Exercise-induced modulation of miR-149-5p and MMP9 in LPS-triggered diabetic myoblast ER stress: licorice glycoside E as a potential therapeutic target

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Author contributions
Yi Du conducted the primary cellular experiments and contributed significantly to the manuscript preparation, while Hong Liu was responsible for conceptualizing the study, overseeing the research process, and finalizing the written document for publication. Both authors collaborated in the analysis and interpretation of the data.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
CCK8, cell counting kit-8; DEGs, differentially expressed genes; DE-miRNAs, differentially expressed micro ribonucleic acids; ER, endoplasmic reticulum; FITC, fluorescein isothiocyanate; LPS, lipopolysaccharide; MMP9, matrix metalloproteinase 9; miRNA, messenger ribonucleic acid; miR-149-5p, microRNA-149-5p; miRNA, micro ribonucleic acid; PPI, protein-protein interaction; PDB, Protein Data Bank; qRT-PCR, quantitative real-time polymerase chain reaction; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database; TIMER, Tumor Immune Estimation Resource; TNF-α, tumor necrosis factor alpha; WB, western blotting; SD, standard deviation; ILG, isoliquiritigenin.

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Abstract

Background: This study explores the relationship between endoplasmic reticulum (ER) stress and diabetes, particularly focusing on the impact of physical exercise on ER stress mechanisms and identifying potential therapeutic drugs and targets for diabetes-related sepsis. The research also incorporates traditional physical therapy perspectives, emphasizing the genomic insights gained from exercise therapy in disease management and prevention.

Methods: Gene analysis was conducted on the GSE168796 and GSE94717 datasets to identify ER stress-related genes. Gene interactions and immune cell correlations were mapped using GeneCard and STRING databases. A screening of 2,456 compounds from the TCMSP database was performed to identify potential therapeutic agents, with a focus on their docking potential. Techniques such as luciferase reporter gene assay and RNA interference were used to examine the interactions between microRNA-149-5p and MMP9.

Results: The study identified 2,006 differentially expressed genes and 616 miRNAs. Key genes like MMP9, TNF-α, and IL1B were linked to an immunosuppressive state. Licorice glycoside E demonstrated high affinity for MMP9, suggesting its potential effectiveness in treating diabetes. The constructed miRNA network highlighted the regulatory roles of MMP9, IL1B, IFNG, and TNF-α. Experimental evidence confirmed the binding of microRNA-149-5p to MMP9, impacting apoptosis in diabetic cells. Conclusion: The findings highlight the regulatory role of microRNA-149-5p in managing MMP9, a crucial gene in diabetes pathophysiology. Licorice glycoside E emerges as a promising treatment option for diabetes, especially targeting MMP9 affected by ER stress. The study also underscores the significance of physical exercise in modulating ER stress pathways in diabetes management, bridging traditional physical therapy and modern scientific understanding. Our study has limitations. It focuses on the microRNA-149-5p-MMP9 network in sepsis, using cell-based methods without animal or clinical trials. Despite strong in vitro findings, in vivo studies are needed to confirm licorice glycoside E’s therapeutic potential and understand the microRNA-149-5p-MMP9 dynamics in real conditions.

Keywords: ER stress; diabetes; physical exercise; gene expression; microRNA-149-5p; MMP9; licorice glycoside E; traditional physical therapy; genomics insights
Gaining increasing attention in the context of chronic illnesses like diabetes, sepsis has increasingly become a focal point of global healthcare discussions [1]. This acute condition, known for its rapid onset and potential to develop into severe complications, poses a unique challenge in diabetic patients. This condition, characterized by its swift onset and potential to escalate into severe complications, has been a persistent challenge for clinicians and researchers alike [2]. The global prevalence of sepsis and its associated high mortality rates not only underscores the clinical urgency of addressing this condition but also highlights the profound socio-economic implications it carries [3]. Diabetic patients with sepsis often experience exacerbated physical and psychological impacts due to the compounded effects of their chronic condition [4]. This intersection also places a substantial economic burden on healthcare systems worldwide, which must navigate the intricacies of treating sepsis in the context of underlying chronic diseases like diabetes.

Historically, the pathophysiological underpinnings of sepsis have been a subject of intense research. While strides have been made in understanding its multifaceted nature, there remain gaps in our comprehension, particularly concerning the intricate cellular mechanisms at play. One such mechanism that has recently come to the forefront of sepsis research is the role of ER stress [5]. The ER, a pivotal cellular organelle, is integral to protein synthesis and folding [6]. However, disruptions in its homeostasis, leading to ER stress, have been implicated in a myriad of diseases, with sepsis being a notable inclusion. This stress response, triggered by the accumulation of misfolded proteins, has been postulated to play a critical role in the pathogenesis of sepsis, offering a novel avenue for therapeutic interventions [7, 8]. Meanwhile, this pathway is also the central feature at the molecular, cellular, and organ level in insulin resistance and type 2 diabetes. It has been reported that ER stress leads to inhibition of the insulin receptor signaling pathway through overactivation of c-Jun N-terminal kinase and subsequent serine phosphorylation of insulin receptor substrate-1 [6]. The ER and related signaling networks are emerging as potential sites for the intersection of inflammatory and metabolic diseases [9]. Özcan et al. found that chemical chaperones that alleviate ER stress in obese and diabetic mice provide a therapeutic pathway for managing complications caused by ER stress, potentially relevant in sepsis [10]. This suggests a potential crossover between ER stress and sepsis pathophysiology in diabetic patients.

Previous studies have indicated the role of microRNA-149-5p (miR-149-5p) and matrix metalloproteinase 9 (MMP9) in diabetes and ER stress. Specifically, miR-149-5p has been reported to regulate pancreatic beta-cell survival and function under ER stress, and MMP9 has been implicated in advanced glycation end products-induced ER stress in diabetics [11]. This means they could be a potential target for the treatment of ER stress-induced sepsis. More specifically, miR-149-5p, a microRNA, has been identified as a regulator of cell proliferation, apoptosis, and migration. It functions either as a tumor suppressor or promoter, depending on the cellular context and target genes. Its interaction with MMP9, an enzyme involved in the degradation of the extracellular matrix, suggests a complex regulatory network affecting tumor growth and metastasis. MMP9's upregulation is associated with enhanced invasive potential of cancer cells and poor prognosis in patients [12, 13]. Within the expansive landscape of therapeutic interventions, physical exercise stands as a time-honored beacon, revered for its multifaceted health-promoting attributes [14]. Additionally, the integration of multi-omics data in traditional physical therapy research, including exercise therapy, has been pivotal in transforming our understanding of these practices. Exercise therapy, a long-standing essential component of holistic healthcare, brings genomic insights to the forefront: exploring how physical exercise influences gene expression and genetic susceptibility in the context of disease management and prevention. This intersection not only places a substantial economic burden on healthcare systems worldwide but also bridges the gap between ancient wisdom and modern science. Historically, the salutary effects of physical exercise have been predominantly associated with cardiovascular enhancements and metabolic regulation [15]. These benefits, ranging from improved cardiac function to optimized glucose metabolism, have been the cornerstone of numerous preventive and rehabilitative programs [16]. However, the therapeutic horizon of physical exercise extends far beyond these conventional domains.

Recent scientific explorations have unveiled a more intricate tapestry of benefits conferred by physical exercise, particularly at the cellular and molecular levels [17, 18]. One of the most intriguing revelations in this context is the potential of exercise to modulate ER stress [19, 20]. The ER, a labyrinthine network within cells, is pivotal for protein synthesis and maturation [21]. Disruptions in its function, leading to ER stress, can set off a cascade of cellular events, potentially culminating in pathological conditions like sepsis [22]. Preliminary studies suggest that physical exercise might act as a counterbalance, attenuating the adverse effects of ER stress and restoring cellular equilibrium [23].

This emerging nexus between physical exercise and ER stress modulation offers a tantalizing prospect for sepsis research [24]. Could exercise, with its ER stress-alleviating properties, serve as a bulwark against sepsis progression? To unravel this intricate puzzle, our research adopts a multifaceted approach. By synergistically leveraging the precision of bioinformatics analysis with the empirical rigor of in vitro cellular experiments, we seek to dissect the intricate interplay between exercise-induced ER stress modulation and its potential ramifications on sepsis [25–27]. Our overarching goal is to elucidate the molecular pathways through which physical exercise might target and ameliorate the deleterious effects of ER stress in the context of sepsis, potentially heralding a novel therapeutic paradigm.

The selection of licorice glycoside E as a potential therapeutic agent is based on its pharmacological properties, including anti-inflammatory, anti-viral, and anti-cancer activities [28, 29]. Given the involvement of miR-149-5p and MMP9 in promoting inflammatory environments and tumor progression, licorice glycoside E's bioactive components may modulate these molecular pathways, offering a rationale for its application in treating conditions where these molecules are implicated. Thus, understanding the roles of miR-149-5p and MMP9 in disease pathogenesis and the therapeutic potential of licorice glycoside E could provide insights into novel treatment strategies.

Our research adopts a multifaceted approach, combining bioinformatics analysis with in vitro cellular experiments, to understand how exercise-induced ER stress modulation affects diabetes-related sepsis. We aim to elucidate the molecular pathways
through which physical exercise might target and ameliorate the adverse effects of ER stress in this context, potentially leading to innovative therapeutic strategies that leverage the benefits of physical exercise in improving outcomes for patients with diabetes-related sepsis. The integration of omics data in traditional physical therapy research, such as exercise therapy, can profoundly change our understanding of these practices, merging ancient wisdom with modern science.

Materials & methods

Data acquisition and key gene selection
Gene expression datasets for sepsis messenger ribonucleic acid (mRNA) and micro ribonucleic acid (miRNA), GSE168796 and GSE94717, respectively, were retrieved from the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/) [30, 31]. Differential analysis was conducted on these datasets using the “limma” package in R, designating genes with a P-value < 0.05 as differentially expressed genes (DEGs) [32]. To investigate the relationship between sepsis, ER stress, and physical exercise, relevant genes were identified via the GeneCard platform (https://www.genecards.org/). Genes overlapping between DEGs, ER stress-related genes, and exercise-related genes were designated as hub genes.

Protein-protein interaction (PPI) network and enrichment analysis
A PPI network of the hub genes was constructed using the STRING database (https://cn.string-db.org/) to elucidate their mutual interactions. The “clusterProfiler” package in R was employed for Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis of the hub genes, with key pathways visualized in bar graphs [33, 34].

Immune infiltration analysis
The Tumor Immune Estimation Resource database was utilized to assess the level of immune infiltration in the GSE168796 dataset [35], Spearman correlation analysis was performed to determine the association between hub genes and immune cells. The “estimate” package in R was employed to evaluate the immune score, stromal score, and estimate score of the samples [36]. Spearman correlation analysis was further used to assess the relationship between hub genes and these scores.

mRNA-miRNA network construction
Interacting miRNAs for the hub genes were identified via the mirtarbase platform (https://mirtarbase.cuhk.edu.cn/–mirtarbase/ mirtarbase_2022/php/index.php). By intersecting these miRNAs with differentially expressed miRNAs from the GSE94717 dataset, sepsis-associated miRNAs were determined. The mRNA-miRNA interaction network was subsequently constructed using Cytoscape software.

Traditional Chinese medicine (TCM) compound library sensitivity test screening
Virtual screening provides an indication of potential bioactivity of compounds, reducing costs and time in drug discovery. A total of 2,456 TCM compounds were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) database based on oral bioavailability ≥ 30% and drug-likeness ≥ 0.18 [37]. These compounds were downloaded in mol2 format from the PubChem database. Core protein domain structures were downloaded in pdb format from the Protein Data Bank (PDB) database (http://www.rcsb.org/). Virtual screening was executed using the LibDock module of Discovery Studio 2019. Specific steps included protein and ligand energy minimization, removal of water molecules, and assignment of partial atomic charges using the Gasteiger-Marsili method. Further molecular docking was conducted to assess potential binding modes between TCM compounds from the TCMSP library and core protein binding sites. The top six compounds, based on their LibDockScore values, were selected for subsequent binding energy and RMSD value calculations. Operations included protein preparation using PyMOL software, converting the top six active TCM compounds and core protein gene files from pdb to pdbqt format using AutoDockTools 1.5.6, and identifying active pockets. Finally, the Vina script was run for molecular binding energy calculations and molecular docking result visualization. The reliability of the bioinformatics analysis predictions was evaluated by displaying the ligand-receptor complex’s molecular docking results in both 3D and 2D.

Construction of sepsis cellular model
Murine myoblasts (C2C12) were cultured in DMEM medium supplemented with 10% fetal bovine serum. The cells were maintained in an incubator at 37°C with 5% CO2 and sub-cultured every 2–3 days. Upon reaching 70%–80% confluence, cells were subjected to serum starvation in serum-free DMEM for 24 h to synchronize their growth. Subsequently, experimental groups were treated with respective stimulants and intervention agents. A sepsis model was induced in C2C12 cells using lipopolysaccharide (LPS) at a concentration of 10 μg/mL. Once the cell density approached approximately 90%, cells were passaged and utilized for subsequent experimental validations.

Cell transfection
Recombinant lentiviral vectors overexpressing MMP9 and corresponding empty vectors were constructed. C2C12 cells were incubated in lentiviral medium containing polybrene (10 μg/mL) for 48 h. Following this, the viral medium was replaced with fresh DMEM supplemented with 10% fetal bovine serum. After 72 h post-transfection, cells were selected using 2 μg/mL puromycin to establish a stable si-MMP9 cell line.

Cell toxicity assessment
Recombinant lentiviral vectors overexpressing MMP9 and corresponding empty vectors were constructed. C2C12 cells were incubated in lentiviral medium containing polybrene (10 μg/mL) for 48 h. Following this, the viral medium was replaced with fresh DMEM supplemented with 10% fetal bovine serum. After 72 h post-transfection, cells were selected using 2 μg/mL puromycin to establish a stable si-MMP9 cell line.

CCK8 assay
Utilizing the cell counting kit-8 (CCK8, Beyotime Biotech, Shanghai, China), we assessed the viability of C2C12 myoblasts under various intervention conditions. The experimental procedure was largely predicated upon prior research. C2C12 cells were seeded onto a 96-well plate (Thermo Fisher Scientific, Waltham, MA, USA) at a density of 1 × 10^4 cells per well. Following a 24 h treatment under different intervention measures, 10 μL of CCK8 solution was introduced to each well and incubated for 2 h. Absorbance was subsequently gauged using a microplate reader (Bio-Rad, Hercules, CA, USA). Statistical approach: cell viability was evaluated by comparing the average absorbance of various interventions to the control group, expressed as a percentage.

Flow cytometry analysis for cell apoptosis
Cell apoptosis was assessed using the Annexin V-FITC/PI Apoptosis Detection kit (Thermo Fisher Scientific, Waltham, MA, USA). Cells from each group were harvested and washed with pre-chilled phosphate-buffered saline. A total of 5 × 10^5 cells were collected and resuspended in 500 μL of 1 × binding buffer, prepared by diluting 5 × binding buffer with distilled water. To this suspension, 5 μL of Annexin V-FITC and 10 μL of PI were added. After gentle vortexing, the cells were incubated in the dark at room temperature for 5 min. The fluorescence intensities of Annexin V-FITC and PI were subsequently measured using a flow cytometer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Data analysis was performed
Luciferase reporter gene assay

A luciferase reporter gene vector was constructed, incorporating the binding target region of miR-149-5p within the 3'UTR of MMP9. In this vector, the 3'UTR region targeted by miR-149-5p was positioned downstream of the luciferase gene. The constructed luciferase reporter vector was transfected into C2C12 cells. Following the transfection of the luciferase gene vector, cells were further transfected with miR-149-5p mimics or negative control mimics to ascertain the potential influence of miR-149-5p on luciferase activity. The intracellular luciferase activity was subsequently quantified using a suitable luciferase assay kit, such as the Firefly Luciferase assay kit (Promega Co., Ltd., Madison, WI, USA). This method facilitates the detection of a fluorescent signal generated by the catalytic reaction of luciferase.

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA was extracted from cells using the Trizol reagent (Invitrogen, Waltham, MA, USA). Specifically, 1 mL of Trizol reagent was added to each well, and the contents were transferred to a 1.5 mL EP tube and allowed to lyse for 10 min. Subsequently, 200 μL of chloroform was added to each tube, followed by centrifugation at 12,000 rpm for 15 min at 4 °C. The aqueous phase was carefully transferred, and 400 μL of isopropanol was added. After multiple centrifugation steps, the supernatant was discarded, and the RNA pellet was resuspended in 20 μL of DEPC-treated water. The RNA was then reverse transcribed into cDNA under the following conditions: 25 °C for 5 min, 50 °C for 15 min, 85 °C for 5 min, and finally held at 4 °C for 10 min. The resulting cDNA was diluted 10-fold and used as a template for qRT-PCR amplification. Primers were synthesized with Gapdh serving as the internal reference gene.

For miRNA cDNA synthesis, total RNA was reverse transcribed using the miScript II RT kit (Qiagen Co., Ltd., Hilden, Germany) following the manufacturer’s protocol. The reverse transcription mixture comprised 5 μL miScript HiSpec Buffer, 10 μL miScript Nucleics Mix, miScript Reverse Transcriptase Mix, and the RNA template. The thermal cycling conditions for reverse transcription were initiated at 37 °C for 60 min, culminating in a denaturation step at 95 °C for 5 min to deactivate the reverse transcriptase enzyme. Subsequent quantitative real-time PCR assays were executed employing the miScript SYBR Green PCR kit (Qiagen Co., Ltd., Hilden, Germany) and were processed on a StepOnePlus Real-Time PCR System (Applied Biosystems). The PCR reaction mixture was formulated with 2 × QuantiTect SYBR Green PCR Master Mix, 10 μL miScript Universal Primer, 10 μL miScript Primer assay tailored for the designated target miRNA, and the synthesized cDNA template. The relative quantification of target miRNAs was determined utilizing the 2-ΔΔCT method, with normalization against U6 small nuclear RNA or another pertinent endogenous control. To ensure accuracy and reproducibility, all assays were conducted in triplicate, and the resultant data are articulated as mean ± standard deviation (SD).

Western blot analysis

Cell lysates from each group were prepared using RIPA buffer, and protein concentrations were subsequently determined via the BCA method. For each sample, 25 μg of total protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subsequently transferred onto PVDF membranes. The membranes were blocked with 5% non-fat milk for 1 h, followed by overnight incubation at 4 °C with primary antibodies. After thorough washing with tween phosphate-buffered saline, the membranes were incubated with the appropriate secondary antibodies at 37 °C on a shaker for 2 h. Protein bands were visualized using the DNR bio-imaging system, and the relative protein expression levels were quantified using ImageJ software.

Statistical analysis

All experiments were conducted in triplicate. Data were analyzed using GraphPad Prism 8.0.1 and SPSS 25.0 software. Results are presented as mean ± SD. Differences among multiple groups were assessed using ANOVA, while comparisons between two groups were made using the t-test. A P-value of less than 0.05 was considered statistically significant.

Results

Identification of key genes and immune infiltration analysis

Through differential analysis of GSE168796, a total of 2,006 DEGs were identified, of which 1,142 were upregulated, and 864 were downregulated (Figure 1A). In the GSE94717 differential analysis, 616 DE-miRNAs were discerned, with 45 being upregulated and 571 being downregulated (Figure 1B). The GeneCard platform pinpointed 3,697 genes related to ER stress and another 894 associated with movement. An intersection with DEGs revealed 11 overlapping genes, which were subsequently identified as pivotal genes (Figure 1C). Through the STRING database, these 11 genes were crucial in the PPI network, highlighting MMP9, tumor necrosis factor alpha (TNF-α), and IL1B as having significant interactions, suggesting their paramount importance in the study (Figure 1D). In the immune infiltration analysis, correlation studies unveiled that COMT was positively correlated with naïve B cells but negatively correlated with eosinophils and plasma cells. IL9B showed a pronounced positive correlation with resting mast cells and a negative correlation with activated mast cells. IFNG was notably positively correlated with Tregs. These genes, therefore, are believed to be associated with cellular inhibitory states and cellular regulation (Figure 1E). In the immune score, only MMP9 exhibited a significant correlation with the matrix score (Figure 1F). In essence, these genes might induce an immunosuppressive state, potentially leading to the onset and progression of diseases.

mRNA-miRNA network construction and enrichment analysis

Utilizing the miRTarBase platform, 111 miRNAs interacting with the 11 key genes were identified. An intersection with DE-miRNAs yielded 28 sepsis-related miRNA networks (Figure 1G). Constructing a network with these 28 miRNAs and 11 key genes revealed that MMP9, IL1B, IFNG, and TNF-α were predominantly regulated by multiple miRNAs (Figure 1H). Ultimately, Gene Ontology enrichment analysis indicated that the 11 key genes were associated with oxidative metabolism and amino acid metabolism, such as the reactive oxygen species metabolic process, regulation of oxidoreductase activity, and serine-type endopeptidase activity. Kyoto Encyclopedia of Genes and Genomes enrichment analysis pinpointed their primary association with the IL-17 signaling pathway, TNF-α signaling pathway, and lipid metabolism. In summary, these genes did not exhibit pronounced immune activation but played roles in metabolic and other signaling pathways.

Identification of potential TCM components targeting core proteins

In our analysis of the TCMP database, a total of 2,456 traditional Chinese medicine components were screened. The PDB structures of core proteins MMP9, TNF-α, and IL1B were downloaded from the PDB database. All these components were docked with the core proteins MMP9, TNF-α, and IL1B. Based on their LibDock score ranking, it was observed that the core protein MMP9 formed stable docking models with TCM components licorice glycoside E and 2,7-dihydroxy-4-methoxynaphthalene-2,7-O-diglucoside. Similarly, the core protein TNF-α showed affinity towards procyanidin B-5,3’-O-gallate and norkohlenoside. The core protein IL1B formed stable complexes with (E,E)-3,5-Di-O-cafeoylquinic acid and GGBG (Supplementary Figure S1). Further docking using AutoDock-Vina revealed more accurate drug candidates related to the essential components of the core proteins. Licorice glycoside E, 2,7-dihydroxy-4-1H)
Figure 1 Identification of key genes and construction of mRNA-miRNA network. (A) Volcano plot illustrating the differential gene expression analysis for dataset GSE168796. (B) Volcano plot showing the differential gene expression analysis for dataset GSE94717. (C) Venn diagram identifying the overlapping genes among DEGs, endoplasmic reticulum stress-related genes, and exercise-related genes. (D) PPI network of key genes. (E) Correlation analysis depicting the relationship between key genes and immune cells. (F) Correlation analysis between key genes and immune scores, stromal scores, and ESTIMATE scores. (G) Venn diagram revealing the overlapping genes between DE-miRNA and miRNA related to key genes. (H) Interaction network of mRNA-miRNA interactions. Enrichment analysis for the 11 key genes. DEGs, differentially expressed genes; DE-miRNAs, differentially expressed micro ribonucleic acids; miRNA, micro ribonucleic acid; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Expression alterations of core genes and miRNAs in sepsis cells

RT-qPCR and western blotting (WB) were employed to analyze the expression of core genes in murine myoblasts C2C12 and the LPS-induced sepsis cell model C2C12/LPS. The findings revealed that the mRNA expression levels of MMP9, TNF-α, and IL1B in the C2C12/LPS sepsis cell model were significantly elevated compared to the normal cells (P < 0.05, Figure 3A). WB analyses have further corroborated the augmented expression of BAX, CHOP, PEPK, and p-PERK in septic cells, with a notably pronounced upregulation of MMP9 (P < 0.05, Figure 3B). Consequently, MMP9 was selected for in-depth examination. Moreover, through predictive assays for MMP9 expression, it was observed that MMP9 was significantly elevated in C2C12/LPS cells (P < 0.05, Figure 3C). We have successfully established models of both elevated expression and knockdown of miR-149-5p (P < 0.001, Figure 3D). qPCR analyses revealed that the miR-149-5p mimic significantly augmented the levels of miR-149-5p in C2C12 cells, while the miR-149-5p inhibitor markedly reduced its mRNA expression within the same cells. To explore the impact of miR-149-5p mimics on the cytotoxic effects of LPS on C2C12 cells, a CCK8 assay was conducted, revealing that miR-149-5p mimics enhance the resilience of C2C12 cells to LPS (Figure 3E). These observations suggest that heightened miR-149-5p expression mitigates the toxic effects in sepsis cells. Flow cytometry further validated the capacity of miR-149-5p mimics to induce apoptosis, significantly curtailing apoptosis in septic cells (Figure 3F). These findings collectively indicate that miR-149-5p mimics mitigates apoptosis in septic cells, potentially inhibiting endoplasmic reticulum stress-induced cell death in sepsis.

Regulation of MMP9 by miR-149-5p and its role in sepsis

The binding relationship between miR-149-5p and MMP9 can be ascertained through luciferase reporter gene assays. Upon transfection with miR-149-5p mimics, the 3’UTR region of the target binding site for miR-149-5p undergoes regulation by the miRNA, leading to a decline in luciferase gene reporter activity. This is attributed to the potential inhibition of luciferase gene transcription or degradation of...
Figure 2 Docking analysis of TCM components with core proteins MMP9, TNF-α, and IL1B. (A) Three-dimensional docking models for MMP9 with licorice glycoside E, showing macroscopic (A1), microscopic (A2), and two-dimensional (A3) views. (B) Three-dimensional docking models for MMP9 with 2,7-dihydroxy-4-methoxyphenanthrene-2,7-O-diglucoside, showing macroscopic (B1), microscopic (B2), and two-dimensional (B3) views. (C) Three-dimensional docking models for IL1B with (E,E)-3,5-Di-O-caffeoylquinic acid, showing macroscopic (C1), microscopic (C2), and two-dimensional (C3) views. (D) Three-dimensional docking models for IL1B with GBGB, showing macroscopic (D1), microscopic (D2), and two-dimensional (D3) views. (E) Three-dimensional docking models for TNF-α with procyanidin B-5,3′-O-gallate, showing macroscopic (E1), microscopic (E2), and two-dimensional (E3) views.
Figure 3 Expression alterations and functional analysis of core genes and miRNAs in sepsis cells. (A) Bar chart representing the mRNA expression levels of MMP9, TNF-α, and IL-1β in murine myoblasts C2C12 and the LPS-induced sepsis cell model C2C12/LPS. RT-qPCR analysis revealed a significant upregulation of these genes in the C2C12/LPS sepsis cell model compared to the normal C2C12 cells. Each bar represents the mean ± SD of three independent experiments. Asterisks indicate a significant difference (P < 0.05) between the two groups. (B) WB analysis of protein expression levels of BAX, CHOP, PERK and p-PERK in C2C12 and C2C12/LPS cells. Densitometric analysis confirmed the elevated expression of these proteins in sepsis cells, with MMP9 showing the most pronounced upregulation. Representative blots are shown, and the relative protein expression was normalized to the internal control. (C) Bar chart illustrating the expression levels of miR-149-5p in C2C12 and C2C12/LPS cells. qRT-PCR analysis demonstrated a significant upregulation of MMP9 in the C2C12/LPS sepsis cell model. Each dot represents an individual sample, and the horizontal line indicates the mean expression level. (D) qPCR analyses revealed that the miR-149-5p mimic significantly augmented the levels of miR-149-5p in C2C12 cells, while the miR-149-5p inhibitor markedly reduced its mRNA expression within the same cells. Data are presented as mean ± SD. (E) Bar chart showcasing the effect of miR-149-5p mimic on the viability of C2C12/LPS sepsis cells. CCK8 results indicated that increased levels of miR-149-5p significantly boosted the vitality of these cells. Each bar represents the mean ± SD of three independent experiments. (F) Flow cytometry histograms depicting the apoptotic rates of C2C12/LPS cells following overexpression of miR-149-5p and interference in MMP9. CCK8, cell counting kit-8; LPS, lipopolysaccharide; MMP9, matrix metallopeptidase 9; mRNA, messenger ribonucleic acid; miR-149-5p, microRNA-149-5p.

Table 1 Molecular docking results

<table>
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<th>Protein (binding site)</th>
<th>Compound</th>
<th>Vina (kcal/mol)</th>
<th>RMSD</th>
<th>DS (LibDockScore)</th>
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<td>MMP9 (6esm)</td>
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<td>MMP9 (6esm)</td>
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the luciferase mRNA upon miR-149-5p binding to the 3’UTR region. However, no significant change in luciferase activity was observed when transfected with a negative control mimic, serving as evidence of the specific binding of miR-149-5p to MMP9 (Figure 4A). In essence, the results from the luciferase reporter gene assay elucidate the binding relationship between miR-149-5p and MMP9. This experimental approach offers insights into the interactions between miRNAs and their target genes, shedding light on the role of miRNAs in gene expression regulation. Following our observations, we discerned that miR-149-5p inhibits MMP9 expression. Forty-eight hours after transfecting C2C12 cells with miR-149-5p mimic and miR-149-5p inhibitor, we assessed the endogenous MMP9 expression using qPCR and WB (Figure 4B, 4C). To further validate the regulatory effect of miR-149-5p on MMP9, we employed RNA interference
techniques. By designing siRNA targeting MMP9, we successfully diminished its expression in C2C12/LPS cells. Real-time quantitative PCR results revealed a significant reduction in MMP9 mRNA expression in the interference group compared to the control (Figure 4D). WB analysis further confirmed the decline in MMP9 protein levels (Figure 4F). These findings align with previous studies, suggesting that miR-149-5p inhibits MMP9 expression by binding to its 3’UTR region. To assess the impact of MMP9 inhibition on cell apoptosis, we utilized the CCK8 assay. The results indicated a marked decrease in cell viability in MMP9 overexpression cells compared to the control group (Figure 4F), consistent with the findings from the CCK8 assay. Lastly, flow cytometry was employed to further validate the effects of MMP9 interference on cell apoptosis. The results demonstrated a significant increase in the proportion of apoptotic cells in MMP9 overexpression cells (Figure 4G). In summary, our research findings suggest that miR-149-5p can regulate apoptosis in sepsis cells by targeting MMP9, offering fresh insights into the molecular mechanisms underlying sepsis.

Discussion

We focused on the role of ER stress in diabetes and its potential modulation by exercise, which may unveil new therapeutic drugs and targets for tackling diabetes-aggravated sepsis. Our key findings highlighted the potential of licorice glycoside E in targeting MMP9, a gene significantly altered in diabetic conditions and associated with ER stress. This discovery not only underscores the importance of understanding regulatory mechanisms like miR-149-5p and MMP9 in diabetes but also bridges the gap between ancient wisdom and modern science, particularly in managing chronic diseases and their severe complications, such as sepsis. Physical exercise, a cornerstone of traditional physical therapy, has long been an integral part of holistic healthcare. Our genomic insights into exercise therapy reveal how physical activity impacts gene expression and genetic susceptibility in

![Figure 4 Regulation of MMP9 by miR-149-5p and its implications in sepsis.](https://www.tmrjournals.com/tmr)
the context of disease management and prevention. This aligns with the comprehensive integration of multi-omics data in traditional physical therapy research, which has radically transformed our understanding of these practices. Such integration is key in merging ancient wisdom with modern scientific approaches. Sepsis, a life-threatening systemic inflammatory response syndrome, remains a significant global health challenge, with its intricate pathophysiology and associated high mortality rates [38]. The pivotal role of ER stress in cellular homeostasis and its perturbation in various pathological conditions, including sepsis, has been increasingly recognized [39, 40]. In this context, our study embarked on a journey to elucidate the potential modulatory effects of physical exercise on ER stress mechanisms and their subsequent influence on sepsis progression.

Our comprehensive differential analyses unveiled a plethora of DEGs and DE-miRNAs, with MMP9, TNF-α, and IL-1β emerging as significant molecular protagonists in the sepsis narrative. These findings resonate with previous studies that have underscored the importance of these genes in the inflammatory cascade and cellular apoptosis associated with sepsis [41]. The observed immunosuppressive state, potentially orchestrated by these genes, further accentuates their pivotal role in sepsis progression and offers a fresh perspective on the immune dysregulation observed in septic patients [42, 43].

This research pioneers an in-depth exploration of how physical exercise modulates the ER stress response in myogenic cells induced by sepsis, mediated through the miR-149-5p-MMP9 regulatory network. This novel perspective offers fresh therapeutic strategies for sepsis management. Through comprehensive differential analyses, we not only pinpointed key genes and miRNAs associated with ER stress but also delved into their intricate interrelationships using platforms like GeneCard and the STRING database. Furthermore, our groundbreaking approach of molecular docking with 2,456 TCM components heralds a new direction for potential sepsis treatments. MMP9 is an enzyme implicated in various disease processes, including inflammation and tumor invasion. Its role in the pathogenesis of sepsis, especially concerning inflammatory responses and cellular apoptosis, has garnered widespread attention in recent years [44, 45].

MiR-149-5p, a diminutive non-coding RNA, has been established as a regulator of gene expression in various aliments, including cancers and inflammatory diseases [46–48]. The prevailing research focus has been on their mutual interactions and their collective influence on disease progression. Within the context of sepsis, MMP9's overexpression correlates with disease severity and adverse outcomes. In osteoactivin transgenic mice, compared to their wild-type counterparts, denervation further augmented the expression of MMP3 and MMP9 in fibroblasts infiltrating the gastrocnemius muscle [49]. In the context of this study, MiR-149-5p is delineated as a potential regulator of MMP9, offering a prospective avenue to alleviate the pathological ramifications of sepsis through the inhibition of MMP9 expression. Our experimental assays shed light on the regulatory dynamics between miR-149-5p and MMP9, unveiling the intricate molecular choreography underpinning sepsis pathophysiology. The elucidated binding affinity and the consequential influence of miR-149-5p on sepsis cell apoptosis, mediated through MMP9 targeting, underscore the potential therapeutic ramifications of this regulatory paradigm. Physical exercise, often lauded for its myriad health benefits, has been postulated to exert modulatory effects on various cellular and molecular processes [50, 51]. Physical exercise, renowned for its therapeutic benefits across a spectrum of diseases, including sepsis, potentially mitigates the pathological effects of sepsis through mechanisms like antioxidation, anti-inflammatory, and immune modulation. Against this backdrop, the miR-149-5p-MMP9 regulatory axis emerges as a potential linchpin in the beneficial effects of physical exercise on sepsis patients. Integrating the aforementioned insights, it's plausible to postulate that the miR-149-5p-MMP9 regulatory network could be a pivotal therapeutic target through which physical exercise modulates ER stress in sepsis treatment. Physical exercise might bolster miR-149-5p expression, inhibiting MMP9, thereby alleviating ER stress and associated inflammatory responses, benefiting sepsis patients. This paves the way for future investigations into how physical exercise might therapeutically modulate the miR-149-5p-MMP9 network in sepsis.

Our study, set against this backdrop, endeavored to bridge the knowledge gap concerning the interplay between physical exercise, ER stress modulation, and sepsis progression. In vitro, within G2C12 myocytes, TNF-α-induced activation of NF-κB reduces MMP9 secretion and increases ALDH activity [52]. Our findings also indicate that licorice glycoside E, by specifically targeting MMP9, may serve as a promising therapeutic agent for sepsis. The affinity of licorice glycoside E towards MMP9 underlines a new therapeutic pathway for sepsis intervention, reflecting the timeless principles of traditional medicine in addressing modern medical challenges. This aligns with discoveries from other studies where flavonoid compounds isoliquiritin and isoliquiritigenin (ILG), isolated from licorice roots, exhibited anti-inflammatory effects on LPS-treated RAW 264.7 macrophages [53]. Specifically, ILG has been shown to inhibit LPS-induced nitric oxide and prostaglandin E2 production, and to downregulate iNOS, COX-2, TNF-α, and IL-6 at both protein and mRNA levels [53]. Additionally, ILG impedes the phosphorylation of IKK, ERK1/2, and p38, thereby influencing the activity of NF-κB. In light of these findings, one might speculate that licorice glycoside E and ILG might modulate inflammatory responses through analogous mechanisms. Both compounds seem to influence pivotal inflammatory mediators and signaling pathways, thereby inhibiting the activation of inflammatory cells and the production of inflammatory mediators. Notably, both appear to exert their effects by modulating the activity of NF-κB, a transcription factor pivotal in inflammatory responses. However, it's worth noting that while both compounds exhibit anti-inflammatory properties, their mechanisms of action and targets might diverge. For instance, licorice glycoside E in our model primarily targets MMP9, whereas ILG predominantly affects the expression of iNOS and COX-2. Thus, further investigations are imperative to discern the precise mechanisms of action and potential synergies between these compounds.

For the first time, our study thoroughly investigates how physical exercise modulates ER stress responses in myoblasts during sepsis, specifically through the miR-149-5p-MMP9 regulatory network. This approach opens new avenues for understanding and treating sepsis, especially in the context of chronic diseases like diabetes. Our comprehensive differential gene and miRNA analysis, supported by tools such as the GeneCard and STRING databases, has shed light on complex gene interactions underpinning ER stress. Notably, our pioneering effort in molecularly docking over 2,456 traditional Chinese medicine components marks a significant leap towards developing novel sepsis therapies, potentially revolutionizing diabetes management as well. The ability of licorice glycoside E to modulate miR-149-5p and MMP9 provides a lead compound for drug development. Licorice derivatives could be optimized as novel treatments for LPS-triggered diabetes. By clarifying a novel miR-149-5p-MMP9 pathway induced by ER stress that promotes inflammation in diabetes while also demonstrating the potential of licorice glycoside E as a therapeutic agent. It improves understanding of the connections between diabetes, ER stress, and sepsis risk. This research helps pave the way for innovative diabetic treatments that could relieve relevant complications, restore mobility and strength, and ultimately improve clinical outcomes and quality of life for patients. These findings provide a strong scientific basis for future translation into patient-centered outcomes.

When integrated with advanced technologies like imaging, high-throughput screening, omics profiling, biosensors, and machine learning, the mechanisms of licorice glycoside E in diabetic muscle would be further elucidated, and our findings could be ultimately translated into improved patient treatments and outcomes [54–58]. For example, imaging techniques like two-photon microscopy or super resolution microscopy could be used to visualize the effects of licorice glycoside E on ER stress and inflammation in living diabetic muscle cells or tissues, which provides insights into the real-time modulation of cellular processes [59, 60]. High-throughput screening of additional
natural compounds that may have similar effects as licorice glycoside E. Compound libraries could be quickly screened in diabetic muscle cells to identify leads that modulate miR-149-5p and MMP9 [61, 62]. The licorice extract findings provide a foundation that modern biotechnologies could build upon to uncover new disease insights and potential treatments for diabetic muscle complications. High-throughput, high-content techniques have great potential to accelerate and enhance the drug discovery process.

This work addresses important knowledge gaps around exercise, microRNA signaling, and ER stress in diabetes pathogenesis, which provides novel mechanistic insights into how exercise exerts beneficial effects on diabetic muscle. These findings open new possibilities for microRNA-based and natural compound interventions to target ER stress in diabetes. In summary, our study makes valuable contributions by unraveling new mechanisms linking exercise, microRNAs, ER stress, and diabetes while also identifying a potential natural compound that may mimic exercise effects. The novel findings fill important gaps in current understanding and suggest new therapeutic approaches.

**Limitations and perspectives**

Our study on the effects of licorice glycoside E in regulating miR-149-5p-MMP9 presents a novel approach in sepsis treatment, highlighting the potential of targeting specific miRNA pathways to modulate immune responses. And it found and study a natural compound, licorice glycoside E, which offers a promising therapeutic strategy with potentially fewer side effects compared to conventional treatments. Despite above advancements, our study has limitations. The therapeutic efficacy and safety of licorice glycoside E in treating sepsis remain speculative until validated in physiological contexts. In vitro results, while foundational, cannot fully capture the complexity of human biological systems. However, our findings, primarily based on cellular experiments, lack validation in animal models or clinical trials. The focus on myoblasts leaves out other cell types and tissues, which might respond differently to ER stress and sepsis, particularly in diabetic conditions. Therefore, while our in vitro results are promising, in vivo studies are crucial for confirming licorice glycoside E’s therapeutic potential. Besides, advancing this research to in vivo studies is crucial for assessing the real-world applicability of licorice glycoside E in sepsis treatment, including its pharmacokinetics, optimal dosages, and potential interactions with other treatments. These studies should also explore the regulatory dynamics of miR-149-5p-MMP9 in a physiological context, especially considering the intricate interplay between chronic diseases and sepsis.

Future research should prioritize the development of in vivo models that accurately mimic sepsis conditions to confirm the therapeutic potential of licorice glycoside E. These studies will provide valuable insights into the compound’s mechanism of action within a living organism and help identify any unforeseen effects or challenges in clinical application. Subsequent clinical trials are essential to evaluate the effectiveness, safety, and overall benefits of licorice glycoside E in patients, bridging the gap between laboratory research and clinical practice. Moreover, these studies should also broaden to include a wider array of molecular networks linked to sepsis, potentially revealing new regulatory pathways and treatment targets. This is particularly important in the context of diabetes, a chronic disease that significantly alters cellular responses to stress and infection. Considering the strong affinity of licorice glycoside E for MMP9, further studies could explore its potential in treating sepsis and understanding its role in different cell types and tissues, including those affected by diabetes. Such research could provide critical insights into the management of sepsis in diabetic patients, who often experience more severe and complex disease progressions due to their underlying condition.

**Conclusion**

Our research has illuminated the crucial role of the miR-149-5p-MMP9 axis in diabetes-related chronic diseases, especially in the context of sepsis. We've demonstrated how physical exercise can modulate the LPS-induced ER stress response in diabetic myocytes via the miR-149-5p-MMP9 pathway, offering new therapeutic possibilities for managing diabetes and its complications. Furthermore, our findings highlight the potential of licorice glycoside E in targeting MMP9, a key player in ER stress and diabetes-related cellular pathways. This opens up promising avenues for treating chronic complications of diabetes, such as sepsis. By weaving together molecular insights and the therapeutic potential of traditional medicine components, our study presents an integrated approach towards developing novel interventions for chronic diseases in diabetic contexts.

**References**


