Original Study

Epidermal Growth Factor Receptor, Excision-Repair Cross-Complementation Group 1 Protein, and Thymidylate Synthase Expression in Penile Cancer

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Abstract

Penile cancer is a rare malignancy with high EGFR expression. In 52 patients, we identified that high EGFR expression was associated with poor tumor differentiation and advanced stage, whereas there was no association of these clinical factors with ERCC1 or TS expression. We identified no KRAS mutations and relatively low expression of ERCC1 and TS compared with other squamous malignancies, which could inform future studies of chemotherapy and targeted therapy.

Objective: To describe the expression of tissue epidermal growth factor receptor (EGFR), excision-repair cross-complementation group 1 protein (ERCC1), and thymidylate synthase (TS) in patients with penile cancer and explore their association with stage and outcome. **Methods:** A total of 52 patients with penile squamous cell cancer who were treated at the University of Southern California from 1995 to 2010 were identified. Paraffin-embedded tissue underwent mRNA quantitation and immunohistochemistry for expression of EGFR, ERCC1, and TS. KRAS mutations were evaluated using polymerase chain reaction–based sequencing. **Results:** EGFR overexpression was common by mRNA (median, 5.09; range, 1.92-104.5) and immunohistochemistry. EGFR expression > 7 was associated with advanced stage and poor differentiation ($P = .01$ and .034 respectively) but not with survival in multivariate analysis. ERCC1 mRNA expression was a median of 0.65 (range, 0.21-1.87). TS expression was a median of 1.88 (range, 0.54-6.47). ERCC1 and TS expression were not associated with grade, stage, or survival. There were no KRAS mutations identified. A total of 17 men received chemotherapy; 8 (47%) had an objective response, including 1 with a pathologic complete response. There was a trend for lower expression of EGFR corresponding to a higher likelihood of response (response rate [RR]) to chemotherapy: 67% RR in EGFR mRNA < 7 versus 33% RR in EGFR > 7 ($P = .31$). **Conclusions:** High expression of EGFR mRNA in squamous cell carcinoma of the penis is associated with advanced stage and poor differentiation, but not survival. In our small heterogeneous subset, molecular marker expression did not show a correlation with the likelihood of chemotherapy response. A prospective evaluation of the role of the EGFR pathway and its regulatory environment in penile cancer is warranted. Given the rarity of this cancer, collaborative prospective cohort evaluations and trials need to be encouraged.

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Introduction

Although squamous cell carcinoma of the penis (PSCC) represents up to 10% of male cancers in Asia and South America, only 1250 new cases are diagnosed each year in the United States and approximately 500 cases are diagnosed each year in the United Kingdom.[1] Because of the limited case numbers, it has been difficult to collect cohorts large enough to facilitate examination of the molecular characteristics of PSCC and explore their relationship with clinical outcomes. P53 expression reportedly is associated with poorer outcomes for patients with stage T1.[2] However, there is a deficit of data with regard to other molecular markers that are prognostic in other solid tumors and may influence response to systemic therapy, such as excision-repair cross-complementation group 1 protein (ERCC1) and thymidylate synthase (TS).

Frequent overexpression of the epidermal growth factor receptor (EGFR) has been documented in PSCC series,[3] although the clinical implications have not been clarified. In head and neck squamous cancers, higher EGFR expression is associated with a higher risk of late relapse, as well as a reduced disease-free and overall survival.[4] In vulvar cancer, EGFR overexpression similarly is associated with decreased survival.[5] Preliminary reports have suggested that PSCC is responsive to therapies that inhibit EGFR.[6] In other malignancies, the ability of EGFR expression by immunohistochemistry (IHC) to predict response has been inconsistent. Alternative techniques, such as fluorescence in situ hybridization, detected gene copy number, and the presence of EGFR or KRAS mutations has been associated with response.[7] The clinicopathologic correlates and frequency of EGFR and KRAS mutations in PSCC have not been delineated, but they have potential as prognostic or predictive markers for EGFR-targeted therapy.

The Los Angeles County University of Southern California (USC) medical center cares for a unique population of underserved indigent and "working poor" patients, and has treated a large number of patients with penile cancer. We undertook a retrospective review of all identified patients treated at the Los Angeles County USC and USC Norris Cancer Center between 1995 and 2010 for whom tissue was available for testing of molecular correlates. The goal of the study was to describe the expression of ERCC1, EGFR, and TS in patients with penile cancer and correlate expression levels with clinical and pathologic characteristics and response to therapy.

Materials and Methods

With institutional review board approval (HS-09-00363), patients with PSCC were identified by searching pathology databases. A total of 74 patients were initially identified; 20 did not have tissue available and 2 did not have follow-up available, leaving 52 patients for the study population. This represents an earlier cohort compared with the full clinical cohort published by our institution.[8] Charts were reviewed for clinical information, and survival was systematically ascertained using the cancer registries at each center.

Tissue blocks were selected by an experienced pathologist (YM) and sectioned in 10-μm sections for laser-captured microdissection of tumor tissue and real-time polymerase chain reaction (PCR) for mRNA expression by Response Genetics, Inc, Los Angeles, California (KD), a Clinical Laboratory Improvement Amendments–certified laboratory. The methodology of extracting RNA and DNA from paraffin-embedded specimens has been described,[9] and patent is pending. Quantitation of RNA was performed using a real-time, fluorescence-based PCR detection method (ABI Prism 7700 Sequence Detection System, Thermo Fisher Scientific, Waltham, MA; TaqMan, Applied Biosystems, Foster City, CA). The output is expression of the gene of interest relative to an internal control gene, β-actin. The DNA extracted from the tumor tissue was added to a 15 μL PCR reaction containing mutation-specific primer/probes, dNTPs, and TaqMan reagents. PCR reactions were run on an ABI Prism 7900HT for 42 cycles with concentrations of reagents and temperatures according to the manufacturer's recommendations. Primer and probes specific for KRAS mutations were purchased from ABI or Sigma (St Louis, MO). Mutations of interest included any of 6 mutations in codon 12 and a single mutation in codon 13, which are known to result in amino acid substitutions. These mutations are as follows: codon 12 (G**G**T>G**A**T), (G**G**T>G**C**T), (G**G**T>G**T**T), (**G**GT>**A**GT), (**G**GT>**C**GT), (**G**GT>**T**GT), and codon 13 (G**G**C>G**A**C). A no template control and extraction control were used as negative controls, and standard positive control was composed of synthetic oligonucleotides mutated for the targeted position.

When there was enough tissue available, additional sections were prepared for IHC with standard deparaffinization and antigen retrieval procedures. Primary antibodies for EGFR and ERCC1 were obtained from AbCam (Cambridge, UK); these were incubated overnight at 3°C and developed using the DAB system (DAKO, Carpinteria, CA). For EGFR, nuclei were lightly counterstained with hematoxylin; for ERCC1, to optimize visualization of nuclear antibody staining, no counterstaining was performed. The intensity of IHC staining was graded by pathologist YM as 0, 1+, or 2+. Normal skin samples initially were used to titrate the antibody concentration to optimize the IHC protocol and later served as positive controls. Most samples were noted to have internal controls, with normal skin next to sections of squamous cancer (Figure 1).

Statistical software package SAS Version 9.2 (SAS Institute Inc, Cary, NC) was used for all of the analyses in this study. Pearson's chi-square or Fisher exact test was used to examine the association between categoric demographic and clinical variables. Wilcoxon rank-sum test was used to test differences in not normally distributed continuous variables between groups or subgroups. Time to overall survival was calculated from the date of diagnosis to the date of death (from any cause) or was censored at the date of last follow-up if the patient was still alive at that time. Kaplan–Meier plots were used to estimate the probabilities of overall survival for every year since diagnosis.[10] The log-rank tests were used to compare the differences in survival between dichotomous molecular biomarker RNA expression subgroups, which were based on the cutoff at the median or 75th percentile for RNA expression level of each biomarker in the dataset. All *P* values reported are 2 sided.

Results

Baseline and demographic characteristics of the study population are summarized in Table 1. The median follow-up is 2.3 years (longest, 16.8 years). At presentation, 6 patients had stage Tis, 10 patients had T1, 23 patients had T2, 12 patients had T3, and 19 patients had pathologic documentation of lymph node involvement