Gynecology and Obstetrics Clinical Medicine 3 (2023) 30–37

Contents lists available at ScienceDirect

Gynecology and Obstetrics Clinical Medicine

journal homepage: www.keaipublishing.com/en/journals/gynecology-and-obstetrics-clinical-medicine

Research Article

Clinicopathologic diagnosis of dVIN related vulvar squamous cell carcinoma: An extended appraisal from a tertiary women's hospital

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ARTICLE INFO

This abstract was presented at the 2020 USCAP Annual meeting in Los Angeles, CA.

Keywords:
Differentiated vulvar intraepithelial neoplasia
Vulvar squamous cell carcinoma
Clinicopathologic features
Immunostaining patterns

ABSTRACT

Background: Differentiated vulvar intraepithelial neoplasia (dVIN) is a non-human papilloma virus (HPV)-related high-grade precursor lesion to vulvar squamous cell carcinoma (vSCCa). Although *TP53* gene mutations have been identified in 80% of dVIN, its role in dVIN pathogenesis as well as malignant transformation is still being poorly understood. Poor reproducible diagnostic criteria and ambiguous p53 immunostaining patterns, along with morphologic discordance still pose a diagnostic challenge.
Methods: A series of 60 cases of dVIN-related vSCCa along with adjacent dVIN were evaluated. Clinicopathological features as well as immunohistochemical results were recorded on the resection-confirmed dVIN-related vSCCa.
Results: The average age of the patients was 71 years. Thirty-five cases (58.4%) of dVIN-related vSCCa were moderately differentiated, fourteen cases (23.3%) were poorly differentiated, and the remaining eleven cases (18.3%) were well-differentiated. Twenty-nine cases (48.3%) were found to have lichen sclerosus adjacent to dVIN. In terms of p53 and p16 expression in dVIN-related vSCCa and the adjacent dVIN, fifty-five (91.7%) dVIN showed mutant p53 immunostaining pattern with strong positive expression in 80% cases (basal/para-basal expression) and null pattern expression in 11.7% cases. Five (8.3%) dVIN showed p53 wild-type staining pattern. The wild-type pattern were seen in 5% of vSCCa and p53 null pattern were seen in 13.3% vSCCa. Six cases demonstrated atypical staining patterns: two cases showed p53 null expression in dVIN but p53 overexpression in invasive carcinoma; three cases exhibited p53 null expression in invasive carcinoma, with the adjacent dVIN showing basal and para-basal mutant (2 cases) and wild-type (1 case) p53 expression patterns. A single case demonstrated p53 wild-type pattern in dVIN and overexpression in invasive carcinoma. In addition, 65% dVIN were p16 negative and 31.7% dVIN had patchy p16 staining.
Conclusion: Clinical and prognostic value of the ambiguous/inconsistent patterns are uncertain and molecular studies are needed for further characterization.

1. Introduction

Differentiated vulvar intraepithelial neoplasia (dVIN) was initially described by Gosling *et al* in 1961 and called "intraepithelial carcinoma, simplex type".[1] This entity was further studied by Hart *et al* who coined the term "differentiated"' to describe the specific morphologic features of dVIN in 1977.[2] In 1986, the International Society for the Study of Vulvar Disease revised the classification, the term "VIN III, severe dysplasia, differentiated type" was recommended for the first time.[3]

Two main pathways, namely Human Papilloma virus (HPV)-related

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https://doi.org/10.1016/j.gocm.2023.01.004
Received 15 September 2022; Received in revised form 17 January 2023; Accepted 18 January 2023
Available online 26 January 2023

and HPV-independent processes, lead to the development of precursor lesions of vulvar squamous cell carcinoma (vSCCa).[4] The HPV-related precursor lesion is the classic/usual vulvar intraepithelial neoplasia, (uVIN/VIN III) and HPV-independent lesions include dVIN and other vulvar aberrant maturation (VAM) conditions including differentiated exophytic vulvar intraepithelial lesion (DEVIL) and vulvar acanthosis with altered differentiation (VAAD). These HPV-independent lesions have been under-recognized in the past, leading to delays in treatment.[5] The 2020 fifth edition of the World Health Organization (WHO) Classification of Female Genital Tumors classified VIN and vSCCa based on its association with HPV infection.[6] This is a crucial distinction to make as the two groups are distinct in terms of prognosis and clinical management.

Clinically, lesions of dVIN are usually unifocal or unicentric, overlap with lichen sclerosus, and appear as grey-white discoloration with a rough surface, as a thick white plaque, or elevated nodule.[7–9] vSCCa arising from an HPV-related process and an HPV-independent disease are distinct with different etiologies, characteristics, underlying oncogenesis, clinical management, and prognosis.[5,10] A populational study by Joura et al. showed an increased incidence of both entities in the past few years ranging from 0.013 per 100,000 (1985–1988) to 0.121 per 100,000 (1994–1997).[11] It is now known that uVIN is primarily associated with high-risk HPV (hrHPV) 16 and 18 infection and responds to multiple treatment regimens such as imiquimod, laser ablation, or surgical excision; whereas dVIN is typically seen in postmenopausal women and associated with chronic inflammatory dermatoses, such as lichen sclerosus and lichen simplex chronicus.[11,12] dVIN demonstrates subtle clinicopathologic features with excision being the preferred management. As opposed to uVIN, dVIN is less radiosensitive, more likely to recur, progresses to vSCCa in a shorter period of time, and has higher disease related mortality than that in uVIN-related vSCCa.[10,13-16]

The rapid progression of dVIN to invasive carcinoma have made it imperative to establish a reproducible clinicopathologic diagnostic criteria for this entity. However, a diagnosis of dVIN remains challenging.[4,5,17] Although *TP53* gene mutations have been identified in 80% of dVIN, its role in dVIN pathogenesis as well as malignant transformation is still being understood. Poor reproducible diagnostic criteria and ambiguous p53 immunostaining patterns along with morphologic discordance still pose a diagnostic dilemma in daily practice. We specifically studied dVIN related vSCCa to establish/confirm the diagnostic criteria and p53/p16/Ki67 immunohistochemical expression patterns in dVIN and dVIN related vSCCa.

2. Materials and methods

2.1. Case selection

The study was approved by the Institutional Review Board Committee (IRB) at our institution. We retrospectively reviewed the archived pathology database to retrieve the cases with a diagnosis of dVIN related vSCCa at our institution from July 2014 to May 2020. The clinicopathological features were documented from the electronic medical records and analyzed.

2.2. Immunohistochemistry

The hematoxylin and eosin (H&E) slides of dVIN related vSCCa cases on biopsy and resections were reviewed by three subspecialty-trained gynecologic pathologists to confirm the diagnosis. The dVIN related vSCCa was diagnosed according to morphologic criteria and immunohistochemistry (IHC) combination in predominantly dVIN. All dVIN related vSCCa were accompanied with adjacent dVIN, which was the reason we call them dVIN related SCCa. Immunohistochemical stains for p53, Ki67, and p16 were performed on all cases. Formalin-fixed, paraffin-embedded tissue sections were deparaffinized, and immunohistochemical staining was performed using protocols optimized for each antibody. p16 (E6H4™ clone, Ventana, Tucson, AZ), Ki67 (30-9 clone, Ventana, Tucson, AZ) P53 (DO-7 clone, Ventana, Tucson, AZ) with appropriate positive and negative controls were used. In situ hybridization (ISH) for high-risk HPV (hrHPV) mRNA was performed (RNAscope® VS Universal Assay, Advanced Cell Diagnostics, Newark, CA). The probe set included the following HPV subtypes: 16, 18, 31, 33 with additional positive and negative control.

2.3. Interpretation and scoring of immunohistochemical preparations

The immunohistochemical staining patterns for p53, Ki67, and p16 were interpreted. Immunostaining composite scores of p53 in vSCCa (range: 1 to 12) were calculated based on a semi-quantitative system by evaluating the extent (based on the percentage of positive cells) and the intensity of the immunostaining. Extent was scored as 0 points: <5%; 1 point: 6–25%; 2 points: 26%–50%; 3 points: 51%–75%; 4 points: 76%–100%. Intensity was arbitrarily scored as weak (1 point), moderate (2 points), or strong (3 points). Intensity was designated as weak when immunostaining was present but only barely detectable. To correlate the extent and the intensity of immunostaining, these values in positive cases were converted into immunostaining composite scores by multiplying the individual scores of extents by intensity (possible range of values from 1 to 12).[18] For example, a case with 26–50% staining (2 points) and strong intensity of immunostaining (3 points) would have an immunohistochemical composite score of $2 \times 3 = 6$. p53 expression in dVIN was categorized into four patterns: pattern 1: basal staining (strongly positive), pattern 2: basal and para-basal staining (strongly positive), pattern 3: null pattern (completely negative), pattern 4: wild-type pattern. The Ki67 proliferative index in dVIN was categorized into two patterns: pattern 1: basal, pattern 2: basal and para-basal. The percentage of Ki67 positive cells per 100 consecutive cells was recorded in vSCCa. p16 immunostaining in both dVIN and dVIN vSCCa was categorized into three categories: negative, patchy staining, and strong block positive.

2.4. Statistical analysis

The differences of the immunohistochemical composite scores and patterns of dVIN-related vSCCa and the adjacent dVIN were documented and analyzed. The Pearson $\chi 2$ test was used for statistical analysis, conducted on an SAS 9.3 software (SAS Institute, Cary, NC). A P value of less than 0.05 was considered statistically significant.

3. Results

A total of 210 cases of vSCCa were retrieved during the study period. Seventy-one cases of dVIN related vSCCa were identified and consisted of biopsies, excisions, or vulvectomy specimens. Five cases with superficial stromal invasion and six cases from outside consultation were excluded from the study due lack of tissue blocks for immunohistochemical study.

3.1. Clinical and demographic findings of dVIN-related vSCCa

Overall, 60 cases of dVIN-related vSCCa were suitable for analysis. The age distribution of 210 cases of vSCCa and 60 cases of dVIN-related vSCCa is demonstrated in Fig. 1. The average age of the 60 patients was 71.2 years (34–92 years) with a median age of 57 years. dVIN-related vSCCa accounted for 38.3% vSCCa (36/94) in patients aged 70 years and older, which was significantly higher than 20.7% (24/116) in patients younger than 70 years of age ($p < 0.01$).

The International Federation of Gynecology and Obstetrics system (FIGO) clinical stages were re-assigned for each case of dVIN related vSCCa based on the American Joint Committee on Cancer (AJCC) 8th edition. Forty-three cases (71.7%) were classified as FIGO stage 1B, twelve cases (20%) were classified as FIGO stage IIIA, three cases were FIGO stage IIIC, and stages IA and IIA had one case in each category, respectively. The depth of invasion for each case correlated with FIGO

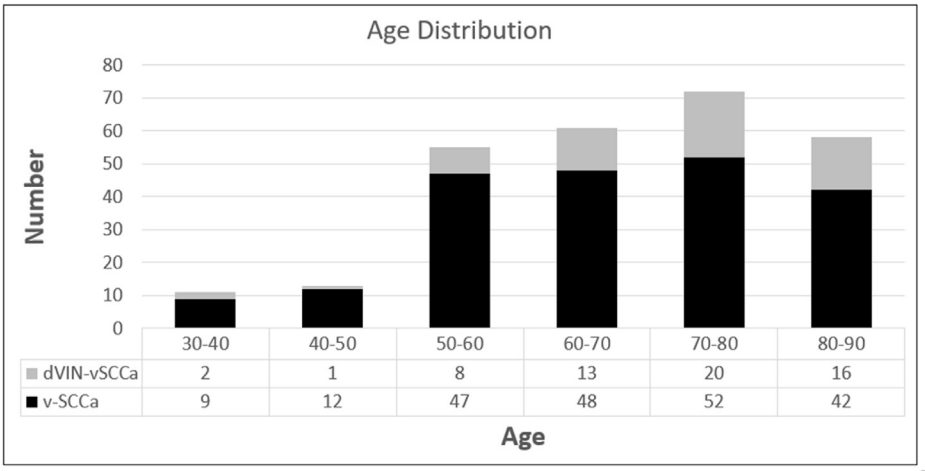

Fig. 1. Age distribution of women with dVIN-related vSCCa and total vSCCa.

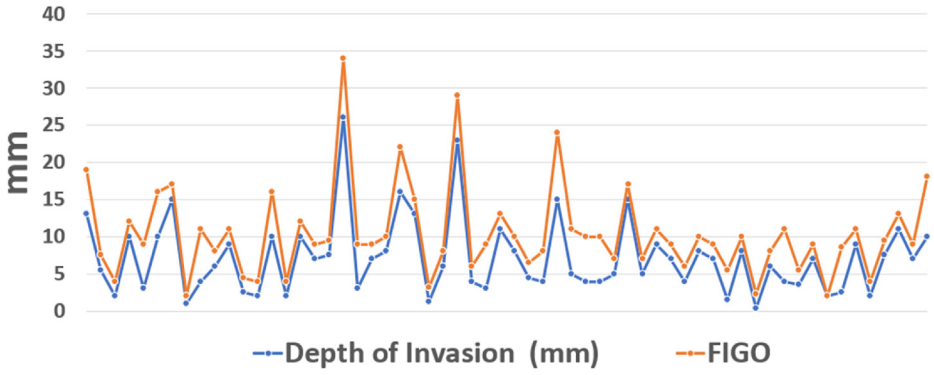

Fig. 2. Depth of invasion and FIGO stage of dVIN-related vSCCa.

stage, as seen in Fig. 2.

FIGO clinical stages were re-assigned for each case of dVIN related vSCCa based on the AJCC 8th edition. Forty-three cases (71.7%) were classified as FIGO stage 1B, twelve cases (20%) were classified as FIGO stage IIIA, three cases were FIGO stage IIIC, and stages IA and IIA had one case in each category, respectively. The depth of invasion for each case correlated with FIGO stage.

3.2. Morphological characteristics of dVIN-related vSCCa

Of sixty cases, thirty-five cases (58.3%) of vSCCa were histologically moderately differentiated, fourteen cases (23.3%) were poorly differentiated, and the remaining eleven cases (18.3%) were well differentiated. Lichen sclerosus was found in the adjacent surface epithelium in 29 cases (48.3%) (Table 1.). Fourteen cases (23.3%) showed lymphovascular invasion either on biopsy or on the resection specimen. Eighteen cases (30%) had lymph node metastasis, with two of them showing extranodal extension.

Table 1
Clinicopathologic characteristics of dVIN-related vSCCa.

A/w Lichen Sclerosus	Histologic Grade No. (%)		
	Well differentiated	Moderately differentiated	Poorly differentiated
29 (48.3)	11 (18.3)	35 (58.3)	14 (23.3)

A/W: associated with; vSCCa: vulvar squamous cell carcinoma; dVIN: differentiated vulvar intraepithelial neoplasia.

3.3. Immunostaining patterns of dVIN-related vSCCa and adjacent dVIN

In the majority of cases, the adjacent dVIN exhibited a basal and para-basal p53 staining pattern (66.7%; 40/60), with 13.3% (8/60) showing a basal pattern, 11.7% (7/60) showing a null pattern, and 8.3% (5/60) showing a wild-type pattern. Most cases showed Ki67 expression in a basal pattern of distribution (61.7%; 37/60), with 38.3% (23/60) of cases showing a basal and para-basal pattern. In vSCCa, most cases showed a strong and diffuse positivity of p53 with score 12 (61.7%; 38/60), with one case having a positive score of 9, five cases with a positive score of 8 and five cases with a positive score of 6. Eight cases showed a null pattern of p53 expression, and three cases demonstrated wild-type expression patterns. Immunostaining for p16 was negative with patchy-type stain in 31.7% (19/60) of dVIN and 18.3% (11/60) in vSCCa. In addition, two of the five p53 wild-type cases of dVIN showed strongly positive staining for p53 in vSCCa (one case had a score of 12 and the other one showed null pattern staining). The remaining three cases showed a consistently p53 wild-type pattern in vSCCa. Four of five p53 wild-type cases in dVIN were p16 completely negative. Three p53 null vSCCa cases showed a basal and para-basal staining pattern in dVIN areas (Table 2).

Figs. 3–9 depict the common staining pattern of mutant p53 and patchy positive p16 in dVIN and dVIN related vSCCa, as well as all atypical staining patterns. Overall, there were 44 cases with a mutant p53 and negative p16 staining in both dVIN and invasive carcinoma areas. A total of five type of p53/p16 staining patterns in dVIN and vSCCa areas were summarized: A. The most common mutant p53 expression pattern in dVIN was basal and para-basal strongly expression (40 cases), with only 8 cases showing a basal-only p53 expression. Most cases showed

Table 2
Summary of p53, p16, and Ki67immunostaining in dVIN and dVIN-related vSCCa.

	IHCs								
	p53 No. (%)				p16 No. (%)			Ki67 No. (%)	
	Basal	B/P	Null	WT	Neg	P-Pos	Pos	Basal	Para-basal
dVIN	8 (13.3)	40 (68.3)	7 (11.6)	5 (8.3)	39 (65)	19 (31.7)	2 (3.3)	37 (61.7)	23 (38.3)
vSCCa	Null 8 (13.3)	Score 6 5 (8.3)	Score 8 5 (8.3)	Score 12 38 (63.3)	WT 3 (5)	Neg 48 (80)	P-Pos 11(18.3)	Pos 1(1.7)	<50% 37 (61.7)	>50% 23 (38.3)

IHC: immunohistochemistry; WT: wild-type; B/P: basal and para-basal; P-Pos: patchy positive.

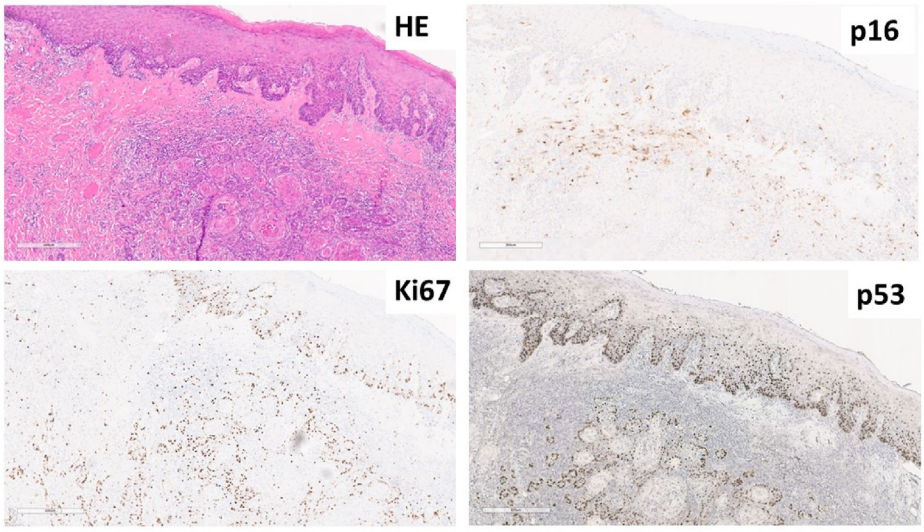

Fig. 3. Example of case reported as "Mutant p53 and negative p16" in dVIN and dVIN related vSCCa, Clinical Stage IB.
dVIN with features of basal cell marked cytologic atypia, elongated rete ridges and disappearing granular cell layer. Associated lichen sclerosus is prominent in the superficial dermis and underneath the epidermis. Keratin pearl in well-differentiated vSCCa on the right (H&E, × 100). Patchy positive p16 staining in both dVIN and vSCCa (IHC, × 100). Ki67 is mostly proliferative in para-basal layer in dVIN and vSCCa with score <50% (IHC, × 100). p53 is overexpressed up to para-basal layer in dVIN as well as overexpressed in vSCCa (score 3x2 = 6) (IHC, × 100).

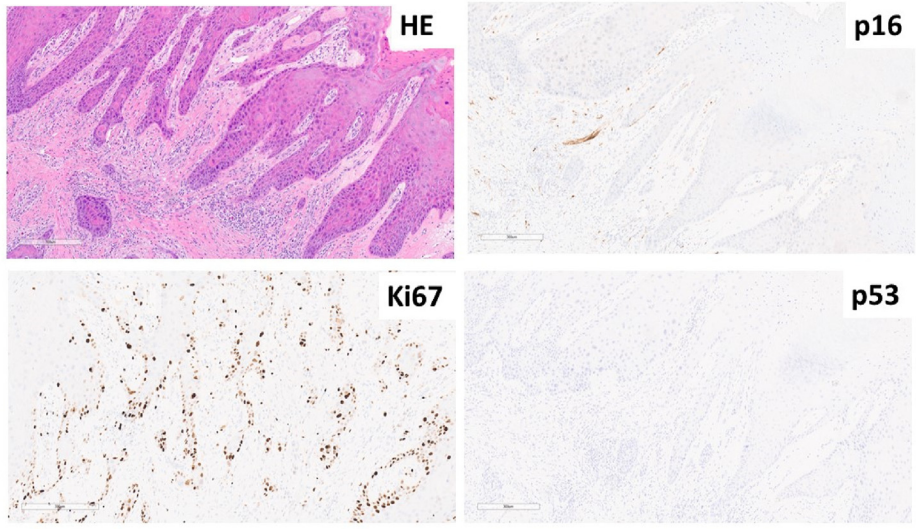

Fig. 4. Examples of case reported as "Null p53 and negative p16" in dVIN and dVIN related vSCCa, Clinical Stage IB.
dVIN with features of basal cell marked cytologic atypia, elongated rete ridges. Associated lichen sclerosus is prominent in the superficial dermis. Tumor nests of well-differentiated vSCCa are scattered in the stroma with inflammatory response (H&E, × 100). Negative p16 in both dVIN and vSCCa (IHC, × 100). Ki67 is mostly proliferative in basal layer in dVIN and vSCCa with score <50% (IHC, × 100). p53 is null pattern in both dVIN as well as vSCCa (IHC, × 100).

entirely negative p16 staining in this pattern, with 19 cases showing patchy positive staining. B. Three cases fit into the second pattern with p53 wild-type pattern and an entirely negative p16 expression. C. The third pattern, showing mutant p53 staining (basal or basal and para-basal strongly positive) and block positive p16 expression, was seen in two cases, both in invasive carcinoma and the adjacent dVIN. D. The fourth pattern was seen in five cases and showed null expression of p53 and completely negative p16 staining. E. Atypical p53 staining patterns were identified in six cases: two cases showed a null p53 pattern in dVIN with p53 overexpression in invasive carcinoma; three cases demonstrated a null p53 pattern in invasive carcinoma with dVIN showing either a basal and para-basal p53 expression (2 cases) or p53 wild-type pattern (1 case). One case demonstrated p53 wild-type pattern in dVIN but overexpression in the invasive carcinoma.

4. Discussion

Poor reproducible diagnostic criteria and ambiguous immunostaining

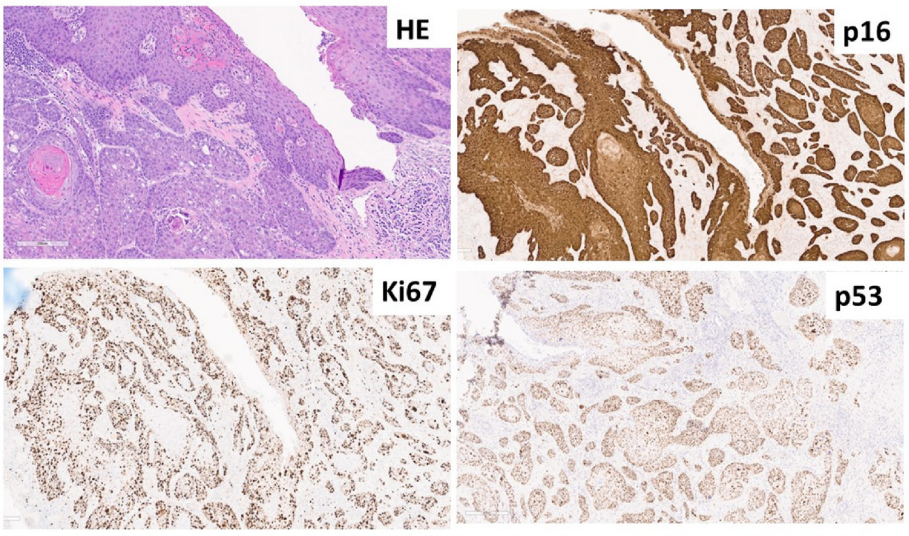

Fig. 5. Example of case reported as "mutant p53 and block positive p16," Clinical Stage IB
dVIN with features of basal cell marked cytologic atypia, elongated rete ridges. Tumor nests of well-differentiated vSCCa are diffusely distributed in the superficial stroma (H&E, × 100). p16 is block positive in both dVIN and vSCCa (IHC, × 100). Ki67 positivity extends to the para-basal layer in dVIN (score 2) and vSCCa with a score >50% (IHC, × 100). p53 is overexpressed in dVIN in up to para-basal layer and overexpressed in vSCCa (score 3 × 4 = 12) (IHC, × 100).

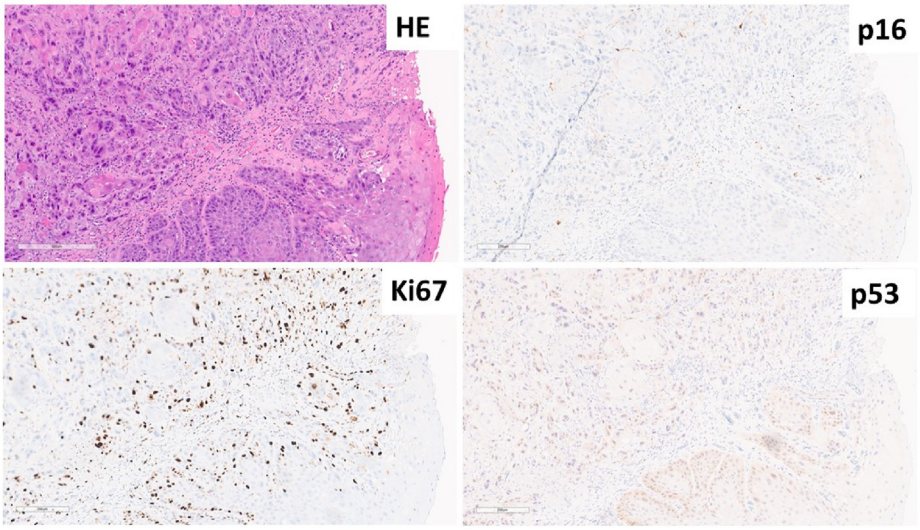

Fig. 6. Example of case reported as "wild-type p53 and negative p16" in dVIN and dVIN related vSCCa. Clinical Stage IIIA.
dVIN with features of basal cell marked cytologic atypia, elongated rete ridges. Small tumor nests or single tumor cells of vSCCa (H&E, × 100). Negative p16 in both dVIN and vSCCa (IHC, × 100). Ki67 is proliferative in basal layer in dVIN and vSCCa with score <50% (IHC, × 100). p53 is wild-type pattern in dVIN and in vSCCa (IHC, × 100).

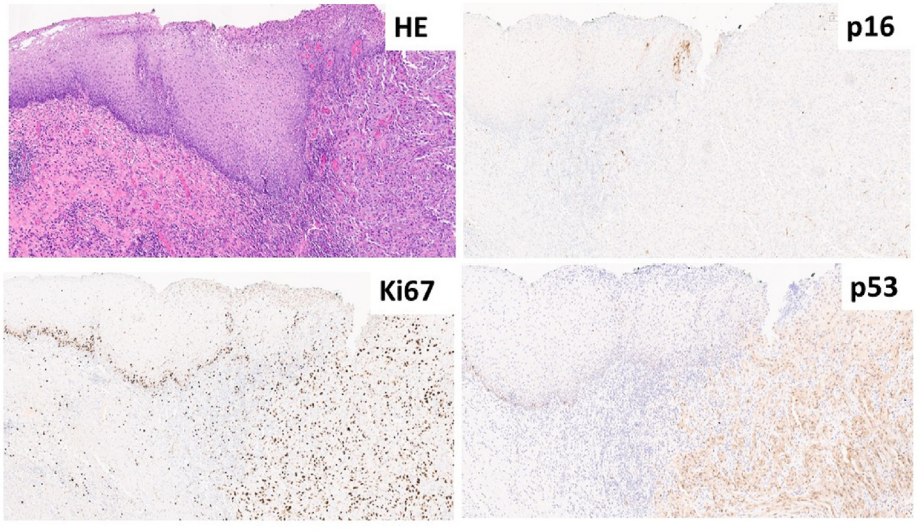

Fig. 7. Example of atypical case reported as dVIN and dVIN related vSCCa, Clinical Stage IB.
dVIN with features of acanthosis, basal cell marked cytologic atypia, eosinophic epidermis. Small tumor nests or single tumor cells of vSCCa on the right (H&E, × 100). Negative p16 in both dVIN and vSCCa (IHC, × 100). Ki67 is proliferative in basal layer in dVIN and vSCCa with score >50% (IHC, × 100). p53 is wild-type pattern in dVIN and overexpressed in vSCCa (score 3x2 = 6) (IHC, × 100).

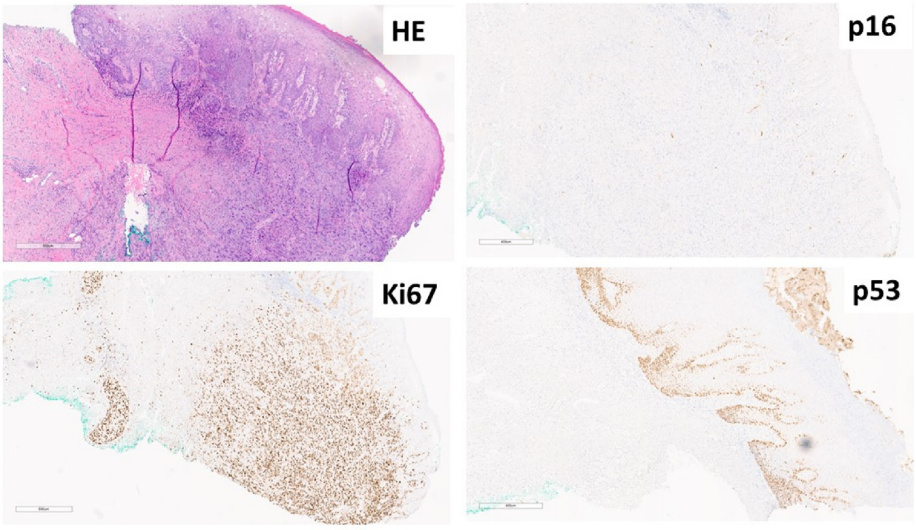

Fig. 8. Example of atypical case reported as dVIN and dVIN related vSCCa, Clinical Stage IB.
dVIN with features of basal cell marked cytologic atypia, elongated rete ridges, acanthosis, and eosinophilic epidermis. Keratinized vSCCa invades into the stroma (H&E, × 100). Negative p16 in both dVIN and vSCCa (IHC, × 100). Ki67 is proliferative in para-basal layer in dVIN and vSCCa with score >50% (IHC, × 100). p53 is overexpressed up to para-basal layer in dVIN but null pattern in vSCCa (IHC, × 100).

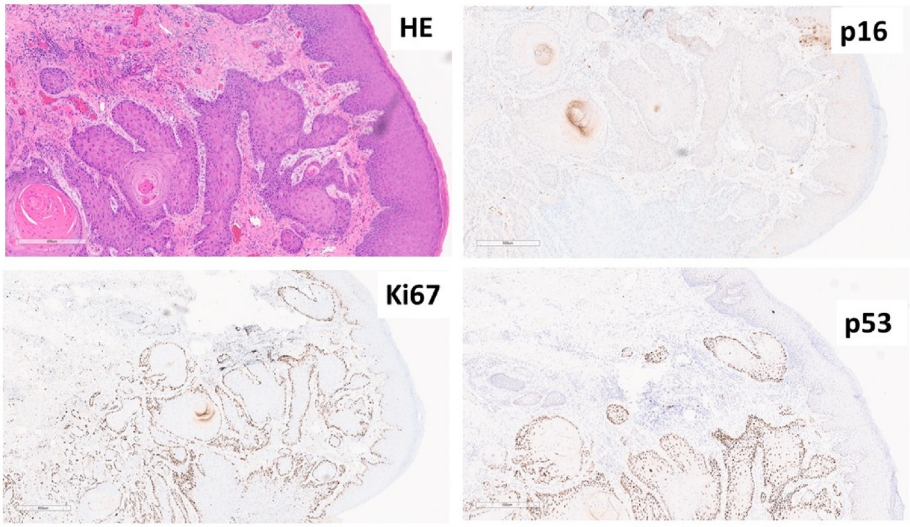

Fig. 9. Example of atypical case reported as dVIN and dVIN related vSCCa, Clinical Stage IIIA.
dVIN with features of basal cell marked cytologic atypia, acanthosis, and eosinophilic epidermis. Keratinized vSCCa invades into the stroma (H&E, × 100). Negative p16 in both dVIN and vSCCa (IHC, × 100). Ki67 is proliferative in basal layer in dVIN and in vSCCa with score <50% (IHC, × 100). p53 is null pattern in adjacent dVIN and overexpressed in vSCCa (score 3 × 3 = 9) (IHC, × 100).

patterns, along with morphologic discordance still affects the accurate diagnosis and classification of vulvar precancer lesions, especially dVIN and dVIN related vSCCa. To address this issue, we studied a series of 60 first-time diagnosed dVIN-related vSCCa from our archived pathologic records. The majority of our cases (80%) are morphologically moderately to poorly differentiated. dVIN-related vSCCa usually occurred in post-menopausal women with peak age of 70–80 years. Forty-six cases (77%) were classified as FIGO stage 1B and above according to the AJCC 8th Edition.

One of the most important morphologic features of dVIN is vulvar basal layer atypia and the high degree of differentiation. By histology, the basal layer of vulvar skin show "cytologic atypia" with vesicular nuclei with thickened, irregular nuclear membranes, coarse chromatin/hyperchromasia, and prominent nucleoli. Other histologic features of dVIN include the increased mitosis, particularly atypical mitosis in the basal cell layer, elongation and anastomoses of rete ridges, dyskeratosis, and prominent acantholysis or spongiosis. Abnormal keratinization and reverse maturation along with basal cell proliferation may also be identified. These features can be extremely subtle, which results in low interobserver agreement even among experienced gynecologic pathologists.[4,19,20] There are some occasions where it can be challenging to distinguish between dVIN and uVIN, especially when dVIN presents with a more basaloid appearance extending beyond the basal layer, architectural disorganization, and undifferentiated keratinocytes.[4,21] In these instances, ancillary immunohistochemical studies can be helpful, such as the hrHPV surrogate marker, p16. In our study, all of the p16 positive cases had negative HPV RNA in-situ hybridization results, regardless of whether p16 was positive in dVIN or vSCCa.

TP53 gene mutation has been reported in up to 80% of dVIN, however its role in dVIN pathogenesis as well as malignant transformation is still being understood. Recently, six p53 immunohistochemical staining patterns have been described, in which the authors have shown its strong correlation with *TP53* mutation status.[22] In this study, the authors identified six major p53 IHC patterns, two wild-type patterns: (1) scattered, (2) mid-epithelial expression (with basal sparing), and four mutant patterns: (3) basal overexpression, (4) para-basal/diffuse overexpression, (5) absent expression, and (6) cytoplasmic expression. These patterns were consistent with *TP53* mutation status in 95% vSCCa and 93% in-situ lesions. They concluded that those exhibited scattered p53 staining and those with a p53 weak basal overexpression could be easily confused. Rakislova *et al*[23] compared patterns of p53 positivity and identified a new six-pattern framework between invasive vSCCa and an adjacent skin

lesion. They found that approximately 74% invasive vSCCa showed abnormal p53 staining and 47% vSCCa with associated skin lesions showed an abnormal p53 stain, with an identical staining pattern between the vSCCa and the adjacent skin lesion in 80% of the cases.

In the present study, we identified four major staining patterns with respect to p53 and p16 expression in dVIN related vSCCa: 1) pattern of "mutant p53 and negative p16" in dVIN and invasive carcinoma (44/60); 2) "wild-type p53 and negative p16" (3/60); 3) "mutant p53 and block positive p16" (2/60); 4) "null p53 and negative p16" (5/60). Additionally, we identified atypical staining patterns in six cases in our cohort: null p53 in dVIN but overexpression in invasive carcinoma (2/60); null p53 in invasive carcinoma with two showing basal and para-basal p53 in dVIN (3/60); wild-type p53 pattern in dVIN (1/60). Only one case demonstrated p53 wild-type pattern in dVIN but overexpression in invasive carcinoma.

TP53 mutations are widely accepted as supportive of a diagnosis of dVIN. Somatic missense, deletion, or truncating mutations of the *TP53* tumor suppressor gene are commonly found in HPV-negative vulvar carcinogenesis, although a subset of them may develop through *TP53*-independent routes. Petitjean *et al* have reported that strong and diffuse p53 expression is a surrogate for *TP53* missense mutation, whereas *TP53* deletion or truncating mutations are associated with a null phenotype,[24] although the null phenotype of p53 has not been well studied in the vulva yet.

Noojj *et al* demonstrated that the *TP53* mutations were much more common in HPV-independent precancer lesions than those related with HPV infection.[25] They further identified a novel, third genetically distinct group of tumors that were HPV-independent but p53 wild-type (so-called p53 wt VIN) but were enriched for somatic mutations in the *NOTCH1* and *HRAS* genes. However, they found no difference in rates of local recurrence or 5-year survival between HPV negative/p53 wt and HPV negative/p53 mutant vSCCa in their study.[26–28]

It may be difficult to interpret p53 expression patterns in some cases. Leigl *et al* have reported the p53 overexpression in lichen sclerosus, therefore p53 is not specific for a diagnosis of dVIN.[28] They also proposed that wild-type p53 protein may accumulate following cell stress or DNA damage, a mechanism postulated to occur in lichen sclerosus where ischemic stress results from the poor oxygenation, further resulting in vasculitis and inflammation.[29] In addition, wild-type and mutant p53 staining pattern may overlap, further complicating interpretation.

This is an observational clinical study with an inclusion of a large number of dVIN-related vSCCa cases from a single academic women's hospital. Both in-situ and invasive carcinoma components along with an immunohistochemical stain panel including p16, p53, and Ki67 were clinicopathologically reviewed. All strongly positive p16 IHC cases were confirmed hrHPV status by RNA in-situ hybridization. Studies have shown that p53 IHC pattern has an excellent correlation with *TP53* sequencing, albeit it was not performed in current study.[24] This study corroborates previous findings that the p53 expression patterns are concordant in dVIN and the subsequent dVIN related vSCCa.[24] However, there is a need for additional studies addressing the mutational profiles of dVIN and dVIN-related vSCCa with discordant or discrepant p53 expression patterns, especially in cases with unusual morphologic features. In this regard, we recommend the clinical correlation and morphologic re-evaluation. An immunohistochemical panel including p53, Ki67, and p16 should be utilized for a diagnosis of dVIN-related vSCCa.

Author contributions

WT: conceptualization, slide review, investigation, data analysis, writing the original draft; VB: slide review, investigation, data analysis, writing the original draft; LH: data analysis, review and edit of the manuscript; TJ: data analysis, review and edit of the manuscript; ZHN: data analysis, review and edit of the manuscript; RB: review of selective slides, data analysis, review and edit of the manuscript; ZCQ: project supervision and conceptualization, review of the slides, data analysis, review and editing of the manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

Ethics approval and consentment

The study was approved by the Institutional Review Board Committee (IRB) of University of Pittsburgh Medical Center. Informed consent was exempt for retrospectively retrieved data from pathology database.

Declaration of competing interest

All authors have no conflict of interests.

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