Network integration of parallel metabolomic-transcriptional data reveals novel metabolic modules regulating divergent macrophage polarization

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We have developed an integrated high-throughput transcriptional-metabolic profiling and analysis pipeline, and applied it to characterize global rewiring during murine macrophage polarization to pro- and anti-inflammatory (M1 and M2) states.

Network based integration of metabolic and transcriptional RNA-seq data allowed us to mitigate problems specific to individual types of data and to obtain a global view of the metabolic changes during macrophage polarization. Metabolic profiling can be directly associated with a well-defined network of biochemical reactions, but it is not thorough: absence of a signal does not imply absence of the metabolite. On the other hand, transcriptional profiling catches all sufficiently expressed genes and this information can be associated with metabolic reactions via enzymes. Thus, we compiled a network of reactions

based on KEGG as a framework to integrate the metabolic and transcriptional profiling data. Next, we adapted BioNet algorithm to weigh the nodes and edges in the network based on the p-value of differential expression (DE) between M1 and M2 conditions. Then we found a most connected subnetwork that contained as much positively scored and as few negatively scored nodes as possible. That led to a set of most important interconnected reactions. As expected this set contained well-known macrophage related pathways such as glycolysis, TCA cycle, etc. However, 1) it showed how these pathways were interacting and 2) it contained modules not described previously.

In M2 macrophages we discovered novel glutamine/glutamate- and UDP-GlcNAc-associated modules, and validated their involvement using isotope labeling studies. Functional importance of these modules was further confirmed by glutamine deprivation and N-glycosylation inhibition experiments. In M1 macrophages we identified a metabolic break at Idh fragmenting the TCA cycle, and validated it using isotope labeling. Label distribution suggested presence of novel variant of aspartate-arginosuccinate shunt. Consistently, inhibition of aspartate-aminotransferase, a key enzyme of the shunt, hindered NO and IL6 production while promoting mitochondrial respiration.

This systems approach provides a highly integrated picture of the physiological modules supporting macrophage polarization, identifying potential pharmacologic control points for both macrophage phenotypes.

1. A.K.Jha et al (2015) Network Integration of Parallel Metabolic and Transcriptional Data Reveals Metabolic Modules that Regulate Macrophage Polarization ,*Immunity* **42**:419-430.