INTRODUCTION

Despite the development of immunosuppressive strategies that have reduced short-term graft survival rates, long-term kidney graft survival remains unchanged. Two major factors contribute: 1. The constant immunological and inflammatory insults to the kidney, and 2. The vast heterogeneity of the individual responses to such an insult. These factors coupled with the fact that there are few adequately powered studies that have looked at the various populations of kidney transplant patients based on their phenotypes have confounded efforts to put a mechanistic handle on the pathogenesis of kidney allograft loss. We report on the global gene expression profiles from ~400 kidney transplant patients encompassing four major phenotypes, well-functioning kidney grafts (TX), Acute Rejection (AR), Chronic Rejection (CR) and grafts that have acute dysfunction but due to causes other than rejection (ADNR). This study used the current “gold standard”, light histology, to test correlations of our molecular phenotypes to graft survival and function. We also mapped gene expression to reveal the biology of AR and CR.

METHODS

274 precisely histology-phenotyped biopsies with 5-year follow-up for outcomes (TGGG database and UNOS) were profiled using Affymetrix microarrays. 4-way classifiers were constructed using multiple tools. Using Nearest Centroids (NC) we designed a threshold-based scoring method calculating differences between the posterior probabilities of histology-processed vs. molecular-predicted phenotypes. ANOVA was scripted using R language compared slopes of 1/creatinine over time (e.g. function as outcome metric). We used our new tool, ImmuneMap, to map differential expression to 60 functional immune pathways linked to transplant rejection in the literature. Pathway mapping was done using Ingenuity Pathway Analysis and public databases such as WikiPathways, PubMed and Pathway Central.

RESULTS

The analysis was driven by use of 4-way classifiers with the NC algorithm as well as other tools for assessing diagnostic metrics in Partek Genomics Suite (version 6.6).

To avoid the potential bias of “over fitting” we used the bootstrapping methodology of Harrell et al. to do 1000 iterations of sampling with replacement of the data to mimic real clinical testing. We also used a “thresholding” algorithm that we developed using the NC tool to reclassify the samples based on the calculated centroid distances. Using the top 200 differentially expressed probesets from a 4-way AR, ADNR, CAN and TX ANOVA with a Nearest Centroid classifier, we are able to molecularly classify the 4 phenotypes at 90-94% Predictive Accuracy in this Discovery cohort (see Tables).

DISCOVERIES: Performance of the 200 probeset 4-way classifier on the 274 AR,ADNR, CR and TX samples.

VALIDATION: Performance of the “locked” 200 probeset 4-way classifier on the 94 AR,ADNR, CR and TX samples from Brazil.

We recently developed a new tool, ImmuneMap, to enhance our ability to map immune/inflammatory pathways in complex gene expression data. There were 40 pathways significantly differentially expressed in the AR biopsies. Of these, 16 pathways (40%) were shared with CR. These represent strong signals for T and B cell-mediated immune activation. Remarkably, not a single immune pathway was uniquely represented in CR suggesting a significant sharing of molecular networks between AR and CAN/IFTA.

CONCLUSIONS

We successfully validated our “thresholded” NC classifiers in an independent cohort from Brazil showing that our molecular phenotypes work despite significantly different racial/ethnic backgrounds. The molecular phenotype was correlated as well or better with both the 1/creatinine over time and graft loss compared to histology phenotypes. Our immune mapping results suggest that CAN/IFTA is actually immune-mediated chronic rejection (CR). We propose that CR is the consequence of inadequate or ineffective long-term immunosuppression.