CD34 and α smooth muscle actin distinguish verrucous hyperplasia from verrucous carcinoma

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Objective. This study evaluated the use of stromal biomarkers CD34 and α smooth muscle actin (α-SMA) to distinguish verrucous carcinoma (VC) from verrucous hyperplasia (VH).

Study Design. Thirteen VH, 15 VC, 20 squamous cell carcinoma (SCC), and 16 of uninvolved adjacent stroma specimens were analyzed for α-SMA and CD34 expression by immunohistochemistry.

Results. Stromal α-SMA positivity was observed in 100% (20 of 20) of the SCC and in 93% (14 of 15) of the VC, whereas none of the VH (0 of 13) or adjacent uninvolved stroma (0 of 16) demonstrated α-SMA reactivity. Stromal CD34 positivity was observed in 100% (13 of 13) of VH and adjacent stroma (16 of 16), while 20% (3 of 15) of VC and 11% (2 of 18) of SCC stroma expressed CD34. The SCC and VC groups differed significantly from the VH and uninvolved stroma groups for both α-SMA and CD34 expression (P < .0001).


Verrucous carcinoma (VC) consists of deceptively bland, broad-based, inwardly undulating epithelium devoid of the usual cytologic criteria for malignancy.1 Verrucous hyperplasia (VH) is described as a lesion that resembles VC but differs in that its base lies above the line connecting the bases of the uninvolved epithelium on both sides of the lesion.2 This feature may be difficult or impossible to evaluate, owing to inadequate segments of surrounding normal tissue or technical difficulties with embedding and cutting. A lesion exhibiting growth both above and below such a line creates further diagnostic confusion. Epithelial features do not clearly resolve the issue, as both lesions lack malignant cytology. The stroma, however, may provide an alternative means by which to distinguish these lesions. The stroma associated with invasive carcinoma is characterized by a loss of CD34+ dendritic cells and a gain of α smooth muscle actin—positive (α-SMA+) myofibroblasts; the opposite is seen in uninvolved adjacent stroma. These principles hold true regardless of anatomic location, as previously described in squamous cell carcinoma (SCC) of the skin,3 cervix,4,5 esophagus,6 and upper aerodigestive tract;7,8 in ductal carcinoma of the breast;9,10 and in adenocarcinoma of the pancreas12 and colon.13 Additionally, a gradation of these stromal alterations has been described in low- to high-risk intraepithelial lesions of the breast,9 cervix,4 and oral mucosa.8

The previous reports in the skin and upper aerodigestive tract include one report of myofibroblasts in oral VC, but VC and VH have not been specifically addressed.3,7,8 This study was performed to compare the stromal reactions among conventional infiltrating SCC, VC, VH, and uninvolved adjacent stroma with regard to CD34 and α-SMA protein expression.

MATERIALS AND METHODS
After institutional review board approval, 13 VH specimens, 15 VC specimens, and 20 conventional SCC specimens from mucocutaneous body sites were retrieved from the pathology archives. Using established criteria,1 histopathologic interpretation of the formalin-fixed, paraffin-embedded, hematoxylin-eosin–stained sections was performed by 3 pathologists (K.P., J.B.T., and M.W.L.) to confirm the diagnosis of VH, VC, or SCC. Among the cases analyzed, 16 contained tumor-free stroma for comparison (5 VC, 4 VH, and 7 SCC). The stroma associated with the lesional epithelium was designated as tumor stroma, whereas the uninvolved adjacent stroma associated with the normal epithelium was designated as tumor-free stroma.

Statement of Clinical Relevance
The differentiation between VH and VC can be challenging in histopathologic diagnosis. This study proposes that stromal expression of CD34 and α smooth muscle actin proteins may aid in distinguishing between these 2 entities.
Immunohistochemistry
Sections were deparaffinized and rehydrated with xylene and serial dilutions of ethyl alcohol to distilled water. Tissue sections were incubated in 1 × sodium citrate buffer at a pH of 6 and heated in a steamer for 20 minutes. Sections were pretreated with DAKO target retrieval solution S1699 (DAKO North America Inc, Carpinteria, CA, USA) for α-SMA. Anti-α-SMA antibody (DAKO North America; M0851, mouse immunoglobulin G [IgG], dilution 1:100) and anti-CD34 antibody (Leica Microsystems Inc, Buffalo Grove, IL, USA; NCL-END, mouse IgG, dilution 1:25) were applied on tissue sections for a 1-hour incubation at room temperature in a humidity chamber. The antigen-antibody binding was detected with labeled antimouse polymer–horseradish peroxidase Envision + system (K4001; DAKO North America) and DAB + chromogen system (K3468; DAKO North America). Tissue sections were briefly immersed in hematoxylin for counterstaining. In all cases, staining of blood vessels served as positive control.

Semiquantitative assessment and statistical analysis
The percentage of immunoreactive tumor-free and tumor-associated stromal cells excluding vessels was recorded as follows: −, no positive cells; +, focal (<50% positive cells); and ++, strong (>50% positive cells), as described by Barth et al. 7 The data were analyzed statistically using STATA 12.1 (StataCorp LP) in the University of Chicago Biostatistics Laboratory. Differences in ordinal immunohistochemical levels among groups were analyzed by the Kruskal-Wallis test followed by the Wilcoxon rank sum test for pairwise comparisons. In addition, because uninvolved adjacent tumor-free and tumor-associated stroma tissues were frequently evaluated in the same patient, proportional odds models were fit with an adjusted variance estimate to allow for the within-patient correlation. Sensitivity and specificity were calculated separately.

RESULTS
One hundred percent (20 of 20) of the tumor-associated stroma from the SCC specimens and 93% (14 of 15) from the VC cases demonstrated α-SMA positivity (Table I; Figures 1 and 2). Strong α-SMA staining was observed in 45% (9 of 20) of SCC and 46% (7 of 15) of VC, whereas focal staining was seen in 55% (9 of 20) of SCC and 46% (7 of 15) of VC. Conversely, none of the VH (0 of 13) or areas of adjacent tumor-free stroma (0 of 16) demonstrated α-SMA reactivity (Figure 3). All VH (13 of 13) as well as the adjacent tumor-free stroma (16 of 16) exhibited CD34 positivity, with 69% (9 of 13) of the VH and 81% (13 of 16) of the adjacent stroma showing strong positivity (Table II). However, only 20% (3 of 15) of VC expressed CD34, with all positive cases being focal. Of note, CD34 was focally lost in association with inflammatory infiltrates (Figure 4). The SCC and VC groups differed significantly from the VH and tumor-free stroma groups for both α-SMA and CD34 (P < .0001). There was no significant difference between SCC and VC (P = .91 and P = .41 for α-SMA and CD34, respectively) or between VH and tumor-free stroma (P = 1.0 and P = .73 for α-SMA and CD34, respectively). Results for within-patient correlation were similar to those of the nonparametric tests (data not shown). Strong α-SMA positivity combined with complete CD34 negativity was 100% specific for carcinoma-associated stroma, whereas diffuse CD34 positivity combined with complete α-SMA negativity was 100% specific for benign-associated stroma. α-SMA was 93% sensitive for VC-associated stroma, and CD34 was 100% sensitive for adjacent stroma.

Table I. Stromal patterns of α-SMA+ myofibroblastic cells

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<th>Pattern</th>
<th>−</th>
<th>+</th>
<th>++</th>
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<tbody>
<tr>
<td>Tumor-free</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Verrucous hyperplasia</td>
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<td>0</td>
</tr>
<tr>
<td>Verrucous carcinoma</td>
<td>1</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0</td>
<td>11</td>
<td>9</td>
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α-SMA, α smooth muscle actin.

DISCUSSION
The present series supports a role for combined CD34 and α-SMA analysis in distinguishing VH from VC. Loss of CD34+ dendritic cells, together with a gain of α-SMA+ myofibroblasts, supports a diagnosis of VC, whereas the opposite reaction supports VH. Diffuse CD34 positivity is strongly indicative of benignity, and its loss is a sensitive marker for malignant stromal alterations. CD34 loss is not specific for malignancy, however, as inflammatory infiltrates were noted to disrupt the dendritic meshwork, a finding that has been previously described. 7 Non specific CD34 loss has also been seen in association with scars and biopsy sites in skin, in breast tissue, and in the upper aerodigestive tract. 7,11,15-17 In the same body sites, α-SMA positivity has been noted in the stroma of granulation tissue. 7,11,15-17 In the present series, positivity for α-SMA was seen only in malignant stroma; however, not all carcinoma cases were positive for α-SMA. Thus, the discriminatory utility of immunohistochemistry for VH and VC hinges on the combined patterns of the 2 markers along with...
contextual prudence in cases with prior surgical manipulation.

In addition to offering a diagnostic adjunct to the current histologic criteria, these results stimulate discourse with respect to the biologic relationship of VH to VC. Whether VH is the same as VC, a precursor to VC, or a separate entity altogether has been a subject of debate. Since it was first described in 1980 by Shear and Pindborg,2 VH has been seen in association with leukoplakia (53%), VC (29%), epithelial dysplasia (66%), and conventional SCC (10%),18 but its placement on a spectrum with these lesions has not been established. Many authors have suggested that VH and VC are the same entity.18-22 Supporting this perspective is the suggestion that anatomic location influences whether a lesion resembles VH or VC.18 Alternatively, VH may represent a precursor to VC or may be an entirely separate entity. The results herein suggest that VH and VC are not the same lesion, as they evoke opposite stromal reactions. Alterations in the stromal phenotype not only provide helpful diagnostic clues in epithelial lesions but also highlight the importance of the stromal-epithelial relationship. In fact, recent evidence suggests that alterations of the stromal compartment alone are sufficient to induce epithelial malignancy.23 During the transition from benign to malignant, resident CD34+ dendritic cells in normal stroma acquire a cancer-activated phenotype characterized by α-SMA expression.24 The acquisition of smooth muscle actin is a feature of myofibroblasts, which are prominent in the desmoplastic reaction to infiltrating solid tumors, and it may be reasonable to attribute the immunostaining pattern for VC to a type of desmoplasia that is below the level of appreciation by routine microscopy. Supplementing routine histologic assessment with these 2 simple and widely available immunostains may contribute diagnostic information. Another diagnostic tool, nuclear cytometry on Feulgen-stained histologic sections, has been reported as useful for differentiating VC from benign lesions.25

SCC

Fig. 1. A-C, Hematoxylin-eosin—stained sections of SCC demonstrating infiltrative islands of squamous epithelium showing cytologic features of malignancy (original magnification × 10, × 50, and × 200, respectively). D-F, Immunohistochemical staining for CD34, demonstrating the absence of a meshwork from the stroma surrounding the tumor cells (original magnification × 10, × 50, and × 200, respectively). G-I, Immunohistochemical staining for α-SMA demonstrating myofibroblasts enveloping the SCC nests (original magnification × 10, × 50, and × 200, respectively). (SCC, squamous cell carcinoma; α-SMA, α smooth muscle actin.)
but to our knowledge, this method is not readily available to most pathologists.

Since its initial description in 1985, proliferative verrucous leukoplakia (PVL) has posed both diagnostic and clinical management challenges. PVL is a condition of unknown etiology that is most often observed in middle-aged women. Initially, the clinical presentation may be of a singular leukoplakic lesion that becomes multifocal over time. Importantly, PVL has been reported to have a high rate of malignant transformation. Although several criteria for PVL have been proposed, the diagnosis of PVL is often made in a retrospective fashion many years later, after the lesion has progressed to SCC. Recently, both DNA aneuploidy and the expression of several candidate protein biomarkers for the prediction of malignant changes in PVL have been investigated for their utility in the diagnosis of PVL. It would be interesting to test the hypothesis that the stromal CD34 and α-SMA expression patterns might aid in both distinguishing between VH and PVL in challenging cases and predicting which PVL cases are at most risk for undergoing malignant transformation.

In conclusion, given that the mainstay of therapy for either of these lesions is complete surgical excision, the distinction between the 2 entities may be more of a histopathologic endeavor rather than a clinical one. For the pathologist, the significance of distinguishing these 2 entities rests on the finding that 20% of VC cases have foci of conventional SCC, for which the term hybrid tumor is used, because these hybrid tumors are treated more aggressively. The biologic relationship between VC and VH has been, and continues to be, a source of a diagnostic dilemma. This study presents a potential additional step in discriminating between them.

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REFERENCES


Table II. Stromal patterns of CD34+ dendritic cells

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<th>Pattern</th>
<th>–</th>
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<tr>
<td>Tumor-free</td>
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<td>Verrucous hyperplasia</td>
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<tr>
<td>Verrucous carcinoma</td>
<td>12</td>
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<td>0</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>18</td>
<td>2</td>
<td>0</td>
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Fig. 3. A-C, Hematoxylin-eosin–stained sections demonstrating VH that is characterized by hyperplastic, undulating epithelium confined to the level of the adjacent uninvolved epithelium (original magnification × 10, × 50, and × 200, respectively). D-F, Immunohistochemical staining for CD34 demonstrates a dendritic meshwork beneath the lesion that is continuous with the uninvolved stroma and consists of triangular cell bodies with delicate, interdigitating cytoplasmic extensions (original magnification × 10, × 50, and × 200, respectively). G-I, Immunohistochemical staining for α-SMA demonstrating negative stromal staining with positive staining of the vasculature (original magnification × 10, × 50, and × 200, respectively). (VH, verrucous hyperplasia; α-SMA, α smooth muscle actin.)

Fig. 4. Immunohistochemical staining for the CD34+ dendritic meshwork demonstrating focal disruption by inflammatory cell infiltrates (asterisk; original magnification × 200).

Fig. 5. Immunohistochemical staining for CD34+ fibrocytes, alpha-smooth muscle antigen-positive myofibroblasts, and CD117 expression in the stroma of invasive squamous cell carcinomas of the oral cavity, pharynx, and larynx. Virchows Arch. 2004;444:231-234.


