DNA Methylation Profiling Reveals Novel Diagnostic Biomarkers In Renal Cell Carcinoma

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Introduction

- Renal cell carcinoma (RCC), while usually fatal when metastatic, is successfully treated with surgery when low volume tumors are confined to the kidney. Because most early stage renal tumors do not result in symptoms, there is a strong need for biomarkers that can detect the presence of the cancer.
- Previous studies indicate DNA methylation changes are early events in carcinogenesis and detection of DNA methylation at RCC candidate loci suggests tumor specific changes could be used diagnostically, but the performance characteristics of these markers have limited utility. Small studies showed simultaneous measurement of several differentially methylated loci could improve performance.
- We hypothesized that better RCC biomarkers could be identified by genome-wide DNA methylation studies. We profiled DNA methylation (Illumina HumanMethylation27 arrays) in tumor and adjacent normal tissue from 98 RCC patients of different histological subtypes utilizing clustering, regression, and classification tools to identify diagnostic biomarker panels.

Identification of Differential Methylation Between Tumor and Benign Adjacent Kidney Tissue

- To identify CpGs with tumor-specific aberrant methylation, we performed linear mixed modeling with paired tumor/normal data at each CpG (including gender and age as covariates), and found 5,880 CpGs significantly different between tumor and normal. Using the most significant CpGs, we performed hierarchical clustering by sample and CpG (Figure 1). We repeated the previous analysis including only the 63 patients with clear cell RCC and found ~85% of the differentially methylated CpGs still significant.

Identification of CpGs Distinguishing Kidney Tumor and Benign Adjacent Normal Tissue

- We used Prediction Analysis of Microarrays (PAM) to identify a set of markers best distinguishing normal from malignant kidney samples. Incorporating all histological subtypes, we identified 20 CpGs (Figure 2 A) and restricting to clear cell RCC patients, we identified 11 CpGs (Figure 2 B). Hierarchical clustering on both samples and CpGs showed almost perfect classification.

RCC Diagnostic Biomarker Models

- With our data as a training set, we built a logistic regression model from the PAM 20 CpG diagnostic list capable of discriminating between tumor and benign adjacent tissues, irrespective of tumor histology. We selected a 5 CpG model with the greatest area under the receiver operating characteristic (ROC) curve and an Akaike Information Criterion (AIC).
- We used publicly available The Cancer Genome Atlas (TCGA) data from over 1,110 tumors as a validation test set. The ROC curve for the test set remained outstanding in all tumor types collectively (Figure 3 A and B), and independently (Figure 3 C, D). Model sensitivity was largely independent of cancer stage and primary tumor size.

Clear Cell RCC Diagnostic Biomarker Models and RNA Expression

- When ccRCC patients were analyzed separately, 4 CpGs produced an optimized diagnostic marker model. TCGA specimens had an outstanding ROC AUC for this model (Figure 4 A). DNA methylation differences between tissues demonstrated good agreement between the datasets (Figure 4 B).
- RNA-seq data was available for some TCGA ccRCC patients, allowing us to investigate whether changes in DNA methylation we observed are correlated with nearby gene transcript levels. Significantly different levels of gene expression were observed in 9 of the 11 genes closest to the significant CpG in the expected direction based on methylation status (Figure 4 C and D).

Conclusions & Future Directions

- We found large scale, genome-wide methylation changes that differentiated malignant from normal kidney tissue and developed panels of CpGs that can discriminate the most common histological subtypes of RCC from normal renal tissue. These methylation differences were validated independently in TCGA data and retained high sensitivity and specificity.
- Because of the high sensitivity and specificity of our biomarkers, these panels are strong candidates for development of a clinical test detecting the major histologic types of RCC. Detection of these methylated sequences could be deployed as a non-invasive detection tool in patient blood or urine for early detection in high risk populations, for monitoring patients after definitive surgical treatment, or possibly in monitoring response to therapy in patients with advanced disease.
- Additional work is necessary to evaluate the performance of these methylation markers in less common RCC histologies, as well as benign entities. Further investigation of the consequences of DNA methylation at these predictive loci, including assessments of gene expression and other DNA alterations, could provide insights into important biological processes common to different RCC histological subtypes.