Transcriptional profiling and network pharmacology analysis identify the potential biomarkers from Chinese herbal formula Huosu-Yangwei formula treated gastric cancer in vivo

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[ABSTRACT] Huosu Yangwei (HSYW) formula is an oriental medicine that has been extensively used to combat chronic atrophic gastritis, precancerous lesions of gastric cancer and advanced gastric cancer clinical therapy. However, the effective compounds of HSYW and its related anti-tumor mechanisms are indistinct. In this study, 160 ingredients of HSYW were identified and 64 effective compounds were screened by the ADMET evaluation. Furthermore, 64 effective compounds and 2579 potential targets were mapped based on public databases. The animal experiment demonstrated that HSYW significantly inhibited tumor growth in vivo. Transcriptional profiles revealed that 81 mRNAs have significantly differential expressed in HSYW-treated N87-bearing Balb/c mice. Network pharmacology and PPI network showed that 12 core genes acted as potential markers to evaluate the curative effects of HSYW. Bioinformatics and q-PCR results suggested that HSYW might regulate the mRNA expression of DNAJB4, CALD, AKR1C1, CST1, CASP1, PREX1, SOCS3 and PRDM1 to against tumor growth in N87-bearing Balb/c mice.

[KEY WORDS] Huosu Yangwei (HSYW) formula; Network pharmacology; protein-protein interaction (PPI) network; Gastric Cancer

Introduction

Gastric cancer is the fifth most frequently malignant tumor and the third leading cause of cancer related mortality in the world [1]. The epidemiology study revealed that the lifestyle and environmental factors are strongly correlated to the etiology of gastric cancer [2]. In the past decades, the occurrence of gastric cancer has reduced dramatically and the survival rate has been increasing particularly [3]. Although a marker of early-malignant lesion was identified from the unique endocrine cells of gastric cancer [4]. However, gastric cancer is remains a major cause of the global burden of cancer.

Chinese herbal medicine has various advantages and achieved the treatment and prevention of tumor such as multiple ingredients, multiple targets and lower side effect [5,6]. Huosu Yangwei (HSYW) formula is an oriental medicine, including Huoxiang, Zisugeng, Baizhu, Zhike, Doukou, Foshou, Wumei, Shengjiang, Dazao, Gancao, Huangqi, Dihuang, Mudanpi, Tianhuafen, Danggui, Ezhu, Gouqizi, Huanglian, Dangsheng, and Pugongying. The previous studies demonstrated that HSYW has been extensively applied for the treatment of chronic atrophic gastritis and precancerous lesions of gastric cancer [7-9]. In advanced gastric cancer treatment, HSYW can significantly relieve clinical symptoms of patients, improve the quality of life, and reduce the incidence of adverse reactions after chemotherapy [10]. Furthermore,
HSYW also can significantly inhibit gastric cancer cells growth in vitro \cite{11}. These studies suggested that HSYW might have worthy anti-tumor values for gastric cancer treatment. However, HSYW contains a total of 21 traditional Chinese herbal, and it is difficult to explore the complex mechanisms of HSYW on gastric cancer by traditional pharmacological methods due to the multi-ingredients, multi-targets and multi-pathways of the formula. Network pharmacology has a unique advantage on Chinese herbal medicine research, which is a systematic approach to disclose the mechanisms of drug \cite{12,13}. Therefore, more and more researchers have begun to use network pharmacology to explore the material basis of TCM, not only reveal the complex interactions between herb ingredients and proteins \cite{14,15}, but also provide an available approach to evaluate the pharmacological effects of Chinese medicine from multiple dimensional perspective \cite{16-19}.

In this study, we identified 160 ingredients of HSYW using the HPLC/MS method, and 64 effective compounds were screened out based on ADMET evaluation. To understand the anti-tumor mechanisms of HSYW, we offer transcriptional profiles of HSYW treated gastric cancer in vivo, and identified several potential markers in the process of curative effects evaluation of HSYW in gastric cancer treatment.

**Materials and methods**

**HSYW ingredients identification**

The HSYW is a hospital preparation of Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, and produced in Shanghai Baolong Pharmaceutical Co., Ltd (Trade Name: Huosu Yangwei Koufuye, Batch Number: Z05050328).

The HSYW solution was activated by 5 mL methanol, and balanced by 60% methanol solution in advance. Then, the sample solution was extracted by 1 mL through SPE column (Waters ACQUITY UPLC® HSS T3, 2.1 × 100 mm, 1.8 µm), eluted by 5 mL 60% methanol solution and collected eluent. Added 10 mL 100% methanol solution to elute again and collected eluent. Combine the twice collected eluents, and concentrate at 40°C until dry. Dissolve again with 1 mL methanol solution, centrifuge (5 min, 12 000 rpm), and take the supernatant to the standard HPLC (Utimite, Themo, US) for fingerprint. Using Sciex Triple TOF 4600 LC/MS instrument, the sample solution was extracted by 1 mL through SPE (Waters ACQUITY UPLC® HSS T3, 2.1 × 100 mm, 1.8 µm), eluted by 5 mL 60% methanol solution to elute again and collected eluent. Added 10 mL 100% methanol solution to elute again and collected eluent. Combine the twice collected eluents, and concentrate at 40°C until dry. Dissolve again with 1 mL methanol solution, centrifuge (5 min, 12 000 rpm), and take the supernatant to the standard HPLC (Utimite, Themo, US) for fingerprint. Using Sciex Triple TOF 4600 LC/MS instrument, the mass spectra of different compounds were achieved.

**Effective compounds evaluation and targets prediction**

Firstly, the ADMET evaluation was used to screen the effective compounds from the identified ingredients of HSYW. The absorption, distribution, excretion and toxicity of each compound was calculated using SwissADME \cite{20} and pkCSM database \cite{21}. Secondly, the TCMSP, TCMIP, TCMD, ZINC and SwissTargetPrediction \cite{22-26} were used to predict the potential targets of effective compounds of HSYW.

**Network construction and Enrichment analysis**

Using the effective compounds and predicted targets, the Compound-Target network (CTN) of HSYW was constructed by Cytoscape software (version 3.8). The nodes represent compounds or targets, edges represent the connection among nodes. Furthermore, the DAVID online \cite{27,28} were used to analyzed the biological functions of potential targets of HSYW, and the significantly terms were defined as P < 0.05, for gene sets containing more than 5 overlapping genes.

**Animal**

Male Balb/c mice (4 weeks old) were purchased from Shanghai Model Organisms Center (SCXK Shanghai 2014-0002), and feeding in SPF Animal Laboratory of Shanghai University (SYXX Shanghai 2014-0008). The mice were housed in polycarbonate cage at a standard air-conditioned room (23 ± 2 °C, 55 + 10% R.H.). All animal procedures were approved by the Shanghai University of Traditional Chinese Medicine Ethical Committee on Animal Experiments, Shanghai, China. The whole studies abide by the 3Rs principle and ARRIVE guidelines of animal experiments.

**Model establishment of GC mice and drug treatment**

**Gastric cancer animal model**

The human gastric cancer cell line N87 was purchased from the Institute of Biochemistry and Cell Biology (Shanghai, China). The cell line N87 was cultured in DMEM medium (Gibco, USA) at 37°C in a humidified 5% CO2 incubator, which was supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and 100 U/mL penicillin-streptomycin mixed antibiotics.

Mice were weighed and randomly divided into 3 groups including the control group (n = 10), model group (n = 10) and HSYW group (n = 10). Logarithmic phase N87 cells (5 × 10^6/mL) were inoculated in the right axillary subcutaneous inoculation and 100μl per mouse.

**Drug treatment**

Mice were treated with the same dose of HSYW as the clinical equivalent of human (body weight, 60 kg), and the conversion coefficient was 10. Finally, the total dose of 20 g mouse was 60 mL/60 kg × 0.02 kg × 10.0 = 0.2 mL every day. The mice in blank groups were treated with 0.1% sodium chloride solution (0.2 mL per mouse, one time per day) for 25 days. One week after inoculation, the mouse weight was weighed every three days. Tumor volumes were measured with vernier caliper, and tumor volume = a × b^2 / 2 (a represents longest diameter, and b represents shortest diameter of tumor).

**Pathological examination**

The hematoxylin and eosin (H.E) staining was performed as indicated in previous reports \cite{29}. The tumor tissue was immersed in 4% paraformaldehyde for 4 h, and transferred to 70% ethanol. Then, these tissues were dehydrated through a series alcohol gradient, embedded into paraffin wax blocks and obtained 5-um-thick tissue sections. Furthermore, sections were dewaxed with xylene and rehydrated by decreasing ethanol concentrations, washed in PBS buffer and...
then stained. After staining, tissue sections were dehydrated using the increasing ethanol concentrations and xylene. Finally, the images were scanned by the pathological image analysis system (Olympus Corporation, Tokyo, Japan).

**Transcriptional profiles detection**

Using RNA sequencing, the transcriptional profiles of HSYW treated gastric cancer mice (HSYW Group, HSYW, n = 3) and gastric cancer mice (Model Group, M, n = 3) were detected, respectively. Total RNA were extracted from the tumor tissues using the mirVana miRNA Isolation Kit (Ambion) following the manufacturer’s protocol. RNA integrity was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The samples with RNA Integrity Number (RIN) ≥ 7 were subjected to the subsequent analysis. The libraries were constructed using TruSeq Stranded, and then were sequenced on the Illumina sequencing platform (HiSeqTM 2500). Raw reads generated during high-throughput sequencing were fastq format sequences. In order to get high-quality reads that could be used for later analysis, raw reads needed to be further quality filtered.

**Differential expressed mRNA analysis**

The mRNAs expression levels were calculated using FPKM (Fragments Per kb Per Million Reads) algorithm. Based on DESeq software, the mRNA counts of all samples were normalized, and the difference significance test of reads was performed by NB (Negative binomial distribution), respectively. Furthermore, R package was used to identify the differentially expressed (DE) mRNAs between the HSYW Group and Model Group (Fold-Change > 1.5, P < 0.05). Heat-map and hierarchical analyses were performed using Cluster 3.0 and Java TreeView programs. The biological functions of DE mRNAs were analyzed using DAVID online, and significance was defined as P < 0.05.

**Protein-protein interaction (PPI) network construction**

To investigate the biological functions of the mRNA profiles of HSYW treated gastric cancer, the DE mRNAs were mapped for compound-target network, and the isolated DE mRNAs were used to construct a PPI network by the IntAct, BioGRID and MINT databases. Furthermore, the consecutive parameters of the network were calculated, including betweenness centrality (BC), closeness centrality (CC), degree (De) and topological coefficient (TC). In this work, the core node of the network was defined as BC ≥ avg (BC), CC ≥ avg (CC), De ≥ avg (De), and TC ≥ avg (TC).

**QRT-PCR validation**

The quantitative reverse transcription-Polymerase Chain Reaction (qRT-PCR) was carried out to identify the mRNA levels of potential markers in HSYW-treated gastric cancer mice. The quantification of mRNA was performed with SYBR Green PCR Master Mixture (TOYOBO, LTD, Japan). Melting curve was used to verify the specificity of each PCR product, and Ct was reckoned as the number of cycle requirement. All mRNAs were validated in triplicate and the mRNA levels were calculated using 2ΔCt. The Student’s t-test was utilized to estimate the mRNAs expression levels, and differences with P value < 0.05 were considered significant.

The primer sequences were designed in the laboratory and synthesized by Generay Biotech (Generay, PRC) based on the mRNA sequences obtained from the NCBI database (Table 1). The expression levels of mRNAs were normalized to ACTB.

**Survival analysis for core genes expression in GC patients**

In this work, the GEPIA database (Gene Expression Profiling Interactive Analysis, http://gepia.cancer-pku.cn/) was used to evaluate the expression levels and survival curves of GC patients of key genes on clinical prognosis, which analyses RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA (The Cancer Genome Atlas) and the GTEx (Genotype-Tissue Expression) projects.

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**Table 1 The primer sequences obtained from the NCBI database**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Forward primer (5’- 3’)</th>
<th>Reverse primer (5’- 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR1C1</td>
<td>ACAATAATGAGGAGCAGGTT</td>
<td>AGGTAGAGGTCACACTAATCC</td>
</tr>
<tr>
<td>ALDH4A1</td>
<td>GGACCTGAAGCCTATTTGC</td>
<td>CGGAAGAAGTCGTAGGTT</td>
</tr>
<tr>
<td>CALD1</td>
<td>CAGAGGAATGAGCATGTAGTA</td>
<td>TTGTGGTGTTGCTTGG</td>
</tr>
<tr>
<td>CASP1</td>
<td>CACATCCTCAGGCTCAGA</td>
<td>CGGTGCTGCTGCTCATT</td>
</tr>
<tr>
<td>CBR3</td>
<td>CAAGCAGTTACTCCGGATA</td>
<td>TCTCCTTCTGTGATGTCT</td>
</tr>
<tr>
<td>CST1</td>
<td>AAGGAGGAGGATAGGATATCA</td>
<td>CTTCATATACGCTGATGG</td>
</tr>
<tr>
<td>DNAJB4</td>
<td>GCTGATGGAAGGAGGTACA</td>
<td>ACAATTCGTCTGGAATACT</td>
</tr>
<tr>
<td>FBXL16</td>
<td>CCTGGACATCTGTGATTC</td>
<td>GACATCTGTCAAGCATACC</td>
</tr>
<tr>
<td>PIGR</td>
<td>TGTTACGGTTGTCACTCA</td>
<td>GAAGCGACATTTCTTCTT</td>
</tr>
<tr>
<td>PRDM1</td>
<td>GGATTCTGGTGGCTGATGG</td>
<td>AACTGTGCTGATGCTGAGTA</td>
</tr>
<tr>
<td>PREX1</td>
<td>CCGAGGAGAATGTCAAGG</td>
<td>CTCGTACACGAGAATTCTT</td>
</tr>
<tr>
<td>SOCS3</td>
<td>CCACCTGGACCTCCTATGA</td>
<td>TTGGCTCTTGTGCTTGT</td>
</tr>
</tbody>
</table>
Statistical analyses

The statistical analysis was conducted by SPSS 23.0 software (IBM Analytics), and all values were presented as mean ± SD with three independent experiments. Using two-tailed Student’s t tests to assess the differences between groups, and P value < 0.05 was considered statistically significant.

Results

Effective compounds identification and potential targets prediction of HSYW

In this work, 160 ingredients of HSYW were identified using the HPLC/MS method (Supplementary Table 1). And then, 64 effective compounds were screened out from the identified ingredients based on the ADMET analysis (Supplementary Table 2). Furthermore, a total of 2579 potential targets of 64 effective compounds were predicted from the databases, and the constructed Compound-Target network (CTN) was shown in Figure 1A.

Enrichment analyses of HSYW targets

The HSYW potential targets were conducted enrichment analysis using DAVID online [27, 28]. In top 20 KEGG pathways, 12 terms were correlated with signaling pathways, such as ErbB signaling pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway, cAMP signaling pathway, Chemokine signaling pathway, Rap1 signaling pathway, T cell receptor signaling pathway, Fc epsilon RI signaling pathway, protein kinase binding, receptor binding, drug binding, and protein binding (Figure 1E).

HSYW inhibit tumor growth in N87-bearing Balb/c mice

To evaluate the effects of HSYW inhibited tumor growth, the N87-bearing Balb/c mice were used to construct...
tumor model and treated by HSYW. After 25 days of HSYW treatment, the tumor volume and weight results revealed that the growth of tumors was significantly inhibited in HSYW treated N87-bearing Balb/c mice (Figure 2A -C). The H.E staining results revealed that the nucleus and cytoplasm of HSYW groups are compact and dark stained, while the models with loose nucleus and pale staining (Figure 2D). It means that the function of tumor cells in the model group was more vigorous than that in HSYW group. These results showed HSYW could be against tumor growth significantly in vivo.

**Transcriptional profiles analysis**

In this study, the mRNA profiles between HSYW treated (n = 3) and N87-bearing Balb/c mice (n = 3) were detected by RNA sequencing to identify the differentially expressed (DE) mRNAs. Using R package, 81 DE mRNAs were identified (fold change > 2, P < 0.05), including 33 up-regulated mRNAs and 48 down-regulated mRNAs in HSYW-treated mice (Figure 2E). The enrichment analysis showed that the Gene Ontology (GO) terms (top 10) mainly associated with regulation of neutrophil degranulation, regulation of neutrophil activation, integrin-mediated signaling pathway, superoxide metabolic process, retinoid metabolic process, collagen metabolic process, diterpenoid metabolic process, myeloid leukocyte migration, lymphocyte differentiation, and T cell differentiation (Figure 2F). The KEGG pathways (top 10) mainly associated with Pertussis, Hedgehog signaling pathway, Staphylococcus aureus infection, Metabolism of xenobiotics by cytochrome P450, Complement and coagulation cascades, Rheumatoid arthritis, Arginine and proline metabolism, Amoebiasis, Legionellosis, and drug metabolism (Figure 2G).

**Compound-DEG network and PPI network construction**

To screen the important targets of HSYW, the DE mRNAs were mapping to the Compound-Target network (CTN). As shown in Figure 3A, the Compound-DEG network was isolated from CTN, which including 47 compounds and 39 DE mRNAs. Furthermore, the 39 DE mRNAs related protein-protein interaction (PPI) network was constructed by the IntAct, BioGRID and MINT databases (Figure 3B). In the PPI network, the consecutive parameters were calculated, respectively, and the core nodes were defined as BC ≥ avg (BC), CC ≥ avg (CC), De ≥ avg (De), and TC ≥ avg (TC). Finally, 12 core clusters were isolated from PPI network, including 6 up-regulated (DNAJB4, CALD1, AKR1C1, CST1, ALDH4A1, and FBXL16) and 6 down-regulated genes (PRDM1, CBR3, PIGR, SOCS3, CASP1, and PREX1) (Figure 3C).

Furthermore, the QRT-PCR was performed to validate the expression levels of 12 core mRNAs between model and HSYW groups. As showed in Figure 3D, the transcriptional...
Expression levels of 12 core genes were significantly regulated between model and HSYW groups ($P < 0.05$). These results suggest that the 12 mRNAs are potential markers in the process of curative effects evaluation of HSYW, and play critical roles in gastric cancer treatment.

Survival analysis for potential markers

Using GEPIA, the expression levels of 12 core genes between gastric cancer ($n = 408$) and normal ($n = 36$) were calculated, respectively, and the results revealed that the expression of DNAJB4, CALD1 and AKR1C1 were significantly decreased, and CST1, CASP1 and PREX1 were significantly increased in tumor tissues (Figure 4A). Furthermore, we also explored the prognostic values of 12 core genes in gastric cancer patients based on the overall survivals (OS) calculation (Supplementary Figure 1). The results showed that SOCS3 and PRDM1 have statically significance ($P = 0.0067$ and 0.02) of OS in gastric cancer patients (Figure 4B).

However, we noticed that the expression levels of SOCS3 and PRDM1 have no difference between gastric cancer and normal. To explore this phenomenon, we further analyzed the expression levels of SOCS3 and PRDM1 among the different tumor grades in gastric cancer. The results showed that SOCS3 and PRDM1 have differential expressions between tumor grade1 and grade3 (Figure 5). In fact, the differential expression levels reveal this gene is associated with tumorigenesis, while the survival rate is correlated with tumor progression. This phenomenon suggested that SOCS3 and PRDM1 are high correlated with cancer progression, but not associated with gastric carcinogenesis.

Discussion

As a traditional Chinese medicine, Huosu Yangwei (HSYW) formula has worthy anti-tumor values for advanced gastric cancer treatment [10]. However, the molecular mechanisms of HSYW treated gastric cancer still unclear. In this work, we firstly identified the main compounds of HSYW based on HPLC/MS method. Then, the transcriptional profiles of HSYW treated N87-bearing Balb/c mice were detected by RNA sequencing. KEGG pathways reveal that the
Hedgehog signaling pathway and Metabolism of xenobiotics by cytochrome P450 might more important for HSYW treated gastric cancer. Hedgehog signaling pathway plays an important role during inflammation and carcinogenesis of gastric epithelial cells \[39\], it can mediate PD-L1 expression level and promote tumor proliferation in gastric cancer \[32\]. Furthermore, Hedgehog signaling pathway is important in the maintenance of CD44 (+) cells, and acts to reverse chemotherapy resistance in these cells and may be beneficial in gastric cancer patients whose tumors express high levels of CD44 \[33\]. Cytochrome P450 enzyme family plays a critical role in the metabolism of various xenobiotics \[34\], especially in chemical carcinogenesis by activating or inactivating carcinogens, which impacts the initiation and promotion of tumors \[35\]. In gastric cancer, the cytochrome P450 1A1 (CYP1A1) expression significantly increases in cancerous tissues \[36\] and cytochrome P450 2A6 (CYP2A6) polymorphisms are associated with the efficacy of S-1 in the adjuvant setting for gastric cancer \[37\]. Our previous study also demonstrated that the HSYW can strongly inhibit a range of human CYPs in a reversible manner \[38\]. These evidences demonstrated that the Hedgehog signaling pathway and Metabolism of xenobiotics by cytochrome P450 might high correlated with gastric cancer process.

Furthermore, 12 core genes were selected based on Compound-DEG network and PPI network. Q-PCR validation showed that the DNAJB4, CALD1, AKR1C1, CST1, ALDHHA1 and FBXL16 was up regulated, and PRDM1, CBR3, PIGR, SOCS3, CASP1 and PREX1 was down regulated in HSYW treated mice. Using GEPIA analysis, the expression levels of DNAJB4, CALD1, AKR1C1, CST1, ALDHHA1 and FBXL16 was up regulated, and PRDM1, CBR3, PIGR, SOCS3, CASP1 and PREX1 was down regulated in HSYW treated mice. Using GEPIA analysis, the expression of potential markers were calculated based on TCGA database. Tumor group, n = 408; Normal group, n = 36); (B) The prognostic value of SOCS3 and PRDM1 in gastric cancer patients by survival analysis.

**Fig. 4** Expression and survival analysis for potential markers based on GEPIA and TCGA databases. (A) Expression levels of 12 potential markers were calculated based on TCGA database. Tumor group, n = 408; Normal group, n = 36); (B) The prognostic value of SOCS3 and PRDM1 in gastric cancer patients by survival analysis.
correlated with cancer progression, but not associated with gastric carcinogenesis. Interestingly, our studies showed that HSYW significantly down-regulated the SOCS3 and PRDM1 expression levels in gastric cancer mice (Figure 3D). This phenomenon indicated that the HSYW can down-regulated the SOCS3 and PRDM1 expression levels and further to improve the survival rate of patients in gastric cancer progression.

Such being the case, DNAJB4, CALD1, AKR1C1, CST1, CASP1, PREX1, SOCS3 and PRDM1 were obtained from the 12 core genes, which were defined as high correlated with gastric cancer progression. In these eight genes, DNAJB4 belongs to the DNAJ (HSP40) family of Heat shock proteins (HSPs) and considered as a tumor suppressor in various tumors [39]. In gastric cancer, DNAJB4 might act as a sensor of E-cadherin structural features and contribute to tumor progression [40]. CALD1 is a calmodulin- and actin-binding protein that has been revealed to display opposite roles in cancer and invasion [41], which is involved with cell proliferation and migration in gastric cancer [42]. AKR1C1 is a member C1 of the human aldo-keto reductase family, and the upregulated AKR1C1 was associated with a variety of cancers [43]. In gastric cancer, activation of the Nrf2/AKR1C axis may contribute to oxaliplatin resistance [44]. CST1 belongs to the cystatin superfamily and inhibits the proteolytic activities of cysteine proteases [45]. It can activate Wnt signaling to promote gastric cancer migration and invasion [46], and is highly involved in tumorigenesis [47]. As a member of the cysteine-aspartic acid protease (caspase) family, CASP1 is involved in diverse cellular processes regulation [48, 49], and higher CASP1 mRNA expression was associated with better overall survival (OS) in gastric cancer patients [50]. Phosphatidylinositol-3,4,5-trisphosphate dependent Rac exchange factor 1 (PREX1), is frequently upregulated in many tumors [51], and the high expression of PREX1 revealed poor prognosis in advanced gastric cancer patients [52]. SOCS3, suppressors of cytokine signaling 3, is a negative regulator of JAK-STAT signaling pathway [53]. In gastric cancer, SOCS3 was identified to be the best predictor of lymph node metastasis [54], and high SOCS3 inhibited cell proliferation, arrested cell cycle and facilitated apoptosis [55]. PRDM1 is a positive regulatory domain zinc finger protein and plays an important role in B and T cell differentiation [56]. Especially, PRDM1 functions as a tumor suppressor and play differential prognostic impact across different cancers [57]. These evidences suggested that the DNAJB4, CALD1, AKR1C1, CST1, CASP1, PREX1, SOCS3 and PRDM1 were high correlated with gastric cancer progression.

In conclusion, we identified 160 ingredients from HSYW and obtained 64 effective compounds based on ADMET analysis. Animal experiment demonstrated that HSYW significantly inhibited tumor growth in vivo. Transcriptional profiles
and network pharmacology showed that 12 core genes acted as potential markers to evaluate the curative effects of HSYW. Bioinformatics analyses revealed that the DNAJB4, CALD1, AKR1C1, CST1, CASP1, PREX1, SOCS3 and PRDM1 were highly correlated with gastric cancer progression, and HSYW might regulate these gene expression levels to against tumor growth in N87-bearing Balb/c mice.

**Authors’ contribution**

Shengquan Fang, Hongmei Ni, Guangbo Ge and Qilong Chen participated in research design; Shengquan Fang, Yuehan Liu, Kunpeng Zhao, Xinghui Zhang, Hongwei Wang, Yuhai Deng and Yuxuan Zhou performed experiments; Shengquan Fang, Yuehan Liu and Qilong Chen wrote or contributed to the writing of the manuscript.

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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