1 **Single cell transcriptome analysis of decidua macrophages in normal and**
2 **recurrent spontaneous abortion patients**

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ABSTRACT

Due to the heterogeneity and different polarization state of decidual macrophages (dMΦ), they play an important role during the pregnancy, but their definition and exact function remain elusive. We isolated CD14⁺CD45⁺ dMΦ from the normal or RSA decidua by flow cytometry, followed by single cell RNA sequencing (scRNA-seq). In total, 23,062 single-cell transcriptomes of macrophage were profiled (12,470 Normal and 10,592 RSA), which were divided into 13 major clusters via T-distributed stochastic neighbor embedding (t-SNE) visualization. We observed that there is higher percentage composition of M1 cells (70.6%) in the normal decidua, and higher percentage composition of M2 cells (68.3%) in the RSA decidua. We identified new markers (M1: S100A8, S100A9, M2: SELENOP, FOLR2, RNASE1) and secreted cytokines (M1: IL1β, TNFSF13B and MMP9; M2: CCL3, CCL4) of the dMΦ. We found that cluster 10 as the specific cluster of dMΦ highly expressing BAG3 in normal group and cluster 7 specific highly expressing CXCL9/10/11 chemokine. After pseudo-time trajectory analysis, we found that the dMΦ formed a continuous “V-shaped” trajectory, with M1 and M2 type cells mainly occupying the two heads. We found that NFκB1, MYC and TCF12 acted as the key transcription factors of dMΦ. Our study redefined the polarization state and physiological characteristic of dMΦ in early normal and RSA pregnancy, which suggests a novel view and therapeutic target for spontaneous abortion prevention.

Keywords: scRNA-seq; Decidua macrophage; Marker gene; Inflammation; Recurrent spontaneous abortion

INTRODUCTION

The microenvironment of normal pregnancy requires dynamics precise regulation of various immune cells during the pregnancy (Erlebacher, 2013). If external stimulation or physiological factors disrupt this precisely regulation, this will lead to pregnancy complications (Perez-Sepulveda, Torres, Khoury, & Illanes, 2014). There are many kinds of immune cells at the maternal-fetal interface. Among them, decidual macrophages (dMΦ) are the second largest group of cells and comprise 20–30% of all leukocytes at the maternal-fetal interface (Gomez-Lopez, StLouis, Lehr, Sanchez-Rodriguez, & Arenas-Hernandez, 2014). Because of this special immune microenvironment, there are many differences in function and characteristics between dMΦ and macrophages from peripheral blood. However, the surface marker genes of macrophages from peripheral blood (such as CD209, CD80) were still used as FACS markers to isolate dMΦ (Svensson et al., 2011). It is necessary to explore the specific new marker of dMΦ using new methods. At present, several research groups have reported the composition of various cell types including dMΦ in the whole maternal-fetal interface (Tsang et al., 2017; Vento-Tormo et al., 2018), but the precise markers of dMΦ under this specific condition need to be furtherly illustrated.

Macrophages display important roles in the pregnancy process via their polarization, through which macrophages can differentiate into specific phenotypes and have specific biological functions in response to microenvironmental stimuli (Aagaard et al., 2014; Raj, Bonney, & Phillippe, 2014). Macrophages can be polarized into classically activated (M1) and alternatively activated (M2) macrophages based on their activation states (Houser, Tilburgs, Hill, Nicotra, & Strominger, 2011). Actually, M1 macrophage are potent effector cells involved in Type 1 T helper (Th1) responses, such as cytotoxicity toward microorganisms and enhanced production of pro-inflammatory cytokines. In contrast, M2 macrophages suppress the inflammatory response, skew the immune response toward Th2-associated immunity, promote tissue remodeling and induce angiogenesis (Svensson-Arvelund & Ernerudh, 2015). Macrophage polarization is crucial for tissue repairing and homeostasis maintenance (PrabhuDas et al., 2015; Tilburgs, Evans, Crespo, & Strominger, 2015). The state of

macrophage activation is related to many kinds of pregnancy complications, during this specific microenvironment of maternal-fetal interface, but whether there are new clusters of macrophages with new characteristic need to be furtherly investigated.

Recurrent spontaneous abortion (RSA) is a common disease for women of childbearing age (Deroux, Dumestre-Perard, Dunand-Faure, Bouillet, & Hoffmann, 2017; Khalaj et al., 2016). At present, it is believed that in addition to fetal chromosomal abnormalities, increasing studies have found that the abnormal immune function of the mother's decidua is also an important factor (Hu, Tang, Mor, & Liao, 2016; Kang, Zhang, & Zhao, 2016). At the maternal-fetal interface, the status of dMΦ inflammatory polarization changed with the development process of pregnancy, the balance of polarization between M1 and M2 macrophages is important for various processes of normal pregnancy, such as trophoblast invasion, spiral artery remodeling, and apoptotic cell phagocytosis (Buckley, Whitley, Dumitriu, & Cartwright, 2016; Li et al., 2016; Ning, Liu, & Lash, 2016). Conversely, the dysregulated polarization of macrophages was associated with inadequate remodeling of the uterine vessels and defective trophoblast invasion and finally led to spontaneous abortion, preeclampsia and preterm birth (Cappelletti et al., 2017; Ferreira, Meissner, Tilburgs, & Strominger, 2017; Triggianese, Perricone, Chimenti, De Carolis, & Perricone, 2016). So, it is very important to study the mechanism of the polarization of dMΦ during normal and pathological pregnancy.

Although increasing evidence has indicated the critical roles of dMΦ in pregnancy-related diseases (Liu et al., 2017), the properties of dysregulated macrophage polarization are still poorly understood. Here, we found that CD45⁺CD14⁺ dMΦ can divided into 13 cluster using t-SNE analysis, and normal pregnancy decidua have more percent composition of M1-type macrophages, but RSA patients have more percent composition of M2-type macrophages, and their expression profile are different from that of peripheral blood macrophages. We found that cluster 10 is the specialized cluster in normal pregnancy and cluster 7 highly expressed CXCL9/10/11, and identified many key transcription factors for each cluster. This will deepen our current knowledge of macrophage polarization involved

103 in physiological or pathological pregnancy processes, and will allow us to develop
104 therapies to improve pregnancy outcomes in the future.
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RESULTS

scRNA-seq analysis of normal dMΦ

To elucidate the cells heterogeneity of dMΦ during normal pregnancy, we performed single-cell RNA-seq (scRNA-seq) on CD45⁺CD14⁺ dMΦ isolated by flow cytometry from decidual mononuclear cells (dMCs) in normal patient. We obtained a total of 14,701 single-cell transcriptomes (10,513 in Normal 1; 4,188 in Normal 2) after quality control (Supplimentary Figure 1 A-C). T-distributed stochastic neighbor embedding (t-SNE) visualization of the combined normal data revealed 17 major clusters based on their marker gene expression, which correspond to macrophages (PTPRC, CD14, MS4A7, CTSS), NKT (PTPRC, NKG7, NCAM1, CD3E, KLRD1), dendritic cells (DCs) (PTPRC, CD1C), Osteoclast & Macrophages (PTPRC, ACP5, RANK, CTSK), T cells (PTPRC, CD3E, CD8A), NK (PTPRC, NKG7, NCAM1, KLRD1), B & Plasma Cells (PTPRC, CD79A, MS4A1), smooth muscle cells (TAGLN, ACTA2, DCN, COL1A1) and endometrial epithelial cells (PAEP, GPX3 and SLPI) (Figures 1A-C and Supplementary Table S1), and each cluster have its own specific marker genes (Figures 1D). We analyzed the percent composition of all kinds of cell types in both Normal 1 and Normal 2 (Supplementary Figure 1D). Macrophages occupied about 82.7% of the total cells in the individual sample (Supplementary Figure 1E), and we next focused on analyzing the function and characterization of dMΦ.

Macrophage cell types were furtherly analyzed in-depth by identifying sub-clusters. After re-clustering, 12,470 macrophage cells (9,005 in Normal 1; 3,465 in Normal 2) in 13 clusters were detected in normal decidua samples (Figure 1E, F and Supplementary Figure 1F-H). Due to that macrophages can be polarized into classically activated (M1) and alternatively activated (M2) macrophages, based on the polarization markers of dMΦ from previously reported (Vento-Tormo et al., 2018) (M1 marker: S100A9, CD68, CD163, CSF1R, IL1β, TNF, IL6, CXCL2, CXCL3, CXCL8; M2 marker: IL10, MRC1, MAF, F13A1, C3, C1QA, C1QB, C1QC, IGF1, IGFBP4), we divided our dMΦ into two parts, M1 cells (5961 in Normal 1 and 2596 in Normal 2) and M2 cells (3044 cells in Normal 1 and 869 cells in Normal 2) (Figure

1G and Supplementary Table S2). Interestingly, the percentage composition of M1 cells (70.6%) were significantly higher than M2 cells (29.4%) in normal decidua (Figure 1H), which is different from the traditional opinion that the higher composition of M1 cells is harmful to normal pregnancy. We also found that the specific marker gene of M1 and M2 (Figure 1I). These observations strongly suggest that normal pregnancy is linked to the relatively high polarization level of M1 macrophages.

For further characterization of M1 or M2 cells in normal decidua, we used Database for Annotation, Visualization and Integrated Discovery (DAVID) to identify KEGG and GO terms enriched among marker genes of M1 or M2. We observed that M1 macrophages might take part in a set of ribosome translational biological processes and several cellular signaling pathways (Ribosome, NOD-like receptor, NF-kappa B and TNF signaling pathway) (Supplementary Figure 2A-C and G), whereas M2 macrophages might take part in a set of antigen processing and presentation biological processes and several cellular signaling pathways (Antigen processing and presentation, lysosome and phagosome signaling pathway) (Supplementary Fig. 2D-F and H). Thus, these results suggest that the marker genes in M1 or M2 play different biological functions in the decidua of early pregnancy.

scRNA-seq analysis of normal and RSA dMΦ

To elucidate the dynamic changes of dMΦ during RSA pathogenesis, we performed scRNA-seq on CD14⁺CD45⁺ dMΦ isolated from RSA patients. We obtained a total of 13,879 single-cell transcriptomes (9,889 in RSA1, 3990 in RSA2) after removing low quality cells (Supplementary Figure 3A-C). After combined with the Normal 14,701 single-cell transcriptomes, T-distributed stochastic neighbor embedding (t-SNE) visualization of the combined normal and RSA data also revealed 17 major clusters, which are consistent with the Normal cells cluster based on marker gene expression (Figures 2A-C and Supplementary Table S3), and each cluster has their specific marker genes (Figure 2D). We analyze the percent composition of all kinds of cells in the four samples (Supplementary Figure 2D). Among these cell types,

macrophages occupied 80.3% of the total cells in the individual sample (Supplementary Figure 2E), and we next focused on analyzing the function and characterization of macrophages.

Macrophage cell types were furtherly analyzed in-depth by identifying sub-clusters. After re-clustering, multiple smaller new clusters emerged, 23,062 macrophage cells (9,005 in Normal 1; 3,465 in Normal 2; 6,993 in RSA1; 3,599 in RSA2) in 13 clusters were detected in normal and RSA decidua samples (Figure 2E, F and Supplementary Figure 3F-H). Based on the macrophage polarization markers from decidua previously reported (Vento-Tormo et al., 2018), we also divided our dMΦ into two parts, M1 cells (5961 in Normal 1, 2596 in Normal 2, 2271 in RSA 1, 1113 in RSA 2) and M2 cells (3044 cells in Normal 1, 869 cells in Normal 2, 4722 RSA 1, 2486 RSA 2) (Figure 2G and Supplementary Table S4). Surprisingly, we observed that the percentage composition of M1 cells in the RSA decidua was significantly lower than that of the normal decidua (31.7% vs. 70.6%), while the percentage composition of M2 cells in the RSA decidua were significantly higher than that of the normal decidua (68.3% vs. 29.4%) (Figure 2H). We also found the specific marker genes of M1 and M2-type macrophages (Figure 2I). These results are contrary to that composition of normal pregnancy, and strongly indicate that M1 polarization level of macrophages plays a very important role in maintaining the normal pregnancy, and much more M2 polarization of macrophages maybe the reason of the RSA pregnancy.

For further characterization of M1 or M2 cells in RSA decidua, we used DAVID to identify KEGG and GO terms enriched among marker genes of M1 or M2. We observed that M1 macrophage might take part in a set of inflammatory response and immune system process biological processes and several cellular signaling pathways (TNF and NOD-like receptor signaling pathway) (Supplementary Figure 4A-C and G), whereas M2 macrophage might take part in a set of immune response biological processes and several cellular signaling pathways (antigen processing and presentation, staphylococcus aureus infection and lysosome signaling pathway) (Supplementary Figure 4D-F and H). Thus, these results suggest that the

characterization of M1 or M2 macrophage from normal or RSA is different, and further reflect the different biological function of M1 and M2 in the decidua of early pregnancy.

Next, we analyzed genes that were differentially expressed in macrophages of normal and RSA decidua. Overall, we observed that numbers of genes were upregulated (102 genes) and downregulated (122 genes) in the RSA group compared with the normal group (Supplementary Table S5). To gain insights into the potential functional differences of the dynamic genes between the two groups, we performed functional enrichment analysis of the genes that were up- or down-regulated in normal and RSA groups. The upregulated genes (Top 5: TMSB4X, RPL18A, RPL39, S100A11, SH3BGRL3) (Supplementary Figure 4I) in the normal group were enriched for the functional categories of Ribosome, Phagosome and HIF-1 signaling pathway, indicating the activation of hypoxia induced genes in early pregnancy. The upregulated genes (Top 5: SELENOP, RNASE1, HLA-DQA1, GNLY, PDK4) (Supplementary Figure 4J) in the RSA group were enriched for the functional categories of Complement and coagulation, Toll-like receptor signaling and Estrogen signaling pathway, indicating the inhibition of inflammatory genes in macrophage of early pregnancy, whether this is related to the RSA requires further study.

New markers and secreted cytokines of the dMΦ

Previous studies labeled dMΦ with markers of peripheral blood macrophages, including classically activated (M1) markers (such as, TLR-2, TLR-4, CD80, CD86, iNOS, and MHC-II (HLA-DRB1)) and alternatively activated (M2) markers (mannitol receptor (ITGAM), CD206 (MRC1), CD163, CD209) (Brown, von Chamier, Allam, & Reyes, 2014). We detected the expression of these molecular in our single cell database, and found that the expression of these molecular (CD80, CD86, ITGAM, CD206 (MRC1), CD163 and CD209) are very low in the dMΦ and they cannot present the polarization state of decidua M1 or M2 (Supplementary Figure 5 A, B), which indicated that dMΦ isolated with classical markers of peripheral blood macrophages cannot reflect the real state of dMΦ in the

maternal-fetal microenvironment.

Is there a more accurate marker for dMΦ in maternal-fetal interface microenvironment? We next intend to examine the new marker of dMΦ, and found that new markers (PTPRC, CD14, MS4A7 and CTSS) can represent the dMΦ (Supplementary Table S1). We also identified the M1 marker genes (S100A8, S100A9, G0S2, S100A12, BCL2A1, FCN1, TIMP1, EREG, CD52 and CCL20) and M2 marker genes (SELENOP, RNASE1, C1QC, FOLR2, C1QB, C1QA, A2M, DAB2, MS4A4A and APOE) of dMΦ, which are easier to distinguish the polarization states of macrophages (Figure 3A, B). These results indicated that the genes (PTPRC, CD14, MS4A7 and CTSS) maybe the ideal new potential markers to isolate the macrophage from the decidua tissue, and M1 or M2 marker genes are easier than the traditional markers to distinguish the phenotypes of macrophages.

We have known that classically M1 and M2 macrophage cells can release various cytokines and chemokines (such as M1: TNFα, IL1α, IL1β, IL6, IL12, CXCL9 and CXCL10; M2: IL10, TGFβ, CCL1, CCL17, CCL18, CCL22 and CCL24) respectively (Brown et al., 2014). However, we find that all these cytokines are not highly expressed in dMΦ, and their expression have no significantly difference between M1 and M2, except for IL-1β, and TGF-β seem more likely highly expressed in M1, but not M2 (Supplementary Figure 5C, D). We found that M2 type dMΦ cells highly expressed CCL3, CCL4, GRN, and M1 type cells highly expressed IL1β, TNFSF13B, CCL3L3, CXCL8, CXCL2, MIF, CCL20, IL1RN and NAMPT (Figure 3A-C). These results indicated that cytokines and chemokines released by dMΦ are different from those of peripheral blood macrophages.

Cluster 10 as the specific cluster of dMΦ in normal group

We believed that dMΦ dysfunction is one of the important reasons that caused RSA, so we speculated that there maybe have some specific clusters disappeared in RSA group compared to normal group. After merged analysis of RSA and normal group by t-SNE, we found that cluster 10 almost disappeared in RSA group (Figure

2E), and this cluster mainly expressed marker genes (ZFAND2A, BAG3, HSPD1 and CACYBP) genes (Figure 4A) and highly expressed cytokines and chemokines (IL1 β , CCL3L3, CCL3, CXCL 8, CXCL2, MIF, NAMPT, IL1RN and CCL4), which specifically expressed TNFSF14 (Figure 4B). To gain insights into the potential functional of this cluster, we performed DAVID software to identify KEGG and GO terms enriched among marker genes of cluster 10. We observed that cluster 10 might take part in a set of cellular component movement and response to unfolded protein process biological processes (Supplementary Figure 6A) and several cellular signaling pathways (glycolysis/gluconeogenesis, ribosome and HIF-1 signaling pathway) (Supplementary Figure 6B). Thus, these results suggest that cluster 10 may take part in regulating hypoxia response or other stimuli, and may play an important role in the implantation process of embryo.

Cluster 7 specifically highly expressed CXCL9/10/11 chemokines

Next, we speculated that there might be some specific clusters in decidua tissue which secrete specific cytokines to maintain pregnancy. After merged analysis of RSA and normal group by t-SNE, we found that although cluster 7 displayed with no difference in cell number between Normal and RSA group (Supplementary Table S4), but this cluster specifically expressed chemokines of CXCL9/10/11 (Figure 5A) and mainly expressed marker genes (GBP1, ISG20, TNFSF10, IFIT3, IFITM1, ISG15, IDO1 and IL4I1) (Figure 5B). To gain insights into the potential functional of this cluster, we performed DAVID software to identify KEGG and GO terms enriched among marker genes of cluster 7. We observed that cluster 7 might take part in a set of biological processes and several cellular signaling pathways, such as type I interferon signaling pathway, cytokine-mediated signaling pathway and immune system process (Supplementary Figure 7A, B). Thus, these results suggest that cluster 7 mainly take part in regulating antiviral immune response.

Single-cell trajectory analysis of dMΦ

We then ordered individual macrophage cells by Monocle2 to pseudo-time

trajectory analysis to reconstruct their differentiation relationship. We found that the macrophage from normal and RSA decidua formed a continuous “V-shaped” trajectory with 7 states (Figure 6A and Supplementary Figure 8A, B). Among them, states 1, 2 and 3 were occupied by M1 type macrophages which included cluster 0, 5, 7, 8, 9, 10, 12 and state 5 was occupied by M2 type macrophage which included cluster 1, 2, 3, 4, 6, 11. M1/M2 macrophage mainly occupied the two heads and mixed macrophages at the turn and tail (Figure 6B and Supplementary Figure 8C, D). States 1, 2 and 3 take part in Ribosome, Parkinson’s disease and inflammation related signaling pathway, while state 5 takes part in antigen processing and presentation signaling pathway (Supplementary Figure 8E-I). The M1/M2 macrophage branch further bifurcated into two subbranches (Figure 6A). One subbranch (State 6) was occupied by cells with highly expressed MALAT1, NEAT1 and S100A6 genes which involved in antigen processing and presentation signaling pathway (Supplementary Figure 8J), whereas the other (State 7) showed strong expression of genes (e.g., HLA-DPB1, HLA-DRA, CLEC10A and RPL15) involved in ribosome signaling pathway (Supplementary Figure 8K). We also found that development process of dMΦ was from M1 to M2 in the early pregnancy (Figure 6C). In short, our dataset enabled the delineation of the macrophage lineage relationship and shed light on the regulatory mechanism of lineage development. These expression patterns were consistent with two different fates of the trajectory, indicating that our scRNA-seq analysis correlated with the macrophage developmental process.

Key transcription factors of dMΦ clusters

Transcription factors (TFs) play key regulatory roles in many biological processes. It is reported that NFκB, STAT1, STAT5, IRF3, and IRF5 as the M1 key transcription factors, and STAT6, IRF4, PPARδ, and PPARγ as the M2 key transcription factors, which have been shown to regulate the expression of M1 or M2 genes. But in our present study, we found that these transcription factors expressed lowly in dMΦ and had no specificity to M1 or M2 type, except for NFκB and STAT1 TFs. It seems that NFκB may be the major TFs involved in M1 macrophage

polarization, and other classic transcription factors maybe have little effect on macrophage polarization (Supplementary Figure 9A-H).

To explore central factors that might contribute to regulate the specific gene expression of each cluster, we firstly investigated possible upstream TFs for these specific genes using TF-binding motif enrichment analysis. We found that top 10 TFs can regulate the gene transcription of normal or combined normal and RSA macrophage (Figure 7A, B). We identified CEBPB, FOSL2, FOXO3, IRF7, NFκB1, PRDM1, MYC and MAFF as the key transcriptional factors for M1 type macrophage including cluster 0, 5, 7, 8, 9, and 10, while EGR1 and TCF12 as the key transcriptional factors for M2 type macrophage, including cluster 2, 6 and 11 (Figure 7C). Therefore, these findings suggested that TFs might play a central role in regulating expression of during human preimplantation development.

DISCUSSION

In this report, we found that normal pregnancy decidua had more percent composition of M1 macrophage, and RSA patient decidua had more percent composition of M2 macrophage, and their characteristics (such as, mRNA expression profile, secreted cytokines and chemokines or transcription factors) were different from those of peripheral blood macrophages. These results will deepen our current knowledge of macrophage activation involved in physiological or pathological pregnancy processes.

The marker genes for isolating dMΦ are very important for studying dMΦ functions. In many previous studies, the surface marker genes of macrophages from peripheral blood, which characterized by the expression of the mannose receptors CD206, CD80, CD209, CD16 or CD163, were utilized as FACS marker to isolate dMΦ, but dMΦ had tissue specificity, and these markers did not separate well for dMΦ (Bockle, Solder, Kind, Romani, & Sepp, 2008; Kammerer et al., 2003; Ono et al., 2018). For exploring more precise macrophage types, we isolated the dMCs using CD45⁺CD14⁺ antibodies, and we found 17 clusters with 9 types of cells. dMΦ occupied up to about 82%. We found that CD45⁺CD14⁺MS4A7⁺ might be the better

combined markers for isolating dMΦ. Furthermore, we identified the new marker genes for dMΦ, but these specific markers need to be further identified.

During early pregnancy, the polarization of dMΦ for success pregnancy is controversial (Ding et al., 2020; Jena, Nayak, Chen, & Nayak, 2019). It is reported that the polarization of dMΦ to an anti-inflammatory state is critical for the success of the pregnancy (Ben Amara et al., 2013). Decidual macrophage activation towards a more M1 phenotype has been associated with the pathology of RSA (Tsao, Wu, Chang, Wu, & Ho, 2018). But we found that the percentage composition of M1 cells in the RSA decidua were significantly lower than M1 cells in the normal decidua (31.7% vs. 70.6%), while the percentage composition of M2 cells in the RSA decidua were significantly higher than M2 cells in the normal decidua (68.3% vs. 29.4%). These results indicate that polarization level of M1 macrophage play a very important role in maintaining the normal pregnancy, and much more polarization of M2 macrophage may be harmful for the early pregnancy. However, our understanding of the function and polarization of dMΦ is still limited and needs to be studied in the future.

The non-pregnant endometrium has a low concentration of macrophages, with fluctuating numbers along the menstrual cycle (Huhn et al., 2020). After fertilization, macrophages represent the second most abundant decidual leukocyte population (around 20–30%) (Yang, Zheng, & Jin, 2019). Human dMΦ may be involved in remodeling the endometrium, trophoblast invasion, and the development of the tolerant milieu required for successful progression of pregnancy (Garcia-Gomez et al., 2019). Macrophage can respond efficiently to environmental signals and change their phenotype and physiology in response to cytokines and extra stimulation (Olmos-Ortiz et al., 2019). According to some reports, dMΦ represent alternatively activated M2 macrophage, particularly since local decidual microenvironments are favorable for alternative activation (Ticconi, Pietropolli, Di Simone, Piccione, & Fazleabas, 2019). The M1 phenotype typically expressed IL-1β, TNF-α, IL-12 and lowly expressed IL-10, whereas the M2 macrophage phenotype typically expressed IL-10 and TGF-β, but lowly expressed IL-12 (Liu et al., 2017; Zhang, He, Wang, &

Liao, 2017). But in our study, we found that M2 type cells highly expressed CCL3, CCL4, GRN, and M1 type cell highly expressed IL1 β , TNFSF13B, and CCL3L3. Previously, it was reported that an inflammatory M1 phenotype was associated with human implantation, characterized by the upregulation of TNF- α , MIP-1 β and GRO- α , together with the secretion of remodeling factors such as matrix metalloproteinases (MMP) -3 and -9, suggesting that decidual M1 macrophages modulate an inflammatory environment that results in increased trophoblastic invasion and spiral artery remodeling.

We found that Cluster 10 as the specific cluster of dM Φ in normal group and almost disappeared in RSA group, and found that this cluster highly expressed inflammation cytokines and chemokines. Cluster 10 might take part in HIF-1 signaling pathway, which suggests that cluster 10 may take part in regulating hypoxia response or other stimuli, and may play an important role in the implantation process of embryo (Macklin, McAuliffe, Pugh, & Yamamoto, 2017; Soares, Iqbal, & Kozai, 2017), We also found that cluster 7 specifically expressed chemokines of CXCL9/10/11 and mainly expressed marker genes of type I interferon signaling pathway, which suggests that cluster 7 mainly take part in regulating antiviral immune response. Whether these clusters are related to the miscarriages requires further study.

MATERIAL AND METHODS

Isolating mononuclear cells from decidua.

Decidua samples were collected from RSA patients or control women undergoing elective terminations of first trimester pregnancies. To isolate decidual mononuclear cells (dMCs), decidual tissue pieces were first washed, remaining tissue was then minced and digested by Collagenase IV (0.1 g/100 mL, sigma) and DNase I (150 U/mL, Sigma) for 40 min at 37°C whilst being gently shaken. The collagenase was quenched by addition of DMEM/F12 with 10% FCS and then filtered through 100 µm and then 40 µm filters. dMCs were isolated following a percoll gradient centrifugation step. This protocol has been adapted from previous reports (Vishnyakova, Elchaninov, Fatkhudinov, & Sukhikh, 2019).

Cell staining and data acquisition by flow cytometry.

dMCs were washed in complete medium. Cells were then counted and distributed at approximately 1×10^6 cells/mL in 200 µL FACS wash (FW, 1×PBS, 2% FCS). Cells were stained with CD14 and CD45 antibody for 15 min at 4 °C. Viability was assessed using DAPI staining incubated for 5 min at 4 °C. Cells were then analyzed on a LSR Fortessa (BD Biosciences). Macrophages were gated on as live CD14⁺CD45⁺ lymphocytes. Further gating on subsets was done as indicated in figure legends. The following antibodies and reagents were used: DAPI (Life Technologies), FITC-CD14 (Biolegend), Cy5-CD45 (BioLegend).

Single-Cell RNA Sequencing (scRNA-seq)

Cells were prepared according to the 10×Genomics® Cell Preparation Guide. The protoplast suspension was loaded into Chromium microfluidic chips with chemistry and barcoded with a 10×Chromium Controller (10×Genomics). RNA from the barcoded cells was subsequently reverse-transcribed and sequencing libraries constructed with reagents from a Chromium Single Cell 30 reagent kit (10×Genomics). Sequencing was performed with Illumina (HiSeq 2000) according to the manufacturer's instructions. All single cell sequencing database deposited under

GEO Accession number (GSE169269).

Single-cell RNA statistical analysis

We applied fastp with default parameters to filter the adaptor sequence and remove the low-quality reads to obtain clean data (Rosenberg et al., 2018). UMI-tools were applied for single cell transcriptome analysis to identify the cell barcode whitelist (Han et al., 2018). The UMI-based clean data was mapped to the human genome (Ensemble version 91) utilizing STAR (Picelli et al., 2014) mapping with customized parameters from the UMI-tools standard pipeline to determine the UMIs counts of each sample. In order to minimize the sample batch, we applied down sample analysis to the samples sequenced according to the mean reads per cell of each sample and achieved a cell expression table with a sample barcode. Cells containing over 200 expressed genes and a mitochondria UMI rate below 20% passed the cell quality filtering and mitochondria genes were removed in the expression table but used for cell expression regression to avoid the effect of the cell status for clustering analysis and marker analysis of each cluster. Seurat package version 2.3.4 (<https://satijalab.org/seurat/>) was used for cell normalization and regression based on the expression table according to the UMI counts of each sample and the percentage mitochondria rate to obtain the scaled data. PCA was constructed based on the scaled data with all highly variable genes. The top 8 principals were used for tSNE construction. Utilizing the cluster method (normal group: k-mean method and K=7; normal group and RSA group: graph cluster method and resolution=1), we acquired the cell cluster result based on the PCA of the top 8 principals and calculated the marker genes using the Find All Markers function with the Wilcoxon Rank-Sum Test under following criteria: LogFC>0.25; p<0.05; min.pct>0.1.

Pseudotime analysis

We applied Single-Cell Trajectories analysis using Monocle2 (<http://cole-trapnellab.github.io/monocle-release>) with DDR-Tree and default parameters. Before Monocle analysis, we selected the marker genes of the Seurat

clustering result. The raw expression counts of the cell passed filtering. Based on the pseudo-time analysis, branch expression analysis modelling (BEAM Analysis) was applied for branch fate determined gene analysis (Vishnyakova et al., 2019).

SCENIC analysis

The SCENIC analysis was run on the cells that passed the filtering, using the 20 thousand motifs database for RcisTarget and GRNboost, as previously described (Sun et al., 2020).

Pathway analysis

Pathway analysis was used to identify the significant pathway of the differential genes according to the KEGG database (Zheng, Hou, Zhou, Li, & Cao, 2017). We used Fisher's exact test to select the significant pathway, where the threshold of significance was defined by the P-value and FDR. The cases were selected when $p < 0.05$.

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AUTHOR CONTRIBUTIONS

Liping Jin and Xiaoping Wan designed the experiments. Fenglian Yang and Yongbo Zhao collected the patient samples with assistance from Haili Gan. Qingliang Zheng and Xiang-Hong Xu carried out the experiments and analyzed the data and interpreted the findings. Liping Jin and Qingliang Zheng wrote the manuscript.

Competing interests

All authors declare no competing interests.

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FIGURE LEGENDS

Figure 1

652

653 **Figure 1** A single-cell analysis of normal human decidual mononuclear cells. (A)
654 t-Distributed stochastic neighbour embedding (t-SNE) analysis of decidual
655 mononuclear cells from the combined Normal 1 and Normal 2 sample. (B) Seventeen
656 healthy human decidual cell clusters. t-SNE analysis of 14,701 decidual mononuclear
657 cells and colored by clustering. (C) Cell type analysis of decidual mononuclear cells
658 annotated by marker genes and colored by cell types. (D) Heatmap analysis of the
659 expression of specific marker genes for each cluster. (E) t-SNE analysis of decidual
660 macrophage from the combined Normal 1 and Normal 2 sample. (F) Thirteen healthy
661 human decidual macrophage clusters. t-SNE analysis of 12,470 decidual macrophage

662 and colored by clusters. (G) M1/2 type analysis of decidual macrophage annotated by
 663 marker genes and colored by cell types. (H) Percent composition analysis of M1/2
 664 cells occupied in Normal 1 and Normal 2. (I) Heatmap analysis of the expression of
 665 M1/2 marker genes.

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Figure 2

Figure 2 A single-cell analysis of combined normal and RSA human decidual mononuclear cells. (A) t-SNE analysis of decidual mononuclear cells from the combined four samples (Normal 1, Normal 2 and RSA1, RSA2). (B) Seventeen human decidual cell clusters. t-SNE analysis of 28,580 decidual mononuclear cells from (A) and colored by clusters. (C) Cell type analysis of decidual mononuclear cells annotated by marker genes and colored by cell types. (D) Heatmap analysis of the expression of specific marker genes for each cluster. (E) t-SNE analysis of decidual macrophages from the combined four samples (Normal 1, Normal 2 and RSA1, RSA2). (F) Thirteen human decidual macrophage clusters. t-SNE analysis of 23,062 decidual macrophages and colored by clustering. (G) M1/2 type analysis of decidual

679 macrophages annotated by marker genes and colored by cell types. (H) Percent
 680 composition analysis of M1/2 cells occupied in Normal 1, Normal 2 and RSA1, RSA2.

681 (I) Heatmap analysis of the expression of M1/2 marker genes in the combined four
 682 samples.

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Figure 3

Figure 3 Identification of the new markers and secreted cytokines of the dMΦ. (A, B) Violin plot showing the new markers of M1 macrophages (A) and M2 macrophages (B). (C) Gene bubble plot showing the secreted cytokines and chemokines of M1 or M2 macrophages.

Figure 5

Figure 5 Characterization of Cluster 7 dMΦ in normal group. (A, B) Violin plot showing the secreted chemokines and the marker genes of cluster 10.

Figure 6 Single-cell trajectory analysis of decidual macrophages. (A-C) Monocle pseudotime trajectory showing the progression of dMΦ.

Figure 7 Identification of the key transcription factors of dMΦ. (A, B) Heatmap analysis of the expression of transcription factor in normal dMΦ (A) and the combined four samples (B). (C) Violin plot showing the expression of transcription factors in each cluster.

1 Supplementary material for “Single cell transcriptome analysis of decidua
2 macrophage in normal and recurrent spontaneous abortion patient”

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4

5 **Figure S1** A single-cell analysis of normal human decidual mononuclear cells. (A-C)
6 t-Distributed stochastic neighbour embedding (t-SNE) analysis of decidual
7 mononuclear cells from the combined Normal 1 and Normal 2 sample on nGene (A),
8 nUMI (B) and percent mitochondrion (C). (D, E) Percent composition analysis of
9 each cell type in Normal 1 and Normal 2 sample. (F-H) t-SNE analysis of decidual
10 macrophage cells from the combined Normal 1 and Normal 2 sample on nGene (F),
11 nUMI (G) and percent mitochondrion (H).

Figure S2 (A-F) GO analysis of the marker genes in M1 (A-C) or M2 (D-F) macrophage. (G,H) KEGG analysis of the marker genes in M1 (G) or M2 (H) macrophage.

16

17 **Figure S3** A single-cell analysis of normal and RSA human decidual mononuclear
18 cells. (A-C) t-SNE analysis of decidual mononuclear cells from the combined four
19 samples (Normal 1, Normal 2 and RSA1, RSA2) on nGene (A), nUMI (B) and
20 pecernt mitochondrion (C). (D, E) Percent composition analysis of each cell type in
21 the combined four samples. (F-H) t-SNE analysis of decidual macrophage cells from
22 the combined four samples on nGene (F), nUMI (G) and pecernt mitochondrion (H).

Figure S4 GO and KEGG analysis of macrophage. (A-C) GO analysis of the marker genes in M1 macrophage from normal and RSA sample. (E-G) GO analysis of the marker genes in M2 macrophage from normal and RSA sample. (D,H) KEGG analysis of the marker genes in M1 (D) or M2 (H) macrophage from normal and RSA sample. (I, J) KEGG analysis of the upregulated (I) or downregulated (J) gene in RSA group compared to the normal group.

Figure S5 The expression analysis of classically markers and secreted cytokines in the four decidua macrophages. (A,B) Violin plot showing the classically markers of M1 macrophage (A) and M2 macrophage (B). (C,D) Violin plot showing the classically secreted cytokines of M1 macrophage (C) and M2 macrophage (D).

Figure S6 GO and KEGG analysis of Cluster 10 as the specific cluster of decidua macrophage in normal group.

Figure S7 GO and KEGG analysis of Cluster 7 decidua macrophage in normal group.

Figure S8 Pseudotime trajectory analysis of decidual macrophage. (A-D) Monocle pseudotime trajectory showing the progression of decidual macrophage. (E-K) KEGG analysis of each state in the pseudotime trajectory analysis.

Figure S9 The expression of classically transcription factors in decidua macrophage samples. (A-D) Violin plot showing the expression of transcription factors in M1 type decidua macrophage. (E-H) Violin plot showing the expression of transcription factors in M2 type decidua macrophage.

57 Table S1 Cluster analysis of decidual mononuclear cells in Normal sample

Cluster	Normal 1	Normal 2	Cell Type	Main Markers
0	1705	41	Macrophage	PTPRC、CD14、MS4A7、CTSS
1	2750	53	Macrophage	PTPRC、CD14、MS4A7、CTSS
2	736	233	Macrophage	PTPRC、CD14、MS4A7、CTSS
3	301	1587	Macrophage	PTPRC、CD14、MS4A7、CTSS
4	1372	491	Macrophage	PTPRC、CD14、MS4A7、CTSS
5	183	794	Macrophage	PTPRC、CD14、MS4A7、CTSS
6	211	183	NKT_NK	PTPRC、NKG7、NCAM1、CD3E、KLRD1
7	744	57	Macrophage	PTPRC、CD14、MS4A7、CTSS
8	419	81	Macrophage	PTPRC、CD14、MS4A7、CTSS
9	749	10	DC	PTPRC、CD1C
10	144	210	Osteoclast	PTPRC、ACP5、RANK、CTSK
11	689	46	Macrophage	PTPRC、CD14、MS4A7、CTSS
12	126	48	NK	PTPRC、NKG7、NCAM1、KLRD1
13	121	299	T Cells	PTPRC、CD3E、CD8A
14	93	16	B&Plasma Cell	PTPRC、CD79A、MS4A1
15	139	38	Smooth muscle cells	TAGLN、ACTA2、DCN、COL1A1
16	31	1	Endometrial epithelial cells	PAEP、GPX3、SLPI

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60 Table S2 M1/M2 analysis of decidua macrophage in Normal sample

Cluster	Normal 1	Normal 2	SubType
0	2995	51	M1
1	1547	86	M2
2	598	478	M2
3	88	1743	M1
4	487	198	M2
5	1320	43	M1
6	320	29	M2
7	477	39	M1
8	69	644	M1
9	271	40	M1
10	464	9	M1
11	92	78	M2
12	277	27	M1

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63 Table S3 Cluster analysis of decidual mononuclear cells in Normal and RSA sample

Cluster	Normal 1	Normal 2	RSA 1	RSA 2	Cell Type	Main Marker
0	1705	41	1953	91	Macrophage	PTPRC、CD14、MS4A7、CTSS
1	2750	53	817	65	Macrophage	PTPRC、CD14、MS4A7、CTSS
2	736	233	1406	840	Macrophage	PTPRC、CD14、MS4A7、CTSS
3	301	1587	386	818	Macrophage	PTPRC、CD14、MS4A7、CTSS
4	1372	491	641	114	Macrophage	PTPRC、CD14、MS4A7、CTSS
5	183	794	409	1211	Macrophage	PTPRC、CD14、MS4A7、CTSS
6	211	183	1477	83	NKT_NK	PTPRC、NKG7、NCAM1、CD3E、KLRD1
7	744	57	466	26	Macrophage	PTPRC、CD14、MS4A7、CTSS
8	419	81	593	170	Macrophage	PTPRC、CD14、MS4A7、CTSS
9	749	10	348	11	DC	PTPRC、CD1C
10	144	210	350	358	Osteoclast	PTPRC、ACP5、RANK、CTSK
11	689	46	107	153	Macrophage	PTPRC、CD14、MS4A7、CTSS
12	126	48	513	17	NK	PTPRC、NKG7、NCAM1、KLRD1
13	121	299	220	12	T Cells	PTPRC、CD3E、CD8A
14	93	16	118	5	B&Plasma Cell	PTPRC、CD79A、MS4A1
15	139	38	22	16	Smooth muscle cells	TAGLN、ACTA2、DCN、COL1A1
16	31	1	63	0	Endometrial epithelial cells	PAEP、GPX3、SLPI

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66 Table S4 M1/M2 analysis of decidua macrophage in Normal and RSA sample

Cluster	Normal 1	Normal 2	RSA 1	RSA 2	SubType
0	2995	51	864	78	M1
1	1547	86	1831	75	M2
2	598	478	259	1701	M2
3	88	1743	146	655	M1
4	487	198	857	564	M1
5	1320	43	655	46	M1
6	320	29	1543	46	M2
7	477	39	365	20	M1
8	69	644	18	107	M1
9	271	40	114	173	M1
10	464	9	21	19	M1
11	92	78	232	100	M2
12	277	27	88	15	M1

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70 Table S5 The genes that upregulated (102 genes) and downregulated (122 genes) in
71 the RSA group compared with the normal group.

Gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj	up_down
TMSB4X	0	-0.26118	0.998	0.999	0	downregulated
RPL18A	0	-0.32367	0.844	0.912	0	downregulated
RPL39	0	-0.32736	0.885	0.936	0	downregulated
S100A11	0	-0.34886	0.819	0.915	0	downregulated
SH3BGRL3	0	-0.40771	0.735	0.864	0	downregulated
RPS10	0	-0.48722	0.695	0.823	0	downregulated
CYBA	0	-0.54804	0.762	0.892	0	downregulated
AP1S2	0	-0.55763	0.464	0.677	0	downregulated
S100A10	0	-0.57411	0.569	0.777	0	downregulated
S100A6	0	-0.59165	0.671	0.827	0	downregulated
CSTA	0	-0.60996	0.487	0.742	0	downregulated
RETN	0	-0.69023	0.049	0.235	0	downregulated
LYZ	0	-0.70282	0.778	0.884	0	downregulated
OLR1	0	-0.70332	0.372	0.641	0	downregulated
S100A4	0	-0.76769	0.693	0.845	0	downregulated
FCN1	0	-0.84552	0.266	0.589	0	downregulated
LGALS2	0	-0.89005	0.095	0.423	0	downregulated
S100A9	0	-0.99828	0.415	0.698	0	downregulated
S100A8	0	-1.15283	0.295	0.619	0	downregulated
TSPO	1.4E-29 4	-0.42342	0.564	0.742	2.5E-290	downregulated
RPL34	1.3E-29 2	-0.28851	0.92	0.954	2.4E-288	downregulated
EREG	2.7E-28 8	-0.67029	0.235	0.463	4.8E-284	downregulated
FCER1A	4.8E-28 5	-0.561	0.046	0.207	8.6E-281	downregulated
OSM	1.1E-28 4	-0.58582	0.188	0.398	1.9E-280	downregulated
MNDA	3.1E-27 4	-0.46768	0.641	0.799	5.6E-270	downregulated
LST1	8.6E-27 2	-0.40264	0.698	0.834	1.5E-267	downregulated
RPL8	2.4E-26 6	-0.30729	0.787	0.88	4.2E-262	downregulated
OAZ1	8.3E-26 3	-0.30498	0.794	0.883	1.5E-258	downregulated
RPL26	9.4E-26 0	-0.28489	0.866	0.922	1.7E-255	downregulated
NOP10	2.5E-25	-0.38349	0.5	0.699	4.4E-252	downregulated

	6					
RPL41	2.2E-25 5	-0.27366	0.945	0.962	3.9E-251	downregulated
GAPDH	5.5E-25 2	-0.29863	0.718	0.855	9.9E-248	downregulated
FTH1	1.8E-24 9	-0.31855	0.997	0.998	3.2E-245	downregulated
VIM	2.2E-24 5	-0.31727	0.702	0.86	4E-241	downregulated
NFKBIA	9.5E-24 5	-0.34568	0.953	0.969	1.7E-240	downregulated
ATP2B1-AS 1	1.5E-24 3	-0.3812	0.499	0.707	2.7E-239	downregulated
PFN1	1.8E-24 2	-0.31315	0.818	0.887	3.2E-238	downregulated
COTL1	2.5E-24 1	-0.36003	0.592	0.767	4.4E-237	downregulated
RPS15	3.3E-23 8	-0.25851	0.856	0.914	6E-234	downregulated
HLA-C	1.9E-23 3	-0.2869	0.851	0.912	3.3E-229	downregulated
RPL7	2.2E-23 3	-0.28598	0.839	0.904	4E-229	downregulated
VCAN	6.5E-22 8	-0.55221	0.156	0.338	1.2E-223	downregulated
G0S2	3.4E-22 6	-0.60025	0.398	0.589	6.1E-222	downregulated
CARD16	4.6E-22 3	-0.38205	0.523	0.703	8.2E-219	downregulated
RPL13	1.5E-22 1	-0.25786	0.907	0.943	2.6E-217	downregulated
PSMB9	1.8E-22 1	-0.35954	0.391	0.579	3.3E-217	downregulated
SERPINA1	1E-217	-0.38346	0.493	0.681	1.9E-213	downregulated
MYL12A	2E-217	-0.31357	0.653	0.797	3.6E-213	downregulated
C14orf2	1.1E-21 4	-0.33563	0.596	0.76	1.9E-210	downregulated
PRELID1	4.1E-21 3	-0.382	0.376	0.561	7.3E-209	downregulated
ACTB	2.9E-21 2	-0.26909	0.959	0.972	5.2E-208	downregulated
RPS13	3.9E-21 1	-0.25634	0.834	0.899	6.9E-207	downregulated
MT2A	1.4E-20	-0.51354	0.372	0.562	2.5E-201	downregulated

	5					
C15orf48	2.7E-20 2	-0.62104	0.327	0.515	4.9E-198	downregulated
RPL35A	3.4E-19 9	-0.25661	0.809	0.894	6E-195	downregulated
ELOB	2.5E-19 7	-0.32927	0.565	0.736	4.5E-193	downregulated
GSTO1	3.6E-19 6	-0.35508	0.47	0.649	6.5E-192	downregulated
LGALS1	2.8E-19 2	-0.33562	0.665	0.81	5E-188	downregulated
BCL2A1	1.6E-19 1	-0.35658	0.577	0.764	2.9E-187	downregulated
RPL21	1.4E-19 0	-0.29317	0.859	0.9	2.5E-186	downregulated
PSME2	1.6E-19 0	-0.33849	0.532	0.7	2.8E-186	downregulated
ATP5L	1.6E-19 0	-0.26733	0.712	0.837	2.8E-186	downregulated
NCF2	1.1E-18 9	-0.31395	0.28	0.461	1.9E-185	downregulated
CDA	3E-189	-0.27338	0.036	0.151	5.3E-185	downregulated
COX7B	3.3E-18 9	-0.3051	0.571	0.729	5.9E-185	downregulated
GLRX	2.9E-18 7	-0.32726	0.4	0.573	5.1E-183	downregulated
CD48	3.6E-18 6	-0.31946	0.306	0.487	6.4E-182	downregulated
S100A12	6.8E-18 5	-0.69062	0.11	0.26	1.2E-180	downregulated
COX6B1	6.4E-18 4	-0.26748	0.664	0.809	1.1E-179	downregulated
CLEC12A	5.4E-17 9	-0.3072	0.251	0.432	9.7E-175	downregulated
PLAUR	3.1E-17 5	-0.31718	0.69	0.833	5.5E-171	downregulated
PLAC8	3.5E-17 3	-0.34694	0.171	0.338	6.2E-169	downregulated
RPL10A	3.6E-17 3	-0.27911	0.705	0.824	6.4E-169	downregulated
CORO1A	3E-168	-0.29251	0.503	0.675	5.3E-164	downregulated
ALDOA	3.2E-16 8	-0.28405	0.571	0.728	5.7E-164	downregulated
SERP1	4.2E-16	-0.28173	0.584	0.744	7.5E-164	downregulated

	8					
LSP1	1.4E-16 7	-0.27108	0.313	0.501	2.5E-163	downregulated
NDUFS5	2.1E-16 6	-0.26964	0.488	0.652	3.7E-162	downregulated
SLC25A6	3.2E-16 6	-0.28004	0.636	0.774	5.7E-162	downregulated
SEC61G	9.5E-16 6	-0.28442	0.48	0.647	1.7E-161	downregulated
RPS27L	1.7E-16 4	-0.32005	0.5	0.659	3.1E-160	downregulated
UQCR11	1.2E-16 3	-0.28267	0.598	0.747	2.2E-159	downregulated
PFDN5	2.5E-16 3	-0.2636	0.774	0.868	4.4E-159	downregulated
COX5B	2.9E-16 0	-0.25493	0.59	0.744	5.3E-156	downregulated
SMIM25	1.1E-15 8	-0.34651	0.14	0.284	2E-154	downregulated
CD44	1.3E-15 8	-0.32179	0.426	0.601	2.2E-154	downregulated
COMMD6	2.7E-15 7	-0.27145	0.559	0.715	4.9E-153	downregulated
HCST	4E-156	-0.29294	0.538	0.698	7.1E-152	downregulated
HBEGF	4.4E-15 4	-0.35951	0.285	0.446	7.8E-150	downregulated
RPS5	3.9E-15 2	-0.26178	0.675	0.793	7E-148	downregulated
TREM1	9.4E-15 0	-0.3272	0.228	0.385	1.7E-145	downregulated
CNBP	1E-149	-0.26629	0.427	0.581	1.8E-145	downregulated
ASGR1	1.6E-14 9	-0.26228	0.148	0.288	2.9E-145	downregulated
TPI1	1.9E-14 6	-0.26807	0.541	0.689	3.4E-142	downregulated
FPR1	2.1E-14 4	-0.28196	0.359	0.524	3.7E-140	downregulated
RBX1	4E-144	-0.25462	0.46	0.616	7.2E-140	downregulated
BST1	3.7E-14 3	-0.25747	0.098	0.218	6.6E-139	downregulated
EMP3	1.4E-14 2	-0.28495	0.572	0.73	2.5E-138	downregulated
GLIPR2	9E-140	-0.27116	0.164	0.301	1.6E-135	downregulated
ANXA2	1.8E-13	-0.26306	0.532	0.685	3.3E-133	downregulated

	7					
AP2S1	4.3E-13 ₆	-0.25715	0.492	0.642	7.7E-132	downregulated
SOD2	5.7E-13 ₆	-0.27257	0.794	0.88	1E-131	downregulated
CTSS	6.5E-13 ₆	-0.28977	0.813	0.878	1.2E-131	downregulated
LDHA	4.1E-13 ₅	-0.29543	0.448	0.606	7.3E-131	downregulated
TKT	1.2E-12 ₆	-0.27763	0.389	0.537	2.1E-122	downregulated
PHPT1	1.4E-12 ₃	-0.2508	0.309	0.453	2.6E-119	downregulated
DEFB1	1E-121	-0.33496	0.03	0.111	1.8E-117	downregulated
DNAAF1	3.6E-12 ₁	-0.27752	0.041	0.127	6.3E-117	downregulated
SLC11A1	2.9E-12 ₀	-0.25588	0.161	0.289	5.2E-116	downregulated
VAMP5	6.1E-10 ₇	-0.31577	0.266	0.393	1.1E-102	downregulated
VEGFA	1.3E-10 ₄	-0.25516	0.238	0.371	2.3E-100	downregulated
SDS	3.1E-10 ₃	-0.26106	0.122	0.23	5.5E-99	downregulated
HLA-DQA2	1.72E-9 ₆	-0.35514	0.469	0.581	3.07E-92	downregulated
HCAR2	4.49E-9 ₆	-0.25879	0.136	0.243	8.02E-92	downregulated
UPP1	1.9E-89	-0.25349	0.263	0.382	3.4E-85	downregulated
TNFAIP6	1.48E-8 ₃	-0.31755	0.061	0.139	2.65E-79	downregulated
CD69	4.74E-7 ₇	-0.28487	0.302	0.423	8.48E-73	downregulated
CCL20	7.56E-7 ₂	-0.43132	0.303	0.411	1.35E-67	downregulated
IFI27	1.81E-5 ₆	-0.35167	0.073	0.138	3.24E-52	downregulated
HSPE1	1.91E-3 ₅	-0.3838	0.566	0.654	3.41E-31	downregulated
HSPA6	4.61E-1 ₂	-0.37895	0.225	0.261	8.23E-08	downregulated
ZFAND2A	0.00029 ₉	-0.30151	0.148	0.164	1	downregulated
SELENOP	0	0.913468	0.713	0.39	0	upregulated

RNASE1	0	0.816963	0.484	0.232	0	upregulated
HLA-DQA1	0	0.771711	0.808	0.684	0	upregulated
GNLY	0	0.764896	0.658	0.422	0	upregulated
PDK4	0	0.719209	0.235	0.054	0	upregulated
C1QB	0	0.683695	0.836	0.603	0	upregulated
APOE	0	0.683174	0.651	0.43	0	upregulated
C1QC	0	0.614516	0.814	0.587	0	upregulated
MMP9	0	0.613143	0.198	0.041	0	upregulated
C1QA	0	0.612532	0.865	0.685	0	upregulated
PSAP	0	0.454332	0.932	0.919	0	upregulated
MALAT1	0	0.40641	1	0.999	0	upregulated
LGMN	2.8E-29 2	0.732768	0.436	0.225	5.1E-288	upregulated
CD74	1.5E-28 3	0.306666	0.996	0.993	2.7E-279	upregulated
EGR1	5.5E-26 9	0.484662	0.728	0.54	9.9E-265	upregulated
A2M	2E-267	0.55409	0.478	0.272	3.5E-263	upregulated
SLCO2B1	3E-267	0.583272	0.412	0.215	5.4E-263	upregulated
SLC40A1	5.8E-26 5	0.657303	0.359	0.17	1E-260	upregulated
STAB1	5.4E-25 3	0.623295	0.466	0.271	9.7E-249	upregulated
CD163	3.7E-23 1	0.597899	0.516	0.349	6.7E-227	upregulated
XCL1	8.4E-21 4	0.389023	0.121	0.019	1.5E-209	upregulated
RGS1	5.2E-20 6	0.347289	0.891	0.774	9.3E-202	upregulated
IFIT2	7.1E-20 5	0.93711	0.295	0.141	1.3E-200	upregulated
XCL2	6E-200	0.379546	0.115	0.019	1.1E-195	upregulated
ITM2B	5.6E-19 4	0.26601	0.923	0.925	9.9E-190	upregulated
KLF6	1.5E-18 6	0.381486	0.942	0.921	2.6E-182	upregulated
LPAR6	1.3E-18 5	0.447135	0.679	0.556	2.3E-181	upregulated
HSPB1	2.6E-18 5	0.384335	0.717	0.594	4.6E-181	upregulated
GZMB	8.2E-18 1	0.418824	0.155	0.045	1.5E-176	upregulated
PLTP	3.5E-17 8	0.56123	0.286	0.143	6.2E-174	upregulated

CCL4L2	1.5E-17 7	0.358592	0.917	0.854	2.6E-173	upregulated
JUN	2.6E-17 2	0.278213	0.903	0.815	4.7E-168	upregulated
PMAIP1	6.5E-17 2	0.742331	0.472	0.345	1.2E-167	upregulated
GPR34	2.5E-16 6	0.460369	0.468	0.319	4.4E-162	upregulated
CSF1R	1.2E-16 4	0.460589	0.566	0.439	2.1E-160	upregulated
PLD3	1.4E-16 4	0.502619	0.459	0.321	2.6E-160	upregulated
STMN1	2.1E-16 1	0.390037	0.337	0.192	3.8E-157	upregulated
NKG7	4.1E-15 3	0.426018	0.22	0.097	7.3E-149	upregulated
MAF	5.7E-14 8	0.440236	0.37	0.224	1E-143	upregulated
FOLR2	2.3E-14 7	0.418758	0.521	0.388	4.1E-143	upregulated
MS4A4A	8.3E-14 4	0.485635	0.526	0.396	1.5E-139	upregulated
CCL4	1.9E-13 9	0.29762	0.969	0.937	3.3E-135	upregulated
MS4A7	7E-138	0.35421	0.775	0.719	1.3E-133	upregulated
FKBP5	7.4E-13 8	0.318166	0.244	0.122	1.3E-133	upregulated
FOSB	7.2E-13 1	0.389312	0.838	0.804	1.3E-126	upregulated
DAB2	7.7E-13 1	0.462853	0.445	0.314	1.4E-126	upregulated
BTG2	5.2E-12 9	0.381731	0.518	0.38	9.3E-125	upregulated
VSIG4	8E-128	0.400143	0.542	0.437	1.4E-123	upregulated
MERTK	4.4E-12 3	0.37953	0.19	0.086	7.9E-119	upregulated
MTSS1	2E-118	0.350248	0.196	0.092	3.6E-114	upregulated
SLC1A3	9.8E-11 1	0.33644	0.216	0.11	1.7E-106	upregulated
MSR1	1.3E-10 9	0.349588	0.467	0.345	2.3E-105	upregulated
LMNA	1.6E-10 6	0.409805	0.254	0.145	2.8E-102	upregulated
EIF4A3	1.4E-10	0.47729	0.601	0.518	2.5E-101	upregulated

	5					
FCGR2A	6.3E-10 3	0.334544	0.689	0.65	1.13E-98	upregulated
PELI1	1.48E-9 8	0.43585	0.314	0.203	2.64E-94	upregulated
KLF2	3.02E-9 5	0.363867	0.475	0.363	5.4E-91	upregulated
DNAJB4	8.25E-9 3	0.367995	0.207	0.112	1.47E-88	upregulated
NRP1	4.05E-9 1	0.316848	0.226	0.129	7.23E-87	upregulated
MMP14	8.65E-9 0	0.317742	0.214	0.119	1.55E-85	upregulated
TSC22D3	4.11E-8 7	0.371407	0.632	0.551	7.35E-83	upregulated
IGF1	8.55E-8 5	0.268461	0.158	0.077	1.53E-80	upregulated
CD81	9.88E-8 5	0.347754	0.383	0.281	1.77E-80	upregulated
FCGRT	1.75E-8 1	0.251694	0.747	0.74	3.13E-77	upregulated
SON	7.25E-7 9	0.298603	0.721	0.68	1.3E-74	upregulated
ARL4C	9.5E-77	0.352745	0.238	0.147	1.7E-72	upregulated
GAL3ST4	1.77E-7 5	0.251777	0.187	0.103	3.16E-71	upregulated
PAEP	7.31E-7 3	0.338709	0.193	0.112	1.31E-68	upregulated
AXL	4.97E-7 2	0.336653	0.288	0.2	8.89E-68	upregulated
CTSW	2.05E-7 0	0.259529	0.106	0.046	3.67E-66	upregulated
GSN	1.23E-6 9	0.329233	0.492	0.42	2.19E-65	upregulated
F13A1	2.6E-67	0.452849	0.394	0.317	4.64E-63	upregulated
XIST	5.8E-66	0.356955	0.542	0.464	1.04E-61	upregulated
LAIR1	1.27E-6 4	0.288541	0.462	0.383	2.28E-60	upregulated
AP2A2	8.55E-6 2	0.265195	0.193	0.118	1.53E-57	upregulated
POLR2J3	1.23E-6 1	0.254658	0.171	0.099	2.2E-57	upregulated
AMD1	4.1E-61	0.337215	0.429	0.345	7.32E-57	upregulated
NRP2	3.88E-5	0.264129	0.235	0.155	6.93E-55	upregulated

	9					
TTYH3	6.51E-5 ₉	0.278148	0.226	0.148	1.16E-54	upregulated
SPATS2L	1.81E-5 ₈	0.267905	0.262	0.18	3.24E-54	upregulated
NR4A2	1.86E-5 ₈	0.299069	0.709	0.665	3.32E-54	upregulated
IFIT3	4.46E-5 ₈	0.27765	0.177	0.106	7.97E-54	upregulated
JUND	6.23E-5 ₈	0.310779	0.765	0.736	1.11E-53	upregulated
HERPUD1	1.9E-50	0.251546	0.664	0.619	3.4E-46	upregulated
CXCL16	3.74E-5 ₀	0.257215	0.454	0.387	6.68E-46	upregulated
TBXAS1	5.25E-4 ₉	0.264461	0.496	0.435	9.38E-45	upregulated
ADAP2	1.55E-4 ₇	0.28704	0.385	0.313	2.77E-43	upregulated
SPP1	2.78E-4 ₇	0.329055	0.207	0.135	4.96E-43	upregulated
EPB41L2	2E-46	0.261669	0.243	0.174	3.57E-42	upregulated
CTS2	7.59E-4 ₆	0.335937	0.506	0.465	1.36E-41	upregulated
ITPR2	8.41E-4 ₆	0.295157	0.277	0.207	1.5E-41	upregulated
PARP14	8.86E-4 ₄	0.293645	0.362	0.291	1.58E-39	upregulated
AKAP9	4.01E-3 ₆	0.25674	0.465	0.408	7.17E-32	upregulated
MRC1	1.18E-3 ₅	0.273246	0.39	0.331	2.1E-31	upregulated
TXNIP	5.82E-3 ₅	0.296059	0.484	0.439	1.04E-30	upregulated
OASL	1.21E-3 ₄	0.250734	0.163	0.11	2.16E-30	upregulated
GOLGA4	4.02E-2 ₆	0.256797	0.326	0.275	7.18E-22	upregulated
VMP1	2.87E-2 ₃	0.256404	0.559	0.538	5.12E-19	upregulated
C1orf56	3.47E-2 ₂	0.280023	0.27	0.224	6.2E-18	upregulated
IFI44L	9.95E-1 ₈	0.273746	0.382	0.356	1.78E-13	upregulated
C12orf57	2.89E-1	0.30924	0.347	0.32	5.17E-09	upregulated

		3					
		4.08E-0					
RASGEF1B		5	0.25127	0.336	0.33	0.728593	upregulated