URINARY RNA SIGNATURES IN RENAL CELL CARCINOMA (RCC)

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Abstract

Background. Renal Cell Carcinoma (RCC) is diagnosed in approximately 65,000 people annually in the United States, accounting for ~3% of all human cancers. The death rate for patients diagnosed with RCC is about 22%. Additionally, recurrence-free survival of patients deemed high-risk using conventional tumor stage and grade criteria is only 44%, and the molecular components that drive high grade, metastatic disease, remain largely unknown. Since tumor histology has limited value in determining patient outcome, there is a clear need for better predictive tools to guide treatment strategies. The ability to predict tumor prognosis at the time of diagnosis could modulate RCC treatment strategies to include more aggressive approaches for patients deemed high risk for metastasis. This personalized approach would help to further stratify patients where traditional histological diagnoses fall short. Urine provides an easily obtainable liquid biopsy that is suitable RNA purification and NextGen sequencing, and can be used to identify biomarkers at the transcript level capable of distinguishing between metastatic and non-metastatic patients.

Methods. Urine was collected pre-surgically at the time of diagnosis from 24 patients whose tumors did not recur (>5 years follow-up) and 28 patients who later experienced metastatic disease. The samples were centrifuged, cellular debris discarded, and RNA was purified from the resulting supernatant. RNA sequencing was carried out on the Illumina HiSeq 2500 platform. ROC, MDS and random forest approaches were used to investigate the predictive accuracy of the 20 urinary transcript molecular signature. ATCG, Protein Atlas, and GEO datasets were queried to investigate the normal/tumor tissue specificity of the signature.

Results. 20 transcripts were identified that reliably classified non-metastatic and metastatic patients using dimensionality reduction. Additionally, a previously unobserved isoform was discovered for one of the gene transcripts. Furthermore, the biomarker panel has promising classification power in publicly available data.

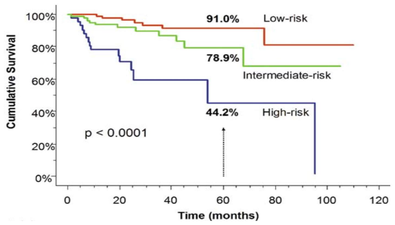
Conclusions. The results of these studies show the feasibility of identifying urinary biomarkers for tumor diagnosis and prognosis. The validation of the reported urinary signature will demonstrate whether it may have significant clinical utility to stratify treatment for patients at high risk for tumor progression, metastasis, and poor outcome.

Background

About 60,000 new cases of renal cell carcinoma occur annually in the United States. 80% of such cases are of the clear cell subtype (ccRCC). 20-30% of patients with ccRCC tumors have metastatic disease at the time of diagnosis, which correlates to overall median survival of less than a year[1].

Using traditional tumor stage and grade criteria, predictions of overall survival in patients deemed high risk is only 44%[2].

Because ccRCC disease outcome cannot be robustly predicted from tumor stage or grade, there is a clear need for biomarkers that can be used in the clinic to help guide treatment strategies.



Experimental Design

Tumor Pathology

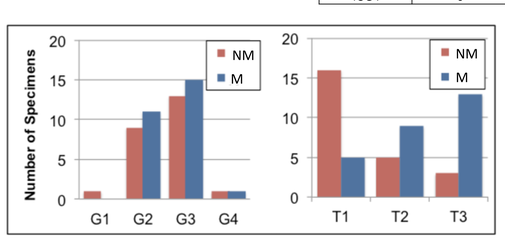
(A) Compilation of RCC tumor stage and grade information associated with the 52 urine samples collected from patients at the time of nephrectomy. Follow-up information permits categorization of these tumors as non-metastatic or as recurrent/metastatic.

(B) Histogram representing the distribution of tumor Grade (G) and Stage (T) associated with the 5 urine 2 samples. Recurrent/metastatic samples are shown as blue bars, and non-metastatic samples are shown as red bars. These plots show that tumor grade does not successfully predict risk of metastasis, and tumor stage was predictive only for high stage tumors.

(A)

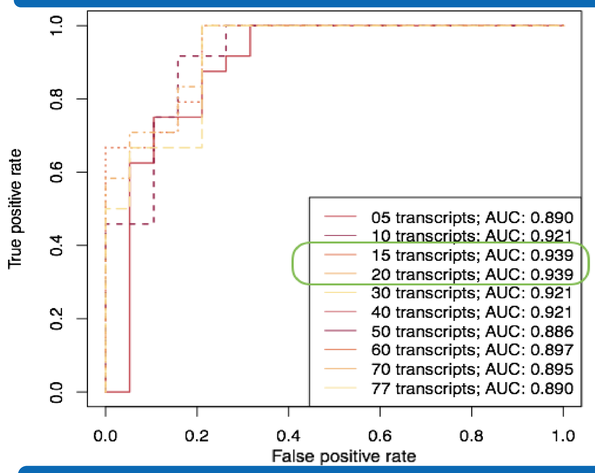
Stage/Grade	Non-Metastatic	Metastatic
T1G?	0	1
T1G1	1	0
T1G2	11	1
T1G3	7	3
T1G4	1	1
T2G1	0	0
T2G2	0	7
T2G2/G3	0	1
T2G3	6	4
T2G4	0	0
T3G1	0	0
T3G2	2	7
T3G3	4	7
T3G4	0	1

(B)



Results

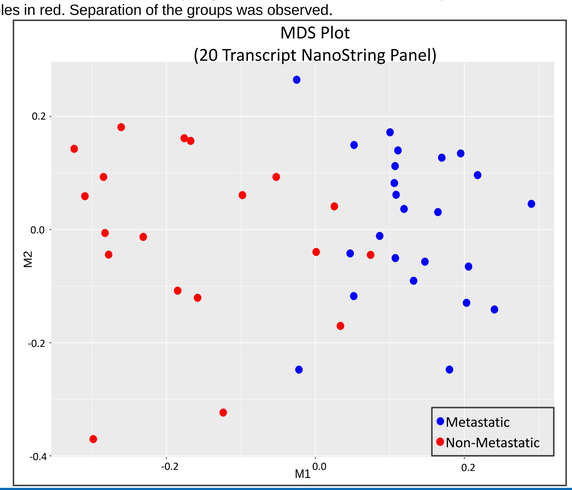
Receiver Operator Curves



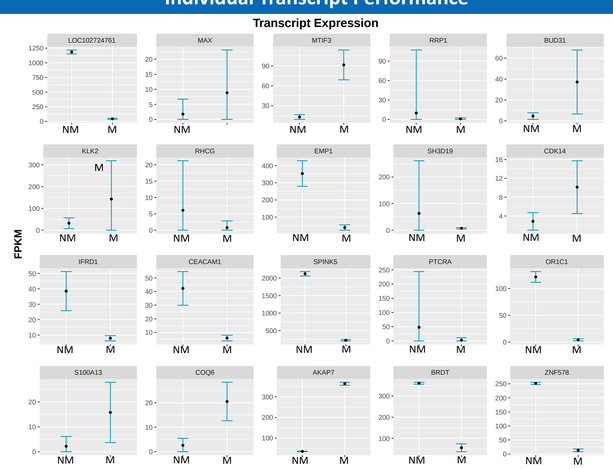
Receiver operator curve (ROC) plotting the true positive rate against the false positive rate. i.e. Sensitivity vs. 1-specificity. This plot shows how well the panel can correctly classify a sample. The greater the area under the curve, the higher the predictive value of the panel. A perfect classifier will have a true positive rate of 1 and a false positive rate of 0. Of the 77 transcripts identified, a smaller subset of 15 to 20 samples correctly classifies these samples with ~94% accuracy. These transcripts will be validated against a new set of ccRCC urine samples using the NanoString platform.

Clustering Based on Differential Transcript Expression

Based on ROC Stratification, the highest performing 20 transcript panel was brought forward from an initial group of 77 transcripts (p=0.005). The candidate 20 transcript signature was clustered in an MDS plot with metastatic samples in blue and non-metastatic samples in red. Separation of the groups was observed.



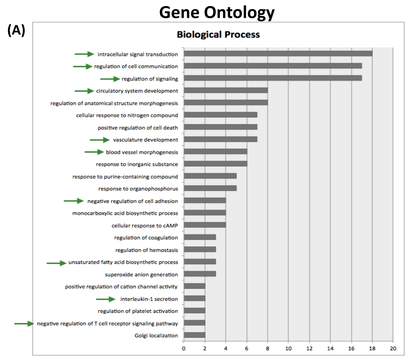
Individual Transcript Performance



Individual transcript expression of the 20 transcript signature. Non-metastatic (NM) and metastatic (M) expression values are shown for each. Dots represents the mean expression values. FPKM expression values are represented on the y-axis and show a wide spread of relative expression between transcripts. Within the 20 transcripts, some are highly expressed in metastatic samples, while others are highly expressed in non-metastatic samples.

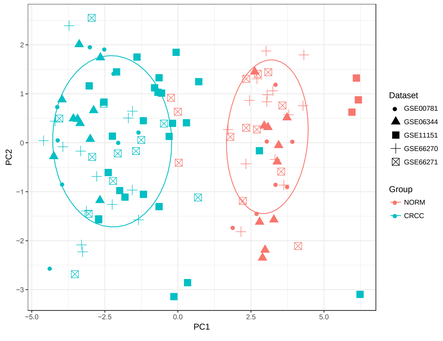
Gene Ontology and Diagnostic Power

(A)



(A) Gene Ontology, showing enrichment of metastatic samples over non-metastatic samples showing enrichment for elements suggestive of an aggressive phenotype. These elements include markers of angiogenesis, epithelial to mesenchymal transition, inflammation, and cell growth. Groups of particular interest are marked with green arrows.

(B)



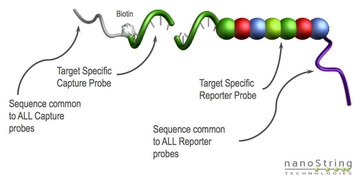
(B) Principal component analysis was used to test whether the Urinary RNA Signature could also classify normal kidney samples from ccRCC samples in public data sets. Successful separation of normal and tumor samples using the selected markers indicate the potential utility of the Signature as a diagnostic tool for RCC.

Conclusions & Future Directions

These data present evidence that human urine can serve as a non-invasively collected biospecimen for RCC RNA biomarker discovery and validation. Furthermore, the reported Urinary RNA Signature demonstrates potential utility for RCC diagnosis and prognosis, and help identify patients at high risk for tumor metastasis at the time of nephrectomy who might benefit from adjuvant radiation or chemotherapeutic treatment.

Future experiments will focus on cross validation studies using additional urine samples using the FDA-approved Nanostring platform.

The urinary transcript signature is the subject of Patent Application **PCT/US2020/046206 Filed to the United States Patent and Trademark Office on August 13, 2020**



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References

[1]Wu, X., Weng, L., Li, X., Guo, C., Pal, S.K., Jin, J.M., Li, Y., Nelson, R. a., Mu, B., Onami, S.H., et al. (2012). **Identification of a 4-microRNA signature for clear cell renal cell carcinoma metastasis and prognosis**. PLoS One *7*, 1–10.

[2]Gabr, A.H., Gdor, Y., Strope, S.A., Roberts, W.W., and Wolf, J.S. (2009). **Patient and pathologic correlates with perioperative and long-term outcomes of laparoscopic radical nephrectomy**. Urology *74*, 635–640.

[3]Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., and Pachter, L. (2012). **Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks.** Nat. Protoc. *7*, 562–578.