Abstract

To apply a network pharmacological approach to explore the targets and possible mechanisms of Kai Yu Zhong Yu Tang (KYZYT) in the treatment of tubal fimbria obstruction. The target information of KYZYT was extracted from TCMSP and HERB database. Genes related to tubal fimbria obstruction were searched using the GENECARD database. Target protein network maps (PPI) were drawn using string database analysis and Cytoscape 3.7.1 software. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and gene function analysis (GO) enrichment analysis were performed with the help of Perl language and biological program package in R language. To explore the multiple pharmacological mechanisms of action of KYZYT in the interventional treatment of tubal fimbria obstruction and to lay the foundation for further experimental validation. Through the collection and analysis of multiple databases, 355 biological targets of KYZYT were identified. 168 targets of tubal fimbria obstruction were obtained from disease database. The “drug-component” and “drug-target” networks of KYZYT were constructed, and the protein interaction network (PPI) of overlapping targets was analyzed to identify the key targets of the drug affecting the disease. In addition, KEGG pathway analysis and GO enrichment analysis were performed on the overlapping targets to explore the mechanism of KYZYT in the treatment of tubal fimbria obstruction. KYZYT has the characteristics of multi-component, multi-target and multi-pathway in the treatment of tubal fimbria obstruction, which provides new ideas and scientific basis for further clarification of the molecular mechanism.

Abbreviation: KEGG = Kyoto Encyclopedia of Genes and Genomes, PPI = protein network maps.

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1. Introduction

Tubal obstruction is the most common cause of female infertility, accounting for approximately 30% to 50% of female infertility.1,2,3 1 million women in the United States will develop pelvic inflammatory disease each year, and 18% of women in Europe and the United States will become infertile after an episode of pelvic inflammatory symptoms. The chance of infertility after one episode of pelvic inflammatory disease is 12%, 23% after two, and increases to 54% after three.1,3,4 In China, the implementation of family planning has led to an increase in uterine procedures such as abortion, leading to a rise in the proportion of infertility caused by pelvic inflammatory disease, which is increasing year on year. Pelvic inflammatory disease often damages the epithelial cells of the fallopian tubes, causing adhesions, obstruction or fluid accumulation at the umbilical end of the tubes, which disrupts the egg-collecting function of the umbilical end of the tubes and leads to infertility.5 Kai Yu Zhong Yu Tang (KYZYT) is made up of Radix Paeoniae Alba, Radix Atractyloides Macrocephalae, Rhizoma Atractylodis Macrocephalae, Dampi, Pollen. The formula uses Bai Shao, which is sour in taste and enters the liver meridian, nourishing liver blood and benefiting liver yin; the subject herb Angelica with its tonic liver body helps the liver to use. The angelica can be regulated in accordance with the nature of the liver, reflecting Fu’s careful use of medicine. The aromatic herbs can clear the depression of liver Qi and calm its reversal; Atractylodes nourishes the Qi and strengthens the spleen, meaning to first strengthen the spleen at the time of liver disease. The combination of Dan Pi and Pollen opens up the depression and disperses the knots, regulating the blockage of Qi in the belt veins. It is now used

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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not only to treat infertility caused by liver Qi blockage, but also in clinical practice to treat breast enlargement, cholecystitis and women’s organ agitation, with satisfactory results. This study initially explored the therapeutic mechanism of KYZYT in treating tubal isthmus obstruction in infertility, laying a preliminary foundation for the subsequent exploration of molecular mechanisms.

2. Materials and Methods

2.1. Screening of active ingredients

HERB database, Swiss database, TCMS database, CTD database and SYMMAP database were utilized to predict the ingredients of KYZYT.

2.2. Prediction of drug-related targets

TCMSP, HERB SWISSTARGET, CTD and SYMMAP database were used to predict the targets of KYZYT, and then the targets were predicted in The Uniport online protein database (Uniprot). To find the corresponding standard gene name, so as to obtain the related target of active ingredients of KYZYT.

2.3. Prediction of disease-related targets

Using GENECARDS database and CTD database to obtain the relevant content of tubal fimbria obstruction and delete the repetitive target gene can obtain the relevant targets of tubal fimbria obstruction.

2.4. Finding common targets of drugs and diseases

By mapping the target information of KYZYT with that of tubal fimbria obstruction, we can obtain the overlapped target Venn map and overlapped target information.

2.5. Construction of active ingredient target network

The active components and overlapping genes of KYZYT can be sorted out by using Cytoscape 3.7.1 software, and the network of active components and overlapping genes of KYZYT can be drawn.

2.6. Construction of drug disease overlapping target protein interaction network (PPI)

The overlapping targets of KYZYT and stroke were imported into string data analysis platform for calculation, and protein network maps (PPI) could be constructed according to the relationship between the targets.

2.7. Enrichment analysis of the Kyoto Encyclopedia of genes and genomes (KEGG) pathway

The overlapping target names of KYZYT and tubal fimbria obstruction were transformed into Entrez gene ID by R language, and then the KEGG related information was obtained by analyzing and calculating with KOBAS database. Then, the P-value was used as the reference value to screen the related targets, and then the R language was used to analyze the related content of pathway enrichment analysis.

2.8. Enrichment analysis of KEGG pathway

The overlapping target names of KYZYT and tubal fimbria obstruction were transformed into Entrez gene IDs using R language, and then analyzed and calculated using the KOBAS database to obtain GO-related information. Then, the P-value was used as the reference value to screen the relevant targets, and then the correlation of functional enrichment analysis was analyzed by R language.

3. Results

3.1. Identification of drug action targets

The chemical composition of the KYZYT formula and the corresponding targets were combined using the TCMSP database (https://tcmspw.com/tcmsp.php), Herb Chinese medicine database (http://herb.ac.cn/), swiss target prediction database (http://www.swissargetprediction.ch/), Comparative Toxicogenomics Database (http://ctdbase.org/) to query the obtained ingredients and corresponding targets and merge them to obtain the chemical composition and corresponding target information of KYZYT formulas. 77 ingredient information and 335 target information were used.

3.2. Predicted results of potential targets of action for drug treatment diseases

The predicted target information of four diseases Blockage of umbrella end of fallopian tube were screened with the help of GENECARD database results and combined and compared to obtain the targets that can jointly affect the four diseases heavily. A total of 168 common disease targets were obtained as a result. Then overlapping comparisons with the KYZYT drug targets obtained above yielded 15 overlapping targets. Topological heterogeneity analysis was performed on the overlapping targets. Among them, TP53, TNF, IL6, CCND1, and MYC were the targets with relatively high Degree values during the effect of KYZYT on selected lung diseases, and this result indicated that KYZYT formulas could exert their effects on the treatment of tubal fimbria obstruction through multiple targets (Fig. 1).

3.3. KEGG pathway enrichment analysis

The overlapping target names were converted to ENTREZ Gene ID using Perl language, and the KOBAS database was used for calculation to obtain 160 KEGG pathway enrichment information, indicating that the active ingredients can be used to treat diseases through multiple pathways. Including AGE-RAGE signaling pathway in diabetic complications, PI3K-AKT signaling pathway, P35 signaling pathway, MAPK signaling pathway, TNF signaling pathway, etc. Then, we used R language to calculate the graph, and the more the color tends to blue, the higher the correlation.

3.4. Functional analysis of GO genes

The overlapping target names were converted into ENTREZ Gene IDs using Perl language, and the KOBAS database was used for computing, and 975 GO gene functions were screened according to the P value top 20, which indicated that KYZYT affects selected diseases can be regulated by participating in a variety of biological functions. Including cytokine-mediated signaling pathway, negative regulation of apoptotic process, positive regulation of peptidyl-serine phosphorylation. Then the operations were performed in R language to obtain the graph, and the more the color tends to blue indicates the higher correlation (Fig. 2).

4. Discussion

Female infertility is one of the most difficult problems in modern medicine, and the trend of female infertility is increasing in recent years, which brings great psychological, family and social pressure to patients. The causes of female infertility include ovulation disorders tubal fimbria obstruction, intrauterine
infection, endometriosis, pelvic inflammatory adhesions, etc. Among them, tubal infertility accounts for about 40% of female infertility factors, and infertility due to proximal blockage of the fallopian tubes has become one of the most important problems in female infertility.[7] In this study, we used the TCM theory to intervene in infertility by using Kai Yu Seeding Yu Tang to explore the molecular mechanism and core protein targets, and to lay the foundation for further research.

Among the active ingredients of KYZYT, the compounds with the highest number of linked targets are glutathione and kaempferol. Pharmacological studies have shown the pharmacological effects of glutathiol and kaempferol through anti-apoptotic, anti-free radical oxidation and inhibition of inflammatory responses.[8]

The 15 targets obtained by combining the component target protein PPI with the disease target protein PPI are the targets corresponding to the chemical components in the drug and the targets related to the disease, so these 22 targets are the core targets of KYZYT for the treatment of tubal fimbria obstruction and influence the treatment of the disease.

The core targets of KYZYT for disease treatment are TNF, TP53, and IL-6, which are highly correlated. It has been shown that TNF-α is associated with inflammatory damage in the
fallopian tube, and the higher its level, the more severe the damage. The possible mechanism of action is that TNF-α has cytotoxic effects, inducing the release of toxic substances from inflammatory cells, stimulating fibroblast proliferation, promoting collagenase release, and participating in the process of tissue damage. Studies have shown that specific TP53 gene polymorphisms are associated with infertility, and tubal fimbria obstruction is more likely in infertility. Elevated IL-6 levels increase the permeability of vascular endothelial cells, leading to pathogenic invasion of the fallopian tubes triggering inflammation and massive infiltration of inflammatory cells.

Based on the enrichment analysis of the KEGG pathway in the core targets, it is clear that MAPK signaling pathway, PI3K-AKT signaling pathway, and P53 signaling pathway are significantly enriched. MAPK signaling pathway and apoptosis in the normal menstrual cycle, there is cyclic and spontaneous apoptosis in the endometrium. Apoptosis is regulated by apoptosis-related genes, including apoptosis-promoting genes, such as Bad, Bax, caspase, etc. The formation of neovascularization is a key step in the formation of EMs. These results suggest that KYZYT may intervene in disease development through multiple biological pathways and core protein activity.

In summary, this study used network pharmacology as the basis, and various software and databases to construct a KYZYT “component-target” network map, and will fully correspond to the target, screen out the therapeutic targets, and conduct KEGG pathway enrichment analysis and GO gene function analysis to systematically study the action pathway and pathway of Scutellaria baicalin for lung cancer treatment. Pathways for the treatment of tubal fimbria obstruction to provide a basis. However, the drawback of this study is that the network pharmacological analysis only provides predictions and needs to be validated by further experiments and clinical trials.

**Author contributions**

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