Differential methylation of enhancers contributes to transcriptional heterogeneity in monocyte-derived macrophages.

Yi Xu[1], Asavela Kama[1], Kelda Perumal[1] and Nikki Gentle[1]
[1]School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg

Macrophages, key players in both innate and adaptive immune responses, exhibit extensive heterogeneity with respect to their gene expression profiles and functions. We sought to establish if this heterogeneity is represented in the myeloid cell line models commonly used to study macrophage differentiation *in vitro* by integrating gene expression (RNA-seq and CAGE-seq), chromatin accessibility (ATAC-seq) and DNA methylation (BS-seq) data obtained from HL-60, U937, and THP-1 cells treated with and without phorbol 12-myristate 13-acetate (PMA) to induce differentiation to a macrophage state. Using RNA sequencing data obtained across three time points, differential gene expression analysis identified genes uniquely expressed in both the differentiated and undifferentiated states of each cell line, as well as a core set of genes that characterise the changes in RNA abundance associated with PMA-induced monocyte-to-macrophage differentiation. PMA treatment was also found to induce distinct changes in gene expression in each individual cell line. Through integrative analysis of time-matched ATAC-seq and BS-seq data, the changes in gene expression observed were then shown to be correlated with increased chromatin accessibility and/or decreased DNA methylation at cell type-specific enhancers (identified based on data from the ENCODE, FANTOM5 and Roadmap Epigenomics projects) in THP-1 cells. Genes identified as belonging to the core set commonly expressed and regulated across all cell lines included known regulators of monocyte-to-macrophage differentiation such as SPI1 (PU.1) and CSF1, while genes found to be commonly expressed but differentially regulated across cell lines included key regulators of immune responses, including members of the NF-κB family and TNF receptor superfamily. These results provide a description of the commonalities and differences in gene expression and regulation observed across cell line models frequently used to study monocyte-to-macrophage differentiation, and have implications for the use of these cell lines in studies relating to immune responses.