Identifying a genetic footprint of RBL cell activation using single-cell gene expression assays

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***Short Abstract* — We present here a mathematical and experimental framework connecting single-molecule, single-cell measurements of RNA production to rat basophil leukemia (RBL) cell activation. We correlate RBL cell morphological changes in response to endotoxin exposure to Interleukin-4 (IL-4) RNA production using a custom, multi-color fluorescence microscope and GPU-accelerated data analysis package. We find that IL-4 production is directly tied to changes in cellular morphology and based on previous works, subsequently tied to RBL cell activation. This work demonstrates the potential for single-cell gene expression assays to predict macroscopic cellular properties, such as a cell morphology or phenotype.**

***Keywords* — single-cell gene expression, IgE signaling, cell-to-cell variability, cell morphology, basophil cells, allergic response**

I. Background

THE level of detail at which one can now investigate biological regulation within individual cells is staggering. New single-cell biological reporter systems, such as fluorescent labeling of individual RNA transcripts,[1] and new single-cell optical measurement techniques, such as super-resolution microscopy,[2] have made large datasets characterizing spatiotemporal cell-to-cell variability in regulatory pathways commonplace.[3] These single-cell techniques provide insight into how changes in gene expression regulate the distribution of cellular response to external stimuli, leading to new discoveries in translational research.[4] Here we focus on the allergic response, particularly the production of histamine by basophil cells in response to an endotoxin. We present recent work correlating Interluekin-4 (IL-4) RNA levels and rat basophil leukemia (RBL) cell activation using single-cell imaging.

Previous studies have connected RBL cell activation to 'membrane ruffling', a distortion of the actin cytoskeleton that leads to higher occurrences of Immunoglobulin-E (IgE) dimerization, leading to the release of histamine by activated RBL cells.[5,6] Additionally, histamine release by active RBL cells is linearly correlated to IL-4 levels,[7] suggesting a key role for IL-4 in RBL cell activation and histamine release.

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II. Results

Utilizing our custom, automated, three-dimensional, multi-color, GPU-accelerated single-molecule microscope,[8] we quantified IL-4 RNA levels and cellular morphology in thousands of RBL cells at multiple times points after exposure to varying levels of a endotoxin. Exploiting the multi-parameter dataset obtained in these experiments, we have created a mathematical framework that ties IL-4 RNA levels to RBL cell morphological changes.

III. Conclusions

We find a direct correlation between IL-4 RNA levels and RBL cell morphology in our experiments. Beyond advances in simultaneously quantifying gene expression and cellular morphology, this result leads to a possible molecular intervention in the allergic response pathway. Further studies are underway to determine if synthetic down-regulation of IL-4 diminishes RBL cell activation and therefore histamine release.

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