Dendritic Cell Subsets: A Characterisation Based On Transcriptomics

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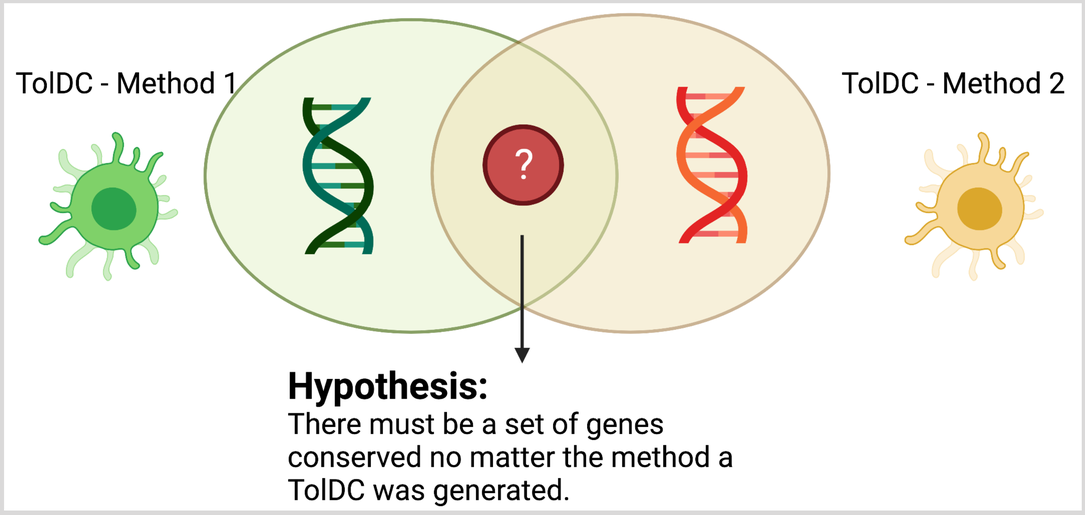
Introduction

Tolerogenic Dendritic Cells are emerging as an exciting cellular therapy for autoimmune conditions. However, to date their cellular phenotype has been characterised based on cell surface marker ratios. This classification method is quite crude and lacks sensitivity. Further, the broad variety of protocols used to generate tolDC in vitro and their functional and phenotypical heterogeneity, are evidencing the need to find robust biomarkers. As a key step towards their translation into therapy and ensuing mechanistic studies[1]. Herein we utilised a meta-analysis like approach to determine genes critical to the tolerogenic dendritic cell. Further, we extend this transcriptomic characterisation to other dendritic cell subsets, while demonstrating the usefulness of our approach.

1. Navarro-Barriuso, J., Mansilla, M. and Martínez-Cáceres, E., 2018. Searching for the Transcriptomic Signature of Immune Tolerance Induction—Biomarkers of Safety and Functionality for Tolerogenic Dendritic Cells and Regulatory Macrophages. Frontiers in Immunology, 9.

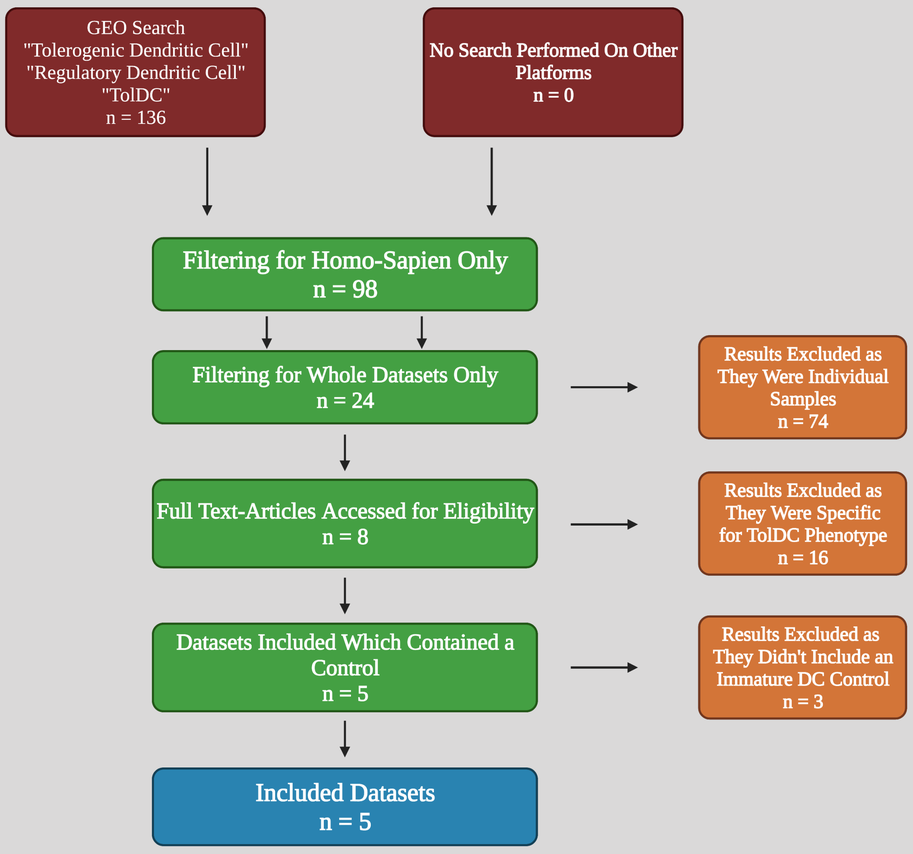


Aims:

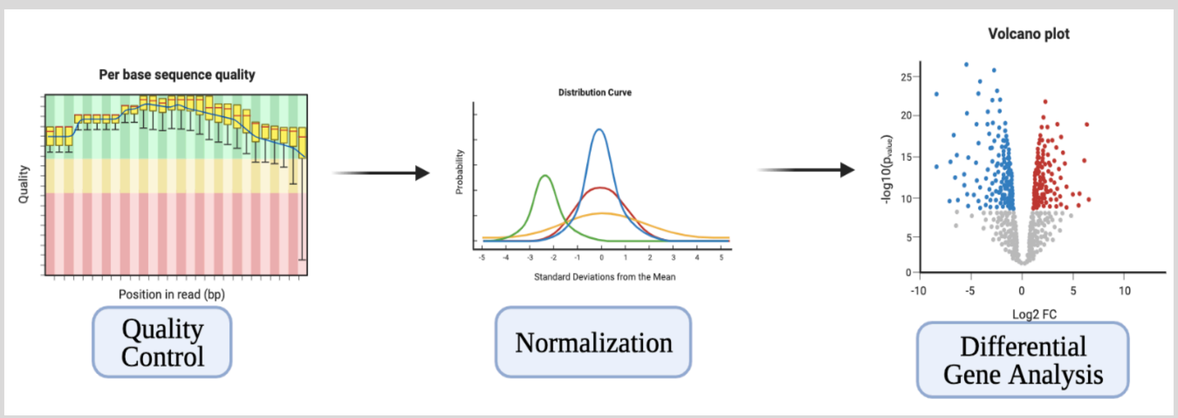
1. Utilise Publicly available gene expression datasets to determine genes critical to the tolerogenic dendritic cell phenotype.
2. Explore the ability of this method to characterise other dendritic cell phenotypes.

Gene Set Discovery Methods/Results

1. Literature Search



2. Processing Methods and Quality Control



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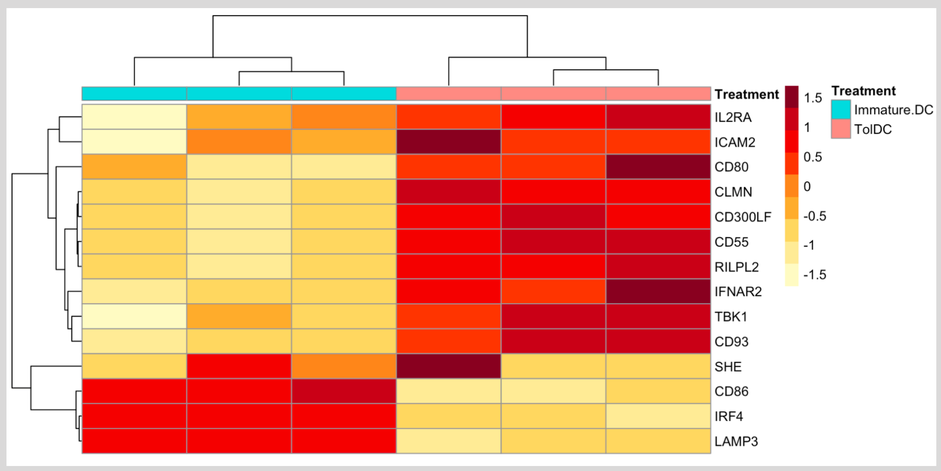
- Each of the datasets included were first examined for quality before being included in further analysis.
- The data was then normalized, to reduce non-biological variability.
- Differential Genes were then determined from each dataset.

3. Aggregating Results



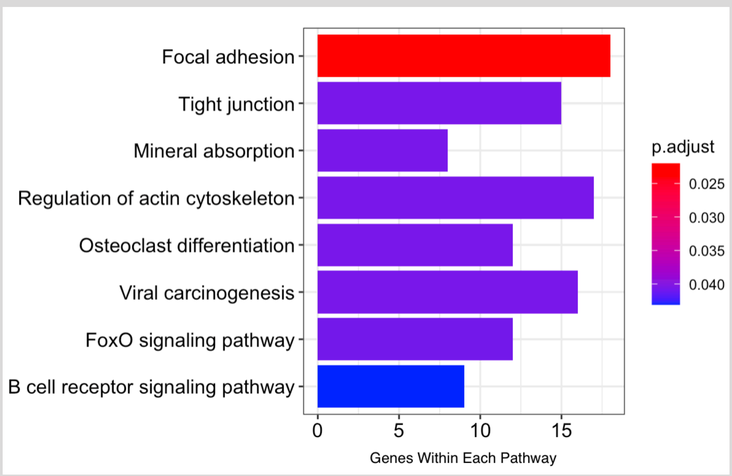
- The results of each differenital gene expression analysis were combined.

- Genes displayed in the heatmap (left) demonstrate the phenotypic change between the immature and tolerogenic dendritic cell phenotypes.

4. Enrichment Analysis

- The genes critical to the tolerogenic dendritic cell phenotype then underwent enrichment analysis.

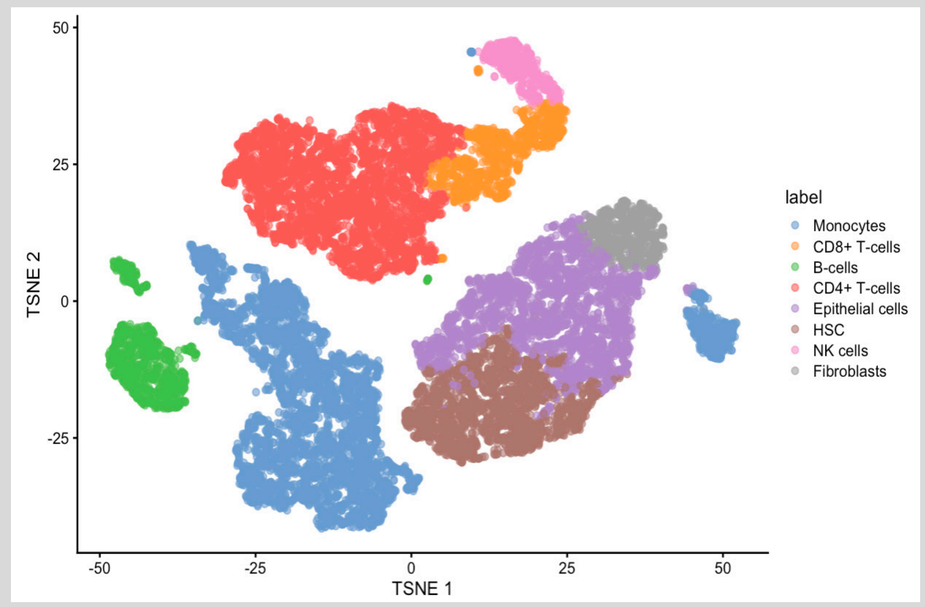
- KEGG pathways were identified and assigned a significance level via a Wilcoxon rank sum test.



Validating Our Gene Signature

- We could not validate the tolerogenic dendritic cell signature due to their small proportions in vivo.
- We then explored how accurate our activated dendritic cell signature was in various other datasets.

1. Annotating scRNAseq Data

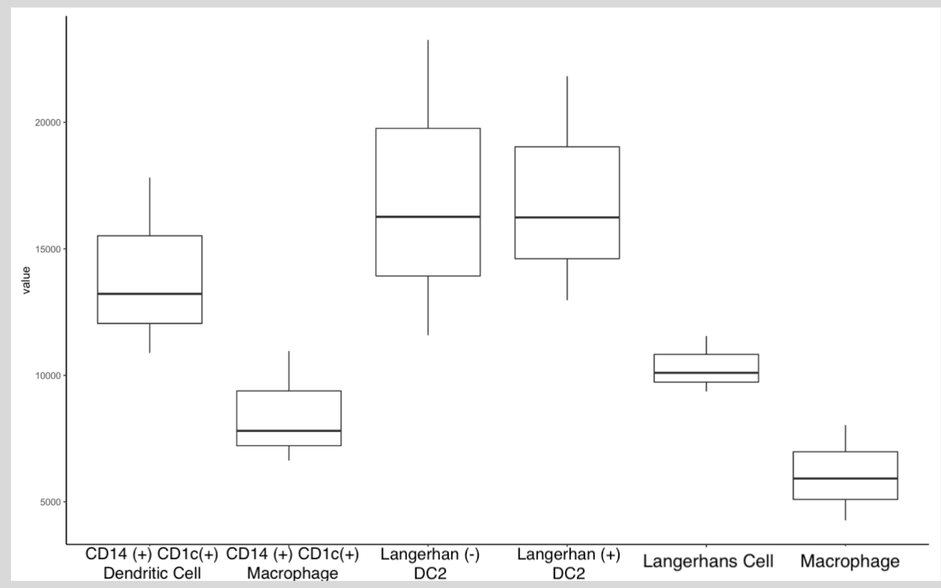


- Healthy PBMCs were isolated and sequenced.

- Cells were clustered and assigned a cell type label base on key markers.

- Dendritic cells were not found due to low proportions.

2. FACS Sorted scRNAseq



- Cells were then sorted via FACS and labelled as a cell phenotype.

- Our gene signature differentiated between dendritic cells and macrophages.

Conclusion & Future Directions

- We successfully determined a transcriptomic signature specific to both the activated and tolerogenic dendritic cell.
- Validating the activated dendritic cell signature in differentiating mononuclear phagocytes.
- The validation for each gene signature is still incomplete.
- Next, we will use gene silencing to examine the ability of both dendritic cell phenotypes in stimualting T-cells. Ideally, neither will stimulate a T-cell effectively.