Network pharmacological analysis of the molecular targets and biological mechanisms associated with the treatment of oligospermic patients with RunJing formula

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Research

Keywords: RunJing Formula, Oligozoospermia, Network Pharmacology, Molecular mechanisms, Pathways

DOI: https://doi.org/10.21203/rs.3.rs-27850/v1

Abstract

Background: Oligozoospermia is a disorder of the male reproductive system defined as the presence of <15 million sperm/ml in a semen sample. This condition is difficult to treat; previous work has shown that neither antioxidants nor antiestrogens can effectively improve sperm count. However, in China, traditional Chinese medicine (TCM) compound prescriptions have achieved good results in the clinical treatment of patients with oligozoospermia. Runjing formula (RJF), an empirical formula first prescribed by Professor Zeng, is widely used in the treatment of oligozoospermic patients. Preliminary unpublished data indicate that this formula can increase sperm count and improve sperm motility. However, the precise pharmacological mechanisms underlying these effects have yet to be elucidated. In this study, we aimed to investigate the molecular mechanisms underlying the effects of Runjing prescription in oligospermic patients. Our analysis focused specifically on network pharmacology and bioinformatic analysis.

Methods: The active components and targets of RJF were screened and identified by means of network pharmacology and bioinformatic analysis, and differentially expressed genes (DEGs) were identified between patients with non-obstructive azoospermia and those with obstructive azoospermia. We then carried out protein-protein interaction (PPI) analysis. The PPI networks of presumptive RJF targets and oligospermia-related targets were visualized and combined to determine potential candidate targets for RJF in oligospermic patients. We also performed gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis and constructed a gene pathway network. This allowed us to screen key target genes and analyze the degree of enrichment in specific signal pathways in order to explore their relevance to RJF and oligospermia.

Results: Analysis showed that 29 target genes were related to the effects of RJF in oligospermic patients and led to the identification of 90 potential molecules. Our analysis showed that the functional annotation of these target genes was related to transcription, cytoplasm, and protein binding. Twenty-one pathways were particularly abundant, including apoptosis, the MAPK signaling pathway, and the Relaxin signaling pathway. *AKT1* was identified as the core gene related to the effects of RJF in oligospermic patients, while *JUN*, *FOS*, *NFKBIA*, and *TNFRSF* represented the key genes in the network of gene pathways.

Conclusion: RJF appears to exert influence on multi-components and multi-targets when used to treat oligozoospermia. The underlying molecular mechanisms appear to be complex and diverse and have direct or indirect relationships with multiple signaling pathways. In this study, bioinformatics and network pharmacology were used to extract the active components of RJF, and the differentially expressed genes associated with oligozoospermia were obtained from the gene expression omnibus (GEO) database. Finally, 29 target genes and 90 active components were identified. GO and KEGG enrichment analysis indicated that RJF mainly acts on oligozoospermia through MAPK and Relaxin signal pathways, and that the key genes involved are *ATK1,JUN, FOS, NFKBIA, and TNFRSF*. These results should help us to design new clinical and experimental research to further expand our understanding of oligospermia.

Background

The World Health Organization (WHO) defines infertility as the inability to conceive after one year of unprotected sexual intercourse (WHO, 2000). Approximately 15% of sexually active couples do not achieve pregnancy within one year and proceed to seek treatment at infertility centers. Previous studies have shown that approximately 50% of infertile cases are associated with a male factor, and often with abnormal semen parameters, although a wide range of conditions can impair male fertility including congenital or acquired urogenital abnormalities, malignancies, infections of the urogenital tract, increased scrotal temperature, endocrine disturbances, genetic abnormalities and immunological factors (Nieschlag et al., 2010). According to WHO criteria, oligozoospermia is defined as a sperm count of < 15 million sperm/ml in a semen sample provided after an appropriate period of abstinence. However, little is known as to how to improve the sperm count in such patients, although previous research has indicated that antioxidants and antiestrogens are not effective. The only available option to create a pregnancy for such patients is the use of assisted reproductive Technology (ART), although this practice is associated with significant financial burden (Jungwirth et al., 2018).

Over recent years, traditional Chinese medicine (TCM) has become known as a potential effective auxiliary strategy with which to treat oligozoospermia. Li et al. (2019) and Mu et al. (2019) both reported the effects of Wuzi Yanzong decoction in the treatment of oligozoospermia. Compared with western medicine, the combination of western medicine and TCM was shown to effectively improve the motility of sperm in infertile patients with kidney deficiency. These authors also reported the positive effects of TCM with regards to sperm deficiency and improving the fertilization rate and the production of high quality embryos in ART cycles. Cheng et al. (2019) further reported that Shizheng decoction improved sperm quality in patients with asthenozoospermia in patients with kidney deficiency and dampness type by blocking sperm apoptosis in response to the upregulated expression of PHB, a mitochondrial membrane protein. This decoction also protected the integrity of the sperm mitochondrial ultrastructure.

Professor Qingqi Zeng, a famous practitioner of TCM in Jiangsu Province, studied successively under Professor Fusong Xu, also a famous practitioner of TCM, and Professor Qi Wang, an academic from the Chinese Academy of Engineering. Runjing formula (RJF) is Professor Zeng's empirical prescription. Preliminary clinical data (unpublished) show that RJF can effectively improve the clinical symptoms of infertile males. RJF is a mixture of 13 Chinese medicine extracts, including Astragali Cpmplanatisemen (Chinese name: shayuanzi), Cuscutae Semen (Chinese name: tusizi), Polygonati Rhizoma (Chinese name: huangjing), Cornus Officinalis Sieb. Et Zucc. (Chinese name: shanzhuyu), Hedysarum Multijugum Maxim. (Chinese name: huangqi), Cinnamomi Ramulu (Chinese name: guizihi), Portulacae Herba (Chinese name: machixian), Citrus Reticulata (Chinese name: chenpi), Radix Salviae (Chinese name: danshen), and Epimrdii Herba (Chinese name: yinyanghuo). RJF has been clinically proven to significantly improve oligozoospermia, although pharmacodynamic aspects of the molecular mechanisms involved have yet to be elucidated. Therefore, there is an urgent need to identify the specific targets of RJF and investigate the potential mechanisms involved. Gaining a more scientific understanding of how RJF exerts influence in oligospermia will help to promote its widespread acceptance as a potential therapeutic intervention.

Network pharmacology is a new field of analysis that is based on the theory of systems biology and analyzes networks of biological systems and selects specific signaling nodes for drugs with multiple targets. The compound prescription of any TCM refers to the mixture of two or more types of TCM; the regulatory network associated with the action of such compounds is therefore complex (Liu et.al, 2016). In a previous study, Sheng et al (2014) constructed a comprehensive and systematic pharmacological model with which to study the multi-factorial pharmacological mechanisms underlying the use of compound Xueshuantong in the treatment of thrombosis. Analysis showed that 22 components and 41 protein targets in compound Xueshuantong were closely related to platelet aggregation and fibrinolysis, thus indicating that many active chemical components may simultaneously interact with the same target. Using network pharmacology, Li et al. (2014) further identified 19 main active components in Gegen Qinlian decoction; 13 of these were identified as anti-diabetic compounds. Of these, the novel anti-diabetic component of puerarin (Ge-Gen) 4-hydroxymephenytoin was shown to increase the insulin secretion of RIN-5F cells and improve the insulin resistance of 3T3-L1 adipocytes (Li et al. 2014).

In the present study, we used network pharmacology to explore the mechanisms and molecular targets of RJF in the treatment of oligozoospermia. The active components and targets of RJF were identified, for the first time, using the TCM Pharmacology Database and Analysis Platform (TCMSP). Next, we identified potential targets for oligozoospermia by identifying and analyzing differentially expressed genes (DEGs) between patients with non-obstructive azoospermia, which results from severe deficits in spermatogenesis that most commonly result from primary testicular dysfunction, and those with obstructive azoospermiaa condition related to a mechanical blockage in the upper or lower reproductive tract**.** Finally, we used Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, and gene ontology (GO) enrichment analyses for biological processes, to identify potential mechanisms for the action of Runjing fomula in the treatment of oligozoospermia.

Materials And Methods

Screening of active ingredients

We searched the TCMSP (http://tcmspw.com/tcmsp.php) for all the ingredients of RJF, focusing on the toxic pharmacokinetics (absorption) of each ingredient (Ru et al., 2014). We then screened the distribution, metabolism, and excretion (ADME) parameters for reach ingredient. The screening conditions were oral bioavailability (OB ≥30%) and drug likeness (DL≥ 0.18)(Tian et al., 2015). A TCMSP target prediction model was used to predict the potential action of each target. In total, 183 eligible compounds and 3408 targets were identified.

Targets related to oligozoospermia

Differentially expressed genes (DEGS) for patients with oligozoospermia were acquired from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds, Series: GSE9210). The following samples (GEO reference numbers) were identified: GSM232970, GSM232971, GSM232972, GSM232973, GSM232974, GSM232975, GSM232976, GSM232977, GSM232978, GSM232979,

GSM232980, GSM232981, GSM232982, GSM232983, GSM232984, GSM232985, GSM232986, GSM232987, GSM232988, GSM232989, GSM232990, GSM232991, GSM232992, GSM232993, GSM232994, GSM232995, GSM232996, GSM232997, GSM232998, GSM232999, GSM233000, GSM233001, GSM233002, GSM233003, GSM233004, GSM233005, GSM233006, GSM233007, GSM233008, GSM233009, GSM233010, GSM233011, GSM233012, GSM233013, GSM233014, GSM233015, GSM233016, GSM233017, GSM233018, GSM233019, GSM233020, GSM233021, GSM233022, GSM233023, GSM233024, GSM233025, GSM233026,and GSM233027). Genes with a P value < 0.005 and a |log 2(fold change)| > 1 were considered to show significant differential expression, and were therefore identified as oligozoospermia- related targets.

Construction of compound networks

Cytoscape software version 3.7.2 (https://cytoscape.org/; Shannon et al., 2003) was used to construct and visualize a compound-target network for RJF. Protein-protein interaction (PPI) data were obtained from The Biological General Repository for Interaction Datasets (Bio GRID), the Database of Interacting Proteins (DIP), the Human Protein Reference Database (HPRD), the Molecular Interaction Database (MINT), the Interaction Molecular Interaction Database (IntAct), and the Biomolecular Interaction Network Database (BIND). These databases were screened with the Bisogenet plugin in Cytoscape software. PPI networks were created for all putative oligozoospermia-related targets and RJF targets and subsequently visualized with Cytoscape software.

Network merging

Next, PPI networks created for putative oligozoospermia-related targets and RJF targets were merged using Cytoscape software. Then, nodes within the interaction network that showed topological importance were screened by calculating degree centrality (DC) and betweenness centrality (BC); for this, we used the Cyto NCA plugin of Cytoscape. Collectively, DC and BC represent key aspects of topological importance; their definitions and computational formulae are heavily used in both network and systems pharmacology (Tang et al., 2015).

Bioinformatic analyses

Gene Ontology (GO) analysis was used to investigate cellular components, biological processes and molecular function. These analyses were performed with the clusterprofiler tool in the R package. Functional categories were defined as being enriched with genes with a false discovery rate (FDR) of <0.05; the top 20 GO functional categories were then selected for further analysis. The DAVID tool was used in conjunction with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database for all pathway analyses. Pathways showing a significant change in FDR (< 0.05) were selected for further analysis. Genes that played a significant regulatory role in pathways were subsequently selected for gene-pathway network analysis. We then created a gene-pathway network in order to screen a series of key target genes that may play a role in the treatment of oligozoospermia by RJF.

Results

Analysis of compound-target networks

In total, our analysis identified 183 compounds from RJF as candidate compounds (Table 1). Analysis of the GEO database identified 1194 potential targets associated with oligozoospermia. Figure 1 shows a volcano plot depicting the distribution of DEGs (shown as red dots in the plot). Figure 2 shows the compound target network created for RJF using the screened compounds. This network featured 119 nodes (90 RJF compounds and 29 compound targets) along with 206 edges that indicate interactions between the compounds and targets.

Analysis of PPI networks

PPI concerns large-scale biological processes, including metabolic control, cell-to-cell interactions, and developmental control. This technique is increasingly becoming regarded as the primary objective of systems biology (Rao et al., 2014). In our study, PPI data were used to create networks of oligozoospermia-related targets and putative RJF targets. The PPI network created for putative RJF targets featured 2682 nodes and 57742 edges, thus representing 2682 interacting proteins and a total of 57742 interactions.

The identification of candidate targets for the action of RJF in the treatment of oligozoospermia

In order to identify the potential mechanisms of action underling the effect of RJF in the treatment of oligozoospermia, we merged the PPI network of RJF putative targets with the PPI network of oligozoospermia-related targets. The resultant network featured 2682 nodes and 95,775 edges (Figure 3A). The median degree of all nodes was 42. Significant targets were defined as all nodes with >84 degrees; this method was in accordance with previously published information (Shen et.al.2019). Next, we constructed a network of significant targets for the action of RJF against oligozoospermia. This network featured 369 nodes and 13354 edges (Figure 3B). Then, we screened these candidate targets further and identified 108 targets with a DC > 84 and a BC > 296.5 (Figure 3C). Overall, out analysis identified 108 target genes that showed association with the action of RJF against oligozoospermia.

GO analysis and pathway enrichment analysis

The Clusterprofiler tool in the R package was used to perform GO and KEGG pathway analysis for the 29 candidate targets. GO analysis was analyzed based on cellular component, biological process, and molecular function. In total, our analysis identified 352 GO terms that showed significant enrichment (FDR < 0.05); 317 of these were associated with biological processes, 8 were associated with cellular components, and 27 were associated with molecular function. Data arising from GO analysis are shown in Supplementary Table 1 and the top 20 GO terms are shown in Figure 4.

Next, KEGG pathway analysis was used to identify pathways that were significantly influenced by RJF in the treatment of oligozoospermia. In total, we identified 58 pathways that were significantly enriched

(FDR < 0.05), including the Relaxin signaling pathway, apoptosis, the AGE–RAGE signaling pathway, endocrine resistance, and pathways associated with prostate cancer. Data arising from KEGG pathway analysis are shown in Supplementary Table 2 and Figure 5.

Gene-pathway network Analysis

Finally, we constructed a gene-pathway network that was based on the significantly enriched pathways, and the genes that regulated these pathways (Figure 6). BC data were used to investigate the topology of the 20 pathways and 21 genes. The network created suggested that *AKT1* had the highest BC and was therefore defined as the core target gene. Several other genes also had a high BC, including *JUN*, *FOS*, *NFkBIA* and *TNFRSF*. It is therefore possible that these are the key target genes responsible for the action of RJF in the treatment of oligozoospermia.

Discussion

For thousands of years, China has formed and developed a unique theory for the treatment and prevention of diseases in traditional Chinese medicine; this theory forms the basis of TCM. In TCM, there is no name for oligozoospermia; rather, we refer to the "Jingshao" and "childless" categories. Because of the theory of "kidney storing essence and dominant reproduction" in TCM, most clinical treatments involve "tonifying kidney and filling essence", “invigorating the spleen”, “removing dampness”, and promoting blood circulation. RJF is an established prescription from Professor Qingqi Zeng, a famous TCM expert from Jiangsu Province. TCM involves treatment that is based on syndrome differentiation from a holistic point of view, and considers that effect requires multiple targets and multiple mechanisms. By constructing multi-level network models, the network pharmacology approach is able to consider TCM from an overall point of view, and is therefore very suitable for the study of unknown components and the mechanisms of action underlying complex TCM prescriptions. Furthermore, this analysis can be supported by various existing databases and software.

In the present study, we created PPI networks of putative RJF targets and oligozoospermia- related targets. These networks were then combined in order to identify candidate targets for the effect of RJF in oligozoospermic patients. In order to identify targets more accurately, we used DC and BC data to filter the nodes and create a new network. Finally, 29 targets were identified and bioinformatics analysis was carried out to study the mechanisms of action that might be responsible for the effect of RJF on oligozoospermia.

Using GO enrichment analysis, we demonstrated that the targets of RJF in the treatment of oligozoospermia were enriched in terms of biological process, cellular composition, and molecular function. These results suggest that RJF regulates a range of relevant biological processes, including gene silencing, gene expression, apoptosis, and signal transduction mediated by the aldehyde ketone reductase superfamily. Spermatogenesis occurs in the testes while sperm maturation occurs in the epididymis. Spermatogonia proliferate in the testis and undergo a series of stepwise changes from primary spermatocytes to mature spermatocytes via division and differentiation in the seminiferous

tubules. The most common cause of oligozoospermia is a reduction in spermatogenic function in the testis (Zhang et al., 2020). The processes of division and differentiation in spermatogenic cells is regulated by a variety of gene and protein signaling pathways, particularly those related to proliferation and apoptosis factors. These processes can have direct effects on the formation and development of spermatozoa (Chen et al., 2017). Therefore, the protein signal pathways and corresponding genes that are involved in aberrant spermatogenesis have become key research areas. A previous study showed that Yijing recipe upregulated the expression of Bcl-2 protein, downregulated the overexpression of Bax protein, and reduced the extent of apoptosis in the testes of adenine-induced infertile rats, thus inhibiting apoptosis in spermatogenic cells (Wang et al., 2014). In another study, Zhao et al. (2019) showed that knockout of the *PGAM1* gene was related to spermatogenic dysfunction induced by Baihuadan, and promoted spermatogenic cell apoptosis by regulating the p53/Caspase3/Caspase6/Caspase9 signaling pathway. Therefore, RJF may regulate the process of apoptosis by interfering with these biological/pathological processes. Previous studies have also shown that oligozoospermia is related to oxidative stress, the androgen receptor, and heat shock (Yan et al. 2018); our present study showed that these factors/processes were significantly enriched.

As a prescription of TCM, RJF has effects multiple pathways, has multiple targets, and features multiple components. Therefore, it follows that RJF can treat oligozoospermia via multiple pathways. In this study, we found that 58 KEGG pathways were enriched, including the MAPK signaling pathway and the relaxin signaling pathway. MAPKs can regulate gene expression, the immune response, cell proliferation, apoptosis, and the response to oxidative stress; these processes are all related to the mechanisms underlying immune regulation (Li et al., 2017). *RLN1* and RXFP1, members of the relaxin family, have both been identified in Sertoli cells; *RLN1* has also been shown to stimulate the proliferation of Sertoli cells (Nascimento et al., 2012) by activating the MEK/ERK1/2 and PI3K/AKT pathways. The expression of *RLN1* and RXFP1 mRNA predominantly occurs in the tubular testis compartment and appears to be involved in the first stage of spermatogenesis (Braun et al., 2015). These findings suggest that *RLN1* may affect spermatogenesis in a direct manner by acting on germ cells, or in an indirect manner by acting on Sertoli cells. In the present study, we also identified significant enrichment in several pathways related to viruses. Virus infection is also related to the occurrence of oligozoospermia (Shao et al., 2020). The autoimmune response caused by virus infection may be regulated in specific ways; for example, the mode of infection varies across different viruses, such as cytomegalovirus, rubella virus, and herpes simplex virus. In this study, we also found that prostate cancer, hepatitis B, the influenza A signaling pathway, and the estrogen signaling pathway, were also significantly abundant, thus suggesting that such pathways may be associated to the effect of RJF on oligozoospermia.

We also constructed a gene-pathway network to identify the core gene and key target genes for the effects of RJF in oligospermic patients. Results suggested that *AKT1* had the maximum BC and was therefore the core target gene. The other top 5 genes (*JUN*, *FOS*, *NFKBIA*, *TNFRSF*, and *CDK4*) were identified as the key target genes. AKT1 (protein kinase B1, protein kinase B1) is an upstream protein in the PI3K/AKT signaling pathway. The process of phosphorylation activates this pathway and regulates cell proliferation and apoptosis; the phosphorylation of P85 is known to be responsible for PI3K

activation. The phosphorylation of AKT1 and P85 may occur together, thus resulting in a cascade reaction, or a cross-linking reaction, with other signaling pathways (Liang et al., 2016; Etemadmoghadam et al., 2014). Genes often work together to regulate cell differentiation, growth, apoptosis and other functions. The *C-FOS* and *C-JUN* genes are pro-apoptotic genes and are one of the earliest 'immediate early' genes. These genes connect extracellular information with the transcription of intracellular target genes; their expression products, FOS and JUN, are DNA binding proteins in the nucleus and are connected by a leucine zipper to form a heterodimer AP-1 complex. TPA response elements are located in the DNA binding region of AP-1; these can bind to DNA through their respective basic amino acid regions, regulate the replication and transcription of DNA, induce the expression of other genes containing TPA response elements, and act as gene regulatory proteins to regulate the transcription of target genes or late-onset genes, thereby inducing cell apoptosis. Apoptosis is mainly mediated by the death receptor pathway (Brandon et al., 2005; Han et al, 2014). *NFKBIA* (NFκB inhibitor α) is a gene that encodes I κ B-α and allows the production of new I κ B-α proteins to terminate NFκB signal transduction by removal from the nucleus. In the death receptor pathway, TNF activates the downstream NFκB pathway, thus leading to the overexpression of NFKBIA. NFKBIA may mediate the inactivation of the p53 gene in HeLa cells. NFKBIA acts as a negative regulator of the classical NFκB pathway through its ability to maintain the presence of NFκB in the cytoplasm (Chen etal.2019). There are two key checkpoints in the cell cycle, the G1/S phase and the G2/M phase. These two checkpoints can smoothly enter the next cycle following interaction with appropriate factors. The smooth progression of the cell cycle is the key to cell proliferation. Cyclin dependent kinase 4 (CDK4is a key regulator that controls the transition from G1 phase to S phase during the cell cycle. The abnormal expression of CDK4 can destroy the homeostasis of cell cycle regulation. CDK4 is known to bind to to Cyclin in the G1 phase to promote cell cycle progression (Gelbert et al., 2014).

Conclusion

In summary, by investigating the study of network pharmacology and bioinformatics, we identified that the RJF exerts effect on oligospermic patients by way of multiple components and multiple targets. The molecular mechanisms underlying these effects are complex and diverse, and can be directly or indirectly related to multiple signaling pathways. Our data will help us to further explore these molecular mechanisms and gain a better understanding of how TCM may be used to treat oligospermia more effectively.

Abbreviations

TCM:traditional Chinese medicine; DEGs:differentially expressed genes;PPI:protein-protein interaction;GO:gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes;GEO:gene expression omnibus;WHO: World Health Organization;ART:assisted reproductive technology;RJF:Runjing formula;TCMSP:TCM Pharmacology Database and Analysis Platform;ADME:distribution, metabolism, and excretion;OB:oral bioavailability;DL:drug likeness;Bio GRID:The Biological General Repository for

Interaction Datasets;DIP:the Database of Interacting Proteins;HPRD:the Human Protein Reference Database;MINT:the Molecular Interaction Database;IntAct:the Interaction Molecular Interaction Database;BIND:the Biomolecular Interaction Network Database;DC:degree centrality;BC:betweenness centrality;FDR:false discovery rate;NFKBIA :NFκB inhibitor α;AKT1 :protein kinase B1, protein kinase B1;CDK4:Cyclin dependent kinase 4.

Declarations

Acknowledgements

Not applicable.

Author contributions

PPD and KY carried out the main analyses and drafted the manuscript. TYZ and YZ designed the research. JL and QQZ helped to draft the introduction and discussion. All authors wrote, read, and approved the manuscript.

Funding

This project was supported by the 2018 Suzhou Science and Technology Plan Development Plan (People's Livelihood Science and Technology-basic Research on Medical and Health Application) [the third batch] (Project No.: SYSD2018174).

Availability of data and materials

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

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1.WHO. WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple. Cambridge University Press: Cambridge. 2000.

2.Andrology. In: Nieschlag E, Behre HM and Nieschlag S, editors. Male reproductive health and dysfunction, in Male reproductive health and dysfunction. Springer Verlag: Berlin. 2010.

3.Jungwirth A, Diemer T, Kopa Z, Krausz C, Minhas S, Tournaye H. EAU Guidelines on Male Infertility. 2018.

4.Mu YY, Hua QX, Yao B, Feng YM, Xie ZY, Gao Z, et al. Effect on sperm quality of asthenospermia and oligospermia treated with grain-moxibustion combined with medicine therapy. Zhongguo Zhen Jiu. 2019;39:843-8.

5.Li JK, Tang YY, Ma YH. Effect of Wuzi Yanzong decoction on embryo quality in assisted reproductive technology in patients with oligoasthenospermia. Yunnan Journal of traditional Chinese Medicine. 2019;40(10):40-42.

6.Cheng WJ, Zhang MJ, Shi YL, Wei XG, Jiang S, Cheng BJ, et al. Effect of Shizheng decoction on mitochondrial membrane protein PHB and ultrastructure of spermatozoa in patients with asthenospermia. J Tradit Chin Med. 2019;18(1):43-46.

7.Liu HP, Zeng LT, Yang KL, Zhang GM. A Network Pharmacology Approach to Explore the Pharmacological Mechanism of Xiaoyao Powder on Anovulatory Infertility. Evid Based Complement Alternat Med. 2016;2016:1-13.

8.Sheng SJ, Wang JX, Wang LR, Liu H, Li PB, Liu MH, et al. Network pharmacology analyses of the antithrombotic pharmacological mechanism of Fufang Xueshuantong Capsule with experimental support using disseminated intravascular coagulation rats. J Ethnopharmacol. 2014;154:735-44.

9.Li HY, Zhao LH, Zhang B, Jiang YY, Wang X, Guo Y, et al. A network pharmacology approach to determine active compounds and action mechanisms of ge-gen-qin-lian decoction for treatment of type 2 diabetes. Evid Based Complement Alternat Med. 2014;2014:495840.

10.Ru JL, Li P, Wang JN, Zhou W, Li BH, Huang C, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminformatics. 2014;6(1):13.

11.Tian S, Wang JM, Li YY, Li D, Xu L, Hou TJ. The application of in silico drug-likeness predictions in pharmaceutical research. Adv Drug Deliv Rev. 2015;86:2-10.

12.Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-504.

13.Tang Y, Li M, Wang JX, Pan Y, Wu FX. CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks. BioSystems. 2015;127:67-72.

14.Rao V Srinivasa, Srinivas K, Sujini G N, Kumar G N Sunand. Protein-protein interaction detection: methods and analysis. Int J Proteomics. 2014;2014:1-12.

15.Shen HB, Zhou YN, Zheng J, Zhu RH. Multi component multi target multi pathway" mechanism of Kuihua Hugan Tablets based on network pharmacology. China J Chin Mater Med. 2019;44:1464-1474.

16.Zhang X, Ning YN, Liu W, Zhang TJ, Qin YY, Zhao SD, et al. Variation analysis of SOX8 gene in Chinese men with non-obstructive azoospermia or oligozoospermia. Andrologia. 2020;2: e13531

17.Chen PD, Li JY, Yang ZX, Chen DN, Zhou WB, Liu F, et al. Research progress on the intervention mechanism of traditional Chinese medicine on spermatogenic cell apoptosis. J Liaoning Univ Tradit Chin Med. 2017;19(03):110-113.

18.Wang L, Chen D, Min Z, Wang JH. Effect of Yijing Recipe on Apoptosis of Testis Spermatogenic Cells and the Expression of Bcl-2 and Bax Protein in Infertility Rats. Chin J Integr Med. 2014;34(5):602-605.

19.Zhao YS, Zhang SB. PGAM1 knockdown is associated with busulfan-induced hypospermatogenesis and spermatogenic cell apoptosis. Mol Med Rep. 2019;19:2497-2502.

20.Yan J, Wang Q, Zhu B, Yan S, Li BY, Zheng YF. Study on the mechanism of Huangjing zanyu capsule in the treatment of oligozoospermia based on network pharmacology. Guiding Journal of Traditional Chinese Medicine and Pharmacy. 2018;24(09):18-22.

21.Li Y, Meng TT, Hao N, Tao HQ, Zou SP, Li MM, et al. Immune regulation mechanism of Astragaloside IV on RAW264.7 cells through activating the NF-kB/MAPK signaling pathway. Int Immunopharmacol. 2017;49:38-49.

22.Nascimento AR, Pimenta MT, Lucas TFG, Royer C, Porto CS, Lazari MFM. Intracellular signaling pathways involved in the relaxin-induced proliferation of rat Sertoli cells. Eur J Pharmacol. 2012;691:283-291.

23.Braun B C, Muller K, Jewgenow K. Expression profiles of relaxin family peptides and their receptors indicate their influence on spermatogenesis in the domestic cat (Felis catus). Domest Anim Endocrinol. 2015;52:25-34.

24.Shao S, Ding T, Jiang M. Advances in relationship between viral infection and male infertility. J Reprod Med. 2020;29(1):120-124.

25.Liang Y, Zhang XB, Yao X, Li Y, Li D. Down-regulating Akt1 expression influences proliferation and apoptosis of cholangiocarcinoma HUCCA-1 cells. Modern Oncology. 2016; 24(03):363-366.

26.Etemadmoghadam D, Bowtell D. AKT1 gene amplification as a biomarker of treatment response in ovarian cancer: mounting evidence of a therapeutic target. Gynecol Oncol. 2014;135:409-10

27.Jeffy BD, Hockings JK, Kemp MQ, Morgan SS, Hager JA, Beliakoff J, et al. An estrogen receptor-alpha/p300 complex activates the BRCA-1 promoter at an AP-1 site that binds Jun/Fos transcription factors: repressive effects of p53 on BRCA-1 transcription. Neoplasia. 2005;7:873-82.

28.Han YY, Cao YL, Liang B, Qu Q, Li NN. Effects of BMSC transplantation on c-fos/c-jun expression of apoptosis-related genes in retinal ischemia-reperfusion injury. New Progress in Ophthalmology. 2014;34(03):209-21.

29.Chen MQ, Jin ZS, Xu CY, Liu RF, Yang G, Dong CJ. Effect of silencing PHF19 gene expression by small interfering RNA on biological behavior of cervical cancer cells. Journal of Mudanjiang Medical College. 2019;2:16-21.

30.Gelbert LM, Cai SF, Lin X, Sanchez-Martinez C, Del PM, Lallena MJ, et al. Preclinical characterization of the CDK4/6 inhibitor LY2835219: in-vivo cell cycle-dependent/ independent anti-tumor activities alone/in combination with gemcitabine. Invest New Drugs. 2014;32:825-37.

Figures

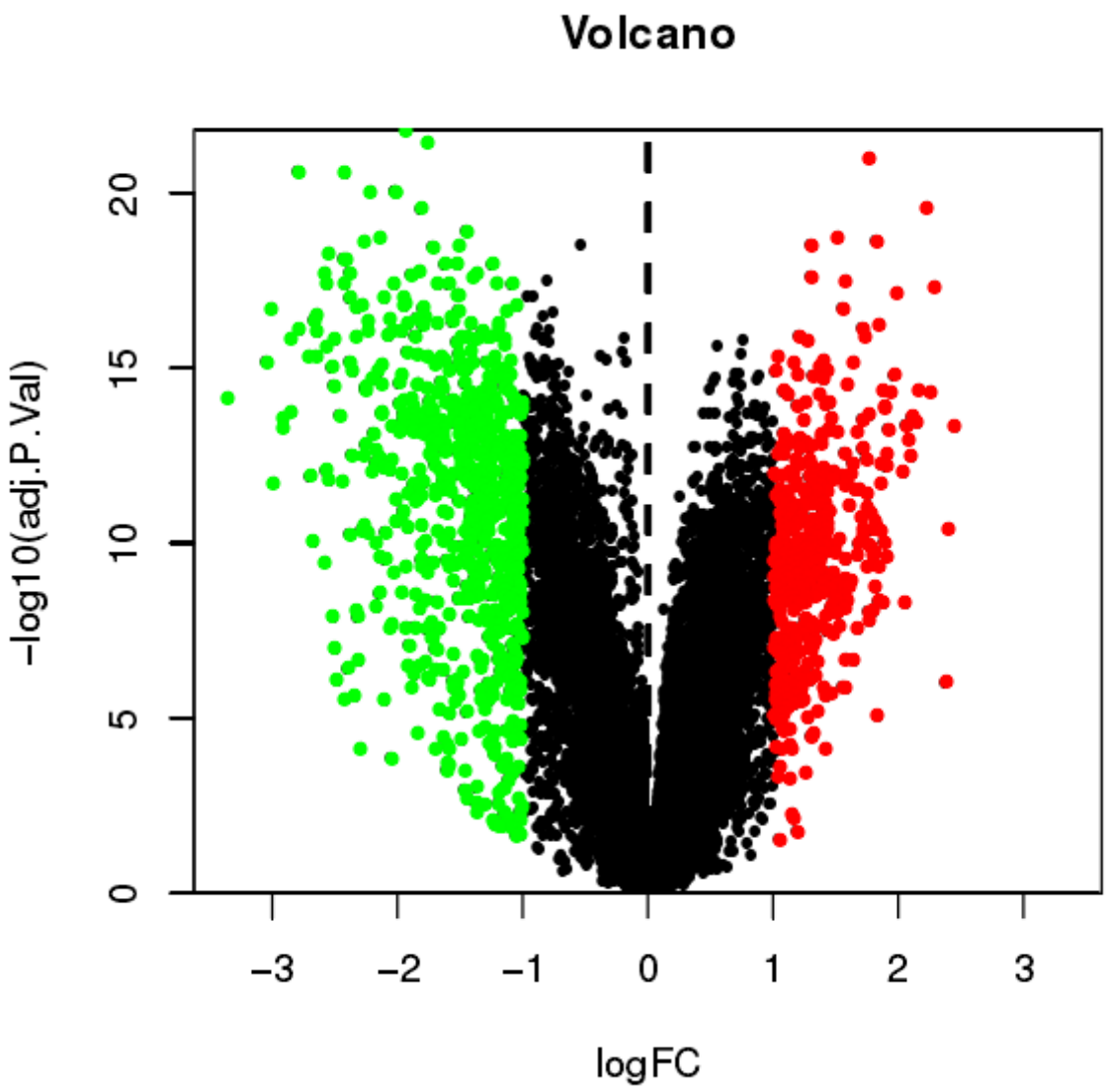

Figure 1

Volcano plot of differentially expressed genes. The abscissa indicates multiple changes in gene expression, while the ordinate indicates the statistical significance of the changes in gene expression. Red dots indicate significantly up-regulated genes, green dots indicate significantly down-regulated genes, and black dots represent non-significant differential expression.

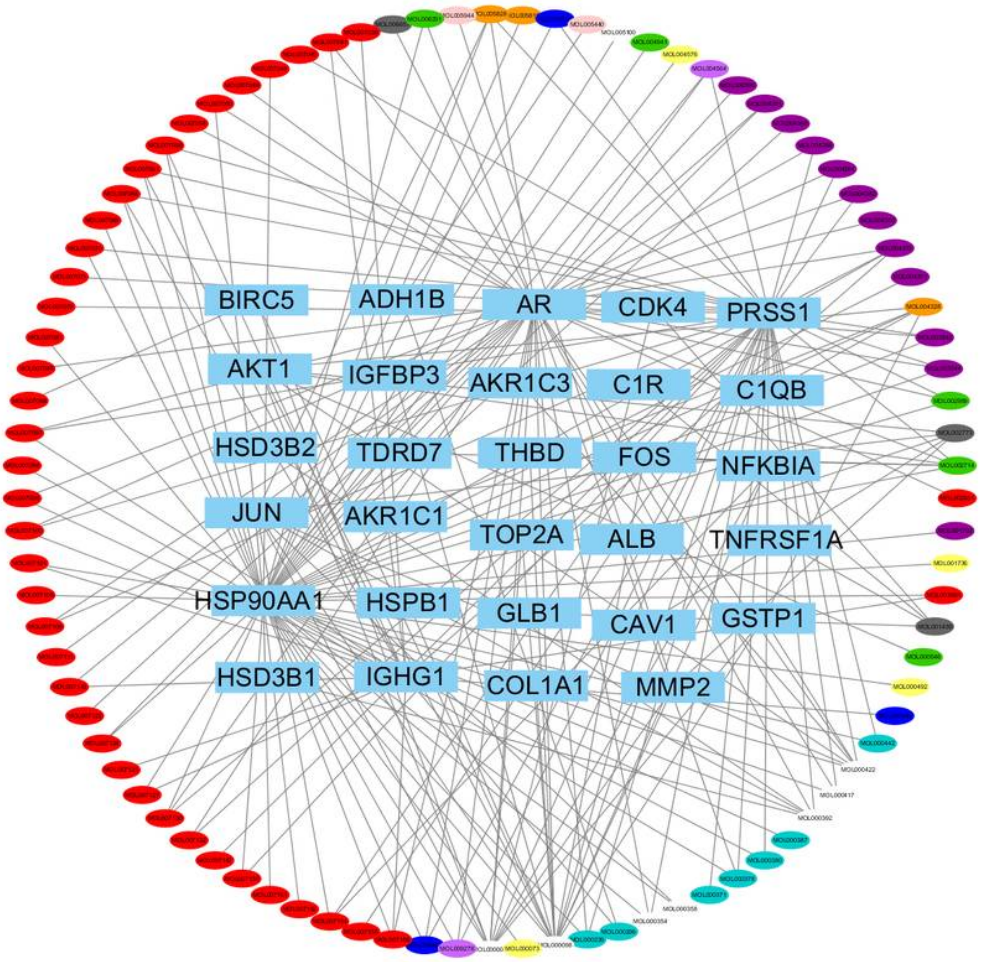

Figure 2

Compound-target network for RJF. Blue rectangles represent targets; oval shapes in different colors represent compounds from different drugs.

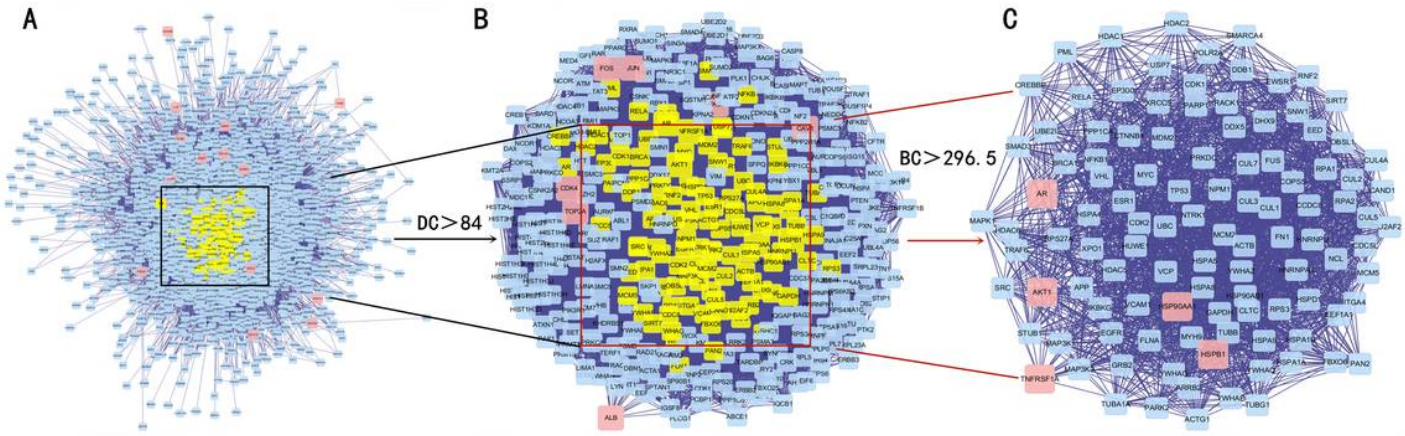

Figure 3

The identification of candidate targets for RJF in oligozoospermia treatment. (A) The interactive PPI network for putative RJF targets and oligozoospermia-related targets. (B) PPI network of significant proteins extracted from the network shown in (A). (C) PPI network of candidate RJF targets for oligozoospermia extracted from the network shown in (B). DC, degree centrality; BC, betweenness centrality

Figure 4

Gene ontology terms of candidate targets for RJF in the treatment of oligozoospermia. The top 20 GO functional categories with a FDR < 0.05 were selected. (A) Biological process, (B) cellular component, and (c) molecular function.

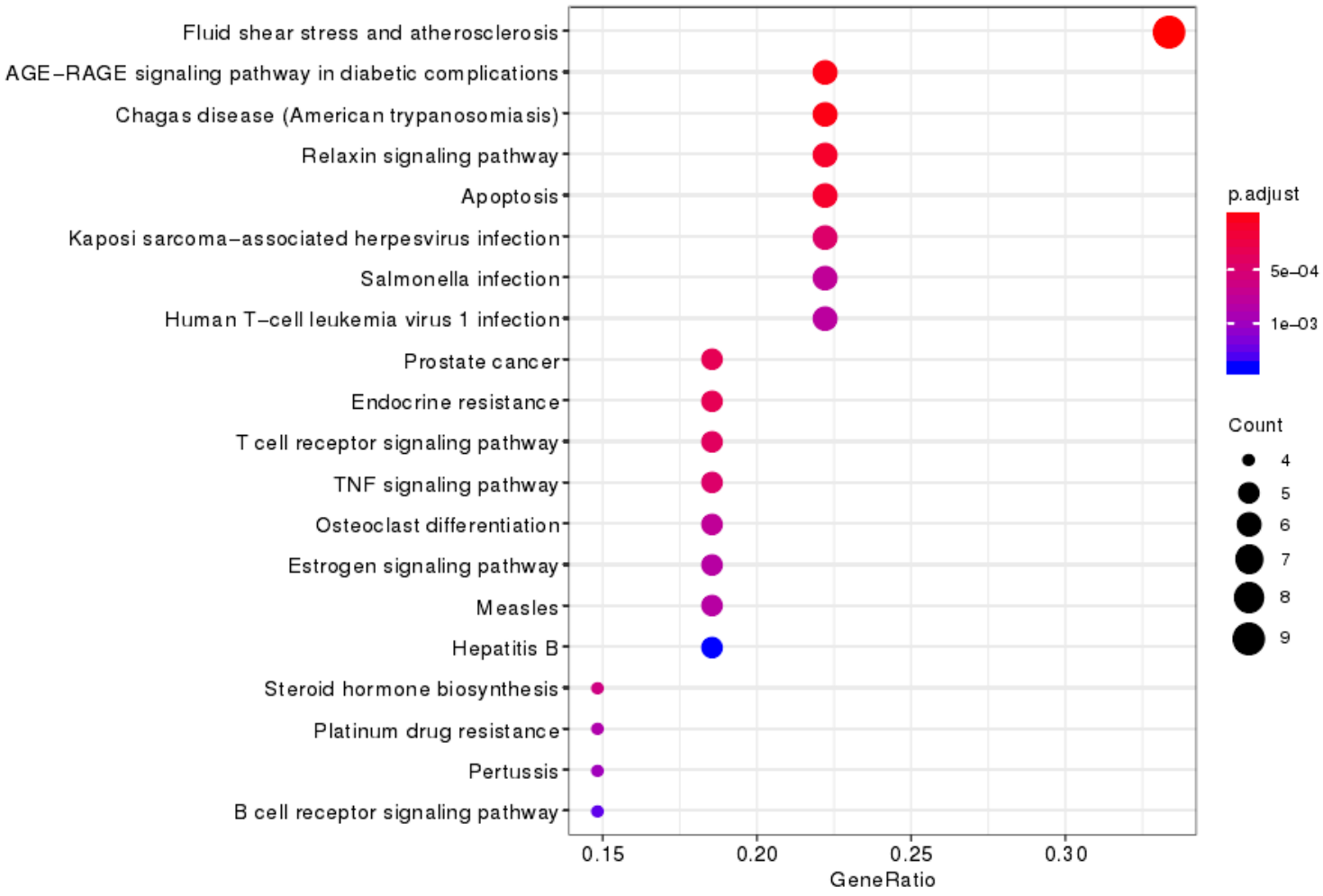

Figure 5

KEGG pathway enrichment of the candidate targets that may be responsible for the effect of RJF in oligospermic patients. Pathways are identified that show a significant change of FDR (<0.05). The relative size of the spots represents the number of genes while the color represents the FDR value.

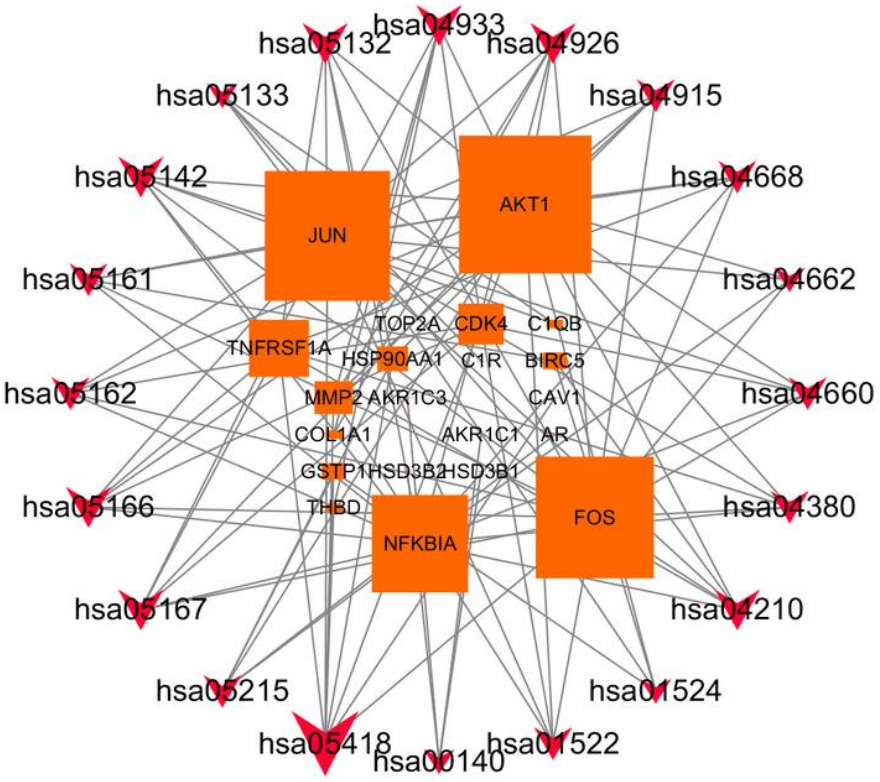

Figure 6

Gene-pathway network for the effect of RJF in oligospermic patients. The topological analysis of 21 pathways and 29 genes was carried out with betweenness centrality. The yellow squares represent target genes while the red V-shapes represent pathways. A large size represents a larger betweenness centrality.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.xls
- SupplementaryTable2.xls