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

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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
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

![Figure 1](image-url)

**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.

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