10X GENOMICS

Research Snapshot:

Gene Expression Variability Across Cells and Species Shapes Innate Immunity

Researchers at Wellcome Sanger Institute (Cambridge, UK) used single cell and bulk RNA sequencing to chart evolution of the innate immune response in mammals. Genes that diverge rapidly between species show high levels of cell-to-cell expression variability and distinct promoter structures. In contrast, genes involved in regulating the response to infection were conserved across species and display low expression variability across cells (1).

SNAPSHOT		10X PRODUCT
RESEARCH AREA	Immunology	• Chromium Single Cell 3' Library and Gel Bead Kit v2 • Chromium Single Cell A Chip Kit • Chromium i7 Multiplex Kit • Cell Ranger Analysis Pipelines
ORGANISM	Human, macaque, mouse, rat, rabbit, pig	
SAMPLE TYPE	Dermal fibroblasts and mononuclear phagocytes	
RESEARCH QUESTIONS	How has the innate immune system evolved mechanisms to fine-tune the pathogen response? How is cell-to-cell transcriptional variability related to response divergence between species?	

Experiment Overview

Gene expression profiling in dermal fibroblasts from primates and rodents, challenged with immune stimuli

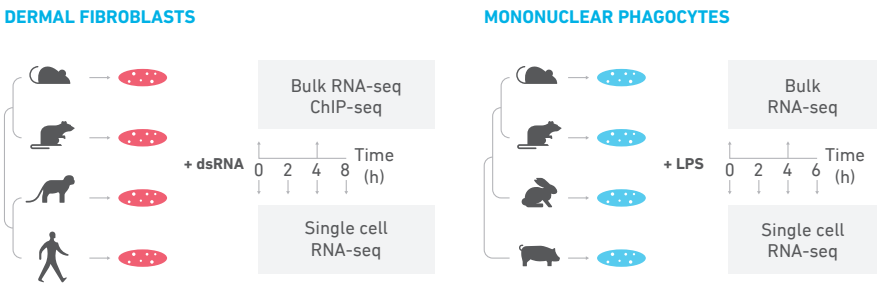
- Primary dermal fibroblasts from human, macque, mouse, and rat
- Challenged with synthetic double-stranded RNA to mimic viral infection
- Bulk RNA-seq after 4 hours
- Single cell RNA-seq over time course at 2, 4, and 8 hours, using fluorescence-activated cell sorting and Smart-seq2 (average 44 cells per time point, per species)
- ChIP-seq to look at promoter region differences among responsive genes
- Fibroblast RNA-seq libraries were sequenced on an Illumina HiSeq 2500 instrument; ChIP-seq libraries were sequenced on an Illumina HiSeq 2000 instrument

Gene expression profiling in mononuclear phagocytes from rodents and other mammal species, challenged with immune stimuli

- Bone marrow-derived mononuclear phagocytes from mouse, rat, rabbit, and pig
- Challenged with lipopolysaccharide (LPS) to mimic bacterial infection
- Bulk RNA-seq after 4 hours
- Single cell RNA-seq over time course at 2, 4, and 6 hours, using the Chromium Single Cell Gene Expression Solution from 10x Genomics (average 2,303 cells per time point, per species)
- Phagocyte RNA-seq libraries were sequenced on an Illumina HiSeq 4000 instrument

Why Single Cell?

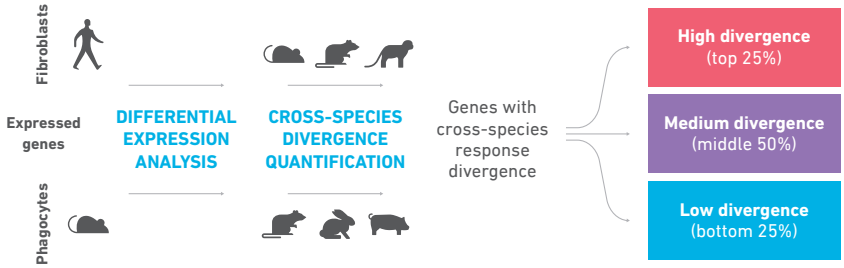
The innate immune response displays high variability across responding cells. While the bulk RNA-seq analysis examined transcriptional divergence in response between species, the single cell RNA-seq looked at cell-to-cell differences. The authors examined single cell gene expression over a time course—including stages of response both earlier and later than the bulk measurement—to include the dynamics and magnitude of response.



Study design

Computational Analysis

Researchers determined genes that were differentially expressed upon immune stimulation. Of these responsive genes, they focused on comparing only genes with one-to-one orthologues across species, then classified these genes as high, medium, or low divergence. To compare cell-to-cell variability, the authors used a Distance to Median analysis approach, which accounts for confounding factors such as gene expression level in single cell data.

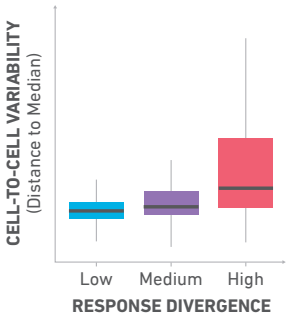


Response divergence across species in innate immune response

Results

Transcriptional profiling identified hundreds of genes that diverge in pathogen response across mammalian species. High divergence genes, including cytokines, have distinct promoter structures, which are dense with transcription factor binding motifs. Genes involved in immune regulation, such as transcription factors and kinases, are relatively conserved in their response to immune challenge.

Single cell analysis revealed that high divergence genes show higher cell-to-cell expression variability than seen with low divergence genes. These findings give clues about the mechanisms that have evolved to fine-tune the pathogen response. For example, cytokines were co-expressed in only a small fraction of responding cells. This pattern of potent-but-limited cytokine expression is conserved across species, suggesting a host strategy to mount effective defense while avoiding tissue damage.



Cell-to-cell variability levels and response divergence across species in response to LPS stimulation in phagocytes. Single cell RNA sequencing data from the Chromium Single Cell Gene Expression Solution.

Reference

[1] Hagai, Chen, Miragaia, Rostom, Gomes, et al., Gene expression variability across cells and species shapes innate immunity. Nature. 563(7730):197-202 (2018) PMID 30356220

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