Anti-rheumatic effects of Tripterygium wilfordii Hook F in a network perspective

Haiyang Fang, Tinghong Yang, Yichuan Wang
Department of Mathematics
Logistical Engineering University
Chongqing, China

Weidong Zhang
Department of Natural Medicinal Chemistry
Second Military Medical University
Shanghai, China

Yang Ga
Tibet Traditional Medical College
Lhasa, China

Jing Zhao*
Department of Mathematics
Logistical Engineering University
Chongqing, China

Yi Zhang
The National Medical College
Chengdu University of TCM
Chengdu, China

* Email: zhaojanne@gmail.com

***Abstract*—Rheumatoid arthritis (RA) is a chronic disease that affects the joints, often those in a person's wrists, fingers, and feet. In contrast to FDA-approved anti-RA drugs, Tripterygium wilfordii Hook F (TwHF), a traditional Chinese medicine (TCM), featured as multi-targeting, have been acknowledged with notable anti-RA effects although the pharmacology is unclear. In this work, we investigated the therapeutic mechanisms of TwHF at protein network level. First, RA-associated genes, the protein targets of FDA approved anti-RA drugs and TwHF were collected. Then we mapped the protein targets of TwHF on the drug-target network of FDA approved anti-RA drugs and KEGG RA pathway, based on these information and resources. Furthermore, we quantitatively analyzed the anti-rheumatic effect of TwHF and compared it with those of FDA approved anti-RA drugs by a network based anti-rheumatic effect score. Our study suggests that TwHF may function as a combination of disease-modifying anti-rheumatic drug and non-steroidal anti-inflammatory drug and its anti-rheumatic power could be comparable with that of anti-inflammatory agents. This study may facilitate our understanding of the RA treatment by TwHF from the perspective of network systems and it may suggest new approach for the study of TCM pharmacology.**

Keywords—Tripterygium wilfordii Hook F; Rheumatoid arthritis; disease gene; drug target; protein-protein interaction network; pathway; therapeutic effect

I. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory joint disorder that principally attacks flexible (synovial) joints, leading to the destruction of articular cartilage and fusion of the joints. It can also affect other tissues throughout the body. RA is considered as a systemic autoimmune disease, whose cause and pathogenesis remain largely unknown.

Currently there is no cure for RA. The aim of treatment is to reduce inflammation, relieve pain, suppress disease activity, prevent joint damage and slow disease progression, so as to maintain the patient's quality of life and ability to function. Clinical treatments for RA include non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs), glucocorticoids, and biological response modifiers.

The herb Tripterygium wilfordii Hook F (TwHF) has long been used in traditional Chinese medicine (TCM) for the treatment of RA. There are some prospective, double-blind, randomized, and controlled trials which have demonstrated significant improvement in RA disease activity by TwHF extract [1].

It has been believed that complex chronic diseases including RA are usually caused by an unbalancing regulating network resulted from the dysfunctions of multiple genes or their products[2-4]. Thus there is a need to study such diseases and their treatment from the viewpoint of network-based systems biology [5-8].

In this work, we studied anti-rheumatic effects of TwHF as compared to FDA-approved anti-RA drugs from network perspective. We first collected genes associated with RA, proteins inhibited by two main active compounds of TwHF, Triptolide and Tripterine, and targets of FDA-approved anti-RA drugs. Then we study the drug targets in the context of RA-associated pathway and protein networks. TwHF's targets were mapped onto the drug-target network of FDA-approved anti-RA drugs and the RA pathway in the KEGG database to investigate their potential anti-RA functions. The network based anti-rheumatic effect score was defined to quantitatively analyze the anti-rheumatic effect of TwHF and compare it with those of FDA approved anti-RA drugs.

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II. MATERIALS AND METHODS

A. Collection of RA-associated genes

We collected genes associated with RA from two resources as follows:

1) the Online Mendelian Inheritance in Man (OMIM) database [9]: we searched the OMIM database with a keyword "rheumatoid arthritis" and found 7 causal genes: CD244, HLA-DR1B, MHC2TA, NFKBIL1, PAD, SLC22A4 and PTPN8.

2) Genetic Association Database(GAD) [10]:we searched the GAD database with a keyword "rheumatoid arthritis" and found 82 genes whose association with RA was shown "Y".

Five of the seven RA causal genes in the OMIM database are also included in the 82 genes collected from the GAD. Thus we have 84 RA associated genes in total.

B. FDA approved anti-RA drugs and their target proteins

The data of FDA approved anti-RA drugs and their targets was downloaded from the DrugBank database [11], which was updated in May of 2013. We searched the DrugBank database with a keyword "rheumatoid arthritis" and extracted all of the FDA approved anti-RA drugs and their corresponding targets (32 drugs and 51 protein targets). Four classes of drugs are used clinically for the treatment of RA. They are non-steroidal anti-inflammatory drugs (NSAID) such as Flurbiprofen, disease-modifying anti-rheumatic drugs (DMARDs) such as Sulfasalazine, glucocorticoids such as Cortisoneacetate, and biological response modifiers such as Etanercept and Abatacept.

C. Target proteins of TwHF's main ingredients

Data about target proteins for TwHF was collected from Herbal Ingredients' Targets Database (HIT) [12], a well-known herb ingredient target database (http:// lifecenter.sgst.cn/hit/), with a keyword "tripterygium wilfordii". According to HIT, TwHF contains two main active components: Triptolide and Tripterine. It was found that Triptolide inhibits 33 target proteins, while Tripterine acts on 9 ones. Since TGFΒ-1 is targeted by both compounds, we totally collected 41 target proteins of TwHF in HIT databas.

D. Protein-protein interaction data

In this research, we used the weighted human protein-protein interaction (PPI) database constructed by Erten et al [13]. Human PPI data of this database was obtained from NCBI Entrez Gene Database [14] . Then using a logistic regression model, which incorporated three features of proteins: gene expression profiles, clustering coefficients of nodes in the PPI network, and subcellular localizations, reliability scores were assigned to each pair of these PPIs. For correlation of gene expression, the expression profiles of normal human tissues measured in the Human Body Index Transcriptional Profiling were used (GSE7307)[15]. This weighted PPI network contains 8959 proteins and 34833 distinct interactions among these proteins. The biggest connected cluster of this network includes 8601 proteins and 34549 distinct interactions, which was used in our analysis.

E. Construction of drug-target network

A drug-target network is defined as a bipartite network for the drug-target associations consisting of two disjoint sets of nodes [16]. One set of nodes corresponds to all drugs under consideration, and the other set corresponds to all the proteins targeted by drugs in the study set. A protein node and a drug node are linked if the protein is targeted by that specific drug according to the DrugBank information.

F. Network Scoring of Anti-rheumatic effects of drugs

1) Scoring network effect of a group of seed nodes

We applied the algorithm of random walk with restart to score the effect of a group of seed nodes on all the nodes in the network under study [17, 18]. Here the network is the weighted human PPI network, while the seeds could be disease-associated genes or protein targets of drugs.

A random walk starts at one of the seed nodes in the set S. At each step, the random walker either moves to a randomly chosen neighbor $u \in N$ of the current node v or it restarts at one of the nodes in the seed set S. The probability of restarting at a given time step is a fixed parameter denoted by r. For each restart, the probability of restarting at $v \in S$ suggests the degree of association between v and the seed set S. For each move, the probability of moving to interacting partner u of the current node v is proportional to the reliability of the interaction between u and v. After a sufficiently long time, the probability of being at node v at a random time step provides a measure of the functional association between v and the genes in seed set S. This process could be denoted as follows:

$$x^{t+1} = (1-r)Px^{t} + rx^{0} \qquad (1)$$

where P is the adjacency matrix of the weighted PPI network, representing the coupling strength of nodes in the network; $r \in [0,1]$ is a parameter denoting the restart probability which needs to be calibrated with real data; xt is a vector in which xt(v) denotes the probability that the random walker will be at node v at time t; x0 is a vector corresponding to the strength of seed nodes. The effect strength of seed set S to each nodes in the network is defined by steady-state probability vector x^{∞} when $x^{t+1} = x^{t}$.

The algorithm of random walk with start has been successfully used in the prioritization of candidate disease genes and r = 0.3 appeared to be a robust choice [19]. We took r = 0.3 in this study.

2) Scoring RA's effect on the human PPI network

In this case the seed nodes are known RA-associated genes. The initial vector x0 was defined as x0(v) = 1 if v is a seed otherwise x0(v) = 0.

Then random walk with restart was used to compute the RA effect score of each node in the human network and get a disease effect vector xRA.

3) Scoring a drug's effect on the human PPI network

In this case the seed nodes are defined as the drug's protein targets.

For a FDA-approved anti-RA drug, the initial vector x0 was defined as x0(v) = 1 if v is a seed, otherwise x0(v) = 0.

Considering that the inhibition potency of natural compounds on protein targets is usually much lower than that of specifically designed drug molecules[20], we defined the initial vector x0 of TwHF as x0(v) = 0.01 if v is a target, otherwise x0(v) = 0.

For each drug, random walk with restart was used to compute its effect score on each node in the human network and get its drug effect vector xdrug.

4) Scoring the anti-rheumatic effects of a drug

We applied the inner product between the vectors of disease effect and drug effect to measure how the drug impacts the human interactome under the influence of the disease[21]. Specifically, $s = < x_{RA}, x_{drugk} >$ is defined as the anti-rheumatic effect score of the kth drug under study. The effect score of TwHF was then compared with that of its random contracts by z-score.

G. Z-score

Z-score was applied to quantify the difference between the anti-rheumatic effect scores of TwHF and its random counterparts:

$$z = \frac{s - \bar{s}_r}{\Delta s_r} \qquad (2)$$

where s is the score of anti-rheumatic effect of TwHF, $\bar{s}_r$ and ΔS_r are the mean and standard deviation of the corresponding metric for the random counterparts. The higher the absolute value of a z-score, the more significant the difference.

III. Results and Discussion

A. Drug-target network for anti-RA drugs under study

It would be interesting to bridge TwHF and existing FDA-approved anti-RA drugs via their common drug targets. This is expected to provide alternative insights for deducing the therapeutic mechanism of TwHF. We constructed the drug-target network for the 32 FDA approved anti-RA drugs included in DrugBank and their corresponding 51 targets and then mapped the 41 targets of TwHF onto this network. As shown in Figure 1, this network shows that the active compounds of TwHF share 4 targets (TNF, PTGS2, CD86 and CD80) with 3 types of anti-RA drugs, in which PTGS2 and TNF are conformed therapeutic targets for non-steroidal anti-inflammatory drugs (NSAID) and biological response modifiers, respectively, suggesting that the effect of TwHF could be a combination of different classes of anti-RA agents.

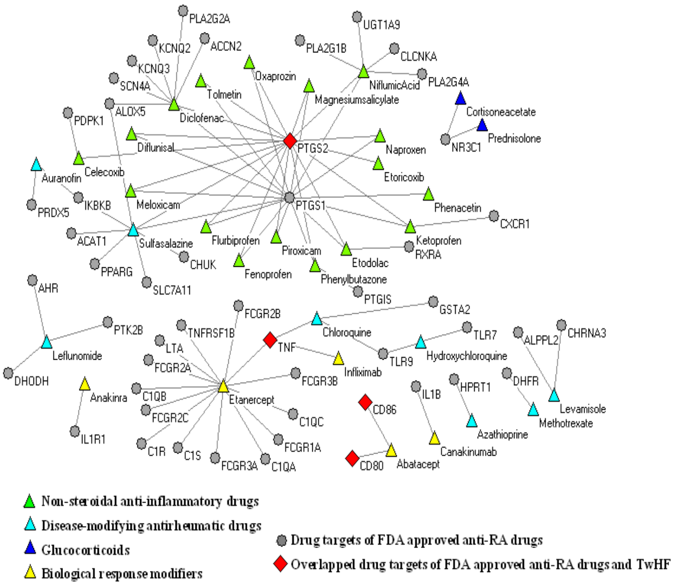

Figure 1. Drug-target network for anti-RA drugs under study. A target protein node and a drug node are linked if the protein is targeted by the corresponding drug. Triangles are drugs, while circles and diamonds are targets.

B. Targets of TwHF's main compounds on the RA pathway in the KEGG database

RA is a systemic autoimmune disease which causes recruitment and activation of inflammatory cells, synovial hyperplasia, and destruction of cartilage and bone. Multiple signaling pathways regulate these different aspects of pathological processes of RA and interact with each other, in which some cytokines such as TNF and ILs play pivotal roles. To explore if TwHF acts on the RA-associated biological processes, we mapped the 41 targets of TwHF on the RA pathway in the KEGG database [22]. It was found that 10 of the 41 targets appear on this pathway (Figure 2). Figure 2 shows that TwHF intervenes in the RA pathway by inhibiting multiple proteins localized at its three distinct but associated developing branches of the disease, thus retarding the processes of inflammatory cell infiltration, inflammatory synovial pannus formation and joint destruction. This suggests the therapeutic effect of TwHF on RA.

C. Anti-rheumatic effects of TwHF compared with those of FDA approved drugs by network scores

To quantitatively compare the anti-rheumatic effect of TwHF with those of FDA approved anti-RA drugs, we chose several representatives from each of the four classes of anti-RA western medicines and then computed the network score for the anti-rheumatic effect of each drug, respectively. The initial vector x0 of drug effect was defined as x0(v) = 1 if node v is a drug target, otherwise x0(v) = 0.

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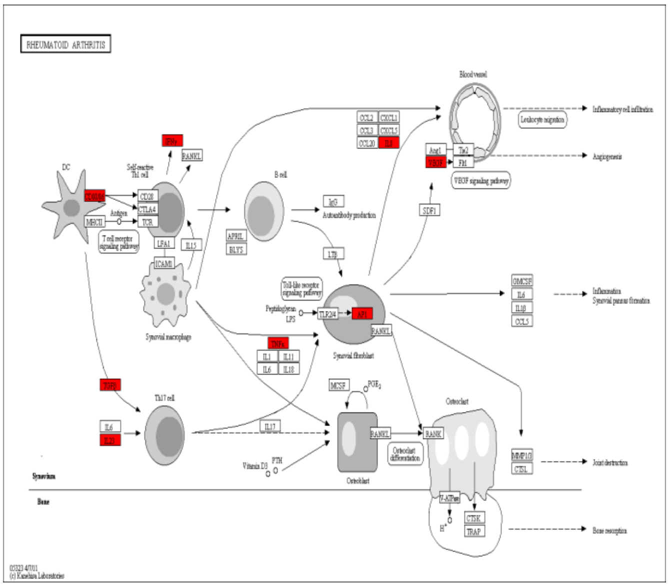

Figure 2. Regulations of TwHF's active compounds on different proteins on RA pathway. Red boxes represent targets of TwHF's active compounds. The original pathway map was downloaded from the KEGG database.

TABLE I. THE ANTI-RHEUMATIC EFFECT SCORES OF REPRESENTATIVE ANTI-RA WESTERN MEDICINES

Class of Drug	Anti-RA drug	Targets	Effect Score
Biological Response Modifiers	Etanercept	FCGR2C,**TNFRSF1B**,**TNF**,**LTA**,FCGR3B,**FCGR3A**,**FCGR2B**,**FCGR2A**,FCGR1A,C1S,C1R,C1QC,C1QB,C1QA	1.186
	Infliximab	**TNF**	0.158
	Abatacept	**CD86**,**CD80**	0.138
	Anakinra	IL1R1	0.075
DMARDs	Chloroquine	TLR9,**TNF**,GSTA2	0.174
	Sulfasalazine	SLC7A11,PTGS2,PTGS1,PPARG, IKBKB,CHUK, ALOX5,ACAT1	0.155
	Leflunomide	PTK2B,DHODH,AHR	0.061
	Auranofin	PRDX5,IKBKB	0.05
	Methotrexate	DHFR	0.017
	Hydroxychloroquine	TLR9,TLR7	0.016
	Azathioprine	HPRT1	0.008
	Levamisole	CHRNA3,ALPP-L2	0.002
Glucocorticoids	Cortisoneacetate	NR3C1	0.044
NSAIAs	Flurbiprofen	PTGS2,PTGS1	0.021

Note: RA-associated disease genes are marked in bold characters.

As shown in Table I, biological response modifiers and disease-modifying anti-rheumatic drugs (DMARDs) get averagely much higher scores than the other two classes of drugs, non-steroidal anti-inflammatory drugs (NSAID) and Glucocorticoids. Actually, biological response modifiers are a new type of DMARDs [23], i.e., biotech agents, while drugs categorized into the class of DMARDs are small molecular compounds. DMARDs target the part of the immune system that is leading to inflammation and joint damage [24]. Thus they can often slow or stop the progression of RA. From Table I we can see that some DMARDs target directly on RA-associated genes such as TNF, CD80 and CD86, supporting their higher anti-rheumatic effects.

Since RA is an inflammatory disease affecting the joints, it gets worse over time unless the inflammation is stopped or slowed. Thus anti-inflammatory is very important in the treatment. Glucocorticoids and NSAIDs are such class of drugs, in which glucocorticoids are steroidal strong anti-inflammatory drugs that can also block other immune responses while NSAIDs work by inhibiting enzymes that promotes inflammation [25]. By reducing inflammation, anti-inflammatory agents help reduce swelling and pain. But they are not effective in reducing joint damage. Thus these drugs alone are not effective in treating the disease and they should be taken in combination with other rheumatoid arthritis medications [26].

We then computed the network score for the anti-rheumatic effect of TwHF. Unlike specifically designed drug molecules, TwHF's two active compounds Triptolide and Tripterine are naturally-occurring substances, thus their inhibition potency on targets could be much weaker. For example, our earlier study found that IC50 of natural compound Astragaloside IV against proteins CN and ACE was approximately two orders higher than corresponding western drugs CsA and enalapril, respectively [20]. Therefore, we defined the initial vector x0 of TwHF as $x0(v) = 0.01$ if node v is a target, otherwise $x0(v) = 0$. In this way, the anti-rheumatic effect score of TwHF was got as 0.0324.

To investigate if the score of TwHF suggests significant anti-rheumatic effect, we generated 3000 random target sets, each of which included same number of proteins as TwHF's targets. It was calculated that the mean effect score of the 3000 random counterparts is 0.007456 and the standard deviation is 0.0000023. Hence the z-score of TwHF's anti-rheumatic effect score is 5.17, suggesting that TwHF exhibits significant anti-rheumatic effect.

Comparison of TwHF's score of 0.0324 with results in Table I suggests that although TwHF's anti-rheumatic effect is much lower than that of most biological response modifiers and disease-modifying anti-rheumatic drugs, it is in the same order as that of anti-inflammatory agents, including Glucocorticoids and NSAIDs.

IV. CONCLUSIONS

We have extracted data related to RA's pathogenesis and treatment — known RA associated genes from the OMIM database and GAD, protein targets of FDA approved anti-RA drugs and TwHF, respectively. First, we constructed Drug-target network for FDA-approved anti-RA drugs. By mapping TwHF's targets on this network, we found that four targets of TwHF, TNF, PTGS2, CD86 and CD80, exist in this network. Then we mapped the targets of TwHF on KEGG RA pathway and found that 10 targets involve in this pathway. These findings indicate that TwHF could intervene in the biological process of the occurrence and development of RA by targeting

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on multiple targets and it may function as a combination of disease-modifying anti-rheumatic drug and non-steroidal anti-inflammatory drug.

At last, we quantitatively analyzed the anti-rheumatic effect of TwHF and compared it with those of FDA approved anti-RA drugs by a network based anti-rheumatic effect score. We got the anti-rheumatic effect score of TwHF as 0.0324, which is significantly higher than that of its random counterparts, suggesting significant anti-rheumatic effect of TwHF. The anti-rheumatic effect score also implies that TwHF's anti-rheumatic power could be comparable with that of anti-inflammatory agents, including glucocorticoids and NSAIDs.

This work applied network approach to explain TwHF's anti-rheumatic effect. It may shed new lights on the study about the TCM pharmacology, and promote the development of nationality medicine.

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