

Original Research Article

Systematic elucidation of the traditional Chinese medicine prescription Danxiong particles via network pharmacology and molecular docking

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Abstract

Purpose: To investigate the pharmacological effect of the traditional Chinese medicine (TCM) prescription Danxiong particles (TDX105) and its mechanism of action.

Methods: The active compound and targets of TDX105 were investigated via network pharmacology. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were enriched, and protein-protein interaction network (PPI) was constructed. A network of 'components-targets-pathways' was developed with Cytoscape 3.8.0 software, while the formation of molecular docking analysis was conducted using Autodock vina software.

Results: There were 304 compounds and 482 targets identified in total. Genes with degree \geq mean node values were selected as the crucial targets, and string database was to be combined to 64 targets identified with cytoscape so as to draw a protein interaction map. A total of 137 pathways were enriched from 64 targets involving mainly 10 pathways, for example, PI3K-Akt signaling pathway, pathways in cancer, human cytomegalovirus infection and focal adhesion. Then, compound-target and compound-target-pathways were constructed using cytoscape (3.8.0). Finally, the five most active compounds, viz, quercetin, myricetin, luteolin, ellagic acid and kaempferol, and the top ten targets AKT1, GAPDH, TP53, ALB, EGFR, MAPK3, JUN, MAPK1, SRC and ESR1 were selected for molecular docking. These targets and compounds had strong interactions through a combination of hydrogen bonds and hydrophobic forces.

Conclusion: The mechanism of action of TDX105 has been successfully explained using the combination of network pharmacology and molecular docking. This may offer a solid foundation to the clinical use of TDX105, and further strengthen the prospects of its development for clinical use.

Keywords: Danxiong particles, Traditional Chinese medicine, Network Pharmacology, Molecular docking

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INTRODUCTION

The traditional Chinese medicine (TCM) Danxiong particles (TDX105) comprises *moutan*

cortex, *chuanxiong rhizoma*, *carthami flos*, *phellodendri chinensis cortex* and *geranium wilfordii maxim*. The pathogenesis of many complex diseases is so complicated that the

therapeutic effect of a single drug may not be appreciable [1]. In a previous study, it was demonstrated that TDX105 was effective against tumors. The TDX105 prescription has been used in TCM for treatment of skin lesions, with good clinical outcomes [2]. The mechanism of action of this prescription, however, has not yet been determined. Therefore, it is necessary to study the mechanism of TDX105 action.

Network pharmacology is a novel science, which has widespread use including to examine multi-target drug, molecular design of biological materials network and identify specific chemical signal nodes. It is established based on the systems biology theory [2]. Several studies have employed network pharmacology to explore the basic pharmacological effects of drugs on diseases, as well as their mechanisms of action [3-6]. Network pharmacology connects diseases and the complex biological relationship of traditional Chinese medicine, and uses network topology for analysis so as to accelerate the development of drugs [7]. Therefore, network pharmacology can be used to fully elucidate the pharmacological mechanism of the TCM prescription *Danxiong* particles (TDX105).

In this study, the active components of TDX105, as well as the targets associated in its pharmacological action, were first collected as well as evaluated via the TCM Systems Pharmacology (TCMSP) platform and PubChem. Then, the top 10 compound targets were chosen based on Degree values. Next, the protein interaction network (STRING) database and Cytoscape 3.8.0 were used to create a network of protein interaction and a visual - target-pathway network diagram. WebGestalt was also applied to forecast targets by GO and KEGG enrichment studies. Lastly, molecular docking was applied to establish the interactions between drugs and targets.

METHODS

Identification and screening of components of TDX105

The platform of Traditional Chinese Medicine Systems Pharmacology (TCMSP, <http://lsp.nwu.edu.cn/tcmsp.php>) is built on pharmacological methods of research that are methodical. It combines the evaluation of bioactive components, relevant targets, linked diseases, and pharmacokinetic data from traditional Chinese medicine. In this study, the five-herbal medicines in TDX105 were input into TCMSP and other databases, and the major compounds were identified, with oral

bioavailability (OB) $\geq 30\%$ and Druglikeness (DL) ≥ 0.18 as the filter standard.

Identification of targets in TDX105

The target of the relevant bioactive components in the five herbs in TDX105 were obtained on the TCMSP platform. The predicted targets were annotated in the Uniprot database (<http://www.uniprot.org/>), meanwhile, the Gene Name was converted to Gene Symbol.

Analysis of protein interaction network

The String database (<https://stringdb.org/>) is used for studying interactions amongst proteins, and for studying protein-protein interactions. The potential targets obtained above were fed into the String database, with *Homo sapiens* as species, and protein parameter score > 0.4 . Then, Cytoscape 3.8.0 was applied for composing a network diagram of protein interaction. Genes with degree \geq average node values were screened as key targets in the plug-in Network Analyzer, and topological analysis was done on the network.

Gene ontology (GO) and KEGG enrichment analysis

WebGestalt (<http://www.webgestalt.org>) is a database for enrichment analysis. It covers multiple databases and it has a variety of algorithms for enrichment analysis. Gene function and pathway-related information were studied in the WebGestalt database. The enrichment analysis method of over representation analysis (ORA) and pathway Functional Database were used to select the gene symbol type and to upload the gene, while keeping other parameters unchanged by default. False discovery rate (FDR) < 0.05 was deemed to be statistically significant.

Network establishment and analysis

Cytoscape is a tool for visualizing network data. In order to systematically explain the pharmacological mechanism of TDX105, 'compound-target-pathway' diagram was built using cytoscape 3.8.0 to illustrate the complex relationship amongst compounds, targets and pathways.

Molecular docking

Molecular docking is a screening strategy that uses ligand selection to find the ideal protein conformation. Firstly, according to, the core bioactive components identified in the "disease-

component-target" network analysis were quercetin, myricetin, luteolin, ellagic acid, kaempferol, while the 10 key targets selected for docking with these components being AKT1, GAPDH, TP53, ALB, EGFR, MAPK3, JUN, MAPK1, SRC, ESR1. Then, according to their molecular identifications, the 3D structures of the small molecules in Mol2 format were downloaded from the TCMSP and integrated into ChemBio3D Ultra 14.0 to energy minimization. After setting the rotatable key, the efficient small molecules were inputted into Autodock Tools 1.5.6 in order to facilitate hydrogenation, charge estimation, charge distribution, and storage. Moreover, Pymol 2.3.0 was used to remove the crystal water in protein and original ligands after the key target proteins were obtained from the PDB database. Thereafter, the protein structure was transferred into AutoDock tools (v1.5.6) where it was subjected to hydrogenation, charge calculation and charge distribution. The types of atoms were specified. Docking was performed by AutoDock Vina 1.1.2. Finally, the link of nodes in the docking findings were explored utilizing PyMOL and Ligplot.

RESULTS

Screened components of TDX105

After screening the herbal components in TDX105 (at OB \geq 30 % and DL \geq 0.18 ADME) with the TCMSP database, three hundred and four (304) compounds were identified. Fifty-nine (59) from *moutan cortex*, 111 from *chuanxiang rhizoma*, 66 from *carthami flos*, 65 from *phellodendri chinensis cortex*, and 30 from *geranium wilfordii maxim*. There were 27 repeated compounds which were removed.

Identified targets in TDX105

Based on the TCMSP database search for targets of the compounds identified, 102 of the compounds could not be mapped to targets, while 482 target genes were identified. The target information obtained was standardized using Uniprot ID, and the 482 genes were mapped for further investigations.

Results from protein interaction network

The Network Analyzer in the Cytoscape 3.8.0 plug-in was performed using topological analysis on the network, and screened out genes with degree \geq average node value (42) as crucial targets. Then, the 64 protein interaction nodes was obtained by STRING website, and the protein interactions network was drawn with Cytoscape 3.8.0 Draw (Figure 1). In the Figure,

the color depth and size of a circle are proportional to the degree value. The higher the degree, the more important the compound in this network.

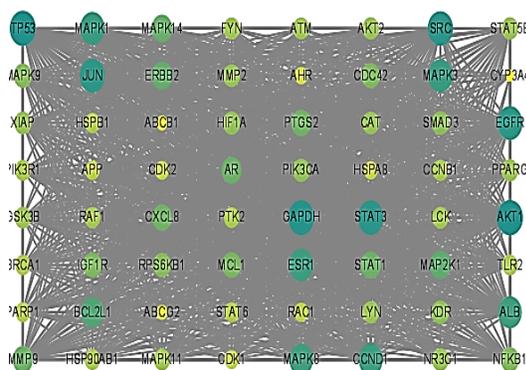


Figure 1: Protein-protein interaction (PPI) network. The nodes represent target. The nodes get larger with higher degree values

Results from GO and KEGG enrichment

The WebGestalt website was used to perform GO and KEGG pathway enrichment analysis to better understand the link between genes and related pathways. The relevant list had 64 unique entrezgene IDs that were unambiguously mapped to 64 user IDs. In the GO Slim summary, the enrichment analysis was based on 64 unique entrezgene IDs that were attributed to the relevant functional categories as well as the reference list. Based on the above conditions, 137 enriched categories were found, with the 40 most significant categories and members in the reduced sets being provided in the chapter. As demonstrated in Figure 2, the top seven functions were biological regulation (64/64), protein binding (64/64), response to stimulus (64/64), metabolic processes (62/64), cell communication (60/64), multicellular organismal process (57/64) and localization (57/64). These GO terms suggest that TDX15 may be closely related to inflammatory factors. As shown in Figure 3, in the pathway enrichment analysis, 132 pathways were mapped, and the first ten pathways were selected for analysis. The enrichment ratio is the number of differentially expressed genes in given pathway divided by the total number of genes. *P* values typically range from 0 to 1. Furthermore, the dots' colors and sizes correspond to the range of *p* values and the number of targets in the indicated pathways, respectively. The *p* value in this investigation, however, was 0. Therefore, there was no color change.

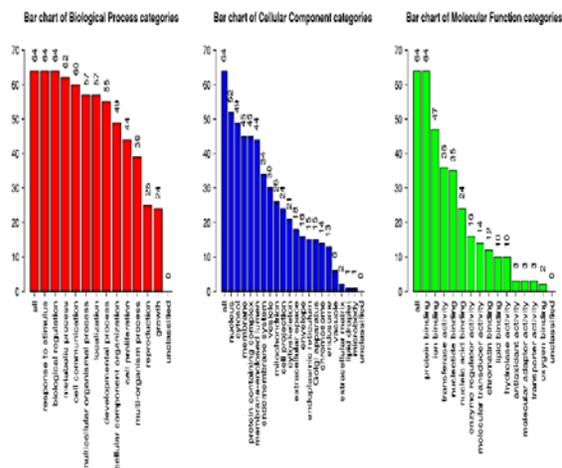


Figure 2: GO map of putative target genes. (a) Biological process categories. (b) Cellular component categories. (c) Molecular function categories

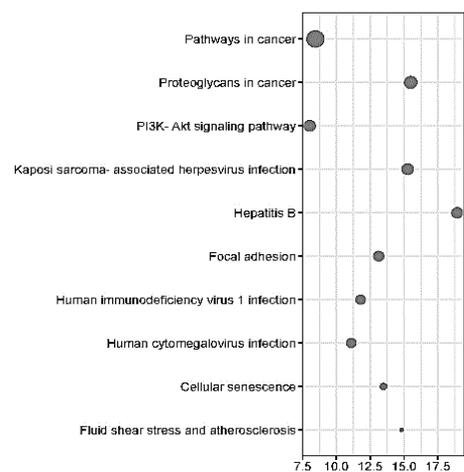


Figure 3: KEGG pathway analysis of target genes. The x-axis represents enrichment ratio, while y-axis represents enriched pathway

Results from network construction and analysis

Cytoscape 3.8.0 software was used to design and analyze a drug-target-pathway diagram to better grasp the complicated relationships between drugs, target genes, and related pathways. This three-layer network has 333 nodes and 2048 sides, as shown in Figure 4. The drug, target symbol, and pathways are represented by the orange ellipse, yellow triangle, and pink V, respectively. Quercetin, myricetin, luteolin, ellagic acid, kaempferol, caffeic acid, adenosine and linolenic acid were present in the 5 herbs in TDX105, and were prominently involved in the interactions.

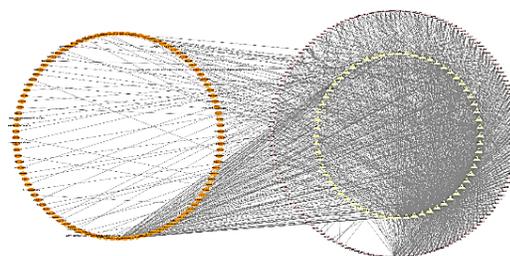


Figure 4: The compound-target-pathway network of TDX105

Based on the above results, the 4 bioactive components, 9 targets, and the top 10 signaling pathways linked to TDX105 were screened to construct a "component-target-pathway" diagram using cytoscape 3.8.0 software. The network diagram in Figure 5 shows that the four bioactive components in TDX105 act on multiple targets in different signal pathways, with intricate relationships amongst components, targets and pathways.

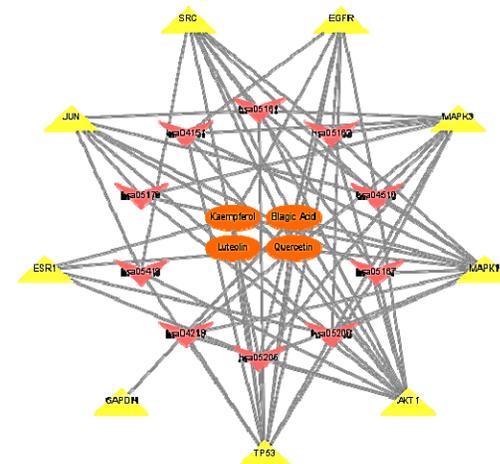


Figure 5: Compound-target-pathway of the TDX105 network. The orange ellipse, yellow triangle and pink V represent drug, target symbol and pathways, respectively

Molecular docking results

This investigation used the AutoDock Vina 1.1.2 molecular docking software to determine the validity of the TCSMP database in predicting molecular targets. A molecule docks with its target by hydrogen bonds in this process. The results are represented in Table 1. The more secure the docking shape, the lesser the docking capability. The docking findings were visually analyzed using PyMOL and Ligplot. Five compounds and ten targets demonstrated strong binding abilities, according to the molecular docking results, (the MOL001002 having the strongest binding ability to the target) as shown

Table 1: Prediction of binding energies of bioactive components of TDX105 with respect to key targets

Compound	MOL ID	AKT1	GAPDH	TP53	ALB	EGFR	MAPK3	JUN	MAPK1	SRC	ESR1
Quercetin	MOL000006	-6.3	-7.5	-7.1	-9.9	-8.8	-9.2	-6	-8.9	-8.9	-7.7
Myricetin	MOL000098	-6.1	-7.5	-6.5	-9.6	-8.7	-9.1	-5.9	-9.1	-8.9	-8.2
Luteolin	MOL000422	-5.9	-7.3	-6.2	-9.9	-8.5	-8.8	-5.8	-9.1	-9	-8
Ellagic acid	MOL001002	-6.4	-8	-6.7	-10	-8.9	-9.8	-5.5	-9.6	-8.7	-6.8
Kaempferol	MOL002008	-6.1	-7.6	-6.4	-8.9	-8.7	-8.9	-5.6	-8.9	-8.9	-7.9

in Figure 6. This indicates that the interaction between MOL001002 and the target was mainly by the creation of hydrogen bonds and hydrophobic forces.

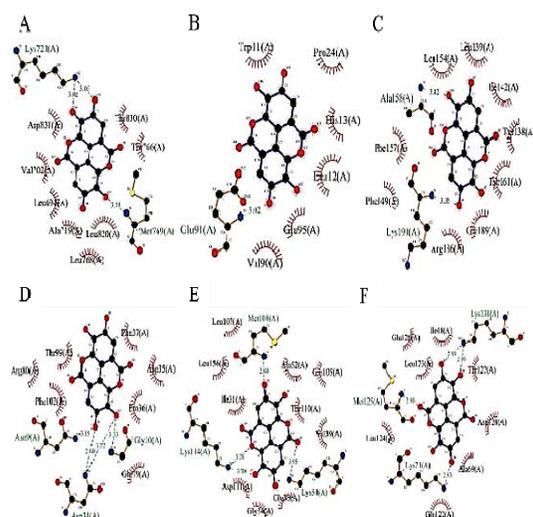


Figure 6: Results of docking of MOL001002 with targets. A: Docking of MOL001002 with EGFR, B: docking of MOL001002 with AKT1, C: docking of MOL001002 with ALB, D: docking of MOL001002 with GAPDH, E: docking of MOL001002 with MAPK1, F: docking of MOL001002 with MAPK3

DISCUSSION

In clinical trials, TDX105 is mainly used for topical treatment of patients with skin injuries, including radiation dermatitis, extravasation and skin damage caused by tumor-targeted drugs [8]. The TDX105 for active components and targets were explored through network pharmacology in order to precisely examine the clinical efficacy of TDX105. Through GO and KEGG enrichment analyses, a PPI network was created, and molecular docking was carried out to systematically unravel the role of TDX105 in the treatment of various diseases. Natural products and many herbal medicines exert pharmacological effects through multiple bioactive components which act on different targets. Network pharmacology was used to investigate the mechanisms of action of essential components of TDX105. The results showed that

through the 5 rules of drug-like screening, a total of 304 bioactive components were obtained after removing duplicates. A total of 64 targets were screened as key targets with degree values ≥ 42 . Those with nodes having degree values greater than 100 (in descending order) were: AKT1, GAPDH, TP53, ALB, EGFR, MAPK3, JUN, MAPK1, SRC and ESR1. The targets involved mainly ten pathways, including PI3K-Akt signaling pathway, pathways in cancer, human cytomegalovirus infection and focal adhesion. Thus, TDX105 affects pathways related to cancer, immune response, inflammatory response, tumor metastasis and wound healing. These results provide a basis for further development of TDX105.

The PI3K-AKT pathway involves various processes such as protein synthesis, cell cycle progression, angiogenesis, cell proliferation, metabolism, DNA repair and cell survival. Network pharmacology analysis revealed that TDX105 contained compounds such as quercetin, kaempferol and ellagic acid. Studies have found that the PTK-mediated pathway is associated with the stimulation of quercetin in epithelial cells, thereby protecting humans from bacterial/viral infection [9]. It has been reported that PTK inhibits serine/threonine protein kinases, a process which is critically dependent on the nature, number, and disposition of phenolic hydroxy or other O-containing substituents [10]. The ERK on the other hand coordinates the proliferation and migration of endothelial cells during angiogenesis [11].

These studies indicate that quercetin and ellagic acid in TDX105 up-regulated the expression of ERK downstream of the PI3K-AKT pathway by inhibiting the up-regulation of PTK, thereby promoting cell proliferation, angiogenesis and DNA repair. In addition, focal adhesion pathway was used for analysis of cell motility, cell proliferation and cell survival. Studies have reported that kaempferol inhibited the proliferation of pancreatic cancer cells, renal cell carcinoma, esophageal squamous cell carcinoma and other cancer cells through EGFR-related pathways [12-14].

This suggests that kaempferol in TDX105 may inhibit the expressions of downstream CycD genes by inhibiting EGFR factor in the focal adhesion pathway, thereby impacting the cell cycle. The results of KEGG and GO enrichment analysis in the present study are in agreement with those obtained in previous research [8].

The enriched pathways have numerous functions in angiogenesis, cell proliferation and DNA repair. In addition, previous research on ellagic acid's anti-angiogenic action revealed that it may be mediated through suppressing MAPK, hypoxia-driven PI3K/Akt/mTOR, and VEGF/VEGFR2 signaling pathways, as well as HDAC6 and HIF-1 responses [15]. However, not much is known on the pharmacological effect of ellagic acid on ALB targets. In all, these results indicate that TDX105 may prevent skin damage through multiple pathways and multiple targets.

Limitations of the study

In this investigation, the active compounds and possible prospective targets of TDX105 were screened through network pharmacology and molecular docking methods, and key pathways for its pharmacological effects were predicted. New ideas and methods were provided for the development and application of TCM compound preparations. However, since the relevant databases and software are regularly updated, the results obtained in this study need to be validated in future research.

CONCLUSION

Multiple signaling pathways and targets are altered by ellagic acid, quercetin, kaempferol, and other bioactive substances in TDX105, such as PI3K-Akt signaling pathway and focal adhesion pathway. In further studies, some investigations will be required to validate these findings to enhance the development of the TDX105 prescription.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Aiping Tian designed the research. Kexin Liu, Ning Li, Mai Yu, Mingjuan Liu, Shani Li and Wei Cai supervised the data collection and data analysis. Kexin Liu and Ning Li wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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