Biologics: Targets and Therapy

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ORIGINAL RESEARCH

STAT5B Suppresses Ferroptosis by Promoting DCAF13 Transcription to Regulate p53/xCT Pathway to Promote Mantle Cell Lymphoma Progression

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Objective: The purpose of this study was to analyze the mechanism by which STAT5B inhibits ferroptosis in mantle cell lymphoma (MCL) by promoting DCAF13 transcriptional regulation of p53/xCT pathway.

Methods: The correlations between STAT5B, DCAF13 and ferroptosis in MCL were analyzed using Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/index.html). The expression levels and pairwise correlations of STAT5B, DCAF13, p53 and xCT in MCL patients were detected, respectively. STAT5B was silenced to confirm their criticality in MCL ferroptosis. the effects of blocking necrosis, apoptosis and ferroptosis on the anti-MCL effects of STAT5B were examined. Cells with STAT5B overexpression and/or DCAF13 silencing were constructed to confirm the involvement of DCAF13 in the STAT5B-regulated p53/xCT pathway. The regulation of p53 ubiquitination was confirmed by DCAF13 overexpression and MG132. The effects of silencing DCAF13 and MG132 on STAT5B overexpression on MCL was clarified by a tumor-bearing nude mouse model.

Results: DCAF13 was overexpressed in MCL and positively correlated with STAT5B, negatively correlated with p53, and positively correlated with xCT. Inhibition of ferroptosis alleviated the inhibitory effects of siSTAT5B on MCL, while inhibition of necrosis and apoptosis had few effects. Silencing of DCAF13 led to the blocking of STAT5B regulation of p53/xCT and ferroptosis. The changes in DCAF13 and the addition of MG132 did not have statistically significant effects on p53 mRNA. Elevation of DCAF13 resulted in downregulation of p53 protein levels, and this inhibition was reversed by MG132. In animal models, the promotion of MCL and the inhibition of ferroptosis by STAT5B. Silencing of DCAF13 blocked STAT5B inhibition of p53 and induction of xCT, GPX4, and GSH.

Conclusion: STAT5B suppresses ferroptosis by promoting DCAF13 transcription to regulate p53/xCT pathway to promote MCL progression.

Keywords: mantle cell lymphoma, STAT5B, DCAF13, ferroptosis

Introduction

Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma, which accounts for 6%~10% of non-Hodgkin lymphomas.[1] The diagnosis of MCL is based on chromosomal translocation t (11;14)(q13;q32) and cyclin D1.[2] The median age of onset of MCL is 60 years, and the overall survival is only 4 to 5 years.[3] The limited understanding of the mechanism behind the occurrence and progression of MCL hinders the development of more effective treatments.

STAT5 is composed of two highly homologous isoforms, STAT5A and STAT5B, but little is known about the role of STAT5B in tumors.[4] A recent study shows that both STAT5A and STAT5B play a crucial role in the survival and proliferation of hematopoietic cells, and their overexpression can cause changes in hematopoietic function and cancer, and in malignant hematopoiesis, the role of STAT5B is greater than that of STAT5A.[5] In prostate cancer, inhibition of

Received: 24 January 2024
Accepted: 21 June 2024
Published: 4 July 2024

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STAT5B increases the sensitivity of cells to enzalutamide, suggesting that STAT5B plays a role in promoting cancer in prostate cancer.[6,7] In addition, STAT5B also plays a role in promoting cancer in pancreatic cancer[8] and osteosarcoma.[9] However, the effects and regulatory mechanism of STAT5B on MCL are still unclear. One of our studies shows that compares with STAT5A, STAT5B has higher research value in MCL. The transcription level of STAT5B is upregulated in MCL, and overexpression of STAT5B promotes the growth of MCL cells and inhibits their apoptosis.[10] However, the regulatory mechanism of STAT5B on MCL is still unclear.

Ferroptosis is a recently discovered form of programmed cell death characterized by peroxidation of unsaturated fatty acids on the cell membrane catalyzed by abnormal ferrous iron, ultimately leading to cell death.[11] GPX4, a selenoprotein that efficiently reduces peroxidized phospholipids, plays a critical role in preventing ferroptosis induced by glutathione (GSH) depletion.[12] When ferroptosis is triggered, it also exerts antitumor effects by inhibiting tumor cell proliferation, reducing drug resistance, and preventing invasion.[13,14] xCT, which is involved in cystine metabolism for GSH synthesis, serves as a negative regulator of ferroptosis, and contributes to cancer development.[15] It is shown that xCT is involved in the regulation of proliferation and apoptosis of primary effusion lymphoma, but research on it in MCL is almost blank.[16] xCT can be negatively regulated by p53 in tumors,[17] which is one of the key suppressor genes of MCL.[18] Interestingly, p53 can be regulated by STAT5B, and it has been found that this regulation mechanism is related to leukemia drug resistance.[19] Curcumin induces p53 expression by regulating STAT5B to exert an anti-colorectal cancer effects.[20] DCAF13 is a functional protein involved in ribosomal RNA processing and is involved in the CUL4-DDB1-related ubiquitination modification process.[21] And DCAF13 is reported to be involved in the regulation of p53-related pathways and is associated with lung cancer development.[22] In breast cancer, DCAF13 is also associated with poor prognosis.[23] However, how STAT5B and DCAF13 regulate p53/xCT ferroptosis and the impact of this mechanism on MCL still needs to be explored.

This study mainly analyzes the mechanism of STAT5B regulating the p53/xCT pathway to inhibit ferroptosis and promote the progression of MCL, in order to provide novel insights into the treatment of MCL.

Materials and Methods

Bioinformatics Analysis

The Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/index.html) database was utilized, and the correlations of STAT5B in peripheral blood with ubiquitinases DCAF13, xCT and GPX4 were revealed by Pearson test.

MCL Sample Collection

From June 2020 to June 2022, four MCL patients from Chongqing University Cancer Hospital were clinically collected, and B cells were isolated from lymph node biopsies. The lymph node tissue form four healthy subjects from Chongqing University Cancer Hospital during the same period were selected as control. Inclusion criteria: (1) MCL diagnosed for the first time; (2) aged between 35 and 70 years old, (3) informed consent. Exclusion criteria: (1) received MCL-related treatment before enrollment; (2) co-infection, other types of hematological diseases, and other types of immune diseases. The content of this study was obtained with the consent of all patients. This study complied with the Declaration of Helsinki and was approved by the Ethics Committee of Chongqing University Cancer Hospital.

Cells Culture

Jeko-1 cells (CL-0128, Priscilla, China) which were validated to be a human MCL cell line and were maintained in DMEM medium (Gibco, USA), which were supplemented with 10% fetal bovine serum (FBS), 100 mg of streptomycin/mL and 100 units of penicillin/mL (Sigma–Aldrich, USA). The cells were cultured in a 5% CO_2 incubator at 37°C and 95% humidity.

To inhibit ferroptosis, Ferrostatin-1 (Fer-1, 1 μmol/L, HY-100579, MedChemExpress) and Liproxstatin-1 (Lip-1, 0.2 μmol/L, HY-132216) were added to pre-culture for 24 h, respectively. Necrostatin-1 (Nec-1, HY-15760) at a final

concentration of 10 μmol/L and Z-VAD-FMK (HY-16658B) at a final concentration of 10 μmol/L were added to the medium to pre-culture for 24 h to inhibit necroptosis and pan-caspase apoptosis, respectively.

In order to block the ubiquitination degradation process, MG132 (HY-13259) with a final concentration of 100 μg/kg was added and incubated for 24 h.

Cells Transfection

Jeko-1 cells were used transfection. STAT5B, siSTAT5B, STAT5B, siDCAF13, DCAF13 and negative control (NC) were from GenePharma Co., Ltd. (China). About 50 pmol (0.67 μg) plasmid was diluted in 25 μL serum-free DMEM as reagent A. 1 μL Entranster^{TM}-R4000 (Engreen) and 24 μL serum-free DMEM were mixed for 25 min as reagent B. 25 μL reagent A and 25 μL reagent B were mixed thoroughly (aspirate 10 times by the pipette), and after standing for 15 min, it was used as a transfection complex. Cells in 0.45 mL complete medium were transfected with 50 μL transfection complex, and the corresponding NC plasmids were used as control.

RT-qPCR

Cells or tissues were placed in 1.5 mL EP tubes, and 1 mL Trizol was added on ice. 0.2 mL of chloroform was added for every 1 mL of Trizol used, shaken vigorously for 15 s, placed at room temperature for 3 min, and centrifuged at 12,000 × *g* for 15 min at 4°C. The upper aqueous phase was transferred to a new tube, the RNA in the aqueous phase was precipitated with isopropanol, and centrifuged under the above conditions for 10 min. After the supernatant was discarded, the RNA pellet was washed with 75% ethanol. At least 1 mL of 75% ethanol was added for every 1 mL of Trizo1 used, centrifuged at 7500 × *g* for 5 min at 4°C. The pellet was left at room temperature to dry the RNA pellet for about 3~5 min. Twenty-five microliters of RNase-free water was added to dissolve the RNA. PrimeScript RT Kit (#DRR037A, Takara) was used for SDC1 mRNA to generate cDNA. SYBR Green reagent (#A2203XL, Yaji, China) was applied to qPCR amplification. STAT5B, DCAF13, p53 and xCT mRNAs were normalized to GAPDH using $2^{-\Delta\Delta Ct}$ method.

Western Blot

About 100 μL lysate + 1 μL protease inhibitor + 1 μL PMSF was used as the lysate to lyse the cells or tissues after removing the culture medium and washing. After centrifugation (12,000 × *g*, 4°C, 15 min), the supernatant was collected into another 1.5 mL tube. 2.5 μL sample and 22.5 μL triple distilled water was applied to measure protein concentration by BCA. 8%~12% SDS–PAGE was used for separation. The gel was cut according to the marker instructions and the position of the target band, the eluted gel was soaked in transfer buffer for 15 min. After washing, 5 mL of milk powder blocking solution was added overnight at 4°C with gentle shaking. Rabbit anti-primary antibody of STAT5B (ab240211, Abcam, USA), DCAF13 (ab240337), p53 (ab32389), xCT (ab175186) and GPX4 (ab125066) were added (4°C, overnight), respectively. Then, it was incubated with secondary antibody (1:2000, ab6721) for 2 h at 37°C. ECL kit (Amersham Biote) and IPP6.0 were used for imprint visualization, and the relative expression level was obtained by the internal reference GAPDH.

CCK-8 Assay

About 100 μL of cell suspension (5×10^4/mL) was added to the wells of 96-well plates. After 24 h, 48 h and 72 h, CCK-8 solution at a volume of 10 μL was added. The plates were gently mixed on an orbital shaker for 1 min at 37°C to ensure uniform mixing. The plates were then incubated for 2 h for the dehydrogenation reaction. Optical density (OD) values at 450 nm wavelength were detected by LEx808 microplate reader (25–315S, Lonza, USA).

EdU Assay

Cells were seeded in 96-well plates overnight at 5000 cells/well. 2 μL of EdU (10 mM) per 1 mL of complete cell culture medium was added. After the cell samples were incubated with EdU and then washed twice with PBS, 100 μL of 4% paraformaldehyde was added, and the samples were fixed at room temperature for 15 min. After washing, 100 μL of 0.3% TritonX-100 was added, and the samples were incubated for 15 min. After washing, 100 μL of reaction solution

was added to each well (protected from light for 30 min). After washing, the Hoechst 33,342 staining solution was diluted with PBS buffer at a ratio of 1:500, added to cover the cells, and incubated for 5 min. The samples were then observed and photographed under a fluorescence microscope.

Elisa

GSH kit (S0052, Beyotime, China) was used to detect GSH concentration. Well settings: blank well (A, blank control well, no sample and enzyme labeling reagent added, with all other steps remaining the same), standard well (B, 50 μL standard), sample well (C, 10 μL sample and 40 μL diluent). After thorough mixing, the enzyme labeling reagent (50 μL, 37°C, 30 min), chromogenic reagents A and B (50 μL each, 37°C, 15 min) were added successively in the dark. The OD was measured at a wavelength of 450 nm, and the concentration of GSH in well C was calculated with hole A as 0 and hole B as the standard.

Immunohistochemistry (IHC) Staining

The fixed tissue samples were treated with 0.01% TritonX-100 for 10 min and rinsed with PBS. The samples were immersed in 0.03% hydrogen peroxide for 10 min, rinsed with PBS for 2 × 3 min. Normal serum was added dropwise and incubated at 37°C for 15 min in a wet box to eliminate non-specific staining. The normal serum was discarded, and the antibodies against STAT5B (ab240211), DCAF13 (ab240337), p53 (ab32049), and xCT (ab307601) were added dropwise at a dilution of 1:100, incubated in a wet box at 37 °C for 1 h, and then overnight at 4°C. The secondary antibody was added, incubated at 37°C for 15 min in a wet box, and rinsed with PBS for 2 × 3 min. DAB color development: 1 drop each of reagents A, B, and C in the DAB color development kit was added to 1 mL of distilled water, mixed well, and added to the slices. Develop color at room temperature, and observed under a microscope to control the reaction time. Counterstain lightly with hematoxylin, immersed in hematoxylin staining solution for 2 min, washed in tap water, rapidly differentiate with hydrochloric acid alcohol solution, turn blue with ammonia solution, and washed in tap water. Stepwise dehydration, clearing and observation by microscopy (DMIRB, Leica).

Xenotransplantation Assay

Six-week-old female nude mice were used for modeling (n = 5). The transfected cells were trypsinized and 1×10^7 cells were taken and dissolved in 150 μL of PBS. Wipe the armpit with alcohol cotton ball, move the needle left and right before injection after the needle was inserted, if there was no resistance, it proved that it was under the skin. The bolus was slow, with a noticeable bulge visible. The syringe should be pulled out slowly, and the alcohol cotton ball should be pressed on the puncture site for about 10 s to prevent the cell suspension from flowing out. Euthanize the mice (cervical dislocation) as soon as the tumor volume reaches 1500 mm^3. All animal experiments were approved by Chongqing University Cancer Hospital.

Statistical Analysis

SAS 6.12 (SPSS Inc., USA) was used to visualize the results (m + SD). Student's *t*-test, one-way analysis of variance (ANOVA) or Tukey's test was performed to analyze the differences of gene level. The correlation was tested by Pearson test. A two-tailed $p < 0.05$ was considered significant.

Results

Expression Characteristics and Correlation of STAT5B, DCAF13, xCT and GPX4 in MCL

Firstly, bioinformatics analysis of MCL patients of GEPIA clarified that STAT5B was not only positively correlated with ubiquitinase DCAF13 (r = 0.53, P < 0.001, Figure 1A), but also positively correlated with ferroptosis mitigating agents xCT (r = 0.59, P < 0.001, Figure 1B) and GPX4 (r = 0.47, P < 0.001, Figure 1C). In addition, MCL lymph node tissues and healthy lymph node tissues were also collected, and compared with normal tissues, STAT5B, DCAF13 and xCT proteins were up-regulated in MCL, while p53 protein was down-regulated (Figure 2A). And STAT5B in MCL tissues

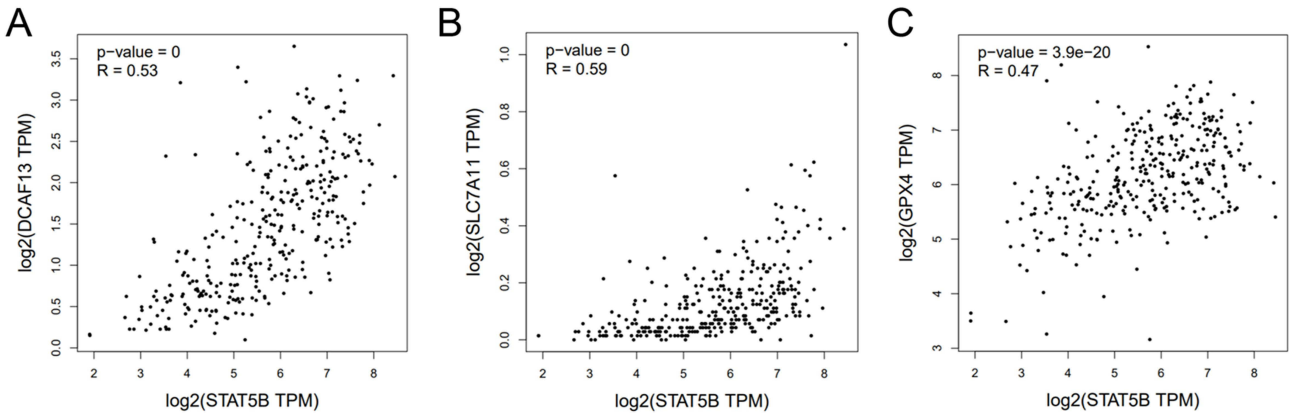

Figure 1 Correlation of STAT5B between DCAF13, xCT and GPX4 in MCL. (**A**) The correlation between STAT5B and DCAF13 in MCL was analyzed by Pearson test based on TCGA database. (**B**) The correlation between STAT5B and xCT (SLC7A11). (**C**) The correlation between STAT5B and GPX4.

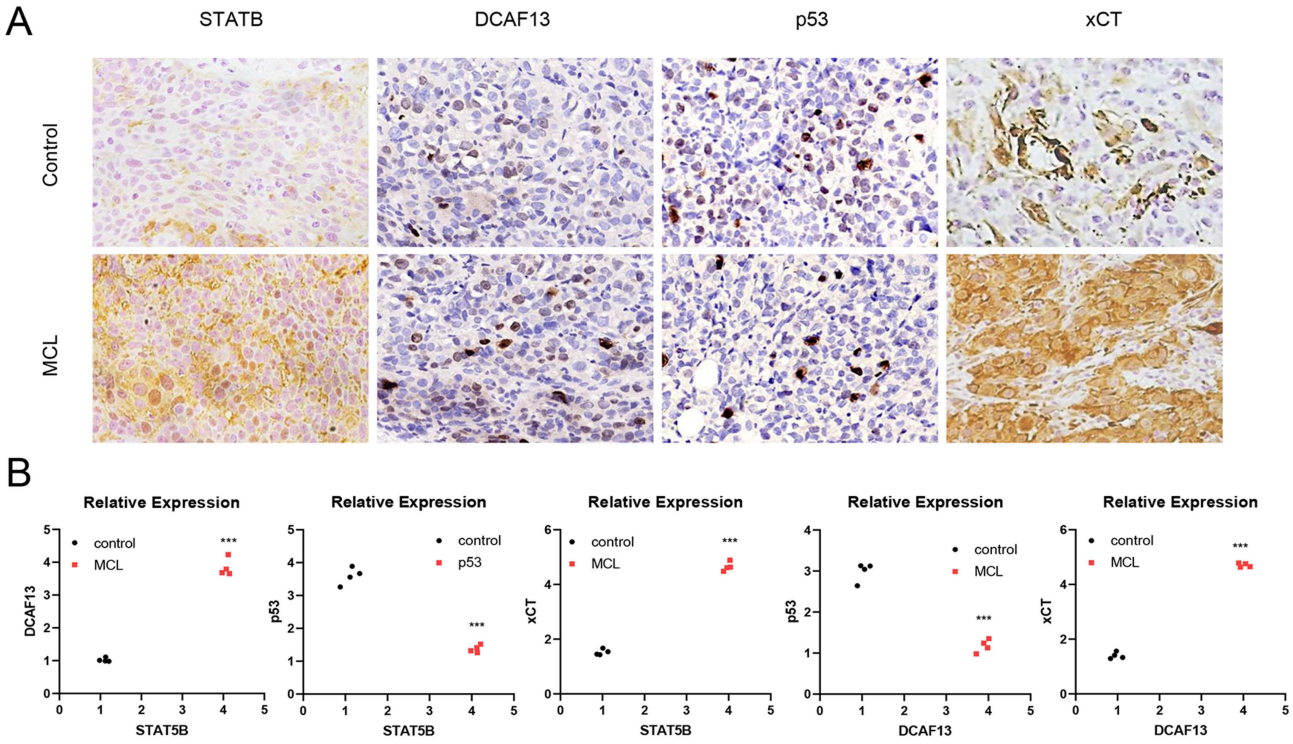

Figure 2 Expression characteristics and correlation of STAT5B, DCAF13, xCT and GPX4 in MCL. (**A**) Immunohistochemical staining to detect the expression of STAT5B, DCAF13, xCT and GPX4 in MCL lymph node tissues and healthy lymph node tissues. (**B**) Pearson was applied to test the association of STAT5B with DCAF13, xCT and GPX4, and the association of DCAF13 with xCT and GPX4. The relative expression level with the control group as 1. ***P < 0.001 vs control.

was positively correlated with DCAF13 and xCT (Figure 2B), respectively, and negatively correlated with p53 (Figure 2B). Similarly, DCAF13 was negatively correlated with p53 and positively correlated with xCT (Figure 2B). This preliminarily suggested that STAT5B might be involved in the regulation of ubiquitinase DCAF13, p53/xCT pathway and ferroptosis in MCL.

STAT5B Regulates p53/xCT Pathway and Promotes Ferroptosis

To preliminarily clarify the effects of STAT5B on MCL ferroptosis, STAT5B was silenced (Figure 3A and B). When STAT5B was inhibited, p53 protein was up-regulated and xCT protein was down-regulated (Figure 3C). In addition, the

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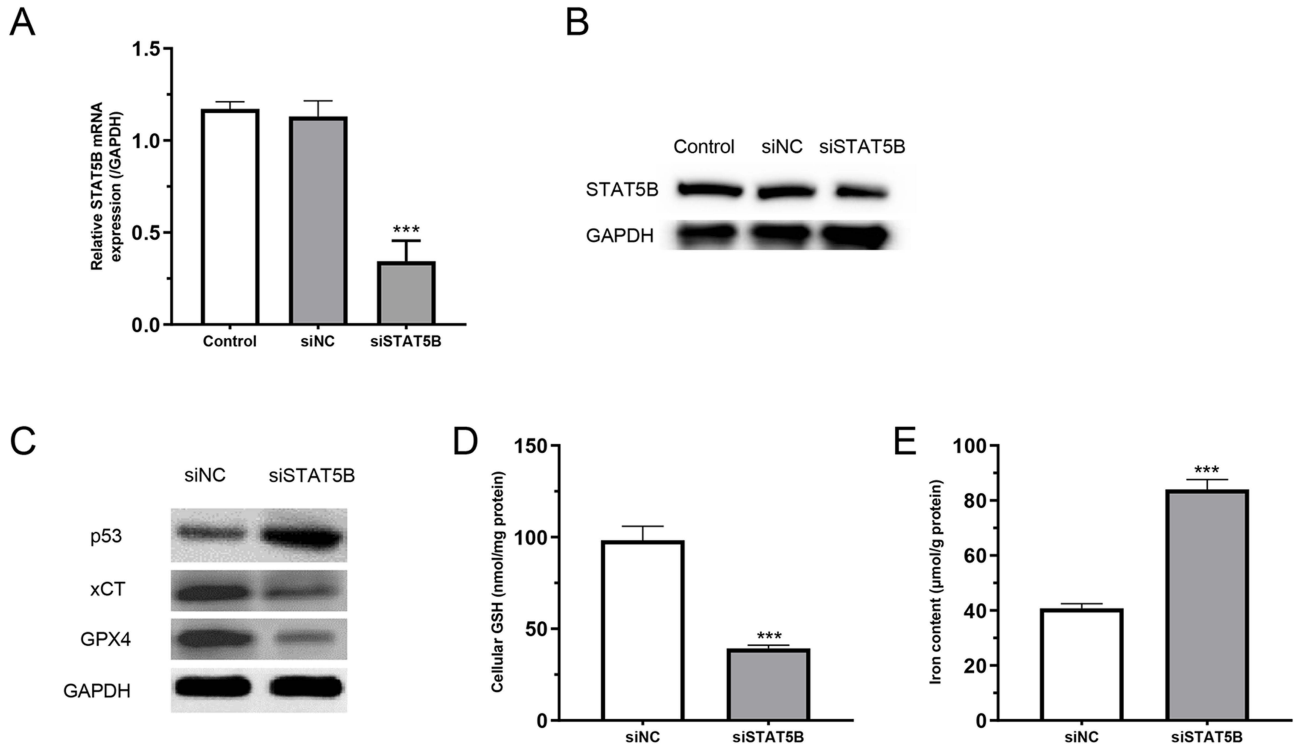

Figure 3 STAT5B regulates p53/xCT pathway and promotes ferroptosis in Jeko-1 cells. (**A** and **B**) Transfection results of Jeko-1 cells silencing STAT5B. (**C**) Effect of silencing STAT5B on p53, xCT and GPX4 protein expression. (**D**) Effect of silencing STAT5B on GSH levels in cells. (**E**) Effects of silencing STAT5B on iron ion levels in cells. ***P < 0.001 vs siNC.

anti-ferroptotic components GPX4 and GSH were also decreased with STAT5B silencing (Figure 3D), and Fe^{2+} levels were increased (Figure 3E). This confirmed the critical role of STAT5B in MCL resistance to ferroptosis.

STAT5B Promotes MCL by Inhibiting Ferroptosis

To confirm the central value of ferroptosis in STAT5B-promoted MCL, Fer-1 and Lip-1 were used to block the ferroptosis process in MCL. Meanwhile, as controls, Nec-1 and Z-VAD-FMK were used to block necrosis and apoptosis, respectively. Silencing of STAT5B resulted in decreased cell viability, which was partially reversed by administration of Fer-1 and Lip-1, whereas blocking necrosis and apoptosis had limited protective effects on MCL (Figure 4A). Blocking ferroptosis also greatly attenuated the impairment of STAT5B silencing on MCL proliferation, whereas Nec-1 and Z-VAD-FMK protected proliferation only slightly (Figure 4B). Inhibition of ferroptosis alleviated the inhibitory effects of siSTAT5B on MCL, while inhibition of necrosis and apoptosis had few effects, indicating that the regulation of STAT5B on mantle cell lymphoma was inseparable from ferroptosis.

STAT5B Regulates p53/xCT Pathway Through DCAF13

The above experiments clarified the regulatory role of STAT5B on ferroptosis and its p53/xCT pathway, and it was necessary to further clarify the regulatory mechanism. DCAF13 mRNA and protein, which were positively correlated with STAT5B, were suppressed with STAT5B silencing (Figure 5A and B). To further analyze the effects of STAT5B and DCAF13 on p53/xCT and GPX4, Jeko-1 cells with STAT5B overexpression and DCAF13 silencing were constructed by transfection (Figure 5C–F). Therefore, MCL cells were divided into four groups: control, STAT5B, siDCAF13, STAT5B +siDCAF13. Changes in STAT5B and DCAF13 had little effect on p53 mRNA (Figure 5G), but p53 protein levels were suppressed with the increase of STAT5B, and this suppression was blocked by silencing of DCAF13 (Figure 5H). Similarly, promotion of xCT and GPX4 by STAT5B was also counteracted by silencing of DCAF13 (Figure 5H).

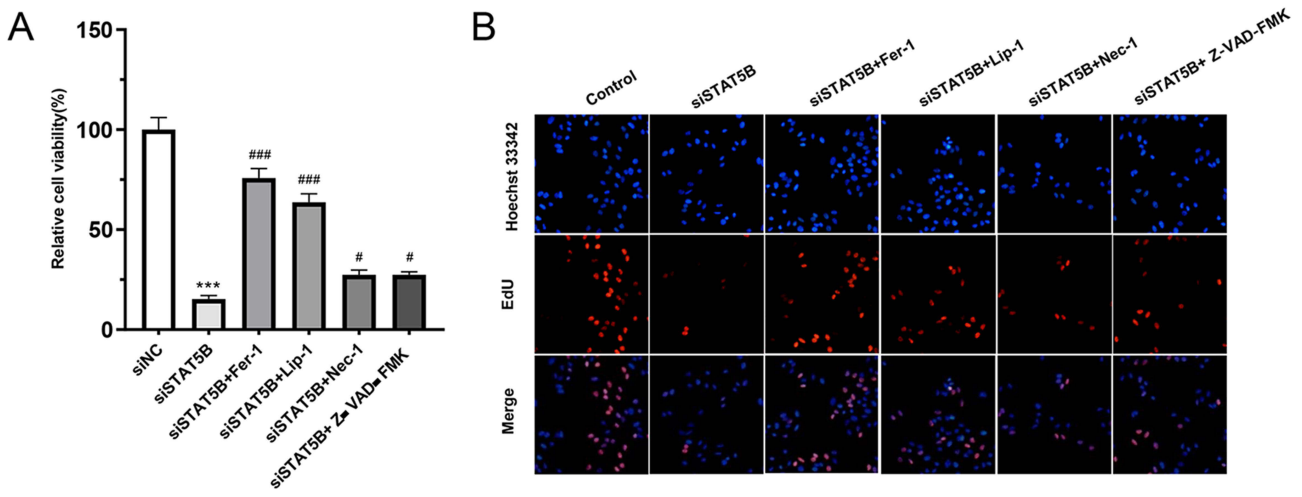

Figure 4 STAT5B promotes Jeko-1 cells proliferation by inhibiting ferroptosis. STAT5B was silenced by transfection, and Fer-1 and Lip-1 were used to block the ferroptosis process in MCL. Meanwhile, as controls, Nec-1 and Z-VAD-FMK were used to block necrosis and apoptosis, respectively. (**A**) Comparison of cell viability in each group. (**B**) Comparison of cell proliferation levels in each group. ***P < 0.001 vs siNC; #P < 0.05, ###P < 0.001 vs siSTAT5B.

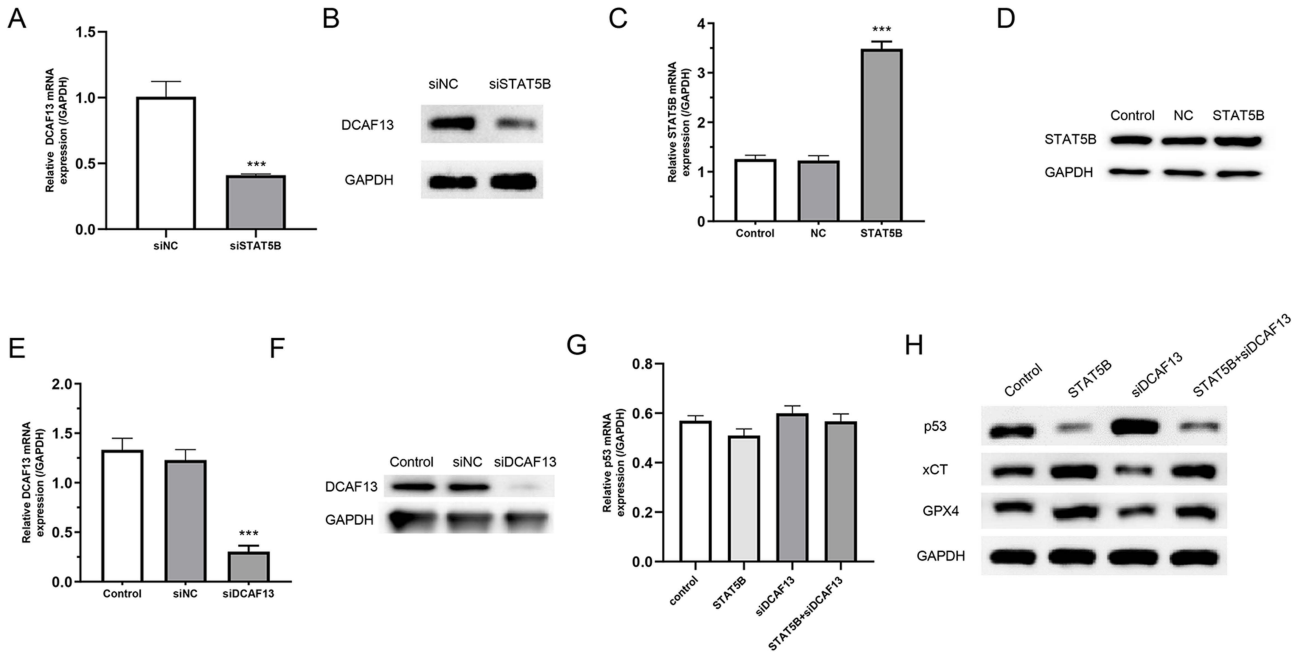

Figure 5 STAT5B regulates p53/xCT pathway through DCAF13 in Jeko-1 cells. (**A**–**B**) Effects of silencing STAT5B on expression of DCAF13 mRNA and protein levels in MCL cells. (**C** and **D**) Transfection results of Jeko-1 cells overexpressing STAT5B. (**E** and **F**) Transfection results of Jeko-1 cells silencing DCAF13. (**G**) Effects of overexpression of STAT5B and silencing of DCAF13 on p53 mRNA levels in MCL. (**H**) Effects of overexpression of STAT5B and silencing of DCAF13 on p53 protein levels in MCL. ***P < 0.001 vs siNC.

At the same time, the proliferation activity MCL cells promoted by STAT5B were also blocked by siDCAF13 (Figure 6A). Compared with the control group, GSH in the STAT5B group increased (Figure 6B), and Fe^{2+} was inhibited (Figure 6C). At the same time, GPX4 and GSH decreased and Fe^{2+} increased in siDCAF13 group (Figure 6B and C). GPX4 and GSH in STAT5B + siDCAF13 group were lower than those in STAT5B group, and p53 was higher than that in STAT5B group (Figure 6B and C). Silencing of DCAF13 led to the blocking of STAT5B regulation of p53/xCT and ferroptosis, confirming that the regulation of STAT5B on p53/xCT pathway and ferroptosis was related to its function of promoting DCAF13 transcription.

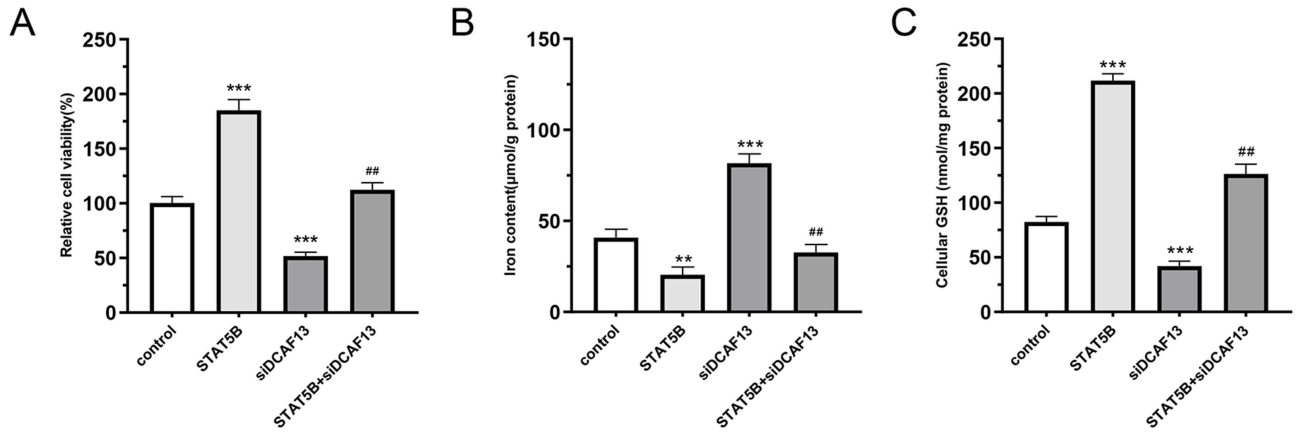

Figure 6 Effect of STAT5B on the level of ferroptosis in Jeko-1 cells through DCAF13. (**A**) Effects of overexpression of STAT5B and silencing of DCAF13 on MCL cell viability. (**B**) Effects of overexpression of STAT5B and silencing of DCAF13 on iron ion level in MCL cells. (**C**) Effects of overexpression of STAT5B and silencing of DCAF13 on GSH in MCL cells. **P < 0.01, ***P < 0.001 vs control; ##P < 0.01 vs STAT5B.

3.5 p53 is Regulated by DCAF13-Associated Ubiquitination

The above experimental results clarified that the transcription and expression of DCAF13 promoted by STAT5B was involved in the regulation of p53 protein, but their impact on p53 mRNA was extremely small. This suggested that DCAF13, regulated by STAT5B, was involved in the regulation of p53 ubiquitination. To clarify this, DCAF13 MCL overexpressing cells were constructed and MG132 was used to block the ubiquitination degradation process. For this purpose, MCL cells were divided into three groups: control, DCAF13, DCAF13 + MG132. It showed that changes in DCAF13 and the addition of MG132 had little effect on p53 mRNA (Figure 7A). Elevation of DCAF13 resulted in downregulation of p53 protein levels, and this inhibition was reversed by MG132 (Figure 7B). Moreover, ubiquitination Western blot further confirmed the regulation of p53 ubiquitination by DCAF13 (Figure 7B). This confirmed that p53 protein in MCL was regulated by a DCAF13-associated ubiquitination mechanism.

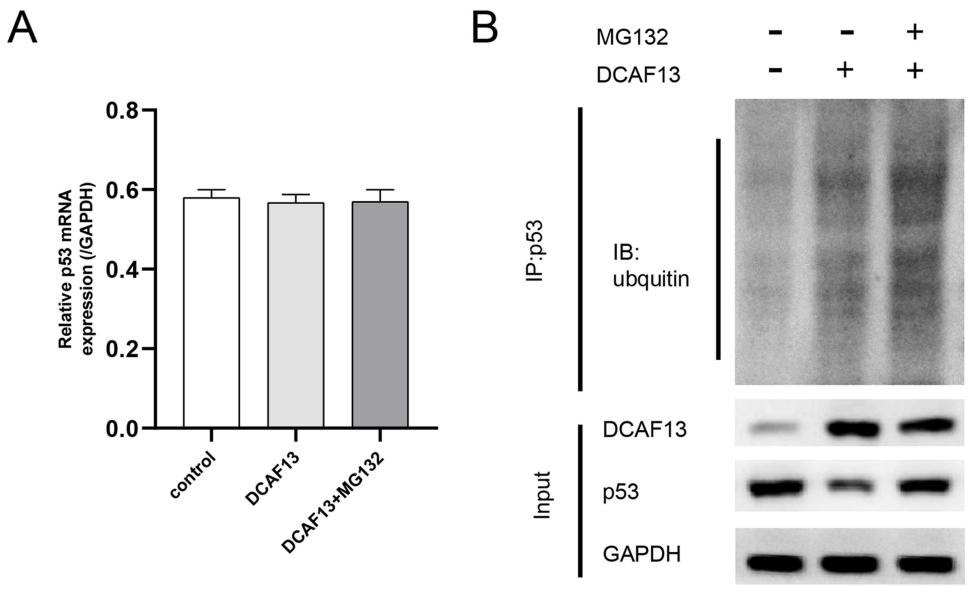

Figure 7 P53 is regulated by DCAF13-associated ubiquitination in Jeko-1 cells. DCAF13 MCL overexpressing cells were constructed and MG132 was used to block the ubiquitination degradation process. (**A**) Effects of overexpression of DCAF13 and MG132 on p53 mRNA. (**B**) IP experiments were used to confirm the modification effect of DCAF13 on the ubiquitination of p53 protein.

STAT5B Inhibits MCL Ferroptosis by Promoting DCAF13 to Regulate p53/xCT Pathway

To analyze the mechanism of STAT5B inhibiting MCL ferroptosis by promoting DCAF13 to regulate p53/xCT pathway in vivo, MCL with overexpression of STAT5B and silence of DCAF13 was used to construct tumor-bearing model, and injected with MG132 to block ubiquitination degradation. Accordingly, the nude mice were divided into four groups: control, STAT5B, STAT5B + siDCAF13, STAT5B + MG132. Elevation of STAT5B promoted tumor growth in vivo, whereas silencing of DCAF13 blocked the MCL-promoting effect of STAT5B (Figure 8A and B). And upregulation of STAT5B inhibited p53 protein and induced xCT expression in MCL tissues, and silencing DCAF13 and MG132 reversed the inhibition of STAT5B on p53 and the promotion of xCT (Figure 8C). More importantly, overexpression of STAT5B in vivo also promoted the expression of anti-ferroptosis factors GPX4 and GSH, and on this basis, silencing of DCAF13 and administration of MG132 reversed the inhibition of STAT5B on MCL ferroptosis (Figure 8C and D). This confirmed in vivo that the mechanism of STAT5B alleviating MCL ferroptosis and regulating p53/xCT pathway was inseparable from ubiquitinase DCAF13 and ubiquitination.

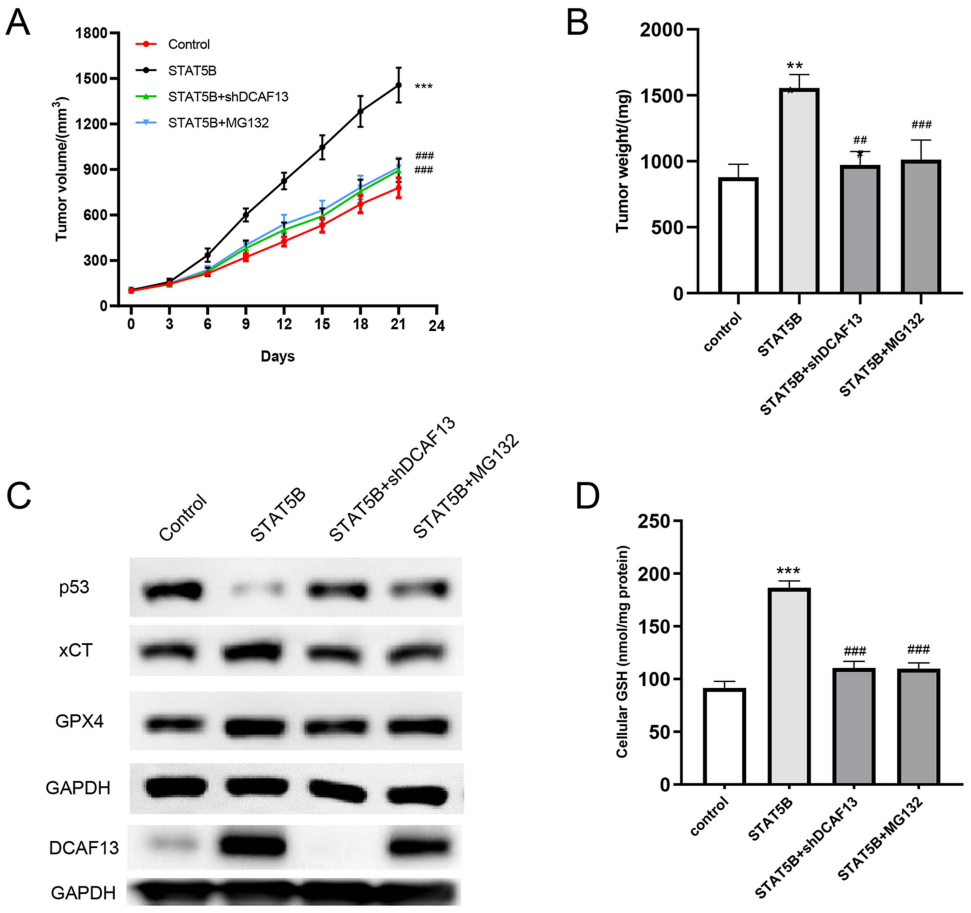

Figure 8 STAT5B inhibits MCL ferroptosis by promoting DCAF13 to regulate p53/xCT pathway in tumor-bearing nude mouse model constructed with Jeko-1 cells. MCL with overexpression of STAT5B and silence of DCAF13 was used to construct tumor-bearing model, and MG132 was applied to block ubiquitination degradation. (**A**) Effects of overexpression of STAT5B, silencing of DCAF13 and MG132 on tumor volume in tumor-bearing nude mice. (**B**) Effects of overexpression of STAT5B, silencing of DCAF13 and MG132 on tumor weight. (**C**) Effects of overexpression of STAT5B, silencing of DCAF13 and MG132 on p53, xCT, GPX4 and DCAF13 protein. (**D**) Effects of overexpression of STAT5B, silencing of DCAF13 and MG132 on GSH in tumor tissues. $^{**}P < 0.01$, $^{***}P < 0.001$ vs siNC; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs STAT5B.

Discussion

The pathogenic factors of MCL are complex, involving environment, heredity, etc., and their interactions.[24] In addition to chemotherapy, although new immunotherapies are constantly being used in clinical practice, the prognosis of MCL patients remains poor.[25,26] The highly anticipated bone marrow transplant remains unaffordable for most people.[27] It is of great significance to analyze the mechanism of MCL progression.

STAT5 has been widely confirmed in cancer, but the specific mechanism of one of its components, STAT5B, has not been fully revealed.[28] Activated JAKs phosphorylate STAT5A/B on key tyrosine residues, inducing a conformational change in the parallel STAT5A/B dimer that exposes the DNA-binding domain. After nuclear translocation, STAT5A/B normally initiates gene transcription at the gamma interferon-activating sequence (GAS) motif.[29,30] STAT5B is the basis of myelopoiesis and lymphoid development, and also plays an important role in maintaining the functions of macrophages, basophils, eosinophils and mast cells.[31,32] STAT5B is expressed at higher levels than STAT5A in all differentiated hematopoietic cell types.[33] STAT5B has a greater role than STAT5A in malignant hematopoiesis.[5] This study also shows the upregulation of STAT5B transcription and protein levels in peripheral blood B cells of MCL patients. However, the mechanism by which STAT5B is regulated in MCL remains unclear.

Compared to normal cells, tumor cells have a higher dependence on the trace element iron for their growth.[34] Excess iron intake has been reported to increase the risk of breast cancer.[35] However, some agents that reduce the levels of iron, such as iron chelators, sulfasalazine, statins, and artemisinin, which cause iron-mediated cytotoxicity, have been shown to have anti-tumor effects.[36,37] It has been confirmed that ferroptosis can have a dual effect on cancer, specifically, moderate levels of ferroptosis can promote the proliferation and metastasis of tumor cells, while excessive ferroptosis can limit or inhibit the abilities of tumor cells to proliferate and metastasize.[38–40] Therefore, ferroptosis is considered as a new direction for the treatment of tumors.

In this study, we found that STAT5B was positively correlated with anti-ferroptosis factors xCT and GPX4 through bioinformatics analysis, and this association was also revealed in MCL tissues. This provides preliminary evidence that STAT5B is related to MCL ferroptosis. To further confirm this link, STAT5B-silenced MCL cell model was constructed, and we found that p53 was elevated along with STAT5B silencing, and we found that, along with STAT5B silencing, p53 was elevated, while xCT and GPX4 were inhibited. More importantly, the use of drugs to inhibit ferroptosis alleviated the inhibitory effects of siSTAT5B on MCL proliferation, while blocking necrosis and apoptosis was difficult to alleviate the damage of STAT5B silencing on MCL. xCT can promote the transfer of cystine, the raw material of GSH, thereby alleviating ferroptosis damage.[41] The transcription and expression of xCT are regulated by p53.[42] Tanshinone IIA-induced reduction of xCT in gastric cancer cells is associated with increased p53 expression.[17] In osteosarcoma and prostate cancer, increasing p53 induces ferroptosis by inhibiting the expression of xCT.[43,44] P53 has been reported to be regulated by STAT5B, in breast cancer and glioblastoma, the inhibition of STAT5B leads to the upregulation of p53.[45,46] Combined with these reports, this study confirmed the role of STAT5B in promoting MCL by regulating p53/xCT pathway-related ferroptosis.

The above experiments clarified the regulatory role of STAT5B in ferroptosis and its p53/xCT pathway. Bioinformatics analysis found that STAT5B was also positively correlated with the ubiquitinase DCAF13. More interestingly, DCAF13 protein, which was up-regulated in MCL, was also negatively correlated with p53 protein and positively correlated with xCT in MCL tissues. Ubiquitination is a process that gradually attaches small molecules of ubiquitin to a specific protein in order to mark the protein, eventually causing it to be degraded or to alter its function and localization. As an E3 ubiquitin ligase, DCAF13 plays a promoting role in various cancers, such as breast,[47] lung[48] and liver cancer.[49] In STAT5B-silenced MCL, both DCAF13 transcription and protein levels were repressed. And silencing DCAF13 attenuated the regulation of p53/xCT pathway and the inhibition of ferroptosis by STAT5B in MCL, as well as the promotion of MCL proliferation. This confirms that the function of STAT5B regulating p53/xCT pathway to inhibit ferroptosis is inseparable from its mechanism of promoting DCAF13.

As an E3 ubiquitin ligase, DCAF13 plays a positive role in cancer by inducing the degradation of tumor suppressor proteins. For example, DCAF13 activates the PI3K pathway that is beneficial for MCL by promoting the ubiquitination and degradation of PTEN.[50,51] In this study, we found that STAT5B and DCAF13 had few effects on p53 mRNA, but

silencing DCAF13 blocked the inhibition of STAT5B on p53 protein. This preliminarily indicates that DCAF13, induced by STATB5, is involved in the ubiquitination regulation of p53 protein. More importantly, in MCL, an elevation of DCAF13 led to downregulation of p53 protein levels, and this inhibition was reversed by MG132. In addition, animal models also confirmed the promotion of MCL and the inhibition of ferroptosis by STAT5B. Silencing of DCAF13 blocked STAT5B inhibition of p53 and induction of xCT, GPX4, and GSH. Blockade of ubiquitination degradation by MG132 also reversed STAT5B's inhibition of p53 and promotion of ferroptosis. This confirmed in vivo that the regulation of p53/xCT by STAT5B in MCL is closely linked to DCAF13-associated ubiquitination".

This study also has some limitations. First, the clinical samples should be expanded to analyze the clinical significance and relevance of STAT5B and DCAF13 in MCL. The molecular mechanism of STAT5B regulating ferroptosis through DCAF13 still needs to be further improved.

Conclusions

In summary, STAT5B promotes the transcription and expression of DCAF13. As a protein related to E3 ubiquitin ligase, DCAF13 promotes the ubiquitination and degradation of p53 protein, which promotes the expression of xCT and GPX4, thereby alleviating ferroptosis in MCL and promoting the progression of MCL. The clinical significance of STAT5B and DCAF13 in MCL ferroptosis deserves further analysis. The mechanism by which STAT5B promotes MCL progression by regulating p53/xCT through DCAF13 remains to be further explored.

Data Sharing Statement

All data and materials are available from the corresponding author with justification.

Ethics Approval and Consent to Participate

All experiments were approved by the Ethics Committee of the Chongqing University Cancer Hospital. For human tissue, all were obtained with the consent of the patient.

Acknowledgments

Wenjun Zhang and Chongling Hu are joint first authors for this study.

Funding

Chongqing Natural Science Foundation. No. cstc2020jcyj-msxmX0535.

Disclosure

The authors report no conflicts of interest in this work.

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