Biomedical Sciences
2023; 9(3): 53-59
http://www.sciencepublishinggroup.com/j/bs
doi: 10.11648/j.bs.20230903.11
ISSN: 2575-3924 (Print); ISSN: 2575-3932 (Online)

Analysis of the Mechanism of *Salvia miltiorrhiza* in the Treatment of Pancreatic Cancer Based on Network Pharmacology

Xueling Tan[1], Jiajun Chen[2], Yuting Bai[3], Xin Chen[4, *]

[1]Department of Clinical Laboratory, Nanchong Central Hospital Affiliated to North Sichuan Medical College, Nanchong, China

[2]Department of Clinical Laboratory, Gaoping District People's Hospital of Nanchong, Nanchong, China

[3]Department of Clinical Laboratory, Affiliated Hospital of North Sichuan Medical College, Nanchong, China

[4]Department of Rehabilitation Medicine, Affiliated Hospital of North Sichuan Medical College, Nanchong, China

Email address:

488874309@qq.com (Xin Chen)

[*]Corresponding author

To cite this article:

Xueling Tan, Jiajun Chen, Yuting Bai, Xin Chen. Analysis of the Mechanism of *Salvia miltiorrhiza* in the Treatment of Pancreatic Cancer Based on Network Pharmacology. *Biomedical Sciences*. Vol. 9, No. 3, 2023, pp. 53-59. doi: 10.11648/j.bs.20230903.11

Received: July 5, 2023; **Accepted**: July 19, 2023; **Published**: July 27, 2023

Abstract: *Objective*: To explore the potential molecular mechanism of *Salvia miltiorrhiza* in the treatment of pancreatic cancer using network pharmacology. *Methods*: The active components and their corresponding core targets in *Salvia miltiorrhiza* were screened by TCMSP database, and the corresponding gene Symbol of core targets was obtained by using Uniprot database. The gene targets for pancreatic cancer were searched from Gene Cards, OMIM, TTD and DrugBank databases. The potential targets of *Salvia miltiorrhiza* were matched with pancreatic cancer gene targets using Venn diagram, and the active components-targets network of *Salvia miltiorrhiza* and the active components-targets network of *Salvia miltiorrhiza* were mapped using Cytoscape software. The DAVID database was used to perform Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis to predict the potential mechanisms of targets. *Results*: A total of 65 active components of *Salvia miltiorrhiza* were screened, corresponding to 118 targets. A total of 2167 pancreatic cancer disease targets were screened, and 75 *Salvia miltiorrhiza*-pancreatic cancer crossover targets were obtained. Six core targets were obtained by PPI network analysis. Luteolin, tanshinone, quercetin and dihydrodanshinolide were the main active components, and ESR1, JUN, CDKN1A, MAPK14, MYC and TP53 were the main core targets. The results of GO analysis showed that the cellular components where the 75 targets of *Salvia miltiorrhiza* for pancreatic cancer therapy function are mainly the nucleus, membrane rafts, spindle, and membrane microdomains. The biological processes are mainly in response to radiation, response to xennobiotic stimulus, gland development, and so on. Molecular functions include DNA binding transcription factors, specific RNA polymerase II binding transcription factors, and regulation of kinase activity. 75 targets of *Salvia miltiorrhiza* for pancreatic cancer treatment are mainly through PI3K/Akt signaling pathway, cancer signaling pathway, pancreatic cancer signaling pathway, HIF-1 signaling pathway and other pathways to regulate tumor immune response, induce apoptosis, promote cell cycle arrest, and tumor metastasis, promote cell cycle arrest and tumor metastasis. *Conclusions*: This study reveals that *Salvia miltiorrhiza* may regulate multiple signaling pathways through multiple targets, thus acting as a therapeutic agent for pancreatic cancer, which also provides theoretical support for the clinical discovery of alternative drugs for pancreatic cancer treatment.

Keywords: Network Pharmacology, Pancreatic Cancer, Mechanism, *Salvia miltiorrhiza*

1. Introduction

Pancreatic cancer is one of the malignant digestive tumours with the highest mortality rate worldwide. It is highly aggressive and difficult to detect at an early stage, and is currently very challenging to treat clinically, accounting for 2.6% of all new cases of malignant tumours in 2020 [1]. Although

surgical resection with post-adjuvant therapy can significantly prolong patient survival, only 13-15% of patients are likely to undergo pancreaticoduodenectomy. Although precision medicine and targeted drugs have been vigorously developed, the efficacy has not met expectations and the 5-year survival rate is still below 10%, urging us to find new therapeutic agents [2]. Botanicals contain a variety of components and targets of action that have outstanding advantages in inhibiting tumour growth and metastasis, and have been used in the treatment of a wide range of cancers. For example, honeysuckle is widely used in the treatment of diseases such as fever and tumours [3, 4], andrographolide reduces oxidative stress and inflammation in arthritis [5, 6], and thujaplicins have anti-inflammatory, antioxidant and anti-cancer effects [7, 8]. Bitter in taste and slightly cold, *Salvia miltiorrhiza* belongs to the heart and liver meridians. It is a classical medicine for activating blood circulation and resolving blood stasis, and the active ingredients in it play an important role in a variety of diseases [9], but there has been a lack of a holistic analysis of the study of *Salvia miltiorrhiza*. In this study, we used network pharmacology as a starting point to find all the targets of the active ingredient and the diseases that these targets may correspond to, by target fishing. Subsequently, a component-target network and PPI network were developed. GO and KEGG analyses of these targets were carried out to analyse the role of *Salvia miltiorrhiza* against pancreatic cancer in terms of biological function and signaling pathway enrichment.

2. Methods

2.1. Screening of Active Components and Targets of Salvia miltiorrhiza

The active ingredients of Salvia miltiorrhiza were searched through the Traditional Chinese Medicine system pharmacology database analysis platform (TCMSP, *http://lsp.nwu.edu.cn/tcmsp.php*) [10]. Due to the complexity of the components of Chinese medicine, not all of them enter the human body and act, the active ingredients that meet both the conditions of oral bioavailability≥30% and drug-like properties≥0.18 were screened, and The targets corresponding to each active ingredient were obtained in TCMSP.

2.2. Screening for Pancreatic Cancer Disease Targets and Pharmacodynamic Targets of Salvia miltiorrhiza for Pancreatic Cancer

The keyword "Pancreatic cancer" was used to obtain pancreatic cancer-related disease targets through four databases: GeneCards (*http://www.gencards.org/*) [11], OMIM (*https://omim.org/*) [12], TTD (*http://db.idrblab.net/ttd/*) [13] and DrugBank (*https://go.drugbank.com/*) [14] to obtain pancreatic cancer-related disease targets. The obtained active ingredients in *Salvia miltiorrhiza* were intersected with the pancreatic cancer target genes by creating a Venn diagram to screen out the common targets as the active ingredients of *Salvia miltiorrhiza* for the treatment of pancreatic cancer.

2.3. Constructing Protein-Protein Interaction Networks to Screen Core Targets

Import the pharmacodynamic targets obtained from the Venn diagram into the String database (*http://string-db.org*) [15], select "Homo sapiens" as the species, set the lowest interaction score to "highest confifence" (0.900), the rest of the parameters were kept unchanged, and the nodes without interactions were removed to construct the target protein-protein interaction network. The network was topologically analyzed by CytoNCA plug-in in Cytoscape 3.8.2 software, and the core targets of *Salvia miltiorrhiza* for pancreatic cancer were selected.

2.4. GO Functional Analysis and KEGG Pathway Enrichment Analysis

To investigate the biological process and the signalling pathways enriched by *Salvia miltiorrhiza* in the treatment of pancreatic cancer, GO analysis and KEGG pathway enrichment analysis were performed using the ClusterProfiler package in R language for the pharmacodynamic targets, and the results of the enrichment analysis were visualised and processed.

3. Results

3.1. Screening of Active Components of Salvia Miltiorrhiza and Their Target Prediction in the Treatment of Pancreatic Cancer

A total of 202 known active components of *Salvia miltiorrhiza* were retrieved from the TCMSP database. Sixty-five active components, corresponding to 118 action targets, were screened according to OB≥30% and DL≥18%. The active components with high connectivity were visualized by Cytoscape software to construct a "*Salvia miltiorrhiza* active component-target" network diagram, in which the sky-blue nodes represent the active components of *Salvia miltiorrhiza* and the pink nodes represent the targets, see Figure 1, The active components with high connectivity include lignan, tanshinone, quercetin and dihydrodanshenolide.

A total of 2167 pancreatic cancer-related disease genes were downloaded from GeneCards, OMIM, TTD and DrugBank databases, as shown in Figure 2A. 65 active ingredients in *Salvia miltiorrhiza* obtained were intersected with pancreatic cancer target genes using Venn diagrams, and 75 shared targets were screened as the active ingredients of *Salvia miltiorrhiza* for anti-pancreatic cancer potency, as shown in Figure 2B.

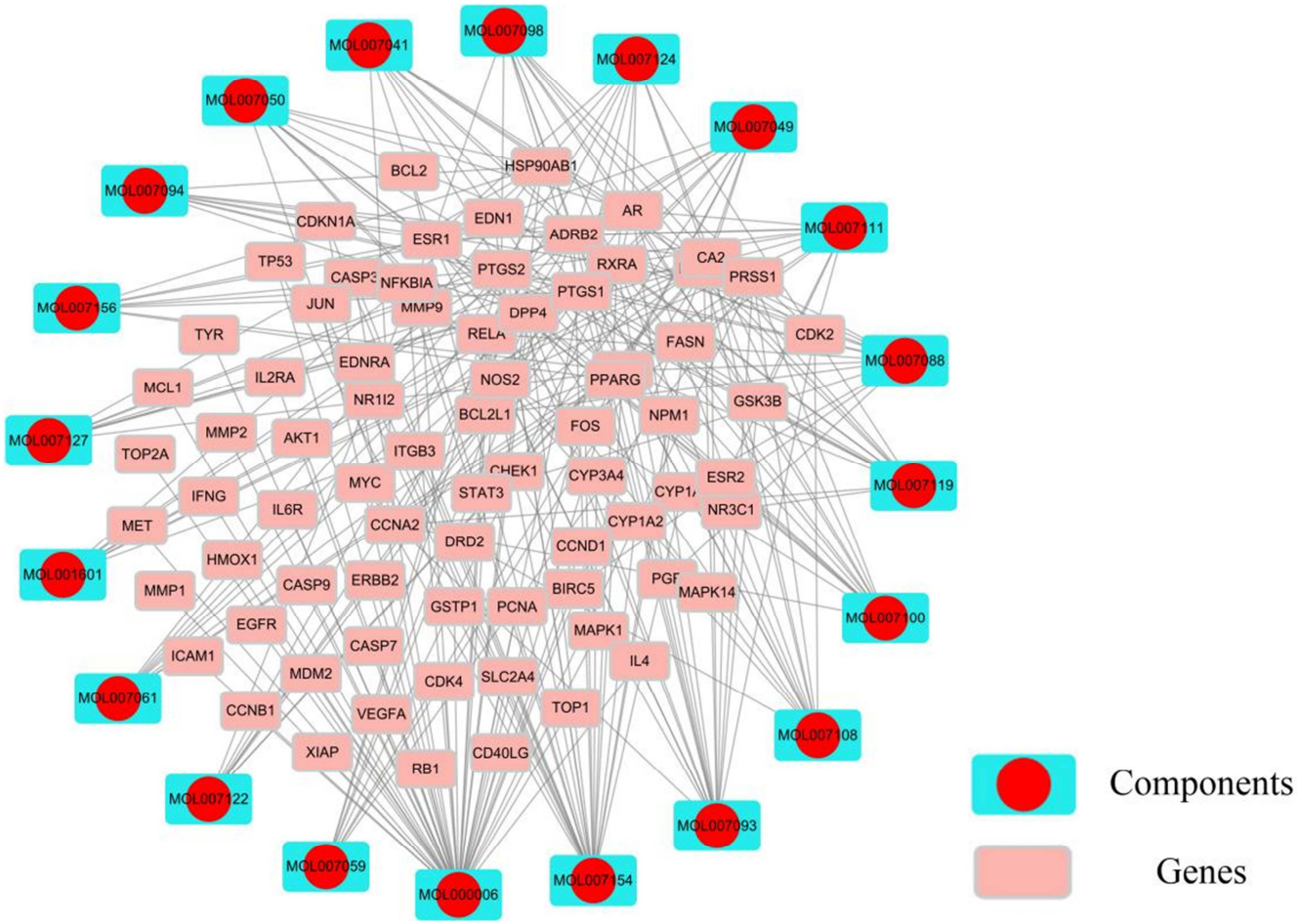

Figure 1. *Active Component-target genes network diagram. The sky-blue rectangle in the outer circle represents the active ingredients of Salvia miltiorrhiza, and the small pink rectangle in the inner circle represents the potential targets of Salvia miltiorrhiza. The grey line indicates the relationship between the active components of Salvia miltiorrhiza and potential targets.*

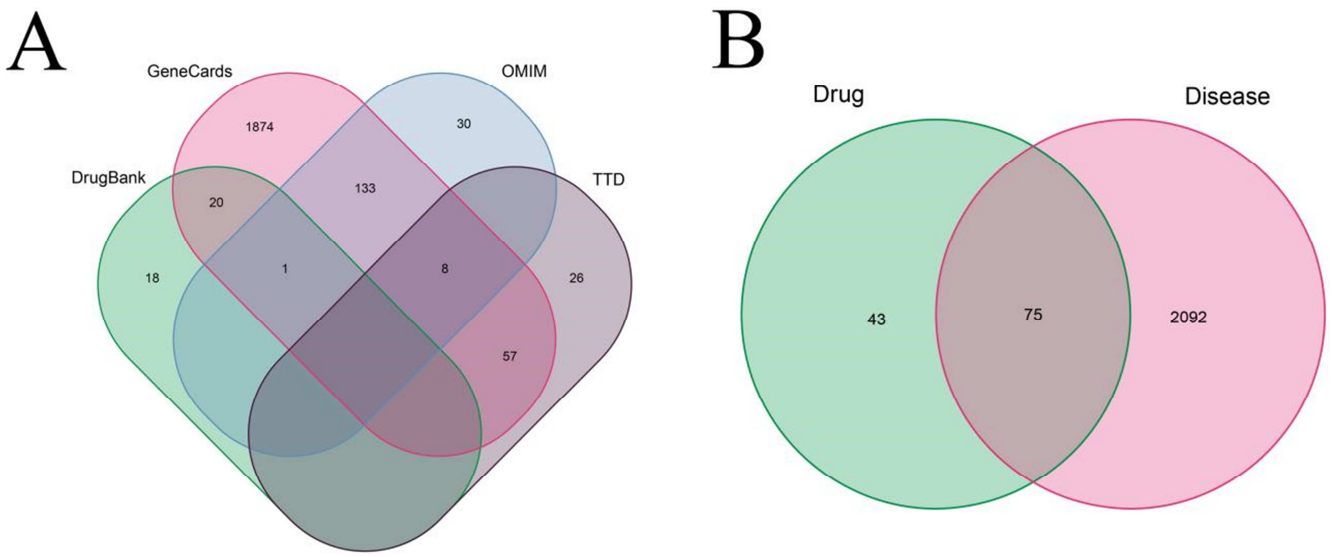

Figure 2. *Salvia miltiorrhiza-pancreatic cancer target map. (A) Summary map of pancreatic cancer-related targets in four databases. (B) Venn diagram of the intersection of Salvia miltiorrhiza target genes and targets of pancreatic cancer.*

3.2. Construction and Analysis of Target Protein Interaction Network of Salvia miltiorrhiza in the Treatment of Pancreatic Cancer

To further understand the functions and mechanisms of the pharmacodynamic targets of *Salvia miltiorrhiza* for pancreatic cancer, the 118 action targets obtained from the above screening were imported into the String database to construct a protein-protein interaction networks, and the topological analysis of the protein interaction network was performed using the CytoNCA, and the genes with BC>116.6, CC>0.51, DC>15.5, EC>0.18, LAC>6.39, NC>9 were taken as intersection and six core targets were identified from the collection, including, see Figure 3.

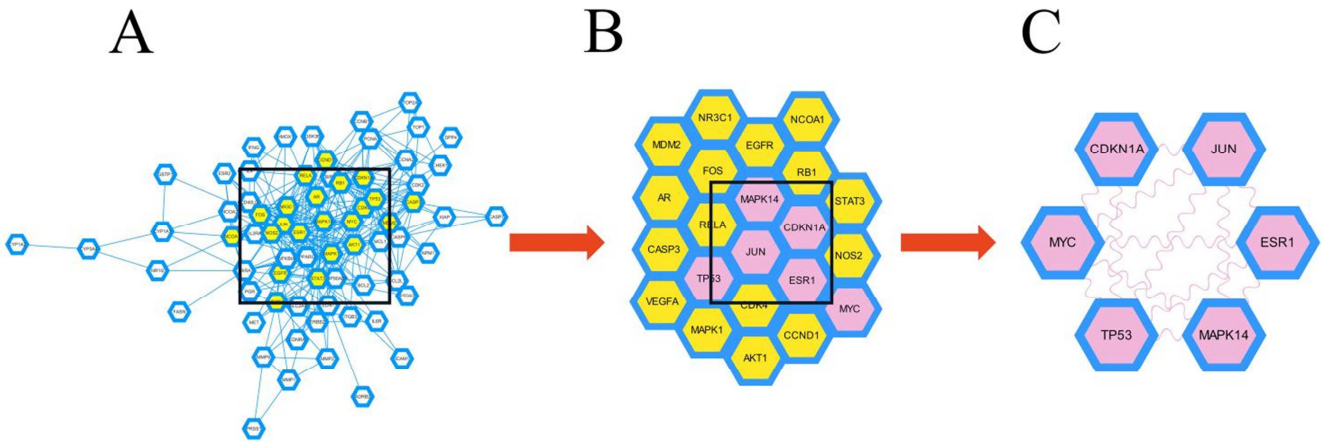

Figure 3. *PPI core network screening flowchart. (A) 118 potential therapeutic targets of Salvia miltiorrhiza. Yellow represents key targets from primary topological analysis. (B) Key targets obtained from first-order topological analysis. Purple represents core key genes acquired by secondary topological molecules. (C) 6 core key targets.*

3.3. GO Analysis and KEGG Pathway Enrichment Analysis of Salvia miltiorrhiza in the Treatment of Pancreatic Cancer

The 75 pharmacodynamic targets of the active ingredient of *Salvia miltiorrhiza* against pancreatic cancer were imported into the DAVID online database for GO analysis and KEGG pathway enrichment analysis, and the threshold value was set at P<0.05. The results of GO analysis showed 2117 entries, including 1937 entries of biological processes (BP), mainly related to response to radiation, response to xennobiotic stimulus, gland development, etc. Cell composition (CC) contains 44 entries, mainly involving nucleus, membrane rafts, spindle, membrane microdomains, etc. The enrichment analysis of KEGG pathways showed that 153 pathways were involved, and the P-value and gene number count were used to measure the enrichment of KEGG and rank the P-value from smallest to largest. In this study, the top 15 signaling pathways were selected for enrichment analysis, among which the potential targets of *Salvia miltiorrhiza* against pancreatic cancer were mainly enriched in PI3K/AKT signaling pathway, cancer signaling pathway, pancreatic cancer signaling pathway, HIF-1 signaling pathway and other signaling pathways, see Figure 4 and Figure 5.

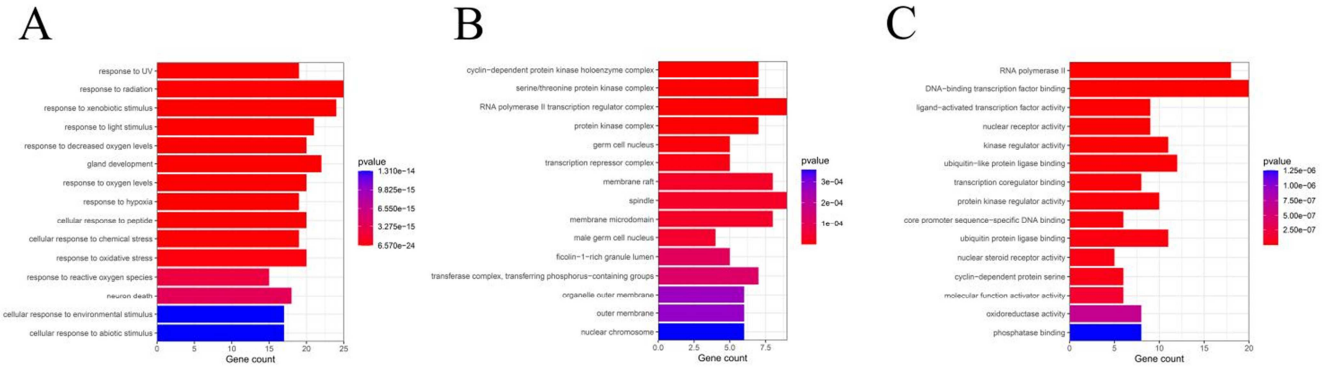

Figure 4. *GO analysis of therapeutic targets of Salvia miltiorrhiza in the treatment of pancreatic cancer. The X axis shows the number of genes enriched in each project, the Y axis indicates the biological process (BP) in which the genes are involved, the cellular component (CC) and the molecular function (MF), and the colour represents the p-value.*

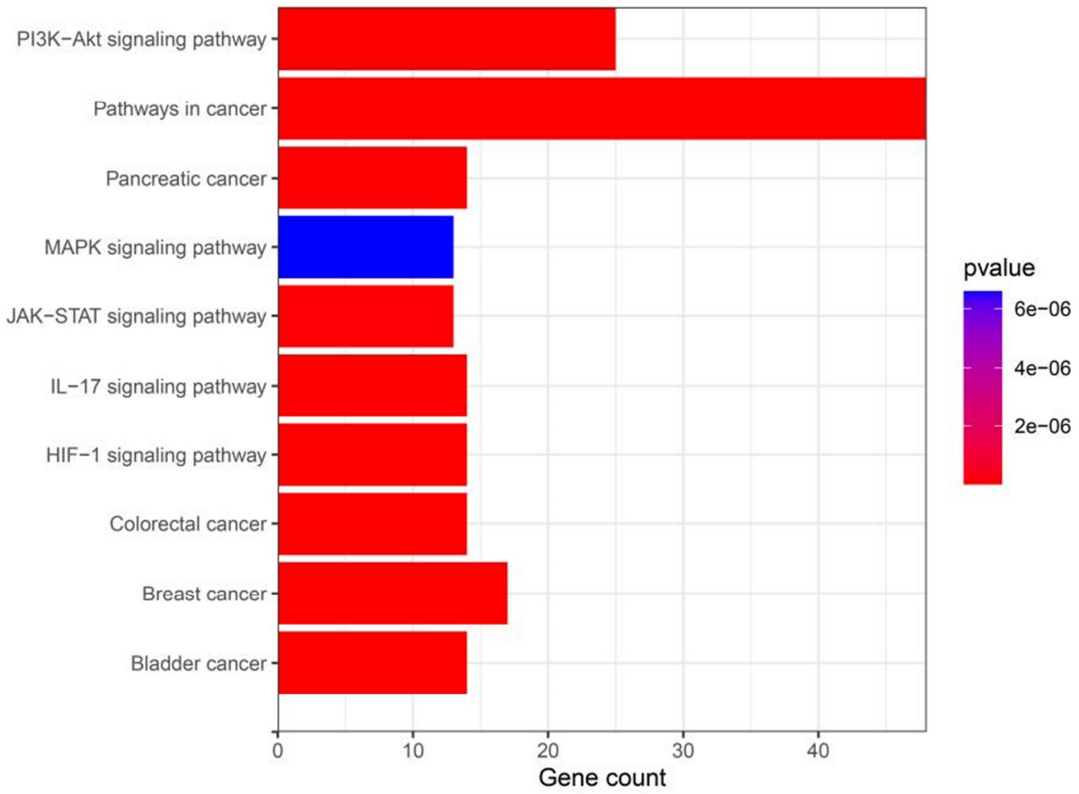

Figure 5. *KEGG pathway enrichment analysis of the therapeutic targets of Salvia miltiorrhiza in the treatment of pancreatic cancer. The X axis indicates the number of genes enriched in each pathway, the Y axis represents the name of the pathways, and the color indicates the p-value.*

4. Discussion

Up to now, although many studies have confirmed the significant antitumor effects of *Salvia miltiorrhiza*, the underlying mechanisms of its antitumor effects have not been fully explored. Cyberpharmacology is an emerging interdisciplinary discipline based on artificial intelligence and big data for the systematic study of drugs [16]. It has been proposed that the TCM network pharmacology approach is expected to shift the paradigm of attacking difficult-to-treat diseases from the traditional one-target-one-drug to network target and multi-component therapy [17], a new research paradigm for the systematic transformation of TCM from empirical medicine to evidence-based medicine [18]. In order to better elucidate the pharmacological mechanisms of *Salvia miltiorrhiza* in anti-pancreatic cancer therapy, we applied a network pharmacology approach to explore the potential mechanisms of *Salvia miltiorrhiza* in the treatment of pancreatic cancer through active ingredient-pancreatic cancer target network construction, PPI network analysis, GO analysis and KEGG enrichment analysis. Currently, there are few pharmacokinetic studies on *Salvia miltiorrhiza*, and in this study, we used OB and DL to screen the active components of *Salvia miltiorrhiza*.

In this study, 65 active components and 75 potential therapeutic targets of *Salvia miltiorrhiza* were obtained through TCMSP database and literature search, which also confirmed the multi-component, multi-target and multi-pathway synergistic characteristics of *Salvia miltiorrhiza* as a Chinese herbal medicine. The active components-target map revealed that luteolin, tanshinone and quercetin play important roles in the treatment of pancreatic cancer with *Salvia miltiorrhiza*. In addition, PPI networks demonstrated information about protein homology and co-expression, and PPI analysis in this study showed that *Salvia miltiorrhiza* exerted significant effects on pancreatic cancer by influencing the entire biological network, including ESR1, JUN, CDKN1A, MAPK14, MYC, TP53, etc. ESR1, estrogen receptor 1, is a transcription factor that promotes cell survival and proliferation, is expressed in approximately 70% of breast cancers, mediates drug resistance in breast cancer, interacts with different protein kinases to produce protein complexes, and stimulates activation of downstream molecules such as Akt during estrogen signaling [19, 20]. The potential role of ESR1 in pancreatic tumors has been debated for many years, but recently it has been reported that ESR1 expression is associated with poor prognosis in pancreatic cancer [21]. JUN is the most widely studied protein in the activator protein-1 (AP-1) complex. Numerous studies have found that phosphorylation of JUN plays an important role in the proliferation and apoptosis of tumor cells, and that phosphorylation of JUN affects its DNA binding ability on the one hand and its transcriptional activation ability on the other [22]. CDKNlA is one of the important proteins in cell cycle regulation and plays an important regulatory role in cell proliferation, differentiation and senescence [23]. Previous studies have suggested that CDKN1A is an important

oncogene, but recent studies have shown that CDKN1A exhibits a two-fold role in tumorigenesis and development, which may either inhibit or promote tumor growth [24]. In some tumors, CDKNlA may play more of its anti-apoptotic and tumor-promoting roles [25, 26].

The results of GO analysis of the pharmacological efficacy of *Salvia miltiorrhiza* against pancreatic cancer showed that the biological processes involved in *Salvia miltiorrhiza* against pancreatic cancer are mainly the response to radiation, response to xennobiotic stimulus, gland development, etc., mainly concentrated in the nucleus, membrane rafts, spindle, membrane microdomains, etc. The results of KEGG signaling pathway enrichment also suggest that the potential mechanism of *Salvia miltiorrhiza* against pancreatic cancer may be due to its coordinated regulation of various cancer-related pathways, such as angiogenesis, cell differentiation, migration, apoptosis, invasion and proliferation. pathways, such as angiogenesis, cell differentiation, migration, apoptosis, invasion and proliferation. In conclusion, the present study analyzed how the active ingredients in *Salvia miltiorrhiza* can fight against pancreatic cancer growth through multiple pathways through a network pharmacology approach, which is complementary to its drug studies against pancreatic cancer.

5. Conclusion

In summary, the potential molecular mechanism of *Salvia miltiorrhiza* for the treatment of pancreatic cancer may act through several core genes such as ESR1, JUN, CDKN1A, MAPK14, MYC, TP53 and regulating various pathways such as PI3K/AKT signaling pathway, cancer signaling pathway, and pancreatic cancer signaling pathway. In this study, a new understanding of the mechanism of action of *Salvia miltiorrhiza* in the treatment of pancreatic cancer was obtained using a network pharmacological approach, and the relationship between the active components of *Salvia miltiorrhiza* and pancreatic cancer was elucidated, providing a scientific basis for its clinical application and mechanism research.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgements

This research was supported by the school-level Scientific Research Project of North Sichuan Medical College-Youth Project (grant number: CBY22-QNA05 and CBY22-QNA45), and the Youth Research Project of the Affiliated Hospital of Sichuan North Medical College (grant number: 2023JC004).

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F: Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021, 71 (3): 209-249.

[2] Siegel RL, Miller KD, Fuchs HE, Jemal A: Cancer Statistics, 2021. *CA Cancer J Clin* 2021, 71 (1): 7-33.

[3] Xu Y, Oliverson BG, Simmons DL: Trifunctional inhibition of COX-2 by extracts of Lonicera japonica: direct inhibition, transcriptional and post-transcriptional down regulation. *J Ethnopharmacol* 2007, 111 (3): 667-670.

[4] Kao ST, Liu CJ, Yeh CC: Protective and immunomodulatory effect of flos Lonicerae japonicae by augmenting IL-10 expression in a murine model of acute lung inflammation. *J Ethnopharmacol* 2015, 168: 108-115.

[5] Mussard E, Jousselin S, Cesaro A, Legrain B, Lespessailles E, Esteve E, Berteina-Raboin S, Toumi H: Andrographis Paniculata and Its Bioactive Diterpenoids Protect Dermal Fibroblasts Against Inflammation and Oxidative Stress. *Antioxidants (Basel)* 2020, 9 (5).

[6] Kumar RA, Sridevi K, Kumar NV, Nanduri S, Rajagopal S: Anticancer and immunostimulatory compounds from Andrographis paniculata. *J Ethnopharmacol* 2004, 92 (2-3): 291-295.

[7] Song X, Zhang Y, Dai E, Du H, Wang L: Mechanism of action of celastrol against rheumatoid arthritis: A network pharmacology analysis. *Int Immunopharmacol* 2019, 74: 105725.

[8] Kashyap D, Sharma A, Tuli HS, Sak K, Mukherjee T, Bishayee A: Molecular targets of celastrol in cancer: Recent trends and advancements. *Crit Rev Oncol Hematol* 2018, 128: 70-81.

[9] Yuan F, Zhao ZT, Jia B, Wang YP, Lei W: TSN inhibits cell proliferation, migration, invasion, and EMT through regulating miR-874/HMGB2/β-catenin pathway in gastric cancer. *Neoplasma* 2020, 67 (5): 1012-1021.

[10] Ru J, Li P, Wang J, Zhou W, Li B, Huang C, Li P, Guo Z, Tao W, Yang Y *et al*: TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J Cheminform* 2014, 6: 13.

[11] Fishilevich S, Nudel R, Rappaport N, Hadar R, Plaschkes I, Iny Stein T, Rosen N, Kohn A, Twik M, Safran M *et al*: GeneHancer: genome-wide integration of enhancers and target genes in GeneCards. *Database (Oxford)* 2017, 2017.

[12] Amberger JS, Hamosh A: Searching Online Mendelian Inheritance in Man (OMIM): A Knowledgebase of Human Genes and Genetic Phenotypes. *Curr Protoc Bioinformatics* 2017, 58: 1.2.1-1.2.12.

[13] Chen X, Ji ZL, Chen YZ: TTD: Therapeutic Target Database. *Nucleic Acids Res* 2002, 30 (1): 412-415.

[14] Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, Chang Z, Woolsey J: DrugBank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 2006, 34 (Database issue): D668-672.

[15] Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P *et al*: STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 2019, 47 (D1): D607-d613.

[16] Nogales C, Mamdouh ZM, List M, Kiel C, Casas AI, Schmidt H: Network pharmacology: curing causal mechanisms instead of treating symptoms. *Trends Pharmacol Sci* 2022, 43 (2): 136-150.

[17] Li S, Zhang B: Traditional Chinese medicine network pharmacology: theory, methodology and application. *Chin J Nat Med* 2013, 11 (2): 110-120.

[18] Zhang B, Wang X, Li S: An Integrative Platform of TCM Network Pharmacology and Its Application on a Herbal Formula, Qing-Luo-Yin. *Evid Based Complement Alternat Med* 2013, 2013: 456747.

[19] Jeselsohn R, Buchwalter G, De Angelis C, Brown M, Schiff R: ESR1 mutations—a mechanism for acquired endocrine resistance in breast cancer. *Nat Rev Clin Oncol* 2015, 12 (10): 573-583.

[20] Omarjee S, Jacquemetton J, Poulard C, Rochel N, Dejaegere A, Chebaro Y, Treilleux I, Marangoni E, Corbo L, Romancer ML: The molecular mechanisms underlying the ERα-36-mediated signaling in breast cancer. *Oncogene* 2017, 36 (18): 2503-2514.

[21] Seeliger H, Pozios I, Assmann G, Zhao Y, Müller MH, Knösel T, Kreis ME, Bruns CJ: Expression of estrogen receptor beta correlates with adverse prognosis in resected pancreatic adenocarcinoma. *BMC Cancer* 2018, 18 (1): 1049.

[22] Behrens A, Jochum W, Sibilia M, Wagner EF: Oncogenic transformation by ras and fos is mediated by c-Jun N-terminal phosphorylation. *Oncogene* 2000, 19 (22): 2657-2663.

[23] Gartel AL, Tyner AL: The role of the cyclin-dependent kinase inhibitor p21 in apoptosis. *Mol Cancer Ther* 2002, 1 (8): 639-649.

[24] Georgakilas AG, Martin OA, Bonner WM: p21: A Two-Faced Genome Guardian. *Trends Mol Med* 2017, 23 (4): 310-319.

[25] Xie D, Lan L, Huang K, Chen L, Xu C, Wang R, Shi Y, Wu X, Wang L, Liu Y *et al*: Association of p53/p21 expression and cigarette smoking with tumor progression and poor prognosis in non-small cell lung cancer patients. *Oncol Rep* 2014, 32 (6): 2517-2526.

[26] Han J, Wang F, Yuan SQ, Guo Y, Zeng ZL, Li LR, Yang J, Wang DS, Liu MY, Zhao H *et al*: Reduced expression of p21-activated protein kinase 1 correlates with poor histological differentiation in pancreatic cancer. *BMC Cancer* 2014, 14: 650.