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Iodine-131 induces ferroptosis and synergizes with sulfasalazine in differentiated thyroid cancer cells via suppressing *SLC7A11*

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Iodine-131 (¹³¹I) plays a key role in the treatment of differentiated thyroid cancer (DTC). Ferroptosis represents a form of regulated cell death that is distinct from necrosis and apoptosis, constituting a unique mode of programmed cell death. In this study, we aimed to ascertain the potential of ¹³¹I to trigger ferroptosis in DTC and to assess the synergistic therapeutic impact of combining ¹³¹I with sulfasalazine (SAS), a ferroptosis inducer, in the context of DTC. The FTC-133 and TPC-1 cell lines were employed to evaluate the impact of ¹³¹I and SAS on cellular functions. Ferrostatin-1 (Fer-1) reversed the cell viability and colony formation ability inhibited by ¹³¹I. ¹³¹I led to an elevation in the levels of malondialdehyde (MDA), reactive oxygen species (ROS), and lipid peroxidation. DTC cells exposed to ¹³¹I displayed characteristic ferroptotic ultrastructure, featuring shrunken mitochondria with increased membrane density. Concurrently, there was a reduction in the content of glutathione (GSH), as well as a downregulation of the expression levels of glutathione peroxidase 4 (GPX4) and solute carrier family 7 member 11 (*SLC7A11*) in the cells treated with ¹³¹I. The CI values for the combination of SAS and ¹³¹I in DTC cells were less than 1, demonstrating that SAS synergized with ¹³¹I. Moreover, the combination of SAS and ¹³¹I significantly increased the MDA levels and lipid peroxidation, decreased the GSH levels, and suppressed the expression of *SLC7A11* and GPX4, while *SLC7A11* knockdown significantly enhanced ferroptosis-related markers in DTC cells. Animal experiments demonstrated that SAS synergized with ¹³¹I resulted in notable decreases in tumor volume and weight. Furthermore, immunohistochemical analyses revealed that the combination of ¹³¹I and SAS significantly downregulated the expression of GPX4 and *SLC7A11* *in vivo*. Taken together, our results suggest that ¹³¹I may induce lipid peroxidation and ferroptosis, and demonstrate the potential for a synergistic therapeutic effect when ¹³¹I is combined with SAS in the treatment of DTC.

KEYWORDS

differentiated thyroid cancer, ferroptosis, Iodine-131, sulfasalazine, *SLC7A11* (xCT)

Introduction

Thyroid cancer is the most prevalent type of endocrine cancer globally. In 2022, it ranked as the seventh most frequently diagnosed cancer worldwide, with its incidence steadily increasing annually (1, 2). The predominant subtype, differentiated thyroid cancer (DTC), generally demonstrates favorable prognosis following standard treatments including surgery and radioactive iodine (RAI) therapy (3). RAI is primarily utilized for three clinical purposes: remnant ablation, adjuvant therapy, and therapeutic intervention in metastatic disease (4, 5).

Ferroptosis, a regulated form of cell death distinct from necrosis and apoptosis in morphology, biochemistry, and genetics, was first conceptualized by Dixon et al. in 2012 (6). This iron-dependent process is driven by lipid peroxidation and has been implicated in both physiological processes and pathological conditions ranging from ischemia-reperfusion injury to neurodegenerative diseases (7–10). Although ferroptosis functions as a natural tumor-suppressive mechanism, its activity is often suppressed in cancer cells (11–13). Given this tumor-suppressive paradox, reactivating ferroptosis represents a promising therapeutic strategy for diverse malignancies. Studies have confirmed that conventional cancer therapies, including radiotherapy, can induce ferroptosis (14, 15). However, the ability of ^{131}I —a widely used internal radiotherapy agent in DTC—to trigger ferroptosis remains underexplored.

GPX4, a central inhibitor of ferroptosis, suppresses lipid peroxidation by catalyzing the reduction of lipid hydroperoxides to lipid alcohols using glutathione (GSH) as a cofactor (16). *SLC7A11* (*xCT*) is a cystine/glutamate antiporter that plays a crucial role in transporting cystine into cells, which is essential for the production of cysteine—a rate-limiting precursor for GSH synthesis (17). Consequently, pharmacological inhibition of *SLC7A11* promotes ferroptosis through dual mechanisms: depleting intracellular GSH and indirectly impairing *GPX4*-mediated lipid repair (18). Recent studies demonstrated that ionizing radiation triggers ferroptosis by downregulating *SLC7A11* expression in solid tumors (15). Sulfasalazine (SAS), an FDA-approved drug for inflammatory bowel disease, has recently been repurposed as a ferroptosis inducer that directly inhibits *SLC7A11* activity, thereby accelerating lipid peroxidation (19). Notably, SAS exhibits radiosensitizing effects by synergistically reducing GSH levels to amplify radiation-induced ferroptosis (20, 21). Moreover, *SLC7A11* overexpression in thyroid cancer tissues suggests its inhibition could offer therapeutic benefits (22–25). Integrating the dual roles of *SLC7A11* in ferroptosis regulation and thyroid cancer progression, we hypothesize that combining SAS with ^{131}I may synergistically enhance treatment efficacy through concerted *SLC7A11* inhibition.

This study aims to investigate the involvement of ferroptosis in ^{131}I -induced cell death in DTC and evaluate the therapeutic potential of combining SAS with ^{131}I .

Materials and methods

Cell culture and reagents

The normal thyroid cell lines Nthy-ori3-1, kindly provided by the Department of Nuclear Medicine, First Affiliated Hospital of Guangdong Pharmaceutical University, and the human thyroid cancer cell lines TPC-1 and FTC-133, obtained from Zhongqiao Xinzhou Biological Co., Ltd. (Shanghai, China), were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (BI, Shanghai, China) and 1% penicillin/streptomycin (BI, Shanghai, China). Cells were maintained at 37°C in a humidified 5% CO_2 atmosphere. Ferrostatin-1 (HY-100579) and sulfasalazine (HY-14655) were procured from MedChemExpress (Monmouth Junction, NJ, USA). Z-VAD-FMK (T7020) and necrostatin-1 (T1847) were obtained from TargetMol (Boston, MA, USA). Sodium iodide-131 (Na^{131}I) was purchased from HTA Co., Ltd. (Beijing, China).

CCK-8 assay

Cell viability of FTC-133 and TPC-1 cells treated with ^{131}I , Ferrostatin-1 (Fer-1), Z-VAD-FMK, necrostatin-1, or SAS was quantified using a CCK-8 assay kit (GIPBIO, Montclair, NJ, USA). Cells were seeded into 96-well plates at a density of 5,000 cells/well and treated with compounds according to experimental protocols. After treatment, the medium in each well was replaced with 100 μL of fresh RPMI-1640 containing 10 μL CCK-8 reagent. Following a 3-hour incubation at 37°C under light-protected conditions, absorbance was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Cell viability (%) was calculated using the formula:

$$\text{Viability (\%)} = \frac{[(\text{OD}_{\text{treated}} - \text{OD}_{\text{blank}})]}{(\text{OD}_{\text{untreated}} - \text{OD}_{\text{blank}})} \times 100\%$$

OD_{blank} refers to wells containing medium without cells

Colony-formation assay

Cells were seeded in 6-well plates at a density of 700 cells/well. The combination group and Fer-1 monotherapy group were pre-treated with 1 μM Fer-1 for 4 hours; subsequently, the combination group and ^{131}I monotherapy group were exposed to ^{131}I (50 $\mu\text{Ci}/\text{mL}$). Cells were cultured in a humidified 5% CO_2 incubator at 37°C until visible colonies formed. Colonies were fixed with 4% paraformaldehyde (PFA) and stained with 0.5% crystal violet (Biosharp, Hefei, Anhui, China) for 30 minutes at room

temperature. Colonies in each well were imaged and quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Transmission electron microscopy

Cells cultured in 100-mm dishes were exposed to ^{131}I (50 $\mu\text{Ci}/\text{mL}$) for 48 h. After treatment, cells were fixed with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C overnight. Subsequently, cells were washed three times with phosphate buffer and post-fixed in 2% osmium tetroxide at room temperature for 1 hour. Following dehydration through a graded acetone series (50%, 70%, 90%, 100%), cells were stained with 1% uranyl acetate, embedded in Epon 812 resin (SPI Supplies, West Chester, PA, USA), and polymerized at 60°C for 48 hours. Ultrathin sections (70 nm) were prepared using an ultramicrotome (Leica EM UC7, Germany) and examined with a transmission electron microscope (HT7800, Hitachi, Tokyo, Japan).

GSH measurement

Intracellular GSH levels were determined using a GSH assay kit (Solarbio Science & Technology Co., Ltd., Beijing, China) according to the manufacturer's protocol. Briefly, cells were seeded in 6-well at a density of 1×10^5 cells/well and treated according to experimental protocols. After treatment, cells were harvested and divided into two equal aliquots: one for protein quantification via the bicinchoninic acid (BCA) assay and the other for GSH analysis. For GSH detection, cells were lysed by sonication after adding Reagent I from the kit. The lysate was centrifuged at $12,000 \times g$ for 10 minutes at 4°C to collect the supernatant. Reagents II and III were sequentially added to the supernatant, mixed thoroughly, and incubated at 25°C for 5 minutes. Absorbance at 412 nm was measured using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Malondialdehyde measurement

Intracellular MDA levels were quantified using an MDA assay kit (Beyotime Biotechnology, Shanghai, China) based on the thiobarbituric acid (TBA) reaction. Cells were seeded in 6-well plates at a density of 1×10^5 cells/well and treated according to experimental protocols. After treatment, cells were harvested, lysed, and centrifuged to collect the supernatant. After protein quantification of the lysates, the MDA working solution was added, and the mixture was heated at 100°C for 15 minutes. The samples were then centrifuged at $1,000 \times g$ for 10 minutes at 4°C. Absorbance of the supernatant was measured at 532 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

ROS detection

Intracellular reactive oxygen species (ROS) levels were detected using the fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). Cells were seeded in 6-well plates at a density of 1×10^5 cells/well. After cell adhesion, the combination group and Fer-1 monotherapy group were pre-treated with 1 μM Fer-1 for 4 hours; subsequently, the combination group and ^{131}I monotherapy group were exposed to ^{131}I (50 $\mu\text{Ci}/\text{mL}$) for 48 h. After treatment, cells were incubated with 10 μM DCFH-DA (prepared in serum-free RPMI-1640) under light-protected conditions for 30 minutes. After washing three times with phosphate-buffered saline (PBS), fluorescence images were acquired using a fluorescence microscope (Axio Observer 7, Carl Zeiss AG, Oberkochen, Germany).

Lipid peroxidation measurement

Cells (2×10^3 cells/well) were seeded in 24-well plates and treated according to experimental groups. Lipid peroxidation levels were quantified using the C11-BODIPY 581/591 fluorescent probe (Beyotime Biotechnology Co., Ltd., Shanghai, China) according to the manufacturer's protocol. Briefly, cells were incubated with 5 μM C11-BODIPY 581/591 under light-protected conditions for 30 minutes. Fluorescence intensity was measured using a microplate reader (Fluoroskan Microplate Reader, Thermo Fisher Scientific, Waltham, MA, USA) (reduced form: Ex/Em 581/591 nm; oxidized form: Ex/Em 488/510 nm). The results were expressed as the ratio of oxidized to reduced fluorescence intensity.

Reverse transcription-quantitative polymerase chain reaction

Total RNA was isolated from FTC-133 and TPC-1 cells using TRIzolTM reagent (Thermo Fisher Scientific, Waltham, MA, USA). Complementary DNA (cDNA) was synthesized from 1 μg RNA using SuperScriptTM II Reverse Transcriptase (Vazyme Biotech Co., Ltd., Nanjing, Jiangsu, China). RT-qPCR amplification was conducted on a LineGene Pro Real-Time PCR System (Bioer Technology, Hangzhou, China) with the following cycling conditions: 95°C for 5 min, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. mRNA expression levels were normalized to the endogenous control gene *GAPDH*. Primer sequences were as follows:

Homo-GPX4-F: 5'-ACATGGTTAACTGGACAAGT ACCG-3'
 Homo-GPX4-R: 5'-GGTCGACGAGCTGAGTGTAG TTTAC-3'
 Homo-SLC7A11-F: 5'-TGGAAGTCTTTGGTCCATTACC AGC-3'
 Homo-SLC7A11-R: 5'-GGTTCCAGAATGTAGCGTCCA AATG-3'

Homo-GAPDH-F: 5'-ACAGCCTCAAGATCATCAGCA-3'
Homo-GAPDH-R: 5'-ATGAGTCCTTCCACGATACCA-3'.

Western blotting

Proteins were extracted from FTC-133 and TPC-1 cells of each experimental group using RIPA lysis buffer (Biosharp Life Sciences, Anhui, China). Protein concentrations were determined using a BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Equal amounts of protein (40 µg per lane) were separated by SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Merck Millipore, Burlington, MA, USA). Membranes were blocked with 5% non-fat milk for 1 h at room temperature, followed by overnight incubation at 4°C with primary antibodies. After washing, membranes were incubated with HRP-conjugated secondary antibodies for 1 h. Protein bands were visualized using an Enhanced Chemiluminescent (ECL) Substrate Kit (Abbkine Scientific Co., Ltd., Wuhan, Hubei, China) and imaged on a ChemiDoc MP Imaging System (Bio-Rad Laboratories, Hercules, CA, USA). Antibody details: Anti-SLC7A11 (1:1,000; HUABIO, Hangzhou, Zhejiang, China), Anti-GPX4 (1:1,000; Cell Signaling Technology, Danvers, MA, USA), Anti-GAPDH (1:10,000; HUABIO, Hangzhou, Zhejiang, China), HRP-conjugated Goat Anti-Rabbit IgG (1:10,000; HUABIO, Hangzhou, Zhejiang, China).

siRNA transfection

SLC7A11-targeting siRNAs were synthesized by IGE Biotechnology Co., Ltd. (Guangzhou, Guangdong, China). Transient transfection was performed using siRNA-Mate Transfection Reagent (GenePharma Co., Ltd., Shanghai, China) following the manufacturer's instructions. FTC-133 cells were transfected with siRNA-*SLC7A11*-1 (5'-GAAGUAAGUUAUGAACUA-3'). TPC-1 cells were transfected with siRNA-*SLC7A11*-2 (5'-GGAAGAGAUUCAAGUAUUA-3'). Cells were harvested 48 h post-transfection for downstream analyses.

Animal experiment

All animal experiments were conducted in accordance with the National Guidelines for Experimental Animal Welfare (the Ministry of Science and Technology of People's Republic Laboratory Animals 2006-09-30) at the Animal Experiment Center of General Hospital of Southern Theater Command (Guangzhou, China). The animal experiments were approved by the Institutional Animal Care and Use Committee of General Hospital of Southern Theater Command. Sixteen BALB/C nude mice (3-4 weeks, 16-18 g) purchased from Guangzhou Ruige Biological Technology Co., Ltd (Guangzhou, China) were used for

the TPC-1 cell xenografts. One week after adaptive feeding, the mice were randomly divided into four groups: control group (nude mice were administered an equivalent volume of saline as the other group), ¹³¹I group (nude mice were administered 7.4 MBq of ¹³¹I once every three days), SAS group (nude mice received 8 mg/kg SAS daily) and combination group (nude mice were administered 7.4 MBq of ¹³¹I once every three days and received 8 mg/kg SAS daily). After three courses of ¹³¹I treatment, mice were euthanized by cervical dislocation. Tumor size was calculated in accordance with the following formula: tumor volume = 1/2 × A × B², where A and B represent the longest and shortest tumor diameters, respectively.

Immunohistochemistry

Tumor tissues removed from mice models were fixed in 4% buffered formalin and embedded in paraffin. After the tumor tissue was sectioned into 5 µm thickness, hematoxylin-eosin staining was performed on the sections. For the purpose of immunohistochemical staining, the primary antibodies, including GPX4 (1:500, CST, USA) and SLC7A11 (1:500, HUABIO, Hangzhou, China), were incubated at 4°C overnight. Images were scanned using 3DHISTECH (PANNORAMIC MIDI, Budapest, Hungary).

Statistical analysis

Quantitative data are presented as mean ± standard deviation (SD) derived from at least three independent biological replicates. Intergroup differences were analyzed by Student's unpaired t-test (two groups) or one-way analysis of variance (ANOVA) with Tukey's *post hoc* test (three or more groups). All analyses were performed using SPSS v26.0 (IBM Corp., Armonk, NY, USA). Statistical significance was defined as follows: *p* < 0.05 (*), *p* < 0.01 (**), and *p* < 0.001 (***) ; nonsignificant (ns) indicates *p* ≥ 0.05.

Results

Fer-1 alleviates cell viability and colony formation ability suppressed by ¹³¹I in DTC cells

Ionizing radiation has been shown to trigger ferroptosis in cancer cells (14). Given that ¹³¹I emits both β- and γ-radiation, we hypothesized it could induce ferroptosis in DTC cells. To test this hypothesis, we first examined the effects of the ferroptosis inhibitor Fer-1 on FTC-133 and TPC-1 cells exposed to grade doses of ¹³¹I. Fer-1 significantly attenuated ¹³¹I-induced cytotoxicity, manifested by increased cell viability (Figure 1A). Colony formation assays corroborated these findings, demonstrating that Fer-1 alleviated ¹³¹I-mediated suppression of clonogenic capacity in both cell lines (Figure 1B). To elucidate the contribution of ferroptosis to

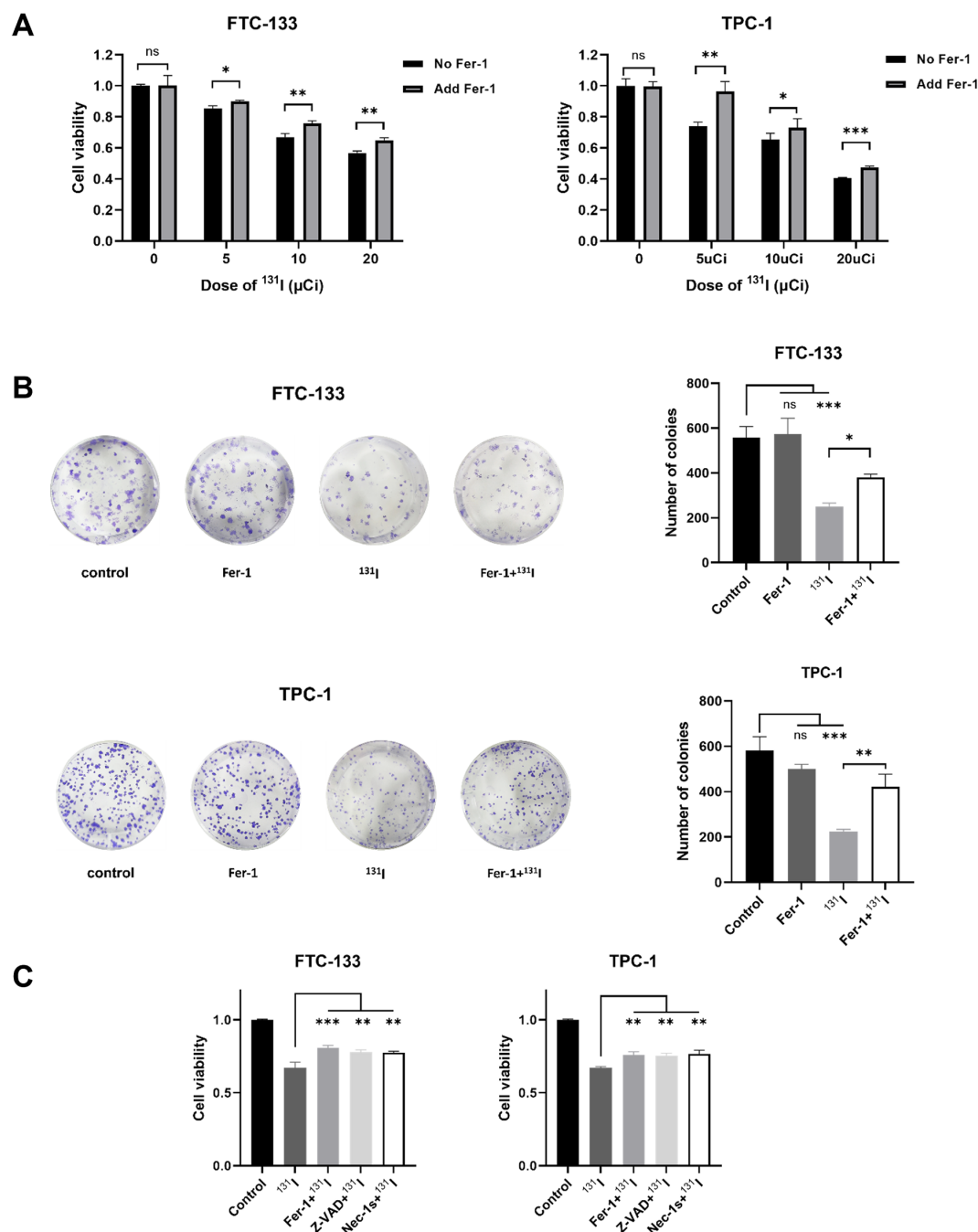


FIGURE 1

Fer-1 alleviates cell viability and colony formation ability suppressed by ^{131}I in DTC cells. (A) Cell viability of FTC-133 and TPC-1 cells that were pretreated with 1 μM Fer-1 for 4 h followed by treatment of 5, 10, 20 μCi ^{131}I for 48 h. (B) Colony formation ability of FTC-133 and TPC-1 cells that were pretreated with 1 μM Fer-1 for 4 h followed by treatment of 50 μCi ^{131}I . (C) Cell viability of FTC-133 and TPC-1 cells that were pretreated with 1 μM Fer-1, 5 μM Necrostatin-1s or 10 μM Z-VAD-fmk for 4 h followed by treatment of 10 μCi ^{131}I for 48 h. Images are representative of at least three independent experiments.

^{131}I -induced cell death, we performed comparative analyses of Fer-1 with apoptosis inhibitor ZVAD-FMK and necroptosis inhibitor necrostatin-1 (Nec-1s). All three inhibitors partially restored viability in ^{131}I -treated DTC cells (Figure 1C), indicating co-activation of ferroptotic, apoptotic, and necroptotic pathway. Based on these findings, we focused subsequent investigations on ferroptosis mechanisms in ^{131}I -treated DTC cells.

Biochemical hallmarks of ferroptosis are observed in ^{131}I -treated DTC cells

We systematically evaluated ferroptosis-associated biochemical alterations in DTC cells following ^{131}I exposure. GSH levels were first quantified in FTC-133 and TPC-1 cells, revealing significant depletion upon ^{131}I treatment (Figure 2A). Consistent with

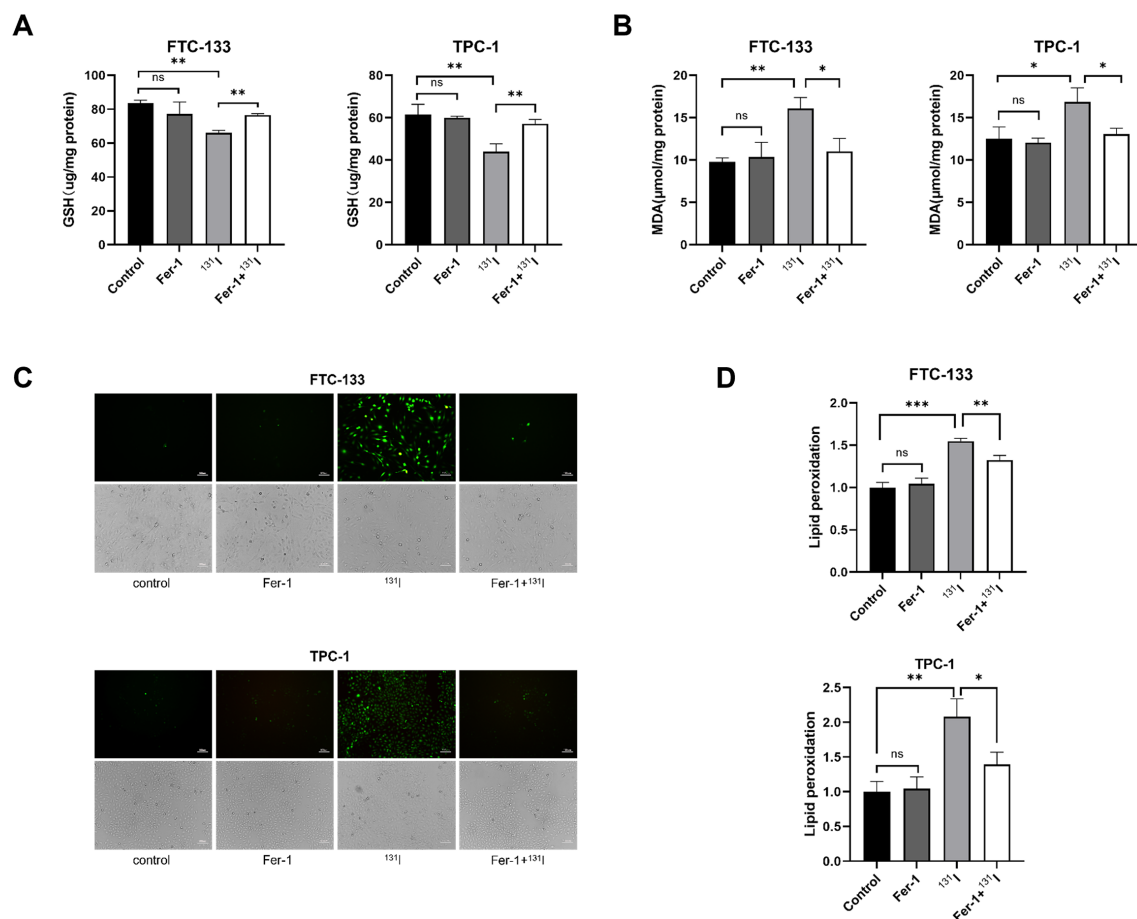


FIGURE 2

Ferroptosis morphological and genetic signatures are observed in ¹³¹I-treated DTC cells. (A) The GSH levels of FTC-133 and TPC-1 cells pretreated with 1 μM Fer-1 for 4 h followed by treatment of 50 μCi ¹³¹I for 48 h. (B) The MDA levels of FTC-133 and TPC-1 cells pretreated with 1 μM Fer-1 for 4 h followed by treatment of 50 μCi ¹³¹I for 48 h. (C) The ROS levels of FTC-133 and TPC-1 cells pretreated with 1 μM Fer-1 for 4 h followed by treatment of 50 μCi ¹³¹I for 48 h. Scale bar = 100 μm. (D) The lipid peroxidation levels of FTC-133 and TPC-1 cells pretreated with 1 μM Fer-1 for 4 h followed by treatment of 50 μCi ¹³¹I for 48 h. Images are representative of at least three independent experiments.

ferroptosis characteristics, MDA—a lipid peroxidation byproduct indicative of ferroptosis (26) —showed marked elevation in ¹³¹I-treated cells versus controls (Figure 2B). ROS production and lipid peroxidation were also substantially amplified in both cell lines post-¹³¹I exposure (Figures 2C, D). Notably, co-treatment with Fer-1 effectively abrogated ¹³¹I-induced perturbations in GSH, MDA, ROS, and lipid peroxidation markers (Figures 2A–D). These data collectively confirm ¹³¹I-triggered ferroptosis activation in DTC cells through canonical biochemical pathways.

Ferroptosis morphological and genetic signatures are observed in ¹³¹I-treated DTC cells

Ferroptosis is characterized by distinct ultrastructural features that distinguish it from apoptosis and necroptosis. To systematically characterize these morphological changes, we performed TEM on ¹³¹I-treated DTC cells. Notably, FTC-133 and TPC-1 cells exposed to ¹³¹I displayed pathognomonic ferroptotic ultrastructure,

featuring shrunken mitochondria with increased membrane density (27) (Figure 3A).

To elucidate ferroptosis-related molecular mechanisms, we quantified *GPX4* and *SLC7A11* expression at both transcriptional and translational levels. RT-qPCR analyses revealed that ¹³¹I treatment significantly downregulated *GPX4* and *SLC7A11* mRNA levels relative to untreated controls (Figure 3B). Protein expression paralleled transcriptional changes, with marked reductions in *GPX4* and *SLC7A11* observed by western blot (Figures 3C, D). These results collectively demonstrate that ¹³¹I triggers ferroptosis in DTC cells through coordinated suppression of *GPX4* and *SLC7A11* and induces mitochondrial remodeling characteristic of ferroptosis in DTC cells.

SAS synergizes with ¹³¹I to promote DTC cells death

Given the observed role of *SLC7A11* suppression in ¹³¹I-induced ferroptosis, we investigated whether pharmacological

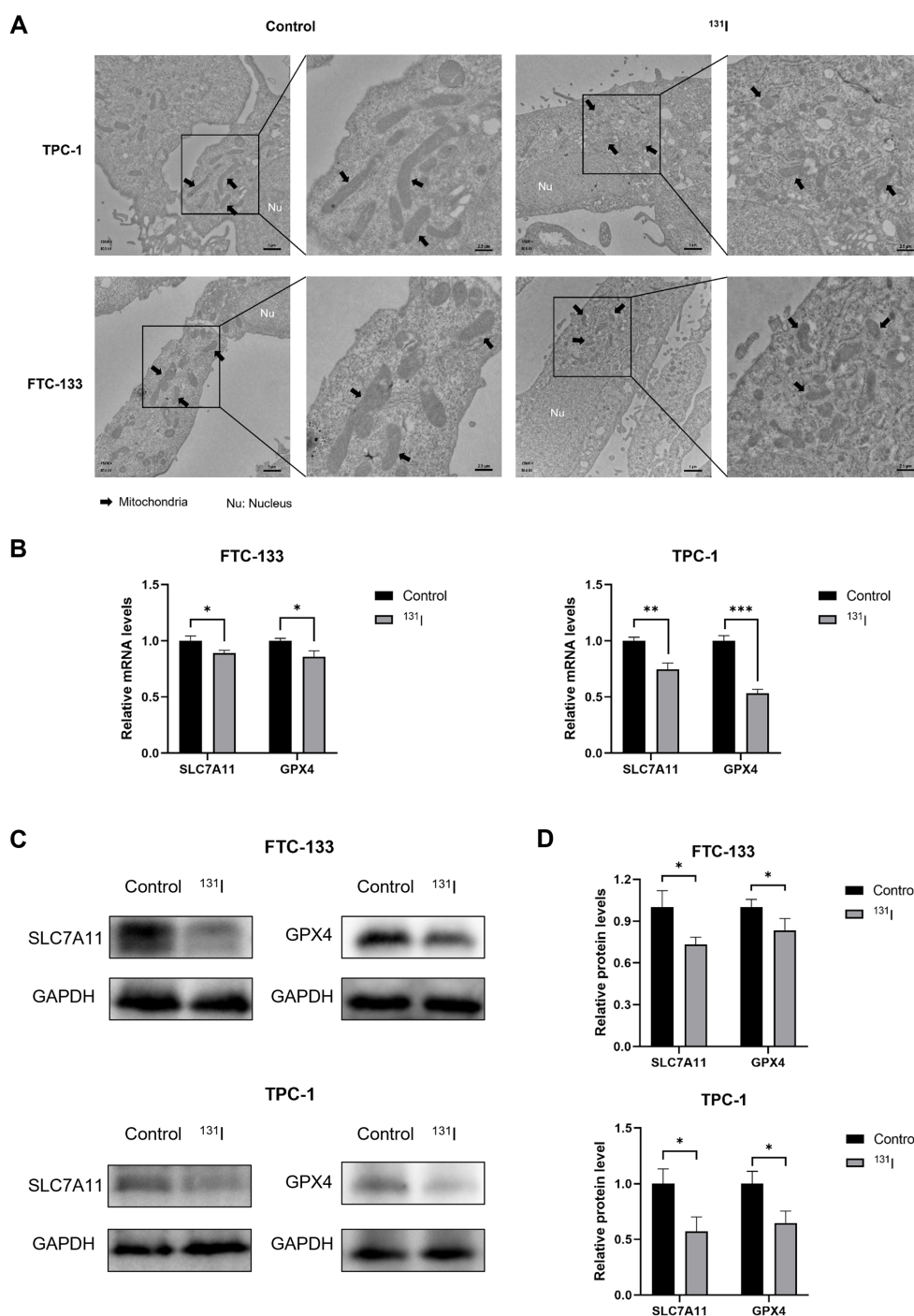


FIGURE 3

Morphological markers and Genetic hallmarks of ferroptosis were observed in ^{131}I -treated DTC cells. **(A)** Transmission electron microscopy images of FTC-133 and TPC-1 cells with or without ^{131}I treatment. Nu, nucleus. Scale bars: left, 1 μm , right, 2.5 μm . **(B)** The relative *SLC7A11* and *GPX4* mRNA expression levels of FTC-133 and TPC-1 cells with or without ^{131}I treatment. **(C)** The relative *SLC7A11* and *GPX4* protein expression levels of FTC-133 and TPC-1 cells with or without ^{131}I . **(D)** The quantitative graph of relative protein expression of *SLC7A11* and *GPX4* using Image J. Images are representative of at least three independent experiments.

SLC7A11 inhibition could enhance ^{131}I efficacy in DTC cells. The system xc^- inhibitor SAS, a class 1 ferroptosis inducer targeting *SLC7A11*, was selected for combination therapy. Dose-response analysis determined IC_{50} values of 0.42 mM for FTC-133 and 0.48 mM for TPC-1 cells (Figure 4A), prompting selection of sub- IC_{50}

SAS concentrations (0.1 mM) for synergy testing. DTC cells were treated with escalating ^{131}I doses (5, 10, 15, 20, 25 μCi) alone, 0.1 mM SAS monotherapy, or their combination. Combination Index (CI) was calculated using the Chou - Talalay method (28), where $\text{CI} > 1$ indicates antagonism; $= 1$ additive effect; < 1 synergy.

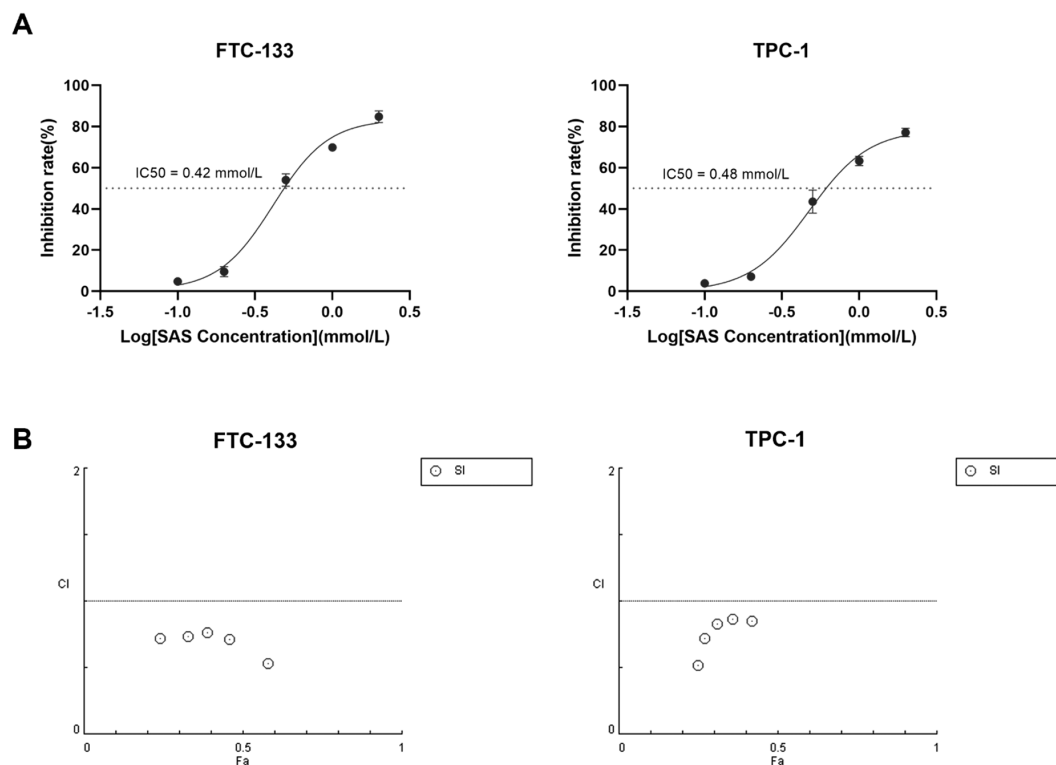


FIGURE 4

SAS synergizes with ^{131}I to promote DTC cells death (A) The IC50 of SAS on FTC-133 and TPC-1 cells for 24 h. (B) Combination Index of sulfasalazine and ^{131}I on FTC-133 and TPC-1 cells (concentration of SAS when treating alone on the cell: 0.1 mM, 0.2 mM, 0.5 mM, 1 mM, 2 mM, concentration of SAS in combination on cells: 0.1 mM, dose of ^{131}I when treating alone and in combination on cells: 5 μCi , 10 μCi , 15 μCi , 20 μCi , 25 μCi , drug treatment time: 24 h). Images are representative of at least three independent experiments.

Quantitative analysis demonstrated the CI values for the combination of SAS and ^{131}I in DTC cells were less than 1, confirming synergistic effects between SAS and ^{131}I in DTC cells (Figure 4B). Additionally, our findings indicated that the combined use of SAS and ^{131}I also inhibits the activity of normal thyroid cells. However, compared to normal thyroid cells, the combined treatment exhibits a significantly more pronounced inhibitory effect on thyroid cancer cells, suggesting a potential therapeutic advantage in thyroid cancer cells while minimizing the toxic effect on healthy tissue (Supplementary Figure S1).

Combination SAS and ^{131}I enhance ferroptosis in DTC cells

To mechanistically delineate the synergistic effects of SAS and ^{131}I combination therapy in DTC cells, we quantified canonical ferroptosis biomarkers (MDA, GSH and lipid peroxidation) using standardized protocols described above. Intriguingly, the combination group exhibited significantly GSH depletion in FTC-133 and TPC-1 cells compared with the other groups (Figure 5A). And the cotreatment with SAS and ^{131}I resulted in a marked elevation in MDA levels compared to the other treatment groups (Figure 5B). Furthermore, the combination of SAS and ^{131}I resulted in a significant induction of lipid peroxidation, which was

significantly higher than that in the other groups (Figure 5C). Moreover, we examined the protein expression levels of GPX4 and SLC7A11 in FTC-133 and TPC-1 cells using Western Blotting. Our results showed that GPX4 and SLC7A11 expression levels were significantly lower in the group treated with the SAS and ^{131}I combination than in those treated with SAS alone, ^{131}I alone, or in the control group. Additionally, GPX4 and SLC7A11 expression levels were significantly downregulated in groups treated with ^{131}I or SAS alone compared to the control group. (Figures 5D, E). The above results indicate that cotreatment with SAS and ^{131}I robustly induces cell death through enhanced lipid peroxidation and activation of ferroptosis.

SLC7A11 knockdown enhances ferroptosis induced by ^{131}I and SAS in DTC cells

Given that the mechanism of action of SAS involves the inhibition of *SLC7A11* activity, and our study has also demonstrated that ^{131}I can downregulate the expression of *SLC7A11*, we posited that their synergistic effects arose from dual targeting of this cystine/glutamate antiporter. To validate this hypothesis, we established *SLC7A11*-knockdown DTC cell models, validated by RT-qPCR and Western blotting (Figures 6A, B). To further elucidate the role of *SLC7A11* in mediating the effects

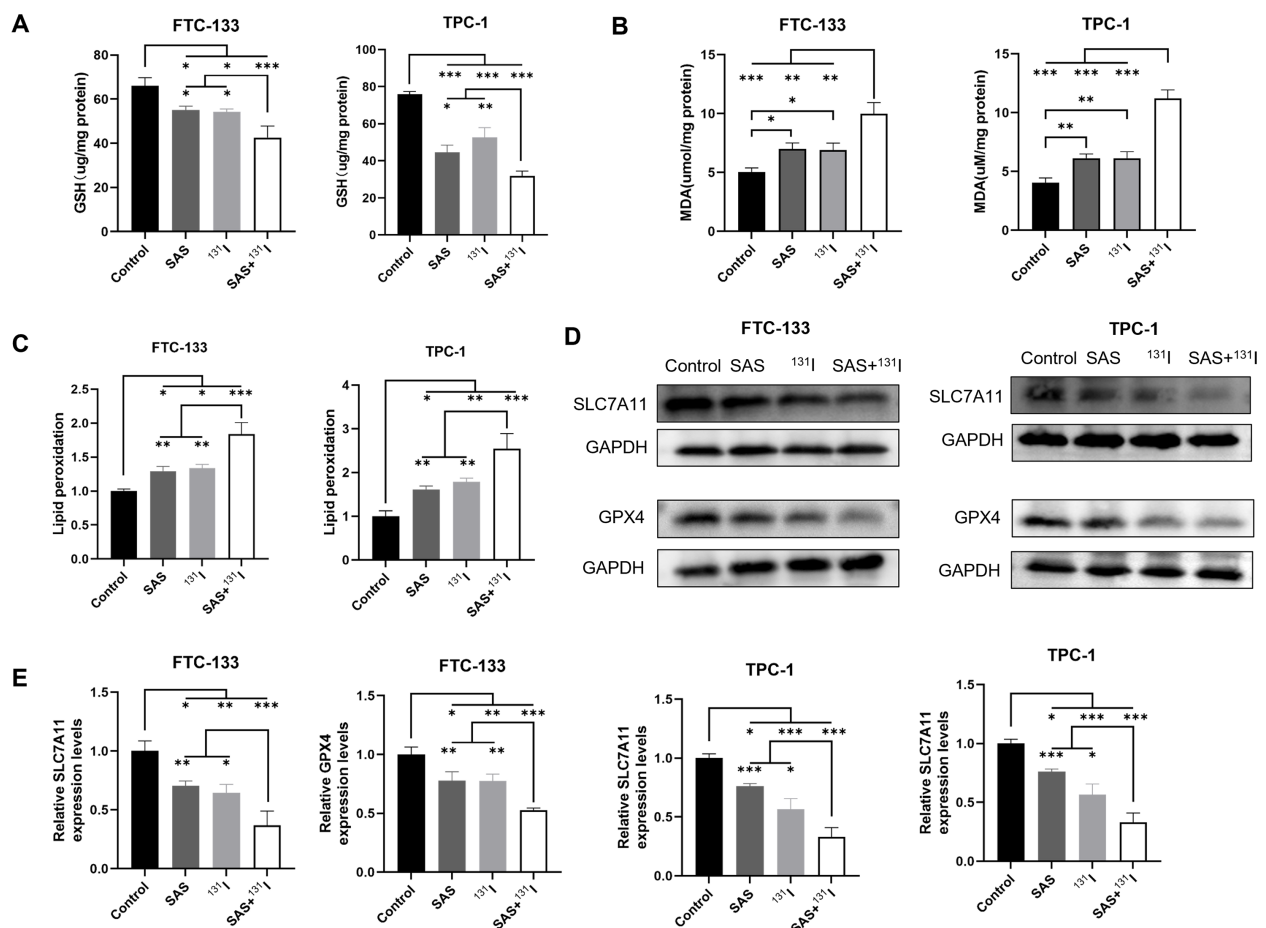


FIGURE 5

Combination SAS and ¹³¹I enhance lipid peroxidation and ferroptosis in DTC cells. (A) The GSH levels of FTC-133 and TPC-1 cells after 0.1 mmol/L SAS and 50 μ Ci ¹³¹I treatment for 24 h. (B) The MDA levels of FTC-133 and TPC-1 cells after 0.1 mM SAS and 50 μ Ci ¹³¹I treatment for 24 h. (C) The lipid peroxidation levels of FTC-133 and TPC-1 cells after 0.1 mM SAS and 50 μ Ci ¹³¹I treatment for 24 h. (D) The GPX4 and SLC7A11 protein expression levels of FTC-133 and TPC-1 cells after 0.1 mM SAS and 50 μ Ci ¹³¹I treatment for 24 h. (E) The quantitative graph of relative protein expression of SLC7A11 and GPX4 using Image J. Images are representative of at least three independent experiments.

of ¹³¹I and SAS on DTC cells, we assessed cell viability, intracellular GSH levels, and lipid peroxidation in SLC7A11-knockdown DTC cells. Our results demonstrated that SLC7A11 knockdown significantly reduced cell viability, irrespective of whether ¹³¹I, SAS, or their combination was applied (Figure 6C). Additionally, SLC7A11 knockdown led to a marked decrease in intracellular GSH levels across all treatment groups, except for the control group (Figure 6D). Moreover, lipid peroxidation levels were significantly elevated in cells treated with ¹³¹I alone, SAS alone, or the combination therapy following SLC7A11 knockdown (Figure 6E). Collectively, these findings highlight the pivotal role of SLC7A11 in the cytotoxic effects of ¹³¹I and SAS on thyroid cancer cells.

SAS synergizes with ¹³¹I in DTC xenograft nude mice model

To validate the therapeutic synergy of SAS and ¹³¹I *in vivo*, TPC-1 xenograft-bearing nude mice were treated with SAS in combination

with ¹³¹I. Post-treatment, significant reductions in tumor volume and weight were observed in the combination-treated group compared to the control group (Figures 7A-C). Additionally, treatment with ¹³¹I alone also resulted in notable decreases in tumor volume and weight compared to the control group. Immunohistochemical analyses revealed that the combination of ¹³¹I and SAS significantly downregulated the expression of GPX4 and SLC7A11 compared to all other group *in vivo* (Figure 7D). Moreover, GPX4 and SLC7A11 expression levels were also reduced in mice treated with ¹³¹I alone or SAS alone compared to the control group. Collectively, these findings suggest that SAS synergized with ¹³¹I to effectively treat DTC *in vivo*, an effect that is associated with enhanced lipid peroxidation and ferroptosis.

Discussion

Ferroptosis, an iron-dependent form of regulated cell death driven by lipid peroxidation (29), functions as a tumor-suppressive

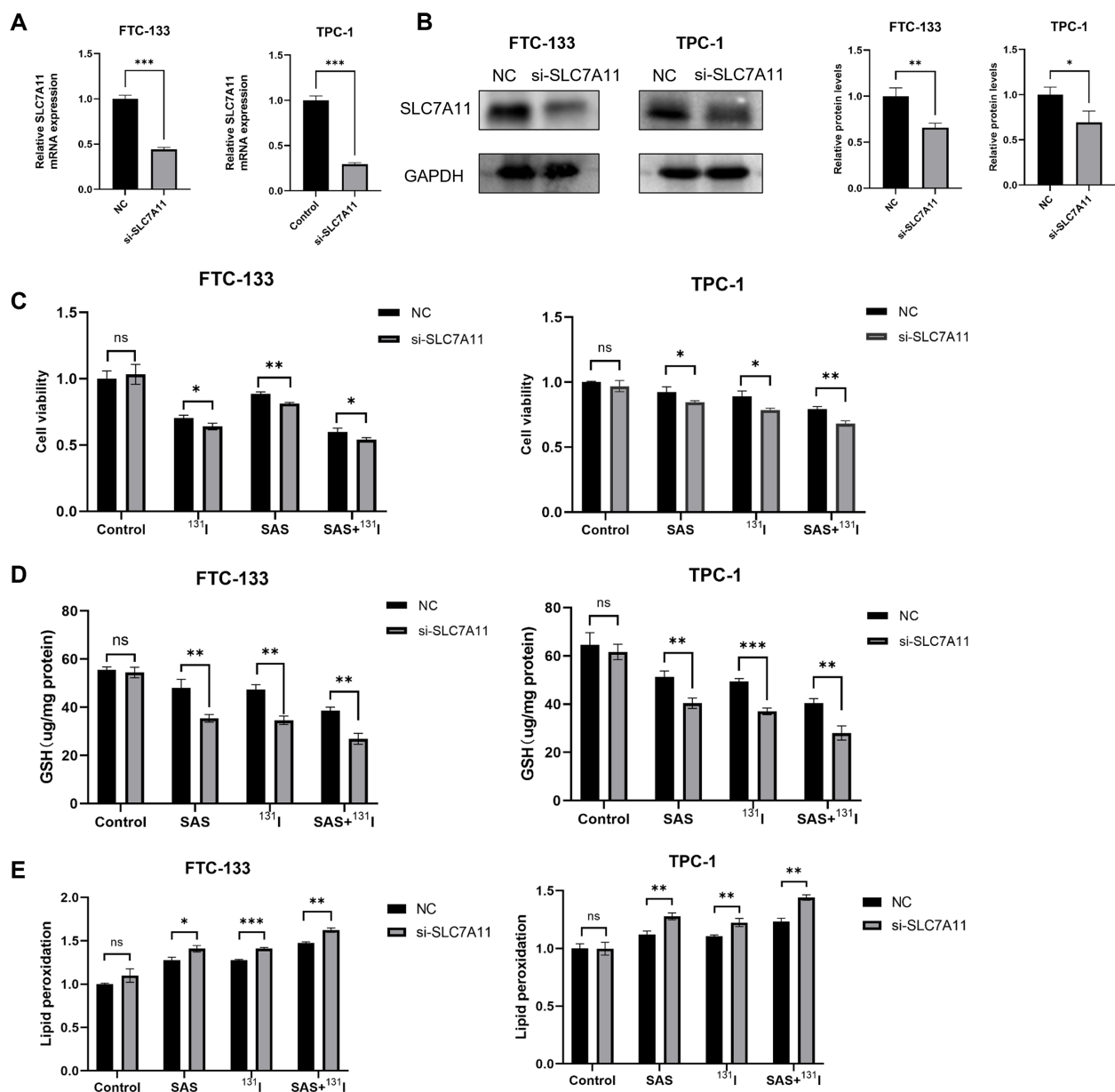


FIGURE 6

SLC7A11 knockdown enhances effects of SAS and 131 I on DTC cells. (A) Relative expression levels of *SLC7A11* mRNA in FTC-133 and TPC-1 cells with or without knockdown of *SLC7A11*. (B) Relative *SLC7A11* protein expression levels in FTC-133 and TPC-1 cells with or without knockdown of *SLC7A11*. (C) Cell viability of DTC FTC-133 and TPC-1 cells treated with 0.1 mmol/L sulfasalazine and 50 μ Ci 131 I for 24 h with or without knockdown of *SLC7A11*. (D) GSH levels of FTC-133 and TPC-1 cells treated with 0.1 mmol/L sulfasalazine and 50 μ Ci 131 I for 24 h with or without knockdown of *SLC7A11*. (E) Lipid peroxidation levels of FTC-133 and TPC-1 cells treated with 0.1 mmol/L sulfasalazine and 50 μ Ci 131 I for 24 h with or without knockdown of *SLC7A11*. Images are representative of at least three independent experiments.

mechanism that is frequently silenced in malignancies (11). Emerging evidence links multiple frontline oncologic therapies—including radiotherapy, endocrine therapy, immune checkpoint blockade, and platinum-based chemotherapy—to ferroptosis potentiation (15, 30–32). The synergistic application of ferroptosis inducer in combination with other therapeutic approaches represents a promising strategy for cancer therapy (33, 34). In this study, we demonstrated that 131 I induces lipid peroxidation and ferroptosis in DTC cells and synergizes with SAS in treating DTC cells. Fer-1 alleviated the inhibitory effects of 131 I on the viability

and clonogenic potential of DTC cells. Through a comprehensive analysis encompassing biochemical, morphological, and genetic perspectives, we have identified that 131 I induces ferroptosis in DTC cells. Specifically, 131 I treatment results in elevated levels of MDA and ROS, concurrent with a reduction in GSH content within DTC cells. Additionally, 131 I induces characteristic morphological changes associated with ferroptosis, including a reduction in mitochondrial volume and contraction of mitochondrial cristae. Furthermore, the expression levels of *SLC7A11* and *GPX4* are downregulated in DTC cells after 131 I treatment.

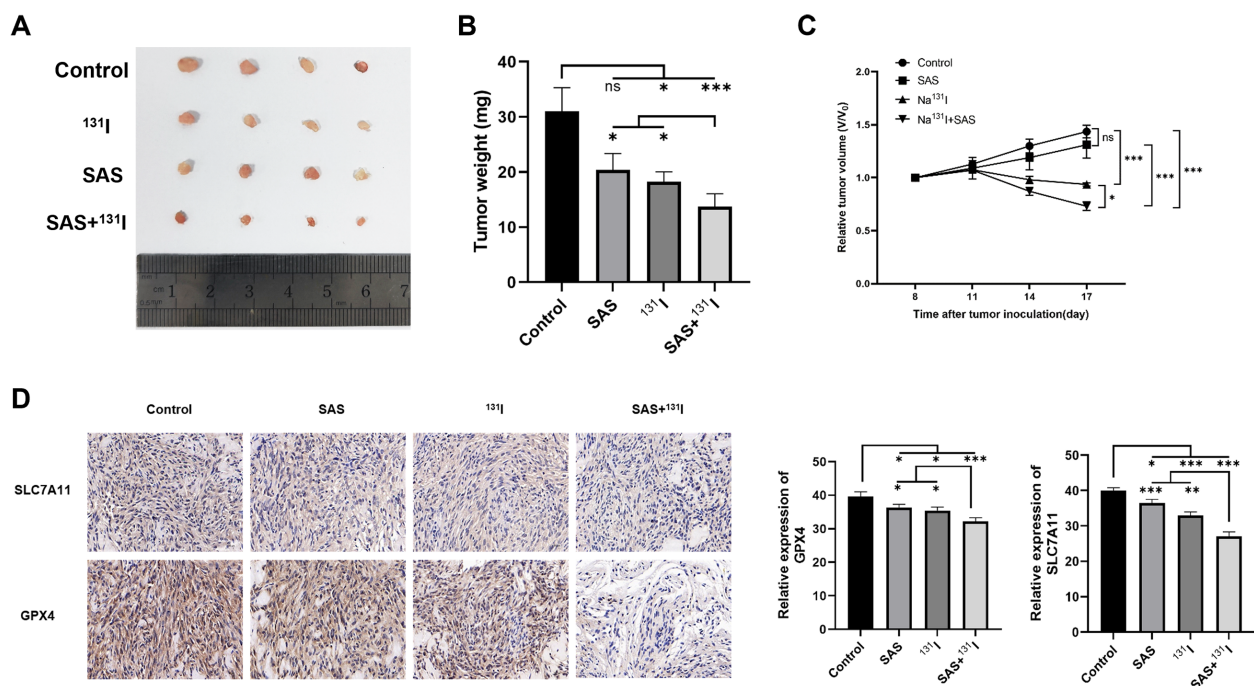


FIGURE 7

SAS synergizes with ¹³¹I in DTC xenograft nude mice model. (A) Image of tumors isolated from different treatment groups. (B) Tumor weights in different treatment groups. (C) Curves of relative tumor volume changes in different treatment groups during the treatment period. (D) Immunohistochemical analysis of *SLC7A11* and *GPX4*.

As a radionuclide, ¹³¹I emits β radiation that induces cell death by damaging DNA and eliciting cytotoxicity. The presence of the NIS on the basal membrane of certain tumor cells, such as those in DTC, cholangiocarcinoma and hepatocellular carcinoma, facilitates iodine uptake and forms the basis for radioiodine therapy (35). However, ¹³¹I can be used for the radiolabeling of molecules, peptides, antibodies and nanomaterials that selectively binding to cancer cells, thereby targeting cancer cells that do not express NIS. For instance, a ¹³¹I-labeled programmed cell death ligand 1 (PD-L1) antibody can target PD-L1 expressed on the cancer cell membrane, inducing ferroptosis in cancer cells (36). Two studies on the synthesis of radiopharmaceuticals using ¹³¹I-conjugated PD-L1 antibodies have demonstrated that these radiotherapeutic agents can induce ferroptosis in tumor cells (36, 37). Moreover, Shen et al. employed 3 nm ultrasmall single-crystal iron nanoparticles as a ferroptosis inducer to synergize with ¹³¹I-PD-L1 antibodies in treating murine breast cancer cells and embryonic fibroblast cells. These findings support our investigation that ¹³¹I can induce lipid peroxidation and ferroptosis in cancer cells. However, given the inability of these cells to uptake ¹³¹I, experiments on the therapeutic application of ¹³¹I as a solitary agent were not conducted in their research. By contrast, the DTC cells used in the present study are capable of iodine uptake, allowing us to directly observe the role of ferroptosis in tumor cells treated with ¹³¹I.

Extensive scholarly research has demonstrated that the induction of ferroptosis enhances the efficacy of radiotherapy in cancer treatment, and can even overcomes the radioresistance of some cancer cells (34, 38–40). SAS has been shown to induce ferroptosis

by inhibiting the *SLC7A11* transporter, a transmembrane protein responsible for cysteine uptake into cells (41). This inhibition leads to a depletion of GSH, a coenzyme essential for the function of *GPX4*. Previous studies have identified that SAS exhibits significant radiosensitizing effect both *in vivo* and *in vitro* (14, 20, 42). However, no research has yet explored whether SAS can synergistically treat DTC by enhancing the ferroptosis-inducing effects of ¹³¹I. In the current study, we treated DTC cells with a combination of ¹³¹I with SAS, and observed that the CI was below 1, indicative of a synergistic interaction. Additionally, cotreatment resulted in the depletion of GSH and the elevation of ROS and MDA levels in DTC cells, along with a marked decrease in the expression of *SLC7A11* and *GPX4*. Our study confirmed that *SLC7A11* is downregulated in DTC cells treated with ¹³¹I. Furthermore, SAS induces ferroptosis by inhibiting the activity of *SLC7A11*. Therefore, we propose that *SLC7A11* plays a crucial role in the synergistic effects of ¹³¹I and SAS on DTC cells. Accordingly, in this study, we knocked down the expression of *SLC7A11* in TPC-1 and FTC-133 cells and subsequently examined the changes in ferroptosis-related markers following exposure to ¹³¹I. The results revealed that, compared with cells without knockdown, cells with *SLC7A11* knockdown exhibited more pronounced ferroptosis-related responses, regardless of whether they were treated with ¹³¹I alone, SAS alone, or a combination of both agents. Furthermore, this study also demonstrated that SAS and ¹³¹I can exert synergistic antitumor effects through inducing ferroptosis *in vivo*. Overall, we suggest that SAS and ¹³¹I can synergistically induce lipid peroxidation and ferroptosis by depleting GSH through the inhibition of *SLC7A11*.

While our study demonstrates the potential of combining ^{131}I and SAS to induce ferroptosis in DTC, we acknowledge that the effects of this combination therapy on normal thyroid cells and GPX4 or SLC7A11 overexpression experiments on DTC cells were not explored. Although ^{131}I is a standard treatment for DTC with a well-established safety profile, and SAS selectively targets ferroptosis in cancer cells, the toxicity of this combination therapy on normal thyroid tissue remains an important consideration. Normal thyroid cells typically express lower levels of GPX4 and SLC7A11 compared to DTC cells, which may reduce their susceptibility to ferroptosis induction (22–25). However, further comparison and *in vivo* studies are required to assess the potential toxicity of ^{131}I and SAS on normal thyroid cells and to investigate effects of GPX4 or SLC7A11 overexpression on DTC cells, ensuring that this combination therapy can be applied safely within a therapeutic window. Additionally, given the crucial role of the NIS in iodine uptake by thyroid cancer cells, future research should focus on whether and how SAS affects NIS expression. This is vital for understanding the synergistic effects of combining ^{131}I and SAS in DTC cells.

Conclusions

Our study highlights that the combination of ^{131}I and SAS can synergistically induce lipid peroxidation and ferroptosis in DTC cells, leading to enhanced therapeutic efficacy. This suggests that targeting ferroptosis pathways, in conjunction with traditional therapies like ^{131}I , may provide a promising approach to improve the treatment of DTC.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Institutional Animal Care and Use Committee of General Hospital of Southern Theater Command (Ethics Approval Number: NBZQZY2024023). The study was conducted in accordance with the local legislation and institutional requirements.

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Author contributions

LL: Conceptualization, Data curation, Methodology, Writing – original draft. JZ: Conceptualization, Supervision, Writing – review & editing. XZ: Formal analysis, Resources, Writing – original draft. PW: Formal analysis, Methodology, Writing – original draft. MM: Software, Writing – original draft. BY: Validation, Visualization, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fonc.2025.1580828/full#supplementary-material>

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