BMC Medical Genomics

RESEARCH

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Bioinformatics analysis of ferroptosis in frozen shoulder

Hongcui Zhang[1], Jiahua Zhou[2], Zhihua Liu[3], Kaile Wang[4] and Hexun Jiang[5*]

Abstract

Objectives Frozen shoulder is a common shoulder disease that significantly affects the patient's life and work. Ferroptosis is a new type of programmed cell death, which is involved in many diseases. However, there have been no studies reporting the relationship between frozen shoulders and ferroptosis. This study identified potential molecular markers of ferroptosis in frozen shoulders to provide more effective strategies for the treatment of frozen shoulders.

Methods GSE238053 was downloaded from the Gene Expression Omnibus (GEO) dataset and intersected with ferroptosis genes to obtain differentially expressed genes (DEGs). The signaling pathways and biological functions of DEGs were performed by WebGestalt and Metascape. The interactions related to these DEGs and the key genes between frozen shoulders and ferroptosis was performed by STRING and Cytoscape. A frozen shoulders rat model was used to validate our predicted genes, Western Blot and qRT-PCR was used to assess the expression levels of our genes of interest.

Results A total of 34 DEGs between GSE238053 and Ferroptosis Database were obtained, most of which were involved in the HIF-1 signaling pathway and inflammatory response. A protein–protein interaction network was obtained by Cytoscape and the key genes (IL-6, HMOX1 and TLR4) were screened by MCODE. Our results of Western Blot showed that the protein expression level of TLR4 and HMOX1 were elevated, and the protein level of IL-6 decreased in frozen shoulders rat model. The mRNA level after frozen shoulders showed that IL-6 was upregulated, whereas TLR4 and HMOX1were downregulated.

Conclusions The results demonstrated that ferroptosis may affect the pathological process of frozen shoulders through these signaling pathways and genes. The identification of IL-6, HMOX1 and TLR4 genes can provide new therapeutic targets for frozen shoulders.

Keywords Frozen shoulders, Ferroptosis, Bioinformatics

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Introduction

Frozen shoulder (FS), also known as adhesive capsulitis, is a chronic condition characterized by pain, stiffness, and restricted range of motion of the shoulder joint [1]. This condition can have a significant impact on patients' quality of life, limiting their ability to perform daily activities and causing significant discomfort [2]. The precise etiology of frozen shoulders remains unclear, however, accumulating evidence suggests that dysregulation of cellular processes, including inflammation and oxidative stress, may play a vital role in its pathogenesis [3].

In recent years, bioinformatics has made significant progress in understanding the molecular mechanisms underlying various diseases [4]. Bioinformatic tools and techniques have enabled the integration of vast amounts of data generated from different experimental platforms, providing valuable insights into the complex biological processes that lead to disease development [5]. Bioinformatics has revealed novel therapeutic targets and potential drug candidates for various diseases by analyzing gene expression profiles, protein networks, and pathway interactions [6].

Ferroptosis is a regulatory cell death process driven by iron-dependent lipid peroxidation and is involved in the pathological and physiological processes of various diseases, including cancer, neurodegenerative diseases, and cardiovascular diseases [7, 8]. In this study, we aim to use bioinformatics analyses to investigate the potential role of ferroptosis in the pathophysiology of frozen shoulders. By integrating transcriptomic data from patients with frozen shoulders, we hope to identify the molecular signatures associated with ferroptosis under these conditions. This approach will enable us to gain a more comprehensive understanding of the underlying mechanisms involved in frozen shoulders and the potential role of ferroptosis in its development and progression.

This is the first study to explore the complex relationship between ferroptosis and frozen shoulders, and our research may lead to the discovery of novel therapeutic targets that can effectively manage this musculoskeletal condition. By understanding the underlying mechanisms that drive ferroptosis in frozen shoulders, innovative treatment strategies that target these specific pathways can be designed. Such targeted approaches may offer patients more personalized and effective treatment options, leading to improved outcomes and quality of life. Furthermore, our findings could contribute to a better understanding of the pathogenesis of frozen shoulders. By clarifying the role of ferroptosis in frozen shoulders, we may gain insights into its etiology and progression, enabling the development of earlier interventions and more effective prevention strategies.

Materials and methods

Animals

Adult male Sprague–Dawley rats ($n=49$, weight 180±20 g) were purchased from the Experimental Animal Center of Qingdao University. These rats were maintained in a controlled environment (22±2℃ room temperature and dark/light cycle 12 h/12 h) with water and chow ad libitum. The animals were randomly assigned into the following two groups: (1) sham group ($n=6$): rats shoulder joint without plaster fixation; (2) FS group ($n=6$): the FS group, animals that received shoulder joint plaster fixation. The protocol for this experimental study was approved by the Animal Care and Use Committee of Qingdao University. Our study met the requirements of the Laboratory Animal Welfare Ethics Committee of Qingdao University. (The approval number: NO.20240415SD1220240520032).

Frozen shoulders model

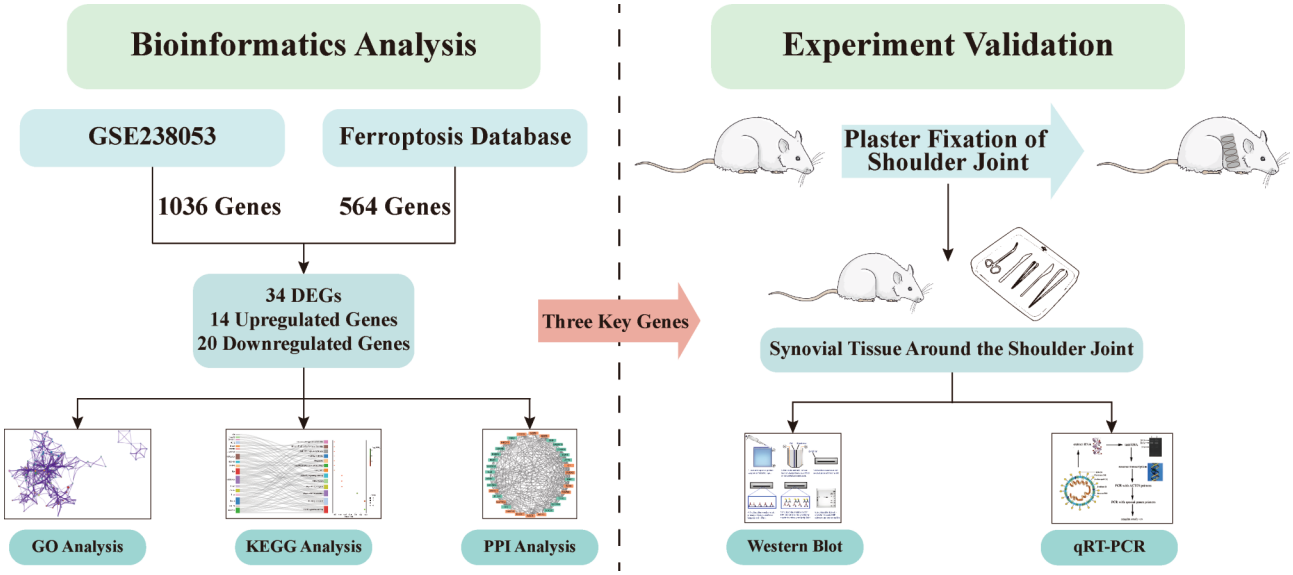
Based on previous studies [9], frozen shoulders was induced using the following methods: maintain one side of the rat's shoulder in a fully adducted and pronated position, while flexing and pronating the elbow joint on the same side. Apply plaster to the entire forelimb and chest, and immobilize one side of the forelimb in the aforementioned position on the chest. After two weeks, the plaster was removed and the frozen shoulders model was successfully established.

Microarray data

The clinical information of patients with frozen shoulders was obtained from Gene Expression Omnibus (GEO). We downloaded the dataset GSE238053 stored by Rosell from GEO. This dataset includes microarray data of synovium tissues from four individuals with frozen shoulders and four healthy controls. This dataset was used for further analysis and mining.

Differential expression analysis

GEO2R, an online analysis tool, was used to analyze differential expression. The expression profiles of synovium tissues in frozen shoulders and healthy controls were compared to identify the differential genes. A volcano plot was produced by GEO2R. Genes retained from diverse samples were selected using the following criteria: a $|\log2\ (\text{fold-change})| > 1.2$ and adjusted P-value<0.05. We also obtained a dataset that included 564 genes from the Ferroptosis Database (FerrDb; zhounan.org) and intersected it with GSE238053 to identify differentially expressed genes (DEGs). The online tool Venny2.1 was employed to generate a Venn diagram of DEGs, and a heat map of DEGs was drawn using HemI Software. The research content of this study is clearly illustrated in Scheme 1.



Scheme 1 The scheme of the study is to clearly illustrate the research content of this study

KEGG enrichment analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was obtained from the GSEA of WebGestalt 2024 (https://www.webgestalt.org/) [10]. KEGG enrichment analysis aims to identify the pathways in which the target gene is involved, and to analyze the significance of each pathway. Gene set enrichment analysis (GSEA) is a method to sort genes according to the degree of differential expression of two samples. It is a method to analyze the whole genome expression profile chip data and compare genes with predefined gene sets. First, the DEGs data were uploaded into WebGestalt to ensure that the data were formatted correctly according to WebGestalt's requirements. Next, successively choose "Homo sapiens" "Gene Set Enrichment Analysis (GSEA)" "pathway" "KEGG" from the dropdown menu. Finally, we submitted the DEGs and obtained the enrichment results.

GO enrichment analysis

Metascape v3.5.20240101 (https://metascape.org/gp/index.html) [11], a widely used tool for gene enrichment analysis, was used in this study. We uploaded the list of DEGs obtained from our analysis into Metascape, an online tool for gene function annotation analysis. Annotation of biological processes, molecular function and cellular components was performed by Metascape, using the genes that were shared between GSE238053 and the ferroptosis dataset.

Protein–protein interaction network analysis

To predict protein–protein interactions (PPIs), STRING v12.0 (https://www.string-db.org/), an online database which can retrieve the interaction between a group of proteins, was utilized in the PPI network analysis. Cytoscape network visualization was obtained with interaction scores > 0.15. The nodes represent genes and the edges represent the links between genes. In addition, the PPI network was built and visualized by Cytoscape v3.8.0 software. Molecular complex detection (MCODE) was used for clustering analysis of gene networks to identify key PPI network modules. The function of MCODE is to select the key subnetworks, that is, modules. A PPI module refers to a PPI module where one module points to one function. Different genes have different module scores, and key genes can be selected according to their scores. In order to identify the key modules, $P<0.05$ was considered to show a significant difference.

Western blot analysis

After two weeks, two groups ($n=3$/group) were killed by decapitation, and synovial tissue around the shoulder joint was quickly removed and stored at -80℃ until use. After adding protease and phosphatase inhibitors, these tissues were homogenized in an ice-cold lysis buffer and measured protein concentrations with a BCA Protein Assay Kit (Solarbio, China). Proteins (30 μg) were separated on a 10% sodium dodecyl sulfate polyacrylamide gel (Sigma, USA) and transferred to polyvinylidene fluoride (PVDF) membranes (Sigma, USA). The membranes were incubated with 5% non-fat dry milk in TBST for 2 h. Then the membranes were incubated with antibodies to TLR4 (diluted 1:1000, Proteintech, Cat No.66350-1-lg), HMOX1 (diluted 1:1000, Proteintech, Cat No.66743-1-lg), IL-6 (diluted 1:1000, Proteintech, Cat No.66146-1-1 g) or β-actin (diluted 1:5000, Proteintech, Cat No.66009-1-lg) overnight at 4 °C. Next, the membranes were incubated with HRP-labeled goat anti-mouse IgG secondary antibody (diluted 1:5000, Proteintech, Cat No.

Table 1 Specific primers used for quantitative real-time PCR

Gene	Forward (5′-3′)	Reverse (5′-3′)
TLR4	GGGCCTAAACCCAGTCTGTTTG	CTTCTGCCCGGTAAGG TCCA
IL-6	AGACTTCACAGAGGATACCAC CCAC	CAATCAGAATTGCCATTG CACAA
HMOX1	CTGGAGGAGGAGATGAGCG	TGGCACTGGCAATGTTGG
GAPDH	AGCCCAGAACATCATCCCTG	CACCACCTTCTTGATG TCATC

RGAM001) for 2 h after washing with TBST. After a final wash in TBST, bands were visualized using an enhanced chemiluminescence ECL reagent (Millipore, USA). Images were analyzed by Image J software.

qRT-PCR analysis

After two weeks, two groups were sacrificed and synovial tissue around the shoulder joint was extracted ($n=3$/group), and qRT-PCR was performed. Briefly, total RNA was collected from the synovial tissue of rats using TRIzol (Invitrogen, USA). RNA purity was tested using Quantus Fluorometer (Promega, USA). First, total RNA was used for reverse transcription reaction. Then, the pre-amplified cDNA samples were mixed with One-Step SYBR PrimeScript PLUS RTPCR Kit (Takara, Japan). Finally, the reaction was conducted on AriaMx HRM (Agilent, USA). All primers are referenced from other studies [12–14]. The thermal cycling conditions comprised 5 min at 95 °C, 45 cycles at 95 °C for 20 s, 56 °C for 15 s, and 72 °C for 20 s. Using ddH_2O instead of cDNA as a negative control. The expression of genes was analyzed using the $2^{-\triangle\triangle}$CT method and normalized using GAPDH. The Primer sequences used for qRT-PCR are listed in Table 1.

Statistical analysis

All data were expressed as mean±S.E.M. in this present study and analyzed by GraphPad Prism 8.0 software (GraphPad Software, CA, USA). T tests were used for analyzing the differences between the groups for the Western Blot and qRT-PCR. In all cases, p-values<0.05 was considered statistically significant.

Results

The analysis of differentially expressed genes

GSE238053, the microarray expression profiling dataset, was downloaded from the GEO database, 17,662 genes were obtained by comparing individuals with frozen shoulders and healthy controls. These genes are presented in the form of volcano plots (Fig. 1). Moreover, we

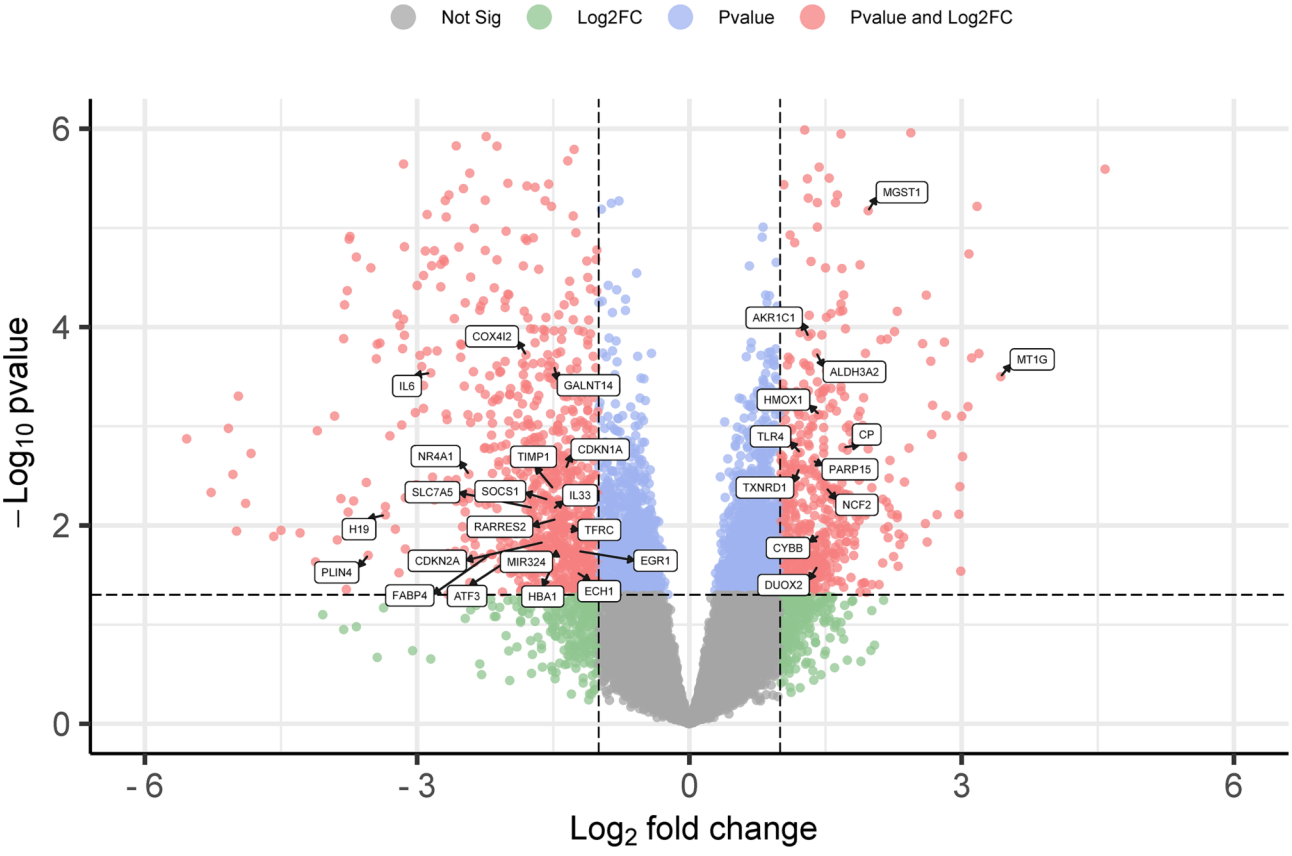


Fig. 1 The 17,662 genes from GSE238053 in the volcano plot

Table 2 Differentially expressed genes

Gene symbol	*P*-value	Fold change	Gene title
Upregulated genes			
EGFR	1.80E-12	1.51	Epidermal growth factor receptor
NQO1	6.37E-07	2.06	NAD(P)H quinone dehydrogenase 1
MGST1	6.71E-06	1.97	Microsomal glutathione S-transferase 1
AKR1C1	1.24E-04	1.31	Aldo-keto reductase family 1 member C1
ALDH3A2	1.83E-04	1.4	Aldehyde dehydrogenase 3 family member A2
MT1G	3.16E-04	3.43	Metallothionein 1G
HMOX1	7.60E-04	1.43	Heme oxygenase 1
CP	1.64E-03	1.69	Ceruloplasmin
TLR4	1.84E-03	1.22	Toll like receptor 4
PARP15	2.22E-03	1.36	Poly(ADP-ribose) polymerase family member 15
TXNRD1	2.68E-03	1.21	Thioredoxin reductase 1
NCF2	4.21E-03	1.51	Neutrophil cytosolic factor 2
CYBB	1.26E-02	1.43	Cytochrome b-245 beta chain
DUOX2	2.59E-02	1.41	Dual oxidase 2
EGFR	1.80E-12	1.51	Epidermal growth factor receptor
NQO1	6.37E-07	2.06	NAD(P)H quinone dehydrogenase 1
Downregulated genes			
IL6	2.90E-04	-2.85	Interleukin 6
CDKN1A	2.65E-03	-1.36	Cyclin dependent kinase inhibitor 1 A
NR4A1	3.05E-03	-2.43	Nuclear receptor subfamily 4 group A member 1
TIMP1	4.25E-03	-1.5	TIMP metallopeptidase inhibitor 1
SOCS1	5.53E-03	-1.55	Suppressor of cytokine signaling 1
SLC7A5	6.66E-03	-1.72	Solute carrier family 7 member 5
IL33	6.81E-03	-1.5	Interleukin 33
H19	7.83E-03	-3.35	H19 imprinted maternally expressed transcript
RARRES2	8.59E-03	-1.46	Retinoic acid receptor responder 2
TFRC	1.07E-02	-1.33	Transferrin receptor
CDKN2A	1.47E-02	-1.6	Cyclin dependent kinase inhibitor 2 A
EGR1	1.81E-02	-1.23	Early growth response 1
MIR324	1.90E-02	-1.44	MicroRNA 324
FABP4	1.94E-02	-2.19	Fatty acid binding protein 4
PLIN4	2.00E-02	-3.54	Perilipin 4
ATF3	2.02E-02	-1.92	Activating transcription factor 3
HBA1	2.80E-02	-1.53	Hemoglobin subunit alpha 1
ECH1	2.97E-02	-1.24	Enoyl-CoA hydratase 1

screened 1036 genes that met the criteria from the sample. We also gained a dataset including 564 genes from the Ferroptosis Database (FerrDb) and intersected them with GSE238053 to identify DEGs. We found altogether 14 upregulated and 20 downregulated genes (Table 2). The heat map and Venn diagram of the DEGs are shown in Fig. 2. The DEGs were further classified as ferroptosis driver, ferroptosis suppressor, and ferroptosis marker via the FerrDb online tool (Table 3).

KEGG enrichment analysis of DEGs

The results of the enrichment gene dataset analysis indicated that the genes significantly enriched were involved in the HIF-1 signaling pathway, pathways in cancer, Hepatocellular carcinoma, microRNAs in cancer, prion disease, PI3K-Akt signaling pathway, ferroptosis, fluid shear stress and atherosclerosis, phagosome, Cushing syndrome, JAK-STAT signaling pathway, human T-cell leukemia virus 1 infection and human cytomegalovirus infection (Fig. 3A and D). KEGG functional analysis revealed that the HIF-1 signaling pathway, PI3K-Akt signaling pathway, ferroptosis, and JAK-STAT signaling pathway were significantly activated in the gene sets (Fig. 3B, C, E and F).

GO enrichment analysis of DEGs

34 DEGs were then uploaded to Metascape, and it was shown that 19 gene ontology were remarkably enriched. The biological pathways were significantly activated in the inflammatory response, inflammatory response to wound, reactive oxygen species metabolic process, positive regulation of cytokine production, response to inorganic substances, positive regulation of reactive oxygen species metabolic process, cellular response to cytokine stimulus, response to oxidative stress, cellular response to abiotic stimulus, cellular response to external stimulus, diterpenoid metabolic process, cellular homeostasis, skeletal muscle cell differentiation, fat cell differentiation, organic anion transport, response to salt, and lytic vacuole. The molecular function was significantly activated in response to oxidoreductase activity and peroxidase activity (Fig. 4).

Protein–protein interaction network analysis of DEGs

We obtained a PPI network that contains 33 nodes and 237 edges. Among the 34 DEGs between individuals with frozen shoulders and healthy controls, one did not form molecular networks with the other molecules. The nodes were used to represent genes and edges to indicate interactions between genes. Upregulated genes were marked in orange, and downregulated genes were labeled in cyan (Fig. 5A). A cluster including 11 downregulated gene (MAPK8) and 5 upregulated genes was obtained by MCODE in Cytoscape. These 16 genes (CDKN2A,

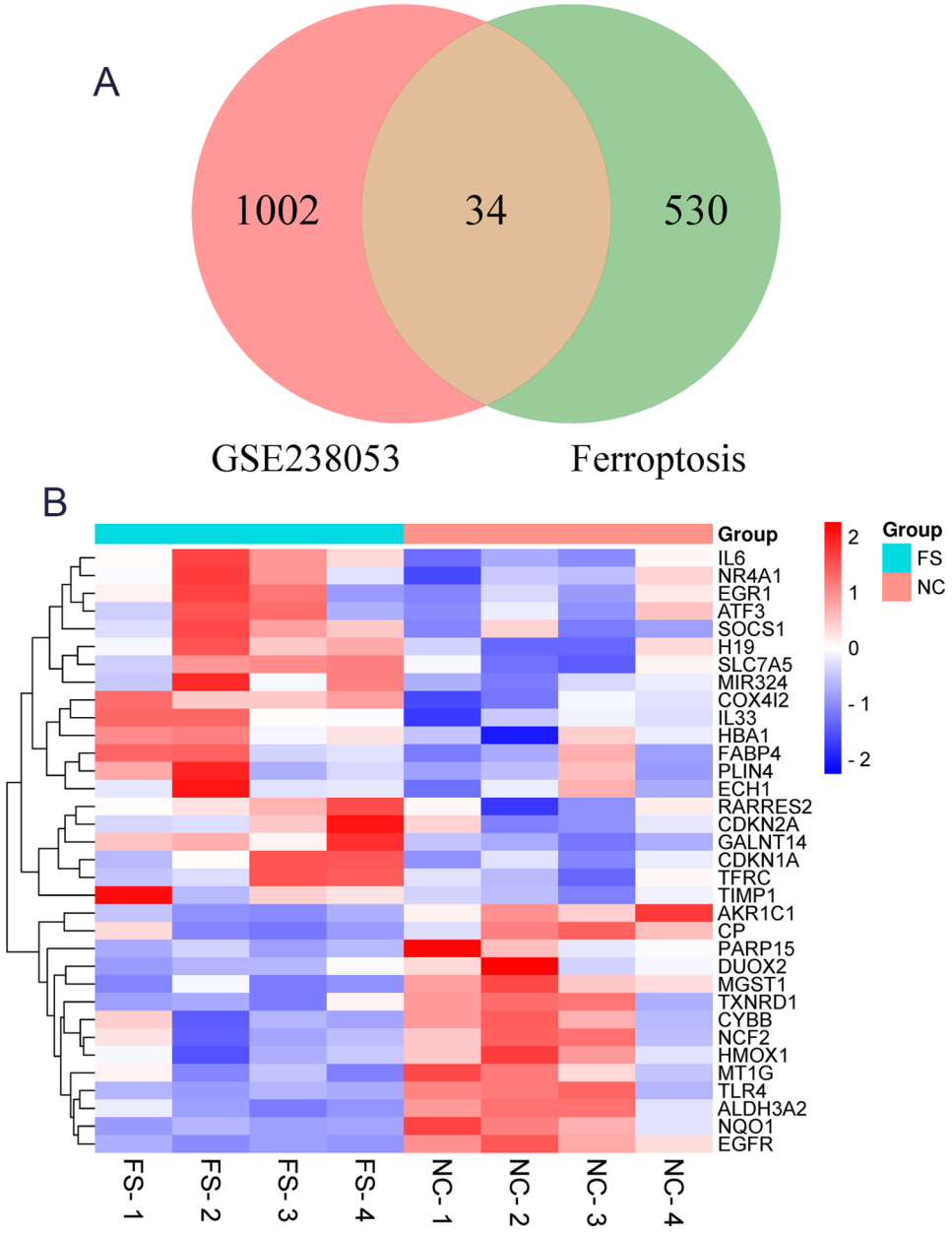


Fig. 2 **A**: Venn diagram of differentially expressed genes. **B**: The 34 differentially expressed genes were shown in the heat map, with red representing significantly up-regulated genes and blue representing significantly down-regulated genes

Table 3 The differentially expressed genes were divided into ferroptosis driver, suppressor, marker and unclassified

Driver	Suppressor	Marker	Unclassified
EGFR, COX4I2, IL6, TLR4, TIMP1, SOCS1, H19, TFRC, CYBB, CDKN2A, EGR1, MIR324, ATF3, DUOX2	NQO1, MGST1, AKR1C1, ALDH3A2, GALNT14, MT1G, HMOX1, CP, PARP15, CDKN1A, NR4A1, RARRES2, FABP4, ECH1		TXNRD1, NCF2, SLC7A5, IL33, PLIN4, HBA1

NR4A1, IL-6, TIMP1, EGR1, NQO1, SOCS1, TFRC, CYBB, FABP4, CDKN1A, HMOX1, TLR4, EGFR, ATF3 and IL33) were the key genes screened by MCODE. The IL-6, HMOX1 and TLR4 genes have higher scores in MCODE (Fig. 5B).

Potential biomarker expression by Western blot and qRT-PCR

Three genes were verified. They had high reliability and were mostly related to frozen shoulders. IL-6, HMOX1 and TLR4 were identified by MCODE as the genes with high MCODE score in the clusters. Then, the filtered

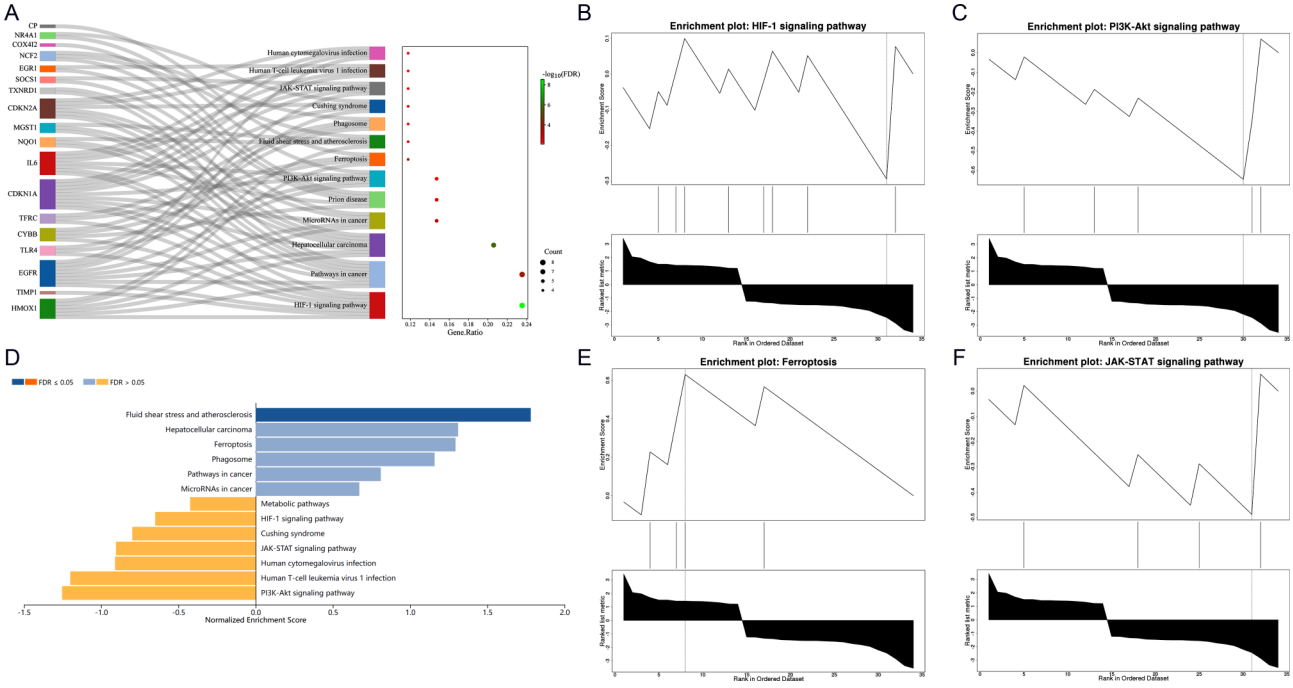


Fig. 3 **A**: Schematic diagram of differential expressed genes and signaling pathways in KEGG enrichment analysis. **B**, **C**, **D**, **E**, **F**: The results of enrichment analysis and GSEA of HIF-1 signaling pathway, PI3K-Akt signaling pathway, ferroptosis, and JAK-STAT signaling pathway by WebGestalt

biomarkers, including IL-6, HMOX1 and TLR4, were verified in FS synovial tissue using Western Blot and qRT-PCR. The results indicated that the expression levels of IL-6 were visibly higher and that the expression of HMOX1 and TLR4 were lower in FS rats than in the sham groups (Fig. 6).

Discussion

Frozen shoulder is the most common self-limiting disease among middle-aged and elderly individuals. The current study investigated and revealed the potential molecular mechanisms underlying frozen shoulders by bioinformatics analysis. We used bioinformatics tools to detect DEGs of ferroptosis and frozen shoulders between the individuals with frozen shoulders and healthy controls. All DEGs were analyzed by KEGG, GO, and PPI network analyses. The study also identified several genes that had not yet been mentioned in the area of frozen shoulders and ferroptosis. These results showed several key pathways and genes that may play pivotal roles in frozen shoulders pathogenesis.

The identification of 14 upregulated and 20 downregulated genes from the intersection of GSE238053 and the Ferroptosis Database demonstrated the dysregulation of specific genes in individuals with frozen shoulders compared to healthy controls. Ferroptosis is characterized by the accumulation of lipid peroxides and iron-dependent oxidative damage, and emerging evidence has implicated its involvement in various disease processes [15]. Frozen shoulder is a condition associated with significant oxidative stress and inflammation within the joint capsule. The increased reactive oxygen species (ROS) can lead to lipid peroxidation, which is a hallmark of ferroptosis [16]. One study by Li et al. investigated the role of ferroptosis in musculoskeletal disorders and highlighted the potential contribution of ferroptosis in the pathogenesis of frozen shoulders. This study demonstrated that key regulators of ferroptosis, such as glutathione peroxidase 4 (GPX4) and system Xc- transporter, may be dysregulated in frozen shoulders, leading to increased susceptibility to iron-mediated oxidative damage and cell death. Additionally, the study identified ferroptosis-related pathways and processes, including lipid peroxidation and redox homeostasis, as potential contributors to frozen shoulders pathology [17]. Inflammatory mediators, which are elevated in frozen shoulders, can induce ferroptosis in cells. For instance, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), commonly seen in inflammatory conditions, have been shown to promote ferroptosis in certain cellular contexts [18]. Another relevant study by Stockwell et al. provided comprehensive insights into the molecular mechanisms of ferroptosis and its implications in disease states. The authors discussed the intricate interplay between iron metabolism, lipid peroxidation, and cellular signaling pathways, underscoring the broader relevance of ferroptosis across diverse pathological conditions [19]. In our study, we found the dysregulation of specific genes associated with ferroptosis,

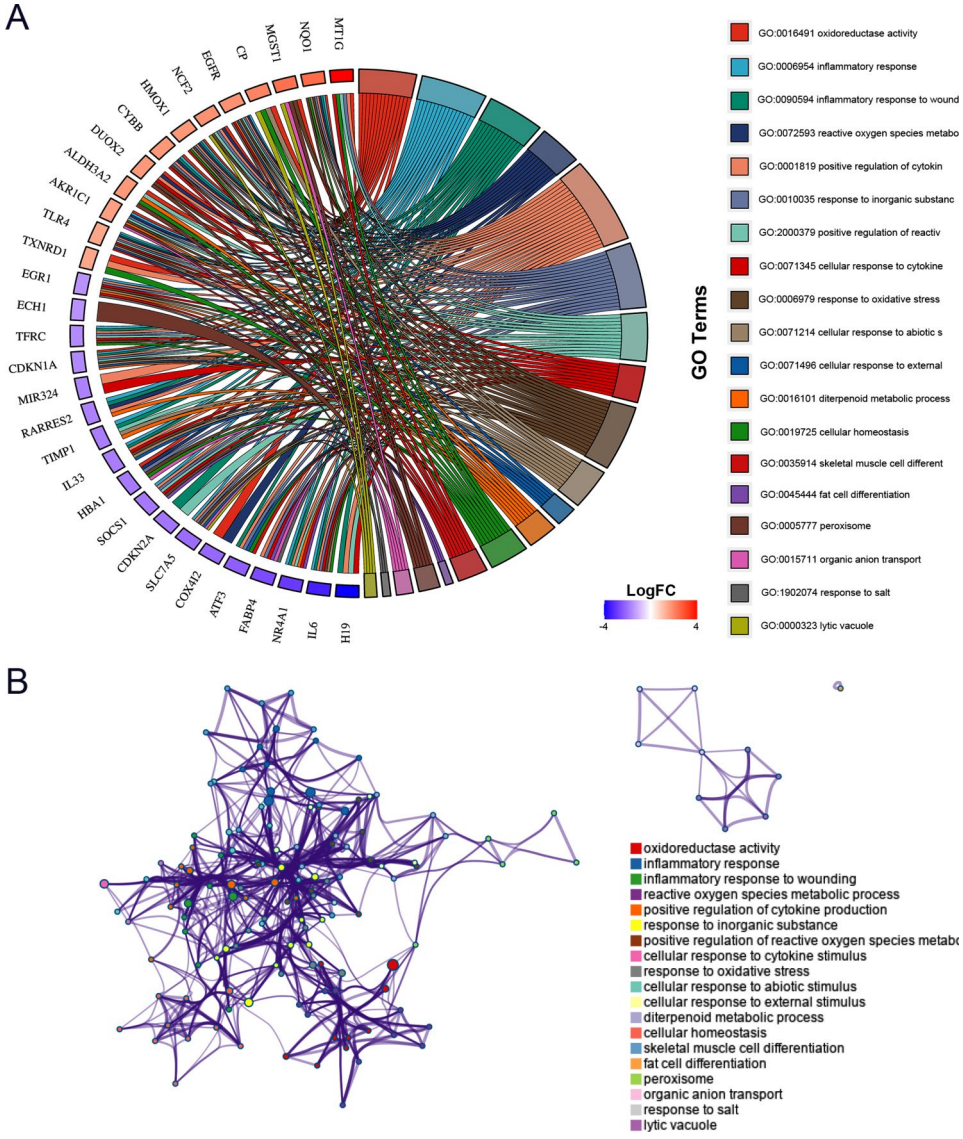


Fig. 4 **A**: Schematic diagram of differential expressed genes and terms in GO enrichment analysis. **B**: Network of enriched terms

suggesting a possible mechanistic connection between frozen shoulders and ferroptosis.

The KEGG enrichment analysis revealed the involvement of several critical signaling pathways, such as the HIF-1, PI3K-Akt, and JAK-STAT pathways, in addition to pathways associated with cancer, hepatocellular carcinoma, and ferroptosis. These findings suggest a complex interplay of molecular pathways that contribute to the pathogenesis of frozen shoulders. The HIF-1 signaling pathway plays a crucial role in cellular responses to hypoxia and has been implicated in various physiological and pathological processes [20]. Our research found that the three key genes we screened, TLR4, HMOX1, and IL6, are enriched in the HIF-1 signaling pathway and may play important roles in regulating cellular processes and stress responses [21]. TLR4 and IL6 may contribute to inflammatory response, tissue damage, and related tissue remodeling, while HMOX1 may be involved in oxidative stress [22–24]. The involvement of these genes may lead to the activation of the HIF-1 signaling pathway under hypoxic conditions, resulting in the development and persistence of frozen shoulders. The genes TLR4 and IL6 are implicated in the pathogenesis of frozen shoulders and are believed to interact with the PI3K-Akt signaling pathway, which plays a crucial role in various cellular processes including cell survival, growth, and metabolism [25]. In frozen shoulderss, TLR4 may contribute to the inflammatory process and tissue damage through the activation of downstream signaling pathways such as PI3K-Akt. Elevated levels of IL6 are often associated with inflammatory conditions and can promote the survival of cells by activating the PI3K-Akt pathway, potentially

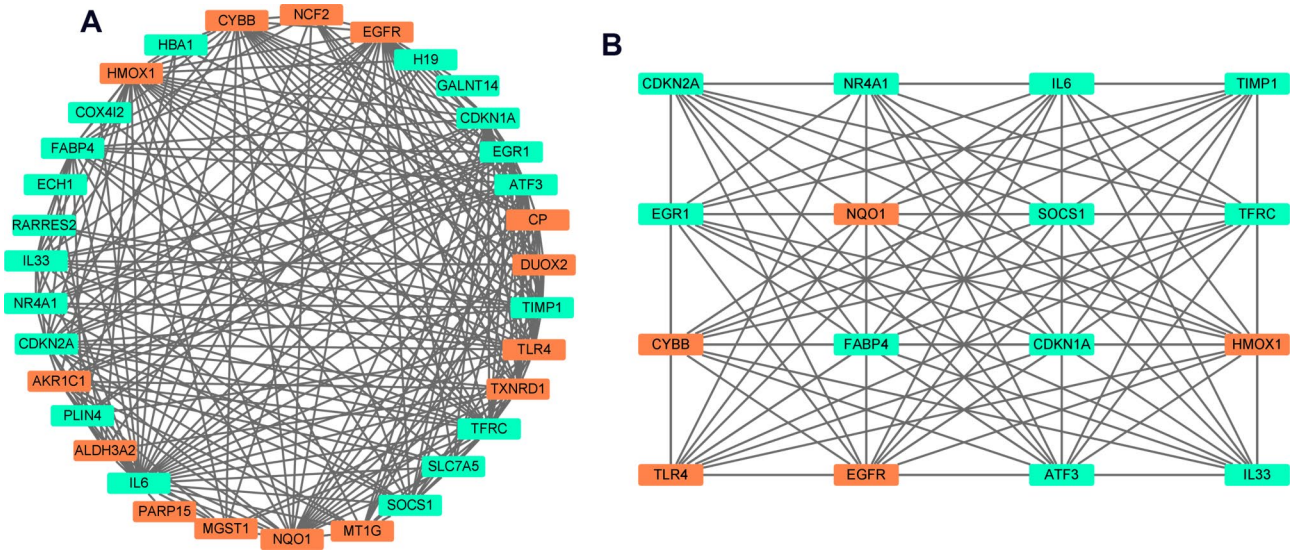


Fig. 5 **A**: PPI network of DEGs. **B**: A cluster was obtained by MCODE in Cytoscape

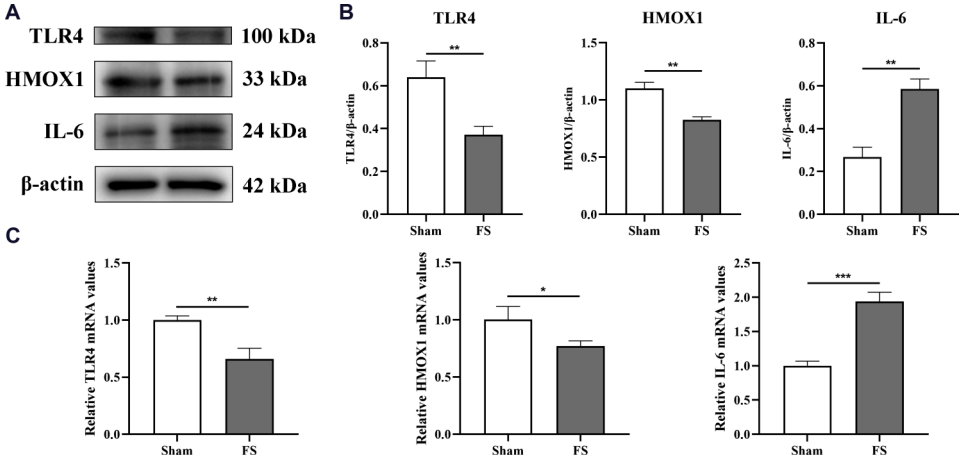


Fig. 6 **A** and **B**: Protein expression of TLR4, HMOX1 and IL-6 in the synovial tissue by Western Blot analysis. **C**: The expression levels of TLR4, HMOX1 and IL-6 in the synovial tissue by qRT-PCR analysis. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ represented comparison of Sham with FS group

influencing the progression of frozen shoulders [26]. Moreover, in addition to key genes, we also found that some DEGs are enriched in the JAK-STAT signaling pathway. The epidermal growth factor receptor (EGFR) gene is a key player in cellular signaling processes, and its interaction with the JAK-STAT signaling pathway has been implicated in various physiological and pathological conditions, including frozen shoulders. EGFR, when activated, can initiate a cascade of events that lead to the activation of the JAK-STAT pathway, which is involved in cell proliferation, differentiation, and survival [27, 28]. frozen shoulders. Additionally, emerging evidence suggests that the JAK-STAT pathway regulates cellular responses to oxidative stress, implicating its potential involvement in ferroptosis-related pathways [29]. In our study, the activation of the HIF-1, PI3K-Akt, and JAK-STAT signaling pathways may indicate their potential as therapeutic targets for modulating the disease process.

Moreover, the GO enrichment analysis highlighted the activation of biological pathways and molecular function related to DEGs. Ferroptosis has been implicated in promoting inflammatory responses, which may contribute to the pathogenesis of frozen shoulders. A study demonstrated that ferroptosis exacerbates inflammation in ischemia-reperfusion injury, highlighting the potential impact of ferroptosis on inflammatory pathways [30]. In frozen shoulders, the interaction between TLR4 and IL-6 could contribute to the chronic inflammatory process observed in the condition [31]. The activation of TLR4 may lead to an increased production of IL-6, which in turn can amplify the inflammatory response, leading to the development of symptoms associated with frozen shoulders. Moreover, the IL-6 trans-signaling via STAT3

has been shown to modulate TLR4-dependent inflammatory responses, suggesting a complex interplay between these two molecules in the regulation of inflammation [32, 33]. In addition, the dysregulation of reactive oxygen species (ROS) metabolic processes mediated by ferroptosis may play a role in the development of frozen shoulders [34]. Doll et al. have elucidated the intricate connections between ferroptosis and ROS metabolism, providing insights into the potential impact of ferroptosis on cellular redox homeostasis [35]. HMOX1 is believed to be upregulated in response to various stress factors, including oxidative stress, as a key protective mechanism against ROS induced cell damage [36, 37]. Ferroptosis may modulate cellular responses to cytokine stimuli, potentially contributing to the pathological changes associated with frozen shoulders. The interplay between ferroptosis and cytokine-mediated signaling revealed the potential impact of ferroptosis on cellular responses to cytokines [38]. Our findings implicate the involvement of immune and oxidative stress-related processes in frozen shoulders, providing potential avenues for further mechanistic investigations and therapeutic interventions.

The generated PPI network and identification of key genes through MCODE analysis offered valuable insights into the interconnectedness of DEGs and potential regulatory hubs within the frozen shoulders molecular landscape. The cluster of 16 key genes represents potential candidates for further experimental validation and functional characterization. The impact of ferroptosis on the development of scapulohumeral periarthritis via the IL-6 gene is a complex process involving multiple cellular and molecular interactions. Ferroptosis can trigger the release of cellular contents, including damage-associated molecular patterns (DAMPs), which can activate immune cells and stimulate the production of cytokines such as IL-6 [39]. This inflammatory response can further exacerbate the condition, leading to the progression of frozen shoulders. Moreover, IL-6 can modulate cellular redox homeostasis, affecting the balance between ROS generation and degradation [40]. Ferroptosis can be considered a consequence of cellular damage due to inflammation, and may further hinder the capacity for tissue regeneration, leading to chronic stiffness and pain. Heme oxygenase-1 (HMOX1), which encodes the enzyme responsible for heme degradation, plays a crucial role in iron metabolism and oxidative stress responses [41]. Recent studies suggest that the interaction between ferroptosis and HMOX1 may significantly influence the development of frozen shoulders. In frozen shoulders, elevated levels of iron and oxidative stress have been observed, suggesting a role for ferroptosis in disease progression. HMOX1 may influence this process by regulating iron availability and oxidative stress levels in the affected tissues [42]. For instance, downregulation of HMOX1 expression may lead to iron accumulation and increased ROS production, thereby promoting ferroptosis and exacerbation of inflammatory responses in the shoulder joint [43]. In addition, TLR4 may regulate genes involved in iron metabolism, leading to increased iron availability and subsequent ferroptosis [23]. Inflammatory cytokines produced downstream of TLR4 signaling may promote oxidative stress and lipid peroxidation, further enhancing ferroptosis to exacerbate the inflammatory processes in frozen shoulders. This vicious cycle between inhibition of TLR4, ferroptosis, and inflammation may contribute to the chronicity and severity of frozen shoulders symptoms [44]. These data suggest that the changes in IL-6, HMOX1 and TLR4 gene expression may be the basis for the interaction between ferroptosis and frozen shoulders. Moreover, our Western Blot and qRT-PCR experimental results are consistent with the expected expression of DEGs. Understanding the interactions and regulatory roles of these key genes may reveal novel targets for therapeutic intervention and provide a more comprehensive understanding of frozen shoulders pathophysiology.

In summary, the current study offers a comprehensive analysis of DEGs, enrichment pathways, and protein interactions between ferroptosis and frozen shoulders, shedding light on potential molecular mechanisms and therapeutic targets. The integration of data and bioinformatics analyses has provided a valuable resource for future exploration and intervention strategies in the context of frozen shoulders pathophysiology.

However, it should be acknowledged that there are limitations inherent to the current study design and several questions remain open. The current conclusion needs to be verified, so the next study will involve a series of experiments designed to validate the interactions between the signaling pathways and the genes identified in this study to provide stronger support for our conclusions and elucidate complex biological processes.

Conclusions

Our study analyzed the GSE238053 and ferroptosis datasets to identify signaling pathways and genes that may affect the pathological process of frozen shoulders, and validated three key genes, IL-6, HMOX1, and TLR4, through experiments, providing new targets for clinical treatment of frozen shoulders.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12920-024-02011-5.

Supplementary Material 1: Is about 1036 genes of GSE238053 and 564 genes of Ferroptosis Database.

Supplementary Material 2: Is about KEGG Enrichment Analysis of DEGs.

Supplementary Material 3: Is about GO Enrichment Analysis of DEGs.

Acknowledgements
Not applicable.

Author contributions
HJ designed the study and wrote the manuscript, HZ executed the study and acquired the data, JZ and ZL interpreted the data, KW analyzed the data and visualized data. All authors contributed to the article and approved the submitted version.

Funding
This study was supported by Weihai Traditional Chinese Medicine Technology Project (No.2023 N-24).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
In this study, the rights and interests of rats were fully protected, which met the requirements of the Laboratory Animal Welfare Ethics Committee of Qingdao University. (The approval number: NO.20240415SD1220240520032)

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

Received: 1 June 2024 / Accepted: 12 September 2024
Published online: 27 September 2024

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