의학석사 학위논문

외음부의 편평세포암과 전암성 병변의 조직학적, 면역화학적 특성 분석

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지도교수 김 규 래

이 논문을 의학석사 학위 논문으로 제출함

2019년 2월

울 산 대 학 교 대 학 원

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신준영의 의학석사 학위 논문을 인준함

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ABSTRACT

Backgrounds. Vulvar squamous cell carcinoma (VSCC) develops from two main subtypes of precancerous lesions, namely usual-type vulvar intraepithelial neoplasia (uVIN), which is associated with human papilloma virus (HPV), and differentiated-type VIN (dVIN), which is HPV-independent. dVIN has a higher rate of recurrence and progression to VSCC than uVIN. It is difficult to make a correct diagnosis of dVIN because the histologic differences between dVIN and normal vulvar epithelium are subtle. **Materials and Methods.** To further define the diagnostic characteristics of the two types of precancerous vulvar lesions, especially dVIN, the histopathologic features and immunohistochemical profiles of 36 lesions were studied. **Results.** In most cases, epithelium adjacent to VSCCs showed the histologic characteristics of either uVIN (20 cases, 56%) or dVIN (11 cases, 31%); however, five cases (14%) had indeterminate histopathology. Nineteen cases (53%) showed block-type immunoreactivity for $p16^{INK4}$ with wild-type p53 expression (probably HPV-related), 13 (36%) showed $p16^{INK4}$ negativity with abnormal p53 expression (HPV-independent), and the remaining four showed negativity (n=3, 11%) or positivity (n=1, 3%) for both markers. All $p16^{INK4}$ block-positive cases were uVINs (n=19) histologically, while $p16^{INK4}$-negative cases were either dVIN or indeterminate. All five indeterminate cases showed abnormal p53 expression, and three of them had cytologic atypia extending up to the midportion of the epidermis. These results suggest that the HPV-independent subtype may have a wider extent of cytologic atypia than previously described. Histologic dVIN (11 cases) showed p53 overexpression in 7 cases (58%), no expression in one case (8%), with the remaining three cases being $p16^{INK4}$-negative/p53-wild-type. These results suggest that dVIN may have a pathogenic mechanism other than abnormal p53. Of 13 cases showing abnormal

p53 expression, two (17%) did not show any appreciable level of cytologic atypia in the basal layer, indicating that p53 mutation may not be predicted by histologic features alone. **Conclusions.** A correlation between histopathologic features and immunohistochemical findings was observed in only 75% of VINs. Therefore, immunohistochemical staining for p53 and $p16^{INK4}$ is required for the correct diagnosis and subtyping of VINs.

Keywords: vulva, vulvar intraepithelial neoplasia, squamous cell carcinoma, $p16^{INK4}$, p53

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INTRODUCTION

Squamous cell carcinoma of the vulva (VSCC) is a relatively uncommon disease, comprising 3–5% of all malignant tumors of the female genital tract (1-5). The incidence rate of invasive vulvar cancer (VSCC) and precancerous lesions (has continuously increased over the past several decades in many countries (6), especially among women under the age 50. The increased incidence of vulvar intraepithelial neoplasia (VIN) in younger women can be explained by increased screening and early detection of precancerous lesions, and increased risk of exposure to human papilloma virus (HPV).

VSCC and precancerous vulvar lesions are classified into two subtypes: human papilloma virus (HPV)-associated (usual-type VIN) and HPV-independent (differentiated-type VIN) (7). HPV infection does not have the same malignant potential in extramucosal sites such as the vulva as it does in mucosal sites. Thus, unlike cervical cancers, only 40–72% of VSCCs are known to be HPV-associated (8,9). The diagnosis of uVIN is usually not problematic since uVIN displays obvious cytologic atypia including hypercellularity, hyperchromasia, anisonucleosis, and abnormal mitotic figures occupying nearly the full-thickness of the epithelium. By contrast, it is not easy to make a correct diagnosis of dVIN, especially in a small biopsy specimen, since the cytological difference between dVIN and normal vulvar epithelium is subtle (8,10,11).

dVIN has a HPV-independent pathogenic mechanism and is frequently associated with chronic dermatosis of the vulva, such as lichen sclerosus, lichen planus or hidradenitis suppurativa (3,4,8,11-14). Because of severe inflammatory cell infiltration in the dermis, it is

difficult to determine whether the cellular atypia is preneoplastic or reactive. Moreover, the threshold of cytologic atypia in basal keratinocytes of the vulva is very subjective. Despite histologic features resembling normal vulvar epithelium, dVIN has a significantly higher risk of local recurrence (15) and progression to invasive carcinoma than uVIN (16). For these reasons, it is important to subtype precancerous lesions by cause and pathogenic mechanism. Immunohistochemical staining for $p16^{INK4}$ and p53 has been used as an ancillary technique (9), however the definition of p53 overexpression in vulvar epithelium has not been clearly determined and varies according to study setting (9,12,13,17,18). This study first examined the expression pattern of p53 in normal vulvar epithelium, and then assessed the correlation between histopathology, $p16^{INK4}$ and p53 in vulvar precancerous lesions to improve diagnostic accuracy.

MATERIALS AND METHODS

Case Selection

Sixty cases of squamous cell carcinoma of the vulva (VSCC) were retrieved from the surgical pathology files of the Department of Pathology, Asan Medical Center, Seoul, Korea during a 16-year period (2000–2016). Of these, 36 cases containing both VSCC and adjacent noncancerous epithelium were selected. The specimens were obtained from incisional biopsy (two cases), simple or wide excision (27 cases) or radical or partial vulvectomy (seven cases). Although the Lower Anogenital Squamous Terminology Standardization Project (LAST) recommends a unified terminology for HPV-associated lesions that includes low-grade

squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL) instead of VIN across lower anogenital sites (19), the uVIN terminology, encompassing vulva intraepithelial neoplasia (VIN) 2 and 3, was used in this study to make a paired contrast with dVIN.

Review of Clinical Findings and Histopathologic Features

The histopathology of 36 VIN lesions was assessed by hematoxylin and eosin (H&E) staining. The slides were reviewed by three pathologists (JYS, COS and K-RK) and classified as usual-type vulvar intraepithelial neoplasia (uVIN) (Figure 1A) or differentiated-type vulvar intraepithelial neoplasia (dVIN) (Figure 1B) according to WHO criteria (7). A case was classified as uVIN if it showed obvious cytologic atypia in at least the lower two thirds of the squamous epithelium, hyperchromasia, anisokaryosis and abnormal mitotic figures. A case was classified as dVIN if it showed thickening of squamous epithelium with abnormal keratinization and obvious basal cell atypia. Cases difficult to classify as either uVIN or dVIN were classified as indeterminate. These included cases with an intermediate degree of cellular atypia (halfway between uVIN and dVIN) (Figure 1C) and cases with questionable cytologic atypia that rendered them indistinguishable from either benign hypertrophic dermatosis or dVIN (Figure 1D). Classification as either uVIN, dVIN or indeterminate was based solely on histopathology as the reviewers who conducted the classification were blinded to any immunohistochemical data.

Dermal inflammation was subdivided into four patterns to assess its contribution to the VIN phenotype: lichen planus-like, lichen sclerosus-like, diffuse, and sparse infiltration. Lichen

planus-like infiltration referred to a dense chronic inflammatory cell infiltrate, typically present in the superficial dermis and obscuring the dermo-epidermal junction (Figure 2A). Lichen sclerosus-like referred to paucicellular edema or dermal hyalinization of variable thickness under the epidermis with a band-like inflammatory infiltrate below the edematous or hyalinized zone (Figure 2B). Diffuse infiltration referred to a diffuse, patternless infiltrate of lymphocytes in the upper dermis with well-preserved epidermal basement membrane (Figure 2C). Sparse inflammation referred to limited inflammatory cell infiltrate in the upper dermis (Figure 2D). Actual cases of lichen planus or lichen sclerosus might be included among the cases classified as lichen planus-like or lichen sclerosus-like, but definitive diagnoses were not rendered because most patients, except for a few, were managed by gynecologic oncologists and dermatologic symptoms were not described precisely in the records.

Clinical information was obtained from medical records and included age, FIGO stage, type of surgery, treatment modality, outcome, such as recurrence, presence or absence of metastasis and metastatic site(s), and cause of death. When the patient was referred from an outside hospital with a recurrent or metastatic tumor after treatment of a primary lesion, the initial FIGO stage was adopted from the medical record of the outside hospital if possible.

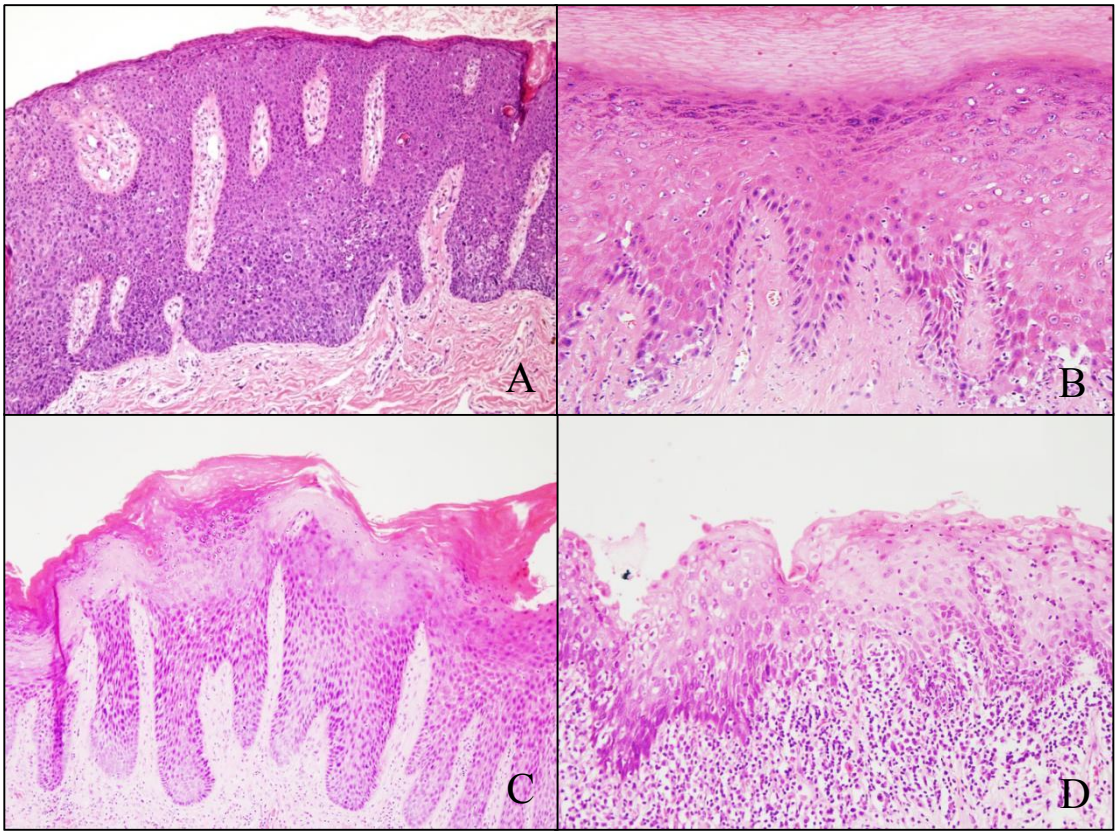

Figure 1. Histopathology of vulvar intraepithelial neoplasia (VIN). (**A)** usual-type VIN (uVIN) showing near full-thickness atypia with hypercellularity, anisonucleosis and abnormal mitotic figures. (**B)** Differentiated VIN (dVIN) showing abnormal thickening of epidermis and basal keratinocyte atypia. (**C–D)** Indeterminate-type VIN including cytologic atypia extending to the midportion of the epithelium (**C**) or questionable cytologic atypia in basal layer (arrows), whether it is a precancerous change or a reactive change to the inflammatory infiltrate (**D**).

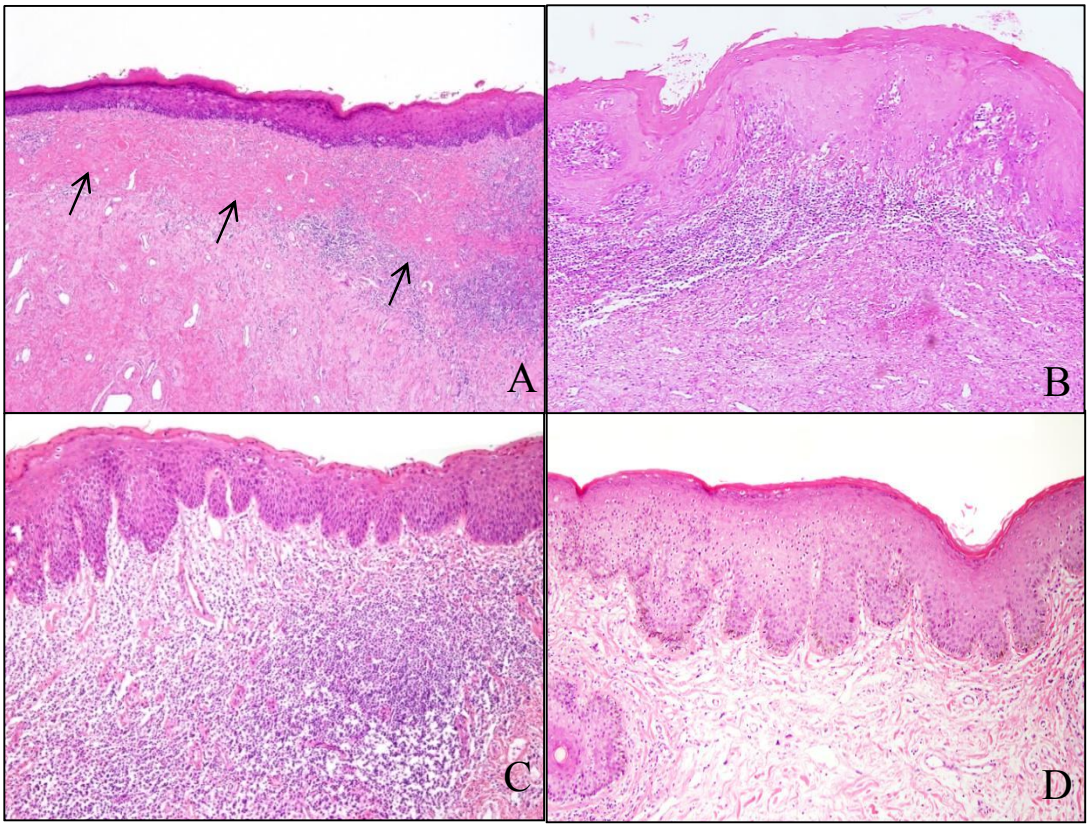

Figure 2. Various degrees and types of dermal inflammation associated with VIN. (A) Lichen sclerosus showing dermal sclerosis below the epithelium (arrows) and inflammatory infiltrate below the sclerotic area. **(B)** Lichen planus-like inflammation showing patchy infiltration of inflammatory cells frequently obscuring the dermo-epidermal junction. **(C)** Diffuse infiltration showing intense infiltration of inflammatory cells in the dermis without obscuring the dermo-epidermal junction. **(D)** Sparse dermal inflammation showing minimal infiltration to none in the dermis.

Immunohistochemistry of Normal Vulvar Epithelium and Vulvar Intraepithelial Neoplasia.

vulvar intraepithelial neoplasia Ten samples of normal vulvar epithelium, obtained from ten patients who received excision for Bartholin's cyst or benign skin adnexal tumor, were used for evaluation of normal expression pattern of p53. Of these 10 cases, nine showed discontinuous weak p53 expression in the nuclei of the basal and suprabasal layers of the vulvar epithelium (Figure 3A), and one case showed stronger but discontinuous nuclear expression in the basal layer (Figure 3B). In all 10 cases, the intensity of nuclear expression was not uniform throughout the keratinocyte population. Subsequently, immunohistochemical staining was conducted for $p16^{INK4}$ and p53 on one representative section of formalin-fixed, paraffin-embedded tissue for each case using a Benchmark XT autoimmunostainer (Ventana Medical System, Tucson, AZ) and the optiview DAB detection kit (Roche Diagnostics, Mannheim, Germany). Each section contained both VSCC and VIN tissue. Briefly, 4-μm-thick tissue sections were transferred onto silanized charged slides, dried for 10 minutes at room temperature, and incubated for 20 minutes at 65°C. After standard heat-mediated epitope retrieval in ethylenediaminetetra-acetic acid (pH 8.0) for 30 minutes in the autostainer, the samples were incubated with primary antibodies to $p16^{INK4}$ (Clone JC8, 1:200; Santa Cruz Biotechnology, Santa Cruz, CA) or p53 (1:1500, DAKO). The sections were subsequently incubated with biotinylated anti-mouse immunoglobulins, peroxidase-labeled streptavidin (LSAB kit; DAKO, Glöstrup, Denmark), and 3,3'-diaminobenzidine. Cervical squamous cell carcinoma and high-grade serous carcinoma were

used as positive controls for $p16^{INK4}$ and p53, respectively. Negative controls were generated by omitting primary antibodies. $p16^{INK4}$ expression was divided into two categories: block-type positive or negative. A sample was considered block-type positive if it showed diffuse strong nuclear and cytoplasmic reactivity in at least the lower two thirds of the epithelium with strong continuous basal and parabasal expression. A sample was considered negative if it showed patchy staining, focally scattered reactivity in a few cells or complete negativity. p53 expression was divided into two categories: wild type or abnormal. Wild-type expression was determined based on the expression pattern of normal vulvar epithelium, and defined as weak and discontinuous reactivity in basal keratinocytes with or without positive cells extending to the upper spinous layers. Abnormal expression was defined as strong continuous nuclear/cytoplasmic immunoreactivity in basal keratinocytes with or without upward extension (overexpression) or completely negative staining (null expression) (Figure 3A). Strong continuous nuclear expression confined to the single layer of basal cells was also regarded as abnormal (Figure 3B). The combined expression of $p16^{INK4}$ and p53 was subdivided into four groups: $p16I^{NK4}$(+)/p53 wild-type, $p16^{INK4}$(-)/abnormal p53, $p16^{INK4}$(+)/abnormal p53, and $p16^{INK4}$(-)/p53 wild-type (Figure 4).

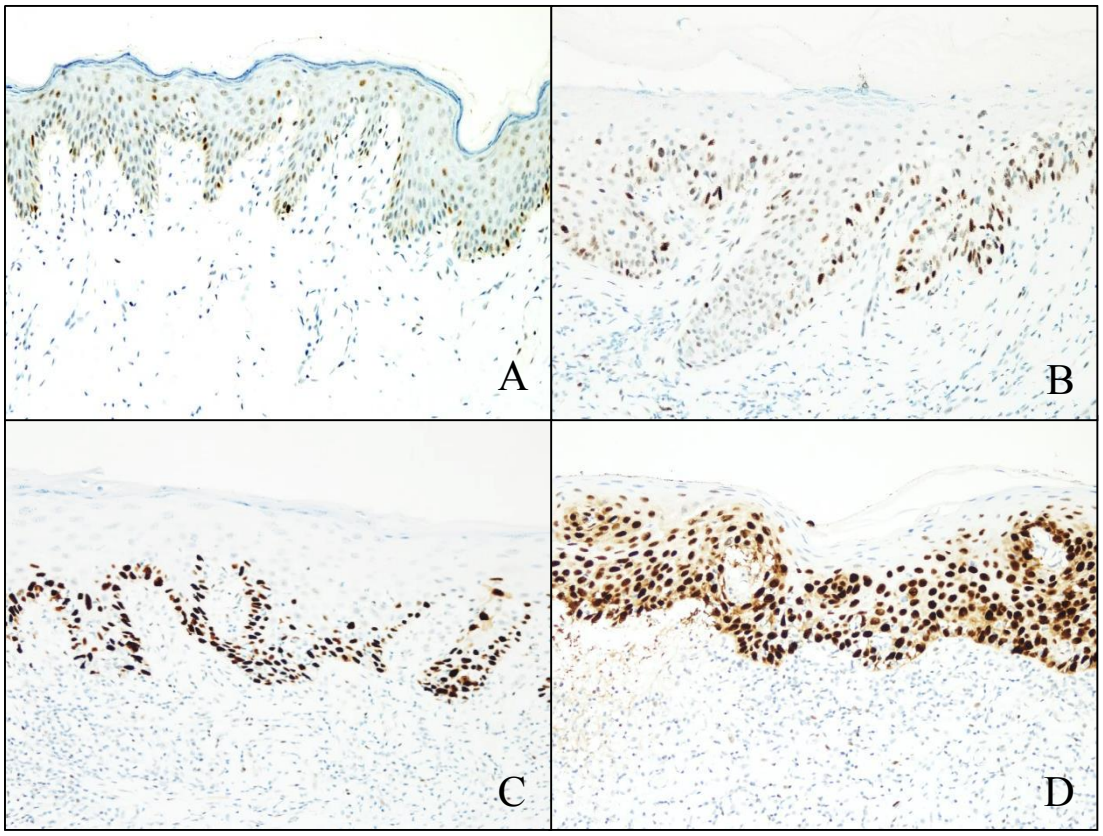

Figure 3. Normal and abnormal p53 expression in vulvar epithelium. Weak and discontinuous expression of p53 scattered throughout entire layers (**A**) or stronger but discontinuous expression of p53 confined to the basal layer (**B**) was defined as wild-type expression, but strong continuous nuclear expression confined to the single layer of basal cells (**C**) or with upward extension (**D**) was regarded as abnormal expression.

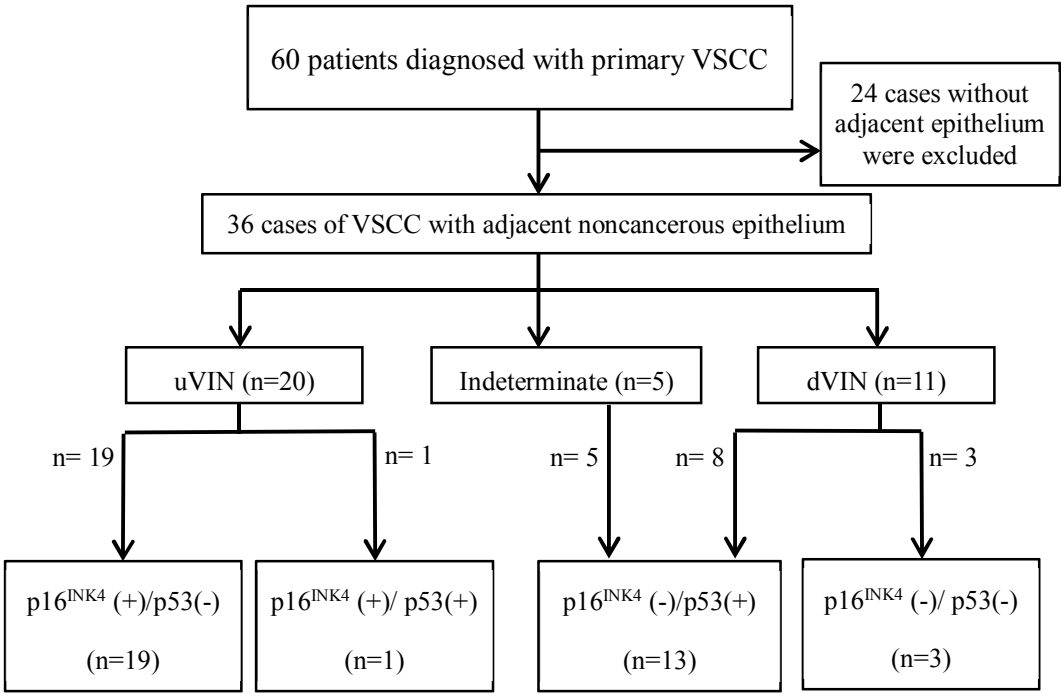

Figure 4. Case selection and immunohistochemical subgroups. Of 60 cases of vulvar squamous cell carcinoma, 24 cases that did not contain adjacent nonneoplastic vulvar epithelium were excluded. The remaining 36 cases were divided based on $p16^{INK4}$ and p53 immunostaining. Three cases in which it was difficult to determine the expression pattern of p53 were subjected to DNA sequencing to assess p53 mutation status. DNA sequencing confirmed these cases had wild-type p53 .

TP53 DNA Sequencing using Capillary Sanger Sequencing

Four cases, including one p53-null case and three cases showing immunonegativity for both $p16^{INK4}$ and p53, were selected for TP53 DNA sequencing to exclude the possibility of false negative p53 immunostaining. The formalin-fixed paraffin-embedded VSCC lesions and adjacent VIN lesions were marked and separately scraped from the glass slides using clean razor blades or glass pipettes. DNA was extracted using the RecoverAll™ Total Nucleic Acid Isolation Kit (Life Technologies, Carlsbad, CA, USA) according to manufacturer instructions. DNA quality was assessed by amplification of the housekeeping gene beta globulin. The Sanger sequencing primers were designed to target mutation hotspots on exons 5, 6, 7 and 8 (Table 1).

Statistical analysis

Statistical analyses were performed using SPSS version 19 (SPSS INc, Chicago, IL). The association between VIN histology, extent of cytologic atypia, dermal inflammatory pattern, progression to carcinoma, recurrence, and mortality between the four immunohistochemical groups was assessed by Chi square and Fisher's exact tests. In this study, $p < 0.05$ was considered statistically significant

Table 1. Primers of *TP53* Sanger Sequencing.

Exon	Direction	Case number (range or %)
5	5’ → 3’	TCACTTGTGCCCTGACTTTC
5	3’ → 5’	AGCTGCTCACCATCGCTATC
6	5’ → 3’	GAGACGACAGGGCTGGTTG
6	3’ → 5’	AGACCCCAGTTGCAAACCAG
7	5’ → 3’	GCCACAGGTCTCCCCAAGG
7	3’ → 5’	CAAGTGGCTCCTGACCTGG
8	5’ → 3’	GGACAGGTAGGACCTGATTTCC
8	3’ → 5’	TCTTGTCCTGCTTGCTTACCTC

RESULT

Clinical Characteristics

Age ranged from 18 to 76 (mean: 56 years). Of the four immunohistochemical groups, the $p16^{INK4}$(+)/p53 wild-type group was significantly younger than the other three groups. Of the 36 VSCC patients, 15 had stage I disease (42%), three stage II (8%), six stage III (17%), four stage IV (11%), and the remaining eight had disease of unknown stage. Thirty-five patients were treated with adjuvant chemotherapy (14 patients) or radiation (21 patients) with additional laser vaporization in five patients. The resection margin was evaluated in 34 specimens. The margin was involved in 15 cases (42%) and clear in 19 cases. The high incidence of positive resection margin was caused by multifocal discontinuous uVIN lesions that were isolated from the main lesion, and by underrecognized dVIN at the margins on frozen sections due to subtle atypia. The size of the tumor was > 2 cm in 21 cases, < 2 cm in 11 cases, and unknown in four cases due to incisional biopsy (2 cases) or resection conducted at another institution (2 cases). The depth of invasion was < 1 mm in nine cases, 1–5 mm in 11 cases, and > 5 mm in 16 cases. Of 36 cases, eight had local recurrence in the perineum (22%). Three of those patients had FIGO stage I, one had FIGO II, three had FIGO III, and one had unknown stage. The surrounding epithelium was classified as uVIN in five cases, dVIN in two, and indeterminate in one. Six patients (17%) had distant metastasis to the pelvic cavity, the lung or the urethra, and subsequently died of the disease (four cases developed from uVIN, one from dVIN, and the other from the indeterminate type), and one patient died of unrelated disease (pancreatic carcinoma) (Table 2). The rates of recurrence

and metastasis were not significantly different between the four immunohistochemical groups (Table 3).

Table2. Clinicopathological Characteristics of VINs

Parameters	Case number (range or %)
Age, median (range)	55.5 (18-76)
Size, median, mm (range)	8 (4-21)
Depth, median, mm (range)	30 (5-55)
Stage	
I	15 (42)
II	3 (8)
III	6 (17)
IV	4 (11)
Not applicable	8 (22)
Procedure	
Radical or partial vulvectomy	7 (19)
Wide exicision	18 (50)
Simple excision or excisional biopsy	9(25)
Punch biopsy	2 (6)
Surgical margin	
Positive	15 (42)
Negative	19 (53)
Adjuvant treatment	
Radiation therapy	20 (56)
Chemotherapy	13 (36)
Laser evapolation	5 (14)
Recurrence	8 (22)
Died of disease	6 (17)

Table 3. Histopathological Characteristics of VSCC and Precursors

Characteristics	Case (%)	Characteristics	Case (%)
Keratinization of VSCC		**Dermal inflammation**	
Keratinizing	21 (58)	LS-like	6 (17)
Non-keratinizing	15 (42)	LP-like	1 (3)
Differentiation of VSCC		Diffuse	20 (56)
WD	26 (72)	Sparse	9 (25)
MD	10 (28)	**Range of cytologicatypia**	
PD	0 (0)	minimal	2 (6)
VIN		<1/3	9 (25)
uVIN	20 (56)	1/3~2/3	8 (22)
dVIN	11 (31)	Full thickness	17 (47)
indeterminate	5 (14)		

WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; VSCC, vulvar squamous cell carcinoma; VIN, vulvar intraepithelial neoplasia; dVIN, differentiated VIN; uVIN usual VIN; LS-like lichen sclerosus like' LP-like, lichen planus like

Expression of $p16^{INK4}$ and p53 in Vulvar Intraepithelial Neoplasia

The number of patients was 19 in the $p16^{INK4}$(+)/p53 wild-type immunohistochemical group (53%), 11 in the $p16^{INK4}$(-)/abnormal p53 group (31%), one in the $p16^{INK4}$(+)/abnormal p53 group (3%), and three in the $p16^{INK4}$(-)/p53 wild-type group (8%) (Figure 4). The $p16^{INK4}$ and p53 immunostaining patterns were concordant in all cases between the VSCC and the adjacent VIN lesion, indicating that the immunoprofile does not change during the progression from VIN to VSCC. Abnormal p53 expression was continuous from dVIN to VSCC without interruption. The VIN subtype, determined histologically, was not always concordant with the expected immunohistochemical profile. Of 20 cases showing histological uVIN, 19 cases (53%) showed $p16^{INK4}$ block-type positivity with wild-type p53, which could probably be interpreted as HPV-associated. Of 11 cases of dVIN, eight showed $p16^{INK4}$(-)/abnormal p53, including a case of $p16^{INK4}$(-)/p53 null expression, which could probably be interpreted as HPV-independent. Three cases of histological dVIN were $p16^{INK4}$(-)/p53 wild-type, and one case of uVIN was classified as $p16^{INK4}$(-)/abnormal p53. Five cases histologically grouped as indeterminate were classified as $p16^{INK4}$(-)/abnormal p53.

TP53 DNA Sequencing using Capillary Sanger Sequencing

Of four cases subjected to DNA sequencing, three cases classified as $p16^{INK4}$(-)/p53 wild-type showed no mutation in TP53. In the fourth case, which was p53-null, the sequencing reaction failed.

Correlation between Histopathology and Immunohistochemistry

The epithelium adjacent to VSCC was histopathologically consistent with uVIN (20 cases, 56%) or dVIN (11 cases, 31%), but 5 cases (14%) had indeterminate features. Those five cases included three cases in which cytologic atypia extended up to the lower 1/3 or midportion of the epidermis (Figure 1C), and two cases showing questionable cytologic atypia that could not be distinguished between benign hypertrophic dermatosis and dVIN (Figure 1D). Among the VSCCs, 21 (58%) were keratinizing and 15 (42%) non-keratinizing. Twenty-six cases (72%) were well-differentiated and 10 (28%) moderately differentiated. There was no poorly differentiated VSCC. The 26 well-differentiated VSCCs were associated with dVIN in 10 cases, uVIN in 12 cases and with the indeterminate type in four cases, suggesting that both uVIN and dVIN may progress to well-differentiated VSCC. In the dermis of adjacent vulvar epithelium, six cases (17%) had lichen sclerosus-like dermal infiltrate, one (3%) lichen planus-like infiltrate, 20 (56%) diffuse infiltrate, and nine (25%) minimal or no inflammatory infiltrate. No significant difference was observed between the four immunohistochemical groups in the intensity or pattern of inflammatory infiltrates, although lichen sclerosus-like and lichen planus-like infiltrates were confined to cases with dVIN and indeterminate histology and to the $p16^{INK4}$(-)/abnormal p53 and $p16^{INK4}$(-)/p53 wild-type immunohistochemical groups (Table 4).

The $p16^{INK4}$ block-type positive cases corresponded histopathologically to uVIN in all cases (n=20), while the $p16^{INK4}$-negative cases were either dVIN (n=11) or indeterminate (n=5), suggesting that $p16^{INK4}$ immunoreactivity is a reliable marker of the HPV-dependent subtype

or uVIN. The histological dVIN cases (11 cases) showed abnormal p53 expression, including overexpression (seven cases, 58%) or no expression (one case, 8%), with linear continuous Ki-67 labeling. The remaining three cases showed non-continuous weak expression (wild-type pattern) with $p16^{INK4}$ (-) basal layer, resembling the expression pattern of normal vulvar epithelium; however, in those three cases, adjacent VSCC also showed the same expression pattern ($p16^{INK4}$ (-) /wild-type p53). Of 13 cases showing abnormal p53 expression, two cases (17%) did not show any appreciable degree of cytologic atypia histologically in the basal layer, suggesting that histologic features alone are not sufficient to diagnose dVIN.

The range of cytologic atypia correlated with the immunohistochemical group ($p < 0.001$); however, there were exceptions. Among 19 $p16^{INK4}$ (+)/p53 wild-type cases, 16 showed cytologic atypia occupying the full thickness of the epidermis, and three showed cytologic atypia confined to the lower 1/3~2/3 of the epidermis. By contrast, of 13 patients with $p16^{INK4}$ (-)/abnormal p53, six showed cytologic atypia confined to the lower 1/3 of the epithelium (37%), four showed cytologic atypia involving 1/3~2/3 of the epithelium (Figure 5A), one (17%) showed atypia nearly in the full thickness, and two (17%) did not have obvious cytologic atypia (Figure 5B). These data suggest that cytologic atypia in the HPV-independent VINs is not confined to the basal layer or lower portion of the vulvar epithelium, but often extends to the upper layers or may occupy nearly the full thickness of the epithelium. Thus, cells with abnormal p53 expression may not always be predicted by histopathology alone. All 19 cases of $p16^{INK4}$ (+)/wild-type p53 had uVIN. Of 13 patients with p16INK4(-)/abnormal p53, eight (62%) had dVIN and five had the indeterminate type.

All patients showing $p16^{INK4}$(+)/wild-type p53 had uVIN, All three patients showing $p16^{INK4}$(-)/wild-type p53 were associated with dVIN, suggesting that dVIN might be caused by a pathogenic mechanism other than abnormal p53.

Among 12 cases with abnormal p53, two did not show recognizable cytologic atypia in basal keratinocytes. Adjacent VSCCs were so well differentiated in those cases that the diagnosis of carcinoma could have been missed if obvious stromal invasion had not been present.

Table 4. Comparisons of Histologic Features and Clinical Outcomes Among the Groups with Different Immunohistochemical Expression

	$P16^{INK4}$(+), p53(wild)	$P16^{INK4}$(-), p53(abormal)	$P16^{INK4}$(+), p53(abnormal)	$P16^{INK4}$(-), p53(wild)	*P*
Case	19 (53)	13 (36)	1 (3)	3 (8)	
Age,median years(range)	52 (18-72)	66 (31-76)	64	58 (49-76)	
VIN					<0.001**
uVIN	19 (100)	0 (0)	1 (100)	0 (0)	
dVIN	0 (0)	8 (62)	0 (0)	3 (100)	
Indeterminate	0 (0)	5 (38)	0 (0)	0 (0)	
Extend of atypia					<0001**
Basal only	0 (0)	2 (17)	0 (0)	0 (0)	
<1/3	0 (0)	6 (37)	0 (0)	3 (100)	
1/3~2/3	3 (16)	4 (25)	1 (100)	0 (0)	
Full thickness	16 (84)	1 (17)	0 (0)	0 (0)	
Inflammation					0.088
LS-like	0 (0)	4 (31)	0 (0)	2 (67)	
LP-like	0 (0)	1 (8)	0 (0)	0 (0)	
Diffuse	13 (68)	6 (46)	1 (100)	1 (33)	
Minimal	6(32)	2(15)	0 (0)	0 (0)	
Recurrence	4 (21)	3 (23)	1 (100)	0 (0)	0.223
Metastasis	1 (5)	1 (8)	0 (0)	0 (0)	0.09
Died of the disease	3 (16)	1 (8)	1 (100)	1 (33)	0.095

VIN: vulvar intraepithelial neoplasia, uVIN: usual-type vulvar intraepithelial neoplasia, dVIN: differentiated-type vulva intraepithelial neoplasia * p <0.05 ; ** p< 0.001

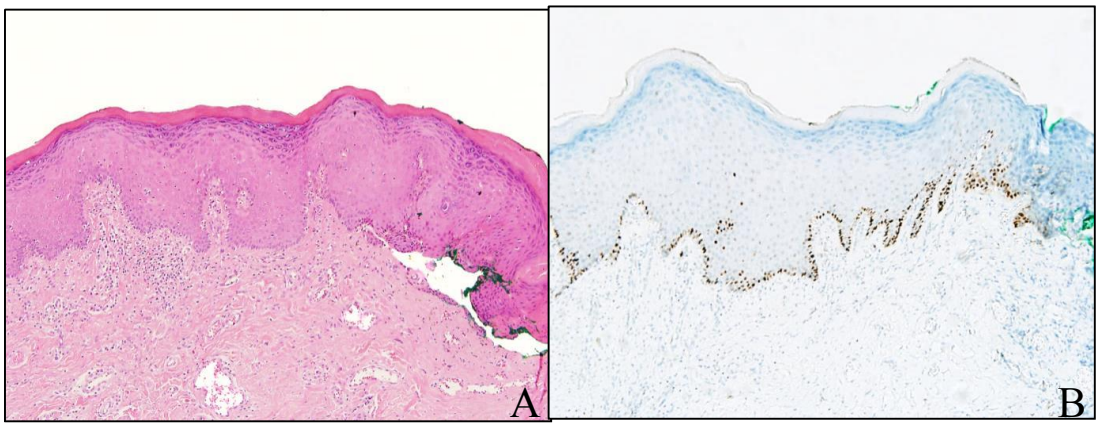

Figure 5. Comparison between histology and p53 overexpression. Some cases showing abnormal p53 expression had cytologic atypia extending up to the midportion or upper layers of the epidermis (**A**) and some cases did not have obvious cytologic atypia (**B**), suggesting that cytologic atypia in HPV-independent VINs is not confined to the basal layer or lower portion of the vulvar epithelium.

DISCUSSION

Precancerous vulvar squamous cell carcinoma (VSCC) lesions are currently subdivided into HPV-associated and HPV-independent subtypes (3), and the proportion of the two subtypes varies between countries. However, the pathogenic mechanism may not be dichotomous, and there could be other mechanisms, such as PTEN mutation and activation of EGFR, causing minor cases of VSCC (9,20,21). Of 11 cases of dVIN, three showed $p16^{INK4}$(-)/p53 wild-type expression by immunohistochemical staining, with confirmed absence of TP53 mutation by DNA sequencing, suggesting that there is another mechanism causing vulvar cancers.

In the histologic diagnosis of VIN, general histologic characteristics of uVIN and dVIN were applied. It is known that uVIN has near full-thickness cytologic atypia, in contrast to dVIN which shows cytologic atypia confined to basal keratinocytes. However, our study showed that the extent and the degree of cytologic atypia are quite variable among dVINs. Two indeterminate cases in our study showed no significant cytologic atypia and abnormal p53 expression in a strong continuous pattern along basal keratinocytes with increased proliferating index, indicating a precancerous lesion.

Histologically, dVIN is characterized by fairly well-differentiated squamous epithelium with basal keratinocyte atypia with or without abnormal keratinization. In our study, the indeterminate type of VIN showing cytologic atypia extending up to the midportion of the epithelium revealed abnormal p53 expression, which was interpreted as HPV-independent. This result suggests that dVIN may have cytologic atypia extending up to the midportion of the epithelium. By contrast, two cases with almost no cytologic atypia in basal keratinocytes,

thus indistinguishable between dVIN and reactive change due to inflammation, also belonged to the HPV-independent subtype based on abnormal p53 expression. These findings suggest that it is difficult to predict p53 mutations based on histology alone. These cases raise the importance of the routine use of p53 immunostaining in suspicious vulvar lesions. This result probably explains why VSCCs have a high frequency of locoregional recurrence (up to 40%) (15,22).

Although a negative resection margin is essential for reducing locoregional recurrence in most tumors, the prognostic relevance of the margin status, and the tumor-free margin distance, remain equivocal in vulvar cancers (22-25). VSCC considered recurrent after margin-free resection can be partly explained by field cancerization, a phenomenon in which second primary tumors arise in a field of cancerization that comprises histologically normal but molecularly changed epithelium. It is known that lichen sclerosus-affected skin and dVIN are important risk factors for recurrence or *de novo* cancer in the anogenital area (15,25). Therefore, in order to prevent local recurrence, in some cases it might be advantageous to detect molecularly altered epithelium before histological changes become apparent, and our case with no apparent cytologic atypia but with p53 overexpression could represent one of those cases.

P53 immunostaining in vulvar epithelium should be carefully interpreted. Although p53 overexpression does not necessarily mean presence of TP53 mutation (13,16), p53 immunostaining retains diagnostic value in small biopsy specimens where routine TP53 sequencing is technically challenging (26). Fallopian tube epithelium may have aberrant p53

expression in histologically benign tubal epithelium (27), yet currently the p53 signature andthe serous tubal intraepithelial lesion are believed to be an early carcinogenic stage of high-grade serous carcinoma having molecularly altered but histologically unchanged epithelium (27,28). Similarly, immunohistochemical staining for p53 and $p16^{INK4}$ may be helpful for the detection of molecularly altered but histologically unchanged vulvar epithelium. Therefore, immunohistochemical staining for p53 and $p16^{INK4}$ needs to be routinely performed to subtype VIN and evaluate the margin status of resected vulvar specimens.

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국문요약

연구배경

외음부에 발생하는 편평세포암(Vulvar squamous cell carcinoma, VSCC)의 발생경로는 크게 두가지로서, 인유두종바이러스 (Human papilloma virus, HPV) 감염에 의해 발생하는 보통 외음부 상피내종양 (usual vulva intraepithelial neoplasia, uVIN)과, 외음부의 만성적인 염증성 피부질환 등이 상피세포의 *TP53* 유전자변이를 유발하여 분화성 외음부 상피내종양 (differentiated vulva intraepithelial neoplasia, dVIN)을 유발하고 이로부터 VSCC로 진행하는 경로를 들 수 있다. 이중 HPV 감염과 연관된 경우에는 뚜렷한 세포학적 변화를 나타내어 진단에 큰 문제가 없으나, HPV 감염과 무관한 경우에는 세포학적 변화가 매우 미미하여 전암성 병변 단계에서 조기 진단이 매우 어렵다. 진단을 보조하는 검사수단으로서 사용되는 면역염색도 판독 기준이 명확하게 정해져 있지 않아 판정에 어려움이 따른다. 본 연구에서는 VSCC와 주변의 전암성 병변을 보다 정확하게 진단하기 위하여, VSCC 진단하에 절제 혹은 생검된 검체에서 종양과 그 주변 전암성 병변을 포함하는 예들의 조직소견을 분석하고, 조직소견과 면역염색 소견을 비교 분석하여 전암성병변, 특히 세포학적 변화가 매우 미미한 dVIN의 진단에 대한 보다 개관적인 소견을 알아보고자 하였다.

연구대상 및 연구방법

2001-2016년까지 서울 아산병원에서 외음부의 편평상피 세포암종의 진단 후 치료를 받았던 증례 가운데 편평상피 세포암종 주변으로 전암성병변이나 비종양성 상피세포를 관찰할 수 있었던 36례를 대상으로 하였다.

각증례의 리뷰를 통해 환자의 임상적인 지표와 병리적인 지표를 평가하였으며, 발병원인을 파악하기위하여 $p16^{INK4A}$과 p53에대한 면역염색을 시행하였다. 면역염색결과에 따라 36 예를 네개의 군(group 1 : $p16^{INK4A}$ (+)p53(-); group 2 : $p16^{INK4A}$ (-)/ p53(+); group 3 : $p16^{INK4A}$ (+)/ p53(+); group 4 : $p16^{INK4A}$ (-)/ p53(-)으로 나누었으며 각 군에서 임상적인 지표와 병리소견을 비교하였다.

결과

36 예 가운데 53%가 $p16^{INK4A}$에 블록양성을 보였으며 HPV 감염과 연관된 종양으로 생각되었고, p53 과발현을 보인 경우는 36%로 HPV와 연관이 없이 독립적인 기전에 의해 발생한 종양으로 생각되었다. $p16^{INK4A}$블록 양성과 p53 과발현은 89%에서 상호배타적인 결과를 보였지만, 두 항체에 대한 염색결과가 모두 양성이거나 음성인 예들도 각각 1례, 3례가 있었다. $p16^{INK4A}$ block positivity, p53 negativity를 보이는 예들은 모두 uVIN의 조직소견을 보였지만 (n=19), $p16^{INK4A}$ 에 음성이었던 증례들 가운데에는 dVIN (62%) 와 조직학적 분류가 어려웠던 5 예 (38%) 가 포함되어있었다. dVIN의 73% 에서 p53 이상발현을 보였으며, 경화성 태선과 편평태선양 염증이 dVIN의 45%, indeterminate type의 40%에서 관찰되었다. 진피내의 염증세포 침윤소견은 dVIN과 uVIN 모두에서 관찰되었고, 경화성태선 혹은 평편

태선과 유사한 염증소견을 보이는 경우는 p53양성/ $p16^{INK4A}$음성군에서 더 흔한 경향을 보였지만 통계적 유의성은 없었다. (*p-value* =0.088) p53 과발현을 보였던 증례 가운데 2 례 (15%)에서는 기저층의 세포학적 비정형성을 전혀 보이지 않아 조직학적 소견만으로 p53 돌연변이를 예측하기는 어려웠다.

결론

외음부의 전암성 병변 가운데 조직학적소견과 발병원인의 일치율은 75% 이었으며, 조직소견만으로 그 발생원인을 추측하는데는 한계가 있었으며, p53 이상 발현 보이는 경우에서도 세포학적 비정형성이 전혀 관찰되지 않는 예들이 있어 조직학적 소견만으로 p53 돌연변이를 예측하기는 어려웠기 때문에 VSCC로 수술을 받았던 여성에서는 좀더 주의 깊은 추적관찰이 필요하다.