Identification of the difference of neutrophils in different locations in RA patients

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Research Article

Keywords: Rheumatoid arthritis, inflammation, KEGG, neutrophils

Posted Date: March 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-308329/v1>

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Abstract

Rheumatoid Arthritis (RA) is a complex systemic disease in which numerous cell types are involved. Neutrophils play an important role in the onset and development of RA. In our study, we aim to identify different functions of neutrophils in different conditions (blood and synovium) of RA patients by using a bioinformatics method to clarify their potential pathogenesis. The gene expression profiles of the GSE154474 dataset were originally produced by using the high-throughput Illumina HiSeq 2000 (Homo sapiens). The biological categories and biochemical pathways were identified and analyzed by the Kyoto Encyclopedia of Genes and Genomes pathway (KEGG), Gene Ontology (GO), and Reactom enrichment. KEGG and GO results showed the biological pathways related to the immune and cellular structure were mainly different. Moreover, we identified several genes including GNB4, RHOA, and TECB2 were involved in the regulation of inflammation. Therefore, this study provides different insights into the pathogenesis of RA.

Introduction

Rheumatoid arthritis (RA) is a systemic disease that can cause the disability of the immune system without proper treatment. It is unclear that the precise pathogenetic mechanisms underlying the development of RA¹. At the onset of RA, patients indicate increased articular inflammation usually in the form of a symmetric polyarthritis². Leukocyte migrates and aggregates in the joint area with early infiltration of neutrophils, T cells, B cells, and plasma cells that produced autoantibodies in the synovium tissue³. Not only the neutrophils, other innate immune cells such as macrophages and natural killer cells are also recruited to the synovium^{4,5}. Finally, the local inflammatory condition contributes to fundamental changes and promotes the formation of hyperplastic, inflammatory synovium^{6,7}.

Neutrophils are the first cell type that respond to acute inflammation and are endowed with the ability of antimicrobial mechanisms⁸. Neutrophils are from bone marrow and respond to granulocyte colony-stimulating factors. It is believed that integrins and selectins are critical in the process of neutrophil egression⁹. Neutrophils also express the C-X-C chemokine receptors. The balance between CXCR2 and CXCR4 and their respective chemokines appears to play a basic role in neutrophil mobilization¹⁰. In the absence of inflammatory stimuli, mature neutrophils locate in the bloodstream and remain in the circulation for a short period⁸. Thus, we consider the neutrophils in the blood as the less active neutrophils than those in the synovium in RA patients.

Here, we studied the relative neutrophil changes between blood and synovium. We identified and analyzed a series of DEGs, the relevant biological processes, and biological functions of neutrophils in the synovium in comparison to those in the blood by using comprehensive bioinformatic analysis¹¹. We performed the signal pathway analysis, the functional enrichment, and protein-protein interaction (PPI) for discovering the features of the neutrophils in the synovium from RA patients. The identified genes and pathways could be critical to favor future clinical and therapeutic studies.

Methods

Data resources

The dataset GSE154474 was obtained from the GEO database (<http://www.ncbi.nlm.nih.gov/geo/>). The data was produced by Illumina HiSeq 2000 (Homo sapiens), Institute of Life Course and Medical Sciences, University of Liverpool, United Kingdom. Bulk RNA-Seq analysis was performed using Neutrophils (purity > 97%) from paired peripheral blood and synovial fluid (SF) from n = 3 patients with severe rheumatoid arthritis.

Data acquisition and preprocessing

The dataset GSE154474 that includes Neutrophils from paired peripheral blood and synovial fluid was analyzed and conducted by R script^{12, 13}. We performed a classical t test to identify DEGs with $P < .01$ and fold change ≥ 1.5 as being statistically significant.

Gene functional analysis

The Gene Ontology (GO) is a functional genomics research that develop a comprehensive model through knowledge-informed computational analysis of biological data. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database is widely used for identifying the high-level functions and utilities of the biological system. The GO analysis and KEGG pathway enrichment analysis were performed by using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (<http://david.ncifcrf.gov/>). $P < .05$ and gene counts > 10 were considered statistically significant.

Module analysis

The Molecular Complex Detection (MCODE) of Cytoscape software was used to analyze the densely connected regions in protein-protein interaction (PPI) networks. The significant modules were from the constructed PPI network using MCODE. The function and pathway enrichment analyses were performed by using DAVID, and $P < .05$ was used as the cutoff criterion.

Reactome pathway analysis

We used the Reactom pathway to obtain the visualisation, interpretation and analysis of potential pathways (<https://reactome.org/>). $P < .05$ was considered statistically significant.

Results

Identification of DEGs of neutrophils from peripheral blood in comparison to synovial fluid in RA patients

The neutrophils were collected from the paired peripheral blood and synovial fluid from patients with severe rheumatoid arthritis. To gain the insights on the different genes, the neutrophils from blood were

compared to those from synovial fluid. A total of 416 genes were identified to be differentially expressed with the threshold of $P < 0.005$. The top 10 up- and down-regulated genes are listed in table 1.

KEGG analysis of DEGs of neutrophils from peripheral blood in comparison to synovial fluid in RA patients

To further identify the biological roles and potential mechanisms of the DEGs of neutrophils from blood and synovial fluid, we performed KEGG pathway and GO categories enrichment analysis (Supplemental Table S1)¹². The KEGG pathway (<http://www.genome.jp/kegg/>) includes curated sets of genes that are to understand the molecular interaction, reaction and relation networks. Our study indicated top five enriched KEGG pathways including “Serotonergic synapse”, “Regulation of actin cytoskeleton”, “Alzheimer's disease”, “Proteoglycans in cancer” and “Pathways in cancer” (Figure 1).

GO analysis of DEGs of neutrophils from peripheral blood in comparison to synovial fluid in RA patients

Gene Ontology (GO) analysis includes cellular components (CC), molecular functions (MF), and biological processes (BP). Here, we identified top five cellular components including “mitochondrial envelope”, “extrinsic component of cytoplasmic side of plasma membrane”, “trans-Golgi network”, “ciliary basal body”, and “axon” (Figure 1). We then identified top five biological processes: “negative regulation of bile acid biosynthetic process”, “epithelial cell proliferation involved in mammary gland duct elongation”, “vocalization behavior”, “regulation of microtubule cytoskeleton organization”, and “keratinocyte differentiation” (Figure 1). We identified top five molecular functions: “formyltetrahydrofolate cyclo-ligase activity”, “calcium: sodium antiporter activity”, “MutL α complex binding”, “extracellular matrix binding”, and “ligand-dependent nuclear receptor binding” (Figure 1).

PPI (protein–protein interactions) network and Module analysis

The PPI networks were constructed to analyze the relationships of DGEs at the protein level. The criterion of combined score > 0.7 was set and the PPI network was created by using the 224 nodes and 320 interactions. Among these nodes, the top ten of most significant genes with highest scores are shown in Table 2. The top two significant modules versus blood samples were selected to indicate the functional annotation (Figure 2).

Reactome Pathway of neutrophils from peripheral blood in comparison to synovial fluid in RA patients

We identified a series of signaling pathways by using Reactome Pathway Database (<https://reactome.org/>). We identified top ten signaling pathways including: “Defective Base Excision Repair Associated with MUTYH”, “Defective MUTYH substrate processing”, “VEGF ligand-receptor interactions”, “VEGF binds to VEGFR leading to receptor dimerization”, “Activation of RAS in B cells”, “RUNX3 regulates RUNX1-mediated transcription”, “Polymerase switching”, “Leading Strand Synthesis”, “Activated NTRK2 signals through FRS2 and FRS3”, and “PTK6 Regulates RHO GTPases, RAS GTPase

and MAP kinases” (Supplemental Table S2). We then constructed the reaction map according to the signaling pathways (Figure 3).

Discussion

Neutrophils are the common cell type in RA synovial fluid and are also detected in RA synovial tissues¹⁴. Various proteases express in neutrophils play crucial roles in joint damage and inflammation⁸. Moreover, these proteases can activate the proinflammatory cytokines, modulate chemokine function and trigger different pathways^{15–18}. Thus, the molecules present in neutrophil granules lead to inflammation and tissue damage¹⁹.

To better understand the different roles of neutrophils in blood and synovium in RA, we analyzed the RNA-seq of peripheral blood and synovial fluid neutrophils from rheumatoid arthritis patients. Thus, by using this, we could learn more about the functions of neutrophils in different conditions and environments. By analyzing the DEGs, we selected 10 proteins that may be critical according to the PPI network analysis. G proteins and Regulator of G protein signaling proteins are widely expressed in various tissues and involved in the immune process^{5, 20, 21}. In our study, the G Protein Subunit Beta 4 was involved in calcium signaling during infection or inflammation²². CXCL12/CXCR4 can activate the RhoA to further promote the inflammation-driven colorectal cancer progression²³. The absence of OA inhibits Th17 cell differentiation and allergic airway inflammation²⁴. TCEB2 is involved in the regulation of apoptosis. TCEB2 is increased in response to the treatment with proteasome inhibitor bortezomib for 24 hours²⁵. Laminin beta 1(LAMB1) is highly expressed in lung tissue and it is critical for both lung morphogenesis and physiological function²⁶. KRAS is the most mutated oncogene in cancer and its receptor AMG 510 contributes the regression of KRAS mutant tumors and improves the anti-tumor efficacy of chemotherapy via formation a pro-inflammatory tumor microenvironment²⁷. F2 accounts for the fate of at least 90% of the prothrombin in plasma, which may affect the blood clotting in RA²⁸. DNMT3a can regulate the transcriptional inhibition on opiate-induced synaptic and behavioral plasticity via UBE2B²⁹. ITGB3 was reported as a hub regulator in the tumor microenvironment, which may regulate the immune system during the tumor genesis³⁰. As a mitochondrial protein, ATP5 proteins are important for the construction of complex V. The loss of ATP5F1D can lead to a metabolic disorder³¹. enhanced ITGA2B was discovered in bone marrow megakaryocytes of sepsis onset. Subsequent upregulation of ITGA2B were seen in circulating platelets³². Circadian clocks play important roles in physiological and pathophysiological processes such as aging³³, bone^{7, 34}, metabolism diseases^{35–38}. Most interestingly, circadian control of neutrophil responsiveness contributes to changes in different location in inflammation condition, which may influence the development of RA³⁹. Interestingly, the genes such as RHOA, and KRAS are critically regulated by circadian clocks^{40, 41}. Thus, these PPI proteins are majorly involved in the inflammation and circulation environment during RA. It is suggested that neutrophils in different places and environments may activate different proteins and play different roles during RA.

KEGG and GO analysis showed that cancer related protein, immune and cell skeletal protein play critical roles by comparing the neutrophils from blood and synovium. The KEGG analysis showed the “Cancer pathways” and “Regulation of actin cytoskeleton” were the major different pathways in neutrophils from blood and synovium. It is suggested that the function of neutrophils from different locations is based on the environment. Neutrophils recognize different substrates of microbial and response by sequestering the cargo via phagocytosis or by releasing bioactive factors outside the cell, thus changing and alerting the environment and bystander leukocytes⁴². Interestingly, the CC of GO analysis showed “Mitochondrial envelop”, “Extrinsic component of plasma membrane”, “Tans-Golgi network” and “B cell activation”, suggesting that the neutrophil components were different between blood and synovium in RA patients. Thus, the microenvironmental condition is crucial for the construction and function of neutrophils.

In summary, we identified different genes in neutrophils from blood and synovium in severe RA patients. Immune dysfunction and microenvironment were two key differences of neutrophils in different locations. This study thus provides further insights into the features of neutrophils from different tissues in RA, which may facilitate the diagnosis and drug development.

Declarations

Declarations of interest: none

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Tables

Due to technical limitations, Table 1 and Table 2 are only available as a download in the supplementary files section.

Figures

Figure 1

The KEGG pathways, biological process, cellular component, and molecular function terms enriched by the DEGs of neutrophils between blood and synovium in RA patients. DEGs =differentially expressed genes, KEGG = Kyoto Encyclopedia of Genes and Genomes.

Figure 2

Top two modules from the PPI network between WT and MM mouse models.

Figure 3

The Reactom pathway visualization. Input genes are represented by the top significantly changed genes obtained from the GSE154474 dataset ($P < 0.01$). The yellow color represents the most relevant signaling

pathways.

Supplementary Files

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

- [TableS1.KEGGandGOanalysis.xlsx](#)
- [TableS2.Rectomeanalysis.csv](#)
- [Tables.docx](#)