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

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Summary 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 $P \leq .05$). 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 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.

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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 to 84 years 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 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 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 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 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 EMT, and some of the most well studied markers include E-cadherin, β-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 EMT.

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.

2. Materials and methods

2.1. Case acquisition

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