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Thesis/dissertation Title

The Analysis of the Phagocytic Heterogeneity in the Macrophage cell line by Single-Cell Transcriptomics

単一マクロファージ株における細胞単位での食作用不均一性に関する１細胞トランスクリプトミクスを用いた分析

Student’s Name

Janewanthanakul Suphamon

Approved Digest

Heterogeneity refers to the concept of variation in similarity. In organisms, heterogeneity can be observed at various levels such as in tissues and cellular heterogeneity. Cellular heterogeneity plays a crucial role in cellular differentiation and immune responses, processes in which the gene expression pattern is regulated. Cellular heterogeneity is also involved in many diseases such as cancer and tumor progression development where the heterogeneity arises from a homogeneous cellular population in tissue and organs. Clonal cell lines can also undergo changes that lead to increased heterogeneity, which is prominent in the macrophage cell line with phagocytic ability. Phagocytosis is a complex process that involves membrane deformation and cytoskeleton rearrangement to engulf foreign particles. Despite its importance, a comprehensive understanding of the full list of genes involved in phagocytosis and the specific alterations remains unclear.

In this study a macrophage cell line known as RAW264.7 was used as a model to study phagocytic heterogeneity. Each macrophage cell line was recorded under the confocal microscope during phagocytosis and then subjected to transcriptomic analysis. After single-cell RNA sequencing, approximately 10,000 gene expression profiles were used to analyze the relationship to phagocytic ability. From differential gene expression analysis, 608 genes were differentially expressed in phagocytic cells where 400 genes were up-regulated, and 208 genes were down-regulated suggesting heterogeneity in the macrophage cells during phagocytosis. The functional enrichment analysis of the upregulated genes revealed the enrichment of immune response, signal transduction, and Fc receptor-mediated phagocytosis. To gain a better understanding of the genes that might play a role in the phagocytic heterogeneity of macrophages, the weighted gene co-expression network

analysis (WGCNA) technique was applied to identify sub-network modules by constructing a co-expression network based on Pearson's correlation. This WGCNA network was divided into sub-networks called gene modules by network connectivity, resulting in 16 modules. Each gene module was then validated for its involvement in phagocytosis. Among 16 modules, 2 modules showed a statistically positive correlation to phagocytosis suggesting that the module was enriched with the genes involved in phagocytosis. The module significance analysis showed that only 1 module revealed a significant correlation between module membership and gene significance. Among 113 genes in the module, 16 genes with high connectivity (hub genes) and with gene expression correlation with phagocytosis were selected to be analyzed for their involvement in phagocytosis by transduction of gRNA in RAW264.7 with stably expressed Cas9 using CRISPR-Cas9. Among the 16 genes, 10 genes (*CCDC162, CNPY4, CXCL2, FAM214A, HCK, KAT8, MMP19, RALGDS TBC1D4, TBC1D25*) were transduced into RAW264.7 with stably expressed Cas9. Out of 10 genes, 6 genes, *CCDC162, CXCL2, FAM214A, HCK, KAT8,* and *MMP19*, were involved in phagocytosis. Among them CCDC162 and FAM214A were discovered to have functional involvement related to phagocytosis. By using graph network analysis of single cell transcriptomics, this study not only identifies novel genes related to phagocytosis but also showcases the use of single-cell transcriptomics and network analysis to study cellular heterogeneity and understand the gene regulation of cellular processes. In summary, this study introduces the application of weight gene co-expression network analysis of single transcriptomics for identifying genes associated with phagocytic heterogeneity in macrophage cell line.