Icariin: A Potential Lipid Metabolism Regulator in Osteoarthritis

Yusheng Li, Juntao Zhang, Aifeng Liu, Chao Zhang, Qi Li, Fangyang Fan, Chenglong Zhang, Qi Liu and Cheng Yang

Abstract

Background: Icariin is a small molecule drug capable of treating osteoarthritis. Additionally, icariin is known to have an excellent ability to regulate lipid metabolism. A growing number of studies have demonstrated that lipid metabolism is related to the pathogenesis of osteoarthritis. Therefore, by regulating lipid metabolism, icariin may have a significant role in osteoarthritis. However, the molecular mechanism by which icariin regulates lipid metabolism in osteoarthritis is currently unknown. Understanding the molecular mechanism would be helpful in the treatment of osteoarthritis. Objective: This study aimed to explore the mechanism of icariin that regulated lipid metabolism in the treatment of osteoarthritis through a combination of molecular docking and network pharmacology. Methods: Firstly, potential targets for icariin were collected from the TCMSp database, Pharm Mapper, and Swiss Target Prediction Server. Targets for osteoarthritis and lipid metabolism were obtained in OMIM, DrugBank, and GeneCards databases. Common targets of icariin, osteoarthritis, and lipid metabolism were acquired by clusterProfiler R package software. We then constructed the drug-target-signaling pathway-disease network after performing GO and KEGG enrichment analyses of common targets. Finally, we performed molecular docking validation. To support our findings, a search of PubMed was performed to find relevant literature published within the last 5 years. Results: We obtained 12 targets that may be important in the regulation of lipid metabolism in osteoarthritis by icariin. Through PPI network analysis, it was determined that 5 core targets, including TNF, PTGS2, CCND1, MMP2, and ESR1, participated in this process. Molecular docking results showed that the icariin had a high affinity to the core target proteins. Relevant studies in the literature suggest that TNF, PTGS2, MMP2, and ESR1 are the core targets. Conclusion: Icariin is a potential modulator of lipid metabolism in osteoarthritis, and the molecular mechanism may be related to core targets such as TNF, PTGS2, MMP2, and ESR1.

Keywords

network pharmacology, molecular docking, icariin, osteoarthritis, lipid metabolism, molecular mechanism

Received: April 26th, 2022; Accepted: August 26th, 2022.

Introduction

The incidence of osteoarthritis increases with advancing age, and it exerts an economic burden on both patients and society. Osteoarthritis is a degenerative joint disease that involves cartilage, subchondral bone, synovium, and systemic inflammatory response. Furthermore, research suggests that osteoarthritis is a systemic disease that involves alterations in lipid metabolism. Currently, therapeutic drugs are unable to stop the progression of osteoarthritis, so better tolerated and safer osteoarthritis remedy medications are essential. Icariin is the primary active ingredient of epimedium (the related information for icariin is shown in Table 1). Numerous studies have shown the positive effects of icariin on osteoarthritis. Additionally, in vitro and in vivo experiments, the biological function of icariin in regulating lipid metabolism has been confirmed. Therefore, icariin may play a crucial role in lipid metabolism in osteoarthritis.

Network pharmacology, including systems biology, network analysis, connectivity, redundancy, and pleiotropy, provides a platform for drug discovery. The TCM network pharmacology has been established by applying the network pharmacology methods in traditional Chinese medicine. It enables us to understand the molecular mechanisms of Chinese medicine better. Furthermore, it helps Chinese medicine transform from empirical therapy to evidence-based medicine.

1 Orthopedics Department, The First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion, Tianjin, China
2 Academy of Medical Engineering and Traditional Medicine, Tianjin University, Tianjin, China

Corresponding Author:
Juntao Zhang, Orthopedics Department, The First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion, Tianjin 300000, China; Academy of Medical Engineering and Traditional Medicine, Tianjin University, Tianjin 300000, China. Email: zhangjuntaoliyan@sina.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access page (https://us.sagepub.com/en-us/nam/open-access-at-sage).
Our study used network pharmacology to elaborate on the potential mechanism of icariin in regulating lipid metabolism in osteoarthritis and intuitively verified its effectiveness through molecular docking. We constructed the PPI network and screened the core targets. Based on the relevant literature available, we aimed to elaborate on the possible mechanisms by which icariin regulates lipid metabolism in osteoarthritis. These early results laid the foundation for subsequent studies.

Materials and Methods

Database and Software-Related Materials

The TCMSP database provides drug targets of active compounds and related diseases to facilitate the analysis of the mechanism of drug action.9

Swiss Target Prediction server provides biologically active small molecules with ligand-based target prediction.10

Pharm Mapper server performs potential drug target prediction for any small molecule and identifies potential candidates from the database within a few hours of run time.11

UniProt database summarizes the information of experimentally validated or computationally predicted protein function.12

OMIM database stores genotypic and phenotypic information.13

The DrugBank database provides detailed drug data and comprehensive drug target information.14

GeneCards database provides complete data on human genes that have been annotated and predicted.15

STRING database allows rapid examination of the potential evidence supporting specific protein-protein association.16

Cytoscape software combines biomolecular interaction networks and provides a unified framework for representing and incorporating network models.17

AutoDock is a computational docking and virtual screening software for interactions between small molecules and receptors.18

AutoDock Vina is a computational program for molecular docking and virtual screening.19

The PDB database is an open-source database providing valuable biological resources. It holds archives of biomolecular structures.20

PubChem is also an open-source database that provides necessary chemical information on cheminformatics, chemical biology, medicinal chemistry, and several other fields.21

PyMol is a biomolecular visualization program for molecular modeling.22

Screen the Targets of Icariin, Osteoarthritis, and Lipid Metabolism

The potential targets of icariin were obtained from the TCMSP database, Swiss Target Prediction server, and Pharm Mapper server. The targets of osteoarthritis and lipid metabolism were obtained in OMIM, Drugbank, and GeneCards databases. Human genes were identified from the target information with the UniProt database. Finally, those targets were combined and deduplicated.

Construct the PPI Network and Screen Core Targets

To gain the common targets, we intersected the targets. We imported the common targets into the STRING database (Table 2). By using the STRING database, we constructed the PPI network, in which the targets separated from the networks were deleted. Cytoscape 3.8.2 software was used to construct the network diagram. The CytoNCA plug-in was used to analyze the topological property of the network, acquiring the important nodes in the PPI network. Nodes in the PPI network represented common targets; the larger the node, the higher the degree value.

GO and KEGG Enrichment Analyses

The clusterProfiler R package was used to conduct GO and KEGG enrichment analyses. The results of the GO and KEGG enrichment analyses were chosen based on $P$ values < 0.05. The top 10 results of the GO analysis for biological processes (BP), cellular components (CC), and molecular function (MF) and the top 20 results from the KEGG analysis are displayed as bar plots and bubble plots, respectively.

Construct the Drug-Target-Signaling Pathway-Disease Network

To visualize the drug-target-signaling pathway-disease network, drugs, common targets, signaling pathways, and disease-related

| Ingredient | Molecular formula | Molecular weight | 2D structure |
|------------|-------------------|-----------------|-------------|
| Icariin    | C$_{33}$H$_{40}$O$_{15}$ | 676.7 | ![2D structure image] |

| Target name | Degree value |
|-------------|--------------|
| TNF         | 10           |
| PTGS2       | 9            |
| CCND1       | 8            |
| MMP2        | 6            |
| ESR1        | 6            |
data were imported into cytoscape3.8.2 software. By constructing the “drug-target-signaling pathway-disease” network, we can better analyze how the medicine affects lipid metabolism in osteoarthritis.

**Molecular Docking**

The targets with degree value greater than the median were chosen as the main targets. First, the 3D structure of the core target protein was obtained from the RCSB PDB database (information about the core target proteins is shown in Table 3), and PubChem was used to retrieve the two-dimensional molecular structures of icariin. Then the structure of icariin was energy minimized in chem3D. AutoDock 4.2 software was used to

**Table 3. Information About the Core Target Proteins.**

| Core target protein name | PDB ID               |
|-------------------------|----------------------|
| TNF                     | 10.2210/pdb5UUI/pdb  |
| PTGS2                   | 10.2210/pdb5F19/pdb  |
| CCND1                   | 10.2210/pdb2W96/pdb  |
| MMP2                    | 10.2210/pdb1GEN/pdb  |
| ESR1                    | 10.2210/pdb3CBP/pdb  |

Figure 1. (A) Common targets of icariin, osteoarthritis, and lipid metabolism, (B) PPI network diagram, and (C) the network map of core targets.
dehydrate and hydrogenate the protein structure of the core target. In this process, the small molecule ligand icariin was hydrogenated, and the ligand flexible bond was configured to be rotatable. Molecular docking simulations and binding energy calculations were performed using the AutoDock vina program. The number of docking poses was set to 10, and the other AutoDock Vina parameters were set to default. Finally, PyMol software was used to visualize the results.

**Literature Validation**

To confirm the accuracy of our findings, we searched PubMed for relevant studies published over the previous 5 years using the keywords “icariin,” “osteoarthritis,” “lipid metabolism,” and “cytokines.” This approach was carried out independently by 2 researchers.

**Results**

***Targets of Icariin, Osteoarthritis, and Lipid Metabolism***

From the TCMSP database, Swiss Target Prediction server, and Pharm Mapper server, a total of 300 targets of icariin were identified. From the OMIM, Drugbank, and GeneCards databases, 1110 osteoarthritis targets were found and 1351 relevant targets for lipid metabolism were obtained.

***The PPI Network Diagram and Core Targets***

There were 12 common targets among icariin, osteoarthritis, and lipid metabolism, as depicted in Figure 1A. A PPI network with 11 nodes and 31 edges was constructed using the STRING database and the Cytoscape software (Figure 1B). The degree value is reflected in the nodes’ color and size. The larger the node and the redder the color, the higher the degree value, which shows that it may be the core target. Information on core targets can be seen in Figure 1C and Table 2.

***GO and KEGG Enrichment Analyses***

In total, 810 biological processes, 17 cellular components, and 63 molecular functions were obtained from the GO enrichment analysis. The results showed that the common targets were primarily related to biological processes, including “tissue...
remodeling” and “steroid hormone response.” Figure 2 shows the top 10 biological processes, cellular components, and molecular functions. Through KEGG enrichment analysis, we discovered 62 signaling pathways. The top 20 KEGG pathways were intercepted from smallest to greatest based on \( P \)-values. KEGG enrichment analysis showed that the “AGE-RAGE signaling pathway in diabetic complications and oxytocin signaling pathway” was the most significantly enriched (Figure 3).

The Drug-Target-Signaling Pathway-Disease Network Diagram

The network diagram of “drug-target-signaling pathway-disease” (Figure 4) was constructed by Cytoscape 3.8.2 software, in which the nodes are represented by different colors and shapes; orange represents common targets, green represents the signaling pathways, red represents the drug, and the purple represents the disease.

Molecular Docking

Icariin was docked ten times with TNF, PTGS2, CCND1, MMP2, and ESR1. All docking binding free energies were smaller than or equal to \(-7\) kcal/mol. It demonstrated that icariin could dock with TNF, PTGS2, CCND1, MMP2, and ESR1 without any external force and develop the optimal chimeric structure. Table 4 shows the minimal free energy of docking binding of icariin to TNF, PTGS2, CCND1, MMP2, and ESR1. Figure 5 shows the molecular docking results.

Results of Literature Validation

We searched the literature and found 38 papers that were relevant to our research. There were 24 studies that involved icariin in the treatment of osteoarthritis; 12 studies that involved the pathogenesis of osteoarthritis and lipid metabolism; and 2 studies that involved icariin in regulating lipid metabolism. TNF\(\alpha\) and IL-1\(\beta\) were the most researched, receiving 6 and 7 mentions, respectively. Figure 6 depicts the findings of the literature validation. Among these targets, TNF\(\alpha\), MMP2, ESR1, and PTGS2 were consistent with our findings, implying that our findings were correct. Icariin may be beneficial in treating lipid metabolism in osteoarthritis by targeting TNF\(\alpha\), MMP2, ESR1, and PTGS2.

Figure 3. KEGG enrichment analyses: top 20 signaling pathways.
Icariin is a Potential Modulator of Lipid Metabolism in Osteoarthritis

Icariin has anti-inflammatory properties.23 Icariin was discovered to suppress chondrocyte autophagy and apoptosis.24 Furthermore, a study on high cholesterol diet-induced atherosclerotic rats revealed that icariin could reduce TC, TG, and LDL-C while increasing HDL-C, significantly improving hyperlipidemia.25 It is reported that icariin can lower blood TC and LDL-C levels among rabbits on a high-cholesterol diet.26 One of the risk factors for the development of osteoarthritis is high serum cholesterol levels.27 Statin lipid-lowering medicines can help slow down the course of osteoarthritis.28 Based on these findings, it is not difficult to draw the following conclusions. Firstly, icariin has excellent anti-inflammatory and lipid-regulating abilities, which may positively affect osteoarthritis and lipid metabolism. Secondly, the development of osteoarthritis is related to lipid metabolism, and the regulation of lipid metabolism is beneficial to the treatment of osteoarthritis.

Table 4. The Minimum Free Energy of Docking Binding.

| Ligand | Docking binding free energy (kcal/mol) |
|--------|-----------------------------------------|
|        | TNF | PTGS2 | CCND1 | MMP2 | ESR1 |
| Icariin | −7.2 | −9.4 | −7.3 | −7.0 | −7.5 |

Discussion

Icariin is a Potential Modulator of Lipid Metabolism in Osteoarthritis

Icariin has anti-inflammatory properties.23 Icariin was discovered to suppress chondrocyte autophagy and apoptosis.24
Thirdly, icariin may be a potential modulator of lipid metabolism in osteoarthritis. After further validation of our study with the literature, we suggest that TNFα, PTGS2, MMP2, and ESR1 are the 4 possible drug targets of icariin in regulating lipid metabolism in osteoarthritis.

Icariin may Regulate Lipid Metabolism in Osteoarthritis by Inhibiting the Inflammatory Response

TNFα is a serum endotoxin-inducing factor, cachexia, and differentiation-inducing factor, mediating inflammatory responses and innate immunity. The significance of TNFα in the pathophysiology of osteoarthritis has been demonstrated through its detection in the synovial fluid of osteoarthritis patients. The level of TNFα also increases as the osteoarthritis score increases. In addition, TNFα levels that are abnormally high in people with osteoarthritis are believed to be the leading cause of cartilage degradation. COX-2 is another name for PTGS2 and is the crucial enzyme that converts arachidonic acid into prostaglandins. During inflammation, COX-2 levels increase, leading to increased prostaglandin production, and subsequent inflammation was significantly reduced with the administration of COX-2-specific inhibitors. COX-2 is highly expressed in synovial vessels, lining cells, and fibroblast-like cells in synovial tissues of patients with various kinds of arthritis. Inflammatory cytokines TNFα and COX-2 have been discovered to be inhibited by icariin. These results suggested that icariin might exert an anti-inflammatory effect by inhibiting TNFα and COX-2.

Obesity is caused primarily by a disorder in lipid metabolism. Moreover, obesity is associated with an increased rate of osteoarthritis. This suggests that disorders of lipid metabolism further increase the risk of osteoarthritis. Higher levels of adipokines, particularly TNFα, will be found in Obesity-related osteoarthritis people. TNFα also stimulates the synthesis of COX-2 and PGE-2, aggravating the inflammatory response and speeding up the osteoarthritis process. Thus, TNF and COX-2 are important inflammatory factors in the lipid metabolism disorder of osteoarthritis. Their high expression in lipid metabolism disorders further aggravates the inflammatory response and accelerates the progression of osteoarthritis. Icariin can regulate osteoarthritis lipid metabolism by inhibiting the inflammatory response induced by TNFα and COX-2. It’s interesting to note that TNFα is a key player in this chain of events, as it activates COX-2 and other enzymes to speed up the inflammatory response. TNFα is probably the primary pharmacological target.

Icariin may Regulate Lipid Metabolism in Osteoarthritis by Inhibiting Extracellular Matrix Breakdown

Adipose tissue secretes leptin which is an adipokine. Leptin is involved in the pathology of osteoarthritis, resulting in chondrocyte enlargement, cartilage degradation, and ossification. Leptin levels are greater than usual in people with osteoarthritis of the knee. Leptin increases MMP-2, MMP-9, and collagen II levels by promoting matrix metalloproteinase expression. Furthermore, higher mechanical stresses produce increased loading on cartilage and subchondral bone in patients with obesity-related osteoarthritis, and the increased joint loading limits cartilage matrix synthesis. MMP-2, a matrix
metalloproteinase, is involved in extracellular matrix remodeling and protein breakdown. Thus, MMP-2 has an irreplaceable role in lipid metabolism in osteoarthritis, as it aggravates cartilage wear by promoting extracellular matrix’s degradation, while MMP-2 levels are further increased in lipid metabolism disorders, which can further accelerate the osteoarthritis’ process. It is reported that icariin can suppress the expression of MMP-2. As a result, icariin may modulate lipid metabolism in osteoarthritis by inhibiting extracellular matrix disintegration.

Estrogen Receptors: New Research Directions in Future

Estrogen receptors were discovered as one of the potential therapeutic targets. Estrogen works by binding to estrogen receptors in the body. Estrogen is one of the first hormones discovered and has an essential role in female reproduction. In addition, estrogen is involved in male reproduction and other systems, including neuroendocrine, vascular, skeletal, and immune systems in both males and females. The incidence of osteoarthritis increases with age and is higher in women than in men, particularly after 50 years, indicating that estrogen plays an important role in the development of osteoarthritis. Estrogen can also regulate lipogenesis and lipolysis in adipose tissue. Furthermore, it inhibits lipid synthesis and promotes lipolysis. The estrogen receptor may be a new direction of research in the regulation of lipid metabolism in osteoarthritis by icariin due to the role of estrogen in osteoarthritis and lipid metabolism.

Conclusion

We investigated the potential molecular mechanism of icariin while regulating lipid metabolism in osteoarthritis. Five core targets, including TNF, PTGS2, CCND1, MMP2, and ESR1 were identified. Further literature research suggests that TNFα, MMP2, PTGS2, and ESR1 may be significant. We suggest that icariin may be a potential therapeutic agent for regulating lipid metabolism in osteoarthritis. Icariin could regulate lipid metabolism in osteoarthritis by inhibiting the inflammatory response. And icariin may regulate lipid metabolism in osteoarthritis via inhibiting extracellular matrix breakdown. Meanwhile, we suggested that ESR1 may be a significantly pharmacological target, and our findings serve as a reference for understanding the therapeutic mechanism.

Acknowledgments

We thank Lin Meng for her contribution to the revision of the manuscript.

Author Contributions

YL and JZ proposed and designed the study. AL drafted the manuscript. CZ and QL helped in revising and improving the manuscript. CZ and FF annotated the images. QL and CY performed the literature validation. All authors discussed and approved the final manuscript.

Ethical Approval and Consent to Participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Natural Science Foundation of China (grant nos. 82074470; 51573137).
References

1. Prieto-Alhambra D, Judge A, Javaid MK, Cooper C, Díez-Perez A, Arden NK. Incidence and risk factors for clinically diagnosed knee, hip and hand osteoarthritis: influences of age, gender and osteoarthritis affecting other joints. *Ann Rheum Dis*. 2014;73(9):1659-1664. doi:10.1136/annrheumdis-2013-203355

2. Litwic A, Edwards MH, Dennison EM, Cooper C. Epidemiology and burden of osteoarthritis. *Br Med Bull*. 2013;105:185-199. doi:10.1093/bmbld/lds038

3. Glyn-Jones S, Palmer AJR, Agricola R, et al. Osteoarthritis. *The Lancet*. 2015;386(9991):376-387. doi:10.1016/S0140-6736(14)60802-3

4. Aspden RM, Scheven BAA, Hutchison JD. Osteoarthritis as a systemic disorder including stromal cell differentiation and lipid metabolism. *The Lancet*. 2001;357(9262):1118-1120. doi:10.1016/S0140-6736(00)04264-1

5. Singh R, Akhtar N, Haqqi TM. Green tea polyphenol epigallocatechin-3-gallate: inflammation and arthritis. [corrected]. *Life Sci*. 2010;86(25-26):907-918. doi:10.1016/j.lfs.2010.04.013

6. Wang M, Gao H, Li W, Wu B. Icariin and its metabolites regulate lipid metabolism: from effects to molecular mechanisms. *Biomed Pharmacother*. 2020;131:110675. doi:10.1016/j.biopha.2020.110675

7. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol*. 2008;4(11):682-690. doi:10.1038/nchembio.1118

8. Li S, Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application. *Chin J Nat Med*. 2013;11(2):110-120. doi:10.1016/S1875-5364(13)60037-0

9. Ru J, Li P, Wang J, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J Cheminform*. 2014;6:13-13. doi:10.1186/1758-2946-6-13

10. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res*. 2019;47(W1):W357-W364. doi:10.1093/nar/gkz382

11. Liu X, Ouyang S, Yu B, et al. Pharmmapper server: a web server for potential drug target identification using pharmacophore mapping approach. *Nucleic Acids Res*. 2010;38(Web Server issue): W609-W614. doi:10.1093/nar/gkq300

12. UniProt C. UniProt: the universal protein knowledge base in 2021. *Nucleic Acids Res*. 2021;49(D1):D480-D489. doi:10.1093/nar/gkaa1100

13. Amberger JS, Bocchini CA, Schiettecatte F, Scott AF, Hamosh A. OMIM.org: online Mendelian inheritance in man (OMIM®), an online catalog of human genes and genetic disorders. *Nucleic Acids Res*. 2015;43(Database issue):D789-D798. doi:10.1093/nar/gku1205

14. Wishart DS, Wu A. Using DrugBank for in silico drug exploration and discovery. *Current Protocols in Bioinformatics*. 2016;54(1):14.4.1-14.4.31. doi:10.1002/cpbi.1

15. Stelzer G, Rosen N, Plaschkes I, et al. The GeneCards suite: from gene data mining to disease genome sequence analyses. *Current Protocols in Bioinformatics*. 2016;54(1):13.30.1-13.30.33. doi:10.1002/cpbi.5

16. Szklarczyk D, Gable AL, Nasut KC, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res*. 2021;49(D1):D605-D612. doi:10.1093/nar/gkaa1074

17. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498-2504. doi:10.1101/gr.129303

18. Forlì S, Huey R, Pique ME, Santer MF, Goodsell DS, Olson AJ. Computational protein-ligand docking and virtual drug screening with the AutoDock suite. *Nat Protoc*. 2016;11(5):905-919. doi:10.1038/nprot.2016.051

19. Trost 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. doi:10.1002/jcc.21334

20. Berman HM, Westbrook J, Feng Z, et al. The protein data bank. *Nucleic Acids Res*. 2000;28(1):235-242. doi:10.1093/nar/28.1.235

21. Kim S, Thiesssen PA, Bolton EE, et al. Pubchem substance and compound databases. *Nucleic Acids Res*. 2016;44(D1):D1202-D1213. doi:10.1093/nar/gkv951

22. Lill MA, Danielson ML. Computer-aided drug design platform using PyMOL. *J Comput-Aided Mol Des*. 2011;25(1):13-19. doi:10.1007/s10822-010-9395-8

23. Xu C-Q, Liu B-J, Wu J-F, et al. Icariin attenuates LPS-induced acute inflammatory responses: involvement of PI3K/Akt and NF-κB signaling pathway. *Eur J Pharmacol*. 2010;642(1):146-153. doi:10.1016/j.ejphar.2010.05.012

24. Mi B, Wang J, Liu Y, et al. Icariin activates autophagy via down-regulation of the NF-κB signaling-mediated apoptosis in chondrocytes. *Front Pharmacol*. 2018;9:605-605. doi:10.3389/fphar.2018.00605

25. Hu Y, Sun B, Liu K, et al. Icariin attenuates high-cholesterol diet induced atherosclerosis in rats by inhibition of inflammatory response and p38 MAPK signaling pathway. *Inflammation*. 2016;39(1):228-236. doi:10.1007/s10753-015-0242-x

26. Zhang W-P, Bai X-J, Zheng X-P, Xie X-L, Yuan Z-Y. Icariin regulates of the NF-κB signaling-mediated apoptosis in chondrocytes. *Biomed Pharmacother*. 2020;136:109935

27. Stelzer G, Rosen N, Plaschkes I, et al. The GeneCards suite: from gene data mining to disease genome sequence analyses. *Current Protocols in Bioinformatics*. 2016;54(1):13.30.1-13.30.33. doi:10.1002/cpbi.5

28. Szklarczyk D, Gable AL, Nasut KC, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res*. 2021;49(D1):D605-D612. doi:10.1093/nar/gkaa1074

29. Sabio G, Davis RJ. TNF and MAP kinase signalling pathways. *Semin Immunol*. 2014;26(3):237-245. doi:10.1016/j.smim.2014.02.009
30. Farahat MN, Yanni G, Poston R, Panayi GS. Cytokine expression in synovial membranes of patients with rheumatoid arthritis and osteoarthritis. *Ann Rheum Dis*. 1993;52(12):870-875. doi:10.1136/ard.52.12.870

31. Özler K, Aktaş E, Atyç Ç, Yılmaz B, Ankan M, Güngör S. Serum and knee synovial fluid matrix metalloproteinase-13 and tumor necrosis factor-alpha levels in patients with late stage osteoarthritis. *Acta Orthop Traumatol Turc*. 2016;50(6):670-673. doi:10.1016/j.aott.2015.11.003

32. Stannus O, Jones G, Cicuttini F, et al. Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. *Osteoarthritis Cartilage*. 2010;18(11):1441-1447. doi:10.1016/j.joca.2010.08.016

33. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem*. 2000;69(1):145-182. doi:10.1146/annurev.biochem.69.1.145

34. Siegle I, Klein T, Backman JT, Nüsing RM, Fritz P. Expression of cyclooxygenase 1 and cyclooxygenase 2 in human synovial tissue: differential elevation of cyclooxygenase 2 in inflammatory joint diseases. *Arthritis Rheum*. 1998;41(1):122-129. <122::AID-ART15>3.0.CO;2-8

35. Zhang W-D, Li N, Du Z-R, Zhang M, Chen S, Chen W-F. IGF-1 receptor is involved in the regulatory effects of icariin and icaritin in astrocytes under basal conditions and after an inflammatory challenge. *Eur J Pharmacol*. 2021;858:172466. doi:10.1016/j.ejphar.2019.172466

36. Xie C, Chen Q. Adipokines: new therapeutic target for osteoarthritis? *Curr Rheumatol Rep*. 2019;21(12):71-71. doi:10.1007/s11926-019-0868-2

37. Berenbaum F, Jacques C, Thomas G, Corvol MT, Béreziat G, Masliah J. Synergistic effect of interleukin-1β and tumor necrosis factor α on PGE2 production by articular chondrocytes does not involve PLA2 stimulation. *Exp Cell Res*. 1996;222(2):379-384. doi:10.1006/excr.1996.0047

38. Fowler-Brown A, Kim DH, Shi I, et al. The mediating effect of leptin on the relationship between body weight and knee osteoarthritis in older adults. *Arthritis Rheumatol*. 2015;67(1):169-175. doi:10.1002/art.38913

39. Wang T, He C. Pro-inflammatory cytokines: the link between obesity and osteoarthritis. *Cytokine Growth Factor Rev*. 2018;44:38-50. doi:10.1016/j.cytogfr.2018.10.002

40. Gabay O, Hall DJ, Berenbaum F, Henrotin Y, Sanchez C. Osteoarthritis and obesity: experimental models. *Joint Bone Spine*. 2008;75(6):675-679. doi:10.1016/j.jbspin.2008.07.011

41. Chowdhury TT, Arghandawi S, Brand J, et al. Dynamic compression counteracts IL-1beta induced inducible nitric oxide synthase and cyclo-oxygenase-2 expression in chondrocyte/agarose constructs. *Arthritis Res Ther*. 2008;10(2):R35-R35. doi:10.1186/ar2389

42. Cui N, Hu M, Khalil RA. Biochemical and biological attributes of matrix metalloproteinases. *Prog Mol Biol Transl Sci*. 2017;147:1-73. doi:10.1016/bs.pmbts.2017.02.005

43. Singh WR, Devi HS, Kumawat S, et al. Angiogenic and MMPs modulatory effects of icariin improved cutaneous wound healing in rats. *Eur J Pharmcol*. 2019;858:172466. doi:10.1016/j.ejphar.2019.172466

44. Hamilton KJ, Hewitt SC, Arao Y, Korach KS. Estrogen hormone biology. *Curr Top Dev Biol*. 2017;125:109-146. doi:10.1016/bs.ctdb.2016.12.005

45. Oliveria SA, Felson DT, Reed JI, Cirillo PA, Walker AM. Incidence of symptomatic hand, hip, and knee osteoarthritis among patients in a health maintenance organization. *Arthritis Rheum*. 1995;38(8):1134-1141. doi:10.1002/art.1780380817

46. Foryst-Ludwig A, Kintscher U. Metabolic impact of estrogen signalling through ERalpha and ERbeta. *J Steroid Biochem Mol Biol*. 2010;122(1):74-81. doi:10.1016/j.jsbmb.2010.06.012