Drug Design, Development and Therapy

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ORIGINAL RESEARCH

Study on the Mechanism of Improving HIV/AIDS Immune Function with Jian Aikang Concentrated Pill Based on Network Pharmacology Combined with Experimental Validation

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Purpose: This study was the first to screen the active compounds of Jian Aikang Concentrated Pill (JAKCP) with network pharmacology, predict its potential targets, screen the signaling pathways, and combine with cellular experimental validation to explore the potential mechanism of JAKCP for the treatment of acquired immunodeficiency syndrome (AIDS).

Methods: The main compounds and targets of Chinese herbs in JAKCP were identified by TCMSP; the targets of AIDS were collected from Genecards, Online Mendelian Inheritance in Man (OMIM), Disgenet, Therapeutic Target Database (TTD) and Drugbank; the network of "Chinese herbs-active compounds-targets" for JAKCP was constructed by Cytoscape, and protein–protein interaction (PPI) network was constructed using STRING to generate the intersection targets, Metascape was conducted to analyze the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), and the network of "main active compounds-core targets-pathways" was constructed by Cytoscape. Finally, the effect of JAKCP on the survival rate of HIV pseudovirus-infected MT-4 cells was investigated by CCK-8 assay, and the predicted targets were verified by ELISA, qPCR and Western blot.

Results: A total of 147 active compounds of JAKCP were screened covering 351 targets and 416 AIDS disease targets were obtained, besides 140 intersection targets and 321 KEGG pathways were collected. Ultimately, quercetin, kaempferol, stigmasterol, beta-sitosterol, epigallocatechin gallate were identified as the important compounds, the core targets are HSP90AA1, IL-10, IL-6, TNF, IL-1β, TP53, and IL-1α, and the biological pathways and processes mainly include T cell activation, regulation of DNA-binding transcription factor activity and apoptotic signaling pathway. Experiments on the targets of "T cell activation" demonstrated that JAKCP promotes the survival of HIV pseudovirus-infected MT-4 cells. Also, JAKCP down-regulated mRNA and protein levels of IL-1α, IL-1β, and IL-6 while up-regulated mRNA and protein levels of IL-2, IL-6ST, and IL-10 in vitro.

Conclusion: JAKCP exerted regulatory immune functions through multi-component, multi-target and multi-pathway, thereby providing novel ideas and clues for the treatment of AIDS.

Keywords: Jian Aikang Concentrated Pill, network pharmacology, HIV/AIDS, multi-pathway, mechanism

Introduction

Acquired immunodeficiency syndrome (AIDS) is an acquired immunodeficiency syndrome caused by human immunodeficiency virus (HIV) infection that severely damages the human immune system. It is usually divided into two types, HIV-1 and HIV-2, based on geographical distribution differences. Among them, HIV-1 is the main pathological type worldwide, and

Received: 14 April 2022
Accepted: 5 August 2022
Published: 18 August 2022

HIV-2 is mainly distributed in West African populations.[1] In recent years, AIDS is in a low epidemic trend with decreasing morbidity and mortality, but the population base still continues to grow,[2,3] life expectancy always lags behind the general population,[4] AIDS still threatens human health.

AIDS is a malignant infectious disease that is transmitted mainly through blood, sexual, mother-to-child and intravenous drug use. Infected infants, intravenous drug users, homosexuals, and patients with hemophilia are at high risk for the disease.[5] With the continuous progress of the disease, AIDS is mostly co-infected with opportunistic infections and malignancies, which seriously threaten human life. There is no curable drug or preventable vaccine for AIDS. Combination of antiretroviral therapy (cART) is currently the most effective regimen for the treatment of AIDS. Clinical combination drugs generally use two nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs) plus protease inhibitors. Abacavir and tenofovir are potent drugs with anti-HIV activity while with significant nephrogenic and osteotoxic characteristics; Ritonavir has the highest genetic activity among the enhanced protease inhibitors with the highest genetic resistance barrier.[6] Clinically, for patients diagnosed with HIV, cART should be started as soon as possible from the same day to 14 days after diagnosis,[7] and patients with advanced HIV are also recommended to start cART quickly to reduce mortality.[8,9] Results of several studies have demonstrated significant viral load suppression 10 or 12 months after early rapid initiation of cART,[10–12] with a viral suppression rate of 85% at 1 year.[13] With the introduction of cART and prophylactic therapy for opportunistic infections, despite the clinical benefits for HIV-infected patients, resulting in significant improvements in life expectancy and quality of life,[14,15] the lifelong persistence, toxicity,[16] long-term adverse effects,[17] drug resistance and high compliance[18] of their medications make clinical treatment more difficult and prognostic. Different from the single target of Western medicine, Traditional Chinese medicine (TCM) often shows different curative effects on various immune diseases by means of "multi-component", "multi-target" and "multi-pathway". So we try TCM, which is a complementary and alternative approach in the treatment of AIDS.

JAKCP consists of five herbs, namely Renshen (RS), Lingzhi (LZ), Aiye (AY), Hongjingtian (HJT) and Gouqizi (GQZ), TCM contains a variety of compounds with medicinal value, pharmacological studies have shown that ginseng, wolfberry can modulate inflammation and immune responses, and are involved in multiple pathways in infectious and immune diseases.[19,20] According to TCM theory, JAKCP is closely related to the spleen and kidney function. Clinically, TCM is effective in the treatment of AIDS,[21] with an 8-year survival rate of 87%.[22] JAKCP has been used to treat thousands of HIV/AIDS patients in "The Project of Treatment of HIV/AIDS Patients with Traditional Chinese Medicine" in Henan Province, China.[23] Our research group has conducted many studies on JAKCP and confirmed that JAKCP has a positive effect on AIDS immune function; however, JAKCP as a traditional Chinese medicine preparation covers compounds with multiple targets, and how these compounds synergistically exert their therapeutic effects are still unclear and in-depth research is required. The holistic view of TCM coincides with network pharmacology, which is characterized by networks and systems, and TCM network pharmacology is a promising methodology, with current research trends centering on the combination of network pharmacology prediction, experimental validation and clinical efficacy.[24]

For the first time, we used an integrated strategy combining network pharmacology and experimental validation to explore the therapeutic potential and mechanism of JAKCP, with the aim of providing a reference for subsequent pharmacological studies and clinical treatment of AIDS.

Materials and Methods

Screening of JAKCP Candidate Active Compounds, Targets Prediction and Network Construction

Through the TCM Systematic Pharmacology Platform (Traditional Chinese Medicine Systems, TCMSP, http://tcmspw.com/tcmsp.php)[25] and relevant literature reports, the chemical composition of the herbs in the JAKCP formulae was collected. TCMSP database is conducted to predict the compounds, set Oral Bioavailability (OB) ≥ 30% and Drug-Likeness (DL)≥ 0.18 to determine the drug-forming properties of the compounds,[26,27] and preliminary screening of active ingredients was performed to obtain active compounds and their protein targets of action. The published literature reports combined with PubChem database,[28] SwissTargetPrediction database were also used to supplement the known targets of

the active compounds which were not predicted.[29] After the screening, the protein target names were standardized on the UniProt protein database (https://www.uniprot.org), restricting humans as species.

The "Chinese herbs-active compounds-targets" network was constructed and analyzed mainly by the software Cytoscape 3.6.0,[30] where the node indicates the component or target, the edge indicates the relationship between them, and the "degree" value of a node demonstrates the number of connections to that node in the network, and the higher the degree value, the more likely the target is to be the key targets for the compound.[31] The network analyzer built into Cytoscape 3.6.0 software was performed to analyze network characteristic parameters, including degree, betweenness and closeness, to investigate the more important components and targets in JAKCP and the relationships between them.

Prediction of AIDS-Related Targets

The prediction of targets of AIDS is available in 5 below disease gene database: the human gene annotation database (GeneCards, https://www. genecards.org),[32] the Online Mendelian Inheritance in Man (OMIM, https://omim.org/),[33] Disgenet (v7.0),[34] Therapeutic Target Database (TTD, http://db.idrblab.net/ttd/)[35] and Drugbank database (https://www.drugbank.ca).[36] Set "Acquired Immunodeficiency Syndrome" as a keyword, the targets were limited to "Homo sapiens", and the search and screening were performed to summarize the obtained disease therapeutic targets and remove duplicate values. The potential target genes in HIV are the intersection of the above 5 databases.

STRING-Based Protein–Protein Interaction (PPI) Network Construction and Intersection Target Screening

The Venny online tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) was selected to calculate the intersection between JAKCP and AIDS targets to further elucidate the potential mechanism of JAKCP on AIDS. PPI networks were constructed by STRING (version 10.5, https://string-db.org/), and the intersection targets of JAKCP and AIDS were input into STRING to generate PPI networks. Then, Cytoscape 3.6.0 was conducted to visualize the network of these targets.[37]

BisoGenet-Based PPI Network Construction and Key Targets Screening

PPI networks were constructed by the built-in plugin BisoGenet in Cytoscape,[38] and the predicted targets of JAKCP and AIDS were imported into BisoGenet separately to generate PPI networks for each. The intersection networks of drug PPI network and disease PPI networks were extracted by the Merge function in Cytoscape 3.6.0 and the attribute values of each node in the intersection network were analyzed using CytoNCA. The median of connectivity M1 is calculated and all nodes with connectivity greater than 3 times M1 are selected as "Hit hubs". The attributes of each node in the Hit hubs network are calculated to archive the 5 median M2, C2, B2, L2 of degree centrality (DC), closeness centrality (CC), betweenness centrality (BC) and local average connectivity (LAC). All nodes whose node attributes satisfy >M2, C2, B2, L2 at the same time are selected, and the final target obtained by screening 2–3 times is used as the key targets.

Gene Ontology (GO) and Pathway Enrichment Analysis

The intersection targets of JAKCP and AIDS were recorded into Metascape (http://metascape.org/gp/index.html), set $P < 0.01$,[39] performed GO analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) of their major biological processes and metabolic pathways and enriched them with the online drawing tool Omicshare (https://www.omicshare.com/) to visualize the data.

Preparation of JAKCP-Containing Serum

JAKCP consisted of 3g rhizomes of Panax Ginseng C. A. Mey, 15g dry fruiting body of Ganoderma, 3g foliage of Folium Artemisiae Argyi, 5g root of Rhodiolae Crenulatae Radix et Rhizoma and 5g fruit of Lycii Fructus, all raw drugs and drug processing were provided by Henan Provincial Institute of Traditional Chinese Medicine. Forty healthy SPF-grade male SD rats were randomly divided into the JACKP-containing serum group and the blank serum control group, with 20 rats in each group. JACKP was administered by gavage to rats at a dose of 6.3g/Kg/d according to the equivalent dose conversion factor for 70 kg adults and rats,[40] and an equal volume of saline was given by gavage to the blank serum control group; the drug

was administered continuously for 7d, divided into two daily doses in the morning and evening with an interval of 12h. After 2h of the last dose,[41] 10% chloral hydrate was injected for anesthesia, and the blood was taken under aseptic conditions via the abdominal aorta. Blood was left at room temperature for 2h, centrifuged at 2500rpm for 30min, the supernatant was taken and mixed with the same group; inactivated at 56°C and 30 min in a water bath, filtered and debacterized by 0.22um microporous filter, and stored in a −80°C refrigerator for backup.

Cell Culture

MT-4 cells and 293T cells were selected for the following experiments, and all cells were derived from our laboratory —"Key Laboratory of Viral Diseases Prevention and Treatment with TCM of Henan Province", and were authenticated by STR profile and kept in our laboratory at 37°C in a 5% CO2 incubator. MT-4 cells were grown in RPMI1640 medium containing 10% FBS and passaged by half change; 293T cells were cultured in DMEM medium supplemented with 10% FBS, 100 U/mL penicillin and 100 mg/mL streptomycin, and passaged once every 1–2 d.

Preparation and Identification of HIV Pseudovirus and Establishment of AIDS Cell Model

The dual plasmid system-PNL4-3-Luc-R-E-plasmid (containing HIV backbone protein gene) and JRFL plasmid (containing HIV envelope protein gene) were used to prepare HIV pseudoviruses. Lip2000 was used for cell co-transfection, and both plasmids were transfected into 293T cells, and supernatants were collected after 48h of culture to obtain transfected virus. After MT-4 cells was infected with this virus, the HIV-specific protein P24 antigen was determined by chemiluminescence, and HIVAg/Ab > 1 indicated that the virus had HIV activity and could identify the virulence of HIV pseudovirus.[42] Finally, the identified identified pseudovirus stock solution and 1640 complete medium containing MT-4 cells were divided into different groups according to the fold dilution method. The expression of fluorescent firefly luciferase gene and the effect on cell viability were measured to determine the optimal modeling concentration for HIV pseudovirus infection of MT-4 cells.[43]

Cell Viability Assay

CCK-8 assay was performed to evaluate the effect of JACKP-containing serum on the viability of HIV pseudovirus-infected MT-4 cells. MT-4 cells were inoculated into 96-well plates at a density of 3×10^4 cells/100 ul/well, incubated for 24 h with HIV pseudovirus 50 ul/well, and incubated successfully for 48 h. Cells were treated with different concentrations of JACKP-containing serum at 5%, 10%, 15% and 20% for 24 h, 48 h and 72 h. Then incubated with 10 μL of CCK-8 solution (5 mL; Solarbio, China) for 120 min. Absorbance was measured at 450 nm using a SpectraMax® i3 multifunctional enzyme marker (Molecular Devices, America). Cell viability was calculated using SPSS 23.0 software.

ELISA Quantitative Analysis

MT-4 cells were inoculated in 6-well plates at a density of 1×10^6 cells/well, incubated at 37°C with 5% CO_2 for 24 h. The supernatant was discarded and 1 mL/well of titrated HIV-1 virus supernatant was added and incubated for 48 h. High, medium and low concentrations of serum containing JACKP and positive control drug lentinan (800ug/mL) 2mL/well were added to each well, then incubated for 24 h. The cells and supernatant of each well were collected, washed with PBS solution and stored at −80°C for analysis. ELISA assay was performed according to the reagent manufacturer's instructions to determine the level of inflammatory cytokine IL-1α (E-EL-H0088c, Elabscience, China), IL-1β (EK101BHS-96, MULTI SCIENCES, China), IL-2 (EK102HS-96, MULTI SCIENCES, China), IL-6 (EK106HS-96, MULTI SCIENCES, China), IL-6ST (E-EL-H6015, Elabscience, China) and IL-10 (EK110HS-96, MULTI SCIENCES, China) in the cells.

qPCR Assay

The supernatant was poured out and washed with PBS solution after collecting each group of cells. First, total RNA was extracted using FastPure® Cell/Tissue Total (Nanjing, China) according to the manufacturer's instructions, and then a nucleic acid protein assay was conducted to assess RNA purity and concentration. Finally, cDNA strands were prepared

from total RNA (1 μg) using HiScript® III RT SuperMix for qPCR (+gDNA wiper) (Nanjing, China). The cDNA was then amplified with specific primers in ChamQ Universal SYBR qPCR Master Mix reagent (Nanjing, China). PCR reaction system consisted of 10 μL 2×ChamQ Universal SYBR qPCR Master Mix, 1 μL each of upstream and downstream primer sequences, 2 μL template cDNA and 6μLRNase-free ddH2O. GAPDH was set as an internal reference and the relative expression of each target was calculated using the 2-ΔΔCt method in ABI Prism SDS 2.0.3.

Western Blot Assay

The cells were lysed with RIPA lysis solution for 30 min and then transferred to centrifuge tubes. 12,000 rpm/min centrifugation was performed for 10 min and the supernatant was extracted. Quantitative protein concentration was determined by BCA protein assay kit. Protein electrophoresis buffer was added to the SDS-PAGE gel in 20 μg spots, and after electrophoresis, the proteins on the SDS-PAGE gel were transferred onto PVDF membranes, sealed with 5% skim milk powder for 1 h at room temperature, and then washed three times with PBST solution. Rabbit anti-IL-1α, IL-1β, IL-2, IL-6, IL-6ST, IL-10 and β-Tubulin antibodies were added respectively, incubated overnight at 4°C, then the membrane was washed again, the corresponding secondary antibodies were added. ECL reagents were used for luminescence development. The protein bands were imaged by chemiluminescence imaging system, and the grayscale values of protein bands were analyzed by Image-Pro Plus 6.0 software, and the relative expression of each group of proteins was compared using β-Tubulin as an internal reference.

Statistical Analysis

All data were expressed as mean ± SD, and the results were analyzed using GraphPad Prism 8 and SPSS 23.0 software. The paired-samples *t*-test was performed to compare quantitative data between groups, and $P < 0.05$ was considered a statistically significant difference between groups.

Results

Network of Chinese Herbs, Compounds and Targets in JAKCP

According to TCMSP and literature reports, the compounds in each Chinese medicine were initially extracted: 190 species in Renshen, 242 species in Lingzhi, 135 species in Aiye, 188 species in Gouqizi and 35 species of Hongjingtian; After screening by conditions (OB≧30%, DL≧0.18) in ADME, A total of 147 active pharmaceutical components were achieved: 22 species of Renshen, 61 species of Lingzhi, 9 species of Aiye, 45 species of Gouqizi and 10 species of Hongjingtian, Quercetin, Kaempferol, Stigmasterol, Beta-sitosterol and Mandenol were the active compounds crossed by more than two herbal medicines. The 147 active compounds are obtained and listed respectively (Supplementary Material 1), some of them are shown in Table 1, and the TCMSP crossover active compounds are reflected and shown in Table 2, Figure 1 and Supplementary Material 2. The action targets of 5 kinds of Chinese herbs are predicted by the method of TCMSP target prediction model combined with literature search: 118 for Renshen, 40 for Lingzhi, 187 for Aiye, 208 for Gouqizi and 254 for Hongjingtian. Finally, information of targets of the 5 Chinese herbs were combined and the duplicate values were removed to archive a total of 351 targets.

The relationship network of active compounds of the 5 Chinese herbs and their action targets was drawn and analyzed using Cytoscape 3.6.0 (Figure 2 and Supplementary Material 3), and a total of 432 nodes (containing 285 targets and 147 active compounds) with 1312 edge relationships were generated. The size of the nodes in the figure represents the corresponding degree values, and the larger area of the nodes represents the larger degree values, indicating the more biological functions involved and their higher biological importance. The top 5 compounds in this network in terms of degree value were Quercetin, Kaempferol, Stigmasterol, Beta-sitosterol and Epigallocatechin gallate, they were identified as important active compounds. In addition, different herbal medicines containing the same active ingredients exhibit more interactions and a considerable number of targets are modulated by multiple compounds. For example, Quercetin, a common active compound of Aiye and Gouqizi, could modulate multiple targets (eg, CASP9, IL10, IL6, PPARG). It is hypothesized that the active compounds of JAKCP may affect multiple targets to effectively treat AIDS. In this network,

Table 1 Information on Some of the 147 Compounds Contained in 5 Chinese Herbs in JAKCP

Herbs	Molecule ID	Molecule Name	OB (%)	DL	CAS Number
RS	MOL005314	Celabenzine	101.8	0.49	53938-08-2
	MOL005308	Aposiopolamine	66.65	0.22	N/A
	MOL005401	Ginsenoside Rg5_qt	39.56	0.79	186763-78-0
	MOL000422	Kaempferol	41.88	0.24	520-18-3
	MOL004492	Chrysanthemaxanthin	38.72	0.58	26989-20-8
LZ	MOL011221	Ganoderic acid V	30.19	0.8	86377-50-6
	MOL000359	Beta-sitosterol	36.91	0.75	5779-62-4
	MOL000279	Cerevisterol	37.96	0.77	516-37-0
	MOL011258	Ganosporelactone B	31.21	0.33	138008-05-6
	MOL011287	Lucidone A	37.22	0.64	97653-92-4
AY	MOL000098	Quercetin	46.43	0.28	73123-10-1
	MOL000449	Stigmasterol	43.83	0.76	83-48-7
	MOL002883	Ethyl oleate (NF)	32.4	0.19	1191-41-9
	MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one	42.36	0.21	N/A
	MOL005720	24-methylenecyloartanone	41.11	0.79	N/A
HJT	MOL000422	Kaempferol	41.88	0.24	520-18-3
	MOL001002	Ellagic acid	43.06	0.43	476-66-4
	MOL013083	Skimmin	38.35	0.32	93-39-0
	MOL000073	Epicatechin	48.96	0.24	35323-91-2
GQZ	MOL000449	Stigmasterol	43.83	0.76	83-48-7
	MOL008400	Glycitein	50.48	0.24	40957-83-3
	MOL003578	Cycloartenol	38.69	0.78	469-38-5
	MOL000098	Quercetin	46.43	0.28	73123-10-1
	MOL008400	Glycitein	50.48	0.24	40957-83-3

Note: N/A represents there was no number of CAS.
Abbreviations: RS, Renshen (Panax Ginseng C. A. Mey); LZ, Lingzhi (Ganoderma); AY, Aiye (Folium Artemisiae Argyi); HJT, Hongjingtian (Rhodiolae Crenulatae Radix et Rhizoma); GQZ, Gouqizi (Lycii Fructus).

the relationship between 5 Chinese herbs, active compounds and targets and the potential pharmacological effects of JAKCP are visualized.

Prediction of AIDS-Related Targets

The number of targets of AIDS retrieved from the 5 disease databases of Genecards, OMIM, Disgenet, TTD and DrugBank were 343, 21, 29, 0 and 80, respectively. The obtained targets were summarized and duplicate targets were removed and 416 targets were obtained finally.

Table 2 Crossover Active Compounds of RS, LZ, AY, HJT and GQZ Herbs in JAKCP

No.	Molecule ID	Cross Compounds	Herbs
A1	MOL000449	Stigmasterol	RS、AY、GQZ
A2	MOL000358	Beta-sitosterol	RS、LZ、AY、GQZ
B1	MOL000422	Kaempferol	RS、HJT
B2	MOL001494	Mandenol	AY、GQZ
C1	MOL000098	Quercetin	AY、GQZ

Note: A1, A2, B1, B2, C1 are the crossover active compounds of 5 kinds of Chinese herbs in JAKCP.
Abbreviations: RS, Renshen (Panax Ginseng C. A. Mey); LZ, Lingzhi (Ganoderma); AY, Aiye (Folium Artemisiae Argyi); HJT, Hongjingtian (Rhodiolae Crenulatae Radix et Rhizoma); GQZ, Gouqizi (Lycii Fructus).

Acquisition of AIDS and JAKCP Intersection Targets

The information of targets of the active compounds in 5 Chinese herbs in JAKCP and the targets of AIDS were genetically mapped through the online Venny 2.1.0 platform to get drug-disease intersection targets, indicating the relationship between the 5 Chinese herbs of JAKCP and AIDS, and identifying 140 target genes which affected by AIDS and regulated by RS, AY, LZ, GQZ and HJT (Table 3, Figure 3 and Supplementary Material 4).

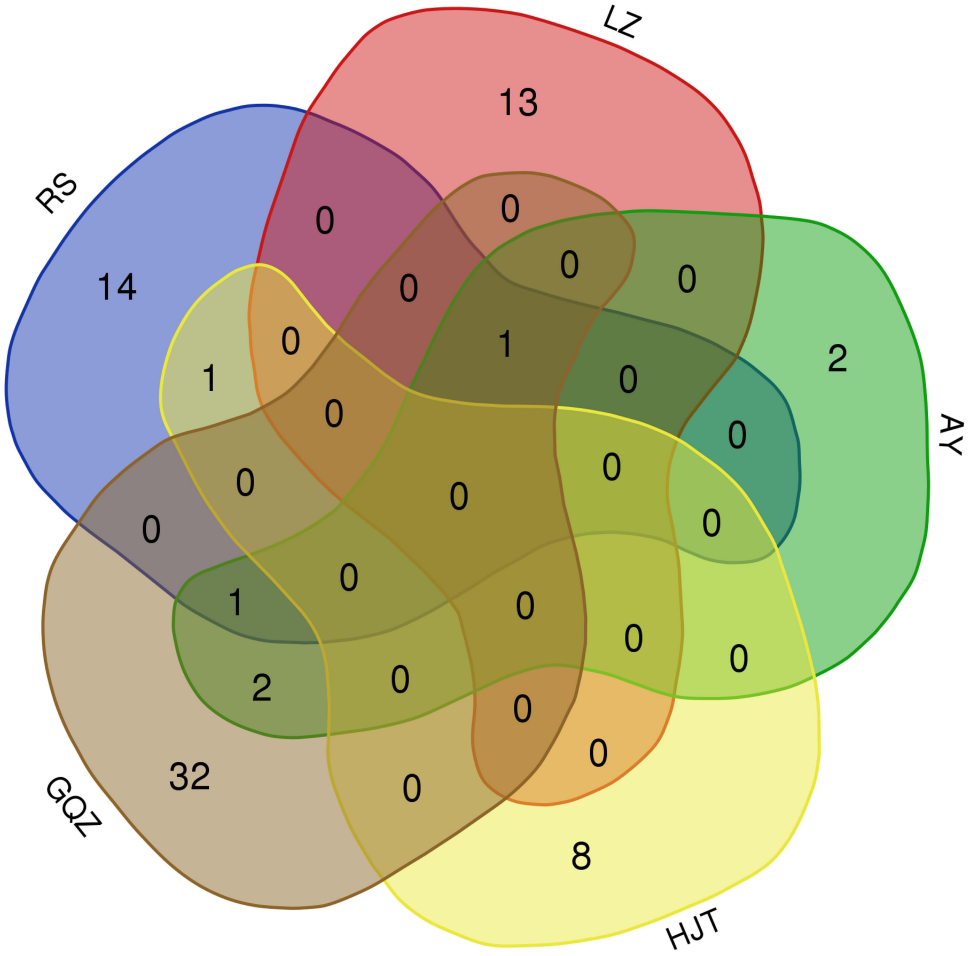

Figure 1 Crossover active compounds of RS (Renshen), LZ (Lingzhi), AY (Aiye), HJT (Hongjingtian) and GQZ (Gouqizi) herbs in JAKCP. In this figure, the blue area represent candidate compounds of RS, the red area represent candidate compounds of LZ, the green area represent candidate compounds of AY, the yellow area represent candidate compounds of HJT, the Orange area represent candidate compounds of GQZ. Finally, Quercetin, Kaempferol, Stigmasterol, Beta-sitosterol and Mandenol were the active compounds crossed by more than two herbal medicines.

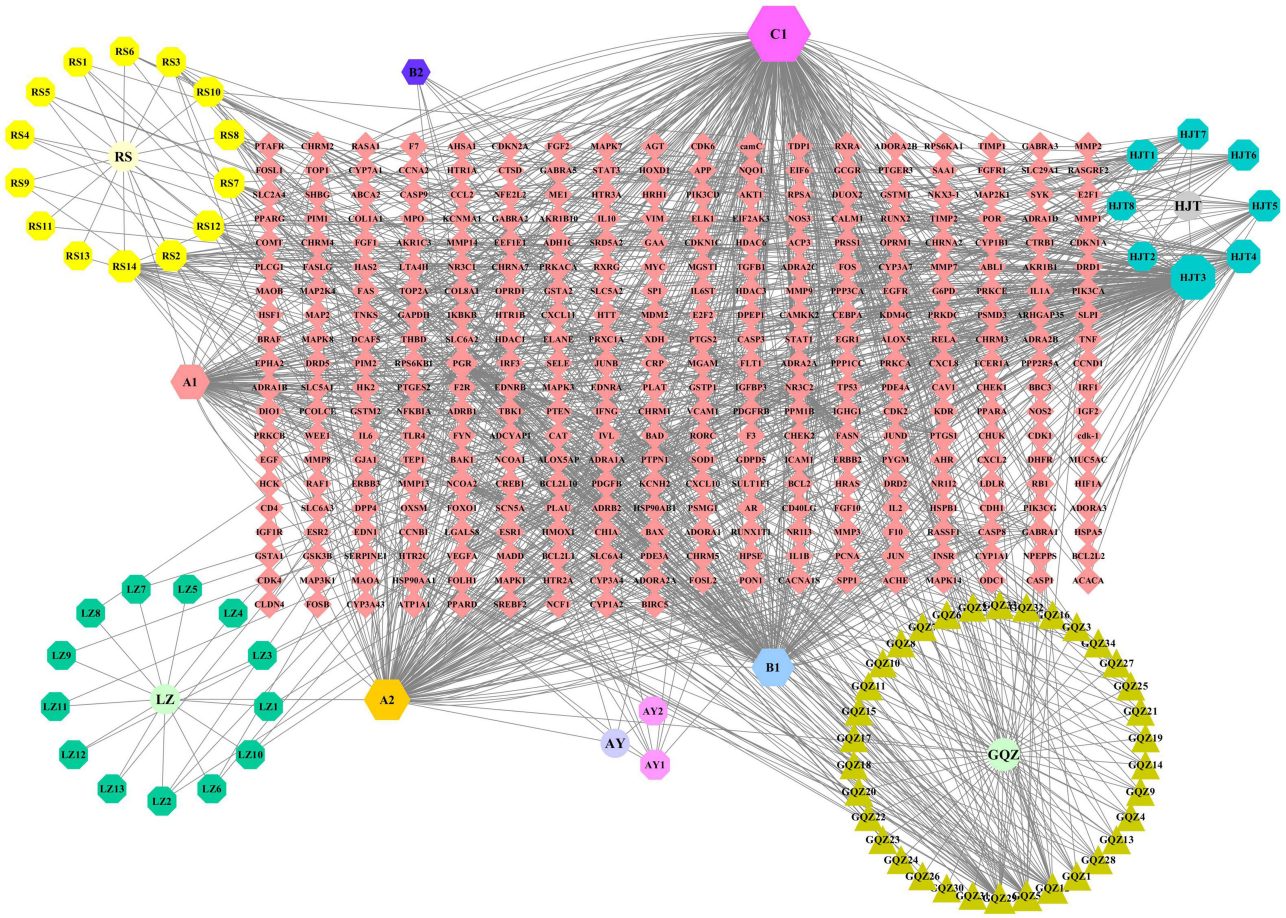

Figure 2 Network of the relationship between five kinds of Chinese herbs, active compounds and targets in JAKCP. All nodes were visualized in degree value, the larger the node, the darker the color and the higher the degree value. The five kinds of Chinese herbs are RS (Renshen), LZ (Lingzhi), AY (Aiye), HJT (Hongjingtian) and GQZ (Gouqizi), among them, the hexagons in the bright yellow area marked with RS represent main compounds contained in RS; the hexagons in the green area marked with LZ represent main compounds contained in LZ; the hexagons in the purple area marked with AY represent main compounds contained in AY; the hexagons in the blue area marked with HJT represent main compounds contained in HJT; the hexagons in the dark yellow area marked with GQZ represent main compounds contained in GQZ; The red diamonds in the middle area represent the targets that all compounds intersect. A1, A2, B1, B2, C1 are the crossover active compounds of five kinds of Chinese herbs in JAKCP.

Construction of PPI Network of Intersection Targets for JAKCP and AIDS by STRING

The 140 intersecting targets of JAKCP and AIDS are demonstrated by STRING (Figure 4 and Supplementary Material 5), which covers 134 nodes and 823 edges, indicating that JAKCP exerts therapeutic effects on AIDS through multiple protein targets, and the first 6 degrees of targets STAT3, PIK3CA, TP53, MAPK3, MAPK1 and AKT1 are the main targets among the intersecting targets.

Construction of PPI Network of the Key Targets for JAKCP and AIDS by BisoGenet

PPI networks of JAKCP and AIDS were constructed by running the BisoGenet function in Cytoscape 3.6.0, and it was found that the potential targets of JAKCP could interact with 9082 targets directly or indirectly, and there were 197,204 interactions between these targets. At the same time, the PPI network of AIDS-related targets was drawn, showing that as many as 9091 targets were directly or indirectly related to them, and as many as 195,305 kinds of interrelationships between these targets, and the intersection network of the two is shown in Supplementary Material 6. The values of network topological characteristics of the above PPI intersection network were calculated, and a total of 122 key targets were obtained through three screening, and the screening strategy is shown in Figure 5 and Supplementary Material 7. Some regions with high density in the PPI complex network are called community or module. Modules are considered to

Table 3 Information on the 140 Intersection Targets of AIDS and JAKCP

No.	Target	UniProt ID	No.	Target	UniProt ID	No.	Target	UniProt ID	No.	Target	UniProt ID
1	VIM	P08670	36	OPRM1	P35372	71	AKT1	P31749	106	CYP1B1	Q16678
2	VEGFA	P15692	37	NR3C1	P04150	72	IKBKB	O14920	107	CYP1A2	P05177
3	VCAM1	P19320	38	NR1I2	O75469	73	IFNG	P01579	108	CXCL8	P10145
4	TP53	P04637	39	NOS3	P29474	74	ICAM1	P05362	109	CXCL10	P02778
5	TNF	P01375	40	NOS2	P35228	75	HSPB1	P04792	110	CTSD	P07339
6	TLR4	O00206	41	NFKBIA	P25963	76	HSPA5	P11021	111	CRP	P02741
7	TGFB1	P01137	42	MYC	P01106	77	HSP90AA1	P07900	112	COMT	P21964
8	STAT3	P40763	43	MPO	P05164	78	SERPINE1	P05121	113	CDKN2A	P42771
9	STAT1	P42224	44	MMP9	P14780	79	HCK	P08631	114	CDKN1A	P38936
10	SP1	P08047	45	MMP2	P08253	80	GSTP1	P09211	115	CDK4	P11802
11	SOD1	P00441	46	MDM2	Q00987	81	FYN	P06241	116	CD40LG	P29965
12	SLPI	P03973	47	MAPK8	P45983	82	FOS	P01100	117	CD4	P01730
13	HIF1A	Q16665	48	MAPK3	P27361	83	FGF2	P09038	118	CCND1	P24385
14	SELE	P16581	49	MAPK14	Q16539	84	FGF1	P05230	119	CCL2	P13500
15	RELA	Q04206	50	MAPK1	Q16539	85	FASLG	P48023	120	CAT	P04040
16	RAF1	P04049	51	MAP2K1	Q02750	86	FAS	P25445	121	CASP9	P55211
17	PTGS2	P35354	52	MAP2	P11137	87	ESR1	P03372	122	CASP8	Q14790
18	PTGS1	P23219	53	KDR	P35968	88	ERBB2	P04626	123	CASP3	P42574
19	PRKACA	P17612	54	JUN	P05412	89	EGFR	P00533	124	CASP1	P29466
20	PPARG	P37231	55	IRF3	Q14653	90	EGF	P01133	125	BCL2L1	Q07817
21	PIK3CG	P48736	56	IL6	P05231	91	DPP4	P27487	126	BCL2	P10415
22	PIK3CA	P42336	57	IL2	P60568	92	DHFR	P00374	127	BAX	Q07812
23	PGR	P06401	58	IL1B	P01584	93	CYP3A7	P24462	128	BAK1	Q16611
24	PDGFB	P01127	59	IL1A	P01583	94	CYP3A4	P08684	129	APP	P05067
25	ALOX5	P09917	60	IL10	P22301	95	ACHE	P22303	130	TOP2A	P11388
26	EDNRB	P24530	61	IL6ST	P40189	96	PLAU	P00749	131	TIMP1	P01033
27	EDN1	P05305	62	HTR2A	P40189	97	PLAT	P00750	132	SPP1	P10451
28	DRD2	P14416	63	HRAS	P01112	98	PDGFRB	P09619	133	SLC6A4	P31645
29	CXCL11	O14625	64	GAA	P10253	99	PCNA	P12004	134	SLC6A3	Q01959
30	CDK1	P06493	65	FLT1	P17948	100	NR3C2	P08235	135	RXRG	P48443
31	CDH1	P12830	66	FGFR1	P11362	101	MMP7	P09237	136	RB1	P06400
32	CCNA2	P20248	67	FASN	P49327	102	MMP1	P03956	137	PTEN	P60484
33	BRAF	P15056	68	F3	P13726	103	LDLR	P01130	138	PRKDC	P78527

(*Continued*)

Table 3 (Continued).

No.	Target	UniProt ID	No.	Target	UniProt ID	No.	Target	UniProt ID	No.	Target	UniProt ID
34	BIRC5	O15392	69	ELANE	P08246	104	INSR	P06213	139	PRKCA	P17252
35	BAD	Q92934	70	AHSA1	O95433	105	AR	P10275	140	AHR	P35869

be biologically significant sets, and the interaction relationships are analyzed by molecular complex detection algorithms to obtain modules (Figure 6 and Supplementary Material 8) after obtaining the core PPI network. According to the P value, the biological processes with three best scores were retained in PPI network and Module respectively and their functions were described, shown in Table 4.

GO and KEGG Pathway Enrichment Analysis

Gene enrichment analysis was performed using Metascape for targets associated with JAKCP and AIDS, including BP (biological process), CC (cellular component), MF (molecular function) and KEGG pathways, results were saved and bubble charted was plotted using Omicshare online platform, more detailed pathway analysis data are provided in Figure 7 and Supplementary Material 9. The top 20 significantly enriched GO terms are shown in Figure 7A, where biological processes closely related to AIDS pathogenesis include apoptotic signaling pathway, T cell activation and positive regulation of cytokine production. These processes directly serve as links to CD4+ T cell differentiation, proliferation and apoptosis. It demonstrates that JAKCP has a direct regulatory role in the production and apoptosis of AIDS T lymphocytes. The top 20 significantly KEGG pathways enrichment are shown in Figure 7D, where signaling pathway closely related to AIDS pathogenesis include cytokine–cytokine receptor interaction, JAK-STAT signaling pathway and Apoptosis.

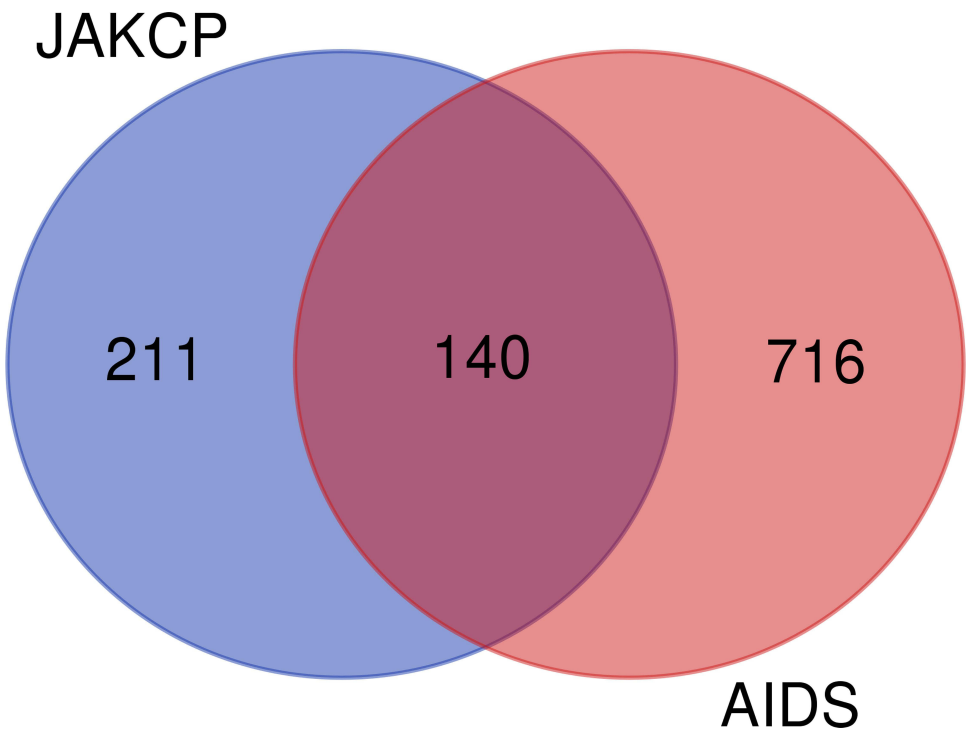

Figure 3 The venn diagram of 140 intersection targets of AIDS (Acquired immunodeficiency syndrome) and JAKCP (Jian Aikang Concentrated Pill).

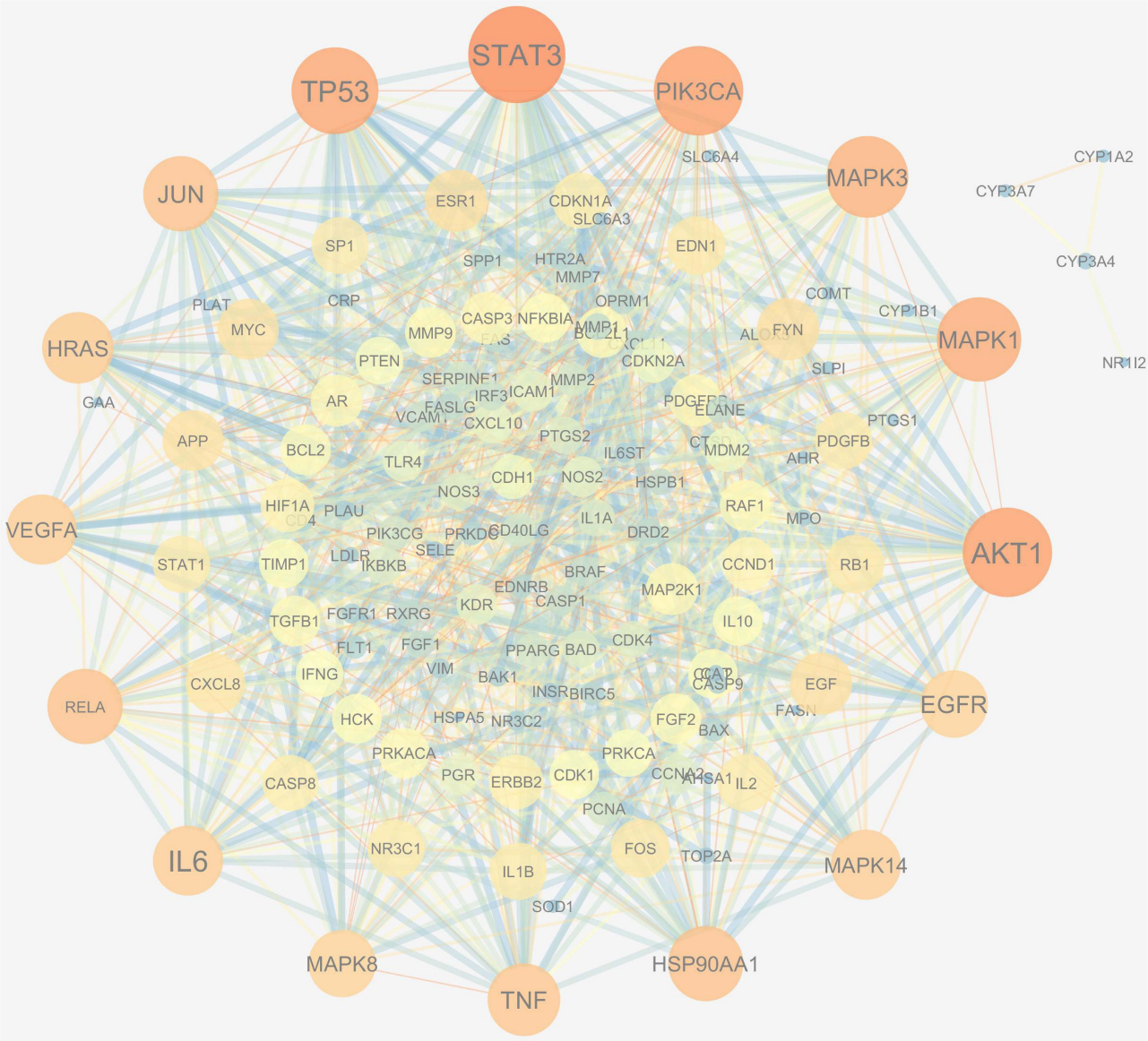

Figure 4 PPI network diagram of intersection targets for JAKCP and AIDS. Edges represent protein–protein interactions, edge thickness indicates the strength of data support, the larger the node, the higher the degree value. The circles with different colors in the figure represent the target names with different degree values.

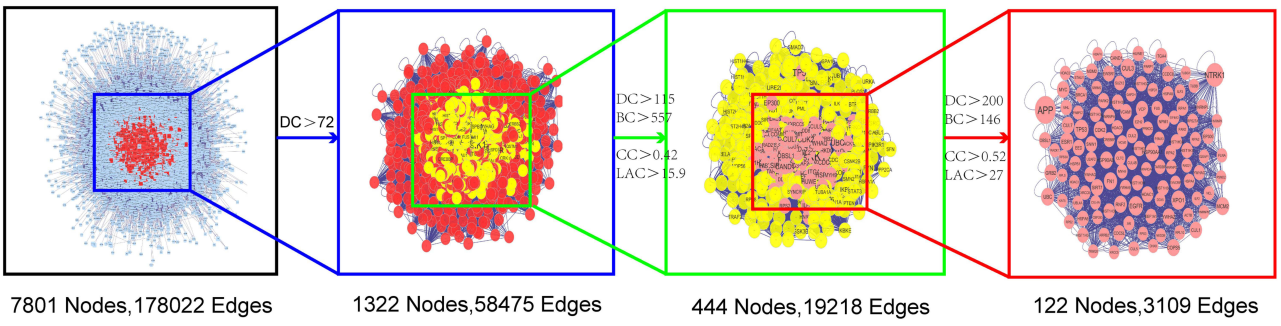

Figure 5 The strategy of key targets in the treatment of AIDS by JAKCP. DC is Degree Centrality, BC is Betweenness Centrality, CC is Closeness Centrality and LAC is Local Average Connectivity-based method, they are four important indicators used in the strategy of screening effective compounds, 122 key targets were finally obtained after three screenings of the above indicators.

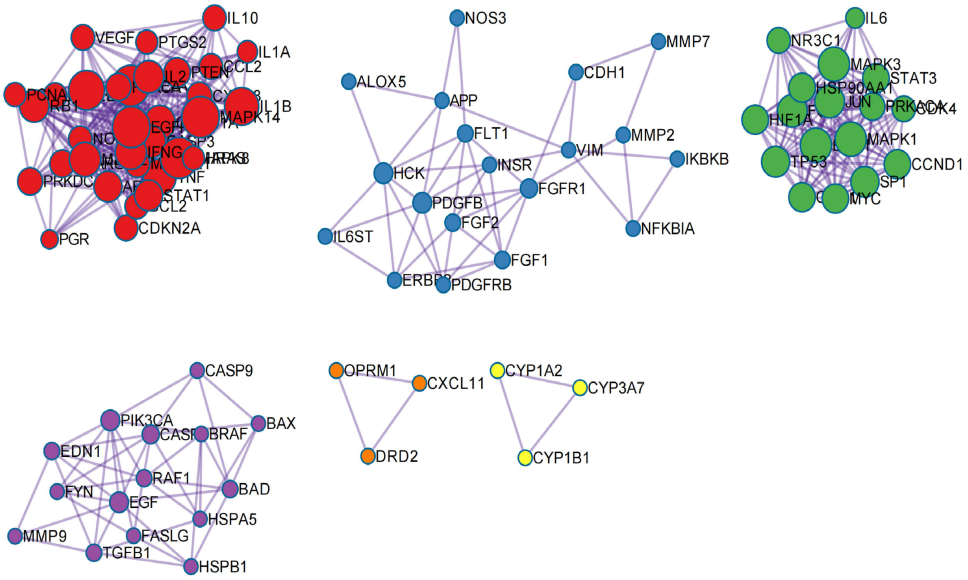

Figure 6 Potential Module network within the core targets-PPI network. Different colored circles represent different target categories. The size of the circular shape represents the magnitude of the degree value, and the lines between them represent the tightness of the connection between different targets.

Construction of "Main Active Compounds-Core Targets-Pathways" Network by Cytoscape

The "main active compounds-core targets-pathways" network diagram is shown in Figure 8 and Supplementary Material 10. The important pathways are "T cell activation, Regulation of DNA-binding transcription factor activity and Apoptotic signaling pathway", according to the degree ranking. The core targets are "HSP90AA1, IL-10, IL-6, TNF, IL-1B, TP53 and IL-1α". Therefore, we selected a certain pathway and target from the "main active compounds-core targets-pathways" system for experimental validation. After screening, the main active compounds in the JAKCP complex were "Quercetin, Ginsenoside rh2 and Epigallocatechin gallate", which could act on "IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10" and regulate the "T-cell activity" pathway. Based on this, we hypothesized that the mechanism of JAKCP for AIDS treatment is through the cytokines IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10 of "T-cell activity" pathway. Consequently, vitro experiments were performed to verify this hypothesis.

Construction of AIDS Cell Model

HIV pseudovirus infected MT-4 cells 48h after P24 antigen was measured by chemiluminescence method, and HIV Ag:1157.31 > 1 was determined, then the virus has the ability to infect MT-4 cells. The optimal HIV pseudovirus titer was determined by the multiplicative dilution method to be 1:4. Thus, an available cell model was constructed at this virus titer.

Effect of JAKCP on the Survival Rate of HIV Pseudovirus Infected MT-4 Cells in vitro

In order to verify the efficacy of JAKCP on AIDS, survival rate of HIV-pseudovirus-infected MT-4 cells treated with different concentrations of JACKP-containing serum was first determined. It demonstrated that JAKCP had

Table 4 Potential Module Function Description of PPI Network of AIDS and JAKCP (Top 3)

Entry	Functional Description	lgp
hsa05167	Kaposi sarcoma-associated herpesvirus infection	−55.2
hsa04060	Cytokine-cytokine receptor interaction	−23.10
hsa04215	Apoptosis	−13.30

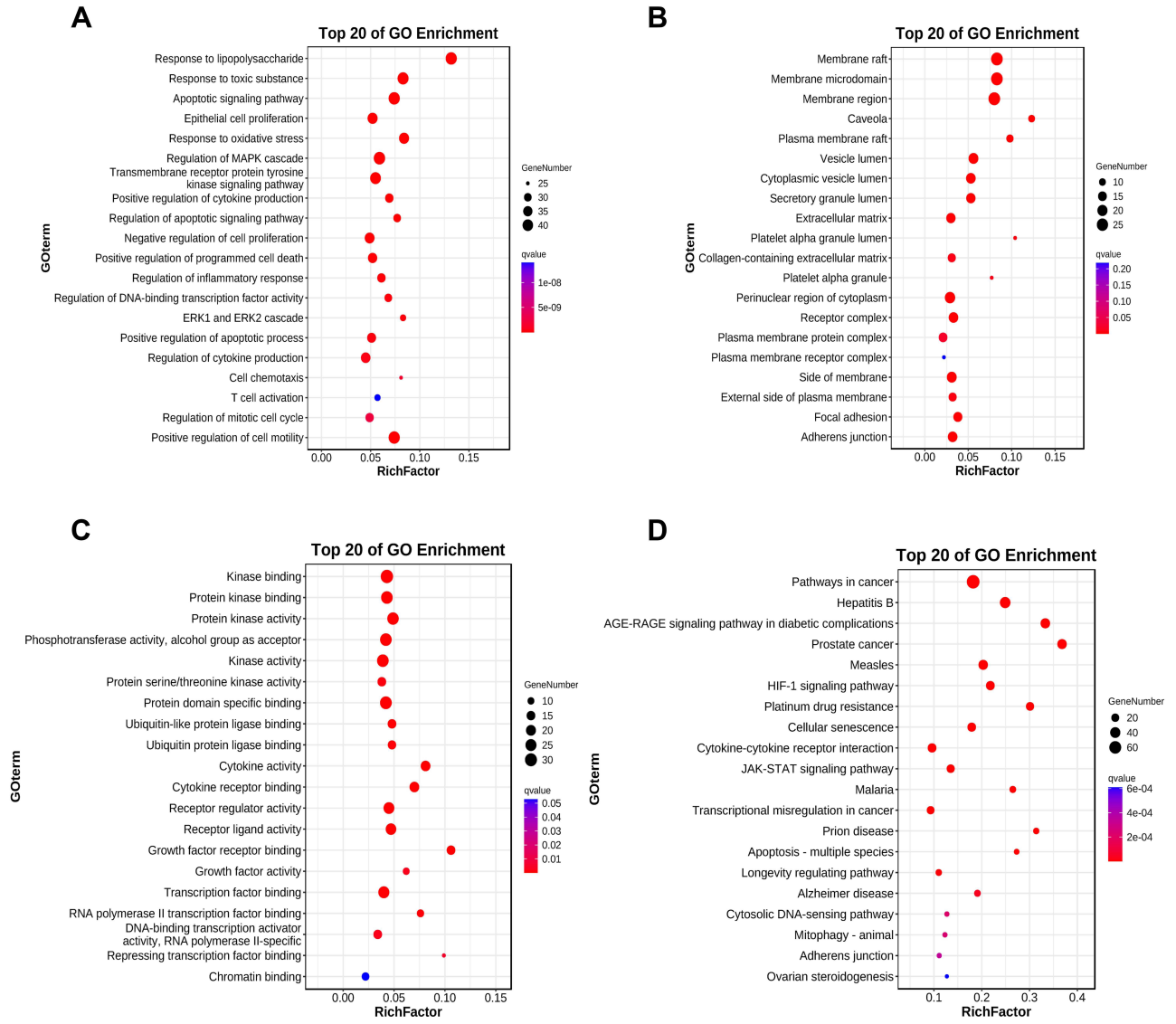

Figure 7 GO and KEGG analysis for the major targets of JAKCP, (**A**) lists the top 20 significantly enriched biological processes in the GO analysis, namely GO-BP; (**B**) lists the top 20 significantly enriched cellular component in the GO analysis, namely GO-CC; (**C**) lists the top 20 significantly enriched molecular function in the GO analysis, namely GO-MF; (**D**) shows the top 20 signaling pathways in the KEGG pathway enrichment analysis. The Y-axis in the figure represents the top 20 biological functions, such as BP, CC, MF and KEGG, the X-axis represents the ratio of the number of target pathway genes to the total number of genes, the size of the bubble area represents the number of enriched genes, and the color of the bubble Represents the significance of enrichment, that is, the magnitude of the q-value value.

a promotional effect on the survival of HIV pseudovirus-infected MT-4 cells in a concentration dependent manner. Cell survival curves are shown in Figure 9 and Supplementary Material 11. Follow-up experiments were performed using High (15%), Medium (10%) and Low (5%) doses of JACKP-containing serum.

Expression of IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10 by ELISA

It was reported that numerous inflammatory cytokines have been reported to be associated with the pathogenesis of AIDS.[44,45] Our ELISA results showed that the expression levels of IL-1α, IL-1β and IL-6 in the JAKCP-containing serum group were significantly lower than those in the model group, while the expression levels of IL-2, IL-6ST and IL-10 were significantly higher than those in the model group (Figure 10 and Supplementary Material 12). These results indicated that JAKCP decreased the expression levels of pro-inflammatory cytokines and increased the expression levels of anti-inflammatory cytokines. JAKCP may play a role in regulation of inflammatory factors.

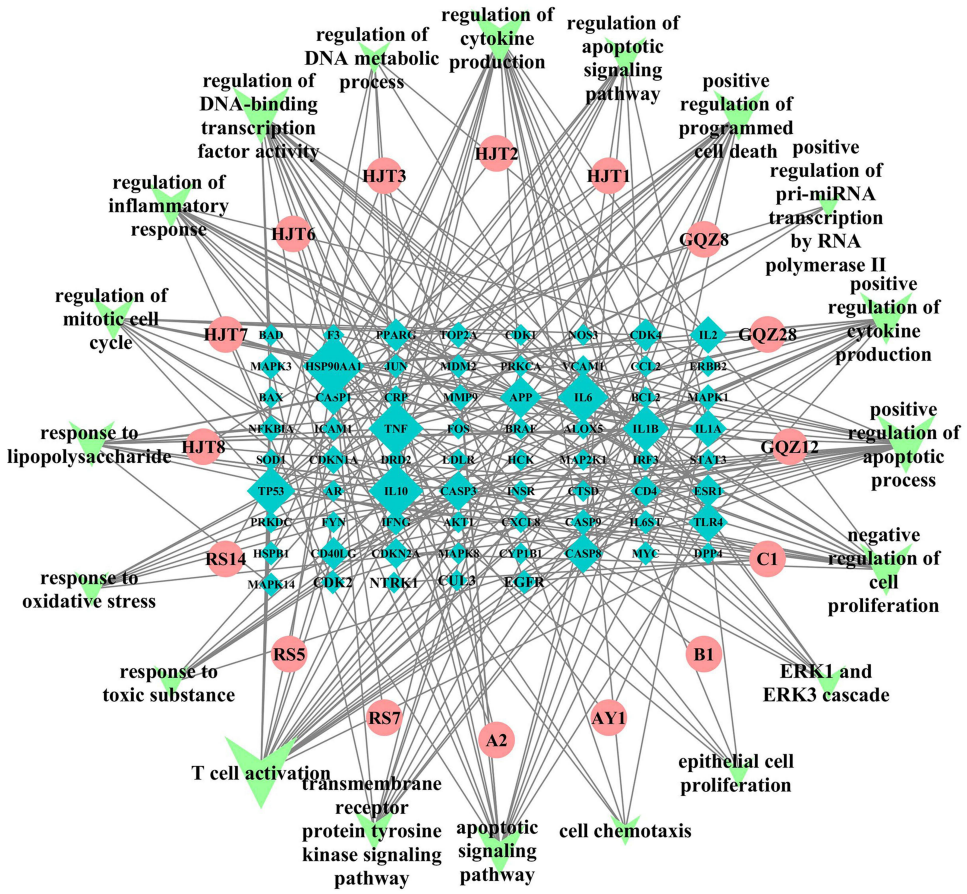

Figure 8 The network of "main active compounds-core targets-pathways". All nodes were visualized in degree value, the larger the node, the darker the color and the higher the degree value. A2, B1, C1 are the crossover active compounds of five kinds of Chinese herbs in JAKCP, A2 is Beta-sitosterol, B1 is Kaempferol, C1 is Quercetin, the other red circles represent the important effective compounds finally screened out, the blue diamonds in the middle represent the corresponding targets, and the green arrows around represent the corresponding pathways.

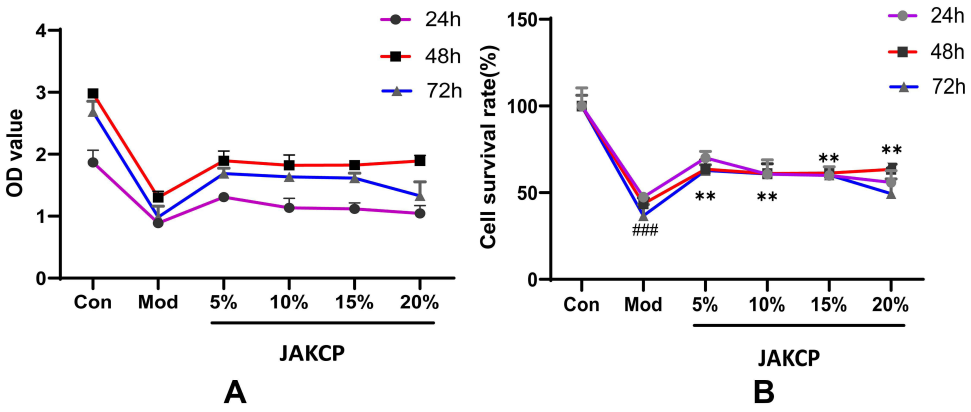

Figure 9 Cell viability effects of JAKCP treatment on MT-4 cells with HIV pseudovirus, Drug concentration-cell viability curves were generated based on the cell viability assay. (**A**) The OD value of JAKCP treatment on MT-4 cells with HIV pseudovirus. (**B**) The cell survival rate of JAKCP treatment on MT-4 cells with HIV pseudovirus. All data was presented as mean ± standard deviation, ###:p<0.001, compared with control group; **:p < 0.01, compared with model group.

mRNA Expression of IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10 by qPCR

The mRNA expression levels of several core targets on the T-cell activity pathway, including IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10, were verified by qPCR. It was found that JAKCP-containing serum decreased the levels of IL-1α, IL-1β and IL-6 and increased the levels of IL-2, IL-6ST and IL-10 (Figure 11 and Supplementary Material 13).

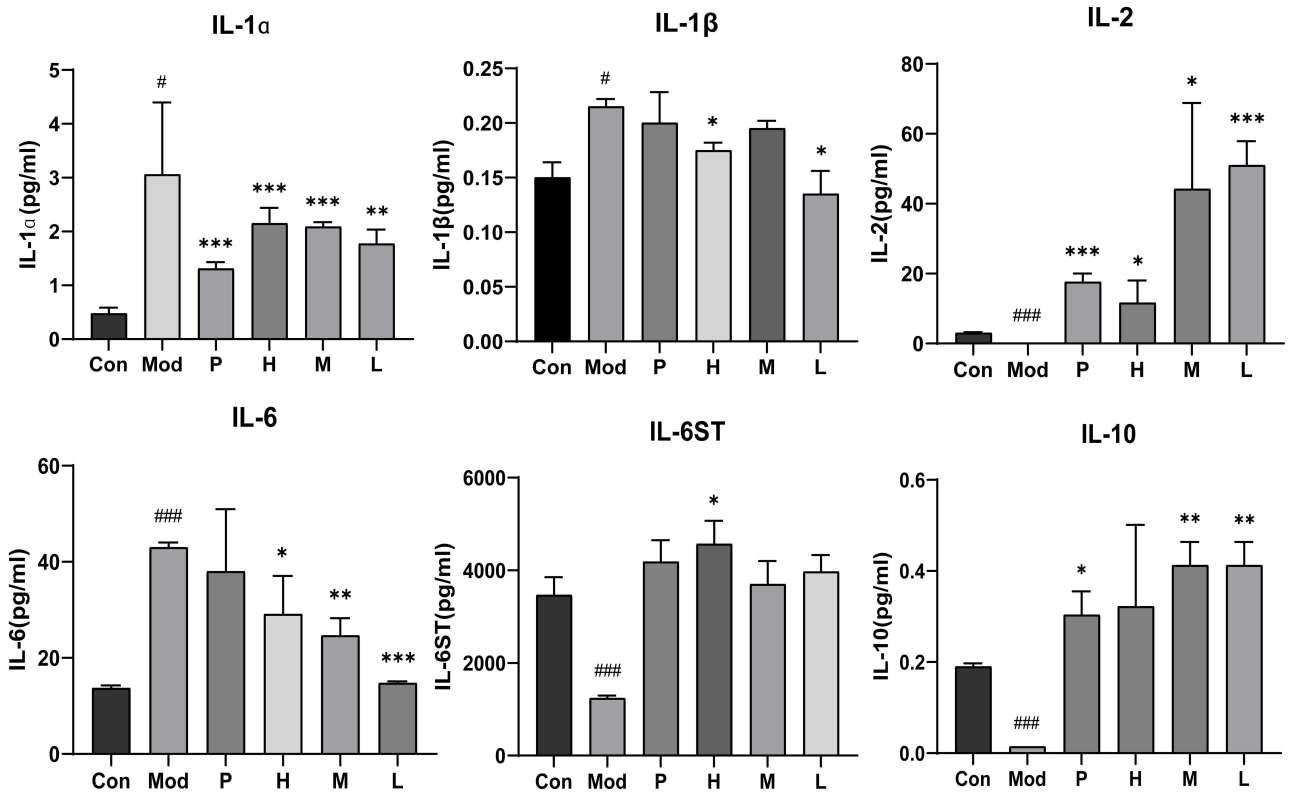

Figure 10 Expression levels of IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10 in serum from JAKCP among groups Con (Control), Mod (Model), P (Positive), H (High), M (Medium) and L (Low). Each histogram is marked with its corresponding target name, which is IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10. All data was presented as mean ± standard deviation, $^{\#}$:p < 0.05,$^{\#\#\#}$:p<0.001, compared with control group;*:p < 0.05,**:p < 0.01, ***:p<0.001, compared with model group.

Protein Expression of IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10 by Western Blot

Targets including IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10 were measured by protein blotting analysis. JAKCP-containing serum decreased the protein expression of IL-1α, IL-1β and IL-6 and increased the protein expression of IL-2, IL-6ST and IL-10 (Figure 12 and Supplementary Material 14).

Discussion

Although cART can reduce AIDS-related morbidity and mortality and has the dual advantages of immunology and virology, cART has also shown some clinical negative effects, which greatly reduce the quality of life of patients.[46] TCM has outstanding advantages in improving immune function,[47] reducing the toxicity and drug resistance rate of antiviral drugs,[48,49] reducing the risk of death[50] and prolonging the survival rate.[23] Therefore, TCM is considered as an effective and safe complementary alternative therapy for the adjuvant treatment of AIDS, however the mechanism is still unclear. In this study, a network pharmacology approach was adopted to describe the relationship between active compounds, targets and signaling pathways and to validate them in combination with in vitro experiments, thus revealing the potential mechanisms of JAKCP.

In this study, 147 active compounds and 351 targets of JAKCP were identified by network pharmacology, which indicated that JAKCP exerted its pharmacological effects on AIDS through multiple pathways and multiple targets. Quercetin, Kaempferol, Stigmasterol, Beta-sitosterol and Epigallocatechin gallate were identified as the top 5 important active compounds. Among them, Quercetin demonstrated excellent antioxidant, anticancer, antiviral, antibacterial and anti-inflammatory activities, and quercetin has potential antiviral and immunomodulatory activities against hepatitis B and C, herpes simplex virus types 1 and 2, influenza virus, human coronavirus and HIV.[51] Quercetin reactivates HIV-1 expression by inducing nuclear translocation of NF-κB, and is less toxic than other HIV-1 activators.[52] Quercetin-3-O-β-

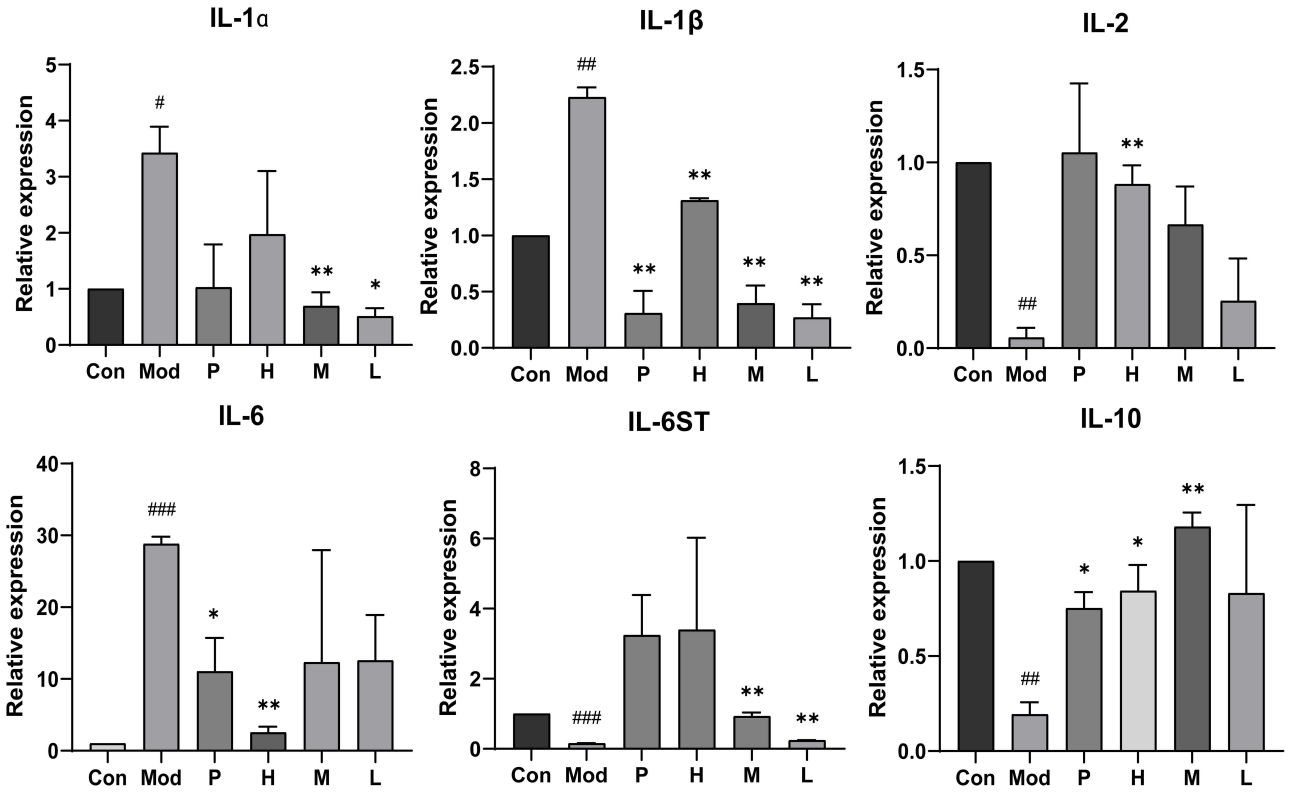

Figure 11 The expression of IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10 mRNA levels were examined by qPCR treated with JAKCP among groups Con (Control), Mod (Model), P (Positive), H (High), M (Medium) and L (Low). Each histogram is marked with its corresponding target name, which is IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10. All data was presented as mean ± standard deviation,$^{\#}$:p < 0.05, $^{\#\#}$:p<0.01, $^{\#\#\#}$:p<0.001, compared with control group; *:p < 0.05,**:p < 0.01, compared with model group.

d- Glucopyranoside and quercetin-3-O-β-d-galactopyranoside have inhibitory activity against HIV-1 IN inhibitors with IC50 values of 19.39 and 21.80 μM, respectively.[53] In addition, Quercetin is a potential HAART adjuvant, and it was found that Quercetin significantly attenuated AZT-induced upregulation of pro-inflammatory cytokines.[54] Kaempferol and Kaempferol-7-O-glucoside have been reported to possess not only potent anti-HSV activity but also anti-HIV-1 reverse transcriptase activity, and a 100ug/mL concentration of kaempferol effectively inhibited HIV-1.[55] As for sitosterol, the main phytosterol in herbs, it has antiviral and immunomodulatory activities.[56] It can be seen that the activity of these single compounds on HIV has been strongly confirmed. In addition to this, there are other compounds with very high biological activity.[57,58] We speculate that JAKCP containing the above compounds is likely to play a role in the treatment of AIDS in a multi-channel and multi-target manner. Therefore, it is the focus of this study to verify whether JAKCP can play a therapeutic role through these active compounds.

GO analysis indicated that JAKCP was associated with some major biological processes, such as T cell activation, Apoptotic signaling pathway and positive regulation of cytokine production. KEGG pathway analysis showed that JAKCP has therapeutic effects on AIDS by regulating cytokine–cytokine receptor interaction, JAK-STAT signaling pathway, apoptosis and other pathways. This study revolves around "T cell activation", which is essential for coordinating immune responses. The formation of immune synapses, molecular components and reorganization of membrane proteins and actin cytoskeletons are the main features of T cell activation. Studies have suggested that reduced T cell receptor (TCR) pool diversity and oligoclonal T cell expansion after long-term ART in HIV-infected patients correlate with elevated CD8+ T cell counts, which correlates with systemic level evidence of sustained T cell activation in the genome-wide blood transcriptome.[59] In addition, cellular experiments confirmed that upregulated miR-124a silences the expression of the target gene SIRT1, thereby regulating the activation of Th2 CD4 + T cells, and activated Th2 CD4 + T cells could secrete IL-10 and TGF-β cytokines involved in the immune response, which in turn enhances the immunity of patients.[60] Another study reported that CD4+ T cell immune activation, IL-2 production and circulating expression

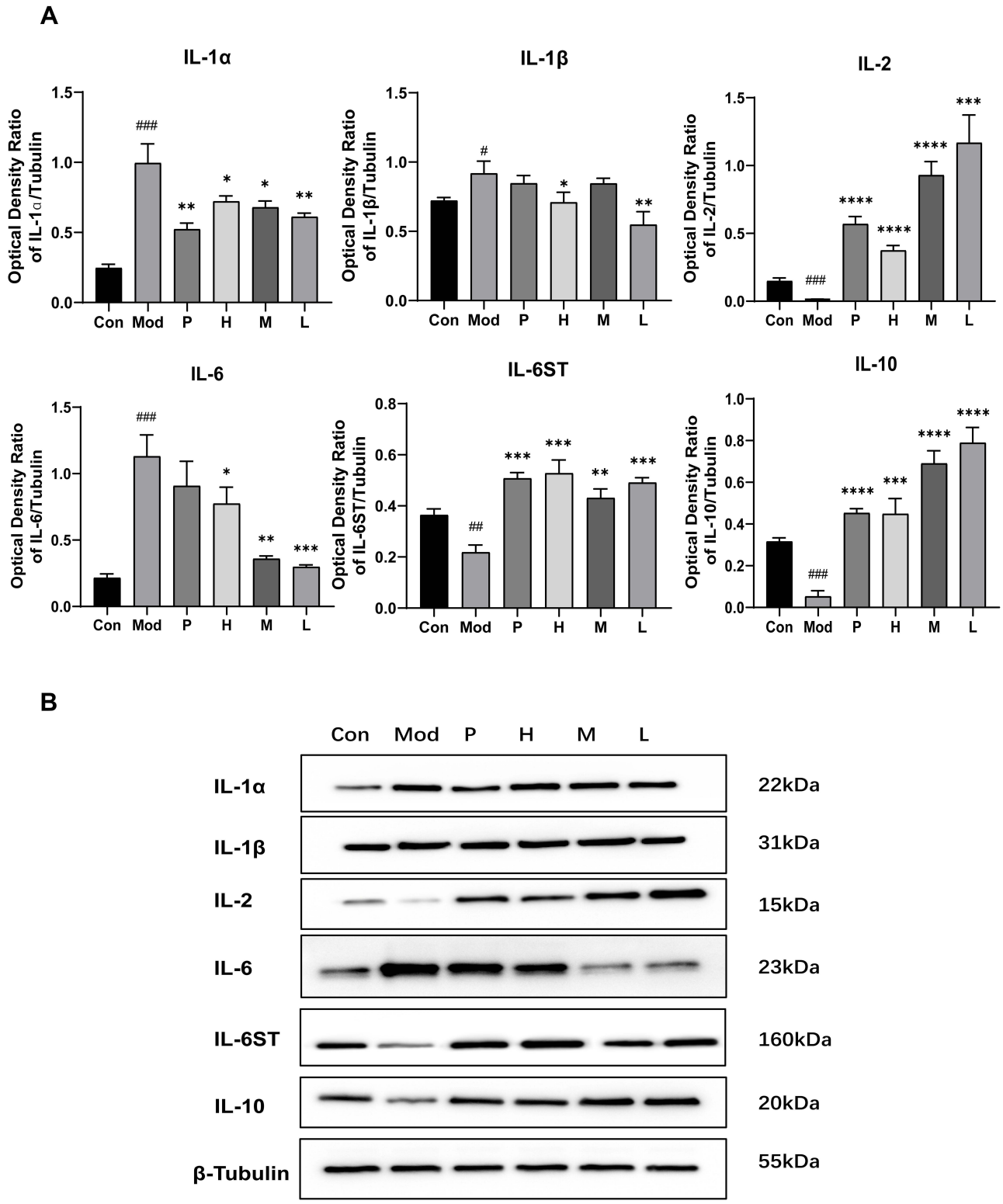

Figure 12 The expression of IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10 protein levels were examined by Western blot among groups Con (Control), Mod (Model), P (Positive), H (High), M (Medium) and L (Low).(**A**) Western blot analysis showed the optical density ratio of IL-1α/Tubulin, IL-1β/Tubulin, IL-2/Tubulin, IL-6/Tubulin, IL-6ST/Tubulin and IL-10/Tubulin on MT-4 cells with HIV pseudovirus among different groups.(**B**) The expression of IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10 protein levels was analyzed by images of the Western-blot results in different groups. All data was presented as mean ± standard deviation, $^{\#}$:p < 0.05, $^{\#\#}$:p<0.01, $^{\#\#\#}$:p<0.001, compared with control group;*:p < 0.05,**:p < 0.01,***:p<0.001,****:p<0.0001, compared with model group.

during acute HIV infection were associated with a decrease in CD4+ T cells 2 years after infection.[61] These all imply that the level of T-cell activation is closely related to the development of AIDS disease. Therefore, we performed cellular experiments to validate this pathway for T-cell activation. As for cytokines, changes in cytokine levels in HIV-infected patients affect the function of the immune system and have the potential to directly influence the course of HIV disease by enhancing or inhibiting HIV replication. In particular, the balance between pro-inflammatory cytokines IL-1, IL-6 and IL-10 may simultaneously play an anti-inflammatory role.[62] This is also consistent with the level of inflammatory cytokine expression in our study. Apoptosis is the main mechanism of progressive loss of CD4+ T cells in HIV-1 disease, and HIV-1 proteins associated with the regulation of apoptosis include gp120, Vpr, Nef, and Tat, which have been confirmed to induce apoptosis by upregulation of the apoptosis effector molecule Fas ligand (FasL), inhibition of manganese-dependent superoxide dismutase expression, and cell cycle protein dependent kinase activation.[63,64] With regard to the JAK/STAT pathway, this includes phosphorylation of STAT3 and STAT1, as well as activation of RAS/MAPK, transduced through signaling by IL-6ST (another name: sGP130).[65] These studies are consistent with the results predicted by our network pharmacology, which further suggest that these pathways may play a role in the process of anti-HIV and regulating immune function. Therefore, we speculate that the effect of JAKCP on AIDS may be related to the above biological process.

In this study, IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10 were identified as 6 hub protein targets in the AIDS "T cell activity" pathway. Experimental verification showed that Jianaikang medicated serum promoted the survival of HIV pseudovirus-infected MT-4 cells in a concentration-dependent manner, and effectively down-regulated the mRNA and protein expressions of the predicted targets IL-1α, IL-1β and IL-6, up-regulated IL-2, IL-6ST and IL-10 mRNA and protein expression. The expression of inflammatory cytokines is regulated by the "T cell activation" signaling pathway, which in turn affects the activity of MT-4 cells. We speculate that stimulating the "T cell activation" pathway can increase the activity of MT-4 cells and reduce cell apoptosis, thereby exerting an immunoregulatory effect, which also implies that JAKCP and its active compounds can promote the survival of immune cells and regulate the balance between cytokines, thereby promoting AIDS immune regulation, and thus, the balance between cytokines is of great significance in HIV disease. Multiple studies have demonstrated overproduction of pro-inflammatory cytokines in HIV-infected individuals with elevated levels of IL-1, IL-6 and TNF-α in serum and culture supernatants, and high levels of IL- 1α and IL-1β, the oversecretion of these three cytokines in turn increases HIV replication.[66,67] Cellular experiments confirmed that acutely HIV-infected cells released significantly higher levels of TNF-α and IL-1β after stimulation with LPS or a combination of LPS plus IFN-γ compared to chronically infected or uninfected cells.[68] These studies are basically consistent with the results of our present study. Our experiments have proved from the cellular level that IL-1 and IL-6 have high expression levels in the AIDS cell model, which is consistent with IL-1α and IL-6 in human HIV-infected patients, and the cytokine decreased after treatment, indicating that Jianaikang medicated serum has a certain regulatory effect on IL-1α and IL-6. Therefore, these pathways could represent therapeutic targets for AIDS.

IL-10 is an important anti-inflammatory and immunoregulatory cytokine that exerts transcriptional and post-transcriptional control on IL-1, TNF-α and IL-6 and other inflammatory cytokines to prevent the production of mRNA and protein. Natural terminator of cytokine synthesis by activated monocytes/macrophages.[69] According to reports, due to the use of different detection methods, the detection amount of IL-10 in serum or in vivo of HIV/AIDS patients has different degrees of sensitivity and specificity, and may be overproduced in some HIV patients,[70] and IL-10 Levels increase as HIV disease progresses, and IL-10 levels in untreated AIDS patients are relatively lower than in treated patients.[71,72] IL-6ST (sGP130) is a stabilizer of the IL-6 receptor complex, and sGP130 production is associated with IL-6. sGP130 is associated with AIDS-related Kaposi's sarcoma AIDS-KS risk was significantly negatively correlated.[73] IL-2 is an important control point in controlling the functional balance of regulatory T cells and effector T cells in vivo, and animal experiments have shown that disruption of the IL-2 pathway leads to lymphoid hyperplasia and autoimmunity, rather than immunodeficiency, suggesting that IL-2 is the main. The physiological function is to limit rather than enhance T cell responses.[74] On the other hand, cellular experiments confirmed that IL-2 production was significantly reduced and immune function was significantly impaired in latent HIV-1-infected cells compared with uninfected parental cells.[59] In addition, cytokines are also involved in the pathogenesis of other immune diseases.[75] In summary, the results of this experiment are consistent with these in vitro and in vivo studies, suggesting that IL-1α, IL-1β, IL-2, IL-6, IL-6ST and IL-10 can directly or indirectly participate in the

pathogenesis of AIDS, Pathological processes and related treatments, therefore, these inflammatory cytokines are potential targets for the treatment of AIDS, and JAKCP may play an immunomodulatory role by regulating the balance between anti-inflammatory cytokines and anti-inflammatory cytokines.

The results of this experiment found that although the expression of most inflammatory cytokines had a certain dose–effect relationship with the concentration of Jianaikang medicated serum, the individual inflammatory cells did not. For example, Figure 10 uses ELISA to detect the expression of IL-6ST in the middle-dose group of Jianaikang medicated serum is lower than that of the low-dose group, Figure 12 uses Western-blot method to detect the IL-1β and low-dose groups in Jianaikang medicated serum, the expression of IL-6ST does not have a certain dose-response relationship. The reason is that, first of all, this phenomenon may be related to the interaction of multiple components of TCM compounds. According to the literature, this phenomenon is relatively common in the research of TCM compounds. Cell experiments by Zeye Zhang and Yanjun Cao on Chinese herbs also yielded similar results to this experiment.[76,77] Secondly, compared with a single compound, TCM compounds have multiple components acting at the same time. This effect may be a combination of positive, negative, transformation and superposition. This hypothesis has been proposed by some Chinese scholars.[78] There is a coordinated and unified relationship of synergy, addition and antagonism. This relationship may be synergistic promotion, inhibition of rejection or intermittent positive and negative synergy or rejection with the change of drug concentration. Finally, the results of this study showed that there was no significant difference in the expression of IL-6ST between the middle and low dose groups ($P = 0.0615$), which means that although there was a difference in the expression of IL-6ST between the middle and low dose groups, this difference is moot. It can be seen that there are many mysteries in the research on the mechanism of action of TCM compounds, which need to be further explored.

To sum up, the experimental verification preliminarily confirmed that our prediction based on network pharmacology on how JAKCP play an immunomodulatory effect may be correct and credible. However, this study still has some limitations, for example, the diversification of computing software makes the screening, integration and processing of data not systematic; the database of TCM is incomplete and there are deviations, and the accuracy and completeness of the collected data cannot be guaranteed.[79] Even so, network pharmacology is still an effective means to study the mechanism of action of TCM compounds. Based on this, our research team carried out a preliminary study on the mechanism of JAKCP regulating AIDS immune function. In the following research work, we will use a combination of mass spectrometry, gas spectrum component detection and network pharmacology prediction to conduct more specific and accurate research on the relevant targets of single Chinese herbs and active compounds in the AIDS cell model. In addition, these and the verification results of TCM compounds can be comprehensively analyzed, so as to clarify how the compound JAKCP exert the therapeutic effect and provide more clues for in-depth mechanism research.

Conclusion

Based on network pharmacology, this study predicted that JAKCP could regulate AIDS immune function through multiple components, multiple pathways and multiple targets. Consistent with the predicted results, the experimental results showed that JAKCP could down-regulated the mRNA and protein levels of IL-1α, IL-1β and IL-6, and up-regulated IL-2, IL-6ST and IL-10 through the "T cell activation" signaling pathway, regulate the balance between pro-inflammatory cytokines and anti-inflammatory cytokines, increase immune cell activity and play an immunomodulatory role. JAKCP may be considered as a good candidate for the treatment of AIDS.

Abbreviations

JAKCP, Jian Aikang Concentrated Pill; AIDS, Acquired immunodeficiency syndrome; TCMSP, Traditional Chinese Medicine Systems; OMIM, Online Mendelian Inheritance in Man; TTD, Therapeutic Target Database; PPI, protein–protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; HIV, human immunodeficiency virus; cART, Combined antiretroviral therapy; NRTIs, nucleotide reverse transcriptase inhibitors; TCM, Traditional Chinese medicine; RS, Renshen; LZ, Lingzhi; AY, Aiye; GQZ, Gouqizi; HJT, Hongjingtian; OB, Oral Bioavailability; DL, Drug-like Properties; TCR, T cell receptor; DC, Degree Centrality; BC, Betweenness Centrality; CC, Closeness Centrality; LAC, Local Average Connectivity-based method.

Ethics Approval

Approval was granted by the Ethics Committee of The First Affiliated Hospital of Henan University of CM (Date 2019.3.25/No.YFYDW2019008).

Acknowledgments

The authors would like to acknowledge the financial supports from the National Natural Science Foundation of China.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by grants from the National Natural Science Foundation of China (NSFC NO. U1604287), the Science and Technology projects in Henan Province (212102311126), the Key Science and Technology Projects in Henan Province (202102310503) and the Key Scientific Research Projects Plan in Colleges and Universities in Henan Province (21A360010).

Disclosure

The author reports no conflicts of interest in this work.

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