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Mechanism of action of celastrol against rheumatoid arthritis: A network pharmacology analysis

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ABSTRACT

Network pharmacology uses bioinformatics to broaden our understanding of drug actions and thereby to advance drug discovery. Here we apply network pharmacology to generate testable hypotheses about the multi-target mechanism of celastrol against rheumatoid arthritis. We reconstructed drug–target pathways and networks to predict the likely protein targets of celastrol and the main interactions between those targets and the drug. Then we validated our predictions of four candidate targets (IKK-β, JNK, COX-2, MEK1) by performing docking studies with celastrol. The results suggest that celastrol acts against rheumatoid arthritis by regulating the function of several signaling proteins, including MMP-9, COX-2, c-Myc, TGF-β, c-JUN, JAK-1, JAK-3, IKK-β, SYK, MMP-3, JNK and MEK1, which regulate the functions of Th1 and Th2 cells, macrophages, fibroblasts and endothelial cells in rheumatoid arthritis. Celastrol is predicted to affect networks involved mainly in cancer, connective tissue disorders, organismal injury and abnormalities, tissue development, cell death and survival. This network pharmacology strategy may be useful for discovery of multi-target drugs against complex diseases.

1. Introduction

Rheumatoid arthritis is an immune-related disease that generally gives rise to continuous joint destruction, decreased life expectancy and working ability, disability, and even elevated mortality [1–3]. The disease is most commonly treated using synthetic and biological disease-modifying anti-rheumatic drugs (DMARDs) [4,5]. These drugs cannot cure the disease, however, and they often cause severe side effects, such as reduced immunity and cancer. Moreover, biological DMARDs place an extreme financial burden on patients without showing correspondingly high efficacy [5–7].

Traditional Chinese medicine may be able to offer more cost-effective alternative treatments against rheumatoid arthritis [8]. For centuries, the principal active ingredient of *Tripterygium wilfordii Hook.f.*, called celastrol, has been used in traditional Chinese medicine to treat inflammation and autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, nephritis, and asthma [9,10]. Celastrol shows anti-inflammatory and immunomodulatory activities, as well as pro-apoptotic effects in many types of cancer, such as ovarian and pancreatic cancers, myeloma, myeloid leukemia, and thyroid carcinoma [11–22]. However, how celastrol exerts therapeutic effects on patients with rheumatoid arthritis is unclear.

Herbal medicines such as celastrol feature multiple components and multiple targets, and analyzing this complexity can be achieved through systems pharmacology as applied in network pharmacology analysis. Systems pharmacology is an emerging field of pharmacology which utilizes network analysis of drug action as one of its approaches and network pharmacology is a method by integrating systems approaches, computational and experimental methods to illuminate the molecular mechanisms of drug [23]. Because network pharmacology can provide a good understanding of the principles of network theory and systems biology, it has been considered to be the next paradigm in drug discovery [24].

In the present study, we explored potential mechanisms of action of celastrol against rheumatoid arthritis using an integrated systems pharmacology approach. First, we identified potential molecular targets of celastrol and the interaction pathways in which those targets play roles. Then we examined overlap in those pathways and the networks they form. We also performed docking studies to predict the interactions that allow celastrol to bind to its predicted targets. Our results may help understanding the mechanisms celastrol treats rheumatoid arthritis and, more generally, to discover natural products against complex diseases.

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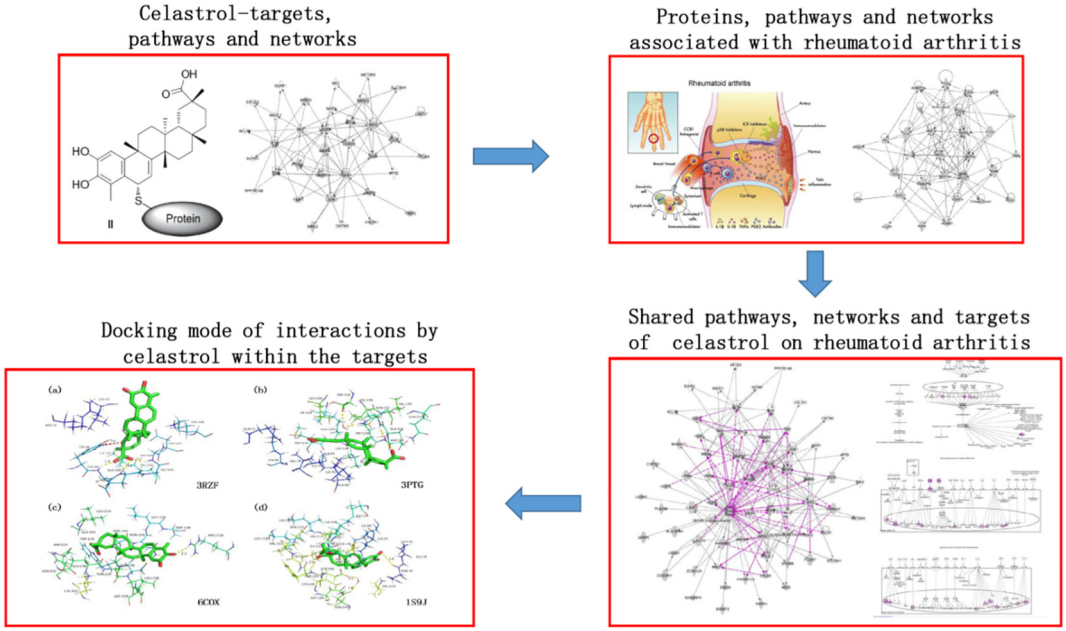

Fig. 1. Workflow in the network pharmacology approach.

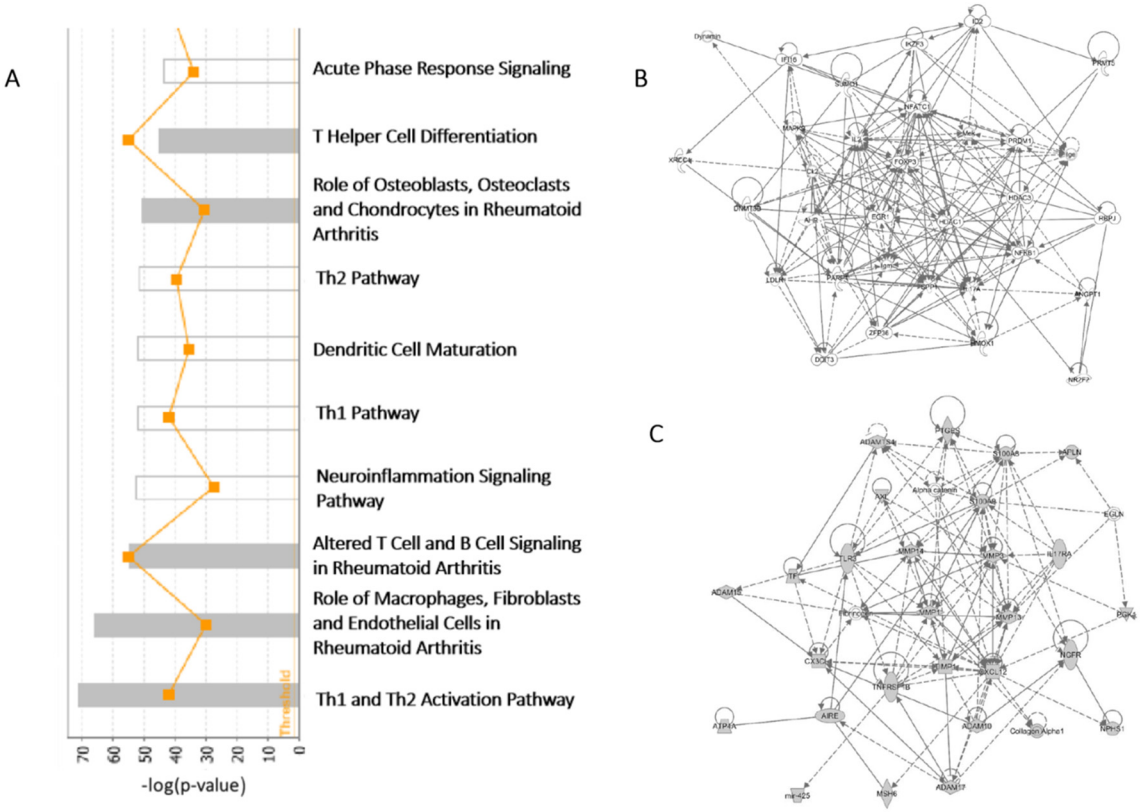

Fig. 2. Pathways and networks associated with rheumatoid arthritis. (A) Pathways formed by proteins associated with rheumatoid arthritis. (B–C) Representative networks formed by proteins associated with rheumatoid arthritis.

2. Materials and methods

2.1. Data preparation

Rheumatoid arthritis-related genes were obtained from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) using the term "rheumatoid arthritis", followed by filtering with the term "*Homo sapiens*". Potential targets of celastrol were obtained in online resources in systems pharmacology (http://lsp.nwu.edu.cn/index.php) and PubChem (https://pubchem.ncbi.nlm.nih.gov/).

A total of 25 human proteins likely targeted by celastrol were obtained from TCMSP (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, http://lsp.nwu.edu.cn/tcmsp.php) and the PubChem database. Their symbols were uploaded

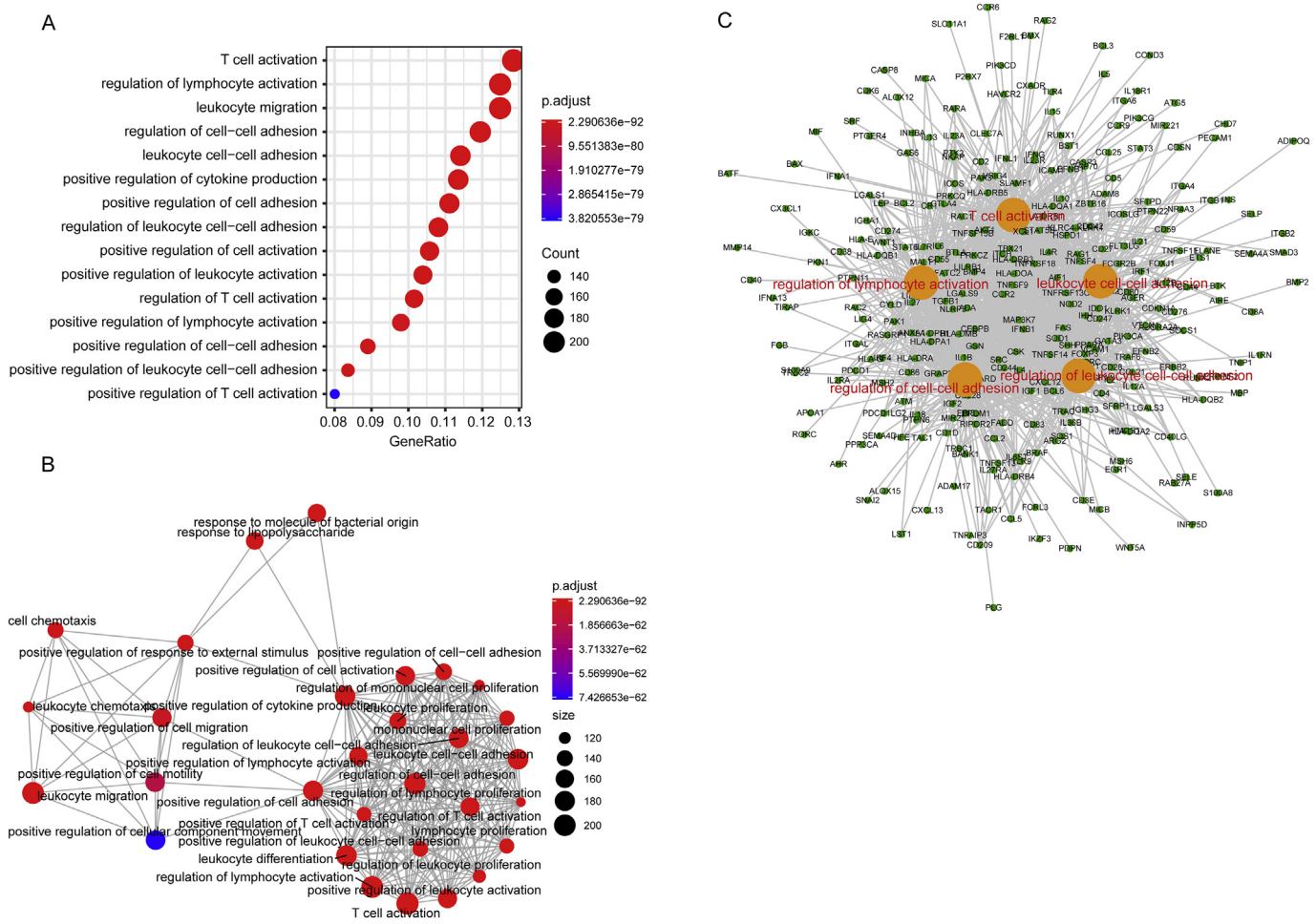

Fig. 3. Gene Ontology (GO) enrichment and network analysis of RA target genes.
(A) Top 15 functionally enriched biological processes with corresponding adjusted *p*-values analyzed by clusterProfiler, which are displayed in a dot plot. The color scales indicated the different thresholds of adjusted p-values, and the sizes of the dots represented the gene count of each term. (B) Interaction networks between enriched biological processes analyzed by enrichMap in the clusterProfiler package. The color scales indicated different thresholds of adjusted p-values, and the sizes of the dots represented the gene count of each term. (C) Sub-network showing important genes in top 5 GO term. The subnetwork depicts the relationships among 5 GO term and RA target genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

into IPA, and 111 pathways and 3 networks were assembled.

2.2. Prediction of pathways and networks affected by celastrol

Pathways and networks were built by Ingenuity Pathway Analysis (IPA, www.ingenuity.com) based on the functions of the human genes related to rheumatoid arthritis and the potential celastrol targets. Enrichment Analysis of RA Target Gene Ontology (GO) Enrichment and Network were performed by R (R 3.6.0 for Windows) and Cytoscape 3.6.1 (http://www.cytoscape.org).

Pathways and networks were ranked according to the amounts of the molecules participating in pathways and networks, respectively. Pathways and networks shared by targets related to rheumatoid arthritis and the potential celastrol targets were identified using the “Compare” module within IPA.

2.3. Binding of celastrol to predicted targets

Docking studies were performed with selected targets using Autodock4.2 based on the crystal structures of the targets as deposited in the RCSB Protein Data Bank (http://www.pdb.org/pdb/home/home.do). A CHARMM force field was employed, and hydrogen atoms were added to the proteins. The binding site was defined as a sphere encompassing protein residues within 12 Å of the original ligand. Default values were used for other parameters, and Genetic Algorithm runs were performed for each ligand. The protocol of the integrated systems pharmacology approach is described in Fig. 1.

3. Results

3.1. Rheumatoid arthritis-related gene pathways and networks

A total of 1175 human genes associated with rheumatoid arthritis were identified in the GenBank database, and the encoded proteins were assembled into a set of 339 pathways and 25 networks using IPA. These pathways involve primarily activation of Th1 and Th2 cells, macrophages and fibroblasts in rheumatoid arthritis; altered T cell and B cell signaling in the disease and signaling pathways during the acute phase of the disease. The networks involve mainly cell movement, immune cell trafficking, hematological system development and function, inflammatory response, connective tissue disorders, organismal injury and abnormalities, as well as cell-to-cell signaling and interactions (Fig. 2).Gene Ontology (GO) Enrichment and Network Analysis showed that T cell activation, regulation of lymphocyte activation, leukocyte migration, regulation of cell-cell adhesion and leukocyte cell-cell adhesion covered the top 3 functions of RA target proteins (Fig. 3).

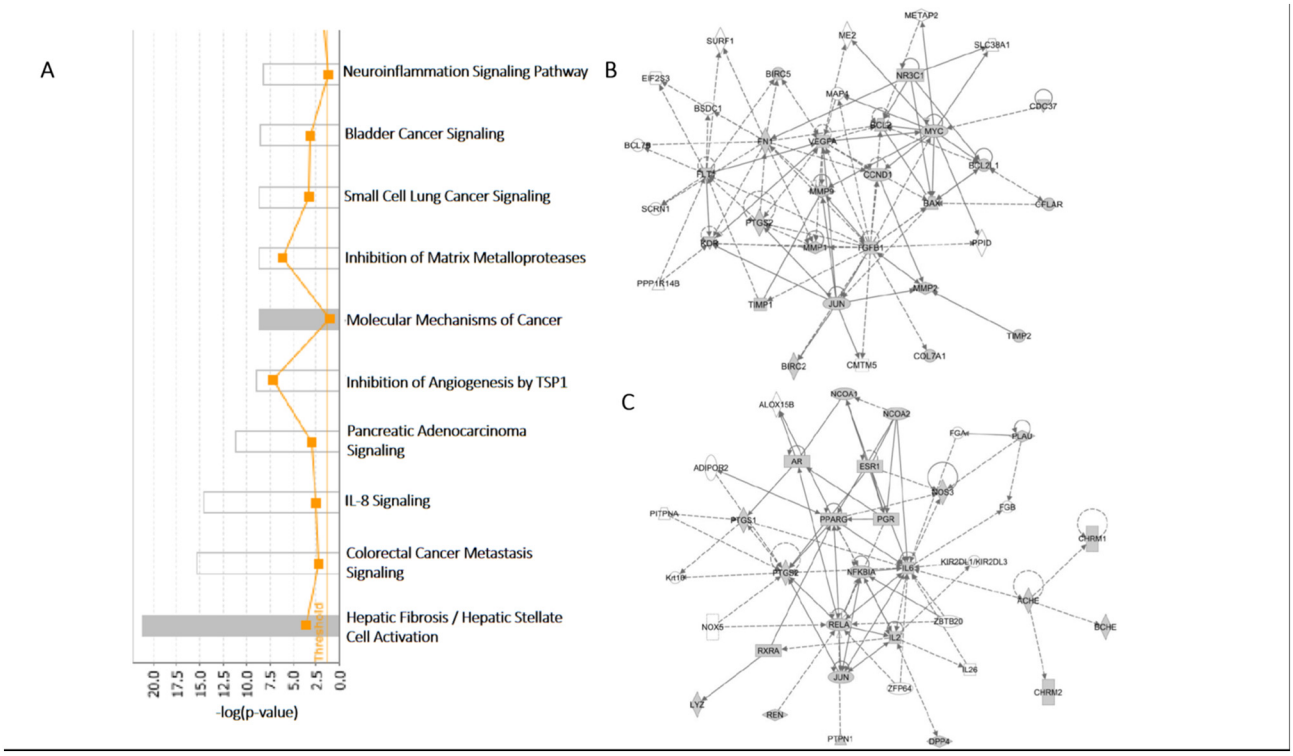

Fig. 4. Pathways and networks by predicted celastrol targets. (A) Pathways involving predicted celastrol targets. (B—C) Representative networks assembled by predicted celastrol targets.

3.2. Networks involving celastrol targets and their functions

The predicted celastrol targets participate primarily in such pathways as hepatic fibrosis, hepatic stellate cell activation, IL-8 signaling, and colorectal cancer metastasis signaling. These networks are involved mainly in cancer, connective tissue disorders, organismal injury and abnormalities, tissue development, cell death and survival, as well as endocrine system development and function (Fig. 4).

3.3. Overlapping networks and special celastrol targeted proteins

228 pathways were identified by “Canonical Pathway” and “Networks” modules of IPA, and 3 shared networks were obtained between the set of predicted celastrol targets and the set of proteins encoded by rheumatoid arthritis-associated genes. These overlapping pathways involve primarily activation of Th1 and Th2 cells, macrophages, fibroblasts and endothelial cells in rheumatoid arthritis. The networks involve primarily cancer, connective tissue disorders, organismal injury and abnormalities, tissue development, cell death and survival.

Based on the canonical pathways and networks, we predicted the following proteins to be the direct targets of celastrol in rheumatoid arthritis: MMP-9, COX-2, c-Myc, TGF-β, c-JUN, JAK-1, JAK-3, IKK-β, SYK, MMP-3, JNK, and MEK1 (Fig. 5).

3.4. Binding mode

Docking studies were performed between celastrol and the following selected potential targets (Fig. 6): IKK-β (PDB: 3RZF), JNK (PDB:3PTG), COX-2 (PDB:6COX) and MEK1 (PDB:1S9J). These potential targets were chosen because they are high-degree nodes in rheumatoid arthritis-associated networks potentially affected by celastrol. High-degree nodes often play more important network roles than low-degree nodes.

The analysis in Fig. 6a predicts that celastrol binds to 3RZF by forming a stable hydrophobic interaction with a binding pocket consisting of GLY-102, GLU-100, CYS-99, TYR-98, LYS-106, ASP-103, GLY-101, VAL-152, ILE-165, ARG-31 and LEU-21. A $\pi - \pi$ interaction is predicted to form between the benzene ring of celastrol and TYR-98, as well as three H-bonds between the oxygen atoms on celastrol and GLY-102 (length: 1.9 Å), GLU-100 (2.2 Å) and CYS-99 (1.8 Å). These interactions anchor celastrol to the binding site in 3RZF.

The analysis in Fig. 6b predicts that celastrol binds tightly to a highly hydrophobic pocket of 3PTG through stable hydrophobic interactions with 19 residues, including LEU-206, ILE-124, SER-193, VAL-196, VAL-197, ASN-152, and ALA-151. The analysis in Fig. 5c predicts that celastrol binds compactly to a pocket in 6COX consisting of 16 residues, including ASP-239, THR-237, ASP-229, TRP-139, ASN-231, GLU-236, LEU-145, SER-146, and ARG-26. Celastrol is also predicted to form an H-bond with ARG-216 (2.2 Å). In Fig. 5d, celastrol is predicted to interact with 1S9J via PHE-209, ASN-78, ILE-139, ILE-99, LYS-97, GLY79, VAL-224, MET-143, and ILE-141. Celastrol is also predicted to form H-bonds with PHE-209 (length: 2.8 Å) and ASN-78 (3.4 Å).

These docking studies provide evidence how celastrol binds to its targets, which may be useful for basic understanding the mechanism of drug action.

4. Discussion

Traditional drug discovery is largely based upon the paradigm of ‘one molecule, one target, one disease’, but there is a growing recognition that drugs work by targeting multiple proteins [25–31]. In addition, biological pathways and networks are abundant and robust, so affecting only a single target can easily fail to produce the desired therapeutic effects [17,32–40]. Therefore, the development of models that can predict multiple drug-target interactions may hold the key to future success in drug discovery against complex diseases such as rheumatoid arthritis.

In the present report, we integrated information from publicly available databases to predict interactions between celastrol and its

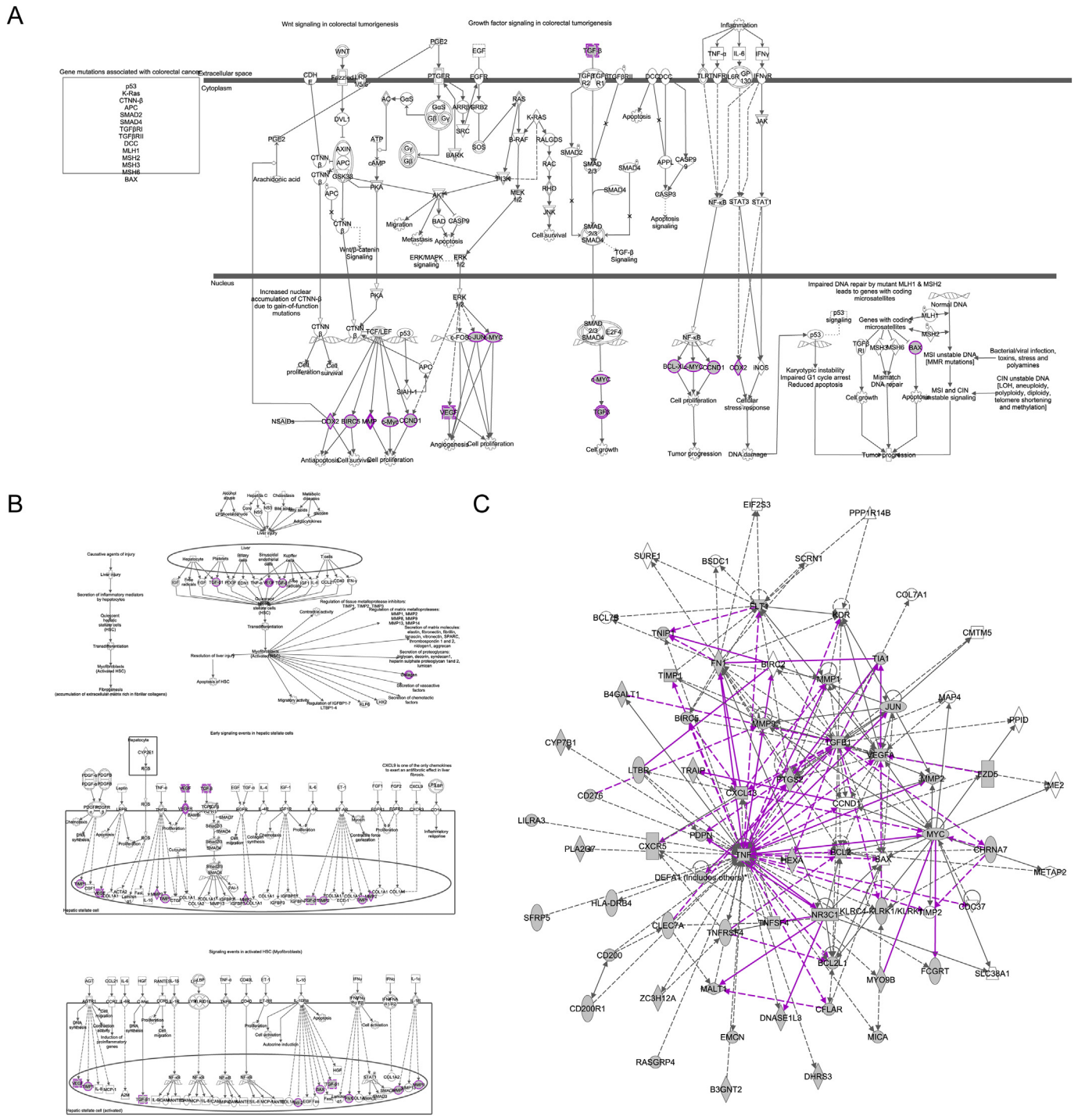

Fig. 5. Representative signaling pathways and overlapped networks linked to rheumatoid arthritis and targeted by celastrol. (A) Colorectal cancer metastasis signaling. (B) Hepatic fibrosis/hepatic stellate cell activation. (C) Merged network involving predicted celastrol targets (purple lines) and proteins encoded by rheumatoid arthritis-associated genes (black lines). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

potential targets in rheumatoid arthritis, as well as the numerous signaling pathways and networks in which celastrol targets participate. We also performed docking studies to predict specific interactions between celastrol and its predicted targets.

Our pathway analysis suggests that celastrol may exert therapeutic effects against rheumatoid arthritis by regulating the functions of Th1 and Th2 cells, macrophages, fibroblasts and endothelial cells. These results are consistent with several studies in vitro and in vivo suggesting that celastrol inhibits several inflammatory chemokines, including mundane T cell expressed and secreted (RANTES), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory proteins (MIP-1a), and growth-regulated oncogene/keratinocyte chemoattractant (GRO/KC); as well as cytokines such as TNF-α and IL-1β [10]. Thus, celastrol suppresses TNF-α-induced production of anti-apoptosis proteins (e.g., Bcl-2, Bcl-XL, survivin), proteins involved in invasion (e.g., MMP-9) [11,41].

Our results provide evidence that celastrol acts on not only progression of rheumatoid arthritis, but also cancer development. The

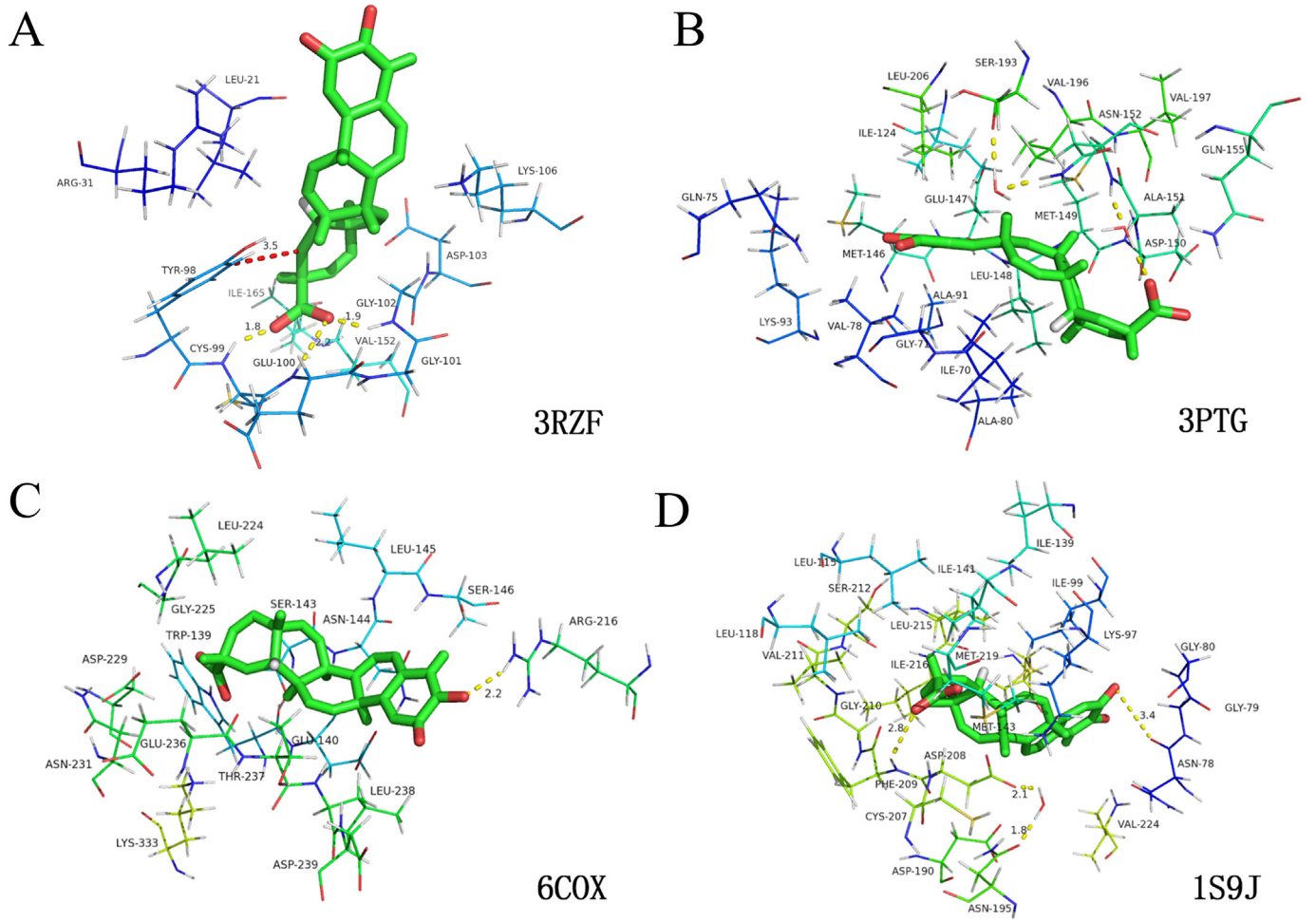

Fig. 6. Molecular models of celastrol binding to the predicted targets (A) 3RZF, (B) 3PTG, (C) 6COX and (D) 1S9J. The yellow dashed lines show H-bonds, and the red dashed lines show π-π interactions, with interaction distances indicated above the lines. The sticks represent the celastrol molecule, and the lines represent residues in the binding sFite. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

networks that we identified as associated with rheumatoid arthritis and affected by celastrol involve primarily cancer, connective tissue disorders, organismal injury and abnormalities, tissue development, cell death and survival. Consistent with our findings, celastrol has been shown to inhibit the proliferation of various cancer cell lines, including C6 glioma, human monocytic leukemia, melanoma, pancreatic cancer, RPMI 8266 myeloma, and K-562 human chronic myelogenous leukemia [42–46].

Though this network pharmacology strategy may be a quick method to predict the targets by drugs on complex diseases, the targets sometimes can be obtained too much. It is necessary to verify the targets by cell experiments.

In particular, our results predict that celastrol exerts therapeutic effects against rheumatoid arthritis at least in part by modulating the function of MMP-9, COX-2, c-Myc, TGF-β, c-JUN, JAK-1, JAK-3, IKK-β, SYK, MMP-3, JNK and MEK1. Our study may inspire and guide further work to establish the molecular targets of celastrol in rheumatoid arthritis and to apply the network pharmacology approach to drug discovery against other inflammatory and autoimmune diseases.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

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