Potential Mechanisms of Yanghe Decoction in the Treatment of Soft Tissue Sarcoma and Arteriosclerosis Obliterans Based on Network Pharmacology

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CMNP 2022;2:e77–e88.

Abstract

Objective The objective of this study was to investigate potential mechanisms of Yanghe Decoction (YHD) in treating soft tissue sarcoma (STS) and arteriosclerosis obliterans (ASO) based on the use of network pharmacology.

Methods Candidate compounds and potential targets were identified through the TCM Systems Pharmacology database and a comprehensive literature search. Related targets of STS and ASO were collected in the GeneCards database, DisGeNET database, and Drugbank database. Furthermore, The STRING 11.0 database was used to determine protein–protein interaction (PPI) networks; common targets were obtained and imported into Cytoscape 3.7.2. Then, a PPI network comprising common targets was drawn, and network topology analysis was performed to screen for key shared targets. Gene ontology functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of key shared targets were performed by using Metascape software. Subsequently, a compound–target–pathway network was constructed via Cytoscape 3.7.2.

Results The following signaling pathways were found to be associated with the mechanisms of YHD in treating STS and ASO: AGE-RAGE signaling pathway, IL-17 signaling pathway; HIF-1 signaling pathway, TNF signaling pathway, interactions between cytokines and cytokine receptors, Th17 cell differentiation, and NOD-like receptor signaling pathway. Among the compounds and targets involved in these pathways, quercetin, luteolin, and kaempferol were found to be core compounds, and TNF, IL-6, and MAPK1 were found to be core targets.

Conclusion Taken together, our findings elucidated that potential mechanisms of YHD in treating STS and ASO involved cellular proliferation/differentiation, angiogenesis, inflammation, immune responses, oxidative stress, and other related signaling pathways.
Introduction

Soft tissue sarcoma (STS) is a malignant solid tumor derived from fat, fascia, muscle, fibers, lymph, and blood vessels. The incidence rate of STS in China is approximately 2.38/100,000 individuals per year, and this rate continues to increase. Although the above findings suggest that YHD may be efficacious in treating both STS and ASO, its underlying mechanisms in this therapeutic process remain unknown. Unfortunately, traditional pharmacological research methods alone are not sufficient to fully elucidate the mechanisms of action of YHD. In contrast, network pharmacology is a powerful tool for investigating the mechanisms of action of TCM compounds. In particular, network pharmacology assesses multilevel relationships of compounds, targets, and pathways to provide insights into the mechanisms of action of TCM compounds.

Materials and Methods

Screening of Chemical Components of YHD
We used the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database and analysis platform and an extensive literature search to identify candidate compounds and targets of the following six key components in YHD: Shu di huang (Rehmannia glutinosa, SD), Rougui (Cinnamomum cassia Presl, RG), Mahuang (Ephedra sinica Stapf, MH), Jiezi (Semen sinapis, JZ), Sheng jiang (Zingiber officinale Rosc, SJ), and Gancao (Glycyrrhiza uralensis Fisch, GC). Lujiao Jiao (Colla Corni Cervi) was excluded since it was not suitable for our present pharmacological study. According to the absorption, distribution, metabolism, and excretion parameters in the TCMSP database, Chinese medicinal compounds with oral bioavailability ≥30% and with a drug-like-index (DL) ≥0.18 were selected as candidate compounds.

Collection and Treatment of Targets in YHD
We obtained potential targets of the candidate compounds through the TCMSP database, uploaded them into the UniProt database (https://www.uniprot.org/), limited the species to “human,” and obtained the genetic information corresponding to each protein target. Then, we imported these data into Cytoscape 3.7.2 to build a compound–target–pathway network diagram.

Acquisition of Common Targets of YHD in the Treatment of STS and ASO
We used “soft tissue sarcoma” and “arteriosclerosis obliterans” as search terms in the GeneCards database (https://www.genecards.org), DisGeNET database (https://www.disgenet.org/), and Drugbank database (https://www.drugbank.ca) to collect disease targets related to STS and ASO. If there were too many disease targets in the database, we defined targets with scores greater than the median score as potential targets. Then, we merged potential targets obtained from each database and removed any duplicates. Subsequently, we determined cross targets, and the cross targets were displayed via a Venn diagram through OmicShare (https://omicshare.com/index.php).

Screening of Key Shared Targets
We entered the obtained common targets into the STRING 11.0 database (https://string-db.org/). The organism category was set to Homo sapiens. We obtained protein–protein interaction networks of the common targets via a combined score >0.4 as the screening criterion. Then, we imported the screened information into Cytoscape 3.7.2 to plot the protein–protein interaction network between the common targets and performed network topology analysis. Key shared targets were selected according to values greater than the median.

GO Function Enrichment and KEGG Signal Pathway Enrichment Analysis
We uploaded the key common targets to Metascape (https://metascape.org/) and set the parameter to H species to obtain the gene ontology (GO) function enrichment analysis results and Kyoto Encyclopedia of Genes and Genomes (KEGG) signal pathway enrichment analysis results. GO functional enrichment analysis includes biological process (BP), cell composition (CC), and molecular function (MF). According to the value of log (10)p, we used the bioinformatics online mapping tool to visualize various top-ranked GO items and filtered out the top 20 signaling pathways according to theirs. OmicShare was used to visualize the results of enrichment analysis of the top 20 KEGG pathways. Finally, we imported these data into Cytoscape 3.7.2 to construct a compound–target–pathway interaction network.
Results

Screening of Chemical Components of YHD
We identified a total of 76 chemical constituents of SD, 103 chemical constituents of RG, 363 chemical constituents of MH, 37 chemical constituents of JZ, 265 chemical constituents of SJ, and 280 chemical constituents of GC. A total of 129 candidate chemical constituents were obtained after screening, including two chemical constituents of SD, three chemical constituents of RG, 23 chemical constituents of MH, three chemical constituents of JZ, five chemical constituents of SJ, and 92 chemical constituents of GC. After deleting duplicates, a total of 118 chemical components were obtained (Table 1).

Collection and Treatment of Targets in YHD
We obtained 28 targets of SD, 153 targets of RG, 209 targets of MH, 17 targets of JZ, 49 targets of SJ, and 213 targets of GC. After deleting duplicates, a total of 233 drug targets were obtained, which were imported into Cytoscape 3.7.2 to construct a drug–compound–target interaction network (Fig. 1).

Acquisition of Common YHD Targets in the Treatment of STS and ASO
We obtained 1,615 STS disease targets and 223 ASO disease targets. The above two groups of disease targets were intersected with 233 compound targets in YHD (Fig. 2), which yielded a total of 43 common targets, as follows: \( \beta \)-NOS3, CXCL10, SERPINE1, VCAM1, IL-10, MAPK1, PLAT, SOD1, IL-2, IFNG, IL-4, ICAM1, HIF1A, \( \beta \)NOS3, PTGS1, F3, TGF-\beta, CCL2, MMP1, STAT1, IL-6, GSR, HMOX1, MMP3, IL-10, MAPK1, PLAT, SOD1, IL-2, IFNG, IL-4, ICAM1, HIF1A, NO53, CXCL10, SERPINE1, VCA1, IL-1B, PTGS1, F3, CYP3A4, and MMP9.

Screening and Analysis of Key Shared Targets
The interaction network between common target proteins is shown in Fig. 3. Through network topology analysis, 20 key common targets were screened out. The specific key common targets were as follows: TNF, IL-6, IL-1\beta, VEGFA, MMP9, CCL2, IL-10, CXCL8, VCAM1, ICAM1, MMP2, SERPINE1, MPO, IL-4, MAPK1, NO53, CRP, HMOX1, STAT3, and IFNG.

GO and KEGG Analyses
A total of 745 BPs were obtained and were mainly related to cell migration, cell apoptosis, cytokine metabolism, and cell response to biological stimuli. A total of 12 CCs were obtained and involved membrane rafts, membrane microdomains, membrane domains, and the extracellular matrix. A total of 14 MFs were obtained and involved cytokine activity, receptor ligand activity, growth factor activity, and chemokine receptor binding. The top-ranked items included cytokine-mediated signaling pathways, positive regulation of cell migration, positive regulation of cell motility, positive regulation of cellular-component movement, positive regulation of locomotion, and other matters (Fig. 4). KEGG pathway enrichment analysis yielded 65 signaling pathways, among which the top 20 pathways included the AGE-RAGE signaling pathway (related to diabetic complications), IL-17 signaling pathway, and HIF-1 signaling pathway and other matters (Table 2). The results of KEGG pathway enrichment analysis of the top 20 KEGG pathways were visualized using OmicShare (Fig. 5) and were then imported into Cytoscape 3.7.2 to build a compound–target–pathways interaction network (Fig. 6).

Discussion
YHD is derived from the *Waike Zhengzi Quansheng Ji*. SD can nourish yin and blood. RG and SJ can warm the yang and dissolve the cold. MH can open the sweat pores of the skin and release pathogens. JZ can dredge collaterals and resolve phlegm. GC can nourish qi and detoxify and harmonize medicines. STS is a type of malignant solid tumor. The formation of STS is due to an imbalance of yin and yang. Yang deficiency leads to cold coagulation, phlegm obstruction, blood stasis, and eventually leads to tumors. Cold dampness pathogen is the superficial reason of ASO, while yang deficiency of spleen and kidney is the root cause. Most of the patients are middle aged and elderly people, who often have the syndromes of deficiency, blood stasis, and phlegm. It can be seen that the above two diseases have yang deficiency, phlegm dampness, and blood stasis in varying degrees. YHD can warm yang to regenerate qi and blood and remove cold to dredge collaterals and disperse accumulation. Therefore, it theoretically explains the reason why YHD can treat STS and ASO.

In the present study, we used network pharmacology to identify signaling pathways involved in the effects of YHD in treating STS and ASO, which included the following: the AGE-RAGE signaling pathway, IL-17 signaling pathway, HIF-1 signaling pathway, TNF signaling pathway, interactions of cytokines and cytokine receptors, Th17 cell differentiation, and NOD-like receptor pathway. The topological properties were analyzed based on the degree values and corresponding intermediary values in the two networks of drug–compound–targets and compound–target–pathways. The most important compounds were determined to be quercetin, luteolin, and kaempferol. Additionally, the most critical targets were found to be TNF, IL-6, and MAPK1. To corroborate these findings, we performed an extensive literature search and further explored the mechanisms of YHD in the treatment of STS and ASO.

Quercetin and luteolin exhibit proteasome inhibitory activity and have significant effects in overcoming multidrug resistances of various tumors such as sarcomas. Quercetin and kaempferol can inhibit tumor invasion and metastasis by inhibiting the activity of MMP-9 in human fibrosarcoma HT1080 cells. Quercetin can induce apoptosis of human liposarcoma SW 872 cells by down-regulating Bcl-2, cleaving PARP, and activating caspase-3, Bax, and Bak. Luteolin can down-regulate \( \beta \)-catenin expression, inhibit Wnt signaling, and reduce the formation of fibromatosis, sarcoma, and...
| Drud | Mol ID       | Molecule Name                                           | OB (%) | DL  |
|------|-------------|---------------------------------------------------------|--------|-----|
| SD   | MOL000359   | Sitosterol                                              | 36.91  | 0.75|
|      | MOL000449   | Stigmasterol                                            | 43.83  | 0.76|
| RG   | MOL000004   | procyandin B1                                           | 67.87  | 0.66|
|      | MOL000422   | Kaempferol                                              | 41.88  | 0.24|
|      | MOL000098   | Quercetin                                               | 46.43  | 0.28|
| MH   | MOL010788   | Leucopelargonidin                                        | 57.97  | 0.24|
|      | MOL002823   | Herbacetin                                              | 36.07  | 0.27|
|      | MOL010489   | Resivit                                                 | 30.84  | 0.27|
|      | MOL004798   | Delphinidin                                             | 40.63  | 0.28|
|      | MOL000006   | Luteolin                                                | 36.16  | 0.25|
|      | MOL000492   | (++)-catechin                                            | 54.83  | 0.24|
|      | MOL001494   | Mandenol                                                | 42     | 0.19|
|      | MOL001506   | Supraene                                                | 33.55  | 0.42|
|      | MOL001755   | 24-Ethylcholest-4-en-3-one                               | 36.08  | 0.76|
|      | MOL002881   | Diosmetin                                               | 31.14  | 0.27|
|      | MOL004328   | Naringenin                                              | 59.29  | 0.21|
|      | MOL004576   | Taxifolin                                                | 57.84  | 0.27|
|      | MOL005043   | Campest-5-en-3β-ol                                       | 37.58  | 0.71|
|      | MOL005190   | Eriodictyol                                              | 71.79  | 0.24|
|      | MOL005573   | Genkwanin                                               | 37.13  | 0.24|
|      | MOL005842   | Pectolinarigenin                                         | 41.17  | 0.3 |
|      | MOL007214   | (+)-Leucocyanidin                                        | 37.61  | 0.27|
|      | MOL011319   | Truflex OBP                                              | 43.74  | 0.24|
| JZ   | MOL010690   | Uniflex BYO                                              | 30.13  | 0.25|
|      | MOL013037   | 2-(2-phenylethyl)-6-[[5S,6R,7R,8S]-5,6,7-trihydroxy-4-keto-2-(2-phenylethyl)-5,6,7,8-tetrahydrochromen-8-yl]oxy]chromone | 31.31 | 0.61 |
|      | MOL001697   | Sinoacutine                                              | 63.39  | 0.53|
| SJ   | MOL000358   | β-sitosterol                                             | 36.91  | 0.75|
|      | MOL001771   | Poriferast-5-en-3β-ol                                    | 36.91  | 0.75|
|      | MOL006129   | 6-methylgingediacetate2                                  | 48.73  | 0.32|
|      | MOL008698   | Dihydrocapsaicin                                         | 47.07  | 0.19|
|      | MOL000392   | Formononetin                                             | 69.67  | 0.21|
|      | MOL000417   | Calycosin                                                | 47.75  | 0.24|
|      | MOL004805   | (2S)-2-[4-hydroxy-3-(3-methylbut-2-enyl) phenyl]-8,8-dimethyl-2,3-dihydropyrano [2,3-f]chromen -4-one | 31.79 | 0.72 |

Table 1: Basic information of 118 candidate compounds of YHD
| Drud     | Mol ID     | Molecule Name                                                                 | OB (%) | DL  |
|----------|------------|-------------------------------------------------------------------------------|--------|-----|
| MOL004806 | Euchrenone  |                                                                              | 30.29  | 0.57|
| MOL004808 | Glyasperin B|                                                                              | 65.22  | 0.44|
| MOL004810 | Glyasperin F|                                                                              | 75.84  | 0.54|
| MOL004811 | Glyasperin C|                                                                              | 45.56  | 0.4 |
| MOL004814 | Isotrifoliol|                                                                              | 31.94  | 0.42|
| MOL004815 | (E)-1-(2,4-dihydroxyphenyl)-3-(2,2-dimethylchromen-6-yl) prop-2-en-1-one      | 39.62  | 0.35|
| MOL004820 | Kanzonols W |                                                                              | 50.48  | 0.52|
| MOL004824 | (2S)-6-(2,4-dihydroxyphenyl)-2-(2-hydroxypropan-2-yl)-4-methoxy-2,3-dihydrofuro[3,2-g]chromen-7-one | 60.25  | 0.63|
| MOL004827 | Semilicoisoavone B|                                                                    | 48.78  | 0.55|
| MOL004828 | Glepidotin A |                                                                              | 44.72  | 0.35|
| MOL004829 | Glepidotin B |                                                                              | 64.46  | 0.34|
| MOL004833 | Phaseolinosflavan |                                                        | 32.01  | 0.45|
| MOL004835 | Glypallichalcone |                                                                   | 61.6   | 0.19|
| MOL004838 | 8-(6-hydroxy-2-benzofuranyl)-2,2-dimethyl-5-chromenol  | 58.44  | 0.38|
| MOL004841 | Licochalcone B|                                                                              | 76.76  | 0.19|
| MOL004848 | Licochalcone G|                                                                              | 49.25  | 0.32|
| MOL004849 | 3-(2,4-dihydroxyphenyl)-8-(1,1-dimethylprop-2-enyl)-7-hydroxy-5-methoxy-coumarin | 59.62  | 0.43|
| MOL004855 | Licoricone     |                                                                              | 63.58  | 0.47|
| MOL004856 | Gancaonin A    |                                                                              | 51.08  | 0.4 |
| MOL004857 | Gancaonin B    |                                                                              | 48.79  | 0.45|
| MOL004860 | Glycyrrhiza uralensis Fisch glycoside E |                                                  | 32.89  | 0.27|
| MOL004863 | 3-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8(3-methylbut-2-enyl) chromone | 66.37  | 0.41|
| MOL004864 | 5,7-dihydroxy-3-(4-methoxyphenyl)-8(3-methylbut-2-enyl) chromone | 30.49  | 0.41|
| MOL004866 | 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6(3-methylbut-2-enyl) chromone | 44.15  | 0.41|
| MOL004879 | Glycyrin       |                                                                              | 52.61  | 0.47|
| MOL004882 | Licocoumarone  |                                                                              | 33.21  | 0.36|
| MOL004883 | Licoisoflavone |                                                                              | 41.61  | 0.42|
| MOL004884 | Licoisoflavone B|                                                                  | 38.93  | 0.55|
| MOL004885 | Licoisoflavanone|                                                                | 52.47  | 0.54|
| MOL004891 | Shinpterocarpin|                                                                | 80.3   | 0.73|
| MOL004898 | (E)-3-[3,4-dihydroxy-5-(3-methylbut-2-enyl)phenyl]-1-(2,4-dihydroxyphenyl) prop-2-en-1-one | 46.27  | 0.31|
| MOL004903 | Liquiritin     |                                                                              | 65.69  | 0.74|
| MOL004904 | Licopyranocoumarin |                                                              | 80.36  | 0.65|
| MOL004905 | 3,22-Dihydroxy-11-oxo-delta(12)-oleanene-27-c-methoxy carbonyl-29-oic acid | 34.32  | 0.55|
| MOL004907 | Glyzglabrin    |                                                                              | 61.07  | 0.35|
| MOL004908 | Glabridin      |                                                                              | 53.25  | 0.47|
| MOL004910 | Glabranin      |                                                                              | 52.9   | 0.31|
| MOL004911 | Glabrene       |                                                                              | 46.27  | 0.44|

(Continued)
| MOL ID       | Molecular Name                                           | OB (%) | DL |
|-------------|----------------------------------------------------------|--------|----|
| MOL004912   | Glabrone                                                 | 52.51  | 0.5|
| MOL004913   | 1,3-dihydroxy-9-methoxy-6-benzofuran[3,2-c]chromenone   | 48.14  | 0.43|
| MOL004914   | 1,3-dihydroxy-8,9-dimethoxy-6-benzofuran[3,2-c]chromenone | 62.9   | 0.53|
| MOL004915   | Eurycaclin A                                             | 43.28  | 0.37|
| MOL004917   | Glycyruside                                              | 37.25  | 0.79|
| MOL004924   | (−)-Medicocarpin                                         | 40.99  | 0.95|
| MOL004935   | Sigmoidin-B                                              | 34.88  | 0.41|
| MOL004941   | (2R)-7-hydroxy-2-(4-hydroxyphenyl) chroman-4-one         | 71.12  | 0.18|
| MOL004945   | (2S)-7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl) chroman-4-one | 36.57  | 0.32|
| MOL004948   | Isoglycyrol                                              | 44.7   | 0.84|
| MOL004949   | Isolicoflavonol                                          | 45.17  | 0.42|
| MOL004957   | HMO                                                      | 38.37  | 0.21|
| MOL004959   | 1-Methoxyphaseollidin                                    | 69.98  | 0.64|
| MOL004961   | Quercetin der                                            | 46.45  | 0.33|
| MOL004966   | 3′-Hydroxy-4′-O-Methylglabridin                           | 43.71  | 0.57|
| MOL004974   | 3′-Methoxyglabridin                                      | 46.16  | 0.57|
| MOL004978   | 2-[(3R)-8,8-dimethyl-3,4-dihydro-2H-pyrano[6,5-f]chromen-3-yl]-5-methoxyphenol | 36.21  | 0.52|
| MOL004980   | Inflacoumarin A                                          | 39.71  | 0.33|
| MOL004985   | Icos-5-enoic acid                                        | 30.7   | 0.2 |
| MOL004988   | Kanzonol F                                               | 32.47  | 0.89|
| MOL004989   | 6-prenylated eriodictyol                                 | 39.22  | 0.41|
| MOL004990   | 7,2′,4′-trihydroxy-5-methoxy-3-arylcoumarin              | 83.71  | 0.27|
| MOL004991   | 7-Acetoxycoumarin-3-yl-5-methylisoflavone                | 38.92  | 0.26|
| MOL004993   | 8-prenylated eriodictyol                                 | 53.79  | 0.4 |
| MOL004996   | Gadelaidic acid                                          | 30.7   | 0.2 |
| MOL005000   | Vestitol                                                 | 74.66  | 0.21|
| MOL005000   | Gancaonin G                                              | 60.44  | 0.39|
| MOL005001   | Gancaonin H                                              | 50.1   | 0.78|
| MOL005003   | Licoagrocarpin                                           | 58.81  | 0.58|
| MOL005007   | Glyasperins M                                            | 72.67  | 0.59|
| MOL005008   | Gyrhrhiza flavonol A                                     | 41.28  | 0.6 |
| MOL005012   | Licoagroisoflavone                                       | 57.28  | 0.49|
| MOL005013   | 18α-hydroxyglycyrrhetic acid                             | 41.16  | 0.71|
| MOL005016   | Odoratin                                                 | 49.95  | 0.3 |
| MOL005017   | Phaseol                                                  | 78.77  | 0.58|
| MOL005018   | Xambioona                                                | 54.85  | 0.87|
| MOL005020   | dehydroglyasperins C                                     | 53.82  | 0.37|

Abbreviation: DL, drug-like-index; GC, Gancao; JZ, Jiezi; MH, Mahuang; OB, oral bioavailability; RG, Rougi; SD, Shudihuang; SJ, Sheng jiang; YHD, Yanghe Decoction.
mesenchymal tumors.\textsuperscript{15} Quercetin,\textsuperscript{16} luteolin,\textsuperscript{17} and kaempferol\textsuperscript{18} all have anticoagulant, antithrombotic, antiplatelet-aggregation, and defibrillating effects. Quercetin can also exert antiarterial effects by inhibiting the expression of SDF-1 and CXCR4 in the sera of APOE mice, regulating blood lipid levels in atherosclerotic rats and interfering with the activities of key proteins in the PI3K/Akt/NF-κb pathway.\textsuperscript{19,20} In addition, luteolin also can reduce atherosclerosis by reducing inflammation in APOE mice.\textsuperscript{21} TNF is a cytokine that can directly kill tumor cells and exerts antitumor effects by activating the immune system. Ubiquitin-specific protease 20 can inhibit smooth-muscle cell inflammation caused by TNF overexpression by deubiquitinating β and relieving atherosclerosis.\textsuperscript{22} IL-6 is a pleiotropic cytokine expressed by immune cells and various tumor cells. IL-6 induces inflammation, promotes cancer cell proliferation, and inhibits apoptosis, thereby promoting chemotherapy resistance. Studies have shown that IL-6 promotes the progression of Ewing’s sarcoma by increasing resistance to apoptosis and promoting metastasis under cellular stress.\textsuperscript{23} Inflammation is a major factor leading to atherosclerosis. IL-6 is an upstream inflammatory cytokine that plays a central role in downstream inflammatory responses leading to atherosclerosis.\textsuperscript{24} MAPK1 is a member of the MAP kinase family and is also known as ERK2. MAPK1/ERK2 is involved in processes such as cellular proliferation, differentiation, and transcriptional regulation. Most patients with angiosarcoma have obvious genetic changes related to the MAPK signaling pathway, which activates the MAPK pathway and increases tumor cell proliferation. In the occurrence and development of alveolar rhabdomyosarcoma, HGF/MET signaling (mainly through ERK2 signaling)

| ID       | Term                                              | p-Value         | Genes                                                                 |
|----------|---------------------------------------------------|-----------------|----------------------------------------------------------------------|
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications | 3.06987E-27     | ICAM1, IL-1β, IL-6, CXCL8, MMP2, NOS3, SERPINE1, MAPK1, CCL2, STAT3, TNF, VCAM1, VEGFA |
| hsa05418 | Fluid shear stress and atherosclerosis            | 3.1339E-20      | HMOX1, ICAM1, IFNG, IL-1β, MMP2, MMP9, NOS3, CCL2, TNF, VCAM1, VEGFA |
| hsa05144 | Malaria                                           | 4.40514E-20     | ICAM1, IFNG, IL-1β, IL-6, CXCL8, IL-10, CCL2, TNF, VCAM1             |
| hsa04657 | IL-17 signaling pathway                           | 2.0254E-17      | IFNG, IL-1β, IL-4, IL-6, CXCL8, MMP9, MAPK1, CCL2, TNF               |
| hsa05142 | Chagas disease (American trypanosomiasis)        | 4.80526E-17     | IFNG, IL-1β, IL-6, CXCL8, IL-10, SERPINE1, MAPK1, CCL2, TNF         |
| hsa05143 | African trypanosomiasis                           | 5.40725E-16     | ICAM1, IFNG, IL-1β, IL-6, IL-10, TNF, VCAM1                         |
| hsa05323 | Rheumatoid arthritis                              | 3.27888E-15     | ICAM1, IFNG, IL-1β, IL-6, CXCL8, CCL2, TNF, VEGFA                  |
| hsa04066 | HIF-1 signaling pathway                           | 8.507E-15       | HMOX1, IFNG, IL-6, NOS3, SERPINE1, MAPK1, STAT3, VEGFA             |
| hsa04668 | TNF signaling pathway                             | 1.47723E-14     | ICAM1, IL-1β, IL-6, MMP9, MAPK1, CCL2, TNF, VCAM1                 |
| hsa05321 | Inflammatory bowel disease                        | 5.51947E-14     | IFNG, IL-1β, IL-4, IL-6, IL-10, STAT3, TNF                        |
| hsa04060 | Cytokine-cytokine receptor interaction            | 3.59495E-13     | IFNG, IL-1β, IL-4, IL-6, CXCL8, IL-10, CCL2, TNF, VEGFA          |
| hsa05164 | Influenza A                                       | 6.88443E-13     | ICAM1, IFNG, IL-1β, IL-6, CXCL8, MAPK1, CCL2, TNF                 |
| hsa05140 | Leishmaniasis                                     | 2.31419E-11     | IFNG, IL-1β, IL-4, IL-10, MAPK1, TNF                             |
| hsa05133 | Pertussis                                         | 2.96757E-11     | IL-1β, IL-6, CXCL8, IL-10, MAPK1, TNF                            |
| hsa05146 | Amoebiasis                                       | 1.24592E-10     | IFNG, IL-1β, IL-6, CXCL8, IL-10, TNF                             |
| hsa05219 | Bladder cancer                                    | 1.66454E-10     | CXCL8, MMP2, MMP9, MAPK1, VEGFA                                  |
| hsa04659 | Th17 cell differentiation                         | 2.41559E-10     | IFNG, IL-1β, IL-4, IL-6, MAPK1, STAT3                            |
| hsa05161 | Hepatitis B                                       | 1.46222E-09     | IL-6, CXCL8, MMP9, MAPK1, STAT3, TNF                            |
| hsa04621 | NOD-like receptor signaling pathway               | 3.97175E-09     | IL-1β, IL-6, CXCL8, MAPK1, CCL2, TNF                             |
| hsa05152 | Tuberculosis                                      | 5.41283E-09     | IFNG, IL-1β, IL-6, IL-10, MAPK1, TNF                             |

Abbreviation: KEGG, Kyoto Encyclopedia of Genes and Genomes.
promotes the cellular motility and participates in the occurrence, invasion, and metastasis of tumor cells.\textsuperscript{25,26} MAPK1 is expressed in platelets and is activated by various agonists. Agonist-induced phosphorylation of MAPK1 can inhibit platelet aggregation; furthermore, loss or down-regulation of MAPK1 up-regulates VCAM-1 expression stimulated by insulin and TNF-\(\alpha\), leading to vascular disease.\textsuperscript{27,28}

The AGE-RAGE signaling pathway not only causes oxidative stress, inflammation, thrombosis, and fibrosis in a variety of cells but also activates a variety of signal transduction pathways related to cellular proliferation and apoptosis. Furthermore, the AGE-RAGE signaling pathway plays an important role in the occurrence, development, and metastasis of tumors.\textsuperscript{29,30} The IL-17 signaling pathway not only participates in autoimmune diseases and chronic inflammatory diseases but also participates in tumor cell survival, angiogenesis, chemokine production, tissue remodeling, and immune modification of the tumor microenvironment, thereby affecting the occurrence and development of STS and ASO.\textsuperscript{31–34} The HIF-1 signaling pathway participates in the regulation of angiogenesis, cellular metabolism, and autophagy, as well as in the occurrence or development of malignant tumors and inflammatory responses. Some studies have shown that the HIF-1 signaling pathway affects cellular metabolism, differentiation, angiogenesis, proliferation, and metastasis and this pathway is related to the prognosis of patients with STS and chondrosarcoma.\textsuperscript{35,36} The HIF-1 signaling pathway can also cause endothelial cell dysfunction, angiogenesis, and inflammation by up-regulating VEGF, NO, ROS and PDGF. Moreover, these responses play a role in the development of ASO.\textsuperscript{37} Cytokines are small polypeptides or glycoproteins that are synthesized and secreted by a variety of cells and include interleukins, interferons,
Fig. 3 Protein–protein interaction network of the common targets of YHD and STS/ASO.

Fig. 4 GO enrichment analysis.
chemokines, growth factors, the tumor necrosis factor superfamily and colony-stimulating factors. Interactions between cytokines and cytokine receptors can regulate the growth and differentiation of cells, regulate immune responses, participate in inflammatory responses, repair damaged tissues, and have regulatory effects on STS and ASO. In addition, TNF signaling pathway, Th17 cell differentiation, and NOD-like receptor signaling pathway are all signal pathways related to immunity and inflammation, which can activate NF-κB, MAPK, and endoplasmic reticulum emergency pathway and promote the release of inflammatory factors such as IL-6 and mediate inflammatory response, which is closely related to tumors, inflammatory diseases, and autoimmune diseases.

Conclusions

In summary, this study reveals that the mechanism of YHD in the treatment of STS and ASO mainly involves cell proliferation, differentiation, angiogenesis, inflammation, immune response, oxidative stress, and other related signal pathways, which is consistent with the current research on the mechanism of STS and ASO. To some extent, it is proved that the results predicted by the network pharmacology method are reliable, but further experimental verification is still needed. This study can not only guide the experimental research in the next stage but can also provide a reliable basis for clinical application and new drug development.

Credit Authorship Contribution Statement

Yiran Zhai: Conceptualization, methodology, data curation, formal analysis, and writing original draft. Binyi Li and Lili Miao: Writing - review & editing. Shanshan Li and Jie Wang: Formal analysis. Shiqing Jiang: Conceptualization, methodology, and supervision.

Funding

This study was supported by 2018 scientific and technological research projects in Henan Province.
Conflict of Interest
The authors declare no conflict of interest.

References
1 Chinese Clinical Oncology Society Guidelines Working Committee. Chinese Clinical Oncology Society (CSCO) Guidelines for the Diagnosis and Treatment of Soft Tissue Sarcoma. Beijing: People’s Medical Publishing House; 2019
2 Vascular Surgery Group, Surgery Branch of Chinese Medical Association. Guidelines for the diagnosis and treatment of arteriosclerosis obliterans of the lower extremities. Chin J Med Sci 2015;95(24):1833–1896
3 Wang HI, Kang F, Huang X. Based on the theory of “Yang Huaqi, Yin Shaping” to discuss the application of warm-yang method in malignant tumors. Chin Basic Med Tradit Chin Med 2019;25(07):999–1002
4 Xu X, Wang S, Zhang MZ, et al. Clinical experience of professor Jiang Shiqing in prescribing Yang He decoction treating for soft tissue sarcoma. Acta Chin Med 2016;31(03):319–321
5 Fan HQ, Zhou I, Liu LF, et al. Based on the theory of “yang qi and yin shaping” to discuss the syndrome differentiation of yam and gangrene diseases. Chin J Basic Med Tradit Chin Med 2019;25(05):685–686
6 Wang ZQ, Qiao KM, Yu YN, et al. Cold and arteriosclerosis obliterans of lower extremities. J Tradit Chin Med 2017;32(04):573–576
7 Chen HB, Zhou HG, Li WT, et al. Network pharmacology: a new perspective of mechanism research of traditional Chinese medicine formula. Zhonghua Zhongyiyao Zazhi 2019;34(05):2873–2876
8 Tao JL, Wang SC, Chen YZ, et al. Review on the research in network pharmacology of traditional Chinese medicine compound. Zhonghua Zhongyiyao Zazhi 2019;34(09):3903–3907
9 Ru J, Li P, Wang J, et al. TC MSP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminform 2014;6(01):13
10 Wang FU. Prescription Science. 2nd ed. Beijing: China Traditional Chinese Medicine Publishing; 2012
11 Zanini C, Giribaldi G, Mandili G, et al. Inhibition of heat shock proteins (HSP) expression by quercetin and differential doxorubicin sensitization in neuroblastoma and Ewing’s sarcoma cell lines. J Neurochem 2007;103(04):1344–1354
12 Shen M, Hang CT, Ping DQ. Targeting tumor ubiquitin–proteasome pathway with polyphenols for chemosensitization. Anti-Cancer Agents in Med Chem 2012;12(08):891–901.
Potential Mechanisms of Yanghe Decoction

13 Lee DE, Chung MY, Lim TG, Huh WB, Lee HJ, Lee KW. Quercetin suppresses intracellular ROS formation, MMP activation, and cell motility in human fibrosarcoma cells. J Food Sci 2013;78(09):H1464–H1469
14 Choi YJ, Lee YH, Lee ST. Galangin and kaempferol suppress phorbol-12-myristate-13-acetate-induced matrix metalloproteinase-9 expression in human fibrosarcoma HT-1080 cells. Mol Cells 2015;38(02):151–155
15 Huang SL, Hsu CL, Yen GC. Growth inhibitory effect of quercetin on SW 872 human liposarcoma cells. Life Sci 2006;79(02):203–209
16 Iftikhar H, Rashid S. Molecular docking studies of flavonoids for their inhibition pattern against β-catenin and pharmacophore model generation from experimentally known flavonoids to fabricate more potent inhibitors for Wnt signaling pathway. Pharmacogn Mag 2014;10(38, Suppl 2):S264–S271
17 Choi JH, Kim KJ, Kim S. Comparative effect of quercetin and quercetin-3-O-β-d-glucoside on fibrin polymers, blood clots, and in rodent models. J Biochem Mol Toxicol 2016;30(11):548–558
18 Choi JH, Kim YS, Shin CH, Lee HJ, Kim S. Antithrombotic activities of luteolin in vitro and in vivo. J Biochem Mol Toxicol 2015;29(12):552–558
19 Choi JH, Park SE, Kim SJ, Kim S. Kaempferol inhibits thrombosis and platelet activation. Biochimie 2015;117:175–186
20 Zha KL, Li JF, Zeng Y. The role of SDF-1/CXCR 4 in the anti-atherosclerosis process of quercetin in Apo E−/− mice. Chongqing Yike DaXue XueBao 2014;39(10):1373–1379
21 Lv L. Anti-Atherosclerosis Study of Quercetin Based on PI3K/Akt/NF-κB Signaling Pathway. Jilin University; 2017
22 Ding X, Zheng L, Yang B, Wang X, Ying Y. Luteolin attenuates atherosclerosis via modulating signal transducer and activator of transcription 3-mediated inflammatory response. Drug Des Devel Ther 2019;13:3899–3911
23 Jean-Charles PY, Wu JH, Zhang L, et al. USP20 (ubiquitin-specific protease 20) inhibits TNF (tumor necrosis factor)-triggered smooth muscle cell inflammation and attenuates atherosclerosis. Arterioscler Thromb Vasc Biol 2018;38(10):2295–2305
24 Lissat A, Joerschke M, Shinde DA, et al. IL6 secreted by Ewing sarcoma tumor microenvironment confers anti- apoptotic and cell-disseminating paracrine responses in Ewing sarcoma cells. BMC Cancer 2015;15:552
25 Hartman J, Frishman WH. Inflammation and atherosclerosis: a review of the role of interleukin-6 in the development of atherosclerosis and the potential for targeted drug therapy. Cardiol Rev 2014;22(03):147–151
26 Murari R, Chandramohan R, Möller I, et al. Targeted massively parallel sequencing of angiosarcomas reveals frequent activation of the mitogen activated protein kinase pathway. Oncotarget 2015;6(34):36041–36052
27 Otabe O, Kikuchi K, Tsuchiya K, et al. MET/ERK2 pathway regulates the motility of human alveolar rhabdomyosarcoma cells. Oncol Rep 2017;37(01):98–104
28 Lee HS, Kim SD, Lee WM, et al. A noble function of BAY 11-7082: inhibition of platelet aggregation mediated by an elevated cAMP-induced VASP, and decreased ERK2/JNK1 phosphorylations. Eur J Pharmacol 2010;627(1–3):85–91
29 Pott GB, Tsurudome M, Bamfo N, Goalstone ML. ERK2 and Akt are negative regulators of insulin and tumor necrosis factor-α stimulated VCAM-1 expression in rat aorta endothelial cells. J Inflamm (Lond) 2016;13:6
30 Yamagishi SI, Matsui T. Role of hyperglycemia-induced advanced glycation end product (AGE) accumulation in atherosclerosis. Ann Vasc Dis 2018;11(03):253–258
31 Zhang X, Liu YJ. Research progress of the anti-tumor effect of AGEs-RAGE system and metformin. Zhongguo Xin Yao Zazhi 2014;23(04):441–444
32 Robert M, Miossec P. Effects of interleukin 17 on the cardiovascular system. Autoimmun Rev 2017;16(09):984–991
33 Guo MX, Zheng YJ, Tang B, et al. Expression of IL-17 signaling pathway related factor mRNA in esophageal tissue of esophageal cancer mice. J Zhengzhou Univ 2019;54(04):492–495(Med Sci)
34 Wang XZ. Exploration of the interaction mechanism between IL-17 and Notch signaling pathway in pancreatic cancer. Beijing: Peking Union Medical College Publishing; 2017
35 Zhang Cj, Yang Pr, Ma Y. New target for autoimmune diseases treatment: act1-mediated IL-17 signalling pathway. Immunol J 2012;28(05):441–444
36 Nyström H, Jönsson M, Werner-Hartlman L, Nilbert M, Carneiro A. Hypoxia-inducible factor 1α predicts recurrence in high-grade soft tissue sarcoma of extremities and trunk wall. J Clin Pathol 2017;70(10):879–885
37 Kouvaras E, Christoni Z, Siassios I, Malizos K, Koukoulis GK, Ioannou M. Hypoxia-inducible factor 1-alpha and vascular endothelial growth factor in cartilage tumors. Biotech Histochem 2019;94(04):283–289
38 Jain T, Nikolaopoulou EA, Xu Q, Qu A. Hypoxia inducible factor as a therapeutic target for atherosclerosis. Pharmacol Ther 2018;183:22–33
39 Zhou W, Yin M, Cui H, et al. Identification of potential therapeutic target genes and mechanisms in non-small-cell lung carcinoma in non-smoking women based on bioinformatics analysis. Eur Rev Med Pharmacol Sci 2015;19(18):3375–3384
40 Huang Y, Yao T, Li X, et al. Bioinformatics analysis of key genes and latent pathway interactions based on the anaplastic thyroid carcinoma gene expression profile. Oncol Lett 2017;13(01):167–176
41 Jiang X, Hao Y. Analysis of expression profile data identifies key genes and pathways in hepatocellular carcinoma. Oncol Lett 2018;15(02):2625–2630
42 Ma Y, Guan RJ. Research progress of stromal cell derived factor1 and its receptor 4 and atherosclerosis. Chin J Evid Based Cardiovasc Med 2013;5(05):323–324
43 Lin QW, Zhang S, Lu WQ. Research progress of NOD-like signaling pathways and the relationship between NOD and tumor. Chin Oncol 2019;29(03):223–228
44 Zhu M, He QH. Research progress of the role of Chinese medicine in autoimmune inflammatory diseases by regulating Th17 cell differentiation. J Hunan Univ Tradit Chin Med 2019;36(08):82–86