

Identification of Key Genes and Metabolic Pathways in Osteoporosis and Sarcopenia Based on Transcriptome Data for Drug Repurposing

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Abstract: Osteoporosis (OP) and sarcopenia (SP) are multifactorial complex disorders with tight bidirectional correlations in their pathogeneses, which creates an urgent demand for therapeutic regimens targeting their shared signaling pathways. This study adopted an integrated bioinformatics strategy to excavate the core molecular pathways common to OP and SP, and screen candidate drugs for repurposing. Seed genes associated with the two disorders were collected from the NCBI database and published literatures. A protein-protein interaction (PPI) network was constructed via STRING and visualized with Cytoscape 3.9.1. Key modules and hub genes were identified through network analysis and Gene Ontology enrichment analysis. miRWalk and miRNet were utilized to predict microRNAs targeting hub genes, and DGIDB database was adopted to retrieve candidate therapeutic agents. Network analysis identified seven immune-related functional modules and ten hub genes including IL6 and TNF, which mainly mediate inflammatory responses and cytokine activities. A total of 20 repurposable drug candidates were obtained, among which nine have been applied for osteoporosis treatment, and the other eleven anti-inflammatory drugs are capable of simultaneously alleviating both OP and SP. Six microRNAs regulating hub genes were also predicted, which require further experimental verification. This research clarifies the shared inflammatory pathways of OP and SP and screens multiple agents with repurposing value, offering novel theoretical references for the combined clinical treatment of the two co-morbid diseases.

Keywords: Osteoporosis; Sarcopenia; Protein-protein interaction network; Hub genes; Drug repurposing; Bioinformatics

1. Introduction

Osteoporosis (OP) is a systemic skeletal disorder characterized by decreased bone mineral density (BMD) and deterioration of bone microstructure, culminating in enhanced bone fragility and fracture risk^[1,2]. OP and sarcopenia (SP) are both complex multifactorial conditions whose pathogenesis involves not only genetic predisposition but also epigenetic regulation and lifestyle factors^[3]. The development of OP is driven by multiple pathological mechanisms that disrupt bone homeostasis. For instance, autoimmune responses can impair this balance through a dual pathway: suppressing osteoblast-mediated bone formation while promoting osteoclast-mediated bone resorption. This imbalance often manifests in conditions like rheumatoid arthritis, which is frequently associated with arthralgia, periarticular bone loss, bone erosion, and systemic osteoporosis^[4-6]. Furthermore, chronic inflammation plays a significant role, as it not only exacerbates bone resorption but also independently increases fracture risk, representing a

major contributor to the destabilization of bone metabolism^[7, 8].

Fragility fractures, a direct consequence of OP, are defined by their occurrence with minimal or no identifiable trauma and pose a serious threat to both the physical and mental well-being of affected individuals^[1]. Key risk factors for OP include advanced age, low body mass index (BMI), and limited outdoor activity time^[9]. Physiologically, peak bone mass is attained around age 30, followed by a gradual decline; it is estimated that individuals may lose approximately 30% of their bone mass between the ages of 30 and 70. Epidemiologically, the global prevalence of OP is 19.7%, with significant geographic variation: rates range from 9% to 38% in women and from 1% to 8% in men. Notably, the age-standardized prevalence in the middle-aged and elderly Chinese population is higher, reaching 33.49% (20.73% in men and 38.05% in women)^[1]. Etiologically, OP is classified into primary and secondary forms. Primary OP, typically age-related, includes idiopathic (IOP), senile (SOP, or age-related), and postmenopausal (PMOP) osteoporosis. Secondary OP refers to bone loss resulting from specific diseases or medications, such as those that impair bone metabolism through effects on non-specific immune pathways^[10].

Sarcopenia (SP) is a progressive, systemic disorder characterized by the loss of muscle strength, function, and mass, attributable to aging or secondary to other diseases^[11]. It is associated with a range of adverse physical and metabolic outcomes and serves as an independent predictor of fracture risk^[12]. The global prevalence of SP is estimated to range from 8% to 36% in individuals under 60 years and from 10% to 27% in those over 60^[13]. In China, the prevalence among adults aged 65 and above is approximately 17.4%^[14].

Notably, SP and OP frequently coexist. In the elderly Chinese population, their comorbidity rate is approximately 17% (95% CI: 14.00–21.00)^[15]. Among adults over 50 years of age, the prevalence of SP is about 14%^[16]. Strikingly, OP is present in 78.1% of individuals diagnosed with SP, compared to only 47.6% in those without sarcopenia. Moreover, patients with both conditions face a 2–3 times higher risk of osteoporotic fractures than those with either condition alone.

The close interrelationship between OP and SP extends beyond comorbidity to shared pathogenesis. Physiologically, muscles exert essential mechanical loading on bones, which is crucial for bone growth and homeostasis. Diminished muscle strength reduces this osteogenic stimulus, thereby disrupting bone metabolism. Conversely, bone-derived factors (e.g., myokines and osteokines) play a role in regulating muscle maintenance and function. Clinically, this interdependence underscores the necessity of an integrated “bone-muscle management” approach. Joint screening of high-risk groups—such as postmenopausal women, the very elderly, and underweight individuals—should be implemented, followed by multidimensional interventions encompassing exercise, nutrition, and pharmacotherapy. This integrated strategy aims to break the vicious cycle between muscle loss and bone fragility, thereby improving patient quality of life.

Conventional drug discovery, which relies heavily on molecular design and clinical trials, is exceedingly costly and time-intensive^[17]. Drug repurposing—identifying new therapeutic uses for existing drugs—offers a promising alternative, as it is generally safer, faster, and more economical than developing new molecular entities^[18]. In this context, network pharmacology represents a potent next-generation paradigm. It integrates reductionist and systems-level approaches with computational and experimental methods, shifting the focus from “single target, single drug” to “network target, multicomponent therapy.” This aligns with a holistic understanding of complex diseases like OP and SP^[19].

Currently, treatment options for SP are limited, and OP is recognized as a chronic inflammatory state that can exacerbate systemic muscle decline. Given the evidence suggesting shared genetic drivers in the onset and progression of both diseases^[20], this study employs network pharmacology to identify key common genes and signaling pathways in SP and OP. The findings will provide a theoretical basis for drug repurposing strategies, the development of targeted nutraceuticals, and novel drug candidates. Furthermore, in light of the current lack of reliable biomarkers for early SP detection, the key genes identified here may also serve as candidate biomarkers to improve screening, diagnosis, and therapeutic monitoring^[20].

2. Materials and Methods

2.1 Identification of Disease-Associated Genes

Seed genes related to sarcopenia (SP) and osteoporosis (OP) were compiled through a comprehensive search of the NCBI database and a review of existing literature^[21-23].

2.2 Construction of the Protein-Protein Interaction (PPI) Network

A PPI network for SP and OP was constructed using the STRING database (version 12.0)^[24]. STRING integrates experimental and computationally predicted interactions to model functional protein networks. The compiled seed gene lists for SP (SGL) and OP (OGL) were uploaded to STRING, which then incorporated additional candidate genes based on evidence channels including co-expression, genomic neighborhood, homology, co-occurrence, and gene fusion. A confidence score threshold of 0.7 was set to filter reliable interactions (excluding low-confidence associations), and an integrated SP-OP PPI network was generated and subsequently visualized and analyzed using Cytoscape software (version 3.9.1)^[25].

2.3 Network Analysis and Functional Enrichment

2.3.1 Module Identification and Enrichment

Key functional modules within the integrated SP-OP network were extracted using the Molecular Complex Detection (MCODE) plugin in Cytoscape with the following parameters: degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and max depth = 100. These parameters were selected to prioritize densely connected gene clusters while excluding isolated or weakly associated nodes^[26].

2.3.2 Hub Gene Identification and Enrichment Analysis

Hub genes, representing highly connected nodes in the PPI network, were identified using the CytoHubba plugin (based on the Maximal Clique Centrality, MCC, method)^[27, 28]. The top 10 genes with the highest MCC scores were selected as hub genes to ensure focus on the most functionally critical nodes. The biological functions and pathways associated with these hub genes were investigated through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses using the g:Profiler and WebGestalt platforms^[29-31]. A false discovery rate (FDR) < 0.05 was set as the statistical significance threshold to avoid false-positive results. GO analysis covers Biological Process (BP), Molecular Function (MF), and Cellular Component (CC). Potential microRNA (miRNA) regulators of the hub genes were predicted using the miRWalk (v3.0) and miRNet (v2.0) databases. The miRWalk score threshold of > 0.8 was used to filter high-confidence miRNA-gene interactions^[32, 33]. Common miRNAs predicted to target both the hub genes and the diseases were used to construct a hub gene-miRNA interaction network in Cytoscape.

2.3.3 Identification of Candidate Drugs for Repurposing

To identify drugs with potential therapeutic relevance, KEGG pathways significantly enriched in the OP-SP network were extracted via g: Profiler. Genes within these pathways were then queried against the Drug-Gene Interaction Database (DGIDB, v5.0.1) to compile a list of known or predicted drug-gene interactions^[34]. From this initial list, candidate drugs were prioritized based on the following criteria: 1) possessing a well-characterized anti-inflammatory mechanism (validated by ≥ 2 independent studies), 2) clinical efficacy supported by human trials or preclinical in vivo models, and 3) holding FDA approval or Phase II/III clinical trial status (retrieved from DGIDB's "drug approval status" annotation). A drug-target gene network was visualized using Cytoscape. The overall workflow for the bioinformatics analysis is summarized in Figure 1.

3. Results and Discussion

3.1 Construction and Analysis of the OP-SP PPI Network

A total of 120 seed genes were initially identified for both OP and SP from the literature and database search. Using the STRING database, 113 and 217 candidate genes were subsequently added to the SP and OP gene lists, respectively. This resulted in final gene sets of 233 genes (Sarcopenia Gene List, SGL) for SP and 337 genes (Osteoporosis Gene List, OGL) for OP. The integrated SP-OP protein-protein

interaction (PPI) network, constructed by combining these lists, contained 3427 edges, with an average node degree of 32.6 and a highly significant PPI enrichment p-value ($< 1.0e-16$), indicating a biologically relevant and densely connected network (Fig.2).

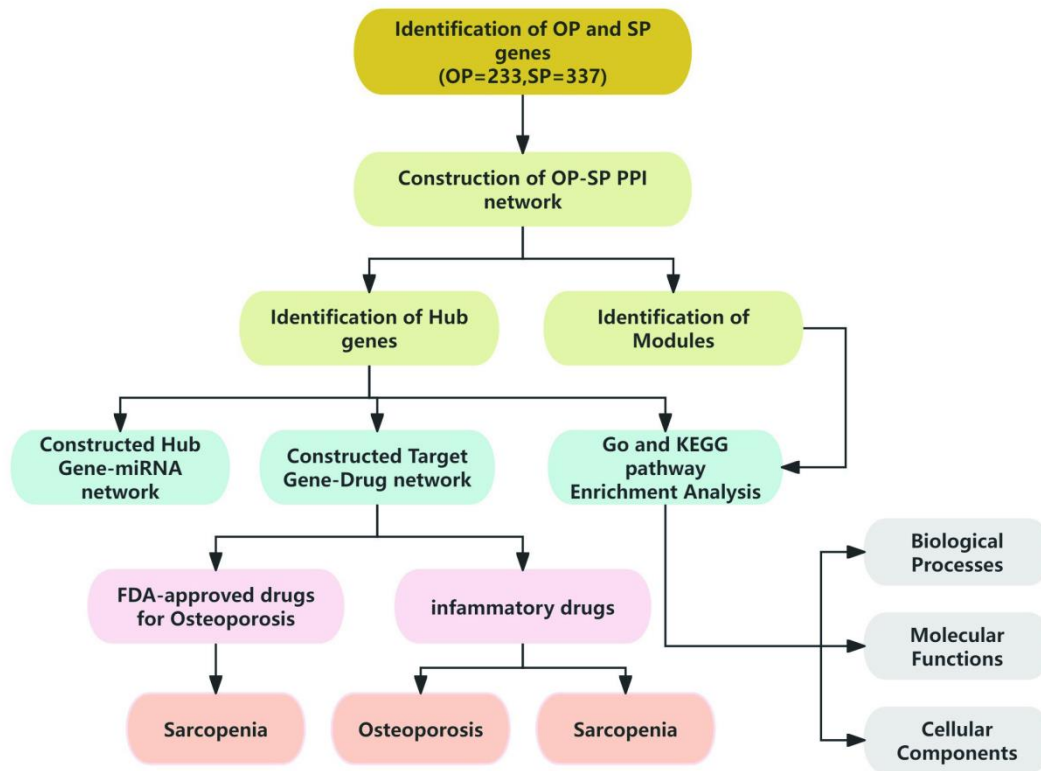


Figure 1 Workflow of the study.

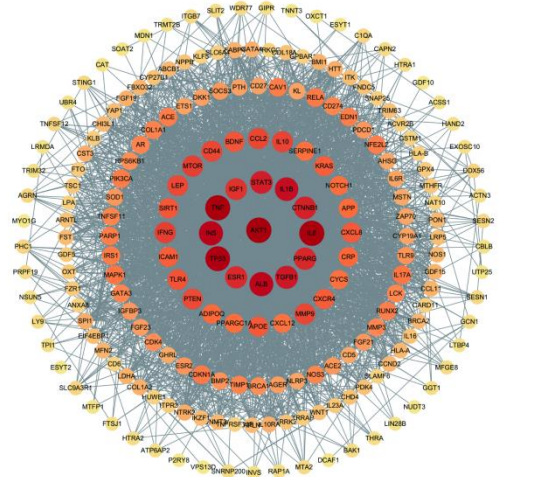


Figure 2 OP-SP network visualized by Cytoscape.

3.2 Identification and Functional Enrichment of Key Network Modules

The PPI network clustering using the MCODE plugin identified seven significant modules (M1-M7), as detailed in Table 1. Functional enrichment analysis performed via WebGestalt revealed their distinct biological themes (Fig. 3A).

Module M1, the largest module with 47 nodes, was predominantly enriched in immune system processes, including immune response and its regulation. All ten subsequently identified hub genes (IL6,

TNF, ALB, IL1B, AKT1, STAT3, INS, PPARG, TLR4, TGFB1) were located within this module. Given the established connection between immune signaling (e.g., the RANK/RANKL/OPG pathway) and bone remodeling, M1 likely represents the core immune-inflammatory axis shared in OP-SP pathogenesis and the molecular signatures in M1 may serve as promising biomarkers for OP-SP.

Module M2 (25 genes) was primarily involved in cell death and apoptosis, processes critical for organismal development, tissue homeostasis, and immune regulation.

Module M3 (8 genes) participated in cell signal transduction, a key process regulating cell proliferation, differentiation, metabolism, and apoptosis.

Module M4 (4 genes) was associated with the regulation of protein binding, fundamental to intracellular signal transduction pathways. Protein-protein interactions (PPIs) constitute the fundamental mechanism by which intracellular signals are propagated. For instance, ligand-receptor binding initiates a cascade of specific PPIs, transducing extracellular signals into the cell to ultimately regulate gene expression and metabolism.

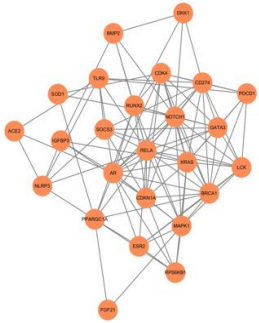
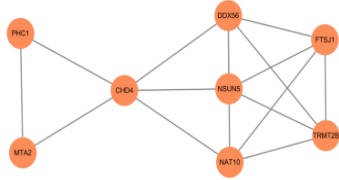
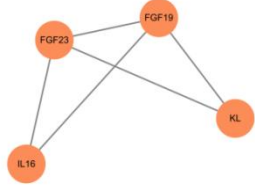
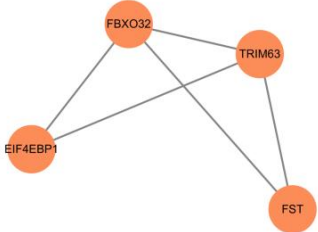
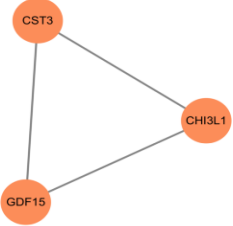
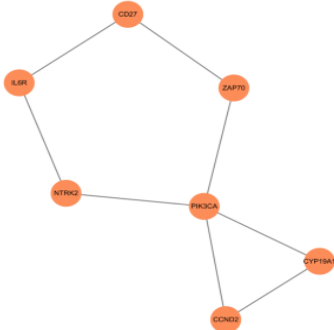
Module M5 is composed of 4 genes and is functionally enriched for processes pertaining to intercellular communication. This fundamental biological process encompasses the transmission of signals between cells and is indispensable for normal physiology. It occurs through two primary modes: direct communication (e.g., via gap junctions that permit the exchange of small molecules and ions) and indirect communication (mediated by secreted signaling molecules such as hormones, neurotransmitters, and cytokines). By coordinating essential cellular behaviors—including proliferation, differentiation, migration, and metabolic activity—intercellular communication plays a pivotal role in overarching physiological processes such as embryonic development, tissue repair, and immune regulation.

Module M6 comprises 3 genes whose functional annotation centers on the regulation of intracellular energy metabolism. This process underpins all vital cellular activities, encompassing major pathways such as glucose metabolism, lipid metabolism, and mitochondrial oxidative phosphorylation. The genes in this module are implicated in fine-tuning the balance of energy production, storage, and utilization. Their potential roles include modulating the activity of core bioenergetic machinery—for instance, regulating the electron transport chain during respiration or participating in rate-limiting steps of glycolysis and the tricarboxylic acid (TCA) cycle. Through these mechanisms, they help ensure that cells dynamically adapt energy flux to meet demands under varying physiological conditions.

Module M7 consists of 7 genes and is functionally annotated to cellular response to external stimuli. The ability to sense and appropriately react to diverse environmental signals is fundamental to cellular homeostasis. Such signals encompass physical stimuli (e.g., mechanical force, temperature fluctuations), chemical stimuli (e.g., hormones, neurotransmitters, nutrients, or pharmaceuticals), and biological stimuli (e.g., pathogen infection). The response mechanism typically initiates with the activation of specific cell-surface receptors, followed by the triggering of downstream signal transduction cascades. This ultimately leads to alterations in the expression of responsive genes, enabling the cell to adapt to changing external conditions and preserve its normal physiological function.

Table 1: List of the modules with RA-OP network constructed via MCODE.

Module	Node	Node Number	Module Figure
1	47	ADIPOQ, ESR1, TP53, INS, IRS1, TNF, IFNG, STAT3, SIRT1, LEP, CD44, BDNF, CCL2, IL10, EDN1, IL6, PPARG, SERPINE1, APP, CXCL8, CXCR4, TGFB1, MMP9, NOS3, TIMP1, PTEN, TLR4, ICAM1, PARP1, TNFSF11, COL1A1, IGF1, ACE, MTOR, IL1B, CTNNB1, CAV1, NFE2L2, IL17A, AKT1, CRP, CYCS, MMP3, CXCL12, AGER, APOE, ALB	

2	25	CDK4, IGFBP3, SOD1, RPS6KB1, DKK1, RELA, CD274, PDCD1, KRAS, LCK, RUNX2, PPARGC1A, BMP2, ESR2, CDKN1A, GATA3, MAPK1, AR, SOCS3, NOTCH1, TLR9, FGF21, ACE2, NLRP3, BRCA1	
3	8	FTSJ1, DDX56, NSUN5, PHC1, NAT10, TRMT2B, CHD4, MTA2	
4	4	FGF23, KL, FGF19, IL16	
5	4	EIF4EBP1, TRIM63, FBXO32, FST	
6	3	GDF15, CST3, CHI3L1	
7	7	CD27, NTRK2, IL6R, ZAP70, CYP19A1, PIK3CA, CCND2	

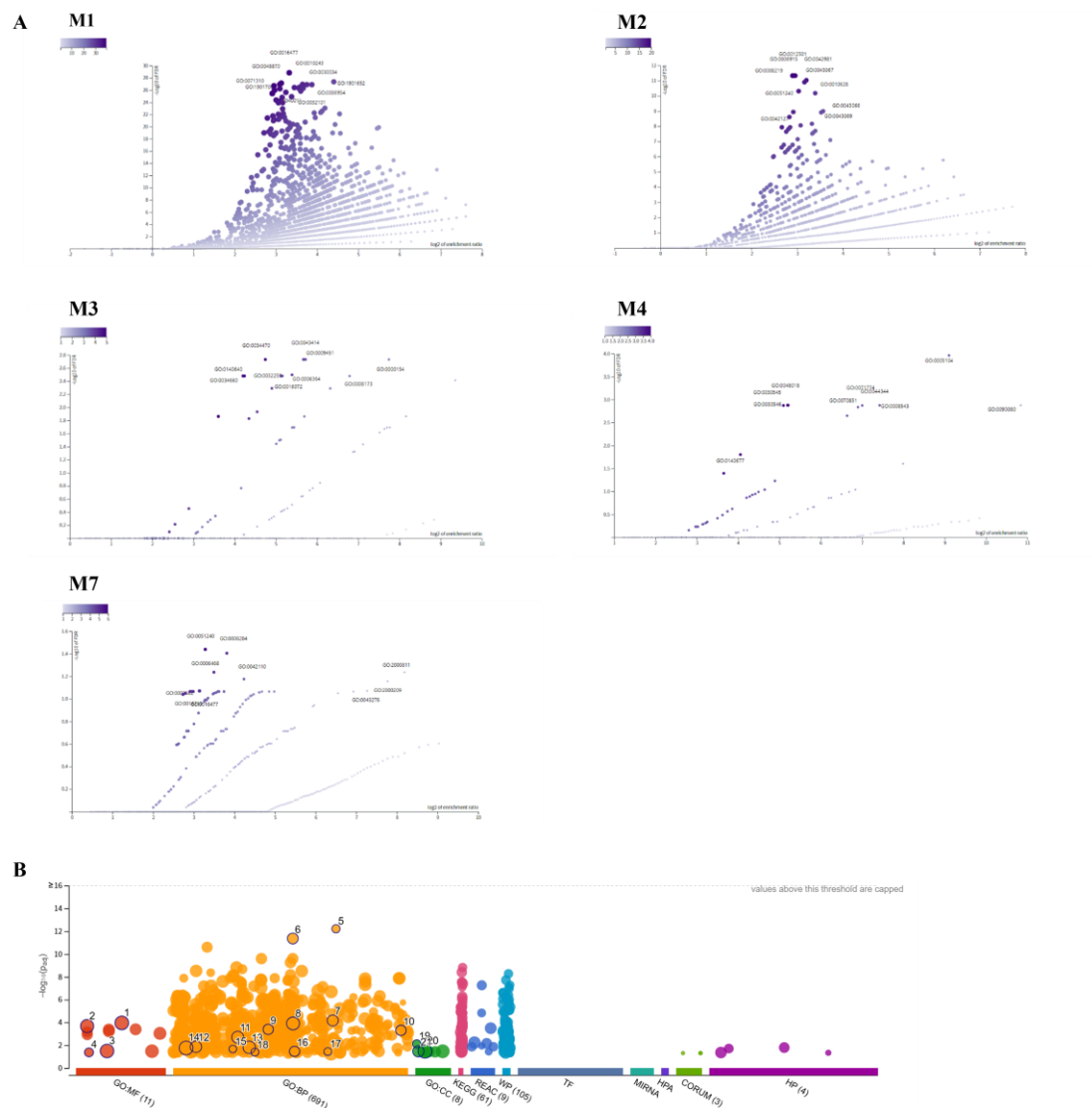


Figure 3 Go Enrichment analysis. (A) Volcano plots were used to visualize enriched biological processes (GO-BP term) M1-M7 modules, via WebGestalt (FDR<0.05). (B) Enriched GO of hub gene via g: Profiler.

3.3 Hub Gene Identification and Functional Analysis

Based on topological properties assessed by the CytoHubba plugin using the MCC ranking algorithm, ten hub genes were identified: IL6, TNF, ALB, IL1B, AKT1, STAT3, INS, PPARG, TLR4, and TGFB1. Their interaction network is shown in Figure 4. The roles of these hub genes are summarized as follows:

STAT3 (Signal Transducer and Activator of Transcription 3) encodes a key transcription factor and central effector of the JAK-STAT signaling pathway. Upon stimulation by cytokines such as IL-6 and IL-10 [35], STAT3 transduces signals from the cell membrane to the nucleus, where it regulates the expression of downstream target genes. Its activation involves phosphorylation-induced dimerization and nuclear translocation, enabling direct involvement in critical biological processes including cell proliferation, apoptosis, immune regulation, metabolic homeostasis, and angiogenesis. Representative target genes of STAT3 encompass anti-apoptotic factors (e.g., Bcl-2) [36], proliferation drivers (e.g., C-MYC) [37], immune modulators (e.g., IL-6, SOCS3) [35, 38], and metabolism-related genes (e.g., GLUT1) [39], highlighting its pleiotropic role in cellular physiology.

ALB: Serum albumin has been suggested as a protective factor and potential predictor for osteoporosis and related fractures [40].

IL1B (Interleukin 1 beta): A pro-inflammatory cytokine mediating chronic inflammatory responses in both OP and SP [41, 42].

AKT1: AKT1 functions as a critical signaling node that links environmental exposures (e.g., bisphenol A) to bone metabolism^[43]. Dysregulation of AKT1—through mutations, reduced phosphorylation, or pathway inhibition—can disrupt bone homeostasis at multiple levels. This positions AKT1 as a central pathogenic mediator in diverse forms of osteoporosis, including age-related, postmenopausal, inflammatory, and metabolic disease-associated bone loss.

TGFB1 (transforming growth factor, beta 1) is a multifunctional cytokine implicated in both skeletal muscle regeneration^[44] and bone metabolism. Accumulating evidence indicates that TGFB1 signaling is closely associated with the pathogenesis and progression of osteoporosis^[45], underscoring its potential role in the interrelated pathophysiology of muscle and bone.

INS encodes insulin, which bidirectionally modulates osteoblast and osteoclast activity via the insulin signaling pathway^[44]. Dysfunctional INS signaling—resulting from insulin resistance, genetic mutations, or age-related decline—disrupts bone homeostasis across molecular, cellular, and systemic levels, thereby constituting a central pathogenic mechanism in osteoporosis associated with diabetes and aging.

PPARG encodes peroxisome proliferator-activated receptor gamma (PPAR- γ), a key nuclear receptor involved in lipid metabolism and inflammation. In the context of glucocorticoid (GC)-induced osteoporosis, the PPAR- γ /SFRP5 axis has been shown to regulate osteogenic differentiation by modulating the Wnt/ β -catenin signaling pathway^[46]. In skeletal muscle, inhibition of PPAR- γ leads to reduced expression of fatty acid oxidation (FAO)-related proteins. This abolishes the beneficial effects of STING inhibition on mitochondrial dynamics (including mitofission and mitophagy) and insulin resistance (IR) in palmitate-treated myotubes, ultimately promoting muscle fiber atrophy^[47]. Thus, PPARG dysfunction may contribute concurrently to bone loss and muscle wasting through distinct yet interconnected metabolic pathways.

TLR4 encodes a pattern recognition receptor that has been implicated in both bone and muscle pathology. Studies indicate that TLR4 activation inhibits the osteogenic differentiation of human bone marrow mesenchymal stem cells, thereby contributing to osteoporosis^[48]. Concurrently, in conditions such as multiple myeloma, TLR4 signaling enhances inflammatory responses and promotes myotube atrophy^[49]. These findings position TLR4 as a shared mediator of bone loss and muscle deterioration, likely through the amplification of pro-inflammatory pathways.

TNF: The TNF (tumor necrosis factor) gene encodes a master regulatory cytokine that orchestrates inflammatory and immune responses, playing a pivotal role in a wide spectrum of both physiological and pathological processes.

IL6 (Interleukin 6): A pleiotropic cytokine with critical roles in inflammation, autoimmunity, and bone loss, whose role is critically shaped by the immunological context^[50, 51]. Notably, chronic inflammatory responses driven by cytokines, particularly IL-6, are central to the pathogenesis of both osteoporosis (OP)^[52] and sarcopenia (SP)^[53].

STAT3 plays a fundamental regulatory role in core cellular processes, including growth, differentiation, and apoptosis, and its dysregulation is strongly implicated in inflammatory and immune pathologies^[54]. The translational relevance of the identified hub genes is supported by external databases. Querying the Therapeutic Target Database (TTD) confirmed TNF and IL6 as established prognostic biomarkers for RA (Biomarker IDs: BM001851, BM001852, BM002809)^[55]. Furthermore, an analysis using the DisGeNET database (v7.0) verified that all ten hub genes in our network are documented biomarkers for osteoporosis (OP). Notably, six of these (TNF, IL6, IL1B, PPARG, TLR4, and TGFB1) are also associated with sarcopenia (SP)^[56], underscoring their shared relevance. Reliable diagnostic biomarkers are crucial for the early and accurate identification of complex, multifactorial diseases like OP and SP, enabling timely intervention^[57]. Therefore, the hub genes prioritized in this study represent strong candidates for dual-purpose screening or diagnostic biomarkers. Moreover, their central network position makes them attractive targets for therapeutic development. Nevertheless, further experimental and clinical studies are required to validate their biomarker utility and therapeutic potential.

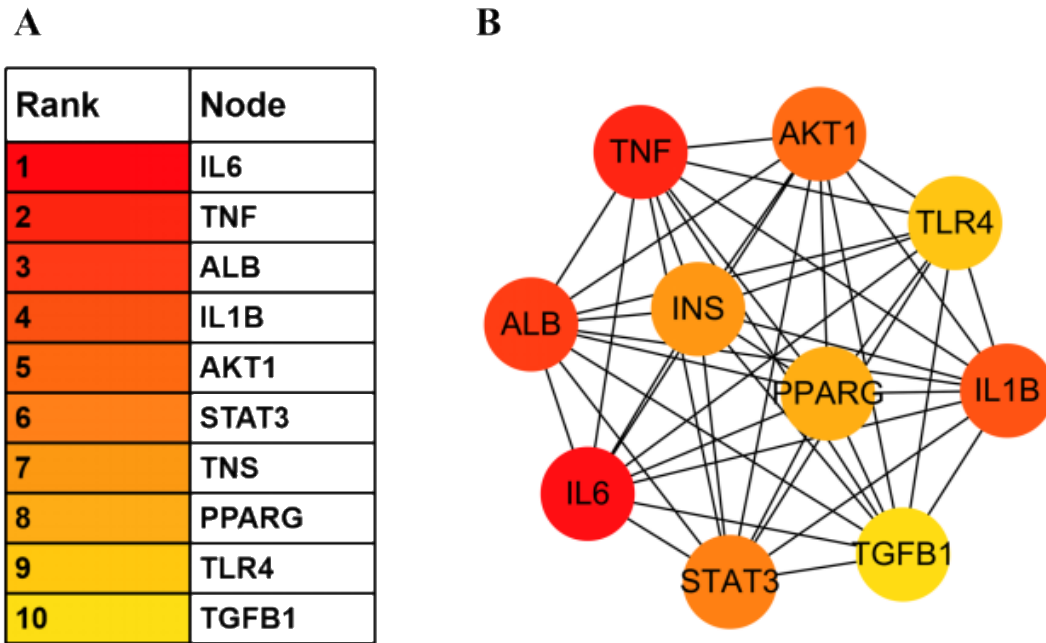


Figure 4 Identification of hub genes. (A) RA-OP hub genes based on the MCC ranking method in Cytohubba plug-in. (B) The rank of hub genes list.

3.3.1 Functional Enrichment and Pathway Analysis of Hub Genes

Gene Ontology (GO) analysis was performed to elucidate the biological functions, molecular activities, and cellular localizations associated with the identified hub genes. Enrichment analysis of GO terms (Biological Process, BP; Molecular Function, MF; Cellular Component, CC) was conducted using g:Profiler, with the results summarized in Figure 3B and the top 10 enriched terms in each category detailed in Table 2.

GO enrichment revealed that the hub genes are significantly involved in key biological processes, including inflammatory response to wounding (GO:0090594), regulation of inflammatory response (GO:0042063), defense response (GO:0031347), regulation of cell development (GO:0060284), and glial cell differentiation (GO:0010001). At the molecular level, the most enriched functions were identical protein binding (GO:0042802), signal receptor binding (GO:0005102), receptor ligand activity (GO:0048018), signal receptor activator/regulator activity (GO:0030546, GO:0030545), cytokine activity (GO:0005125), and molecular function regulator activity (GO:0140677). Cellular component analysis indicated that these genes are predominantly localized to the phagocytic cup (GO:0001891).

KEGG pathway analysis further demonstrated that the hub genes are enriched in several disease-related and metabolic pathways. The most significant pathways included Systemic Lupus Erythematosus (KEGG:05321), Type 2 Diabetes Mellitus (KEGG:04932), the AGE-RAGE signaling pathway in diabetic complications (KEGG:04933), Chagas disease (KEGG:05142), and Lipid and atherosclerosis (KEGG:05417).

Table 2: Top 10 GO terms of hub genes.

GO ID	GO terms	Description	Count	P-adj
GO:0090594	BP	inflammatory response to wounding	6	6.022×10^{-13}
GO:0051091	BP	positive regulation of DNA-binding transcription factor activity	8	4.301×10^{-12}
GO:0010001	BP	glial cell differentiation	8	2.523×10^{-11}
GO:0042063	BP	gliogenesis	8	2.532×10^{-10}
GO:0051090	BP	regulation of DNA-binding transcription factor activity	8	2.532×10^{-10}
GO:0050727	BP	regulation of inflammatory response	8	5.498×10^{-10}
GO:0031347	BP	regulation of defense response	9	1.487×10^{-9}
GO:0032755	BP	positive regulation of interleukin-6 production	6	2.117×10^{-9}
GO:0006954	BP	inflammatory response	9	3.018×10^{-9}
GO:0060284	BP	regulation of cell development	9	3.148×10^{-9}

GO:0042802	MF	identical protein binding	8	1.103×10^{-4}
GO:0005102	MF	signaling receptor binding	7	2.081×10^{-4}
GO:0048018	MF	receptor ligand activity	5	4.030×10^{-4}
GO:0030546	MF	signaling receptor activator activity	5	4.268×10^{-4}
GO:0030545	MF	signaling receptor regulator activity	5	5.934×10^{-4}
GO:0005125	MF	cytokine activity	4	6.571×10^{-4}
GO:0140677	MF	molecular function activator activity	6	9.149×10^{-4}
GO:0005126	MF	cytokine receptor binding	4	1.162×10^{-3}
GO:0019899	MF	enzyme binding	6	3.190×10^{-2}
GO:0098772	MF	molecular function regulator activity	6	3.320×10^{-2}
GO:0001891	CC	phagocytic cup	2	7.630×10^{-3}
GO:0031410	CC	cytoplasmic vesicle	6	3.376×10^{-2}
GO:0097708	CC	intracellular vesicle	6	3.421×10^{-2}
GO:0005788	CC	endoplasmic reticulum lumen	3	3.423×10^{-2}
GO:0034774	CC	secretory granule lumen	3	3.720×10^{-2}
GO:0060205	CC	cytoplasmic vesicle lumen	3	3.823×10^{-2}
GO:0031983	CC	vesicle lumen	3	3.858×10^{-2}
GO:0031093	CC	platelet alpha granule lumen	2	4.421×10^{-2}

GO ID: Gene ontology ID from g: Profiler web server; GO term: PB = Biological Processes, MF = Molecular Function, CC = Cellular Component; Count: Number of hub genes in each term of GO.

3.3.2 Construction of a Bipartite Hub Gene-miRNA Network

Potential microRNA (miRNA) regulators of the hub genes and those associated with OP or SP were predicted using miRWalk and miRNet, respectively. A Venn diagram (Fig. 5) identified 10 miRNAs common to both diseases, six of which—*hsa-miR-212-3p*, *hsa-miR-501-5p*, *hsa-miR-6851-5p*, *hsa-miR-204-5p*, *hsa-miR-4521*, and *hsa-miR-6792-3p*—were also predicted to target the hub genes (Table 3). These findings suggest a potential regulatory link between these miRNAs and the core network of OP-SP pathogenesis. A bipartite hub gene-miRNA interaction network was subsequently visualized using Cytoscape (Fig. 6).

It is important to note that while the PPI network integrates known biological interactions, the hub genes were prioritized primarily based on topological properties (e.g., the MCC algorithm). The identification of shared miRNAs that preferentially target these topologically central genes reinforces their biological relevance and provides a post-transcriptional regulatory context. Therefore, these hub genes represent promising candidates for further investigation into disease mechanisms, prognostic biomarker development, and therapeutic targeting. Further experimental studies are warranted to validate the regulatory roles of these miRNAs and their utility as biomarkers bridging OP and SP.

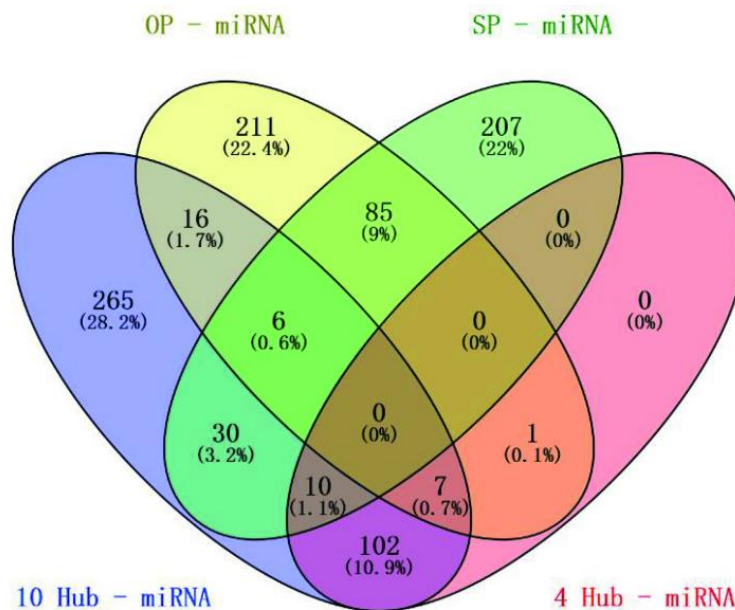


Figure 5 Venn diagram of the miRNAs.

Table 3 List of the common miRNAs related to hub genes and RA-OP disease.

miRNA	Hub Genes
hsa-miR-212-3p	IL6, TNF, ALB, AKT1, INS
hsa-miR-501-5p	IL6, TNF, AKT1, STAT3, INS, PPARG, TLR4
hsa-miR-6851-5p	IL6, TNF, ALB, AKT1, INS, PPARG, TLR4
hsa-miR-204-5p	IL6, TNF, STAT3, INS
hsa-miR-4521	IL6, TNF, STAT3, INS, PPARG, TLR4, TGFB1
hsa-mi-6792-3p	TNF, ALB, STAT3, INS

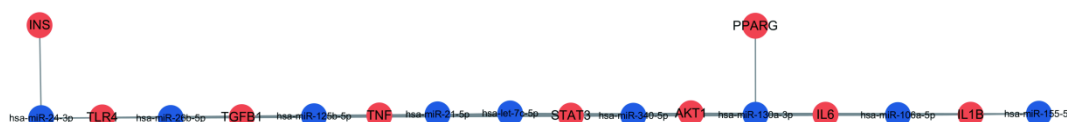


Figure 6 The bipartite hub gene-miRNAs interaction network.

3.3.3 Construction of the Drug-Target Interaction Network

To identify candidate drugs for repurposing, we focused on hub genes enriched in two key KEGG pathways relevant to OP-SP pathogenesis: the “MAPK signaling pathway” (KEGG:04068, involved in cellular stress and proteostasis) and “Osteoclast differentiation” (KEGG:04380).

Genes associated with these pathways were prioritized for drug targeting. Within the MAPK signaling pathway, three hub genes (TNF, IL6, and AKT1) were enriched. The osteoclast differentiation pathway contained five hub genes (TNF, IL1B, AKT1, TLR4, and IL6). Notably, four hub genes—TNF, IL1B, IL6, and AKT1—were central to both pathways and ranked among the highest in the overall hub gene list, underscoring their central role in the shared pathogenesis of OP and SP, particularly in the process of osteoclast differentiation. The four genes were thus prioritized as therapeutic targets.

Osteoclasts, the principal bone-resorbing cells, are derived from monocyte-macrophage lineage precursors and are essential for bone remodeling. Their differentiation and activation are central to bone metabolism and can be influenced by mediators such as the neuropeptide Substance P (SP) [58]. Mature osteoclasts secrete specific enzymes, including cathepsin K, matrix metalloproteinase 9 (MMP9), and tartrate-resistant acid phosphatase (TRAP), which degrade the bone matrix. In pathological states, osteoclasts are typically localized to sites of active bone resorption, such as the periosteum and joint margins.

Querying the Drug-Gene Interaction Database (DGIDB) with these four genes yielded 265 potential drug candidates. This initial list was refined by excluding drugs with primary indications unrelated to musculoskeletal or inflammatory diseases. Subsequent evaluation identified 20 promising candidates (Table 4).

Among these, nine drugs (Golimumab, Adalimumab, Certolizumab Pegol, Siltuximab, Infliximab, Etanercept, Rilonacept, Canakinumab, and Capivasertib) are already FDA-approved, primarily for inflammatory conditions often associated with bone loss (e.g., rheumatoid arthritis), supporting their repurposing potential for OP and, by shared target mechanism, for SP.

Table 4 List of the drugs interacting with target hub genes.

Genes	Drugs
TNF	NERELIMOMAB, CROMOLYN SODIUM, PLACULUMAB, METHYLENE BLUE, ALTEPLASE, CARBOPLATIN, GENTAMICIN, GOLIMUMAB, BUPIVACAINE, PENTOXIFYLLINE, BENZO[E]PYRENE, THALIDOMIDE, PYRAZINAMIDE, GLIMEPIRIDE, OMEPRAZOLE, LENERCEPT, ADALIMUMAB, PRAZOSIN HYDROCHLORIDE, REMTOLUMAB, MIDOSTAURIN, LENABASUM, MAGNESIUM SULFATE ANHYDROUS, PICIBANIL, FLUOCINOLONE ACETONIDE, MILTEFOSINE, HOMIDIUM BROMIDE, OZORALIZUMABPEGSUNERCEPT, CERTOLIZUMAB PEGOL, THERAPEUTIC HORMONE, NAFAMOSTAT, FOLIC ACID, ABBV-257, RISPERIDONE, COVA322,

	HYDROXYCHLOROQUINE,CERTOLIZUMAB, AN0128, 5,7-DIHYDROXY-4-METHYLCOUMARIN, SORAFENIB,AFELIMOMAB, PLACULUMAB, INFLIXIMAB, DIDANOSINE, ISIS 104838,SOBLIDOTIN, SPIRONOLACTONE,MEROPENEMANHYDROUS,ETHAMBUTOLHYDROCHLORIDE,MYCOPHENOLATE MOFETIL, MASOPROCOL, DERSALAZINE, AVX-470, AME-527,CYCLOSPORINE, ONERCEPT, CYT007-TNFQB, PROPYLTHIOURACIL, TIMOLOL MALEATE,ABBV-3373, METHIMAZOLE, PF-04236921, LACTULOSE, HALOFUGINONE,PROTON PUMP INHIBITOR, MITOMYCIN C ANALOG KW-2149, ATORVASTATIN CALCIUM TRIHYDRATE,DIGOXIN, PROCARBAZINE HYDROCHLORIDE, LAPACHONE, ORTATAXEL, IMMUNOSTIMULANT,RABEPRAZOLE, RIFAMPIN, PNEUMOCOCCAL POLYVALENT VACCINE, CEFOTAXIME SODIUM,ETANERCEPT, AZ9773, TETRANDRINE, REMTOLUMAB, CELASTROL, LENALIDOMIDE,HUANG LIAN, ANESTHETIC AGENT, STAVUDINE, CARBAMAZEPINE, AMPHOTERICIN B LIPOSOMAL,INSULIN, REGULAR, HUMAN,BACILLUS CALMETTE-GUERIN ANTIGEN, RUTIN,VITAMIN B6, MIDAZOLAM HYDROCHLORIDE, GEMCITABINE
IL1B	ASPIRIN, MAFOSFAMIDE, DIACETYLRHEIN, INFLIXIMAB, LANSOPRAZOLE TILUDRONIC ACID, GEVOKIZUMAB, LUTIKIZUMAB, CELASTROL, CYTARABINE,RILONACEPT, TT-301,METFORMIN, ANTICONVULSANT AGENT, ANTIDEPRESSANT AGENT,ACITRETIN, ERYTHROMYCIN, ALTEPLASE, OFLOXACIN, PENTOXIFYLLINE,USTEKINUMAB, LITHIUM, RALOXIFENE HYDROCHLORIDE, MITOGEN-ACTIVATED PROTEIN KINASE INHIBITOR, DRUGSATFDA.NDA:008378, NICARDIPINE, GLUCOSAMINE,BECLOMETHASONE DIPROPIONATE, PAREGORIC, CEFACLOX ANHYDROUS, IBUDILAST,MELATONIN, PENTAMIDINE, PRAVASTATIN SODIUM, VERAPAMIL, THYROGLOBULINRISEDRONIC ACID, AUTOANTIGEN, CDP-484, ETIDRONIC ACID, CHINESE HERBS,CANAKINUMAB, RESVERATROL, NIMUSTINE, FLUTICASONE, GALLIUM NITRATEHYDROQUINONE, POLYVALENT VACCINE, CLODRONIC ACID, ECHINACEA PREPARATION,HYDROCORTISONE BUTYRATE
IL6	CISPLATIN, ZILTIVEKIMAB, CHINESE HERBS, PF-04236921, MRA 003 US,SIGNAL TRANSDUCTION INHIBITOR, BINETRAKIN, IBUDILAST, INSULIN, REGULAR,HUMANSILTUXIMAB, METRONIDAZOLE, CLAZAKIZUMAB, GALLIUM NITRATE,INTERFERON ALFA-2B, DULOXETINE HYDROCHLORIDE, MEDI-5117, GEMFIBROZIL,BIAFINE CREAM, ETANERCEPT, ELSILIMOMAB, SAQUINAVIR, INFLIXIMAB,LINEZOLID, IFOSFAMIDE, OLOKIZUMAB, HMG-COA REDUCTASE INHIBITOR,MIDOSTAURIN, SELENIUM, SIRUKUMAB, PEGINTERFERON ALFA-2B,ECHINACEA PREPARATION, ENZYME INHIBITOR, FENOFIBRATE MICRONIZED, VITAMIN K,PEGINTERFERON ALFA-2A, RIBAVIRIN, VITAMIN A PALMITATE, ARSENIC TRIOXIDE,DIHYDROSPHINGOSINE, LEVOFLOXACIN ANHYDROUS, YSIL6, NELFINAVIR,RITUXIMAB, CLAZAKIZUMAB, SELECTIVE ESTROGEN RECEPTOR MODULATOR,IMIPENEM-CILASTATIN SODIUM, FENTANYL CITRATE, ZILTIVEKIMAB, ADALIMUMAB
AKT1	TAS0612, GEDATOLISIB, IPATASERTIB, EVEROLIMUS, PHA-767491,OMIPALISIB, GSK-690693, BARDOXOLONE METHYL, GSK-269962A, MK-2201,UPROSERTIB, CANERTINIB DIHYDROCHLORIDE, ANDROGRAPHOLIDE, WORTMANNIN,XL-418, BAY-1125976, CHEMBL:CHEMBL1086397,DESIPRAMINE,NELFINAVIR,RESVERATROL, AKT INHIBITOR

VIII, MIRANSERTIB, PERIFOSINE, VEMURAFENIB, AFURESERTIB, CANTHIN-6-ONE, PAN-AKT INHIBITOR ARQ751, SR-13668, CHEMBL:CHEMBL480356, FEXAGRATINIB, ENZASTAURIN, PIFUSERTIB, CYC-116, CLOMIPRAMINE, DOXEPIN HYDROCHLORIDE, LY-2780301, TRIMIPRAMINE, XL418, LOVASTATIN, TOPOTECAN HYDROCHLORIDE, CEP-11981, GAMBOGIC AMIDE, AFURESERTIB HYDROCHLORIDE, NORTRIPTYLINE, GIGANTOL, DABRAFENIB, (2S)-1-(1H-INDOL-3-YL)-3-([5-(3-METHYL-1H-INDAZOL-5-YL)PYRIDIN-3-YL]OXY)PROPAN-2-AMINE, FASUDIL, FULVESTRANT, NINTEDANIB ESYLATE, ARCHEXIN, CMX-2043, AMITRIPTYLINE HYDROCHLORIDE, SOTRASTAUIN, 5-METHOXYCANTHINONE, MK-2206, CHEMBL:CHEMBL257158, WITHAFERIN A, LAUROGUADINE, BMS-754807, M-2698, CENISERTIB, AKT INHIBITOR MK2206, CI-1040, LY-294002, CAPIVASERTIB, TRICIRIBINE, TRIFLUOPERAZINE, EUPALININ A, MONOAMINE OXIDASE INHIBITOR, TRICIRIBINE PHOSPHATE

In the context of osteoclast activation and differentiation, a pro-inflammatory milieu—driven by cytokines such as IL-1 β , IL-6, TNF- α , and RANKL—promotes osteoclastogenesis and enhances bone resorption. A persistent inflammatory response is a key risk factor for low bone mineral density in OP. Accordingly, controlling inflammation is crucial for mitigating disease activity and inhibiting bone loss. Studies by Xu et al. reinforce that pivotal genes, including TNF, IL1B, IL6, and TGFB1, orchestrate processes spanning immune regulation, bone remodeling, and inflammatory responses. Indeed, inflammation in OP can activate osteoclasts via mediators like TNF- α , IL-1, IL-6, and the RANKL and Wnt/DKK1/sclerostin pathways, directly contributing to bone loss. Notably, IL-6 and its signaling pathway represent promising targets for treating inflammatory and autoimmune disorders^[52, 59].

Given the central role of these genes in inflammatory pathology, we identified 11 additional drugs—Certolizumab, Lenercept, SIRUKUMAB, OLOKIZUMAB, ELSILIMOMAB, GEVOKIZUMAB, LUTIKIZUMAB, IPATASERTIB, TRICIRIBINE, PERIFOSINE, and WORTMANNIN—with well-defined anti-inflammatory mechanisms. While not currently FDA-approved for OP, their potent immunomodulatory profiles support their investigation for SP. Since suppressing inflammation is a cornerstone therapeutic strategy for both conditions, these agents represent viable candidates for the combined management of OP and SP (Table 5).

Table 5 List of the proposed drugs.

Genes	Group 1	Group 2
TNF	PEGOL, SILTUXIMAB, INFLIXIMAB	Certolizumab, Lenercept
IL1B	ETANERCEPT, RILONACEPT	GEVOKIZUMAB, LUTIKIZUMAB
IL6	GOLIMUMAB, ADALIMUMAB, CERTOLIZUMAB	SIRUKUMAB, OLOKIZUMAB, ELSILIMOMAB
AKT1	CANAKINUMAB, INFLIXIMAB, CAPIVASERTIB	IPATASERTIB, TRICIRIBINE, PERIFOSINE, WORTMANNIN

A bipartite drug-target network was constructed to visualize these interactions (Figure 7). This network-based approach enables the systematic exploration of known or novel therapies that target shared OP-SP pathways. Identifying and repurposing drugs that act on common key genes offers a rational and efficient strategy for developing novel treatments for OP and SP.

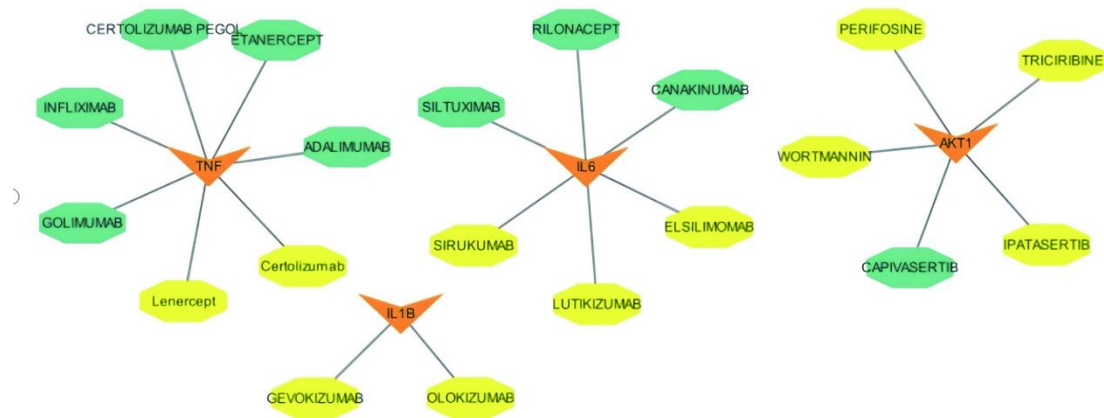


Figure 7 The drug-target genes interaction network constructed by Cytoscape. Orange points represent target hub genes, green points represent the candidate drug for repurposing for OP, and yellow points represent the candidate drugs for repurposing for both SP and OP.

4. Conclusion

Osteoporosis (OP) and sarcopenia (SP) are complex multifactorial disorders whose growing clinical association has garnered significant research interest. OP is a systemic skeletal disease characterized by reduced bone mineral density, often driven by chronic inflammatory processes. SP involves the progressive loss of skeletal muscle mass, strength, and function, leading to increased fall risk and diminished quality of life. Beyond genetic predisposition, epigenetic regulation and lifestyle factors contribute critically to the pathogenesis of both conditions.

This study employed an integrated bioinformatics approach to explore shared molecular mechanisms and identify drug repurposing opportunities for OP and SP. Genes associated with each disease were curated, and a combined OP-SP protein-protein interaction (PPI) network was constructed and analyzed using STRING and Cytoscape. Hub genes and functional modules were identified and functionally characterized via WebGestalt and g: Profiler. Candidate drugs and regulatory miRNAs were retrieved using DGIDB, miRWalk, and miRNet.

Network clustering revealed seven significant modules, with the largest and most connected module (M1) heavily enriched for immune system processes. Ten hub genes were prioritized: IL6, TNF, ALB, IL1B, AKT1, STAT3, INS, PPARG, TLR4, and TGFB1. These genes are predominantly involved in inflammatory responses, cytokine receptor binding, and were notably localized to the phagocytic cup. Screening of drug-gene interactions yielded 20 repurposable candidates. Among these, nine drugs (GOLIMUMAB, ADALIMUMAB, CERTOLIZUMAB PEGOL, SILTUXIMAB, INFLIXIMAB, ETANERCEPT, RILONACEPT, CANAKINUMAB, and CAPIVASERTIB) are already FDA-approved for inflammatory conditions related to OP and target pathways central to osteoclast differentiation, making them strong candidates for SP. An additional 11 drugs (Certolizumab, Lenercept, SIRUKUMAB, OLOKIZUMAB, ELILIMOMAB, GEVOKIZUMAB, LUTIKIZUMAB, IPATASERTIB, TRICIRIBINE, PERIFOSINE, and WORTMANNIN) were selected for their potent anti-inflammatory effects and represent novel candidates for the combined management of OP and SP, given the central role of inflammation in both diseases.

Notably, this work not only provides a list of core shared genes but also offers a network perspective and mechanism-driven drug repurposing blueprint, advancing our understanding of bone-muscle crosstalk. However, several limitations should be acknowledged. First, the study relies solely on public transcriptome data and bioinformatics predictions, lacking experimental validation of hub gene functions and miRNA regulatory roles in clinical samples or in vitro/in vivo models. Second, the analysis did not account for the interactive effects of epigenetic modifications (e.g., DNA methylation, histone acetylation) and environmental factors (e.g., diet, exercise) on the shared pathogenic network. Third, drug repurposing was based on molecular interaction data without considering pharmacokinetic/pharmacodynamic properties or potential off-target effects. Fourth, the PPI network did not distinguish cell-type-specific gene expression patterns, which may obscure tissue-specific mechanisms in bone and muscle.

To address these limitations and extend the findings, future research will focus on the following directions: 1) Collect clinical samples from OP, SP, and comorbid patients to validate the expression levels of hub genes (IL6, TNF, AKT1, etc.) and candidate miRNAs (hsa-miR-204-5p, hsa-miR-212-3p) via quantitative real-time PCR and Western blotting. 2) Perform in vitro experiments using osteoblasts, myoblasts, and bone marrow mesenchymal stem cells to verify the regulatory roles of hub genes in cell proliferation, differentiation, and apoptosis. 3) Conduct in vivo studies using age-related OP/SP mouse models or ovariectomized mice to evaluate the therapeutic efficacy of candidate drugs (e.g., Sirukumab, Ipatasertib) on bone mineral density and muscle function. 4) Integrate single-cell RNA sequencing data to dissect cell-type-specific molecular mechanisms underlying OP-SP crosstalk. 5) Incorporate epigenetic data (e.g., DNA methylation profiles) and gut microbiota sequencing to construct a more comprehensive multi-omics regulatory network. 6) Collaborate with clinical teams to design pilot clinical trials for repurposed drugs, assessing their safety and efficacy in comorbid patients.

In summary, this study delineates a shared inflammatory network underlying OP and SP pathogenesis and proposes mechanistically supported drug candidates for repurposing. The identified limitations and targeted future plans enhance the rigor of the work, while the proposed research directions may facilitate the translation of bioinformatics findings into clinical applications, ultimately improving the diagnosis and treatment of OP and SP.

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