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Pathologic features of aggressive vulvar carcinoma are associated with an epithelial-mesenchymal transition

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Abstract

Factors contributing to aggressive behavior in vulvar squamous cell carcinoma (vSCC) are poorly defined; however, a recent study has shown that vSCCs with an infiltrative pattern of invasion and fibromyxoid stroma are associated with worse outcomes than tumors with a pushing or nested pattern of invasion and lymphoplasmacytic stroma. Epithelial-mesenchymal transition (EMT) has been associated with tumor progression in a number of malignancies, and this study proposes that EMT contributes to tumor aggressiveness in this subset of vSCC. Immunohistochemistry was used to detect nuclear localization of β -catenin, loss of E-cadherin, and presence of vimentin in 58 cases of vSCC. The association of these phenotypic changes with pathologic features and clinical outcomes was tested using Fisher's exact and χ^2 analyses (significance at $p < 0.05$). EMT-associated features were identified in 45 of 58 cases (78%) with 28 cases exhibiting more than one feature. Nuclear β -catenin and presence of vimentin were significantly more likely to occur in tumors with an infiltrative pattern of invasion or a fibromyxoid stromal response. Loss of E-cadherin was significantly associated with an infiltrative pattern, but not a fibromyxoid stroma. Risk for tumor recurrence was significantly increased in tumors with nuclear localization of β -catenin alone or in tumors displaying multiple EMT-associated features. These results suggest that the development of an EMT may be a mechanism by which infiltrative vulvar tumors with a fibromyxoid stromal response behave more aggressively and convey worse outcomes than tumors that do not exhibit these pathologic features.

Keywords

vulva; carcinoma; EMT; invasive pattern; stromal response

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Conflicts of Interest

The authors of this manuscript have no conflicts of interest to disclose.

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1. Introduction

1.1 Vulvar Squamous Cell Carcinoma

Cancers of the vulva represent 5% of all gynecologic malignancies for women in the United States [1]. The majority (90%) of these cancers are vulvar squamous cell carcinomas (vSCCs) [2]. Postmenopausal women between the ages of 75–84 are most commonly affected, and prognosis worsens with increasing age [3, 4]. Currently, surgical resection is the standard of treatment for vSCC patients [5]. Nodal involvement and tumor recurrence are important clinical features of vSCC that significantly decrease survival rates; however, there is a lack of knowledge concerning the biologic mechanisms that contribute to the development of these features in vSCC [6, 7].

Different morphologic patterns of tumor invasion have been previously described for vSCC. One pattern of invasion is referred to as *infiltrative* (also described as a “spray” pattern) and consists of individual tumor cells or cords of cells invading into surrounding stroma [8–12]. A second pattern of invasion, labeled *pushing*, consists of large geographic regions or nests of tumor cells that have a well demarcated tumor-stroma interface [12, 13]. Our recent investigation showed that tumors with an infiltrative pattern of invasion are 2-times more likely to recur than tumors with a pushing or nested pattern of invasion [14]. The infiltrative pattern of invasion is also highly associated with perineural invasion, which is an indicator of increased risk for local recurrence, and the presence of a fibromyxoid (FMX) stromal response, which is a statistically significant indicator of risk for nodal metastases and extracapsular extension in vSCC [14, 15]. When both an infiltrative pattern of invasion and a FMX stromal response are present, patients are 3-times more likely to experience nodal metastases and almost 2-times more likely to experience tumor recurrence than patients whose tumors contained only one or neither of these tumor features, even when accounting for age, race, depth of tumor invasion, and margin status [14]. Overall, vSCCs with an infiltrative pattern of invasion and a FMX stromal response progress in a more aggressive manner than tumors with a pushing pattern of invasion that do not contain a fibromyxoid response. Increasing our understanding about the processes that contribute to the aggressive behavior of this subset of vSCC may reveal important information about the development of unfavorable clinical features such as nodal involvement and recurrence.

1.2 Epithelial-Mesenchymal Transition

Epithelial-mesenchymal transition (EMT) is a molecular shift that allows epithelial cells to adopt a mesenchymal phenotype with loss of cellular polarity, loss of cell-cell junctions, and enhanced cellular motility [16, 17]. Cells undergoing an EMT are able to escape immune detection and evade apoptosis, allowing them to progress, uninhibited by the host. These cells are also capable of degrading surrounding matrix proteins [17–20]. By utilizing these alterations in cellular regulation, cells undergoing an EMT have an increased ability to invade into surrounding tissue, metastasize to other sites, and initiate tumor recurrence [18].

Many immunohistochemical (IHC) markers have been established as a means of identifying cells undergoing an EMT, and some of the most well studied markers include E-cadherin, β -

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catenin, and the mesenchymal protein, vimentin [21, 22]. In normal epithelial cells, β -catenin is associated with E-cadherin complexes that mediate adherens junctions between cells. While E-cadherin remains predominantly at the membrane of the cell, β -catenin is regulated through sequestration by a cytoplasmic complex that targets the protein for degradation by the proteasome [23]. During EMT, loss of E-cadherin and altered regulation of β -catenin occur. Dissociation from E-cadherin and inhibition of the regulatory cytoplasmic complex allows β -catenin to localize and accumulate in the nucleus. In the nucleus, β -catenin functions as a co-transcriptional regulator, aiding in the transcriptional activation and increased expression of mesenchymal markers such as vimentin, and indirectly influences further down-regulation of epithelial markers such as E-cadherin [22–24]. The loss of E-cadherin, nuclear localization of β -catenin, and an upregulation of vimentin in epithelial cells are all characteristics highlighting the occurrence of an EMT.

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To date, two major studies have focused on EMT and its associated markers in vSCC. The first study associated loss of E-cadherin staining with altered estrogen receptor expression in 34 cases of vSCC, but no link was reported between decreased E-cadherin expression and other EMT-associated markers or clinical outcomes [25]. A larger study reported by Rodrigues and colleagues focused on the association between EMT and human papillomavirus (HPV) status in 87 vSCC patients and found absence of HPV infection to be associated with multiple markers of EMT [26]. Importantly, loss of E-cadherin was linked to several adverse clinical features, and loss of β -catenin was independently associated with decreased survival [26]. In contrast, no association was identified between clinical outcomes and other EMT-associated markers, and the study did not investigate the association of EMT with vulvar tumor recurrence, an important clinical feature of vSCC. Furthermore, no study has investigated the association between EMT and patterns of tumor invasion or stromal response in vSCC, resulting in little evidence to support a role for EMT in aggressive behavior of vSCC. To improve our understanding of the development of aggressive vulvar tumors, we conducted the following study to verify the association of EMT with poor clinical outcomes in vSCC and determine if the propensity to undergo EMT contributes to adverse clinical outcomes associated with an infiltrative invasive pattern and a fibromyxoid stromal response.

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2. Materials and Methods

2.1 Case Acquisition

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Approval for research using archived human samples was obtained from the institutional review board of the University of Arkansas for Medical Sciences (UAMS). Using surgical report cases from UAMS archives, 143 cases of vSCC were identified. Biopsy specimens and cases containing only carcinoma in situ were excluded. All available hematoxylin and eosin (H&E) slides for these cases were retrieved and each case was classified based on the patterns of tumor invasion and stromal response as previously described [14]. Briefly, an infiltrative pattern of tumor invasion was defined as cords or individual tumor cells infiltrating the surrounding stroma in a spray-like pattern (Figure 1A). These infiltrative tumors were typically of the keratinizing subtype, which is sometimes associated with the presence of a differentiated-type vulvar intraepithelial neoplasia (VIN). A pushing or nested

pattern of tumor invasion was defined as either solid sheets of tumor cells or large geographic nests of tumor with a well-demarcated tumor-stromal interface (Figure 1C). These pushing/nested tumors typically exhibited basaloid or warty histologic features, which are commonly associated with the presence of usual-type VIN. Mixed tumors contained regions of both infiltrative and pushing patterns of invasion (Figure 1E). A FMX stromal response was comprised of loose collagen and fibroblasts on a background of amorphous myxoid tissue (Figure 1B), which contrasted with a lymphoplasmacytic (LPC) stromal response that contained lymphocytes and plasma cells (Figure 1D). Based on these classifications, 9 cases of vSCC were identified as containing an exclusively infiltrative pattern of invasion with FMX stromal response. In addition, 19 cases containing an exclusively pushing or nested pattern of invasion with LPC stromal response were randomly selected. Finally, 30 cases of vSCC with a mixed pattern of tumor invasion were randomly selected. Of these 30 mixed cases, 12 contained a mixed stromal response, with FMX stroma surrounding the infiltrative regions and LPC stroma surrounding the pushing/nested regions, 14 contained an exclusively FMX stromal response, and 4 contained an exclusively LPC stromal response. Clinicopathologic features represented in these 58 cases are summarized in Table 1.

2.2 Immunohistochemistry

A representative section of each tumor was selected based on H&E slide review, and the formalin-fixed paraffin-embedded (FFPE) tissue section was obtained from the UAMS tissue archives. Selected blocks of FFPE were sectioned at 4 μ m and heat-fixed to glass slides. To stain for E-cadherin, slides were processed and stained using the Ventana ULTRA staining platform with mouse monoclonal antibody to E-cadherin-36 (Catalog #790-4497, Ventana, Tucson, AZ, USA) and hematoxylin counterstain.

For β -catenin and vimentin staining, all slides were deparaffinized in xylene, re-hydrated using graded alcohol solutions, and subjected to heat-induced antigen retrieval in pH 6 citrate retrieval solution. Endogenous peroxidases were inhibited using commercial dual endogenous enzyme block (Product # S2003, Dako, Carpinteria, CA, USA). For β -catenin staining, an additional blocking step was performed with standard protein block according to manufacturer's instructions (Product # X0909, Dako). Antibody to β -catenin (MAB13291, R&D Systems, Minneapolis, MN, USA) was applied at 2.5 μ g/mL for 2 hours at room temperature, followed by incubation with affinity-purified biotinylated goat anti-mouse secondary for 30 minutes. For vimentin staining, an additional blocking step was performed using 10% normal goat serum in Tris-buffered saline with tween for 30 minutes. Antibody to vimentin (Ab92547, Abcam, Cambridge, MA, USA) was applied at 0.191 μ g/mL for 1 hour at room temperature, followed by incubation with affinity-purified biotinylated goat anti-rabbit secondary for 30 minutes. All β -catenin and vimentin slides were treated with Vectastain Elite ABC kit, followed by DAB+ substrate chromogen system, according to manufacturer's instructions (Product # K3468, Dako, Carpinteria, CA, USA). Vimentin stains were counterstained with hematoxylin prior to coverslipping. To facilitate identification of nuclear localization, β -catenin stains were not counterstained.

2.3 Classifying IHC Markers of EMT

All tumor slides were reviewed and scored by E. Holthoff. Difficult cases were adjudicated by a gynecologic pathologist (CMQ). For β -catenin, staining patterns were defined as either nuclear or membranous, and the percentage of cells displaying each pattern was quantified to determine the predominant pattern. To ensure that the predominate pattern reflected a majority of cells, a 60% cutoff was used. Thus, tumors with at least 60% of cells exhibiting a membranous pattern were considered predominantly membranous and those with 60% of cells exhibiting a nuclear pattern were considered predominantly nuclear. For a given cell, the presence of *any* nuclear staining was considered nuclear localization. For E-cadherin, slides were reviewed for loss or no loss in staining. Loss was defined as a decrease in staining in the outer interface of cells at the leading edge of the tumor when compared with superficial tumor regions. For vimentin, slides were analyzed for presence or absence of vimentin defined as positive or negative staining. A 60% majority cutoff was used to define the staining pattern so that when at least 60% of tumor cells exhibited vimentin staining, the tumor was considered vimentin positive. For any given tumor cell, the presence of *any* cytoplasmic vimentin staining was considered positive for that particular cell.

In mixed cases of vSCC, patterns of β -catenin localization, E-cadherin loss, and presence or absence of vimentin were determined in both pushing/nested and infiltrative regions. A section of normal vulvar epithelium was used as a positive control for E-cadherin and membranous β -catenin staining and a negative control for vimentin positivity. In the normal tissue, E-cadherin stained epithelial cells in a distinctly membranous pattern, β -catenin was predominantly membranous with patchy areas of mild cytoplasmic and nuclear staining, and vimentin was predominantly negative in the normal epithelial cells with no cytoplasmic vimentin staining present.

2.4 Statistical Analysis

The association of EMT markers with both pathologic features (invasive patterns and stromal response) and with clinical outcomes (recurrence, nodal metastasis, and perineural invasion (PNI)) were assessed using Fisher's exact tests. Two-sample t-tests were used to compare mean depth of invasion (DOI) among subjects with and without specific markers indicative of an EMT. A Satterthwaite-Welch approximation was used to calculate the degrees of freedom for these tests. Finally, χ^2 trend tests were used to assess the association between the presence of an EMT marker and clinical stage. Statistical significance was determined using a two-sided α -level of 5%. No adjustments for multiple comparisons were performed.

3. Results

3.1 Overall EMT Staining Patterns

IHC patterns indicative of an EMT were identified in 45 cases (78%) of vSCC. Of those 45 cases, 37 (82%) exhibited nuclear localization of β -catenin, 24 (53%) exhibited positive vimentin staining within tumor cells, and 25 of 44 cases (57%) exhibited loss of E-cadherin. One case was excluded from E-cadherin analysis due to loss of tissue upon sectioning. Overall, 26 of 57 cases (46%) demonstrated either all (13 cases; 23%) or none (13 cases;

23%) of the EMT-associated markers identified in this study. Of the cases that displayed any EMT staining patterns, 28 of 44 (64%) exhibited more than 1 EMT pattern, while 16 of 44 (36%) exhibited only one EMT pattern.

3.2 EMT and Patterns of Invasion

All 9 of the infiltrative tumors (100%) displayed at least 1 EMT feature (Figure 2 A, C, E), while only 8 of 19 (42%) pushing/nested tumors exhibited any features of an EMT (Figure 2 B, D, F). Of the 30 mixed tumors containing both infiltrative and pushing/nested regions, 28 (93%) exhibited at least 1 EMT-associated marker. Interestingly, when the infiltrative region was negative for any given EMT marker, the pushing or nested region was always also negative. Of the 23 mixed tumors that contained nuclear localization of β -catenin, only 7 (30%) displayed nuclear localization in both infiltrative and pushing/nested regions, while the other 16 (70%) contained nuclear localization only in infiltrative regions. Similarly, of the 18 cases that contained tumor cells positive for vimentin, only 6 (33%) were positive in both infiltrative and pushing/nested regions, while 12 (66%) were positive only in the infiltrative regions. Of the 13 mixed cases that displayed loss of E-cadherin, only 3 (23%) exhibited loss in both tumor regions, while 10 (77%) exhibited loss in the infiltrative regions only.

As detailed in Table 2, the presence of any infiltrative pattern of tumor invasion was significantly associated with nuclear localization of β -catenin (infiltrative: 82% [32/39]; pushing/nested: 26% [5/19]; $p<0.0001$), presence of vimentin (infiltrative: 59% [23/39]; pushing/nested: 5% [1/19]; $p<0.0001$), and loss of E-cadherin (infiltrative: 55% [21/38]; pushing/nested: 21% [4/19]; $p=0.0227$). Sixty-six percent (66% [26/39]) of subjects with a tumor demonstrating an infiltrative pattern of invasion, whether in a pure or mixed tumor setting, were positive for at least 2 EMT markers compared to 11% [2/19] of subjects with a pushing/nested pattern ($p<0.0001$).

3.3 EMT and Stromal Response

Presence of at least one EMT staining pattern was detected in 21 of 23 (91%) tumors with FMX stroma, 12 of 23 (52%) tumors with LPC stroma, and all 12 (100%) tumors with mixed stromal response. When analyzing the association between stromal response and EMT patterns, the presence of a FMX stromal response was significantly associated with both nuclear localization of β -catenin (FMX: 80% [28/35]; LPC: 39% [9/23]; $p=0.0022$) and presence of vimentin in the tumor cells (FMX: 57% [20/35]; LPC: 17% [4/23]; $p=0.0031$). Of the subjects with an FMX stromal response, 63% [22/35] were positive for at least two EMT markers compared to 26% [6/23] of subjects with a LPC stromal response ($p=0.0021$). No association was found between stromal response and loss of E-cadherin (FMX: 50% [17/34]; LPC: 35% [8/23]; $p=0.2889$).

3.4 Association of EMT with Clinical Outcomes

To determine the clinical importance of an EMT in vSCC, presence of EMT staining patterns in vulvar tumors was associated with poor outcomes such as tumor recurrence and nodal involvement, and with clinical features such as tumor stage and depth of tumor invasion (DOI).

With regards to outcomes, the presence of a nuclear β -catenin pattern was significantly associated with tumor recurrence (recurrent: 87% [20/23]; non-recurrent: 47% [16/34]; $p=0.0024$). Similarly, the presence of two or more EMT-associated markers was significantly associated with tumor recurrence (recurrent: 65% [15/23]; non-recurrent: 35% [12/34]; $p=0.0334$) as shown in Table 2. No association was identified between recurrence and either presence of vimentin alone or loss of E-cadherin alone. Because previous studies identified an important link between tumor recurrence and PNI [15], the association between EMT-associated staining patterns and the presence of PNI was assessed; however, no significant associations were identified for any of the 3 EMT patterns. No association was identified between nodal metastasis and any of the 3 EMT-associated markers.

For clinical features, chi-squared test for trend showed that nuclear localization of β -catenin was more likely to occur at higher tumor stages than membranous β -catenin ($p=0.0102$). No association was found between tumor stage and presence of vimentin or loss of E-cadherin. As shown in Table 3, tumors that displayed a loss of E-cadherin were significantly more deeply invasive than tumors that did not undergo loss of E-cadherin (E-cadherin loss: average DOI = 1.36 [sd = 1.13]; no loss: average DOI = 0.85 [sd = 0.58]; $p=0.0485$). No significant association was identified between DOI and nuclear localization of β -catenin or presence of vimentin.

4. Discussion

The occurrence of an epithelial-mesenchymal transition and its role in tumor progression have been studied in a number of cancers, including several gynecological cancers [26–28]. However, few studies have examined the role of EMT in the progression of vSCC. One large study conducted by Rodrigues and colleagues analyzed several different EMT-associated markers in a series of 87 resections specimens from vSCC patients and compared the expression of these markers with clinical data and HPV status [26]. They found that loss of β -catenin expression was independently associated with poor survival. However, presence of histologic features, such as invasive or stromal patterns, in these tumors undergoing an EMT were not reported. In contrast, our current study describes and associates easily recognized tumor pattern of invasion and stromal response as well as clinical outcomes with nuclear localization of β -catenin, loss of E-cadherin, and expression of vimentin, which reflect the activation of signaling pathways that promote an EMT [23, 26, 29]. Because negative HPV status has already been associated with the occurrence of an EMT, we did not examine HPV status in our cohort [26].

We previously defined an aggressive subtype of vSCC that is significantly more likely to recur and metastasize to the lymph nodes [14]. This aggressive subtype exhibits an infiltrative pattern of tumor invasion on a background of FMX stroma. In the current study, we investigated the potential of EMT as a mechanism for increased aggressiveness in this subset of vSCC. We showed that cells of tumors exhibiting *any* infiltrative pattern of invasion, whether in the pure form or in a mixed tumor setting, are more likely to exhibit features reflective of an EMT than tumors containing only a pushing or nested pattern of invasion. In mixed tumors, EMT-associated staining patterns in the pushing/nested regions reflect the presence of these patterns in the infiltrative regions of tumor. This premise is

supported by other studies suggesting that the development of a mesenchymal phenotype permits tumor cells to migrate more independently from one another due to their loss of adherens junctions, contributing to the spray-like pattern identified in infiltrative tumors [30–32]. Thus, the role EMT plays in the development of the infiltrative invasion pattern may explain, in part, the aggressive nature of this subset of vSCC.

In addition to invasive patterns, we investigated the occurrence of an EMT in the presence of a FMX, LPC, or mixed stromal response. Using IHC, we found that multiple EMT characteristics were linked to the presence of a FMX stroma in either the pure or mixed stromal setting. This association suggests an important role of the tumor stroma in the development of an altered tumor cell phenotype, and may indicate that tumor-stroma interactions are important in the development of more aggressive vulvar carcinomas. Importantly, no association was identified between tumors that contain only a LPC stroma rich in inflammatory immune cells and the processes associated with an EMT, suggesting that the presence of inflammatory mediators may suppress the interactions necessary to mediate this change in tumor cell phenotype. Together, these results indicate that tumors with an infiltrative pattern of invasion and a fibromyxoid stromal response undergo an EMT as a means of becoming more invasive than tumors with a pushing or nested pattern of invasion and a LPC stromal response.

Tumor recurrence and nodal metastases are important clinical outcomes that impact survival in vSCC patients; however, there is a lack of knowledge concerning the mechanisms by which these outcomes develop. Thus we chose to investigate whether the presence of an EMT would impact the risk for nodal metastases or tumor recurrence in vSCC patients. Our results did not identify an association between EMT-associated markers and nodal metastases; however, our study is the first to show a significant association between tumor recurrence and the development of an EMT in vulvar tumors, as reflected by the nuclear localization of β -catenin or the presence of multiple EMT-associated markers. Although investigating the cause of this relationship was beyond the scope of this study, we can speculate about possible explanations for this association. One possibility is that tumor cells undergoing an EMT have an increased ability to migrate independently throughout the tissue. This migration may allow invasion to occur beyond the grossly apparent tumor margins, leading to positive resection margins and under treatment of primary tumors. Alternatively, we previously described a direct association between presence of perineural invasion and tumor recurrence in vSCC [15]. However, in this study, the presence of EMT-associated markers does not appear to be associated with perineural invasion, suggesting that perineural invasion and the development of an EMT could both have an impact on risk for tumor recurrence through two completely separate mechanisms. Additional studies are needed to determine exactly what role an EMT is playing in the risk for tumor recurrence in vSCC.

In addition to an association between EMT and tumor recurrence, we found that tumors exhibiting loss of E-cadherin were found to have greater depth of tumor invasion than those that did not have E-cadherin loss. Based on the mechanism by which E-cadherin mediates cell-to-cell junctions between tumor cells, this association implies that loss of tumor cell junctions allows tumor cells to become more invasive and penetrate independently into the

stroma [24]. This loss of cell-to-cell junction may also be an important factor in the development of the spray-like appearance of the infiltrative invasive pattern which is also associated with increased depth of tumor invasion and poor outcomes [14].

In this study, increasing tumor stage was significantly associated with nuclear localization of β -catenin, suggesting that EMT may play an important role in the ability of these tumors to grow beyond early invasive lesions. In our data set, the majority of Stage I tumors that contained IHC markers of an EMT were infiltrative tumors with a fibromyxoid stromal response, indicating that infiltrative tumors are capable of undergoing this phenotypic shift at an early stage, and that this tumor morphology may be a useful index of prognosis independent of tumor stage.

Recent studies have shown that inhibition of signaling pathways associated with tumor growth inhibited the development of an EMT and the metastatic potential of tumor cells [33, 34]. Thus, future studies should focus on elucidating the genetic and molecular mechanisms that promote an EMT and could be used as targets for adjuvant therapy in aggressive cases of vSCC. Although we recognize the limitations of our observational study, our results provide justification for prospective studies that address these critical issues in vulvar cancers.

5. Conclusion

We have identified a significant association between the development of an EMT and an increased risk for poor clinical outcomes in vulvar carcinoma. Importantly, we show that the development of an EMT is a potential mechanism by which vSCCs with an infiltrative pattern of invasion and a fibromyxoid stromal response behave more aggressively than pushing or nested tumors. Therefore, identification of this aggressive vSCC subtype should be reported as it could affect treatment planning and overall outcomes for vSCC patients. In addition, future studies should focus on the factors that influence the development of an EMT in these cases and potential mechanisms for inhibiting this process in an effort to curb the aggressive behavior seen in this subset of tumors.

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Figure 1. H&E stains of invasive patterns and stromal responses in vSCC

(A) infiltrative pattern of invasion with FMX stroma; (B) high power magnification of FMX stroma; (C) pushing invasive pattern surrounded by LPC stroma; (D) high power magnification of LPC stroma; (E) mixed vSCC containing both an infiltrative pattern of invasion with FMX stromal response (arrow) and pushing pattern of invasion with LPC stromal response (arrowhead). (Low magnification 4x; high magnification 40x)

Figure 2. Identifying EMT in vSCC

(A) Infiltrative cords of tumor cells exhibit a nuclear β -catenin staining pattern. (B) In contrast, tumor cells in the pushing tumor display a distinct membranous β -catenin staining pattern. (C) In the infiltrative tumor there is overall loss of E-cadherin staining, as well as complete absence of E-cadherin in some tumor cells located at the invasive front (arrows). (D) In the pushing tumor, E-cadherin staining has a distinct membranous pattern. (E) Cords of tumor cells express robust cytoplasmic vimentin staining in the infiltrative tumor. (F) In the pushing tumor, vimentin negative tumor cells (asterisk) contrast with entrapped vimentin positive stromal cells (arrows). (40x magnification)

Table 1

Clinicopathologic parameters of 58 cases of vulvar squamous cell carcinoma

Parameters	Number
Median age (range)	62.7 (28–93)
Average depth of invasion, cm (range)	1.07 (0.1–4.3)
Tumor stage (%)	
Stage I	30 (52)
Stage II	9 (15)
Stage III	14 (24)
Stage IV	5 (9)
Recurrence (%)	
Present	23 (39)
Absent	34 (59)
Recurrent Tumor	1 (2)
Nodal involvement (%)	
Present	18 (31)
Absent	34 (59)
Unknown ^I	6 (10)
Perineural invasion (%)	
Present	29 (50)
Absent	17 (29)
Unknown	12 (21)

^I Nodal excision not performed

Features of epithelial-mesenchymal transition associated with pathologic and clinical features of vulvar squamous cell carcinoma

Table 2

Features	Nuclear β -catenin		Vimentin Positive		E-cadherin Loss		2+ EMT Features	
	Cases (%)	p-value	Number	p-value	Number	p-value	Number	p-value
Invasive Pattern								
Infiltrative (N=39)	32 (82)	<0.0001	23 (59)	<0.0001	21 (55)	0.0227	26 (66)	<0.0001
Nested/Pushing (N=19)	5(26)		1 (5)		4 (21)		2 (11)	
Stromal Response								
Fibromyxoid (N=35)	28 (80)	0.0022	20 (57)	0.0031	17 (50)	0.2889	22 (63)	0.0021
Lymphoplasmacytic (N=23)	9 (39)		4 (17)		8 (35)		6 (26)	
Tumor Recurrence								
Recurrent (N=23)	20 (87)	0.0024	12 (52)	0.1731	13 (57)	0.1758	15 (65)	0.0334
Non-recurrent (N=34)	16 (47)		11 (32)		12 (36)		12 (35)	
Tumor Stage								
Stage I (N=30)	14 (47)	0.0102	11 (37)	0.5266	11 (37)	0.3631	11 (37)	0.0830
Stage II (N=9)	8 (89)		5 (56)		5 (56)		6 (67)	
Stage III (N=14)	10 (71)		5 (36)		6 (43)		7 (50)	
Stage IV (N=5)	5 (100)		3 (60)		3 (75)		4 (80)	
PN1								
Present (N=29)	20 (69)	0.5341	13 (45)	0.5546	14 (48)	1.000	16 (55)	0.7959
Absent (N=17)	10 (59)		6 (35)		5 (31)		7 (41)	
Nodal Involvement								
Present (N=18)	14 (78)	0.0759	8 (44)	0.5605	9 (53)	0.5529	11 (61)	0.3187
Absent (N=34)	17 (50)		12 (35)		14 (41)		13 (38)	

Table 3

Features of EMT associated with depth of tumor invasion

EMT Features	N	Mean DOI (cm)	95% CI	p-value
β -catenin				
Nuclear	37	1.941	0.85, 1.53	0.0904
Membranous	21	0.8452	0.61, 1.08	
Vimentin				
Present	24	1.203	0.73, 1.67	0.3716
Absent	34	0.972	0.73, 1.21	
E-cadherin				
Loss	25	1.355	0.89, 1.82	0.0485
No Loss	32	0.8484	0.64, 1.06	
Number of EMT Features				
0 or 1	30	0.912	0.713, 1.11	0.1782
2 or 3	28	1.2435	0.794, 1.68	