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Network pharmacology-based screening of the active ingredients and mechanisms of Huangqi against aging

Siyu Lan, MM[a], Jie Duan, MM[b], Nan Zeng, MD[c], Bin Yu, MM[a,*], Xuping Yang, MD[d], Hong Ning, MM[a], Yilan Huang, MM[d], Youyi Rao, MM[a]

Abstract

Studies have shown that Huangqi (HQ) has anti-aging efficacy. However, its active ingredients and mechanisms for anti-aging are still unclear. In this study, we will systematically screen the active ingredients of HQ and explore the possible mechanism of HQ in prevention from aging through network pharmacology technology.

The main active ingredients of HQ were obtained from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). The possible targets were predicted by TCMSP. The related targets for aging were obtained from GeneCards (The Human Gene Database) and Online Mendelian Inheritance in Man (OMIM) database. The common targets of HQ and aging were obtained using R 3.6.3 software. The protein–protein interaction (PPI) network and the ingredient-target-disease network were constructed using Cytoscape 3.7.2 software for visualization. In addition, the Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotation of potential targets were performed using R 3.6.3 software.

Based on the screening conditions, 16 active ingredients and 28 drug targets were obtained. The PPI network contained 29 proteins, including PTGS2, AR, NOS2, and so on. GO functional enrichment analysis obtained 40 GO items ($P < .05$). KEGG pathway enrichment analysis obtained 110 aging related pathways ($P < .05$), including hypoxia inducible factor 1 signaling pathway, PI3K-Akt signaling pathway, AGE-RAGE signaling pathway in diabetic complication, among others.

Sixteen effective ingredients of HQ and 28 targets against aging were identified through network pharmacology. Multiple pathways were involved in the effect of HQ on preventing aging.

Abbreviations: ADME = absorption, distribution, metabolism and elimination, AR = androgen receptor, AR = androgen receptors, CHRM3 = muscarinic acetylcholine receptor M3, DL = drug similarity, ESR1 = estrogen receptor 1, GO = Gene Ontology, HQ = Huangqi, KEGG = Kyoto Encyclopedia of Genes and Genomes, NOS2 = nitric oxide synthase2, OB = oral bioavailability, OMIM = Online Mendelian Inheritance in Man, PPARG = peroxisome proliferator-activated receptor gamma, PPI = protein-protein interaction, PTGS2 = prostaglandin G/H synthase2, TCM = traditional Chinese medicine, TCMSP = Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform.

Keywords: active ingredients, aging, huangqi (HQ), network pharmacology

1. Introduction

Aging is defined as the gradual decline of internal physiological functions, which increases the age-specific mortality and age-specific fertility decline.[1] Multiple strategies were explored to prevent aging, including natural molecules,[2] the vitamin C, vitamin E, raspberry leaf cell culture extract,[3] Fillerina (one commercially available dermocosmetic filling treatment),[4] and coenzyme Q10.[5] In traditional Chinese medicine (TCM), some

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[a] *Department of Pharmacy, Mianyang Central Hospital, Mianyang,* [b] *Department of Clinical Pharmacy, Pidu District People's Hospital,* [c] *School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu,* [d] *Department of Pharmacy, The Affiliated Hospital of Southwest Medical University, Luzhou, China.*

[*] *Correspondence: Bin Yu, Department of Pharmacy, Mianyang Central Hospital, No. 12, Changjia Alley, Jingzhong Street, Mianyang 621000, China (e-mail: yubin202008@163.com).*

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herbals have also been used for anti-aging, such as *Lycium barbarum*.[6]

HQ, also known as *Astragalus membranaceus*, is one of the most important qi tonic drugs in TCM.[7] HQ has the functions of protecting liver,[8] diuresis,[9] eliminating fatigue,[10] and enhancing human immunity.[1] Recent studies show that HQ and its ingredients activate telomerase and inhibit the process of replicating aging.[11] It also has antioxidant activity and neuroprotective effect.[11,12] Therefore, HQ has the great potential to prevent aging. However, the active components and of HQ are still unclear. Due to the complexity of TCM ingredients, exploring the possible mechanisms is still difficult.

Network pharmacology is based on the interdisciplinary theory of pharmacology and biology, which employs a variety of cutting-edge technologies such as omics, high-throughput screening, and Network analysis.[13] It can reveal the complex network relationship between "ingredient-gene-target-disease," helps to understand the molecular basis of disease from multi-dimensional, and predict the potential pharmacological mechanism of drugs.[14] In this study, we will systematically screen the active ingredients of HQ and explore the possible mechanism of HQ in prevention from aging through network pharmacology technology.

2. Materials and methods

Ethical approval was waived or not necessary, all procedures performed in studies do not involve human participants or annimals.

2.1. Screening active ingredients

The flowchart of this study is shown in Figure 1. The active ingredients of HQ were firstly collected from TCM for Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmspw.com/tcmsp.php), a unique system pharmacology platform constructed for Chinese Herbal Medicines.[15]

First, we input the name of the herbal medicine to record the related ingredients. Then, the plasma absorption components were predicted by setting thresholds (oral bioavailability [OB] ≥ 30% and drug similarity [DL] ≥ 0.18) in the comprehensive absorption, distribution, metabolism, and elimination screening system.[16] OB is an important indicator for identifying the nature of classified drugs, representing the percentage of unaltered drugs that reach the systemic circulation, and can be used to determine the dosing regimen.[17,18] DL estimates the "drug-like" degree of a hypothetical ingredient, which helps to optimize its pharmacokinetics and drug properties.[19]

2.2. Screening targets for aging

In GeneCards platform (https://www.genecards.org/) and OMIM platform (https://www.omim.org/), aging, as the key word, was used to get the target of aging. Two copies intersection of data were used to get relevant targets of aging.

2.3. Target prediction and validation

Target prediction can help to elucidate the underlying mechanisms of HQ. We used TCMSP and DrugBank databases (https://www.drugbank.ca/) to search and verify the relevant targets of the ingredients. The UniProt database (https://www.uniprot.org/) was employed to converse the target name to the official name.

2.4. Network construction

To analyze the interaction between targets, we uploaded the relevant targets of HQ against aging to the STRING (https://string-db.org/) platform. Species column was set as Homo sapiens. We performed combined score screening to get protein

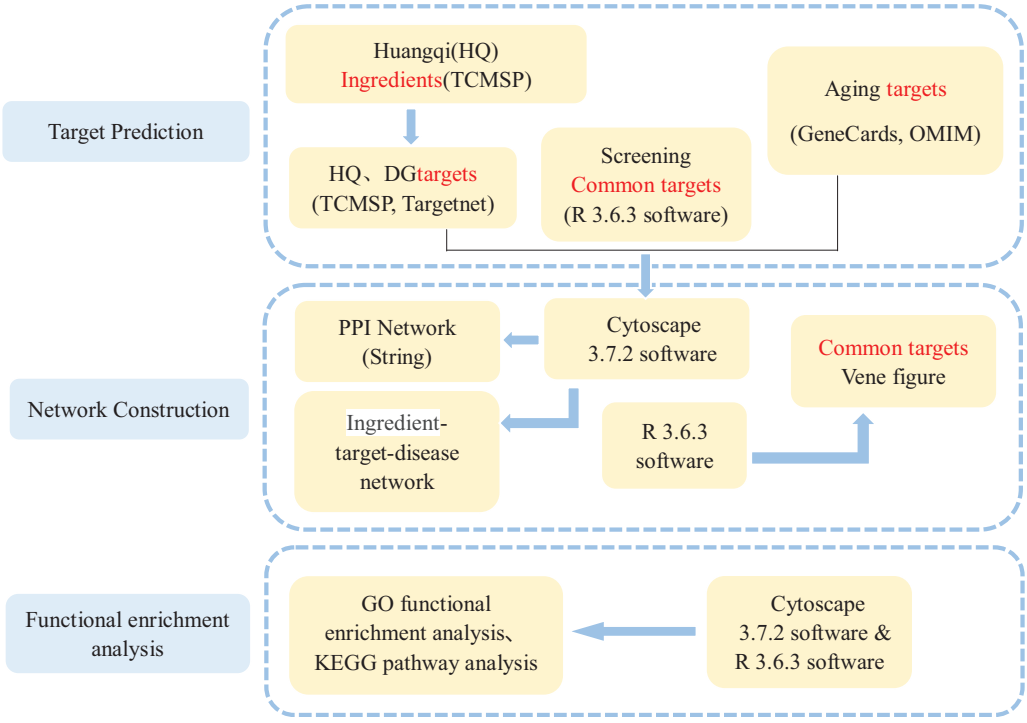

Figure 1. The flowchart of this study.

Table 1

The main active components of HQ.

No.	Mol ID	Mol name	Related targets	OB%	DL
1	MOL000211	Mairin	PGR	55.38	0.78
2	MOL000239	Jaranol	NOS2, PTGS1, AR, SCN5A, PTGS2, ESR2, CHEK1, PRSS1, NCOA2	50.83	0.29
3	MOL000296	Hederagenin	PGR, NCOA2, CHRM3, CHRM1, CHRM2, ADRA1B, GABRA1, GRIA2, ADH1B, ADH1C, LYZ, PTGS1, SCN5A, PTGS2, RXRA, SLC6A2	36.91	0.75
4	MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	PGR	36.23	0.78
5	MOL000354	Isorhamnetin	NOS2, PTGS1, ESR1, AR, PPARG, PTGS2, ESR2, MAPK14, GSK3B, PRSS1, CCNA2, NCOA2, PYGM, PPARD, CHEK1, AKR1B1, NCOA1, F7, ACHE, GABRA1, MAOB, GRIA2	49.60	0.31
6	MOL000371	3,9-di-O-methylnissolin	NOS2, PTGS1, CHRM3, CHRM1, ESR1, ADRB1, SCN5A, PTGS2, HTR3A, ADRA2C, RXRA, ACHE, ADRA1B, ADRB2, ADRA1D, OPRM1, GABRA1, PRSS1, NCOA2	53.74	0.48
7	MOL000378	7-O-methylisomucronulatol	NOS2, PTGS1, CHRM3, KCNH2, CHRM1, ESR1, AR, ADRB1, SCN5A, PPARG, CHRM5, PTGS2, ADRA2C, CHRM4, RXRA, OPRD1, ADRA1A, CHRM2, ADRA1B, SLC6A3, ADRB2, ADRA1D, SLC6A4, ESR2, GABRA1, MAPK14, GSK3B, CHEK1, RXRB, PRSS1, CCNA2, NCOA2	74.69	0.30
8	MOL000379	9,10-dimethoxypterocarpan-3-O-beta-D-glucoside	PTGS2, NCOA2	36.74	0.92
9	MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	NOS2, PTGS1, CHRM3, CHRM1, ESR1, SCN5A, PTGS2, HTR3A, RXRA, ACHE, ADRA1B, ADRB2, ADRA1D, GABRA1, PRSS1, NCOA2, NCOA1, CHRM4	64.26	0.42
10	MOL000387	Bifendate	PTGS2, KDR, MET, PTGS1	31.10	0.67
11	MOL000392	Formononetin	NOS2, PTGS1, CHRM1, ESR1, AR, PPARG, PTGS2, RXRA, ADRA1A, SLC6A3, ADRB2, SLC6A4, ESR2, MAPK14, GSK3B, MAOB, CHEK1, PRSS1, CCNA2, PKIA, ACHE, JUN, ATP5F1B, ND6	69.67	0.21
12	MOL000417	Calycosin	NOS2, PTGS1, ESR1, AR, PPARG, PTGS2, RXRA, ESR2, MAPK14, GSK3B, CHEK1, PRSS1, CCNA2, NCOA2, ADRB2	47.75	0.24
13	MOL000422	Kaempferol	NOS2, PTGS1, AR, PPARG, PTGS2, NCOA2, PRSS1, PGR, CHRM1, ACHE, SLC6A2, CHRM2 ADRA1B, GABRA1, F7, BCL2, JUN, MAPK8, MMP1, HMOX1, CYP3A4, CYP1A2, SELE, VCAM1, ALOX5, GSTP1, AHR, INSR, PPP3CA, GSTM1, GSTM2, AKR1C3	41.88	0.24
14	MOL000433	FA	GSK3B	68.96	0.71
15	MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	PTGS2, RXRA, PRSS1	39.05	0.48
16	MOL000098	Quercetin	PTGS1, AR, PPARG, PTGS2, NCOA2, AKR1B1, PRSS1, KCNH2, SCN5A, ADRB2, MMP3, F7, RXRA, ACHE, GABRA1, MAOB, EGFR, VEGFA, BCL2, PLAU, MMP2, MAPK1, EGF, RB1, TNFSF15, JUN, IL6, TP63, POR, ODC1, TOP1, SOD1, MMP1, ACACA, HMOX1, CYP3A4, CYP1A2, F3, GJA1, IL1B, CCL2, SELE, VCAM1, PTGER3, SULT1E1, MGAM, IL2, PLAT, THBD, COL1A1, IFNG, ALOX5, MPO, GSTP1, NQO1, AHR, COL3A1, INSR, ACPP, CTSD, ACPP, CTSD, GSTM1, GSTM2	46.43	0.28

AR = androgen receptor, DL = drug similarity, HQ = Huangqi, NOS2 = nitric oxide synthase2, OB = oral bioavailability, PPARG = peroxisome proliferator-activated receptor gamma.

interaction PPI network. Cytoscape 3.7.2 software was used to construct the HQ active ingredient-target-disease network.

2.5. GO Function and KEGG pathway enrichment analysis

To further understand the function of the core target genes and the main pathways of HQ against aging, The DAVID (https://david.ncifcrf.gov/) database, the Cytoscape 3.7.2 software, and R 3.6.3 software were used to perform GO function and KEGG pathway enrichment analysis on the targets at $P < .05$.

3. Results

3.1. Active ingredients of GQ

Eighty-seven ingredients of HQ were retrieved through the TCMSP platform. According to screening conditions (OB > 30% and DL > 0.18), 20 main active ingredients were screened, and 4 of them failed to find relevant targets. The targets were further analyzed through DrugBank and UniProt databases to check the correlation with aging. Specific details on these active ingredients and related targets of HQ were shown in Table 1.

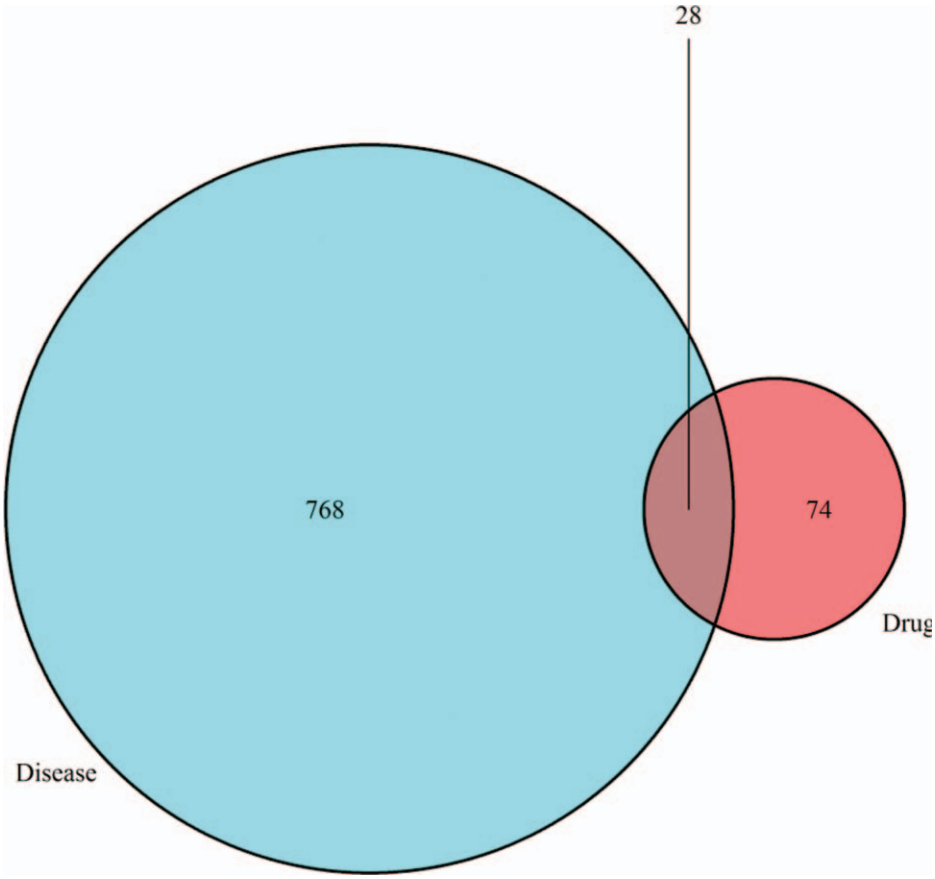

Figure 2. The vene diagram of HQ therapy and prevention of aging-related targets.

3.2. Targets of aging

Through the GeneCards platform (https://www.genecards.org/) and the OMIM platform (https://www.omim.org/), 23,993 targets for aging were identified. According to the relevance score of GeneCards platform, 184 targets with a score of ≥30 points were selected. A total of 623 targets were obtained from OMIM platform. The duplicate targets were eliminated. Finally, 796 targets related to aging were identified. The venn diagram of the drug and disease co-acting target was shown in Figure 2 and we identified 28 targets for the common action of drug and disease.

3.3. Network construction and analysis

3.3.1. PPI Network and active ingredient-target-disease network. PPI network is constructed based on protein interaction. There were 29 protein nodes and 182 interaction lines (Fig. 3A). The *P* value of PPI enrichment is 1.0e-16 ($P < .05$). We established an active ingredient-target-disease network to facilitate the exploration of the overall regulatory mechanism of HQ in aging prevention. HQ can prevent aging through multiple ingredients and multiple targets, which was shown in (Fig. 3B).

3.4. GO function enrichment analysis

The Cytoscape 3.7.2 software and R 3.6.3 software were used to analyze the 62 potential targets of HQ against aging using GO annotation analysis. We obtained 40 related biological processes, molecular functions, and cellular components. The GO bar plot and bubble plot of the first 20 entries was shown in Figure 4. GO function enrichment analysis showed that the main biological processes of active ingredients of HQ were cytokine activity, growth factor receptor binding, cytokine receptor binding, receptor ligand activity, phosphatase binding, hormone binding, nuclear receptor activity, transcription factor activity, direct ligand regulated sequence-specific DNA binding, heme-binding, growth factor binding, protein phosphatase binding, tetrapyrrole binding, steroid hormone receptor activity, RNA polymerase II transcription factor binding, growth factor activity, platelet-derived growth factor binding, antioxidant activity, steroid hormone receptor binding, RNA polymerase II basal transcription factor binding, and protease binding.

3.5. KEGG pathway enrichment analysis

The Cytoscape 3.7.2 software and R 3.6.3 software were used to analyze the 62 potential targets of HQ against aging using KEGG pathway enrichment analysis. One hundred ten related signaling pathways were obtained. The bar plot and dot plot of the first 20 entries was shown in Figure 5. KEGG pathway enrichment analysis showed that the main signaling pathways and diseases of active ingredients of HQ were hypoxia inducible factor 1 signaling pathway, AGE-RAGE signaling pathway in diabetic complications, relaxin signaling pathway, IL-17 signaling

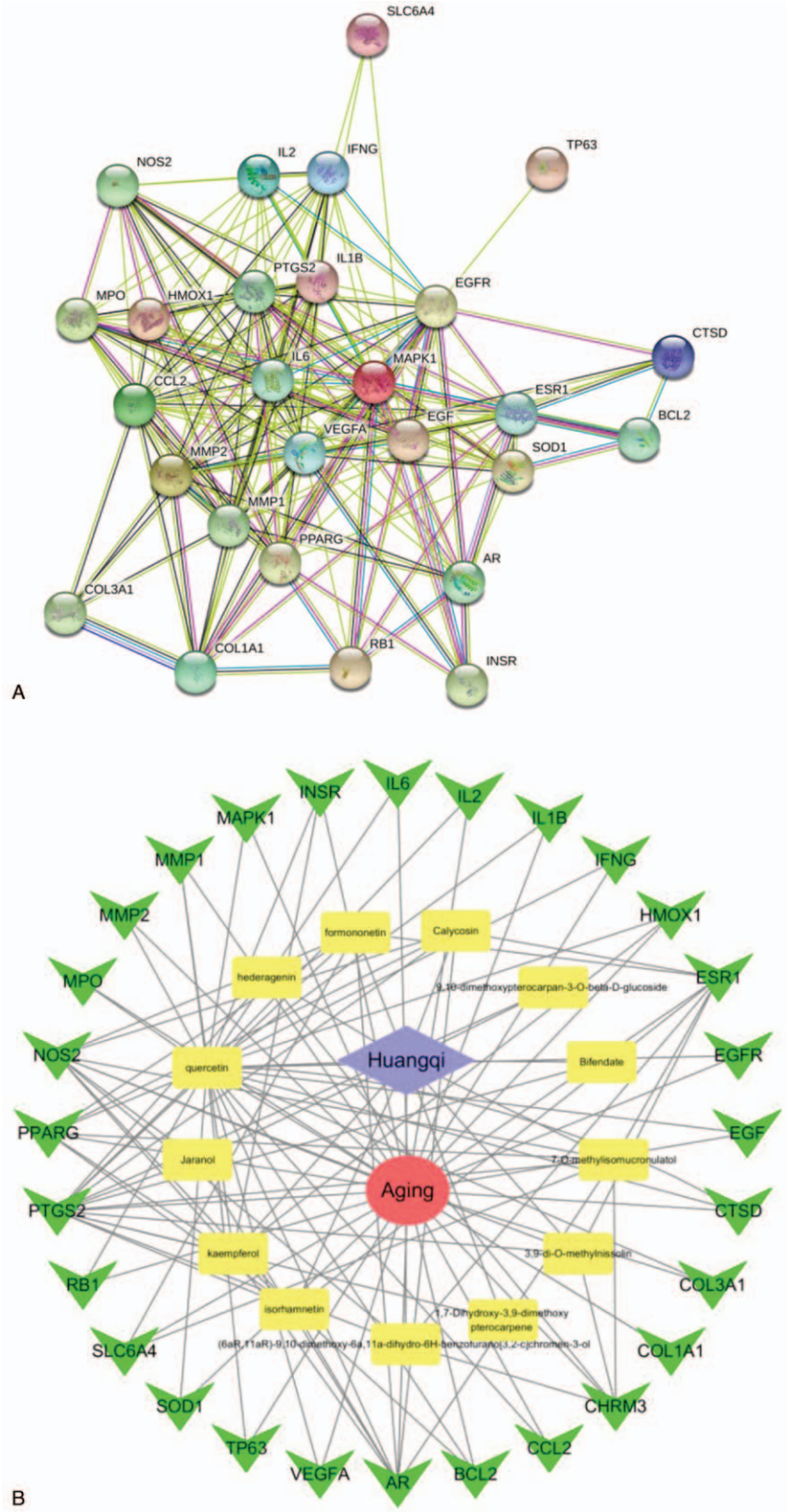

Figure 3. PPI network of HQ active ingredients and Ingredient-Target-Disease network between HQ and aging. Blue represents HQ, red represents aging, yellow represents the effective ingredients of HQ, and green represents the common targets of the 2. HQ = Huangqi, PPI = protein–protein interaction.

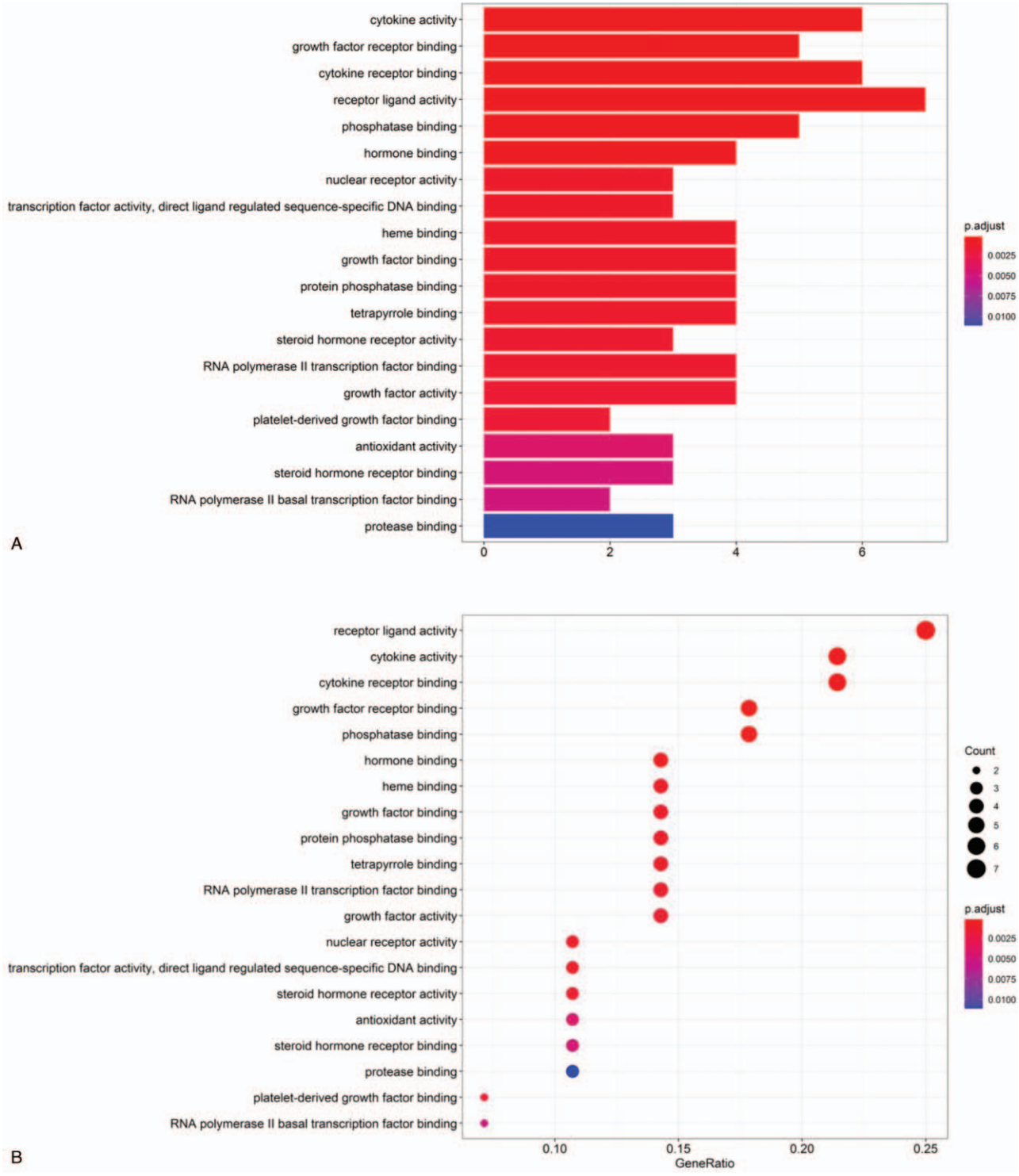

Figure 4. The bar plot of HQ active ingredients GO function enrichment analysis. GO = Gene Ontology, HQ = Huangqi.

pathway, PI3K-Akt signaling pathway, estrogen signaling pathway, bladder cancer, chagas disease (American trypanosomiasis), fluid shear stress and atherosclerosis, human cytomegalovirus infection, prostate cancer, endocrine resistance, EGFR tyrosine kinase inhibitor resistance, rheumatoid arthritis, amoebiasis, tuberculosis, pancreatic cancer, prion diseases, leishmaniasis, and graft-versus-host disease.

4. Discussion

Many studies have shown that HQ has significant preventive effects on aging.[20–22] However, the pharmacological mechanism of HQ against aging has not been clearly clarified. In this study, we used network pharmacology strategies to address this problem. After screening and verification, 16 potential active ingredients and 28 corresponding potential targets were retained.

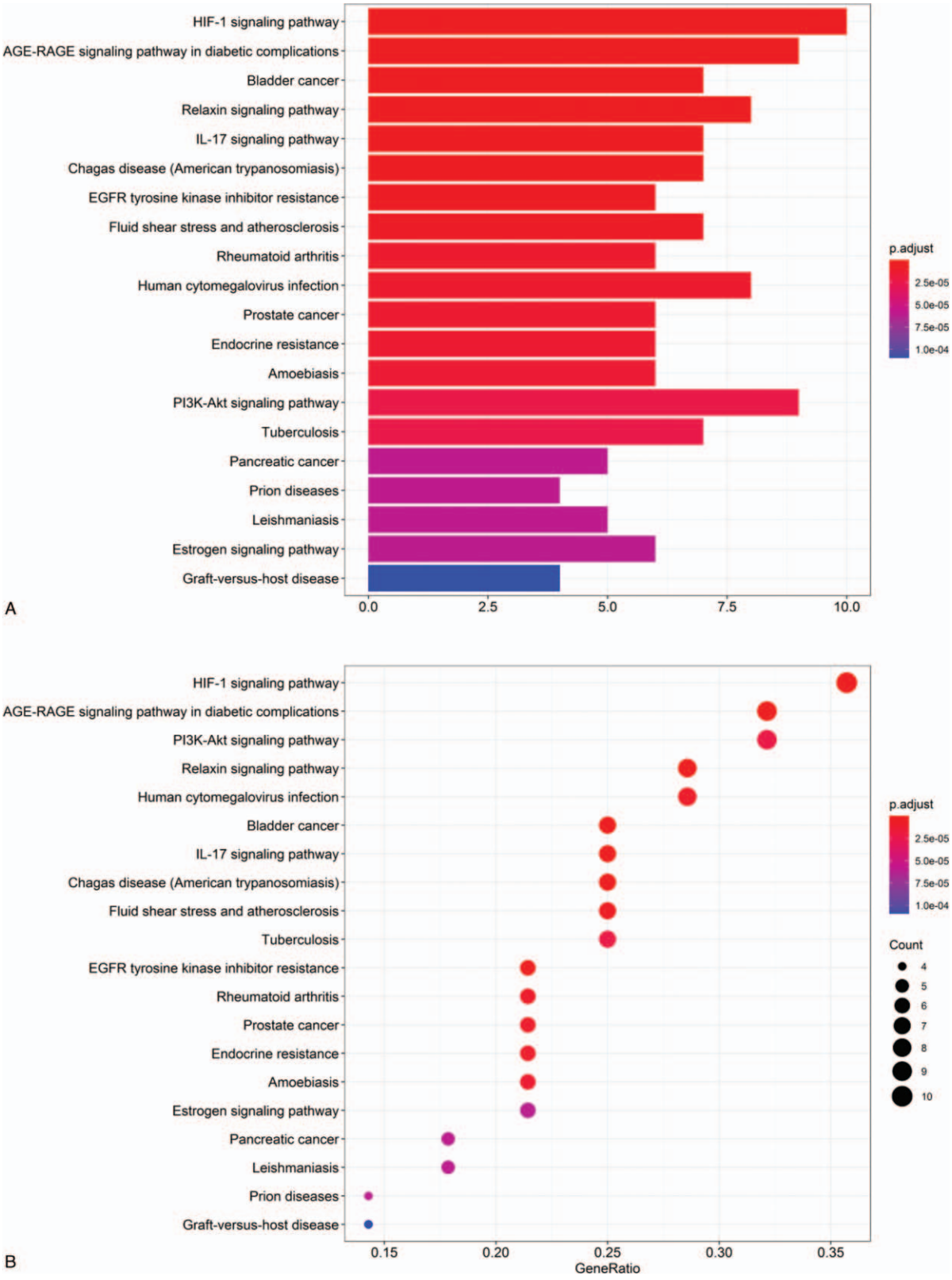

Figure 5. The bar plot of HQ active ingredients KEGG pathway enrichment analysis. HQ = Huangqi, KEGG = Kyoto Encyclopedia of Genes and Genomes.

We also established the PPI network and ingredient-target-disease network, and conducted GO and KEGG enrichment analysis to systematically and comprehensively understand potential preventive mechanism of HQ on aging.

PPI network analysis showed that multiple active ingredients interacted tightly with multiple targets. According to degree ranking of main targets, prostaglandin G/H synthase2 (PTGS2), androgen receptor (AR), nitric oxide synthase2 (NOS2), peroxisome proliferator-activated receptor gamma (PPARG), estrogen receptor 1 (ESR1), and muscarinic acetylcholine receptor M3 (CHRM3) are the important targets involved in HQ against aging. PTGS2, encoding for prostaglandin-endoperoxide synthase2, cyclooxygenase-2, or COX-2, plays indispensable roles in premature aging.[23,24] Low et al also identified the age-related changes of testosterone and androgen receptors (ARs) in male rats, and the serum content of androgen receptor immunoreactivity is decreased with the age of rats.[25] Moxibustion can inhibit reproductive aging by increasing the expression of AR.[26] Therefore, we speculate that AR has a greater impact on male aging than females, and may be negatively correlated with growth of age. NOS2 has also been shown to be closely related to human skin aging.[27] Interestingly, we reported that the 3 human genes (*NOS1*, *-2*, *-3*) coding for the 3 isoforms of the NADPH-dependent enzymes had an effect on common age related phenotypes and longevity in humans.[28] PPARG is an important metabolic control factor that can extend lifespan. In mouse models, decreased expression of PPARG reduces lifespan.[29] Studies showed that ESR1 regulated female premature ovarian failure and provided a new entry point for revealing the genetic mechanisms involved in ovarian reserve and oocyte aging.[30,31] Hardy et al found that CHRM3 seemed to decline with maturation and aging and to be less expressed in males.[32] There are few studies on the prevention of aging by CHMR3. So, the role of this target is still unclear. In total, HQ may prevent aging through targeting PTGS2, AR, NOS2, and ESR1 PPARG and CHRM3.

According to degree ranking in ingredient-target-disease network, the top 5 active ingredients are quercetin, formononetin, calycosin, kaempferol, and isorhamnetin. Quercetin is a natural ingredient with antioxidant potential, and aging is a deteriorating process that reduces physical and functional potential due to oxidative stress.[33,34] Quercetin supplements are considered as promising natural protective ingredients that can be used to delay the aging process and maintain human health.[35] Formononetin has protective effect on the skin against UV-induced oxidation.[36] Formononetin can penetrate into the epidermis and dermis and has the potential to prevent skin aging in cosmetics.[37] In Hsu and Chiang's research,[38] they found that calycosin had skincare functions, but the mechanism remains unknown. Zhang et al showed that calycosin alone does not promote the proliferation of aging hematopoietic stem cells, but it can significantly promote the proliferation of aging hematopoietic stem cell in combination with calycosin-7-glucoside. In addition, calycosin, formononetin, astragalus glycosides, and ferulic acid have a synergistic effect in promoting cell proliferation and inhibiting cell senescence.[39] Therefore, we speculate that calycosin alone has no obvious effect on aging, but it may enhance the anti-aging effect of other substances. Kaempferol is a natural flavonoid and present in different plant species. It has the therapeutic properties of anti-inflammatory, antioxidant and anti-cancer. Kaempferol can alleviate aging by reducing oxidative stress and improving mitochondrial function.[40] Hussin et al[41] found that isorhamnetin had anti-aging properties using proton nuclear magnetic resonance methods. Totally, these 5 substances are the main ingredients of HQ, and they all have certain anti-aging effects.

The enrichment of GO biological processes in prevention of aging by HQ mainly involves cytokine activity, cytokine receptor binding, receptor ligand activity, and so on. Cytokines are proteins secreted by cells and play important roles in maintaining normal cell function.[42] Cytokines play important roles in inflammation. Inflammaging is also a key to understand aging. So, the cytokines may be the potential target of HQ against aging.[43]

The KEGG pathway enrichment analysis showed that the prevention of HQ on aging mainly involves hypoxia inducible factor 1 signaling pathway, PI3K-Akt signaling pathway, and AGE-RAGE signaling pathway. Studies have shown that hypoxia potentially contributes to functional decline during the aging process.[44] PI3K and AKT are novel targets for chemical modulation of telomere protection against aging.[45] AGE/RAGE also mediate cellular dysfunction and affect the aging process.[46] So, these signal pathways may also be the targets of HQ against aging.

5. Conclusion

In this study, we used network pharmacology to analyze the effective ingredients and potential targets of HQ in preventing aging. Sixteen effective ingredients of HQ and 28 targets were identified. Multiple ingredients, multiple targets, and multiple pathways were involved in the effect of HQ on preventing aging.

Author contributions

Conceptualization: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Data curation: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Formal analysis: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Funding acquisition: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Investigation: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Methodology: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Project administration: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Resources: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Software: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Supervision: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Validation: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Visualization: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Writing – original draft: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.
Writing – review & editing: Siyu Lan, Jie Duan, Nan Zeng, Bin Yu, Xuping Yang, Hong Ning, Yilan Huang, Youyi Rao.

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