Microarray vs. Next-Generation Sequencing Comparison of Gene Expression Signatures for Subclinical Acute Rejection

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INTRODUCTION

Several studies have described gene expression-based signatures for clinical diagnostics based on DNA Microarrays. Though they are the method of choice for global gene expression, microarrays lack advantages offered by newer technologies such as Next Generation Sequencing (NGS) including more accurate quantification, the ability to multiplex clinical samples as well as the unique capability to study microRNAs, long non-coding RNAs and novel transcripts. We compare gene expression signatures obtained from peripheral blood and biopsies using both global profiling platforms to evaluate their diagnostic capabilities to differentiate subclinical Acute Rejection (subAR) from clinical Acute rejection (cAR) and Transplant eXcellent subjects (TX). A second objective was to provide a validation of our array-based gene expression diagnostics using an orthogonal RNA expression technology.

METHODS

We profiled 69 biopsy documented subjects (cAR=21, subAR=23 and TX=25). Matching blood and biopsy samples were studied using microarrays (Affymetrix HT U133 Plus PM arrays; n=67). Signals < Log2 of 4.14 filtered for low signal intensities. Analysis on 27,980 probesets (~13,900 genes). NGS was done on the Ion Torrent Proton System (n=68). Samples were aligned using the STAR aligner and differential expression of normalized (DESeq2 Values) was done using ANOVA. Runs with >10 million reads were used for analysis (average 16 million reads). Samples were filtered for a minimum of 5 normalized cpm per gene. All analyses were done in Partek Genomics Suite 6.6 with Bonferroni corrections. Predictions were done with the Nearest Centroid Algorithm.

RESULTS

A 2-step approach was used for predictive algorithms, where subAR+cAR vs. TX was used to clearly separate a subAR or cAR from TX as the 1st step. The 2nd step was using the subAR vs. cAR genes to separate the subARs from the cARs. Overall predictive accuracies for the microarrays and NGS was 94% and 91% for the biopsies and 91% and 89% for the PAXgene samples respectively.

***DISCLOSURES:** ***Kurian, SM., Salomon, DS. Friedewald, J. Abecassis, MM*: Stockholders, TGI.**

Diagnostic metrics for Biopsy Samples - Microarrays

METHOD	CLASSIFIES	% PREDICTIVE ACCURACY	SENSITIVITY (%)	SPECIFICITY (%)	POSITIVE PREDICTIVE VALUE (%)	NEGATIVE PREDICITVE VALUE (%)	AUC
Nearest Centroid (2 Step Prediction)	TX vs. cAR	97%	100%	94%	95%	100%	0.965
	TX vs. subAR	95%	100%	90%	91%	100%	0.947
	cAR vs. subAR	86%	90%	81%	81%	90%	0.862

Diagnostic metrics for Biopsy Samples - NGS

METHOD	CLASSIFIES	% PREDICTIVE ACCURACY	SENSITIVITY (%)	SPECIFICITY (%)	POSITIVE PREDICTIVE VALUE (%)	NEGATIVE PREDICITVE VALUE (%)	AUC
Nearest Centroid	TX vs. cAR	95%	100%	88%	92%	100%	0.943
	TX vs. subAR	100%	100%	100%	100%	100%	1.000
	cAR vs. subAR	79%	76%	83%	83%	72%	0.792

Diagnostic metrics for PAXgene Samples - Microarrays

METHOD	CLASSIFIES	% PREDICTIVE ACCURACY	SENSITIVITY (%)	SPECIFICITY (%)	POSITIVE PREDICTIVE VALUE (%)	NEGATIVE PREDICITVE VALUE (%)	AUC
Nearest Centroid (1 Step Prediction)	TX vs. cAR	90%	95%	85%	86%	94%	0.898
	TX vs. subAR	91%	95%	87%	86%	95%	0.912
	cAR vs. subAR	91%	88%	94%	95%	85%	0.905

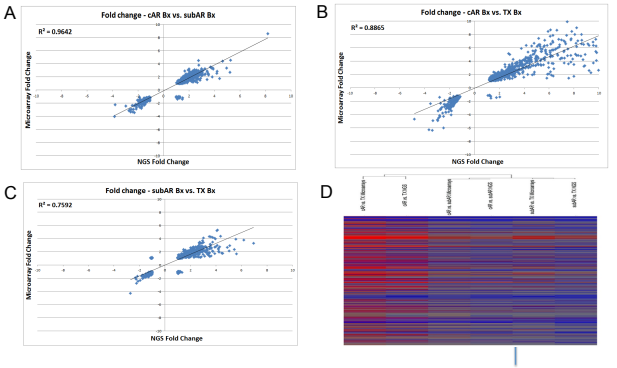
Diagnostic metrics for PAXgene Samples - NGS

METHOD	CLASSIFIES	% PREDICTIVE ACCURACY	SENSITIVITY (%)	SPECIFICITY (%)	POSITIVE PREDICTIVE VALUE (%)	NEGATIVE PREDICITVE VALUE (%)	AUC
Nearest Centroid	TX vs. cAR	92%	95%	90%	90%	95%	0.921
	TX vs. subAR	83%	83%	82%	83%	82%	0.829
	cAR vs. subAR	93%	94%	95%	94%	95%	0.943

1066 genes that were common to microarrays and NGS (both differentially expressed at FDR < 1%) were plotted for correlation analyses of directionality of fold change and the correlation of absolute fold changes.

Differential Expression - Fold Change Agreement between Microarrays and NGS

	Significant DE Genes (< 1% FDR)	All Genes (above 5 cpm threshold)
cAR vs. TX	1063/1066 (99.8%)	5747/7076 (81.2%)
cAR vs subAR	1063/1066 (99.8%)	6080/7076 (85.2%)
subAR vs. TX	1042/1066 (97.8%)	5652/7076 (79.8%)



A, B and C) Correlation plots of the absolute fold changes of 1066 differentially expressed genes between cAR, subAR and TX. *From 96-75% r^2 correlations are seen between array and NGS results for all 3 comparisons.* **D)** Heatmap of the 1066 genes showing the clustering by clinical phenotypes rather than the technology platforms. *Demonstrates that with unbiased clustering of the array vs. NGS data that samples cluster by the phenotypes, not the technologies.*

CONCLUSIONS

- Both microarray and NGS platforms perform similarly on matched clinical biopsy and blood samples with respect to predictions of clinical rejection phenotypes based on standard diagnostic metrics of sensitivity, specificity, positive and negative predictive values and area under the curve (AUC).
- Both platforms also demonstrate very high correlations with respect to fold-change directionality both amongst significantly differentially expressed genes as well as all genes detected above specified thresholds.
- The premise of our work is that global gene expression profiling of blood and biopsy samples reveal diagnostic signatures for clinical phenotypes (cAR, subAR and TX). In this study we demonstrate that a completely orthogonal global RNA expression technology (NGS RNAseq) provides the same highly correlated phenotyping of clinical samples as our microarray technology.