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Network pharmacology dissection of multiscale mechanisms of herbal medicines in stage IV gastric adenocarcinoma treatment

Li Gao, PhD[a], Jian Hao, PhD[b], Yang-Yang Niu, MD[b], Miao Tian, BS[c], Xue Yang, PhD[b], Cui-Hong Zhu, BS[b], Xiu-Li Ding, MD[b], Xiao-Hui Liu, MD[b], Hao-Ran Zhang, MD[b], Chang Liu, BS[b], Xue-Mei Qin, PhD[a], Xiong-Zhi Wu, PhD[a,*]

Abstract

Increasing evidence has shown that Chinese Herbal Medicine (CHM) has efficient therapeutic effects for advanced gastric adenocarcinoma, while the therapeutic mechanisms underlying this treatment remain unclear.

In this study, the Kaplan–Meier method and Cox regression analysis were used to evaluate the survival benefit of CHM treatment, and correlation analysis was applied to identify the most effective components in the formulas. A network pharmacological approach was developed to decipher the potential therapeutic mechanisms of CHM.

CHM treatment was an independent protective factor. The hazard ratio was 0.364 (95% CI 0.245–0.540; $P < 0.001$). The median survival time was 18 months for patients who received CHM treatment, while for patients without CHM treatment was decreased to 9 months ($P < 0.001$). Thirteen out of the total 204 herbs were significantly correlated with favorable survival outcomes ($P < 0.05$), likely representing the most effective components in these formulas. Bioinformatics analyses suggested that the simultaneous manipulation of multiple targets in proliferation pathways (such as epidermal growth factor receptor, fibroblast growth factor receptor 2, human epidermal growth factor receptor 2, proliferating cell nuclear antigen, and insulin like growth factor 2) and the process of cancer metastasis (collagen families, fibronectin 1 and matrix metalloproteinases families) might largely account for the mechanisms of the 13 herbs against gastric adenocarcinoma.

A network pharmacology method was introduced to decipher the underlying mechanisms of CHM, which provides a good foundation for herbal research based on clinical data.

Abbreviations: CHM = Chinese Herbal Medicine, DL = drug-likeness, EGFR = epidermal growth factor receptor, ERBB2 (HER2) = human epidermal growth factor receptor 2, FGFR2 = fibroblast growth factor receptor 2, GEO = gene expression omnibus, MMP = matrix metalloproteinase.

Keywords: Chinese herbal medicine, clinical retrospective trial, gastric adenocarcinoma, network pharmacology

1. Introduction

Gastric carcinoma is the 5th most common cancer and the 3rd most frequent cause of cancer death worldwide.[1] The vast majority of gastric cancers are adenocarcinomas.[2] Without effective treatment, patients with advanced gastric cancer have a poor prognosis, with a median survival time of 4.3 months, and the median survival time with chemotherapy increases to 8.7 months.[3–6] Although the role of palliative gastrectomy in patients with advanced gastric cancer is unclear, several studies have suggested that resection might provide some survival benefit.[7–10] However, both chemotherapy and palliative operation are associated with significant morbidity and poor quality of life due to the high risk of complications.

In China, Chinese Herbal Medicine (CHM) treatment is one of the most frequently used complementary and alternative medicine therapies. According to preliminary studies, CHM therapy may help sustaining a reduced tumor size and relieving tumor-related symptoms.[11,12] Multimodality treatment using chemotherapy, palliative operation, and CHM has gained increasing favor for the treatment of gastric cancer patients in recent years.[13–15] Chinese herbal formula is a multicomponent and thus likely multitarget therapeutic, that can be included into the treatment protocols of integrative oncology. However, a herbal formula comprising of numerous compounds is too complex to be detected solely using conventional experimental

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[a] Modern Research Center for Traditional Chinese Medicine, Shanxi University, Taiyuan, [b] Key Laboratory of Cancer Prevention and Therapy, National Clinical Research Center for Cancer, Tianjin Medical University Cancer Institute and Hospital, Tianjin, [c] College of Chemistry and Chemical Engineering, Shanxi University, Taiyuan, P.R. China.

[*] Correspondence: Xiong-Zhi Wu, Zhong-Shan-Men Inpatient Department, Key Laboratory of Cancer Prevention and Therapy, National Clinical Research Center for Cancer, Tianjin Medical University Cancer Institute and Hospital, Huan-Hu-Xi Road, Ti-Yuan-Bei, He-Xi District, Tianjin 300060, P.R. China (e-mail: wuxiongzhi@163.com).

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methods. Thus, there is an urgent need to develop new and appropriate approaches to address this problem.

Network pharmacology, which clarifies the synergistic effects and the underlying mechanisms of multicomponent and multi-target agents through the analysis of networks, provides an understanding of the underlying complex relationships between CHM and diseases through the bridge "target."[16–18]

In the present study, as shown in Fig. 1, we investigated 154 patients with stage IV gastric adenocarcinoma to evaluate the efficiency of CHM treatment. We further identified effective prescription herbs that are closely correlated with survival using correlation analysis. Furthermore, we investigated the pharmacological mechanisms of the effective prescription herbs acting on gastric adenocarcinoma using bioinformatics approaches.

2. Methods

2.1. Patient characteristics

This study was performed under the approval of the Ethics Committee of Tianjin Medical University. CHM treatment can only be administered by doctors who have CHM prescription right. Therefore, in this retrospective study, the CHM-treated patients were recruited from Zhong-Shan-Men Inpatient Department of Tianjin Medical University Cancer Institute and Hospital. Patients in the non-CHM group were recruited from the Department of Gastrointestinal Cancer Surgery, where doctors were not permitted to prescribe Chinese herbal medication.

The major inclusion criteria were as follows: age 18 or older; diagnosed by biopsy specimen obtained during gastroscopy or surgery; received chemotherapy no less than 3 times; and patients in the CHM group received CHM treatment ≧2 months. The major exclusion criteria were as the following: serious complications (massive hemorrhage of the gastrointestinal tract, complete obstruction of the digestive tract, gastrointestinal perforation, severe cardiac or pulmonary diseases, etc.), radiotherapy or immunotherapy, concurrent cancer, incomplete medical records, lack of accurate documentation of recurrence time, and loss to follow-up. The medical records of 247 patients with stage IV gastric adenocarcinoma between March 2009 and October 2011 were retrospectively reviewed. A total of 93 patients were excluded for the following reasons: loss to follow-up or incomplete medical records (n = 53), 2nd primary malignancy (n = 5), serious comorbidities (n = 7), using herbal treatment provided from other hospital CHM specialists for patients in the non-CHM group (n = 5), less than 3 months (6 courses) of CHM treatment for patients in CHM group (n = 5), and with radiotherapy or immunotherapy (n = 18). A total of 154 patients who received conventional chemotherapy no less than 3 times were included. Fifty eight patients who received CHM treatment after or during chemotherapy were included in the CHM group, and 96 patients without any CHM treatment were included in the non-CHM group.

2.2. Treatments

CHM was administered according to the syndrome differentiation. In general, the formula used in the present study contained 20 to 30 types of Chinese medicinal substances. The formulas were administered orally 3 times a day, 30 minutes after meals for 2 months, or more. Every 2 weeks, some medicinal substances might be changed based on the symptoms of patients. CHM formulas were documented in the electronic prescription system. The patient formulas were collected every 2 weeks, starting from the date CHM treatment was received to the date the patients died or the time of data closure. By the end of the follow-up period, patients in the CHM-group received CHM treatment for 7376 days, with 204 types of medicinal substances including 185 plants, 15 animal products, and 4 mineral substances. Roots of 54 herbs, stems of 27 herbs, leaves of 8 herbs, flowers of 5 herbs, and seeds or fruits of 45 herbs were used. The whole plants of 46 herbs were used.

These Chinese medicinal substances were included for further analysis by the following criteria: single medicinal substance frequency/total frequency >10%; medicinal substances administered to patients surviving longer than 12 months (the median survival time of all patients); and correlation coefficient ≧0.25 and P-value < 0.05, based on the results of the correlation analysis.

2.3. Collection of significant genes associated with gastric adenocarcinoma

Significant genes associated with gastric adenocarcinoma were collected from the gene expression omnibus (GEO) database[19] using "gastric adenocarcinoma" as key words, and the relative literatures were simultaneously referenced.[20–23] The interrelations of these genes and their functional groups were analyzed

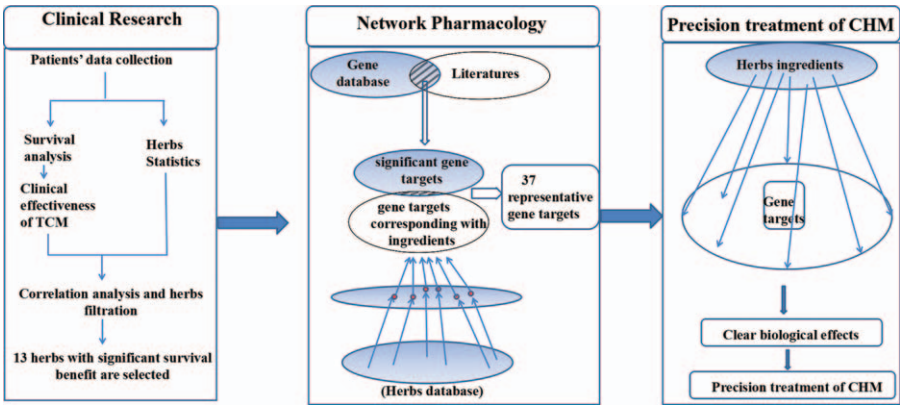

Figure 1. Process overview.

using Cytoscape plugin ClueGO,[24] and the genes involved in the functions of proliferation or cancer metastasis were selected. Subsequently, these genes were screened according to reported frequencies in Google Scholar to select genes highly correlated with gastric adenocarcinoma.

2.4. Herb formulation ingredient collection and target fishing

The chemical ingredients were collected from the Traditional Chinese Medicine Systems Pharmacology Database[25] (http://lsp.nwsuaf.edu.cn/tcmsp.php), and subsequently screened according to drug-likeness (DL) values, considering both pharmacodynamic and pharmacokinetic properties. The ingredients with DL values higher than 0.18 were retained for further investigation.

Target fishing was performed to identify or predict the potential targets of the chemical ingredients. The validated targets were extracted from the Herbal Ingredients' Targets Database[26] (http://lifecenter.sgst.cn/hit/). The predicted targets were obtained using ChemMapper[27] (http://lilab.ecust.edu.cn/chemmapper/), an online tool for predicting targets based on 3D similarity. The scores for the validated targets were defined as 1, and the scores for the predicted targets were determined using ChemMapper and ranged from 0 to 1. The proliferation (or metastasis) function scores of each herb were calculated by adding up the scores of all ingredients acting on all proliferation (or metastasis)-associated genes.

2.5. Network construction and analysis

Both validated and predicted targets were used to construct the networks. The ingredients-targets networks of herbs and herbs-targets networks were constructed using Cytoscape software (Version 3.2.1).[28] The networks were analyzed using Cytoscape plugin CentiScaPe to calculate topological parameters, including degree, betweenness, and closeness. The larger the node's degree/betweenness/closeness is, the more important the node is in the network.[28] For each herb, the top 15% ranked ingredients/targets (up to 6) were considered as putative major ingredients and targets.

2.6. Statistical analysis

Survival time was considered as the time from the day stage IV gastric adenocarcinoma was histologically confirmed or the date of diagnosis of recurrent/metastatic disease, until death from cancer or the last day the patient was witnessed alive. The clinicopathological features were based on computed tomography and endoscopy findings or pathologic diagnoses. The baseline comparison was obtained using the χ^2 test. Kaplan–Meier curves and multivariate Cox regression analysis were used to evaluate the differences in survival time. Spearman bivariate correlation analysis was used to determine the correlation between herbs and survival time. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 19.0.

3. Results

3.1. Survival characteristics

This study included 154 analyzed patients with stage IV gastric adenocarcinoma. All patients received conventional chemotherapy no less than 3 times, and 58 patients received CHM treatment. The median survival time of the 154 patients was 12 months (95% confidence interval 10.6–13.4). The univariate analysis revealed that the primary tumor size ($P = 0.044$), ascites ($P = 0.023$), weight loss ($P = 0.031$), Karnofsky performance status (KPS) of 80 or less ($P < 0.001$), primary tumor differentiation ($P = 0.008$), and distant spread patterns ($P < 0.001$) were significantly associated with the reduced median survival time. The Cox regression analysis showed that Karnofsky performance status KPS, ascites, poor tumor differentiation, and distant spread patterns were independent predictors of poor survival. Palliative operation and CHM treatment were independent protective factors. The hazard ratio (HR = Exp[β]) of CHM treatment was 0.364 and the associated 95% confidence intervals ranged from

Table 1

Univariate and multivariate analyses of variables assessing for impact on survival.

	Univariate analysis		Multivariate analysis		
Variable	***P***	**N**	**Exp (β)**	**95% CI for exp (β)**	***P***
Age, year (≤60/>60)	0.321	83/71	–	–	–
Gender (male/female)	0.906	105/89	–	–	–
Primary tumor size	*0.044*		–	–	0.126
≤4		37	0.559	0.301–1.039	0.066
5–10		98	0.564	0.316–1.007	0.053
>10 cm		19	1	1	–
KPS ≤ 80 (yes or no)	*<0.001*	19/105	1.944	1.270–2.976	***0.002***
Weight loss (yes or no)	*0.031*	52/102	–	–	0.794
Ascites (yes or no)	*0.023*	40/114	1.830	1.140–2.938	***0.012***
Primary tumor location (cardia; body; pylorus)	0.134	47/34/73	–	–	–
Primary tumor differentiation (high and moderate vs poor)	*0.008*	58/96	1.999	1.340–2.980	***0.001***
The pattern of distant spread	*<0.001*		–	–	***0.006***
Hematogenous		56	1.799	1.077–3.003	0.025
Local		49	0.749	0.433–1.295	0.300
Implantational		19	1.277	0.642–2.537	0.486
Lymphatic circulation	–	30	1	1	–
Chemotherapy regimens	0.975	–	–	–	–
Palliative operation (yes or no)	*0.001*	55/99	0.506	0.340–0.754	***0.001***
CHM (yes or no)	*<0.001*	58/96	0.364	0.245–0.540	***<0.001***

CI = confidence interval, CHM = Chinese Herbal Medicine, KPS = Karnofsky performance status.

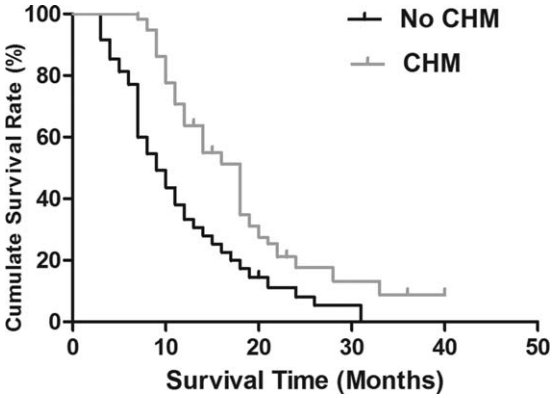

Figure 2. Kaplan–Meier curve between CHM and non-CHM groups. Patients with CHM treatment had a longer median survival time than those without CHM treatment (18 vs 9 months, $P < 0.001$). CHM = Chinese Herbal Medicine.

0.245 to 0.540 ($P < 0.001$). Detailed data are presented in Table 1. The baselines of the patient demographics were equal between patients with and without CHM treatment (Table S1, http://links.lww.com/MD/B218). Patients in the CHM group had a longer median survival time (18 months) compared with the non-CHM group (9 months). The 1- and 2-year survival rates for the CHM and non-CHM groups were 63.8%, 17.6% and 33.3%, 8.0%, respectively ($P < 0.001$; Fig. 2).

Patients in CHM-group received a total of 7376 days of herbal treatment. Treatment was continued for a minimum of 90 days and a maximum of 584 days. Patient who had the longest medicine-taken time survived 24 months. The average treatment time for patients who survived longer than 12 months (the median survival time of all patients) was 151 days. The average treatment time for patients who survived shorter than 12 months was 93 days.

3.2. Candidate genes associated with gastric adenocarcinoma

A total of 338 genes differentially expressed or harboring genetic variations in gastric adenocarcinoma were obtained from the GEO database after removing redundant entries (Table S2, http://links.lww.com/MD/B218) and categorized according to different functions using ClueGO (Table S3, http://links.lww.com/MD/B218). As shown in Fig. 3, these genes were primarily involved in cancer metastasis and cell proliferation, including the regulation of cell adhesion, cell migration, extracellular matrix disassembly, blood vessel development, apoptotic signaling pathway, response to growth factor, positive regulation of cell proliferation, etc. Other biological functions were important for development and metabolism. After gene screening, 37 genes highly correlated with metastasis and cell proliferation in gastric adenocarcinoma were selected for further analysis.

3.3. Candidate herbs associated with gastric adenocarcinoma and their putative major ingredients and targets

A total of 13 types of herbs were significantly associated with the survival of patients with stage IV gastric adenocarcinoma based on the correlation analysis ($P < 0.05$ for all). The ingredient-target networks of the herbs are illustrated in Fig. 4. The putative major ingredients were obtained after analyzing the topological parameters of the networks (Table 2). The major functional scores of herbs on proliferation and metastasis are also shown in Table 2. The number of ingredients, the serial number of each ingredient, DL values, and number of validated/predicted targets are shown in Table S4, http://links.lww.com/MD/B218. The detailed target information for the ingredients is shown in Table S5, http://links.lww.com/MD/B218.

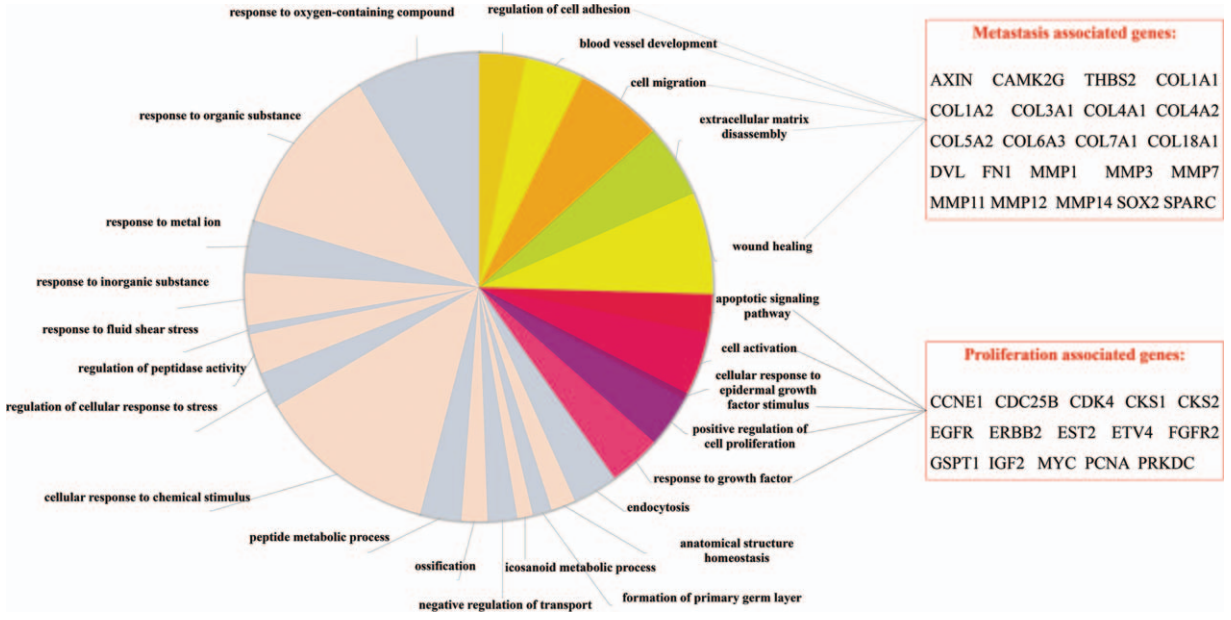

Figure 3. Categorization of all of the genes obtained from the ClueGO analysis according to biological process and molecular function.

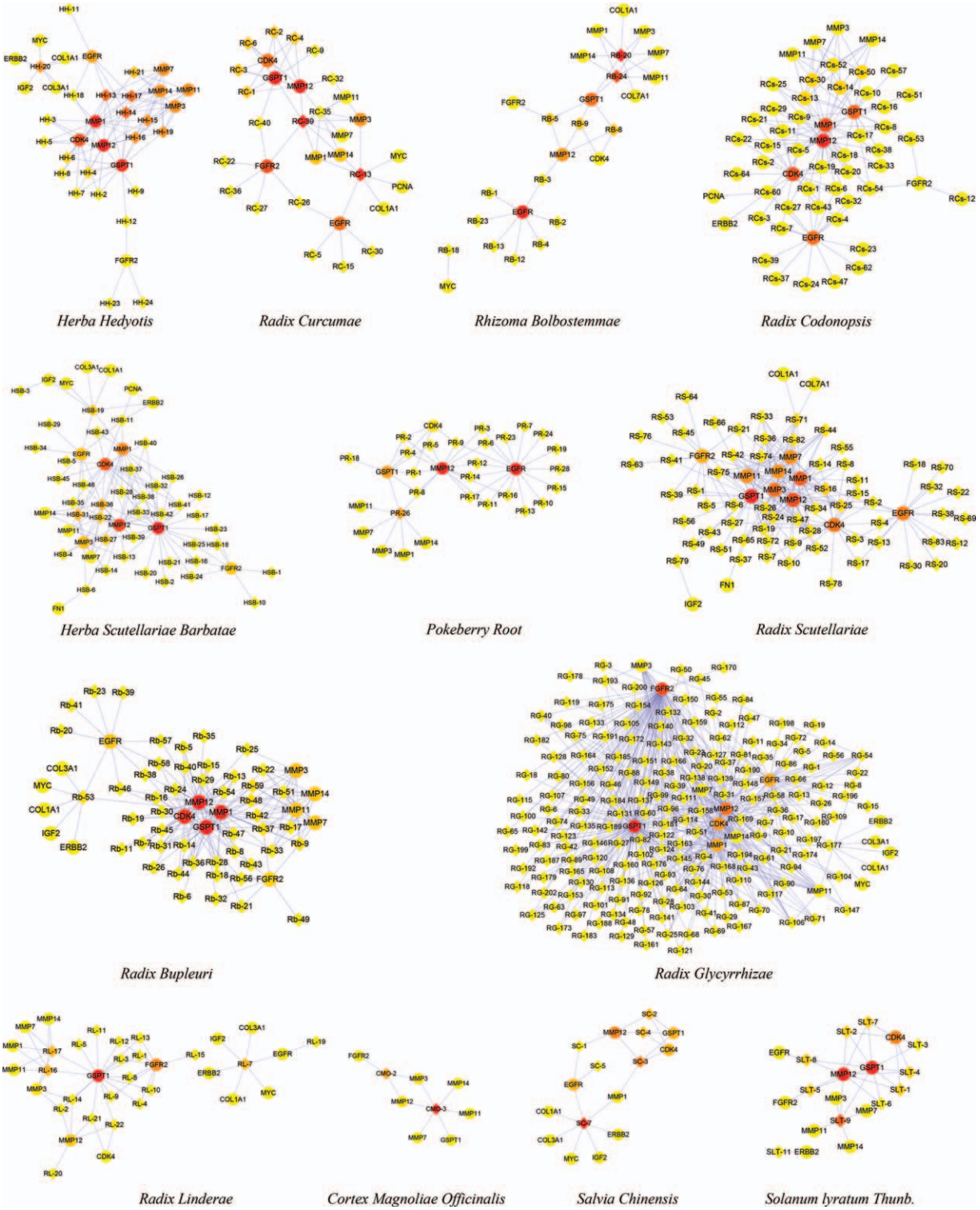

Figure 4. The ingredient-target networks. The diamond nodes represent ingredients; the circular nodes represent targets; and the colors of the nodes are illustrated from red to yellow in descending order of degree values.

3.4. Integrative analysis, pathway, and network of candidate herbs associated with gastric adenocarcinoma

The 13 herbs and 37 genes highly correlated with the development and progression of gastric adenocarcinoma were used to construct an integrative network. The synthetic herb-target network is shown in Fig. 5. In the present study, 18 cancer-related genes involved in different stages of oncogenesis were identified as targets of 13 herbs using network analysis. Interestingly, GSPT1 and matrix metalloproteinase (MMP)12 are predicted targets shared among all 13 herbs. Epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR)2, MMP3, MMP7, MMP11, and MMP14 are shared among 12 herbs. To better understand the gene functions

Table 2

Correlation coefficients of herbs to survival, and the function scores, putative major ingredients of 13 herbs.

Chinese name	Latin name	Function scores on proliferation	Function scores on metastasis	Correlation to survival	Major ingredients
甘草	*Radix Glycyrrhizae*	39.775	19.572	0.380	(2S)-7-Hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl)chroman-4-one (RG-168) 8-Prenylated eriodictyol (RG-138) Vitexin (RG-54) Glabranin (RG-169) Glepidotin B (RG-158) Glepidotin A (RG-157)
半枝莲	*Herba Scutellariae Barbatae*	13.321	8.773	0.293	(3S,8S,9S,10R,13R,14S,17R)-10,13-Dimethyl-17-[(E,1R,4R)-1,4,5-trimethylhex-2-enyl]-2,3,8,9,11,12,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3-ol (HSB-36) (8S,9S,10R,13R,14S,17R)-10,13-Dimethyl-17-[(E,1R,4R)-1,4,5-trimethylhex-2-enyl]-2,3,8,9,11,12,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthrene (HSB-31) Rivularin (HSB-27) 5,6,2'-Trihydroxy-7,8-dimethoxyflavone (HSB-22) Quercetin (HSB-19) Luteolin (HSB-11)
黄芩	*Radix Scutellariae*	11.206	6.900	0.360	3,4',5,7-Tetrahydroxyflavone-3-L-rhamnoside (RS-16) Sucrose (RS-71) Catalpol (RS-25) Salidroside (RS-82) Paniculatin (RS-44) Rivularin (RS-36)
党 参	*Radix Codonopsis*	8.615	5.401	0.390	Fritillaziebinol (RCs-50) 11-Hydroxyrankinidine (RCs-30) Poriferasta-7,22E-dien-3beta-ol (RCs-14) Spinasterol (RCs-13) Tangshenoside ii (RCs-52) Luteolin (RCs-60)
柴 胡	*Radix Bupleuri*	7.495	8.037	0.344	Isorhamnetin-3-mono-beta-D-glucoside (Rb-9) Spinasterol (Rb-59) Quercetin (Rb-53) Saikosaponin e_qt (Rb-48) PLO (Rb-42) Chikusaikoside II_qt (Rb-37)
白花 蛇舌草	*Herba Hedyotis*	6.171	6.295	0.458	Scandoside methyl ester (HH-13) Deacetyl asperulosidic acid methyl ester (HH-14) Geniposide (HH-17)
石见 穿	*Salvia Chinensis*	5.586	4.404	0.306	Quercetin (SC-7)
乌 药	*Radix Linderae*	5.085	3.481	0.322	Quercetin (RL-7) Boldine (RL-17) Norboldine (RL-16)
醋商陆	*Pokeberry Root*	4.368	0.732	0.380	2-[[(1R,4aS,8aR)-5,5,8a-Trimethyl-2-methylene-decalin-1-yl]methyl]hydroquinone (PR-26) Jaligonic acid (PR-9) Esculentic acid (PR-4)
郁金	*Radix Curcumae*	4.255	4.636	0.417	4,5-Epoxy-12-acetoxy-7a,11a-Dihydrogermacradien-8-one (RC-39) Curcumin (RC-13) Curcumenolactone C (RC-35)
土贝母	*Rhizoma Bolbostem-mae*	2.283	3.183	0.399	Sucrose (RB-20) 37417-41-7 (RB-24)
白英	*Solanum Lyratum Thunb.*	1.492	0.509	0.301	Volon (SLT-9)
厚朴	*Cortex Magnoliae Officinalis*	0.146	0.033	0.401	Eucalypto (CMO-2) Neohesperidin (CMO-3)

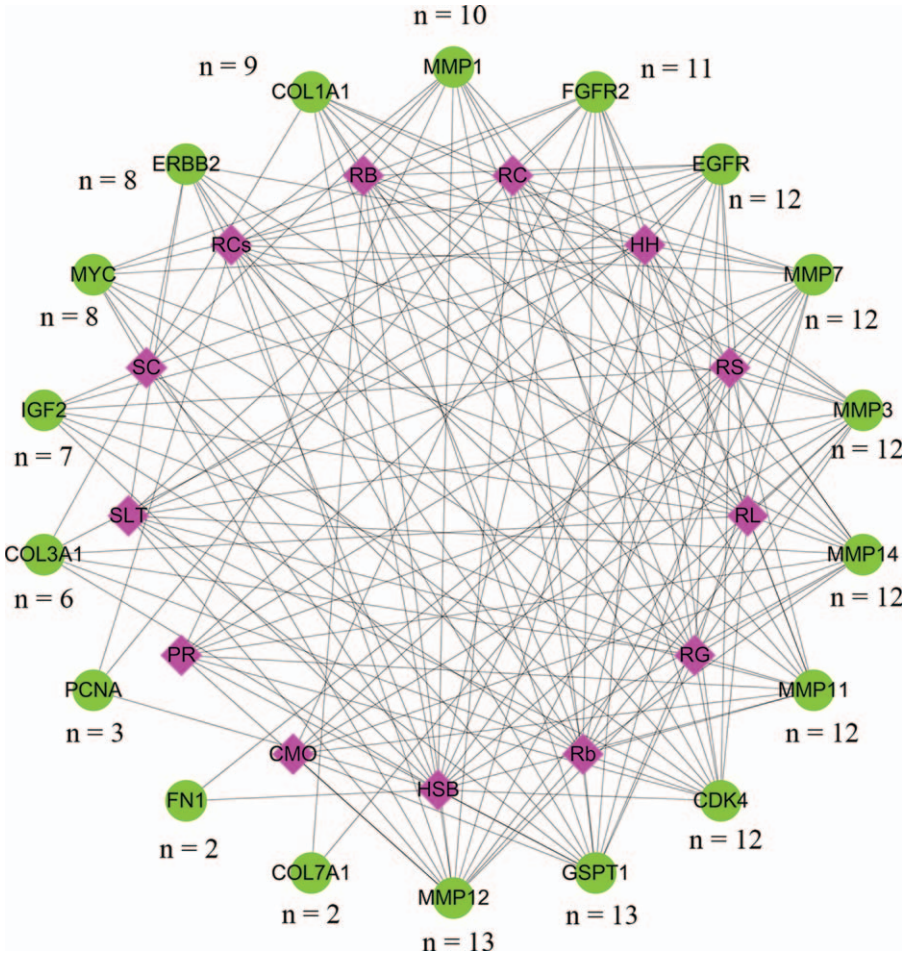

Figure 5. The herb-target networks of the 13 herbs. The diamond nodes represent herbs, and the circular nodes represent targets. The targets distributed in a circle indicate that these components function in the same number of herbs, which illustrated as "n."

associated with 13 herbs, we mapped the 18 genes to the canonical signaling pathways identified in the Kyoto Encyclopedia of Genes and Genomes and summarized the most relevant pathways (Fig. 6).

4. Discussion

Current therapeutic methods for stage IV gastric cancer include cytoreductive surgery, chemotherapy, and biotherapy. Compared with best supportive care, palliative operation and systematic chemotherapy prolonged the survival time of patients with stage IV gastric cancer.[3–10] However, the prognosis and quality of life of stage IV gastric cancer patients treated by these Western treatments are still below expectations. As one of the most frequently used complementary and alternative medicine therapies in China, CHM treatment may contribute to sustaining a reduced tumor size and relieving tumor-related symptoms.[11,12] In China, multimodality treatment using chemotherapy, palliative operation, and CHM has gained favor for the treatment of gastric cancer patients in recent years.[13–16] The results of the present study showed encouraging results of CHM treatment for gastric cancer patients. The results of the present study showed the efficacy of CHM treatment for gastric cancer patients. Patients in the CHM group had a longer median survival time (18 months) than those in the non-CHM group (9 months), with 63.8% 1-year and 17.6% 2-year survival rates.

Considering the multicomponent and multitarget characteristics of CHM herbal formulas, which are too complex to detect solely with conventional experimental methods, we 1st proposed a new analysis method to identify herbs significantly associated with survival. The analysis was realized as follows. First, all the prescriptions were collected, and the most frequently used herbs with high correlation coefficients in survival were included for further analysis. The pharmacological mechanisms of the effective herbs were further investigated using a network approach. Notably, most of the related herbs in conventional network pharmacology have been mined in previous wide-scale studies. The selected herbs might depart from practice without clinical information. The significance and largest difference between the present and conventional network pharmacology is that the present study is based on clinical data and is a true reflection of clinical medication.

Gastric adenocarcinoma, accounting for approximately 95% of gastric cancers, results from a combination of environmental and lifestyle factors as well as the accumulation of generalized and specific genetic alterations.[29] A sequence of molecular events for gastric adenocarcinoma have been proposed, including genes and molecules, such as growth factors and their receptors, cell-cycle regulators, cell-adhesion molecules, and matrix-

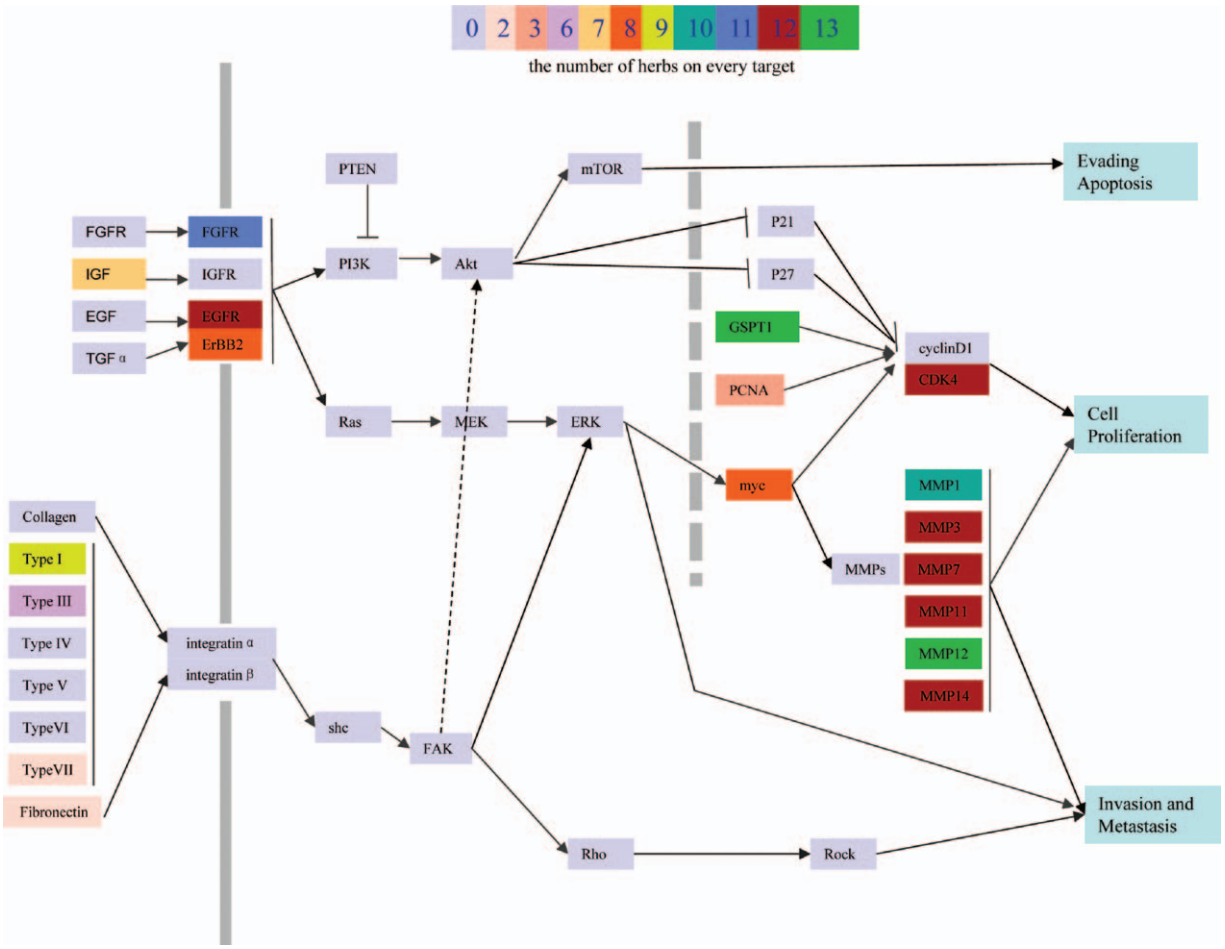

Figure 6. Associated pathways of the 13 herbs against gastric adenocarcinoma. The colored nodes are potential protein targets. Different colors represent different potentials. The light blue nodes are relevant targets in the pathway.

degrading enzymes, which participate in proliferation and metastasis and might be good prognostic factors.[20–23]

In the present study, a total of 338 genes expressed differentially or harboring genetic variations in gastric adenocarcinoma were obtained from the GEO database and categorized according to molecular function. The metastatic process included tumor cell detachment, local invasion, motility, angiogenesis, vessel invasion, survival in the circulation, adhesion to endothelial cells, extravasation, and regrowth in different organs.[30,31] Interestingly, 25.5% of these genes were primarily involved in cell adhesion, cell migration, extracellular matrix disassembly, blood vessel development, and wound healing associated with gastric adenocarcinoma metastases, such as collagen families,[20,23,32,33] fibronectin 1,[20,23,34] and MMPs families.[20,23,35–39] Some cell proliferation-related genes, such as MYC,[20,23,40] cyclin E1,[20,23,41] CDC28 protein kinase regulatory subunit 1,[20,42,43] CDC28 protein kinase regulatory subunit 2,[23,43] cyclin-dependent kinase 4,[20,23,44] and proliferating cell nuclear antigen,[20,44,45] EGFR,[20,44,46] FGFR4,[20,23] insulin like growth factor 2,[20] cell division cycle 25B,[47] human epidermal growth factor receptor 2,[44,45] and protein kinase, DNA-activated, catalytic polypeptide,[48] have been previously reported as showing extremely high levels of expression in gastric adenocarcinoma. A total of 37 genes, established in a large number of experimental and clinical studies, were selected for further study.

The clinical findings demonstrated that 13 herbs were significantly associated with the survival of patients with stage IV gastric adenocarcinoma. A total of 626 composite ingredients contained in these 13 herbs, with DL values above 0.18, were retained for further research. The ingredient-target herb networks showed that Chinese herbs play a role in the treatment of gastric adenocarcinoma in a biological network-level manner. Major ingredients with high-degree distributions were identified in these 13 herbs. Surprizingly, quercetin, a flavonoid presents in many vegetables and fruits, inhibits the proliferation of a wide range of cancers,[49–53] and is the major ingredient of 4 out of our 13 herbs (*Radix Linderae*, *Salvia Chinensis*, *Radix Bupleuri*, and *Herba Scutellariae Barbatae*). Similar studies have demonstrated that quercetin inhibits cell proliferation and induces protective autophagy in gastric cancer cells.

These 4 herbs also share common targets, including GSPT1 and MMP-12. MMP-12 facilitates the initiation and progression of tumor invasion and metastasis through the degradation of a broad spectrum of extracellular matrix (ECM) components.[54] Increased MMP-12 expression has been associated with tumor invasion, lymph node metastasis, distant metastasis, and TNM stage in gastric cancer.[39] Although quercetin inhibited MMP-12 expression and activity in response to inflammation both in vivo and in vitro,[55,56] it remains unknown whether quercetin effectively functions against gastric cancer through MMP-12.

Radix Glycyrrhizae, one of the most commonly prescribed herbs in China for the treatment of various diseases, ranging from microbial infections to cancer, showed the highest scores in both antiproliferation and antimetastasis functions. *Radix Glycyrrhizae* inhibits the growth of gastric cancer cells by arresting cell cycle progression and inducing apoptosis.[57] Vitexin, a major ingredient of *Radix Glycyrrhizae*, has also been shown to induce antitumor effects and cytotoxic activities through proapoptotic processes, mediated by a decreased Bcl-2/Bax ratio and the activation of caspases.[58] However, the effects of other major *Radix Glycyrrhizae* ingredients (RG-168, 8-prenylated eriodictyol, vitexin, glabranin, glepidotin B, and glepidotin A) on gastric adenocarcinoma have not been studied until recently.

Herba Hedyotis, with the closest association to survival, scored high in both antiproliferation and antimetastasis functions. *Hedyotis diffusa* is a well-known traditional Chinese herb, long used as a major component in CHM formulas for the clinical treatment of various types of cancers, including colon carcinoma, breast cancer, and hepatocellular carcinoma.[59–63] However, the mechanisms of *Hedyotis diffusa* in gastric adenocarcinoma have not been deeply researched.

Currently, the network pharmacology method provides a straightforward way to identify the intricate connections between herbal therapeutic spots and diseases. In the present study, 18 cancer-related genes involved in different stages of oncogenesis were included in the targets of 13 herbs through network analysis. To better understand the gene functions associated with these 13 herbs, we analyzed the 18 genes and summarized the related pathways. As shown in the network (Fig. 6), the 13 herbs might realize antigastric-adenocarcinoma activity mainly through 3 routes: the disruption of the MAPK signaling pathway, leading to the inhibition of cell proliferation in gastric adenocarcinoma; and the inhibition of the disruption of the PI3K signaling pathway to escape apoptotic effects. Both routes were activated through proliferation-related growth factors and their receptors. Expectedly, the 13 herbs suppressed the growth factors or receptors contributing to proliferation. Moreover, the 13 herbs might intercept the tumorigenesis of gastric adenocarcinoma through the disruption of tumor metastasis, which is regulated by MMPs family and collagens. GSPT1 and MMP-12 are predicted targets shared among all 13 herbs. EGFR, FGFR2, MMP-3, MMP-7, MMP-11, and MMP-14 are shared among 12 herbs. Interestingly, some targets of the 13 herbs, such as FGFR,[64] collagens,[65] and MMPs[66] signaling require the assistance of coreceptors, which are primarily sulfate proteoglycans. In a previous study, we reported that the sulfated glycopeptide derived from Gekko swinhonis Guenther blocks formation of HS/FGF/FGF receptor complex and interferes with bFGF bioactivity, thereby inhibiting tumor angiogenesis and tumor growth.[67] These coincidences might provide some evidence for future analyses of these molecular mechanisms.

Although network pharmacology is a promising method for identifying potential targets and active ingredients, there are some drawbacks affecting the analysis results. The ingredients of herbs were screened based on DL values, which might be inconsistent with the precise ingredients; the validated targets of these ingredients might be influenced by highly related studies; and the accuracy of target prediction depends on the target prediction tools.

5. Conclusion

In the present study, we showed that CHM treatment was associated with survival benefit for patients with stage IV gastric adenocarcinoma and identified 13 herbs beneficial to survival through correlation analysis. Moreover, we introduced a network pharmacological method to decipher the underlying mechanisms, which provides a good foundation for herbal research based on clinical data.

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