# Lack of P16$^{ink4a}$ Over Expression in Penile Squamous Cell Carcinoma is Associated with Recurrence after Lymph Node Dissection

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**Purpose:** There have been conflicting data in studies on the prognostic role of high risk human papillomavirus in penile squamous cell carcinoma. Using P16$^{ink4a}$ over expression as a surrogate marker for high risk human papillomavirus, we evaluated high risk human papillomavirus status with respect to various clinical features, including recurrence and overall survival, among others.

**Materials and Methods:** P16$^{ink4a}$ over expression was evaluated by immunohistochemistry for 119 consecutive patients with penile squamous cell carcinoma. Several variables were recorded including age, stage, histological grade, lymph node status, lymphovascular invasion, metastasis and recurrence. Median followup was 30 months.

**Results:** P16$^{ink4a}$ over expression was detected in 49.5% (59 of 119) of samples. There was no significant difference between P16$^{ink4a}$ negative and P16$^{ink4a}$ positive tumors in terms of stage ($p = 0.518$), histological grade ($p = 0.225$), lymphovascular invasion ($p = 0.388$), overall survival ($p = 0.156$) or lymph node metastasis ($p = 0.748$). P16$^{ink4a}$ negative tumors were more likely to recur overall ($p = 0.04$), especially if patients had positive lymph nodes at diagnosis ($p = 0.002$).

**Conclusions:** These data suggest that P16$^{ink4a}$/high risk human papillomavirus status is associated with recurrence, especially in patients with positive lymph nodes at diagnosis. Thus, patients with P16$^{ink4a}$ negative penile cancer, particularly those with lymph node metastases, may warrant closer observation after surgery.

**Key Words:** penis; carcinoma, squamous cell; genes, p16; recurrence

**Abbreviations and Acronyms**

CT = computerized tomography

HPV = human papillomavirus

HR = high risk

TMA = tissue microarray

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Study received institutional review board approval.

* Financial interest and/or other relationship with Genoptix.

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PENILE cancer is a rare neoplasm in developed countries, representing 0.4% to 0.6% of all malignant neoplasms in the United States and Europe.[1] The majority are squamous cell carcinomas with numerous histological variants.[2–5] The 2 etiologic pathways recently established for penile cancer are HR-HPV induced and HPV negative.[6] HR-HPV induced penile carcinomas have a prevalence of approximately 50% worldwide.[7–10] In the absence of HR-HPV infection, malignancy may develop from chronic inflammatory diseases such as lichen sclerosis and lichen planus.[11–13]

HPV is a nonenveloped DNA virus. Although about 200 genotypes have

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been described, specific genotypes such as HPV-16 and 18 are associated with an increased risk of malignant transformation. Malignancy is thought to be secondary to HPV genome integration into the host genome,[14] which results in disruption of the E2 gene and leads to over expression of viral oncogenes E6 and E7.[14] The binding of E6 to P53 and E7 to hypophosphorylated Rb ultimately leads to accumulation of $P16^{ink4a}$.

Recently $P16^{ink4a}$ over expression by immunohistochemistry has been shown to be a specific and reliable surrogate marker for HR-HPV induced penile carcinoma.[6,15] Mannweiler et al showed that $P16^{ink4a}$ over expression correlated with the detection of HR-HPV DNA with 100% specificity, and it was independent of the HR-HPV genotype and the presence of single or multiple HR-HPV genotypes.[6] Similar to cervical and oropharyngeal HR-HPV induced carcinomas, $P16^{ink4a}$ over expression by immunohistochemistry has also been shown to be a reliable marker to identify HR-HPV induced penile cancers.

Although there have been several attempts to study the prognostic significance of $P16^{ink4a}$ over expression in penile cancer, the results have been somewhat conflicting, in part because many studies are underpowered due to the rarity of the disease.[9,15–18] In addition, the definition of $P16^{ink4a}$ over expression varied, especially in early studies. For a valid definition of the lesion as HR-HPV induced, a correct interpretation of $P16^{ink4a}$ staining is critical. Recent studies have demonstrated that only diffuse, continuous and strong nuclear and cytoplasmic $P16^{ink4a}$ staining is correlated with HR-HPV induced lesions,[6,19] and it is also consistent with the recent recommendation by the CAP/LAST (Lower Anogenital Squamous Terminology) Standardization project on HPV associated lesions.[20]

As $P16^{ink4a}$ over expression can be reliably used as a surrogate marker for HR-HPV, we studied its significance in penile squamous cell carcinoma in terms of various clinical parameters such as lymph node metastasis, survival and recurrence. To our knowledge this is the largest clinical outcome study investigating penile cancer stratified by $P16^{ink4a}$ over expression with the recommended staining interpretation guidelines.

## MATERIALS AND METHODS

### Patients

This study was approved by the institutional review board at Vanderbilt University. Between 1998 and 2013, 127 patients were treated and followed at our institution for penile squamous cell carcinoma. Pathological material from 8 cases was not available for this study, including 5 for which tissue blocks were not available and 3 for which the representative tumor was not present on the tissue microarray. Therefore, 119 patients were included in this study. The clinical data collected from medical chart review included age, tumor localization, treatment, lymph node metastases and recurrence.

Recurrence was defined as the return of local, regional or distant disease found via physical examination or imaging at least 6 months after diagnosis. Local recurrence was defined as recurrent tumor on the penis, regional recurrence as recurrent disease in the inguinal and/or pelvic lymph nodes, and distant recurrence as distant metastasis.

Followup was defined as the time from diagnosis until the last recorded clinical followup or death. Patients alive and/or recurrence-free at the last followup were censored. The American Joint Committee on Cancer TNM staging system for penile cancer (7th edition, 2010) was used for staging.

### Surgical Procedures

The primary tumors were removed via excisional biopsy, circumcision, or partial or total penectomy. Standard inguinal lymphadenectomies performed included superficial and deep nodes. The decision of inguinal or pelvic lymphadenectomy was related to multiple factors such as staging, comorbidity and intraoperative findings, and was decided on a case by case basis. The breakdown of lymphadenectomy in relation to stage, histological grade and $P16^{ink4a}$ status is summarized in table 1.

### TMA Building and $P16^{ink4a}$ Immunohistochemistry

TMAs were constructed from 119 penile cancer cases using a manual arrayer (Beecher Instruments, Sun Prairie, Wisconsin). The original hematoxylin and eosin stained slides were reviewed by a genitourinary pathologist (LLG). Formalin fixed, paraffin embedded tissue blocks were retrieved from the pathology archive. Two tissue cores (1.0 mm each) from each tissue block were used for the TMA.

Immunohistochemical analyses were performed on the Leica Bond-MAX platform (Leica Microsystems, Buffalo Grove, Illinois). Antigen retrieval was performed using the Bond Epitope Retrieval Solution 1. Slides were then incubated with monoclonal antibody to $P16^{ink4a}$ (mouse

**Table 1.** *Lymph node dissection at diagnosis*

| | No Dissection | Dissection | Totals | p Value |
|---|---|---|---|---|
| Overall | 69 | 50 | 119 | |
| P16 status: | | | | |
| Neg | 34 (49.2) | 26 (52) | 60 | 0.913 |
| Pos | 35 (50.2) | 24 (48) | 59 | |
| Pathological T classification: | | | | |
| Tis + Ta | 22 (31.8) | 1 (2) | 23 | <0.001 |
| T1 | 26 (37.6) | 16 (32.6) | 42 | |
| T2 or greater | 21 (30.4) | 33 (66) | 54 | |
| Histological grade: | | | | |
| Well differentiated | 12 (17.39) | 8 (16) | 20 | 0.002 |
| Moderately differentiated | 24 (35.7) | 26 (52) | 50 | |
| Poorly differentiated | 11 (15.9) | 14 (28) | 25 | |
| Unavailable | 22 (31.8) | 2 (4) | 24 | |
| Lymphovascular invasion: | | | | |
| No | 67 (97.1) | 37 (74) | 104 | <0.001 |
| Yes | 2 (2.9) | 13 (26) | 15 | |