Transcriptional Subsetting of SLE Patient Cohorts Based on Metabolic Pathway Activity

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ACR Convergence

Poster Session B

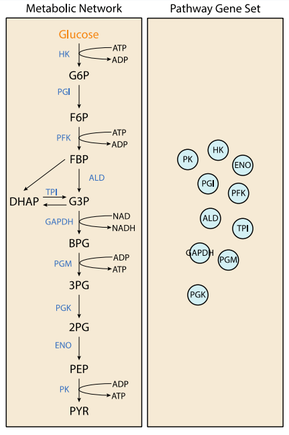
Poster No. 0523

Background/Purpose

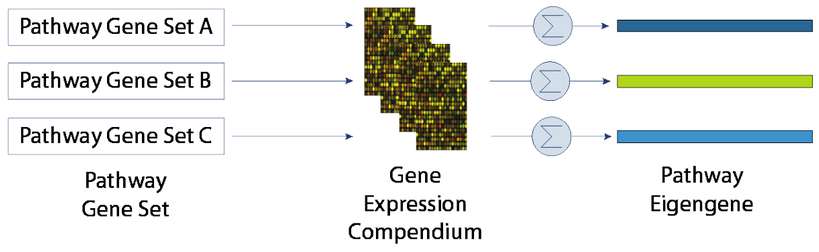
Systemic lupus erythematosus (SLE) is a highly complex, heterogeneous, autoimmune disorder, with diverse clinical presentation. The autoimmune process in SLE is driven by a complex interplay between genetics, epigenetics and the environment. To identify new therapeutic targets, predict patient responsiveness, discover biomarkers, and ultimately transform the treatment of SLE there is a need to mechanistically untangle patient heterogeneity. Since metabolic processes integrate signaling, functional genomics, and environmental cues, analyzing metabolic pathway activity can be a more powerful approach to define drivers of heterogeneity in SLE compared with traditional whole transcriptome analyses. Here we showcase how metabolic pathway activity, as defined by a mathematical transformation of well curated pathway gene sets, can effectively subset patients into discrete clusters driven by immunopathological features of the disease.

Metabolic Activity Can Be Expressed Using Eigengenes

Given that metabolism modulates signaling, transcriptional, and epigenetic events in immune cells, we hypothesized that metabolic activity would be sufficient to subset patient cohorts in SLE. To test this hypothesis, we collected a set of 84 canonical metabolic pathway gene sets from the public domain (KEGG, Reactome), encompassing 1,692 protein-coding genes. These gene sets represent metabolic pathway composition in the transcriptional space. In the example to the right we see how the glycolytic pathway is defined in terms of a pathway gene set.

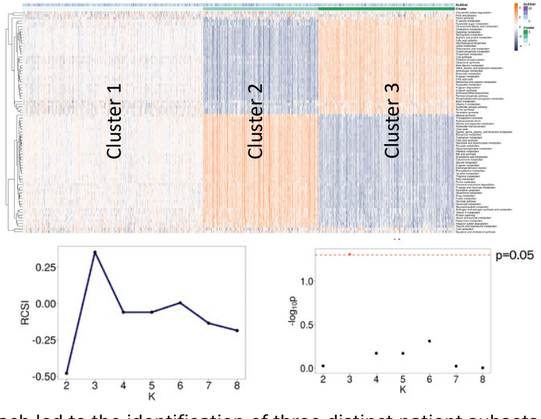


Using these metabolic pathway gene sets and gene expression data from well-curated data sets, we define an eigengene[1] as a summarization of the expression of the genes in a given gene set. Thus, the eigengenes provide a representation of the pathway activity for a given pathway. This framework allows us to define pathway activities in patient cohorts, which we can then use to perform patient stratification.



SLE Patient Subsetting Based On Metabolism

We tested the ability of metabolic pathway analysis to define mechanistically-relevant patient subsets using the ILLUMINATE-1 whole blood expression data set[2], comprised of 879 patients with moderate to severe SLE. We calculated metabolic pathway eigengenes and used an unsupervised consensus clustering algorithm to identify patient subsets.



This approach led to the identification of three distinct patient subsets (clusters 1-3). Performing this analysis on random gene sets did not reveal any clear patient subsets, supporting the hypothesis that a key parameter driving patient subsetting is metabolic pathway activation.

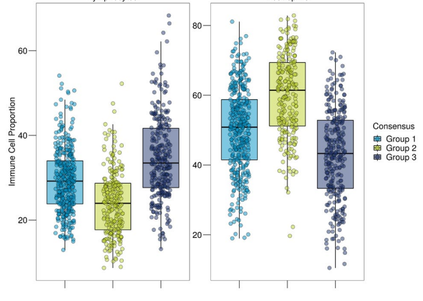
Metabolically-defined Subsets Are Biologically Significant

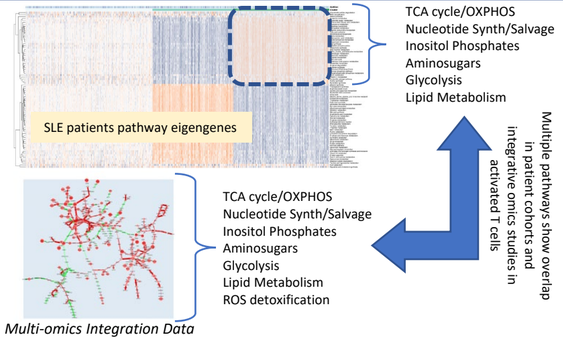
We explored the biological significance of the clusters in several ways:

- We deconvoluted the expression data into immune cell proportions.[3] Clusters 2 & 3 revealed enrichment of neutrophils (N) and lymphocytes (L) respectively. L: N ratios correlate with key immunopathological feature of SLE, e.g. renal involvement (see figure below).[7]

Additionally:

- Clusters were independent of SLEDAI and steroid treatment (data not shown)
- An independent longitudinal study showed Clusters 2 &3 to be time stable (data not shown).





Further exploration revealed that the metabolic pathways up-regulated in Cluster 3 corresponded to the core metabolic pathways that comprise an "anabolic hub" coordinating immune activation in vitro across multiple cell types. E.g., integrated-omics analysis[6] of primary human memory T cells activated with anti-CD3/CD28/CD2 revealed engagement of the same pathways that define the patient cluster (see figure above). We found the convergence of these in vitro data with patient metabolic subsetting data to be striking.

These direct connections of patient subsets with coordinated metabolic hubs enable selection of novel drug targets for modulation of immune cell activation in pre-defined patient subsets.

Conclusions

- Metabolic information *alone* is sufficient to define patient subpopulations in SLE.
- The data are consistent with known immune cell metabolic reprogramming in autoimmune diseases[4, 5].
- Patient subsets can be linked to distinct metabolic hubs, the orchestrated metabolic events that control immune cell function.
- These studies will facilitate discovery of novel targets that impact the disease-associated metabolic programming of immune cell drivers of SLE.

These studies further provide a framework for metabolic precision medicine approaches to assign individual patients to disease subgroups that would best benefit from drugs modulating those targets.

References

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