$p16^{INK4a}$ is a Marker of Good Prognosis for Primary Invasive Penile Squamous Cell Carcinoma: A Multi-Institutional Study

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Purpose: We assessed the prognostic role of $p16^{INK4a}$ expression in penile cancer with respect to cancer specific survival.

Materials and Methods: Based on a multi-institutional collaboration wax embedded tissues from 92 surgically treated patients, including 27 with total and 65 with partial penectomy, were retrospectively evaluated. After a central histopathological review by 1 pathologist a tissue microarray was constructed for $p16^{INK4a}$ immunostaining. Two independent pathologists evaluated $p16^{INK4a}$ expression, which was correlated with cancer specific survival. The κ statistic was used to assess interobserver variability. Univariate and multivariate Cox proportional hazards analysis was applied to assess the independent effects of prognostic factors on cancer specific survival during a median postoperative followup of 32 months (IQR 6–66).

Results: The κ statistic revealed excellent interobserver agreement (κ 0.934, $p < 0.001$). Two and 5-year cancer specific survival rates for the entire study cohort were 86% and 74%, respectively. The 2 and 5-year rates for patients without and with $p16^{INK4a}$ expression differed significantly (73% and 57% vs 95% and 85%, respectively, $p = 0.011$). Univariate analysis revealed $p16^{INK4a}$ expression as a significant prognostic factor with respect to cancer specific survival ($p = 0.018$). Multivariate analysis identified koilocytosis (HR 0.24, 95% CI 0.07–0.83, $p = 0.024$), $p16^{INK4a}$ expression (HR 0.44, 95% CI 0.23–0.84, $p = 0.013$), and histological stage (HR 3.54, 95% CI 1.88–6.67, $p < 0.001$) and grade (HR 2.47, 95% CI 1.00–6.09, $p = 0.049$) as independent prognostic factors for cancer specific survival.

Conclusions: Results show that $p16^{INK4a}$ seems to be a prognostic parameter for primary invasive penile cancer with excellent interobserver reproducibility. At pathology laboratories without antibodies against $p16^{INK4a}$ conventional histological determination of koilocytosis by the pathologist also appears to provide important prognostic information for cancer specific survival.

Key Words: penis; carcinoma, squamous cell; cyclin-dependent kinase inhibitor p16; prognosis; mortality

Abbreviations and Acronyms

CSS = cancer specific survival
HCI = Harrell concordance index
HPV = human papillomavirus
PC = penile cancer
SCC = squamous cell carcinoma

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PENILE cancer accounts for approximately 0.1 to 0.9 new cases per 100,000 males annually in the Western world.[1] In clinical practice 3 nomograms using conventional histopathological parameters have been developed to predict survival.[2–4] However, additional prognostic biomarkers are desirable to refine the individual prognosis.

HPV positivity was linked to a favorable outcome in subsets of genital and extragenital human carcinomas,

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although this association was not confirmed by others.[5–9] Recently Cubilla et al reported that $p16^{INK4a}$, a tumor suppressor that induces cell cycle arrest and prevents cell division by cyclin-dependent kinase 4 and 6 inhibition, is a reliable marker for high risk HPV infection in penile SCC cases.[10] These data provide the basis for the hypothesis that $p16^{INK4a}$ might be a prognostic marker for penile PC.

To our knowledge we present the first comprehensive study of the prognostic role of $p16^{INK4a}$ for CSS. We retrospectively evaluated a study cohort of 27 patients treated with total penectomy and 65 treated with partial penectomy.

MATERIALS AND METHODS

A retrospective, computerized database analysis identified 110 consecutive patients with PC that was surgically treated between January 1993 and December 2010, including total and partial penectomy in 27 and 77, respectively, and excisional biopsy in 6. Archival wax embedded specimens were drawn from the pathology files affiliated with 6 Charité-University Medicine Teaching Hospitals in Brandenburg, Germany.

Pathology handling of surgical specimens was unique at these 6 institutions. Briefly, maximum tumor diameter was measured grossly before fixation and its distance from the surgical margin and gross appearance were recorded. Pathological dissection followed anatomical landmarks.[11] After separating the foreskin a horizontal cut perpendicular to the urethra was made at the coronary sulcus. The sample was then divided into 4 parts of equal size, which were entirely embedded for histological examination. The glans was cut sagittally and each section was subdivided into a superior and an inferior portion. Multiple contiguous histological sections were taken during dissection and fixed with neutral formalin for 12 hours.

Institutional review board approval to evaluate CSS by death certificates was obtained from the Federal State of Brandenburg medical ethics committee.

Central Histopathological Review

All retrieved slides were histologically reexamined by 1 clinical pathologist (SG) to standardize classification by the TNM classification system, 7th edition, and histological grading by the Broders classification.[12,13] Basaloid, spindle cell and any other SCC subtypes harboring any portion of anaplastic cells were considered high grade while papillary, warty and verrucous carcinomas were considered low grade.[14]

We recorded the results of evaluating conventionally stained sections, anatomical tumor site, lymphovascular invasion and tumor thickness, defined as the vertical

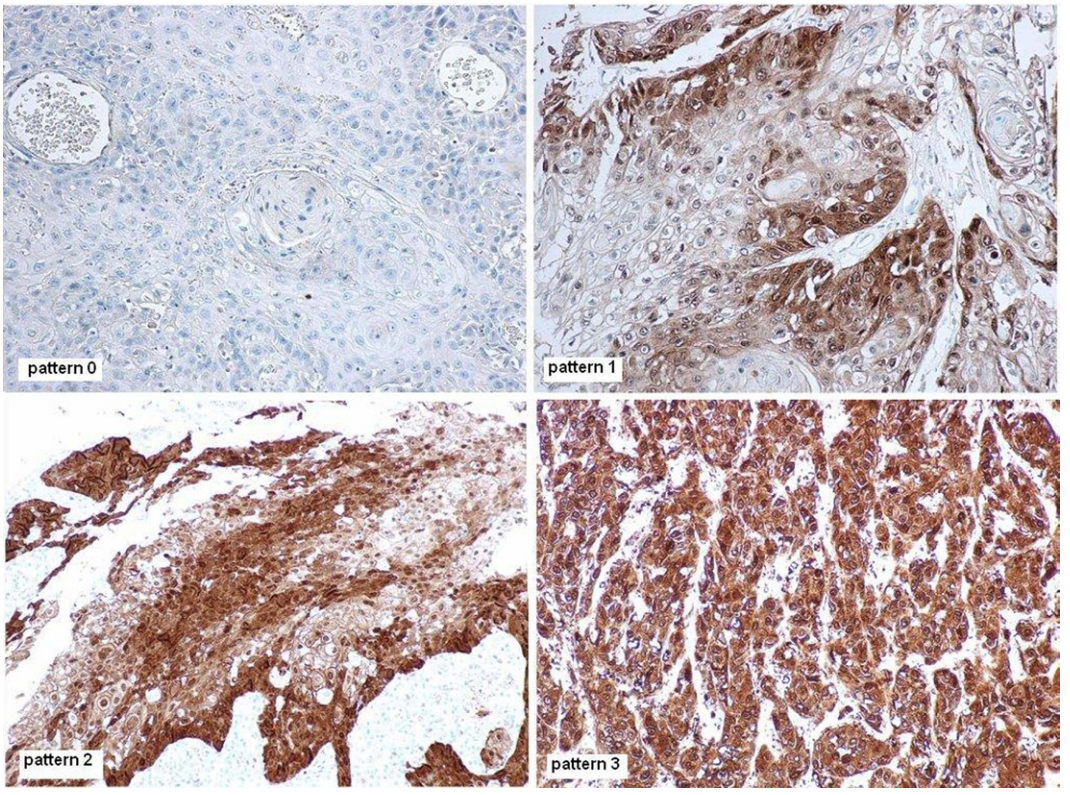

Figure 1. Patterns of $p16^{INK4a}$ expression noted in study cohort penile SCC samples, including pattern 0—penile SCC with complete absence of $p16^{INK4a}$ expression in all epithelial cells, pattern 1—penile SCC with spotty, patchy and discontinuous immunostaining for $p16^{INK4a}$ in suprabasal epithelial cells (pattern 1+), pattern 2—more extensive but discontinuous suprabasal $p16^{INK4a}$ expression compared with pattern 1 and pattern 3—full-thickness, continuous $p16^{INK4a}$ immunostaining in all epithelial cells. Reduced from ×10.