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A Multigene Signature Based on Cell Cycle Proliferation Improves Prediction of Mortality Within 5 Yr of Radical Nephrectomy for Renal Cell Carcinoma

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Abstract

Background: There is a critical need for improved prognostic discrimination in patients with renal cell carcinoma (RCC) given the increasing awareness that some patients may be managed with active surveillance, while others with higher-risk disease might benefit from adjuvant therapy following surgery.
Objective: To determine whether a multigene proliferation signature predicts long-term oncologic outcomes in surgically resected RCC.
Design, setting, and participants: The cell cycle proliferation (CCP) score was determined after radical nephrectomy for localized clear cell, papillary, or chromophobe RCC in 565 patients.
Outcome measurements and statistical analysis: The primary end point was disease-specific mortality (DSM), and disease recurrence was a secondary end point. Association with outcomes was evaluated by Cox proportional hazards survival analysis. The CCP score was compared with the Karakiewicz nomogram, and a composite (R-CCP) score was developed.
Results and limitations: A total of 68 patients (12%) recurred and 32 (6%) died of disease within 5 yr of nephrectomy. The CCP score was an independent predictor of recurrence (hazard ratio [HR] 1.50, 95% confidence interval [CI] 1.07–2.09) and DSM (HR 2.49, 95% CI 1.53–4.04) after adjusting for clinical variables using the baseline nomogram. The composite R-CCP score gave a Harrell's concordance index of 0.87 and stratified patients into low- (n = 338) and high-risk (n = 202) categories with 99% and 84% cancer-specific survival probabilities, respectively ($p < 0.001$).
Conclusions: The CCP score is a significant, independent predictor of long-term oncologic outcomes in patients who have undergone nephrectomy for RCC. Combining the molecular classifier with baseline clinical variables allows for accurate, patient-specific risk assessment for use in guiding clinical management.
Patient summary: In this study, we sought to understand how well gene expression information from individual kidney tumors can predict cancer recurrence and death following surgical removal. We found that the combination of the gene expression test and clinical characteristics provides an accurate prognostic assessment to help inform clinical decisions.

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1. Introduction

Despite the increase in tissue-based molecular classifiers for cancer risk stratification [1–4], none have been utilized for patients with renal cell carcinoma (RCC). This is despite substantial recent advances in the understanding of key RCC progression drivers, with mutations in genes such as *BAP1, SETD2*, and *PBRM1* associated with significant survival differences [5–7]. Despite this, the prognostic accuracy of individual somatic mutations is often limited in localized malignancies, and measurement of gene expression across a panel of genes is more frequently utilized clinically [8,9]. An example of this is the cell cycle progression (CCP) score, an RNA expression assay that measures the activity of genes involved in cellular proliferation currently utilized clinically as a prognostic marker in men with prostate cancer [4,10,11].

Current clinical tools to stratify patients with RCC are restricted to a limited set of variables such as tumor size. While renal mass biopsy can be utilized in patients with untreated renal masses to assist with management decisions, it provides relatively inaccurate data regarding tumor grade [12–15]. Even when surgical pathology is available, prognostic assessment relies on a small number of clinical and pathologic factors in order to identify patients at a high risk of recurrence. Importantly, there is likely a subset of patients with more aggressive disease who could benefit from adjuvant systemic therapy following surgery [16].

Prior data, including an initial pilot study, support an association between cell cycle regulators and oncologic outcomes in RCC [17,18]. We therefore hypothesized that the established CCP gene signature would provide additional prognostic information beyond standard clinical parameters, offering a new approach for risk stratification. In this multisite study, we sought to determine the performance of the CCP classifier for predicting disease-specific mortality (DSM) in patients undergoing radical nephrectomy for localized RCC and develop a novel composite score combining gene expression with baseline clinical information.

2. Patients and methods

2.1. Patient cohort

Tumor tissue and clinicopathologic data were obtained from 670 patients who underwent radical nephrectomy for RCC at Massachusetts General Hospital (MGH) from 2000 to 2007 and at the University of Michigan (UM) from 2000 to 2009. Eligible patients had localized pT1–T3 clear cell, papillary, or chromophobe RCC. Patients were not eligible if they received any neoadjuvant therapy or had bilateral, sarcomatoid, collecting duct, node-positive tumors, or any clinical evidence of metastatic disease. Patients were also required to have a $\geq$37 d (0.1 yr) follow-up, rendering this a landmark analysis. Five patients were excluded due to the identification of sarcomatoid or unclassified RCC histology on pathology re-review, and 100 patients were excluded due to insufficient tumor content and/or extracted RNA, leaving 565 patients in the final cohort.

2.2. Sample preparation and CCP score

Formalin-fixed paraffin-embedded blocks were re-reviewed by a genitourinary pathologist at each center (R.M. and C-L.W.) to ensure accurate histologic classification. A representative area of the highest grade region of viable tumor was then identified from each patient, and $5 \times 10\ \mu m^2$ thickness sections were cut sequentially onto slides for processing [4]. Samples were deparaffinized, and total RNA was extracted using miRNeasy (Qiagen). Isolated total RNA was treated with DNase I (Sigma), and High-capacity cDNA Archive Kits (Applied Biosystems) was used to convert total RNA into single-strand cDNA. After preamplification, samples were loaded onto Taqman low-density arrays (TLDAs; Applied Biosystems) to measure gene expression [4]. All samples were run in duplicate, at a minimum.

Using the TLDA platform, CCP score was calculated based on the unweighted average expression of 31 cell cycle genes normalized to the expression of 15 housekeeping genes. Higher scores represent greater expression of cell cycle genes. A one-unit change in score represents doubling of gene expression. All molecular data were generated blinded to clinical data and matched only when the data and final analytic plan were locked.

2.3. Calculation of Karakiewicz nomogram and R-CCP scores

To control for baseline patient characteristics, Karakiewicz nomogram point totals were calculated as previously described, and the patient data included all necessary information for calculation of this score [19]. This model was selected based on its end point (DSM) and inclusion of clear cell, papillary, and chromophobe histologic subtypes in the development and validation of the nomogram. The prognostic accuracy of the Karakiewicz and CCP scores were compared, and a combined score (R-CCP) consisting of a weighted average of the CCP and Karakiewicz scores was developed. The weights were determined according to the log hazard ratios (HRs) from a Cox model with the CCP and Karakiewicz scores as covariates and with DSM as the end point, censored at 5 yr. Additionally, a prespecified cutpoint was delineated in order to categorize patients into low- and high-risk groups according to the R-CCP score. The high-risk threshold was prespecified as the risk of DSM corresponding to the lowest 10th percentile of patients with pT3 or high grade (Fuhrman 3–4) disease, meaning that 90% of patients with high-risk pathology would be expected to fall above this threshold. The R-CCP score associated with this risk level was established as the cutpoint for categorization. That is, given the absence of a currently validated risk threshold, we selected a risk cutoff that would otherwise (in the absence of additional molecular information) encompass the vast majority of patients with adverse pathology at the time of nephrectomy.

2.4. Statistical analysis

The statistical analyses followed a prespecified and signed statistical analysis plan. Follow-up time was calculated as the time from surgery, and all patients who were not lost to follow-up and did not have an adverse event were censored at 5 yr. The primary end point was DSM, and the primary aim was to determine the association between the CCP score and DSM after adjusting for clinicopathologic characteristics. Secondary objectives included assessment of the correlation between CCP and recurrence-free survival (RFS), and the performance of the combined score and combined score categories for predicting DSM.

Chi-square tests were used to compare clinical and pathologic characteristics between the two sites, and Cox proportional hazards models were used to evaluate the association of the CCP score with recurrence and death. CCP was modeled as a numeric variable, and the test statistic was based upon the partial likelihood ratio test, whose value is given by the change in the deviance of the full model versus the

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