Single-cell RNA sequencing reveals activated status of peripheral blood monocytes in tissue homeostasis

Jiarui Lu (✉ lujr5@mail2.sysu.edu.cn)

Sun Yat-Sen University Guanghua School of Stomatology

Junpan Luo

Sun Yat-Sen University Guanghua School of Stomatology

Junbing Guo

Sun Yat-sen University First Affiliated Hospital

Jie Zeng

Third Affiliated Hospital of Guangzhou Medical College

Xiaolei Zhang

Sun Yat-Sen University Guanghua School of Stomatology

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Abstract

Osteoclasts are giant multinucleated cells responsible for bone resorption, derived from myeloid cells of the monocyte/macrophage lineage. Peripheral blood monocytes have increased osteoclast differentiation potential, but only a small proportion of monocytes can differentiate into osteoclasts. The characteristics of osteoclast precursors remain unknown. The aim of this study is to explore the heterogeneity of human blood monocytes from healthy donors by use of single cell RNA sequencing (scRNA-seq), and infer the osteoclastogenic potential of the newly identified subsets with the bioinformatics approach analysis. In this study, magnetic cell separator was used to collect CD14⁺ monocytes from human peripheral blood, and scRNA-seq was applied as an unbiased analysis strategy to identify and analyze human blood monocyte subtypes at the single-cell resolution. Bioinformatics analysis was subsequently performed based on the differentially expressed genes (DEGs) of each cluster. Six monocyte clusters of human blood were identified, which were named of, "Initial MONO", "Antigen presenting MONO", "Inflamed MONO", "Bacteriolytic MONO", "Non-classical MONO", and "Antivirus MONO". Each cluster exhibited unique transcriptional profile and distinct pathway enrichments, revealing the steady-state activation of monocytes. These data provided new opportunities and insight to explore unique populations of monocytes and their different potential, and further conducive to the development of more effective anti-bone resorptive therapy.

Introduction

The balance of the bone resorption by osteoclasts and bone formation of osteoblasts is critical for bone development and the maintenance of homeostasis [1]. Osteoclasts are giant multinucleated cells, constituting the main class of cells that are responsible for bone resorption [2]. For osteoclasts to be formed, both precursor cells, the myeloid cells of the monocyte/macrophage lineage, and regulation from the microenvironment are necessary [3].

Studies have shown increased osteoclast differentiation potential of peripheral blood monocytes compared to bone marrow monocytes [4]. Monocytes of peripheral blood are heterogeneous and consist of several subsets. For human beings, blood monocytes are suggested to be subdivided on the basis of surface expression of CD14 and CD16 proteins, into 3 subsets, that is, CD14⁺⁺CD16⁻ (classical monocyte), CD14⁺CD16⁺ (intermediate monocyte) and CD14⁺CD16⁺⁺ (non-classical monocyte) [5, 6]. Numerous studies have shown that each subset has distinct functions. Classical monocytes are rapidly recruited into inflamed tissues, and promote immune responses during injury or inflammation, such as the identification and removal of microbes and dying cells [7]. Intermediate monocytes are raised in a later stage of inflammation, and their presence increased with more advanced stages in some chronic infection diseases [8–10]. Non-classical monocytes are characterized by their unique function of patrolling the vascular endothelium and removing damaged cells and debris from the vasculature [11].

While compared with the bulk of the human peripheral blood monocytes, only a small proportion of monocytes can differentiate into osteoclasts [12]. In previous study, it was found that the purified

CD16⁺ human peripheral blood monocyte subset from healthy donors, differentiates into osteoclasts by stimulation with macrophage colony-stimulating factor (M-CSF or CSF-1) and receptor activator of nuclear factor-kappaB ligand (RANKL) [13]. Another study has shown that human peripheral blood osteoclast precursors reside in the proliferative monocytes' subpopulation, which is capable of proliferating *in vitro* [14]. Since the choice of surface markers for cell subpopulation requires sufficient knowledge and validation, it is suggested to apply functional criteria, such as PM status, rather than surface marker phenotyping, to avoid the difficulty in defining [14].

Transcriptome analysis provides a comprehension of gene structure and function, as well as allows us an understanding of the human genome at the transcription level [15]. Nowadays, single-cell transcriptomics using next-generation transcriptional sequencing has been a powerful tool to analyze cell-to-cell variability on a genome scale [16]. The advance of single-cell technology provides an unbiased, optional workflow that allows cells to be sequenced without prior knowledge of target genes and proteins, and cells are grouped according to their transcriptional characteristics [17]. The recent breakthrough of scRNA-seq allows the study of the transcriptomic landscape at single-cell resolution within a given population [18]. It offers the possibility of enhancing detection of cell types with low frequencies and blurred intermediate cell states based on transcriptome of each cell [19]. It is expected to map cell phenotypic heterogeneity and to identify previously undefined cell populations as well as their gene expression characteristics [20].

The aim of the present study is to explore the heterogeneity of human blood monocytes from healthy donor by use of scRNA-seq, and infer the osteoclastogenic potential of the newly identified subsets with the bioinformatics approach analysis. Compared with the current classification of monocyte defined by surface makers, the results of this study were expected to provide a more unbiased and comprehensive map of human blood monocytes. This exploration may facilitate the identification of osteoclast precursors and discovery of a beneficial approach as an anti-bone resorptive therapy.

Materials And Methods

Tissue collection and materials.

The fresh blood sample for the scRNA-seq was obtained from representative healthy donors with informed consent. The representative donors are healthy and able to represent basic blood condition of most Chinese human beings. Monocytes of healthy donors have been collected from CD14⁺ cell separation and osteoclast differentiation before as previously demonstrated [21]. This study was conducted in compliance with the Declaration of Helsinki.

Cell separation and cell sorting.

The peripheral blood of the volunteer (100 mL) was collected into BD vacutainer cell preparation tubes (CPT) with sodium citrate (BD) and centrifuged at high speed (1,500 g, 4 °C) for 30 minutes. After centrifugation, the mononuclear cells in the middle layer of the tube were sucked out and suspended in

the complete medium (α -MEM, Thermo Fisher Scientific, USA; 10% fetal bovine serum, FBS, Gibco, USA). Mononuclear precipitation was obtained by low-speed centrifugation (1300 rpm, 4°C) for 10 minutes. After the cell precipitation was eluted three times with CD14⁺ separating solution (0.5% FBS, Gibco, USA; 2 mM Ethylene Diamine Tetraacetic Acid, EDTA, Thermo Fisher Scientific, USA, pH = 8) for 5 minutes each time and magnetic coated with MACS CD14 human microbeads (MiltenyiBiotec, Bisley, UK) for 15 min at 4°C, CD14⁺ monocytes were collected by magnetic cell separator as previously reported [22].

10x sample processing and cDNA library preparation.

Samples were prepared as described by the 10x Genomics Single Cell 3' v2 Reagent Kit user guide. After reverse transcription, cDNA was recovered using the Recovery Agent provided by 10x, and then cleaned up by Silane DynaBead (Thermo Fisher Scientific, USA) as described in the user guide. Prepare the cDNA library following the method outlined in the Single Cell 3'Reagent Kits v2 User Guide, and modify the PCR cycle appropriately based on the calculated cDNA concentration (as recommended by 10X Genomics).

Sequencing.

The molarity of each library was calculated based on library size as measured by a bioanalyzer (Agilent Technologies, USA) and qPCR amplification data. Samples were pooled and normalized to 10 nM, then diluted to 2 nM using elution buffer with 0.1% Tween20 (Sigma-Aldrich, St Louis, MO). Samples were sequenced on a Novaseq 6000 with the following run parameters: Read 1—26 cycles, read 2—98 cycles, index 1—8 cycles. A median sequencing depth of 50,000 reads/cell was targeted for each sample.

Cell clustering, differential expression, and pathway analysis.

Raw sequencing data (bcl files) were converted to fastq files with Illumina bcl2fastq, v2.19.1 and aligned to the human genome reference sequence. The Cell Ranger analysis pipeline was used to generate a digital gene expression matrix from the data. Then, the data were further analyzed with the filtered barcode matrix files using the Seurat package, v2.3.4 (<http://seurat.r-forge.r-project.org/>, RRID:SCR_007322). The raw digital gene expression matrix (unique molecular identifiers, UMI counts per gene per cell) was filtered, normalized, and clustered using R 2 (<http://www.r-project.org/>, RRID:SCR_001905). Cell and gene filtering was performed as follows: Cells with a very small library size (< 1500) and a very high (> 0.5) mitochondrial genome transcript ratio were removed. Genes detected (UMI count > 0) in less than three cells were removed. Normalization was performed in the scan R package using the default implementation of their pool and deconvolute normalization algorithm. After normalization, clustering was performed using standard package procedures. Cell clusters were visualized using t-SNE. The major cell type-specific markers were screened by Seurat's Bimod likelihood ratio statistical test. The differential gene between the specified cell population and the remaining cell population satisfies $P\text{-value} \leq 0.05$ and the differential expression range greater than or equal to 2 times. These markers are further subjected to bidirectional hierarchical clustering of genes and samples. According to log2FC (FoldChange), a heat map of the top 10 genes unique to each cluster was shown.

Gene ontology and pathway enrichment analysis of DEGs.

Gene ontology (GO, <http://www.geneontology.org/>, RRID:SCR_002811) analysis is a common useful method for annotating genes and gene products and for identifying characteristic biological attributes for high-throughput genome or transcriptome data [23, 24]. Kyoto Encyclopedia of Genes and Genomes (KEGG) Expression Database (<http://www.genome.jp/kegg/expression/>, RRID:SCR_001120) is a knowledge information base that systematically analyzes gene functions and links genomic information to advanced functions [25]. Comprehensively, mapping of user's gene to the relevant biological annotation in the DAVID database (<http://david.abcc.ncifcrf.gov/>, RRID:SCR_001881) is an essential foundation for success of any high-throughput gene functional analysis [26]. In order to analyze the DEGs at the functional level, GO enrichment and KEGG pathway analysis were performed using clusterProfiler, v3.6.0 (<http://bioconductor.org/packages/release/bioc/html/clusterProfiler.html>, RRID:SCR_016884). $P < 0.05$ was considered statistically significant.

Gene Set Variation Analysis.

For Gene set variation analysis (GSVA, <http://www.broadinstitute.org/gsea/>, RRID:SCR_003199), the Hallmark and Immunologic gene set used for the functional analysis was downloaded from the Molecular Signatures Database (MSigDB, <http://software.broadinstitute.org/gsea/msigdb/index.jsp>, RRID:SCR_016863) [27], a widely used and comprehensive database. GSVA was performed using GSVA (v1.32.0). $P < 0.05$ was considered statistically significant.

Monocle2 trajectories analysis.

In order to predict cellular differentiation, cells were ordered in pseudo-time using Monocle2, v2.6.4 (<http://cole-trapnell-lab.github.io/monocle-release/docs/>, RRID:SCR_016339) [28]. The top 1000 DEGs in the six clusters (ranked by lowest q value) were used to sort the cells. The BEAM method in the Monocle2 package was used to uncover genes with significantly different expression levels across branch points [28].

Results

scRNA-seq analysis revealed distinct human blood monocyte subsets.

To define the heterogeneity of monocytes, scRNA-seq experiments were performed by use of CD14⁺ mononuclear cells. Within the filtered population, most cells were CD14 positive (Fig. 1a). As for CD14 negative cells, CD14 transcripts may not be well detected because of the relatively small number of genes in a single cell obtained on the 10X platform. Initial clustering resulted in 7 distinct clusters of sequenced cells (Fig. 1b). After the QC filtering, a total of 7408 cells were obtained (Fig. 1c).

As expected, the majority cells of all the clusters were CD14 positive (Fig. 2a). The markers of CD3, CD56 (NCAM1), CD19 were used to further detect the purity of monocytes (Fig. 2a). Overlaying the markers with

our initial clustering revealed that cluster #6 were not monocytes. A high expression of T cell-specific markers (CD3D/E/G) was found in cluster #6 (Fig. 2a). Therefore, cluster#6 was excluded from further analyses of monocyte-lineage cells.

In the remaining clusters, 7352 monocytes from the blood were kept (Fig. 1d). These monocytes were grouped into 6 clusters visualized using a t-distributed stochastic neighbor embedding (t-SNE) plot where each dot represents a single cell (Fig. 2b). Based on the cluster-defining differentially expressed genes (DEGs) (Figs. 2c), GO (Fig. 3a) and KEGG (Fig. 3b) analysis of DEGs of each cluster, GSVA(Fig. 4), trajectory analysis (Fig. 5), the clusters from the sample were named as “Initial MONO”, “Antigen presenting MONO”, “Inflamed MONO”, “Bacteriolytic MONO”, “Non-classical MONO”, and “Antivirus MONO” respectively (Fig. 2b). Basic information of these nomenclatures would be discussed in details later.

The Non-classical MONO formed a homogeneous population.

We observed six clusters and found cluster #4 mapped mostly close to the well-established monocyte subsets, non-classical (CD14⁺CD16⁺⁺) monocytes (based on the post hoc overlap of transcript and surface marker expression). We referred to this subset henceforth as “Non-classical MONO”. Non-classical MONO highly expressed FCGRF3A (CD16), and expression of CD14 was relatively lower, compared with other clusters.

GO analysis results showed that DEGs were significantly enriched in molecular function (MF), including oxidoreductase activity (acting on a heme group of donors, oxygen as acceptor), heme-copper terminal oxidase activity and cytochrome-c oxidase activity (Fig. 3a). For biological process (BP), the DEGs were enriched in co-translational protein targeting to membrane, establishment of protein localization to endoplasmic reticulum (Fig. 3a). In addition, GO cell component (CC) analysis displayed that DEGs were significantly enriched in the ficolin-1-rich granule (Fig. 3a). Enrichment analysis using the KEGG pathway databases (Fig. 3b) showed the most significantly enriched pathways of DEGs were ribosome and proteasome pathways.

Antivirus MONO and Inflamed MONO were two activated monocyte subsets.

One of the clusters that we found was the “Antivirus MONO” cluster. The top 10 marker genes associated with this cluster included *IFI44L*, *IFIT1*, *MX1*, *ISG15*, *IFI44*, *IFIT2*, *HERC5*, and indicating stimulation of monocytes presumably by interferon (Fig. 2c). The Hallmark gene set collection further supported this notion with enrichment of interferon- α and interferon- β response (Fig. 4). GO analysis (Fig. 3a) results showed that DEGs were significantly enriched in BP, including negative regulation of viral genome replication, negative regulation of viral process and negative regulation of viral life cycle. For MF, RAGE receptor binding was particularly enriched. Enrichment analysis using the KEGG pathway databases (Fig. 3b) showed that DEGs in Antivirus MONO were associated with Cytosolic DNA-sensing pathway. All these implied an activated response to vaccine and an ongoing antivirus function of this cluster.

Interestingly, we found a second cluster with cells of activated status, labeled as the “Inflamed MONO”. Unlike the “Antivirus MONO” cluster, we discovered the Inflamed MONO cluster highly expressed the up-regulated DEGs associated with chemokine, including *CCL4*, *CCL3*, *CCL3L3*, *CCL4L2*, *CXCL2* and *CXCL8*. In addition, this cluster had a high expression of *IL1B*, encoding a member of the interleukin 1 cytokine family, which is an important mediator of the inflammatory. *TNFAIP3*, a gene whose expression is rapidly induced by the tumor necrosis factor (TNF), were also up-regulated. Compared with the rest of the monocytes, GO, KEGG and HALLMARK analysis (Fig. 3,4) revealed that the “Inflamed MONO” cluster were: (1) enriched in protein phosphatase activator activity and MAP kinase tyrosine/serine/threonine phosphatase activity, and cellular response to interleukin-1; (2) activated in several signaling pathways including TNF- α signaling via NF κ B, inflammatory response, *IL2-STAT5* signaling, *IL6-JAK-STAT3* signaling; and (3) stimulated by various stimuli or receptors including *TNF*, *IL-1*.

Antigen presenting MONO and Bacteriolytic MONO exhibited unique transcriptional profile.

A slightly higher level of expression of MHC class II genes was found in cluster #1, including *HLA-DRA*, *HLA-DPB1*, *HLA-DRB1*, *HLA-DQB1* and *HLA-DPA1* (Fig. 2b), so we named this cluster as “Antigen presenting MONO”. GO term analysis (Fig. 3a) revealed enrichment of expected CC such as MHC class II protein complex, MHC protein complex, and MF such as MHC class II receptor activity, MHC class II protein complex binding in this cluster. KEGG analysis (Fig. 3b) showed that the most significantly enriched pathways were Asthma, Allograft rejection, Graft-versus-host disease, leading us to postulate that these cells were disease-related.

Another subset of monocytes was the “Bacteriolytic MONO” cluster. This cluster was featured with high expression of *LYZ* and antisense to *LYZ*, which encodes human lysozyme with a primarily bacteriolytic function. We also found high expression of proto-oncogene involved in control of cellular proliferation such as *C1orf56*. Notably, *CDC42SE1*, which may play a role in early contractile events in phagocytosis in macrophages, was also up-regulated in this cluster. The results of GO analysis (Fig. 3a) showed that “Bacteriolytic MONO” enriched BP of DNA deamination and CC of Arp2/3 protein complex, implying the functional attributes of this cluster.

Cell state displayed a developmental course of monocyte.

Using scRNA-seq analysis, it is possible to simulate the development trajectories of different heterogeneous populations based on transcriptional changes during cell differentiation. We used Monocle2 pseudo-time trajectory analysis to reveal the development of monocytes. As shown in Fig. 4, monocytes exhibited a relatively linear developmental process with one branch. Cluster # 0 and the “Non-classical MONO” cluster were the main groups located at the two ends of the trajectory, respectively. Based on the current human blood monocytes development model, we set cluster # 0 as the most immature branch in pseudo-time and named it “initial MONO”. Analyzing significantly different genes in the initial MONO, we found a high expression of *S100A8*, *S100A9*, *S100A12*, matching with the characteristics of genes involved in regulating many cellular processes such as cell cycle and differentiation. This DEGs of this cluster and Antigen-presenting MONO were enriched in many identical

GO (Fig. 3a) and pathways (Fig. 3b), suggesting that these two subgroups might be highly similar. GSVA (Fig. 4) shows that the multiple pathways of Initial MONO were down-regulated, further hinting its initial state.

Next, we focused on analyzing the branches of the trajectory. In the sample, branch point 1 divided the trajectory into 3 states (Fig. 5a). When we plotted the composition of each state in each cluster (Fig. 5b), it was clear that the non-classical MONO cluster was dominated by one state, while the initial MONO was dominated by two states. However, the remaining clusters were distributed in all three states, further indicating that the transcriptional profile of these clusters was induced by secondary stimuli. We believed that these four clusters may be a mixture of cells from different developmental stages, which were stimulated to form a unique transcriptome map.

Discussion

ScRNA-seq can provide more insight into cells itself for more synthesized observation and analysis. Here, we reported comprehensive single-cell expression profiling of human blood monocytes using this technology. We identified novel distinct subsets of human blood monocytes at the transcriptome level at single-cell resolution, and genes of major cell type-specific markers were defined on different clusters respectively. Bioinformatics analysis was subsequently performed on these genes. The clusters defined by unsupervised classification algorithms exhibited different functions and pathway enrichments. Furthermore, our study expanded our understanding of the mechanisms of monocyte differentiation, and prompted that each subset exhibits a different response for stimuli.

In the present study, we revealed significant heterogeneity than cell surface marker-defined monocyte subtypes, consistent with previous conclusions of a single-cell transcriptional profiling on the basis of 339 monocytes [19]. Specifically, our study found that human blood monocytes could be unbiasedly grouped into six clusters. Based on the cluster-defining DEGs, function enrichment analysis and pseudo-time trajectory analysis, we named each cluster in the sample: "Initial MONO", "Antigen presenting MONO", "Inflamed MONO", "Bacteriolytic MONO", "Non-classical MONO" and "Antivirus MONO".

Non-classical MONO maps were mostly close to the well-established monocyte subsets, non-classical (CD14⁺CD16⁺⁺) monocyte. This cluster highly expressed genes which are negative regulators of cell proliferation and transcription repressor. The DEGs enriched in molecular function of oxidase activity and biological process of protein translation and localization. Initial MONO was the least mature cluster in the pseudo-time and had high expression of *S100A8*, *S100A9* and *S100A12*. In addition, a slightly higher expression of MHC class II genes was found in Antigen presenting MONO. DEGs of this cluster enriched in molecular function of MHC class II receptor activity, MHC class II protein complex binding, and cell component of MHC class II protein complex, MHC protein complex. It was worth noting that Initial MONO shared similar DEGs enrichment with Antigen presenting MONO, indicating that the two clusters might be highly similar. Two clusters, Inflamed MONO and Antivirus MONO, demonstrated unique transcriptional features. Inflamed MONO was featured with high expression of chemokine, including *CCL4*, *CCL3*,

CCL3L3, *CCL4L2*, *CXCL2*, and *CXCL8*. Moreover, GSVA showed multiple up-regulated pathways, such as *TNF-α* signaling via *NFκB*, inflammatory response, *IL2-STAT5* signaling, *IL6-JAK-STAT3* signaling. Antivirus MONO expressed interferon induced proteins, such as *IFI44L*, *IFIT1*, *MX1*, *ISG15*, *HERC5* and *IFIT2*. GSVA also showed a strong response to interferon-α and interferon-β. DEGs are enriched in biological process of negative regulation of viral process, indicating its significant anti-virus function. Finally, Bacteriolytic MONO featured with high expression of *LYZ* and antisense to *LYZ*.

In our scRNA-seq analysis, we found discrete developmental stages among these six subsets. The prevailing idea is that monocytes differentiate from $CD14^{++}CD16^{-}$ via $CD14^{+}CD16^{+}$ to $CD14^{+}CD16^{++}$ [29]. We found a bona fide “ $CD16^{++}$ Monocyte” cluster in the scRNA-seq dataset, comprising 7% of total monocytes, and we named it Non-classical MONO. Non-classical MONO cluster was believed to be the terminal stage of monocyte, due to its up-regulated expression of negative regulators of cell proliferation. On the other hand, an Initial MONO subset was found mainly occupying the least mature branch in the pseudo-time. Many transitional populations between Initial MONO and Non-classical MONO were observed. These transitional populations were not dominated by one cell state, and may contain cells from different developmental stages, which were stimulated to display this unique transcriptome map.

The presence of these monocyte clusters revealed the steady-state activation of monocytes, which may have a considerable impact on the survival, proliferation or differentiation of monocytes. The unique transcription profiles of each subgroup have caused us to think more about their different functions and potentials. Among them, Inflamed MONO attracted our great attention. This cluster had high expression of chemokine in comparison other clusters, especially *CCL4*, *CCL3*. Moreover, the up-regulated pathways in GSVA, such as *TNF-α* signaling via *NFκB*, inflammatory response, *IL2-STAT5* signaling, *IL6-JAK-STAT3* signaling, showed the state in which the group responded to inflammation. It is reported that chemokine signals are crucial for enhanced differentiation of circulating osteoclast progenitor cells [30]. *CCL4* was considered to promote the migration and viability of preosteoclast cells [31]. And inhibition of *CCL3* abrogated precursor cell fusion and bone erosions in human osteoclast cultures [32]. These results indicated that this cluster might have increased capacity to attract immune cells with high expression of chemokines, and increased potential to differentiate into osteoclasts.

Although none of the marker genes is sufficient to distinguish the newly defined populations at cell surface level, more studies are needed to reveal whether each subset exhibits a different response for inflammatory and osteoclastogenic stimuli. It was hypothesized human osteoclast formation can be inhibited at an early stage of differentiation by interfering with the expression of pro-inflammatory cytokines, thus inhibiting the signaling pathways involved in inflammation mediated stimulation [33]. We sought to evaluate whether the enriched genes in this cluster are associated with the regulation of monocyte development and differentiation. In this way, we can find new targets to inhibit pathological bone resorption caused by abnormal osteoclast formation.

To sum up, this study reported comprehensive profiling of gene expression of human blood monocytes at single cell resolution, and empirically inferred cell subtypes. The specific expression signatures and

enriched pathways for each monocyte subtype were sorted, providing a resource for further understanding of subtype functions and ontogeny. We realized that in the steady state of healthy people, monocytes are stimulated to display unique transcriptional profiles, and thus change their differentiation potential. Though it was regrettable that only a blood sample from a donor has been collected, existing data can still provide new opportunities and insight to explore unique populations of monocytes and their exact functions, and further conducive to the development of more effective anti-bone resorptive therapy.

Declarations

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Availability of data and materials

The datasets analyzed during the current study are available in the Sequence Read Archive (SRA) with accession number of SRR11411797.

Authors' contributions

XLZ and JZ conceived, designed the experiments, and performed funding acquisition. JRL and JPL performed the experiments. JPL and GJB contributed reagents/materials/analysis tools. JRL wrote the paper. All authors read and approved the final manuscript.

Compliance with ethical standards

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the local ethical committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The fresh blood sample for the scRNA-seq was obtained from a donor with informed consent. Collection, distribution and use of the human materials has been approved by the Ethics Committee of the Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen University, China(No: AF/SC-09/02.0).

Informed consent

Informed consent was obtained from all individual participants included in the study.

Abbreviations

scRNA-seq	Single-Cell RNA sequencing
DEGs	differentially expressed genes
GO	Gene ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
GSVA	Gene set variation analysis
CC	cell component
BP	biological progress
MF	molecular function

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Figures

Figure 1

CD14 expression and initial clustering of sequenced cells. (a) Most of cells within the filtered population expressed CD14 protein; (b) Initial clustering resulted in 7 distinct clusters of sequenced cells; (c) Number and proportion of cells in each cluster after initial clustering; (d) Number and proportion of cells in each cluster after excluding cluster#6

Figure 2

Unbiased clustering of human blood monocytes. (a) Violin plots demonstrated the expression of CD14, CD16, CD19, CD56, CD3(D/E/G) of each cluster. The y-axis represents log-normalized expression value; (b) Six distinct human blood monocyte clusters were numbered, named, and displayed with a t-SNE plot; (c) Top 10 up-regulated DEGs (ranked by log2 fold change) of each cluster and CD14 were plotted using heatmap

Figure 3

GO and KEGG analysis of DEGs of each cluster. (a) GO term enrichment analysis of DEGs identified in each cluster; (b) Significantly enriched pathway terms using the KEGG pathway databases of DEGs in each cluster

Figure 5

Single-cell trajectory analysis revealed a linear monocyte developmental pathway. (a) Pseudo-time trajectory of blood monocyte with pseudo-time (left). The trajectory was separated into three cell states by branch point 1(middle). All clusters were demonstrated in the trajectory(right); (b) Individual cluster demonstrated in the trajectory

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [DEGs.xlsx](#)
- [ImmuGeneGSVA.csv](#)
- [hallmarkGSVA.csv](#)