

Th17/IL-17F Activates the TP53/Nrf2 Signaling Pathway-Mediated Ferroptosis and Plays an Important Role in Acute-on-Chronic Liver Failure

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Abstract

Liver failure is a critical condition caused by various factors, leading to extensive hepatocyte necrosis, severe hepatic dysfunction, and ultimately systemic multi-organ failure. Acute-on-chronic liver failure (ACLF) is the most prevalent form of liver failure in Asia. The pathogenesis of ACLF is complex. Transcriptomic sequencing revealed that the Th17 signaling pathway is closely associated with ACLF, and TP53 is a core gene implicated in liver failure. In addition, ferroptosis also plays an important role in ACLF. In vitro, we found that reactive oxygen species (ROS) levels and iron content were significantly increased in IL-17-treated L-02 cells compared with untreated controls. Compared with the control group, the expression levels of SLC7A11 and GPX4 were significantly decreased, while ACSL4 and TRIM25 were significantly increased in the IL-17 treatment group. We also observed a significant upregulation of P53 and a downregulation of Nrf2. This study suggests that TP53 may play a key role in ACLF by mediating ferroptosis. Experimental results confirmed that IL-17 stimulation induced ferroptosis-related oxidative stress, structural alterations in cells, and differential expression of ferroptosis-related genes. Most importantly, changes in P53 and Nrf2 expression following IL-17 treatment suggest that Th17/IL-17 may promote ferroptosis through the TP53/Nrf2 signaling pathway and thereby contribute to the pathogenesis and progression of liver failure.

Introduction

Liver failure refers to severe hepatic impairment resulting from various factors such as viral infections, alcohol consumption, drug toxicity, surgical complications, metabolic disorders, and circulatory dysfunction, leading to the inability to maintain essential physiological functions. Based on medical history, clinical manifestations, and disease progression, liver failure can be classified into acute, subacute, chronic, and acute-on-chronic types (subacute)¹. Liver failure is associated with an extremely high mortality rate. The 30-day mortality rate for patients with acute liver failure (ALF) is 26.7%, and ACLF, the most prevalent type of liver failure in Asia, has a mortality rate ranging from 30–70%². The etiologies of liver failure are diverse, its prognosis is poor, the mortality rate is high, and the underlying pathogenesis remains complex. Over the past few decades, numerous studies have focused on elucidating the mechanisms of liver failure.

Persistent inflammation and immune activation are key mechanisms in the development of liver failure. Th17 cells, a newly identified subset of helper T cells differentiated from CD4⁺ T lymphocytes, have been shown to alleviate acute liver injury by regulating Th17 cell proliferation and cytokine secretion³. Studies have demonstrated that an increased frequency of Th17 cells is positively correlated with the severity of liver injury and ACLF progression, and their activation can exacerbate inflammatory responses associated with ACLF^{4,5}.

Furthermore, hepatocellular necrosis can trigger secondary immune responses that promote the release of inflammatory mediators and aggravate liver failure⁶. Ferroptosis, first described by Dixon et al. in 2012, is a form of iron-dependent, non-apoptotic cell death characterized by intracellular iron

accumulation and lipid peroxidation⁷. his distinct mode of cell death is driven by iron-dependent phospholipid peroxidation and regulated by various cellular metabolic processes, including redox homeostasis, iron metabolism, mitochondrial function, and the metabolism of amino acids, lipids, and carbohydrates, as well as multiple disease-related signaling pathways^{8,9}. Ferroptosis contributes to a wide range of pathological conditions, including neurodegenerative diseases such as Alzheimer's and Parkinson's diseases⁹, cancers¹⁰, ischemia-reperfusion injury¹¹, and kidney diseases like acute kidney injury (AKI) and chronic kidney disease (CKD)¹². Moreover, ferroptosis plays an important role in drug-induced liver injury, non-alcoholic fatty liver disease, and hepatic fibrosis^{13–15}. Studies have found that mitochondrial dysfunction and excessive oxidative stress are key contributors to acute liver failure and that liver function can be improved through antioxidant therapy¹⁶, suggesting that ferroptosis may be a critical mechanism involved in liver failure.

Regulatory T cells (Tregs) can induce high expression of GPX4 under co-stimulation of the T cell receptor (TCR) and CD28 to maintain immune homeostasis. Deletion of the GPX4 gene leads to the excessive accumulation of lipid peroxides in Tregs, resulting in ferroptosis, which in turn promotes the production of IL-1 β and enhances the response of T helper 17 (Th17) cells^{17,18}. These findings suggest that Th17 cells may be closely associated with ferroptosis. However, it remains unclear whether Th17 cells influence the occurrence and progression of ferroptosis in liver failure and the mechanisms involved. In this study, we collected blood samples from patients with ACLF and performed transcriptome sequencing to identify signaling pathways and genes most closely associated with the condition. We subsequently established a cellular injury model to verify the connection between Th17 cells and ferroptosis, exploring the expression of key ferroptosis-related genes and possible signaling pathways. These findings offer new insights into the pathogenesis and potential therapeutic targets of acute-on-chronic liver failure.

Materials and Methods

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of the First Affiliated Hospital of Kunming Medical University (Approval No. 2022-L-41). Written informed consent for treatment and subsequent anonymous data processing was obtained from all participants and, in the case of vulnerable individuals, from their next of kin.

Transcriptome Sequencing and Bioinformatics Analysis

Peripheral blood samples were collected from ACLF patients and healthy controls for transcriptome sequencing. Differentially expressed genes were identified using DESeq2 or edgeR, and visualized via volcano plots and heatmaps. GO and KEGG enrichment analyses were conducted using the *clusterProfiler* package, with results displayed as bubble plots and enrichment networks. Principal component analysis (PCA), clustering, and expression heatmaps were generated in R to assess inter-sample variation and gene expression patterns.

Cell Viability

L-02 cells (Shanghai Institute of Cell Biology, China) were seeded in 96-well plates at a density of 1×10^4 cells/well. IL-17 (100 ng/ml) and Erastin (10 μ M) were added to experimental wells and incubated for 24, 48, and 72 h. Cell viability was measured using CCK-8 reagent (Dojindo, Japan); 10 μ l was added to each well, followed by 2 h incubation in the dark. Absorbance was measured at 450 nm using a microplate reader (BioTek ELx800, USA). Cell viability (%) was calculated as: $(OD_{\text{experiment}} - OD_{\text{blank}}) / (OD_{\text{control}} - OD_{\text{blank}}) \times 100\%$.

Detection of Intracellular ROS Content

Intracellular ROS levels were measured using the DCFH-DA fluorescent probe. DCFH-DA is a non-fluorescent molecule that can permeate cell membranes and be hydrolyzed by intracellular esterases into 2',7'-dichlorofluorescein (DCF), which is subsequently oxidized by ROS to produce a strong fluorescent signal¹⁹. L-02 cells were seeded in 6-well plates at a density of 2×10^5 cells/well. When cell confluence reached approximately 50%, the medium was replaced with complete medium lacking antibiotics, and cells were assigned to different treatment groups: control, IL-17 (100 ng/ml), or Erastin (10 μ M). After 48 h, 1 ml of DCFH-DA (10 μ M/L) working solution, diluted 1:1000 in serum-free medium, was added to each well and incubated at 37°C for 30 minutes. Cells were washed three times with PBS, and fluorescence intensity was measured using a fluorescence microplate reader with excitation/emission wavelengths of 485 nm and 530 nm.

Intracellular Iron Content Detection

Intracellular iron content was assessed using the PGSK fluorescent probe. PGSK is a specific probe for divalent iron (Fe^{2+}), forming a fluorescent product upon binding²⁰. L-02 cells were seeded in 96-well plates at a density of 1×10^4 cells/well and cultured at 37°C with 5% CO_2 . Once cell confluence reached about 50%, the medium was replaced with complete medium without antibiotics, and cells were divided into control, IL-17 (100 ng/ml), and Erastin (10 μ M) treatment groups. PGSK (5 μ M/L) working solution, diluted 1:1000 in serum-free medium, was added (1 ml/well), followed by 30 min incubation in the dark at 37°C. Cells were then washed three times with PBS, and fluorescence intensity was measured using a fluorescence microplate reader at excitation/emission wavelengths of 490 nm and 520 nm.

Electron Microscopy

Cells were fixed with pre-cooled 2.5% glutaraldehyde (in PBS, pH 7.4) for 4 h, followed by three 10-minute PBS washes. Samples were then dehydrated, embedded, sectioned, and stained with uranyl acetate and lead citrate. Mitochondrial ultrastructure was examined using a transmission electron microscope (Hitachi HT7700, Japan). Random fields were imaged to assess mitochondrial number, morphology, cristae integrity, and membrane density.

Western Blot

L-02 cells were seeded in 6-well plates at a density of 2×10^5 cells/well and treated with IL-17 (100 ng/ml) or Erastin (10 μ M) for 24 h. Cells were lysed using RIPA buffer (Beyotime, China), and protein concentrations were measured using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific). Equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes (Millipore). Membranes were blocked and incubated overnight at 4°C with primary antibodies: anti-GPX4 (1:1000, Abcam), anti- β -actin (1:5000, Proteintech), among others. After washing, membranes were incubated with HRP-conjugated secondary antibody (Goat Anti-Rabbit IgG-HRP, 1:5000, Jackson ImmunoResearch) at room temperature for 1 h. Protein bands were visualized using the SuperPico ECL Chemiluminescence Kit (Vazyme), and band intensities were quantified using ImageJ software.

Measurement of Malondialdehyde (MDA) and Glutathione (GSH) Levels

L-02 cells were seeded in 6-well plates at a density of 2×10^5 cells/well. After 24 h of culture, cells were divided into three groups: control, IL-17 (100 ng/ml), and Erastin (10 μ M). Cells were collected into centrifuge tubes, and the supernatant was removed following centrifugation. For every 5×10^6 cells, 1 ml of extraction buffer was added, and cells were lysed using ultrasound in an ice bath. After centrifugation at 8000g for 10 minutes at 4°C, the supernatant was collected and kept on ice. MDA and GSH levels in the supernatant and plasma were measured using a malondialdehyde assay kit (Cat. S0131S, Beyotime).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 9.0 software (GraphPad Software, San Diego, CA, USA). Prior to conducting parametric tests, the normality of data distribution was assessed. Comparisons between two groups were evaluated using two-tailed Student's *t*-tests, while one-way analysis of variance (ANOVA) was employed for comparisons among three or more groups. When ANOVA indicated significant differences, Bonferroni's post hoc test was subsequently applied to perform pairwise comparisons. A *P*-value of less than 0.05 was considered indicative of statistical significance.

Results

Bioinformatics Analysis

We collected peripheral blood samples from patients with acute-on-chronic liver failure (ACLF) and healthy volunteers to conduct transcriptomic sequencing. The analysis revealed a total of 1,312 genes exhibiting significant differential expression between the two groups. As illustrated in the volcano plot (Fig. 1A), among these, 693 genes were upregulated and 619 genes were downregulated in ACLF patients compared to healthy controls. A heatmap displaying the top 20 differentially expressed genes is

shown in Fig. 1B. Gene Ontology (GO) enrichment analysis of biological processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed significant enrichment of the Th17 signaling pathway in the pathogenesis of ACLF, as presented in Figs. 1C and 1D. To further investigate the molecular mechanisms involved, protein-protein interaction (PPI) network analysis was conducted. We found that 25 differentially expressed genes overlapped with known ferroptosis-related genes (Fig. 2A). Based on these overlapping genes, a PPI network was constructed to identify core genes involved in ferroptosis in ACLF. Among them, TP53 emerged as the most prominent hub gene (Figs. 2B–D). These findings suggest a strong association between the Th17 signaling pathway and the development of ACLF and identify TP53 as a key regulatory gene involved in ferroptosis during the progression of this disease.

Cell Viability and Intracellular ROS Content Detection

Cell viability was evaluated using the CCK-8 assay to assess the effects of different treatments on L-02 cells. As shown in Fig. 3A, the control group maintained stable cell viability at 24, 48, and 72 hours, indicating good cell growth under normal culture conditions. In contrast, Erastin treatment led to a significant reduction in cell viability, with the degree of inhibition becoming more pronounced over time. Similarly, IL-17 treatment markedly suppressed cell viability compared to the control group, and the inhibitory effect also intensified with extended treatment duration. These results indicate that both IL-17 and Erastin exert significant inhibitory effects on the viability of L-02 cells.

To assess intracellular oxidative stress, we measured reactive oxygen species (ROS) levels using the DCFH-DA fluorescent probe. As shown in Fig. 3B, L-02 cells in the control group exhibited low baseline ROS levels, reflecting minimal oxidative stress under standard conditions. In the Erastin treatment group, ROS levels were significantly elevated, indicating that Erastin, as a known ferroptosis inducer, triggered intracellular oxidative stress and promoted ROS production. Similarly, IL-17 treatment caused a marked increase in ROS levels, demonstrating that IL-17 also induces a strong oxidative stress response, leading to substantial ROS accumulation. These findings confirm that both IL-17 and Erastin significantly elevate ROS levels in L-02 cells and can effectively induce oxidative stress.

Intracellular Iron Content Detection

Iron overload, a hallmark of ferroptosis, leads to intracellular accumulation of iron and promotes lipid peroxidation²¹. To evaluate this, intracellular iron levels were detected using the PGSK fluorescent probe. As shown in Fig. 3C, L-02 cells in the control group maintained low levels of intracellular iron, with correspondingly low PGSK fluorescence intensity, indicating that iron content remained at a physiological baseline under normal culture conditions. However, in the Erastin-treated group, there was a marked increase in intracellular iron content, as evidenced by significantly elevated PGSK fluorescence. This effect was more pronounced than in the IL-17 group. IL-17 treatment also resulted in a substantial increase in PGSK fluorescence intensity, suggesting that IL-17 promotes intracellular iron accumulation through a specific, yet undetermined mechanism. Collectively, these findings indicate that both IL-17 and Erastin significantly increase intracellular iron levels in L-02 cells.

Mitochondrial Structural Changes

To explore the morphological characteristics associated with ferroptosis, we examined mitochondrial ultrastructure using transmission electron microscopy. Mitochondrial changes are a defining feature of ferroptosis and are typically characterized by reduced volume, increased electron density, and loss or disintegration of cristae²². As shown in Fig. 4, in both the Erastin and IL-17 treatment groups, mitochondria exhibited substantial shrinkage or atrophy. The number of mitochondrial cristae was significantly reduced, and their structure appeared disorganized or completely absent. In addition, the integrity of the mitochondrial membrane was disrupted, and in some cases, mitochondria appeared fragmented. These ultrastructural changes are consistent with classic features of ferroptosis and indicate that both Erastin and IL-17 induce mitochondrial damage associated with this form of cell death.

Ferroptosis-Related Gene and Protein Expression

In the control group of L-02 cells, the expression level of SLC7A11 was relatively high. Treatment with Erastin significantly reduced SLC7A11 expression compared to the control group ($p < 0.05$). Similarly, the expression of SLC7A11 in the L-02 + IL-17 group was markedly lower than in the control group ($p < 0.001$). These results demonstrate that both Erastin and IL-17 can suppress the expression of SLC7A11, with IL-17 exhibiting a stronger inhibitory effect ($p < 0.01$), as shown in Fig. 5A.

The expression of GPX4 was also elevated in the control group. After Erastin treatment, GPX4 expression was significantly decreased ($p < 0.05$). Likewise, IL-17 treatment resulted in a notable reduction in GPX4 expression compared to the control group ($p < 0.05$). However, there was no statistically significant difference in GPX4 expression between the L-02 + Erastin and L-02 + IL-17 groups (ns.), indicating that both agents suppress GPX4 expression to a similar extent, as illustrated in Fig. 5B.

The expression of ACSL4 in the L-02 control group was used as the baseline. Erastin treatment significantly elevated ACSL4 expression compared to the control group ($p < 0.01$). IL-17 also induced a significant increase in ACSL4 expression ($p < 0.05$). Furthermore, ACSL4 expression in the L-02 + IL-17 group was lower than that in the L-02 + Erastin group, and the difference between the two was statistically significant ($p < 0.05$). These findings indicate that both IL-17 and Erastin can promote ACSL4 expression, although Erastin exerts a stronger effect (Fig. 5C).

Compared with the L-02 control group, Erastin treatment significantly increased TRIM25 expression ($p < 0.05$). Similarly, IL-17 treatment led to a significant upregulation of TRIM25 expression compared with the L-02 control group ($p < 0.001$). Furthermore, TRIM25 expression in the L-02 + IL-17 group was higher than in the L-02 + Erastin group, with the difference between the two reaching statistical significance ($p < 0.001$). These results demonstrate that both IL-17 and Erastin can promote TRIM25 expression, as shown in Fig. 5D.

As shown in Fig. 6A, compared with the L-02 control group, the expression level of P53 in the L-02 + Erastin group was significantly upregulated ($p < 0.05$). IL-17 treatment also significantly increased P53 expression compared with the L-02 control group ($p < 0.01$). Moreover, P53 expression in the L-02 + IL-17 group was higher than that in the L-02 + Erastin group, with the difference between the two being statistically significant ($p < 0.05$). These results indicate that both IL-17 and Erastin can upregulate P53 expression, with IL-17 exhibiting a stronger effect.

As shown in Fig. 6B, Compared with the L-02 control group, the expression of Nrf2 in the L02 + Erastin group was significantly decreased, and the difference was statistically significant compared with the L-02 control group ($p < 0.001$). The expression of Nrf2 in the L02 + IL-17 group was also decreased, and the difference was statistically significant compared with the L-02 control group ($p < 0.01$). The results showed that the expression of Nrf2 was downregulated in both the IL-17 and Erastin groups.

The levels of MDA and GSH in each group were determined using assay kits. As shown in Fig. 7, MDA levels in the L-02 + Erastin group were significantly elevated compared with the L-02 control group ($p < 0.001$). MDA levels were also significantly increased in the L-02 + IL-17 group relative to the L-02 control group ($p < 0.05$). Furthermore, MDA levels in the L-02 + Erastin group were higher than those in the L-02 + IL-17 group, and the difference between the two was statistically significant ($p < 0.01$). GSH levels were significantly decreased in the L-02 + Erastin group compared with the L-02 control group ($p < 0.01$). Similarly, GSH levels in the L-02 + IL-17 group were significantly reduced compared with the L-02 control group ($p < 0.05$). In addition, GSH levels in the L-02 + Erastin group were lower than those in the L-02 + IL-17 group, although the difference between the two groups was not statistically significant (ns.).

Discussion

Acute-on-chronic liver failure (ACLF) refers to the acute deterioration of liver function triggered by acute insults (such as infection, alcohol consumption, or drug toxicity) superimposed on an underlying chronic liver disease (such as cirrhosis). This condition is frequently accompanied by multiple organ failure and carries a high short-term mortality rate. Persistent immune dysregulation and a sustained inflammatory response are regarded as the core pathogenic mechanisms of ACLF. Among the immune cell populations, CD4⁺ T cells play a pivotal role in mediating the adaptive immune response in ACLF, with Th17 cells representing a critical subset derived from CD4⁺ T lymphocytes. Under the influence of cytokines such as TGF- β , IL-6, and IL-23, the key transcription factor for Th17 cell differentiation—retinoic acid receptor-related orphan receptor gamma t (ROR γ t)—is activated, thereby driving the polarization of CD4⁺ T cells into Th17 cells^{23–25}. Th17 cells are known to exert potent pro-inflammatory effects by enhancing immune responses and promoting the development of inflammation. Their hallmark function lies in the secretion of IL-17 family cytokines, including IL-17A and IL-17F²⁶. Accumulating evidence indicates that Th17 cells are implicated in the pathogenesis of various liver diseases, including hepatocellular carcinoma (HCC) and alcoholic liver disease²⁷. Several studies have reported a positive correlation between the frequency of circulating Th17 cells and disease severity in patients with chronic hepatitis B (CHB), suggesting that Th17 cells play a role in disease progression. Furthermore, Th17 cell

levels are significantly elevated in ACLF patients compared to those with CHB alone, indicating their involvement in the progression of ACLF²⁸. Mitochondrial dysfunction and excessive oxidative stress have been shown to be key contributors to liver failure, with ferroptosis emerging as a novel and critical form of regulated cell death implicated in this process¹⁶. Ferroptosis is characterized by iron-dependent lipid peroxidation and has been increasingly recognized as a potential driver of liver injury. Previous studies have shown that ferroptosis can activate the immune system and regulate immune responses^{29–31}. In the present study, we sought to investigate the role of the Th17/IL-17 signaling axis in modulating hepatocyte ferroptosis and its potential contribution to ACLF. Using bioinformatics analysis combined with experimental validation, we examined how IL-17 may influence ferroptotic pathways. Transcriptomic analysis of clinical liver tissue samples from patients with ACLF and healthy controls revealed significant differences in gene expression between the two groups. GO and KEGG pathway enrichment analysis demonstrated that the differentially expressed genes were significantly enriched in Th17-associated signaling pathways. Further analysis using a protein–protein interaction (PPI) network identified 25 genes closely associated with ferroptosis, among which TP53 emerged as the most prominent hub gene. Nevertheless, the precise mechanism by which the Th17 signaling pathway activates TP53 remains to be fully elucidated. To address this question, we conducted a series of preliminary intervention experiments on hepatocytes.

Ferroptosis is closely linked to disturbances in iron metabolism. Iron, an essential trace element, participates in numerous physiological and biochemical processes. In its trivalent (Fe^{3+}) form, iron binds to transferrin and enters cells via transferrin receptors, where it is reduced to divalent iron (Fe^{2+}) by the metalloreductase STEAP3. The resulting Fe^{2+} then engages in various intracellular reactions. When iron homeostasis is disrupted, excessive Fe^{2+} can participate in Fenton reactions, producing reactive oxygen species (ROS), such as hydroxyl radicals. These ROS interact with polyunsaturated fatty acids in cellular membranes, leading to lipid peroxidation, loss of membrane integrity, and ultimately ferroptotic cell death^{32,33}. Mitochondrial changes, including shrinkage, cristae loss, and increased membrane density, are hallmark features of ferroptosis²².

In this study, we observed that both ROS and intracellular iron levels were elevated in L-02 hepatocytes following IL-17 treatment. Transmission electron microscopy revealed classic morphological features of ferroptosis in these cells, including reduced mitochondrial volume, increased membrane density, and the disappearance of cristae. These findings indicate that IL-17 may promote ferroptosis in hepatocytes, thereby contributing to liver injury and possibly the progression to ACLF. Additionally, we examined the expression of several key ferroptosis-regulatory molecules, including SLC7A11, ACSL4, and GPX4, to further characterize the involvement of IL-17 in this process.

SLC7A11 (solute carrier family 7 member 11) is a critical component of system Xc⁻, responsible for transporting cystine into cells and exporting glutamate. Inhibition of SLC7A11 disrupts cystine uptake, leading to reduced glutathione (GSH) synthesis, which in turn diminishes the activity of glutathione peroxidase 4 (GPX4), ultimately resulting in lipid peroxidation and the induction of ferroptosis^{7,9}. Thus,

SLC7A11 is a key regulatory target in ferroptosis. Notably, in cancer therapy, suppressing SLC7A11 expression can effectively trigger ferroptosis in tumor cells³⁴.

ACSL4 (long-chain acyl-CoA synthetase 4) catalyzes the conjugation of long-chain polyunsaturated fatty acids (PUFAs) with coenzyme A to form acyl-CoA, which serves as a primary substrate for lipid peroxidation. ACSL4 promotes the esterification and accumulation of PUFAs in cellular membranes, thereby heightening sensitivity to lipid peroxidation. Its overexpression facilitates ferroptosis, while its knockout or inhibition can significantly attenuate this process³⁵.

Recent studies further highlight the essential role of ACSL4 in cancer cell ferroptosis, showing that ACSL4-mediated production of 5-hydroxyeicosatetraenoic acid (5-HETE) contributes directly to the induction of ferroptosis. ACSL4 is therefore not only a sensitive indicator but also a critical driver of ferroptosis³⁶.

GPX4 (glutathione peroxidase 4) is a vital antioxidant enzyme that reduces lipid hydroperoxides to non-toxic lipid alcohols using GSH as a cofactor, thereby safeguarding cells from oxidative stress-induced damage. As a core regulator of ferroptosis, GPX4 inhibition—via agents such as RSL3—leads to the accumulation of lipid peroxides and triggers ferroptotic cell death, whereas GPX4 overexpression can protect against ferroptosis³⁷.

TRIM25 (tripartite motif-containing 25) is an E3 ubiquitin ligase involved in diverse cellular functions including immune regulation, cell death, and metabolism through ubiquitination and protein-protein interactions. In recent years, TRIM25 has emerged as a modulator of ferroptosis. Inhibition of the TRIM25/Keap1 axis impairs SLC7A11 expression³⁸, and a small molecule compound known as N6F11 has been found to bind TRIM25 and promote GPX4 degradation³⁹. These findings suggest that TRIM25 plays an active role in the regulatory network of ferroptosis. In our study, we observed that in L-02 cells treated with IL-17, SLC7A11 levels were decreased, ACSL4 expression was elevated, GPX4 expression was reduced, and TRIM25 levels were increased, compared with the control group. The consistent and significant alterations in these key ferroptosis regulators further support the hypothesis that the Th17/IL-17 axis may induce ferroptosis via specific signaling pathways, thereby contributing to liver injury and acute-on-chronic liver failure (ACLF). To further elucidate the potential signaling mechanisms involved, and based on our bioinformatics analysis, we investigated the expression of p53 in hepatocytes following IL-17 intervention.

The TP53 gene, also known as the p53 gene, is one of the most crucial tumor suppressor genes in cell biology. It loses partial or complete function in more than 50% of human cancers⁴⁰. The p53 protein acts as a transcription factor and tumor suppressor that regulates cell cycle arrest, DNA repair, and apoptosis. It prevents the proliferation of cells with damaged or mutated DNA by initiating transcriptional programs that promote cell cycle arrest or trigger apoptosis, thereby maintaining genomic stability and preventing tumorigenesis⁴¹. Furthermore, studies have demonstrated that p53 can modulate oxidative stress responses, cell proliferation, and oncogenesis⁴². Ferroptosis, a regulated form of cell death

dependent on iron and characterized by lipid peroxidation, is closely linked to various metabolic pathways. Several p53 targets—such as SLC7A11, VKORC1L1, GLS2, and PLTP—are directly involved in regulating ferroptosis^{34,43–47}. Increasing evidence highlights p53 as a promising therapeutic target in cancer treatment due to its influence on the ferroptotic pathway. In fact, p53-mediated ferroptosis has been proposed as a powerful anti-tumor mechanism⁴⁸. Notably, some studies also report that elevated TP53 expression can promote lipid peroxidation and ferroptosis through the SLC7A11–ALOX12 axis, in a manner that is independent of GPX4 regulation⁴⁹.

Baicalin, a natural compound with antioxidant, antiviral, and anti-inflammatory properties, has been shown to significantly alleviate hepatocyte oxidative stress (OS) damage and DNA damage induced by aflatoxin B1 (AFB1), a highly hepatotoxic compound. This protective effect on hepatocytes is closely associated with the TP53-mediated ferroptosis pathway. Baicalin physically interacts with p53, resulting in the inhibition of GPX4 and SLC7A11 expression, thereby suppressing ferroptosis and exerting a protective effect on hepatocytes⁵⁰. These findings collectively suggest that TP53 may promote the progression of liver failure by inducing ferroptosis. In a study of doxorubicin-induced chronic cardiomyopathy (DICC), deficiency of interleukin-12p40 was found to prevent the condition by inhibiting Th17 differentiation and IL-17 production. IL-17A plays a critical role in cardiomyocyte ferroptosis in DICC by activating the TNF receptor-associated factor 6 (TRAF6)/mitogen-activated protein kinase 1 (MAPK)/p53 signaling pathway (TRAF6/MAPK/p53)⁵¹. Moreover, particulate matter 2.5 (PM2.5) can induce ferroptosis in CD4⁺ T cells in experimental autoimmune uveitis (EAU) mice, potentially promoting IL-17 production and increasing Th17 cell frequency, thereby exacerbating EAU severity. Inhibition of ferroptosis not only alleviates disease severity in PM2.5-exposed mice but also reduces the frequency of Th17 cells⁵². Consistent with these findings, our study demonstrated elevated p53 expression following IL-17 intervention, confirming the link between Th17/IL-17 and p53 signaling pathways. To further elucidate the mechanisms by which Th17/IL-17 induces ferroptosis, we examined Nrf2 expression and found a significant reduction. Nuclear factor erythroid 2-related factor 2 (Nrf2) is the principal adaptive regulator involved in oxidative stress responses⁵³. Extensive research has established that Nrf2 plays a pivotal role in the regulation of ferroptosis^{54–57}. In a study involving patients with triple-negative breast cancer (TNBC), early-stage mutations in the TP53 tumor suppressor gene were identified. Mutant p53 conferred protection against ferroptosis by regulating Mgst3 and Prdx6 in an NRF2-dependent manner; these genes encode glutathione-dependent peroxidases that detoxify lipid peroxides. Thus, mutant p53 contributes to ferroptosis resistance in TNBC⁵⁸. Our study also implicates the TP53/Nrf2 signaling pathway as a potential mediator of ferroptosis.

In conclusion, transcriptome sequencing of peripheral blood from ACLF patients, combined with GO and KEGG pathway enrichment and PPI network analyses, revealed that the onset and progression of liver failure are closely linked to inflammatory immunity, particularly enriched in the Th17 signaling pathway, with TP53 identified as a core gene associated with liver failure. Further experiments using L-02 hepatocytes treated with IL-17 showed increased intracellular ROS and iron levels. Compared to controls, SLC7A11 expression decreased, ACSL4 expression increased, GPX4 expression decreased, and TRIM25

expression increased. To further investigate the signaling pathways by which Th17/IL-17 induces ferroptosis, we assessed the expression of p53 and Nrf2 after IL-17 intervention in hepatocytes, observing a significant increase in p53 and a significant decrease in Nrf2. These results suggest that Th17/IL-17 may promote ferroptosis through the TP53/Nrf2 signaling pathway, playing a crucial role in liver failure development.

However, our study did not include cell or animal models of liver failure. In future research, we plan to utilize an acute-on-chronic liver failure model to verify whether Th17/IL-17 influences the p53/Nrf2 signaling pathway and consequently ferroptosis in liver failure, to further elucidate the pathogenesis of ACLF.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethic committee of the First Affiliated Hospital of Kunming Medical University, with approval No. 2022-L-41. Written informed consents were obtained from the participants and next of kin for all vulnerable participants.

Conflict of Interest

There is no conflict of interest.

Funding

Declaration

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Author Contribution

Author Contributions Ye Liu conceived and designed the study, and drafted the original manuscript. Zihong Bao supervised the project and contributed to project administration. Ju Zhou and Chunxia Yang performed the experiments and collected the data. Chunmei Chao contributed to data validation and curation. Wu Li conducted data analysis and prepared the visualizations. Dingchun Li contributed to methodology development and critical revision of the manuscript. All authors discussed

the results, contributed to the interpretation, revised the manuscript, and approved the final version for submission.

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Figures

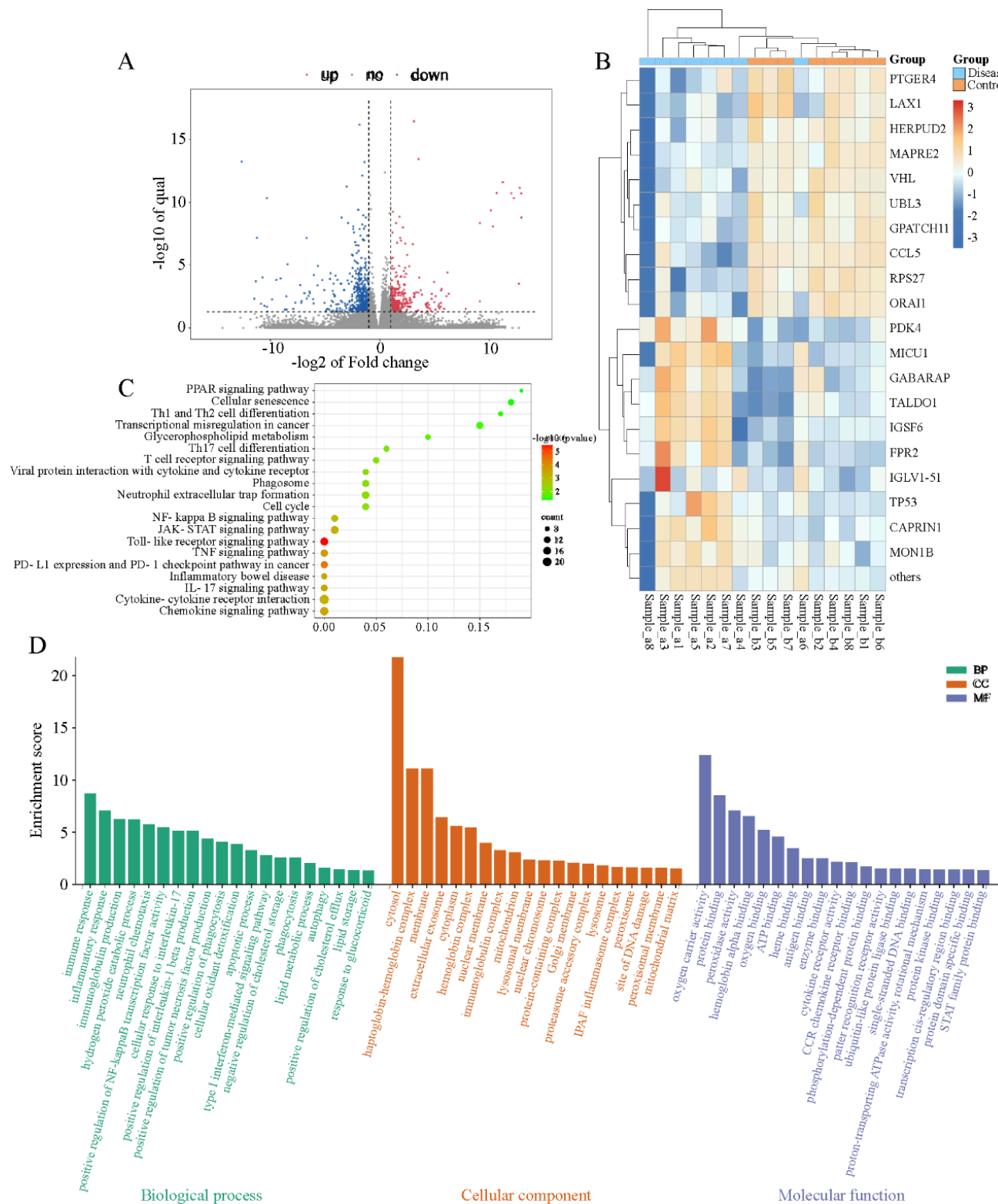


Figure 1

Transcriptome analysis of samples from acute-on-chronic liver failure and control groups. (A) Volcano plot of differentially expressed genes between the two groups; (B) Heat map of the 20 most significantly differentially expressed genes; (C–D) GO biological function enrichment analysis and KEGG signaling pathway enrichment analysis of differentially expressed genes.

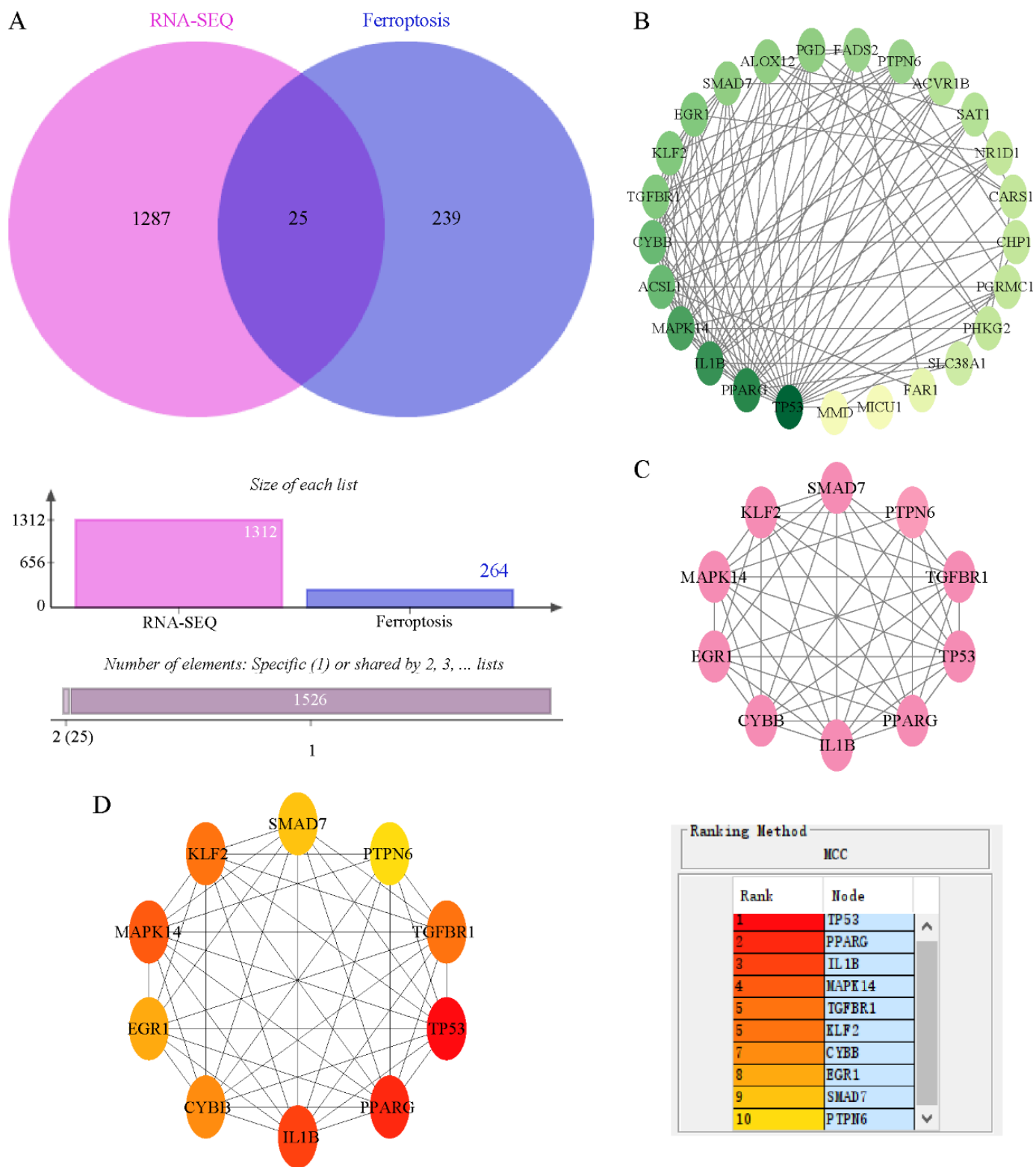


Figure 2

PPI network analysis. (A) Analysis between differentially expressed genes and ferroptosis dataset; (B–D) PPI network diagrams of ferroptosis-related differentially expressed genes.

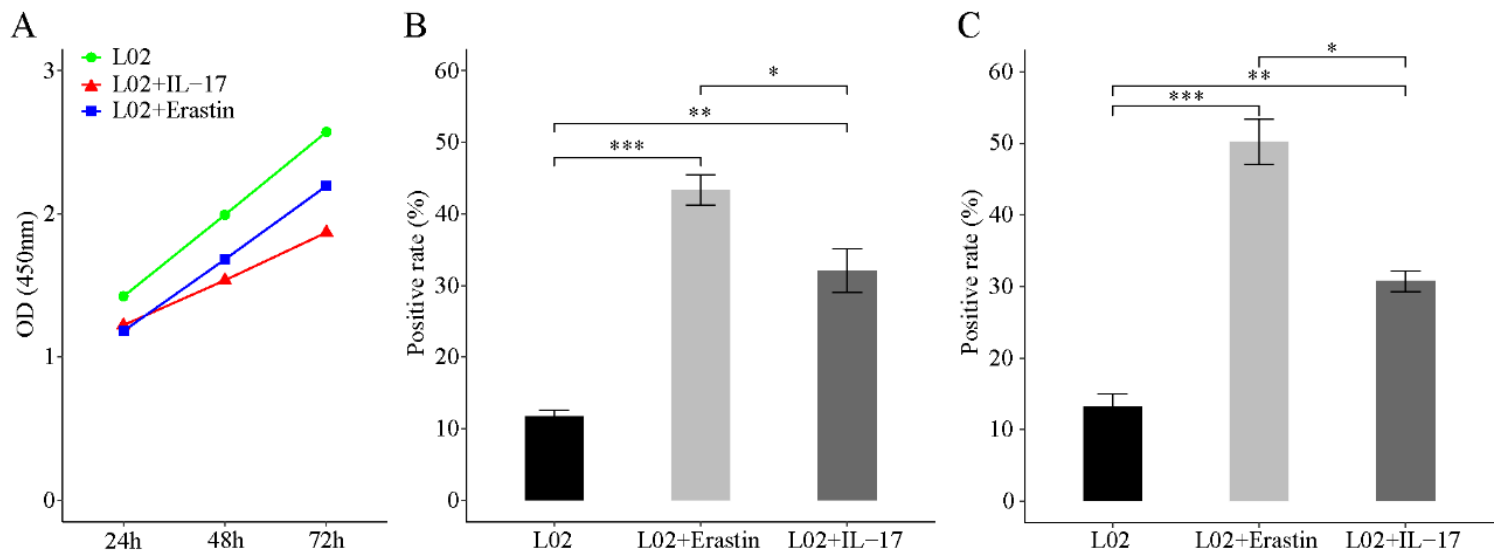


Figure 3

(A) Cell viability in each group; (B) Intracellular ROS levels in each group; (C) Intracellular iron levels in each group. * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.001 .

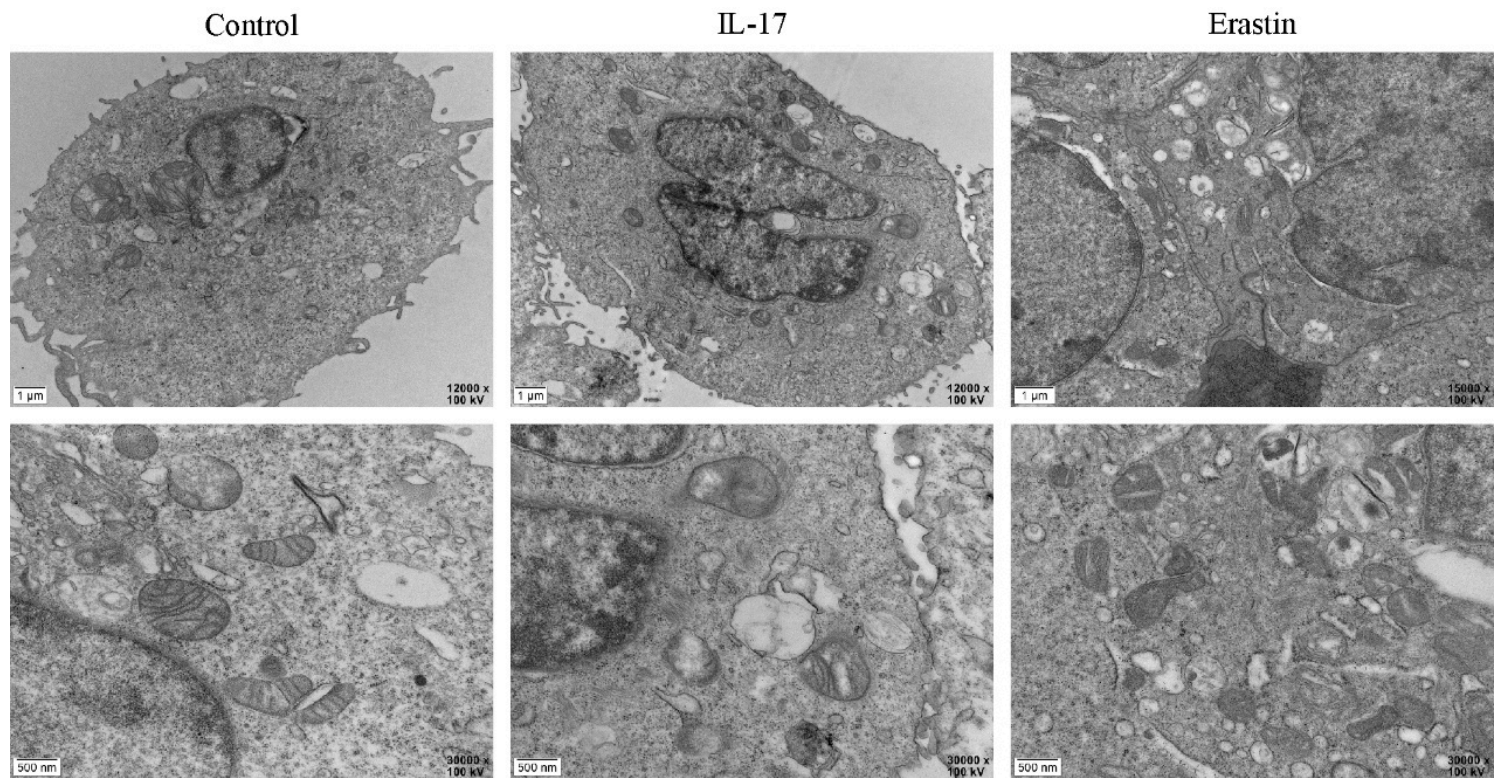


Figure 4

Mitochondrial structural changes in each group.

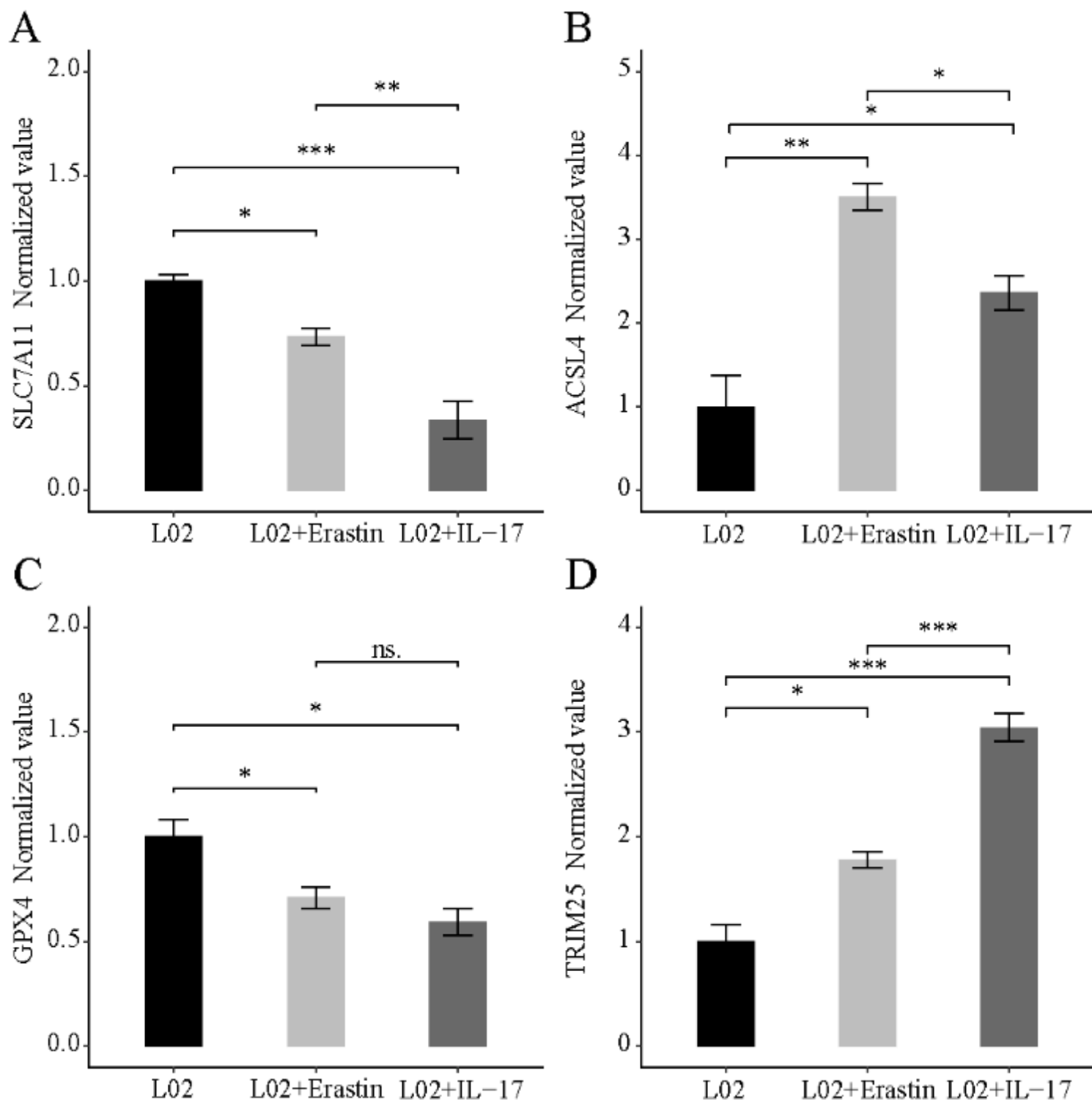


Figure 5

Expression levels of SLC7A11, GPX4, ACSL4, and TRIM25 in each group. * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.001 .

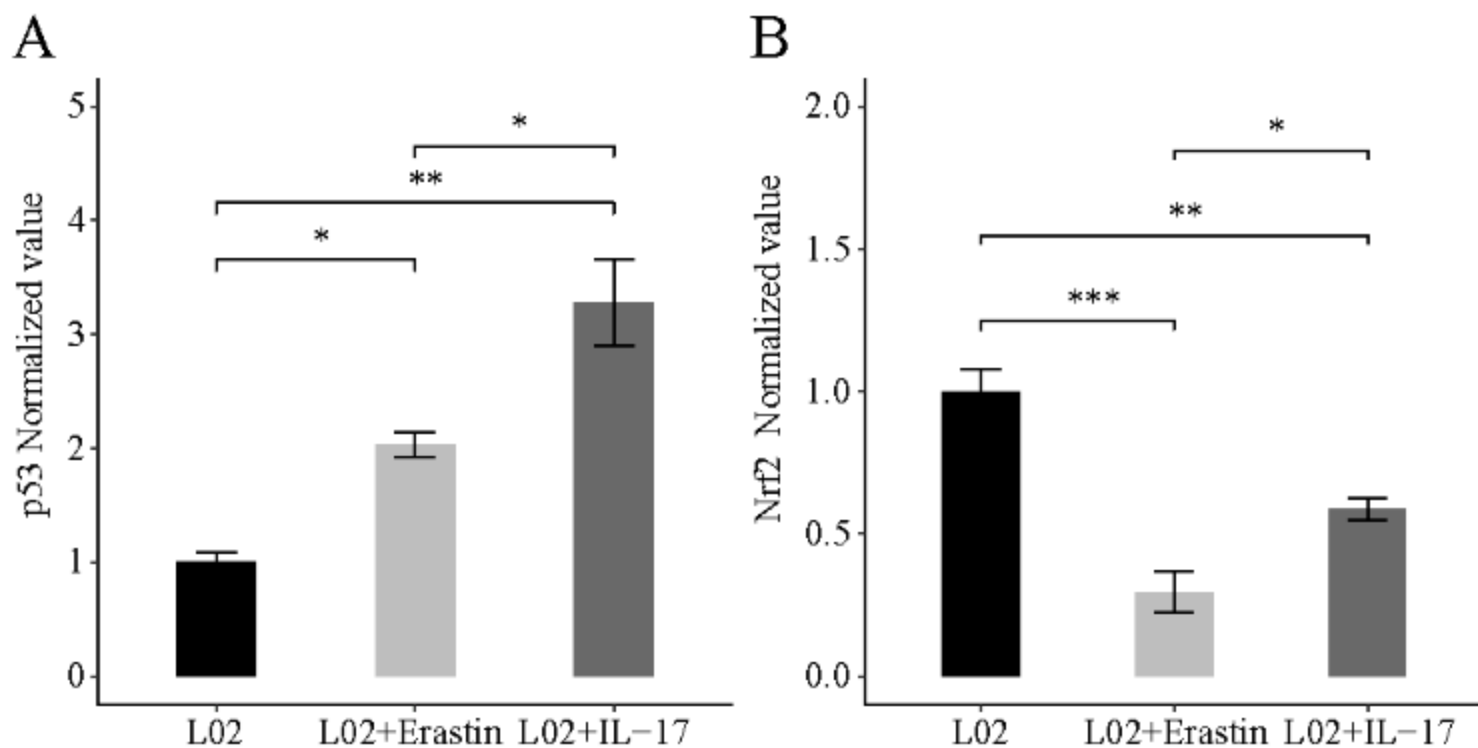


Figure 6

Expression levels of p53 and Nrf2 in each group. * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.001 .

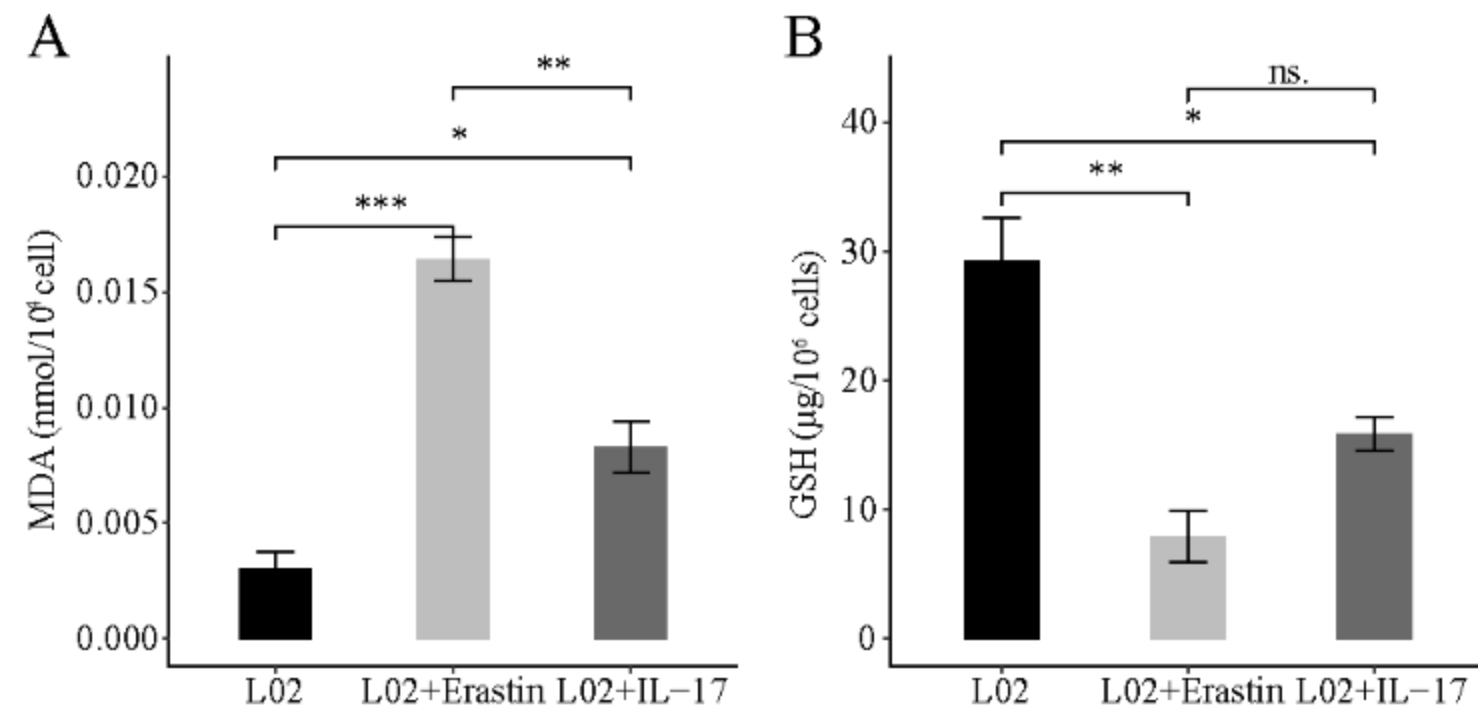


Figure 7

MDA and GSH level in each group. * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.001 .