Molecular mechanism of an aspirin compound for alleviation of osteoporosis using nerwork pharmacology and molecular docking

Cơ chế phân tử của hợp chất aspirin giảm loãng xương sử dụng dược lý mạng và gắn kết phân tử

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Abstract: The aspirin compound (AC) is commonly found to have a wide range of pharmacological activities. This study aimed to investigate the underlying mechanism of the anti-osteoporotic (anti-OP) activity of AC using network pharmacology and molecular docking approaches. First, AC targets were identified using the GeneCards database, and second, OP-related targets were mined using a combination of the GeneCards and DisGeNet databases. The intersection targets from the Genecards, AC, and OP databases were considered candidate targets and were utilized to calculate protein-protein interactions between targets. We discovered a C4A intersection target in the Genecards, AC, and OP databases. This is useful for molecular binding. In addition, we obtained 11 additional prospective targets that may be used to attach the AC molecule to these targets. At the intersection region of the AC and OP target groups are the target genes HLA-DQA1, HLA-DQB1, RPL31, SATB2, SP1, and WNT1. And at the intersection region of the GeneCards and OP databases, we discovered the anti-OP target genes CTSK, PDIA2, RARG, and TBC1D8. They are crucial binding targets that interact with other protein targets. According to the protein-protein interaction network, C4A showed the highest binding capacity with other proteins. The gene ontology (GO) analysis performed in this work found the 10 biological processes, 10 cellular components, 9 molecular activities, and 13 biological pathways. Furthermore, C4A and 10 candidate targets are associated with homologous genes mainly involved in the signaling pathways of the Kyoto Encyclopedia of Genomics and Genomics (KEGG). The AC molecule was found to be highly bound to 11 target candidates. This study identified candidate targets for AC to alleviate OP using the search for protein-protein interactions and the associated signaling pathways of the targets to endorse AC against OP. This may help us understand the mechanism of action of AC during OP treatment.

Keywords: *Aspirin derivative (AC); protein-protein interactions; osteoporosis (OP) ; network pharmacology ; molecular docking calculation*

Tóm tắt: Hợp chất aspirin (AC) thường được tìm thấy có nhiều hoạt tính dược lý. Nghiên cứu này nhằm mục đích làm rõ cơ chế cơ bản của hoạt động chống loãng xương (OP) của AC bằng cách sử dụng dược lý mạng và phương pháp gắn kết phân tử. Đầu tiên, các mục tiêu AC được xác định bằng cách sử dụng cơ sở các dữ liệu GeneCards và thứ hai, các mục tiêu liên quan đến OP được khai thác bằng cách sử dụng kết hợp các cơ sở dữ liệu GeneCards và DisGeNet. Các mục tiêu trong vùng giao nhau của cơ sở dữ liệu Genecards,

AC và OP được coi là mục tiêu ứng viên và được sử dụng để tính toán tương tác protein-protein giữa các mục tiêu. Chúng tôi đã phát hiện ra mục tiêu C4A trong vùng giao nhau của cơ sở dữ liệu Genecards, AC và OP. Điều này rất hữu ích cho gắn kết phân tử. Ngoài ra, chúng tôi đã thu được 11 mục tiêu tiềm năng bổ sung có thể được sử dụng để gắn phân tử AC vào các mục tiêu này. Tại vùng giao nhau của nhóm mục tiêu AC và OP là 6 gen mục tiêu HLA-DQA1, HLA-DQB1, RPL31, SATB2, SP1 và WNT1. Tại khu vực giao nhau của hai cơ sở dữ liệu GeneCards và OP, chúng tôi đã phát hiện ra 4 gen mục tiêu chống OP là CTSK, PDIA2, RARG và TBC1D8. Chúng là những mục tiêu quan trọng tương tác với các mục tiêu protein khác. Theo mạng lưới tương tác protein-protein, C4A cho thấy khả năng liên kết cao nhất với các protein khác. Phân tích bản thể gen (GO) được thực hiện trong công trình này đã tìm thấy 10 quá trình sinh học, 10 thành phần tế bào, 9 hoạt tính phân tử và 13 con đường sinh học. Hơn nữa, C4A và 10 mục tiêu ứng viên được gắn kết với các gen tương đồng chủ yếu liên quan đến các lộ trình truyền tín hiệu KEGG. Phân tử AC được phát hiện là có liên kết cao với 11 ứng viên mục tiêu. Nghiên cứu này đã xác định được các mục tiêu ứng viên gắn kết AC để giảm bớt OP bằng cách sử dụng các tương tác protein-protein và các lộ trình truyền tín hiệu liên quan của các mục tiêu gắn kết. Điều này có thể giúp chúng ta hiểu được cơ chế hoạt động của AC trong quá trình điều trị OP.

Từ khóa: *Hợp chất Aspirin (AC); tương tác protein – protein; loãng xương (OP); dược mạng; tính toán lắp ghép phân tử*

1. Introduction

According to historical National Health Interview Survey (USA) data, 19% of 27,157 adults aged 18 or older regularly consume aspirin. The number of habitual consumers has increased by 57% due to the favorable impact on the cardiovascular system. Aspirin seems to inhibit the prostaglandin E2 (PGE2) gene from making cyclooxygenase (COX), which is one of the parts that controls bone metabolism, in older people who are at risk for osteoporosis [1]. It is essential for the growth of osteoblasts and bone formation. In osteocytes, PGE2 is also necessary for mechanical signaling [2,3,4]. Three different types of osteocytes from various lineages control bone homeostasis. Osteogenic osteoblasts derived from mesenchymal stem cells express Runt 2 and osteox-related transcription factors. Osteoclasts are derived from hematopoietic progenitor cells and express markers such as the calcitonin receptor (CTR), tartrate-resistant acid phosphatase (TRAP), and cathepsin-K (CTSK). Osteocytes are terminally differentiated osteoblasts found in the bone matrix; they can form and decompose bone and influence the behavior of other bone cells. Osteoblasts generate RANKL, which binds to RANK on osteoclast precursors and facilitates their differentiation into osteoclasts [1,5]. Osteoblasts also secrete osteoprotegerin (OPG), which inhibits osteoblast differentiation by binding to RANKL.

Aspirin is one of the precursors to non-steroidal anti-inflammatory drugs (NSAIDs), which have antipyretic, analgesic, and anti-inflammatory effects. By forming an irreversible

covalent bond with the hydroxyl group of serine 530 (carrying out the acetylation reaction), it inhibits all isomeric forms of COX and blocks enzyme access to arachidonic acid. Aspirin is supplanted with a selective COX-2 inhibitor in the treatment of fever, malaise, and inflammation due to the risk of gastrointestinal bleeding [1,5,6]. Due to its antiplatelet effect, it is routinely used in moderate quantities to prevent cardiovascular events in high-risk individuals. According to some studies, low-dose aspirin may also reduce the incidence of colorectal cancer.

The use of aspirin by the elderly may increase the risk of osteoporosis. In addition, it inhibits the production of prostaglandin E2, an essential component for bone remodeling. A literature search on the effects of aspirin on bone health was conducted using a comprehensive scientific database. According to in vitro studies, aspirin increases the viability of bone marrow mesenchymal stem cells, the precursors of osteoblasts, and stimulates osteoblast differentiation [5]. Aspirin can prevent bone loss in osteoporosis animal models. While aspirin enhanced bone mineral density, it did not reduce the risk of fracture. Even one study indicated that aspirin use increased the risk of fractures. Aspirin can increase bone mineral density, but its impact on fracture prevention is ambiguous. To determine the effects of aspirin on human bone health, more data is required.

Although osteoporosis and heart failure (HF) are common among the elderly, patients with HF have a higher chance of acquiring osteoporosis. The relationship between heart failure and osteoporosis varies by gender and heart failure severity. Both heart failure and osteoporosis share the same risk factors, drug use, and pathogenic mechanisms [6]. Age, vitamin D deficiency, kidney disease, and diabetes are all common risk factors for these two diseases. Aspirin, spironolactone, thiazide diuretics, and nitric oxide donors are potential osteoporosis preventatives. Heart failure and osteoporosis appear to share a pathogenesis that may involve activation of the renin-angiotensin-aldosterone system, elevated parathyroid hormone levels, and/or oxidative/nitrosative stress. A significant risk factor for post-fracture mortality is heart failure. To reduce the risk of osteoporosis-related fractures, it is essential to execute a comprehensive assessment of osteoporosis in HF patients.

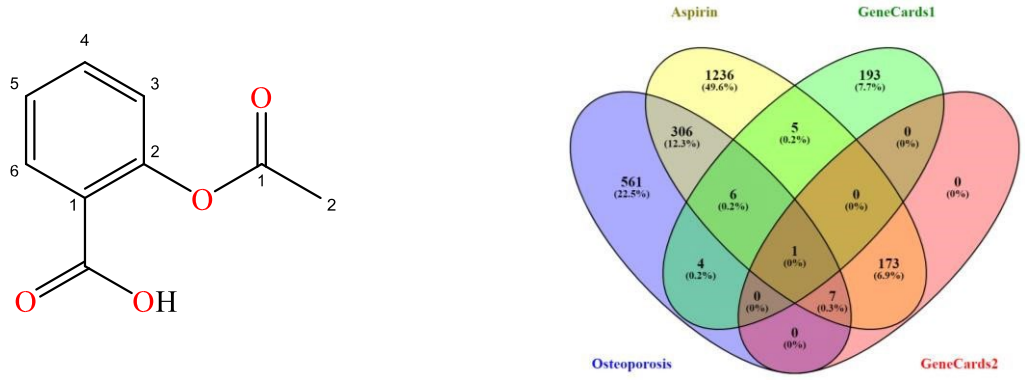
Many studies have investigated the association between cardiovascular diseases and osteoporosis [7]. Many clinical trials and animal studies have demonstrated the effectiveness of incorporating Jin Hong Tang into the diet for the treatment of common osteoporosis [8]. The mechanism of osteoporosis is based on Jin Hong Tang data, and the scientific literature contains only a few scattered preliminary reports. This study aimed to search for the AC molecular mechanisms underlying the treatment of

OP osteoporosis using network pharmacology, GO gene, KEGG analysis, and molecular docking.

2. Materials and methods

2.1. Identifying the candidate targets

Figure 1a depicts the chemical structure of the aspirin compound (AC) obtained from PubChem. We searched the Gene Database (NCBI), GeneCards, and DisGeNet using the search terms "osteoporosis" and “Homo sapiens” to identify the genes associated with osteoporosis (OP) [6]. GeneCards were utilized to seek out AC target proteins. As shown in Figure 1b, we then used Venny diagram to determine potential AC targets for OP reduction.



a) b)

Figure 1. a) Chemical structure of AC; b) Venn diagram of targets relative to AC and OP. A total of 561 targets are included in OP (left), 1236 targets are included in AC (right), 193 targets are GeneCards1, 6 targets (AC-OP-GeneCards1 intersection) in the center, and 1 target (AC-OP-GeneCard2 intersection

2.2. Analyzing the Protein-Protein Interactions

To analyze the protein interaction network of candidate targets, the species option was set to Homo sapiens and the confidence score was set to greater than 0.7 [12].

2.3. Enrichment Analysis

The aforementioned candidate targets underwent gene ontology (GO) functional enrichment analysis and concomitant genomic and metabolic pathway (KEGG) enrichment analysis [11]. The species preference was set to Homo sapiens, and cellular components, molecular functions, biological processes, and signaling pathways associated with the targets were analyzed. The diagrams were created using the bioinformatics website.

2.4. Docking calculation

Autodock can be used to evaluate the binding efficiency between AC and candidate target proteins. The RCSB Protein Data Bank (http://www.pdb.org) was used to determine the X-ray crystal structures of the predicted targets.

ChemDraw also allows for the reconstruction of AC's 3D structure [10]. Then, edit candidate target proteins located at the intersection of AC and OP using MOE and Autodock.

Water removal, ligand removal, hydrogen addition, partial charge correction, and protein optimization were structural modifications applied to candidate proteins. The connection between AC and candidate targets was evaluated using Autodock. Considered accurate are the top docking models with RMSD 2. Using MOE for protein-ligand interactions, docking results were visualized.

3. Results and discussion

3.1. Identifying the candidate targets

As illustrated in Figure 1b, after removing redundant information, we obtained 561 OP-related therapeutic targets and 1236 AC-related therapeutic targets, of which 6 were overlapped between AC-OPGenesCards1, 4 targets in the junction between OP and GeneCards1, and 1 target in the junction between AC-OP-GeneCards1-GeneCards2. Human (Homo sapiens) candidate protein targets are located within the junction region. We selected one common candidate C4A target for AC-OP-GeneCards1-GeneCards2 and six additional targets, including HLA-DQA1, HLA-DQB1, RPL31, SATB2, SP1, and WNT1 intersection of AC and OP [10,11]. These targets are potential molecular targets that mediate the anti-OP effects of AC.

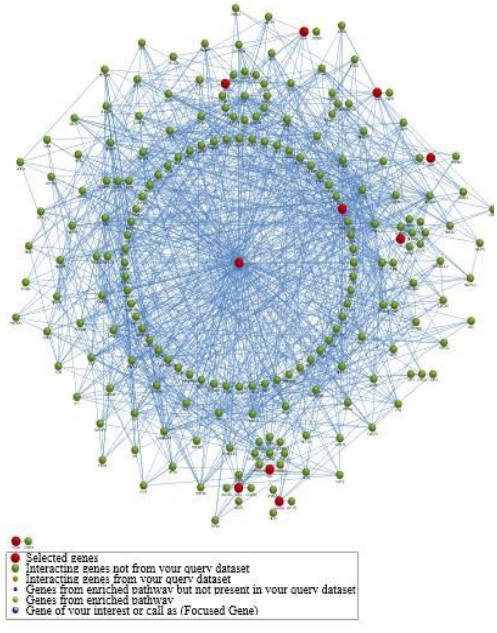
3.2. Interaction of the candidate targets

A protein-protein interaction network was made using a candidate C4A target of AC-OP-GenesCards1 and GenesCards2, six candidate protein targets of AC-OP (HLA-DQA1, HLA-DQB1, RPL31, SATB2, SP1, and WNT1), and four candidate protein targets of OP-GenesCards (CTSK, PDIA2, RARG, and TBC1D8). The ten target proteins are described in detail in Table 1.

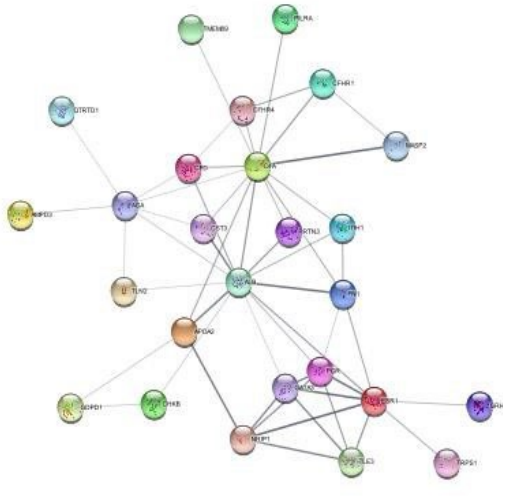
Table 1. Target Proteins of AC against OP in the Protein Interaction Network

no.	protein names	gene symbol	no.	protein names	gene symbol
1	Cathepsin K	CTSK	6	ribosomal protein L31	RPL31
2	Major Histocompatibility Complex, Class II, DQ Alpha 1	HLA-DQA1	7	SATB homeobox 2	SATB2
3	Major histocompatibility complex, class II, DQ beta 1	HLA-DQB1	8	Sp1 transcription factor	SP1
4	protein disulfide isomerase family A member 2	PDIA2	9	TBC1 domain family member 8	TBC1D8
5	retinoic acid receptor gamma	RARG	10	WNT family member 1	WNT1

Figure 2a shows how the 11 candidate protein genes C4A, HLA-DQA1, HLA-DQB1, RPL31, SATB2, SP1, WNT1, CTSK, PDIA2, RARG, and TBC1D8 interact with other protein genes. There are several target proteins with the greatest number of protein interactions. We found that the C4A protein gene with the most possible interactions is the best candidate for osteoporosis therapy [12]. Figures 2b and 2c indicate that the molecular target C4A is located in the crossover region of the four target groups. It may be a potential candidate target for OP-reduced AC activity in the protein-protein interaction network. As shown in Figure 2b, the interactions between C4A protein units include: Number of nodes: 21 (proteins), number of edges: 167 (protein interactions), mean node degree: 15.9, system average number of local clustering: 0.888, expected number of edges: 22. Nodes with the greatest degree of connectivity with other gene symbols represent nodes in the entire network and are also the most promising drug targets [13]. These targets were also associated with other proteins with different confidence probabilities (p). Figure 2d shows the distribution of similar proteins, with 11 genomic proteins selected as candidate protein targets. C4A is required for the covalent propagation of immunoglobulins and immune complexes, and it increases the solubility of immune aggregates. So, C4A may be what makes binding work well and is needed for amide bonds to form with immune aggregates. It can also speed up the metabolism of the carbonyl thioester group.



a)



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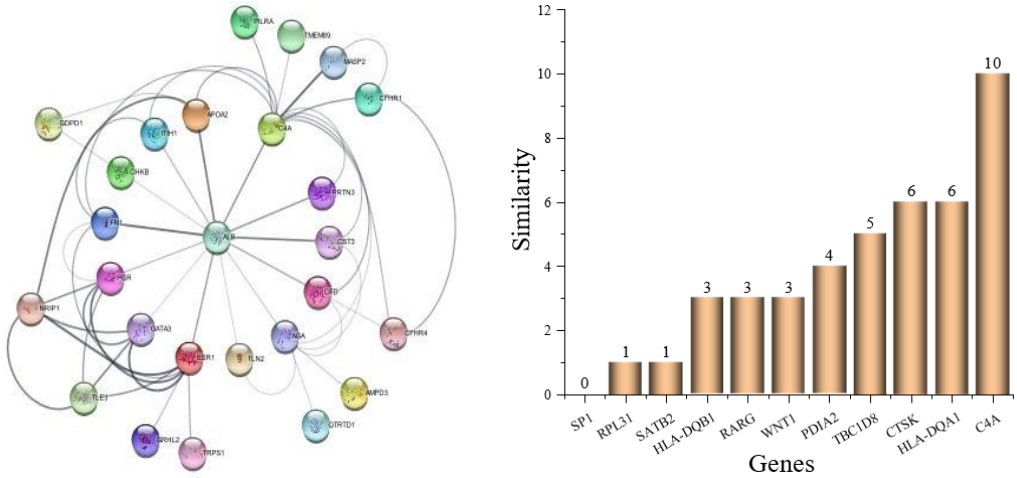


c) d)

Figure 2. a) protein-protein interaction network between 11 candidate targets for the treatment of OP; b) and c) protein-protein interaction network: 21 nodes (proteins) and 167 edges (protein interactions) for the anti-OP effect of AC in the protein interaction network; d) the distribution of similarity proteins.

3.3. Enrichment Analysis

3.3.1. GO analysis

GO analysis to identify biological processes and pathways with a significance level of 0.05. The histogram columns represent the number of enriched genes, while the lower P-values indicate a higher level of confidence in the enrichment results. By binding to the transcriptional core regulator, DNA-binding transcription factors, RNA polymerase II, and upregulating small molecule metabolism. They are functionally molecularly improved in the gene ontology (GO). In Figure 3, the GO analysis graph clarifies the relationship between each target, cellular component, biological process, molecular function, and biological pathway in ascending P-value order.

At the intersection of the AC and OP target groups are the cellular components, biological processes, molecular functions and biological pathways of eleven target proteins, which include six genes (HLA-DQA1, HLA-DQB1, RPL31, SATB2, SP1 and WNT1) and four genes (CTSK, PDIA2, RARG and TBC1D8). They serve as important engagement targets. Figure 3 depicts the process of analyzing biological systems. Positive regulation of transcription from the RNA polymerase II promoter, and transcriptional positive regulation (DNA template) and negative regulation of the process are the three most important processes. Positive regulation of gene expression, a transcription-initiating hormone-mediated signaling pathway from the RNA polymerase II promoter regulates biological processes and pathways [14]. Molecular function also describes responses to lipopolysaccharides, which are active regulators of nitric oxide biosynthesis, as well as responses to glucocorticoids, antibiotics, hydrogen peroxide, blood pressure regulators, and cellular senescence. The majority of them are highly correlated with the pathogenesis of OP.

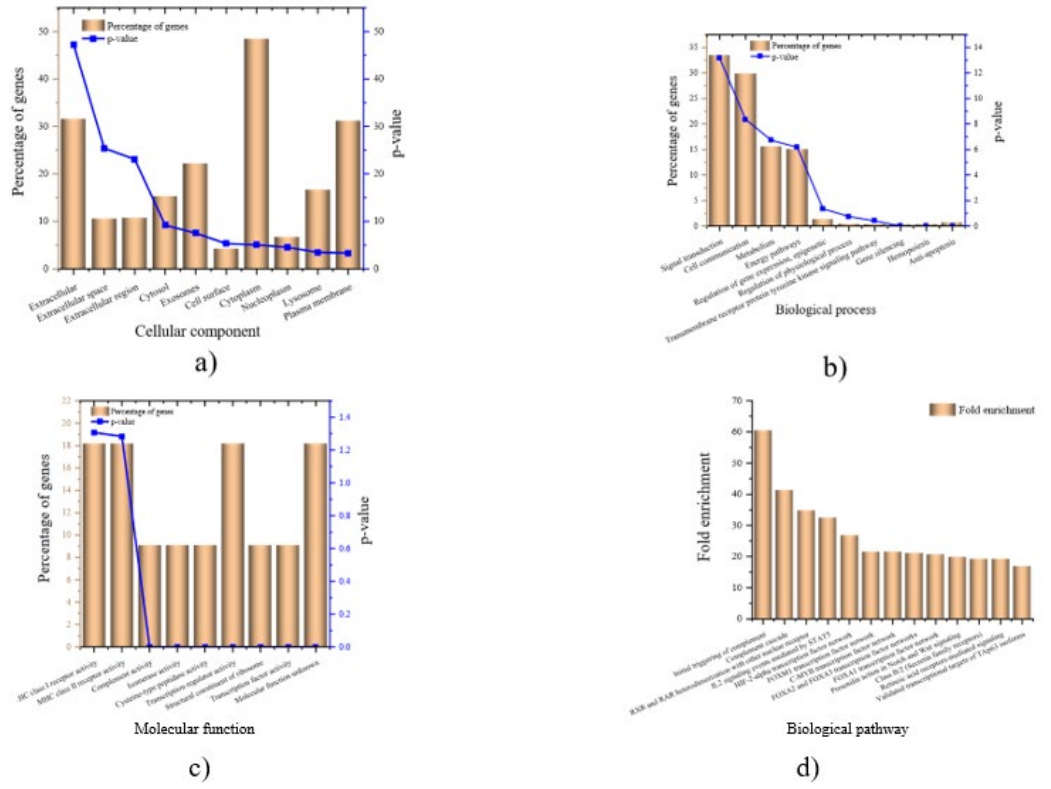


Figure 3. GO analysis represent the cellular composition, biological processes, molecular function and Biological pathway of the 11 target proteins at the p confidence level: a) cellular component;b) biological process; c) Molecular function; d) Biological pathway

In addition, the adjusted P value of 0.01 revealed that nine cellular components were involved in the antiOP effect of AC, including the cytoplasm, cytoplasmic nucleus, lethal signaling complex, nucleus, cytoplasm, neuronal cell body, protein complex, cavernous, and endoplasmic reticulum (Figure 3). As depicted in Figure 3, GO analysis identified a total of 9 major molecular functions, 4 being steroid hormone receptor activity, sequence-specific DNA binding, enzyme binding, transcription factor binding, drug binding, active RNA polymerase II transcription factor (activated sequence-specific DNA binding), identical protein binding, protein complex binding, and protein binding [15].

In general, GO analysis revealed that positive transcriptional regulation from the RNA polymerase II promoter has the greatest number of targets among biological processes. Regarding molecular function, protein binding has a greater number of targets.

This gene codes for the acidic complement factor form. These protein genes can be expressed as a singlechain precursor that is proteolytically cleaved into a third of the alpha, beta, and gamma chains before secretion. This gene was found to have two transcriptional variants that encode distinct isoforms.

3.3.2. KEGG pathway analysis

A Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was done to find other possible

pathways that could be involved in the anti-OP effects of AC. The KEGG pathway enrichment analysis showed that 11 interferon targets are mostly found in signaling pathways. This suggests that they treat osteoporosis by interacting with multiple sequenced pathways, which are shown in Figure 4 in ascending and descending order by the P-value and the number of interleaves, respectively, based on the objectives [14]. This suggests that these pathways play an important role in the treatment of osteoporosis. Figure 4 is a diagram of the KEGG analytical signal pathway. It shows the relationship between the enrichment of each target and the leading pathways, with the size of the graph representing the quantity[13].

The pathways of fluid shear stress and atherosclerosis, as well as hypoxia-inducible pathways, in which the highlighted genes are genes in the PPI network of common targets, indicate that these pathways may mediate AC activity to treat OP.

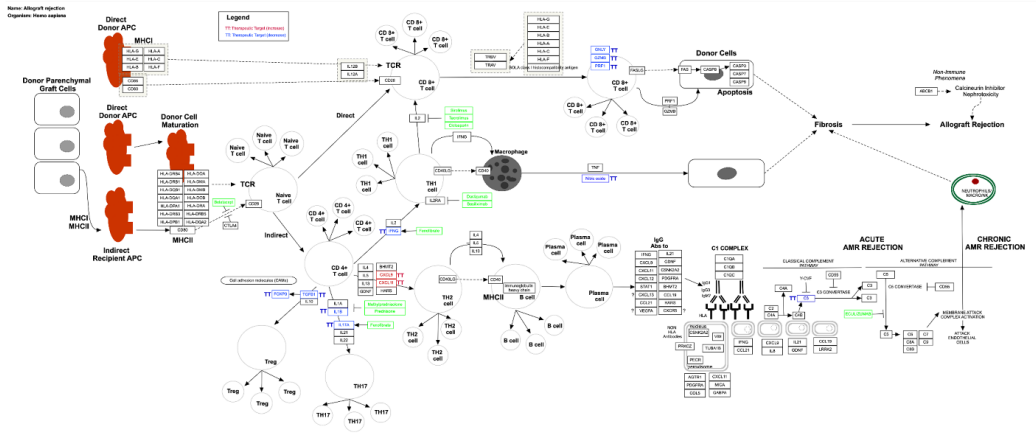


Figure 4. Signaling pathways involved in C4A and candidate targets

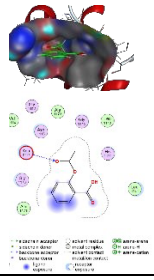
This pathway depicts the molecular interactions involved in the adaptive immune response underlying allogeneic graft destruction. Antigen-presenting cells (APCs) from either the donor (direct pathway) or the acceptor (indirect pathway) can activate T cells, which makes both CD8+ and CD4+ T cells mature. Allogeneic donor cells die when CD8+ T cells stimulate them, while CD4+ T cells develop into TH1, TH2, T17, and Treg cells. Activated TH1 produces TNFA and NO, which are cytotoxic to donor graft cells. TH2 cells activate B cells. When B cells are activated, plasma cells, IgG antibodies, and the complement cascade pathway are made. This happens in both antibody-mediated acute rejection (AMR) and chronic AMR. C3 is the therapeutic target in these two types of AMR. Exogenous YCF treatment inhibits C3, preventing AMR. Eculizumab binds to C5 and stops C5a and the cell membrane attack complex from being made. C3a and C35 are powerful chemotactic factors that stimulate the infiltration of proinflammatory cells. Belatacept inhibits the binding of CD80 and CD86 to CD28. Corticosteroids inhibit anti-inflammatory cytokines.

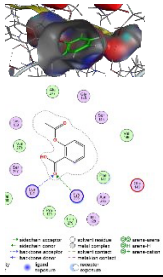
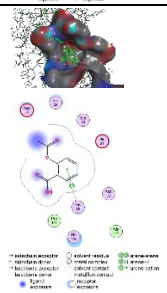
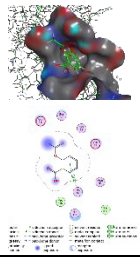
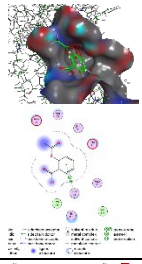
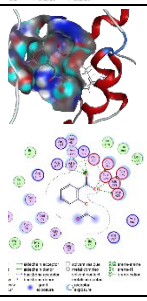
Immunosuppressive corticosteroids like CTLA4 inhibit T-cell activation.

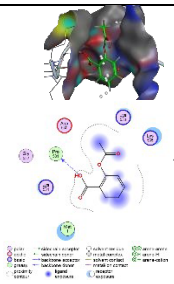
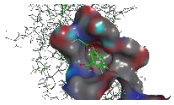
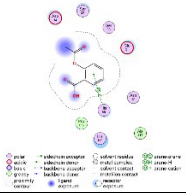
3.4. Docking calculation

To consider the binding capacity of AC to targets, the selection of binding targets that strongly interact AC with proteins, such as molecular target C4A, is a result of the intersection of four AC-OP-target groups. GenesCards1-GenesCards2 may provide treatment for OP. In addition, six target genes, HLA-DQA1, HLADQB1, RPL31, SATB2, SP1, and WNT1, were also obtained from the intersection region of the AC and OP target databases, and four target genes, CTSK, PDIA2 , RARG, and TBC1D8, were obtained from the intersection of the AC-OP-GenesCards1-GenesCards2 databases. To assess AC binding, the GeneCards1 and OP targets were used. Table 2 illustrates that AC binds to C4A and the seven most important selected target proteins through hydrogen bond formation and hydrophobic interactions, as shown in Table 2. The calculation results indicate that AC can bind to the binding pocket of C4A and other target proteins through pi-H interactions, hydrophobicity, and hydrogen bonding [14]. We can assume that the bond between AC and protein is stronger when the binding energy (kcal/mol) is lower. The docking information is presented in Table 2 and the affinities of the eight target proteins for AC are all less than -5 kcal/mol, indicating that all bindings are significant. Notably, C4A has the strongest affinity for AC, representing the highest binding capacity with the lowest binding energy. We found that the results correctly predicted the docking of ACs into the binding pocket of each of the eight candidate target proteins. The binding capacity of AC is used in consideration for the treatment of osteoporosis and to confirm the efficacy of osteoporosis treatment. This possibility is also related to the bone index, which partly reflects the quality of osteoporosis treatment. In contrast, the dry-wet bone ratio increased significantly with AC treatment. The binding results of AC-H are very similar to those of other target protein-interacting groups. In addition, when treating osteoporosis, AC may tend to elevate the femoral and brachial indexes. Results may depend on the duration of the AC treatment.

Table 2. The docking results are illustrated for AC bonds to the most important target proteins

Gene symbol	Interaction	Score (S) kcal/mol	RMSD	Ligand Interactions				
				Ligand	Receptor	Interaction	Distance (Å)	E (kcal/mol)
C4A		-5.830	0.558	O4	N ASP 1117(A)	H-acceptor	3.15	-0.70
				O3	N HIS 1172 (A)	H-acceptor	2.99	-3.00

CTSK		-5.159	1.268	O3	N LYS 131 (A)	H-acceptor	2.81	-2.10
				O3	CE LYS 295 (A)	H-acceptor	3.19	-1.50
HLA-DQA1		-5.251	1.074	O2	O ASN14 (A)	H-donor	3.03	-1.60
				6-ring	CG1 VAL13 (A)	pi-H	4.18	-0.60
HLA-DQB1		-5.687	0.881	6-ring	CA ASP35 (A)	pi-H	4.27	-0.70
RPL31		-5.395	1.627	O3	NZ LYS70 (S)	H-acceptor	2.94	-3.50
SATB2		-5.631	1.427	O2	OE1 GLU25 (A)	H-donor	2.84	-4.00
				O3	NE ARG77 (A)	H-acceptor	3.34	-1.40
				O3	NH2 ARG77 (A)	H-acceptor	3.43	-1.40

SP1		-5.231	1.010	O2	O PRO531 (A)	H-donor	2.99	-2.70
WNT1		-5.583	1.1624	6-ring	CE1 HIS88 (A)	pi-H	3.65	-0.50
				O3	HE1 HIS88 (A)	H-acceptor	2.61	-1.50

3.5. Discussion

AC or its derivatives may exist in nature and they may be extracted from different plants. It has been demonstrated that ACs have the ability to cure conditions linked to calcium imbalance and osteoporosis in addition to their anti-inflammatory, anti-cancer, and antidiabetic capabilities. Additionally, AC can influence late osteoclastogenesis and promote osteoblast differentiation. This provides additional evidence that AC may have a bone-protective effect in osteoporosis. A sustained AC supply can be made possible by new extraction and synthetic pretreatment techniques for naturally existing active substances. Despite limitations in medication and treatment, osteoporosis (OP) remains a widespread chronic illness of concern in today elderly society. A current research goal is the creation of new drugs for the treatment of OP, and In this regard, AC derivatives may have intriguing potential [14]. The interactions between biological macromolecules and chemical compounds are reflected by the network pharmacology technique utilized in this study. This innovative research strategy may help in the quest for straightforward new drugs. Using target network building and GO analysis, we discovered important potential targets involved in the treatment of OP with AC derivatives.

Through network-based pharmacological research, a new potential activity was found. In order to treat OP, the results collected suggested eleven significant possible candidate targets for AC. Through interactions between proteins, these eleven protein targets were found to be the main molecular targets of AC derivatives that inhibit OP from happening. Highly enriched pathways in the findings of the KEGG analysis pointed to the C4A protein gene target. This has highlighted their crucial role in mediating OP-

inhibitory AC activity. The results of GO analysis also showed that OP-reducing AC activity was primarily related to biological processes such as inflammation, aging, reactions to glucocorticoids, and responses to hydrogen peroxide [15]. Recent studies have shown a clear connection between OP and the inflammatory response brought on by the immune system.

Cytokines (COX) are key players in the inflammatory response and contribute to the pathological phase of OP by promoting bone resorption in osteoblasts. It can also stop osteoblastic osteoclastogenesis. This could upset the balance of bone turnover and osteoclast activity. Additionally, OP acceleration, osteoblast activation, and bone marrow stem cell differentiation into osteoblasts are all significantly influenced by aging. Both cause a reduction in bone mass and muscle strength. Calcium absorption is decreased by aging-related increases in intracellular calcium concentrations and a narrowing of calcium distribution across cell membranes. Osteoporosis is also exacerbated by long-term calcium deficiency. The results of the GO analysis show that AC has the ability to regulate these important protein targets in the network to regulate OP-related pathogenic processes. The bulk of enrichment pathways are linked to OPs, such as receptor signaling pathways, according to findings from KEGG pathway analysis of target proteins.

Molecular docking studies provide a more intuitive explanation of the interaction between AC and its predicted protein targets in relation to OP [16]. Docking analysis demonstrated that all eight major proteins are targets of AC in osteoporosis. In addition, the results indicate that hydrophobic interactions and hydrogen bonds are the predominant forms of interaction, which is suggestive of the molecular mechanism underlying the AC treatment of OPs. Future research may see if a specific technique that can determine the water content of AC before testing can yield more accurate results.

3. Conclusion

From the above study results, we can conclude that this study received reliable results from centralized screening from the AC, OP, and GenesCards databases. We have obtained a valuable molecular mechanism of action using GO and KEGG pathway analyses for OP-reduced AC targets and activity based on network pharmacology techniques. The mechanism of AC action to treat OP was elucidated by the results of this study. We found that AC activity can alleviate OP through multiple targets and multiple pathways. The results of the study here show the potential to treat OP and provide a possible mechanism of action to treat OP using AC. It could become the reference for AC validation tests based on these protein candidate targets.

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