Validation of GEMCaP as a DNA Based Biomarker to Predict Prostate Cancer Recurrence after Radical Prostatectomy

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Purpose: We aimed to validate GEMCaP (Genomic Evaluators of Metastatic Cancer of the Prostate) as a novel copy number signature predictive of prostate cancer recurrence.

Materials and Methods: We randomly selected patients who underwent radical prostatectomy at Cleveland Clinic or University of Rochester from 2000 to 2005. DNA isolated from the cancer region was extracted and subjected to high resolution array comparative genomic hybridization. A high GEMCaP score was defined as 20% or greater of genomic loci showing copy number gain or loss in a given tumor. Cox regression was used to evaluate associations between the GEMCaP score and the risk of biochemical recurrence.

Results: We report results in 140 patients. Overall 38% of patients experienced recurrence with a median time to recurrence of 45 months. Based on the CAPRA-S (Cancer of the Prostate Risk Assessment Post-Surgical) score 39% of the patients were at low risk, 42% were at intermediate risk and 19% were at high risk. The GEMCaP score was high (20% or greater) in 31% of the cohort. A high GEMCaP score was associated with a higher risk of biochemical recurrence (HR 2.69, 95% CI 1.51–4.77) and it remained associated after adjusting for CAPRA-S score and age (HR 1.94, 95% CI 1.06–3.56). The C-index of GEMCaP alone was 0.64, which improved when combined with the CAPRA-S score and patient age (C-index = 0.75).

Conclusions: A high GEMCaP score was associated with biochemical recurrence in 2 external cohorts. This remained true after adjusting for clinical and pathological factors. The GEMCaP biomarker could be an efficient and effective clinical risk assessment tool to identify patients with prostate cancer for early adjuvant therapy.

Abbreviations and Acronyms

aCGH = array comparative genomic hybridization

BCR = biochemical recurrence

CAPRA-S = Cancer of the Prostate Risk Assessment Post-Surgical

CNA = copy number aberration

FFPE = formalin fixed, paraffin embedded

GEMCaP = Genomic Evaluators of Metastatic Cancer of the Prostate

GS = Gleason score

PSA = prostate specific antigen

RP = radical prostatectomy

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In 2015 WHO estimated a diagnosis of 1.1 million new prostate cancer cases, resulting in 300,000 deaths worldwide and 27,540 deaths in the United States.[1] An estimated 130,000 RPs are performed each year and RP remains a curative procedure for early stage prostate cancer.[2–4] Depending on tumor grade and stage 20% to 35% of patients experience biochemical disease progression within 5 years of local treatment.[5,6] In addition to pathological grading and staging, PSA remains the most widely used and validated biomarker to predict the risk of BCR after local therapy. There is still a degree of clinical uncertainty when deciding which subsets of patients are at risk for recurrence, especially in the setting of intermediate clinicopathological risks.

Using traditional clinicopathological features alone to direct adjuvant therapy following surgery for prostate cancer results in overtreatment of some patients and under treatment of others.[7] Giving adjuvant therapy to all patients with adverse pathological features is not ideal due to side effects of therapy while withholding adjuvant therapy from all patients is not ideal due to the possibility of missing a window for cure. Advances in genomics, high resolution arrays and our increased understanding of the molecular biology of tumor aggressiveness has allowed for the development of new tissue based biomarkers to move beyond the traditional clinical and pathological features used to risk stratify patients for adjuvant therapy.[8] Tissue based biomarkers could give patients a more personalized risk assessment based on their unique tumor profile and reduce uncertainty in treatment decision making.

We previously reported the discovery of a suite of DNA based biomarkers associated with prostate cancer recurrence and metastasis using aCGH to analyze regions of CNA (gain or loss) in tumor genomes.[9–12] They mapped to a set of loci termed GEMCaP (supplementary Appendix, http://jurology.com/). A positive GEMCaP signature, defined as 20% or greater of the loci with CNAs, outperformed a traditional nomogram when combined with clinicopathological characteristics.

In this study we aimed to validate the GEMCaP assay as a DNA based biomarker which is independent of traditional risk assessment by CAPRA-S score, relatively easy to obtain, inexpensive to assay and simple to interpret by patients and clinicians to predict disease progression after RP. Using high resolution aCGH we performed genome-wide analysis of copy number alterations in 2 independent cohorts of randomly selected men at low, intermediate or high clinical recurrence risk who underwent RP and received no further treatment until the time of metastasis. GEMCaP performance was compared to the CAPRA-S score, a current validated standard risk model.[13] To evaluate the independence of the clinical usefulness of GEMCaP to predict BCR the predictive performance was assessed after adding the GEMCaP score to the CAPRA-S risk model.

PATIENTS AND METHODS

Patient Selection and Study Design

We obtained institutional review board approval to retrospectively identify a total of 203 patients who underwent RP at Cleveland Clinic or University of Rochester from 2000 to 2005 and had tissue available for research. Patients were selected based on preoperative D'Amico low to high risk groups using clinical stage, PSA and GS at diagnosis. Study inclusion criteria included RP with standard pelvic lymph node dissection for prostate cancer with post-RP PSA less than 0.1 ng/ml, adequate tissue for aCGH and clinical data available for analysis. We excluded patients with persistent disease after surgery in the form of detectable PSA after RP, unknown nodal stage (pNx), neoadjuvant or adjuvant androgen deprivation therapy or radiotherapy, distant metastatic disease and incomplete followup. In addition, 22 of 203 patients (11%) with inadequate DNA for aCGH analysis and 38 (21%) in whom a combination of Agilent® and manual aCGH quality control failed were excluded from study. This resulted in 140 patients in the final analysis.

A high GEMCaP score was defined as 20% or more of the genomic loci showing copy number gain or loss in a given tumor as in previous studies.[12] We used available clinical and postoperative pathological staging to calculate the postoperative CAPRA-S score. The primary goal was to validate GEMCaP as a DNA biomarker for disease recurrence after RP.

Sample Preparation

All tumor tissues were evaluated from FFPE prostatectomy specimens by a single genitourinary pathologist (CM-G or JJ-G) at each institution to avoid variability. Pathological TNM stage and Gleason score were assigned based on the 2005 modified Gleason criteria. Tumor tissue from the highest Gleason areas representative of the Gleason score was macrodissected from FFPE RP specimens (approximately 10 slides at 15 μm) to maximize tumor content. They were subjected to DNA extraction using the QIAamp® DNA FFPE Tissue Kit according to the manufacturer protocol.

Array Comparative Genomic Hybridization

We applied aCGH to identify CNA regions (gain and loss) in the genome using an oligonucleotide microarray