

Deciphering the potential mechanism of Siwu decoction for treating cancer-related anemia based on network pharmacology and molecular docking technology

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Author contributions

Li ZY and Song SJ conducted the study and wrote the original draft; Zeng KW and Lu ZY designed the study; Li ZY, Song SJ, Zhong CL, Li HJ, Wang L, Zhao HX, Zeng KW, and Lu ZY discussed and analyzed the data; Li ZY, Zeng KW, and Lu ZY reviewed and edited the paper. All authors have read and approved the final manuscript.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

SWD, Siwu decoction; CRA, cancer-related anemia; TCM, traditional Chinese medicine; PPI, protein-protein interaction; GO, gene ontology; KEGG, Kyoto encyclopedia of Genes and Genomes; OB, oral bioavailability; ROS, reactive oxygen species; IL-6, interleukin-6; IL-1 β , interleukin-1 β ; TNF- α , tumor necrosis factor- α ; CRP, C-reactive protein; ESA, erythropoiesis-stimulating agents; NP, network pharmacology; DL, drug-likeness; BP, biological process; CC, cellular component; MF, molecular function; EPO, erythropoietin.

Citation

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Abstract

Background: Siwu decoction (SWD) is a traditional Chinese herbal decoction commonly used for treating various symptoms of blood deficiency and blood stasis, including cancer-related anemia (CRA). However, due to its complex composition, the key active ingredients and underlying mechanisms of its therapeutic effects often remain unknown. This research aims to use network pharmacology and molecular docking technology to systematically elucidate the potential mechanisms underlying the efficacy of SWD in treating cancer-related anemia. **Methods:** The key constituents of SWD were procured from the TCMSP database. Leveraging the Swiss ADME and Swiss Target Prediction databases, potential targets were recognized. Disease-related targets were assembled via the GeneCards and DrugBank databases. Constructing the PPI network involved the utilization of the STRING database, followed by visualization through Cytoscape 3.9.1 software. Subsequently, GO and KEGG enrichment analysis was conducted utilizing the DAVID database, with visual analysis performed on the macrobiotic platform. For molecular docking, the Autodock software was employed, and the molecular docking outcomes were visualized using the PyMOL software. **Results:** In this investigation, a comprehensive revelation of 18 primary active compounds and 511 associated targets linked to CRA was accomplished. The outcomes of protein-protein interaction (PPI) analysis unequivocally identified AKT1, EGFR, SRC, VEGFA, HRAS, MAPK3, and STAT3 as pivotal proteins within the SWD's framework for effective CRA intervention. Notably, signaling pathways such as the HIF-1, JAK-STAT, TNF- α , and PI3K-Akt pathways, intricately involved in hematopoietic stem cell proliferation, differentiation, and inflammatory response, emerged as closely intertwined with the therapeutic application of SWD for CRA treatment. The congruence between these potential targets and SWD's primary therapeutic constituents for CRA treatment was substantiated by the outcomes of molecular docking analysis. **Conclusion:** This work provided a reference for further fundamental research by outlining the primary active ingredients and putative molecular mechanisms of SWD in the treatment of CRA.

Keywords: Siwu decoction; cancer-related anemia; network pharmacology; molecular docking

Introduction

Cancer-related anemia is a multifactorial condition frequently observed across various types of tumors, displaying a heightened incidence during tumor development. It commonly co-occurs in patients diagnosed with cancer [1, 2]. The occurrence of cancer-related anemia extends beyond the consequences of anti-tumor interventions, such as chemotherapy and radiation therapy. Instead, it is predominantly linked to the low-grade chronic inflammation induced by the tumor [3]. The pathogenesis of CRA is quite complex, involving the interaction of multiple factors. For example, the presence of cancer can slowly activate the immune response, trigger a sustained immune system response, and thus cause CRA. This activation arises due to the direct and indirect inhibitory effects of cytokines on erythropoiesis. In addition, elevated levels of reactive oxygen species (ROS) in cancer patients usually lead to increased release of cellular inflammatory factors. This elevation can occur either as a constituent of the immune response or as a result of heightened metabolism [4]. Reactive oxygen species (ROS) have the potential to impede erythropoiesis, disrupt nutritional equilibrium, and worsen anemia [5]. It is worth noting that there is a close link between anemia and inflammatory markers. In the development and progression of anemia, various inflammatory markers, including interleukin-6 (IL-6), interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), C-reactive protein (CRP), fibrinogen, hepcidin, erythropoietin, reactive oxygen species, and erythrocyte sedimentation rate, play a crucial role. These markers are instrumental in influencing the occurrence and advancement of anemia, forming a complex network of interactions within the physiological processes related to hematopoiesis and red blood cell regulation. Their collective impact underscores the intricate relationship between inflammatory pathways and the intricate mechanisms governing anemia's pathophysiology. The levels and activities of these inflammatory markers can affect the generation and life span of red blood cells, thus affecting the frequency and severity of anemia. In addition, the stage of cancer is also one of the important factors that affect the frequency and severity of anemia [6, 7].

An optimal treatment strategy for patients with cancer-related anemia (CRA) should focus on addressing the underlying factors contributing to anemia. In recent times, novel approaches have emerged in the treatment of cancer and chemotherapy-induced anemia. These methodologies involve the utilization of erythropoiesis-stimulating agents (ESA), blood transfusions, and iron supplementation to uphold hemoglobin levels in cancer patients. The objective is not only to ameliorate anemia symptoms but also potentially decrease the necessity for blood transfusions, consequently reducing treatment-related risks. Each of these interventions has a certain complexity, involving multiple factors and possible side effects. Additionally, anemia itself and the interventions deployed to mitigate it can carry substantial financial implications and revenue considerations.

The use of traditional Chinese medicine (TCM) for the treatment of CRA has recently piqued the interest of researchers because of its multiple targeting effects, lack of toxins and side effects, and affordability. Cancer-related anemia is categorized as “gas-blood deficiency syndrome” in traditional Chinese medicine. In accordance with the holistic theory, Traditional Chinese Medicine (TCM) employs three potential therapeutic mechanisms—namely, the “nourishing gas and blood,” the “detoxifying and enhancing effect,” and the “invigorating health and boosting essence”—in the treatment of CRA. Siwu decoction (SWD) is a traditional herbal formula that consists of four main ingredients: *Angelica Sinensis Radix*, *Chuanxiong Rhizoma*, *Paeoniae Radix Alba*, and *Rehmanniae Radix Praeparata*. *Angelica Sinensis Radix* is primarily used in clinical practice to address Qi deficiency (Qi is a positive substance present within the human body. The balance of Qi is closely associated with both health and the occurrence of diseases), while *Chuanxiong Rhizoma*, *Paeoniae Radix Alba*, and *Rehmanniae Radix Praeparata* are known for their benefits in

treating blood deficiency conditions [8]. Given the complex nature of herbal remedies, a promising approach to unraveling the molecular evidence behind their effects is the emerging field of network pharmacology (NP) [9]. NP utilizes existing extensive and fragmented data to provide a holistic perspective, making it a powerful tool for studying herbal formulas such as Siwu decoction [10]. In this study, the network pharmacology method was used to construct a network model of cancer-related anemia related factors and analyze their potential mechanisms and targets. Through this method, we can comprehensively evaluate the therapeutic effect and mechanism of Siwu decoction on cancer-related anemia, and provide new ideas and methods for clinical treatment.

Methods

Screening the active ingredients and targets of SWD

With oral bioavailability (OB) $\geq 30\%$ and drug-likeness (DL) ≥ 0.18 as the standard, “*Angelica*, Sichuan lovage rhizome, *Radix paeonies alba*, and *Rehmannia glutinosa*” were searched by TCMSP (<http://tcmsp.com/tcmssp.php>), and screened for active-active ingredients and their corresponding target proteins. Then, according to the published literature supplement undetected target point. After completing the above steps, it is in the Uniprot database (<https://www.uniprot.org/>) and the Swiss TargetPrediction Database (<http://www.swisstargetprediction.ch/>) for standardized conversion of proteins and genes.

Collection of predicted targets of CRA

Firstly, we used the Gene Cards Database to search the predicted targets, and “cancer-related anemia” was used as the keyword. The website is as followed: (<https://www.genecards.org/>). Simultaneously, the DrugBank database (<https://go.drugbank.com>) was utilized to supplement disease-related gene information. Following the removal of duplicate values, a set of targets associated with Cancer-Related Anemia (CRA) was derived.

Establishment of component-target network and identification of key active components

Next, in order to consolidate the shared targets linked to both drugs and diseases, we integrated the potential targets of active ingredients for both Siwu decoction (SWD) and Cancer-Related Anemia (CRA) using the Venn database (<https://bioinfo.gp.cnb.csic.es/tools/venny/>), facilitating the identification of common targets between the two entities. The subsequent step involved aligning the acquired shared target data with the constituents of Siwu decoction (SWD). Employing the “Compounds-targets” network within Cytoscape 3.9.1 software, the active ingredients and their shared targets were meticulously portrayed as individual nodes and interconnected edges, respectively, within the network. The complex relationships between these ingredients and their associated targets were visually represented. Rigorous topological scrutiny of this network was conducted using the “network analyzer” function of the software. Following an in-depth analysis of the outcomes, the compounds deemed pivotal within SWD, integral to the effective treatment of Cancer-Related Anemia (CRA), were successfully pinpointed and identified.

Establish the construction of the protein-protein interaction network

The shared targets identified in the previous steps were integrated into the STRING database (<https://www.string-db.org/>) to construct a comprehensive protein-protein interaction (PPI) network. In this network, a focus was maintained on protein species of human origin, and a stringent interaction score criterion of “highest confidence (0.900)” was applied. To enhance network clarity, unconnected nodes were excluded, resulting in a refined PPI network. The resultant dataset of the PPI network was then imported into Cytoscape 3.9.1 software for in-depth analysis. Leveraging the powerful “network analyzer” feature, nodes exhibiting degree and betweenness centrality values surpassing the mean were singled out as pivotal targets. It's

noteworthy that, within this network, nodes represent either compounds or targets, and a higher degree value implies an elevated probability of a compound or target exerting a significant influence on its surroundings within the network structure. This analytical approach adds granularity to the understanding of the intricate relationships within the PPI network, thereby offering insights into potential key targets crucial for the effective treatment of Cancer-Related Anemia (CRA).

Gene ontology and pathway enrichment analysis

Our study involved Gene Ontology (GO) functional enrichment and KEGG pathway enrichment analyses, leveraging the David database (<https://david.ncicrf.gov/>). The GO analysis encompassed a comprehensive exploration of three fundamental components: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). This systematic examination allowed for a detailed understanding of the functional roles and associations of the identified genes, shedding light on their involvement in diverse biological processes, cellular structures, and molecular functions. The integration of GO functional enrichment with KEGG pathway analysis offers a holistic perspective on the potential mechanisms and pathways implicated in the context of our study, contributing valuable insights into the biological significance of the identified targets and compounds. To enhance the specificity of our analysis, we chose the “Homo species” option on the platform and applied a significance threshold of P value < 0.01. For visual representation and further insights, we employed the Cytoscape 3.9.1 plug-ins CluoGO, in conjunction with the online platform available at <http://www.sangerbox.com/>. This strategic integration of tools facilitated a comprehensive exploration of functional enrichments and

pathways, yielding valuable insights into the biological context of the studied elements.

Molecular docking validation of key components and key targets

The six main compounds were extracted and stored as files in mol2 format within the TCMSP database (<https://tcmspw.com/tcmsp.php>). Following this, the identification of the six most crucial targets within the Protein-Protein Interaction (PPI) network was performed, and their respective molecular structures were obtained from the PDB database (<http://www.rcsb.org>). Molecular docking studies involving the essential active compounds and their respective targets were subsequently conducted using the Autodock VINA software. The binding energies, serving as indicators of binding strength, were meticulously evaluated. The outcomes of these docking studies were then visualized using PyMOL software, providing a comprehensive understanding of the molecular interactions between the identified compounds and their corresponding targets.

Results

The active components and effective targets of SWD

Following the elimination of duplicate entries among the initial ingredients and adherence to the criteria outlined by TCMSP and the SwissADME system, a comprehensive set of 18 chemical constituents sourced from SWD was identified. These constituents comprise 2 from Danggui, 2 from Shudi, 11 from Baishao, and 7 from Chuanxiong (as presented in Table 1). As per the projections made by TCMSP and the SwissADME system, a total of 511 target interactions involving the 18 chemical components within SWD were anticipated.

Table 1 The active ingredients of SWD

Drugs	Components	MOL ID	MW	ID
CX BS SD	sitosterol	MOL000359	414.79	M3
DG SD	Stigmasterol	MOL000449	412.77	M1
DG BS	beta-sitosterol	MOL000358	414.79	M2
CX	FA	MOL000433	441.45	CX1
CX	Perlolyrine	MOL002140	264.3	CX2
CX	senkyunone	MOL002151	326.52	CX3
CX	wallichilide	MOL002157	412.57	CX4
CX	Mandenol	MOL001494	308.56	CX5
CX	Myricanone	MOL002135	356.45	CX6
BS	paeoniflorgenone	MOL001918	318.35	BS1
BS	albiflorin_qt	MOL001928	318.35	BS9
BS	Mairin	MOL000211	456.78	BS2
BS	(+)-catechin	MOL000492	290.29	BS3
BS	Paeoniflorin	MOL001924	480.51	BS4
BS	Lactoferrin	MOL001921	462.49	BS5
BS	Kaempferol	MOL000422	286.25	BS7
BS	benzoyl paeoniflorin	MOL001930	584.62	BS8
BS	(3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,4,8,10,14-penta-methyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione	MOL001919	358.52	BS6

Related targets for CRA

GeneCards and Drugbank were employed to retrieve pertinent targets associated with CRA. Through a harmonization of outcomes from both databases and the subsequent exclusion of duplicated targets, a total of 1851 distinct disease-related targets were amassed.

To construct the component-target network and pinpoint crucial active components, a systematic process was initiated. This process led

to the discovery of 253 shared targets within the intersection of 259 drug targets and 1598 disease targets (depicted in Figure 1). These common targets were then linked back to the constituent elements of SWD. Subsequently, the component-target network diagram was structured employing Cytoscape 3.9.1 software (as depicted in Figure 2). The Network Analyzer plug-in was employed to compute essential network topology parameters.

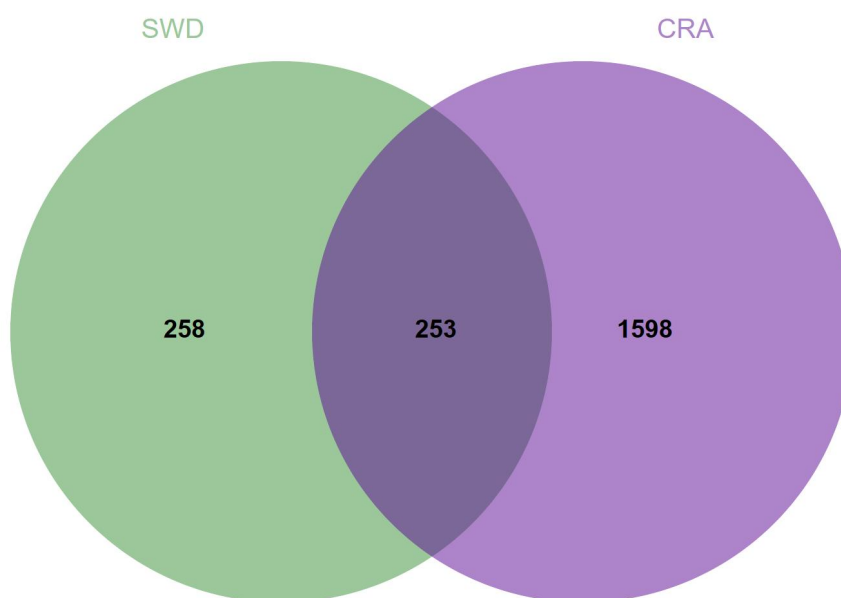


Figure 1 Venn diagram of the target of SWD and the target of CRA. SWD, Siwu Decoction; CRA, cancer-related anemia.

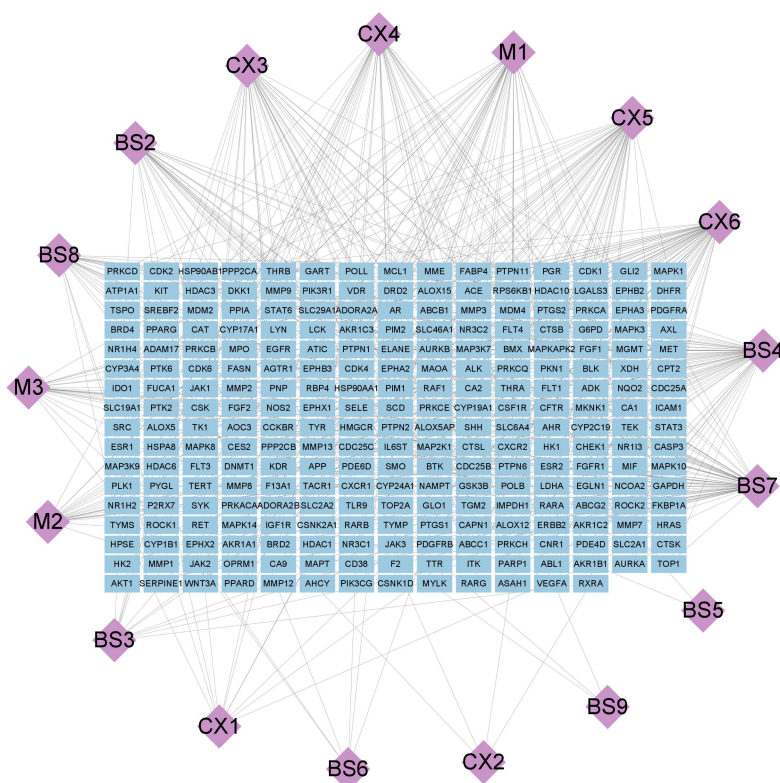


Figure 2 Compound-target network of SWD-CRA

Construction of PPI network and core targets analysis

The establishment of the Protein-Protein Interaction (PPI) network, which encompasses shared targets, was accomplished through the utilization of the STRING database. The intricate network structure is visually represented in Figure 3A, and a comprehensive visual analysis of this network was conducted using Cytoscape 3.9.1 software. In this graphical representation, it's noteworthy that the size and color intensity of nodes are indicative of their respective degree values,

while the thickness and color of edges are proportional to the combined score values, as clearly illustrated in Figure 3B. By adopting a meticulous degree-ranking approach, we were able to pinpoint the top 10 critical targets, namely AKT1, GAPDH, EGFR, SRC, VEGFA, HRAS, MAPK3, HSP90AA1, STAT3, and CASP3. This strategic identification of targets underscores their potential significance as core entities in the context of treating Cancer-Related Anemia (CRA) using Siwu decoction (SWD), as elucidated in detail in Table 2.

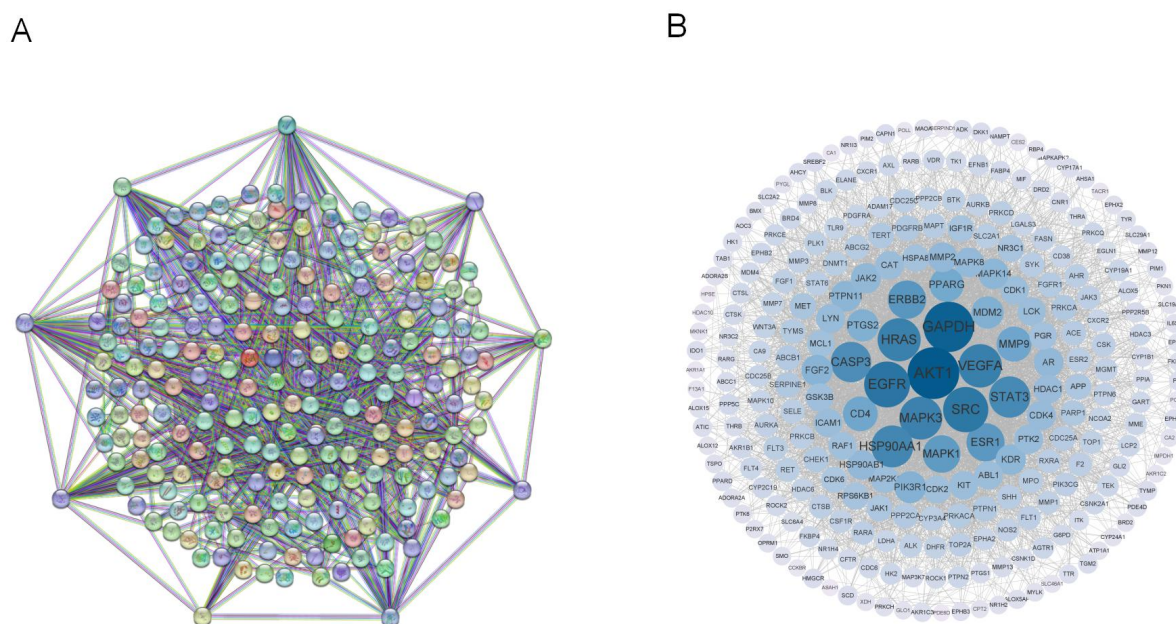


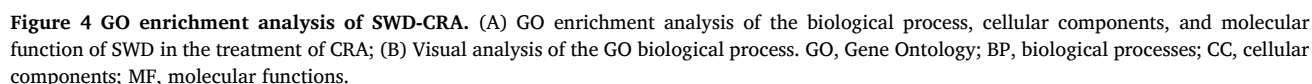
Figure 3 SWD-CRA intersection target PPI network. (A) The PPI network of SWD in the treatment of CRA; (B) Visual network diagram from A. PPI, protein-protein interaction.

Table 2 The binding energy of compound and core targets (kcal/mol)

	Kaempferol	Paeoniflorin	Myricanone	Mandenol	Stigmasterol	Wallichilide
AKT1	-6.28	-5.19	-6.89	-4.58	-7.43	-5.97
EGFR	-5.58	-5.92	-7.60	-3.49	-8.48	-7.37
SRC	-5.82	-5.51	-6.80	-3.41	-7.40	-6.22
VEGFA	-5.94	-5.48	-6.44	-4.92	-7.33	-8.01
HRAS	-7.81	-6.93	-7.15	-5.19	-8.23	-6.05
MAPK3	-6.44	-5.87	-7.09	-4.56	-7.86	-6.73

To delve into the dynamic activities of Siwu decoction (SWD) within the context of Cancer-Related Anemia (CRA), the DAVID database was employed to augment our comprehension of the Gene Ontology (GO) bioprocesses associated with the 253 common targets. A stringent filtering criterion was implemented, employing False Discovery Rate (FDR) and P -values < 0.01 . The resultant outcomes of the GO enrichment analysis encompassed 851 biological processes, 206 molecular functions, and 98 cellular components. To enhance clarity,

these findings were visually represented through the Sangerbox website (<http://sangerbox.com>), illustrating the intricate landscape of biological processes, molecular functions, and cellular components, as depicted in [Figure 4A](#). Additionally, for a nuanced interpretation of gene cluster enrichment patterns, a visual gene cluster enrichment analysis was conducted using ClueGO, an integrated plug-in within Cytoscape, as vividly illustrated in [Figure 4B](#). This comprehensive approach adds depth to our understanding of the multifaceted biological implications of SWD in addressing CRA.



KEGG analysis

In order to delve into the functional implications of the 253 potential gene targets within the context of treating breast cancer-associated CRA through SWD, the pathways module of the DAVID database facilitated KEGG pathway enrichment analysis. This endeavor resulted in the identification of 161 pathways exhibiting significant

differences, as indicated by FDR values below 0.01 and *P*-values below 0.01. The most noteworthy pathways are visually depicted using a bubble plot in Figure 5. The outcomes of the KEGG enrichment analysis illuminated that SWD's therapeutic effects might be attributed to its participation in the modulation of signaling pathways such as PI3K-Akt, HIF-1, JAK-STAT, and others.

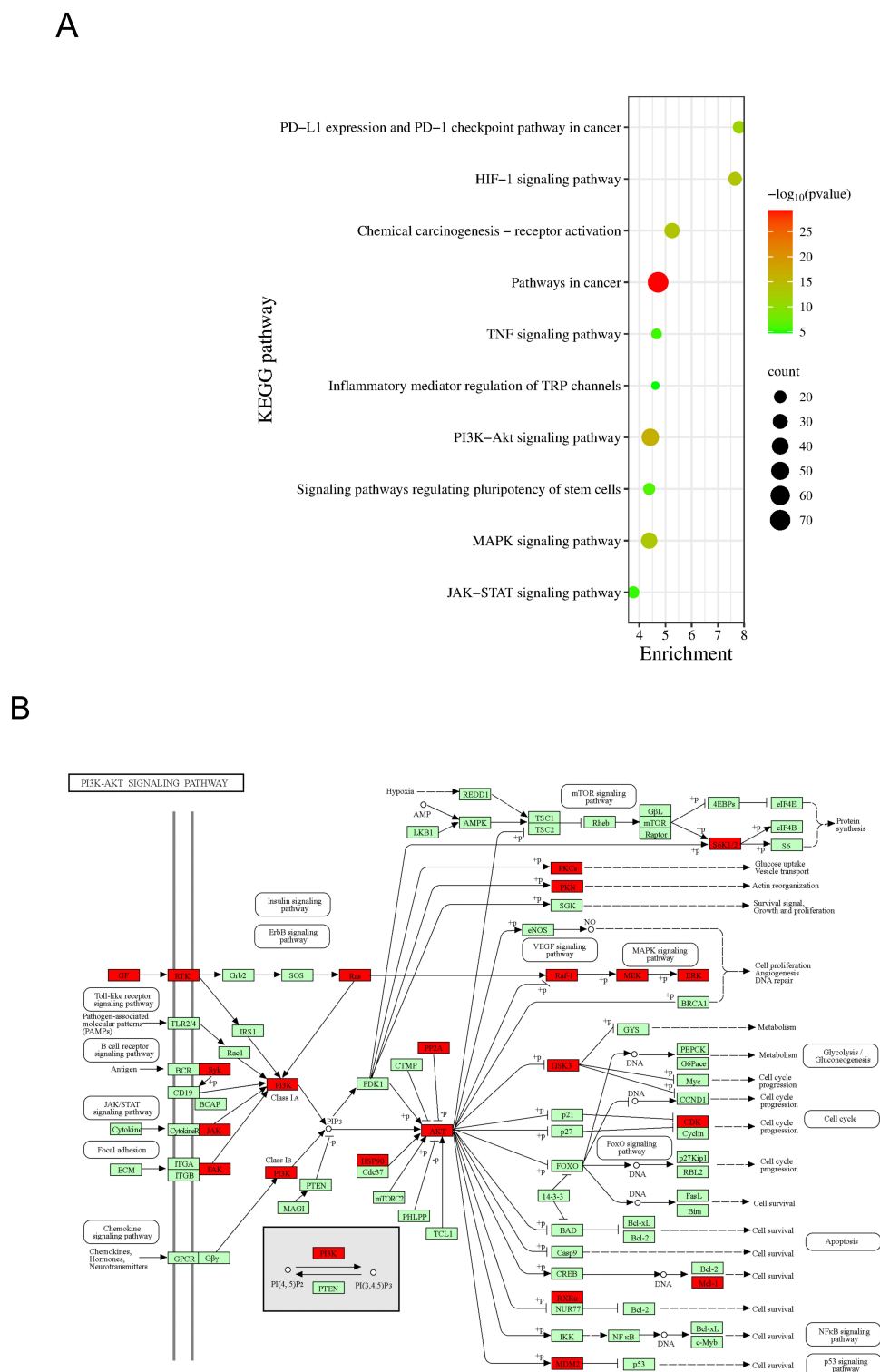


Figure 5 KEGG analysis of targets of SWD in the treatment of CRA. (A) KEGG bubble diagram of potential SWD-CRA targets; (B) PI3K-Akt signaling pathway. KEGG, Kyoto encyclopedia of genes and genomes.

Drawing insights from existing literature, we curated a selection of ten pathways profoundly associated with Cancer-Related Anemia (CRA) to elucidate target genes enriched within KEGG pathways and active components. Utilizing Cytoscape 3.9.1 software, we created an interaction network, intricately capturing components, targets, and pathways. The integrated plug-in for degree analysis was instrumental in scrutinizing network centrality, visually depicted in [Figure 6A](#). From these analyses, the top six pivotal components within Siwu decoction (SWD) for addressing CRA emerged: Kaempferol, Paeoniflorin, Myricanone, Mandenol, Stigmasterol, and Wallichilide. Subsequently, we delved into the identification of their key targets and corresponding signaling pathways, visually represented through the construction of a Sankey diagram ([Figure 6B](#)). The outcomes unveiled that these core components primarily influence critical signaling pathways such as the HIF-1 signaling pathway, JAK-STAT signaling pathway, TNF- α signaling, and PI3K-Akt signaling pathway, shedding light on their potential mechanisms in addressing CRA.

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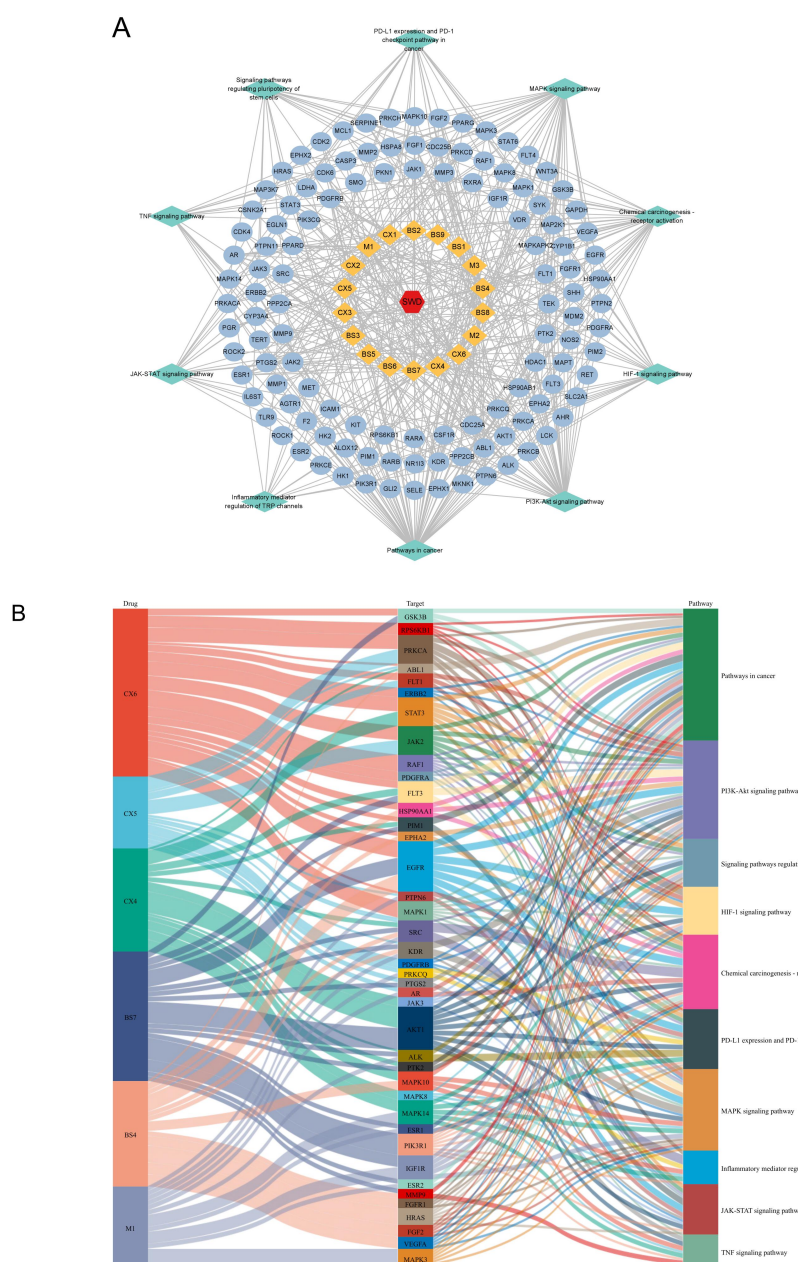


Figure 6 Analysis of the “component-target-pathway” network. (A) “Herb-compound-target-function” network of SWD for CRA. (B) Sankey diagram of the compound-target-pathway.

Molecular docking analysis

Using AutoDock and Pymol software to protect the core active ingredients Kaempferol, Paeoniflorin, Myricanone, Mandenol, Stigmasterol, Wallichilide and core targets AKT1, EGFR, SRC, VEGFA, HRAS, MAPK3 underwent molecular docking (Table 2), of which Stigmasterol and EGFR had the strongest binding capacity of -8.48 kJ·mol⁻¹, followed by Stigmasterol and HRAS, and wallichilide and VEGFA (Figure 7). The consensus in the field suggests that a lower binding energy signifies a more stable interaction between a protein and a molecule. Specifically, a binding energy below -5.0 kJ/mol indicates a favorable potential for combination, with a binding energy below -7.0 kJ/mol suggesting excellent binding capacity. The outcomes of our molecular docking analyses consistently revealed that the majority of proteins and molecules exhibited noteworthy binding abilities, as evidenced by their generally low binding energies. This observation underscores the robust and potentially effective interactions between the identified proteins and the active molecules under investigation.

Discussion

Malignant neoplasm development and occurrence pose a significant risk to public health. One of the main causes of problems and death in cancer patients is thought to be cachexia, which is brought on by the progression of the disease to advanced stages. Cancer and cancer cachexia frequently coexist with cancer-related anemia, which worsens patients' suffering and hastens the growth of malignant tumors [11, 12]. Cancer-related anemia is not an independent disease but rather a clinical syndrome that often leads to clinical symptoms such as dizziness, fatigue, difficulty breathing, cognitive impairment, poor concentration, and depression [13]. Compared to patients with tumors without anemia, patients with anemic tumors have an overall

increased risk of death by nearly 65%. Therefore, effective treatment of cancer-related anemia is an essential step in the treatment of cancer patients.

The prevailing standard of care for individuals with cancer-related anemia predominantly centers on enhancing erythropoiesis through the administration of erythropoietin (EPO), which may further promote the growth of tumors, and result in the occurrence of thrombus [14]. Lately, researchers have shown increased interest in the application of traditional Chinese medicine (TCM) for addressing Cancer-Related Anemia (CRA). This attention is attributed to TCM's multi-targeting effects, reduced toxicity, and cost-effectiveness [15]. Liu employed network pharmacology and molecular docking methodologies to elucidate the principal active components and potential molecular mechanisms associated with the use of cooked *rehmannia* in treating Cancer-Related Anemia (CRA) [16]. Similarly, Li reveals the mechanism of action of *Angelica* in the treatment of CRA [17]. Siwu decoction is a traditional blood tonic medicinal meal, consisting of *Rehmanniae Radix Praeparata*, *Angelicae Dahuricae Radix*, *Ligusticum chuanxiong hort*, and *Cynanchum otophyllum Schneid*. It has been used in the treatment of a variety of anemias, and its specific molecular mechanisms have been widely studied. Despite the common use of first-line treatments, such as erythropoiesis-stimulating agents (ESAs) and iron preparations like iron polysaccharide complex and ferrous sulfate, for the management of cancer-related anemia, adverse reactions often arise. These range from mild symptoms like decreased appetite and dark stools to more severe consequences, including an increased susceptibility to infections, accelerating tumor progression, and posing a serious threat to patients' overall health. Additionally, contemporary medical approaches to treating tumor-related anemia frequently encounter diminishing therapeutic effects, exacerbated by escalating drug dosages, which, in turn, elevate the occurrence of the aforementioned adverse reactions.

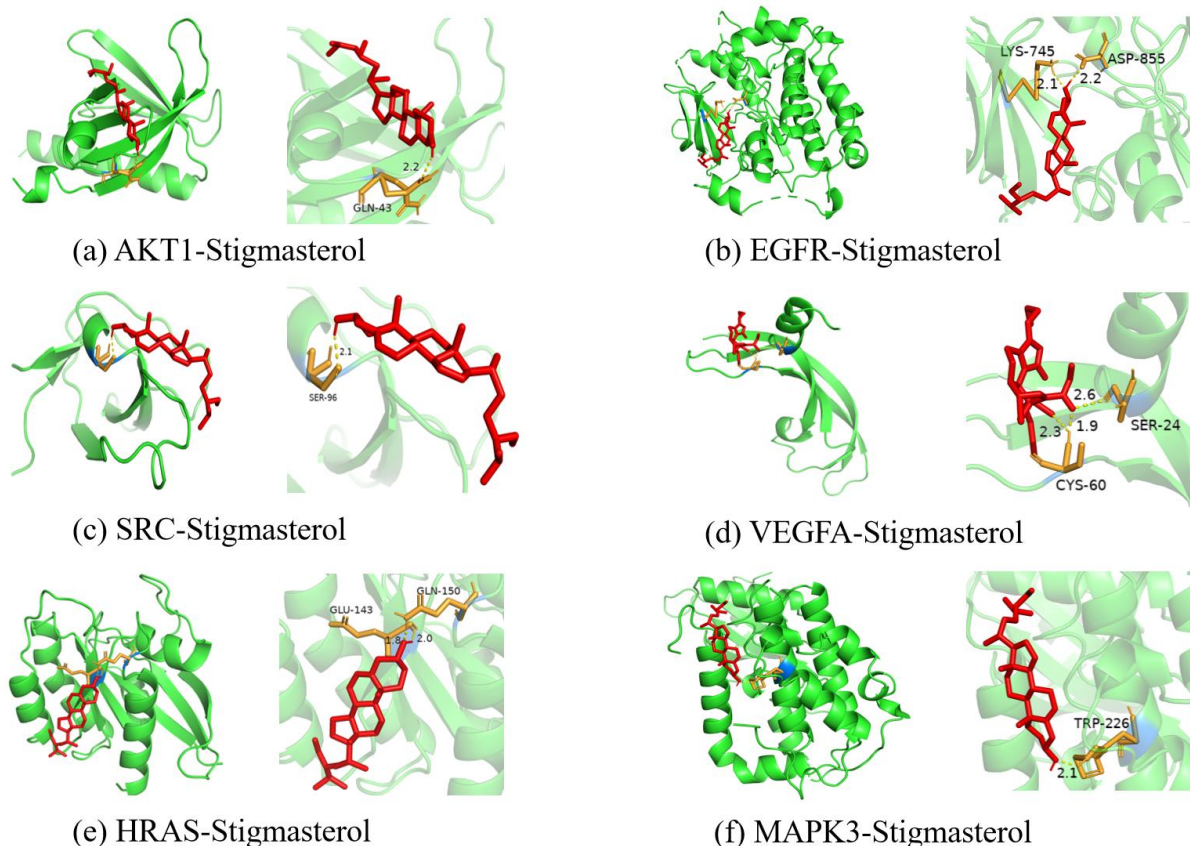


Figure 7 Molecular docking diagram of key active ingredient Stigmasterol and core target

In contrast, Traditional Chinese Medicine's Siwu decoction (SWD) commonly employed in China and several Asian countries, demonstrate considerable advantages in addressing anemia and gynecological disorders. When compared to conventional pharmaceuticals used in modern medicine for cancer-related anemia, SWD exhibit several noteworthy benefits. Firstly, it exerts a multifaceted impact by modulating pathways such as PI3K-Akt, HIF1, and JAK-STAT, promoting the generation and differentiation of hematopoietic stem cells to ameliorate anemia [18]. Additionally, it regulates inflammation by influencing factors like TNF, thereby improving the tumor microenvironment. Secondly, the adverse reactions associated with SWD are relatively minor. To date, there is no evidence indicating an impact on tumor progression, and the risk of infection associated with injections is circumvented. Thirdly, these Traditional Chinese Medicine components are cost-effective, composed mainly of readily available medicinal herbs, resulting in a more economical option for patients and alleviating their financial burden. However, the mechanism of action in CRA is still not well understood. Herein, in order to unveil the complicated connection between CRA and SWD, We used network pharmacology to explore the potential role of SWD in the treatment of CRA, and molecular docking methods to verify the binding of active ingredients in SWD with target proteins. The PI3K pathway is found in a variety of cells, affects the metabolism of cancer cells and hematopoietic stem cells, and also promotes red blood cell maturation [19, 20].

According to the study's findings, there are 18 chemical ingredients from SWD were obtained on cancer-related anemia, and these primary components have 253 CRA intervention targets. With the creation of the effective components-targets-pathways network connection, as well as using network pharmacology, we are anticipated to figure out the primary active components. As for exploring the potential targets, molecular docking technology were used as well. In light of the outcomes from enrichment analysis, our findings indicate that Siwu decoction, in its therapeutic approach to cancer-related anemia, significantly influences 3 key signaling pathways, specifically the PI3K-Akt, HIF-1, and JAK-STAT pathways, which play crucial roles in the regulation of hematopoietic stem cell proliferation and differentiation. The PI3K-Akt signaling pathway is frequently over-altered or activated in various cancer types, exerting a crucial role in the survival, proliferation, growth, and metabolic processes of erythroid progenitor cells. Its pervasive activation across cancers underscores its significance in influencing key cellular functions associated with the progression and maintenance of cancer, particularly impacting the delicate balance of erythroid progenitor cell survival and proliferation [21]. The PI3K-Akt signaling pathway undergoes activation through phosphorylation, where the activated AKT exerts its influence on downstream proteins. This regulatory cascade, in turn, governs essential cellular processes, including cell cycle modulation, apoptosis inhibition, and facilitation of erythrocyte maturation. This mechanistic insight underscores the pivotal role of the PI3K-Akt pathway in orchestrating key cellular events, presenting a potential avenue for therapeutic exploration in manipulating erythrocyte development and survival, with implications for conditions such as cancer-related anemia [22]. The HIF-1 pathway is intricately associated with critical aspects of cancer biology, including cancer cell invasion, resistance to chemotherapy, and the initiation of metastasis. As a regulatory factor in hypoxia-related adaptive responses within tumor cells, HIF-1 emerges as a potential therapeutic target for tumor treatment [23]. The JAK-STAT pathway holds a crucial significance in governing the processes of cell growth, differentiation, and the survival of immune cells within the hematopoietic system. This intricate signaling pathway orchestrates fundamental cellular activities, influencing the intricate balance essential for the functioning and maintenance of hematopoietic elements. Also, the JAK-STAT signaling pathway mostly mediates a huge range of processes related with immunomodulatory, for example, involvement in tumor cell recognition and tumor-driven immune evasion [24]. The outcomes of molecular docking revealed robust binding of the core active ingredient, with notable affinity

observed between Stigmasterol and EGFR, indicating a significant interaction at the key target site. The binding energy is $-8.48 \text{ kJ}\cdot\text{mol}^{-1}$, the affinity is good, and EGFR is a signaling molecule in the PI3K, JAK-STAT, HIF-1 signaling pathway. Hence, the potential therapeutic impact of Siwu decoction on anemia appears to be predominantly linked to the interaction between stigmasterol and EGFR within the aforementioned signaling pathway. However, additional confirmation is imperative to solidify these findings. Further validation through comprehensive investigations and experiments is warranted to establish the efficacy and mechanisms underlying the proposed treatment approach.

Conclusion

This investigation sheds light on the potential role of SWD in addressing CRA. The pivotal constituents driving SWD's efficacy are identified as Stigmasterol and wallichilide. The molecular mechanisms underlying its effects appear to be intertwined with the modulation of key signaling pathways, notably the PI3K-Akt, HIF1, and JAK-STAT pathways influencing hematopoietic stem cell proliferation and differentiation. Additionally, the TNF signaling pathways related to inflammation and inflammatory anemia are also implicated. Collectively, our study establishes a solid basis for comprehending the beneficial impacts of CRA treatment.

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