplasia

Table 2.4 Percentage frequency of somatic mutations in penile SCC

Gene	Frequency
TP53	32%
CDKN2A	25%
NOTCH1	17%
PIK3CA	13%
FAT1	25%
CASP8	17%
FBXW7	11%

Molecular Alterations in Penile Cancer

In recent years there has been significant interest in the molecular alterations in penile SCC and several studies have been published highlighting the extent and frequency of these genetic changes. These have been summarised in a recent review paper [31] and the main findings are listed below:

Somatic Mutations

The presence of somatic mutations (Table 2.4) in penile SCC has been extensively examined and involves several genes including tumour suppressor genes (TP53 and CDKN2A), genes involved in cell signalling (NOTCH1, PIK3CA and FAT1) and those involved in apoptosis (CASP8) or acting as cell cycle regulators (FBXW7).

Copy Number Variations

Gains in the MYC gene at 8Q24 have been found in 32% of penile SCC and gains in the EGFR gene at 7p12 have been seen in about 25% of cases. Similar findings have also been reported in head and neck SCC.

Genes Related with HPV Status

Studies have shown a lower mutational load in penile SCC that is HPV-related when compared to cases that are independent of HPV. Furthermore, there appears to be an inverse correlation between HPV positivity and mutations in TP53 and CDKN2A.

Potential Therapeutic Targets

Although many genes have been found to be altered in the pathogenesis of penile SCC, these have been challenging to target separately. Potential therapeutic interventions are typically based on a combination of treatments based on an interaction between a number of signalling pathways. These include:

1. mTOR inhibitors in patients with TP53 mutation and stimulated PI3K signalling pathway.
2. PARP inhibitors in patients with defective DNA repair pathways.
3. Combination of PI3K and mTOR inhibitors in NOTCH1 mutated tumours.

Markers of Penile Cancer Progression and Metastasis

The genetic alterations seen in penile SCC may be involved either in primary carcinogenesis, tumour progression or metastatic spread [32]:

1. Primary carcinogenesis
   A. Genes upregulated by inflammation – COX2, PGE2
   B. Tumour suppressor genes – p53, p16, PTEN
   C. Oncogenes – HPV E6 / 7, MYC
   D. Apoptosis – bcl2, p53, telomerases
2. Proliferation and invasion
   A. Growth factors / receptors- EGFR, Her3, VEGF, PI3K, PTEN, AKT
   B. Epithelial / mesenchymal - MMP2 / MMP9, E cadherin, Glut1
   C. Cell cycle – Ki67
3. Metastasis
   A. Metastasis suppressor genes – KAI1, Nm23H1

The identification of the stage of tumorigenesis in which these particular genes are involved may result in new small molecular target agents that may stop the local

growth or spread of penile SCC and allow the prospect of curative treatment in affected patients.

Summary

In conclusion, surgeons need to be aware of the updated changes in the classification of penile tumours, the significance and nomenclature of PeIN, the pathological risk factors and the differences highlighted in the updated AJCC and UICC stagings. Patients with penile cancer should be referred to a specialist centre, with any diagnostic slides and/or blocks made available for expert review prior to subsequent treatment planning by the specialist team to ensure correct diagnosis, subtyping, grading, and staging. Standardized structured reporting guidelines and discussion at the supra-regional multidisciplinary team meetings lead to optimal management decisions taken and subsequently better oncological outcomes and patient survival. Communication with high quality reports and understanding of clinicians of what constitutes an adequate report, is the key to ensure proper management of these patients.

Key Points

- Due to its rarity, it is important to have specialist pathological expertise available to evaluate histopathological, immunohistochemical and molecular genetic features of penile specimens.
- Important pathological features to consider include presence of precursor lesions and predisposing lesions such as lichen sclerosus and condylomatous changes.
- Knowledge of subtyping of both HPV and non-HPV related penile cancers is important due to their differing prognostic profiles.
- Other important prognostic features on pathology include grade, stage, presence of perineurial, lymphovascular invasion and excision margins.
- Various potential biomarkers exist including Ki67, SCC antigen e cadherin and p53.

Revisions Questions

Multiple Choice Questions

1. What is the causative agent for condyloma acuminatum?
   (a) HSV
   (b) HPV 6 and 11
   (c) E. coli
   (d) Schistosoma

2. Which of the following are HPV related pre-cancerous conditions (Choose all that apply)?
   (a) Warty undifferentiated PeIN.
   (b) Basaloid undifferentiated PeIN
   (c) Differentiated PeIN
   (d) Paget's disease
3. Which immunohistochemical marker is a surrogate for HPV in the tissue
   (a) P53
   (b) P63
   (c) P16
   (d) KI-67
4. Which of the following is recommended for pathological staging of penile squamous cell carcinoma and considers pathological risk factors for appropriate management decisions?
   (a) AJCC 8th edition
   (b) AJCC 7th edition
   (c) UICC 8th edition
   (d) All of the above
   (e) None of the above

Viva Cases

Case 1
A 51-year-old man presents with a flat white lesion with irregular and indistinct borders on the glans. Histopathology shows hyperkeratosis, parakeratosis, and prominent cytological atypia in the basal keratinocytes. The background skin shows features in keeping with lichen sclerosus. The atypia extends to the peripheral limit.

A. What are the differential diagnoses and key points indicated in the history and pathology?

Case 2
Following a previous diagnosis of squamous cell carcinoma which was treated by glans resurfacing, a 75-year-old man presents with a recurrent ulcerated fungating tumour of the neo-glans involving the coronal sulcus. Sectioning of the specimen received in pathology shows deep invasion of the cavernosum. Histopathology shows a poorly differentiated grade 3–4 squamous cell carcinoma composed predominantly of spindle cells with increased atypical mitosis, necrosis, multinucleated cells, and multiple foci of perineurial and lymphovascular invasion.

A. What is the subtype of cancer in discussion and the key points related to this subtype?

Answers

Multiple Choice Questions

1. **B.** Condyloma acuminatum in the majority of cases is caused by low risk HPV genotypes 6 and 11
2. **A and B.** Undifferentiated PeIN has been very strongly related to oncogenic HPV infection resulting from the integration of viral genome into the DNA of the host cell, leading to oncogene overexpression and cell proliferation. This is morphologicaly of three types; warty, basaloid and warty-basaloid.
3. **C.** Strong and diffuse nuclear expression of p16 is a strong predictor for the presence of HPV (high risk) in penile neoplasia.
4. **A**. Except for the modification of the T2 and T3 stages, which are the same in UICC 8 and AJCC 8, the advances documented in the literature do not seem to have been considered in UICC 8.

Viva Cases

Case 1

A. Important Discussion Points to cover:
   - Type of pre-cancerous and predisposing lesions
   - Differentiated versus undifferentiated PeIN (HPV related)
   - Association of lichen sclerosus and differentiated PeIN
   - The value of p16 (negative in differentiated PeIN) and Ki-67 (overexpression in the basal and parabasal layers) in the diagnosis of pre-cancerous lesions
   - Follow up and close surveillance advised in cases of margin involvement. Asses for presence of multifocal lesions.
   - Concomitant penile carcinoma if present, is usually low grade of ususal, verrucous, papillary and pseudohyperplastic type.

Case 2

A. Important discussion points to cover include:
   - Sarcomatoid carcinoma is a rare and aggressive variant of squamous cell carcinoma.
   - Presence of adjacent squamous cell carcinoma or PeIN helps establish a diagnosis.
   - Spindle cell should comprise 30% of the tumour to qualify for the diagnosis.
   - Any history of previous treatment (radiotherapy) should be documented.
   - This tumour is pT3 and shows perineurial and lymphovascular invasion which are all adverse pathological risk factors.
   - These cases have a high mortality rate with increased risk of haematogenous and lymph node metastases as well as local recurrence.

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