Integrating Data Mining, Network Pharmacology, and Molecular Docking Verification to Investigate the Molecular Mechanism of Traditional Chinese Medicine Prescriptions for Treating Male Infertility

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Abstract

Background: Male infertility (MI) affects almost 5% adult men worldwide, and 75% of these cases are unexplained idiopathic. There are limitations in the current treatment due to the unclear mechanism of MI, which highlight the urgent need for a more effective strategy or drug. Traditional Chinese Medicine (TCM) prescriptions have been used to treat MI for thousands of years, but their molecular mechanism is not well defined.

Methods: Aiming at revealing the molecular mechanism of TCM prescriptions on MI, a comprehensive strategy integrating data mining, network pharmacology, and molecular docking verification was performed. Firstly, we collected 289 TCM prescriptions for treating MI from National Institute of TCM Constitution and Preventive Medicine for 6 years. Then, Core Chinese Materia Medica (CCMM), the crucial combination of TCM prescriptions, was obtained by the TCM Inheritance Support System from China Academy of Chinese Medical Sciences. Next, the components and targets of CCMM in TCM prescriptions and MI-related targets were collected and analyzed through network pharmacology approach.

Results: The results showed that the molecular mechanism of TCM prescriptions for treating MI are regulating hormone, inhibiting apoptosis, oxidant stress and inflammatory. Estrogen signaling pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway, and TNF signaling pathway are the most important signaling pathways. Molecular docking experiments were used to further validate network pharmacology results.

Conclusions: This study not only discovers CCMM and the molecular mechanism of TCM prescriptions for treating MI, but may be helpful for the popularization and application of TCM treatment.

Background

Male infertility (MI), characterized by the abnormal semen quality or defective sperm transport [1], has become a disease with high incidence, and 75% of these cases are unexplained idiopathic [2]. The main types of MI are azoospermia, oligozoospermia, asthenospermia and teratospermia [3], which is considered to be caused by varicocele, idiopathic, obstruction, cryptorchidism, immunologic, ejaculatory dysfunction, testicular failure, drug effects/radiation, endocrinology, and all others [4]. Besides, a most recent study showed that sperm fertility could be affected by the testicular-borne factors [5, 6]. Currently, the most common method to treat MI is assisted reproductive technology (ART), which has successfully improved the pregnancy rate of infertile couples [7]. However, unsolved problems of ART are still prevailed, such as associated high-cost, potential risk to safety, uncertainty about treatment. Besides, ART could not fundamentally improve the sperm quality of MI patients [8]. Moreover, Coronavirus Disease 2019 is the most serious disease in 2020, and SARS-CoV-2 was confirmed as the causal virus. [9]. To be noted, some studies have shown that SARS-CoV-2 could directly damage the testicular functions, or by secondary inflammatory and immunological responses, finally leading to the emerging of MI [10-16]. Thus, it is urgent to find a more effective therapy or drug to cure MI.

Traditional Chinese Medicine (TCM) can be effective in the treatment of many diseases, including MI [17]. The characteristic of TCM treatment involves formulating different TCM prescriptions based on the constitutional signs and symptoms of the patients [18, 19]. In TCM prescriptions, Core Chinese Materia Medica (CCMM) plays a crucial role in the medical cases, which is used to find the underlying laws and associations of TCM treatment. For MI, various TCM prescriptions have been used, but its molecular mechanism is still unclear, which limits the clinical application of TCM. Therefore, in order to accelerate the TCM clinical application, it is essential to explore CCMM the underlying molecular mechanism of TCM prescriptions for treating MI.

The concept of holism of TCM has much in common with the major points of network pharmacology, where the general “one target, one drug” mode is shifted to a new “network target, multi-components” mode [20, 21]. In such a mode, the combination of network pharmacology and TCM prescriptions would create a novel direction for discovering bioactive components and potential targets, revealing the molecular mechanism, and examining scientific evidence of numerous herbs in TCM prescriptions based on complex biological systems of human body. Molecular docking, a method of predicting the binding sites, is performed to estimate the associations between components and targets [22]. Correspondingly, this study not only uncovers CCMM and the underlying molecular mechanism of TCM prescriptions for treating MI, but may be helpful for the popularization and application of TCM treatment.

In view of the “multi-component”, “multi-target”, and “multi-pathway” characteristics of TCM prescriptions [23], we adopted a comprehensive approach integrating data mining, network pharmacology, and molecular docking verification. First, 6 years of TCM prescriptions for treating MI were come from the medical record of outpatient departments in National Institute of TCM Constitution and Preventive Medicine affiliated to Beijing University of Chinese Medicine. Next, TCM Inheritance Support System (TCMISS) software was utilized to screen and discover CCMM in TCM prescriptions. Then, the components and targets of CCMM in TCM prescriptions, and the MI targets were obtained from various databases, respectively. Subsequently, network pharmacology was used to deeply investigate the core targets and signaling pathways in the mechanism of CCMM in TCM prescriptions against MI. In order to estimate the network pharmacology results, molecular docking approach was performed to investigate the interactions between representative components and key targets of CCMM in TCM prescriptions on MI.

Materials And Methods

TCM prescriptions analysis

TCM prescriptions used to treat MI were obtained from the outpatient departments of National Institute of TCM Constitution and Preventive Medicine affiliated to Beijing University of Chinese Medicine (date: from January 2013 to June, 2019), which were prescribed by Professor Qi Wang. The inclusive criterion of TCM prescriptions are (1) the patient was first diagnosed as MI, including azoospermia, oligozoospermia, asthenospermia and teratospermia; (2) the patient was older than 23; (3) the patient has been married for

more than 1 year and had normal sex without contraception for 12 months, but the woman was un-pregnant due to the male factors; (4) the patient has no family history related to MI. The exclusive criterion is that the patient's wife has a disease that makes it difficult to conceive. TCMISS software (V2.5) is provided by China Academy of Chinese Medical Sciences. It is specially focuses on data mining and analysis of TCM prescriptions, finally uncovers the core law and combination [24, 25]. The software contains six functional modules: clinical collection, platform management, data management, knowledge retrieval, statistical report, and data analysis. Three graduate students were responsible for the accuracy of prescriptions collection. One of them used the “clinical collection” function to collect the prescriptions, the others used the “platform management” function to check the data. The “data analysis” function was used to analysis the frequency of Chinese Materia medica (CMM) in TCM prescriptions, then the combinations of CCMM were obtained. The support degree was 140 and the confidence score was greater than or equal to 0.95.

Components of CCMM in TCM prescriptions

Two TCM databases, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, <http://tcmospw.com/tcmosp.php>) [26] and Traditional Chinese Medicine Integrative Database (TCMID, <http://119.3.41.228:8000/tcmid/>), were used to obtain the components of CCMM [27] (Additional file 1: Table S1). Then, two absorption, distribution, metabolism, and excretion (ADME)-related models [28, 29], oral bioavailability (OB) $\geq 30\%$ and drug-likeness (DL) ≥ 0.18 [30], were the screening criteria of the bioactive components using Venn diagram [31]. Next, the structure information of the bioactive components was searched from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) [32] and ALOGPS2.1 (<http://www.vcclab.org/lab/alogps/>) [33] (Additional file 2: Table S2).

Targets of bioactive components of CCMM in TCM prescriptions

Swiss Target Prediction (<http://www.swisstargetprediction.ch/>) [34] was used to obtain the targets of bioactive components, with the species limited to “Homo sapiens” and probability value > 0 . Finally, the names of targets were standardized by UniProtKB (<https://www.uniprot.org/>) [35] (Additional file 3: Table S3). The CCMM component-target network was constructed using Cytoscape (<http://www.cytoscape.org>, version 3.8.0) [36]. The degree value of network was calculated by Network Analyzer [37], a plugin of the Cytoscape software.

Targets of MI

The key words “male infertility”, and “infertility, male” were used to search the MI-related targets from 4 different databases, including DisGeNET database (<https://www.disgenet.org/>) [38], Comparative Toxicogenomics Database (CTD, <http://ctdbase.org/>) [39], Online Mendelian Inheritance in Man (OMIM, <http://omim.org/>) [40], and GeneCards (<https://www.genecards.org/>, updated on Mar. 11, 2020) [41]. Then, the targets were standardized using UniProtKB (Additional file 5: Table S5).

CCMM-MI common-target network

The common targets of CCMM and MI were obtained by Venn diagram (Additional file 7: Table S7), then CCMM-MI common-target network was constructed using the Cytoscape software. In addition, the topological properties of the network were calculated by Network Analyzer (Additional file 8: Table S8).

GO and KEGG pathway enrichment analyses

The Database for Annotation Visualization and Integrated Discovery (DAVID, <https://david.ncifcrf.gov/>, version 6.8) [42] was used to conduct the Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses for the targets of CCMM in TCM prescriptions, the MI-related targets, and the CCMM-MI common targets. The screening criteria is $P \leq 0.05$ using the Bonferroni correction [43] (Additional file 4, 6, 9: Table S4, 6, 9). Furthermore, we used the BiNGO plug-in (<http://apps.cytoscape.org/apps/bingo>) [44] and ClueGO plug-in (<http://apps.cytoscape.org/apps/cluego>) [45] to perform the GO and KEGG pathway enrichment analyses for the key targets of CCMM and MI.

PPI network construction and evaluation

The protein-protein information (PPI) of the common targets of CCMM and MI was obtained from STRING database v11.0 (<http://string-db.org>) [46], with the confidence score equal or greater than 0.4. We imported the PPI information to TSV format, then constructed PPI network by Cytoscape. The topology parameters, including degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC), were used to screen the key targets (Additional file 10: Table S10).

Molecular docking verification

Protein Data Bank (PDB) (<http://www.rcsb.org/>) [47] were used to obtain the X-ray crystal structures of the targets, including AKT1 (PDB ID: 3QKK), MAPK3 (PDB ID: 6GES), MAPK1 (PDB ID: 4QYY), EGFR (PDB ID: 5X2K), GAPDH (PDB ID: 6ADE), TNF (PDB ID: 1FT4). Then, PyMOL 2.4 (<https://pymol.org/2/>) [48] was applied to remove water molecules and pro-ligand small molecules. The protein receptor files and ligand files were processed and then converted to pdbqt format using AutoDock Tools 1.5.6. Each grid box was centered on ligand. Finally, molecular docking calculations were performed using Autodock Vina 1.1.2 [49]. The docking results were visualized and displayed as 3D diagrams and 2D diagrams by using PyMOL 2.4 and ligplus.

Results

Results of data mining by TCMISS

We collected 289 TCM prescriptions and 149 CMM used to treat MI. The top 20 high frequent CMM of the prescriptions are shown in Table 1, including *Morindae Officinalis Radix*, *Cuscutae Semen*, *Lycii Fructus*, *Mori Fructus*, *Angelicae Sinensis Radix*, *Hedysarum Multijugum Maxim.*, *Swim bladder*, *Plantaginis Semen*, *Hirudo*, *Cyperi Rhizoma*, *Lysimachiae Herba*, *Hordei Fructus Germinatus*, *Endothelium corneum*, *Crataegus pinnatifida Ege.*, *Sojae Semen Praeparatum*, *Hippocampus japonicus*, *Fraxini Cortex*, *Oysters*,

Placenta Hominis, and Panax Ginseng C. A. Mey. The frequency of the top 6 CMM is greater than 170, with a percentage greater than 60%, indicating that they are CCMM in the prescriptions on MI. The combinations of CCMM in TCM prescriptions are shown in Table 2. In addition, the application mode of CCMM in TCM prescriptions was virtualized as a network using the TCMISS software (Figure 1).

Table 1: The top 20 high frequent CMM in TCM prescriptions.

Number	Name of CMM	Frequency	Percentage
1	Morindae Officinalis Radix	225	77.85%
2	Cuscutae Semen	208	71.97%
3	Lycii Fructus	194	67.13%
4	Mori Fructus	188	65.05%
5	Angelicae Sinensis Radix	183	63.32%
6	Hedysarum Multijugum Maxim.	176	60.90%
7	Swim bladder	156	53.98%
8	Plantaginis Semen	123	42.56%
9	Hirudo	112	38.75%
10	Cyperi Rhizoma	108	37.37%
11	Lysimachiae Herba	71	24.57%
12	Hordei Fructus Germinatus	68	23.53%
13	Endothelium corneum	54	18.69%
14	Crataegus pinnatifida Ege.	43	14.88%
15	Sojae Semen Praeparatum	40	13.84%
16	Hippocampus japonicus	34	11.76%
17	Fraxini Cortex	33	11.42%
18	Oysters	31	10.73%
19	Placenta Hominis	29	10.03%
20	Panax Ginseng C. A. Mey.	24	8.30%

Table 2: The combinations of CCMM in TCM prescriptions.

Number	The combinations of CCMM	Frequency
1	Cuscutae Semen, Morindae Officinalis Radix	188
2	Cuscutae Semen, Lycii Fructus	178
3	Cuscutae Semen, Angelicae Sinensis Radix	153
4	Cuscutae Semen, Mori Fructus	165
5	Cuscutae Semen, Hedysarum Multijugum Maxim.	153
6	Morindae Officinalis Radix, Lycii Fructus	176
7	Morindae Officinalis Radix, Angelicae Sinensis Radix	170
8	Morindae Officinalis Radix, Mori Fructus	167
9	Hedysarum Multijugum Maxim., Morindae Officinalis Radix	167
10	Angelicae Sinensis Radix, Lycii Fructus	148
11	Lycii Fructus, Mori Fructus	155
12	Hedysarum Multijugum Maxim., Lycii Fructus	148
13	Hedysarum Multijugum Maxim., Angelicae Sinensis Radix	171
14	Cuscutae Semen, Morindae Officinalis Radix, Lycii Fructus	163
15	Cuscutae Semen, Morindae Officinalis Radix, Angelicae Sinensis Radix	148
16	Cuscutae Semen, Morindae Officinalis Radix, Mori Fructus	148
17	Cuscutae Semen, Hedysarum Multijugum Maxim., Morindae Officinalis Radix	148
18	Cuscutae Semen, Lycii Fructus, Mori Fructus	143
19	Cuscutae Semen, Hedysarum Multijugum Maxim., Angelicae Sinensis Radix	149
20	Morindae Officinalis Radix, Angelicae Sinensis Radix, Lycii Fructus	143
21	Morindae Officinalis Radix, Lycii Fructus, Mori Fructus	141
22	Hedysarum Multijugum Maxim., Morindae Officinalis Radix, Lycii Fructus	143
23	Hedysarum Multijugum Maxim., Morindae Officinalis Radix, Angelicae Sinensis Radix	163
24	Hedysarum Multijugum Maxim., Angelicae Sinensis Radix, Lycii Fructus	146
25	Cuscutae Semen, Hedysarum Multijugum Maxim., Morindae Officinalis Radix, Angelicae Sinensis Radix	144
26	Hedysarum Multijugum Maxim., Morindae Officinalis Radix, Angelicae Sinensis Radix, Lycii Fructus	141

CCMM component-target network

The components and targets of CCMM in TCM prescriptions were collected from TCMSPP, TCMID, and Swiss Target Prediction databases. A total of 20 components and 359 targets from Morindae Officinalis Radix, 13 components and 302 targets from Cuscutae Semen, 47 components and 446 targets from Lycii Fructus, 7 components and 157 targets from Mori Fructus, 6 components and 296 targets from Angelicae Sinensis Radix, 22 components and 436 targets from Hedysarum Multijugum Maxim, were obtained. Then, we constructed a CCMM component-target network using the 98 components and 816 targets (Figure 2). We found that beta-sitosterol, sitosterol, quercetin, kaempferol, isorhamnetin, CLR, campesterol, Stigmasterol, and beta-carotene are repeated more than once in CCMM. The structure, OB, and DL of these duplicate components are shown in Table 3. So, we thought that these duplicate components should be further explored in the next experiment.

Table 3: The components with more than one occurrence in different herbs of CCMM in TCM prescriptions.

Mol ID	Molecule Name	Structure	OB (%)	DL	Herb
MOL000358	beta-sitosterol		36.91	0.75	Morindae Officinalis Radix Cuscutae Semen Lycii Fructus Mori Fructus Angelicae Sinensis Radix Hedysarum Multijugum Maxim.
MOL000359	sitosterol		36.91	0.75	Morindae Officinalis Radix Lycii Fructus Hedysarum Multijugum Maxim.
MOL000098	quercetin		46.43	0.28	Cuscutae Semen Lycii Fructus Mori Fructus Hedysarum Multijugum Maxim.
MOL000422	kaempferol		41.88	0.24	Cuscutae Semen Mori Fructus Hedysarum Multijugum Maxim.
MOL000354	isorhamnetin		49.6	0.31	Cuscutae Semen Hedysarum Multijugum Maxim.
MOL000953	CLR		37.87	0.68	Cuscutae Semen Lycii Fructus
MOL005438	campesterol		37.58	0.71	Cuscutae Semen Lycii Fructus
MOL000449	Stigmasterol		43.83	0.76	Lycii Fructus Angelicae Sinensis Radix
MOL002773	beta-carotene		37.18	0.58	Lycii Fructus Mori Fructus

GO and KEGG enrichment analyses of the targets of CCMM in TCM prescriptions

The GO enrichment analysis contains three sections, including biological process, cellular component, and molecular function. We found that CCMM could inhibit apoptosis, promote cell proliferation, and regulate the cytosolic calcium ion concentration through negative regulation of apoptotic process (GO:0043066), positive regulation of cytosolic calcium ion concentration (GO:0007204), positive regulation of cell proliferation (GO:0008284). Additionally, Prostate cancer (hsa05215), HIF-1 signaling

pathway (hsa04066), Progesterone-mediated oocyte maturation (hsa04914), and Acute myeloid leukemia (hsa05221) are related to male reproductive function, which was shown in Figure 3.

MI-related targets

A total of 671 targets of MI were collected from four different databases. Among these, 225 targets were from CTD, 210 targets were from DisGeNET, 197 targets were from GeneCards, 181 targets were from OMIM (Figure 4A). Subsequently, we performed GO and KEGG enrichment pathway analyses on MI-related targets (Figure 4B and C). The results showed that pathways in cancer (hsa05200), PI3K-Akt signaling pathway (hsa04151), MAPK signaling pathway (hsa04010) were the most significant signaling pathways. Moreover, positive regulation of transcription from RNA polymerase II promoter (GO:0045944), response to drug (GO:0042493), negative regulation of apoptotic process (GO:0043066), spermatogenesis (GO:0007283) were the most significant terms in biological process. According to the above results, we suggest that MI is related to apoptosis and spermatogenesis.

CCMM-MI common-target network

Based on the results of Venn diagram, we obtained 127 common targets of CCMM and MI (Figure 5). Then, we established a CCMM-MI common-target network, including 90 components and 127 targets (Figure 6). Especially, it suggested that Jaranol, kaempferol, (6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol, isoflavanone, quercetin, 1-hydroxy-3-methoxy-9,10-anthraquinone, 3,9-di-O-methylnissolin, isorhamnetin, morin, (3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol, Cnidilin, Sitosterol alpha1, citrostadienol, beta-sitosterol, and NSC63551 are the top 15 components with high degree value in the process of CCMM against MI. The structure and degree value of these components were shown in Table 4. To be noted, kaempferol, quercetin, isorhamnetin, and beta-sitosterol were the duplicate components in CCMM. As shown in Table 5, Aromatase (CYP19A1), Androgen receptor (AR), Estrogen receptor beta (ESR2), Estrogen receptor (ESR1), Acetylcholinesterase (ACHE), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), Sex hormone-binding globulin (SHBG), Steroid 17-alpha-hydroxylase/17,20 lyase (CYP17A1), Cholinesterase (BCHE), Peroxisome proliferator-activated receptor alpha (PPARA), Nuclear receptor subfamily 1 group I member 3 (NR1I3), Squalene monooxygenase (SQLE), Peroxisome proliferator-activated receptor gamma (PPARG), Nitric oxide synthase, inducible (NOS2), and Glucocorticoid receptor (NR3C1) were the top 15 CCMM-MI common targets with high degree value.

Table 4: The top 15 components with high degree value acted on the CCMM-MI common targets.				
Num ber	Molecule Name	Structure	Deg ree	Herb
1	Jaranol		28	Hedysa rum Multiju gum Maxim.
2	kaempferol		28	Cuscut ae Semen Mori Fructus Hedysa rum Multiju gum Maxim.
3	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol		27	Hedysa rum Multiju gum Maxim.
4	isoflavanone		27	Hedysa rum Multiju gum Maxim.
5	quercetin		26	Cuscut ae Semen Lycii Fructus Mori Fructus Hedysa rum Multiju gum Maxim.
6	1-hydroxy-3-methoxy-9,10-anthraquinone		25	Morind ae Officin alis
7	3,9-di-O-methylnissolin		24	Radix Hedysa rum Multiju gum Maxim.
8	isorhamnetin		24	Cuscut ae Semen Hedysa rum Multiju gum Maxim.
9	morin		23	Mori Fructus
10	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol		22	Hedysa rum Multiju gum Maxim.
11	Cnidilin		22	Angelic ae Sinensi s Radix
12	Sitosterol alpha1		21	Lycii Fructus
13	citrostadienol		21	Lycii Fructus
14	beta-sitosterol		21	Morind ae Officin alis Radix Cuscut ae Semen Lycii Fructus Mori Fructus Angelic ae Sinensi s Radix Hedysa rum Multiju gum Maxim.
15	NSC63551		21	Cuscut ae Semen

Table 5: The top 15 CCMM-MI common targets with high degree value.

Number	Gene name	Protein name	Degree
1	CYP19A1	Aromatase	56
2	AR	Androgen receptor	50
3	ESR2	Estrogen receptor beta	50
4	ESR1	Estrogen receptor	46
5	ACHE	Acetylcholinesterase	42
6	HMGCR	3-hydroxy-3-methylglutaryl-coenzyme A reductase	40
7	SHBG	Sex hormone-binding globulin	40
8	CYP17A1	Steroid 17-alpha-hydroxylase/17,20 lyase	39
9	BCHE	Cholinesterase	38
10	PPARA	Peroxisome proliferator-activated receptor alpha	33
11	NR1I3	Nuclear receptor subfamily 1 group I member 3	32
12	SQLE	Squalene monooxygenase	31
13	PPARG	Peroxisome proliferator-activated receptor gamma	31
14	NOS2	Nitric oxide synthase, inducible	27
15	NR3C1	Glucocorticoid receptor	22

GO and KEGG pathway enrichment analyses of the CCMM-MI common targets

Figure 7A indicated that CCMM could inhibit apoptosis in the treatment of MI, via negative regulation of apoptotic process (GO:0043066). In addition, CCMM can reduce oxidant stress through oxidation-reduction process (GO:0055114), and positive regulation of nitric oxide biosynthetic process (GO:0045429). CCMM can also promote cell proliferation for treating MI, through positive regulation of cell proliferation (GO:0008284). Besides, CCMM can regulate male reproductive function on MI, through positive regulation of ERK1 and ERK2 cascade (GO:0070374). As shown in Figure 7B and 7C, CCMM mainly regulated hormones in the treatment of MI, via steroid hormone receptor activity (GO:0003707), and thyroid hormone receptor activity (GO:0004887). The main KEGG signaling pathways were PI3K-Akt signaling pathway, Estrogen signaling pathway, HIF-1 signaling pathway, and TNF signaling pathway, demonstrating that CCMM could treat MI via regulating hormone, reducing apoptosis, oxidant stress, and inflammatory (Figure 7D).

PPI network analysis

PPI network was constructed to analyze the core proteins of the CCMM-MI common targets (Figure 8). The results showed that Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), RAC-alpha serine/threonine-protein kinase (AKT1), ESR1, Mitogen-activated protein kinase 3 (MAPK3), Epidermal growth factor receptor (EGFR), Tumor necrosis factor (TNF), Mitogen-activated protein kinase 1 (MAPK1), Heat shock protein HSP 90-alpha (HSP90AA1) are the core proteins in the PPI network under the screening criteria of $DC \geq 60$, $BC \geq 0.025$, and $CC \geq 0.651$ (Figure 8C).

GO and KEGG pathway enrichment analyses of the key targets

Furthermore, GO and KEGG enrichment analyses were performed on the key targets of the PPI network (Figure 9). The results showed that Estrogen signaling pathway is the most significant signaling pathway, and DNA damage induced protein phosphorylation is the most significant GO term. The common targets

between the related targets in PI3K-Akt signaling pathway, Estrogen signaling pathway, HIF-1 signaling pathway, TNF signaling pathway, and the key targets in the PPI network are AKT1, MAPK3, MAPK1, EGFR, GAPDH, and TNF (Table 6, Figure 10-13).

Table 6: The most significant KEGG signaling pathways and key targets.

Classification	KEGG signaling pathway	Key targets
Hormone regulation	Estrogen signaling pathway	AKT1 (Akt) MAPK3/1 (ERK1/2) EGFR
Apoptosis	PI3K-Akt signaling pathway	AKT1 (AKT) MAPK3/1 (ERK)
Oxidant stress	HIF-1 signaling pathway	AKT1 (AKT) MAPK3/1 (ERK) GAPDH
Inflammatory	TNF signaling pathway	AKT1 (Akt) MAPK3/1 (ERK1/2) TNF

Molecular docking

In this study, we chose kaempferol, quercetin, isorhamnetin, beta-sitosterol from CCMM as small molecules (ligands), AKT1, MAPK3, MAPK1, EGFR, GAPDH, and TNF as proteins to perform the molecular docking (Table 7). Kaempferol was docked with sixteen residues to form hydrophobic interactions in AKT1 (Gly 294, Leu181, Gly162, Lys179, Gly159, Asp274, and Ser7) and 4 hydrogen bonds (kaempferol₀₅: Asp292_O (2.9 Å), kaempferol₀₅: Leu195_N (2.8 Å), kaempferol₀₄: Phe161_N (3.1 Å), kaempferol₀₄: Thr160_N (2.8 Å)) (Figure 14A, B). In addition, kaempferol was predicted to interact with MAPK3 via Gly262, Leu284, Asn255, Leu258, Gly259, Lys287 and form 2 hydrogen bonds with the residues Pro285 (3.1 Å) and Ser263 (3.0 Å) (Figure 14C, D). Kaempferol could bind to MAPK1 by forming hydrophobic interactions with the neighboring residues Asp165, Ala50, Leu105, Leu154, Ile29, Val137, Lys52, Tyr34 and 2 hydrogen bonds with Asp104 (2.7 Å) and Met106 (3.1 Å) (Figure 14E, F). Besides, kaempferol bound to a pocket in EGFR, which was composed of Ala743, Leu792, Leu718, Cys797, Arg841, Leu844, and Gly796. The hydrogen bonds formed by kaempferol₀₅ and Met793_N (3.0 Å), kaempferol₀₆ and Asp855_{OD2} (2.9 Å), kaempferol₀₆ and Asn842_{OD1} (2.8 Å), further enhanced the interaction between the ligand and the AKT1 protein (Figure 14G, H). Furthermore, kaempferol was docked to GAPDH by forming hydrophobic interactions with the neighboring residues (Ile38, Phe37, Thr99, Val101, Phe102, Arg80, Pro36, Asp35, Gly12) and 2 hydrogen bonds with Asn34 (3.1 Å) and Asn9 (3.2 Å) (Figure 14I, J). kaempferol was also predicted to interact with TNF via Leu67, Ala62, Phe60, Leu71, Asn65 and form a hydrogen bond with the residue Glu64 (3.0 Å) (Figure 14K, L).

As shown in Figure 15A and B, quercetin was observed to interact with AKT1 via Lys276, Glu278, Leu295, Gly294, His194, Phe161, Glu191, Ile186, Asp292 and form 3 hydrogen bonds with Thr5 (2.9 Å), Asp274 (2.9 Å), and Ser7 (2.9 Å). According to the analysis results shown in Figure 15C and D, quercetin forms hydrophobic interactions with 7 residues in MAPK3 (Leu258, Gly262, Leu284, Asn255, Gly259, Lys287, and Pro285) and a hydrogen bond (quercetin₀₄: Ser263_{OG} (2.7 Å)). Figure 15E and F shows that quercetin

was predicted to interact with MAPK1 via Gly167, Tyr34, Gln103, Ala50, Val37, Ile54, Glu69 and Thr66, and formed two hydrogen bonds with Asp165 (2.9 Å) and Lys52 (3.1 Å). In addition, the action modes of quercetin and EGFR were shown in Figure 15G and H. Quercetin binds to a pocket in AKT1, composing of Gly796, Ala743, Leu792, Val726, Leu844, Leu718 and Cys797. Three hydrogen bonds, quercetin_{O5}: Asp800_{OD2} (2.9 Å), quercetin_{O7}: Met793_N (3.1 Å) and Gln791_O (2.7 Å), further enhance the interactions between the ligand and the EGFR protein. As shown in Figure 15I and J, quercetin was predicted to interact with GAPDH via Asp35, Phe37, Arg80, Val101, Phe102, Thr99, Pro36, Gly12 and formed two H-bonds with the residues Asn9 (3.1 Å) and Asn34 (3.1 Å). Quercetin could bind to TNF by forming hydrophobic interactions with the neighboring residues Asn65, Leu71, Phe60, Ala62, Leu67 and a hydrogen bond with Glu64 (2.8 Å) (Figure 15K, L).

According to Figure 16A and B, isorhamnetin was observed to form hydrophobic interactions with nineteen residues in AKT1 (Phe438, Gly157, Leu156, Thr291, Met227, Val164, Met281, Gly159) and 3 hydrogen bonds (isorhamnetin_{O4}: Glu234_{OE2} (2.8 Å), isorhamnetin_{O7}: Gly162_N (3.0 Å) and Phe161_N (3.2 Å)). As shown in Figure 16C and D, isorhamnetin was observed to interact with MAPK3 via Pro264, Lys287, Leu284, Asn255, Gly259, Leu258, Gly262 and Pro285 and form a H-bond with the residue Ser263 (2.8 Å). Figure 16E and F showed that isorhamnetin could bind to MAPK1 by forming hydrophobic interactions with the surrounding residues (Lys52, Gln103, Ile82, Ala50, Leu154, Ile29, Val37, Tyr34 and Glu69) and 2 hydrogen bonds (Asp165_{OD2} (2.8 Å), Asp104_O (2.6 Å)). Moreover, isorhamnetin was predicted to interact with EGFR via Leu792, Gly796, Leu844, Cys797, Arg841, Ala743, Met790 and Val726 and formed 3 hydrogen bonds with the residues Met793_N (3.1 Å), Asn842_{OD1} (2.9 Å), Asp855_{OD2} (2.8 Å) (Figure 16G and H). Besides, isorhamnetin was observed to form hydrophobic interactions with thirteen residues in GAPDH (Gly12, Phe11, Gly10, Pro36, Thr99, Phe37, Val101, Ser98, Ile14 and Ala183), and formed 2 hydrogen bonds with the residues Arg13 (3.3 Å) and Asp35 (3.0 Å) (Figure 16I and J). Isorhamnetin was also interacted with TNF via Asn65, Ala62, Phe60, Leu71, Leu67 and form a H-bond with the residue Glu64 (2.9 Å) (Figure 16K, L).

The action modes of beta-sitosterol and AKT1 are shown in Figure 17A and B. Beta-sitosterol bound to a pocket in AKT1, composing of Val164, Asn279, Gly294, Leu295, Phe161, Glu191, His194, Leu181, Thr195, Glu198, Lys179, Asp292 and a hydrogen bond, Glu234 (2.9 Å). As shown in Figure 17C and D, beta-sitosterol was predicted to interact with MAPK3 via Ser283, Asn255, Pro285, Leu285, Gly259, Tyr280, Leu284. According to the analysis results shown in Figure 17E and F, beta-sitosterol was observed to form hydrophobic interactions with 14 residues in MAPK1 (Ala50, Leu154, Ile82, Met106, Lys52, Asp104, Ile29, Cys164, Gln103, Asp165, Tyr34, Ile54, Arg65 and Glu69) and 2 H-bond with the residue Tyr62 (3.0 Å) and Thr66 (2.9 Å). Beta-sitosterol could bind to EGFR by forming hydrophobic interactions with the surrounding residues Cys775, Leu844, Ala743, Lys745, Leu718, Asp800, Arg841, Cys797, Val726, Met790, Thr854 and a H-bond with Gln791 (2.9 Å) (Figure 17G, H). Moreover, beta-sitosterol was observed to GAPDH by forming hydrophobic interactions with the surrounding residues Cys152, Ala183, Arg13, Gly12, Gly10, Asp35, Phe37, Thr99, Ser98, Ile14, Ser122 (Figure 17I, J). Beta-sitosterol was also interacted with

TNF via Asn65, Ala62, Phe60, Asp93, Ser72, Leu71, Leu67, Lys32 and a hydrogen bond with His66 (3.1 Å) (Figure 17K, L).

Table 7: The binding energy of molecular docking between ligands and proteins.

Ligand	Proteins	Affinity (kcal/mol)	Dist from best mode	
			rmsd l.b.	rmsd u.b.
kaempferol	AKT1	-7.5	0.000	0.000
	MAPK3	-5.4	0.000	0.000
	MAPK1	-8.1	0.000	0.000
	EGFR	-6.6	0.000	0.000
	GAPDH	-6.5	0.000	0.000
	TNF	-4.4	0.000	0.000
quercetin	AKT1	-7.7	0.000	0.000
	MAPK3	-5.3	0.000	0.000
	MAPK1	-8.1	0.000	0.000
	EGFR	-6.5	0.000	0.000
	GAPDH	-6.5	0.000	0.000
	TNF	-4.3	0.000	0.000
isorhamnetin	AKT1	-7.5	0.000	0.000
	MAPK3	-5.4	0.000	0.000
	MAPK1	-8.1	0.000	0.000
	EGFR	-6.7	0.000	0.000
	GAPDH	-6.6	0.000	0.000
	TNF	-4.4	0.000	0.000
beta-sitosterol	AKT1	-9.8	0.000	0.000
	MAPK3	-5.3	0.000	0.000
	MAPK1	-9.5	0.000	0.000
	EGFR	-7.2	0.000	0.000
	GAPDH	-7.5	0.000	0.000
	TNF	-5.9	0.000	0.000

Discussion

With the changes in people’s living habits, environmental pollution, and psychological factors, the rising incidence rate of infertility has become a serious problem , where male infertility accounts for 50% of the cases [50]. TCM prescriptions have been used for treating MI for thousands of years, but its molecular mechanism remains unclear. In this study, we first collected TCM prescriptions from the outpatient departments of National Institute of TCM Constitution and Preventive Medicine. So, the quality and reliability of the prescriptions could be guaranteed. CCMM in TCM prescriptions for treating MI were screened by TCMISS, which were Morindae Officinalis Radix, Cuscutae Semen, Lycii Fructus, Mori Fructus, Angelicae Sinensis Radix, Hedysarum Multijugum Maxim. Then we obtained the components and targets of CCMM in TCM prescriptions, and the MI-related targets from different databases. The

Venn diagram was utilized to find the common targets between CCMM and MI. We performed GO and KEGG enrichment pathway analyses for CCMM targets, MI-related targets, and the CCMM-MI common targets. It showed that regulating hormone, inhibiting apoptosis, oxidant stress and inflammatory are the molecular mechanism of TCM prescriptions against MI. The vital signaling pathways are Estrogen signaling pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway, and TNF signaling pathway. Finally, the molecular docking approach was conducted to verify the strong interactions between the representative components and key targets, indicating the reliability of the network pharmacology results (Figure 18).

In detail, kaempferol could protect sperm from estrogen-induced oxidative DD [51]. An in vitro study has shown that kaempferol restored motility of aluminum-exposed human sperm cells and decreased the levels of malondialdehyde (MDA) production, a lipid peroxidation marker [52]. Quercetin was confirmed to indirectly affect the stimulation of the sex organs, both at the cellular and organ levels [53], and show outstanding beneficial effects on the serum total testosterone [54]. Isorhamnetin is a kind of flavonoid and a direct metabolite of quercetin. Isorhamnetin maintained longer than quercetin in plasma [55]. It has the anti-inflammatory, and antioxidant effects [56, 57]. Beta-sitosterol is the natural occurring phytosterols having steroidal moiety, which can inhibit tumor growth, modulates immune response, and has antioxidant capacity. Beta-sitosterol is regarded as a potential chemo preventive agent for treating a variety of cancer, including prostatic carcinoma and breast cancer [58].

For the obtained signaling pathways, Estrogen signaling pathway is the most notable signaling pathway. Estrogens are involved in the pathophysiology of varicocele-associated male infertility [59]. Estrogen stimulation can directly affect the apoptosis of germ cells, and it can also change the communication between germ cells to change their apoptosis [60], which may have a profound impact on MI. The aberrant activation of PI3K-Akt signaling pathway may contribute to increase cell invasiveness and facilitate prostate cancer progression [61]. Hypoxia-Inducible Factor (HIF)-1 plays an integral role in responding to low oxygen concentrations or hypoxia in human [62]. TNF family is regarded to stimulate NF- κ B, thus implicating in varicocele-mediated pathogenesis [63].

AKT1 is considered as the moderator of cellular growth, survival, metabolism and proliferation [64]. AKT1 also suppress radiation-induced germ cell apoptosis in vivo [65] and enhance the effects of thyroid hormone on postnatal testis development [66]. The MAPKs has been linked to disturbances in spermatogenesis and dysfunction of germ cells and Sertoli cells, resulting in reduced semen quality and male reproductive dysfunction [67]. In human, MAPK3 and MAPK1 may play a crucial role in cell cycle progression and apoptosis [68]. In the capacitation process, the EGFR is partially activated by protein kinase A (PKA), resulting in phospholipase D (PLD) activation and actin polymerization [69]. In the testis, GAPDH is of particular importance for spermatogenesis, and reduced sperm motility induced by male infertility [70].

Conclusion

In summary, based on the comprehensive approach integrating data mining, network pharmacology, and molecular docking verification, we found that *Morindae Officinalis Radix*, *Cuscutae Semen*, *Lycii Fructus*, *Mori Fructus*, *Angelicae Sinensis Radix*, *Hedysarum Multijugum Maxim* are CCMM in TCM prescriptions for treating MI. The molecular mechanism of TCM prescriptions against MI is regulating hormone, inhibiting apoptosis, oxidant stress and inflammatory. The most representative signaling pathways are Estrogen signaling pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway, and TNF signaling pathway. According to the network pharmacology results, molecular docking verified the strong interactions of the representative components and key targets of CCMM in TCM prescriptions on MI. This study not only discovers CCMM and the molecular mechanism of TCM prescriptions for treating MI, but may be helpful for the popularization and application of TCM treatment.

List Of Abbreviations

Abbreviation	Full name
MI	Male infertility
TCM	Traditional Chinese Medicine
CCMM	Core Chinese Materia Medica
ART	Assisted Reproductive Technology
TCMISS	TCM Inheritance Support System
CMM	Chinese Materia medica
TCMSP	Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform
ADME	absorption, distribution, metabolism, and excretion
OB	oral bioavailability
DL	drug-likeness
CTD	Comparative Toxicogenomics Database
OMIM	Online Mendelian Inheritance in Man
DAVID	Database for Annotation Visualization and Integrated Discovery
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
PPI	protein-protein information
DC	degree centrality
BC	betweenness centrality
CC	closeness centrality
PDB	Protein Data Bank
CYP19A1	Aromatase
AR	Androgen receptor
ESR2	Estrogen receptor beta
ESR1	Estrogen receptor
ACHE	Acetylcholinesterase
HMGCR	3-hydroxy-3-methylglutaryl-coenzyme A reductase
SHBG	Sex hormone-binding globulin
CYP17A1	Steroid 17-alpha-hydroxylase/17,20 lyase

BCHE	Cholinesterase
PPARA	Peroxisome proliferator-activated receptor alpha
NR1I3	Nuclear receptor subfamily 1 group I member 3
SQLE	Squalene monooxygenase
PPARG	Peroxisome proliferator-activated receptor gamma
NOS2	Nitric oxide synthase, inducible
NR3C1	Glucocorticoid receptor
GAPDH	dehydrogenase
AKT1	RAC-alpha serine/threonine-protein kinase
MAPK3	Mitogen-activated protein kinase 3
EGFR	Epidermal growth factor receptor
TNF	Tumor necrosis factor
MAPK1	Mitogen-activated protein kinase 1
HSP90AA1	Heat shock protein HSP 90-alpha

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

XB and ZQL proposed the conception; XB, GML, DL, and XLF designed and investigated the study analysis; XB wrote the manuscript; YBT, QL, ZJL, SJY, TT, SYW, LRL, and KLZ contributed to supervising the manuscript; YFZ and ZQL funded the study. All authors read and approved the final manuscript.

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References

1. Agarwal A, Parekh N, Panner Selvam MK, Henkel R, Shah R, Homa ST, Ramasamy R, Ko E, Tremellen K, Esteves S et al: Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. *World J Mens Health* 2019, 37(3):296-312.
2. Okada H, Tajima A, Shichiri K, Tanaka A, Tanaka K, Inoue I: Genome-wide expression of azoospermia testes demonstrates a specific profile and implicates ART3 in genetic susceptibility. *PLoS Genet* 2008, 4(2):e26.
3. Huynh T, Mollard R, Trounson A: Selected genetic factors associated with male infertility. *Hum Reprod Update* 2002, 8(2):183-198.
4. Machen GL, Sandlow JL: Causes of Male Infertility. In: *Male Infertility*. Springer; 2020: 3-14.
5. Kiyozumi D, Noda T, Yamaguchi R, Tobita T, Matsumura T, Shimada K, Kodani M, Kohda T, Fujihara Y, Ozawa M: NELL2-mediated lumicrine signaling through OVCH2 is required for male fertility. *Science* 2020, 368(6495):1132-1135.
6. Lord T, Oatley JM: Testicular-borne factors affect sperm fertility. *Science* 2020, 368(6495):1053-1054.
7. Qin J, Sheng X, Wang H, Liang D, Tan H, Xia J: Assisted reproductive technology and risk of congenital malformations: a meta-analysis based on cohort studies. *Arch Gynecol Obstet* 2015, 292(4):777-798.
8. Rhoton-Vlasak A, Kramer JM, Plasencia E: Assisted Reproductive Technology and Its Impact on Male Infertility Management. In: *Male Infertility*. Springer; 2020: 309-320.
9. Romagnoli S, Peris A, De Gaudio AR, Geppetti P: SARS-CoV-2 and COVID-19: From the Bench to the Bedside. *Physiological reviews* 2020, 100(4):1455-1466.
10. Singh B, Reschke L, Segars J, Baker VL: Frozen-thawed embryo transfer: the potential importance of the corpus luteum in preventing obstetrical complications. *Fertility and sterility* 2020, 113(2):252-257.
11. Illiano E, Trama F, Costantini E: Could COVID-19 have an impact on male fertility? *Andrologia* 2020:e13654.

12. Wang S, Zhou X, Zhang T, Wang Z: The need for urogenital tract monitoring in COVID-19. *Nature Reviews Urology* 2020:1-2.
13. Segars J, Katler Q, McQueen DB, Kotlyar A, Glenn T, Knight Z, Feinberg EC, Taylor HS, Toner JP, Kawwass JF: Prior and novel coronaviruses, COVID-19, and human reproduction: what is known? *Fertility and Sterility* 2020.
14. Xu J, Qi L, Chi X, Yang J, Wei X, Gong E, Peh S, Gu J: Orchitis: a complication of severe acute respiratory syndrome (SARS). *Biol Reprod* 2006, 74(2):410-416.
15. Youssef K, Abdelhak K: Male genital damage in COVID-19 patients: Are available data relevant? *Asian Journal of Urology* 2020.
16. Aitken RJ: COVID-19 and human spermatozoa—potential risks for infertility and sexual transmission. *Andrology* 2020:1-5.
17. Zhou SH, Deng YF, Weng ZW, Weng HW, Liu ZD: Traditional Chinese medicine as a remedy for male infertility: a review. *The world journal of men's health* 2019, 37(2):175-185.
18. Li S, Zhang Z, Wu L, Zhang X, Li Y, Wang Y: Understanding ZHENG in traditional Chinese medicine in the context of neuro-endocrine-immune network. *IET systems biology* 2007, 1(1):51-60.
19. Li H, Zhao L, Zhang B, Jiang Y, Wang X, Guo Y, Liu H, Li S, Tong X: A network pharmacology approach to determine active compounds and action mechanisms of ge-gen-qin-lian decoction for treatment of type 2 diabetes. *Evidence-based complementary and alternative medicine* 2014, 2014.
20. Hopkins AL: Network pharmacology: the next paradigm in drug discovery. *Nature chemical biology* 2008, 4(11):682.
21. Shao L, ZHANG B: Traditional Chinese medicine network pharmacology: theory, methodology and application. *Chinese journal of natural medicines* 2013, 11(2):110-120.
22. Ping QX, Zhi DH, Jing XJ, Ying HX, Feng GX, Yao W, Qi ZY, Wei JJ, Gong LL, Tao YS: Novel quinoline compound derivatives of NSC23925 as potent reversal agents against P-glycoprotein-mediated multidrug resistance. *Frontiers in chemistry* 2019, 7:820.
23. Zhang D, Zhang Y, Gao Y, Chai X, Pi R, Chan G, Hu Y: Translating traditional herbal formulas into modern drugs: a network-based analysis of Xiaoyao decoction. *Chinese medicine* 2020, 15(1):1-11.
24. Wu Z, Yang L, He L, Wang L, Peng L: Systematic Elucidation of the Potential Mechanisms of Core Chinese Materia Medicas in Treating Liver Cancer Based on Network Pharmacology. *Evidence-Based Complementary and Alternative Medicine* 2020, 2020:1-13.
25. Wu D, Zhang X, Liu L, Guo Y: Key CMM combinations in prescriptions for treating mastitis and working mechanism analysis based on network pharmacology. *Evidence-Based Complementary and Alternative Medicine* 2019, 2019:1-11.
26. 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.
27. Huang L, Xie D, Yu Y, Liu H, Shi Y, Shi T, Wen C: TCMID 2.0: a comprehensive resource for TCM. *Nucleic Acids Res* 2018, 46(D1):D1117-D1120.

28. Xu X, Zhang W, Huang C, Li Y, Yu H, Wang Y, Duan J, Ling Y: A novel chemometric method for the prediction of human oral bioavailability. *Int J Mol Sci* 2012, 13(6):6964-6982.
29. Walters WP, Murcko MA: Prediction of 'drug-likeness'. *Adv Drug Deliv Rev* 2002, 54(3):255-271.
30. Feng W, Ao H, Yue S, Peng C: Systems pharmacology reveals the unique mechanism features of Shenzhu Capsule for treatment of ulcerative colitis in comparison with synthetic drugs. *Sci Rep* 2018, 8(1):16160.
31. Bardou P, Mariette J, Escudié F, Djemiel C, Klopp C: jvenn: an interactive Venn diagram viewer. *BMC bioinformatics* 2014, 15(1):1-7.
32. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B et al: PubChem 2019 update: improved access to chemical data. *Nucleic Acids Res* 2019, 47(D1):D1102-D1109.
33. Tetko IV, Poda GI: Application of ALOGPS 2.1 to predict log D distribution coefficient for Pfizer proprietary compounds. *Journal of medicinal chemistry* 2004, 47(23):5601-5604.
34. Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V: SwissTargetPrediction: a web server for target prediction of bioactive small molecules. *Nucleic Acids Res* 2014, 42(Web Server issue):W32-38.
35. UniProt C: UniProt: a hub for protein information. *Nucleic Acids Res* 2015, 43(Database issue):D204-212.
36. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T: Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003, 13(11):2498-2504.
37. Assenov Y, Ramirez F, Schelhorn SE, Lengauer T, Albrecht M: Computing topological parameters of biological networks. *Bioinformatics* 2008, 24(2):282-284.
38. Pinero J, Bravo A, Queralt-Rosinach N, Gutierrez-Sacristan A, Deu-Pons J, Centeno E, Garcia-Garcia J, Sanz F, Furlong LI: DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Res* 2017, 45(D1):D833-D839.
39. Davis AP, Grondin CJ, Johnson RJ, Sciaky D, McMorran R, Wiegiers J, Wiegiers TC, Mattingly CJ: The Comparative Toxicogenomics Database: update 2019. *Nucleic Acids Res* 2019, 47(D1):D948-D954.
40. 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.
41. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, Stein TI, Nudel R, Lieder I, Mazor Y et al: The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. *Curr Protoc Bioinformatics* 2016, 54:1 30 31-31 30 33.
42. Sherman BT, Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature protocols* 2009, 4(1):44-57.
43. Chen H, Wang N, Zhao X, Ross CA, O'shea KS, McInnis MG: Gene expression alterations in bipolar disorder postmortem brains. *Bipolar disorders* 2013, 15(2):177-187.

44. Maere S, Heymans K, Kuiper M: BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics* 2005, 21(16):3448-3449.
45. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman WH, Pages F, Trajanoski Z, Galon J: ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 2009, 25(8):1091-1093.
46. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP et al: STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res* 2015, 43(Database issue):D447-452.
47. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE: The Protein Data Bank. *Nucleic Acids Res* 2000, 28(1):235-242.
48. Yuan S, Chan HCS, Filipek S, Vogel H: PyMOL and Inkscape Bridge the Data and the Data Visualization. *Structure* 2016, 24(12):2041-2042.
49. Trott O, Olson AJ: AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 2010, 31(2):455-461.
50. Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, Schlegel PN, Howards SS, Nehra A, Damewood MD et al: Best practice policies for male infertility. *Fertil Steril* 2002, 77(5):873-882.
51. Anderson D, Schmid TE, Baumgartner A, Cemeli-Carratala E, Brinkworth MH, Wood JM: Oestrogenic compounds and oxidative stress (in human sperm and lymphocytes in the Comet assay). *Mutation Research/Reviews in Mutation Research* 2003, 544(2-3):173-178.
52. Jamalan M, Ghaffari MA, Hoseinzadeh P, Hashemitabar M, Zeinali M: Human sperm quality and metal toxicants: protective effects of some flavonoids on male reproductive function. *International journal of fertility & sterility* 2016, 10(2):215.
53. Taepongsorat L, Tangpraputgul P, Kitana N, Malaivijitnond S: Stimulating effects of quercetin on sperm quality and reproductive organs in adult male rats. *Asian journal of andrology* 2008, 10(2):249-258.
54. Khaki A, Fathiazad F, Nouri M, Khaki A, Maleki NA, Khamnei HJ, Ahmadi P: Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. *Phytotherapy Research* 2010, 24(9):1285-1291.
55. Lee H-J, Lee H-J, Lee E-O, Ko S-G, Bae H-S, Kim C-H, Ahn K-S, Lu J, Kim S-H: Mitochondria-cytochrome C-caspase-9 cascade mediates isorhamnetin-induced apoptosis. *Cancer letters* 2008, 270(2):342-353.
56. Boesch-Saadatmandi C, Loboda A, Wagner AE, Stachurska A, Jozkowicz A, Dulak J, Döring F, Wolfram S, Rimbach G: Effect of quercetin and its metabolites isorhamnetin and quercetin-3-glucuronide on inflammatory gene expression: role of miR-155. *The Journal of nutritional biochemistry* 2011, 22(3):293-299.
57. Dong G-Z, Lee J-H, Ki SH, Yang JH, Cho IJ, Kang SH, Zhao RJ, Kim SC, Kim YW: AMPK activation by isorhamnetin protects hepatocytes against oxidative stress and mitochondrial dysfunction. *European Journal of Pharmacology* 2014, 740:634-640.

58. Patel K, Kumar V, Verma A, Rahman M, Patel DK: β -sitosterol: bioactive compounds in foods, their role in health promotion and disease prevention “a concise report of its phytopharmaceutical importance”. *Current Traditional Medicine* 2017, 3(3):168-177.
59. Guido C, Perrotta I, Panza S, Middea E, Avena P, Santoro M, Marsico S, Imbrogno P, Andò S, Aquila S: Human sperm physiology: Estrogen receptor alpha (ER α) and estrogen receptor beta (ER β) influence sperm metabolism and may be involved in the pathophysiology of varicocele-associated male infertility. *Journal of cellular physiology* 2011, 226(12):3403-3412.
60. Alves MRC: Estrogens regulate the survival and death communication between Sertoli and germ cells: a clue for male infertility? : Universidade da Beira Interior; 2013.
61. Shukla S, MacLennan GT, Hartman DJ, Fu P, Resnick MI, Gupta S: Activation of PI3K-Akt signaling pathway promotes prostate cancer cell invasion. *International journal of cancer* 2007, 121(7):1424-1432.
62. Velickovic LJ, Stefanovic V: Hypoxia and spermatogenesis. *International urology and nephrology* 2014, 46(5):887-894.
63. Celik O, Kutlu O, Tekcan M, Celik-Ozenci C, Koksall IT: Role of TNF-related apoptosis-inducing ligand (TRAIL) in the pathogenesis of varicocele-induced testicular dysfunction. *Asian journal of andrology* 2013, 15(2):269.
64. Poplinski A, Tüttelmann F, Kanber D, Horsthemke B, Gromoll J: Idiopathic male infertility is strongly associated with aberrant methylation of MEST and IGF2/H19 ICR1. *International journal of andrology* 2010, 33(4):642-649.
65. Rasoulpour T, DiPalma K, Kolvek B, Hixon M: Akt1 suppresses radiation-induced germ cell apoptosis in vivo. *Endocrinology* 2006, 147(9):4213-4221.
66. Santos-Ahmed J, Brown C, Smith SD, Weston P, Rasoulpour T, Gilbert ME, Hixon ML: Akt1 protects against germ cell apoptosis in the postnatal mouse testis following lactational exposure to 6-N-propylthiouracil. *Reproductive Toxicology* 2011, 31(1):17-25.
67. Li MW, Mruk DD, Cheng CY: Mitogen-activated protein kinases in male reproductive function. *Trends in molecular medicine* 2009, 15(4):159-168.
68. Cocchia N, Pasolini M, Mancini R, Petrazzuolo O, Cristofaro I, Rosapane I, Sica A, Tortora G, Lorzio R, Paraggio G: Effect of sod (superoxide dismutase) protein supplementation in semen extenders on motility, viability, acrosome status and ERK (extracellular signal-regulated kinase) protein phosphorylation of chilled stallion spermatozoa. *Theriogenology* 2011, 75(7):1201-1210.
69. Breitbart H, Etkovitz N: Role and regulation of EGFR in actin remodeling in sperm capacitation and the acrosome reaction. *Asian journal of andrology* 2011, 13(1):106-110.
70. Gunnarsson D, Nordberg G, Selstam G: Differential effects of cadmium on the gene expression of seven-transmembrane-spanning receptors and GAPDH in the rat testis. *Toxicology letters* 2007, 168(1):51-57.

Figures

Figure 1

CCMM network.

Figure 2

CCMM component-target network. Purple nodes stand for herbs of CCMM. Pink nodes represent bioactive components from each herb. Yellow nodes indicate bioactive components that appear more than once from different herbs. Blue nodes stand for targets.

Figure 3

GO and KEGG enrichment analyses of the CCMM targets ($p\text{-value} \leq 0.05$). (A) The top 20 GO enrichment terms, including biological process, cellular component, and molecular function. The bar plot represents the GeneRatio (%) of the top 20 significant enrichments. (B) The top 20 KEGG pathways. The color scales indicate the different thresholds for the p-values, and the sizes of the dots represent the number of targets corresponding to each term.

Figure 4

GO and KEGG enrichment analyses of the MI-related targets ($p\text{-value} \leq 0.05$). (A) Venn diagram: the number of MI-related targets from the four different databases are 225, 204, 197, and 181. (B) The top 20 KEGG pathways. (C) The top 20 GO enrichment terms, including biological process, cellular component, and molecular function. The bar plot represents the GeneRatio (%) of the top 20 significant enrichments.

Figure 5

Common targets of CCMM and MI. Intersection of Venn diagram: 127 targets are common to CCMM and MI.

Figure 6

CCMM-MI common-target network. Purple nodes stand for herbs of CCMM. Pink nodes represent bioactive components from each herb. Yellow nodes indicate bioactive components that appear more than once from different herbs. Blue nodes stand for the common targets of CCMM and MI. The size of the circle represents the node degree of the target protein.

Figure 7

GO and KEGG pathway enrichment analyses of the CCMM-MI common targets ($p\text{-value} \leq 0.05$). (A) The top 20 biological processes. (B) Nineteen cellular components. (C) The top 20 molecular functions. (D) The top 20 KEGG pathways. The color scales indicate the different thresholds for the p-values, and the sizes of the dots represent the number of targets corresponding to each term.

Figure 8

The PPI network. (A) The PPI network of CCMM and MI. (B) The PPI network by the screening criteria of $DC \geq 41$. (C) The PPI network by the screening criteria of $DC \geq 60$, $BC \geq 0.025$, and $CC \geq 0.651$. The size and color of the node represent the degree of the target protein. The width and color of the edge represent the combined score of the target protein.

Figure 9

GO and KEGG pathway enrichment analyses of the key targets ($p\text{-value} \leq 0.05$). (A) GO enrichment of the key targets using BiNGO, including biological process, cellular component, and molecular function. (B)

KEGG pathway enrichment of the key targets using ClueGO.

Figure 10

Estrogen signaling pathway. The green rectangle represents the targets related to the CCMM-MI common-target network. The red rectangle represents the key targets of the PPI network.

Figure 11

PI3K-Akt signaling pathway. The green rectangle represents the targets related to the CCMM-MI common-target network. The red rectangle represents the key targets of the PPI network.

Figure 12

HIF-1 signaling pathway. The green rectangle represents the targets related to the CCMM-MI common-target network. The red rectangle represents the key targets of the PPI network.

Figure 13

TNF signaling pathway. The green rectangle represents the targets related to the CCMM-MI common-target network. The red rectangle represents the key targets of the PPI network.

Figure 14

Molecular models of the binding of kaempferol from CCMM to the predicted targets (A, B) AKT1, (C, D) MAPK3, (E, F) MAPK1, (G, H) EGFR, (I, J) GAPDH, and (K, L) TNF shown as 3D diagrams and 2D diagrams.

Figure 15

Molecular models of the binding of quercetin from CCMM to the predicted targets (A, B) AKT1, (C, D) MAPK3, (E, F) MAPK1, (G, H) EGFR, (I, J) GAPDH, and (K, L) TNF shown as 3D diagrams and 2D diagrams.

Figure 16

Molecular models of the binding of isorhamnetin from CCMM to the predicted targets (A, B) AKT1, (C, D) MAPK3, (E, F) MAPK1, (G, H) EGFR, (I, J) GAPDH, and (K, L) TNF shown as 3D diagrams and 2D diagrams.

Figure 17

Molecular models of the binding of beta-sitosterol from CCMM to the predicted targets (A, B) AKT1, (C, D) MAPK3, (E, F) MAPK1, (G, H) EGFR, (I, J) GAPDH, and (K, L) TNF shown as 3D diagrams and 2D diagrams.

Figure 18

The experimental flow of this study. TCM, Traditional Chinese Medicine; MI, male infertility; TCMISS, TCM Inheritance Support System; CCMM, Core Chinese Materia Medica; PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

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