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Original Article

Exploration of the mechanism of tetramethoxyflavone in treating osteoarthritis based on network pharmacology and molecular docking

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Abstract

Objectives: This study aimed to explore the potential mechanisms of TMF (5,7,3',4'-tetramethoxyflavone) in treating osteoarthritis (OA) using network pharmacology and molecular docking. **Materials and Methods:** Databases including SwissTargetPrediction, BATMAN-TCM, PharmMapper, TargetNet, SuperPred, and SEA were utilized to screen the targets of TMF. "OA" was used as the disease keyword to predict OA-related genes through GeneCards, Therapeutic Target Database, PharmGKB, Online Mendelian Inheritance in Man, and Comparative Toxicogenomics Database. The Venn diagram was employed to identify the intersection of predicted targets between TMF and OA as potential targets for TMF in treating OA. The intersection targets were input into the STRING 12.0 online database to construct a protein–protein interaction (PPI) network and identify core targets. Subsequently, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the Metascape V3.5 online database platform. Finally, molecular docking between TMF and core targets was conducted using AutoDockTools 1.5.6. **Results:** A total of 228 intersection targets for TMF treating OA were obtained, and PPI network analysis identified 5 core targets: STAT3, SRC, CTNNB1, EGFR, and AKT1. GO enrichment analysis yielded 2736 results, while KEGG analysis identified 203 pathways. Most elated GO and KEGG items of TMF in treating OA may include hormonal responses, antiviral and anticancer effects, anti-inflammation, phosphorus metabolism, phosphate metabolism, nitrogen compound responses, cancer-related pathways, PI3K-Akt signaling pathway, and MAPK signaling pathway. Molecular docking revealed good binding affinities between TMF and all core targets except STAT3. **Conclusion:** TMF might act on multiple targets and activate diverse pathways to intervene in OA, revealing the molecular processes involved in TMF treatment of OA.

Keywords: *5,7,3',4'-tetramethoxyflavone, Molecular docking, Network pharmacology, Osteoarthritis*

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Introduction

Murraya exotica L. belongs to the Rutaceae family and is widely used for rheumatoid arthritis, anti-inflammatory and antimicrobial purposes, pain relief, reduction of body swelling, and anticancer and antidiarrheal effects [1,2]. 5,7,3',4'-tetramethoxyflavone (TMF), as a flavonoid compound, is one of the major components found in the leaves of *Murraya exotica L.* [3]. TMF's anti-inflammatory and analgesic effects stem from its ability to inhibit endoplasmic reticulum stress-induced chondrocyte apoptosis, significantly downregulating the production of nitric oxide (NO) and interleukin-6 (IL-6) [4-6]. With widespread cultivation, good quality, high yield, and numerous pharmacological properties and clinical applications, TMF presents a promising candidate for drug development.

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by the destruction of joint cartilage, often leading to joint pain and functional impairment. Patients commonly experience clinical symptoms such as joint stiffness, swelling, deformity, and restricted movement [7]. Surveys indicate a 46.3% incidence rate of OA among the middle-aged and elderly population in China, with knee OA prevalence reaching as high as

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15.6% [8]. However, to date, the treatment outcomes for OA have not been notably effective. According to the 2022 Annual Review of OA, issues persist regarding the need for enhanced evidence in OA treatment, the lack of efficacy of most intra-articular or acupuncture therapies, the limited benefits of oral medications, and the absence of a particularly superior treatment [9]. Thus, research on OA holds crucial significance for human well-being.

Studies have shown significant effectiveness of TMF in treating OA [10,11]. However, the pathogenesis of OA remains unclear, and there is still a lack of effective drugs and methods to alleviate pain in the middle-aged and elderly populations, thereby reducing the occurrence of disabilities. Hence, investigating the mechanism of action for OA becomes particularly important. Network pharmacology, integrating pharmacology, systems biology, and computational analysis techniques, elucidates the connections between ingredients and disease targets. By analyzing targets related to the components and diseases, conducting enrichment analysis, and identifying pathways and biological processes (BPs) for traditional Chinese medicine in treating diseases, network pharmacology aids in understanding the mechanism of action for traditional medicine [12]. As shown in Figure 1, this study employs network pharmacology combined with molecular docking to explore the mechanism of TMF in treating OA, providing a theoretical foundation for subsequent basic research.

Materials and Methods

Acquisition of target points for TMF

Retrieve the canonical SMILES, two-dimensional structure, and three-dimensional (3D) structure of TMF from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Use "5,7,3',4'-tetramethoxyflavone" as a search term or input TMF's SMILES to obtain TMF's target points from the following databases: (1) SwissTargetPrediction (http://swisstargetprediction.ch/), (2) Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM, http://bionet.ncpsb.org/batman-tcm/), (3) PharmMapper (http://lilab-ecust.cn/pharmmapper/index.html), (4) TargetNet (http://targetnet.scbdd.com/), (5) SuperPred (https://prediction.charite.de/index.php), and (6) Similarity Ensemble Approach (SEA, https://sea.bkslab.org/). For standardized final target selection, utilize the UniProt protein database (https://www.uniprot.org), limit to "Human," convert the target points into standardized targets, and merge and deduplicate all standardized protein targets to obtain the final target points for TMF.

Retrieval of osteoarthritis-related targets

Using "OA" as the disease search term, OA-related targets were obtained from the following databases: (1) GeneCards (https://www.genecards.org/), (2) Therapeutic Target Database (TTD, https://db.idrblab.net/ttd/), (3) PharmGKB (https://www.pharmgkb.org/), (4) Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/), and (5) Comparative Toxicogenomics Database (CTD, https://ctdbase.org/). Targets with relevance scores >10 in GeneCards and CTD databases were selected. All targets from the above databases were collated, standardized via the UniProt protein database, and merged to remove duplicates, resulting in the final action targets for OA. Simultaneously, in the Gene Expression Omnibus (GEO, https://www.ncbi.

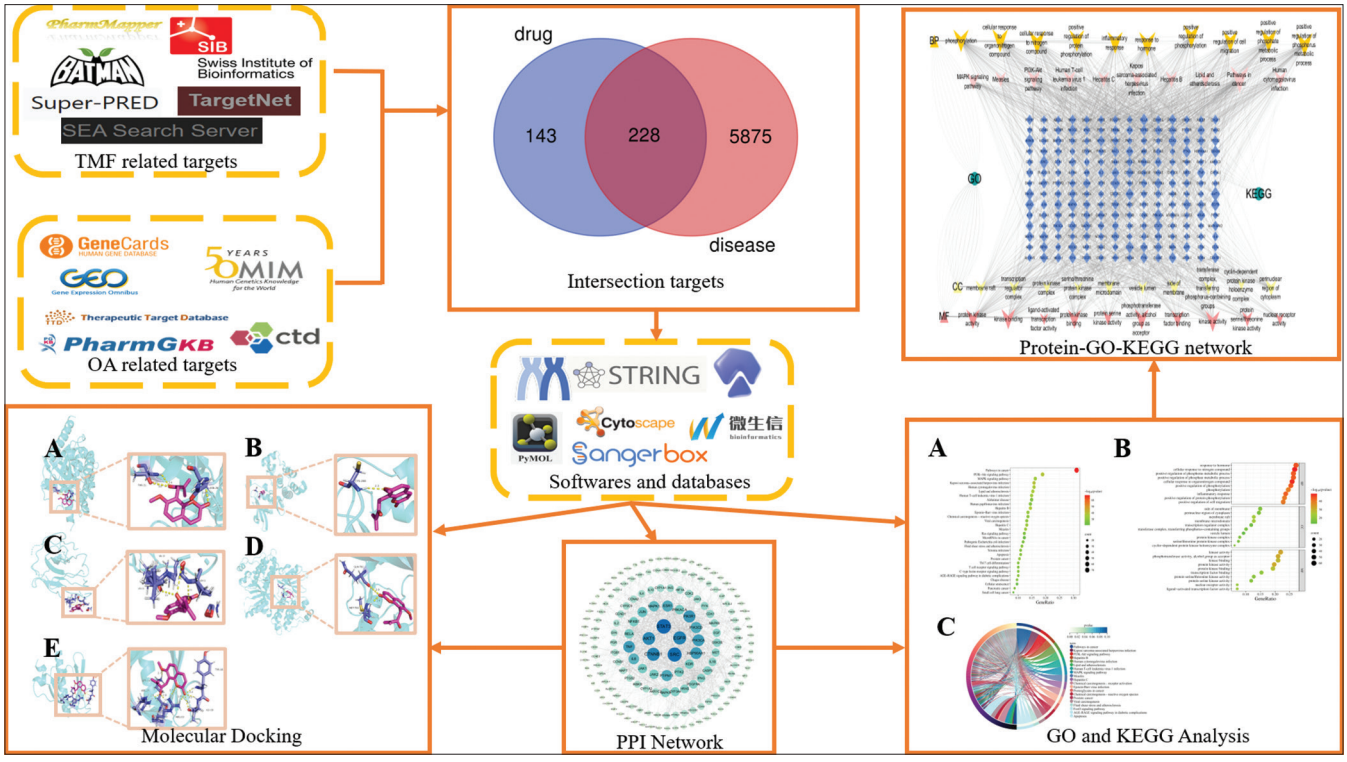

Figure 1: The flowchart of the entire investigation. OA: Osteoarthritis, GO: Gene Ontology, KEGG: Kyoto Encyclopedia of Genes and Genomes. PPI: Protein–protein interaction

nlm.nih.gov/gds/) database, under "Expression profiling by array," OA-related targets were obtained using the GEO2R algorithm. The selection criteria were $P < 0.05$ and $|\log2FC|>2$ for targets, followed by generating a volcano plot using the bioinformatics platform (http://www.bioinformatics.com.cn/).

Intersection targets of TMF and osteoarthritis

Through the Draw Venn Diagram online platform (http://bioinformatics.psb.ugent.be/webtools/venn/), intersection targets between TMF and OA were obtained.

Construction of protein–protein interaction (PPI) Network and Selection of Core Targets

The STRING12.0 online database (https://cn.string-db.org/) was used to construct the PPI network. "Multiple Proteins" were selected, and the intersection targets were inputted. Under "Organism," "*Homo sapiens*" was chosen, and the "minimum required interaction score" was set to > highest confidence (0.900). Subsequently, Cytoscape 3.7.1 software (https://cytoscape.org/) was utilized for visual analysis of the PPI network. NetworkAnalyzer in Cytoscape 3.7.1 was employed to calculate the degree values in the PPI network, considering targets with values higher than the central value as core targets.

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analysis

In the Metascape V3.5 database platform (https://www.metascape.org), the intersected gene targets were inputted as species "*H. sapiens*." Custom Analysis was performed for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, Gene Ontology (GO) molecular function (MF), GO cellular component (CC), and GO BP. The top 30 ranked GO terms and KEGG pathways with a threshold of $P < 0.05$ were selected and visualized through bubble plots using the bioinformatics online platform. KEGG pathway analysis results were organized, and a KEGG bubble diagram was generated swiftly using the enrichment analysis circle plot tool in the SangerBox biomedical data analysis toolbox (http://sangerbox.com/tool.html).

Construction of protein-GO-KEGG network

Cytoscape 3.7.1 software was utilized to construct the protein-GO-KEGG network. The top 10 entries of KEGG and GO with the most significant P values were selected by Metascape, and their related proteins were collected into Excel files. These files were imported into Cytoscape 3.7.1 for visualization and adjustment to represent the results effectively.

Molecular docking

Molecular docking enables a more visual understanding of the binding patterns and interactions between protein macromolecules and small-molecule compounds. TMF underwent molecular docking with the top five ranked core targets. OpenBabel 2.4.1 (https://openbabel.org/) software was used to convert the 3D structures downloaded from the PubChem database in SDF format to mol2 format for further use. Spatial structures of target proteins were obtained from the PDB database (https://www.rcsb.org/), selecting "*Homo sapiens*" as the species, aiming for lower resolution and more recent structures whenever possible. In addition, structures with longer chains and lower resolution from the UniProt database's "Structure" section were selected. PyMOL software (https://pymol.org/2/) was employed to remove all water molecules and original ligands from the macromolecular spatial structures, saving them in PDB format. Furthermore, AutoDockTools (Vina 1.5.6, http://autodock.scripps.edu/) was used to convert the small-molecule ligands and processed target protein receptors into PDBQT file format. GridBox enclosed the protein macromolecules, defining the docking area, and the "Genetic Algorithm" was selected for the subsequent molecular docking. Finally, PyMOL software was utilized again for visual analysis of the docking results.

Results

Potential action targets of TMF in treating osteoarthritis

All protein targets of TMF were sourced from six open-source databases: Swiss, BATMAN-TCM, PharmMapper, TargetNet, SuperPred, and SEA. After eliminating duplicate targets, a total of 371 relevant TMF targets were obtained, as shown in Figure 2a. OA-related targets were derived from five open-source databases: CTD, TTD, OMIM, GeneCards, and PharmGKB, with quantities of 6544, 33, 30, 47, and 4, respectively. After removing duplicate targets, a total of 6103 targets were obtained, as illustrated in Figure 2b. From the GEO database, a total of 14738 OA-related targets were acquired, consisting of 1366 upregulated genes and 1497 downregulated genes, visualized in the form of a volcano plot, as depicted in Figure 2c. Ultimately, 228 intersection targets between TMF and OA were collected, as illustrated in Figure 2d.

Construction of protein–protein interaction and selection of core targets

The intersection targets of TMF and OA were inputted into the STRING online platform with an interaction score set at 0.9 and free nodes hidden. This process resulted in a PPI network graph displaying 228 nodes and 619 edges, indicating the interactions among protein targets. Larger and darker nodes in the PPI network represent more critical protein targets. In Figure 3, the top five targets based on degree values are STAT3 (degree value = 31), SRC (degree value = 30), CTNNB1 (degree value = 27), EGFR (degree value = 27), and AKT1 (degree value = 26). Subsequently, STAT3, SRC, CTNNB1, EGFR, and AKT1 were identified as pivotal targets for TMF in treating OA and were subjected to molecular docking with TMF.

Gene Ontology enrichment analysis and Kyoto Encyclopedia of Genes and Genomes pathway analysis

The intersection targets underwent GO analysis using the Metascape V3.5 database platform, resulting in three sets: BP, MF, and CC. Upon filtering the results with $P < 0.05$, 2386, 225, and 125 results were respectively obtained, totaling 2736 GO analysis results. The top 10 entries from each category were visualized on the bioinformatics platform, forming 30 entries, as depicted in Figure 4a. The top 10 of BP were hormone response, cellular response to nitrogen compounds, positive regulation of phosphate metabolic process, positive

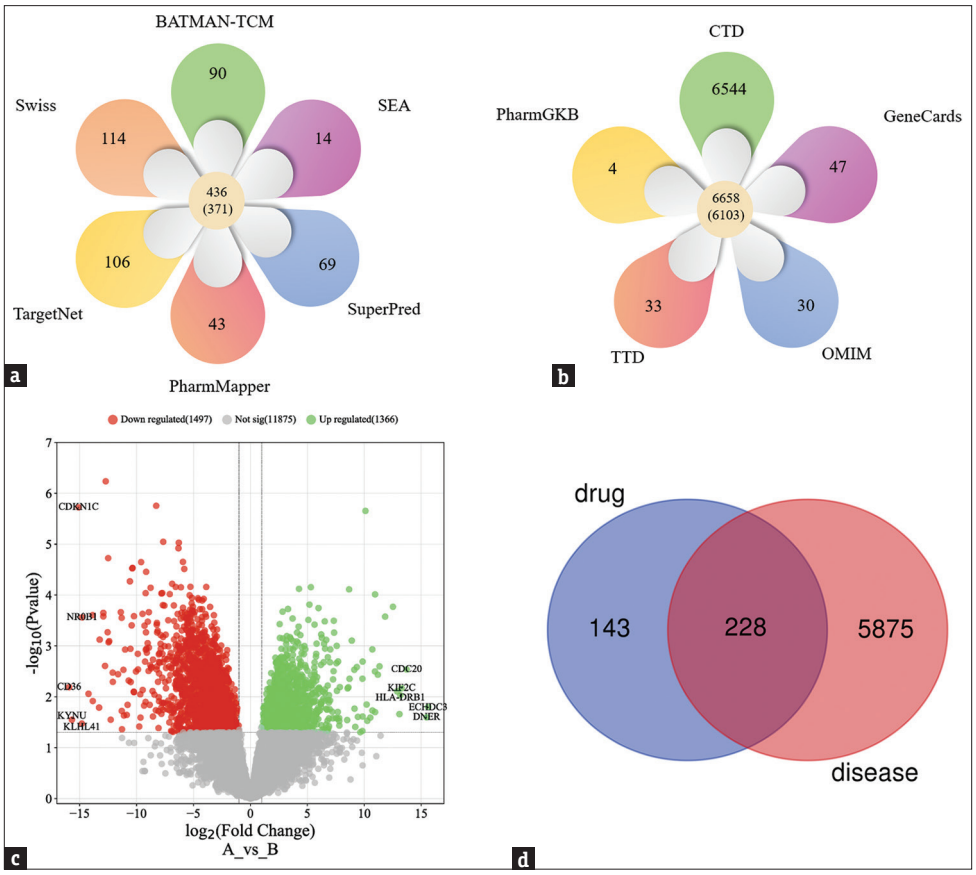

Figure 2: Targets of TMF or/and osteoarthritis (OA). (a) TMF targets in six open-source databases; (b) Targets of OA in five open-source databases; (c) Volcano diagram of targets of OA; (d) The intersection of TMF and OA

regulation of phosphate-containing compound metabolic process, cellular response to organic nitrogen compounds, positive regulation of phosphorylation, phosphorylation, inflammatory response, positive regulation of protein phosphorylation, and positive regulation of cell migration. The top 10 of CC were lipid raft, membrane microdomain, cyclin-dependent protein kinase holoenzyme complex, lateral side of membrane, protein kinase complex, serine/threonine protein kinase complex, transferase complex, perinuclear region of cytoplasm, transcription regulation complex, and vesicle lumen. The top 10 of MF were kinase activity, protein kinase activity, transferase activity-alcohol groupas acceptor, kinase binding, protein kinase binding, nuclearreceptor activity, ligand-activated transcription factor activity, transcription factor binding, protein serine/threonine kinaseactivity, and protein serine kinase activity.

In addition, KEGG pathway analysis was also performed on the intersection targets using Metascape V 3.5, resulting in 203 outcomes after filtering ($P < 0.05$). The top 30 entries were visualized on the bioinformatics platform, and the results are shown in Figure 4b, revealing pathways such as cancer pathways, PI3K-Akt signaling pathway, MAPK signaling pathway, human cytomegalovirus infection, lipid and atherosclerosis, human T-cell leukemia virus 1 infection, Alzheimer's disease, hepatitis B, human papillomavirus infection, Kaposi sarcoma-associated herpesvirus infection, EB virus infection, and chemical carcinogenesis-reactive oxygen species. This indicates that TMF's anti-inflammatory, anticancer, and antiviral properties are important targets or pathways in treating OA. The top 20 pathways based on *P* values were further used to generate a KEGG circle diagram in the SangerBox biomedical data analysis tool, providing a more intuitive view of pathway proportions and their association with multiple genes in Figure 4c.

Construction of protein GO KEGG network

As depicted in Figure 5, the protein-GO-KEGG network graph involves a total of 241 nodes and 1631 edges. Among them, the intersection genes between GO and KEGG pathways amount to 196, represented by diamond shapes. V-shaped nodes indicate KEGG signaling pathways, and triangular nodes signify GO enrichment results, encompassing BP, CC, and MF. Connections between nodes represent a certain level of association, where larger nodes denote stronger correlations. This graph illustrates that in treating OA, TMF acts on multiple targets, resulting in various biological effects and multiple mechanisms. The top 10 proteins based on degree value among the intersection genes are AKT1, MAPK1, IKBKB, PIK3CA, JAK2, SRC, CCND1, EGFR, PRKACA, and GSK3B. Notably, AKT1, SRC, and EGFR align with the core targets previously identified in the PPI network, suggesting that these proteins merit focused attention for further investigation.

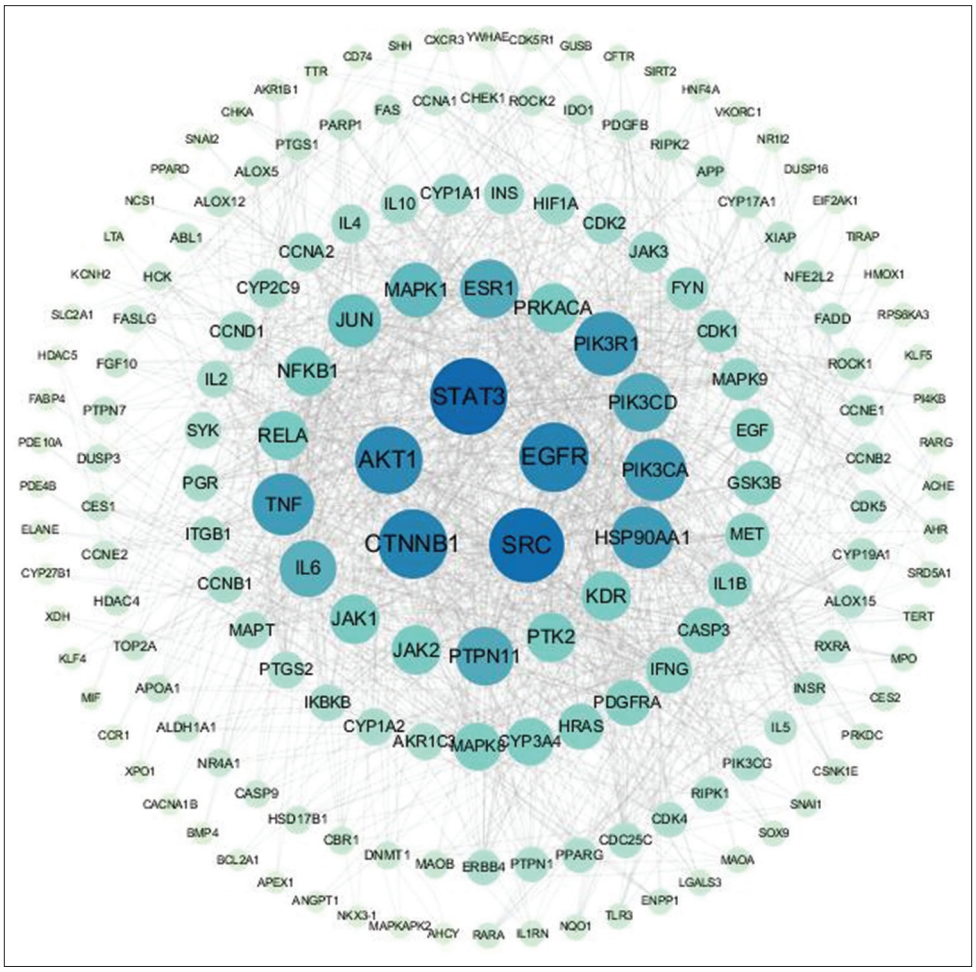

Figure 3: Protein–protein interaction network graph of intersection genes

Molecular docking

Lower binding energies are generally considered indicative of better binding activity between large and small molecules. Binding energies below −4.25 kcal/moL suggest a certain level of affinity between the large and small molecules, while those below −5 kcal/moL indicate relatively stronger affinity [13,14]. We performed molecular docking between TMF and the top five high-degree targets (STAT3, SRC, CTNNB1, EGFR, and AKT1) in the protein interaction network. Most targets exhibited notably strong binding energies: −4.9 kcal/mol for STAT3, −5.18 kcal/moL for SRC, −7.24 kcal/moL for CTNNB1, −5.13 kcal/moL for EGFR, and − 5.13 kcal/moL for AKT1, as illustrated in Figure 6. Specific amino acid residues of these proteins, such as THR 31and ASN 26 of STAT3, CYS 280 of SRC, LUE 11, VAL 12 and GLN 13 of CTNNB1, GLN 791 and MET 793 of EGFR, and ARG 25, LYS 14, ARG 23, ILE 19 and TYR 18 of AKT1, formed tight hydrogen bonds with TMF.

Discussion

OA is a joint-related disease initially characterized by metabolic disturbances in joint tissues, leading to molecular disruptions. This eventually results in physiological disruptions marked by features such as cartilage degeneration, joint inflammation, bone remodeling, and loss of normal joint function. The most prominent aspect of cartilage degeneration is the loss of matrix components, including type II collagen, aggrecan, and matrix-degrading enzymes such as metalloproteinases [15]. Given TMF's diverse biological properties encompassing anti-inflammatory, anticancer, and antiviral characteristics, we employed bioinformatics and systems pharmacology to study TMF's potential role and mechanism in OA. This exploration holds the promise of significantly improving the survival rates of OA patients, sparing them from pain. Numerous therapeutic targets associated with OA have been reported in multiple studies, such as the Hippo pathway, IL-6 receptor antagonism, WNT signaling, miR-146a-5p, controlled release of corticosteroids, TRPV2 protein, TGF-β/TAK1-FoxO1 signaling pathway, PI3K/Akt signaling pathway, and IL-1β inhibitors, among others [16-24]. The variation in methodologies results in different involved targets and molecular mechanisms.

In our research, we identified 228 potential protein targets for TMF therapy in OA through intersection analysis and acquired five core targets (STAT3, SRC, CTNNB1, EGFR, and AKT1). Enrichment analyses unveiled 2736 entries in GO analysis and 203 entries in KEGG analysis. These results indicated TMF's involvement in anticancer,

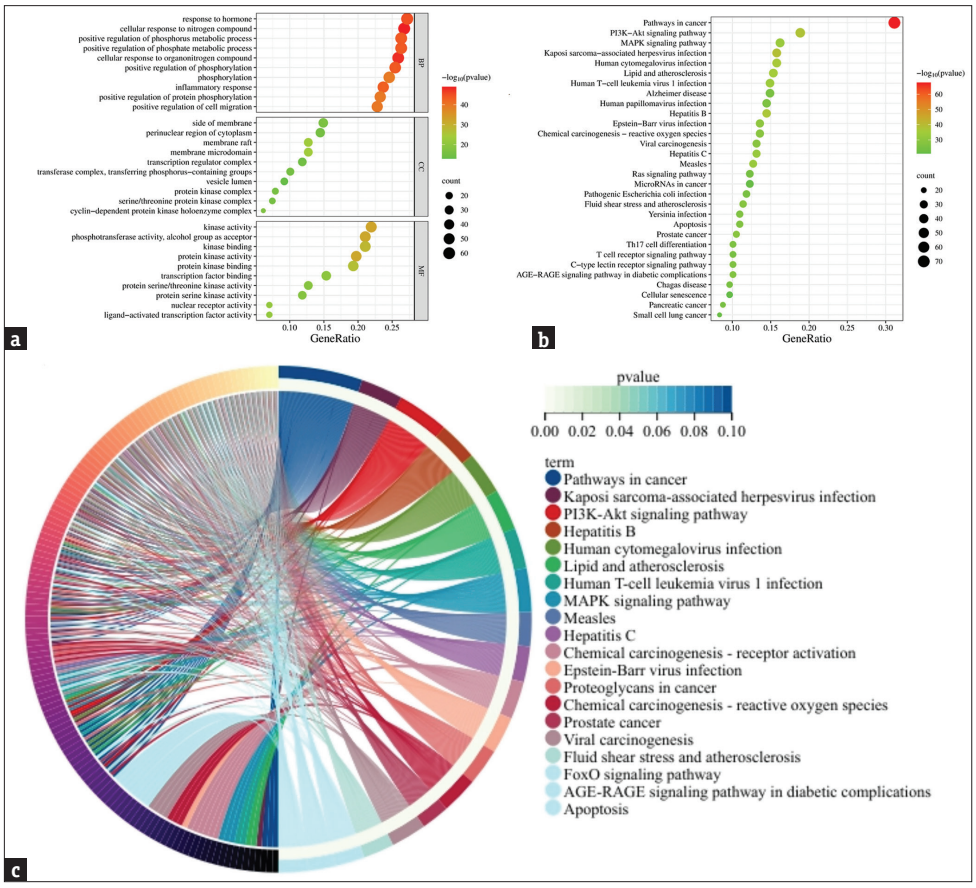

Figure 4: Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of intersection genes between tetramethoxyflavone and osteoarthritis. (a) GO analysis; (b) KEGG analysis; (c) KEGG circle diagram

antiviral, anti-inflammatory responses, hormone reactions, cell apoptosis, PI3K-Akt, and MAPK signaling pathways for treating OA. In addition, molecular docking exhibited that TMF displayed binding activity with only the STAT3 target in OA, albeit at a moderate level, while most other target proteins exhibited favorable binding capabilities. Therefore, research suggests a significant promise for TMF in treating OA. Within the PPI network derived from our study, the 228 shared targets of TMF and OA yielded 228 nodes and 619 edges. Furthermore, we conducted molecular docking between TMF and the top five protein targets (STAT3, SRC, CTNNB1, EGFR, and AKT1) in the PPI network, revealing CTNNB1 to exhibit the lowest binding energy at −7.24 kcal/moL. β-catenin, a multifunctional protein, plays a vital role in tissue cell organization and maintenance. As an effector of WNT signaling pathway, it regulates cell proliferation and gene expression during development. In addition, β-catenin is a component of cell adhesion complex, regulating cell sorting and tissue [25]. As a key gene in the Wnt/β-catenin pathway, CTNNB1 has a significant impact on chondrogenesis and mature cartilage formation and can be targeted for treating OA by regulating the Wnt/β-catenin signaling pathway [26-28]. Chondrocyte apoptosis serves as a primary cause of cartilage degeneration in OA. AKT1, a member of the serine/threonine protein kinase family, can control cartilage calcification in OA, demonstrating potential therapeutic effects [29-31]. Autophagy, a highly conserved mechanism maintaining bodily equilibrium, can be relieved to aid OA's future development. AKT1, as a related gene in the autophagy pathway, can reduce NO production, enhance the expression of health markers, and lower OA indicators [32]. In addition, targeting AKT1 and modulating the PI3K/AKT/mTOR signaling pathway can activate chondrocyte autophagy, consequently reducing osteoarthritic pain [33,34]. STAT3, a member of the STAT family, acts as a transcription factor under various pathological conditions [35]. Evidence suggests that the RORα plays a crucial role in cartilage development and OA pathology. Blocking RORα elevates the expression of cartilage matrix components such as type II collagen and aggrecan while significantly downregulating the IL-6/STAT3 pathway, reversing cartilage damage [36-38]. STAT3 is associated with both IL-6-induced cartilage damage and IL-1β-induced inflammatory reactions. These findings illustrate STAT3's role as a major signaling pathway involved in cartilage injury, where IL-6 induces cartilage degradation through the STAT3 pathway, while inhibiting STAT3/NF-κB signaling alleviates IL-1β-induced inflammation, relieves cartilage degeneration, and promotes local autophagy, exhibiting its promising therapeutic effects for OA [39,40]. These results further support TMF as an effective drug target for treating OA.

According to the GO and KEGG enrichment analysis, TMF treatment for OA is closely associated with antiviral,

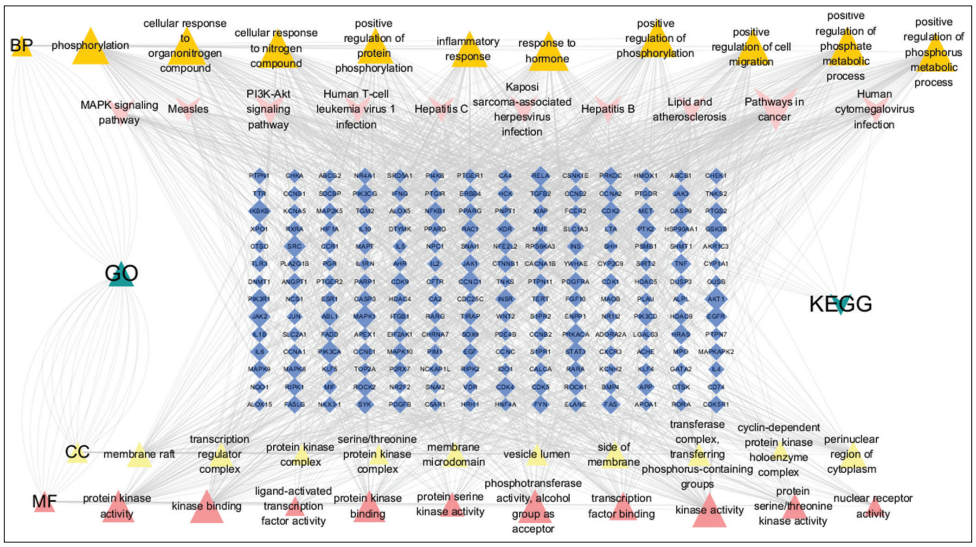

Figure 5: Protein-Gene Ontology-Kyoto Encyclopedia of Genes and Genomes network. BP: Biological process, GO: Gene Ontology, KEGG: Kyoto Encyclopedia of Genes and Genomes, CC: Cellular component, MF: Molecular function

anticancer, anti-inflammatory, hormone response, phosphorus metabolism processes, phosphate metabolism processes, nitrogen compound response processes, cancer pathways, PI3K-Akt signaling pathways, MAPK signaling pathways, and more. Hormones play a crucial role in regulating the body, as the positive and negative feedback of certain hormones maintains the harmony and balance of our organism. OA is more common in middle-aged and older adults and menopausal women. The common factor between these groups is hormonal imbalance, which in older adults can easily lead to hormonal disruption, resulting in metabolic abnormalities, obesity, and other symptoms. Menopausal women might experience accelerated aging, obesity, and menopausal issues due to hormonal imbalances. OA is highly correlated with unhealthy metabolic types such as obesity and hormonal imbalances, along with factors such as genetics, age, and trauma (chronic, acute, and accidents), all of which contribute to the risk of OA [41]. There are various hormones associated with OA, such as sex hormones, estrogen, thyroid hormones, endogenous melatonin, and 19-carbon steroid hormones, among others. Sex hormones, particularly estrogen, have long been considered a potential factor in systemic OA, especially in women. Estrogen, one of the sex hormones, disrupts cartilage through receptor-mediated mechanisms, making women more susceptible to OA. Hormone replacement therapy targeted at women's vulnerability to OA can alleviate the adverse effects of menopause. Replacing menopausal estrogen can prevent arthritis in major joints [42-44]. Predicting thyroid-sensitive indicators such as TSHI, TT4RI, TFQI, and FT3/FT4 shows a close relationship with OA. Among these indices, TFQI can serve as a useful predictor for OA and offer new approaches to OA treatment [45]. Endogenous melatonin acting on the MT1 receptor reverses OA-induced pathological conditions, reduces the expression levels of inflammatory factors, participates in antioxidant and anti-inflammatory activities, and aids in OA treatment [46]. 19-carbon steroid hormones actively regulate the balance between synthesis and breakdown factors, suppress degradation signaling pathways, inhibit pro-inflammatory factors, and play a protective role in cartilage [47]. NO, an inorganic nitrogen compound, is also a type of inflammatory mediator closely associated with the apoptosis of chondrocytes. Chondrocytes, under OA stimulation, become cells involved in catabolic metabolism, producing enzymes for degradation. This imbalance leads to the degenerative changes in joint cartilage. Consequently, the apoptosis of chondrocytes is often linked to the onset of OA. The synthesis of reactive nitrogen species like NO and its derivatives primarily depends on NO synthase. They function as critical cellular messengers in proper gene regulation, signal transduction, and cell cycling [48]. In inflammatory diseases, NO functions as a double-edged sword. Under normal physiological conditions, NO serves as an anti-inflammatory agent. Various cytokines promote the activity of NOS, resulting in the production of abundant NO in cells. Subsequently, NO scavenges free radicals and further eradicates microbes, thereby preventing cellular damage. However, on the other hand, NO acts as a pro-inflammatory factor. Excessive production may lead to cytotoxicity as NO reacts with superoxide to form salts like peroxynitrite. Secondary chain reactions generate NO2 and hydroxide, exacerbating toxicity, causing cellular damage, and increasing inflammatory responses [49]. The biological effects of NO on chondrocytes are intricate, influenced by multiple factors, and involved in various BPs. NO holds promise as a new therapeutic target for treating OA. The calcium–phosphorus ratio has a close risk relationship with pain and disability indicators in OA [50]. Literature suggests that the release of calcium and phosphorus is related to thyroid hormones. When calcium decreases, it stimulates the secretion of thyroid hormones. The function of thyroid hormones is to maintain calcium homeostasis by dissolving bone minerals, inducing renal calcium reabsorption, and phosphorus excretion, indicating that lowering the calcium–phosphorus ratio and increasing parathyroid hormone levels might be appropriate tools for diagnosing OA diseases apart from radiology and MRI imaging [51]. The metabolism of phosphates is also related to OA. When changes occur in extracellular Ca^{2+} levels,

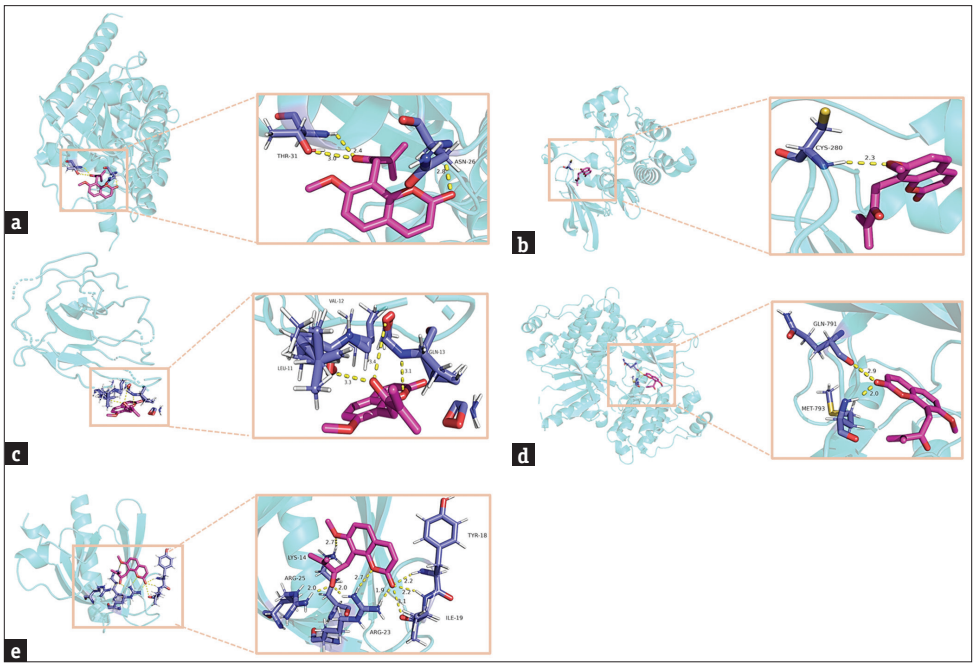

Figure 6: Molecular docking of TMF with STAT3, SRC, CTNNB1, EGFR, and AKT1. (a) Protein binding of TMF with STAT3; (b) Protein binding of TMF with SRC; (c) Protein binding of TMF with CTNNB1; (d) Protein binding of TMF with EGFR; (e) Protein binding of TMF with AKT1

disturbances in pyrophosphate and phosphate metabolism, and imbalances between noncollagen protein inhibitors and promoters, pathological calcification occurs in the body. In most OA joints, basic calcium phosphate (BCP) crystals are associated with severe degenerative changes. BCP crystals enhance signaling through growth factors like Wnt3a, promote cartilage deposition, stimulate cartilage cell enlargement, and activate inflammatory bodies, fostering a pro-inflammatory environment. This explanation can help understand the relationship between phosphates and OA, although the specific mechanisms are yet to be investigated [52-54]. According to information presented by GCBI, the cancer pathway is upstream of the MAPK signaling pathway, which in turn is upstream of the apoptosis pathway. Therefore, both the cancer pathway and the MAPK signaling pathway have some effect on the apoptosis pathway. Since cancer-related pathways mainly feature promoting cell proliferation and/or inhibiting cell differentiation and death, these pathways are highly regulated in OA synovial tissue, exhibiting high cell turnover, rapid cell proliferation, differentiation, and death. This phenomenon is rational because continuous knee joint activity leads to substantial cell death [55,56]. The PI3K/Akt signaling pathway is gradually emerging as a new target for treating OA. PI3K is a protein with certain catalytic activity found widely in various body cells, involved in activities such as cell proliferation, migration, and apoptosis, serving as the initiating factor for the PI3K/Akt pathway. Akt, a target protein with three subtypes and a molecular weight of approximately 57 kDa, displaying high consistency and sequence homology, is the primary downstream effector of PI3K. The PI3K/Akt signaling pathway occupies a significant position as a cellular autophagy pathway, mainly responsible for regulating cell activities such as growth, metabolism, and apoptosis. It inhibits the activity of autophagy-related protein targets, affects downstream effector activity, and reduces cellular autophagy capacity. At present, the molecular mechanisms of treating OA via the PI3K/Akt pathway primarily fall into two categories: first, inhibiting the PI3K/Akt pathway can affect chondrocyte autophagy, maintaining cartilage homeostasis, suppressing inflammation, and thereby reducing osteoarthritic pain; second, activating the PI3K/Akt signaling pathway promotes chondrocyte proliferation, reduces cell apoptosis, and exerts an anti-arthritic effect [57,58]. In summary, TMF can modulate the biological processes of hormone response, cell response to nitrogen compounds, phosphorus metabolism, and phosphate metabolism, and the pathways of cancer pathways, PI3K/AKT pathway, and MAPK pathway, and exhibits anti inflammatory, antiviral and anticancer effects, thereby alleviating OA's clinical symptoms. Furthermore, molecular docking shows that apart from STAT3, the remaining four core targets exhibit good binding capabilities with TMF, indicating TMF's effective binding with specific proteins associated with OA. According to network pharmacology, TMF can be used to treat OA patients. However, more experiments and clinical studies are required to confirm the specific scenarios.

Conclusion

A total of 228 intersection targets for TMF treating OA were obtained, and PPI network analysis identified 5 core targets: STAT3, SRC, CTNNB1, EGFR, and AKT1. GO enrichment analysis yielded 2736 results, while KEGG analysis identified 203 pathways. GO and KEGG results suggested that TMF in treating OA may involve various pathways including hormonal responses, antiviral and anticancer effects, anti-inflammation, phosphorus metabolism, phosphate metabolism, nitrogen compound responses, cancer-related pathways, PI3K-Akt signaling pathway, and

MAPK signaling pathway. Molecular docking revealed good binding affinities between TMF and 4 core targets (SRC, CTNNB1, EGFR, and AKT1). TMF might act on multiple targets and activate diverse pathways to intervene in OA, revealing the molecular processes involved in TMF treatment of OA. However, further clinical validation is needed to confirm these specific results.

Data availability statement

The essential data can be found in the article, and some other data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of interest

There are no conflicts of interest.

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