Heliyon 10 (2024) e29062

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Identification of ferroptosis-associated genes and potential pharmacological targets in sepsis-induced myopathy

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ARTICLE INFO

Keywords:
Sepsis
Sepsis-induced myopathy
Ferroptosis
Enrichment analysis
Drug-gene prediction

ABSTRACT

Background: The role of Ferroptosis in the course of sepsis-induced myopathy is yet unclear. The objective of our work is to identify key genes connected with Ferroptosis in sepsis-induced myopathy and investigate possible pharmaceutical targets related to this process. This research aims to provide new insights into the management of sepsis-induced myopathy.

Methods: We got the GSE13205 dataset from the Gene Expression Omnibus (GEO) and extracted Ferroptosis-associated genes from the FerrDb database. After conducting a functional annotation analysis of these genes, we created a protein-protein interaction network using Cytoscape software to identify important genes. Subsequently, we employed CMap to investigate prospective pharmaceuticals that could target these crucial genes.

Results: A total of 61 genes that are expressed differently (DEGs) have been found concerning Ferroptosis. These genes are involved in a wide range of biological functions, including reacting to signals from outside the cell and the availability of nutrients, programmed cell death, controlling apoptosis, and responding to peptides, chemical stressors, and hormones. The KEGG pathway study revealed that these pathways are involved in Ferroptosis, autophagy, P53 signaling, PI3K-Akt signaling, mTOR signaling, HIF-1 signaling, endocrine resistance, and different tumorigenic processes. In addition, we created a network that shows the simultaneous expression of important genes and determined the top 10 medications that have the potential to treat sepsis-induced myopathy.

Conclusion: The bioinformatics research undertaken sheds insight into the probable role of Ferroptosis-associated genes in sepsis-induced myopathy. The identified critical genes show potential as therapeutic targets for treating sepsis-induced myopathy, offering opportunities for the development of tailored medicines.

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https://doi.org/10.1016/j.heliyon.2024.e29062
Received 31 October 2023; Received in revised form 28 March 2024; Accepted 28 March 2024
Available online 30 March 2024

1. Introduction

Currently, sepsis is characterized as a severe impairment of organ function caused by an unbalanced immune response to infection [1]. With developments in medical technology and care, there has been a dramatic improvement in the overall survival rate of sepsis patients [2]. Nevertheless, a significant proportion of patients continue to experience sepsis-induced myopathy, which is defined by the wasting (reduced cross-sectional area) and debilitation (decreased muscle strength) of the limbs and respiratory muscles [2,3]. This myopathy not only extends the duration of bed rest and significantly diminishes the quality of life, but also leads to higher rates of readmission and mortality within a span of five years [4]. Regrettably, there is currently a shortage of effective treatment options for sepsis-induced myopathy, apart from rehabilitation therapy and nutritional support [5].

In the last ten years, a unique type of programmed cell death called ferroptosis has become recognized, existing alongside apoptosis and necroptosis. Ferroptosis is distinguished by abnormal iron metabolism and lipid peroxidation, which are controlled by different metabolic pathways [6]. Through the Fenton reaction, ferrous iron interacts with PUFAs on the cell membrane, triggering lipid peroxidation by generating LOOHs. Cell death through ferroptosis is initiated when the antioxidant system, comprised of GPX4 and glutathione, is compromised, leading to the excessive buildup of LOOHs [6]. Lately, there has been a significant focus on the significance of ferroptosis in the development and advancement of many diseases, including infections and malignancies. During bacterial infections, cellular ferroptosis occurs, leading to the release of intracellular iron that promotes bacterial multiplication. Additionally, the infection itself supplies substrates, such as fatty acids and ROS, which further contribute to lipid peroxidation and worsen the infection [7]. Ferroptosis has been identified as a factor in the development, treatment, and medication resistance of hepatocellular carcinoma, gastric cancer, and breast cancer in the field of oncology [8]. It is worth mentioning that immune cells, including macrophages, T cells, and B cells, are prone to ferroptosis, which leads to a decrease in their quantity and functionality. As a result, this triggers inflammatory responses or particular immunological reactions [9]. Mounting evidence suggests that iron ions play a crucial role in regulating inflammation and sepsis. Drugs that target iron-associated molecules, such as iron chelators, have shown promising results in managing sepsis. Ferroptosis is acknowledged as a fundamental process that contributes to the development of multiple organ failure in sepsis [10].

The importance of ferroptosis in inflammatory diseases, sepsis, and other infection-related conditions has garnered significant attention in recent years [10,11]. Recent research indicates that the inhibition of ferroptosis can relieve sepsis-induced multi-organ dysfunction in animals, including damage to the heart [12,13]. Nevertheless, the specific mechanisms and genes involved in the occurrence of ferroptosis in sepsis-induced myopathy have not been fully understood. In this study, we applied bioinformatics analysis to explore public databases, intending to uncover Ferroptosis-related genes and pathways likely connected with sepsis-induced myopathy. Additionally, we tried to discover prospective therapeutic medicines, delivering useful insights for further research and subsequent therapy of sepsis-induced myopathy.

2. Experimental procedures

2.1. Collection and processing of microarray datasets

We retrieved microarray datasets from the Gene Expression Omnibus (GEO) by searching for “sepsis” and “skeletal muscle.” The search criteria used were: (“sepsis-induced myopathy” [MeSH Terms] or “sepsis” [All Fields]) and (“skeletal muscle atrophy” [MeSH Terms]) in “*Homo sapiens*” [organism], along with the filters “GSE” and “Expression profiling by an array.” Subsequently, we downloaded the GSE13205 dataset, which consists of 13 skeletal muscle biopsy samples from sepsis patients and 8 control samples, from the GEO website.

2.2. Identifying genes with differential expression related to ferroptosis

We utilized the Affymetrix Human Genome U133 Plus 2.0 Array platform (GPL570) to purchase a roster of 388 Ferroptosis-associated genes sourced from the “FerrDb” database. Using the “limma” package in the R software, we conducted a differential expression analysis comparing sepsis and control cohorts. We established adjusted cutoff thresholds at a p-value of less than 0.05 and a fold change (FC) greater than 1. The intersection of differentially expressed genes (DEGs) and Ferroptosis-associated genes was visualized using a Venn diagram.

2.3. Analysis of functional enrichment and protein-protein interaction networks

We performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses on differentially expressed genes (DEGs) linked with Ferroptosis. We used the “clusterProfiler” package in R for this analysis. The GO analysis included three main categories: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). KEGG analysis was undertaken to reveal probable pathways. To analyze the protein-protein interaction (PPI) network of differentially expressed genes (DEGs) relevant to ferroptosis, we employed the STRING online database. Subsequently, we imported the obtained results into Cytoscape (version 3.8.2) for additional investigation. The cytoHubba plugin was utilized to identify key subnetworks. In this process, the top 10 genes with the greatest scores in each subnetwork were identified as hub genes, based on the maximum correlation criterion (MCC algorithm).

2.4. Pharmacological target screening

We employed the CMap database to identify possible pharmaceutical targets. This collection contains data on changes in gene expression profiles caused by 33,609 small-molecule compounds in different cell lines. Connectivity scores within the range of −100 to 100 were obtained. Positive scores indicate similar changes caused by the compound and the uploaded genes, while negative scores indicate conflicting changes caused by the compound. Compounds that have connection scores below −90 were considered to have a good predictive value.

2.5. Model of sepsis caused by cecal ligation and puncture (CLP)

The cecal ligation and puncture (CLP) protocol was used to produce a sepsis model. Concisely, male mice who were 7–8 weeks old and weighed between 22 and 26 g were given anesthesia with an intraperitoneal injection of pentobarbital sodium (40 mg/kg) before completing the CLP process. Sham-operated mice underwent comparable procedures without CLP induction. Post-surgery, mice were revived with subcutaneous injection of sterile saline (50 mL/kg). Gastrocnemius muscle samples were taken for RT-PCR analysis 96 h post-procedure.

2.6. Real-time quantitative polymerase chain reaction (qRT-PCR)

We employed a PT 10/35 Polytron homogenizer (manufactured by Kinematica AG, located in Lucerne, Switzerland) to homogenize gastrocnemius tissue in RNA STAT-60 (produced by Tel-Test, based in Friendswood, TX) at a temperature of 4 °C for RNA isolation. The cDNA synthesis was conducted using the RT kit (Takara, Dalian, China) according to the manufacturer's instructions. SYBR-Green (Takara, China) was utilized for qRT-PCR investigation. The $2-\Delta\Delta CT$ technique was utilized to ascertain the relative levels of expression. PCR primers, provided by Beijing Qingdao Biotechnology Co., LTD, are detailed in Table 1.

2.7. Statistical analysis

The data are reported as the mean ± standard error of the mean (s.e.m.) derived from six replicate trials. The PCR data was analyzed by a researcher who was unaware of the group assignments. T-tests were used for statistical comparison. The data was analyzed and presented graphically using GraphPad Prism 6 for Windows (San Diego, CA, USA). A significance level of $P < 0.05$ was considered to have a statistically significant result.

3. Results

3.1. Detection of genes with altered expression levels in skeletal muscles

Using GEO2R analysis, we discovered 2232 differentially expressed genes (DEGs) in dataset GSE13205. This set includes 1469 genes that were upregulated and 763 genes that were downregulated. The volcano plot in Fig. 1 visually represents the differentially expressed genes (DEGs), with upregulated genes indicated by red dots and downregulated genes indicated by blue dots.

3.2. Differentiation of ferroptosis-associated DEGs

A grand number of 388 genes associated with Ferroptosis were obtained from the "FerrDb" database. The analysis of the DEGs from GSE13205 identified Ferroptosis-associated DEGs near the intersection. The Venn diagram depicts 327 genes that are exclusively related to Ferroptosis on the left side, 61 genes that are associated with Ferroptosis in the middle, and 1645 genes that are uniquely differentially expressed in GSE13205 on the right side (Fig. 2A). The expression pattern of these Ferroptosis-associated DEGs was shown using a heatmap. Among these genes, 51 showed upregulation while 10 displayed downregulation (Fig. 2B). We extensively

Table 1
List of primers used in qRT-PCR.

Gene	Sequence (5′–3′)	
	Forward primer	Reverse primer
CD44	ACCTTGGCCACCACTCCTAATA	TTGACTTGGATGGTTGTTGTGG
CDKN1A	GGGTGAGGAGGAGCATGAAT	AAAGTTCCACCGTTCTCGGG
HMOX1	GGAAATCATCCCTTGCACGC	TGTTTGAACTTGGTGGGGCT
MTOR	CTCTTGCCCTTCGAACCCTT	ATGCCAAGACACAGTAGCGG
KRAS	GTCAGAATCCTCACTCTCACAGA	CCAGGACACTCACCACCATT
MDM2	CTGCTTTGTTAACGGGGCCT	GGTATTGCACATTGGCCTGG
PTEN	AGGATACGCGCTTGGGC	ACAGCGGCTCAACTCTCAAA
STK11	CCGACAGATTAGGCAGCACA	TCGCATGCATCCTCGCTAAG
TP53	GTGCTCACCCTGGCTAAAGT	AGGAGGATGAGGGCCTGAAT
TP63	ACAGCAGAACTGACAGGAGC	ATTCTTACCAGCTGGGCACC

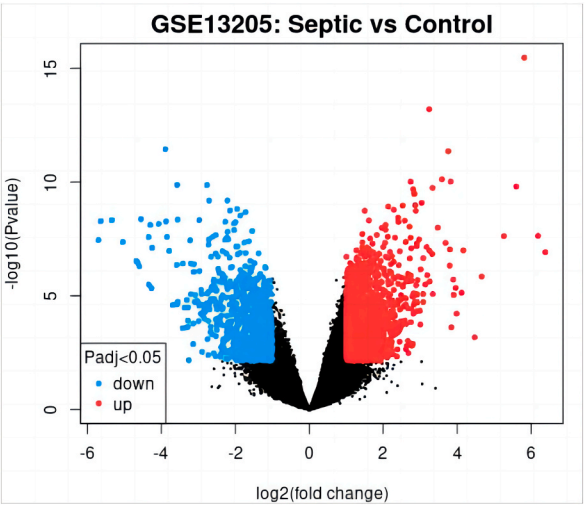

Fig. 1. Analysis of Differential Gene Expression (DEG) in Sepsis. (A) Volcano plot displaying gene expression in sepsis patients from dataset GSE13205.

classified Ferroptosis-associated Differentially Expressed Genes (DEGs) using the "FerrDb" database. The results are presented in Table 2.

3.3. GO and KEGG enrichment analysis of ferroptosis-associated DEGs

We performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment studies on differentially expressed genes (DEGs) linked with Ferroptosis. These analyses were conducted using the Metascape database. The Molecular Functions (MF) that are worth mentioning include oxidation-reduction reactions, NDPDH effect, binding to P53, flavin adenine dinucleotide binding, nuclear receptor binding, and protein domain binding (Fig. 3A). The Enriched Biological Processes (BP) encompassed many cellular responses such as replies to external signals, regulation of cell death and apoptosis, and responses to peptides, chemical stressors, and hormones (Fig. 3A). The prominent cellular components (CC) include cytoplasmic membranes, peroxisomal membranes, microtubules, mitochondrial outer membranes, and transcriptional regulatory complexes (Fig. 3A). The KEGG enrichment analysis identified several relevant pathways, including Ferroptosis, autophagy, p53 signaling, PI3K-Akt signaling, mTOR signaling, HIF-1 signaling, endocrine resistance, and numerous tumor-related pathways (Fig. 3B).

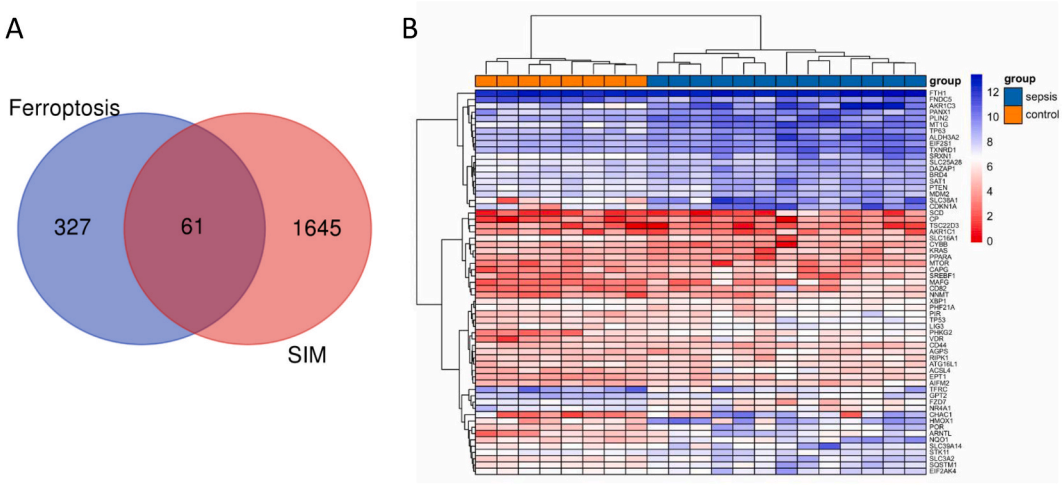

Fig. 2. Identification of Genes Associated with Ferroptosis and Venn Diagram Analysis. A Venn diagram is used to visually represent the genes that are connected to Ferroptosis among the DEGs (Differentially Expressed Genes). A heatmap illustrating the expression levels of genes associated with Ferroptosis.

Table 2
61 ferroptosis-related differently expressed genes (FRDEGs).

Symbol	Classification	Log FC	Adj.P.Val	Description
CD44	Suppressor	−1.2255989	4.58E-02	CD44 molecule
PANX1	Driver	1.3266896	1.68E-02	Pannexin 1
PTEN	Driver	1.1789928	9.14E-04	Phosphatase and tensin homolog
DAZAP1	Suppressor	1.2796452	2.14E-04	DAZ associated protein 1
PIR	Suppressor	1.1924413	1.77E-0	Pirin
EPT1	Driver	1.2047228	1.16E-03	Ethanolamine phosphotransferase 1
PHKG2	Driver	2.1990329	1.18E-03	Phosphorylase kinase catalytic subunit gamma 2
SLC39A14	Driver	1.4037619	3.46E-02	Solute carrier family 39 member 14
SQSTM1	Suppressor	1.4905747	1.41E-03	Sequestosome
ALDH3A2	Suppressor	1.4041188	9.28E-03	Aldehyde dehydrogenase 3 family member A2
PLIN2	Suppressor	1.3132135	7.28E-03	Perilipin 2
CD82	Driver	1.8943271	8.24E-03	CD82 molecule
TSC22D3	Unclassified	1.0465409	1.19E-02	TSC22 domain family member 3
KRAS	Driver	1.5494956	1.14E-03	KRAS proto-oncogene, GTPase
AGPS	Driver	1.7417282	8.46E-0	Alkylglycerone phosphate synthase
MT1G	Suppressor	2.8337876	8.36E-07	Metallothionein 1
MTOR	Suppressor	1.122992	7.22E-03	Mechanistic target of rapamycin
BRD4	Suppressor	1.081167	6.17E-04	Bromodomain containing 4
FTH1	Marker	1.1582855	1.88E-02	Ferritin heavy chain 1
FZD7	Suppressor	−1.886953	4.06E-02	Frizzled class receptor 7
SCD	Suppressor	2.6827402	2.89E-02	Stearoyl-CoA desaturase
EIF2S1	Unclassified	1.1462236	2.32E-04	Eukaryotic translation initiation factor 2 subunit alpha
CAPG	Unclassified	1.469307	3.37E-02	Capping actin protein, gelsolin like
POR	Driver	2.0527337	5.07E-04	Cytochrome p450 oxidoreductase
TFRC	Marker	−1.699993	1.48E-02	Transferrin receptor
ARNTL	Suppressor	2.6138233	1.16E-03	Aryl hydrocarbon receptor nuclear translocator like
NNMT	Unclassified	1.6352288	1.42E-02	Nicotinamide N-methyltransferase
CHAC1	Driver, Marker	3.8436779	5.13E-03	ChaC glutathione specific gamma-glutamylcyclotransferase 1
VDR	Suppressor	1.8741741	7.62E-03	Vitamin D (1,25- dihydroxyvitamin D3) receptor
HMOX1	Driver	2.9166101	1.73E-03	Heme oxygenase 1
PPARA	Suppressor	−1.176100	3.98E-02	peroxisome proliferator activated receptor alpha
SRXN1	Unclassified	1.9768304	1.40E-03	Sulfiredoxin 1
AIFM2	Suppressor	1.0713214	2.05E-02	Apoptosis inducing factor, mitochondria associated 2
MDM2	Driver	2.3431006	5.67E-02	MDM2 proto-oncogene
NQO1	Suppressor	2.924495	1.02E-03	NAD(P)H quinone dehydrogenase 1
NR4A1	Suppressor	−1.102334	2.43E-03	Nuclear receptor subfamily 4 group A member 1
FNDC5	Suppressor	−1.485953	7.01E-03	Fibronectin type III domain containing 5
RIPK1	Unclassified	1.0570921	3.41E-02	Receptor interacting serine/threonine kinase 1
TXNRD1	Unclassified	1.2861945	1.43E-03	Thioredoxin reductase 1
CYBB	Driver	−1.223086	4.70E-02	Cytochrome b-245 beta chain
SLC3A2	Suppressor	1.8040127	2.67E-04	Solute carrier family 3 member 2
XBP1	Unclassified	1.0837335	9.20E-03	X-box binding protein
EIF2AK4	Unclassified	1.0176844	1.54E-02	Eukaryotic translation initiation factor 2 alpha kinase 4
TP53	Driver, Suppressor	1.1104206	7.58E-03	Tumor protein p53
MAFG	Unclassified	1.8704537	2.80E-02	MAF bZIP transcription factor
STK11	Suppressor	1.4479413	4.61E-02	Serine/threonine kinase 11
AKR1C1	Suppressor	2.8235375	4.99E-02	Aldo-keto reductase family 1 member C1
SLC38A1	Driver	4.1678237	3.82E-05	Solute carrier family 38 member 1
CDKN1A	Suppressor	3.082594	9.99E-04	Cyclin dependent kinase inhibitor 1A
SLC25A28	Driver	1.3432447	3.73E-04	Solute carrier family 25 member 28
AKR1C3	Suppressor	3.3411724	6.08E-04	Aldo-keto reductase family 1, member C3
GPT2	Unclassified	−1.349294	1.47E-03	Glutamic–pyruvic transaminase 2
SREBF1	Suppressor	1.6725466	1.41E-02	Sterol regulatory element binding transcription factor 1
ATG16L1	Driver	1.011825	3.34E-02	Autophagy related 16 like 1
LIG3	Driver	1.319350	7.17E-02	DNA ligase 3
CP	Suppressor	1.3374469	3.60E-02	Ceruloplasmin (ferroxidase)
SLC16A1	Suppressor	−1.305147	2.79E-03	Solute carrier family 16 member 1
ACSL4	Driver	2.250323	6.84E-03	Acyl-CoA synthetase long-chain family member 4
TP63	Suppressor	1.1180772	3.10E-03	Tumor protein p63
SAT1	Driver	1.5574782	1.68E-03	Spermidine/spermine N1-acetyltransferase 1
PHF21A	Driver	1.2633848	3.73E-02	PHD finger protein 21A

3.4. Construction of PPI network and identification of hub genes

A Protein-Protein Interaction (PPI) network was created by utilizing the STRING database. The network was generated by setting an interaction score threshold of greater than 0.4, resulting in a total of 57 nodes and 362 edges. Significantly, out of the 61 differentially expressed genes (DEGs) related to Ferroptosis, four genes displayed no connections with other genes and did not contribute to

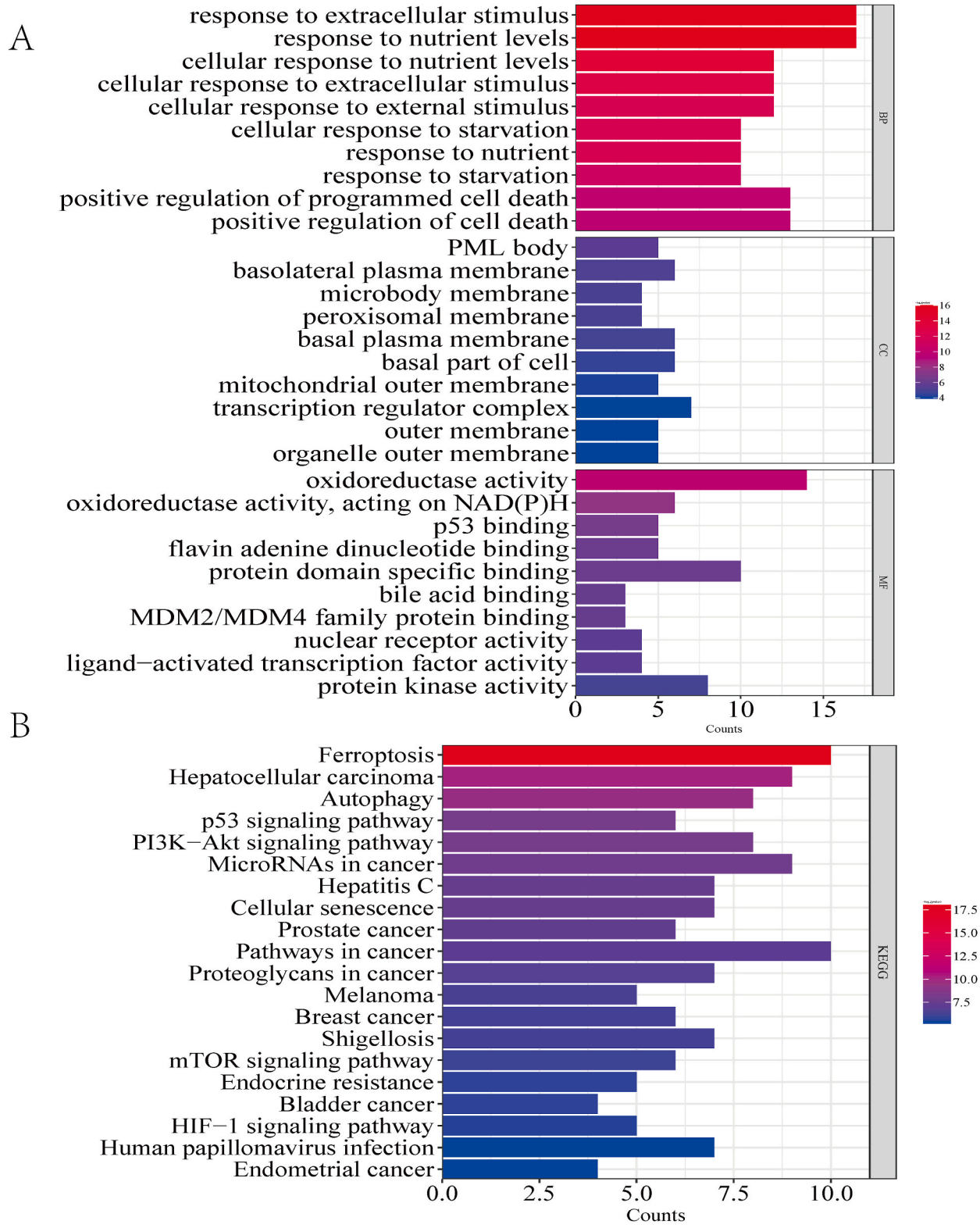

Fig. 3. Perform GO and KEGG enrichment analysis of differentially expressed genes (DEGs) linked with ferroptosis using the Metascape database. GO enrichment analysis. (B) Enhancement of Biological Processes (BP) pathways. (C) Cellular Components Enrichment (CC). (D) KEGG enrichment analysis.

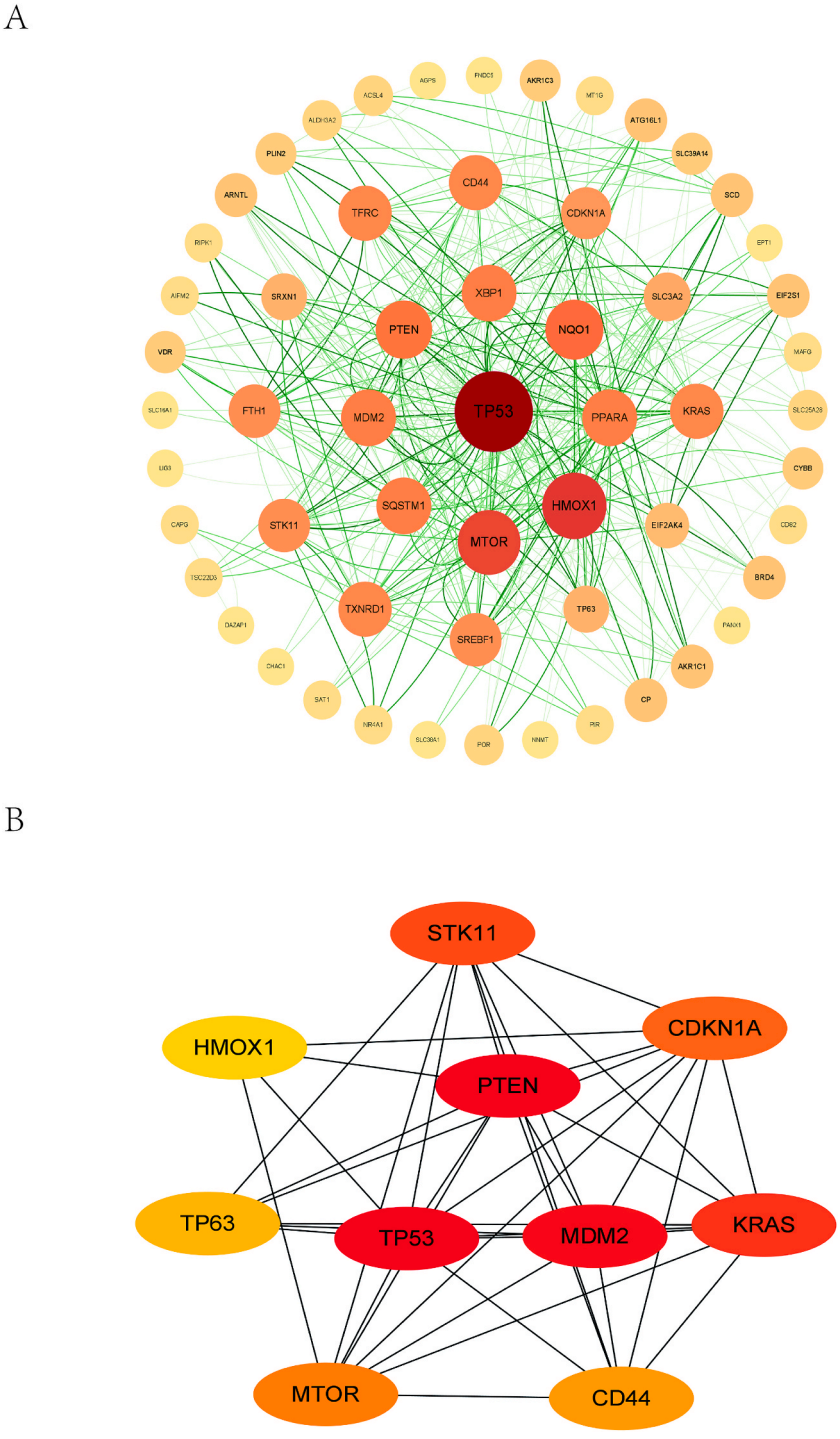

Fig. 4. Creating a Protein-Protein Interaction (PPI) network using the STRING Database (4A). Identification of hub genes was performed using the Cyto-Hubba plugin in Cytoscape (4B).

molecular networks, as shown in Fig. 4A. Subsequently, Hub genes were discovered using the Cyto-Hubba plugin in Cytoscape, selecting the top 10 genes with the highest MCC algorithm scores as Hub genes (Fig. 4B).

3.5. Validation of hub gene expression differences

We obtained expression matrices of Hub genes from the GEO database and visualized their expression differences using violin plots. Using GSE13205 as the training set, sepsis patients showed markedly increased expression levels of TP53, PTEN, MDM2, KRAS, STK11, CDKN1A, MTOR, TP63, and HMOX1 in skeletal muscles compared to the control group. In contrast, the expression of CD44 showed a notable decrease in patients with sepsis (Fig. 5A–J). In addition, we collected total RNA from the gastrocnemius muscles of both the CLP group (4 days after CLP) and the sham group, each consisting of six mice. The subsequent qRT-PCR analysis demonstrated an

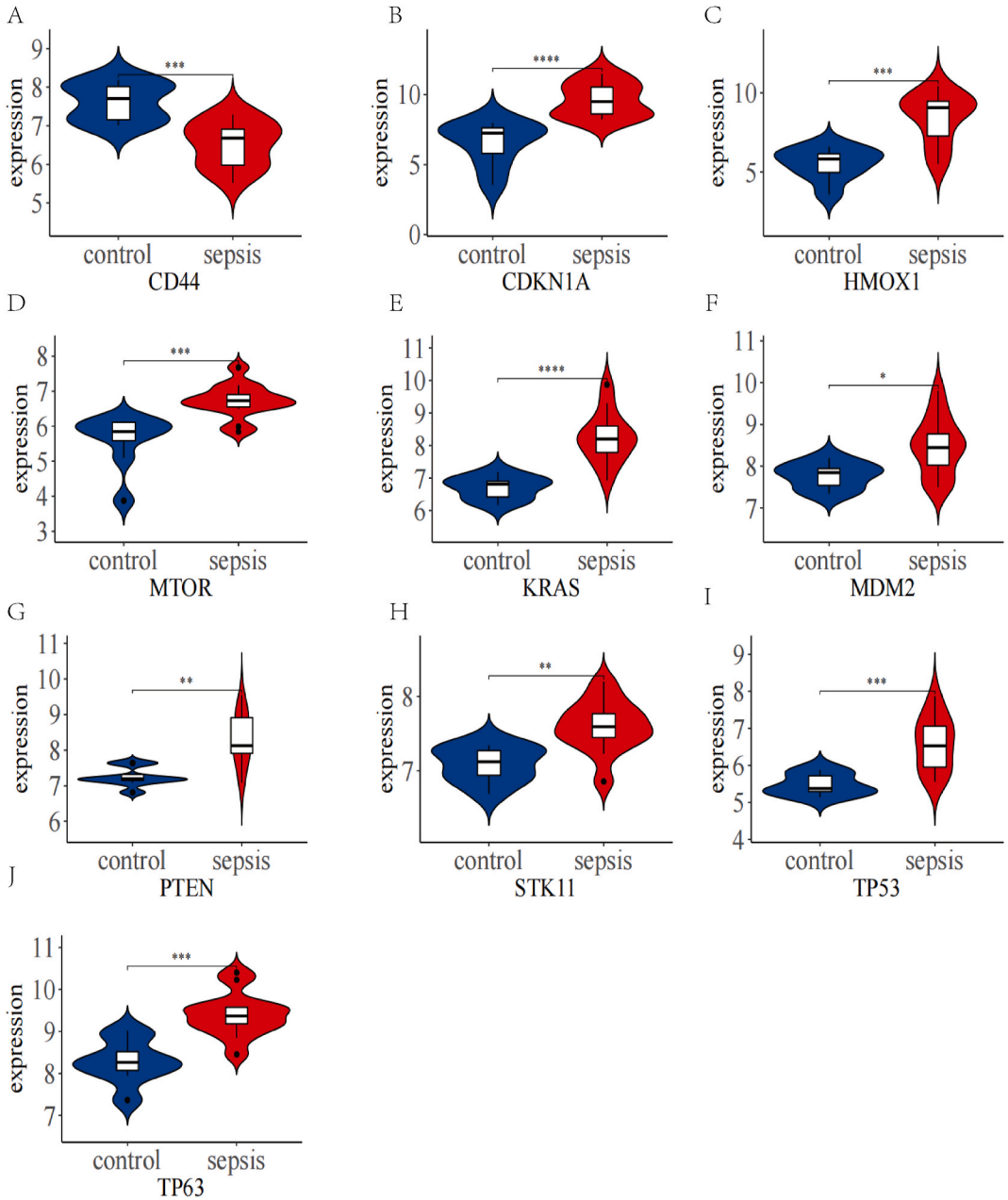

Fig. 5. The expression levels of CD44(A), CDKN1A(B), HMOX1(C), MTOR(D), KRAS(E), MDM2(F), PTEN (G), STK11(H), TP53(I), and TP63(J) were compared between the sepsis and control groups in the GSE13205 dataset. *P < 0.05 compared to control; **P < 0.01 compared to control; ***P < 0.001 compared to control.

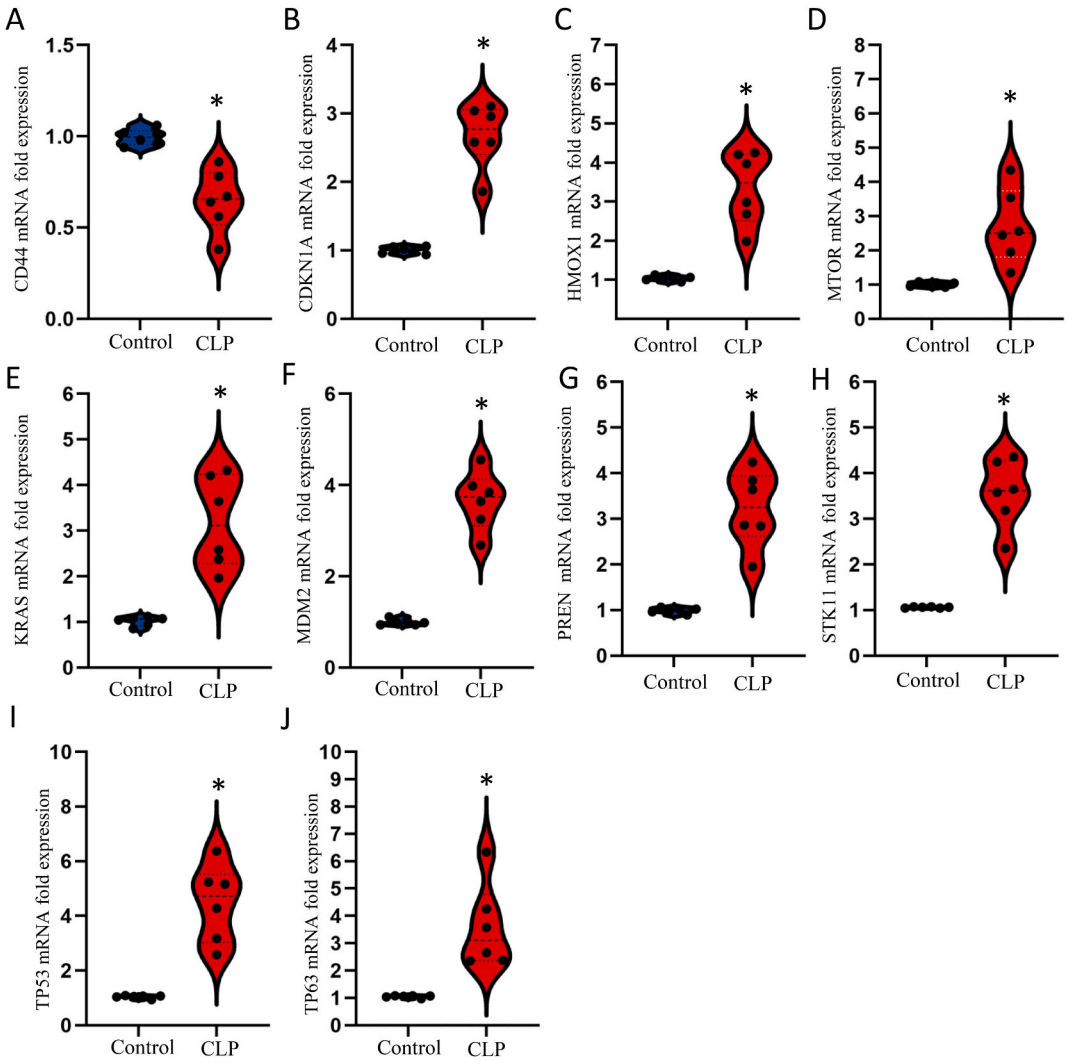

Fig. 6. Mouse gastrocnemius muscles were sampled 96 h after the CLP or Sham procedure to extract total RNA. The expression of Hub genes was evaluated by RT-qPCR array analysis. The mRNA levels of CD44(A), CDKN1A(B), HMOX1(C), MTOR(D), KRAS(E), MDM2(F), PTEN(G), STK11(H), TP53(I), and TP63(J) were compared between the CLP and Sham mice groups. The data shown is the average value plus or minus the standard error of the mean from six mice in each group. The observed difference is statistically significant ($p < 0.05$) when compared to the sham group.

Table 3
List of top 10 small molecular compound provided by CMap to FRDEGs.

CMap name	ID	Score	Description
Varenicline	BRD-K18855837	−99.93	Acetylcholine receptor agonist
Zalcitabine	BRD-K85925969	−99.93	Nucleoside reverse transcriptase inhibitor
Zebularine	BRD-A01145011	−99.93	DNA methyltransferase inhibitor
FTI-276	BRD-K75532464	−99.93	Farnesyltransferase inhibitor
9-methyl-5H-6-thia-4,5-diaza-chrysene-6,6-dioxide	BRD-K14696368	−99.93	NF-kB pathway inhibitor
Peucedanin	BRD-K72034655	−99.93	Apoptosis stimulant
Tofacitinib	BRD-K31283835	−99.93	JAK inhibitor
Curcumin	BRD-K69690935	−99.89	Cyclooxygenase inhibitor
L-BSO	BRD-A47706533	−99.82	Glutathione transferase inhibitor
Fatostatin	BRD-K99696746	−99.82	SREBP inhibitor

increase in the expression of P53, PTEN, MDM2, KRAS, STK11, CDKN1A, MTOR, TP63, and HMOX1, and a decrease in the expression of CD44 in the CLP group compared to the sham group (Fig. 6A–J).

3.6. Pharmacological target screening

We evaluated and refined the outcomes of the screening process for possible pharmaceutical targets from the CMap database, using drug connectivity scores as a criterion. The medications suggested for sepsis-related myopathy that stood out among the top-ranking ones were Varenicline, Zalcitabine, Zebularine, FTI-276, 9-methyl-5H-6-thia-4,5-diaza-chrysene-6,6-dioxide, Peucedanin, Tofacitinib, Curcumin, L-BSO, and Fatostatin (Table 3). These medications show potential as possible therapies for sepsis-induced muscle weakness.

4. Discussion

The progress in medical technology and care has resulted in a progressive enhancement in the overall survival rate of people with sepsis. However, there has been a surge in the prevalence of ICU-acquired weakness (ICU-AW) or sepsis-induced muscle weakness [14]. Sepsis-induced myopathy is a disease characterized by the degeneration and debilitation of skeletal muscles [2]. Sepsis-induced myopathy not only prolongs hospitalization and complicates rehabilitation but also strongly adds to late mortality among sepsis survivors [2,4]. The current treatment strategies for sepsis-induced myopathy primarily concentrate on enhancing inflammatory disorders, delivering nutritional support, and implementing rehabilitation therapy. However, the current therapeutic options are still not sufficient in terms of their effectiveness [2]. Hence, it is imperative to investigate novel therapeutic strategies.

The progress of personalized and precision medicine offers hopeful opportunities for treating sepsis-induced myopathy. This involves combining genomes and transcriptomics with therapy approaches that are customized to the specific metabolic characteristics of each individual. This work commenced by analyzing the gene expression patterns of patients with sepsis-induced myopathy and healthy persons to discover genes that are differently expressed and linked with ferroptosis. The research revealed 61 genes that were expressed differently in sepsis-induced myopathy and were associated with ferroptosis. Among these genes, 51 were upregulated and 10 were downregulated. These data indicate that Ferroptosis-associated genes may have a role in the development of sepsis-induced myopathy, which might be targeted by drugs.

Additionally, further GO enrichment analysis indicated the role of these genes in numerous biological processes, including oxidative stress response, NADPH effect, P53 binding, flavin adenine dinucleotide binding, nuclear receptor binding, and protein domain binding. Oxidative stress plays a significant role in the development of skeletal muscle wasting and weakening during sepsis [15], making it an attractive target for pharmacological intervention [16,17]. Phenolic substances have been found to reduce sepsis-induced skeletal muscle atrophy by reducing oxidative stress [18]. In septic rats, an increased NAD(P)H oxidase complex is associated with elevated ROS [19]. P53 is a crucial component in the cellular aging process of sepsis. It acts as a target for metformin, a therapeutic agent that reduces senescence in mouse myocytes and skeletal muscle cells [20]. These genes were discovered to be linked to a range of biological processes, including reactions to stimuli from outside the cell, the body's nutrition levels, programmed cell death (apoptosis), and responses to peptides, chemical stressors, and hormones. These findings align with earlier studies. Enhancing the nutritional intake is seen as a top priority for addressing the muscle loss associated with sepsis [21]. Apoptosis, necroptosis, and ferroptosis are all important mechanisms and drug targets in sepsis-induced organ dysfunction [22,23]. Apoptosis and necroptosis have received significant attention in the study of sepsis-induced myopathy [5,24], whereas research on ferroptosis remains limited. Moreover, numerous peptides and hormones have been identified to exert diverse biological effects on sepsis-induced muscle wasting [25]. For instance, glucocorticoids have a direct catabolic effect and lead to muscle protein loss, while GH, IGF-I, and androgens are major regulators of anabolic metabolism [25,26].

The KEGG pathway analysis showed that the differentially expressed genes associated with Ferroptosis in sepsis-induced myopathy mainly contribute to pathways such as ferroptosis, autophagy, the p53 signaling pathway, the PI3K-Akt signaling pathway, the mTOR signaling pathway, the HIF-1α signaling pathway, endocrine resistance, and several cancer-related pathways. Autophagy has been recognized as a major regulatory mechanism and therapeutic target for reducing sepsis-induced muscle atrophy [27,28]. The activation of the PI3K-Akt signaling pathway has been shown to mitigate oxidative stress and decrease inflammation, consequently relieving muscle wasting caused by sepsis [16,29]. The mTOR pathway, an evolutionarily conserved protein kinase, finely regulates protein metabolism in skeletal muscle [30,31]. In addition, HIF-1α has a crucial function in promoting inflammation and cell death, and inhibiting HIF-1α has been demonstrated to improve diaphragmatic dysfunction caused by mechanical ventilation in a mouse model of endotoxemia [32,33]. These observations serve as a basis for the creation of specific therapies for sepsis-induced muscle weakness.

Through our analysis, we have constructed a network that shows the interactions between proteins related to Ferroptosis and genes that are expressed differently. Within this network, we have found 10 key genes that are directly connected to the occurrence of ferroptosis in sepsis-induced myopathy. The following genes are included: TP53, PTEN, STK11, MTOR, HMOX1, CDKN1A, TP63, MDM2, KRAS, and CD44. In addition to its function as a crucial tumor suppressor, TP53 also plays a substantial role in the development of skeletal muscle atrophy. By inhibiting the expression of SLC7A11, it can cause ferroptosis in muscle cells, which hinders the transformation of C2C12 cells from myoblasts to myotubes [34]. PTEN, also known as Phosphatase and tensin homolog, functions as a crucial inhibitory controller of the PI3K/Akt pathway. Elevated PTEN expression suppresses PI3K/Akt signaling activity, intensifying muscle disease. PTEN suppression greatly increases skeletal muscle mass and strength while improving several dystrophic muscle features, such as myofiber inflammation, necrosis, and fibrosis [35]. STK11, also known as Serine/Threonine protein kinase 11, is

involved in critical biological processes such as cell proliferation, division, and differentiation. It has a significant impact on the way glucose is used, the breakdown of fatty acids, and the control of the growth, multiplication, and specialization of skeletal muscle precursor cells [36]. MTOR activates the process of adding phosphate groups to specific molecules that control the production of proteins, while also preventing the breakdown of proteasomes and autophagy proteins. This helps regulate inflammation and the loss of muscle mass caused by sepsis [37]. HMOX1, also known as Heme oxygenase 1, is an essential enzyme that plays a critical role in multiple cellular biological processes. Downregulation of HMOX1 has been demonstrated to enhance cardiac muscle cell viability in mice exposed to LPS [38]. CDKN1A, also known as p21 (cyclin-dependent kinase inhibitor 1A), is a CDK inhibitor that contributes to the regulation of the cell cycle and apoptosis in skeletal muscle cells [39]. Transcript variations of TP63, also known as Tumor Protein 63, have contrasting effects on skeletal muscle development [40].

The CMap database contains more than one million gene expression fingerprints acquired by perturbing a wide range of cell types using different agents, such as small-molecule drugs, gene overexpression, and knockdown reagents. This database makes a connection, varying in strength, between diseases, genes, and prospective therapies based on how cells respond to disturbance. We inputted Ferroptosis-associated DEGs that were strongly expressed into the CMap database in our analysis. The differential gene expression signatures were compared to all perturbational signatures in the database. Subsequently, small molecule drugs substantially linked with the expression of these genes in sepsis-induced myopathy were discovered. Varenicline and nine additional chemical compounds demonstrated a connective score of less than −90, indicating a robust negative connection between the expression of these genes in cells treated with these compounds and their counterpart in sepsis-induced myopathy. Tofacitinib is a selective inhibitor of the JAK pathway, which can affect the metabolism and function of skeletal muscles, resulting in muscular atrophy [41]. Furthermore, research has demonstrated that Curcumin can ameliorate myopathy induced by atorvastatin [42]. FTI276 can inhibit the activation of Ras and the phosphorylation of ERK1/2, which are known to be negative regulators of the Ras-ERK pathway. These molecules have been shown to have a role in skeletal myocyte development, as documented in studies [43,44].

However, our study has several drawbacks. Initially, we planned to examine many datasets, but we were only able to incorporate the GSE13205 expression profiling datasets for analysis due to challenges in obtaining specimens and the lack of extensive research on sepsis-induced myopathy. Furthermore, while we employed the gastrocnemius muscle of CLP mice to confirm the expression disparities of the ten identified hub genes, we did not have the necessary pathways associated with signal processes to conduct additional validation. In addition, although we investigated small molecule drugs that could potentially inhibit these hub genes using the cMap database, their effectiveness has not been confirmed. To address these limitations, our next basic experimental studies will focus on completely studying the complicated link between these Ferroptosis-associated genes and sepsis-induced myopathy.

5. Conclusion

The bioinformatics analysis conducted in this study has shed light on the potential role of Ferroptosis-associated genes in the development of sepsis-induced myopathy. The identification of crucial genes indicates potential targets for therapeutic intervention in the treatment of sepsis-induced myopathy, thereby creating opportunities for the creation of focused therapeutic agents.

Ethics statement

This study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Approval No. TJ-IRB20230214).

Data availability statement

The data and material used during the current study are available from the corresponding author upon reasonable request.

Consent statement

Not applicable. No individual personal data are included in the study.

Funding

The research was financially sponsored by the Hubei Provincial Department of Science and Technology (No. 2023AFB825) and Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (No. 2023A15).

CRediT authorship contribution statement

Dongfang Wang: Writing – original draft, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ligang Xu:** Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Yukun Liu:** Writing – original draft, Visualization, Validation, Supervision, Project administration, Investigation, Data curation. **Chuntao Wang:** Formal analysis, Data curation, Conceptualization. **Zhikai Xu:** Validation, Resources, Project administration, Methodology. **Fan Yang:** Investigation. **Zhanfei Li:** Investigation, Funding acquisition, Formal analysis, Data curation. **Xiangjun Bai:** Writing – review & editing, Validation, Supervision, Project administration. **Yiliu Liao:** Writing

– review & editing, Software, Formal analysis. **Xiangping Liu:** Writing – review & editing. **Yuchang Wang:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank the reviewers for their helpful comments on this article.

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