



A single-cell survey of *Drosophila* blood

Sudhir Gopal Tattikota^{1*}, Yanhui Hu^{1,5}, Yifang Liu^{1,5}, Bumsik Cho^{2,5}, Victor Barrera³, Michael Steinbaugh³, Sang-Ho Yoon², Aram Comjean¹, Fangge Li¹, Franz Dervis¹, Ruei-Jiun Hung¹, Jin-Wu Nam², Shannan Ho Sui³, Jiwon Shim² and Norbert Perrimon^{1,4*}

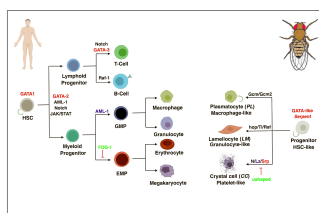
¹ Department of Genetics, Blavatnik Institute, Harvard Medical School, Boston, USA; ² Department of Life Sciences, Hanyang University, Seoul, Korea; ³ Department of Biostatistics, Harvard TH Chan School of Public Health, Boston, USA; ⁴ Howard Hughes Medical Institute, Boston, USA; ⁵ Equal authors;

* Corresponding authors: sudhir_gt@hms.harvard.edu; perrimon@genetics.med.harvard.edu



INTRODUCTION

Drosophila blood cells, called hemocytes, are classified into plasmatocytes (PM), crystal cells (CC), and lamellocytes (LM) based on the expression of a few marker genes and cell morphologies, which are inadequate to classify the complete hemocyte repertoire. Here, we used single-cell RNA sequencing (scRNA-seq) to map hemocytes across different inflammatory conditions in larvae. We discovered rare subsets within CCs and LMs that express the FGF ligand *branchless* and receptor *breathless*, respectively. Our scRNA-seq analysis reveals the diversity of hemocytes and provides a rich resource of gene expression profiles for a systems-level understanding of their functions.



Drosophila blood cells

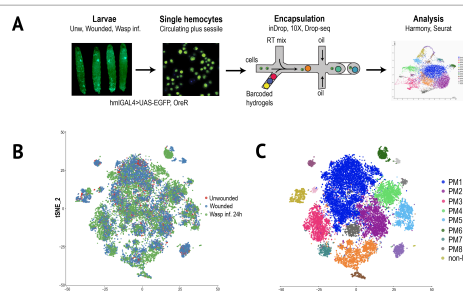
Mechanisms of hematopoiesis are highly conserved in humans and *Drosophila*. The three major classes of fly blood cells—PMs, CCs, and LMs, are functionally similar to macrophages, platelets, and granulocytes, respectively, in humans.

HIGHLIGHTS

1. scRNA-seq of *Drosophila* blood recovers PMs, CCs, and LMs
2. scRNA-seq identifies hemocyte states
3. Pseudotemporal ordering identifies CC and LM intermediates
4. scRNA-seq uncovers a novel role for FGF signaling in inter-hemocyte crosstalk

RESULTS

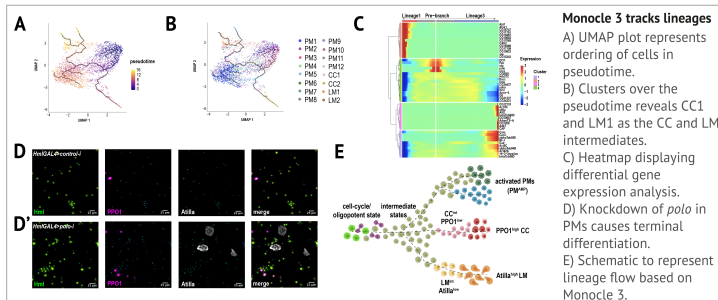
1. scRNA-Seq of *Drosophila* blood



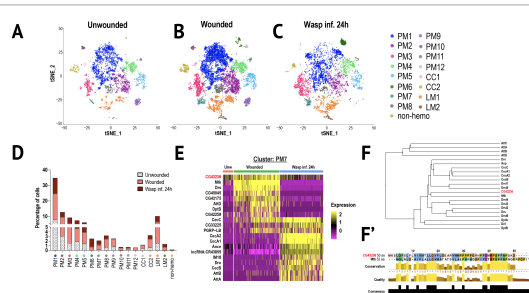
scRNA-seq of hemocytes

A) Schematic representing the scRNA-seq workflow. B) t-Stochastic Neighbor Embedding (t-SNE) plot displaying the datasets after batch correction using Harmony. C) t-SNE plot representation of ~20K hemocytes across Unwounded (Unw), wounded, and wasp infested (24 h post infestation) conditions. Each dot represents a cell.

2. Pseudotemporal ordering of hemocytes identifies CC, LM intermediates



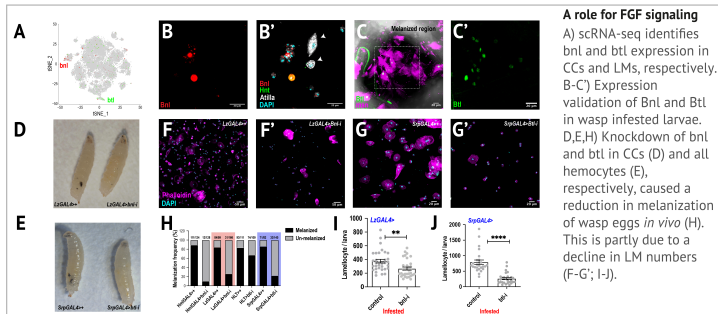
3. Cell composition changes and identification of a novel Mtk-like AMP



Mtk-like AMP

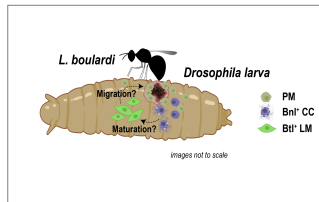
A-C) t-SNE plots representing cell compositions within each cluster in Unwounded (A), wounded (B), and wasp infested 24h (C) conditions. D) Cell fraction changes in the three conditions. E) Heatmap representing differential gene expression of PM7: PM7^{AMP}. F) Phylogenetic (F) and global alignment (F') of AMP sequences reveal CG43236 as a potentially novel Mtk-like AMP.

4. scRNA-Seq implicates a novel role for FGF signaling in inter-hemocyte crosstalk



SUMMARY

In this study, we used inDrops, Drop-seq, and 10X genomics-based scRNA-Seq platforms to characterize the blood cell transcriptome repertoire in *Drosophila*. Using different inflammatory conditions including mechanical injury and wasp infestations, we identified several states of PMs that are enriched in mitotic, metabolic, and antimicrobial gene modules. We demonstrate that the FGF components, Btl and Bnl, are required for mediating effective immune responses against parasitoid wasp eggs, highlighting a novel role for FGF signaling in inter-hemocyte crosstalk.



FUTURE DIRECTIONS

- We are now addressing the following aspects pertaining to scRNA-Seq:
1. Demonstrate the contribution of oligopotential states to terminal lineages
 2. Identify the role of Mtk-like CG43236 in immune response
 3. Cross species comparison: Common cell types between flies and humans

ACKNOWLEDGMENTS

We thank the Single Cell Core (SCC) members: Mandovi Chatterjee, Alex Ratner and Sarah Boswell for their expert help with inDrops.