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Bioinformatics Analysis Identifies Hub Genes and Molecular Pathways Involved in Sepsis-Induced Myopathy

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Background: Sepsis-induced myopathy (SIM) is a complication of sepsis that results in prolonged mechanical ventilation, long-term functional disability, and increased patient mortality. This study aimed to use bioinformatics analysis to identify hub genes and molecular pathways involved in SIM, to identify potential diagnostic or therapeutic biomarkers.

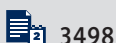
Material/Methods: The Gene Expression Omnibus (GEO) database was used to acquire the GSE13205 expression profile. The differentially expressed genes (DEGs) in cases of SIM and healthy controls, and the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the limma R/Bioconductor software package and clusterProfiler package in R, respectively. The protein-protein interaction (PPI) network data of DEGs was retrieved using the STRING database and analyzed using the Molecular Complex Detection (MCODE) Cytoscape software plugin.

Results: A total of 196 DEGs were obtained in SIM samples compared with healthy samples, including 93 upregulated genes. The DEGs were significantly upregulated in mineral absorption, and the interleukin-17 (IL-17) signaling pathway and 103 down-regulated genes were associated with control of the bile secretion signaling pathway. A protein-protein interaction (PPI) network was constructed with 106 nodes and 192 edges. The top two important clusters were selected from the PPI by MCODE analysis. There were 16 hub genes with a high degree of connectivity in the PPI network that were selected, including heme oxygenase 1 (HMOX1), nicotinamide adenine dinucleotide phosphate quinone dehydrogenase 1 (NQO1), and metallothionein (MT)-1E.

Conclusions: Bioinformatics network analysis identified key hub genes and molecular mechanisms in SIM.

MeSH Keywords: **Gene Expression Profiling • Intensive Care Units • Sepsis**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/919665>



Background

Sepsis-induced myopathy (SIM) is a complication of sepsis that results in prolonged mechanical ventilation, long-term functional disability, and increased patient mortality [1]. In patients with SIM, quality of life is seriously affected by reduced peripheral muscle strength, including the weakness of respiratory muscles. Because the balance between the work of breathing and the burden on respiratory muscles is affected in SIM, patients may develop respiratory failure. Reduced peripheral muscle strength adversely affects the daily activities of patients, which can result in increased mortality for patients with heart failure.

Previous clinical studies on the management of SIM mainly focussed on the reduction in mortality from sepsis. With increasing developments and improvements in life support systems used in the intensive care unit (ICU), the mortality rates from sepsis have been significantly reduced, resulting in the increased prevalence of SIM among survivors [2].

Cytokines, including interleukins and tumor necrosis factor (TNF), are small protein molecules with cell signal transduction functions that activate and phosphorylate downstream target cells and are involved in the progression of sepsis [3]. The host responses in SIM are maintained in a dynamic balance between pro-inflammatory and anti-inflammatory factors at an early stage. Pro-inflammatory cytokines include TNF- α , interleukin-1 β (IL-1 β), and interferon regulatory factor 3 (IRF3). These cytokines are activated by pattern recognition receptors, pathogen-associated molecular patterns (PAMPs), and endogenous damage-associated molecular patterns (DAMPs), which results in the activation of downstream signaling or cytokine cascades [4,5]. Recent studies have shown that anti-inflammatory cytokines, including IL-10, IL-4, interleukin-1 receptor antagonist (IL-1RA), and TNF- β block the action of pro-inflammatory cytokines responses [4,5]. The stress response to SIM has a progressive time course. Following organ failure and tissue damage caused by infection, patients then suffer from the effects of the activation of cytokines [4,5]. However, the molecular mechanisms of SIM remain unclear.

Bioinformatics and network analysis of gene microarrays is an effective way to explore gene expression profiles in the pathogenesis of disease. Therefore, this study aimed to use bioinformatics analysis to identify hub genes and molecular pathways involved in SIM, to identify potential diagnostic or therapeutic biomarkers. Differentially expressed genes (DEGs) were compared between SIM samples and healthy samples using bioinformatics analysis. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were used to construct the protein-protein interaction (PPI) network. Also, in this study, an analysis was performed using Molecular Complex Detection (MCODE) software [6].

Material and Methods

Microarray data

The expression profile GSE13205 was downloaded from the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo) and was used in this study. The dataset consisted of 13 sepsis-induced myopathy (SIM) samples and eight healthy samples. The muscle biopsies were taken from the lateral portion of the vastus lateralis muscle, 10–20 centimeters above the knee in patients with or without SIM. The platform used was the Affymetrix Human Genome U133 Plus 2.0 Array (GPL570, HG-U133_Plus_2) (ThermoFisher Scientific, Waltham, MA, USA).

Identification of differentially expressed genes (DEGs)

The limma R/Bioconductor software package was used to perform the identification of DEGs between SIM samples and healthy samples in R (Version 3.5.3) [7]. The cutoff criteria were $|\log_{2}FC| \geq 2$ (\log_{2} fold change) and a P-value < 0.05 . Finally, 196 DEGs were screened out, including 93 upregulated genes and 103 down-regulated genes.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs

GO enrichment analysis was commonly used to annotate the degree of gene function terms in DEGs, which included molecular function (MF), biological process (BP), and cellular component (CC). In this study, attention was given to the enrichment results of BP to illustrate the pathophysiology process of SIM from a larger biological perspective. KEGG enrichment analysis was used to demonstrate enriched signaling pathways in DEGs [8]. The clusterProfiler was used to perform GO and KEGG enrichment analysis of DEGs [9]. $P < 0.05$ was considered to represent statistical significance.

Protein-protein interaction (PPI) network and subcluster analysis

To ensure the optimal graphical display of protein interactions of DEGs, the Search Tool for the Retrieval of Interacting Genes (STRING) database (<http://string-db.org/>) was used to generate the PPI network dataset. A minimum required interaction score ≥ 0.4 was selected as the cutoff value. The ggnet network package was used to visualize the PPI network [10]. The Molecular Complex Detection (MCODE) Cytoscape software plugin (Version 3.7.1) was used to create subclusters in the PPI network of SIM [6,11]. The advanced options were set at: degree cutoff=2; node score cutoff=0.2; and K-Core=2.

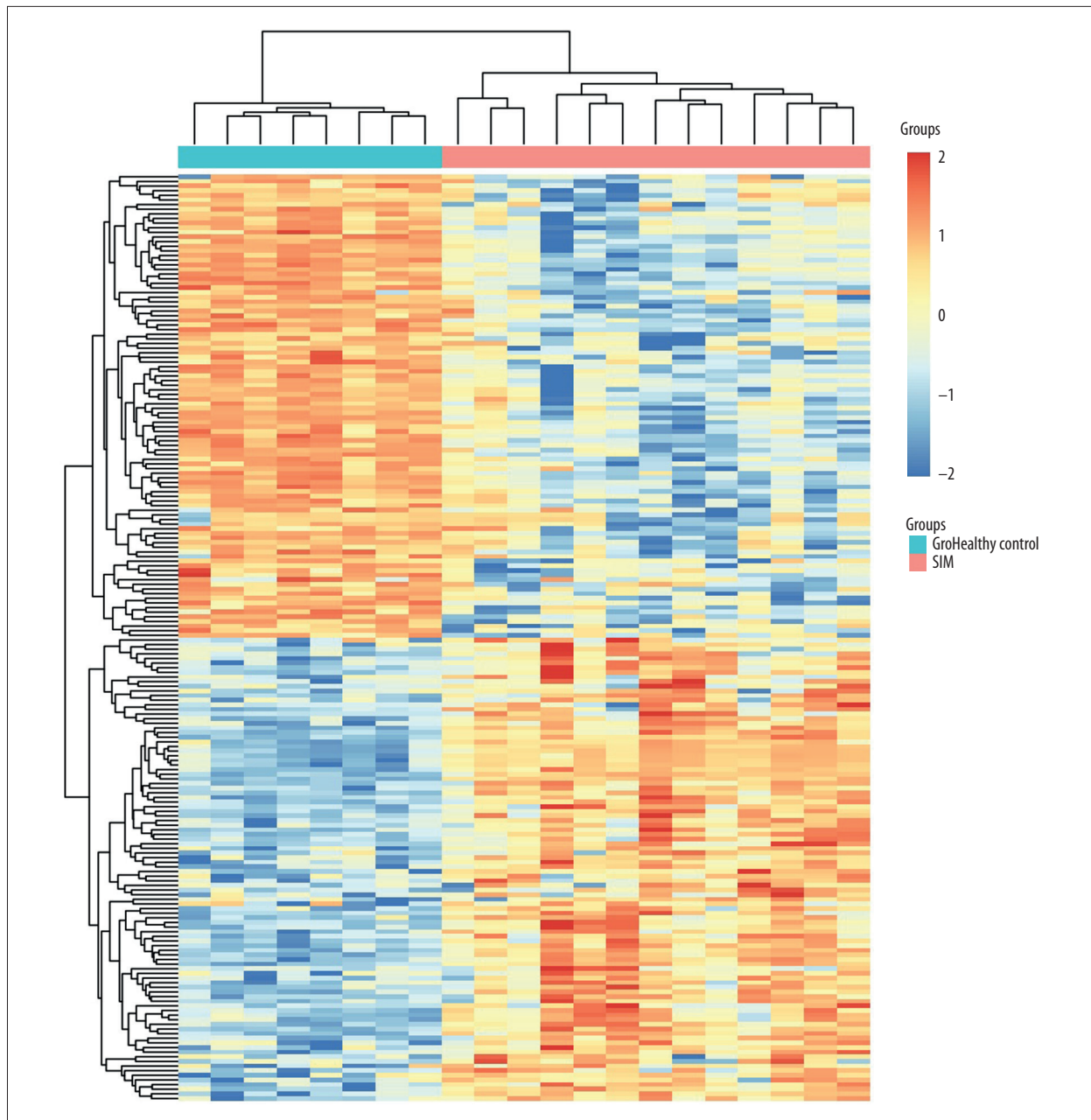


Figure 1. Heatmap of the top 100 upregulated and down-regulated genes of the expression dataset for sepsis-induced myopathy (SIM) initially analyzed using the limma R/Bioconductor software package.

Results

Identification of differentially expressed genes (DEGs)

In total, 13 samples from cases of sepsis-induced myopathy (SIM) and eight healthy samples were analyzed. The heatmap of the top 100 upregulated and down-regulated genes of the expression dataset that were preliminarily processed by the limma R/Bioconductor software package are shown in Figure 1. After the set threshold of P-value and logFC, there

were 93 upregulated genes and 103 down-regulated genes in the DEGs (Figure 2). The upregulated DEGs and down-regulated DEGs are listed in Table 1.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs

GO enrichment analysis of the biological process (BP) showed that upregulated DEGs were significantly enhanced for detoxification of copper ion, the stress response to copper ion,

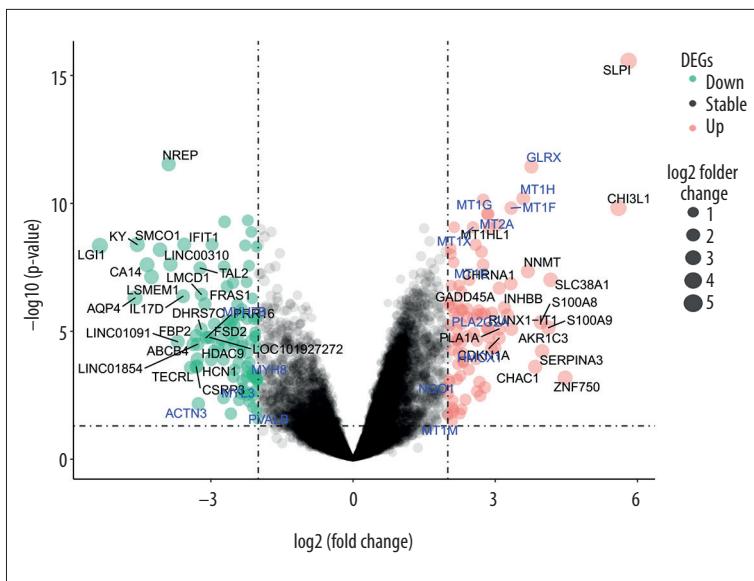


Figure 2. Volcano plot of the differentially expressed genes (DEGs) in sepsis-induced myopathy (SIM). Genes with a log fold change (logFC) >3 are labeled in black, and genes of the top two subclusters are labeled in blue.

Table 1. The 196 differentially-expressed genes (DEGs) screened from microarray data, including 93 upregulated genes and 103 down-regulated genes in the cases of sepsis-induced myopathy (SIM) compared with the healthy controls.

DEGs	Gene names
Upregulated DEGs	SLPI, CHI3L1, ZNF750, SLC38A1, S100A8, SERPINA3, S100A9, CHAC1, GLRX, NNMT, MT1H, AKR1C3, MT1F, CHRNA1, RUNX1-IT1, INHBB, GADD45A, CDKN1A, PLA1A, MT1HL1, TOGARAM2, PLA2G2A, HMOX1, RRAD, MYC, MT2A, MAFF, MT1G, AKR1C1, PNPLA3, ZNF653, ERV3-2, MT1E, IL6R, CGREF1, SCD, CES4A, ETV5, LINC01554, REPS2, ARNTL, FASN, HDAC4, AKR1C2, PFKP, MT1X, DPP3, HK2, ERRF1, SLC5A6, HMGB3, FCMR, LIPG, HMGB3P1, EFCAB7, TRIM62, CHMP1B, MYBPH, FOSL2, NLE1, SLC22A5, PCK1, MMP3, ETS2, SLC16A6, LMNB2, IFFO2, IFITM10, SELL, PHKG2, ERVK13-1, DHCR24, SMAP2, CTPS1, ZNHIT2, GYPE, KDM7A, AREG, LRRN3, BEST3, TRAF3IP2, LOC105374325, MT1M, BYSL, SLC7A6, PSMD8, POR, LINC01996, CMSS1, NQO1, NT5C, ARHGAP20, CXCL2
Down-regulated DEGs	LGI1, AQP4, KY, CA14, LSMEM1, SMC01, NREP, LINC00310, LINC01091, IL17D, IFIT1, TECRL, ABCB4, FBP2, CSRP3, HCN1, HDAC9, LINC01854, ACTN3, TAL2, DHRS7C, LMCD1, FRAS1, PRR16, FSD2, LOC101927272, SLC1A7, ATP2B2, C1orf127, CTXN3, KCNN2, MYOZ3, LSMEM2, ASB15, METTL21C, NPY6R, LINC00312, SHISA2, IRS1, NOV, SNX20, TTTY14, LOC646736, MYH7B, PDP1, IL12B, SEMA6D, WEE2-AS1, PGAM2, SFRP4, LUM, OTUD1, LINC01279, OLFML1, CLSTN2, RGS3, PDZRN3, TYRP1, C10orf71-AS1, E2F8, WDR62, METTL21EP, MIR1-1HG, LINC01405, XPO4, C12orf75, PPP1R3A, PDZRN3-AS1, FNDC1, MXRA5, LMOD1, CALML6, TET1, DIXDC1, MYL3, SNAI3, PALM2, TM6SF1, RNF150, EML1, OPCML, SEC14L5, FAM83B, UPK3A, EYA1, MYLK3, C20orf197, MFAP4, TMEM182, OR7E47P, CAPN6, CX3CR1, ANGPT1, ATP1B4, SLC25A30, COQ10A, KLHL31, PVALB, APBA1, NKAIN2, OGN, MYH8, KCNS3

The upregulated and down-regulated genes arranged in descending order according to the absolute value of the log fold change (logFC). DEGs – differentially expressed genes; FC – fold change.

detoxification of inorganic compounds, cellular zinc ion homeostasis, and the stress response to metal ions. Down-regulated DEGs were enriched for striated muscle contraction, muscle system process, muscle filament sliding, actin-myosin filament sliding, and granulocyte-macrophage colony-stimulating factor (GM-CSF) production (Table 2).

KEGG enrichment analysis showed that upregulated DEGs were significantly enhanced for mineral absorption, the IL-17 signaling pathway. Down-regulated DEGs were significantly enhanced for the bile secretion signaling pathway (Table 3).

Protein-protein interaction (PPI) network and subcluster analysis

A total of 192 interaction pairs of the DEGs were identified using the STRING database. A PPI network with 106 nodes of the DEGs was constructed based on these pairs (Figure 3). Analysis using the Molecular Complex Detection (MCODE) Cytoscape software plugin was used to create subclusters for the PPI networks. A total of nine subclusters were created (Figure 4). Genes were selected with subclusters with the MCODE score >5 as hub genes. In total, 16 genes were screened from two

Table 2. Top five Gene Ontology (GO) terms for sepsis-induced myopathy (SIM) enriched by upregulated and down-regulated genes, respectively.

DEGs	ID	Term	Count	P-value	Genes
Upregulated	GO: 0046916	Cellular transition metal ion homeostasis	12	3.69E-13	HMOX1/MT1E/MT1F/MT1G/MT1H/MT1HL1/MT1M/MT1X/MT2A/MYC/S100A8/S100A9
	GO: 0006882	Cellular zinc ion homeostasis	10	8.69E-16	MT1E/MT1F/MT1G/MT1H/MT1HL1/MT1M/MT1X/MT2A/S100A8/S100A9
	GO: 0055069	Zinc ion homeostasis	10	1.97E-15	MT1E/MT1F/MT1G/MT1H/MT1HL1/MT1M/MT1X/MT2A/S100A8/S100A9
	GO: 0071276	Cellular response to cadmium ion	10	1.97E-15	AKR1C3/HMOX1/MT1E/MT1F/MT1G/MT1H/MT1HL1/MT1M/MT1X/MT2A
	GO: 0010273	Detoxification of copper ion	8	9.64E-17	MT1E/MT1F/MT1G/MT1H/MT1HL1/MT1M/MT1X/MT2A
Down-regulated	GO: 0003012	Muscle system process	8	1.53E-03	ACTN3/CSRP3/KCNN2/LMCD1/LMOD1/MYH8/MYL3/PGAM2
	GO: 0006936	Muscle contraction	7	1.89E-03	ACTN3/CSRP3/KCNN2/LMOD1/MYH8/MYL3/PGAM2
	GO: 0014706	Striated muscle tissue development	7	2.71E-03	ACTN3/CSRP3/EYA1/HDAC9/METTL21C/MYL3/MYLK3
	GO: 0006941	Striated muscle contraction	6	2.35E-04	ACTN3/CSRP3/KCNN2/MYH8/MYL3/PGAM2
	GO: 0007519	Skeletal muscle tissue development	5	1.64E-03	ACTN3/CSRP3/HDAC9/METTL21C/MYL3

GO – Gene Ontology.

Table 3. The top five Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched in sepsis-induced myopathy (SIM) by upregulated and down-regulated genes, respectively.

DEGs	ID	Pathway	Count	P-value	Genes
Upregulated	hsa04978	Mineral absorption	9	1.30E-10	HMOX1/MT1E/MT1F/MT1G/MT1H/MT1HL1/MT1M/MT1X/MT2A
	hsa04657	IL-17 signaling pathway	5	4.41E-04	CXCL2/MMP3/S100A8/S100A9/TRAF3IP2
	hsa00140	Steroid hormone biosynthesis	3	1.40E-03	AKR1C1/AKR1C2/AKR1C3
	hsa04066	HIF-1 signaling pathway	5	1.80E-03	CDKN1A/HK2/HMOX1/IL6R/PFKP
	hsa05216	Thyroid cancer	3	3.55E-03	CDKN1A/GADD45A/MYC
Down-regulated	hsa04976	Bile secretion	4	1.10E-04	ABCB4/AQP4/ATP1B4/KCNN2
	hsa04022	cGMP-PKG signaling pathway	5	9.54E-04	ATP1B4/ATP2B2/CALML6/IRS1/MYLK3
	hsa04970	Salivary secretion	3	4.20E-03	ATP1B4/ATP2B2/CALML6
	hsa04971	Gastric acid secretion	3	4.20E-03	ATP1B4/CALML6/MYLK3
	hsa04261	Adrenergic signaling in cardiomyocytes	4	5.21E-03	ATP1B4/ATP2B2/CALML6/MYL3

KEGG – Kyoto Encyclopedia of Genes and Genomes; DEGs – differentially expressed genes.

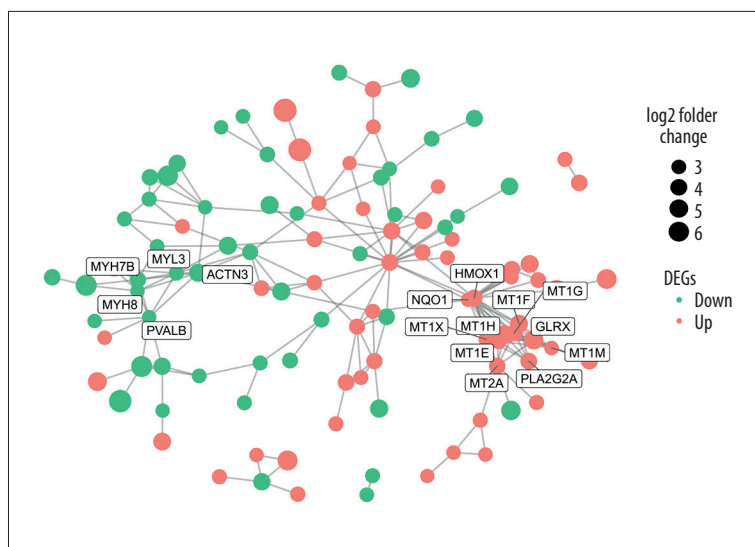


Figure 3. The protein–protein interaction (PPI) network for the differentially expressed genes (DEGs) in sepsis-induced myopathy (SIM). Genes of the top two subclusters are labeled separately.

subclusters. Hub genes of the top two subclusters contained in the top 5 GO enrichment analysis terms were processed by the GOplot package (Figure 5) [12]. These hub genes included heme oxygenase 1 (HMOX1), nicotinamide adenine dinucleotide phosphate quinone dehydrogenase 1 (NQO1), metallothionein (MT)-1E, MT1G, MT1H, myosin light chain 3 (MYL3), parvalbumin (PVALB), actinin alpha 3 (ACTN3), myosin heavy chain (MYH)-7B, and MYH8 genes. Almost all of the upregulated genes identified were associated with GO terms that included metal ions, while most of the down-regulated hub genes were associated with terms related to skeletal muscle.

Discussion

Sepsis accounts for a significant proportion of deaths in the intensive care unit (ICU), and has become a significant public health issue. Currently, due to the early recognition of sepsis, as well as the improvements of early control of infection, fluid resuscitation, life support, and other supportive treatments, patient mortality from sepsis has reduced [2]. With the increasing number of survivors from sepsis, one of the most common sequelae include sepsis-induced myopathy (SIM). This condition is characterized by extensive weakness of the limbs or respiratory muscles that occur while the patient is in the intensive care unit (ICU), which cannot be explained by causes other than severe sepsis. In the present study, data mining and bioinformatics analysis were performed to identify the differentially expressed genes (DEGs) from muscle biopsy samples from patients with SIM and healthy controls. This study approach identified the possible hub genes that were highly correlated with the protein–protein interaction (PPI) network to identify the target genes involved in the pathogenesis of SIM.

Hub genes of the top two subclusters that were identified using Molecular Complex Detection (MCODE) analysis and showed a high degree of connectivity in the PPI network are shown in Table 4. These hub genes included HMOX1, NQO1, MT1E, MT1G, MT1H, GLRX, MT1F, MT1X, MT2A, MT1M, PLA2G2A, MYL3, PVALB, ACTN3, MYH7B, and MYH8.

The HMOX1 gene encodes the enzyme, heme oxygenase 1, which mediates the process of heme catabolism and converts heme to biliverdin. Larsen et al. validated that knockdown of HMOX1 resulted in death in mouse models of sepsis, which indicate the protective effect of HMOX1 in the pathogenesis of sepsis [13]. These previous studies have supported the protected effects of HMOX1 expression in the prevention of tissue damage from free heme [13]. Also, a previous study showed that HMOX1 was required for IL-10 expression in sepsis, which supported that HMOX1 was involved in the regulation of anti-inflammatory cytokines [14].

The NQO1 gene encodes the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) dehydrogenase quinone 1. Previous studies have shown that NQO1 is a downstream gene of nuclear factor-erythroid-2-related factor 2 (NRF2), which is involved in the suppression of inflammation [15]. Also, overexpression of NQO1 and HMOX1 alone, or in combination, can reduce the expression of pro-inflammatory factors, including TNF- α and IL-1 [16].

The metallothionein (MT) family of cysteine-rich, low molecular weight proteins, are located on the membrane of the intracellular Golgi apparatus. The ability to bind physiological and heterogeneous metal ions with cysteine residues of MT is the mechanism that mitigates metal toxicity and oxidative stress and mediates the regulation of zinc and copper. MT1E, MT1G, MT1H, MT1F, MT1X, MT2A, and MT1M are subtypes or

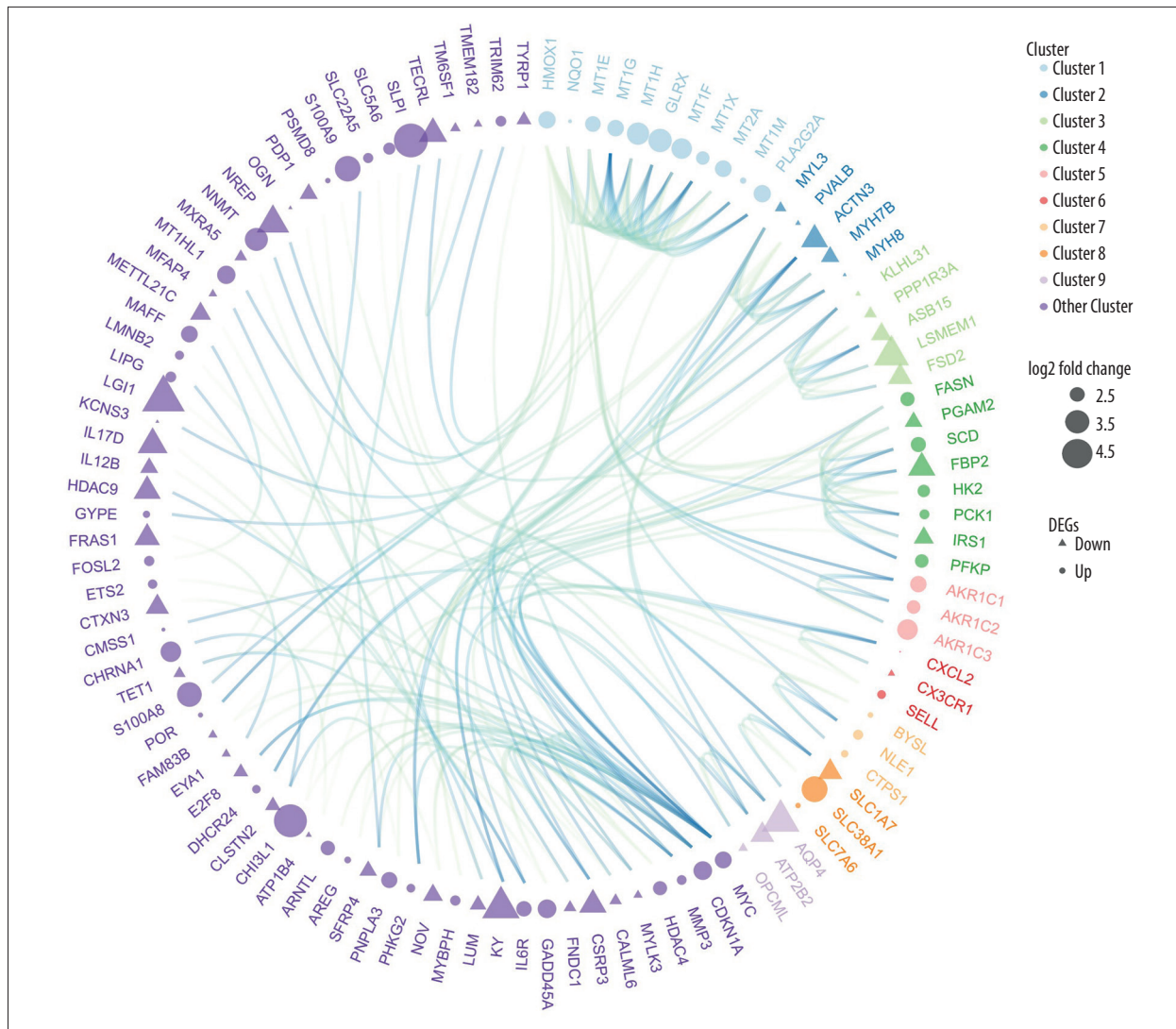


Figure 4. Subclusters created by Molecular Complex Detection (MCODE) analysis for sepsis-induced myopathy (SIM). Genes of the top two subclusters show an increased degree of connectivity.

isoforms of MT. MT has a protective effect on acute lung injury induced by lipopolysaccharide (LPS) in a mouse model [17]. MT2 plays a protective role by mediating the AKT pathway, reducing inflammation [18].

Glutaredoxin-1 is an enzyme located in the cytosol that is encoded by the GLRX gene, and is associated with inflammation induced by LPS. However, unlike the other genes identified in this study, inflammation induced by LPS is reduced by the down-regulation of GLRX [19]. Therefore, GLRX may be a product of positive feedback in the regulation of SIM.

PLA2G2A is an isoform of the family of phospholipase A2 (PLA2). A previous study has shown a close relationship with inflammation and PLA2G2A in inflammatory diseases, including sepsis, as the serum levels of PLA2G2A was positively correlated

with the degree of inflammation [20]. In the current study, the expression of PLA2G2A was down-regulated in SIM samples. In the later stages of sepsis, the mRNA level of TNF- α in the skeletal muscle in ICU acquired weakness (ICUAW) patients were shown to be lower than that of healthy controls [21]. The pathological features of ICUAW and SIM are similar, and the expression level of PLA2G2A in inflammatory disease is mainly regulated by pro-inflammatory factors, including TNF- α [21]. Therefore, the expression level of PLA2G2A in SIM may be mediated by TNF- α .

Myosin light chain 3 is a protein that is found mainly in the heart and type I skeletal muscle fibers and is encoded by the MYL3 gene [22]. The PVALB gene encodes parvalbumin, a small calcium-binding albumin protein, which is closely associated with skeletal muscle contraction, mainly in fast-twitch muscle

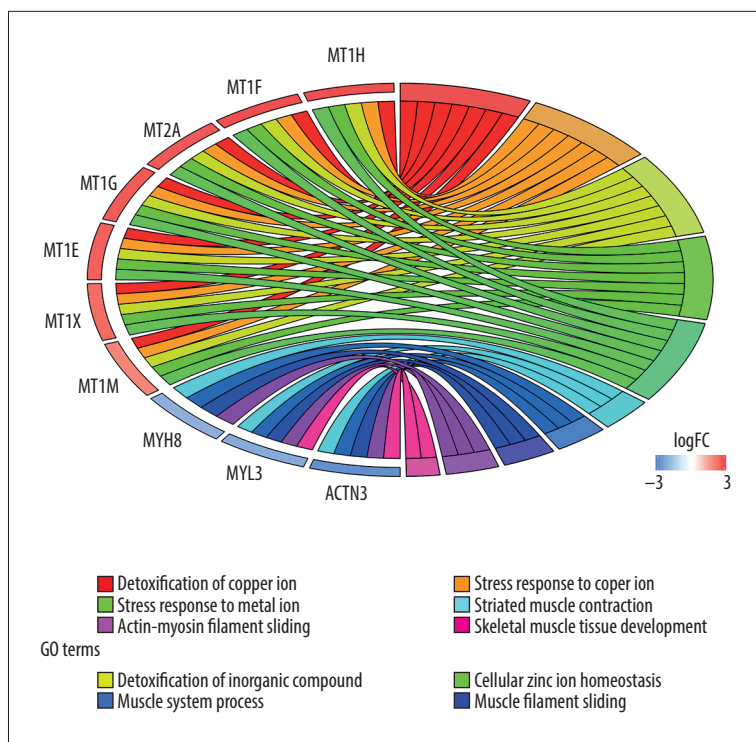


Figure 5. Hub genes of the top two subclusters contained in the top ten terms of Gene Ontology (GO) enrichment analysis of biological processes in sepsis-induced myopathy (SIM).

Table 4. Genes of the top two subclusters identified for sepsis-induced myopathy (SIM) by Molecular Complex Detection (MCODE) analysis with a high degree of connectivity in the protein–protein interaction (PPI) network.

Gene	Degree	DEGs	logFC	Subcluster
HMOX1	13	Up	2.92	Cluster 1
NQO1	12	Up	2.03	Cluster 1
MT1E	10	Up	2.74	Cluster 1
MT1G	10	Up	2.83	Cluster 1
MT1H	10	Up	3.59	Cluster 1
GLRX	9	Up	3.76	Cluster 1
MT1F	9	Up	3.33	Cluster 1
MT1X	9	Up	2.53	Cluster 1
MT2A	9	Up	2.85	Cluster 1
MT1M	5	Up	2.09	Cluster 1
PLA2G2A	5	Up	2.93	Cluster 1
MYL3	8	Down	-2.19	Cluster 2
PVALB	8	Down	-2.03	Cluster 2
ACTN3	7	Down	-3.26	Cluster 2
MYH7B	7	Down	-2.55	Cluster 2
MYH8	6	Down	-2.01	Cluster 2

MCODE – Molecular Complex Detection; PPI – protein–protein interaction; logFC – log fold change.

fibers. In a mouse model of age-induced sarcopenia, PVALB expression was shown to play an important role in contractile dysfunction [23]. Alpha-actinin-3 is an actin-binding protein encoded by the ACTN3 gene. ACTN3 expression has a role in fast-twitch muscle fibers [24]. MYH7B and MYH8 are isoforms of the motor protein in the myosin heavy chain family. MYH7 is mainly distributed in the heart and skeletal muscles (slow-twitch muscle fibers), while MYH8 has an extensive role in almost all muscle fibers. Several types of myosin heavy chain isoforms are associated with the physiological characteristics of different muscle fiber types [25]. The pathogenesis of SIM is often accompanied by a severe and sustained loss of skeletal muscle, which is mainly caused by reduced muscle synthesis and an increase in muscle degeneration.

In the present study, almost all the enrichment terms of up-regulated genes in the Gene Ontology (GO) enrichment analysis were related to a large class of metal ions. Trace elements are necessary to maintain cellular homeostasis, as are the microorganisms that cause infection. During the pathogenesis of sepsis, the endogenous equilibrium of metal ion homeostasis is often abnormal [26,27]. Oxidative stress also occurs in sepsis with the excess generation of reactive oxygen species (ROS), including superoxide. Superoxide is converted to hydrogen peroxide under the action of superoxide dismutase and is then converted to water by catalyzing with metal ions [28]. In an LPS-induced mouse model of sepsis, the serum zinc levels decreased together with an increase in TNF- α and other cytokines, which may be due to the cytokine-mediated redistribution [29]. Similar trends were found in patients with sepsis who were admitted to the intensive care unit (ICU) [30–32]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway mineral absorption, which was enriched by upregulated genes, was consistent with the hypothesis that metal ions may participate in the regulation of SIM. Although the role of metal ions as antioxidants in the treatment of sepsis remains controversial, the role of selenium, for example, has been shown [26].

All the enrichment terms of down-regulated genes in the GO enrichment analysis were associated with skeletal muscle. The characteristic of muscular atrophy in patients with SIM is significant and is consistent with the loss of myosin and myosin-associated proteins, resulting in an increased rate of muscle

degradation compared with muscle protein synthesis. The ubiquitin-proteasome system plays an important role in muscle proteolysis in the process of ICUAW [33]. Clarke et al. showed that treatment with a ubiquitin-proteasome system inhibitor could prevent proteolysis of MYH, which indicated that MYH was degraded via the ubiquitin-proteasome system [34,35].

This study had several limitations. ICUAW is a broad category of disease characterized by respiratory muscle or peripheral skeletal muscle weakness due to nerve injury or muscle wasting that cannot be explained except by critical disease [36]. Immobilization, application of corticosteroid, insulin resistance, and infection are closely associated with ICUAW [33,37]. Therefore, in clinical practice, skeletal muscle atrophy is also affected by other factors, which means that ICUAW affected by sepsis alone was virtually absent. Also, there are no recognized clinical diagnostic criteria for SIM, and all the factors described above, and in this study, will affect the specificity of the patient samples used for the study of SIM. As several of the hub genes identified in the present study had not been previously associated with SIM, further *in vitro* and functional studies and studies using knockout mice are needed to verify the molecular biological mechanisms of these genes.

Conclusions

This study aimed to use bioinformatics analysis to identify hub genes and molecular pathways involved in sepsis-induced myopathy (SIM), to identify potential diagnostic or therapeutic biomarkers. Sepsis is closely associated with the expression of pro-inflammatory factors, trace metal ions, and myosin family proteins and genes. Further studies are needed to validate the associated hub genes and their functional roles in SIM.

Acknowledgments

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Conflict of interest

None.

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