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Original contribution

Immunohistochemical expression of ARID1A in penile squamous cell carcinomas: a tissue microarray study of 112 cases☆

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Summary *ARID1A*, a member of the chromatin remodeling genes family, has been suggested as a novel tumor suppressor gene in gynecologic malignancies. However, its role in penile cancer has yet to be determined. This study assesses the immunohistochemical expression of ARID1A in penile squamous cell carcinoma (SCC) and its association with pathologic features, human papillomavirus (HPV) status, and previously reported mammalian target of rapamycin pathway markers in the same cohort. Four tissue microarrays were constructed from 112 cases of formalin-fixed, paraffin-embedded penile SCC from Paraguay. Each tumor was sampled 3 to 12 times. ARID1A expression was evaluated by immunohistochemistry using a polyclonal rabbit anti-ARID1A (BAF250A) antibody. An *H* score was calculated in each spot as the sum of expression intensity (0-3+) by extent (0%-100%). Median *H* score per case was used for statistical analysis. ARID1A expression was observed in all cases, ranging from 3% to 100% of tumor cells (median, 95%). In 96 cases (86%), ARID1A expression was observed in 90% or more tumor cells. HPV DNA was detected in 20 (38%) of 52 analyzed samples. There was a significant trend of association between ARID1A and histologic grade. ARID1A expression was not associated with histologic subtype ($P = .61$) or HPV status ($P = .18$). ARID1A expression decreased with decreasing levels of PTEN expression ($P = .01$). ARID1A was expressed in penile SCC, in most cases at high levels. A significant trend of association was found between histologic grade and ARID1A expression, with lower ARID1A expression, lower histologic grades, and decreased PTEN expression.

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1. Introduction

The incidence of penile cancer varies worldwide, with the highest rates seen in developing countries, particularly in Africa and South America, suggesting a relationship between epidemiologic factors and cancer development. Squamous cell carcinoma (SCC) accounts for most of penile cancer, and about half of them are classified as usual SCC [1]. Penile SCC is associated with several established risk factors including phimosis, chronic inflammation, poor hygiene, and smoking. Human papillomavirus (HPV) is an additional risk factor for the development of penile SCC. Its presence has been reported in 20% to 40% of penile cancer. Different subtypes are known to have distinctive clinicopathologic features. HPV-related tumors are more frequently seen in younger patients with basaloid and warty carcinomas [2].

Penile cancer is primarily treated with surgical resection [3]. Locally advanced or metastatic disease often requires a multidisciplinary approach, involving both surgery and systemic therapy [3,4]. Despite improvements in cancer therapy, mortality and morbidity rates remain high for patients with advanced penile cancer [5]. Regional lymph node metastasis is followed by distant metastasis and is the most important prognostic factor for survival. Detection of patients with higher risk for lymph node disease is crucial for appropriate clinical management [6,7]. Reliable prognostic factors for the development of lymph node metastasis are being sought. Histologic features such as pathologic tumor stage, grade, and vascular invasion have been suggested to predict lymph node metastasis [8]. In addition, several biomarkers have been investigated for their potential prediction of lymph node metastasis. The association between p53 expression and lymph node metastasis has been observed [9]. In addition to p53 expression, Ferrandiz-Pulido et al [10] have recently suggested a role for the mammalian target of rapamycin (mTOR) pathway in the development of lymph node metastasis.

Recently, inactivating mutations in *AT-rich interactive domain 1A* (*ARID1A*) have been recognized in several tumors [11,12], suggesting that it is a tumor suppressor gene in many different cell types, including ovarian and endometrial epithelium [13,14]. However, its role in penile cancer has yet to be determined.

The aim of this study is to evaluate the ARID1A immunohistochemical expression in a cohort of penile SCC. Furthermore, the association with pathologic features, HPV status and mTOR pathway markers is assessed.

2. Materials and methods

The current study was approved by the institutional review board at the Johns Hopkins School of Medicine (Baltimore, MD).

2.1. Case selection, tissue microarray construction, morphologic evaluation, and HPV detection

The present study includes tissue samples from 112 patients with invasive SCCs of the penis diagnosed at the Instituto de Patología e Investigación (Asunción, Paraguay) between 2000 and 2011. Cases were selected based on availability of formalin-fixed, paraffin-embedded tissue blocks. From each case, 1 to 4 blocks were selected. Four tissue microarrays (TMAs) were built at the Johns Hopkins TMA Lab Core (Baltimore, MD) using a previously described procedure [15]. Three tissue cores of 1 mm each were obtained per block, giving a representation of 3 to 12 TMA spots per case.

Histologic subtyping was performed in whole-tissue sections using previously published morphologic criteria [16]. Histologic grading was performed spot by spot using previously published and validated criteria [17]. For statistical analysis, the highest grade at the TMA spots was assigned as the histologic grade of the case.

In 52 cases, HPV detection was done by SPF_{10} polymerase chain reaction and DEIA, a DNA enzyme-immunoassay for general HPV detection, as previously described [18]. Tissue samples (5% of the total) in which no HPV presence was expected were used as controls.

2.2. ARID1A expression and scoring system

ARID1A expression was evaluated by immunohistochemistry on 5-μm TMA sections using a polyclonal rabbit (BAF250A) anti-ARID1A antibody (HPA005456; Sigma-Aldrich, St Louis, MO), whose specificity has been confirmed by Western blot [19]. Antigen retrieval was performed by submerging the tissue sections in citrate buffer (pH 6.0) and then in a steamer for 10 minutes. The sections were then incubated with the rabbit antibody at a dilution of 1:200 at 4°C overnight. A positive reaction was detected by the EnVision+ System (Dako, Carpinteria, CA). Only nuclear staining was scored, and tumor stromal cells served as positive internal controls.

An *H* score was assigned in each TMA spot as the sum of the products of the intensity (0 for negative, 1 for weakly positive, 2 for moderately positive, and 3 for strongly positive) multiplied by the extent of immunoexpression (0%-100%), obtaining a value ranging from 0 to 300. For each case, the pooled median of the TMA *H* scores was used for statistical analyses.

2.3. mTOR pathway markers

The mTOR pathway biomarkers (PTEN, phospho-AKT, phospho-mTOR, and phospho-S6) were previously reported on this cohort [20]. Immunohistochemistry was performed using the PowerVision Poly-HRP IHC Detection System (Leica Microsystems, Bannockburn, IL). Sections were deparaffinized, rehydrated, and subjected to heat-induced