

Investigation of key pathogenic mechanisms and potential intervention targets of *Eucommia ulmoides* in the treatment of sarcopenia based on network pharmacology and molecular docking technology

Yuejiao Zhong¹, Zhaoshi Bai², Qiang Wei³, Bin Zhou⁴, Siwen Liu¹, Lingxiang Chen¹ (✉)

¹Department of Medical Oncology, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing 210009, China

²Department of pharmacy, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing 210009, China

³Department of ultrasonic, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing 210009, China

⁴Department of General surgery, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing 210009, China

Received: 21 April 2025 | Revised: 6 June 2025 | Accepted: 26 June 2025

ABSTRACT

This study aims to identify molecular targets and potential mechanisms of action of *Eucommia ulmoides* in the treatment of sarcopenia through network pharmacology and bioinformatics analysis. The active components of *E. ulmoides* with a drug similarity score of ≥ 0.18 and an oral bioavailability of $\geq 30\%$ were screened from the TCMSp platform, and the target genes of active compounds were determined from the DrugBank database. Using the lateral femoral muscle sample dataset GSE1428 from the GEO database, differentially expressed genes (DEGs) were identified using GEO2R with $|\log FC| > 0.5$ and $p < 0.05$ as the criteria, and candidate molecular targets were determined through Venn diagrams. Enrichment analyses of the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were carried out. Protein-protein interaction (PPI) networks and transcription factor (TF)-DEG-miRNA networks were constructed using the STRING tool and DMNC algorithm to screen key genes. Twenty-eight active compounds of *E. ulmoides*, involving 217 genes, were identified in the TCMSp database. The Venn diagram presents 13 common genes. KEGG pathway analysis showed that differentially expressed genes (DEGs) were mainly enriched in multiple signaling pathways. Finally, the key module and key gene HSPB1 were determined through PPI network and TF-DEG-miRNA network analysis. Molecular docking showed that the affinity of HSPB1 with quercetin was -9.1 , and the binding force was strong. HSPB1 can be used as a novel predictive biomarker for sarcopenia. *E. ulmoides* may exert therapeutic effects on sarcopenia through targeting HSPB1 via multiple pathways.

KEYWORDS

traditional Chinese medicine, *Eucommia ulmoides*, sarcopenia, HSPB1, network pharmacology, molecular docking, bioinformatics

1 Introduction

With the aging population in China, health problems among the elderly have become increasingly prominent [1]. Among these, muscle strength and muscle mass tend to decrease with age starting in middle adulthood [2]. Studies have shown that muscle strength in the legs decreases by 1.5%–5% annually

and muscle mass decreases by 1%–2% each year in individuals over 50 years old, with muscle mass potentially decreasing by 15% every decade after the age of 70, and some individuals eventually developing sarcopenia [3, 4]. Sarcopenia, also known as muscle wasting syndrome, is a progressive and systemic skeletal muscle disease characterized by the accelerated loss of muscle mass and function [5]. According to

© The Author(s) 2025. *Aging Research* published by Tsinghua University Press. The articles published in this open access journal are distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

✉ Address correspondence to Lingxiang Chen, bg4wfc@126.com

Cite this article as Zhong, Y. J., et al. *Aging Research*, 2025, 3: 9340050.

the Asian Sarcopenia Working Group, the prevalence of sarcopenia in elderly Chinese populations (average age = 72 years) is 3.1%. Sarcopenia increases the risk of falls, fractures, disability, and death [6]. Current conventional pharmaceutical treatments for sarcopenia include neurotrophic drugs, corticosteroid replacement therapy, insulin therapy, and glucocorticoid replacement therapy [7]. However, the efficacy of these treatments is limited due to the risk of drug side effects, uncertainties in treatment outcomes, the limitations of drug use, and economic burdens. Traditional Chinese medicine (TCM) has played a widespread and important role in the treatment of non-neoplastic diseases. It works by regulating imbalanced physiological functions, enhancing the body's self-repair capacity, and improving overall symptoms, effectively alleviating various non-neoplastic diseases and improving the quality of life for patients.

From the perspective of TCM theory, sarcopenia can be classified under terms such as “wei syndrome” or “xu lao”, with its pathogenesis closely related to deficiencies in the spleen and kidney, insufficient qi and blood, and malnutrition of the muscles and tendons [8]. Muscle loss reflects not only the depletion of the body's material foundation but also the decline in organ function and insufficient qi and blood generation. Therefore, TCM treatment for sarcopenia follows the principle of pattern differentiation and treatment, using herbs to tonify the spleen and kidney, replenish qi and blood, strengthen muscles and bones, and restore organ function, ultimately increasing muscle mass, improving muscle function, and enhancing overall physical strength [9, 10]. *Eucommia ulmoides*, a traditional Chinese medicinal herb, has sweet and warm properties and is considered as nourishment to the liver and kidney. It is known for its effects in tonifying the liver and kidney, strengthening the muscles and bones, and regulating the Chong and Ren meridians [11]. Based on pharmacological analysis, *E. ulmoides* exhibits multiple bioactivities, including promoting protein synthesis, enhancing muscle strength, improving bone density, antioxidant activity, and immune modulation [12]. The choice to study *E. ulmoides* in the treatment of sarcopenia is based on the integration of TCM theory and the pharmacological effects of *Eucommia*. Its ability to tonify the liver and kidney and strengthen muscles and bones directly targets the pathogenesis of sarcopenia according to TCM theory. By promoting protein synthesis and enhancing muscle strength, *E. ulmoides* offers a unique advantage for treating sarcopenia. Through systematic investigation of its pharmacological mechanisms and specific applications in sarcopenia, this research aims to open up a new therapeutic approach for patients with sarcopenia. This study, based on network pharmacology, explores the role of *E. ulmoides* in the treatment of sarcopenia and uses bioinformatics methods to preliminarily explore the underlying mechanistic networks, providing a reference for the development of new drugs and clinical applications based on *E. ulmoides*.

2 Results

2.1 Active compounds and target genes of *E. ulmoides*

In the TCMSP database, a total of 28 active compounds were

identified in *E. ulmoides* (as shown in Table 1). Additionally, target genes for these active compounds were obtained from the DrugBank database, resulting in 217 distinct target genes.

2.2 Screening of differentially expressed genes (DEGs)

DEGs were screened from the Gene Expression Omnibus (GEO) database dataset GSE1428, and volcano plots, PCA plots, heatmaps, and sample normalization box plots were constructed (Figs. 1A–1D). The target genes corresponding to the active compounds of *E. ulmoides* were intersected with the DEGs from the dataset, and a Venn diagram was generated, resulting in 13 candidate target genes. These genes are as follows: *DRD1*, *HTR2A*, *ADRA1B*, *BAX*, *INSR*, *DIO1*, *CXCL11*, *CDKN1A*, *HSPB1*, *NCF1*, *NFKBIA*, *ERBB2*, and *ALB* (Fig. 1E).

2.3 Functional enrichment analysis of DEGs

DEGs were significantly enriched in biological processes (BPs), including regulation of calcium ion chelation, phosphatidylinositol 3-kinase signaling, positive regulation of MAPK cascade, peptide tyrosine modification, response to temperature stimulus, adrenergic receptor signaling, regulation of synaptic vesicle exocytosis, establishment of protein membrane localization, glucose import, regulation of fibroblast proliferation, and p53-mediated intrinsic apoptotic signaling pathways (Fig. 2A). Cellular components (CC) were enriched in plasma membrane rafts, membrane rafts, membrane microdomains, protein kinase complexes, NADPH oxidase complexes, secondary lysosomes, transferase complexes that transfer phosphorus-containing groups, and cyclin-dependent protein kinase holoenzyme complexes (Fig. 2B). In molecular functions (MFs), DEGs were mainly involved in serine/threonine kinase inhibitor activity, G protein-coupled amine receptor activity, transmembrane receptor protein tyrosine kinase activity, protein kinase inhibitor activity, insulin receptor substrate binding, phosphatidylinositol-3,4-bisphosphate binding, cyclin-dependent serine/threonine kinase regulatory activity, DNA-binding transcription factor binding, copper ion binding, and postsynaptic neurotransmitter receptor activity (Fig. 2C). KEGG pathway enrichment analysis showed that DEGs were primarily enriched in the calcium signaling pathway, HIF-1 signaling pathway, chemokine signaling pathway, p53 signaling pathway, MAPK signaling pathway, Toll-like receptor signaling pathway, neurotrophin signaling pathway, PI3K-Akt signaling pathway, osteoclast differentiation, neuroactive ligand-receptor interaction, FoxO signaling pathway, and apoptosis (Fig. 2D). The top five results are shown in Table 2.

2.4 Construction of PPI network for DEGs and TF-DEGs-miRNA network

A protein-protein interaction (PPI) network was constructed using the STRING database to illustrate the interactions among proteins encoded by the DEGs (Fig. 3A). When there are hundreds of genes involved, it is necessary to use a plugin with topological network algorithms to assign values to each gene and rank them to identify key genes. Based on the DMNC algorithm, the most important module was selected,

Table 1 Active compounds of *E. ulmoides* in the TCMSP database (OB $\geq$ 30% and DL $\geq$ 0.18)

Mol ID	Molecule name	OB	DL
MOL002058	40957-99-1	57.2	0.61
MOL000211	Mairin	55.37	0.77
MOL000358	β -Sitosterol	36.91	0.75
MOL000422	Kaempferol	41.88	0.24
MOL004367	Olivil	62.22	0.4
MOL000443	Erythraline	49.17	0.55
MOL005922	Acanthoside B	43.35	0.76
MOL006709	AIDS214634	92.42	0.54
MOL007059	3- β -Hydroxymethylenetanshinone	32.16	0.4
MOL000073	ent-Epicatechin	48.95	0.24
MOL007563	Yangambin	57.52	0.8
MOL009007	Eucommin A	30.51	0.84
MOL009009	(+)-Medioresinol	87.18	0.61
MOL009015	(-)-Tabernemontanine	58.66	0.6
MOL009027	Cyclopamine	55.42	0.82
MOL009029	Dehydroiconiferyl alcohol 4, gamma'-di-O-beta-D-glucopyranoside_qt	51.44	0.39
MOL009030	Dehydrodieugenol	30.1	0.23
MOL009031	Cinchonan-9-al, 6'-methoxy-, (9R)-	68.21	0.4
MOL009038	GBGB	45.57	0.82
MOL009042	Helenalin	77.01	0.19
MOL009047	(+)-Eudesmin	33.28	0.62
MOL009053	4-[(2S,3R)-5-[(E)-3-hydroxyprop-1-enyl]-7-methoxy-3-methylol-2,3-dihydrobenzofuran-2-yl]-2-methoxy-phenol	50.75	0.39
MOL009055	Hirsutin_qt	49.81	0.37
MOL009057	liriodendrin_qt	53.13	0.79
MOL000098	Quercetin	46.43	0.27
MOL002773	β -Carotene	37.18	0.58
MOL008240	(E)-3-[4-[(1R,2R)-2-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-1-methylol-ethoxy]-3-methoxy-phenyl]acrolein	56.31	0.36
MOL011604	Syringetin	36.82	0.37

which includes eight genes (Fig. 3B), and their scores are shown in Table 3. Among them, HSPB1 had the highest score, indicating it is the most likely key gene to play a crucial role. To investigate the functional roles of the DEGs, we explored the potential regulatory relationships between DEGs, transcription factors (TFs), and miRNAs. The miRNA-DEG interactions were identified using the miRTarBase database for network analysis. The potential regulatory relationships between DEGs and TFs were identified based on TF binding site data and genetic coordinate information provided by ENCODE. The study did not find any miRNAs interacting with HSPB1, but the mRNAs interacting with HSPB1 are shown in Fig. 3C.

2.5 Molecular docking

In the process of screening DEGs, the candidate gene *HSPB1* was identified, which is present in the compound quercetin from the TCM *E. ulmoides*. The three-dimensional (3D) structure of quercetin was retrieved from the PubChem database, and the receptor protein structure of the key target was obtained from the Protein Data Bank (PDB) database. Molecular docking was conducted using the DockThor

platform. The study found that HSPB1 has an affinity of -9.1 with quercetin, indicating a strong binding affinity between the two. Further visualization of the docking interaction was performed using the CB-DOCK2 platform (<https://cadd.labshare.cn/cb-dock2/>) (Fig. 4).

3 Discussion

Sarcopenia is a complex degenerative disease characterized by the loss of muscle strength and function, with progressive muscle mass reduction as people age. It commonly occurs in the elderly and severely impacts physical health and quality of life [13]. TCM has long played an important role in the treatment of various non-cancer diseases. However, due to the multi-component and multi-target nature of TCM, elucidating its mechanisms of action is challenging. Network pharmacology, an emerging research method based on systems biology, provides a systematic approach to exploring the mechanisms of TCM and its multi-component, multi-target effects, offering new insights into TCM research [14].

In this study, we used network pharmacology and bioinformatics techniques to identify the core targets and

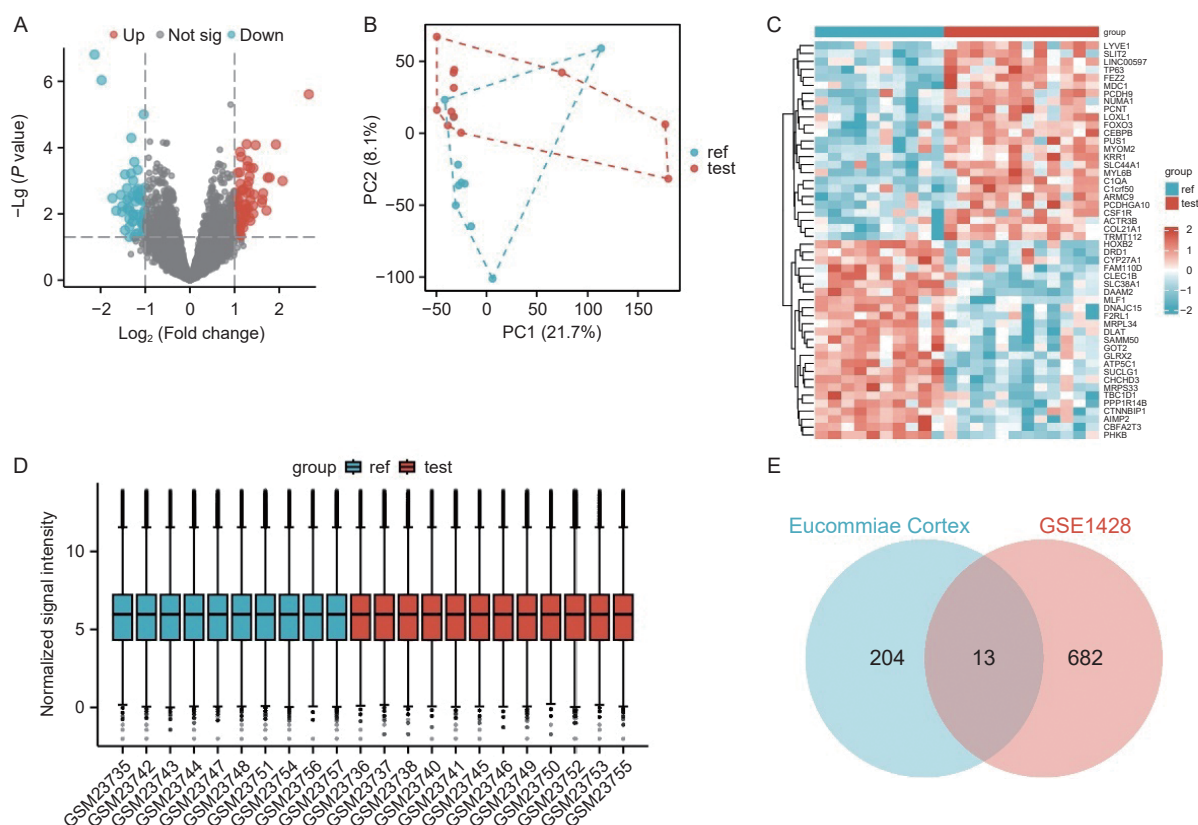


Figure 1 Screening of DEGs. (A) Volcano plot of dataset GSE1428, (B) PCA plot, (C) heatmap, and (D) sample normalization box plot; (E) Venn diagram of the target genes of active compounds in *E. ulmoides* intersected with DEGs from dataset GSE1428.

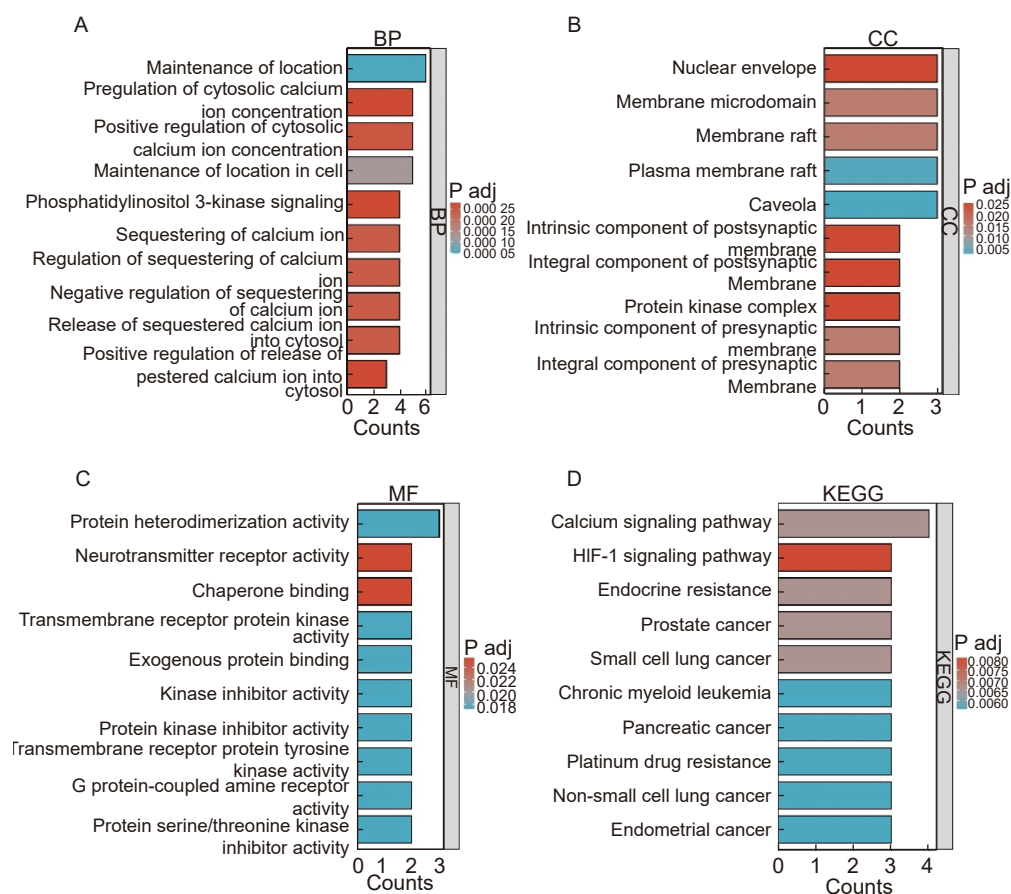


Figure 2 GO and KEGG enrichment analysis of differentially expressed genes (DEGs): (A) Enrichment results for biological processes. (B) Enrichment results for cellular components. (C) Enrichment results for molecular functions. (D) Enrichment results for KEGG signaling pathways.

Table 2 Top 5 results from GO and KEGG enrichment analysis

Ontology	ID	Description	GeneRatio	BgRatio	p value	Adjusted p value
BP	GO:0051235	Maintenance of location	6/13	331/18800	4.4e-08	4.61e-05
BP	GO:0051651	Maintenance of location in cell	5/13	219/18800	2.44e-07	0.0001
BP	GO:0051209	Release of sequestered calcium ion into cytosol	4/13	121/18800	1.12e-06	0.0002
BP	GO:0051283	Negative regulation of sequestering of calcium ion	4/13	122/18800	1.15e-06	0.0002
BP	GO:0051282	Regulation of sequestering of calcium ion	4/13	124/18800	1.23e-06	0.0002
CC	GO:0005901	Caveola	3/13	82/19594	1.96e-05	0.0015
CC	GO:0044853	Plasma membrane raft	3/13	113/19594	5.12e-05	0.0020
CC	GO:0099056	Integral component of presynaptic membrane	2/13	67/19594	0.0009	0.0153
CC	GO:0098889	Intrinsic component of presynaptic membrane	2/13	74/19594	0.0011	0.0153
CC	GO:0045121	Membrane raft	3/13	326/19594	0.0012	0.0153
MF	GO:0030291	Protein serine/threonine kinase inhibitor activity	2/13	33/18410	0.0002	0.0173
MF	GO:0008227	G protein-coupled amine receptor activity	2/13	51/18410	0.0006	0.0173
MF	GO:0004714	Transmembrane receptor protein tyrosine kinase activity	2/13	60/18410	0.0008	0.0173
MF	GO:0004860	Protein kinase inhibitor activity	2/13	70/18410	0.0011	0.0173
MF	GO:0019210	Kinase inhibitor activity	2/13	74/18410	0.0012	0.0173
KEGG	hsa05213	Endometrial cancer	3/13	58/8164	9.25e-05	0.0056
KEGG	hsa05223	Non-small cell lung cancer	3/13	72/8164	0.0002	0.0056
KEGG	hsa01524	Platinum drug resistance	3/13	73/8164	0.0002	0.0056
KEGG	hsa05212	Pancreatic cancer	3/13	76/8164	0.0002	0.0056
KEGG	hsa05220	Chronic myeloid leukemia	3/13	76/8164	0.0002	0.0056

mechanisms of *E. ulmoides* in treating sarcopenia. Furthermore, molecular docking confirmed the direct interaction between the key gene HSPB1 and the effective compound quercetin (a flavonoid) from *E. ulmoides*. HSPB1, also known as heat shock protein family B (small) member 1, encodes a protein that is part of the heat shock protein family. Its primary function is as a molecular chaperone involved in various biological processes, such as thermotolerance, inhibition of apoptosis, regulation of cell development and differentiation, and signal transduction. HSPB1 is widely expressed in various types of cells, particularly in muscle cells, where it is highly expressed during different stages of cell differentiation and development [15, 16]. There is existing research on the role of HSPB1 in sarcopenia. For example, Chen, et al. [17] used bioinformatics analysis to identify that the HSPB1 gene is closely related to ferroptosis in sarcopenia and showed high sensitivity and specificity for sarcopenia diagnosis. He, et al. [18] identified DNA methylation differences in genes associated with sarcopenia (HSPB1, PBX4, CNKSR3, ORMDL3, MIR10A, ZNF619, and CRADD) in blood samples from elderly women, enhancing the understanding of the epigenetic mechanisms of sarcopenia. In this study, we found that the compound quercetin binds to HSPB1, which is a plant-derived flavonoid with various biological activities, such as antioxidant, anti-inflammatory, antiviral, anti-tumor, hypolipidemic, hypoglycemic, antibacterial, and immunoregulatory effects [19, 20]. Hour, et al. [21] described how quercetin can induce early myogenic differentiation by activating p-IGF-1R, with AKT activation being essential for this process. Additionally, quercetin was found to promote myoblast migration by regulating the ITGB1 signaling pathway and activating FAK and paxillin

phosphorylation. Kim, et al. [22] also confirmed the therapeutic potential of quercetin as an antioxidant for bone-muscle-tendon regeneration and aging. In this study, molecular docking showed that the binding affinity between HSPB1 and quercetin is -9.1, indicating that the lower the binding energy is, the more stable the interaction will be, which provides valuable reference data for subsequent experimental exploration.

The molecular mechanisms of sarcopenia involve various factors, including age-related physiological changes, neuromuscular dysregulation, inflammation, and immune factors [23]. Based on the molecular characteristics of the key gene HSPB1 and the KEGG pathways predicted in this study, we can speculate on the potential mechanisms related to age-related physiological changes as follows: (1) Regarding the role of HSPB1 in apoptosis or muscle homeostasis, previous studies have shown that HSPB1, as a member of the heat shock protein family, plays a critical role in cellular stress response, protein folding, and cell protection. These functions directly or indirectly affect the homeostasis and apoptosis process of muscle cells [17, 18]. Calcium ions, as important second messengers within cells, play a central role in processes such as muscle contraction, cell proliferation, and differentiation. Existing literature suggests that HSPB1 may regulate the physiological functions of muscle cells by modulating the function of calcium ion channels or related proteins, affecting intracellular calcium ion concentration [24, 25], maintaining calcium balance and mitigating muscle dysfunction due to calcium signaling disturbances. (2) Hypoxia-inducible Factor (HIF-1) pathway: In elderly individuals, HIF-1 activity may change, impacting muscle energy metabolism and oxygen supply [26]. HSPB1 may help

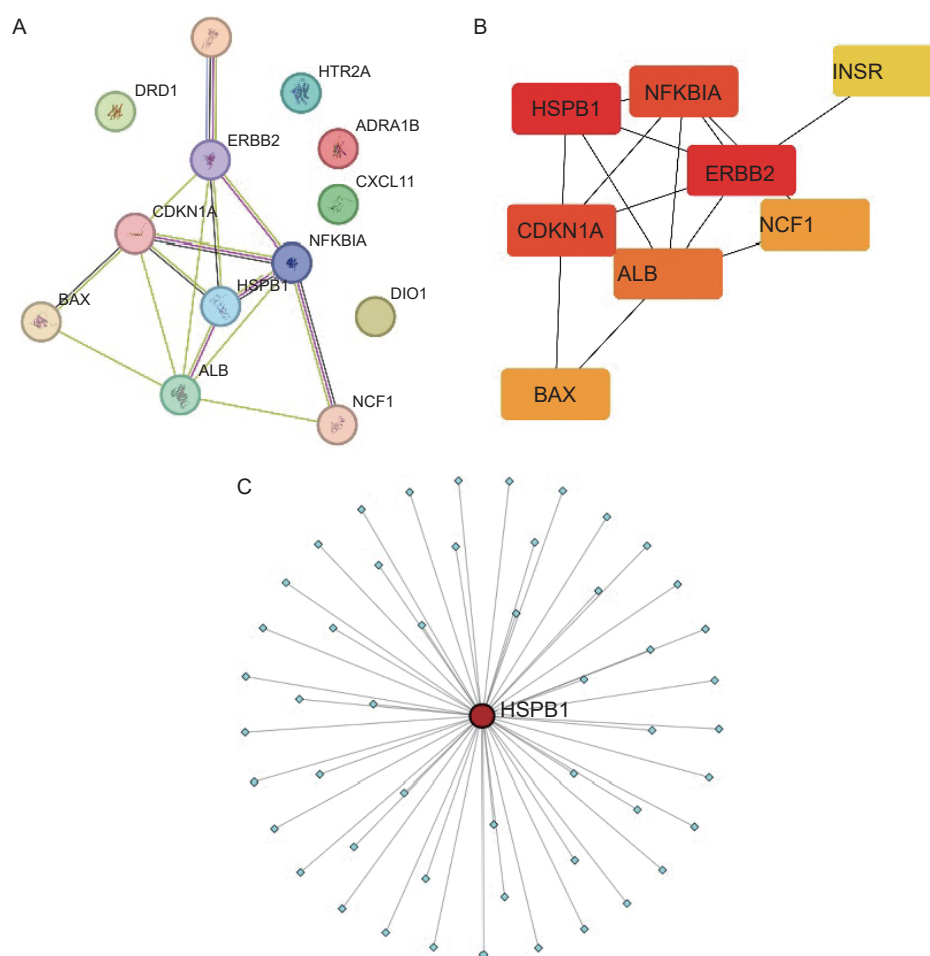


Figure 3 PPI network construction and TF-DEG-miRNA network construction. (A) PPI network of DEGs constructed using STRING. (B) The most important module obtained from the PPI network using the DMNC algorithm in Cytoscape. (C) mRNA network construction showing interactions with HSPB1.

Table 3 Scores of the 8 key genes filtered according to the DMNC algorithm

Gene name	HSPB1	ERBB2	CDKN1A	NFKB1A	ALB	BAX	NCF1	INSR
Score	0.568393712	0.568393658	0.453783847	0.453783847	0.380393302	0.307786103	0.307786103	0

muscle cells adapt to hypoxic environments through its stress-protective properties, mitigating muscle loss due to metabolic dysfunction. (3) As for the association between HSPB1 and the FoxO signaling pathway, the FoxO transcription factor family plays a key role in processes such as cell apoptosis, metabolic regulation, and muscle regeneration [27]. Research has shown that HSPB1 can interact directly or indirectly with FoxO protein, affecting its transcriptional activity and regulating the expression of downstream target genes [17]. In the context of sarcopenia, abnormal activation of the FoxO signaling pathway is closely related to increased apoptosis of muscle cells, and HSPB1 may exert anti-apoptotic effects by inhibiting excessive activation of FoxO, protecting muscle cells from damage.

Neuromuscular dysregulation mechanisms may involve the following: (1) Neuromuscular communication: Effective communication between nerves and muscles relies on the precise interaction between neurotransmitters and receptors [28]. HSPB1 may influence the expression or function of neurotransmitter receptors, improving the efficiency of neuromuscular junction transmission and alleviating muscle

dysfunction caused by neural regulation imbalances. (2) Neurotrophic factors: neurotrophic factors are crucial for neuronal growth and survival [29]. HSPB1 may regulate neurotrophic signaling pathways, promoting neuronal health and indirectly maintaining normal muscle function.

Inflammation and immune factors may involve the following: (1) Toll-like receptor (TLR) pathway: TLRs are an important component of the innate immune system, involved in pathogen recognition and initiation of inflammatory responses [30]. HSPB1 may regulate TLR signaling, reducing muscle damage and catabolism caused by chronic low-grade inflammation. (2) Chemokine pathway: Chemokines guide immune cell migration to sites of inflammation [31]. HSPB1 may affect chemokine signaling, regulating immune cell migration and activation, thus controlling the extent of inflammation in muscle tissues. (3) MAPK signaling pathway: The MAPK pathway plays a central role in cellular stress responses [32]. As a stress-response protein, HSPB1 may regulate the MAPK pathway, enhancing muscle cell resistance to internal and external stresses and delaying the development of sarcopenia.

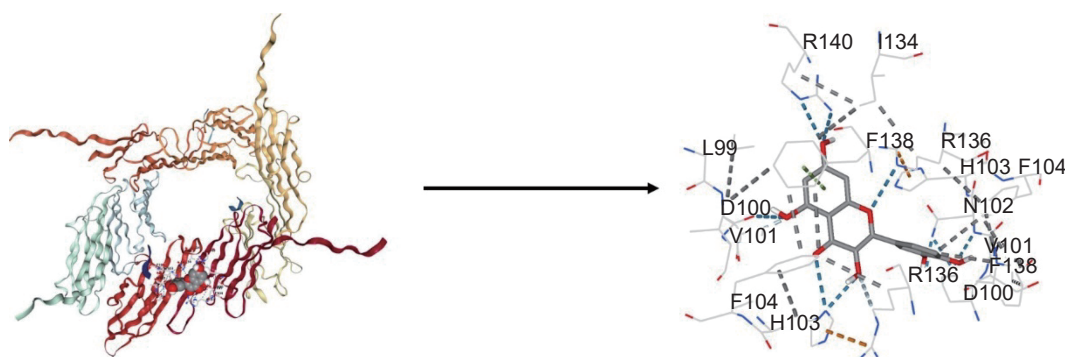


Figure 4 Molecular docking diagram of the active compound quercetin from the Chinese medicine *E. ulmoides* with the core target HSPB1.

Based on these findings, we can gain a more comprehensive understanding of the potential mechanisms through which *E. ulmoides* may treat sarcopenia. These pathways may interact with each other and form a complex regulatory network. The effective compound quercetin from *E. ulmoides* may exert its therapeutic effects through single-target and multi-pathway interactions.

4 Conclusions

HSPB1 could serve as a novel predictive biomarker for sarcopenia patients. *E. ulmoides* may exert therapeutic effects on sarcopenia by targeting HSPB1 through multiple pathways, providing a theoretical foundation for further *in vitro* experimental validation. The results of this study are based on data mining and analysis through network pharmacology, offering some predictive value. However, experimental validation is needed to further elucidate the molecular mechanisms involved.

5 Methodology

5.1 Screening of active ingredients and target genes

The active ingredients of *E. ulmoides* were screened using the TCMSP platform with the criteria: drug-likeness (DL) score ≥ 0.18 and oral bioavailability (OB) $\geq 30\%$ [33, 34]. Predicted target genes for the compounds were then retrieved from the DrugBank database (<https://www.drugbank.ca/>). The target information was also compared with data from the UniProt database (<https://www.uniprot.org/>).

5.2 Screening of DEGs

Global gene expression profiles of the vastus lateralis muscle from 10 young male participants (aged 19–25 years) and 12 elderly male participants (aged 70–80 years) were obtained from the GEO database (dataset GSE1428). DEGs were screened based on the criteria $|\log FC| > 0.5$ and $p < 0.05$. The data were normalized and preprocessed. According to the annotation information from the GEO database, gene symbols were assigned to all IDs, and duplicate gene names were removed using the mean method. The intersection of the targets of *E. ulmoides* and the DEGs in this dataset was used to identify the final candidate targets.

5.3 Functional enrichment analysis

The functional enrichment analysis of DEGs was performed using the clusterProfiler package in R. Based on the expression

levels of key genes, the DEGs were divided into high-expression and low-expression groups. Gene Set Enrichment Analysis (GSEA) v4.2.3 was then used to analyze biological differences between the two groups. The Molecular Signatures Database (MSigDB) v7.5.1 was used for background gene set data. Specifically, the C2 KEGG subset and C5 GO subset were used for functional enrichment analysis. A false discovery rate (FDR) $< 25\%$ and $p < 0.05$ were considered statistically significant.

5.4 Construction of PPI network and identification of Hub genes

A PPI network was constructed using the online STRING database (<https://string-db.org/>). The candidate target genes were input to generate the PPI network diagram. The results were then imported into Cytoscape for visualization and correlation analysis. The key protein molecules were screened using the DMNC algorithm.

5.5 Construction of transcription factor (TF)-DEGs-miRNA network

MiRTarBase v8.0 and ENCODE databases were used to retrieve the TF–DEG and miRNA–DEG interaction data. DEGs that show interaction with TFs and miRNAs were selected, and the corresponding miRNAs and TFs that regulate them were extracted. The integrated TF–DEGs–miRNA network was then visualized using Cytoscape.

5.6 Molecular docking

The 3D structures of the active compounds were retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). The receptor proteins of the key targets were obtained from the Protein Data Bank (PDB ID: 6GJH, resolution: 2.10 Å, DOI: <https://doi.org/10.2210/pdb6GJH/pdb>). The 3D structures of the active compounds and receptor proteins were uploaded to the DockThor platform (<https://dockthor.incc.br/v2/>) for molecular docking analysis. According to the DockThor calculations, the lower the binding energy between the ligand and receptor, the more stable the binding and the better the effect. Finally, the results were visualized using the CB-DOCK2 database (<https://cadd.labshare.cn/cb-dock2/>).

5.7 Statistical analysis

GraphPad Prism 7.0 software was used to create graphs and perform statistical analysis. All data are presented as mean $\pm$ standard deviation (SD). For DEG analysis, *t*-tests were used to determine the *p* values and adjusted *p* values, with *p* values

being adjusted by FDR. A p value < 0.05 was considered statistically significant.

Ethics approval

This study does not involve human subjects or animal experiments and does not require ethical approval.

Data availability

The data and analysis code generated in this study can be obtained from the corresponding author or the first author upon reasonable request.

Originality and reproducibility statement

The results of this study have not been published in full text or in part in other journals, and there is no issue of multiple submissions. All authors confirm that the content of the paper is original and are responsible for the authenticity and completeness of the data.

Author contributions

Yuejiao Zhong and Lingxiang Chen drafted the manuscript and designed the study. Yuejiao Zhong, Zhaoshi Bai, Qiang Wei, and Bin Zhou performed the data analysis. Lingxiang Chen and Siwen Liu critically revised the study. The final version of the manuscript has been approved by all authors.

Conflict of interests

All authors declare that there is no conflict of interest. This study did not accept any commercial sponsorship or other interest associations that might affect the research results. The author has no financial or non-financial interests with suppliers of research materials, drug manufacturers, etc.

References

- [1] Wang, H., Qin, D., Fang, L., Liu, H., Song, P. Addressing healthy aging in China: Practices and prospects. *Bioscience Trends*, **2024**, 18(3): 212–218. <https://doi.org/10.5582/bst.2024.01180>
- [2] Shen, J. B., Ma, Y. Y., Niu, Q. A study on the physical aging characteristics of the older people over 70 years old in China. *Frontiers in Public Health*, **2024**, 12: 1352894. <https://doi.org/10.3389/fpubh.2024.1352894>
- [3] Huang, S., Chen, X., Ding, H., Dong, B. The relationship between low and asymmetric handgrip strength and low muscle mass: Results of a cross-sectional study on health and aging trends in western China. *BMC Geriatrics*, **2024**, 24(1): 650. <https://doi.org/10.1186/s12877-024-05199-4>
- [4] Cao, M., Lian, J., Lin, X., Liu, J., Chen, C., Xu, S., Ma, S., Wang, F., Zhang, N., Qi, X. et al. Prevalence of sarcopenia under different diagnostic criteria and the changes in muscle mass, muscle strength, and physical function with age in Chinese old adults. *BMC Geriatrics*, **2022**, 22(1): 889. <https://doi.org/10.1186/s12877-022-03601-7>
- [5] Abe, T., Yoshimura, Y., Sato, Y., Nagano, F., Matsumoto, A. Sarcopenia as a robust predictor of readmission within 6 months among individuals experiencing acute stroke. *Annals of Geriatric Medicine and Research*, **2024**, 28(3): 307–314. <https://doi.org/10.4235/agmr.24.0017>
- [6] Weng, S. E., Huang, Y. W., Tseng, Y. C., Peng, H. R., Lai, H. Y., Akishita, M., Arai, H., Hsiao, F. Y., Chen, L. K. The evolving landscape of sarcopenia in Asia: A systematic review and meta-analysis following the 2019 Asian working group for sarcopenia (AWGS) diagnostic criteria. *Archives of Gerontology and Geriatrics*, **2025**, 128: 105596. <https://doi.org/10.1016/j.archger.2024.105596>
- [7] Smith, C., Woessner, M. N., Sim, M., Levinger, I. Sarcopenia definition: Does it really matter? Implications for resistance training. *Ageing Research Reviews*, **2022**, 78: 101617. <https://doi.org/10.1016/j.arr.2022.101617>
- [8] Wang, C., Zhang, H., Nie, X., Ding, F., Liu, Q., Hou, L., Deng, Y., Ye, W., Yue, J., He, Y. Traditional Chinese medicine constitution and sarcopenia: A cross-sectional study. *Frontiers in Public Health*, **2024**, 12: 1368933. <https://doi.org/10.3389/fpubh.2024.1368933>
- [9] Zhang, Y., Liu, K., Zhan, Y., Zhao, Y., Chai, Y., Ning, J., Pan, H., Kong, L., Yuan, W. Impact of Chinese herbal medicine on sarcopenia in enhancing muscle mass, strength, and function: A systematic review and meta-analysis of randomized controlled trials. *Phytotherapy Research*, **2024**, 38(5): 2303–2322. <https://doi.org/10.1002/ptr.8154>
- [10] Zhou, C., Zhou, C., Ma, H., Liu, C., Yang, L. Exploring traditional Chinese medicine as a potential treatment for sarcopenia: A network pharmacology and data mining analysis of drug selection and efficacy. *Medicine (Baltimore)*, **2023**, 102(41): e35404. <https://doi.org/10.1097/MD.00000000000035404>
- [11] Wang, C. Y., Tang, L., He, J. W., Li, J., Wang, Y. Z. Ethnobotany, phytochemistry and pharmacological properties of eucommia ulmoides: A review. *The American Journal of Chinese Medicine*, **2019**, 47(2): 259–300. <https://doi.org/10.1142/S0192415X19500137>
- [12] Song, L., Yin, H., Han, R., Li, J., Ma, N., Wang, Y., Guo, H. Metabolism of du Zhong formula in rats using UPLC-Q-TOF/MS. *Journal of Mass Spectrometry*, **2022**, 57(1): e4795. <https://doi.org/10.1002/jms.4795>
- [13] Matsumoto, A., Yoshimura, Y., Nagano, F., Bise, T., Kido, Y., Shimazu, S., Shiraishi, A. Statin use impairs muscle strength recovery in post-stroke patients with sarcopenia. *Geriatrics & Gerontology International*, **2023**, 23(9): 676–683. <https://doi.org/10.1111/ggi.14646>
- [14] Chen, X., Wang, Y., Liu, M., Song, X., Wang, D., Zhang, J. Network pharmacology-based analysis of the effects of puerarin on sarcopenia. *Annals of Translational Medicine*, **2022**, 10(12): 671. <https://doi.org/10.21037/atm-22-2360>
- [15] Chen, K., Hou, C., Xu, L., Peng, H., He, C., Liu, J., Wang, G., Huang, S., Liu, X. HSPB1 regulates autophagy and apoptosis in vascular smooth muscle cells in arteriosclerosis obliterans. *Cardiovascular Therapeutics*, **2022**, 2022: 3889419. <https://doi.org/10.1155/2022/3889419>
- [16] Esteller, D., Morrow, J., Alonso-Pérez, J., Reyes, D., Carbayo, A., Bisogni, G., Cateruccia, M., Monforte, M., Tasca, G., Alangary, A. et al. Muscle magnetic resonance imaging of a large cohort of distal hereditary motor neuropathies reveals characteristic features useful for diagnosis. *Neuromuscular Disorders*, **2023**, 33(10): 744–753. <https://doi.org/10.1016/j.nmd.2023.08.010>
- [17] Chen, Y., Zhang, Y., Zhang, S., Ren, H. Molecular insights into sarcopenia: Ferroptosis-related genes as diagnostic and therapeutic targets. *Journal of Biomolecular Structure and Dynamics*, **2024**: 1–19. <https://doi.org/10.1080/07391102.2023.2298390>
- [18] He, L. X., Khanal, P., Morse, C. I., Williams, A., Thomis, M. Differentially methylated gene patterns between age-matched sarcopenic and non-sarcopenic women. *Journal of Cachexia, Sarcopenia and Muscle*, **2019**, 10(6): 1295–1306. <https://doi.org/10.1002/jcsm.12478>
- [19] Georgiou, N., Kakava, M. G., Routsis, E. A., Petsas, E., Stavridis, N., Freris, C., Zoupanou, N., Moschovou, K., Kiriakidi, S., Mavromoustakos, T. Quercetin: A potential polydynamic drug. *Molecules*, **2023**, 28(24): 8141. <https://doi.org/10.3390/molecules28248141>
- [20] Radzka, J., Łapińska, Z., Szwedowicz, U., Gajewska-Naryniecka, A., Gizak, A., Kulbacka, J. Alternations of NF- κ B signaling by natural compounds in muscle-derived cancers. *International Journal of Molecular Sciences*, **2023**, 24(15): 11900. <https://doi.org/10.3390/ijms241511900>

- [21] Hour, T. C., Vo, T. C. T., Chuu, C. P., Chang, H. W., Su, Y. F., Chen, C. H., Chen, Y. K. The promotion of migration and myogenic differentiation in skeletal muscle cells by quercetin and underlying mechanisms. *Nutrients*, **2022**, 14(19): 4106. <https://doi.org/10.3390/nu14194106>
- [22] Kim, J. G., Sharma, A. R., Lee, Y. H., Chatterjee, S., Choi, Y. J., Rajvansh, R., Chakraborty, C., Lee, S. S. Therapeutic potential of quercetin as an antioxidant for bone-muscle-tendon regeneration and aging. *Aging and Disease*, **2024**, 16(3): 1414–1437. <https://doi.org/10.14336/AD.2024.0282>
- [23] Wu, J., Ding, P., Wu, H., Yang, P., Guo, H., Tian, Y., Meng, L., Zhao, Q. Sarcopenia: Molecular regulatory network for loss of muscle mass and function. *Frontiers in Nutrition*, **2023**, 10: 1037200. <https://doi.org/10.3389/fnut.2023.1037200>
- [24] Yogendran, V., Mele, L., Prysyazhna, O., Budhram-Mahadeo, V. S. Vascular dysfunction caused by loss of Brn-3b/POU4F2 transcription factor in aortic vascular smooth muscle cells is linked to deregulation of calcium signalling pathways. *Cell Death & Disease*, **2023**, 14(11): 770. <https://doi.org/10.1038/s41419-023-06306-w>
- [25] Pereyra, A. S., Fernandez, R. F., Amorese, A., Castro, J. N., Lin, C. T., Spangenburg, E. E., Ellis, J. M. Loss of mitochondria long-chain fatty acid oxidation impairs skeletal muscle contractility by disrupting myofibril structure and calcium homeostasis. *Molecular Metabolism*, **2024**, 89: 102015. <https://doi.org/10.1016/j.molmet.2024.102015>
- [26] Nguyen, T. H., Conotte, S., Belayew, A., Declèves, A. E., Legrand, A., Tassin, A. Hypoxia and hypoxia-inducible factor signaling in muscular dystrophies: Cause and consequences. *International Journal of Molecular Sciences*, **2021**, 22(13): 7220. <https://doi.org/10.3390/ijms22137220>
- [27] Ye, Y. L., Kuai, Z., Qian, D. D., He, Y. T., Shen, J. P., Wu, K. F., Ren, W. Y., Hu, Y. GLP-2 ameliorates D-galactose induced muscle aging by IGF-1/Pi3k/Akt/FoxO3a signaling pathway in C2C12 cells and mice. *Archives of Gerontology and Geriatrics*, **2024**, 124: 105462. <https://doi.org/10.1016/j.archger.2024.105462>
- [28] Gafurova, C. R., Tsentsevitsky, A. N., Fedorov, N. S., Khaziev, A. N., Malomouzh, A. I., Petrov, A. M. β 2-adrenergic regulation of the neuromuscular transmission and its lipid-dependent switch. *Molecular Neurobiology*, **2024**, 61(9): 6805–6821. <https://doi.org/10.1007/s12035-024-03991-2>
- [29] Xu, X., Song, L. L., Li, Y. Y., Guo, J., Huang, S., Du, S., Li, W. Z., Cao, R. J., Cui, S. S. Neurotrophin-3 promotes peripheral nerve regeneration by maintaining a repair state of Schwann cells after chronic denervation via the TrkC/ERK/c-Jun pathway. *Journal of Translational Medicine*, **2023**, 21(1): 733. <https://doi.org/10.1186/s12967-023-04609-2>
- [30] Cappelletti, C., Brugnani, R., Bonanno, S., Andreetta, F., Salerno, F., Canioni, E., Vattemi, G. N. A., Tonin, P., Mantegazza, R., Maggi, L. Toll-like receptors and IL-7 as potential biomarkers for immune-mediated necrotizing myopathies. *European Journal of Immunology*, **2023**, 53(11): 2250326. <https://doi.org/10.1002/eji.202250326>
- [31] Chen, J. W., Zhao, X. X., Wang, Z. X., Sun, L., Tian, Y. Causal association of circulating cytokines with sarcopenia-related traits: A Mendelian randomization study. *Cytokine*, **2024**, 180: 156643. <https://doi.org/10.1016/j.cyto.2024.156643>
- [32] Mano, Y., Tsukamoto, M., Wang, K. Y., Nabeshima, T., Kosugi, K., Tajima, T., Yamanaka, Y., Suzuki, H., Kawasaki, M., Nakamura, E. et al. Oxidative stress causes muscle structural alterations via p38 MAPK signaling in COPD mouse model. *Journal of Bone and Mineral Metabolism*, **2022**, 40(6): 927–939. <https://doi.org/10.1007/s00774-022-01371-1>
- [33] Arif, R., Bukhari, S. A., Mustafa, G., Ahmed, S., Albeshr, M. F. Network pharmacology and experimental validation to explore the potential mechanism of *Nigella sativa* for the treatment of breast cancer. *Pharmaceuticals (Basel)*, **2024**, 17(5): 617. <https://doi.org/10.3390/ph17050617>
- [34] Hou, Y., Zhang, L., Li, H., Zhang, R., Sun, J., Jia, H., Xu, H. Exploration of key pathogenic mechanisms and potential intervention targets of the traditional Chinese medicine *Coptis chinensis* in the treatment of cervical cancer based on network pharmacology and molecular docking techniques. *Translational Cancer Research*, **2025**, 14(1): 626–639. <https://doi.org/10.21037/tcr-2024-2608>