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Clinical Investigation: Genitourinary Cancer

Prognostic Utility of Cell Cycle Progression Score in Men With Prostate Cancer After Primary External Beam Radiation Therapy

Stephen J. Freedland, MD,[*,‡,§] Leah Gerber, MS,[*,‡,§] Julia Reid, MStat,[¶] William Welbourn, PhD,[¶] Eliso Tikishvili, MD,[¶] Jimmy Park, BS,[¶] Adib Younus, BS,[¶] Alexander Gutin, PhD,[¶] Zaina Sangale, MD,[¶] Jerry S. Lanchbury, PhD,[¶] Joseph K. Salama, MD,[†,||] and Steven Stone, PhD[¶]

*Departments of *Surgery and [†]Radiation Oncology, Durham VA Medical Center, Durham, North Carolina; Departments of [‡]Surgery (Urology), [§]Pathology, and [||]Radiation Oncology, Duke University School of Medicine, Durham, North Carolina; and [¶]Myriad Genetics, Inc, Salt Lake City, Utah*

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Summary

This study evaluated whether an mRNA-based diagnostic assay (CCP score) can be used as a prognostic indicator in EBRT-treated prostate cancer patients. The CCP score was significantly predictive of biochemical recurrence even after adjustment for standard clinical parameters. This is also the first study that evaluated the prognostic utility of CCP score in African American patients.

Purpose: To evaluate the prognostic utility of the cell cycle progression (CCP) score, a RNA signature based on the average expression level of 31 CCP genes, for predicting biochemical recurrence (BCR) in men with prostate cancer treated with external beam radiation therapy (EBRT) as their primary curative therapy.

Methods and Materials: The CCP score was derived retrospectively from diagnostic biopsy specimens of men diagnosed with prostate cancer from 1991 to 2006 (n = 141). All patients were treated with definitive EBRT; approximately half of the cohort was African American. Outcome was time from EBRT to BCR using the Phoenix definition. Median follow-up for patients without BCR was 4.8 years. Association with outcome was evaluated by Cox proportional hazards survival analysis and likelihood ratio tests.

Results: Of 141 patients, 19 (13%) had BCR. The median CCP score for patient samples was 0.12. In univariable analysis, CCP score significantly predicted BCR ($P = .0017$). The hazard ratio for BCR was 2.55 for 1-unit increase in CCP score (equivalent to a doubling of gene expression). In a multivariable analysis that included Gleason score, prostate-specific antigen, percent positive cores, and androgen deprivation therapy, the hazard ratio for CCP changed only marginally and remained significant ($P = .034$), indicating that CCP provides prognostic information that is not provided by standard clinical parameters. With 10-year censoring, the CCP score was associated with prostate cancer-specific mortality ($P = .013$). There was no evidence for interaction between CCP and any clinical variable, including ethnicity.

Conclusions: Among men treated with EBRT, the CCP score significantly predicted outcome and provided greater prognostic information than was available with clinical parameters. If

Reprint requests to: Stephen J. Freedland, MD, Division of Urology, Duke University Medical Center, Box 2626, Duke University School of Medicine, Durham, NC 27710. Tel: (919) 668-5946; E-mail: steve.freedland@duke.edu

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validated in a larger cohort, CCP score could identify high-risk men undergoing EBRT who may need more aggressive therapy.

Introduction

For men with newly diagnosed prostate cancer, accurate risk stratification enables appropriate clinical management. Currently, clinical parameters such as Gleason score, serum prostate-specific antigen (PSA) level, and clinical T stage provide some prognostic information. For men identified with low-risk disease, active surveillance or other deferred intervention regimens may be the best choice (1). Alternatively, for men with intermediate- and high-risk disease, curative therapy is warranted. However, within all of these risk groups there is heterogeneity of clinical outcomes. Therefore, improved discrimination would be useful to determine optimal therapy for all patients.

For men treated with external beam radiation therapy (EBRT), existing prognostic models are hindered by the fact that the tumor is not removed, precluding accurate ascertainment of stage, grade, and tumor volume (2). Moreover, no prognostic biomarkers with the exception of PSA have proven sufficiently informative to impact clinical care. Approximately 30% of the patients treated with definitive ERBT progress biochemically. If these patients could be identified at diagnosis, they might benefit from more aggressive therapies (eg, EBRT with brachytherapy boost) (3) and/or concurrent administration of systemic therapies like androgen deprivation therapy (ADT). However, because more-intensive therapies also result in unwanted morbidities, they should be avoided in men who are likely to be controlled after EBRT alone. As such, accurate assessment of the risk of biochemical progression is crucial to provide optimal clinical care.

Recently we developed a prognostic RNA signature that helps characterize prostate cancer aggressiveness (4, 5, 10). The signature is based on determining the expression levels of cell cycle progression (CCP) genes and likely measures the fraction of tumor cells that are actively dividing. Because the signature is based on fundamental cancer biology, it potentially provides prognostic information in many different clinical settings. In fact, the signature has been associated with adverse outcome in conservatively managed cohorts from the United Kingdom and in surgically treated cohorts from the United States (4, 5, 10). However, its ability to predict outcome after EBRT is untested. Here we evaluated the prognostic utility of the CCP score for predicting biochemical recurrence (BCR) in men treated with EBRT as their primary curative therapy. We hypothesized that high CCP score would be correlated with poor outcome (ie, BCR) and that this association would hold even after controlling for standard clinical characteristics.

Methods and Materials

Cohort

Patients were included if they underwent diagnostic biopsy for prostate cancer between 1991 and 2006 and were treated with definitive EBRT (either alone or in combination with ADT). Patients without available formalin-fixed and paraffin-embedded blocks containing their original diagnostic biopsy were excluded. Additional predefined exclusion criteria were pretreatment PSA level >100 ng/mL and patients who began treatment >2 years after diagnostic biopsy. Finally, patients with follow-up data for <3 years who had not developed BCR within this time frame were excluded.

Sample preparation and real-time polymerase chain reaction

Formalin-fixed and paraffin-embedded biopsy tumor blocks underwent pathologic evaluation. The original diagnostic hematoxylin and eosin-stained tissue sections from each block were evaluated for tumor content. The tumor area was identified, measured (length in millimeters), and circled. On the basis of the tumor length, additional unstained 10-μm sections of tissue were cut so that at least 20 mm of total tumor (millimeters on hematoxylin and eosin × number of slides) were used for subsequent RNA isolation.

Selected tumor regions were removed from the unstained slides by macro-dissection according to pathologist's instructions. The tumor region was dissected directly into a centrifuge tube and the paraffin removed using xylene and washed with ethanol. Samples were treated overnight with proteinase K digestion at 55°C. Total RNA was extracted using miRNeasy (Qiagen, Valencia, CA) as described by the manufacturer (with the exception of the extended proteinase K digestion). Isolated total RNA was treated with DNase I (Sigma, St. Louis, MO) before complementary DNA (cDNA) synthesis. A high-capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) was used to convert total RNA into single-strand cDNA as described by the manufacturer. Before measuring expression levels, the cDNA was preamplified with a pooled reaction containing 31 CCP and 15 housekeeping gene TaqMan assays. Preamplification reaction conditions were 95°C for 10 minutes, 95°C for 15 seconds, and 60°C for 14 cycles and dilute 1:20 using 1× Tris-EDTA buffer before loading on Taqman Low Density Arrays (Applied Biosystems) to measure gene expression. All samples were run in triplicate.

CCP score calculation

The CCP score was calculated from the expression data of 31 CCP genes normalized by the expression of 15 housekeeping genes as previously described (4). The CCP scores were rejected if more than 9 CCP genes were missing ($n=5$) or if the standard deviation of the CCP scores within the triplicate was >0.5 ($n=2$; these 2 CCP scores were also part of the group of CCP scores rejected because of missing genes).

Statistical analysis

Survival analysis was carried out using Cox proportional hazards models, to assess the association between the CCP score as a continuous variable and risk of BCR. The primary endpoint was time to BCR event. Biochemical recurrence was defined as