Discovery and validation of a urinary exosomes mRNA signature for the diagnosis of human kidney transplant rejection.

Poster # 443

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Introduction:

Patients with end stage renal disease usually undergo transplantation, however as many as 10-15% of these patients develop acute kidney rejection. Methods for monitoring clinical rejection includes increase in serum creatinine and urinary protein secretion. These methods are not very accurate and may not reflect subclinical rejection. Currently, the patients are often monitored by repeat biopsies that may result in increased complications and cost. An accurate, non-invasive method would allow for earlier diagnosis and minimize the amount of immunosuppression needed to manage these patients. Extracellular vesicles such as exosomes are a promising new platform for biomarkers and can be used to monitor RNA and protein expression. Exosomes shed from the rejected kidney into the urine are likely originating from glomerular podocytes, renal tubular cells and from immune cells activated during rejection.

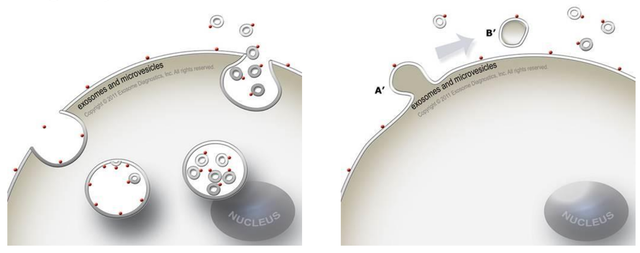
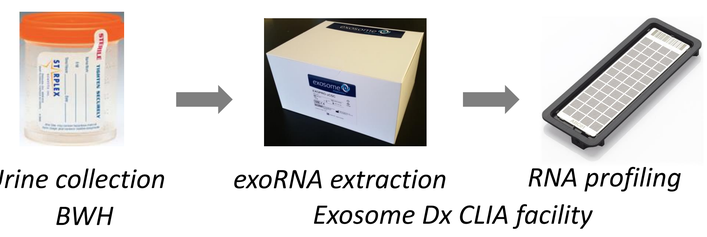


Fig 1. Exosome/microvesicle biogenesis
Exosomes and other vesicles can be released by (A) multivesicular body pathway or through (B) direct budding at the plasma membrane.

Methods

Urine samples were collected from patients undergoing a transplant kidney biopsy for clinical indications. A total of 66 urine samples across two cohorts (38 rejections, 28 non-rejections) were collected. RNA from both the urinary cell pellets and exosomes were isolated from up to 20mls urine for expression profiling. Two patient cohorts were screened, first to generate a candidate marker panel (training) and a second to verify the performance of the smaller panel (test). ExoRNA was reverse transcribed and pre-amplified prior to analysis of RNA signature using the OpenArray® Human Inflammation Panel. OpenArray® is a TaqMan qPCR array. Human Inflammation Panel consists of 586 target and 21 endogenous control assays.



Urine collection BWH → *exoRNA extraction Exosome Dx CLIA facility* → *RNA profiling*

Fig 2. Workflow of urine exoRNA isolation and expression profiling

Table 1. Urine samples from kidney transplant patients (training cohort)

Rejection Criteria	Number of samples
Cellular rejection including borderline rejection	7
Antibody mediated rejection (AMR): acute or chronic active	7
No rejection	14
Total	28

Results:

We detected the expression of 207 (34%) to 518 (85%) genes. Two samples were excluded from analysis due to low RNA yield. Analysis of mRNA expression in urinary pellets and exosomes, from the training cohort samples, identified genes that were differentially regulated. The exosome samples identified 23 significantly differentially expressed genes (figure 3). The genes identified from exosomal RNA performed significantly better in correctly differentiating between rejection and non-rejection compared to the cell pellet RNA (data not shown).

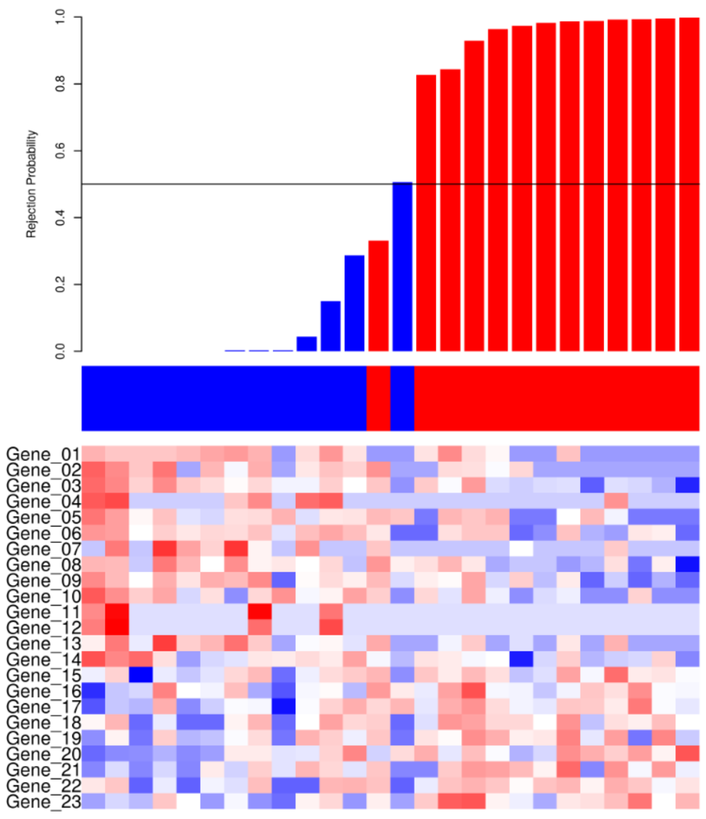


Fig 3. Gene signature identified in training cohort differentiating between kidney rejection and non-rejection. *Boxplot:* Height of each bar is the rejection probability estimated from gene-expression (Red bars - rejection samples, blue bars - non-rejection samples). *Heatmap*: Marker expression levels (blue tones indicate higher Crt values relative to quantile normalization value, and thus lower relative expression levels).

Table 2. Urine samples from kidney transplant patients (test cohort)

Rejection Criteria	Number of samples
Cellular rejection including borderline rejection	14
Antibody mediated rejection (AMR): acute or chronic active	4
Cellular and AMR	6
No rejection	14
Total	38

In the second, test cohort, the extracted samples were again run on the OpenArray® Human Inflammation Panel. One sample was excluded due to low RNA yield. The performance of the 23-gene signature was evaluated (figure 4). ROC analysis of the signature demonstrated an AUC of 0.853 (figure 5)

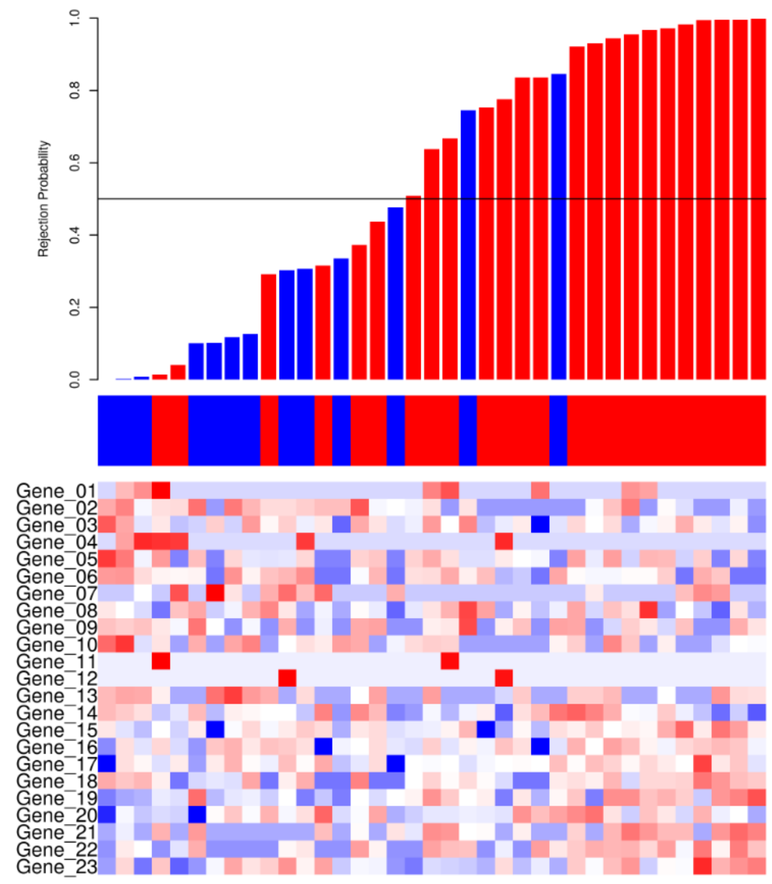


Fig 4. Performance of 23-gene signature in test cohort. *Boxplot:* Height of each bar is the rejection probability estimated (Red bars - rejection samples, blue bars - non-rejection samples). *Heatmap*: Marker expression levels (blue tones indicate higher Crt values relative to quantile normalization value, and thus lower relative expression levels).

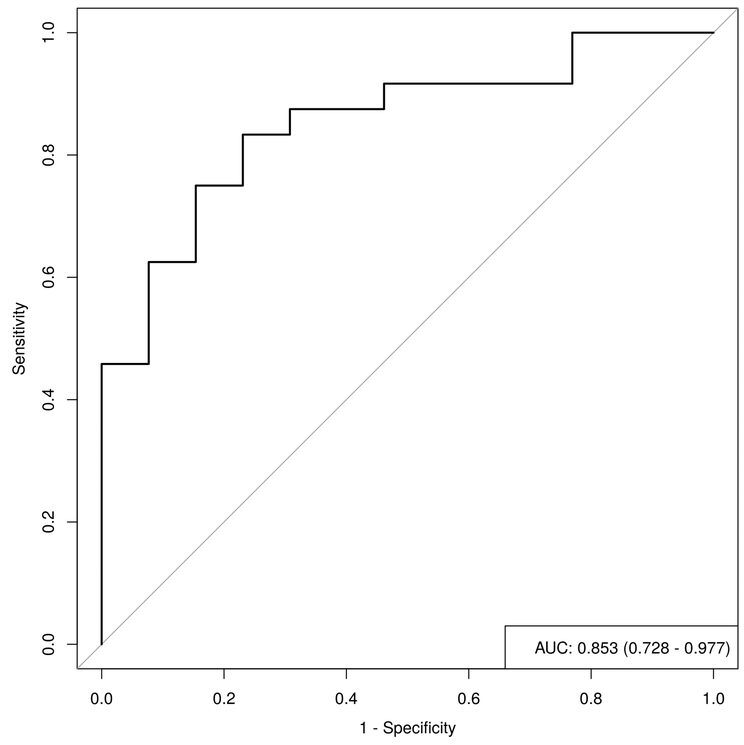


Fig 5. ROC curve analysis of the 23-gene signature in test cohort

Conclusions

We have identified a 23-gene signature in urine exosomes that could characterize patients with kidney rejection. Analysis of cellular RNA from urine was unable to generate such a signature. Further experiments are required to fully validate this signature

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