

Original Article

Curcumin suppresses head and neck squamous cell carcinoma progression via NCOA4-FTH1-mediated ferroptosis

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Received 31 December 2025 Accepted 8 May 2026 Published 27 May 2026

Abstract

Head and neck squamous cell carcinoma (HNSCC) remains a prevalent malignancy with limited therapeutic options. Ferroptosis is an iron-mediated type of programmed cell death, and it has surfaced as a viable oncology treatment approach. Curcumin (Cur) is a natural polyphenol that exhibits potent anti-tumor properties; however, the underlying molecular mechanisms regarding cell death modalities in HNSCC remain underexplored. The results of this study show that Cur is an effective ferroptosis inducer in HNSCC based on transcriptomic sequencing analysis. Cur treatment triggers hallmark ferroptosis characteristics that include iron accumulation, lipid peroxidation, radical oxygen species generation, and mitochondrial damage. These effects are all reversed by the ferroptosis inhibitor ferrostatin-1. Furthermore, we discover that Cur promotes the lysosomal degradation of ferritin heavy chain 1 (FTH1). Specifically, Cur enhances the interaction between the cargo receptor, nuclear receptor coactivator 4 (NCOA4), and FTH1, facilitating ferritinophagy. NCOA4 knockdown blocks FTH1 degradation and rescues HNSCC cells from Cur-induced ferroptosis. These results demonstrate that Cur triggers ferroptosis in HNSCC through NCOA4-mediated ferritinophagy. The results of this study highlight the NCOA4-FTH1 axis as a promising therapeutic target and support Cur as a potential candidate for HNSCC treatment.

Key words ferroptosis, curcumin, FTH1, NCOA4, head and neck squamous cell carcinoma

Introduction

Head and neck squamous cell carcinoma (HNSCC) accounts for approximately 90% of all head and neck cancers. It is the most prevalent type of malignant tumor and primarily affects individuals with histories of tobacco and alcohol use, although its incidence is rapidly rising in young adults due to human papillomavirus infection [1]. The current HNSCC therapeutic strategies include surgical resection, radiotherapy, and concurrent chemotherapy [2] to improve locoregional control and organ preservation. However, the non-selective nature of radiotherapy and chemotherapy invariably damages surrounding healthy tissues. This can lead to severe dysphagia, mucositis, and xerostomia [3,4]. Additionally, HNSCC has a high propensity for recurrence and metastasis, with 5-year survival rates for advanced-stage disease remaining as low as 40%–60% [5]. Therefore, it is important to find novel therapeutic

agents for HNSCC therapy.

Curcumin (Cur; Figure 1A) is derived from the turmeric plant *Curcuma longa* [6]. It has been widely studied for its broad-spectrum anti-tumor effects in many types of cancers, as it possesses a favorable safety profile [7–10]. These anti-cancer effects are achieved through multiple mechanisms, such as inhibiting nuclear factor kappa B (NF-κB) signaling and its regulated genes that induce cell cycle arrest and suppress proliferative signals such as the PI3K/Akt pathway [11]. Additionally, Cur was found to inhibit angiogenesis by decreasing vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) [12,13], as well as inhibiting metastasis. Notably, Cur can also cross the blood-brain barrier and has exhibited therapeutic potential against neurological disorders [14,15]. However, the specific anti-tumor effects and underlying mechanisms of Cur against HNSCC have not

yet been revealed.

Ferroptosis is a distinct form of regulated cell death characterized by lipid peroxidation, and it differs mechanistically and morphologically from apoptosis, autophagy, and necrosis [16,17]. Its occurrence relies on intracellular iron metabolism and redox homeostasis. Additionally, it is marked by specific mitochondrial changes that include shrinkage and reduced or absent cristae [18]. An accumulating amount of evidence has implicated dysregulated ferroptosis in the pathogenesis of multiple human cancers, where it plays a context-dependent role in either inducing or suppressing tumorigenesis [19,20]. Given its potential as a tumor-suppressive mechanism, ferroptosis induction has emerged as a promising therapeutic strategy, particularly for malignancies resistant to conventional treatments [21]. Ferritin heavy chain 1 (FTH1) is a key molecular iron metabolism regulator, a core component of iron storage that maintains cellular iron homeostasis [22]. Crucially, FTH1 stability is tightly regulated by nuclear receptor coactivator 4 (NCOA4). NCOA4 functions as a selective autophagy cargo receptor that targets ferritin for lysosomal degradation, a process termed “ferritinophagy” [23]. NCOA4 promotes the autophagic turnover of ferritin and facilitates the release of intracellular labile iron, thereby sensitizing cancer cells to ferroptosis. However, the specific role of the NCOA4-FTH1 axis in ferroptosis regulation within HNSCC remains poorly understood, limiting the development of targeted therapies.

In this study, we demonstrate that Cur significantly suppresses HNSCC proliferation both *in vitro* and *in vivo*. Transcriptome sequencing analysis revealed that this anti-tumor effect is mediated by ferroptosis induction. Furthermore, we elucidate the underlying mechanism by which Cur promotes NCOA4-mediated ferritinophagy to trigger ferroptosis in HNSCC.

Materials and Methods

Cell culture and treatment

The HNSCC cell lines HSC-3 and SCC-7 were purchased from the Cell Bank of Type Culture Collection, Chinese Academy of Sciences (Shanghai, China). HSC-3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Waltham, USA). SCC-7 cells were cultured in RPMI-1640 (Gibco) supplemented with 10% fetal bovine serum (FBS; C04001; Viva Cell, Shanghai, China) at 37°C with 5% CO₂. Cur (458-37-7), ferrostatin-1 (Fer-1; 347174-05-4) and bafilomycin A1 (Baf-A1; 88899-55-2) were purchased from MedChemExpress (Monmouth Junction, USA).

Cell viability analysis

HSC-3 and SCC-7 cells were seeded in 96-well plates at a density of 5000 cells per well and treated with Cur (0, 5, 10, or 15 μM) or Fer-1 (10 μM) for 24 h to assess cell proliferation. Next, the cells were added to 10 μL of Cell Counting Kit-8 (CCK-8) reagent (B34304; Selleck Chemicals, Houston, USA) for 2 h and assessed by measuring the absorbance at 450 nm using a microplate reader (Molecular Devices, San Jose, USA).

Colony formation assay

Cells were seeded in 6-well plates at a density of 1×10^3 cells per well. The culture medium with or without drugs was replenished every 2–3 days. Colonies were fixed and stained with 0.1% crystal violet, and the number of colonies was counted in five random fields under a microscope (Olympus, Tokyo, Japan).

RNA sequencing (RNA-seq)

Total RNA was extracted from control and curcumin-treated HSC-3 cells ($n = 3$ per group) using TRIzol reagent (Invitrogen, Carlsbad, USA). RNA quality and concentration were assessed before library preparation. After quality inspection, cDNA synthesis and PCR amplification were performed, and subsequent sequencing was conducted by Novogene (Beijing, China). Gene expression was quantified by featureCounts and normalized as FPKM. Gene expression levels were calculated, and differentially expressed genes between control and curcumin-treated cells were identified for further analysis. Differentially expressed genes identified from RNA-seq analysis were subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using the “clusterProfiler” package in R software. The protein-protein interaction (PPI) network was analyzed using the STRING database (<https://string-db.org/>) and visualized with Cytoscape software (Cytoscape 3.9.1).

Viral infection

Lentiviruses encoding a negative control (Vector) or FTH1 (oe-FTH1) were used to infect HSC-3 and SCC-7 cells *in vitro*. All viruses were provided by Genechem Biosciences (Shanghai, China).

H&E staining

Tumor tissues were harvested, washed with PBS, and immediately fixed in 4% paraformaldehyde (PFA; abs9179; Absin, Shanghai, China) at room temperature for 24 h. The fixed tissues were dehydrated through a graded ethanol series and embedded in paraffin. The paraffin-embedded tumor blocks were sectioned into 5-μm-thick slices using a microtome (Leica, Wetzlar, Germany) and mounted on glass slides. The sections were deparaffinated, dehydrated, and subjected to antigen retrieval. The sections were then blocked and incubated with primary antibodies or a specific dye that included Ki67 (1:100; MA5-14520; Thermo Fisher Scientific, Waltham, USA), FTH1 (1:100; 11682-1-AP; Proteintech, Rosemont, USA), 4-HNE (1:100; bs-6313R; Bioss Antibodies, Beijing, China), NCOA4 (1:500; 10968-1-AP; Proteintech), SQSTM1 (1:200; 18420-1-AP; Proteintech), and LC3B (1:500; 81004-1-RR; Proteintech). Finally, these slices were stained with 3,3'-diaminobenzidine (DAB; P0202; Beyotime, Shanghai, China). Images were then generated using a microscope (Olympus) for analysis.

Co-immunoprecipitation (Co-IP)

Lysates of the cells or tissues generated by IP lysis buffer were treated with the addition of primary antibodies or IgG (as a negative control; 30000-0-AP; Proteintech). They were then reacted with protein A/G beads (PR40025; Proteintech) overnight at 4°C. The beads with protein were then processed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analyses.

Western blot analyses

Cells were lysed using radioimmunoprecipitation assay buffer (RIPA) containing a complete protease inhibitor cocktail (Roche, Basel, Switzerland). Protein concentrations were determined using a bicinchoninic acid assay (BCA) protein assay kit (23227; Thermo Fisher Scientific) and then electrophoresed by 12% SDS-PAGE and transferred to a polyvinylidene fluoride membrane (88520; Thermo Fisher Scientific). After incubation in 5% non-fat milk, the

membranes were incubated with the following primary antibodies: anti-FTH1 (1:1000; 3998S; Cell Signaling Technology, Danvers, USA), anti-SQSTM1 (1:1000; MA5-31499; Thermo Fisher Scientific), anti-NCOA4 (1:1000; ab86707; Abcam, Cambridge, UK), and anti-GAPDH (1:10,000; MA5-15738; Thermo Fisher Scientific). Subsequently, the membrane was incubated at room temperature with an HRP-labeled secondary antibody (1:5000; Proteintech) for 60 min. After washing with TBST for 3 times, the Clix imaging system (Clix, Shanghai, China) was used to detect the chemiluminescence signal.

Reactive oxygen species (ROS) and lipid ROS assays

The intracellular and lipid ROS levels were determined using dihydrofluorescein diacetate (DCFH-DA; S0033S; Beyotime) staining and Liperfluo (L248; Dojindo, Kumamoto, Japan), respectively. The cells were washed and incubated with DCFH-DA for 20 min at 37°C in the dark. The cells were then collected, washed, and resuspended in a phosphate buffer solution (PBS). A flow cytometer (BD Biosciences, Franklin Lakes, USA) was used to detect the ROS levels.

Iron assay

Intracellular iron (Fe^{2+}) was detected using FerroOrange (F374; Dojindo) according to the manufacturer's instructions. In brief, cells were seeded in 24-well plates and incubated overnight. The cells were then treated with the indicated agents and stained with a final concentration of 1 μM FerroOrange for 30 min at 37°C and 5% CO_2 . Images were acquired using a confocal microscope (Nikon A1R; Nikon, Tokyo, Japan).

Small interfering RNA (siRNA) transfection

For transfection, the riboFECT CP reagent (C10502-05; RiboBio, Guangzhou, China) was used following the manufacturer's protocol, with si-NC serving as a negative control. The siRNA sequences for silencing *NCOA4* were synthesized by RiboBio and shown as follows: *Homo-siNCOA4*: forward 5'-GAGAAGUGGUUAUAUCGAA TT-3', reverse 5'-UUCGAUAUAACCACUUCUCTT-3'; *Mus-siNCOA4*: forward 5'-GGUUAUCAAGCUCCUACATT-3', reverse 5'-UGUAAG GAGCUUGAUAACTT-3'; and siRNA-NC: forward 5'-UUCUCCGAA CGUGUCACGUTT-3', reverse 5'-ACGUGACACGUUCGAGAATT-3'.

Immunofluorescence staining

For the immunofluorescence analysis, cells were cultured on glass coverslips, washed with PBS, and fixed with 4% formaldehyde. After fixation, the coverslips were washed with PBS and permeabilized with 0.1% Triton X-100 in PBS for 10 min. After permeabilization, the cells were blocked with 5% bovine serum albumin (BSA) in PBS containing 0.1% Triton X-100 for 1 h. The cells were then incubated overnight at 4°C with anti-FTH1 (Cell Signaling Technology), anti-NCOA4 (Abcam). After washing three times with PBS, the cells were incubated with fluorescently labeled secondary antibodies (Beyotime) for 2 h at room temperature. Nuclear counterstaining was performed using 4',6-diamidino-2-phenylindole (DAPI; D9542-1MG; Thermo Fisher Scientific). Fluorescence imaging was performed using a confocal microscope (Nikon A1R).

Malondialdehyde (MDA) and glutathione (GSH) assays

MDA and reduced GSH are important markers of ferroptosis. MDA

(S0131S; Beyotime) and GSH (S0053; Beyotime) assay kits were used to detect the MDA and GSH levels, respectively, in accordance with the manufacturer's protocol.

LysoTracker staining

Cells were seeded onto sterile coverslips in 24-well plates at a density of 5×10^4 cells/well and cultured overnight. Following the indicated treatments, cells were stained with Lyso-Tracker Red (C1047S; Beyotime) at a final concentration of 50 nM according to the manufacturer's protocol. Subsequently, cells were washed, fixed, and counterstained with DAPI as described previously. Images were captured using a laser scanning confocal microscope (Nikon A1R).

JC-1 staining

Cells were seeded in 6-well plates at a density of 4×10^5 cells/well and cultured overnight. Following the indicated treatments, mitochondrial membrane potential was evaluated using a JC-1 assay kit (C2006; Beyotime) according to the manufacturer's protocol. After washing twice with the provided JC-1 buffer, fluorescence images were immediately captured using a fluorescence microscope (Olympus). Green fluorescence from JC-1 monomers and red fluorescence from JC-1 aggregates were detected.

Wound healing assay

Cells were seeded into 6-well plates and grown to 90%–100% confluence. A “#”-shaped scratch was created in the cell monolayer using a sterile 200- μL pipette tip. The detached cells were gently washed away with PBS, after which the remaining cells were treated with the indicated treatments. Images were captured immediately after scratching (0 h) and again at 24 h and 48 h using a microscope (Olympus) at the same fields. The wound area was quantified using ImageJ software (NIH, Bethesda, USA) to calculate the wound closure rate.

Transwell assay

A total of 5×10^4 cells suspended in 200 μL of DMEM containing 1% FBS and the indicated treatments were seeded into the upper chamber of a Transwell insert (8.0 μm pore size; PTEP24H48; Corning, New York, USA). The insert was then placed into a 24-well plate pre-filled with 600 μL of DMEM supplemented with 10% FBS as a chemoattractant. After 24 h of incubation, non-migrated cells on the upper surface of the membrane were removed. Migrated cells on the lower surface were fixed, stained with crystal violet (C0121; Beyotime), and photographed under a microscope (Olympus). The number of migrated cells was counted in three randomly selected fields per well using ImageJ software.

Transmission electron microscopy

Following the indicated treatments, cells were collected and gently washed with pre-cooled PBS. The samples were fixed with 2.5% glutaraldehyde at 4°C overnight, followed by post-fixation with 1% osmium tetroxide at room temperature for 1–2 h. After dehydration through a graded acetone series, the samples were embedded in Epon-812 epoxy resin (Shell Chemical, London, UK). The sections were prepared using an ultramicrotome and mounted on copper grids, followed by sequential staining with uranyl acetate for 10 min and lead citrate for 1 min at room temperature. Finally, images were

acquired using a JEM-1400FLASH transmission electron microscope (JEOL, Tokyo, Japan).

Subcutaneous tumor model

All male C57BL/6 mice (4 weeks old) were procured from GemPharmatech (Nanjing, China). The SCC-7 cell line was used to establish the subcutaneous tumor model. The SCC-7 cells were resuspended in serum-free medium at a density of 2×10^7 cells/mL and mixed with a matrix at a ratio of 1:1. Next, 100 μ L of the cell suspension was injected subcutaneously into the mice. When the tumors were macroscopical, the mice were divided into the following four groups: saline, Baf-A1 (1 mg/kg), Fer-1 (0.1 mg/kg), and Cur (50 mg/kg) groups. These four treatment types were administered once every 2 days for a total of six times. The tumor tissues were generated, weighed, and fixed, and proteins were extracted 22 days after injection. All of the mice used in this research were housed under specific pathogen-free (SPF) conditions in the animal facility at the SPF-level Laboratory Animal Room of the Chengdu Medical College in accordance with Animal Care and Ethical Regulations (No. 20230327128).

Statistical analysis

GraphPad Prism 9.0 was used for the statistical analysis. All values are shown as the mean $\pm$ SD. Student's *t* test was used to compare two groups, and multi-group comparisons were performed using one-way analysis of variance (ANOVA). *P* < 0.05 was considered significantly different.

Results

Cur inhibits HNSCC proliferation *in vitro* and *in vivo*

To identify whether Cur exhibits anti-tumor efficacy in HNSCC, we examined the cytotoxicity of Cur in HSC-3 (derived from humans) and SCC-7 (derived from mice) cells. Cur inhibited the viability of HSC-3 and SCC-7 cells under a concentration gradient treatment (Figure 1B). In addition, a colony formation assay showed a decreased proliferative ability of the HSC-3 and SCC-7 cells (Figure 1C). A cell viability/cytotoxicity assay (calcein/PI) also suggested that Cur caused cytotoxicity in a dose-dependent manner in both HSC-3 and SCC-7 cells (Figure 1D). Furthermore, Cur inhibited the migration ability of HSC-3 and SCC-7 cells according to wound healing (Supplementary Figure S1A) and transwell assay (Supplementary Figure S1B). To further verify the anti-tumor effects of Cur *in vivo*, we established subcutaneous HNSCC tumor models. Figure 1E shows that Cur treatment significantly reduced solid tumor tissues. The tumor volumes (Figure 1F) and weights (Figure 1G) were also inhibited after Cur treatment. The immunohistochemical staining results showed that Cur reduced Ki67 expression (a proliferation marker) (Figure 1H).

Taken together, these results suggest that Cur inhibits the proliferation and migration of HNSCC cells *in vitro* and *in vivo*.

Cur-induced cytotoxicity in HNSCC cells is dependent on ferroptosis

To identify how Cur induces cytotoxicity in HNSCC cells, we generated HSC-3 cells with Cur treatment or nothing to conduct RNA-seq. KEGG analysis of upregulated genes induced by Cur showed that ferroptosis was the most significant enriched pathway (Figure 2A). This result indicated that Cur may induce cytotoxicity in HNSCC cells due to ferroptosis. Next, to verify whether Cur-

induced cytotoxicity is dependent on ferroptosis, we treated HSC-3 and SCC-7 cells with Cur and Fer-1. The results showed that Fer-1 reversed Cur-induced cytotoxicity according to the CCK-8 assay (Figure 2B). Colony formation, wound healing, and Transwell assays also showed that the reduced proliferation and migration abilities caused by Cur were reversed by the addition of Fer-1 (Figure 2I and Supplementary Figure S2A,B). These results indicated that Cur-induced cytotoxicity is dependent on ferroptosis.

We next determined whether Cur induces ferroptosis and Fer-1 reverses it. We analyzed key ferroptosis process markers, including GSH depletion, ROS accumulation, lipid peroxidation, Fe²⁺ accumulation, and mitochondrial damage. The data showed that Cur treatment reduced the GSH/GSSG ratio, while Fer-1 restored the reduction in HSC-3 and SCC-7 cells (Figure 2C). Additionally, Cur treatment led to increased ROS levels (Figure 2D), increased lipid ROS levels (Figure 2E,F), Fe²⁺ accumulation (Figure 2G), and mitochondrial damage according to JC-1 staining (Figure 2H), which were restored by Fer-1.

The pathway enrichment analysis implicated the lysosome pathway (Figure 2A); hence, we hypothesized that Cur might regulate lysosomal function and autophagy. To test this hypothesis, we utilized LysoTracker staining to monitor the lysosomal status. The results revealed that Cur treatment effectively enhanced lysosome formation (Supplementary Figure S3A). Furthermore, western blot analysis corroborated these results, showing significant changes in the protein levels of the lysosomal LAMP2 marker and the key autophagy indicator SQSTM1 (P62) following Cur administration (Supplementary Figure S3B). These data collectively indicated that Cur activated the autophagy-lysosome pathway.

Taken together, these results indicate that Cur-induced cytotoxicity is dependent on ferroptosis and that Fer-1 reverses Cur-induced ferroptosis in HNSCC cells.

Cur causes ferroptosis in HNSCC cells through FTH1 downregulation

FTH1 is an important component of iron storage proteins, and its decreased expression leads to an accumulation of free iron within cells, thereby promoting lipid peroxidation and ultimately exacerbating ferroptosis [24]. Lysosomal activity is critical for ferritinophagy, a process in which ferritin is degraded to release free iron and promote ferroptosis; hence, we hypothesized that Cur might regulate FTH1 stability via the lysosomal pathway. To test this hypothesis, we performed immunofluorescence staining. The results revealed a distinct co-localization between FTH1 and the lysosomal marker (Figure 3A), suggesting the translocation of FTH1 to lysosomes. Furthermore, western blot analysis demonstrated a dose-dependent decrease in FTH1 protein levels following treatment with Cur (Supplementary Figure S4A). We then further verified that FTH1 overexpression (Supplementary Figure S4B,C) reversed the Cur-induced reduction in cytotoxicity (Figure 3B), increased GSH levels (Figure 3C), increased ROS levels (Figure 3D), increased lipid peroxidation, and increased lipid ROS levels (Figure 3E,F). In addition, Fe²⁺ FerroOrange and JC-1 (for detection of mitochondrial membrane potential) fluorescent analyses showed that Cur-induced mitochondrial damage and Fe²⁺ accumulation could be destroyed by FTH1 overexpression (Figure 3G,H). Calcein/PI staining also showed that FTH1 overexpression could alleviate damage caused by Cur in both HSC-3 and SCC-7 cells (Supplementary Figure S4D).

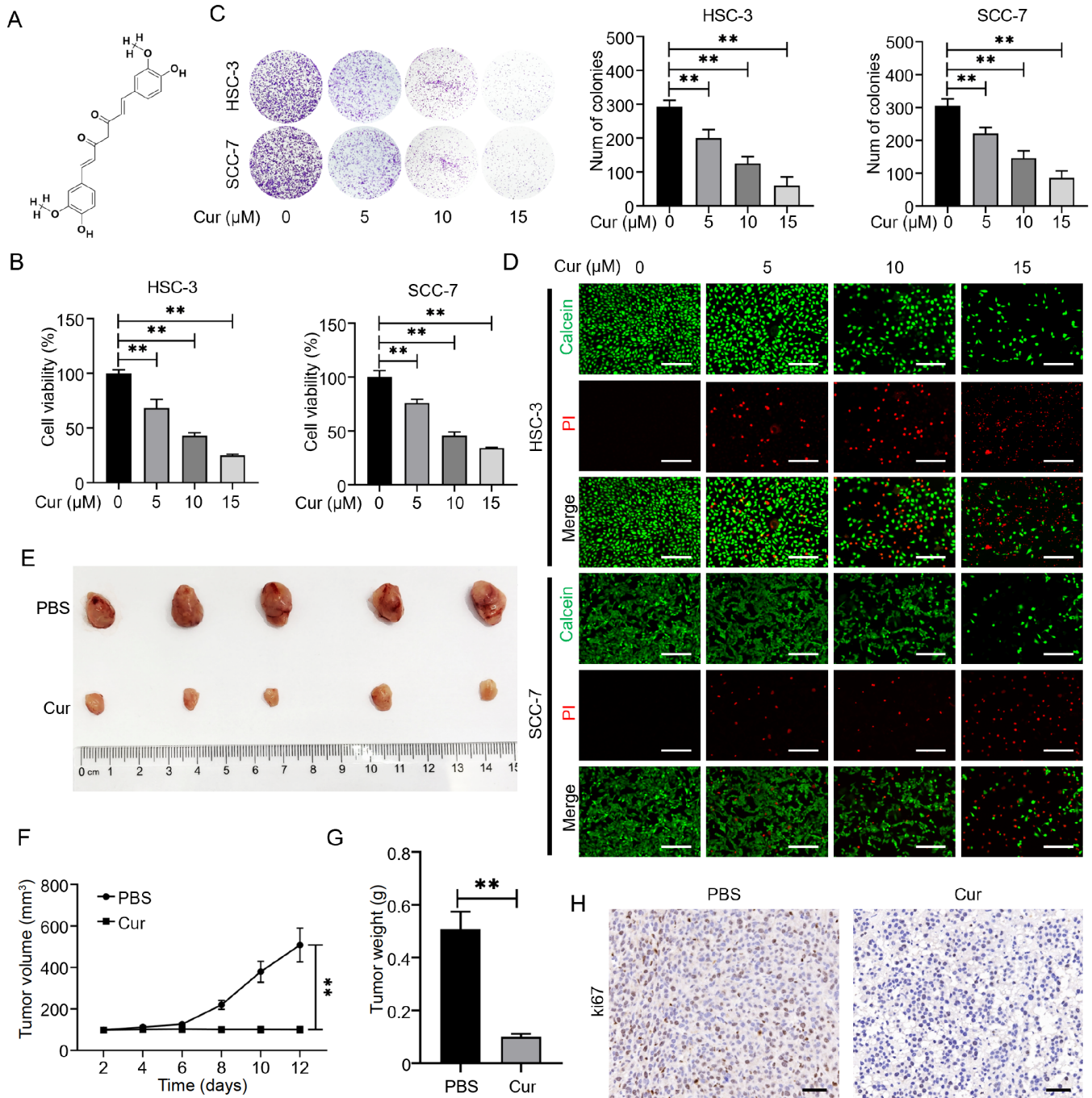


Figure 1. Cur suppresses proliferation of HNSCC cells *in vitro* and *in vivo* (A) The chemical structure diagram of curcumin. (B) Cell viability detection of HSC-3 and SCC-7 cells treated with 0, 5, 10, and 15 μM Cur. (C) Representative images of the colony formation and quantitative analysis of HSC-3 and SCC-7 cells treated with 0, 5, 10, and 15 μM Cur. (D) Representative images of HSC-3 and SCC-7 cells stained with calcein/PI and treated with 0, 5, 10, and 15 μM Cur (scale bar: 100 μm). (E) Representative images of HNSCC tumor tissues treated with Cur ($n = 5$). (F) Tumor volume. (G) Tumor weight. (H) IHC staining of Ki67 in tumor tissues (scale bar: 100 μm). Data are presented as the mean $\pm$ SD. ** $P < 0.01$.

Overall, these results suggest that Cur-induced ferroptosis is dependent on FTH1 downregulation in HNSCC cells.

Cur reduces FTH1-NCOA4 interactions in HNSCC cells via FTH1 autophagy-lysosome degradation

To investigate the mechanism by which Cur reduces FTH1 expression, we analyzed the protein-protein interaction network.

We found differentially expressed proteins that demonstrated interactions between FTH1 and NCOA4 (Figure 4A). Previous seminal studies have established NCOA4 as a selective cargo receptor that targets ferritin for lysosomal degradation, a process termed ferritinophagy [23]. We therefore performed a Co-IP assay to investigate whether Cur-induced FTH1 degradation is mediated by NCOA4. Cur treatment significantly upregulated NCOA4 expression

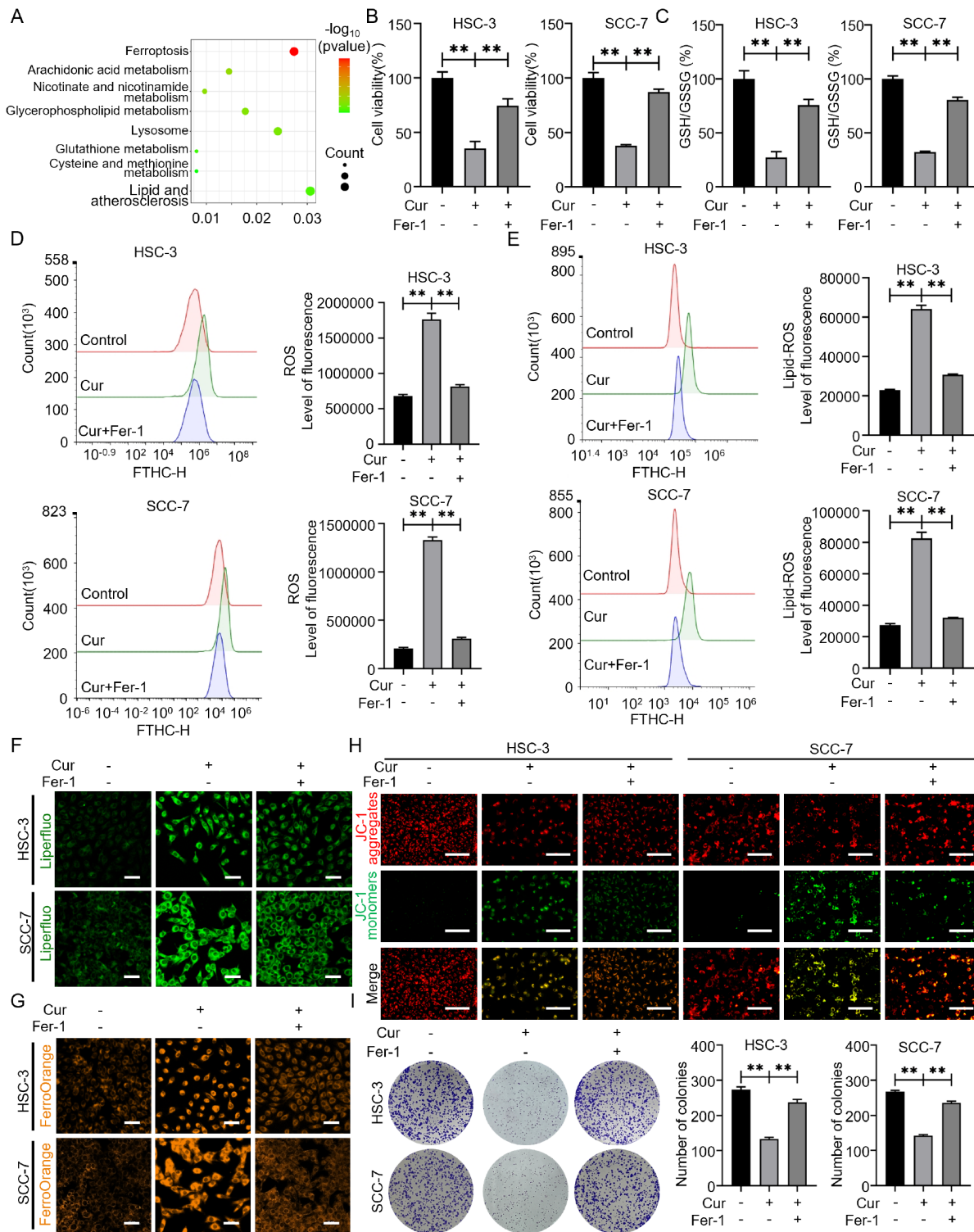


Figure 2. Cur-induced cytotoxicity in HNSCC cells is dependent on ferroptosis (A) Dot plot depicting KEGG results of upregulated genes in the Cur group compared with the control group. (B) HSC-3 and SCC-7 cells were treated with Cur (10 μM) alone or in combination with Fer-1 (10 μM), and their viability was evaluated using the CCK-8 assay. (C) Detection of the GSH/GSSG ratio of HSC-3 and SCC-7 cells treated with Cur (10 μM) alone or in combination with Fer-1 (10 μM). (D) Analysis of ROS levels in HSC-3 and SCC-7 cells treated with Cur (10 μM) alone or in combination with Fer-1 (10 μM). (E,F) Analysis of Liperfluor levels in HSC-3 and SCC-7 cells treated with Cur (10 μM) alone or in combination with Fer-1 (10 μM) (scale bar: 50 μm). (G) Detection of intracellular Fe^{2+} levels by FerroOrange staining in HSC-3 and SCC-7 cells treated with Cur (10 μM) alone or in combination with Fer-1 (10 μM) (scale bar: 50 μm). (H) Representative images of cellular JC-1 fluorescence in HSC-3 and SCC-7 cells treated with Cur (10 μM) alone or in combination with Fer-1 (10 μM) (scale bar: 100 μm). (I) Representative images of colony formation and quantitative analysis. ** $P < 0.01$.

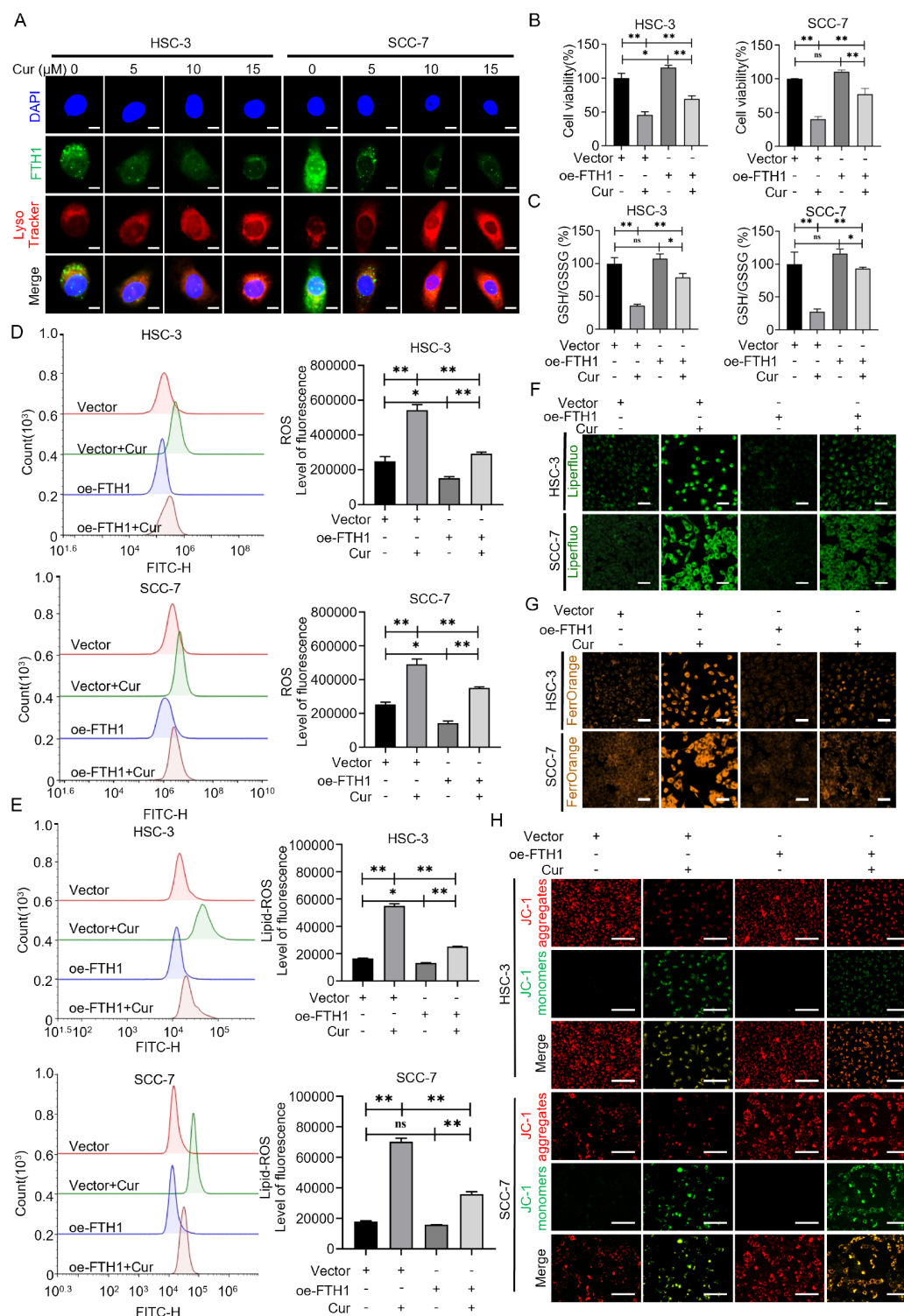


Figure 3. Cur causes ferroptosis in HNSCC cells by downregulating FTH1 (A) Co-localization of DAPI (blue), FTH1 (green) and LysoTracker (red) in HSC-3 and SCC-7 cells. The fluorescence-integrated density was measured by ImageJ software (scale bar: 10 μ m). (B) Detection of cell viability in HSC-3 and SCC-7 cells. (C) Analysis of the GSH/GSSG ratio in HSC-3 and SCC-7 cells transfected with empty vector or FTH1, with or without Cur (10 μ M) treatment. (D,E) Analysis of total ROS (D) and lipid ROS (E) levels in HSC-3 and SCC-7 cells transfected with empty vector or FTH1, with or without Cur (10 μ M) treatment. (F) Analysis of Liperfluo staining in HSC-3 and SCC-7 cells transfected with empty vector or FTH1, with or without Cur (10 μ M) treatment (scale bar: 50 μ m). (G) Detection of intracellular Fe^{2+} levels by FerroOrange staining in HSC-3 and SCC-7 cells transfected with empty vector or FTH1, with or without Cur (10 μ M) treatment (scale bar: 50 μ m). (H) Representative images of cellular JC-1 fluorescence in HSC-3 and SCC-7 cells transfected with empty vector or FTH1, with or without Cur (10 μ M) treatment (scale bar: 100 μ m). * $P < 0.05$, ** $P < 0.01$. ns, not significant.

in a dose-dependent manner. Notably, there was a marked enhanced physical interaction between NCOA4 and FTH1 following Cur treatment, suggesting that Cur promotes FTH1 by NCOA4 recruitment for subsequent autophagic degradation (Figure 4B).

To further verify that Cur-induced ferroptosis depends on autophagy-lysosome-mediated ferritin degradation, we applied the autophagy inhibitor Baf-A1 to identify whether it reverses Cur-induced ferroptosis. The results showed that Cur treatment significantly increased the co-localization of NCOA4 with FTH1 (Figure 4C) and FTH1 with lysosomes (Figure 4D). This result indicated the formation of the ferritinophagy complex. However, the blockage of autophagic flux by Baf-A1, as confirmed by an accumulation of autophagy markers in the western blot analysis (Figure 4E and Supplementary Figure S5), effectively reversed these effects. Critically, the functional assays demonstrated that Baf-A1 co-treatment significantly alleviated Cur-induced cytotoxicity. Compared to the Cur-only group, the Cur + Baf-A1 group exhibited restored cell viability (Figure 4F,G) and colony-forming ability (Figure 4H).

Overall, these results demonstrate that Cur triggers ferroptosis by promoting NCOA4-dependent ferritinophagy.

NCOA4 mediates Cur-induced ferroptosis by targeting FTH1

Based on the above results that demonstrated that Cur regulated the FTH1-NCOA4 interaction and autophagy-lysosome degradation of FTH1 (Figure 4), we next investigated whether NCOA4 is required during Cur-induced ferroptosis. First, we established NCOA4-knockdown cell lines using small interfering RNA (si-NCOA4). The knockdown efficiency was verified by western blot analysis (Figure 5A and Supplementary Figure S6). In the negative control (siNC) group, Cur treatment significantly downregulated FTH1 while activating autophagy (indicated by increased LAMP2 and decreased SQSTM1). However, in the si-NCOA4 group, NCOA4 deficiency markedly impaired the Cur-induced degradation of FTH1, despite the activation of upstream autophagy signals (Figure 5A and Supplementary Figure S6). This result indicates that NCOA4 acts as an essential bridge connecting autophagy to ferritin degradation in response to Cur treatment.

We then evaluated whether blocking the NCOA4-mediated ferritinophagy axis could rescue cells from Cur-induced ferroptosis. NCOA4 silencing significantly attenuated Cur cytotoxicity. Cells in the si-NCOA4 + Cur group exhibited restored cell viability (Figure 5B) and colony-forming capacity (Figure 5C) compared to those in the si-NC + Cur group. Furthermore, we analyzed a panel of ferroptosis-related markers (Figure 5D–H) that consistently showed that the hallmarks of ferroptosis, such as lipid peroxidation and iron accumulation, were effectively reversed by NCOA4 knockdown.

Collectively, these genetic loss-of-function assays provide compelling evidence that Cur triggers ferroptosis specifically through the NCOA4-FTH1 axis.

Cur inhibits HNSCC progression through ferroptosis induction

To verify the antitumor effects of Cur and the role of ferroptosis *in vivo*, subcutaneous tumor SCC-7 cell models were established. After 12 days, Cur was administered alone or with the addition of saline, Baf-A1, or Fer-1 once every 2 days until day 22 (Supplementary Figure S7A). In this tumor model, the tumor size (Figure

6A), volume (Figure 6B), and weight (Figure 6C) were suppressed by Cur treatment, while the addition of Fer-1 and Baf-A1 reversed these changes to some extent. There was no difference in the mouse body weights (Figure 6D) following Cur treatment. Furthermore, Cur treatment increased the Fe²⁺ level (Figure 6E), reduced the GSH levels (Figure 6F), increased MDA production (Figure 6G), and increased mitochondrial dysfunction (Figure 6H). All of these results indicated ferroptosis induction. However, the addition of Baf-A1 or Fer-1 reversed these key ferroptosis events (Figure 6E–H). Additionally, immunohistochemistry and western blot analyses of the tumor tissues showed lower expression levels of Ki67, FTH1, and SQSTM1 along with increased NCOA4, 4-HNE, and LAMP2 levels in the Cur-treated group. These effects were reversed by the addition of Baf-A1 or Fer-1 (Figure 6I,J and Supplementary Figure S7B). H&E staining was performed on the major organs, and histological examination revealed no significant toxicity or tissue damage attributable to Cur (Supplementary Figure S7C).

Collectively, these results indicate that Cur suppresses HNSCC progression *in vivo*, which is associated with ferroptosis induction mediated by the FTH1-NCOA4 interaction and autophagy-lysosome degradation of FTH1.

Discussion

HNSCC is a common oral malignant tumor worldwide, with an oral malignant tumor incidence rate of over 90% [1]. Clinically, a combination of various intervention measures is often adopted for treatment, including surgical resection as the main approach. This is typically supplemented by radiotherapy, chemotherapy, and biological therapy [4,25,26]. However, the 5-year survival rate for patients with HNSCC remains less than 50% [3,27]. In this study, we identified Cur as a potent HNSCC progression suppressor and elucidated a novel mechanism linking autophagy-dependent iron metabolism to ferroptosis. We demonstrated that Cur inhibited HNSCC growth *in vitro* and *in vivo* by triggering ferroptosis, driven by NCOA4-mediated ferritinophagy.

Initially, we confirmed the robust anti-tumor efficacy of Cur. Cur dose-dependently inhibited the proliferation and migration of HSC-3 and SCC-7 cells and suppressed tumor growth in xenograft models, and this result was consistent with the results of previous studies on natural compounds. To decode the underlying mechanism, RNA-seq was performed. This pinpointed ferroptosis as the most significantly enriched pathway. Ferroptosis is an iron-dependent form of regulated cell death that is characterized by an accumulation of lipid peroxides [28]. Our data validated that Cur treatment led to GSH depletion, excessive ROS production, lipid peroxidation, and mitochondrial membrane potential collapse. Importantly, the ferroptosis inhibitor Fer-1 effectively reversed this Cur-induced cytotoxicity and restored the cellular redox balance, solidifying the conclusion that Cur exerts its anti-tumor effects primarily through ferroptosis.

A critical prerequisite for ferroptosis is the accumulation of labile Fe²⁺. This fuels the Fenton reaction to generate ROS [16]. However, the source of this iron overload in Cur-treated cells was unclear. We focused on ferritinophagy, a selective form of autophagy in which the iron storage protein ferritin (FTH1) is degraded in lysosomes to release free iron [24,29,30]. Our results showed that Cur treatment promoted FTH1 translocation to lysosomes and its subsequent degradation. This process was dependent on the autophagy machinery, as the lysosomal inhibitor Baf-A1 prevented FTH1

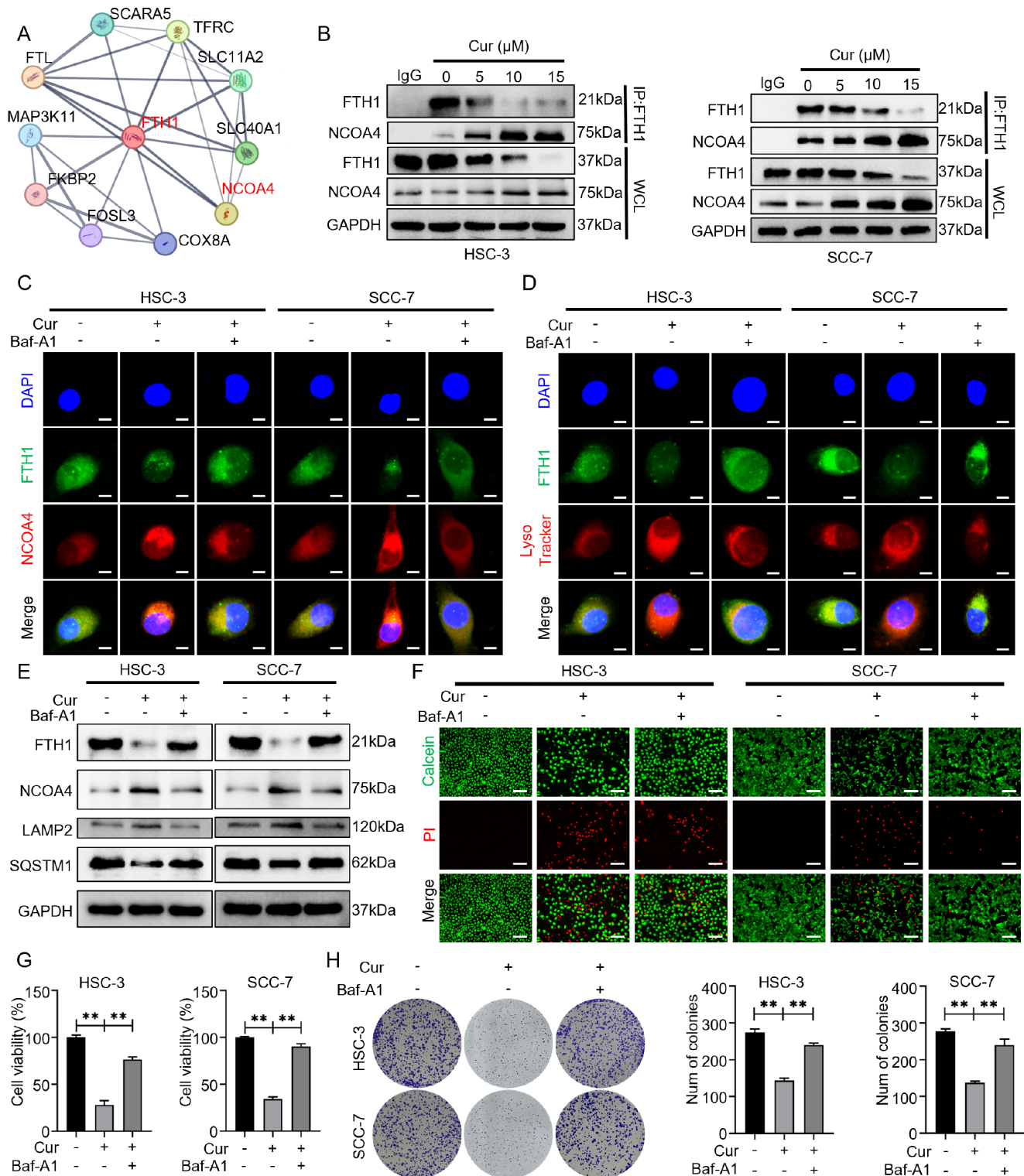


Figure 4. Cur facilitates the FTH1-SQSTM1 interaction and autophagy-lysosome degradation of FTH1 in HNSCC cells (A) The PPI network of FTH1 was constructed with the String database. (B) NCOA4 was immunoprecipitated with FTH1 antibody and examined by immunoblotting in HSC-3 and SCC-7 cells. (C) HSC-3 and SCC-7 cells were treated with Cur (10 μ M) alone or in combination with Baf-A1 (1 μ M), and representative images of the co-localization of FTH1 (green) with NCOA4 (red) are shown (scale bar: 10 μ m). (D) Representative image of the co-localization of FTH1 (green) with lysosomes (red) (scale bar: 10 μ m). (E) Western blot analysis of the expressions of FTH1, NCOA4, LAMP2, and SQSTM1 in HSC-3 and SCC-7 cells after treatment with Cur (10 μ M) alone or in combination with Baf-A1 (1 μ M). (F) Representative images of HSC-3 and SCC-7 cells stained with calcein/PI and treated with Cur (10 μ M) alone or in combination with Baf-A1 (1 μ M) (scale bar: 100 μ m). (G) Cell viability detection of HSC-3 and SCC-7 cells treated with Cur (10 μ M) alone or in combination with Baf-A1 (1 μ M). (H) Representative images of colony formation and quantitative analysis. ** P < 0.01.

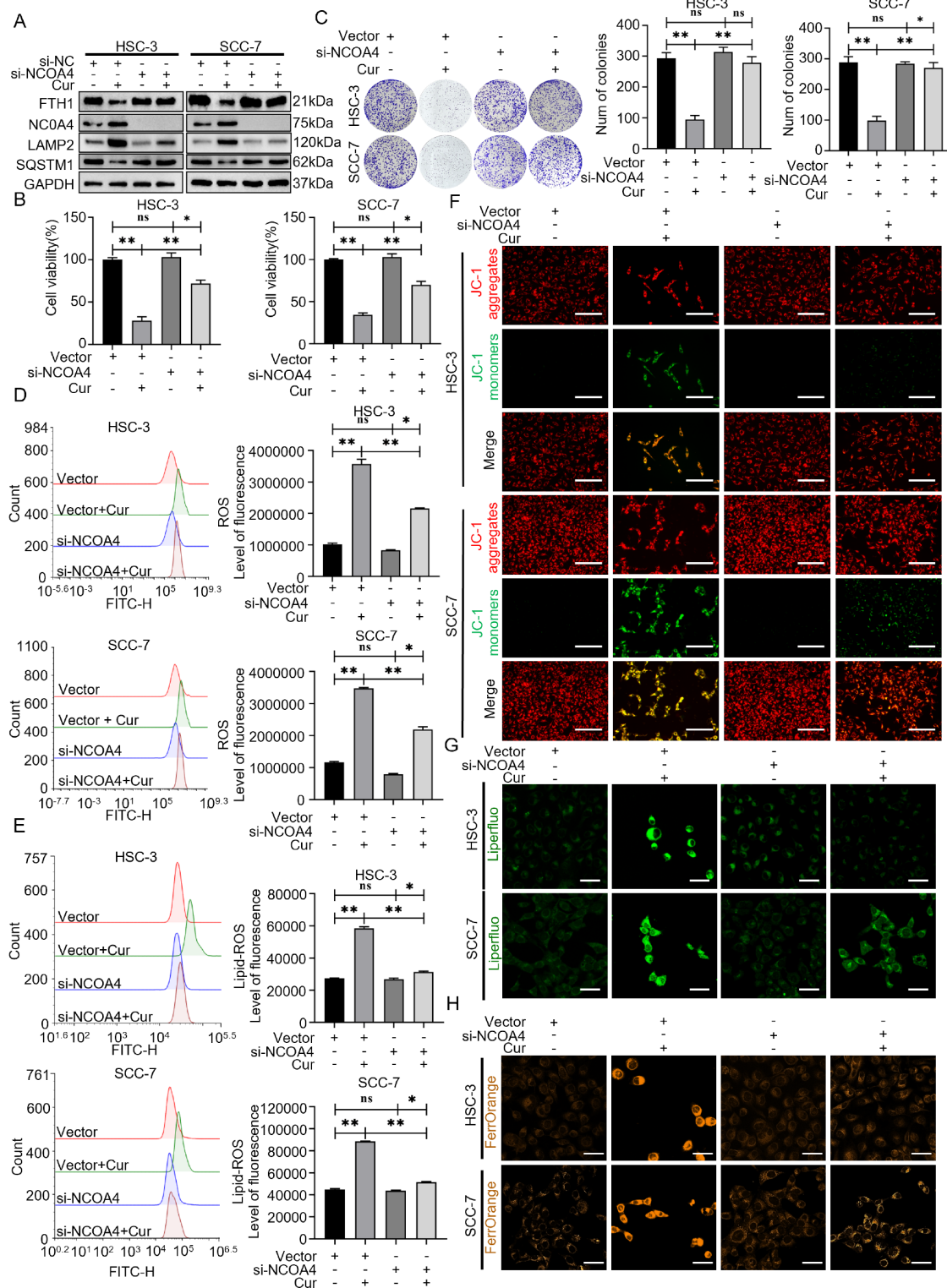


Figure 5. Cur regulates FTH1 and ferroptosis through NCOA4 (A) Western blot analysis of NCOA4 from siRNA interference cells treated with or without Cur (10 μ M). (B) Cell viability was determined by a CCK-8 kit in HSC-3 and SCC-7 cells. (C) Colony formation assays and quantitative analysis of HSC-3 and SCC-7 cells transfected with si-NC or si-NCOA4, with or without Cur (10 μ M) treatment. (D,E) Analysis of ROS levels (D) and lipid-ROS levels (E) in HSC-3 and SCC-7 cells transfected with si-NC or si-NCOA4, with or without Cur (10 μ M) treatment. (F) Representative images of cellular JC-1 fluorescence in HSC-3 and SCC-7 cells transfected with si-NC or si-NCOA4, with or without Cur (10 μ M) treatment (scale bar: 100 μ m). (G) HSC-3 and SCC-7 cells transfected with si-NC or si-NCOA4, with or without Cur (10 μ M) treatment, and Liperfluo levels were detected (scale bar: 50 μ m). (H) Detection of intracellular Fe^{2+} levels by FerroOrange staining in HSC-3 and SCC-7 cells transfected with si-NC or si-NCOA4, with or without Cur (10 μ M) treatment (scale bar: 50 μ m). * P < 0.05, ** P < 0.01. ns, not significant.

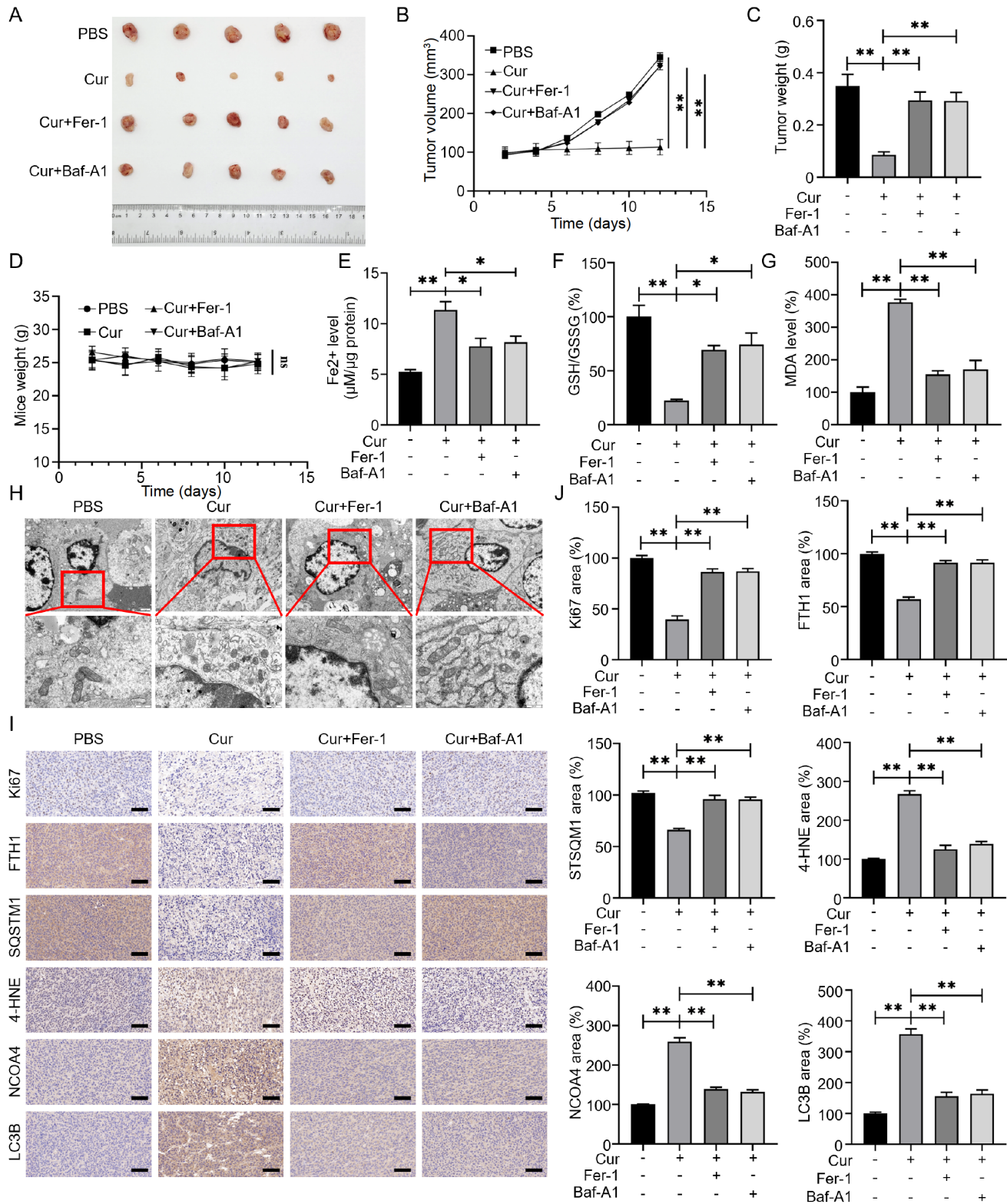


Figure 6. Cur inhibits HNSCC progression through the induction of ferroptosis (A) Representative photograph of tumor samples from each group (Cur: 40 mg/kg; Baf-A1: 1 mg/kg; and Fer-1: 5 mg/kg). (B) Tumor volume. (C) Tumor weight. (D) Mouse weight detection after tumor model establishment and treatment. (E) Analysis of Fe²⁺ levels in the tumor tissues. (F) Analysis of the GSH/GSSG ratio in the tumor tissues. (G) Analysis of MDA levels in tumor tissues. (H) Representative images of transmission electron microscopy of tumor tissues. Scale bar: 1 μm (top); scale bar: 500 nm (bottom). (I) IHC was used to determine the levels of Ki67, FTH1, SQSTM1, 4-HNE, LC3B and NCOA4 in tumor tissues. Scale bar: 200 μm. (J) Quantitative analysis of the IHC results of proteins including Ki67, FTH1, SQSTM1, 4-HNE, LC3B and NCOA4. Data are presented as the mean ± SD. **P* < 0.05, ***P* < 0.01. ns, not significant.

degradation and attenuated Cur-induced cell death. These results agreed with the growing consensus that autophagy serves as an upstream ferroptosis driver by regulating cellular iron availability.

Furthermore, we identified NCOA4 as a key molecular bridge during this process. NCOA4 acts as a selective cargo receptor that tethers FTH1 to autophagosomes [23,31–34]. We observed that Cur upregulated NCOA4 expression and enhanced the physical interaction between NCOA4 and FTH1. Crucially, silencing of *NCOA4* via siRNA impaired FTH1 degradation and significantly abrogated Cur-induced ferroptosis. This result suggested that the NCOA4-FTH1 axis is the specific target through which Cur disrupts iron homeostasis. By hijacking this physiological iron-recycling mechanism, Cur forced cancer cells into a state of lethal iron overload.

Here, our study provides the first evidence that Cur induces ferroptosis in HNSCC via the NCOA4-ferritinophagy axis. By promoting the autophagic degradation of ferritin, Cur elevates the labile iron pool. This leads to catastrophic lipid peroxidation and tumor cell death. These results not only unraveled a new molecular mechanism of Cur but also suggested that targeting NCOA4-mediated ferritinophagy could be a viable therapeutic strategy for patients with HNSCC. Future studies may explore the synergistic effects of Cur with existing chemotherapies to further enhance clinical outcomes.

In conclusion, we report that Cur inhibits HNSCC growth by inducing ferroptosis. This process is driven by NCOA4-mediated ferritinophagy, where Cur promotes the lysosomal degradation of FTH1 to unleash intracellular iron and catalyze lipid peroxidation. Our study reveals the NCOA4-FTH1 axis as a vulnerable target in HNSCC, and the results support the potential of Cur as a ferroptosis-inducing agent for cancer therapy.

Supplementary Data

Supplementary data are available at *Acta Biochimica et Biophysica Sinica* online.

Data Availability

The raw RNA sequencing data generated in this study have been deposited in the NCBI SRA under BioProject accession number PRJNA1342507. All other data supporting the findings of this study, including raw data and original images, are available from the corresponding author upon reasonable request.

Author Contributions

Yao Liu and Junli Ning designed and performed a majority of the experimental work. Yao Liu wrote the manuscript with extensive input from all authors. Ye Zhao and Ge Zhang conceived of the project and supervised and designed all the research. Zegang Li, Hong Yang, Xin Li, and Zhangyong Tang performed the experiments and prepared the mouse samples. All authors have read and approved the final version of the manuscript.

Funding

This work was supported by the grants from the Science and Technology Program of Sichuan Province (No. 2026NSFSC1833), Chengdu Medical College-Xindu District People's Hospital Joint Research Project (No.2022LHXD-05), Open funding from the Sichuan Provincial Key Laboratory of Development and Regeneration (No. SYS23-07), Chengdu Medical College (No. CYZYB22-04), and School of Laboratory Medicine of Chengdu

Medical College (No. JYZK202201).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Mody MD, Rocco JW, Yom SS, Haddad RI, Saba NF. Head and neck cancer. *Lancet* 2021, 398: 2289–2299
2. Lin P, Xie W, Li Y, Zhang C, Wu H, Wan H, Gao M, *et al.* Intratumoral and peritumoral radiomics of MRIs predicts pathologic complete response to neoadjuvant chemioimmunotherapy in patients with head and neck squamous cell carcinoma. *J Immunother Cancer* 2024, 12: e009616
3. Ma J, Liu Y, Huang XL, Zhang ZY, Myers JN, Neskey DM, Zhong LP. Induction chemotherapy decreases the rate of distant metastasis in patients with head and neck squamous cell carcinoma but does not improve survival or locoregional control: a meta-analysis. *Oral Oncol* 2012, 48: 1076–1084
4. Bhatia A, Burtneess B. Treating head and neck cancer in the age of immunotherapy: a 2023 update. *Drugs* 2023, 83: 217–248
5. Zou Q, Zhou X, Lai J, Zhou H, Su J, Zhang Z, Zhuang X, *et al.* Targeting p62 by sulforaphane promotes autolysosomal degradation of SLC7A11, inducing ferroptosis for osteosarcoma treatment. *Redox Biol* 2025, 79: 103460
6. Kotha RR, Luthria DL. Curcumin: biological, pharmaceutical, nutraceutical, and analytical aspects. *Molecules* 2019, 24: 2930
7. Arena A, Romeo MA, Benedetti R, Masuelli L, Bei R, Gilardini Montani MS, Cirone M. New insights into curcumin- and resveratrol-mediated anti-cancer effects. *Pharmaceuticals* 2021, 14: 1068
8. Abadi AJ, Mirzaei S, Mahabady MK, Hashemi F, Zabolian A, Hashemi F, Raei P, *et al.* Curcumin and its derivatives in cancer therapy: potentiating antitumor activity of cisplatin and reducing side effects. *Phytother Res* 2022, 36: 189–213
9. Giordano A, Tommonaro G. Curcumin and cancer. *Nutrients* 2019, 11: 2376
10. Ma W, Xu D, Zhao L, Yuan M, Cui YL, Li Y. Therapeutic role of curcumin in adult neurogenesis for management of psychiatric and neurological disorders: a scientometric study to an in-depth review. *Crit Rev Food Sci Nutr* 2023, 63: 9379–9391
11. Ren B, Zhang Y, Liu S, Cheng X, Yang X, Cui X, Zhao X, *et al.* Curcumin alleviates oxidative stress and inhibits apoptosis in diabetic cardiomyopathy via Sirt1-Foxo1 and PI3K-Akt signalling pathways. *J Cell Mol Med* 2020, 24: 12355–12367
12. He Y, Wang H, Lin S, Chen T, Chang D, Sun Y, Wang C, *et al.* Advanced effect of curcumin and resveratrol on mitigating hepatic steatosis in metabolic associated fatty liver disease via the PI3K/AKT/mTOR and HIF-1/VEGF cascade. *Biomed Pharmacother* 2023, 165: 115279
13. Shanmugam MK, Rane G, Kanchi MM, Arfuso F, Chinnathambi A, Zayed ME, Alharbi SA, *et al.* The multifaceted role of curcumin in cancer prevention and treatment. *Molecules* 2015, 20: 2728–2769
14. Zhao C, Zhou X, Cao Z, Ye L, Cao Y, Pan J. Curcumin and analogues against head and neck cancer: from drug delivery to molecular mechanisms. *Phytomedicine* 2023, 119: 154986
15. Mohseni M, Sahebkar A, Askari G, Johnston TP, Alikiaii B, Bagherniya M. The clinical use of curcumin on neurological disorders: an updated systematic review of clinical trials. *Phytother Res* 2021, 35: 6862–6882
16. Hadian K, Stockwell BR. SnapShot: ferroptosis. *Cell* 2020, 181: 1188–1188.e1
17. Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. *Nat Rev Mol Cell Biol* 2021, 22: 266–282
18. Mou Y, Wang J, Wu J, He D, Zhang C, Duan C, Li B. Ferroptosis, a new

- form of cell death: opportunities and challenges in cancer. *J Hematol Oncol* 2019, 12: 34
19. Zhang C, Liu X, Jin S, Chen Y, Guo R. Ferroptosis in cancer therapy: a novel approach to reversing drug resistance. *Mol Cancer* 2022, 21: 47
 20. Zhou Q, Meng Y, Li D, Yao L, Le J, Liu Y, Sun Y, *et al.* Ferroptosis in cancer: from molecular mechanisms to therapeutic strategies. *Sig Transduct Target Ther* 2024, 9: 55
 21. Chen X, Kang R, Kroemer G, Tang D. Broadening horizons: the role of ferroptosis in cancer. *Nat Rev Clin Oncol* 2021, 18: 280–296
 22. Park JM, Su YH, Fan CS, Chen HH, Qiu YK, Chen LL, Chen HA, *et al.* Crosstalk between FTH1 and PYCR1 dysregulates proline metabolism and mediates cell growth in KRAS-mutant pancreatic cancer cells. *Exp Mol Med* 2024, 56: 2065–2081
 23. Mancias JD, Wang X, Gygi SP, Harper JW, Kimmelman AC. Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. *Nature* 2014, 509: 105–109
 24. Deng Y, Zeng L, Liu H, Zuo A, Zhou J, Yang Y, You Y, *et al.* Silibinin attenuates ferroptosis in acute kidney injury by targeting FTH1. *Redox Biol* 2024, 77: 103360
 25. Burtneiss B, Harrington KJ, Greil R, Soulières D, Tahara M, de Castro Jr G, Psyrris A, *et al.* Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. *Lancet* 2019, 394: 1915–1928
 26. Solomon B, Young RJ, Rischin D. Head and neck squamous cell carcinoma: genomics and emerging biomarkers for immunomodulatory cancer treatments. *Semin Cancer Biol* 2018, 52: 228–240
 27. Xu P, Liu D, Zhou J, Tang Z, Wang S, Huang Y, Feng M, *et al.* Survival analysis of patients with metastatic head and neck squamous cell carcinoma treated with metastasis-directed radiotherapy and immunotherapy. *Radiat Oncol* 2025, 20: 31
 28. Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, *et al.* Regulation of ferroptotic cancer cell death by GPX4. *Cell* 2014, 156: 317–331
 29. Liu Y, Sun Q, Guo J, Yan L, Yan Y, Gong Y, Lin J, *et al.* Dual ferroptosis induction in N2-TANs and TNBC cells via FTH1 targeting: a therapeutic strategy for triple-negative breast cancer. *Cell Rep Med* 2025, 6: 101915
 30. Mi Y, Wei C, Sun L, Liu H, Zhang J, Luo J, Yu X, *et al.* Melatonin inhibits ferroptosis and delays age-related cataract by regulating SIRT6/p-Nrf2/GPX4 and SIRT6/NCOA4/FTH1 pathways. *Biomed Pharmacother* 2023, 157: 114048
 31. Fang Y, Chen X, Tan Q, Zhou H, Xu J, Gu Q. Inhibiting ferroptosis through disrupting the NCOA4-FTH1 interaction: a new mechanism of action. *ACS Cent Sci* 2021, 7: 980–989
 32. Luo C, Liang H, Ji M, Ye C, Lin Y, Guo Y, Zhang Z, *et al.* Autophagy induced by mechanical stress sensitizes cells to ferroptosis by NCOA4-FTH1 axis. *Autophagy* 2025, 21: 1263–1282
 33. Wang Y, Zhang Y, Gao M, Chen Z, Lu J, Li Y, Di Y, *et al.* Lipocalin-2 promotes CKD vascular calcification by aggravating VSMCs ferroptosis through NCOA4/FTH1-mediated ferritinophagy. *Cell Death Dis* 2024, 15: 865
 34. Wu H, Liu Q, Shan X, Gao W, Chen Q. ATM orchestrates ferritinophagy and ferroptosis by phosphorylating NCOA4. *Autophagy* 2023, 19: 2062–2077