Division of Rheumatology, Department of Medicine, Feinberg School of Medicine

Transcriptomic Profiling of Arthritic (CD11c-Flip-KO/HUPO) Mice

Shang-Yang Chen, Qi-Quang Huang, MD, Deborah R. Winter, PhD, Richard M. Pope, MD

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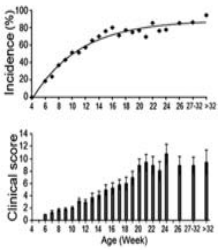
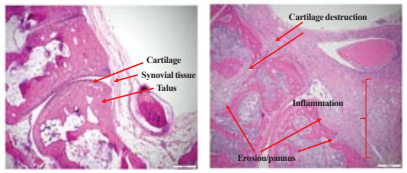
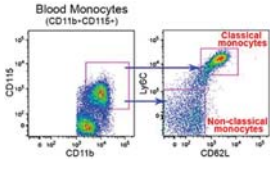
Background

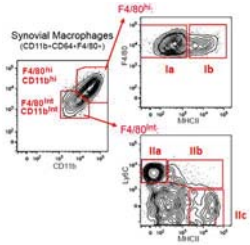


- Spontaneously develop inflammatory arthritis that resembles rheumatoid arthritis



- Synovial macrophage populations



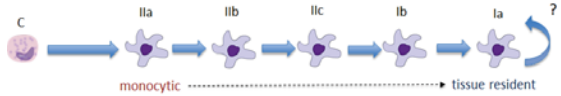


C: Ly6C^{hi}, Classical monocytes
NC: Ly6C^{lo}, Non-classical monocytes

Ia: F4/80^{hi}, MHCII^{-} macrophages
Ib: F4/80^{hi}, MHCII^{+} macrophages
IIa: F4/80^{int}, Ly6C^{+} MHCII^{-} mono/macrophages
IIb: F4/80^{int}, Ly6C^{+} MHCII^{+} macrophages
IIc: F4/80^{int}, Ly6C^{-} MHCII^{+} macrophages

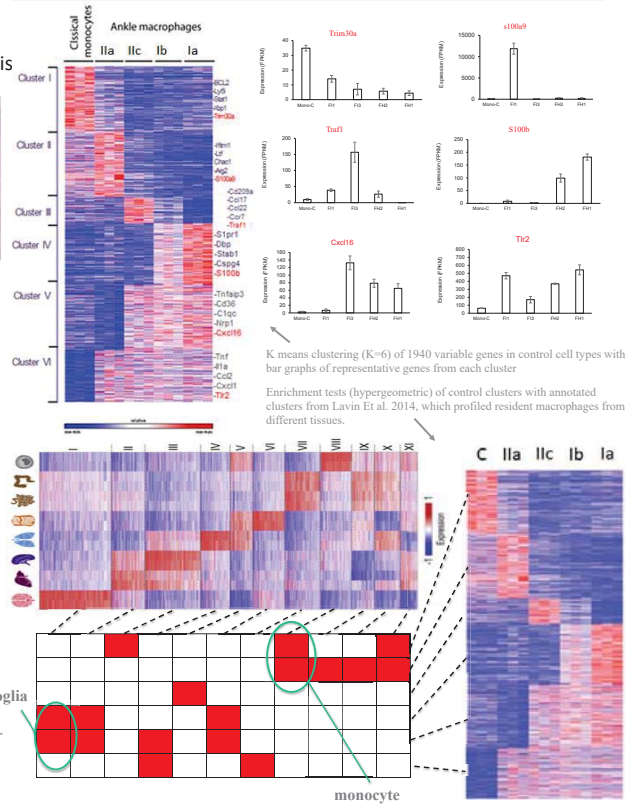
Methods & Research Objectives

- RNA-seq on control and HUPO synovial macrophage populations
  - ➢NEBNext full length prep protocol
  - ➢Processed and normalized into FPKM values
- Interrogate the relationships between synovial macrophage populations.



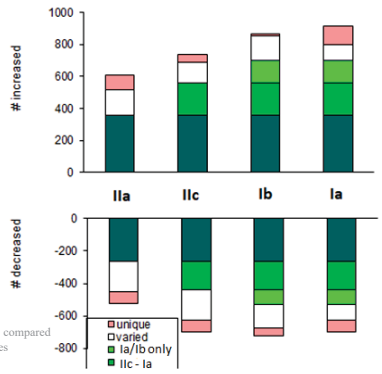
- Understand how inflammatory arthritis (HUPO) alters
  - ➢the differentiation process
  - ➢individual cell populations

Figure 1. The transcriptome of control samples



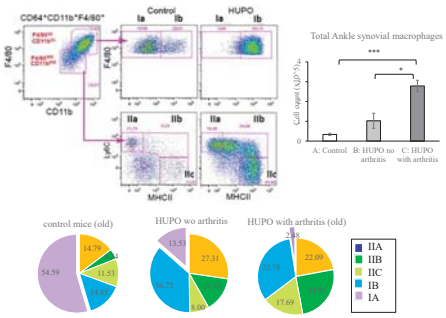
K means clustering (K=6) of 1940 variable genes in control cell types with bar graphs of representative genes from each cluster

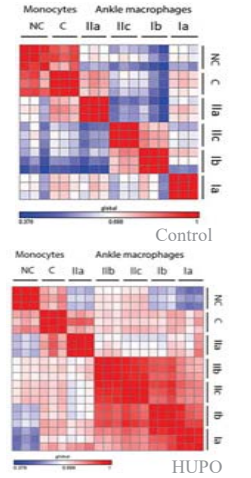
Enrichment tests (hypergeometric) of control clusters with annotated clusters from Lavin Et al. 2014, which profiled resident macrophages from different tissues.



Number of differentially expressed genes compared to classical monocytes in control cell types

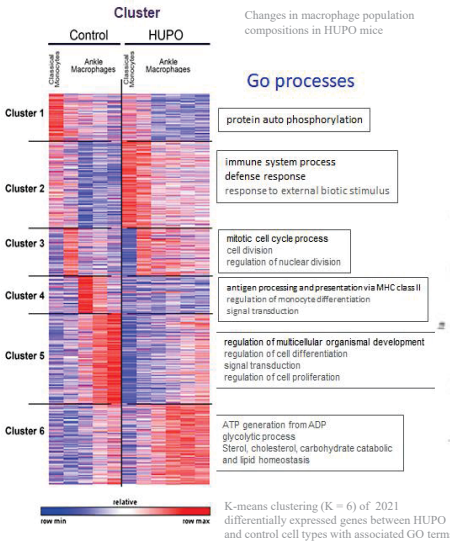
Figure 2. Control vs HUPO samples





Changes in macrophage population compositions in HUPO mice

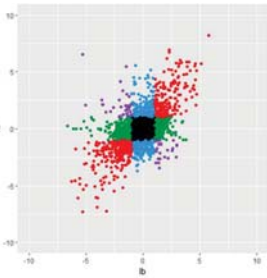
Expression correlation plots of control and HUPO cell types



Go processes

- protein auto phosphorylation
- immune system process
  defense response
  response to external biotic stimulus
- mitotic cell cycle process
  cell division
  regulation of nuclear division
- antigen processing and presentation via MHC class II
  regulation of monocyte differentiation
  signal transduction
- regulation of multicellular organismal development
  regulation of cell differentiation
  signal transduction
  regulation of cell proliferation
- ATP generation from ADP
  glycolytic process
  Sterol, cholesterol, carbohydrate catabolic and lipid homeostasis

K-means clustering (K = 6) of 2021 differentially expressed genes between HUPO and control cell types with associated GO terms



Gene expression foldchanges in Ia & Ib cells in response to HUPO

Results

- Each cell type in control samples has a distinct transcriptional profile
- Ia & Ib specific genes are significantly enriched with tissue-residency signatures
- Loss of Ia cells only in HUPO
- All cell types became more similar to monocytes in HUPO => loss of tissue residency?

Future Directions

- Obtain co-regulated modules of genes using network analysis methods
- Identify key transcription factors and pathways involved in the pathogenesis of arthritic (HUPO) mice