Regular article

Network pharmacology to decipher the mechanism of Danggui Longhui Wan against chronic myeloid leukemia

Xuehan Ma, Qiulin Yan, Shaojiang Song*, Guodong Yao*

Key Laboratory of Computational Chemistry Based Natural Antitumor Drug Research & Development, Liaoning Province, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China

Abstract

Chronic myeloid leukemia (CML) is a hematopoietic myeloproliferative disorder. The Chinese prescription Danggui Longhui Wan (DGLHW) has been utilized in CML treatment, but its underlying mechanisms remain unclear. In this study, we gathered 794 constituents, 1249 drug targets, 1654 disease genes and 129 intersection genes. GO and KEGG were used to analyze the function of these genes. Compatibility of prescription study showed that monarch drug, minister drug, assistant and guide drug played a synergistic role in the treatment of CML. In addition, we obtained 20 hub genes and 12 key components. Molecular docking indicated that the main compounds and core proteins had good binding ability. The results of this study also showed that DGLHW might play a role in the treatment of CML by affecting MAPK, PI3K/AKT, FoxO and p53 signaling pathways.

Keywords: chronic myeloid leukemia; Danggui Longhui Wan; network pharmacology; compatibility analysis

1 Introduction

Chronic myeloid leukemia (CML) is the most common chronic leukemia, with an annual incidence of 16-20% worldwide, accounting for about 15% of adult leukemia. It is a clonal myeloproliferative disorder of hematopoietic stem cells [1]. The hallmark of CML is the acquired reciprocal translocation between the long arms of chromosomes 9 and 22, cytogenetically visible as the Philadelphia chromosome (Ph) [2] and the fusion gene BCR-ABL, by which the encoded BCR/ABL fusion protein can improve the tyrosine kinase activity [3]. With the advent of tyrosinase inhibitors (TKIs) [4], the overall 10-year survival rate of patients with CML has increased from 50% to 85-90% [5]. Despite significant progress in CML treatment with TKIs in recent years, 20% to 30% of patients still suffer adverse reactions or develop secondary drug resistance [6].

Traditional Chinese medicine (TCM) has obvious advantages and significant curative effects in the treatment of CML. With the development of traditional Chinese medicine, many prescriptions or single drugs have been found to treat CML. The prescription Danggui Longhui Wan (DGLHW)

* Author to whom correspondence should be addressed. Address: School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, 103 Wenhua Rd., Shenyang 110016, China; Tel.: +86-24-23986510; E-mail: songsj99@163.com (Shaojiang Song); guodong_yao@163.com (Guodong Yao).

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has been used for years in the treatment of CML [7]. It is commonly prescribed to alleviate symptoms associated with excessive fire in the liver and gallbladder [8]. Besides, it can improve *Qi* stagnation and blood stasis, excessive heat and toxin, constipation and other symptoms caused by CML. Interestingly, it does not inhibit bone marrow or reduce platelet levels, and it has few side effects. This prescription consists of eleven ingredients from traditional Chinese medicinal herbs, including *Indigo naturalis* (Chinese name is QingDai, QD), *Angelica sinensis* (Chinese name is DangGui, DG), *Aloe vera* (Chinese name is LuHui, LH), *Moschus* (Chinese name is SheXiang, SX), Phellodendri Chinensis Cortex (Chinese name is HuangBai, HB), Aucklandiae Radix (Chinese name is MuXiang, MX), Coptidis Rhizoma (Chinese name is HuangLian, HL), Gardeniae Fructus (Chinese name is ZhiZi, ZZ), Scutellariae Radix (Chinese name is HuangQin, HQ), Rhei Radix et Rhizoma (Chinese name is DaHuang, DH) and Gentiana scabra Bunge (Chinese name is LongDan, LD) [9]. In this prescription, DH, LH and LD are monarch drugs. QD, HB, HL, ZZ and HQ are minister drugs. DG, SX and MX are assistant and guide drugs. Due to the complex chemical composition of traditional Chinese medicine compounds, their pharmacological action is characterized by multi-target and multi-level effects. The specific mechanism by which DGLHW treats CML is not yet fully understood.

Network pharmacology is an interactive network based on the drug-target-gene-disease, including bioinformatics, network biology and pharmacology [10]. It helps to identify the bioactive compounds in traditional Chinese medicine formulas and clarify their complex pharmacological mechanisms from systemic and holistic perspectives [11]. In order to lay a foundation for the clinical study of DGLHW in treating CML, we employed network pharmacology to investigate the multi-component and multi-target characteristics of prescription, and to explore how the chemical active composition of DGLHW alleviate the disease progression of CML.

2 Methods

2.1 Compound database building

The chemical constituents of eleven drugs in the DGLHW were obtained from the literature. Swiss Target Prediction database (https:// www.swisstargetprediction.ch/) was used to predict the targets of compounds. Targets whose probability > 0 were selected in the prediction results for further analysis [12]. The retrieved results were combined to construct a chemical composition database of DGLHW.

2.2 Screening potential targets for CML

We gathered CML disease-related targets from Gene Expression Omnibus (GEO, https://portal.gdc.cancer.gov/) database [13]. GEO is an international overt repository that archives and freely distributes high-throughput gene expression and other functional genomics datasets [14]. In order to determine the decontrolled gene expression, the differentially expressed genes (DEGs) were identified using the "limma" package of R software. Genes with a log2-fold change |log2FC| ≥3 and *P* value < 0.01 were considered DEGs.

2.3 Protein-protein interaction (PPI) analysis

PPI data were derived from String (https://cn.string-db.org/, ver.12.0) [15], an online database of known and predicted protein-protein interactions with the species limited to "Homo sapiens" and a confidence score > 0.4.

2.4 Network construction

All visualized networks were constructed using software Cytoscape (http://cytoscape.org/, ver. 3.9.1) [16], an open software platform for visualizing biological pathways, molecular interaction networks, and integrating these networks with annotations, gene expression profiles and other state data. We constructed the three dominating networks by Cytoscape.

2.5 Enrichment analysis

To explore the function of potential genes, the Database for Annotation, Visualization and Integrated Discovery (DAVID; http://david.abcc.ncifcrf.gov/) was employed in GO and KEGG pathway enrichment analysis. We selected the top 10 GO terms and the top 20 KEGG pathways with the smallest *P* value and import them into the bioinformatics mapping website (http://www.bioinformatics.com.cn/) and ChiPlot (https://www.chiplot.online/) [17] to draw enrichment diagram.

2.6 Molecular docking

Through the network pharmacological analysis, molecular docking of core components with the key targets was performed. The crystal structures of CA4, PTGS2, MMP9, CA6, CDK1, CCNB1, MMP8, PTGES, PYGL, LCK, MPO and NR3C2 (PDB code: 3fw3, 5fdq, 4hma, 3fe4, 1x8b, 6gu2, 1bzs, 8pyv, 3ddw, 1qpc, 4p04 and 1y9r) were obtained from RCSB PDB database (https://www.rcsb.org/). The structures of the compounds were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Molecular docking was performed using iGEMDOCK (ver. 2.1), and the docking results were visualized and analyzed using Discovery Studio 2020 Client.

3 Results

3.1 Identification of ingredients in DGLHW and targets collection

Firstly, the chemical components of 11 traditional Chinese medicines in the formula were collected from the published literature. There are 106 components in DH, 24 components in LD, 47 components in LH, 74 components in HL, 46 components in HQ, 69 components in HB, 88 components in ZZ, 6 components in QD, 139 components in MX, 187 components in DG, and 24 components in SX. Among them, 50 components were repeated. Swiss Target Prediction database was used to predict the targets. Finally, after data collation, 552 targets were found in DH, 951 targets in DG, 869 targets in HB, 847 targets in HL, 477 targets in HQ, 326 targets in LD, 382 targets in LH, 785 targets in MX, 435 targets in QD, 338 targets in SX, and 711 targets in ZZ. Altogether, 1249 targets were identified after removing the duplicate data.

In order to better understand the relationship between each drug and its target, we created a compound-target network. (Fig. 1). Notably, a compound can regulate multiple targets, and conversely, a target can be regulated by multiple compounds. For example, chrysophanic acid, rhein, citreorosein, physcion 8-*O*-*β*-D-glucopyranoside, sennidin C and other compounds co-regulat MMP9. Many components interact and regulate multiple target genes in the network.

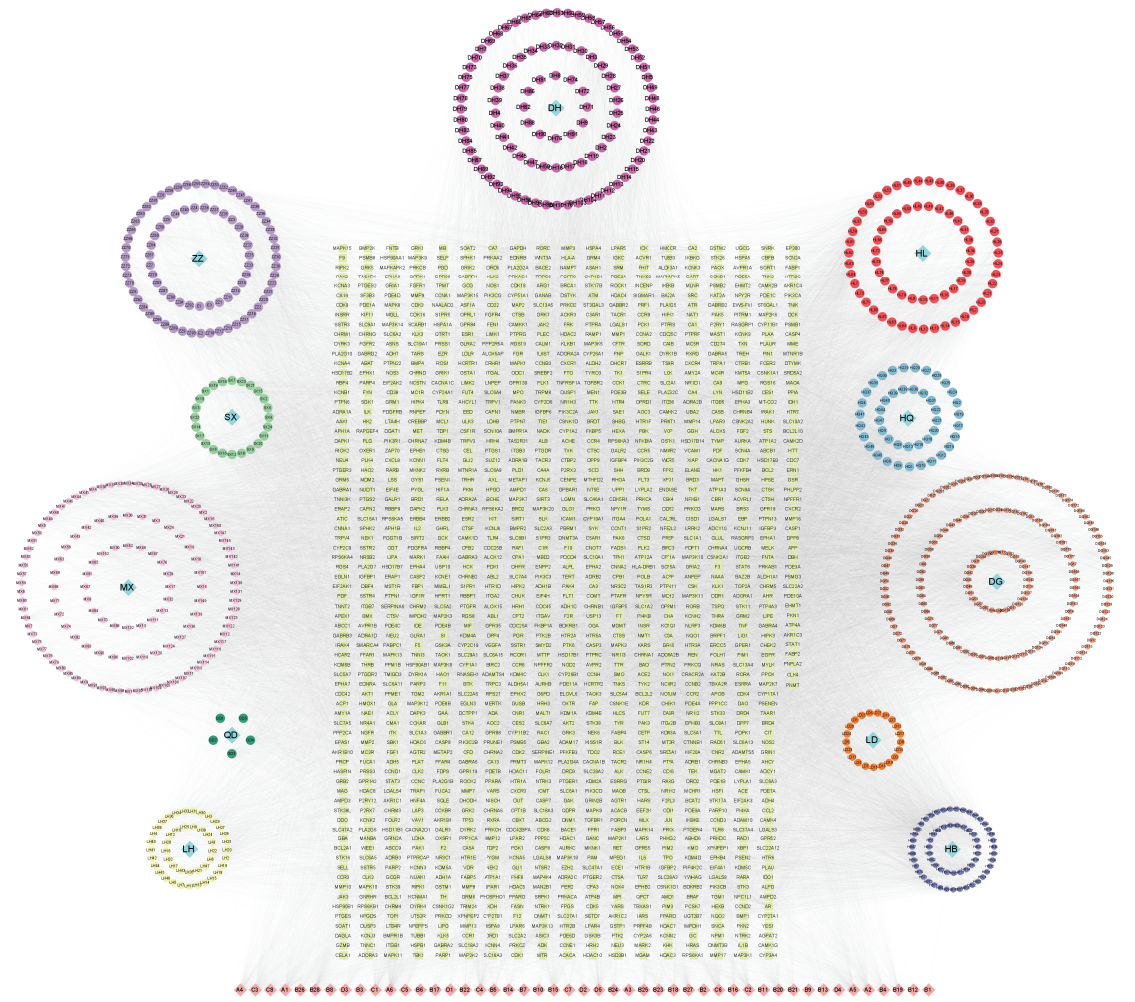

Fig. 1 Compound-Target Network of potential targets in DGLHW (The blue diamond represents TCM, the pink diamond represents the repeated ingredients in each herb, circles represent compounds, and yellow squares represent targets.)

3.2 Disease target analysis

Through the GEO database, the results were analyzed to obtain the 1783 CML-related disease targets (GSE100026). By analyzing the disease targets, we found that CD8A, IFNB, GADPH, CDK1, CD19, CXCL8, FCGR3B, CCNB1 and GZMB had higher degrees in the disease PPI analysis. It means they may play a part in the development of the disease.

3.3 GO and KEGG analysis of intersection targets

The intersection targets of DGLHW and CML were obtained by venn diagram (Fig. 2a). We then constructed the PPI network to reveal the relationship between these proteins. (Fig. 2b). In order to further explore the multiple mechanisms of DGLHW in CML at the system level, functional enrichment analysis was performed on the target genes. We input the 129 intersection genes into the

DAVID database for analysis. The results of analysis are relevant to the development of CML.

GO analysis results show that the functions of these potential targets are related to many biological processes and molecular function, which is important for the development of CML (Fig. 2c), 52 biological processes were enriched, and the top 10 biological processes were selected for analysis, such as protein phosphorylation, protein autophosphorylation, peptidyl-tyrosine phosphorylation, G2/M transition of mitotic cell cycle, transmembrane receptor protein tyrosine kinase signaling pathway, chemotaxis, peptidyl-serine phosphorylation, cell chemotaxis, calcium-mediated signaling, and chemokine-mediated signaling pathway. They play an important role in cell signaling pathways, including cell proliferation, migration, apoptosis, differentiation and cell cycle. This means that DGLHW influences the disease process of CML by regulating these biological processes.

In molecular function analysis, top 10 GO terms indicated the targets were related to protein tyrosine kinase activity, ATP binding, C-C chemokine receptor activity, C-C chemokine binding, chemokine receptor activity, and transmembrane receptor protein tyrosine kinase activity. These chemokines help regulate the migration and activation of immune cells in response to inflammation and infection [18]. Besides, we found that many molecular functions were related to protein kinase activities, including tyrosine kinase activity. According to the literature, TKIs, such as imatinib, play a vital role in the treatment of CML diseases and TKIs are still the main therapeutic drug for the treatment of CML.

To further reveal the potential mechanism of the DGLHW on the effect of CML, we conducted KEGG pathway enrichment analysis and screened out top 20 pathways based on the threshold of $P < 0.01$ (Fig. 2d), such as cell cycle, viral protein interaction with cytokine and cytokine receptor, cellular senescence, Th17 cell differentiation, calcium signaling pathway, T cell receptor signaling pathway, cytokine-cytokine receptor interaction, chemokine signaling pathway, phospholipase D signaling pathway, p53 signaling pathway, FoxO signaling pathway, IL-17 signaling pathway and so on. These signaling pathways are closely related to many biological processes such as cell growth, proliferation, differentiation and energy metabolism.

Furthermore, PI3K pathway inhibits the FoxO family of transcription factors. Inactivation of FoxO results in enhanced proliferation and reduced apoptosis in CML cells. Besides, T cell function is central to immune reconstitution and control of residual chronic myeloid leukemia [19]. Also, we found p53, FoxO and Ras signaling pathway were related to the constitutive activation of BCR-ABL1 [20], the central player in the pathogenesis of CML.

Our screened targets are consistent with those in the literature reports, indicating that DGLHW can play a therapeutic role in CML by regulating tyrosine kinase activity, inhibiting pathways associated with CML and restraining cell proliferation.

3.4 Compatibility of DGLHW prescription

In order to study the role of monarch drug, minister drug, assistant and guide drug in the formula, we analyzed the compatibility of the DGLHW prescription. We firstly constructed the venn diagram of intersection targets of monarch drug, minister drug, assistant and guide drug with CML, respectively. (Fig. 3a).

We found that kaempferol, chrysophanic acid, vanillic acid and other 173 compounds played an important role in the effect of the monarch drug. These ingredients in the monarch drug co-regulate CA1, CDK1, CCNB1, GAPDH, MMP9, CXCL8 and other proteins. By analyzing these genes, we found that monarch drug related targets

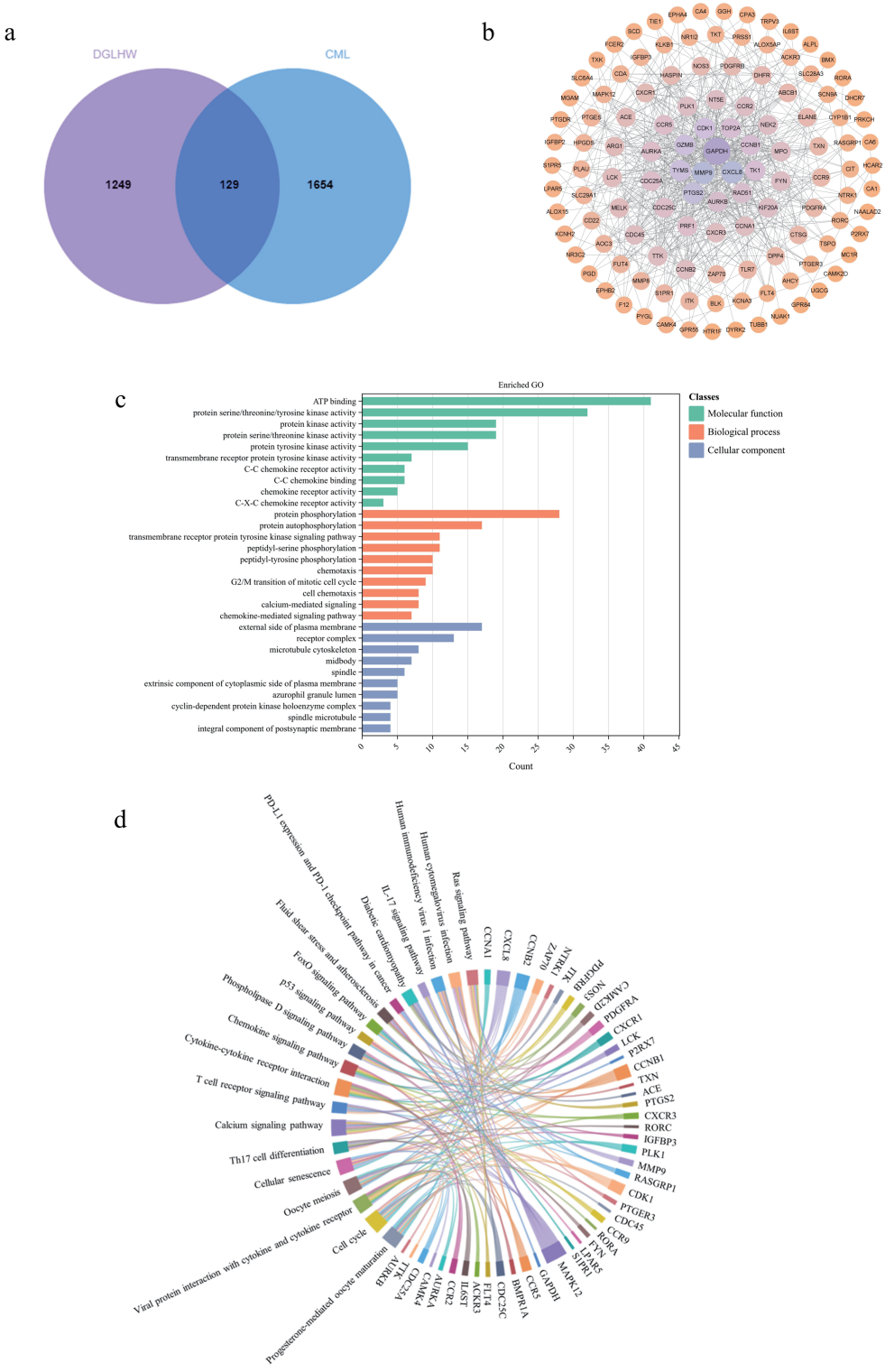

Fig. 2 a. Venn diagram of ingredients and CML targets; b. PPI network of intersection genes; c. GO enrichment analysis of intersection targets; d. KEGG pathway analysis of intersection targets

were enriched into cell cycle, cellular senescence, p53 signaling pathway, FoxO signaling pathway, pathways in cancer, PI3K-Akt signaling pathway, MAPK signaling pathway, and so on. Through GO analysis (Fig. 3b), we identified some biological processes, such as peptidyl-tyrosine phosphorylation, G2/M transition of mitotic cell cycle, protein autophosphorylation, transmembrane receptor protein tyrosine kinase signaling pathway and positive regulation of kinase activity. In molecular function, we obtained top 10 GO terms, such as protein serine / threonine / tyrosine kinase activity, ATP binding, protein tyrosine kinase activity, protein kinase activity, protein serine/threonine kinase activity and carbonate dehydratase activity. These shows that monarch drug may play a major role in the treatment of CML.

Analysis of minister drugs showed that berberine, palmatine, magnoflorine, beta-sitosterol and other 275 components might play an important role. Together, these components regulate GAPDH, MMP9, CXCL8, PTGS2, GZMB, TYMS, TK1, CCNB1 and other proteins. The intersection genes of minister drugs were analyzed. Through KEGG analysis, numerous pathways for potential target genes were identified, such as viral protein interaction with cytokine and cytokine receptor, cytokine-cytokine receptor interaction, chemokine signaling pathway, cell cycle, p53 signaling pathway, calcium signaling pathway, Ras signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway and rap1 signaling pathway (Fig. 3c). Through GO analysis, top 10 GO terms in biological process were identified, such as protein phosphorylation, protein autophosphorylation, peptidyl-tyrosine phosphorylation, transmembrane receptor protein tyrosine kinase signaling pathway, G2/M transition of mitotic cell cycle, chemotaxis, cell chemotaxis and calcium-mediated signaling. Molecular function analysis showed these genes played a role in protein serine/threonine/tyrosine kinase activity, ATP binding, protein kinase activity, non-membrane spanning protein tyrosine kinase activity, protein serine/threonine kinase activity, and transmembrane receptor protein tyrosine kinase activity. The analysis results of minister drug in GO and KEGG were consistent with that of monarch drug, indicating that minister drug assisted monarch drug in the treatment of CML to a certain extent.

By analyzing the 347 ingredients of the assistant and guide drug, we found that most components had heat clearing, detoxifying, anti-inflammatory and antioxidant effects. They could relieve fever, pain, bleeding and other symptoms caused by CML. Assistant and guide drug work together to regulate GAPDH, MMP9, CXCL8, PTGS2, TYMS, CDK1, TK1, CCNB1, TOP2A and other key proteins. By KEGG analysis, we found these genes are implicated in cell cycle, p53 signaling pathway, Chemokine signaling pathway, phospholipase D signaling pathway, PI3K-Akt signaling pathway, rap1 signaling pathway, MAPK signaling pathway (Fig. 3d). GO analysis showed that assistant and guide drug related genes were enriched into protein phosphorylation, chemotaxis, peptidyl-tyrosine phosphorylation, protein autophosphorylation, calcium-mediated signaling, G2/M transition of mitotic cell cycle and peptidyl-serine phosphorylation in biological process. Besides, molecular function results showed that the genes were closely related in protein serine/threonine/tyrosine kinase activity, protein tyrosine kinase activity, ATP binding, chemokine receptor activity, protein kinase activity and serine-type endopeptidase activity. The analysis of GO and KEGG of assistant and guide drug showed that it could help monarch drug and minister drug in the treatment effect of CML.

On the whole, 11 Chinese medicines cooperate with each other, exhibit synergistic effect, and jointly play a role in the treatment of CML. The results of compatibility analysis of DGLHW showed that

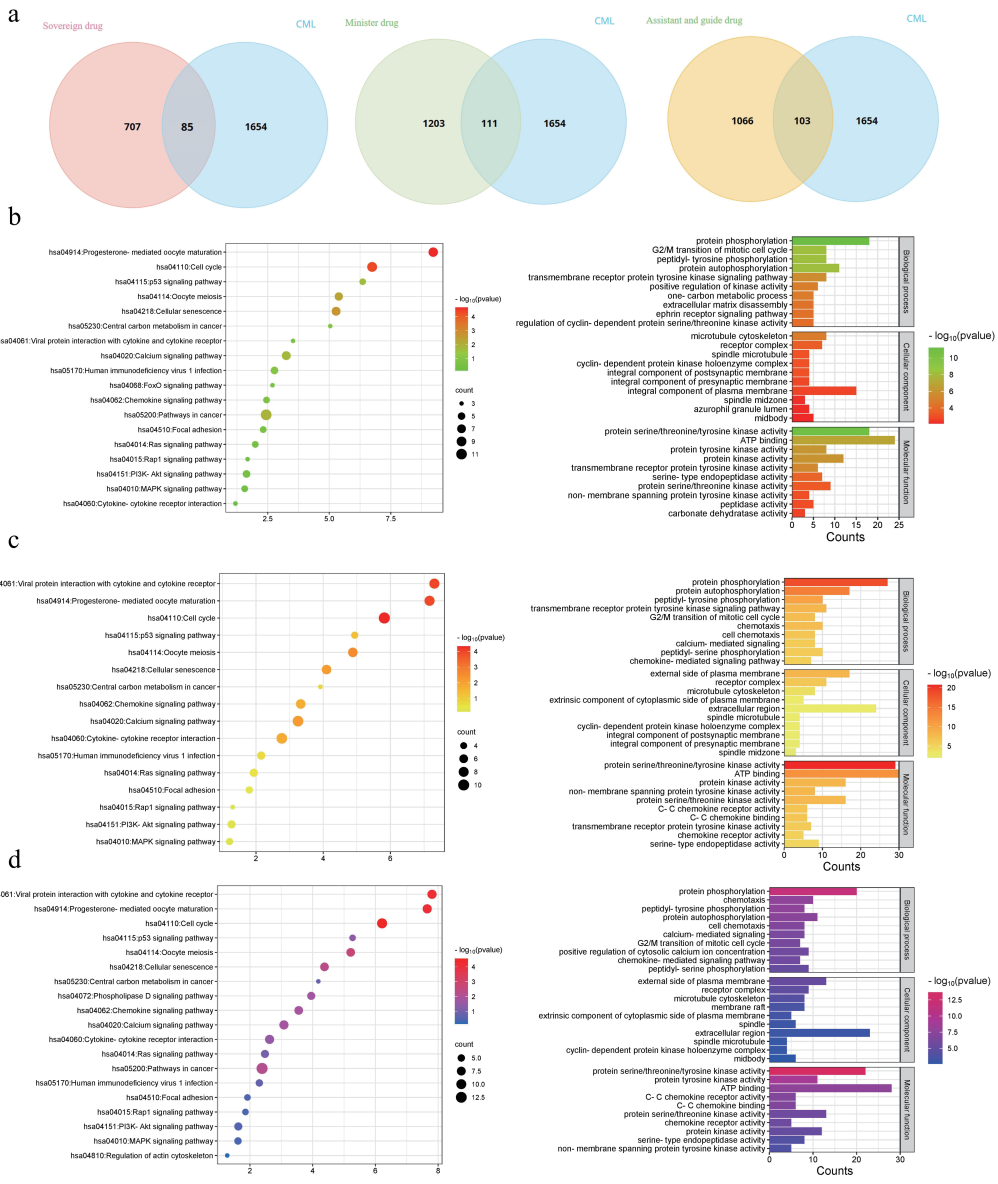

Fig. 3 a. Common targets of monarch drug, minister drug, assistant and guide drug and CML, respectively; b. GO and KEGG analysis of monarch drug and CML common genes; c. GO and KEGG analysis of minister drug and CML common genes; d. GO and KEGG analysis of assistant and guide drug and CML common genes

monarch drug, minister drug, assistant and guide drug were enriched in the p53 signaling pathway, calcium signaling pathway, Ras signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway, and other important pathways. In addition, the molecular function was enriched in tyrosine kinase inhibitors and energy metabolism. These are closely related to the development of CML disease. Therefore, we believe that the drugs in the compound have a synergistic effect in the treatment of CML.

3.5 Core gene and key component analysis

Based on the results of the above investigations, the possible molecular mechanism of DGLHW in

the treatment of CML was preliminarily clarified. In order to further study the compatibility and synergistic mechanism of DGLHW from the molecular mechanism, we studied the core genes of the prescription. We selected the intersection targets of monarch drug, minister drug, assistant and guide drug and disease through the venn diagram, obtaining 73 core targets (Fig. 4a). GO analysis showed that these genes were enriched in tyrosine kinases, energy metabolism, cell cycle and other molecular functions related to CML (Fig. 4b). Besides, enrichment analysis of these genes was performed to investigate the functions of these genes. KEGG analysis (Fig. 4c) indicated these genes were enriched in p53 signaling pathway, FoxO signaling pathway, MAPK signaling pathway and other signaling pathways associated with CML. The results of the analysis of these genes were generally consistent with the compatibility analysis. This suggests that these genes play a key role in regulating the effect of the monarch drug, minister drug, assistant and guide drug.

We constructed PPI network to analyze the relationship between 73 genes and Chinese medicine ingredient (Fig 4d). Among them, the top 20 genes with higher degree were identified as the hub genes. They are CA1, CA4, PTGS2, MMP9, CA6, SLC6A4, CDK1, ABCB1, CCNB1, MMP8, CDC25A, PTGES, P2RX7, ELANE, PYGL, LCK, CCNB2, SLC29A1, MPO and NR3C2. Meanwhile, we constructed heat maps of repeated compounds with 11 herbs (Fig. 4e). The 12 components with the highest number of repetitions were identified as the key compounds. They are beta-sitosterol, linoleic acid, stigmasterol, vanillin, protocatechuic acid, chlorogenic acid, palmitic acid, myristic acid, myrcene, chrysophanic acid, kaempferol and berberine.

3.6 Molecular docking simulation

Molecular docking is an important means to understand the interaction mechanism between drugs and target proteins. Previously, we have investigated the underlying mechanism of DGLHW in the treatment of CML. We selected 12 key ingredients in the formula for molecular docking with 12 hub proteins involved in the development of CML disease. The energy of each compound docking with the protein was plotted as a heat map (Fig. 5a). The compounds showed relatively high binding potential to the active site of the target and the docking energies were -149.428, -141.901, -136.447, and -133.722 kcal/mol, respectively (Fig. 5b - 5e).

4 Discussion

Chronic myeloid leukemia (CML) is a hematopoietic stem cell disorder characterized by BCR-ABL1, an oncogenic fusion gene arising from the Philadelphia chromosome. The development of tyrosine kinase inhibitors (TKIs) to overcome the constitutive tyrosine kinase activity of the BCR-ABL protein has dramatically improved disease management and patient outcomes over the past decades [21]. In this study, we analyzed the potential molecular mechanism of DGLHW in treating CML. Studies have shown that the DGLHW can enhance the synergistic effect of monarch, minister, assistant and guide through multi-component, multi-target and multi-pathway, so as to treat CML, and may also play a preventive and therapeutic role in other diseases.

According to the literature, we have identified some key proteins that regulate the pathological process of CML. MMP9 has the ability to degrade the extracellular matrix components and plays an important role in the pathophysiological functions. Overexpression of MMP9 is related to various diseases [22]. We found that MMP9 was related to different phases of CML [23], and the expression level of MMP9 was significantly decreased in CML patients treated with TIKs. Ubiquitous BCR-ABL

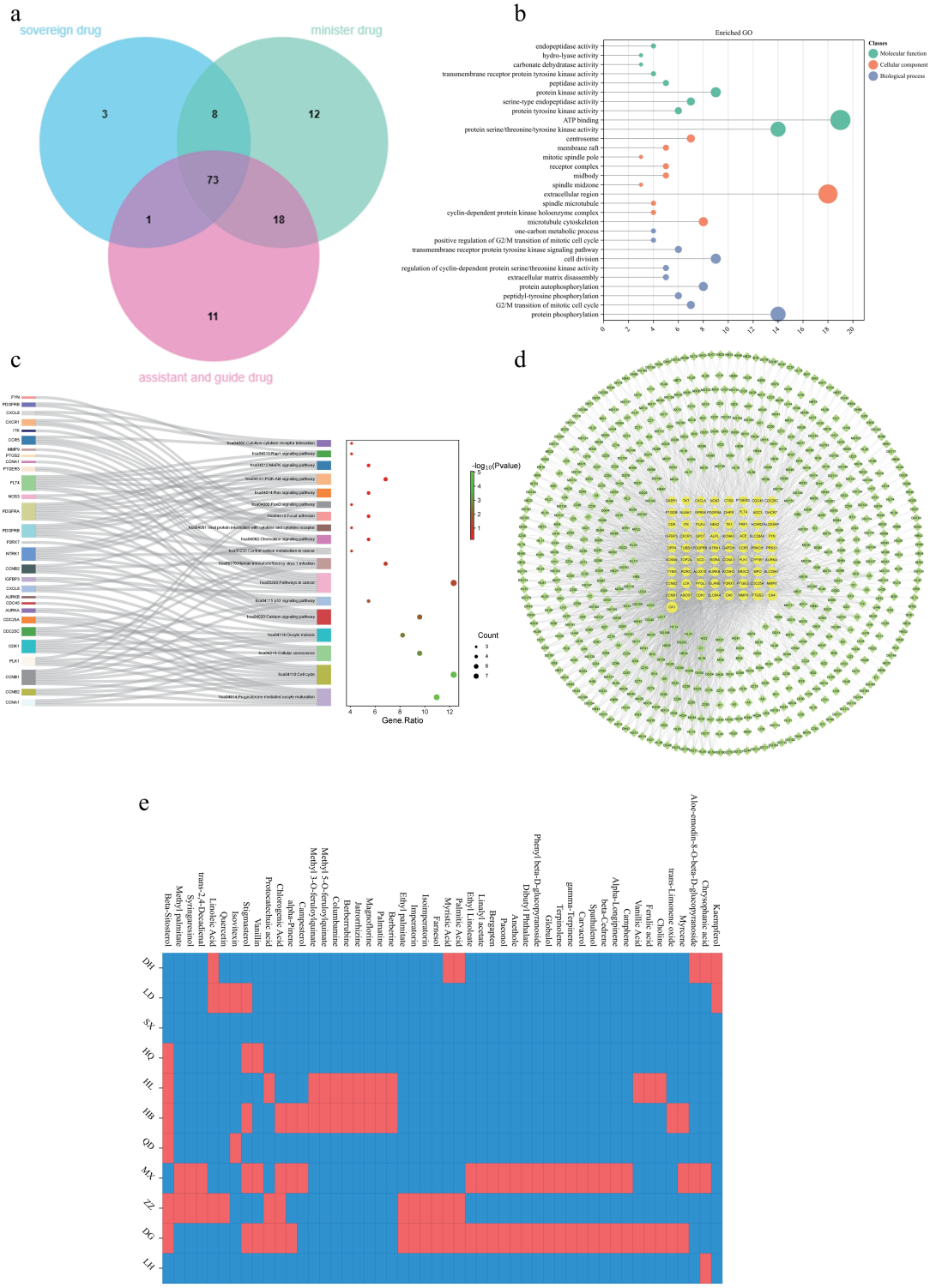

Fig. 4 a. Common targets of monarch drug, minister drug, assistant and guide drug and CML; b. The KEGG analysis of core genes; c. The GO analysis of core genes; d. Network of core gene and DGLHW ingredients; e. Volcanic map of repeated ingredients and Chinese medicine (Red represents compounds present in the Chinese medicine. Blue indicates that the compound is not present in the Chinese medicine.)

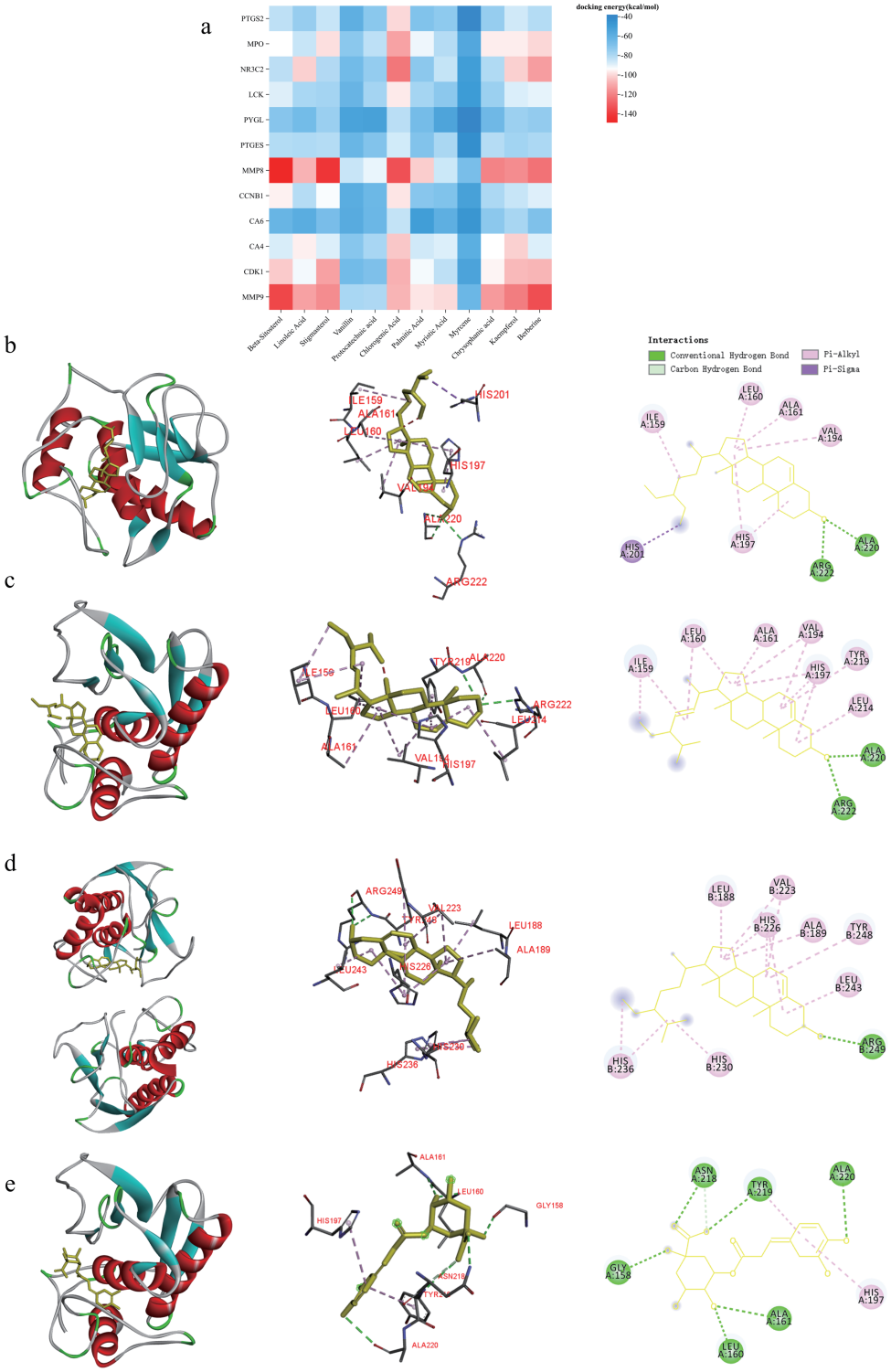

Fig. 5 a. Heat map of molecular docking of key components and hub proteins; b. Molecular docking of compound beta-sitosterol and the MMP8 protein; c. Molecular docking of compound stigmasterol and the MMP8 protein; d. Molecular docking of compound beta-sitosterol and the MMP9 protein; e. Molecular docking of compound chlorogenic acid and the MMP8 protein

expression stimulates CML by activating CDK1 and cyclin B1, promoting pro-apoptotic, inhibiting antiapoptotic marker expression and regulating the activation of Ras pathway. Thus, CDK1 is of great importance in antileukemic treatment [24].

In addition, some components in the prescription also showed the effect of treating CML. For instance, indirubin, the active component of DGLHW, a traditional Chinese medicine formulation, has shown promising clinical results. In the 1980s, chronic myeloid leukemia patients treated with indirubin demonstrated encouraging results [25]. The mechanism of its action in the treatment of chronic myeloid leukemia mainly lies in the influence on chromosome DNA synthesis and energy metabolism of tumor cells, but it has no obvious inhibitory effect on bone marrow. It can inhibit the cell proliferation and induce cell differentiation and cell death [26]. Kaempferol, a flavonoid compound, can enhance the TRAIL-induced cytotoxicity and apoptosis in human CML cell line K-562. Therefore, the pharmacological effect of kaempferol can be regarded as a way to treat clinical CML [27]. Berberine is a quaternary ammonium alkaloid isolated from *Coptis chinensis*. It is involved in leukemia stem cell-related pathways by targeting JAK2 and MCL1. Besides, it can inhibit the cell viability and colony formation of BCR-ABL1 independent imatinib-resistant cells in vitro, while extending the survival time of transplanted CML mice and transplanted CML-like mouse models *in vivo* [28]. We also found chlorogenic acid can inhibit the phosphorylation of BCR-ABL and lead to apoptosis of CML cell lines. Meanwhile, ROS induced by chlorogenic acid can directly induce apoptosis by destroying mitochondrial membrane potential and activating caspase [29]. Quercetin can increase the expression levels of Bax, Caspase-3 and Caspase-8 in CML cells to induce apoptosis and inhibit cell proliferation [30]. Therefore, various components in the compound synergy and efficiency together play the role of anti-CML, which also reflects the holistic principle of TCM compound in treating diseases.

Thus, we analyzed the mechanism of action of DGLHW in CML disease by applying multiple active ingredients to multiple targets, and the results were consistent with the literature.

5 Conclusion

In summary, DGLHW has significant advantages in the treatment of CML, which is consistent with the previous studies. Also, we reveal and predict the main biological information and pharmacological mechanisms of DGLHW treatment of CML by network pharmacological method. Results show that it plays a role in the treatment of CML by regulating CA1, PTGS2, MMP9, CDK1 and other proteins to affect MAPK, PI3K/AKT, FoxO and p53 signaling pathways (Fig. 6). The analysis of the results of compatibility study found that it had the same effect as the formula on the whole. It is of significant value to provide theoretical basis for clinical treatment CML. Although DGLHW is a promising drug for treatment of chronic myeloid leukemia, further research is needed to prove it.

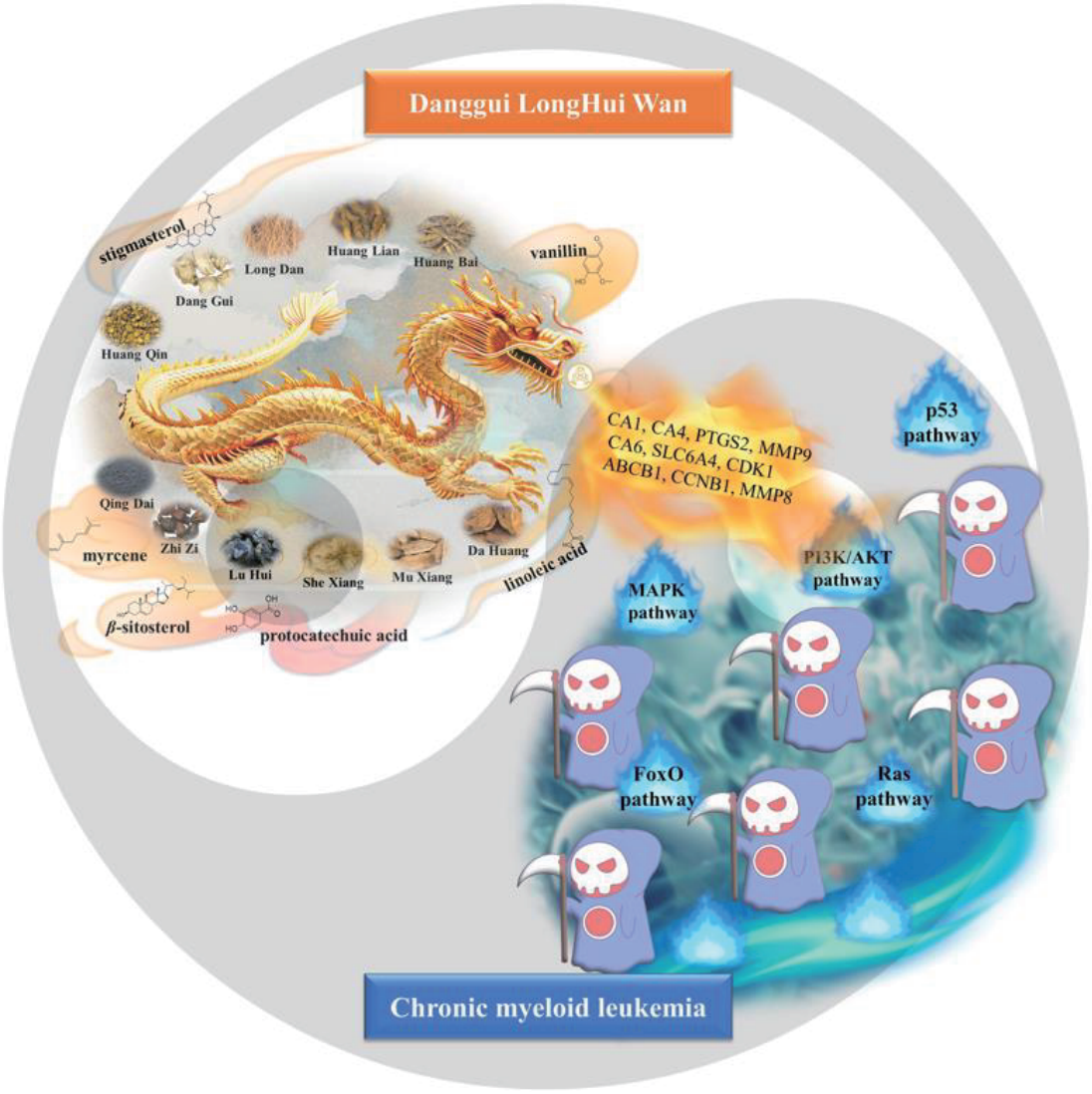

Fig. 6 Potential mechanisms of DGLHW in the treatment of CML

Conflict of interest

The authors declare no conflict of interest.

References

[1] Flis S, Chojnacki T. Chronic myelogenous leukemia, a still unsolved problem: pitfalls and new therapeutic possibilities. Drug Des Devel Ther, 2019, 13: 825-843.

[2] Osman AEG, Deininger MW. Chronic Myeloid Leukemia: Modern therapies, current challenges and future directions. Blood Rev, 2021, 49: 100825.

[3] Sattler M, Griffin JD. Molecular mechanisms of transformation by the BCR-ABL oncogene. Semin Hematol, 2003, 40: 4-10.

[4] Barreto Vianna DR, Gotardi J, Baggio Gnoatto SC, et al. Natural and Semisynthetic Pentacyclic Triterpenes for Chronic Myeloid Leukemia Therapy: Reality, Challenges and Perspectives. Chem Med Chem, 2021, 16: 1835-1860.

[5] Younes S, Ismail MA, Al-Jurf R, et al. Management of chronic myeloid leukaemia: current treatment options, challenges, and future strategies. Hematology, 2023, 28: 2196866.

[6] Alves R, Gonçalves AC, Rutella S, et al. Resistance to Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia-From Molecular Mechanisms to Clinical Relevance. Cancers (Basel), 2021, 13: 4820.

[7] Perabo FG, Frössler C, Landwehrs G, et al. Indirubin-3'-monoxime, a CDK inhibitor induces growth inhibition and apoptosis-independent up-regulation of survivin in transitional cell cancer. Anticancer Res, 2006, 26: 2129-2135.

[8] Yu XG, Qiao J, Wang C, et al. Determination of 3 components in Danggui Longhui Pills by RP-HPLC. Northwest Pharm J, 2021, 36: 386-389.

[9] Gaboriaud-Kolar N, Myrianthopoulos V, Vougogiannopoulou K, et al. Natural-Based Indirubins Display Potent Cytotoxicity toward Wild-Type and T315I-Resistant Leukemia Cell Lines. J Nat Prod, 2016, 79: 2464-2471.

[10] Guo MF, Dai YJ, Gao JR, et al. Uncovering the Mechanism of Astragalus membranaceus in the Treatment of Diabetic Nephropathy Based on Network Pharmacology. J Diabetes Res, 2020, 2020: 5947304.

[11] Ni M, Liu X, Meng Z, et al. A bioinformatics investigation into the pharmacological mechanisms of javanica oil emulsion injection in non-small cell lung cancer based on network pharmacology methodologies. BMC Complement Med Ther, 2020, 20: 174.

[12] Gfeller D, Grosdidier A, Wirth M, et al. Swiss Target Prediction: a web server for target prediction of bioactive small molecules. Nucleic Acids Res, 2014, 42: W32-W38.

[13] Clough E, Barrett T. The Gene Expression Omnibus Database. Methods Mol Biol, 2016, 1418: 93-110.

[14] Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets--update. Nucleic Acids Res, 2013, 41: D991-D995.

[15] Szklarczyk D, Franceschini A, Wyder S, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res, 2015, 43: D447-452.

[16] Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res, 2003, 13: 2498-2504.

[17] Ji X, Tang J, Zhang J. Effects of Salt Stress on the Morphology, Growth and Physiological Parameters of Juglansmicrocarpa L. Seedlings. Plants (Basel), 2022, 11: 2381.

[18] Märkl F, Huynh D, Endres S, et al. Utilizing chemokines in cancer immunotherapy. Trends Cancer, 2022, 8: 670-682.

[19] Naka K, Hoshii T, Muraguchi T, et al. TGF-beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. Nature, 2010, 463: 676-680.

[20] Minciacchi VR, Kumar R, Krause DS. Chronic Myeloid Leukemia: A Model Disease of the Past, Present and Future. Cells, 2021, 10: 117.

[21] Bugler J, Kinstrie R, Scott MT, et al. Epigenetic Reprogramming and Emerging Epigenetic Therapies in CML. Front Cell Dev Biol, 2019, 7: 136.

[22] Mondal S, Adhikari N, Banerjee S, et al. Matrix metalloproteinase-9 (MMP-9) and its inhibitors in cancer: A minireview. Eur J Med Chem, 2020, 194: 112260.

[23] He ZK, Xue S, Zhang YH, et al. Expression Levels of JARID1B, Hes1 and MMP-9 Genes in CML Patients Treated with Imatinib Mesylate. Zhongguo Shi Yan Xue Ye Xue Za Zhi, 2019, 27: 1071-1076.

[24] Rao Q, Xie K, Varier KM, et al. Design, Synthesis, and Antileukemic Evaluation of a Novel Mikanolide Derivative Through the Ras/Raf/MEK/ERK Pathway. Front Pharmacol, 2022, 13: 809551.

[25] Blažević T, Heiss EH, Atanasov AG, et al. Indirubin and Indirubin Derivatives for Counteracting Proliferative Diseases. Evid Based Complement Alternat Med, 2015, 2015: 654098.

[26] Xiao Z, Hao Y, Liu B, et al. Indirubin and meisoindigo in the treatment of chronic myelogenous leukemia in China. Leuk Lymphoma, 2002, 43: 1763-1768.

[27] Saraei R, Rahman HS, Soleimani M, et al. Kaempferol sensitizes tumor necrosis factor-related apoptosis-inducing ligand-resistance chronic myelogenous leukemia cells to apoptosis. Mol Biol Rep, 2022, 49: 19-

29.

[28] Huang G, Yin Z, Wang X, et al. System analysis of Huang-Lian-Jie-Du-Tang and their key active ingredients for overcoming CML resistance by suppression of leukemia stem cells. Phytomedicine, 2023, 117: 154918.

[29] Rakshit S, Mandal L, Pal BC, et al. Involvement of ROS in chlorogenic acid-induced apoptosis of Bcr-Abl+ CML cells. Biochem Pharmacol, 2010, 80: 1662-1675.

[30] Hassanzadeh A, Hosseinzadeh E, Rezapour S, et al. Quercetin Promotes Cell Cycle Arrest and Apoptosis and Attenuates the Proliferation of Human Chronic Myeloid Leukemia Cell Line-K562 Through Interaction with HSPs (70 and 90), MAT2A and FOXM1. Anticancer Agents Med Chem, 2019, 19: 1523-1534.