ORIGINAL RESEARCH

A network pharmacology approach to determine the synergetic mechanisms of herb couple for treating rheumatic arthritis

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Purpose: The purpose of this study was to investigate the therapeutic mechanism(s) of *Clematis chinensis* Osbeck/*Notopterygium incisum* K.C. Ting ex H.T (CN).

Methods: A network pharmacology approach integrating prediction of ingredients, target exploration, network construction, module partition and pathway analysis was used.

Results: This approach successfully helped to identify 12 active ingredients of CN, interacting with 13 key targets (Akt1, STAT3, TNFsf13, TP53, EPHB2, IL-10, IL-6, TNF, MAPK8, IL-8, RELA, ROS1 and STAT4). Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis indicated that CN-regulated pathways were mainly classified into signal transduction and immune system.

Conclusion: The present work may help to illustrate the mechanism(s) of action of CN, and it may provide a better understanding of antirheumatic effects.

Keywords: targets prediction, pathways analysis, action mechanism, *Clematis chinensis* Osbeck, *Notopterygium incisum* K.C. Ting ex H.T. Chang

Introduction

RA is a chronic autoimmune disease influenced by genetic factors, environmental factors and interaction.[1] The prevalence of RA is ~1% in the adult population, with a higher incidence in the elderly and women.[2] In addition to disability and joint destruction,[3] patients with RA have a higher risk of dying prematurely from cardiovascular diseases.[4] Consequently, prevention and treatment of RA are critical in clinical therapy. Therapeutic agents for RA include NSAIDs, glucocorticoids, DMARDs, biologic DMARDs and, most recently, small molecular signal inhibitors.[5] However, most of current drugs, which play an important role in treating RA, have severe adverse effects, including gastrointestinal irritation, kidney injury, cardiovascular risk and even the so-called Cushing's syndrome.[6] Consequently, TCMs, with clinical application for thousands of years, have recently attracted more and more attention due to prominent effectiveness and less side effects.[7] CC and NI, as TCMs, have been frequently used to treat RA for their anti-inflammatory activity.[8–10] Our previous study has reported that CN has evident anti-rheumatic effects in adjuvant-induced arthritis in rats.[11] However, the molecular mechanism(s) of CN in the treatment of RA remains to be elucidated. Network pharmacology is an efficient tool to clarify targets and mechanisms of TCMs.[12] The methodologies of network pharmacology highlight the paradigm shift from "one drug, one target" to "multicomponent therapeutics, biological network".[13] TCMs have the advantages of multiple components and targets, which

correspond to the methodologies of network pharmacology. Thus, network pharmacology is desirable for exploring the mechanisms of TCMs. In the present study, we respectively collected the information of targets from active ingredients in CN and targets of RA from several databases for the first time. In order to uncover the rationality of CN, network construction and topological structural analysis were established, which offered underlying synergistic mechanisms of CN for treating RA.

Methods
Building database of ingredients
All the chemical ingredients' data of CC and NI were derived from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://lsp.nwu.edu.cn/tcmsp.php). TCMSP is a unique system pharmacology platform of TCMs that is capable of providing the relationship between drugs, targets and diseases.

Screening of active ingredients
The active constituents from CC and NI were filtered by integrating OB and DL. DL helps to describe pharmacokinetic and pharmaceutical properties of compounds, such as solubility and chemical stability. Usually, the selection criterion for the "drug-like" compounds in TCMs is 0.18.[14] OB represents the relative amount of an oral drug that is absorbed into the blood circulation.[15] Since low OB is the primary reason responsible for the development of TCMs into therapeutic drugs, it is vital to conduct OB screening criterion. Based on literatures and suggestions in TCMSP, we selected OB≥30% and DL≥0.18 as a screening threshold.[16,17] The ingredients conforming to both standards mentioned earlier will be preserved for further analysis.

TCM-associated target prediction
Three databases are combined to predict relevant targets of active ingredients in CC and NI comprehensively. GeneCards database (http://www.genecards.org/) automatically integrates gene-centric data from ~125 web sources, while BATMAN-TCM (http://bionet.ncpsb.org/batman-tcm) ranks potential drug–target interactions based on their similarity to the known drug–target interactions. STITCH database (http://stitch.embl.de/) integrates many sources of experimental and manually curated evidence with text-mining information and interaction predictions. First, the active constituents were severally entered into GeneCards, BATMAN-TCM and STITCH. Then, duplications and unified names were removed from the targets obtained from the aforementioned three tools. Noteworthy, only the targets of *Homo sapiens* were kept for further study.

RA-associated target prediction
Different genes associated with RA were collected from DisGeNET (http://www.disgenet.org/web/DisGeNET). DisGeNET is a useful platform providing the search of the molecular underpinnings of diseases, the analysis of disease genes, the validation of predicted genes and so on.

Network construction and node screening
The different targets from CC, NI and RA were submitted to Agilent Literature Search 3.1.1 (LitSearch version 2.69). Based on the human targets, we set "Max Engine Matches" as 10 and searched through the whole text. Then, the protein–protein interaction network was visualized by Cytoscape 3.5.1 software. Finally, we severally selected the top 30 targets of high-node degree as key targets for further analysis.
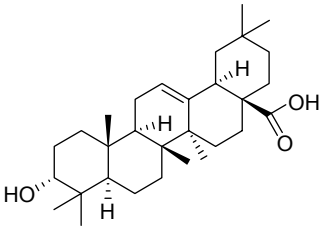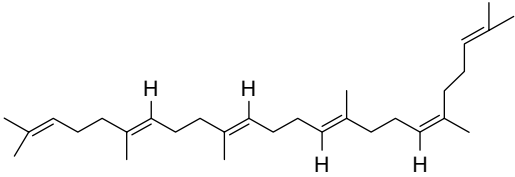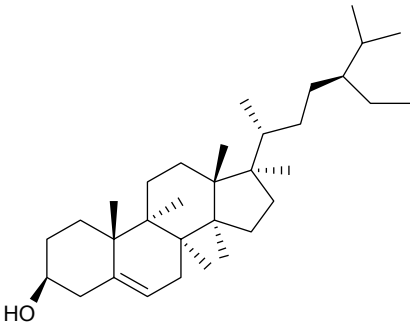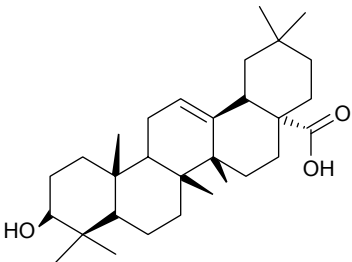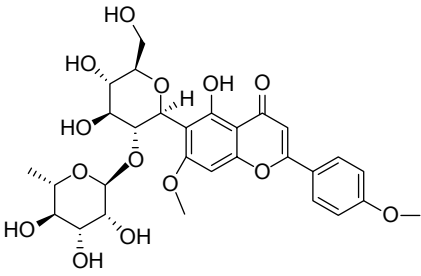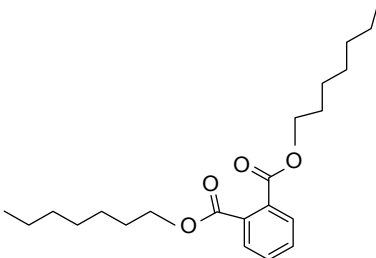
Module partition and KEGG pathway analysis
MCODE was applied to identify the molecular network for module identification according to the clustering of genes in the network. Then, main modules obtained from CC, NI and RA were submitted to DAVID Bioinformatics Resources 6.8 software (https://david.ncifcrf.gov/) to carry out GO functional enrichment analysis. "*Homo Sapiens*" was also limited to identify KEGG pathways that were significantly enriched in the identification module. Of note, P-value was implemented to explore the statistical significance of the modules. KEGG pathways with $P<0.05$ (P-values were corrected using the Benjamini–Hochberg procedure) are significant signaling pathways.

Results
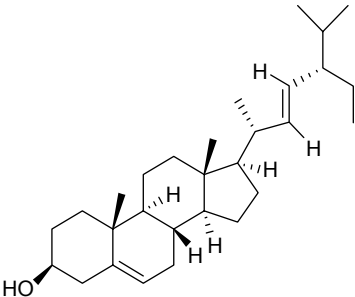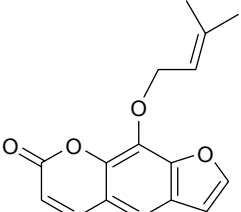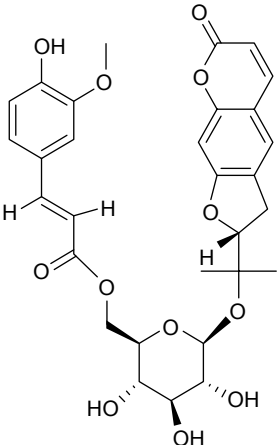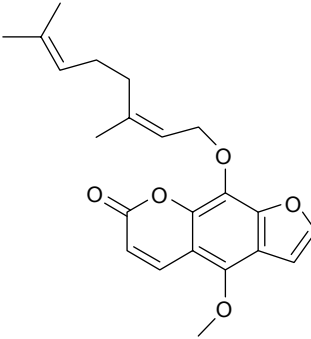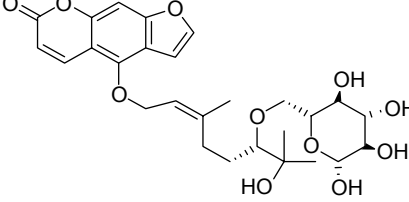### Active ingredients of CC and NI
A total of 484 ingredients of CN were retrieved from TCMSP, including 114 ingredients of CC and 370 ingredients of NI. In this study, 21 active compounds from 484 compounds met both the requirements, OB≥30% and DL≥0.18 (Table 1). It has been validated experimentally that some ingredients possess pharmacological activities. For example, β-sitosterol (OB=36.91, DL=0.75) plays a

Table 1 Active ingredients of CC and NI

Molecule ID	Molecule name	Structure	OB (%)	DL	Herb
MOL001663	(4aS,6aR,6aS,6bR,8aR,10R,12aR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydropicene-4a-carboxylic acid		32.03	0.76	CC
MOL002372	(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene		33.55	0.42	CC
MOL000358	β-Sitosterol		36.91	0.75	CC, NI
MOL005594	Clematoside A′_qt		37.51	0.76	CC
MOL005598	Embinin		33.91	0.73	CC
MOL005603	Heptyl phthalate		42.26	0.31	CC
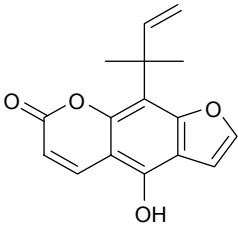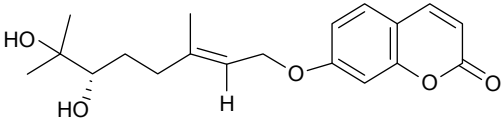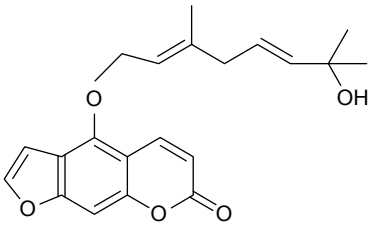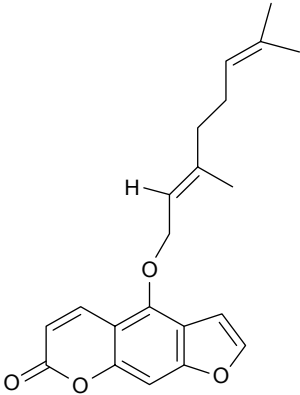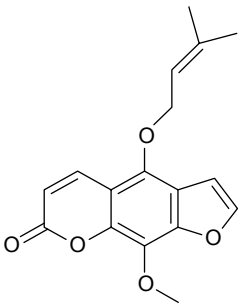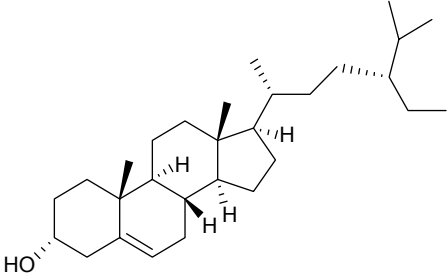
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Table 1 (*Continued*)

Molecule ID	Molecule name	Structure	OB (%)	DL	Herb
MOL000449	Stigmasterol		43.83	0.76	CC
MOL001941	Ammidin		34.55	0.22	NI
MOL011962	6′-Feruloylnodakenin		32.02	0.67	NI
MOL011963	8-Geranoxy-5-methoxypsoralen		40.97	0.50	NI
MOL011968	Coumarin glycoside		33.07	0.78	NI
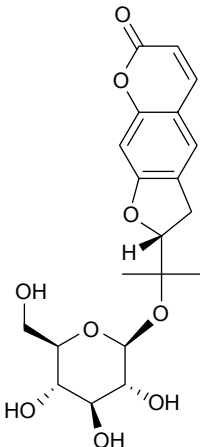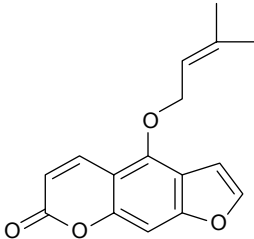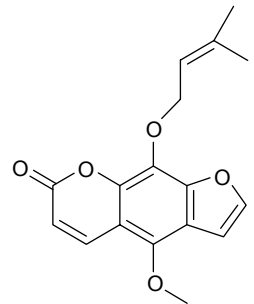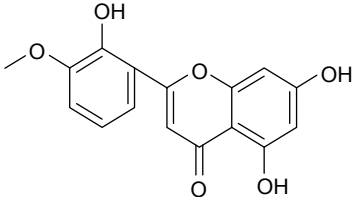
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Table 1 (*Continued*)

Molecule ID	Molecule name	Structure	OB (%)	DL	Herb
MOL011969	Demethylfuropinnarin		41.31	0.21	NI
MOL011971	Diversoside_qt		67.57	0.31	NI
MOL011975	Notoptol		62.97	0.48	NI
MOL001951	Bergapten		41.73	0.42	NI
MOL001956	Cnidilin		32.69	0.28	NI
MOL000359	Sitosterol		36.91	0.75	NI

(*Continued*)

Table 1 (*Continued*)

Molecule ID	Molecule name	Structure	OB (%)	DL	Herb
MOL004792	Nodakenin		57.12	0.69	NI
MOL001942	Isoimperatorin		45.46	0.23	NI
MOL002644	Phellopterin		40.19	0.28	NI
MOL002881	Diosmetin		31.14	0.27	NI

Abbreviations: CC, *Clematis chinensis* Osbeck; NI, *Notopterygium incisum* K.C. Ting ex H.T. Chang; OB, oral bioavailability; DL, druglikeness.

significant role in anti-inflammation,[18] anti-tumor[19] and anti-hyperlipidemia.[20] Moreover, nodakenin (OB=57.12, DL=0.69) plays an important therapeutic effect in inflammatory disorders and has been regarded as one of the standard ingredients of NI in Chinese Pharmacopoeia.[10,21]

Target prediction

TCMs give play to their pharmacological effects through multiple ingredients and targets. Thus, besides predicting

ingredients, it is also necessary for the exploration of targets. However, searching for targets through literatures is time consuming and labor intensive. In the present work, predictive models including GeneCards, BATMAN-TCM and STITCH were used to predict 301 targets, which interacted with 12 active ingredients. It is interesting that another nine ingredients were removed for having no relevant targets. In addition, the DisGeNET database was also used to predict 1,869 targets associated with RA.

Figure 1 Top 30 targets of CC and NI.
Note: Green nodes are shared targets from RA and herb couple, red ones are shared targets from RA and CC and purple ones are shared targets from RA and NI.
Abbreviations: CC, *Clematis chinensis* Osbeck; NI, *Notopterygium incisum* K.C. Ting ex H.T. Chang; RA, rheumatoid arthritis.

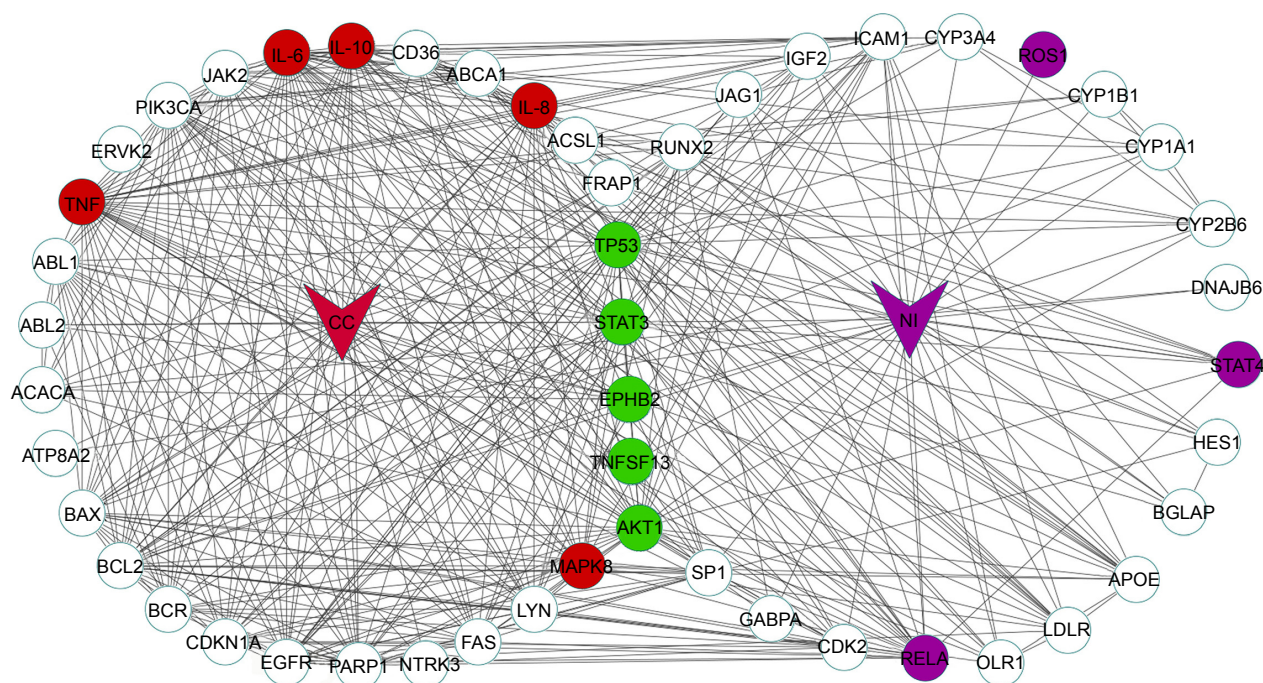## Network construction and node screening

Network construction was automatically performed after searching by Agilent Literature Search. Molecular network of CC, NI and RA separately consisted of 781, 267 and 746 nodes and was connected by 2,873, 633 and 2,303 edges, respectively. The topological parameters of CC, NI and RA show that node-degree distribution obeys the power law distribution. To further investigate the synergistic mechanisms of herb couple, the top 30 targets of high-node degree were severally chosen. As shown in Figure 1, the herb couple shares 13 targets (Akt1, STAT3, TNFsf13/APRIL, TP53, EPHB2, IL-10, IL-6, TNF, MAPK8/JNK, IL-8, RELA, ROS1 and STAT4) with RA, in which five targets (STAT3, Akt1, TP53, TNFSF13 and EPHB2) are the overlapped targets in CC and NI and another five targets (IL-10, IL-6, TNF, MAPK8 and IL-8) and three targets (RELA, ROS1 and STAT4) are separate in CC and NI.

Module partition and KEGG pathway analysis
Module partition
MCODE software was used to analyze the molecular network. A total of 122 modules were identified from original networks with 47 modules from CC, 25 modules from NI and 50 modules from RA. Top 10 modules with more nodes were respectively selected for KEGG pathway analysis.

KEGG pathway analysis
In order to deduce the potential pathways affected by herb couple, DAVID Bioinformatics Resources 6.8 software was used to perform pathway enrichment analysis. Since diseases arise from the dysfunctions of basic biological functions, we removed the KEGG pathway section of human diseases. We found that herb couple could totally affect 35 signal pathways, including 16 pathways from both CC and NI, 17 pathways solely from CC and 2 pathways solely from NI.

As shown in Table 2A, herb couple acts on six pathways in signal transduction, such as PI3K–Akt signaling pathway and JAK–STAT signaling pathway. Immune system and endocrine system, respectively, cover three pathways such as NOD-like receptor signaling pathway and GnRH signaling pathway. Furthermore, the herb couple also regulates other pathways in cell process, development and nervous system. Signal pathways solely from CC are shown in Table 2B and indicated that CC has the possibility of being associated with the immune system, signal transduction, endocrine system and cell process. Compared with the signal pathways in CC, NI solely regulates cytosolic DNA-sensing pathway and cell process, which are associated with signal transduction and cell process, respectively (Table 2C).

Discussion
RA is a chronic autoimmune disease that is implicated in inflammation, angiogenesis, bone destruction and the

Table 2 (A) Signal pathways of herb couple, **(B)** individual signal pathways of CC and **(C)** individual biological pathways of NI

A

Pathway class	Pathway name	CC's targets on pathway	NI's targets on pathway
Signal transduction	FoxO signaling pathway	IL-6, HRAS, SGK1, TGFB3, IGF1, FOXO1, IL-7R, IL-10, STAT3, PCK1, AKT1, MAPK1, NRAS, G6PC, CDKN1A, KRAS, CDKN2B, MAPK9, PIK3CA, MAPK8, CAT, EGF	AKT1, IL-6, SMAD4, PIK3CA, IL-10, STAT3, CDK2
	PI3K–Akt signaling pathway	FGFR1, HRAS, KITLG, NFKB1, COL2A1, BCL2L1, KIT, IL-7R, ATF2, AKT1, KRAS, BCL2, PIK3CA, EGF, CSF1R, IL-4, IL-3, IL-6, SGK1, IL-2RB, HSP90AA1, RELA, TP53, IGF1, KDR, PCK1, NRAS, MAPK1, CDKN1A, G6PC, IFNB1, VEGFA, PDGFRA, JAK1, PDGFRB, JAK2, EPOR	IBSP, AKT1, IL-6, IFNA1, BCL2, RELA, TP53, KITLG, PIK3CA, COL2A1, TLR4, CDK2, SPP1
	NF-κB signaling pathway	MAP3K7, IRAK1, TNF, TNFSF13B, LYN, BCL2, RELA, BCL2A1, IL-1B, NFKB1, BCL2L1, BIRC3, TRAF6, CXCL12, BLNK	ICAM1, TNF, TNFSF11, PTGS2, RELA, BCL2, TLR4
	TNF signaling pathway	CSF2, IL-6, TNF, SOCS3, RELA, NFKB1, CX3CL1, BIRC3, MMP3, ATF2, MAP3K7, AKT1, MAPK1, FOS, IL-1B, PIK3CA, MAPK9, MAPK8, FAS, MAP2K7	AKT1, ICAM1, IL-6, TNF, CCL2, PTGS2, RELA, PIK3CA
	Sphingolipid signaling pathway	HRAS, TNF, RELA, TP53, NFKB1, AKT1, NRAS, MAPK1, KRAS, BAX, BCL2, PIK3CA, MAPK9, MAPK8, DEGS1	AKT1, TNF, RELA, BAX, BCL2, TP53, PIK3CA
	HIF-1 signaling pathway	IL-6, ERBB2, RELA, IGF1, NFKB1, STAT3, AKT1, MAPK1, CDKN1A, BCL2, VEGFA, CAMK2D, PIK3CA, NOS2, EGF	AKT1, IL-6, RELA, BCL2, PIK3CA, TLR4, NOS2, STAT3, RBX1
Endocrine system	Prolactin signaling pathway	HRAS, SOCS3, RELA, NFKB1, STAT3, AKT1, NRAS, MAPK1, FOS, KRAS, SLC2A2, MAPK9, PIK3CA, MAPK8, JAK2	AKT1, TNFSF11, RELA, PIK3CA, STAT3
	Insulin resistance	PPARA, IL-6, TNF, SOCS3, RELA, FOXO1, NFKB1, PPARGC1A, CPT1A, STAT3, PTPN11, PCK1, AKT1, G6PC, CD36, SLC2A2, MAPK9, PIK3CA, MAPK8	AKT1, PPARA, IL-6, TNF, CD36, RELA, PIK3CA, STAT3
	Adipocytokine signaling pathway	PPARA, TNF, SOCS3, RELA, NFKB1, PPARGC1A, CPT1A, STAT3, PCK1, PTPN11, AKT1, G6PC, ACSL1, CD36, MAPK9, MAPK8, JAK2	AKT1, PPARA, TNF, CD36, RELA, ADIPOQ, STAT3
Cell process	Apoptosis	AKT1, IL-3, TNF, AIFM1, BCL2, RELA, NTRK1, BAX, TP53, PIK3CA, NFKB1, FAS, BCL2L1, BIRC3	AKT1, TNF, RELA, BAX, BCL2, TP53, PIK3CA
	Cytokine–cytokine receptor interaction	CSF2, TNF, TGFB3, KITLG, IL-13, TNFSF13, KIT, CX3CL1, IL-7R, CXCL12, IL-10, IL-1B, FAS, EGF, CSF1R, IL-4, IL-3, IL-2RB, IL-6, FLT3, KDR, TNFSF8, ACVR2A, TNFSF13B, IFNB1, VEGFA, PDGFRA, PDGFRB, EPOR	TNFRSF11B, IL-6, IFNA1, TNF, CCL2, TNFSF11, KITLG, TNFSF13, IL-10
Development	Osteoclast differentiation	TNF, SOCS3, RELA, PPARG, NFKB1, MAP3K7, TYK2, AKT1, FOS, MAPK1, IFNB1, MAPK9, JAK1, IL-1B, PIK3CA, MAPK8, TRAF6, MAP2K7, BLNK, CSF1R	AKT1, TYK2, TNFRSF11B, TNF, TNFSF11, SQSTM1, RELA, PIK3CA
Immune system	Toll-like receptor signaling pathway	IRAK1, IL-6, TNF, RELA, NFKB1, MAP3K7, AKT1, MAPK1, FOS, IFNB1, IL-1B, PIK3CA, MAPK9, MAPK8, TRAF6, MAP2K7	AKT1, IL-6, IFNA1, TNF, IRF5, RELA, IRF7, PIK3CA, TLR4, SPP1, TLR9
	RIG-I-like receptor signaling pathway	MAP3K7, TNF, IFNB1, RELA, MAPK9, NFKB1, MAPK8, TRAF6	IFNA1, TNF, ATG5, RELA, IRF7
	JAK–STAT signaling pathway	IL-4, CSF2, IL-3, IL-6, IL-2RB, SOCS3, IL-13, BCL2L1, IL-24, IL-7R, IL-10, STAT3, PTPN11, TYK2, AKT1, STAT4, IFNB1, JAK1, PIK3CA, EPOR, JAK2	AKT1, TYK2, IL-6, STAT4, IFNA1, PIK3CA, IL-10, STAT3
Nervous system	Neurotrophin signaling pathway	IRAK1, HRAS, RELA, TP53, NFKB1, PTPN11, NTRK3, AKT1, NRAS, MAPK1, BDNF, KRAS, BCL2, BAX, NTRK1, CAMK2D, SH2B3, MAPK9, PIK3CA, MAPK8, ABL1, TRAF6, MAP2K7	AKT1, RELA, BAX, BCL2, TP53, PIK3CA

B

Immune system	NOD-like receptor signaling pathway	MAP3K7, MAPK1, IL-6, TNF, HSP90AA1, RELA, MAPK9, IL-1B, NFKB1, MAPK8, TRAF6, BIRC3
	B-cell receptor signaling pathway	AKT1, MAPK1, NRAS, FOS, HRAS, KRAS, LYN, RELA, PIK3CA, NFKB1, BLNK
	Chemokine signaling pathway	ITK, HRAS, LYN, RELA, NFKB1, CX3CL1, CXCL12, STAT3, AKT1, NRAS, MAPK1, KRAS, PTK2B, PIK3CA, JAK2
	Intestinal immune network for IgA production	IL-4, IL-6, TNFSF13B, TNFSF13, CXCL12, IL-10
	Fc epsilon RI signaling pathway	IL-4, CSF2, IL-3, HRAS, TNF, LYN, IL-13, AKT1, NRAS, MAPK1, KRAS, MAPK9, PIK3CA, MAPK8, MAP2K7
	Hematopoietic cell lineage	IL-4, CSF2, IL-3, IL-6, TNF, FLT3, KITLG, ANPEP, KIT, IL-7R, CD36, MS4A1, IL-1B, EPOR, CSF1R
	T-cell receptor signaling pathway	IL-4, PTPRC, ITK, CSF2, HRAS, TNF, RELA, CBL, NFKB1, IL-10, MAP3K7, AKT1, NRAS, MAPK1, FOS, KRAS, PAK4, PIK3CA, MAP2K7
Endocrine system	Progesterone-mediated oocyte maturation	AKT1, MAPK1, HSP90AA1, KRAS, PIK3CA, MAPK9, IGF1, MAPK8
	GnRH signaling pathway	MAPK1, NRAS, HRAS, KRAS, PTK2B, CAMK2D, MAPK9, MAPK8, MAP2K7
	Estrogen signaling pathway	AKT1, MAPK1, NRAS, FOS, HRAS, HSP90AA1, KRAS, SP1, FKBP5, PIK3CA, ATF2
Signal transduction	Rap1 signaling pathway	FGFR1, HRAS, KITLG, IGF1, CDH1, KIT, KDR, CTNNB1, AKT1, NRAS, MAPK1, KRAS, VEGFA, PDGFRA, PIK3CA, PDGFRB, EGF, CSF1R
	MAPK signaling pathway	FGFR1, HRAS, TNF, TGFB3, NFKB1, ATF2, MAP3K7, AKT1, FOS, BDNF, KRAS, IL-1B, FAS, EGF, TRAF6, MAP2K7, RELA, NF1, TP53, DUSP5, NRAS, MAPK1, NTRK1, MAPK8IP2, PDGFRA, MAPK9, PDGFRB, MAPK8
	VEGF signaling pathway	AKT1, MAPK1, NRAS, HRAS, KRAS, VEGFA, PIK3CA, KDR
	ErbB signaling pathway	HRAS, ERBB2, CBL, AKT1, NRAS, MAPK1, CDKN1A, KRAS, PAK4, CAMK2D, MAPK9, PIK3CA, MAPK8, ABL1, EGF, MAP2K7, ABL2
	Ras signaling pathway	FGFR1, HRAS, KITLG, NFKB1, KIT, BCL2L1, AKT1, KRAS, PAK4, PIK3CA, EGF, CSF1R, RELA, NF1, IGF1, KDR, PTPN11, NRAS, MAPK1, RASSF1, VEGFA, PDGFRA, PDGFRB, MAPK9, MAPK8, ABL1, ABL2
Cell process	Focal adhesion	HRAS, ERBB2, IGF1, COL2A1, BIRC3, KDR, CTNNB1, AKT1, MAPK1, BCL2, PAK4, VEGFA, PDGFRA, MAPK9, PIK3CA, PDGFRB, MAPK8, EGF
	Signaling pathways regulating pluripotency of stem cells	FGFR1, HRAS, TBX3, IGF1, STAT3, CTNNB1, AKT1, ACVR2A, INHBA, WNT1, NRAS, MAPK1, KRAS, PIK3CA, JAK1, JAK2, APC

C

Signal transduction	Cytosolic DNA-sensing pathway	IL-6, IFNA1, RELA, IRF7, IL-33
Cell process	Cell cycle	HDAC1, TP53, SMAD4, CHEK1, CUL1, CDK2, RBX1

Abbreviations: CC, *Clematis chinensis* Osbeck; NI, *Notopterygium incisum* K.C. Ting ex H.T. Chang.

immune regulation. Therapeutic agents for RA, including NSAIDs, glucocorticoids and DMARDs, are limited due to their side effects.[22] TCMs are common drugs for the treatment of RA, with clinical effectiveness and less adverse effects. Our prior study has noted that herb couple of CC and NI was experimentally validated possessing antirheumatic effects.[11] However, TCM, as a multi-component synergistic system agent, is comprehensive and abstruse. Therefore, their research method is different from chemical drugs. In the present study, to better recognize the drug combination of CN, we proposed a network pharmacology approach integrating prediction of ingredients and pathway analysis strategy of targets for CN. The method was applied to explore the potential regulation of inflammatory response, immune system and angiogenesis of CN and provide a new sight for the treatment of RA. Nonetheless, our method still has some limitations and needs to further improve. The approach just predicts and analyzes the potential synergetic mechanism of CC and NI from the perspective of biological network. Clinical and experimental trials are required to be further validated.

Targets analysis of herb couple
Targets related to RA
Synovial inflammation is a basic pathological change in RA and results in swelling and pain in the joints of RA patients. Thus, anti-inflammation is critical in the treatment of RA. Results of key targets' analysis found that RA and CN shared a total of 13 targets. Among these targets, IL-6, IL-8 and IL-10 all belonged to the IL family. In RA, IL-6 can be released by monocytes, macrophages and endothelial cells and influences T-cell development, which indirectly promotes the production of Th1, Th2 and Th17 cells with proinflammatory properties. IL-6 can also increase the level of VEGF in synovial fibroblasts, aggravating joint inflammation and damage.[23] In addition to inflammation, IL-6 increases osteoclast recruitment by acting on hematopoietic stem cells, leading to joint damage in RA.[24,25] Therefore, blockade of IL-6 action is effective to reduce both inflammation and joint destruction in RA.[26] The anti-inflammatory response is essential to control the degree and duration of the inflammatory response in RA. In macrophages, the anti-inflammatory response relies on IL-10/JAK/STAT3 signaling pathway. IL-10 signaling cascade starts upon IL-10 binding to IL-10R and activates STAT3 via the JAK1 kinase.[27] STAT3 stimulates the transcription of specific genes and in turn represses proinflammatory cytokines such as IL-1, IL-6, IL-12 and TNF-α. Moreover, MAPK8, also known as JNK, is activated

in RA synovium and mediates joint destruction in adjuvant arthritis of rats.[28] MAPK8 signalosome represents a target to prevent joint destruction.[29] TNFSF13 sustains B-cell activation and thus enhances autoimmune diseases. It also regulates synovial inflammation in RA.[30] Therefore, TNFSF13 could also be a therapeutic strategy aimed at downregulating synovial inflammation. Furthermore, other targets are also involved in the process of RA, such as STAT4,[31] EphB2,[32] TP53,[33] Akt1[34] and RELA.[35] Upregulation or downregulation of abovementioned targets contributes to treating RA. On the other hand, ROS1, highly expressed in a variety of tumor cell lines, is used as a drug target to suppress tumors clinically.[36] Based on our predictions, we speculate that ROS1 may have some relevance with RA, and the result would be validated in our future study.

Pathway analysis of herb couple
Pathways related to immune response
As shown in Table 2, many signal pathways are classified into immune system and regulate the balance of the immune system. Innate immune is the first line of defense against foreign pathogens and is the basis and initiator of adaptive immunity. B and T cells are well known to be related to adaptive immune response.[37] Table 2B shows that CC is associated with B-cell receptor signaling pathway and T-cell receptor signaling pathway, indicating that CC may have a play in the adaptive immune response. In addition, we also found that CN could regulate some proinflammatory molecule-involved pathways such as chemokine signaling pathway and Fc epsilon RI signaling pathway. In addition, cytosolic DNA-sensing pathway, MAPK signaling pathway and apoptosis are highly associated with the function of immune response, although not classified into the immune system.[38,39] Considering the effects of immune pathways on disease progress and joint destruction, modulation of these pathways may have important implications for treating RA.

Pathways related to inflammation
Another large category of signal pathways is signal transduction, including a number of well-known signal pathways that are related to inflammation, such as JAK–STAT signaling pathway, NF-κB signaling pathway and TNF signaling pathway. For example, canonical NF-κB signaling pathway is critical for the regulation of the inflammation response. Although less extensively studied, non-canonical pathway plays an indirect role in synovial inflammation via the high expression of its activators such as CD40L, CD40, BAFF/BAFF-R and RANKL in RA synovium.[40] Since both

canonical and non-canonical NF-κB signaling pathways participate in inflammatory response and the pathogenesis of RA, inhibitors of these two pathways can play a role in the treatment of RA. TNF, as the upstream target of the NF-κB pathway, has already been regarded as a therapeutic target in RA.[41] Moreover, the JAK–STAT pathway is an important pathway for the transduction of cytokines associated with RA and is regarded as a target in inflammatory and autoimmune diseases.[42] Tofacitinib, a JAK inhibitor, proves effective in the treatment of RA through reducing the expression of metallo-proteinase and interferon-regulated gene in RA synovium.[43]

Pathways related to angiogenesis

Angiogenesis is a complex process involving the growth of new blood vessels and plays an important role in the growth, metastasis and prognosis of tumor. It is accompanied by the entire process of RA and can foster the infiltration of inflammatory cells into the joints, leading to synovial hyperplasia and progressive bone destruction.[44] Ras signaling pathway and VEGF signaling pathway regulated solely by CC are related to angiogenesis. VEGF signaling pathway plays an important role in promoting the proliferation of vascular endothelial cells and the formation of new blood vessels,[45,46] while Ras signaling pathway is related to tumor angiogenesis and vascular permeability.[47] Certainly, inhibiting VEGF signaling is a feasible antiangiogenic and anti-inflammatory therapeutic strategy in RA.[48]

Conclusion

TCMs usually exert a multicomponent and multi-pathway synergetic efficacy in the treatment of various diseases. Therefore, the research approach applied to TCMs should correspond to the mechanisms of synergy. In this study, we applied a network pharmacology approach to identify the RA-related targets and signal pathways of CN, making it possible to connect genomic space to pharmacological space. In summary, we predicted the action mechanism(s) of herb couple for treating RA through the analysis of key targets and KEGG pathways.

Abbreviations

RA, rheumatoid arthritis; CC, *Clematis chinensis* Osbeck; NI, *Notopterygium incisum* K.C. Ting ex H.T. Chang; CN, *Clematis chinensis* Osbeck/*Notopterygium incisum* K.C. Ting ex H.T. Chang; TCM, traditional Chinese medicine; NSAIDs, nonsteroidal anti-inflammatory drugs; DMARDs, disease-modifying antirheumatic drugs; TCMSP, traditional Chinese medicine systems pharmacology database and analysis platform; OB, oral bioavailability; DL, druglikeness; KEGG, Kyoto Encyclopedia of Genes and Genomes; Akt1/PKB, protein kinase B; TNFsf13/APRIL, proliferation-induced ligand; TP53, tumor protein p53; EPHB2, EPH receptor B2; IL-10, interleukin-10; IL-8, interleukin-8; NF-κB3, nuclear factor-κB3; ROS1, c-ros oncogene 1 receptor tyrosine kinase; GnRH, gonadotropin-releasing hormone; CD40L, CD40 ligand; CD40, CD40 molecule; BAFF, B-cell-activating factor belonging to the TNF family; RANKL, receptor activator for nuclear factor-κB ligand; JAK, janus kinase; STAT, signal transducing activator of transcription; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappaB; MAPK8, mitogen-activated protein kinase 8; TNF, tumor necrosis factor; IL-6, interleukin-6; VEGF, vascular endothelial growth factor; BATMAN-TCM, bioinformatics analysis tool for molecular mechanism of TCM; GO, gene ontology.

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Disclosure

The authors report no conflicts of interest in this work.

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