Evaluation of the Prognostic Significance of Altered Mammalian Target of Rapamycin Pathway Biomarkers in Upper Tract Urothelial Carcinoma

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OBJECTIVE To evaluate the prognostic value of altered mammalian target of rapamycin (mTOR) pathway biomarkers in upper tract urothelial carcinoma (UTUC).

MATERIALS AND METHODS We performed a multi-institutional review of clinical and pathologic information on patients receiving extirpative surgery for UTUC from 1990 to 2008. Immunohistochemistry for phosphorylated-S6, mTOR, phosphorylated-mTOR, PI3K, phosphorylated-4EBP1, phosphorylated-AKT, PTEN, HIF-1a, raptor, and cyclin D was performed on tissue microarrays from radical nephroureterectomy (RNU) specimens. Prognostic markers were identified and the significance of altered markers was assessed with the Kaplan-Meier analysis and the Cox regression analysis.

RESULTS Six hundred twenty patients were included. Over a median follow-up of 27.3 months, 24.6% of patients recurred and 21.8% died of UTUC. On multivariate analysis, PI3K (odds ratio, 1.28; $P = .001$) and cyclin D (odds ratio, 3.45; $P = .05$) were significant predictors of clinical outcomes. Cumulative marker score was defined as low risk (no altered markers or 1 altered marker) or high risk (cyclin D and PI3K altered). Patients with high-risk marker score had a significantly higher proportion of high-grade disease (91% vs 71%; $P < .001$), non–organ-confined disease (61% vs 33%; $P < .001$), and lymphovascular invasion (35% vs 20%; $P = .001$). The Kaplan-Meier analysis demonstrated a significant difference in cancer-specific mortality (CSM) based on the risk groups. On Cox regression multivariate analysis for CSM incorporating non–organ-confined disease, grade, lymphovascular invasion, tumor architecture, and marker score, high-risk biomarker score was an independent predictor of CSM (hazard ratio, 1.5; 95% confidence interval, 1.04-2.3; $P = .03$).

CONCLUSION Alterations in mTOR pathway correlate with established adverse pathologic features and independently predict inferior oncologic outcomes. Incorporation of mTOR-based marker profiles may allow for enhanced patient counseling, risk stratification, and individualized treatment regimens. UROLOGY 84: 1134–1140, 2014. © 2014 Elsevier Inc.

Upper tract urothelial carcinoma (UTUC) comprises 7%-8% of all renal tumors and 5% of all urothelial malignancies.[1,2] A significant amount of information regarding the prognostic variables and the natural history of UTUC has surfaced over the last several years because of multi-institutional, collaborative investigations.[3,4] In an international study of >1300 patients treated with radical nephroureterectomy (RNU), 5-year survival rate was excellent for patients with low-stage disease (>90%) but dramatically declined for patients with locally advanced or lymph node (LN)–positive disease (5%-10%).[4]

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Improved clinical outcomes may be expected with an earlier detection, enhanced risk stratification, and incorporation of effective systemic therapies. However, screening for UTUC is precluded by the low incidence; prognostic models currently have only approximately 75% accuracy for predicting clinicopathologic outcomes; and neoadjuvant and adjuvant chemotherapies are administered to <15% of patients.[3,5] Despite the increased use of cross-sectional imaging, refinements in surgical technique, and increased use of perioperative chemotherapy over the last 3 decades, no significant improvements in the survival rate for patients with UTUC have been observed.[6]

Prognostic biomarker analysis on biopsy specimens or RNU specimens may improve outcomes by allowing for enhanced risk stratification and individualized patient counseling regarding incorporation of systemic chemotherapy. In addition, biomarker-based identification of biologic pathways involved in UTUC carcinogenesis can be used to predict the response to systemic or targeted therapy. The PI3K/AKT/mTOR pathway is one such promising pathway that has been investigated in bladder urothelial carcinoma as well as other malignancies.[7-12] Basic and translational investigations suggest that mammalian target of rapamycin (mTOR) inhibitors, such as everolimus, may have a role in the treatment of patients with urothelial carcinoma and that the response to mTOR inhibitors may be predicted by gene expression.[13,14] We report systematic evaluation of aberrantly expressed biomarkers within the mTOR pathway in an international multicenter cohort of patients with UTUC. We hypothesize that altered mTOR constituent expression may provide clinically useful information in patients with UTUC.

MATERIALS AND METHODS

Institutional review board approval was obtained before this investigation. A previously described cohort of patients that received RNU or partial ureterectomy (PU) for the management of UTUC from the multi-institutional Upper Tract Urothelial Carcinoma Collaboration was used for analyses.[4] Of the >1300 patients in the original data set, pathologic specimens for tissue microarray (TMA) construction were obtained for 620 patients from RNU or PU specimens, and this subgroup comprised our study cohort. Surgical technique and indication for RNU vs PU was based on surgeon preference. The extent and completion of lymphadenectomy were based on surgeon discretion.

In general, patients were seen at least every 3-4 months in the first postoperative year, semiannually for the second year, and then annually. Comprehensive clinical and pathologic data were maintained in an institutional review board–approved database. Multiple data reviews and quality assurance checks were performed throughout the data collection process to ensure accuracy and completeness of data points.

All surgical specimens were processed according to the standard pathologic procedures. Pathologic variables were prospectively rereviewed by a genitourinary pathologist (P.K.), who was blinded to clinical outcomes, using defined uniform criteria. Tumors were staged according to the *American Joint Committee on Cancer–Union Internationale Contre le Cancer Tumor-Node-Metastasis Classification 2010, seventh edition*.[15] Final specimen tumor grading was assessed according to the 1998 World Health Organization/International Society of Urological Pathology Consensus Classification system.[16] In addition, all specimens were evaluated for tumor location, pattern of tumor growth (papillary vs sessile), presence of lymphovascular invasion (LVI), tumor necrosis, concomitant carcinoma in situ, and pathologic LN status.

TMA staining for mTOR constituents were constructed using a previously described procedure.[17,18] Briefly, immunohistochemical analysis was performed for the following mTOR and related pathway members: phosphorylated-S6 ribosomal protein (Ser 235/236), and phosphorylated-4EBP1 (Thr 37/46), phosphorylated-mTOR, mTOR, PTEN, PI3K, phosphorylated-AKT, raptor, HIF-1α, and cyclin D. Immunostaining was performed using the Dako Autostainer (DakoCytomation, Carpinteria, CA) for PTEN, Discovery XT (Ventana Medical Systems) for phosphorylated-AKT, and BenchMark XT automated stainer (Ventana Medical Systems) for all other antibodies. The staining process has been previously described.[18] Appropriate positive and negative controls were used for each run of immunostains and checked for validation of the assay.

The extent of staining (percentage of positive cells) and intensity of staining were evaluated by an experienced genitourinary pathologist (P.K.). An H score was assigned to each TMA spot as the product of extent of immunoexpression (0 for no cells staining, 1 for <25% cells staining, 2 for 26%-69% cells staining, and 3 for >70% cells staining) intensity and staining (0 for negative, 1 for weakly positive, 2 for moderately positive, and 3 for strongly positive) for an H score range between 0 and 9.[18] Triplicate staining was performed; the mean extent and intensity were used for the H score calculation.

STATISTICAL ANALYSIS

Hospital charts were reviewed, data points entered into standardized forms, and compiled into 1 database. Outcomes were measured by time-to-disease recurrence or to UTUC-specific mortality from the time of surgery. Statistical analyses were performed using SPSS, version 19 (IBM, Chicago, IL) and R package (R Core Team, 2014).

To initially screen for markers, patients were separated into 2 groups based on whether they had an event at 2 years (recurrence and/or cancer-specific death). Prognostic markers were identified by conducting univariate and multivariate analyses for event at 2 years. Consistency was demonstrated by assessing prognostic significance of screened markers for 4 related variables (event at 2 years, recurrence, cancer-specific mortality [CSM], and all-cause mortality). Once prognostic markers were identified, marker status (altered vs unaltered) was decided based on natural integer cutpoints for probability of event at 2 years.

Patients were separated into 2 groups based on whether they had an altered markers status or not. The Fisher exact test and the chi-square test were used to evaluate the association between groups and clinicopathologic