Gene Regulatory Network Reveals the Landscape of Myeloid Dendritic Cell Differentiation

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BACKGROUND

Most immune cells, originated in the bone marrow, develop from hematopoietic stem cells, which follow one of two major differentiation tracks – myeloid and lymphoid lineages and fully develop into immune cells by specific molecular cues in the bone marrow. Dendritic cells (DCs), one of the immune cells in myeloid lineage, are specialized in presenting antigens to naïve T cells, and are commonly divided into cDC1 (CD8α+ DC) and cDC2 (CD11b+ DC) subpopulations based on their lineage, surface phenotype and characteristics. In some studies, DC is further subdivided into monocyte-derived DC, plasmacytoid DC, and inflammatory DC, and recent studies revealed two subpopulations in cDC2 referred to as DC2 and DC3 by single-cell technologies. However, the heterogeneity of DC makes their precise classification and elucidation of differentiation pathway complex. Through unbiased bioinformatics analysis, we assess the whole transcriptomic profiles of myeloid cells in the bone marrow and systematically identify their functional and phenotypic heterogeneity.

METHODS

Bone marrow cells were collated from three single-cell RNA sequencing studies (Hu et al. 2022, You et al. 2022, Oetjen et al. 2018). Quality control metrics for each dataset were used as documented, and processed datasets were integrated using the Seurat R package. Cells were clustered based on the graph-based clustering approach and visualized in two dimensions by uniform manifold approximation and projection (UMAP). Pre-ranked enrichment test of the DEGs onto pathway terms obtained from MsigDBGene Ontology was performed using FGSEA R package. Trajectory analysis was performed using Monocle R versions 3 based on the gene expression profiles. The regulon matrix representing the regulatory interactions between transcription factors (TFs) and their target genes were inferred using the SCENIC R package. Co-expression network analysis of interconnected genes in biologically enriched modules was identified using hdWGCNA R package.

RESULTS

A total of 81,867 bone marrow cells were collated and classified into 5 major cell lineages (**Figure 1a**). Based on the expression of canonical marker genes, we defined them as hematopoietic stem/progenitor (HSPC) lineage (CD34 high), myeloid lineage (CD33 high), T/NK lineage (CD7 high), B cell Lineage (CD19 high), and erythroid/megakaryocyte (EM) lineage (GATA1 high) (**Figure 1b**). Identified HSPC were re-clustered into subpopulations, and trajectory analysis showed that HSCs primarily diverege into two developmental branches - myeloid fate, lymphoid fate, and LMPP in myeloid fate is divided into EM fate and granulocyte-monocyte fate (**Figure 1c**).

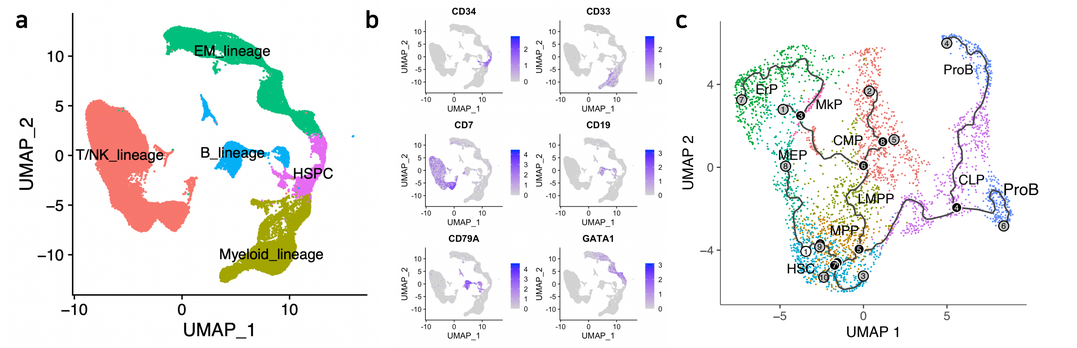

Figure 1. Transcriptomic landscape of human bone marrow cells. a. UMAP visualization of main lineages in bone marrow based on single-cell transcriptomics. EM erythroid/megakaryocyte; HSPC hematopoietic stem/progenitor **b.** UMAP visualization of the expression of lineage-specific marker genes. **c.** Differentiation trajectories of HSPC populations.

We integrated HSPCs in granulocyte-monocyte fate with myeloid lineage cells and re-clustered into subpopulations (**Figure 2a**). We collated 15,437 cells in the developing myeloid system, and trajectory inference identified that monocytes and DCs share common differentiation pathway originating from the HSC and diverge in the stage of MDP (**Figure 2b, 2c**). Monocytes are the direct progeny of MDP and mature into two subpopulations of CD14+ monocyte and CD16+ monocytes. In contrast, DC is further diverged from MDP to CDP, and mature into two subpopulations of pDC and cDC. DEGs between MDP and CDP showed significant enrichment for several biological pathways related to biosynthesis of macromolecules and organization of cytoskeletal structures (**Figure 2d, 2e**). DEGs up-regulated in MDP were highly associated with the biosynthesis of amide and peptide molecules, as MDP suppresses T cell responses through the production of nitrogen monoxide. DEGs up-regulated in CDP showed enrichment with microtubule and cytoskeleton organization, revealing a process of dendritic spine morphogenesis.

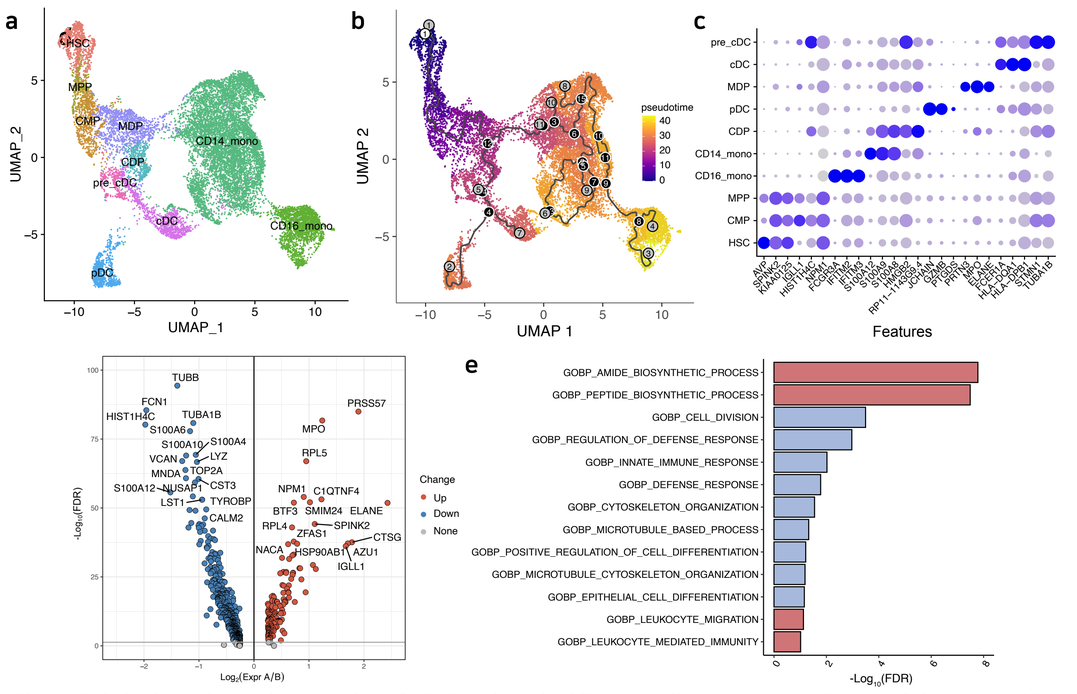

Figure 2. Sub-clustering and Integration of HSPC and Myeloid lineage cells. a. UMAP visualization of clusters in HSPC and myeloid-lineage cells. HSC hematopoietic stem cell; MPP multipotential progenitor; CMP common myeloid progenitor; MDP monocyte-dendritic cell progenitor; CDP common dendritic cell progenitor; mono monocyte. **b.** Differentiation trajectories of HSPC and myeloid-lineage cells colored in pseudotime. **c.** Gene expression of top 5 up-regulated cell type-specific markers. **d.** Volcano plot showing the fold change of DEGs in MDP compared to CDP. **e.** Functional annotations for DEGs showing signific-antly enriched biological pathways (FDR ≤ 0.1). Red and blue colors indicates up- and down-regulations.

Trajectory analysis revealed that CDP differentiates into pre-cDC, which expresses HMGB2, a selectively expressed gene in highly proliferative cells undergoing differentiation, and fully diverge into cDCs (**Figure 3a, 3b, 3c**). Through gene regulatory network analysis, the cell states of each subpopulation were identified based on key regulon activity (**Figure 3d**). After entering the intermediate state of cDC (cDC_gr0), which has relatively weak regulon activity and gene expression, cells fully diverge into 3 groups of cDCs - cDC_gr1, cDC_gr2, cDC_gr3. cDC_gr1 and cDC_gr2, sharing similar activity of regulons required for cDC2 development such as IRF2 and CEBPB, are divided by the expression of genes such as S100A8, S100A9, VCAN, and FCN1 (**Figure 3e**). In contrast, cDC_gr3 shows strong activity of IRF1, the key driver gene for the function of cDC1.

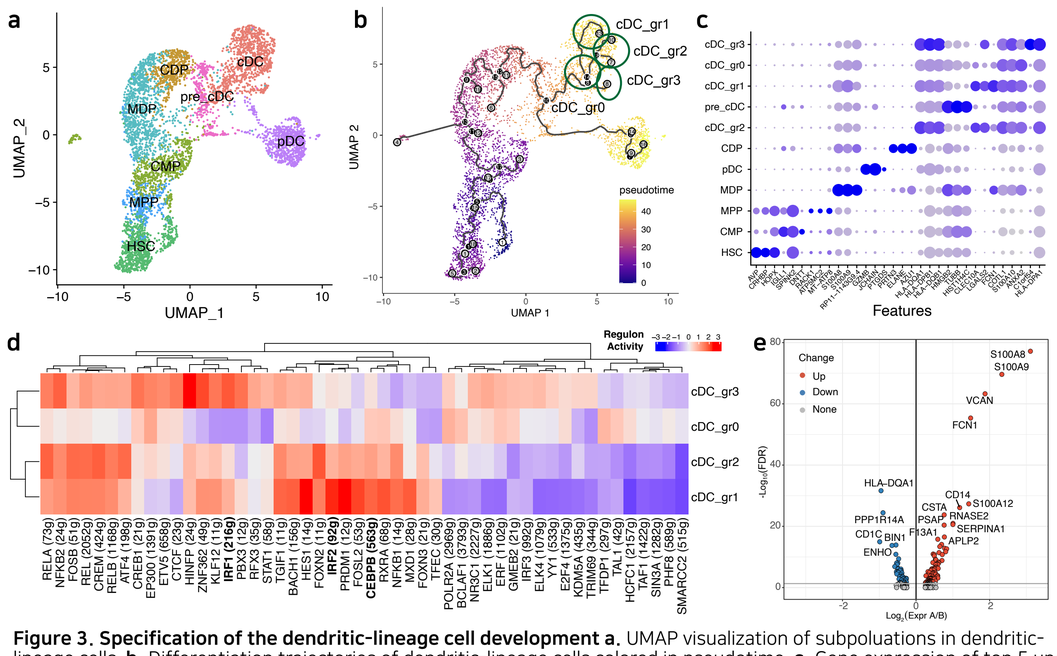

Figure 3. Specification of the dendritic-lineage cell development a. UMAP visualization of subpoluations in dendritic-lineage cells. **b.** Differentiation trajectories of dendritic-lineage cells colored in pseudotime. **c.** Gene expression of top 5 up-regulated cell type-specific markers. **d.** Heatmap of the regulon activity for cDC subpopulations inferred by SCENIC. **e.** Volcano plot showing the fold change of DEGs in cDC_gr1 compared to cDC_gr2.

To further delineate the biological distinction of the cDC2 subpopulations, we analyzed gene co-expression network analysis of dendritic-lineage cells (**Figure 4a**). As a result, we found 4 co-expression modules showing difference in expression levels between cDC_gr1 and cDC_gr2 (**Figure 4b, 4c**). cDC_gr1 shows higher expression of module Brown and Black, which is characterized by ATP synthesis and cellular junctions. cDC_gr2 shows higher expression of module Blue and Yellow, characterized by T cell differentiation and MHC protein assembly.

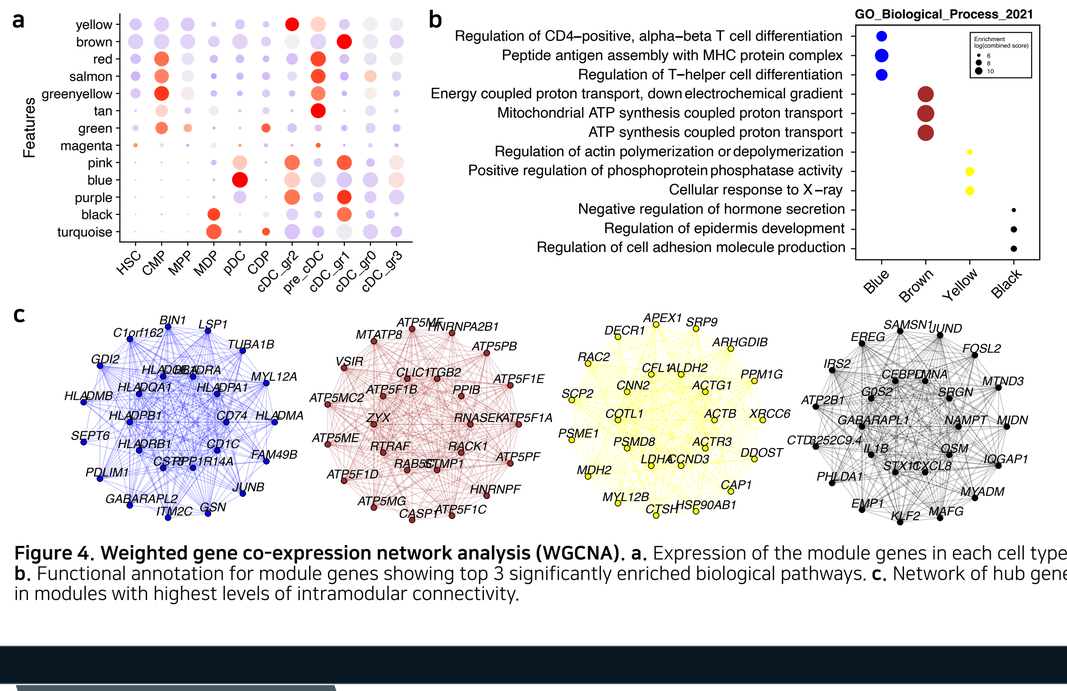

Figure 4. Weighted gene co-expression network analysis (WGCNA). a. Expression of the module genes in each cell type. **b.** Functional annotation for module genes showing top 3 significantly enriched biological pathways. **c.** Network of hub genes in modules with highest levels of intramodular connectivity.

DISCUSSIONS

We provided a high-resolution transcriptome map of bone marrow myeloid cells and deciphered the major changes in transcriptional profiles on myeloid divergence. Our results suggest that the myeloid-biased differentiation initiates from the stage of LMPP, and further diverge into DC and monocyte in the stage of MDP. Moreover, we identified different regulatory activities of IRF1 and IRF2 between cDC subpopulations, and observed biological alterations in cDC2 represented as enrichment with cellular adhesion or T cell differentiation pathways. These findings will further provide insights into understanding the heterogeneity of myeloid DC populations at transcript level.

REFERENCES

1. Tonglin H, Yanna Z, Xiaoling Y, Ruilan G et al. Single-Cell RNA-Seq of Bone Marrow Cells in Aplastic Anemia. Front Genet 2021;12:745483.
2. You G, Zhang M, Bian Z, Guo H et al. Decoding lymphomyeloid divergence and immune hyporesponsiveness in G-CSF-primed human bone marrow by single-cell RNA-seq. Cell Discov 2022 Jun 22;8(1):59.
3. Oetjen KA, Lindblad KE, Goswami M, Gui G et al. Human bone marrow assessment by single-cell RNA sequencing, mass cytometry, and flow cytometry. JCI Insight 2018 Dec 6;3(23).

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