Transcriptomics of liver tissue, PBMCs, and monocytes in alcoholic hepatitis

Trina M. Norden-Krichmar[1], Christophe Magnan[1], Zhang-Xu Liu[2], Jon Jacobs[3], Timothy Morgan[4], and the Southern California Alcoholic Hepatitis Consortium (SCAHC)

[1]University of California, Irvine, CA, USA; [2]University of Southern California, Los Angeles, CA, USA; [3]Pacific Northwest National Laboratory, Richland, WA, USA; [4]VA Long Beach Healthcare System, Long Beach, CA, USA

Introduction

- Alcoholic Hepatitis (AH) is an alcohol-induced inflammatory liver disease with high mortality and morbidity rates.

- The factors that determine the progression to AH and the subsequent prognosis are not well understood.

- This study used RNA sequencing for gene expression profiling of human liver tissue, peripheral blood mononuclear cells (PBMCs), and monocytes from patients with AH and healthy controls.

Methods

- **Sample ascertainment.**
  - The blood samples and liver biopsies in this study were collected at baseline by the Southern California Alcoholic Hepatitis Consortium (SCAHC) from Alcoholic Hepatitis patients (AH, n=15), and from normal healthy controls (normal, n=15). For 7 of the AH patients, a liver tissue biopsy was also collected at baseline.
  - As normal liver tissue controls, RNA sequencing data of normal liver samples (n=3) from the Human Protein Atlas study (www.proteinatlas.org and Kampf et al., FASEB, 2014) were downloaded from EMBL-EBI (http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-1733/samples/).

- **RNA sequencing.**
  - PBMCs and monocytes were isolated by the Liu lab (USC) from the blood samples into cell pellets. RNA was extracted from the liver biopsies and cell pellets.
  - RNA was sequenced at 2x100 paired-end on an Illumina HiSeq.
  - The RNAseq data was aligned to the human genome (hg19) using Tophat2 alignment software (Kim et al., Genome Biol. 2013).

- **Differential gene expression analysis.**
  - Differential expression (DE) analysis was performed with the Cufflinks software (Trapnell et al., Nature Methods, 2012), using upper quartile normalization between the data files.
  - Normalized DE between the groups was filtered for FPKM>=2, abs(log2(fold change)) >= 1.2, and that were significant at FDR-adjusted p-value <= 0.05.
  - Heatmaps were constructed with cummeRbund (Goff et al., 2012) to display a subset of the differentially expressed genes that met the thresholds listed above.

- **Proteomics**
  - Proteomics data was provided by the Jacobs lab (PNNL) from a high mass accuracy LC-MS/MS platform. Data was filtered by abs(fold change) > 0.2, and p-value <= 0.05.

- **Pathway Analysis.**
  - Ingenuity Pathway Analysis IPA software (QIAGEN) was used to determine the top canonical pathways and for comparison to proteomics data.

Contact Information   Email: tnordenk@uci.edu

Conclusions

- Differences in gene expression were observed between the AH and normal samples, with the most significantly enriched pathways related to immune and inflammatory function. Comparison with proteomics data confirmed enrichment in these pathways.

- The gene expression profiles of PBMCs and monocytes were similar to each other, when compared with the liver tissue samples. However, differences in gene expression and enriched pathways were also revealed when focusing upon the PBMCs and monocytes.

- The preliminary results suggest that there are characteristic gene expression profiles of alcoholic liver disease that may be detected by PBMCs and monocytes in the blood, in addition to that seen in the liver tissue.

- The ability to detect differentially expressed genes may point to treatment options and disease progression monitoring for alcoholic hepatitis.

Results

- **PBMCs at baseline for Alcoholic Hepatitis vs. Healthy Normal Controls**:
  Gene expression profiles, some of the top canonical pathways, and comparison with proteomics data
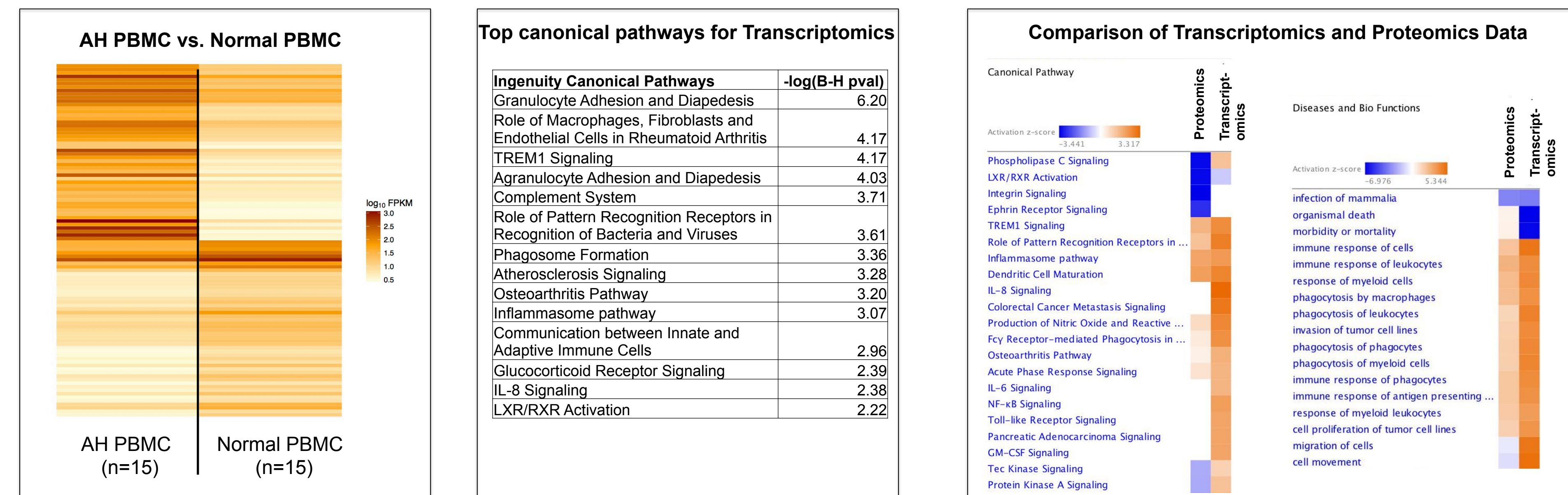
- **Monocytes at baseline for Alcoholic Hepatitis vs. Healthy Normal Controls**:
  Gene expression profiles, some of the top canonical pathways, and comparison with proteomics data

Similar significant top canonical pathways
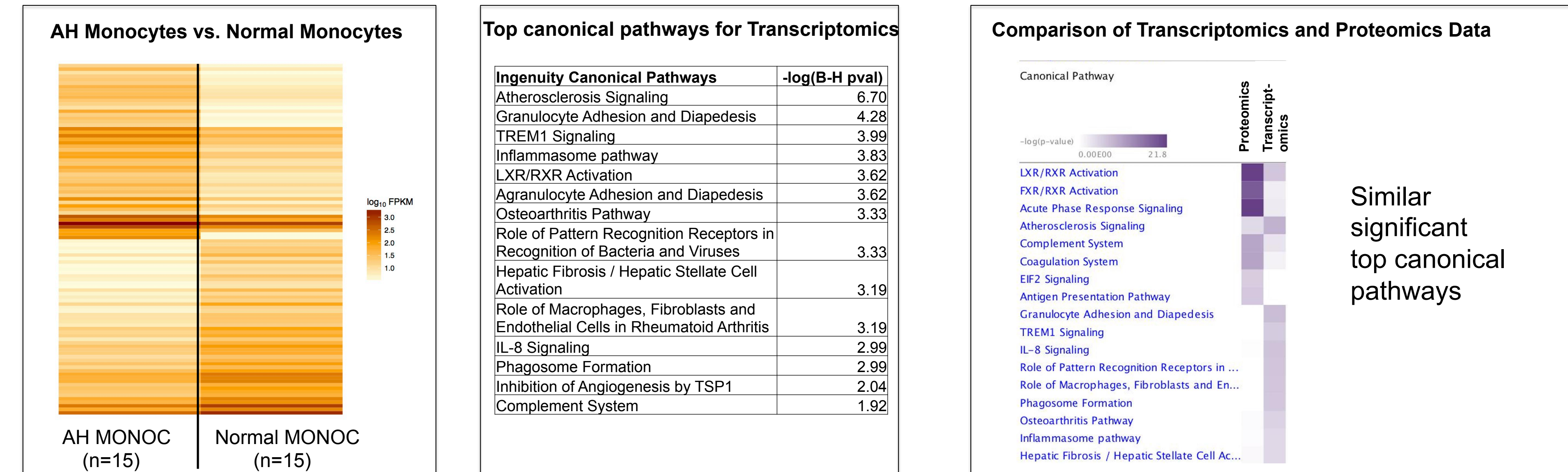
- **Gene expression profiles of liver tissue, PBMCs, and monocytes from seven AH patients**

- The first heatmap of this section accentuates the gene expression differences between tissue, PBMCS, and monocytes.

- However, in the second heatmap, similar gene expression trends can be discovered by comparing AH to Normal samples. Some genes have higher expression in AH vs. Normal across the sample types.

- Selecting several genes and finding genes with similar expression patterns, suggests that a panel of such genes may be used to profile AH vs. Normal gene expression using blood-based samples, such as PBMCs and monocytes.