HPV-negative Penile Intraepithelial Neoplasia (PeIN) With Basaloid Features

José Guerrero, MD,* Isabel Trias, MD,* Luis Veloza, MD, PhD,† Marta del Pino, MD, PhD,‡
Adriana Garcia, MD, PhD,* Lorena Marimon, BSc,§ Sherley Diaz-Mercedes, MD,*
Maria T. Rodrigo-Calvo, MD,* Silvia Alós, BSc,* Tarek Ajami, MD,‖
Rafael Parra-Medina, MD, PhD,¶ Antonio Martinez, MD, PhD,*# Oscar Reig, MD, PhD,#**
Maria J. Ribal, MD, PhD,‖ Juan M. Corral-Molina, MD, PhD,‖ Jaume Ordi, MD, PhD,*§
Inmaculada Ribera-Cortada, MD, PhD,* and Natalia Rakislova, MD, PhD*§

Abstract: Most human papillomavirus (HPV)-independent penile squamous cell carcinomas (PSCCs) originate from an intraepithelial precursor called differentiated penile intraepithelial neoplasia, characterized by atypia limited to the basal layer with marked superficial maturation. Previous studies in vulvar cancer, which has a similar dual etiopathogenesis, have shown that about one fifth of HPV-independent precursors are morphologically indistinguishable from high-grade squamous intraepithelial lesions (HSILs), the precursor of HPV-asssociated carcinomas. However, such lesions have not been described in PSCC. From 2000 to 2021, 55 surgical specimens of PSCC were identified. In all cases, thorough morphologic evaluation, HPV DNA detection, and p16, p53, and Ki-67 immunohistochemical (IHC) staining was performed. HPV-independent status was assigned based on both negative results for p16 IHC and HPV DNA. Thirty-six of the 55 PSCC (65%) were HPV-independent. An intraepithelial precursor was identified in 26/36 cases (72%). Five of them (19%) had basaloid features, morphologically indistinguishable from HPV-associated HSIL. The median age of the 5 patients was 74 years (range: 67 to 83 y). All 5 cases were p16 and DNA HPV-negative. Immunohistochemically, 3 cases showed an abnormal p53 pattern, and 2 showed wild-type p53 staining. The associated invasive carcinoma was basaloid in 4 cases and the usual (keratinizing) type in 1. In conclusion, a small proportion of HPV-independent PSCC may arise on adjacent intraepithelial lesions morphologically identical to HPV-associated HSIL. This unusual histologic pattern has not been previously characterized in detail in PSCC. p16 IHC is a valuable tool to identify these lesions and differentiate them from HPV-associated HSIL.

Key Words: penile cancer, differentiated penile intraepithelial neoplasia, p53, p16, HPV

(*Am J Surg Pathol* 2022;00:000–000)

Penile cancer is a rare malignancy,[1] and penile squamous cell carcinomas (PSCCs) represent >90% of the malignancies of this organ. Classically, PSCC had been subclassified based on its histologic features.[2,3] Growing evidence showing that PSCC may arise following 2 distinct etiopathogenic pathways, one associated with human papillomavirus (HPV) and a second independent of HPV[4] has led to a significant switch in the classification of these tumors, which in the last revision of the World Health Organization (WHO) are primarily subclassified according to their association or not with HPV.[5] Significant epidemiological differences have been identified between these 2 main types of PSCC, with HPV-associated neoplasms usually arising in men with a history of smoking habit and multiple sexual partners,[6] while the HPV-independent tumors are frequently associated with chronic inflammatory conditions, lichen sclerosus, and phimosis.[1] However, unlike other anatomic sites with HPV-associated

and HPV-independent cancers, in which the former have consistently shown to have a better prognosis than the latter,[7] it is still unclear whether this prognostic implication also applies to PSCC.[8,9] Recently, a few studies have shown a trend towards longer disease-free survival in patients with HPV-associated PSCC.[10,11]

Similar to vulvar squamous cell carcinomas,[12] the majority of PSCC develop from premalignant lesions generically named penile intraepithelial neoplasia (PeIN).[13] Remarkably, each etiopathogenic type is associated with a type of PeIN lesion with specific morphologic features. The PeIN lesion on which HPV-associated PSCC develop was previously known as Bowenoid or undifferentiated PeIN, and after the launch in 2012 of the lower anogenital squamous terminology is also referred to as penile high-grade squamous intraepithelial lesion (HSIL).[14] Penile HSILs are etiologically and morphologically identical to the HSIL of the vulva, uterine cervix, or anus and typically show basaloid or warty features, with obvious architectural and cytologic disarray involving the whole thickness of the epithelium.[13] Characteristically, penile HSIL and HPV-associated PSCC show p16 overexpression[15] resulting in "block-type" immunohistochemical (IHC) staining and wild-type pattern of p53 IHC expression. The PeIN lesion on which HPV-independent PSCC originate is called simplex or differentiated penile intraepithelial neoplasia (dPeIN), a lesion that has marked similarities with differentiated vulvar intraepithelial neoplasia (dVIN), the precursor of HPV-independent vulvar squamous cell carcinoma.[16] Both dPeIN and dVIN are characterized by acanthosis, prominent intercellular bridges, large keratinocytes, and atypia limited to the basal layer with marked maturation in the superficial layers of the epithelium.[16,17] Presence of inflammatory conditions, such as lichen sclerosus and lichen simplex chronicus, is also frequently associated with dPeIN.[17] dPeIN is typically negative for p16, and about half of them show abnormal p53 IHC expression,[18] similarly to dVIN.[19]

Our group described in 2009 an unusual morphologic variant of dVIN that mimicked HSIL.[20] Recent studies have shown that this variant accounts for about one fifth of the precursors of HPV-independent vulvar squamous cell carcinomas.[21] In the penis, a few studies have occasionally briefly mentioned PeIN lesions showing mixed differentiated and basaloid or differentiated and warty features.[17,22,23] However, this HSIL-like pattern of dPeIN has not been thoroughly characterized in terms of HPV testing and p16 and p53 IHC staining patterns. In this study, we aimed to describe, in a series of well-characterized HPV-independent PSCC (negative for HPV DNA and p16), the prevalence and the histologic features of dPeIN with HSIL-like morphology, a lesion that can be easily misdiagnosed as HPV-associated penile HSIL.

MATERIALS AND METHODS

Case Selection and Histologic Evaluation of the Invasive Carcinoma

A computer-based search was conducted on the pathology database of the Hospital Clinic of Barcelona to retrieve all patients surgically treated of PSCC from January 1, 2000, through December 31, 2021. Two expert pathologists (I.R.-C. and I.T.) and 1 trainee (J.G.) reviewed all the available slides. Five to 34 slides were available per case for revision (mean: 12 slides). Morphologic evaluation was performed on hematoxylin and eosin glass slides. The histologic subtype of PSCC was established following the WHO 2016 classification.[24] Histologic evaluation was blind to IHC and HPV DNA detection results.

Clinical variables, including treatment and outcome, were collected from the medical records. The TNM staging was performed according to the eighth edition of the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) staging manual.[25] All samples and data were used in accordance with the ethical standards of 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Institutional Review Board of the Hospital Clínic of Barcelona (Protocol code HCB.2020.1207).

Histologic Evaluation of the Adjacent Skin

The skin adjacent to the invasive carcinoma was carefully evaluated in search of (1) associated premalignant (PeIN) and (2) inflammatory lesions. One to 15 slides with adjacent skin were available per case for revision (median: 5 slides). Following the criteria of WHO 2016 classification,[24] PeIN were further classified based on pure morphologic criteria as dPeIN (HPV-independent PeIN) and HSIL (HPV-associated PeIN). A distance of at least 1 cm away from the invasive carcinoma was required to categorize a case as PeIN.

The diagnosis of dPeIN (HPV-independent PeIN) was based on the presence of moderate to marked basal atypia, dyskeratosis, and elongated and anastomosing rete ridges, along with preserved maturation of the upper layers of the epithelium.[17] HSIL (HPV-associated PeIN) was diagnosed on the basis of evident cytologic and architectural atypia involving the whole thickness of the epithelium with the absence of maturation and basaloid looking or koilocytic-like (warty) features.

Inflammatory lesions such as lichen simplex chronicus and lichen sclerosus were also recorded if present. Lichen simplex chronicus was diagnosed in the presence of acanthosis, spongiosis, and hyperkeratosis, along with papillary dermal fibrosis and interstitial inflammation with histiocytes and lymphocytes.[26] Lichen sclerosus was identified by a thickened basement membrane, as well as subepidermal edema and dense fibrosis.[26]

Immunohistochemistry

A representative paraffin block was selected from each case for p16 and p53 IHC staining. The selection was based on the presence in the block of invasive PSCC, as well as adjacent skin, including, if present, premalignant and/or inflammatory lesions. The automated BenchMark ULTRA platform (Ventana, Tucson, AZ) was used for the IHC techniques, following the manufacturer's protocol. For each biomarker, the evaluation was performed

separately in the invasive carcinoma and in the adjacent premalignant lesion, the inflammatory lesion (if present), and the normal epithelium.

p16 staining (clone E6H4; Roche) was performed in all cases in the invasive tumor as well as in the adjacent skin lesions (PeIN and inflammatory lesions). Only diffuse and continuous cytoplasmic and nuclear staining in a group of contiguous cells at the basal and parabasal layers (block staining) was considered positive for p16. The absence of staining or patchy staining were considered as a negative result for p16.

Staining for p53 (clone DO-7; Roche) was conducted in all cases in the invasive tumor and in the premalignant and/or inflammatory lesions (if present). The p53 6-pattern framework recently introduced in vulvar squamous cell carcinoma was used to evaluate p53 staining.[27,28] Two staining patterns were classified as "normal," indicative of wild-type protein: (1) scattered and (2) mid-epithelial. The scattered pattern was defined as weak or moderate heterogenous nuclear staining in isolated cells, while the mid-epithelial pattern consisted of strong staining in mid-epithelial cells, with sparing of the basal and lower parabasal cells. Four patterns were considered as "abnormal" indicative of mutated p53 protein: (1) basal overexpression (at least 80% of the cells in the basal layer), (2) parabasal/diffuse overexpression (positive staining in at least 80% of basal cells with extension to cells in the superficial layers), (3) null pattern (complete absence of staining), and (4) cytoplasmic expression (with or without nuclear staining).[21] Normal adjacent skin, stromal, or inflammatory cells served as an inner staining control.

Ki-67 IHC analysis was performed with the monoclonal antibody (clone 30-9; Roche).

HPV Analysis

In all cases, the same block of formalin-fixed, paraffin-embedded tissue used for IHC including both the PSCC and the adjacent skin (with a premalignant and inflammatory lesion, if present), was selected. No microdissection was conducted. DNA extraction was performed on two 10 μm whole tissue sections. Paraffin sections without tissue were cut before and after each carcinoma sample to avoid contamination. The microtome blade was replaced after each case. The DNA was extracted after 1-hour incubation in 20 μL of proteinase K solution (1 mg/mL) at 56°C. Subsequently, proteinase K was heat-inactivated at 95°C for 1 hour. The DNA was isolated using a commercial kit (QIAamp Tissue Kit; Qiagen, Hilden, Germany) according to the manufacturing instructions.

A volume of 10 μL of isolated DNA was used for PCR amplification, using the SPF10-LiPA system (Fujirebio, Gent, Belgium). HPV genotyping was performed using INNO-LiPA HPV Genotyping Extra II kit (Fujirebio). This system allows the amplification and genotyping of high-risk HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; of the probable high-risk HPV 26, 53, 66, 70, 73, and 82; and of low-risk HPVs 6, 11, 40, 42, 43, 44, 54, 61, 62, 67, 81, 83, and 89. Quality in each run was ensured with both positive and negative controls for DNA isolation, amplification, hybridization, and genotyping.

RESULTS

A total of 55 PSCC were evaluated; 36 (55%) were HPV DNA-negative and p16-negative and thus were classified as HPV-independent PSCC. Among these 36 HPV-independent PSCC, PeIN was identified in the adjacent skin in 27 cases (75%), 5 cases (14%) showed only inflammatory lesions with no atypia (4 lichen simplex chronicus and 1 lichen sclerosus and lichen simplex), while 4 cases (11%) showed normal skin. Twenty-two of 27 PeIN (82%) showed classic differentiated features with marked basal atypia and thus were classified as dPeIN. The remaining 5 of the 27 PeIN lesions (18%) were deemed unusual due to clear and extensive basaloid traits,

TABLE 1. Clinical and Pathologic Characteristics of the 5 Unusual, HSIL-like dPeIN Lesions

Case No.	Age (y)	Location of PeIN	Histologic Subtype		Associated Lesions	Surgery	IHC Results				Size (Invasive Tumor) (mm)	TNM Stage
			PeIN	Invasive Carcinoma			HPV DNA	p16	Ki-67 (%)	p53 IHC Pattern		
1	70	Glans and foreskin	Basaloid	Usual type (keratinizing)								
2	72	Foreskin	Basaloid	Basaloid	—	Partial penectomy	Negative	Negative	20	Mutated-type (null pattern)	23	pT1aN0M0
3	67	Foreskin	Basaloid	Warty-basaloid	—	Shaving	Negative	Negative	20	Mutated-type (basal overexpression)	11	pT1aN0M0
4	76	Glans	Basaloid	Basaloid	LSC	Glandectomy	Negative	Negative	30	Wild-type (scattered staining)	5	pT1aN0M0
5	83	Glans and foreskin	Basaloid	Basaloid	—	Partial penectomy	Negative	Negative	10	Wild-type (mid-epithelial staining)	20	pT1aNoM0

LSC indicates lichen simplex chronicus.

morphologically indistinguishable from HPV-associated PeIN (HSIL). Table 1 outlines the clinical and pathologic characteristics of the 5 unusual, HSIL-like dPeIN lesions. The procedure for the selection of cases for this study is shown in Figure 1.

Clinical Features

The median age at diagnosis of the 5 patients with unusual HSIL-like dPEIN was 74 years (range, 67 to 83 y). Three patients were consulted for phimosis (cases 1, 2, and 4). The average size of the invasive carcinoma was 17 mm (range: 5 to 28 mm). Two lesions (40%) were located in the inner foreskin, 1 (20%) in the glans, and the remaining 2 (40%) compromised both inner foreskin and the glans. At presentation, 4 patients were at clinical stage I and 1 at stage IIIa.

In 2 cases, the limits between dPeIN and invasive carcinoma were clinically distinguishable (cases 2 and 4). In both cases, PeIN was described as a persistent erythematous lesion with superficial ulceration, unifocal (case 2), or multifocal (case 4). Patient 2 had unsuccessfully been treated with imiquimod. In 3 cases (cases 1, 3, and 5), the PeIN lesion had not been identified clinically. In case 3, the entire lesion was described as a giant exophytic lesion reminiscent of giant condyloma, and no distinction between PeIN and invasive carcinoma was made. The lesion had been treated with topical imiquimod without clinical response.

Only 1 of the patients (case 3) had a documented history of cigarette smoking and alcohol consumption. None of the patients referred multiple sex partners. No synchronous or metachronous anogenital tumors were observed in any of the 5 patients.

Histologic Characteristics

Figure 2 shows the typical histologic characteristics of the HSIL-like HPV-independent precursor. All 5 unusual dPeIN displayed evident HSIL-like basaloid morphologic traits. The epidermis was entirely occupied by undifferentiated, basaloid-like keratinocytes with an increased nucleus-to-cytoplasmic ratio. Loss of maturation ("wind-blown" pattern) and moderate to severe cellular atypia were evident throughout the epithelium. Prominent mitoses were identified in all 5 cases (Fig. 2). In 1 of the cases (case 4), the adjacent skin additionally showed the presence of lichen simplex chronicus (case 4). None of the patients had adjacent lichen sclerosus.

In 1 patient (case 1), the adjacent invasive carcinoma of usual keratinizing type, 3 patients (cases 2, 3, and 5) showed basaloid type carcinoma, and 1 (case 4) mixed basaloid-warty carcinoma. Areas of associated dPeIN with typical features (prominent intercellular bridges, large keratinocytes, and abnormal maturation with basal atypia) were identified in case 1.

IHC Findings

The characteristic IHC features of HPV-independent lesions mimicking HSIL are shown in Figure 3. p16 IHC was completely negative in the 5 intraepithelial lesions, as well as in the adjacent invasive PSCC. Three cases showed abnormal p53 IHC staining (suggestive of mutation): 2 of them showed diffuse overexpression (cases 1 and 3), and 1 null pattern (case 2). Two lesions (cases 4 and 5) showed wild-type p53 staining. Ki-67 staining ranged from 5% to 30% of the cells in the HSIL-like lesion. All 5 lesions showed a low index of proliferation, with a mean of 17% (range: 5% to 30%).
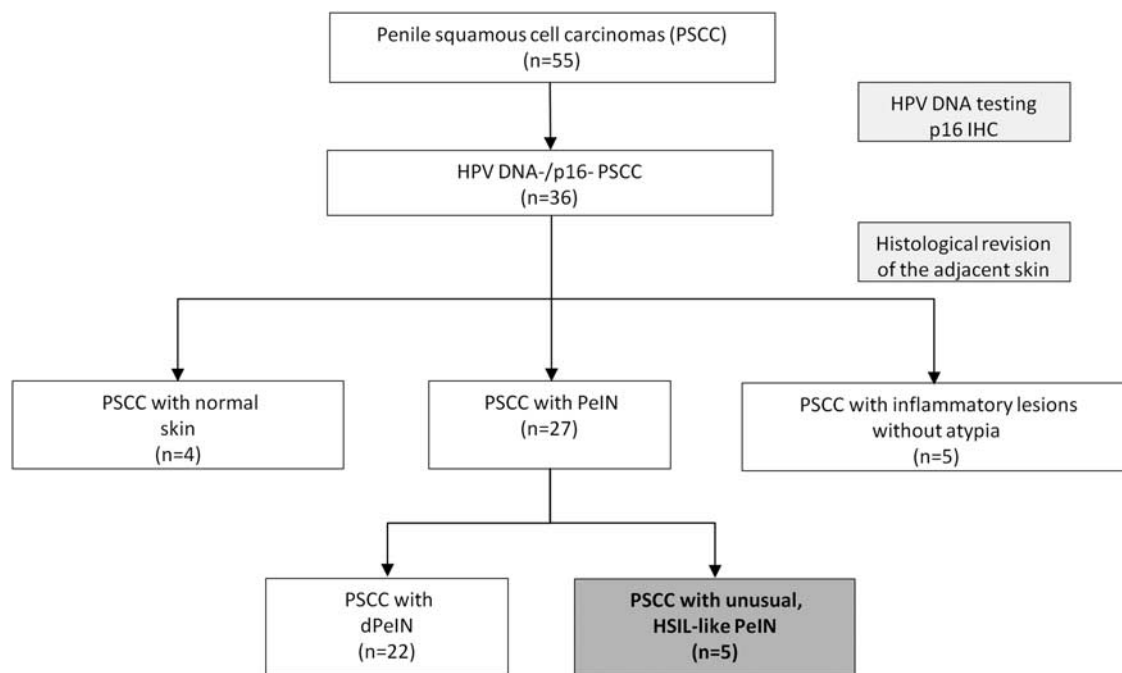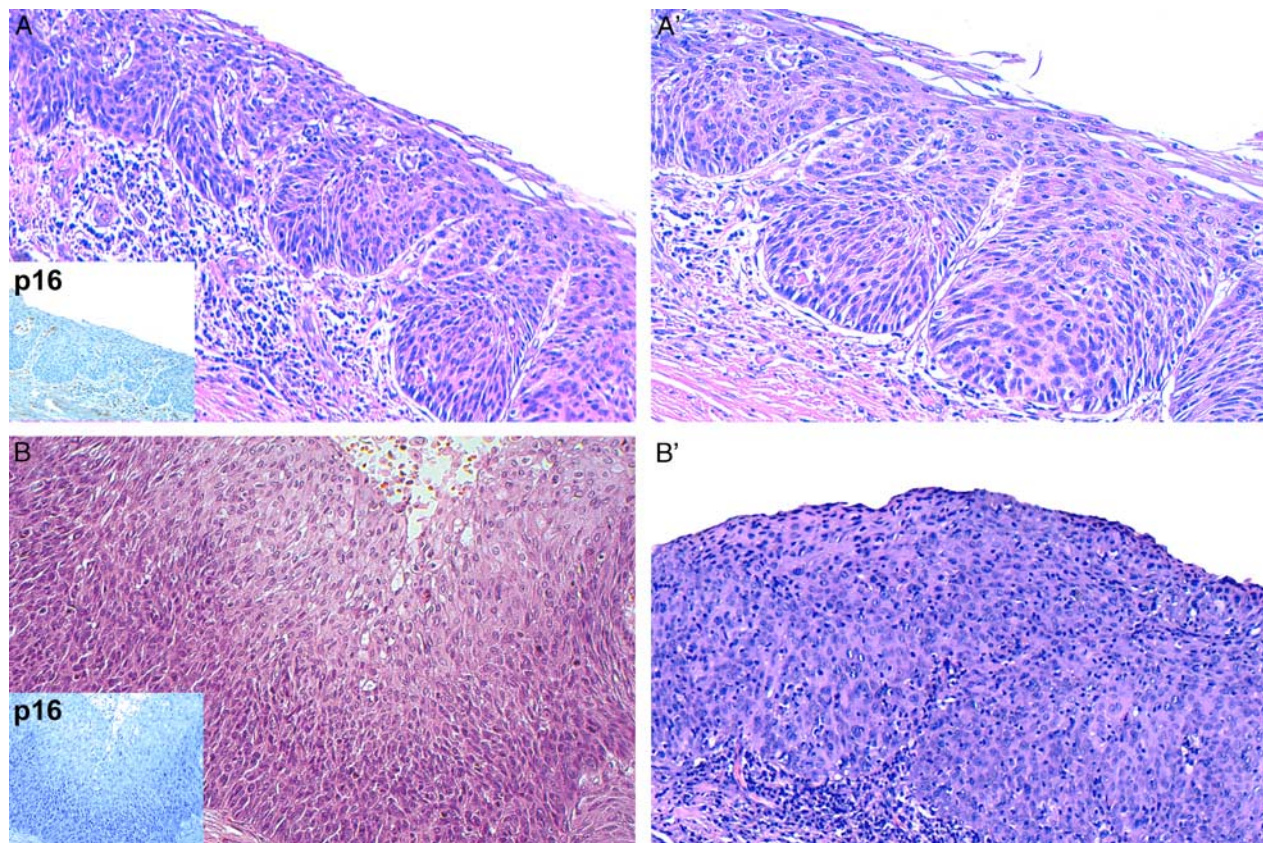

FIGURE 1. Study algorithm.

FIGURE 2. Two cases (cases 1 and 3) with penile HPV-independent PeIN simulating HPV-associated PeIN (HSIL). Case 1 (A, A′), The epidermis is markedly disorganized, showing the "wind-blown" appearance, abundant mitotic features and atypical, slightly enlarged keratinocytes. Only minimal maturation is observed in the superficial layer. p16 staining is negative. Case 3 (B, B′), The epidermis is thickened and entirely replaced by small, undifferentiated, basaloid-like keratinocytes disposed in a disorganized manner. Mitotic figures are evident throughout the epithelium. p16 is negative in the lesion.

HPV DNA Testing

All the 5 unusual PeIN as well as their associated invasive carcinoma were negative for both low-risk and high-risk HPV types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74).

DISCUSSION

In this study, we describe a subset of well-characterized, HPV-independent dPeIN lesions (HPV-negative and p16-negative) with histologic features indistinguishable from typical HPV-associated PeIN (HSIL). All 5 lesions displayed unequivocal traits typical of HPV-associated lesions (HSIL) such as architectural disarray, loss of maturation, and altered nucleus-to-cytoplasmic ratio evident throughout all levels of the epithelium. Negativity for both HPV DNA and p16 in the invasive PSCC as well as in the adjacent PeIN allowed ruling out any role of HPV in the pathogenesis of these tumors. Interestingly, all 5 lesions showed low proliferative index with Ki-67 staining, which is in keeping with the findings reported in HPV-associated precursors throughout the anogenital tract.[29,30] To the best of our knowledge, this unusual histologic pattern of HPV-negative PeIN has not been reported in detail in PSCC, although a few dPeIN lesions with mixed basaloid and differentiated features have occasionally been mentioned in a few reports.[17,22,23]

IHC stains were supportive of an HPV-independent oncogenic pathway involved in these cases. Expression of p16 was consistently negative in all 5 cases, both in the basaloid dPeIN and the associated PSCC. The staining of p53 showed an abnormal (suggestive of mutation) pattern in 3 cases. Considering that *TP53* mutations are far more common in HPV-independent PSCC,[31] the unusual lesions described in this study probably comprise a morphologic spectrum of dPeIN.

A few cases of HPV-associated invasive PSCC with p16-negative IHC result have been previously identified by Chahoud et al.[32] These tumors had nonsense *CDKN2A* mutations, which could explain the p16 IHC-negative result. In our series, the negative result for HPV DNA, in addition to the negative staining for p16, strongly favors the absence of any carcinogenic role of HPV in these cases.

Interestingly, similar HPV-independent lesions with HSIL-like, basaloid pattern have been described.[20,21] Indeed, we described in 2009 for the first time a subset of 4 HPV DNA-negative (and p16-negative) cases of vulvar intraepithelial
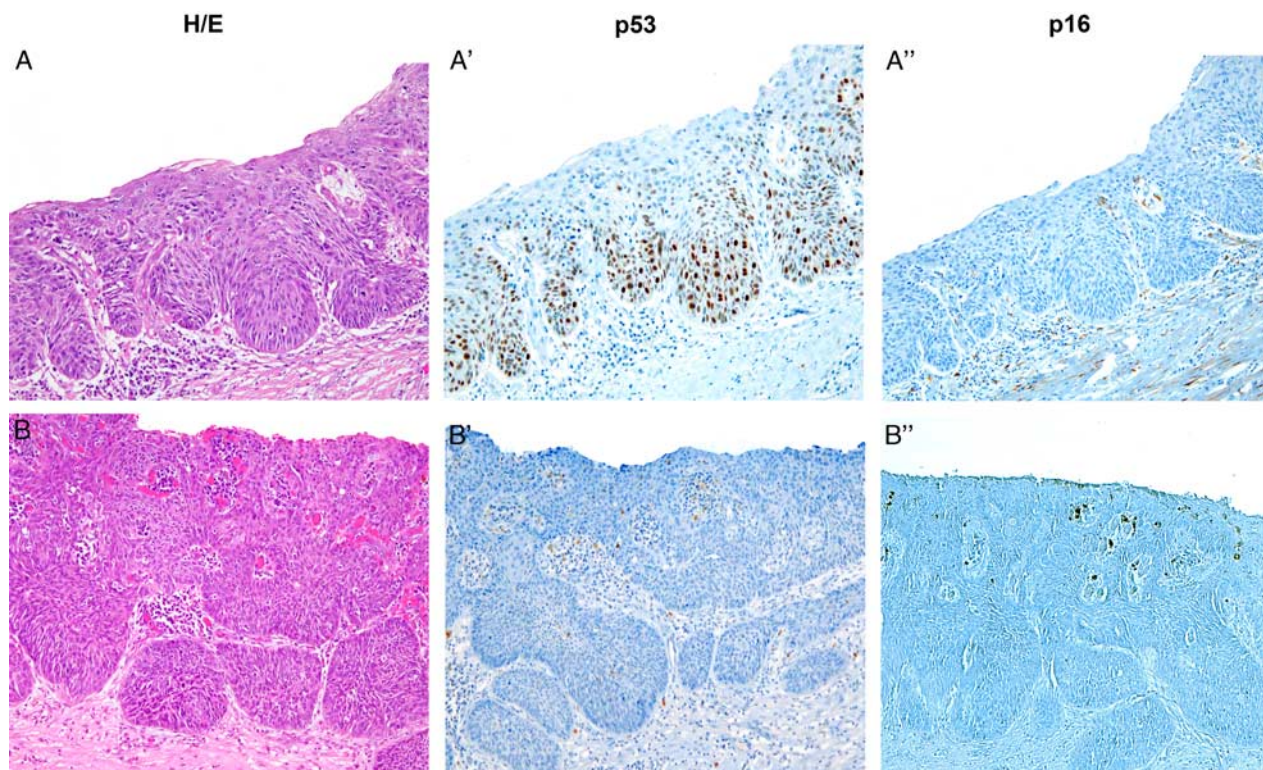
FIGURE 3. Histologic and IHC features of HPV-independent lesions mimicking HSIL. Case 1. A, The epidermis shows marked architectural and cellular atypia. A′, p53 stain showing suprabasal overexpression. A″, Negative p16 stain. Case 2. B, Acanthotic epidermis filled with small, basaloid-like cells. B′, p53 stain showing absence of expression (null pattern), with conserved staining in the inflammatory cells. B″, Negative p16 stain. H/E indicates hematoxylin and eosin.

neoplasia, morphologically indistinguishable from HSIL.[20] In 2020, we further characterized these unusual precursors of vulvar cancer in a larger international multicenter cohort study[21] showing that 6% of all dVIN showed definite basaloid and/or warty features. The basaloid histology of these unusual vulvar lesions was identical to the 5 lesions identified in this series. Remarkably, vulvar squamous carcinoma is a rare neoplasm with dual etiopathogenesis (HPV-associated and HPV-independent) and a highly similar genomic landscape (frequent *TP53*, *CDKN2A*, and *NOTCH-1* mutations).[33,34] Thus, it is plausible that both types of carcinomas also share unusual HPV-negative precursors, mimicking HPV-associated PeIN.

Several clinical characteristics of the 5 cases presented in this study might be considered as additional evidence of a carcinogenic origin independent of HPV. First, most of the patients did not have lifestyle risk factors for HPV-associated neoplasm, such as multiple sex partners, smoking, and alcohol consumption history. Second, the absence of any response to imiquimod, a treatment that has shown to be effective in HPV-associated lesions.[28] Third, the foreskin was compromised in most of the cases, in accordance with the evidence accumulated for dPeIN,[17] and contrarily to HPV-associated HSIL, which mostly affects the glans.[35] Unfortunately, the clinical behavior of these lesions is unknown. However, the appropriate classification of these lesions as HPV-independent might be clinically relevant in similarity with HPV-

independent squamous premalignant lesions of the vulva which have proven to be more aggressive in the vulva.[36] Thus, the patients with HSIL-like HPV-independent lesions of the penis will probably have to undergo a stricter follow-up than patients with penile HSIL.

In summary, our study shows that HPV-independent precursors may have a broader morphologic spectrum than originally thought, featuring occasionally a basaloid morphology identical to HPV-associated HSIL/PeIN. Immunostaining for p16 is a reliable tool in differential diagnosis.

REFERENCES

1. Thomas A, Necchi A, Muneer A, et al. Penile cancer. *Nat Rev Dis Primers*. 2021;7:12.
2. Chaux A, Velazquez E, Algaba F. Developments in the pathology of penile squamous cell carcinomas. *Urology*. 2010;76:S7–S14.
3. Chaux A, Reuter V, Lezcano C, et al. Comparison of morphologic features and outcome of resected recurrent and nonrecurrent squamous cell carcinoma of the penis: a study of 81 cases. *Am J Surg Pathol*. 2009;33:1299–1306.
4. Mannweiler S, Sygulla S, Winter E, et al. Two major pathways of penile carcinogenesis: HPV-induced penile cancers overexpress p16ink4a, HPV-negative cancers associated with dermatoses express p53, but lack p16ink4a overexpression. *J Am Acad Dermatol*. 2013;69:73–81.
5. Cubilla AL, Velazquez EF, Amin MB, et al. The World Health Organisation 2016 classification of penile carcinomas: a review and

update from the International Society of Urological Pathology expert-driven recommendations. *Histopathology*. 2018;72:893–904.

6. Vieira CB, Feitoza L, Pinho J, et al. Profile of patients with penile cancer in the region with the highest worldwide incidence. *Sci Rep*. 2020;10:2965.

7. Kühn JP, Schmid W, Körner S, et al. HPV status as prognostic biomarker in head and neck cancer—which method fits the best for outcome prediction? *Cancers (Basel)*. 2021;13:4730.

8. Kidd LC, Chaing S, Chipollini J, et al. Relationship between human papillomavirus and penile cancer-implications for prevention and treatment. *Transl Androl Urol*. 2017;6:791–802.

9. Chipollini J, Pollock G, Hsu C, et al. National trends and survival outcomes of penile squamous cell carcinoma based on human papillomavirus status. *Cancer Med*. 2021;10:7466–7474.

10. Lont AP, Kroon BK, Horenblas S, et al. Presence of high-risk human papillomavirus DNA in penile carcinoma predicts favorable outcome in survival. *Int J Cancer*. 2006;119:1078–1081.

11. Ashley S, Shanks JH, Oliveira P, et al. Human papilloma virus (HPV) status may impact treatment outcomes in patients with pre-cancerous penile lesions (an eUROGEN Study). *Int J Impot Res*. 2021;33:620–626.

12. del Pino M, Rodriguez-Carunchio L, Ordi J. Pathways of vulvar intraepithelial neoplasia and squamous cell carcinoma. *Histopathology*. 2013;62:161–175.

13. Velazquez EF, Chaux A, Cubilla AL. Histologic classification of penile intraepithelial neoplasia. *Semin Diagn Pathol*. 2012;29:96–102.

14. Darragh TM, Colgan TJ, Thomas Cox J, et al. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Int J Gynecol Pathol*. 2013;32:76–115.

15. Cubilla AL, Lloveras B, Alejo M, et al. Value of p16(INK)4 389 (a) in the pathology of invasive penile squamous cell carcinomas: a report of 202 cases. *Am J Surg Pathol*. 2011;35:253–261.

16. Reutter JC, Walters RA, Selim MA. Differentiated vulvar intraepithelial neoplasia : what criteria do we use in practice? *J Low Genit Tract Dis*. 2016;20:261–266.

17. Chaux A, Velazquez EF, Amin A, et al. Distribution and characterization of subtypes of penile intraepithelial neoplasia and their association with invasive carcinomas: a pathological study of 139 lesions in 121 patients. *Hum Pathol*. 2012;43:1020–1027.

18. Chaux A, Pfannl R, Lloveras B, et al. Distinctive association of p16INK4a overexpression with penile intraepithelial neoplasia depicting warty and/or basaloid features: a study of 141 cases evaluating a new nomenclature. *Am J Surg Pathol*. 2010;34:385–392.

19. Pinto AP, Miron A, Yassin Y, et al. Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carcinoma. *Mod Pathol*. 2010;23:404–412.

20. Ordi J, Alejo M, Fusté V, et al. HPV-negative vulvar intraepithelial neoplasia (VIN) with basaloid histologic pattern: an unrecognized variant of simplex (differentiated) VIN. *Am J Surg Pathol*. 2009;33:1659–1665.

21. Rakislova N, Alemany L, Clavero O, et al. HPV-independent precursors mimicking high-grade squamous intraepithelial lesions (HSIL) of the vulva. *Am J Surg Pathol*. 2020;44:1506–1514.

22. Alvarado-Cabrero I, Sanchez DF, Piedras D, et al. The variable morphological spectrum of penile basaloid carcinomas: differential diagnosis, prognostic factors and outcome report in 27 cases classified as classic and mixed variants. *Appl Cancer Res*. 2017; 37:3.

23. Oertell J, Caballero C, Iglesias M, et al. Differentiated precursor lesions and low-grade variants of squamous cell carcinomas are frequent findings in foreskins of patients from a region of high penile cancer incidence. *Histopathology*. 2011;58:925–933.

24. Moch H, Cubilla AL, Humphrey PA, et al. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs—Part A: Renal, Penile, and Testicular Tumours. *Eur Urol*. 2016;70:93–105.

25. Sanchez DiF, Fernandez-Nestosa MJ, Canete-Portillo S, et al. What is new in the pathologic staging of penile carcinoma in the 8th Edition of AJCC TNM Model: rationale for changes with practical stage-by-stage category diagnostic considerations. *Adv Anat Pathol*. 2021;28:209–227.

26. Chan MP, Zimarowski MJ. Vulvar dermatoses: a histopathologic review and classification of 183 cases. *J Cutan Pathol*. 2015;42:510–518.

27. Kortekaas KE, Solleveld-Westerink N, Tessier-Cloutier B, et al. Performance of the pattern based interpretation of p53 immunohistochemistry as a surrogate for tp53 mutations in vulvar squamous cell carcinoma. *Histopathology*. 2020;77:92–99.

28. Tessier-Cloutier B, Kortekaas KE, Thompson E, et al. Major p53 immunohistochemical patterns in in situ and invasive squamous cell carcinomas of the vulva and correlation with TP53 mutation status. *Mod Pathol*. 2020;33:1595–1605.

29. Simionescu C, Mărgăritescu CL, Stepan A, et al. The utility of p16, E-cadherin and Ki67 in cervical squamous intraepithelial lesions diagnosis. *Rom J Morphol Embryol*. 2010;51:621–626.

30. Walts AE, Lechago J, Bose S. P16 and Ki67 immunostaining is a useful adjunct in the assessment of biopsies for HPV-associated anal intraepithelial neoplasia. *Am J Surg Pathol*. 2006;30:795–801.

31. Kashofer K, Winter E, Halbwedl I, et al. HPV-negative penile squamous cell carcinoma: disruptive mutations in the TP53 gene are common. *Mod Pathol*. 2017;30:1013–1020.

32. Chahoud J, Gleber-Netto FO, McCormick BZ, et al. Whole-exome sequencing in penile squamous cell carcinoma uncovers novel prognostic categorization and drug targets similar to head and neck squamous cell carcinoma. *Clin Cancer Res*. 2021;27:2560–2570.

33. Salama AM, Momeni-Boroujeni A, Vanderbilt C, et al. Molecular landscape of vulvovaginal squamous cell carcinoma: new insights into molecular mechanisms of HPV-associated and HPV-independent squamous cell carcinoma. *Mod Pathol*. 2022;35:274–282.

34. Carreras-Dieguez N, Guerrero J, Rodrigo-Calvo M, et al. Molecular landscape of vulvar squamous cell carcinoma. *Int J Mol Sci*. 2021; 22:7069.

35. Gregoire L, Cubilla AL, Reuter VE, et al. Preferential association of human papillomavirus with high-grade histologic variants of penile-invasive squamous cell carcinoma. *J Natl Cancer Inst*. 1995;87:1705–1709.

36. Voss FO, Thuijs NB, Vermeulen RFM, et al. The vulvar cancer risk in differentiated vulvar intraepithelial neoplasia: a systematic review. *Cancers (Basel)*. 2021;13:6170.