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Kidney Cancer

Higher Expression of Topoisomerase II Alpha Is an Independent Marker of Increased Risk of Cancer-specific Death in Patients with Clear Cell Renal Cell Carcinoma

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Abstract

Background: Tumor-based biomarkers of outcome for patients with clear cell renal cell carcinoma (ccRCC) remain limited, especially for those with low-risk disease. Type IIa topoisomerase (TOPOIIa) is a well-known biomarker of DNA replication and a target for antineoplastic agents, but it has not been evaluated as a biomarker of ccRCC outcome.
Objective: To evaluate the association of TOPOIIa expression in ccRCC and risk of cancer-specific death following surgery.
Design, setting, and participants: Two independent cohort studies were studied in tertiary referral urology practices in the United States. We identified cohorts of 1378 (analytic) and 279 (validation) patients who underwent nephrectomy for clinically localized ccRCC and had paraffin tumor tissue available. TOPOIIa expression was assessed using immunohistochemistry and scored as the number of positive cells per square millimeter.
Outcome measurements and statistical analysis: Our primary end point was cancer-specific survival (CSS). We evaluated TOPOIIa expression as a continuous variable and dichotomized as low versus high. For associations with CSS, we used Kaplan-Meier curves and Cox regression models.
Results and limitations: In both cohorts, patients who had high TOPOIIa expression were approximately three times more likely to experience ccRCC death than those with low expression (hazard ratio [HR]: 2.75; 95% confidence interval [CI], 2.12–3.56; $p = 1.79E\text{-}14$ and HR: 3.45; 95% CI, 1.34–8.88; $p = 0.0104$, respectively). Multivariable adjustment for pathologic features of aggressiveness did not explain these associations, and stratified analysis suggests that the association is more pronounced among patients with low-risk disease as defined by the Mayo Clinic SSIGN (stage, size, grade, and necrosis) score.
Conclusions: Higher TOPOIIa expression is independently associated with increased risk of cancer death among patients undergoing surgery for ccRCC, and the prognostic value is pronounced among patients with low-risk disease. Evaluation of TOPOIIa in ccRCC provides the opportunity to help guide postsurgical surveillance for ccRCC patients as well as inform the design of more targeted clinical trials and novel treatment strategies.

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1. Introduction

Mortality rates for renal cell carcinoma (RCC) have been rising steadily for >3 decades [1]. During the same time period, there has been little change in 5-yr survival for patients diagnosed with RCC (approximately 65%) [2]. Moreover, the small observed increases in survival can be attributed in part to a lead-time bias associated with a rise in the incidental detection of small, clinically dubious tumors [3,4]. Taken together, these trends underscore the need to continue efforts to improve our understanding of the factors that predict RCC aggressiveness, particularly among the growing number of individuals diagnosed with low-risk RCC.

DNA topoisomerases are enzymes that manage the topologic state of DNA in the cell by introducing temporary single- or double-strand breaks in the DNA [5]. Through these strand breaks, the topoisomerase enzymes allow for a wide variety of essential DNA metabolic reactions including replication, transcription, recombination, and chromatin remodeling [6,7]. Several investigative teams have reported that higher intratumor expression levels of topoisomerase enzymes are an indicator of poor prognosis in a variety of human cancers [8–10]. Of interest, drugs targeting topoisomerase enzymes have been developed and represent some of the most successful drugs used to treat human malignancies [11]. Despite this well-known association with cancer aggressiveness, the potential role of topoisomerases in the pathogenesis and prognosis of RCC remains unknown.

Motivated by this gap in understanding, we used two large independent cohort studies to analyze and validate the hypothesis that higher tumor protein expression levels of the type IIa topoisomerase (TOPOIIa) are associated with increased risk of cancer-specific death following surgery for localized clear cell RCC (ccRCC). Moreover, we explore the specific hypothesis that this association is more pronounced among patients with low-risk ccRCC.

2. Patients and methods

2.1. Patient selection

After institutional review board approval, we identified 1663 patients treated with radical nephrectomy or nephron-sparing surgery (NSS) for unilateral, sporadic, noncystic, organ-confined (ie, N0 or Nx, M0) ccRCC between 1990 and 2006 from the Mayo Clinic Rochester Nephrectomy Registry. Of these, 1464 patients (88%) had paraffin-embedded tissue blocks available for immunohistochemical (IHC) staining and available outcome data, and this group represents our analytic cohort. For our validation cohort, we identified 415 patients from the Mayo Clinic Florida Nephrectomy Registry treated with radical nephrectomy or NSS for unilateral, sporadic, noncystic ccRCC between 2000 and 2011. Of these, 337 (81%) had tissue blocks and outcome data available, and this group represents our validation cohort. We discuss further loss of cases in both cohorts resulting from failure of IHC staining in the Results section. Of note, the underlying patient catchment areas for Mayo Rochester and Mayo Florida are separated by >1000 miles (1600 kilometers) and as such represent geographically and culturally unique populations within the United States.

2.2. Data collection

For both cohorts, we abstracted follow-up data from the registry efforts at each institution. Briefly, these data are routinely updated and maintained through a combination of active (mail-out questionnaires) and passive (medical record, linkage to national databases) surveillance by experienced clinical coordinators [12]. Loss to follow-up is <5% for both registry efforts. In addition, we abstracted data on relevant clinicopathologic covariates including age at surgery, gender, symptoms at presentation, Eastern Cooperative Oncology Group performance status, the 2010 American Joint Committee on Cancer (AJCC) primary tumor classification, regional lymph node involvement, distant metastases, the 2010 AJCC TNM stage groupings, tumor size, nuclear grade, and presence of coagulative tumor necrosis. To obtain the pathologic features in a standardized fashion, one urologic pathologist at each site (J.C.C. and K.J.W.) centrally reviewed the microscopic hematoxylin and eosin slides from all specimens without knowledge of patient outcome.

2.3. Type IIa topoisomerase expression

We identified a paraffin-embedded block with representative tumor tissue for each patient in both cohorts and obtained a 5-μm-thick slide for IHC. Technicians in our core facility performed IHC staining for TOPOIIa using a monoclonal antibody and the respective protocol from Leica Microsystems (Buffalo Grove, IL, USA). One of our study pathologists (J.C.C.) trained a certified cytotechnologist (T.H.) to review the stained slides to determine TOPOIIa expression in each tumor. The staining pattern was recorded as the average of the number of positive tumor cells in each of five representative high-powered fields using a Leica DMR microscope (Leica Microsystems, Wetzlar, Germany). With a 10/25 eyepiece and a ×40 objective, the Leica DMR has an object field diameter of 0.625 mm^2, resulting in a high-powered field of 0.307 mm^2. As such, TOPOIIa expression was quantified as the number of positive tumor cells per square millimeter. For the purposes of evaluating intrarater reliability, we selected a random sample of 50 cases from the analytic cohort for re-review by the same cytotechnologist. Similarly, to assess interrater agreement, we randomly sampled 100 cases from the analytic cohort for independent review by a urologic pathologist (K.J.W.).

2.4. Statistical methods

For our analysis in both cohorts, we explored the magnitude of the association of continuous TOPOIIa expression and RCC-specific death by using Cox proportional hazards regression models and summarized the results with hazard ratios (HRs) and 95% confidence intervals (CIs). Smoothing splines were used to explore the functional form of the continuous TOPOIIa, which was quantified as the number of positive cells per square millimeter, and it was determined that the square-root transformation of TOPOIIa had a linear relationship with cancer-specific survival (CSS). Thus, for both the analytic and validation cohorts, the continuous TOPOIIa variable was quantified as the number of positive cells per millimeter in the Cox regression models. In the Cox models, we first estimated the age-adjusted association of TOPOIIa expression with time to RCC-specific death. Then, to assess the association of TOPOIIa expression with RCC-specific death after controlling for other known predictors of ccRCC outcome, we constructed Cox models that adjusted for individual pathologic features of aggressiveness as well as a composite scoring system (Mayo Clinic stage, size, grade, and necrosis [SSIGN] score). We also evaluated TOPOIIa expression as a dichotomized variable (ie, high vs low). To estimate a cut point for dichotomizing TOPOIIa expression into high-versus-low expression, we used the analytic cohort and chose the cut point that maximized the concordance index. As a result, tumors with TOPOIIa expression <16.6 positive cells per square millimeter categorized as "low"; those