“HPV-independent precursors mimicking high-grade squamous intraepithelial lesions (HSIL) of the vulva”

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ABSTRACT

Two etiopathogenic types of vulvar squamous cell carcinoma (VSCC) have been described, human papillomavirus (HPV)-associated and HPV-independent. Precursor lesions, frequently identified in the adjacent skin, are also distinct in the two types of VSCC: high-grade squamous intraepithelial lesions (HSIL) in HPV-associated, and differentiated vulvar intraepithelial neoplasia (dVIN) or vulvar acanthosis with altered differentiation in HPV-independent VSCC. Although HPV-independent precursors mimicking HSIL have been described in the vulva, their frequency and morphological spectrum have not been completely characterized. We explored, in a large series of HPV-independent VSSC, the frequency and the histological features of precursors mimicking HSIL. We included 779 DNA HPV-negative/p16-negative VSCC with at least 1 cm of adjacent skin. We evaluated the histological and immunohistochemical (p16 and p53) characteristics of the intraepithelial lesions, focusing on precursors mimicking HPV-associated vulvar HSIL. 254 tumors (33%) had adjacent premalignant lesions. Of them, 186 (73%) had dVIN, 22 (9%) vulvar acanthosis with altered differentiation, and 46 (18%) lesions that mimicked HSIL. The mean age of the patients with these HSIL-like lesions was 72 ± 15 years. Twenty-six of these HSIL-like lesions had basaloid morphology, 13 warty and 7 mixed basaloid/warty features. All the HSIL-like precursors were DNA HPV-negative/p16-negative, 74% of them showed p53 abnormal staining, and 35% of them had areas of conventional dVIN. In conclusion, about one-fifth of the HPV-independent precursors mimic HSIL, showing either basaloid or warty features. Older age, the presence of areas of typical HPV-independent intraepithelial lesions, together with p16 negativity should raise suspicion of HPV-independent etiology.

INTRODUCTION

Two distinct etiopathogenic types of vulvar squamous cell carcinomas (VSCC) are recognized, human papilloma virus (HPV)-associated, and HPV-independent [1,2]. It is widely recognized that most VSCC arise from intraepithelial lesions that precede the development of invasive neoplasm for a variable period of time, and each etiopathogenic type of VSCC arises on a specific variety of intraepithelial lesion [1,3,4].

HPV-associated VSCCs develop from an intraepithelial precursor designated as high-grade squamous intraepithelial lesion (HSIL), etiologically and morphologically similar to the more frequent lesions of the uterine cervix. These HSIL lesions typically show nuclear abnormalities and abnormal maturation involving the full thickness of the epithelium. Characteristically, HSIL exhibits a marked p16 overexpression involving a continuous area of the epithelium, resulting in the so-called “block-type” immunohistochemical (IHC) staining.

A significant number of HPV-independent VSCCs develop from an intraepithelial precursor named differentiated vulvar intraepithelial neoplasia (dVIN), frequently in a background of chronic inflammatory skin disorders, especially lichen sclerosus (LS) and lichen simplex chronicus (LSC) [3]. In contrast with the sharply defined pathogenesis of the HPV-associated precursors and VSCC, with a specific cause -HPV- involved in the malignant transformation, the pathogenesis of the HPV-independent VSCC and dVIN is poorly understood, and probably includes more than a single etiological factor. In this regard, although p53 mutations are detected in over 70% of HPV-independent VSCC and its precursors, approximately 30% of the VSCC show a wild-type *TP53* status [5]. In the last few years other distinctive precursors of HPV-independent VSCC have been described: vulvar acanthosis with altered differentiation (VAAD) and differentiated exophytic vulvar intraepithelial lesion (DEVIL), which has overlapping histological features with VAAD, but is characterized by more

prominent exophytic features [6]. Both lesions are defined by prominent acanthosis, parakeratosis, verruciform architecture and lack of significant basal atypia. They are commonly associated with LSC and are considered precursors of verrucous carcinoma [7]. However, to date, both entities are still controversial due to their non-specific histological features which overlap with LSC, and their uncertain prognosis [8,9].

Initial reports suggested that the two etiopathogenic types of VSCC (HPV-associated and HPV-independent) could be separated using pure histological criteria. However, recent evidence has shown that a considerable morphological overlap exists between the two types of VSCC [10] and that, consequently, the distinction can only be reliably made using ancillary techniques such as p16 IHC and/or molecular HPV testing. This morphological overlap has also been observed for the intraepithelial lesions: some HPV-associated precursors simulate HPV-independent lesions (dVIN and/or LS) [11,12], and, contrarily, some HPV-independent intraepithelial precursors may have features of HSIL [13,14]. Thus, there is increasing evidence indicating that the histological characteristics of the intraepithelial lesions are not entirely reliable [11,13,15]. Nevertheless, this evidence is based on few isolated descriptions, and the frequency of these HPV-independent precursors mimicking HSIL is poorly known. Moreover, the morphological spectrum of these HSIL-like lesions has not been completely characterized and may be broader than commonly thought.

In this study, we aimed to explore, in a large series of well-characterized HPV-independent VSCC (HPV DNA negative and p16-negative), the frequency, histological and immunohistochemical (IHC) features of the adjacent intraepithelial lesions, focusing on unusual lesions mimicking HSIL, i.e. HPV-associated precursor.

MATERIALS AND METHODS

Case selection

We reviewed a series of 1709 invasive VSCCs included in the VVAP study (International Survey on HPV prevalence and type distribution in vulvar, vaginal, anal, penile neoplasms) coordinated by the Catalan Institute of Oncology (ICO, Barcelona-Spain) in collaboration with DDL Diagnostics Laboratory (Rijswijk, the Netherlands). The study was approved by the local and ICO Ethics Committees.

We selected all cases fulfilling the following inclusion criteria: (1) presence of invasive VSCC; (2) negative result for HPV detection by polymerase chain reaction (PCR); (3) negative result for p16 IHC in the invasive tumor; and (4) presence of at least 1 cm of skin surrounding or overlying the invasive tumor.

Tissue preparation, nucleic-acid isolation and HPV DNA detection

DNA extraction was performed on whole sections of formalin-fixed paraffin-embedded tissue from surgical specimens or vulvar biopsies as previously described [10]. No microdissection was performed in any of the cases, and in all cases the analyzed tissue included the invasive tumor and the adjacent skin with the inflammatory and/or premalignant lesions, if present. Sectioning and sample preparation were carried out with the highest safety measures to avoid cross-contamination. Processing and pathology diagnosis were done by the ICO laboratory. In all cases the tissue analyzed included the invasive tumor and, when present, the adjacent intraepithelial and/or inflammatory lesion.

HPV DNA detection and typing were performed using SPF10 PCR, DEIA and the LiPA25 system (version 1, Laboratory Biomedical Products, Rijswijk, The Netherlands) as previously described [10]. Briefly, LiPA25 can be used to detect 25 high-risk and low-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). Each run contained negative and positive controls to

monitor the efficiency of DNA isolation, PCR amplification, hybridization, and genotyping procedures.

Histological evaluation

A single histological slide of each tumor was available for review. The VSCC were classified into keratinizing, basaloid, warty, non-keratinizing subtypes, or other less frequent subtypes following the WHO 2014 classification [16].

The squamous epithelium adjacent to the neoplasms was carefully evaluated in search of associated precursor lesions. In order to establish the diagnosis of intraepithelial lesion, the lesion had the peripheral extension for at least 1 cm away from the invasive carcinoma to rule out possible peripheral intraepithelial extension of the invasive tumor.

The diagnosis of dVIN required the presence of atypical keratinocytes limited to the basal and parabasal layers in the context of a fully differentiated epithelium [3,4]. LS was diagnosed on the basis of homogenization of the collagen of the papillary dermis, alongside with other features described elsewhere [3]. Other inflammatory/reactive skin conditions included LSC, squamous hyperplasia, lichen planus, and other non-specific inflammatory and/or reactive abnormalities. VAAD was diagnosed by absence of atypia and the presence of three main features [7]: 1) prominent acanthosis with variable verruciform architecture 2) loss of the granular cell layer with superficial epithelial cell pallor, and 3) multilayered parakeratosis. DEVIL was diagnosed based on the same criteria as VAAD, in addition to at least focal preservation of granular layer and more striking parakeratosis [6]. The adjacent skin was considered normal in the absence of dVIN and/or LS, VAAD, DEVIL, and of any other premalignant or inflammatory/reactive skin lesions.

The HPV-independent lesions with HSIL-like features were further sub-classified as basaloid, warty, and mixed basaloid/warty subcategories. Briefly, the basaloid HSIL-

like pattern was characterized by small atypical cells throughout the epidermis, with a marked architectural disarray [4]. Warty type HSIL-like pattern was characterized by epidermis with wide and deep rete ridges, moderate to marked pleomorphism and easily recognizable koilocytic-like changes.

All 1709 cases included in the VVAP study were evaluated independently by two pathologists (N.R. and J.O.). This evaluation was blind to the HPV detection and IHC results. All discrepant results were reviewed in an adjudication meeting and a final diagnosis was established by consensus between the reviewers.

p16 IHC

All cases were stained with a p16 monoclonal antibody using the CINtec Histology Kit (clone E6H4; Roche-mtm-Laboratories, Heidelberg, Germany) [17]. In addition to the initial evaluation performed in the invasive tumor, the p16 IHC staining was evaluated independently in the adjacent skin. Lesions showing strong and diffuse cytoplasmic and nuclear or only cytoplasmic block-like staining were considered as positive (p16 upregulation), whereas patchy or completely negative p16 staining was considered as p16 negative.

p53 IHC

p53 was detected with the monoclonal antibody (clone DO-7; Dako, Carpinteria, CA). The staining was evaluated separately in the invasive tumor and in the adjacent skin. The evaluation was performed following recent p53 IHC pattern-based interpretation framework [5,18]. Briefly, p53 staining were classified into six major categories: two normal (wild-type) and four abnormal (mutant) patterns.

Wild-type patterns included: scattered and mid-epithelial (basal sparing) staining. The p53 IHC wild-type staining was further classified into scattered and mid-epithelial. Scattered pattern was defined as heterogeneous, disperse nuclear staining in the basal and/or parabasal layer. Mid-epithelial pattern was diagnosed when there was moderate

to strong nuclear p53 staining in the parabasal layers staining, with notable basal sparing.

Abnormal (mutant) p53 staining patterns were classified into: basal overexpression, parabasal (diffuse) overexpression, null and cytoplasmic patterns. Basal pattern was defined as continuous, strong nuclear staining of basal layer. The same basal staining but with suprabasal extension was classified as p53 parabasal (diffuse). The p53 null pattern was characterized by a complete absence of staining in the tumor and/or intraepithelial lesion. Finally, diffuse cytoplasmic staining with or without nuclear expression was classified as a cytoplasmic pattern. The null and cytoplasmic patterns required an intrinsic positive control (non-lesional skin, stromal or inflammatory cells) [19].

RESULTS

From 1709 cases included in the previous study, 556 tested positive for HPV and were excluded from the present study. From 1153 HPV-negative cases, 93 VSCC showed p16 positivity in the invasive tumor and were consequently excluded. From 1060 DNA HPV- and p16- negative tumors, 281 were excluded as no adjacent skin was available for evaluation. Overall, 779 cases fulfilled the inclusion criteria. Figure 1 shows the study algorithm.

From 779 HPV DNA- and p16-negative VSCC, with at least 1 cm of skin, 329 (42%) cases showed normal adjacent skin and 450 (58%) cases showed at least one skin abnormality. Intraepithelial precursors were identified in 254 cases (33%). From the 254 VSCC with adjacent precursors lesions, 186 (73%) had dVIN (including 49 cases of dVIN arising in a background of LS), 22 (9%) had VAAD, and 46 (18%) cases had precursors that closely simulated HSIL [HSIL-like lesions] (Figure 1). Inflammatory skin lesions not associated with intraepithelial precursors were identified in 196 cases

(25%); 36 cases showed LS and 160 other non-specific inflammatory/reactive skin lesions including LSC.

p16 staining was negative in all the identified skin lesions adjacent to the VSCC and in the normal skin. p53 staining was abnormal in 172/254 (68%) intraepithelial precursors and in 38/196 (19%) inflammatory lesions. Among dVIN, 129/186 (69%) lesions showed p53 abnormal pattern (114 overexpression; 66 of them parabasal and 48 basal; 13 null pattern and 2 cytoplasmic staining). Among VAAD, 9/22 (41%) showed p53 abnormality (9 overexpression; 4 of them parabasal and 5 basal). Among HSIL-like lesions, 34/46 (74%) showed p53 abnormality (see the detailed data below). Among LS, 9/36 (25%) showed p53 abnormality (6 basal overexpression and 3 null pattern). Finally, 29/160 (18%) inflammatory/reactive lesions showed p53 abnormalities (29 overexpression;15 of them parabasal and 14 basal). All the lesions with wild-type expression showed scattered staining.

Characteristics of the HSIL-like lesions

Lesions with HSIL-like features were identified in 6% (46/779) of the HPV-independent VSCC. All of the 46 HSIL-like lesions showed cytological atypia and architectural abnormalities with nuclear hyperchromasia, high nuclear/cytoplasmic ratio, mitoses and loss of maturation. Twenty-six of them mimicked basaloid vulvar HSIL; these cases were characterized by small to medium-sized undifferentiated cells replacing the epidermis (Figures 2 and 3). Thirteen cases mimicked the warty variant of vulvar HSIL; they showed epithelial thickening with papillomatosis, koilocytic-like changes and moderate to marked pleomorphism (Figure 4). Mixed basaloid and warty features were observed in seven cases.

The mean age (± standard deviation [SD]) of the patients with HSIL-like precursors was 72 ± 15 years (range, 37-94).

p16 and p53 staining of the HSIL-like lesions

All the HSIL-like lesions were p16-negative. Among 34 HSIL-like lesions with p53 IHC abnormal patterns, 29 cases displayed p53 overexpression patterns (27 parabasal and 2 basal) (Fig 2 B), four cases showed p53 null pattern (Fig 2 E), and one case showed cytoplasmic staining (Fig 2 H), both in the intraepithelial lesion and in the invasive tumor. Table 1 shows the age, histological sub-typing and other vulvar skin lesions (if any) in the 34 patients with HSIL-like lesions that showed p53 IHC abnormal patterns. Figure 2 shows three cases with HSIL-like lesions with abnormal (mutant) p53 patterns.

Twelve lesions showed p53 wild-type pattern of staining; 11 scattered pattern (Fig 3 B) and 1 mid-epithelial pattern (Fig 3 E). In seven of them, the adjacent invasive tumor showed p53 overexpression (6 parabasal and 1 basal), whereas in 5 cases the invasive carcinoma showed also the same p53 wild-type pattern. Table 2 shows the age, histological sub-typing and other vulvar skin lesions (if any) in the 12 patients with HSIL-like lesions that showed p53 IHC wild-type patterns. Figure 3 shows two cases with HSIL-like lesions with normal (wild-type) p53 patterns.

Association of HSIL-like precursors with other intraepithelial lesions and subtype of the adjacent invasive carcinoma

The association of HSIL-like precursors with other intraepithelial and inflammatory skin lesions, as well as the subtype of the invasive carcinoma is shown in tables 1 and 2. In 16/46 cases (35%), small areas of conventional dVIN adjacent to areas of HSIL-like lesions were identified (Fig 2 F and 3 F). In two cases VAAD/DEVIL lesions were identified adjacent to the HSIL-like precursor, one of them had areas of dVIN as well. In 8/46 (17%) cases, LS was identified in combination with or adjacent to the precursor lesion (Fig 3 C). LSC was identified in 14/46 (30%) cases. In one case, both LS and LSC were observed alongside HSIL-like precursor.

The invasive carcinoma was of keratinizing type in 21/46 (46%) cases, of non-keratinizing type in 7/48 (15%), of basaloid type in 11/46 (24%), of warty type in 3/46 (6%), and of mixed basaloid/warty type in 4/46 (9%) cases.

DISCUSSION

In this study, we confirm that a subset of HPV-independent VSCC (6%) arise on intraepithelial lesions that closely mimic the HPV-associated HSIL lesions. In all these cases, the HPV-independent nature of the lesion was strongly established by HPV negative testing result using a highly sensitive PCR technique and p16 IHC negativity in the invasive tumor, i.e. a negative result for two of the more specific markers of HPV infection, which, when negative, are considered the “gold standard” for excluding HPV-association [10,20]. Remarkably, not only the invasive tumor but also the intraepithelial lesion were negative for these two methods, which allowed to exclude a role of HPV in their pathogenesis.

We have previously reported that these unusual morphological variants were rare, occurring in 4% of all HPV-independent tumors [13]. In this larger series of HPV-independent VSCC, the proportion of these unusual vulvar HSIL-like lesions was 6%, slightly higher than in the previous study [13]. It is noteworthy, however, that the HSIL-like lesions represented 18% of all the premalignant lesions identified in the study. One possible explanation for this increase is that we became more aware of any unusual morphology in our routine diagnostic practice. Indeed, in our previous study we only identified dVIN as other associated intraepithelial lesion [13], whereas in the present study, however, we also identified other HPV-negative precursors such as VAAD or DEVIL.

The histological features of HSIL-like precursors were obvious in all cases: significant architectural disarray, nuclear atypia, and mitotic activity with abnormal mitotic figures extending to the upper layers of the epithelium [3]. As previously

described [13], most of these lesions mimicked basaloid vulvar HSIL, with small to medium-sized undifferentiated cells replacing the epidermis. However, approximately one-third of the cases mimicked the warty variant of vulvar HSIL, showing acanthosis with papillomatosis, and moderate to marked pleomorphism. Similarly to conventional vulvar HSIL, a subset of unusual lesions reported herein showed mixed basaloid and warty changes [12]. As far as we are aware, these unusual HSIL-like vulvar lesions with pure warty-like morphology and combined basaloid/warty-like features have not been previously reported. These findings show that the morphological spectrum of HSIL-like HPV-independent lesions is broader than previously thought.

Several additional histological and clinical findings strongly suggest that the lesions presented in this report are indeed HPV-independent precursors. Importantly, in more than one-third of our cases areas of conventional HPV-independent lesions (dVIN and/or LS) were identified. These data are in keeping with those reported in larger series of HPV-independent VSCCs [21]. Interestingly, a phenomenon of overlapping between dVIN-like and HSIL-like morphology has been previously described in occasional cases [22]. Moreover, the mean age of the patients with these HSIL-like lesions (72 years) is similar to the age of the patients with conventional HPV-independent precursors (73 years). Finally, more than two-thirds (74%) of these unusual lesions in our series showed p53 abnormal patterns, a percentage similar to that observed in conventional dVIN [23], whereas the typical vulvar HSIL commonly shows a p53 wild-type scattered or mid-epithelial patterns [5,18].

One of the HSIL-like cases showed mid-epithelial p53 pattern, which has been recently described in HPV-associated lesions [15,18]. Indeed, the characteristics of this case (p53 wild-type pattern, non-keratinizing features of the invasive tumor, basaloid features in the intraepithelial precursor) suggest that the case might be a false negative HPV DNA testing result. On the other hand, the age of the patient of the patient [71

years], and the presence of conventional dVIN in the adjacent skin suggest HPV-independent etiology.

Interestingly, 12 out of 46 HSIL-like precursors (26%) of the present series showed p53 wild-type pattern on IHC. These findings also expand the profile of HSIL-like lesions reported in our previous study, all of them displaying p53 parabasal overexpression [13] and shows that the spectrum of p53 IHC findings in HSIL-like precursors is broader than initially considered. These findings are interesting, as *TP53*-wild type vulvar tumors are increasingly being recognized, and recent studies [6,12] suggest that these lesions could have better prognosis than those VSCC harboring *TP53* mutations [12]. Interestingly, in seven of the 12 cases of HSIL-like lesions with wild-type p53 staining, the VSCC associated with the intraepithelial lesion showed a clear p53 IHC overexpression. The most likely explanation for these discordant results between the intraepithelial lesion (p53 IHC wild-type) and the invasive tumor (p53 IHC mutant) is that the *TP53* mutations in these VSCC were acquired as a late event [24]. In addition, our results indicate that p53 IHC is not entirely reliable to identify the HSIL-like lesions.

The main strength of this study is the high number of VSCC analyzed. Furthermore, in all the cases a thorough HPV DNA analysis, as well as p16 IHC was performed to exclude HPV infection. The main limitation of the study is that only one paraffin block containing both invasive tumor and intraepithelial lesion was available for each case. Thus, only lesions that already had progressed to carcinoma were available for analysis. In addition, it cannot be excluded that the association with conventional dVIN, LS or LSC is more common than described in this report. On the other hand, the frequency of these HSIL-like lesions can be underestimated. A second limitation is that mutational analysis of these unusual cases was not performed. A third limitation is the subjective nature of the histological evaluation, and the possible influence of the presence of the invasive carcinoma and the precursor lesion on the same slide. In

order to minimize these biases, all cases were evaluated independently by two pathologists, blindly to HPV and IHC (p16 and p53). Lastly, the almost complete absence of follow-up data precludes any evaluation of the prognostic relevance of these unusual HSIL-like precursors.

In conclusion, our study highlights that at least one-fifth of all HPV-independent vulvar precursors may closely mimic the HPV-associated HSIL. Similarly to conventional vulvar HSIL, these HSIL-like lesions have basaloid and/or warty features. The presence or overlapping features of other typical HPV-negative precursors should raise suspicion of HPV-independent etiology. p16 IHC staining may help to correctly classify them as HPV-independent more reliably than histological criteria and p53 IHC. Older age could be another diagnostic clue. Possibly, women with these lesions will need to undergo stricter follow-up than patients with conventional HPV-associated HSIL. Thus, HPV detection tests and/or IHC (p16 and p53) should be performed routinely on premalignant vulvar lesions to accurately distinguish HPV-positive and -independent precursors. Additionally, if the morphology is discordant with p16 staining, HPV molecular testing is highly recommended. Large-scale research using whole exome/genome or next generation sequencing, and including cases with clinical and follow-up data, is warranted to provide more insight on the molecular abnormalities and the clinical behavior of these unusual lesions.

Disclosure/Conflict of interest

No sources of support, such as sponsorship were employed. The authors do not have any conflicts of interest

FIGURE LEGENDS

Figure 1. Study algorithm.

Footnote: DEVIL: differentiated exophytic vulvar intraepithelial lesion; dVIN: differentiated vulvar intraepithelial neoplasia; HSIL: high-grade squamous intraepithelial lesion; LS: lichen sclerosus; LSC: lichen simplex chronicus; VAAD: vulvar acanthosis with altered differentiation.

Figure 2. Three cases with HPV-independent precursors simulating high-grade squamous intraepithelial lesion (HSIL) of basaloid type, showing abnormal (mutant) p53 patterns.

(A) The epidermis is replaced by a homogeneous population of small, "undifferentiated" keratinocytes with scant cytoplasm, showing only minimal maturation in the superficial layer. **(B)** Strong positive nuclear staining for p53 in the basal cells with suprabasal extension (parabasal pattern). **(C)** Another area from the same case showing HSIL-like lesion (on the right) giving rise to well-differentiated, keratinizing vulvar squamous carcinoma (VSCC) (on the left).

(D) The epidermis shows acanthosis, and is replaced by homogenous, small keratinocytes. There are abundant pigmented melanocytes throughout epidermis. The degree of atypia is mild. **(E)** p53 shows completely absent staining, concordant with p53 null pattern. The normal epithelium on the right of the lesion shows wild-type p53 expression. **(F)** A different area from the same case showing conventional differentiated vulvar intraepithelial neoplasia (dVIN), with atypia restricted to the basal layer and marked differentiation in the upper layers, as well as abrupt keratinization and anastomosing rete ridges.

(G) There is architectural disarray in the two lower thirds of the epidermis, and maturation with hyperkeratosis in the superficial layer. The degree of atypia is moderate **(H)** p53 shows faint cytoplasmic staining, with focal nuclear expression in the

intraepithelial lesion. There is moderate nuclear staining in the few stromal cells in the dermis stratus (control staining). **(I)** Another area from the same case showing invasive carcinoma with mixed basaloid and keratinizing features, with some koilocytic-like changes on the left. (A), (C), (D), (F) and (G), (I) hematoxylin and eosin stain; (B), (E), (H) p53 immunohistochemical stain.

Figure 3. Two cases with HPV-independent lesions simulating high-grade squamous intraepithelial lesion (HSIL) of basaloid type, showing normal (wild-type) p53 patterns. **(A)** The epidermis shows prominent architectural disarray; “wind-blown pattern”, being entirely replaced by medium size keratinocytes. **(B)** p53 shows scattered, heterogeneous staining (p53 wild-type scattered pattern). **(C)** Another area from the same case showing low-grade SIL (on the right) and lichen sclerosus (on the left). **(D)** The epidermis is totally replaced by small (right side) to medium size (left side) keratinocytes. **(E)** p53 shows moderate nuclear staining in the upper layers, leaving negative staining in the basal layer. **(F)** Another area from the same case showing conventional differentiated vulvar intraepithelial neoplasia (dVIN), with atypia restricted to the basal layer, marked differentiation in the upper layers, as well as markedly elongated and anastomosing rete ridges. The lesion shows, however, some basaloid features in the basal layer (A), (C), (D), (F) hematoxylin and eosin stain; (B), (E) p53 immunohistochemical stain.

Figure 4. A case with HPV-independent lesion mimicking high-grade squamous intraepithelial lesion (HSIL) of warty type. The epidermis shows superficial hyperkeratosis and acanthosis, together with exophytic, papillary features, obvious architectural disarray with mild cellular pleomorphism and easily recognizable koilocytic-like changes. The p53 shows strong, positive nuclear staining in the basal layer with slight suprabasal extension (parabasal pattern), whereas p16 staining is

completely negative. **(A)** and **(C)** hematoxylin and eosin stain; **(B)** p53 immunohistochemical stain; **(D)** p16 immunohistochemical stain.

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