GEF TRANSCRIPTS EXPRESSED IN PERIPHERAL BLOOD OF PATIENTS WITH LUPUS NEPHRITIS IDENTIFY NOVEL MECHANISMS AND THERAPEUTIC TARGETS

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Background Lupus nephritis affects more than one-half of patients with SLE and is the most common serious manifestation of the disease. Lupus nephritis is more common in Hispanic and African-American patients than in those of European ancestry, and class III and IV nephritis progresses to end-stage renal disease in 10%–15% of patients within 15 years of diagnosis. Identification of markers and mechanisms of lupus nephritis could provide new approaches to predict and treat disease.

Methods To identify blood cell transcriptome biomarkers that differentiate renal and non-renal disease we performed RNA sequencing on peripheral blood samples from 15 patients with lupus nephritis and 14 patients with non-nephritis manifestations of SLE (samples represented each patient during flaring and quiescent disease states) and from 3 healthy donors. To relate gene expression to activity of nephritis, 216 longitudinal samples from 30 patients with lupus nephritis covering a median time frame of 28 months were analyzed using the Illumina HT-V4 Bead array. Serum albumin levels were documented at the time of each visit.

Results Principal component analysis of RNA sequencing data clearly differentiated SLE patients with nephritis from those without nephritis, and linear models for microarray (limma) analysis identified 153 gene transcripts differentially expressed between the two patient groups (fold change >1.5; p<0.05). U1 and U3 RNA transcripts were increased in lupus nephritis samples, and the most highly expressed transcript based on fold change was TREML4, encoding a protein previously identified as amplifying TLR7 signaling and promoting type I interferon production. Analysis of longitudinal microarray data in relation to serum albumin identified 120 transcripts. Those most significantly correlated with lupus nephritis activity were pituitary tumor-transforming gene 1 (PTTG1), recently identified as polymorphic and associated with SLE, uridine cytosine kinase 2 (UCK2), thioredoxin (TXN), and RNASE2. Expression of PTTG1 fluctuated over time, with elevated levels preceding the time of peak renal disease activity.

Conclusions Spliceosome-associated RNAs and TREML4, a TLR7-associated gene product, may represent biomarkers of lupus nephritis, and PTTG1, the product of a lupus-associated gene reported to be involved in epithelial-mesenchymal transition, may be a novel therapeutic target associated with active nephritis. These studies provide a rich data set stimulating new understanding of mechanisms contributing to lupus nephritis.

Acknowledgements This work was supported by the Lupus Research Alliance, Pfizer-Centers for Therapeutic Innovation, and the Emerald Foundation.

A NOVEL APPROACH TO ANALYZE SINGLE CELL RNA-SEQ DATA FROM LUPUS NEPHRITIS SAMPLES

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Background Single-cell RNA-Seq (scRNA-seq) has the potential to increase our understanding of cell populations in lupus. Recently, kidney scRNA-Seq data from lupus nephritis (LN) patients has provided the opportunity to determine the heterogeneity of cells within the affected kidney. However, since individual cells were not identified phenotypically, it is necessary to identify populations computationally. The unique technical challenges of scRNA-Seq data make it difficult to approach this analysis with conventional unsupervised bioinformatics techniques. The implementation of natural language processing (NLP) -inspired techniques, however, makes it possible to identify meaningful clusters of cells without prior knowledge of the cell types present in the sample.

Methods We have developed a recursive, unsupervised, heuristic technique (StarShipTM) to dynamically perform top-down, divisive clustering on scRNA-Seq data. First, the cells are mapped onto an n-dimensional unit sphere, where n is the number of available genes. The angles between all cells are used to construct a cosine distance metric: 1-cos(θ). The cosine distance is used to carry out k-means or k-medoids clustering, with k set to 2 for each iteration. At each split of the data, the algorithm evaluates whether it has sorted the remaining cells into meaningful populations and stops making splits when a user-defined criterion is met. Once all clusters are finalized, a Mann-Whitney U test determines genes that distinguish clusters or groups of clusters from other cells. This algorithm was validated using publicly available peripheral blood mononuclear cell (PBMC) scRNA-Seq data from 10X Genomics and tested in scRNA-Seq data from LN patients from the NIAMS AMP RA/SLE initiative. Adjusted Rand Index (ARI) was used to compare generated partitions to known cell types in the PBMC data.