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Evaluation of Invasive Squamous Cell Carcinoma, Seborrheic Keratosis and Verruca Vulgaris in Superficial Shave Biopsies Using p16, p53, p63, and PHLDA1 Immunohistochemistry

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Abstract

Occasionally, the distinction between malignant and benign is challenging in superficial shave biopsies of squamoproliferative lesions. This phenomenon is compounded by the increasing prevalence of conditions encountered that weaken the immune system, such as chemotherapy, immune deficiency diseases, and anti-rejection medications for organ transplantation that have all been shown to increase the risk of the development of squamous cell carcinoma. We collected 30 cases (10 invasive SCC, 10 SK and 10 VV) and performed immunohistochemical staining using a panel approach composed of markers important for proliferation and the cell cycle, including Ki-67, p16, p53, and PHLDA1. The results demonstrate that the invasive SCC group was enriched for high PHLDA1 (80% with PHLDA1 score=3, 100% with PHLDA1 score ≥ 2) and high p53 (50% of SCC with p53 score ≥ 2 vs. 60% of SK and 90% of VV with p53 score=1). The SK group was enriched for low p16 (100% with p16 score ≤ 1) and high p63 scores (100% with p63 score=3). A panel approach may be utilized to help in the distinction between benign keratoses and carcinoma and may be increasingly critical to promote quality care.

Keywords: Squamous cell carcinoma; Verruca vulgaris; Seborrheic keratosis; Benign keratosis; p16; p53; PHLDA1

Introduction

Excision specimens of squamous cell carcinoma (SCC), verruca vulgaris (VV), and seborrheic keratosis (SK) demonstrate classic histopathologic features especially along the base of the lesion, precluding the necessity for ancillary diagnostic techniques such as immunohistochemistry (IHC). However, pathologists are often presented with superficial shave biopsies, some lacking basal epithelium, rendering distinction of these squamoproliferative lesions with overlapping histologic features challenging. An established immunohistochemical panel does not yet exist for this differential diagnosis. Therefore, our goal was to evaluate the diagnostic utility of immunohistochemical stains p16, p53, p63, PHLDA1, and the proliferation marker Ki-67 in differentiating SCC, VV, and SK. Although p16 has been used as a diagnostic adjunct for diagnosing human papillomavirus (HPV)-related intraepithelial neoplasia of the genital and oropharyngeal mucosa, its applications in diagnosing cutaneous SCC have not been extensively studied [1,2]. Mutations in the tumor suppressor p53 are noted to occur early and often in skin carcinogenesis [3,4]. p63 mutations have not frequently been implicated in cancers, but one of its isoforms is often upregulated in neoplasia and is expressed in SCC [3]. PHLDA1 is a hair follicle bulge marker that has demonstrated utility in differentiating basal cell carcinoma (BCC) from trichoepithelioma and trichoblastomas [5-10]. Given the increased incidence of squamoproliferative lesions in patients treated with BRAF inhibitors [11-14], it is increasingly important to distinguish benign keratoses (SK, VV) from SCC. We evaluate the utility of IHC to assist in the distinction of these lesions and provide pathologists with a tool in approaching this potentially difficult diagnostic differential in shave biopsy specimens.

Materials and Methods

We searched the Department of Pathology database for shave biopsy cases received for evaluation between 2012 and 2014. 30 cases (10 invasive SCC, 10 SK and 10 VV) were randomly selected. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections sliced at 5 μm using Ki-67 (Dako monoclonal mouse anti-human antibody, 1:200 dilution, Leica instrument), p16 (Ventana E6H4, 1:2 dilution, Leica instrument), p53 (Ventana DO-7, 1:400 dilution, Ventana instrument), p63 (Biocare Medical BC4A4, 1:100 dilution, Leica instrument), and PHLDA1 (Santa Cruz Biotech RN-6E2, 1:400 dilution, Leica instrument) IHC. Positive controls were performed for each antibody with demonstration of the appropriate staining pattern.

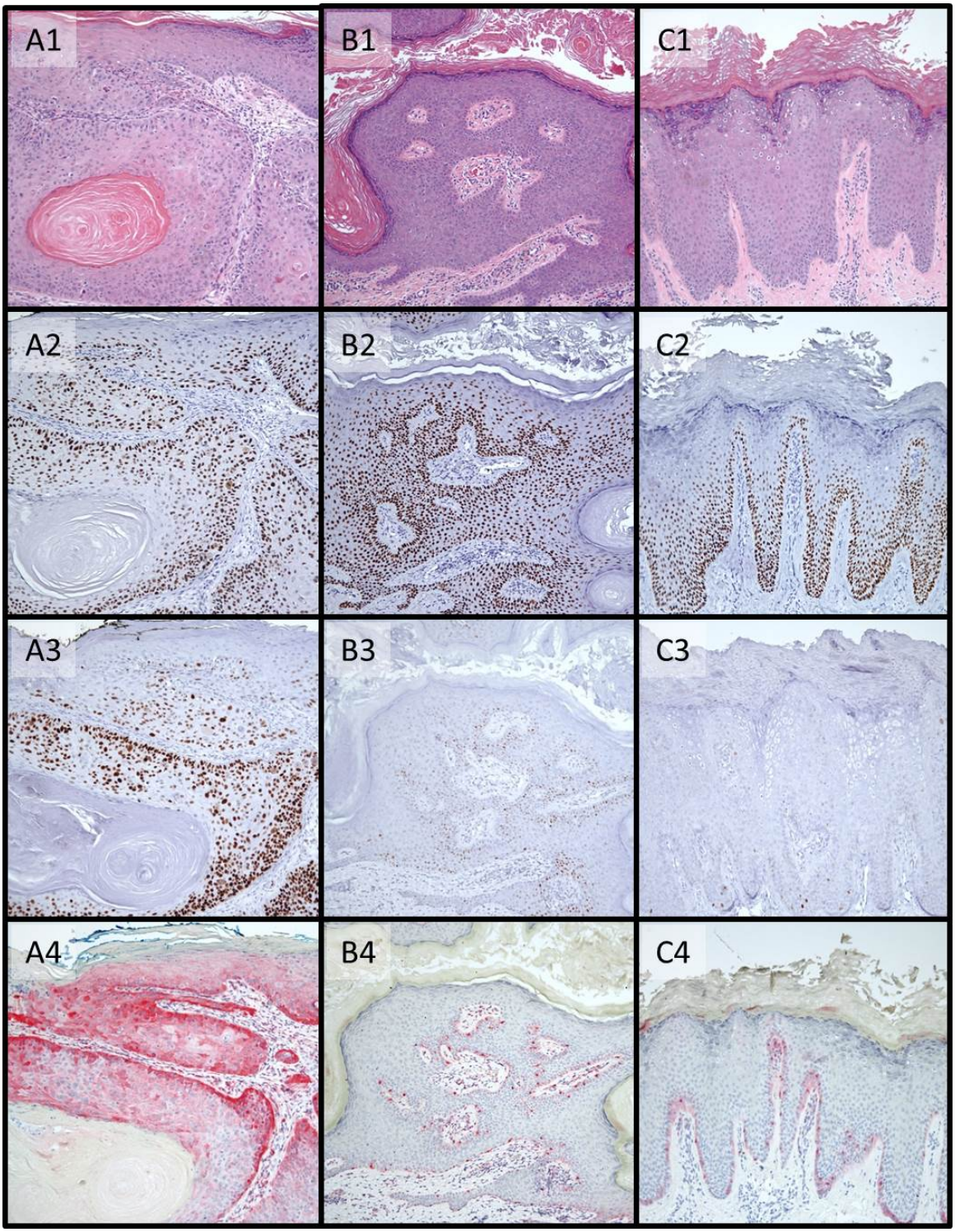

Figure 1: Histology and immunohistochemistry of squamoproliferative lesions. Staining of invasive SCC Case 7 (A), SK Case 15 (B), and VV Case 27 (C) with H&E (1), p63 (2), p53 (3), and PHLDA1 (4) immunohistochemistry. Original magnification 100X.

Each stain was assessed for percent lesional expression and, when applicable, distribution of staining. Stains were scored without knowledge of the original diagnosis as 1, 2, or 3 (p53, p63, PHLDA1, Ki-67) or 0, 1, or 2 (p16) based on the criteria detailed in Table 1 (RB and JK). The authors then unblinded themselves to and confirmed agreement with the original histologic diagnoses. Sensitivity,

specificity, positive predictive value, and negative predictive value for each diagnosis were calculated with various combinations of staining results.

Stain	p16	p53	p63*	PHLDA1	Ki-67
Score=0	<1% of cells	N/A	N/A	N/A	N/A
1	1-30% of cells	<5% of cells	<50% of thickness	<10% of cells	<5% of cells or staining restricted to basal layer
2	>30% of cells	5-30% of cells	50-90% of thickness	10-40% of cells	5-30% of cells or extending above basal layer but less than 30% of cells
3	N/A	>30% of cells	>90% thickness	>40% of cells	>30% of cells
*As measured beneath the granular layer. N/A: Not Applicable.					

Table 1: Immunohistochemical scoring criteria.

Results

The results of p16, p53, p63, PHLDA1, and Ki-67 staining are shown in Tables 2-4 with histology and IHC of representative SCC, VV, and SK cases shown in Figure 1.

The invasive SCC group was enriched for high PHLDA1 (80% with PHLDA1 score=3, 100% with PHLDA1 score ≥ 2).

Stain	p16	p53	p63	PHLDA1	Ki-67
Intensity	Weak to strong	Strong	Strong	Weak to strong	Weak to strong
Distribution	Nuclear and cytoplasmic lesional staining	Nuclear lesional staining	Nuclear lesional staining	Cytoplasmic lesional staining with background stromal staining	Nuclear lesional staining

Table 2: Overall immunohistochemical stain expression patterns.

Invasive SCC	p16	p53	p63	PHLDA1	Ki-67
Case 1	1	3	2	3	2
Case 2	1	1	2	3	1
Case 3	2	1	2	3	1
Case 4	1	1	2	2	1
Case 5	2	1	1	3	1
Case 6	2	2	1	3	1
Case 7	1	3	2	3	2
Case 8	2	3	3	3	2
Case 9	2	3	3	3	3
Case 10	1	1	1	2	1
SK	**p16**	**p53**	**p63**	**PHLDA1**	**Ki-67**
Case 11	1	2	3	2	1
Case 12	0	1	3	2	1
Case 13	1	2	3	2	2
Case 14	1	2	3	1	1
Case 15	1	1	3	1	1
Case 16	0	1	3	1	1
Case 17	0	2	3	1	1
Case 18	0	1	3	1	1
Case 19	1	1	3	1	1
Case 20	0	1	3	2	1
VV	**p16**	**p53**	**p63**	**PHLDA1**	**Ki-67**
Case 21	0	2	3	1	1
Case 22	0	1	2	1	1
Case 23	2	1	1	1	1
Case 24	1	1	2	1	1
Case 25	0	1	3	1	1
Case 26	0	1	3	1	2
Case 27	1	1	2	1	1
Case 28	0	1	2	1	1
Case 29	1	1	2	2	1
Case 30	1	1	3	1	1
SSC: Squamous Cell Carcinoma; SK: Seborrheic Keratosis; VV: Verruca Vulgaris					

Table 3: Immunohistochemical scores by lesion type.

Compared to VV and SK, the SCC group also showed high p53 (50% of SCC with p53 score ≥ 2 vs. 60% of SK and 90% of VV with p53 score=1). The SK group was enriched for low p16 (100% with p16 score ≤ 1) and high p63 scores (100% with p63 score=3).

Invasive SCC	**p16**	**p53**	**p63**	**PHLDA1**	**Ki-67**
Score=0	0/10	N/A	N/A	N/A	N/A
1	5/10	5/10	3/10	0/10	6/10
2	5/10	1/10	5/10	2/10	3/10
3	N/A	4/10	2/10	8/10	1/10
SK	**p16**	**p53**	**p63**	**PHLDA1**	**Ki-67**
Score=0	5/10	N/A	N/A	N/A	N/A
1	5/10	6/10	0/10	6/10	9/10
2	0/10	4/10	0/10	4/10	1/10
3	N/A	0/10	10/10	0/10	0/10
VV	**p16**	**p53**	**p63**	**PHLDA1**	**Ki-67**
Score=0	5/10	N/A	N/A	N/A	N/A
1	4/10	9/10	1/10	9/10	9/10
2	1/10	1/10	5/10	1/10	1/10
3	N/A	0/10	4/10	0/10	0/10
SCC: Squamous Cell Carcinoma; SK: Seborrheic Keratosis; VV: Verruca Vulgaris; N/A: Not Applicable.					

Table 4: Immunohistochemical score frequency by lesion type.

Sensitivity, specificity, positive predictive value, and negative predictive value for selected staining results are displayed in Table 5.

Lesions included in analysis	**SK, VV , Invasive SCC**			**SK, Invasive SCC**			**SK, VV**		
Criteria for diagnosing SK	p16<2 and p63=3	p63=3	p16<2	p16<2 and p63=3	p63=3	p16<2	p16<2 and p63=3	p63=3	p16<2
Sensitivity	1	1	1	1	1	1	1	1	1
Specificity	0.8	0.7	0.3	1	0.8	0.5	0.6	0.6	0.1
PPV	0.71	0.63	0.42	1	0.83	0.67	0.71	0.71	0.53
NPV	1	1	1	1	1	1	1	1	1
Lesions included in analysis	SK, VV, Invasive SCC			SK, Invasive SCC			Invasive SCC, VV		
Criteria for diagnosing Invasive SCC	PHLDA1=3 and p53=3	PHLDA1 =3	p53=3	PHLDA1=3 and p53=3	PHLDA1=3	p53=3	PHLDA1=3 and p53=3	PHLDA1=3	p53=3
Sensitivity	0.4	0.8	0.4	0.67	0.8	0.4	0.4	0.8	0.4
Specificity	1	1	1	1	1	1	1	1	1
PPV	1	1	1	1	1	1	1	1	1
NPV	0.77	0.91	0.77	0.83	0.83	0.63	0.63	0.83	0.63
Lesions included in analysis	SK, VV, Invasive SCC			SK, VV			Invasive SCC, VV		
Criteria for diagnosing VV	PHLDA1=1 and p53=1	PHLDA1 =1	p53=1	PHLDA1=1 and p53=1	PHLDA1=1	p53=1	PHLDA1=1 and p53=1	PHLDA1=1	p53=1

Sensitivity	0.8	0.9	0.9	0.8	0.9	0.9	0.8	0.9	0.9
Specificity	0.8	0.7	0.45	0.6	0.4	0.4	1	1	0.5
PPV	0.67	0.6	0.45	0.67	0.6	0.6	1	1	0.64
NPV	0.89	0.93	0.9	0.75	0.8	0.8	0.83	0.91	0.83
SK: Seborrheic Keratosis; VV: Verruca Vulgaris; SCC: Squamous Cell Carcinoma; PPV: Positive Predictive Value; NPV: Negative Predictive Value.									

Table 5: Diagnostic statistics by stain criteria and differential diagnosis.

Discussion

p53 is a tumor suppressor that promotes cell cycle exit, senescence or apoptosis in response to DNA damage [3,15]. Soini and colleagues found an association between increased p53 expression and Ki-67 expression in benign skin lesions, including seborrheic keratosis [16]. They also noted clustering of p53 positive keratinocytes in areas of damage and inflammation. [16] Ko et al. found p53 expression in inverted follicular keratosis as well as SK [17]. Hussein et al. noted significantly increased p53 expression in invasive and in situ SCC compared to normal skin [18]. p53 expression in SK was also increased but to a lesser degree [18]. Another study found that p53 expression was highest in SK with less expression in malignant and pre-malignant squamous epithelial lesions, including SCC [19]. Bito et al. found the incidence of p53 expression to be greater in SCCs arising in sun-damaged skin [20], and p53 expression has frequently been noted in actinic keratosis (AK). A study by Gouvêa found strong p53 expression in oral SCC arising in patients with a history of proliferative verrucous leukoplakia [21]. Other studies have replicated the finding of more frequent p53 expression in SCC as compared to SK, with some evidence for increasing expression of p53 with higher grade histology. [22,23] Our results further support the finding of enriched p53 expression in invasive squamous cell carcinoma compared to SK and VV.

p63, a p53 homologue, is normally expressed in the nuclei of the basal epidermis, cells of the germinative hair matrix, and hair follicle external root sheath, and plays a role in regulating keratinocyte-specific gene expression [24-27]. Chang et al. were able to differentiate pagetoid SCC in situ from primary extramammary Paget's disease by the strong p63 positivity present in the former [28]. Takeuchi et al. demonstrated strong p63 expression in both SK and poorly differentiated SCC [29]. Other studies have noted similar findings of p63 expression in SK and SCC [30]. A trend towards increased p63 expression in less differentiated cells of invasive SCC has been noted [24]. Increased p63 expression in SK has been supported by microarray analysis [31]. Although we demonstrated p63 expression in VV and invasive SCC, only SK showed diffuse and strong positivity for p63.

PHLDA1 is a marker of matrical differentiation with demonstrated utility in identifying a subset of tumors of the hair follicle [5-10]. We found that a high level of PHLDA1 expression was moderately sensitive and highly specific for invasive SCC compared to SK and VV.

p16 expression has been described as a diagnostic tool for differentiating SCC in situ from actinic keratosis and benign squamous cutaneous lesions [32]. Hodges et al. found increasing p16 expression in the progression from AK to SCC in situ to invasive SCC [33]. Beyond its association with HPV-related oropharyngeal SCC, p16 expression in other cutaneous head and neck SCC with lymph node metastasis can be frequent [34]. Bai and colleagues noted that a small number of vulvar and non-vulvar SKs demonstrated p16 expression with poor correlation with HPV-DNA status [35]. A study by Nakamura et al. found a subset of SK with p16 staining in all lesional cells [36]. Our findings demonstrated low p16 expression in all SK and most VV. Recently, diffuse cyclin D1 and p16 expression was demonstrated more frequently in SCCIS and SCC than in AK. Although the invasive SCC group was enriched for high p16 expression, this marker did not improve diagnostic yield above that offered by PHLDA1 and p53. Several studies have documented higher Ki-67 expression in SCC compared to non-malignant lesions including inflammatory dermatoses and SK [19,37]. Although increased Ki-67 staining was noted in a subset of invasive SCC, this marker failed to add additional diagnostic information in our cohort of cases [38,39].

This limited collection of cases provides support for the utilization of diffuse and strong p63 staining as a sensitive marker for SK with potential use of low to no p16 staining for its specificity for SK when invasive SCC is the primary differential diagnostic consideration. The combination of high PHLDA1 and p53 staining appeared highly specific for invasive SCC compared to SK and VV. Low PHLDA1 and p53 staining demonstrated moderate sensitivity for VV in this small collection of cases, but a specific marker for VV was not apparent from the markers tested [40]. Although our sample size is limited and does not include in situ SCC cases, our findings provide some support for utilization of IHC when a superficial shave biopsy specimen prompts the differential diagnosis of invasive SCC, SK, and VV. Additional studies with more cases are merited to validate these findings.

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