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THU0029

IDENTIFICATION AND COMPARING OF HUB GENES IN SYSTEMIC LUPUS ERYTHEMATOSUS T AND B CELLS BY BIOINFORMATIC ANALYSIS

Q. Zhou¹, L. Long¹. ¹Sichuan Provincial People's Hospital, Department of Rheumatology and Immunology, Chengdu, China

Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder that can virtually involve any organ system of the body. Many efforts have been made to elucidate the pathogeny, but the molecular mechanisms are still not understood. Lymphocytes are considered to play an important role in SLE pathogenesis. Aberrantly activated T cells mediate inflammatory responses and activate B cells to differentiate and produce autoantibodies, resulting in multisystem manifestations [1, 2]. With the wide use of gene technique, more genetic studies on SLE were performed and differentially expressed genes (DEGs) were identified. Integrating and re-analyzing these data can help us understand the molecular mechanisms and identify diagnostic and therapeutic targets of SLE.

Objectives: In this study, we downloaded the microarray datasets GSE4588 and GSE10325 from Gene Expression Omnibus (GEO) database to identify the candidate genes in T and B cells respectively.

Methods: Datasets GSE4588 and GSE10325 were downloaded from GEO (<http://www.ncbi.nlm.nih.gov/geo>). The DEGs between T or B cells and control samples were screened using GEO2R (<http://www.ncbi.nlm.nih.gov/geo/geo2r>). logFC lfold changel >1 and P-value <0.05 were considered statistically significant. To analyze the function of DEGs, biological analyses were performed using DAVID database (<http://david.ncicrf.gov>). P<0.05 was considered statistically significant. The PPI networks of DEGs were constructed using STRING database, and an interaction with a combined score >0.4 was considered statistically significant. The PPI networks were drawn using Cytoscape and the most significant module was identified using MCODE. The criteria for selection were: MCODE scores >5, degree cut-off=2, node score cut-off=0.2, Max depth=100 and k-score=2. The hub genes were selected with degrees ≥10.

Results: After standardization of the microarray results, DEGs in T and B cells were identified respectively (Fig. 1).

Figure 1. Venn diagram, PPI network and the most significant module of DEGs in SLE. (A) The overlap between 2 datasets contained 56 genes in T cells and 83 in B cells. (B) PPI networks were constructed using Cytoscape. (C) The most significant module in T cells had 17 nodes and 193 edges, while that in B cells had 19 nodes and 171 edges. Upregulated genes are in red; downregulated genes are in blue.

Changes in biological processes in T and B cells were both mainly enriched in type I interferon signalling pathway, defense response to virus, and negative regulation of viral genome replication. Changes in cell component in T cells was enriched in the cytosol while in B cells it was in cytoplasm. KEGG pathway analysis revealed that the DEGs of T cells were mainly enriched in influenza A, measles, herpes simplex infection and hepatitis C, while DEGs of B cells were mainly enriched in measles. Changes in molecular function were not listed because the p values were ≥0.05.

4 genes were identified as hub genes (2 in each cell population). In T cells, the hub genes are PLSCR1 and GINS2. PLSCR1 may contribute to the prothrombotic tendency in SLE. GINS2 is involved in the initiation of DNA replication and cell cycle progression. In B cells, the hub genes are ISG15 and TOP2A. Increased ISG15 is correlated with lymphocytopenia in SLE patients. TOP2A encodes a DNA topoisomerase and anti-topoisomerase II antibody could be found in SLE.

Conclusion: In our study, 2 mRNA microarray datasets were analyzed to obtain DEGs between SLE T and B cells versus healthy controls. A total of 56 DEGs were identified in T cells and 83 in B cells. Most of the DEGs were upregulated. Changes in biological processes in T and B cells were mainly related to type I interferon signalling pathway and anti-virus function. KEGG also showed the same. PLSCR1 and GINS2 were hub genes in T cells while ISG15 and TOP2A

were hub genes in B cells. Overexpression of these genes might play an important role in the pathogenesis of SLE.

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Adaptive immunity (T cells and B cells) in rheumatic diseases

THU0030

DISTINCT EFFECTS OF FIVE JAK INHIBITORS IN THE MODULATION OF HUMAN B CELL ACTIVATION

N. Frede¹, J. Hueppe¹, R. Lorenzetti¹, A. Troilo¹, M. T. Schleyer¹, R. Voll¹, J. Thiel¹, N. Venhoff¹, M. Rizzi¹. ¹Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg im Breisgau, Germany

Background: JAK inhibitors have been successfully introduced in the treatment of rheumatoid arthritis (RA) and psoriatic arthritis and are in clinical trials for numerous other autoimmune diseases. JAK inhibition effectively reduces cytokine-mediated activation and survival of pathology-driving immune cells by targeting signaling downstream of cytokine receptors. The outcome of such immunomodulation hence will largely depend on the intrinsic expression of the four different JAKs, the cytokine environment and the targeted cell type. Comparative studies investigating the effect on B cells are lacking. In light of the use of JAK inhibitor treatment in autoantibody mediated diseases, the study of the B cell compartment represents a milestone to assess their potential.

Objectives: We thus aimed to study the B cell compartment as well as B cell function under JAK inhibition in RA patients and to compare the specific effect the JAK inhibitors tofacitinib (pan-JAK), baricitinib (JAK1/2), ruxolitinib (JAK1/2), upadacitinib and filgotinib (selective JAK1) on in vitro B cell activation, differentiation, proliferation, and class switch.

Methods: B cell subpopulations in RA patients treated with baricitinib or tofacitinib was assessed by flow cytometric analysis of peripheral blood mononuclear cells. For in vitro studies, magnetically isolated total B cells from healthy donors were stimulated T-cell -independently with CpG and treated with scalar doses of the JAK inhibitors tofacitinib, baricitinib, ruxolitinib, upadacitinib and filgotinib. Flow cytometric analysis was performed on days 0, 3 and 6. Cytokine secretion was measured by Cytokine Multiplex Assay.

Results: B cell phenotyping of RA patients treated with JAK inhibitors baricitinib or tofacitinib showed an increase in marginal zone (MZ) B cells. To investigate this further, we turned to an in vitro model of T-cell-independent B cell activation with CpG via TLR9, known to support MZ B cell expansion. Here, JAK1/2 and selective JAK1 inhibitor treatment led to a dose-dependent decrease of total B cell numbers. When assessing B cell-subpopulations, we observed an altered B cell differentiation with a significant increase in MZ-like B cells under JAK inhibition, which led to a subsequent increase in plasma-blast differentiation in the first days. This effect was more pronounced upon pan-JAK inhibitor treatment than JAK1 or JAK1/2 inhibition, indicating that broader JAK inhibition is associated with a stronger effect (tofa > rufo > bari > upa > filgo).

Notably, we further detected a significant dose-dependent reduction of switched memory formation, strongest with JAK1/2 inhibition (upa > rufo > bari > tofa > filgo). Consistent with this finding, we observed decreased AID expression under JAK inhibition. Concomitantly, induction of STAT3 expression and STAT3 phosphorylation were reduced under JAK inhibition, suggesting that downstream signalling was abrogated.

To assess the role of autocrine signaling in this system, we measured cytokine secretion upon JAK inhibition and found that JAK2 inhibition led to reduced IL10 secretion. This in turn resulted in an increase of inflammatory cytokines such as IL6, TNF, highlighting the importance of B cell as cytokine-secreting cell type.

Conclusion: In a T-independent in vitro B cell model JAK inhibition led to a reduced total B cell number as well as reduced switched memory development, whereas MZ-like B cells were increased. Especially JAK2 inhibition strongly impaired switched memory formation. JAK inhibition does not only impact cytokine signalling but also leads to changes in cytokine secretion dynamics and amounts, potentially impacting other cell types.

In conclusion, JAK inhibition has a major effect on B cell activation and maturation, with differential outcomes between JAK inhibitors hinting towards distinct and unique effects on B cell homeostasis.