Letter

DOI: 10.5582/bst.2021.01046

ChIP-sequencing analysis of E2F transcription factor 2 reveals its role in various biological processes of rheumatoid arthritis synovial fibroblasts

Luwen Li[1,2,3], Yihang Zhang[1,2,3], Lin Wang[1,2,3], Jihong Pan[1,2,3,*]

[1] Biomedical Sciences College & Shandong Medicinal Biotechnology Centre, Shandong First Medical University & Shandong Academy of Medical Sciences, Ji'nan, China;
[2] Key Lab for Biotech-Drugs of National Health Commission, Ji'nan, China;
[3] Key Lab for Rare & Uncommon Diseases of Shandong Province, Ji'nan, China.

SUMMARY The development and progression of rheumatoid arthritis (RA) are complex and the pathogenesis of this disease is not fully understood. E2F transcription factor 2 (E2F2) affects the development and progression of many diseases. To identify the mechanisms underlying the role of E2F2 in RA, chromatin immunoprecipitation was performed followed by sequencing (ChIP-seq) using the E2F2 antibody. Gene Ontology (GO) analysis of differentially expressed genes (DEGs) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of captured downstream target genes and Metascape analysis of 22 protein molecules partly elucidated the mechanism by which E2F2 affects the progression of RA. Results indicated that E2F2 affects the metabolism of RASFs and the development of ribosome synthesis as well as the stress response. Results indicated that E2F2 can affect multiple biological processes involving RASFs and indicate a unique possibility of targeting E2F2 in the treatment of RA.

Keywords E2F2, ChIP-seq, metabolism, stress response, synthesis of ribosomes

Rheumatoid arthritis (RA) is a chronic autoimmune disease mainly characterized by erosive and symmetrical polyarthritis (*1*). Factors such as heritability, hormones, and the environment are involved in the pathogenesis of RA (*2*); however, its etiology and pathogenesis are complex and have not been fully elucidated. E2F2 is a member of the E2F family of transcription factors and affects the development and progression of many diseases. E2F2 also affects various cellular processes, such as the cell cycle, proliferation, apoptosis, invasion, and migration (*3*). E2F2 promotes the secretion of inflammatory factors in RA synovial fibroblasts (RASFs) and influences the development of RA (*4*). However, additional mechanisms underlying the role of E2F2 in the development and progression of RA need to be elucidated.

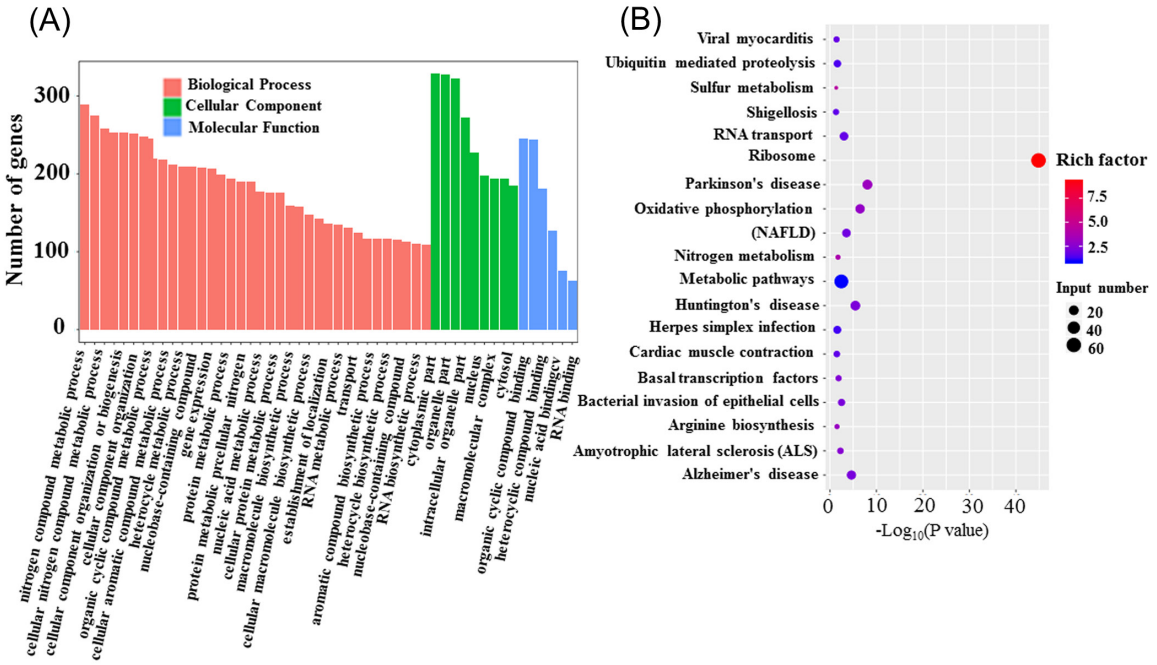
The current authors conducted a study to further identify the mechanisms of E2F2 in RASFs in order to study RA in-depth. The synovial tissue of a patient with RA (female, 56 years of age, DAS28: 3.7; RF: 56; CRP: 59 mg/L; anti-CCP: 26 E/mL; and time of onset: 16 months) was collected during knee replacement surgery. The synovial tissue was collected at Shandong Provincial Hospital (Ji'nan, China), and informed consent was obtained from the patient. The research plan was approved by the Ethics Committee of the Shandong Academy of Medical Sciences. The patient met the American College of Rheumatology diagnostic criteria for RA. Fetal calf serum was purchased from Invitrogen, Carlsbad, CA, USA. Anti-E2F2 antibody (SAB2108118) was purchased from Sigma-Aldrich, St. Louis, MO, USA.

As were used in previous studies, type II and III collagenases were used to digest synovial tissue for 6 hours (*5*) and RASFs were cultured. RASF cells (1×10^6) were harvested and crosslinked with formaldehyde. Cells were then lysed and sonicated, and anti-E2F2 antibody was used in immunoprecipitation (IP). This was followed by water bath decrosslinking, DNA purification, construction of a sequencing library, amplification, and high-throughput sequencing. The protocol is as described by Park (*6*).

Gene Ontology (GO) was used to perform enrichment analysis on the functions of peak-related genes, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to perform significant

enrichment analyses on the pathway functions of related genes. Finally, Metascape was used to analyze protein molecules.

GO function annotation and enrichment analysis of KEGG metabolic pathways were performed on high-throughput E2F2 ChIP-seq results. GO annotation indicated that differentially expressed genes were mainly enriched in the nitrogen compound metabolic process, cellular nitrogen compound metabolic process, cellular component organization, or biogenesis (Figure 1A). KEGG pathway annotation indicated that the genes participated in 19 metabolic pathways including ribosomes, Parkinson's disease, and oxidative phosphorylation (Figure 1B).To further explore the mechanism by which E2F2 influences the progression of RA, protein molecules from ChIP-seq were screened and enriched for E2F2. Twenty-two proteins (Figure 1C) were identified, and enrichment analysis was performed with Metascape. Metascape indicated that the main pathways included localization, the response to stimulus, the metabolic process, and biological regulation (Figure 1D). These results are in accordance with the results described earlier and further illustrate that downstream genes regulated by E2F2 are closely related to the metabolic process. The current results also indicate that E2F2 may affect the body's stress response. Interestingly, two of the 22 proteins, thrombospondin-1 (THBS1) and derlin-1, are related to endoplasmic reticulum (ER) stress. Moreover, THBS1 may be a biomarker of early RA (*7*). Abnormal ER stress may promote the



(C)

RAC1	GPR37L1	PLA2G12B	BRD9	THBS1
EYA3	ERVV-2	TBC1D12	EPRS1	ADCK1
CIAO3	MED4	DERL1	ECSIT	VANGL1
MBOAT1	SOCS2	SRSF4	OR6C1	RFESD
CLPTM1	ERAS			

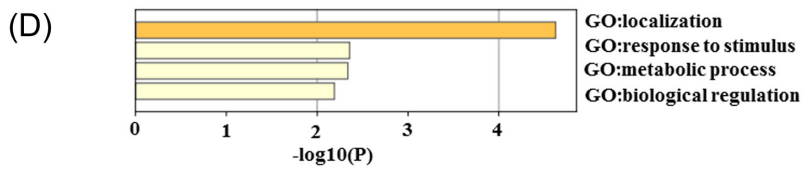


Figure 1. Enrichment of differential genes. (A) Number of genes identified for specific GO annotation terms. **(B)** KEGG pathway enrichment indicating the significance of enrichment. The size of the dot indicates the number of genes in the KEGG pathway, and the shade of the dot indicates the degree of rich factor enrichment. **(C)** The 22 proteins identified by screening and enrichment from E2F2 ChIP-seq. **(D)** Metascape was used to enrich the 22 corresponding genes; enrichment terms are represented in terms of their degree of enrichment. This is expressed as a -log10 [*P*-value], where a higher value indicates more significant enrichment **(B,D)**.

pathogenesis of RA through abnormal cell proliferation and production of pro-inflammatory cytokines (*8,9*). The 22 identified genes also encoded some proteins related to cellular processes (RAC1, CLPTM1, and ERAS), signal conversion-related proteins (GPR37L1.ECSIT), transcription-related proteins (MED4 and BRD9), and ubiquitin ligase in protein degradation. These molecules represent different aspects of the complexity of the pathogenesis of RA.

In summary, the current results indicated that E2F2 influences metabolism, the stress response, and ribosome synthesis of RASFs, thereby affecting the development and progression of RA. Therefore, a promising strategy would be to intervene in metabolism, the stress response, and ribosome synthesis to combat the progression of RA. Accordingly, E2F2 is likely to serve as a potential target in the treatment of RA.

Funding: This research was supported by a grant from the National Sciences Fund Committee (NSFC) of China (Grant No. 81671624).

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Liu Y, Jin J, Xu H, Wang C, Yang Y, Zhao Y, Han H, Hou T, Yang G, Zhang L, Wang Y, Zhang W, Liang Q. Construction of a pH-responsive, ultralow-dose triptolide nanomedicine for safe rheumatoid arthritis therapy. Acta Biomater. 2021; 121:541-553.
2. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet. 2016; 388:2023-2038.
3. Evangelou K, Havaki S, Kotsinas A. E2F transcription factors and digestive system malignancies: How much do we know? World J Gastroenterol. 2014; 20:10212-10216.
4. Zhang R, Wang L, Pan JH, Han J. A critical role of E2F transcription factor 2 in proinflammatory cytokines-dependent proliferation and invasiveness of fibroblast-like synoviocytes in rheumatoid Arthritis. Sci Rep. 2018; 8:2623.
5. Wang L, Zheng Y, Xu H, Yan X, Chang X. Investigate pathogenic mechanism of TXNDC5 in rheumatoid arthritis. PLoS One. 2013; 8:e53301.
6. Park PJ. ChIP-seq: Advantages and challenges of a maturing technology. Nat Rev Genet. 2009; 10:669-680.
7. Rahmati M, Moosavi MA, McDermott MF. ER stress: A therapeutic target in rheumatoid arthritis? Trends Pharmacol Sci. 2018; 39:610-623.
8. Yamasaki S, Yagishita N, Tsuchimochi K, Nishioka K, Nakajima T. Rheumatoid arthritis as a hyper-endoplasmic-reticulum-associated degradation disease. Arthritis Res Ther. 2005; 7:181-186.
9. Park YJ, Yoo SA, Kim WU. Role of endoplasmic reticulum stress in rheumatoid arthritis pathogenesis. J Korean Med Sci. 2014; 29:2-11.

Received January 27, 2021; Revised March 26, 2021; Accepted April 22, 2021.

**Address correspondence to:*
Jihong Pan, Biomedical Sciences College & Shandong Medicinal Biotechnology Centre, Shandong First Medical University & Shandong Academy of Medical Sciences, No. 6699 Qingdao Road, Ji'nan 250117, China.
E-mail: panjihong@sdfmu.edu.cn

Released online in J-STAGE as advance publication April 29, 2021.