

Norcantharidin induces ferroptosis via the suppression of NRF2/HO-1 signaling in ovarian cancer cells

XIAOYAN ZHU¹, XIAOHONG CHEN², LONGSHAN QIU¹, JIANHUA ZHU¹ and JIANCAI WANG¹

¹Department of Obstetrics and Gynecology, Jianhu Hospital Affiliated to Nantong University, Yancheng, Jiangsu 224700;

²Department of Gynecology, People's Hospital of Gansu Province, Lanzhou, Gansu 730000, P.R. China

Received January 7, 2022; Accepted May 19, 2022

DOI: 10.3892/ol.2022.13479

Abstract. Increasing evidence has indicated a crucial role of ferroptosis in ovarian cancer (OC). Norcantharidin (NCTD), a normethyl compound of cantharidin, is extensively used in clinical practice as an optional anticancer drug. However, whether NCTD leads to ferroptosis in OC has not been previously explored, at least to the best of our knowledge. In the present study, the effect of NCTD on SKOV3 and OVCAR-3 cells was evaluated. The experimental data of the present study revealed that NCTD significantly suppressed SKOV3 and OVCAR-3 cell viability in a concentration- and time-dependent manner. The results of *Cell Counting Kit-8* assay revealed that NCTD treatment decreased SKOV3 and OVCAR-3 cell viability. In comparison, pre-incubation with ferrostatin-1 (Fer-1) significantly reversed the NCTD-induced reduction in SKOV3 and OVCAR-3 cell viability; however, no changes in cell viability were observed when the SKOV3 and OVCAR-3 cells were treated with NCTD, in combination with the apoptosis inhibitor, Z-VAD-FMK, the ferroptosis inhibitor, necrostatin-1, and the autophagy inhibitor, 3-methyladenine. Additionally, it was observed that NCTD markedly enhanced reactive oxygen species production and malondialdehyde and ferrous ion levels in the SKOV3 and OVCAR-3 cells; however, pre-incubation with Fer-1 abolished these effects. Flow cytometry also demonstrated a significant increase in cell death following treatment of the SKOV3 and OVCAR-3 cells with NCTD; however, pre-incubation with Fer-1 also reversed these effects. *In vivo* experiments demonstrated that NCTD significantly reduced tumor volume and weight. More importantly, it was revealed that nuclear factor erythroid 2-related factor 2 (NRF2), heme oxygenase 1 (HO-1), glutathione peroxidase 4 (GPX4) and solute carrier family 7 member 11 (xCT) expression levels were significantly decreased following NCTD treatment.

Collectively, NCTD may represent a potent anticancer agent in OC cells, and NCTD-induced ferroptotic cell death may be achieved by inhibiting the NRF2/HO-1/GPX4/xCT axis.

Introduction

Ovarian cancer (OC) poses a serious threat to women's health and the incidence of ovarian cancer has increased to 11.7 per 100,000 women per year. Moreover, 30% of patients are diagnosed with advanced illness due to the recurrence of cancer and chemotherapy resistance (1). Tumor metastasis is one of the main characteristics of OC and the most important cause of mortality in patients bearing advanced tumors (2). Among patients with OC, ~90% do not survive due to metastasis-related complications (3). However, patients often suffer from cancer recurrence due to chemotherapy resistance after the initial treatment (4). Therefore, elucidating the underlying mechanisms through which cancer metastasis is regulated is of utmost importance (5).

Ferroptosis is an iron-dependent, lipid peroxidation-driven cell death cascade that is critical for the progression of anti-cancer therapies (6,7). Prostaglandin-endoperoxide synthase 2 (PTGS2), also known as cyclooxygenase-2 (Cox-2), is the key enzyme that catalyzes prostaglandin biosynthesis (8). It mainly functions both as a peroxidase and a dioxygenase (8). During ferroptosis, the significant upregulation of PTGS2 has been identified (9). Glutathione-specific γ -glutamylcyclotransferase 1 (CHAC1) has been reported to decrease glutathione (GSH) levels and enhance cystine-starvation-induced ferroptosis (10). Both PTGS2 and CHAC1 are markers of ferroptosis (8).

The transcription factor, nuclear factor erythroid 2-related factor 2 (NRF2), has been suggested as a major regulator of intracellular oxidation homeostasis and lipid peroxidation (11,12). There is increasing evidence to indicate that NRF2 is strongly associated with the process of ferroptosis (11,12). The silencing of NRF2 may significantly reduce the expression of solute carrier family 7 member 11 (SLC7A11; xCT) and heme oxygenase 1 (HO-1) (12,13). xCT is a key gene that has been suggested to contribute to 'iron overload-ferroptosis' (12). The knockdown of xCT expression has been reported to result in reduced cystine-dependent GSH peroxidase activity and increased reactive oxygen species (ROS) and malonaldehyde (MDA) production, subsequently resulting in cellular ferroptosis (13). GSH is a necessary substrate for

Correspondence to: Dr Jiancai Wang, Department of Obstetrics and Gynecology, Jianhu Hospital Affiliated to Nantong University, 666 Nanhuan Road, Jianhu, Yancheng, Jiangsu 224700, P.R. China
E-mail: 2016150068@jou.edu.cn

Key words: ovarian cancer, norcantharidin, ferroptosis, nuclear factor erythroid 2-related factor 2

glutathione peroxidase 4 (GPX4), which is a key antioxidant enzyme (14). The depletion of GPX4 has been demonstrated to disrupt the balance of oxygen homeostasis and result in ferroptosis (14). In addition, NRF2 has also been revealed to directly interact with GPX4, which ultimately results in ferroptosis, by inducing intracellular antioxidant system damage (15). Therefore, targeting NRF2 may be useful for the induction of ferroptosis-related therapy in cancer patients.

Norcantharidin (NCTD), a normethyl compound of cantharidin, is extensively used in clinical practice as an optional anticancer drug in China, considering advantages of easy synthesis, potent activity as compared with cantharidin, and limited side-effects (16,17). In recent years, increasing evidence has demonstrated that NCTD significantly suppresses tumor cell proliferation and migration both *in vitro* and *in vivo* (16,18). However, whether NCTD suppresses OC via ferroptosis has not been previously reported, at least to the best of our knowledge.

In the present study, the specific function and underlying mechanisms of NCTD were first explored in SKOV3 and OVCAR-3 cells. The findings presented herein may prove to be of therapeutic value for patients with OC.

Materials and methods

Cells and cell culture. SKOV3 and OVCAR-3 human OC cell lines were purchased from Procell Life Science & Technology Co., Ltd (cat. nos. CL-0215 and CL-0178). Short tandem repeat (STR) analysis was performed to confirm cell line authentication. SKOV3 or OVCAR-3 cells were cultured in McCoy's 5A or RPMI-1640 culture (Cytiva) supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc.), streptomycin (100 mg/ml; Invitrogen; Thermo Fisher Scientific, Inc.) and penicillin (100 U/ml; Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C in a humidified atmosphere, containing 5% CO₂.

Cell Counting Kit-8 (CCK-8) assay. In brief, the SKOV3 and OVCAR-3 cells were seeded in 96-well plates at a density of 2,000 cells/well, overnight. The SKOV3 and OVCAR-3 cells were then incubated with 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 µg/ml NCTD (cat. no. C7632-25MG; Sigma-Aldrich; Merck KGaA) for 24 h at 37°C. Subsequently, 10 µl CCK-8 reagent (Beijing Solarbio Science & Technology Co., Ltd.) were added to each well and the cells were incubated for 4 h at 37°C. Cell viability was determined at OD_{450 nm} using a microplate reader (NanoDrop OneC; Thermo Fisher Scientific, Inc.). Each sample evaluation was performed in triplicate. For the negative control (NC) group, only 200 µl PBS (Beijing Solarbio Science & Technology Co., Ltd.) were added to each well in the 96-well plate. The cell survival rate was calculated as follows (19): (Test group-NC)/(control group-NC) x100. The concentration that caused 50% growth inhibition (IC₅₀) was calculated according to a previous study (20).

In addition, the SKOV3 and OVCAR-3 cells were pre-incubated with different inhibitors to explore which type of cell death was mainly induced by NCTD, including 20 µM of the apoptosis inhibitor, Z-VAD-FMK (cat. no. HY-16658B; MedChemExpress), 1 µM of the ferroptosis inhibitor, ferrostatin-1 (Fer-1; cat. no. HY-100579;

MedChemExpress), 10 µM of the necrosis inhibitor, necrostatin-1 (Nec-1; cat. no. HY-15760; MedChemExpress), and 10 µM of the autophagy inhibitor, 3-methyladenine (3-MA; cat. no. HY-19312; MedChemExpress), for 1 h at 37°C. Subsequently, the SKOV3 and OVCAR-3 cells were treated with 10 and 20 µg/ml NCTD for an additional 24 h at 37°C. CCK-8 assay was carried out as described above.

2',7'-Dichlorofluorescein diacetate (DCFH-DA) staining. In brief, the SKOV3 and OVCAR-3 cells were seeded in 6-well plates at a density of 10⁵ cells/well overnight. The SKOV3 and OVCAR-3 cells were then pre-incubated with or without 1 µM Fer-1 (cat. no. HY-100579; MedChemExpress) at 37°C for 1 h followed by treatment with 10 or 20 µg/ml NCTD for a further 24 h at 37°C. Furthermore, the SKOV3 and OVCAR-3 cells were incubated with 20 µM H₂O₂ for 24 h at 37°C. Subsequently, 1 ml DCFH-DA (cat. no. HY-D0940; MedChemExpress) was added to each well at a final concentration of 10 µM for 20 min at 37°C. After washing with 2 ml serum-free DMEM culture medium (HyClone; GE Healthcare Life Sciences), 1 ml of DAPI (Beijing Solarbio Science & Technology Co., Ltd.) was added to each well at a final concentration of 100 ng/ml for 20 min at 37°C. Subsequently, all cells were washed with 2 ml serum-free DMEM culture medium (HyClone; GE Healthcare Life Sciences) and the fluorescence was observed under a fluorescence microscope (x20; Olympus Corporation).

Flow cytometric assay. The SKOV3 and OVCAR-3 cells were seeded in 6-well plates at a density of 10⁵ cells/well overnight. The SKOV3 and OVCAR-3 cells were then pre-incubated with or without 1 µM Fer-1 at 37°C for 1 h, followed by treatment with 10 or 20 µg/ml NCTD for an additional 24 h at 37°C. Subsequently, cell death was analyzed using an Annexin V/7-AAD double staining kit (cat. no. KGA1026; Nanjing KeyGen Biotech Co., Ltd.) according to the manufacturer's instructions. Briefly, the SKOV3 and OVCAR-3 cells were centrifuged at 1,000 x g for 3 min at 4°C and washed with PBS (Beijing Solarbio Science & Technology Co., Ltd.) thrice. The cells were then re-suspended using 500 µl 1X Annexin V Binding Buffer (cat. no. KGA1026; Nanjing KeyGen Biotech Co., Ltd.). Subsequently, 5 µl of the Annexin V/7-AAD reagent were added, and the cells were incubated for 10 min at room temperature. Following a 1-h incubation, cell death was determined using a BD FACSCalibur system (BD Biosciences), and data were analyzed using ModFit software version 4.1 (Verity Software House, Inc.). Annexin V⁺/7-AAD⁻ cells were considered alive cells (Q4), Annexin V⁺/7-AAD⁺ cells were considered early apoptotic cells (Q3), and Annexin V⁺/7-AAD⁺ cells were considered late apoptotic and necrotic cells (Q2).

Quantification of MDA, ROS, GSH and ferrous ion (Fe²⁺) contents. The SKOV3 and OVCAR-3 cells were seeded in 6-well plates at a density of 1x10⁵ cells/well overnight. The SKOV3 and OVCAR-3 cells were then pre-incubated with or without 1 µM Fer-1 at 37°C for 1 h, followed by treatment with 10 or 20 µg/ml NCTD for a further 24 h at 37°C. Subsequently, 10⁶ cells were treated in 100 µl RIPA buffer (Beijing Solarbio Science & Technology Co., Ltd.) at 4°C for 15 min. The intracellular MDA and Fe²⁺ contents were then

quantified using a Micro MDA assay kit (cat. no. BC0025; Beijing Solarbio Science & Technology Co., Ltd.) and Iron assay kit (cat. no. MAK025; Sigma-Aldrich; Merck KGaA), respectively, according to the manufacturer's instructions.

For the tumor tissues, MDA, ROS, GSH and Fe²⁺ levels were quantified using a Micro MDA assay kit (cat. no. BC0025; Beijing Solarbio Science & Technology Co., Ltd.), a Reactive Oxygen Species assay kit (cat. no. CA1401; Beijing Solarbio Science & Technology Co., Ltd.), a Micro Reduced Glutathione assay kit (cat. no. BC1175; Beijing Solarbio Science & Technology Co., Ltd.) and an Iron assay kit (cat. no. MAK025; Sigma-Aldrich; Merck KGaA), respectively, according to the manufacturer's instructions.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from the SKOV3 and OVCAR-3 cells using RNAVzol (Vigorous Biotechnology Beijing Co., Ltd.), according to the manufacturer's protocol. The concentration and purity of the RNA samples were determined by measuring the optical density ratio, OD260/OD280 using a Nanodrop One Ultra Micro Ultraviolet Spectrophotometer (ND-ONE-W(A30221); Thermo Fisher Scientific, Inc.). RT-qPCR was performed using a Takara PrimeScript™ One Step RT-PCR kit Ver 2.0 (cat. no. RR055A; Takara Bio, Inc.) according to the manufacturer's instructions. The following PCR reagents were used: 20 µl RNase Free ddH₂O, 25 µl 2X 1 step buffer, 2 µl PrimeScript 1 step enzyme mix, 1 µl forward primer, 1 µl reverse primer and 1 µl RNA template. The cycling conditions were as follows: 50°C for 30 min, 94°C for 2 min, 30 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min, followed by 72°C for 10 min. GAPDH was used as an internal control. Relative mRNA expression was normalized to GAPDH using the 2^{-ΔΔC_q} method (21). The primers used in the present study are listed in Table I.

Western blot analysis. Proteins were isolated from the SKOV3 and OVCAR-3 cells using a total protein extraction kit (Beijing Solarbio Science & Technology Co., Ltd.). A BCA protein assay kit (Pierce; Thermo Fisher Scientific, Inc.) was used to quantify the protein concentration. Protein samples (30 µg/lane) were then loaded onto 12% SDS-PAGE gels, and proteins were then transferred onto polyvinylidene difluoride (PVDF) membranes. Subsequently, protein was blocked with 8% skim milk (Pierce; Thermo Fisher Scientific, Inc.) in 0.1% Tris buffered saline with Tween-20 (TBST; OriGene Technologies, Inc.) for 2 h at room temperature. After washing with TBST thrice (5 min/wash), the membranes were incubated with primary antibodies against NRF2 (cat. no. 12721), HO-1 (cat. no. 26416), xCT (cat. no. 12691), GPX4 (cat. no. 52455), PTGS2 (cat. no. 12282) and GAPDH (cat. no. 5174), all of which were purchased from Cell Signaling Technology, Inc., and CHAC1 (cat. no. MA5-26311; Thermo Fisher Scientific, Inc.), overnight at 4°C. Primary antibodies were diluted with antibody diluent reagent solution (1:1,000; cat. no. 003218; Thermo Fisher Scientific, Inc.). The membranes were then incubated with anti-rabbit IgG-conjugated secondary antibody (1:5,000 dilution; cat. no. ZB-2301; OriGene Technologies, Inc.) at room temperature, for 1 h. Immobilon Western Chemilum HRP (cat. no. WBKLS0500; MilliporeSigma) was used to visualize the antibody-antigen interactions. ImageJ

Table I. Sequences of primers used in the present study.

| Primer name | Sequence (5'-3') |
|-------------|------------------------|
| CHAC1-Fw | AGCAGATATGGTGGGTGGCT |
| CHAC1-Rv | GGAATTCCTCCAGGGCTATGGA |
| PTGS2-Fw | GAGGGATCTGTGGATGCTTCG |
| PTGS2-Rv | AAACCCACAGTGCTTGACAC |
| NRF2-Fw | TGCCCCCTGGAAGTGTCAAAC |
| NRF2-Rv | CCCCTGAGATGGTGACAAGG |
| HO-1-Fw | AGGGAATTCTCTTGGCTGGC |
| HO-1-Rv | GCTGCCACATTAGGGTGTCT |
| xCT-Fw | TCTCCCTATGCCAAACAGGTG |
| xCT-Rv | TTCCCACTGGGCTAAATGGAC |
| GPX4-Fw | GTTTTCGCCCAAGGACATCG |
| GPX4-Rv | TGAGGAACTGTGGAGAGACG |
| GAPDH-Fw | TTCAACAGCGACACCCACTC |
| GAPDH-Rv | CTGGTGGTCCAGGGGTCTTA |

Fw, forward; Rv, reverse; PTGS2, prostaglandin-endoperoxide synthase 2; CHAC1, glutathione-specific γ-glutamylcyclotransferase 1; NRF2, nuclear factor erythroid 2-related 2; HO-1, heme oxygenase 1; xCT, solute carrier family 7 member 11; GPX4, glutathione peroxidase 4.

software version 1.8.0 (National Institutes of Health) was also applied for densitometric analysis.

Human cell line xenografts. Athymic nu/nu female mice aged 6-8 weeks (n=9; mean weight, 20.21±1.54 g) were purchased from the specific pathogen SPF (Beijing) Lab Animals Technology Co. Ltd. Mice were housed in a temperature- and humidity-controlled environment (20-24°C, 45-55% humidity), with free access to food and water and in groups of three. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC ID: 17-3256) at Nantong University and performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Briefly, the mice were anesthetized with isoflurane (Sigma-Aldrich; Merck KGaA) inhalation at a concentration of 2.5% for anesthetic induction and then at 1.5% for anesthetic maintenance (22-24). SKOV3 cells (10⁶ cells per mouse in 100 µl PBS) were implanted subcutaneously into the right flanks of 6- to 8-week-old female nu/nu mice. Animal health and behavior were monitored each day. The mice were randomly divided three treatment groups (n=3 mice in each group, n=9 in total) as follows: i) The vehicle control [4% DMSO with 30% polyethylene glycol (PEG) 300 and double distilled H₂O]; ii) NCTD (100 mg/kg/day); and iii) NCTD (200 mg/kg/day) for 4 weeks. Tumor weight and tumor volume were determined at the end of a 4-week period. To ensure the mice were fully anesthetized, regular and even breathing was looked for. All mice were euthanized by decapitation under deep isoflurane anesthesia (5%) (25). The successful induction of anesthesia was confirmed by observation of the following parameters: respiration decreased in frequency and increased in depth, eyelid and cornea reflexes disappeared, muscle tension and the reflex response reduced, and no response to pain or other stimulation. Death was confirmed by a cessation of breathing.

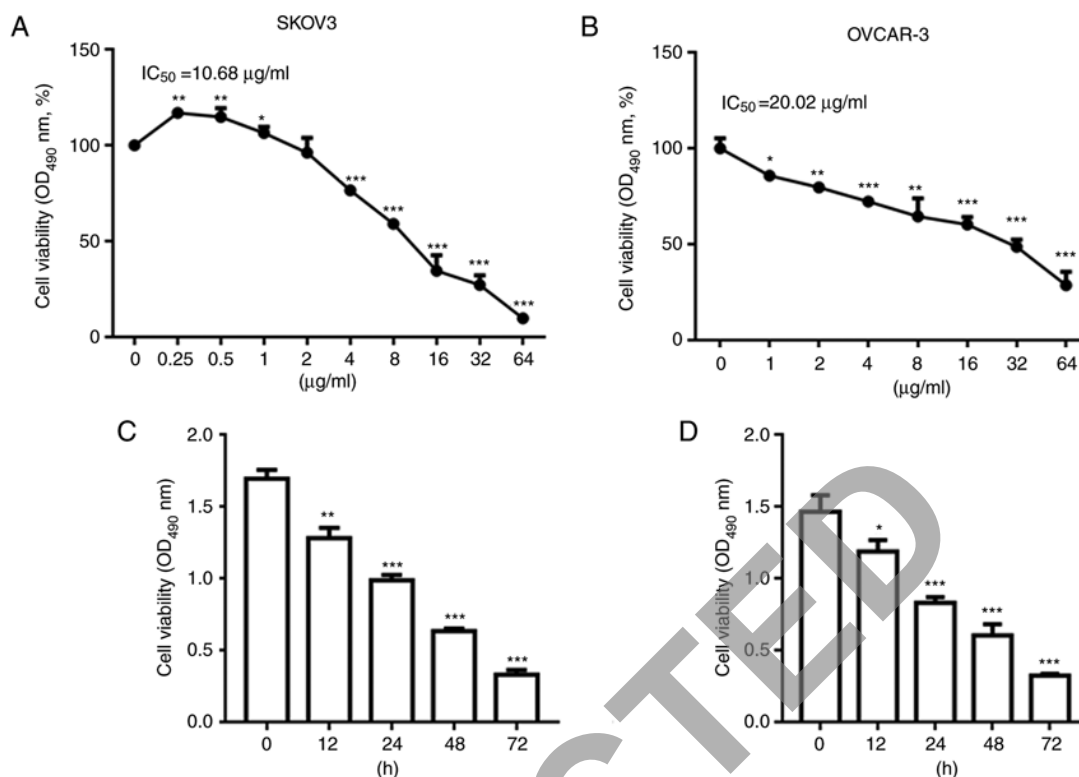


Figure 1. NCTD decreases ovarian cancer cell viability in a concentration- and time-dependent manner. SKOV3 and OVCAR-3 cells were treated with NCTD at 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 $\mu\text{g/ml}$ for 24 h. NCTD treatment decreased (A) SKOV3 and (B) OVCAR-3 cell viability in a concentration-dependent manner. SKOV3 and OVCAR-3 cells were treated with 10 and 20 $\mu\text{g/ml}$ NCTD for 0, 12, 24, 48 and 72 h. The results of CCK-8 assay demonstrated that NCTD reduced (C) SKOV3 and (D) OVCAR-3 cell viability in a time-dependent manner. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. * $P < 0.05$ and ** $P < 0.01$ and, *** $P < 0.001$ vs. control (no treatment). NCTD, norcantharidin.

Tumor volumes were calculated using the following formula: $a^2 \times b \times 0.4$, where 'a' corresponds to the smallest diameter and 'b' to the diameter perpendicular to 'a'. GSH, ROS, Fe^{2+} and MDA levels were also assessed in the tumor tissues with the aforementioned kits.

Statistical analysis. Statistical analysis was performed with SPSS version 13.0 (SPSS Inc.), and all data were quantitative. Statistical analysis was performed using an unpaired Student's t-test for comparisons between two groups and one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for comparisons of more than two groups. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

NCTD decreases OC cell viability in a concentration- and time-dependent manner. Firstly, the SKOV3 and OVCAR-3 cells were treated with NCTD at concentrations of 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 $\mu\text{g/ml}$ for 24 h. As depicted in Fig. 1A and B, treatment with NCTD decreased SKOV3 and OVCAR-3 cell viability in a concentration-dependent manner. However, NCTD increased cell viability at 0.25 $\mu\text{g/ml}$ in comparison with the untreated sample. We hypothesize that at 0.25 $\mu\text{g/ml}$, NCTD promoted SKOV3 cell proliferation due to a stress response. The IC_{50} values of NCTD were 10.68 and 20.02 $\mu\text{g/ml}$ for the SKOV3 and OVCAR-3 cells, respectively. Subsequently, the SKOV3 and OVCAR-3 cells were treated with 10 and 20 $\mu\text{g/ml}$ NCTD. The results of CCK-8 assay revealed

that NCTD reduced SKOV3 and OVCAR-3 cell viability in a time-dependent manner at 12, 24, 48 and 72 h (Fig. 1C and D).

NCTD induces ferroptosis in OC cells. SKOV3 and OVCAR-3 cells were pre-incubated with different inhibitors, including Z-VAD-FMK (an apoptosis inhibitor), Fer-1 (a ferroptosis inhibitor), Nec-1 (a necrosis inhibitor) and 3-MA (an autophagy inhibitor), and then treated with NCTD. The results of CCK-8 assay revealed that treatment with NCTD significantly decreased SKOV3 and OVCAR-3 cell viability (Fig. 2A and B). In comparison, pre-incubation with Fer-1 significantly reversed the NCTD-induced reduction in SKOV3 and OVCAR-3 cell viability. However, no changes in cell viability were observed when the SKOV3 and OVCAR-3 cells were treated with NCTD in combination with Z-VAD-FMK, Nec-1 and 3-MA (Fig. 2A and B). Additionally, DCFH-DA staining indicated that H_2O_2 increased ROS accumulation (Fig. 2C and D). NCTD markedly enhanced ROS production in the SKOV3 and OVCAR-3 cells, with the pre-incubation with Fer-1 abolishing these effects (Fig. 2C and D). Flow cytometric assays also demonstrated a significant increase in cell death following treatment of the SKOV3 and OVCAR-3 cells with NCTD. However, cell death significantly decreased when the SKOV3 and OVCAR-3 cells were pre-incubated with Fer-1 (Fig. 2E and F). These observations indicated that NCTD mainly contributed to OC cell death by inducing ferroptosis.

NCTD increases intracellular MDA and Fe^{2+} levels. The intracellular MDA and Fe^{2+} contents were then quantified. The

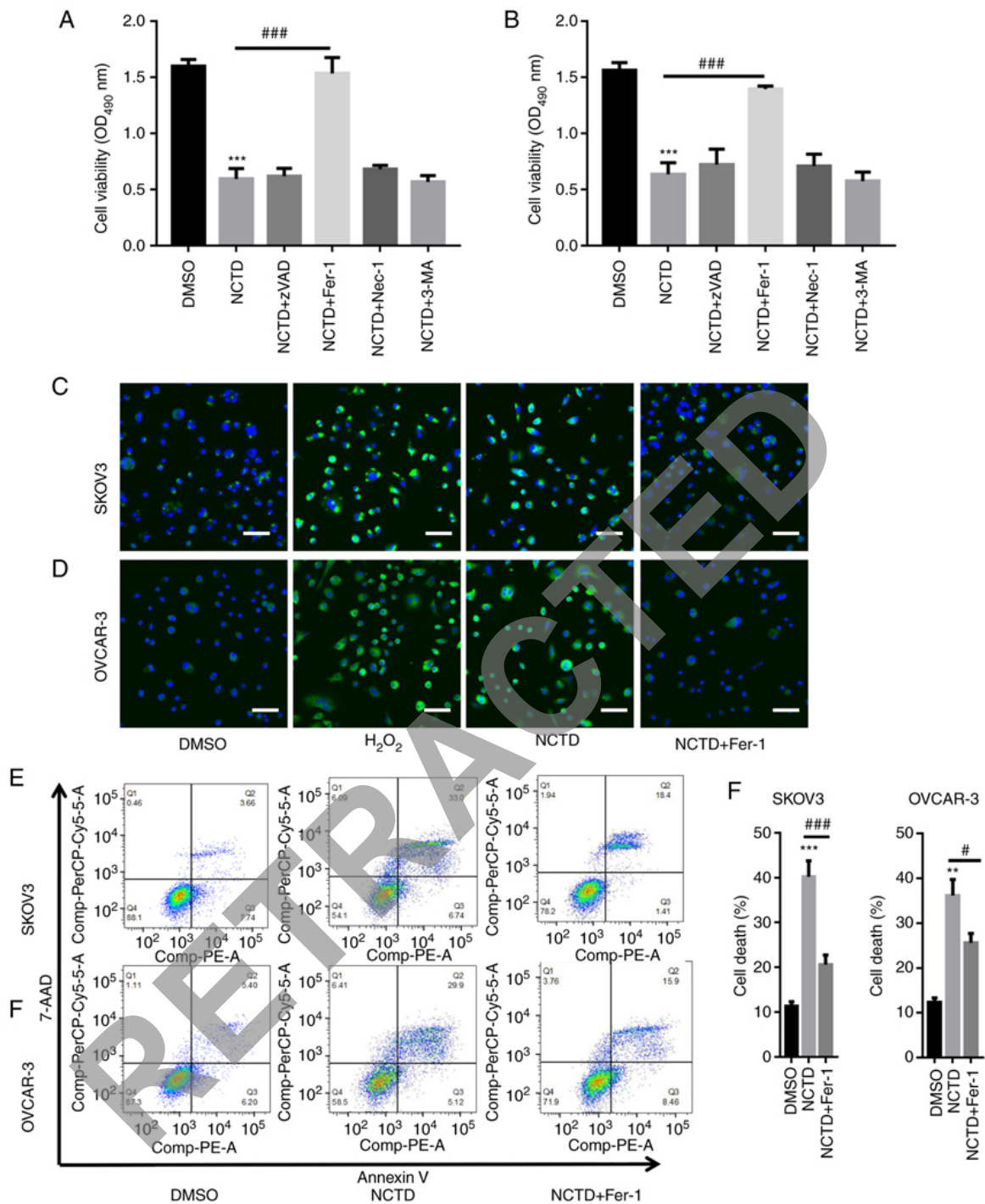


Figure 2. NCTD induces ferroptosis in ovarian cancer cells. SKOV3 and OVCAR-3 cells were pre-incubated with various inhibitors, including 20 μ M Z-VAD-FMK (an apoptosis inhibitor), 1 μ M Fer-1 (a ferroptosis inhibitor), 10 μ M Nec-1 (a necrosis inhibitor) and 10 μ M 3-MA (an autophagy inhibitor), for 1 h. Subsequently, SKOV3 and OVCAR-3 cells were treated with 10 and 20 μ g/ml NCTD for an additional 24-h time period. The results of CCK-8 assay depicted that pre-incubation with Fer-1 significantly reversed the NCTD-induced downregulation of (A) SKOV3 and (B) OVCAR-3 cell viability. DCFH-DA staining indicated that NCTD evidently enhanced reactive oxygen species production in (C) SKOV3 and (D) OVCAR-3 cells, with Fer-1 pre-incubation abolishing these effects (scale bar, 50 μ m). Flow cytometry also revealed a significant increase in cell death following treatment of the SKOV3 and OVCAR-3 cells with NCTD, with the effects decreasing when (E) SKOV3 and (F) OVCAR-3 cells were pre-incubated with Fer-1. One way ANOVA followed by Tukey's post hoc test was applied for statistical analysis. ** P <0.01 and *** P <0.001 vs. control (DMSO); * P <0.05 and *** P <0.001 vs. NCTD. NCTD, norcantharidin; Fer-1, ferrostatin-1; Nec-1, necrostatin-1; 3-MA, 3-methyladenine; DCFH-DA, 2',7'-dichlorofluorescein diacetate.

results revealed that NCTD significantly increased the intracellular MDA and Fe²⁺ levels in the SKOV3 and OVCAR-3 cells (Fig. 3A-D). However, following pre-incubation with Fer-1, the MDA and Fe²⁺ levels were significantly decreased in the SKOV3 and OVCAR-3 cells (Fig. 3A-D). PTGS2 and

CHAC1 ferroptosis marker mRNA levels were also quantified. The results of RT-qPCR analysis indicated that NCTD significantly increased the PTGS2 and CHAC1 mRNA levels in SKOV3 and OVCAR-3 cells. By contrast, pre-incubation with Fer-1 significantly reversed these effects (Fig. 3E-H).

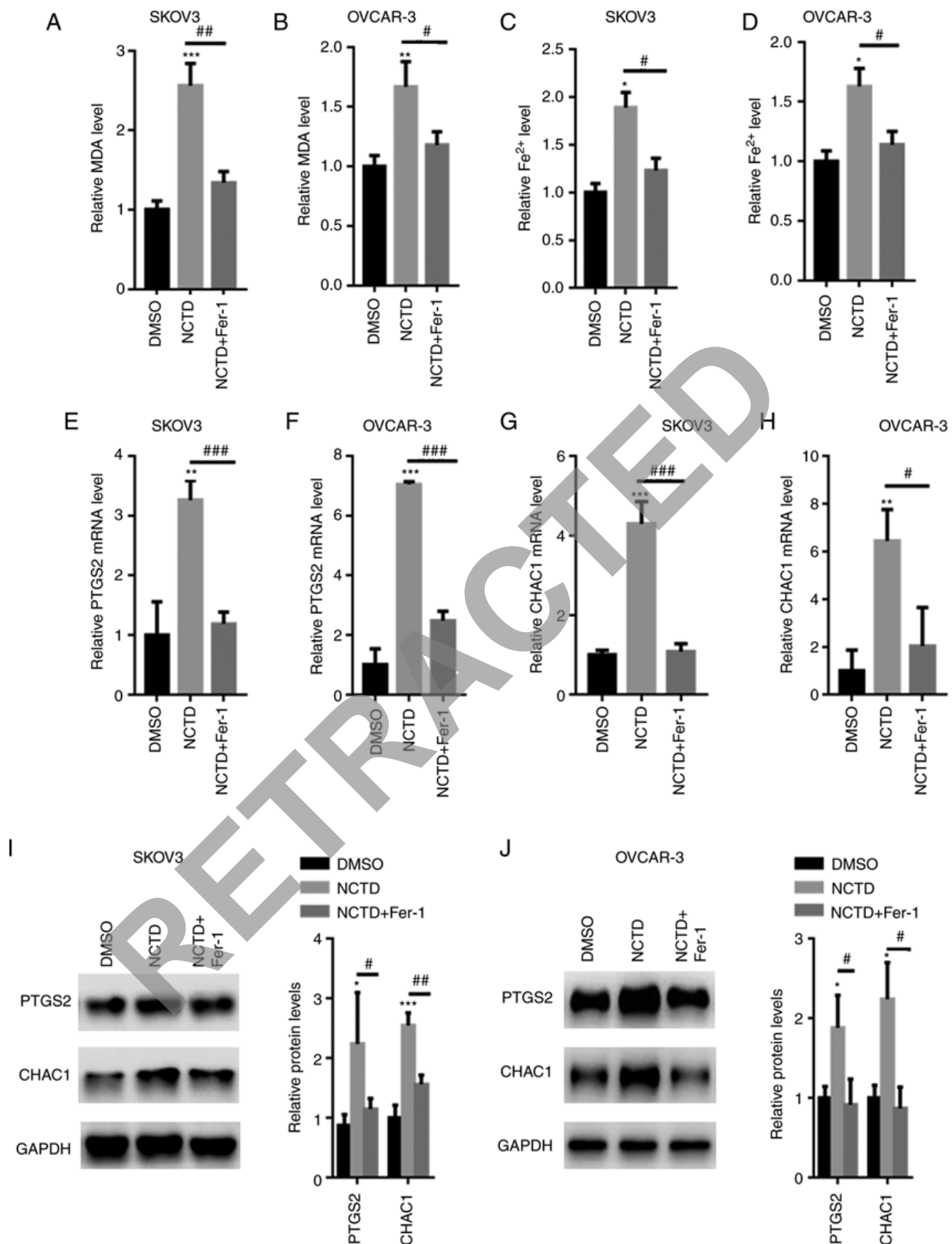


Figure 3. NCTD increases the intracellular Fe²⁺ content in SKOV3 and OVCAR-3 cells. SKOV3 and OVCAR-3 cells were pre-incubated with 1 μ M Fer-1 for 1 h. The SKOV3 and OVCAR-3 cells were then treated with 10 and 20 μ M NCTD for an additional 24-h time period. Pre-incubation with Fer-1 reduced the NCTD-induced upregulation of MDA contents in (A) SKOV3 and (B) OVCAR-3 cells. Fer-1 decreased the NCTD-induced elevation in the Fe²⁺ contents in (C) SKOV3 and (D) OVCAR-3 cells. Reverse transcription-quantitative PCR analysis demonstrated that the PTGS2 and CHAC1 mRNA levels were decreased in the (E and G) SKOV3 and (F and H) OVCAR-3 cells, pre-incubated with Fer-1. Western blot analysis indicated that the expression of PTGS2 and CHAC1 was enhanced in (I) SKOV3 and (J) OVCAR-3 cells treated with NCTD, compared with that of the control. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. * P <0.05, ** P <0.01 and *** P <0.001 vs. control (DMSO); # P <0.05, ## P <0.01 and ### P <0.001 vs. NCTD. NCTD, norcantharidin; Fer-1, ferrostatin-1; MDA, malonaldehyde; Fe²⁺, ferrous ion; PTGS2, prostaglandin-endoperoxide synthase 2; CHAC1, glutathione-specific γ -glutamylcyclotransferase 1.

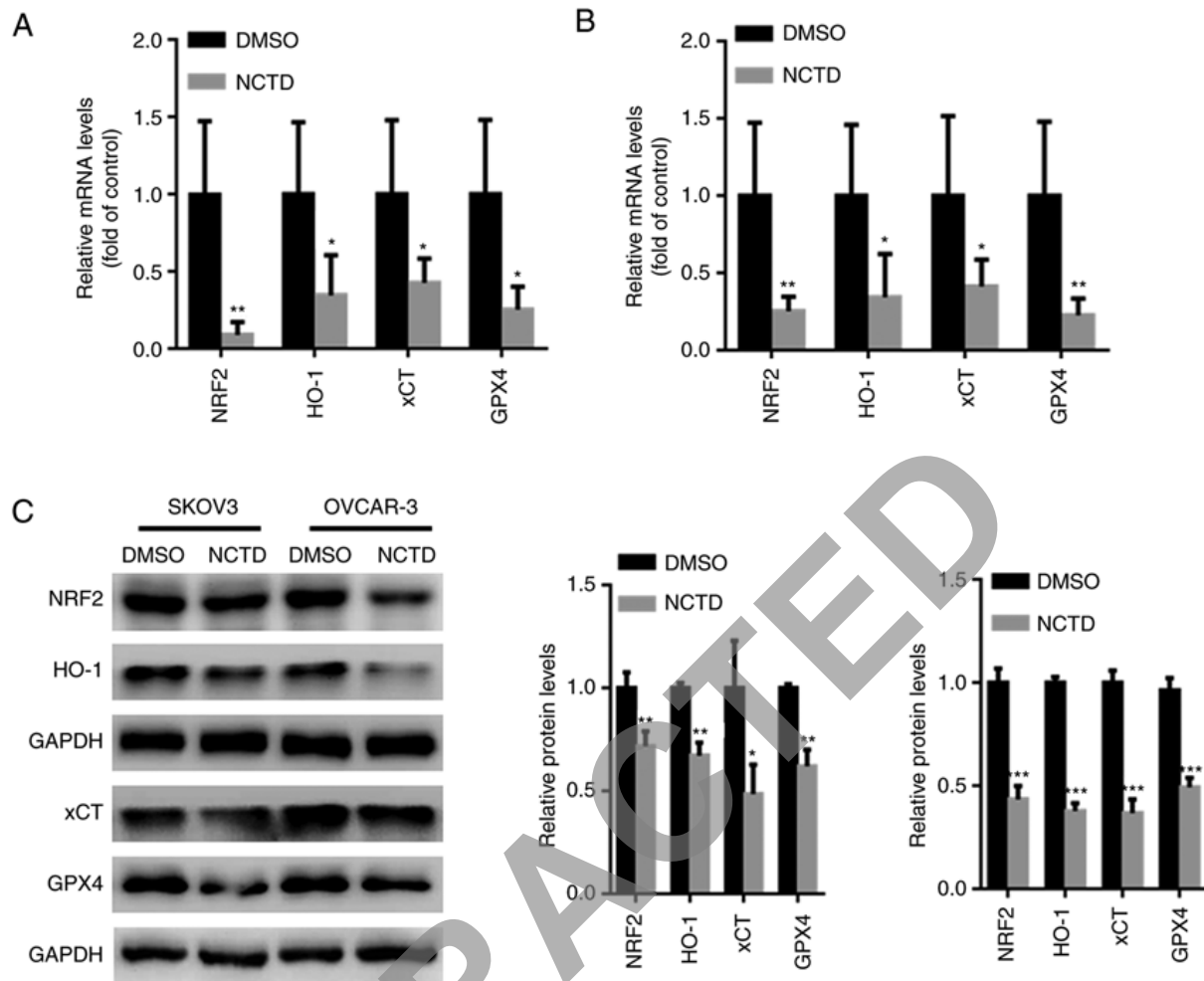


Figure 4. NCTD suppresses NRF2/HO-1 signaling in ovarian cancer cells. SKOV3 and OVCAR-3 cells were treated with 10 and 20 μ M NCTD for 24 h. Reverse transcription-quantitative PCR analysis indicated that the mRNA levels of NRF2, HO-1, xCT and GPX4 were significantly suppressed in the (A) SKOV3 and (B) OVCAR-3 cells treated with NCTD. (C) Western blot analysis demonstrated that the NRF2, HO-1, xCT and GPX4 protein levels were significantly suppressed in SKOV3 and OVCAR-3 cells incubated with NCTD. An unpaired Student's t-test was used for statistical analysis. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. control (DMSO). NCTD, norcantharidin; NRF2, nuclear factor erythroid 2-related 2; HO-1, heme oxygenase 1; xCT, solute carrier family 7 member 11; GPX4, glutathione peroxidase 4.

Western blot analysis also revealed that PTGS2 and CHAC1 expression levels were increased in the SKOV3 and OVCAR-3 cells treated with NCTD, compared with those of the untreated control, with the application of Fer-1 eliminating these effects (Fig. 3I and J).

NCTD suppresses NRF2/HO-1 signaling in OC cells. Previous studies have indicated that NRF2 may be critical for ferroptosis-related cancer cell death (26,27). Hence, the effects of NCTD on NRF2 activation in SKOV3 and OVCAR-3 cells were examined in the present study. The results of RT-qPCR revealed that the NRF2, HO-1, xCT and GPX4 mRNA levels were significantly decreased in the SKOV3 and OVCAR-3 cells treated with NCTD (Fig. 4A and B). Moreover, western blot analysis demonstrated that the NRF2, HO-1, xCT and GPX4 protein levels were significantly suppressed in the SKOV3 and OVCAR-3 cells incubated with NCTD (Fig. 4C).

NCTD suppresses tumor growth in vivo. The *in vivo* experimental results demonstrated that treatment with 100 and 200 mg/kg NCTD for 4 weeks significantly

suppressed tumor volume and weight (Fig. 5A-C). Treatment with 100 and 200 mg/kg NCTD also significantly decreased the GSH content in the tumors (Fig. 5D). In comparison, the MDA, ROS and Fe^{2+} contents were significantly increased in the tumor tissues of SKOV3-injected nu/nu female mice following treatment with 100 and 200 mg/kg NCTD treatment for 4 weeks (Fig. 5E-G). Western blot analysis also demonstrated that NCTD decreased the NRF2, HO-1, xCT and GPX4 protein levels, whereas it increased the PTGS2 and CHAC1 expression levels in SKOV3 tumor xenografts (Fig. 5H).

Discussion

OC is the leading cause of gynecological malignancy-related mortality worldwide, and >75% of affected women are diagnosed at advanced stages of OC, presenting with indistinct and non-specific symptoms (28). Following diagnosis, the 5-year survival rate of patients with late-stage disease is limited to less than one-third (29). Metastatic disease following surgery and intensive platinum-taxane chemotherapy is suggested to be the major cause of mortality (29).

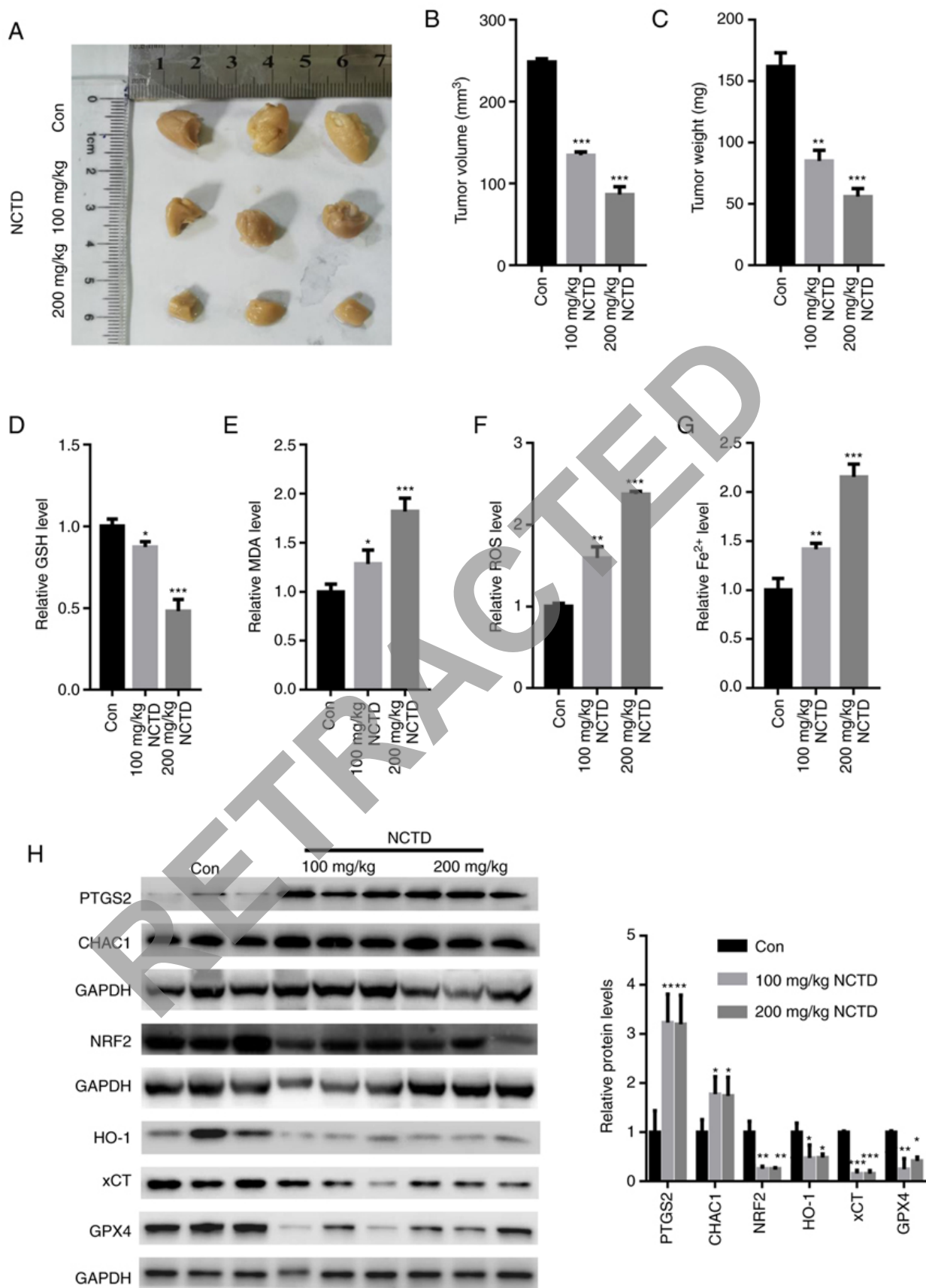


Figure 5. NCTD suppresses tumor growth *in vivo*. (A) Representative tumor images. Treatment with 100 and 200 mg/kg NCTD for 4 weeks significantly suppressed (B) tumor volume and (C) weight. (D) Treatment with 100 and 200 mg/kg NCTD significantly increased the GSH content in the tumors. The (E) MDA, (F) ROS and (G) Fe²⁺ contents were significantly decreased following treatment with 100 and 200 mg/kg NCTD for 4 weeks. (H) The results of western blot analysis demonstrated that NCTD reduced the NRF2, HO-1, xCT and GPX4 protein levels, elevating the expression of PTGS2 and CHAC1 in SKOV3-derived xenografts. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. *P<0.05, **P<0.01 and ***P<0.001 vs. control. Con, control; NCTD, norcantharidin; ROS, reactive oxygen species; MDA, malondialdehyde; Fe²⁺, ferrous ion; NRF2, nuclear factor erythroid 2-related 2; CHAC1, glutathione-specific γ -glutamylcyclotransferase 1; HO-1, heme oxygenase 1; xCT, solute carrier family 7 member 11; GPX4, glutathione peroxidase 4.

Previous studies have confirmed that ferroptosis-induced cell death plays a crucial role in the development of OC (30-32). NCTD prevents tumorigenesis by suppressing cell proliferation, and inducing apoptosis and cell cycle arrest in various tumors, including hepatocellular carcinoma, breast cancer and urinary bladder carcinoma (33-35). However, whether NCTD induces ferroptosis in OC has not been explored to date, at least to the best of our knowledge. In the present study, the results of CCK-8 assay indicated that NCTD suppressed OC cell viability in a time- and concentration-dependent manner. In the SKOV3 cells, a slight increase in cell viability was observed at 1 $\mu\text{g/ml}$. Hence, 0.25 and 0.5 $\mu\text{g/ml}$ NCTD were used for a detailed exploration. However, in the OVCAR-3 cells, a stable downward trend of cell viability was noted following treatment with 1 $\mu\text{g/ml}$ NCTD. In a previous study, a slight increase in cell viability following NCTD treatment was also observed in human MG63 osteosarcoma cells (36). In line with this finding, it was revealed that at a concentration of 0.25 and 0.5 $\mu\text{g/ml}$, the SKOV3 cells exhibited enhanced proliferation, statistically significant as compared with the control group. It is suggested that this may be attributed to not having reached the dose-effect association in SKOV3 cells. Evidently, at low concentrations (0.25 and 0.5 $\mu\text{g/ml}$), NCTD did not inhibit the proliferation of SKOV3 cells. Apart from the dose-effect association, it was hypothesized that the SKOV3 cells may activate a stress reaction in response to low concentration of NCTD treatment, thereby promoting cell proliferation. However, this phenomenon was observed in the OVCAR-3 cells. These data indicated that OVCAR-3 cells were more sensitive to NCTD treatment.

Multiple mechanisms underlying ferroptotic cell death have been reported (37,38). In non-small cell lung cancer, NCTC has been demonstrated to trigger apoptotic cell death, by activating mitophagy-mediated autophagy signaling (39). In prostate cancer cells, NCTD has been revealed to induce endoplasmic reticulum stress-mediated apoptosis via suppressing SIRT1 (40). However, NCTD has not been previously reported to induce ferroptosis in other cell types. To explore which form of cell death was induced by NCTD, in the present study, NCTD was combined with different cell death inhibitors, including z-VAD, Nec-1, 3-MA and Fer-1. The results of the present study revealed that only Fer-1, a ferroptosis inhibitor, abolished NCTD-induced cell death in SKOV3 and OVCAR-3 cells, whereas the other inhibitors did not. Furthermore, NCTD significantly increased ROS, MDA and Fe^{2+} production. However, pre-incubation with Fer-1 abolished these effects. Flow cytometry also confirmed that NCTD increased SKOV3 and OVCAR-3 cell death. In contrast to other types of cell death, including apoptosis and necrosis, it is suggested that NCTD may have activated ferroptosis in OC cells due to the characteristics of iron dependence and accumulation of lipid ROS.

Ferroptosis is a new form of regulated cell death in the intracellular microenvironment that is induced by redox state disorder regulated via NRF2, a key regulator in iron chelators and lipophilic antioxidants (11,12). NRF2 contributes to ferroptosis by regulating its downstream target genes, including HO-1, xCT and GPX4 (12,13). One of the roles of GPX4 is to remove lipid ROS production, and GPX4 suppression results in the accumulation of lipid ROS, thereby inducing ferroptosis in

HEK293T cells and rat intestinal epithelial IEC-6 cells (41,42). xCT is composed of a cystine/glutamate transporter, and it mainly acts to provide a substrate for GSH synthesis (43). xCT inhibition decreases the capacity of GPX4 to clear lipid ROS via inadequate glutathione synthesis and ultimately induces cell death (43,44). The present study demonstrated that NCTD suppressed the expression of NRF2, HO-1, GPX4 and xCT, suggesting that NCTD-induced ferroptotic cell death may be achieved by inhibiting the NRF2/HO-1/GPX4/xCT axis.

The role of NCTD in ovarian tumor growth was also explored *in vivo*. It was demonstrated that NCTD significantly suppressed tumor volume and weight. The GSH contents were significantly increased following NCTD treatment, whereas the ROS, MDA and Fe^{2+} levels were significantly reduced. Consistent with *in vitro* findings, the expression of NRF2, HO-1, GPX4 and xCT was significantly suppressed in nude mice treated with NCTD compared with that in control mice.

However, there are limitations to the present study. For instance, NRF2 overexpression or knockdown experiments are required to verify its involvement in NCTD-induced ferroptosis. Furthermore, concerning animal experiments, a control drug should have been used as a positive control. For instance, positive drugs, including erastin, should be used, which is a well-known ferroptosis activator and has been reported to inhibit OC cell growth via inducing ferroptosis *in vitro* and *in vivo* (31,45,46).

In conclusion, NCTD may represent a potent anticancer agent in OC cells and may induce OC cell death by blocking NRF2-related ferroptosis.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Jiangsu Maternal and Child Health Research Project (Project no. f201629).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

XZ performed the experiments, analyzed the data and wrote the manuscript. XC, LQ and JZ performed a part of the RT-qPCR experiments. JW designed the experiments, analyzed the data and gave final approval of the version to be published. All authors read and approved the final manuscript. XZ and JW confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC ID: 17-3256) at Nantong University and performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Wang Z, Guo E, Yang B, Xiao R, Lu F, You L and Chen G: Trends and age-period-cohort effects on mortality of the three major gynecologic cancers in China from 1990 to 2019: Cervical, ovarian and uterine cancer. *Gynecol Oncol* 163: 358-363, 2021.
- Liu Y, Ren CC, Yang L, Xu YM and Chen YN: Role of CXCL12-CXCR4 axis in ovarian cancer metastasis and CXCL12-CXCR4 blockade with AMD3100 suppresses tumor cell migration and invasion in vitro. *J Cell Physiol* 234: 3897-3909, 2019.
- Xu Y, Ma YH, Pang YX, Zhao Z, Lu JJ, Mao HL and Liu PS: Ectopic repression of receptor tyrosine kinase-like orphan receptor 2 inhibits malignant transformation of ovarian cancer cells by reversing epithelial-mesenchymal transition. *Tumour Biol* 39: 1010428317701627, 2017.
- Yang C, Xia BR, Zhang ZC, Zhang YJ, Lou G and Jin WL: Immunotherapy for ovarian cancer: Adjuvant, combination, and neoadjuvant. *Front Immunol* 11: 577869, 2020.
- Sarkar S, Malekshah OM, Nomani A, Patel N and Hatefi A: A novel chemotherapeutic protocol for peritoneal metastasis and inhibition of relapse in drug resistant ovarian cancer. *Cancer Med* 7: 3630-3641, 2018.
- Zhu L, Chen D, Zhu Y, Pan H, Xia D, Cai T, Lin H, Lin J, Jin X, Wu F, *et al*: GPX4-regulated ferroptosis mediates S100-Induced experimental autoimmune hepatitis associated with the Nrf2/HO-1 signaling pathway. *Oxid Med Cell Longev* 2021: 6551069, 2021.
- Zhang Z, Fu C, Liu J, Sai X, Qin C, Di T, Yang Y, Wu Y and Bian T: Hypermethylation of the Nrf2 promoter induces ferroptosis by inhibiting the Nrf2-GPX4 axis in COPD. *Int J Chron Obstruct Pulmon Dis* 16: 3347-3362, 2021.
- Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, Clemons PA, Shamji AF, Clish CB, *et al*: Regulation of ferroptotic cancer cell death by GPX4. *Cell* 156: 317-331, 2014.
- Xiao X, Jiang Y, Liang W, Wang Y, Cao S, Yan H, Gao L and Zhang L: miR-212-5p attenuates ferroptotic neuronal death after traumatic brain injury by targeting Ptg2. *Mol Brain* 12: 78, 2019.
- Chen MS, Wang SF, Hsu CY, Yin PH, Yeh TS, Lee HC and Tseng LM: CHAC1 degradation of glutathione enhances cystine-starvation-induced necroptosis and ferroptosis in human triple negative breast cancer cells via the GCN2-eIF2 α -ATF4 pathway. *Oncotarget* 8: 114588-114602, 2017.
- Dong H, Xia Y, Jin S, Xue C, Wang Y, Hu R and Jiang H: Nrf2 attenuates ferroptosis-mediated IIR-ALI by modulating TERT and SLC7A11. *Cell Death Dis* 12: 1027, 2021.
- Feng L, Zhao K, Sun L, Yin X, Zhang J, Liu C and Li B: SLC7A11 regulated by NRF2 modulates esophageal squamous cell carcinoma radiosensitivity by inhibiting ferroptosis. *J Transl Med* 19: 367, 2021.
- Dong H, Qiang Z, Chai D, Peng J, Xia Y, Hu R and Jiang H: Nrf2 inhibits ferroptosis and protects against acute lung injury due to intestinal ischemia reperfusion via regulating SLC7A11 and HO-1. *Aging (Albany NY)* 12: 12943-12959, 2020.
- Song X and Long D: Nrf2 and ferroptosis: A new research direction for neurodegenerative diseases. *Front Neurosci* 14: 267, 2020.
- Ma CS, Lv QM, Zhang KR, Tang YB, Zhang YF, Shen Y, Lei HM and Zhu L: NRF2-GPX4/SOD2 axis imparts resistance to EGFR-tyrosine kinase inhibitors in non-small-cell lung cancer cells. *Acta Pharmacol Sin* 42: 613-623, 2021.
- Zhou J, Ren Y, Tan L, Song X, Wang M, Li Y, Cao Z and Guo C: Norcantharidin: Research advances in pharmaceutical activities and derivatives in recent years. *Biomed Pharmacother* 131: 110755, 2020.
- Zeng D, Xiao Z, Xu Q, Luo H, Wen L, Tang C, Shan Y, Tian J, Wei J and Li Y: Norcantharidin protects against renal interstitial fibrosis by suppressing TWEAK-mediated Smad3 phosphorylation. *Life Sci* 260: 118488, 2020.
- Wang Y, Jiang W, Li C, Xiong X, Guo H, Tian Q and Li X: Autophagy suppression accelerates apoptosis induced by norcantharidin in cholangiocarcinoma. *Pathol Oncol Res* 26: 1697-1707, 2020.
- Guan Z, Chen J, Li X and Dong N: Tanshinone IIA induces ferroptosis in gastric cancer cells through p53-mediated SLC7A11 down-regulation. *Biosci Rep* 40: BSR20201807, 2020.
- Matsumura T, Kasai M, Hayashi T, Arisawa M, Momose Y, Arai I, Amagaya S and Komatsu Y: a-glucosidase inhibitors from paraguay natural medicine, nangapiry, the leaves of *Eugenia uniflora*. *Pharm Biol* 38: 302-307, 2000.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402-408, 2001.
- Baird RC, Li S, Wang H, Naga Prasad SV, Majdalany D, Perni U and Wu Q: Pregnancy-associated cardiac hypertrophy in corin-deficient mice: Observations in a transgenic model of preeclampsia. *Can J Cardiol* 35: 68-76, 2019.
- Sun J, Hao W, Fillmore N, Ma H, Springer D, Yu ZX, Sadowska A, Garcia A, Chen R, Muniz-Medina V, *et al*: Human Relaxin-2 fusion protein treatment prevents and reverses isoproterenol-induced hypertrophy and fibrosis in mouse heart. *J Am Heart Assoc* 8: e013465, 2019.
- Sundaresan NR, Gupta M, Kim G, Rajamohan SB, Isbatan A and Gupta MP: Sirt3 blocks the cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense mechanisms in mice. *J Clin Invest* 119: 2758-2771, 2009.
- Kopeček JA, McTiernan CF, Chen X, Zhu J, Mburu M, Feroze R, Whitehurst DA, Lavery L, Cyriac J and Villanueva FS: Ultrasound and microbubble-targeted delivery of a microRNA inhibitor to the heart suppresses cardiac hypertrophy and preserves cardiac function. *Theranostics* 9: 7088-7098, 2019.
- Roh JL, Kim EH, Jang H and Shin D: Nrf2 inhibition reverses the resistance of cisplatin-resistant head and neck cancer cells to artesunate-induced ferroptosis. *Redox Biol* 11: 254-262, 2017.
- Gai C, Liu C, Wu X, Yu M, Zheng J, Zhang W, Lv S and Li W: MT1DP loaded by folate-modified liposomes sensitizes erastin-induced ferroptosis via regulating miR-365a-3p/NRF2 axis in non-small cell lung cancer cells. *Cell Death Dis* 11: 751, 2020.
- Jayson GC, Kohn EC, Kitchener HC and Ledermann JA: Ovarian cancer. *Lancet* 384: 1376-1388, 2014.
- Doubeni CA, Doubeni AR, and Myers AE: Diagnosis and management of ovarian cancer. *Am Fam Physician* 93: 937-944, 2016.
- Li L, Qiu C, Hou M, Wang X, Huang C, Zou J, Liu T and Qu J: Ferroptosis in ovarian cancer: A novel therapeutic strategy. *Front Oncol* 11: 665945, 2021.
- Cheng Q, Bao L, Li M, Chang K and Yi X: Erastin synergizes with cisplatin via ferroptosis to inhibit ovarian cancer growth in vitro and in vivo. *J Obstet Gynaecol Res* 47: 2481-2491, 2021.
- Ye Y, Dai Q, Li S, He J and Qi H: A Novel Defined risk signature of the ferroptosis-related genes for predicting the prognosis of ovarian cancer. *Front Mol Biosci* 8: 645845, 2021.
- Yeh CB, Hsieh MJ, Hsieh YH, Chien MH, Chiou HL and Yang SF: Antimetastatic effects of norcantharidin on hepatocellular carcinoma by transcriptional inhibition of MMP-9 through modulation of NF- κ B activity. *PLoS One* 7: e31055, 2012.
- Liu D, Shi P, Yin X, Chen Z and Zhang X: Effect of norcantharidin on the human breast cancer Bcap-37 cells. *Connect Tissue Res* 53: 508-512, 2012.
- Yu CC, Ko FY, Yu CS, Lin CC, Huang YP, Yang JS, Lin JP and Chung JG: Norcantharidin triggers cell death and DNA damage through S-phase arrest and ROS-modulated apoptotic pathways in TSGH 8301 human urinary bladder carcinoma cells. *Int J Oncol* 41: 1050-1060, 2012.
- Mei L, Sang W, Cui K, Zhang Y, Chen F and Li X: Norcantharidin inhibits proliferation and promotes apoptosis via c-Met/Akt/mTOR pathway in human osteosarcoma cells. *Cancer Sci* 110: 582-595, 2019.
- Yi R, Wang H, Deng C, Wang X, Yao L, Niu W, Fei M and Zhaba W: Dihydroartemisinin initiates ferroptosis in glioblastoma through GPX4 inhibition. *Biosci Rep* 40: BSR20193314, 2020.

38. Li X, Zou Y, Xing J, Fu YY, Wang KY, Wan PZ and Zhai XY: Pretreatment with roxadustat (FG-4592) attenuates folic acid-induced kidney injury through antiapoptosis via Akt/GSK-3 β /Nrf2 pathway. *Oxid Med Cell Longev* 2020: 6286984, 2020.
39. Liu Z, Li B, Cao M and Jiang J: Norcantharidin triggers apoptotic cell death in non-small cell lung cancer via a mitophagy-mediated autophagy pathway. *Ann Transl Med* 9: 971, 2021.
40. Wu MH, Hui SC, Chen YS, Chiou HL, Lin CY, Lee CH and Hsieh YH: Norcantharidin combined with paclitaxel induces endoplasmic reticulum stress mediated apoptotic effect in prostate cancer cells by targeting SIRT7 expression. *Environ Toxicol* 36: 2206-2216, 2021.
41. Vuckovic AM, Bosello Travain V, Bordin L, Cozza G, Miotto G, Rossetto M, Toppo S, Venerando R, Zaccarin M, Maiorino M, *et al*: Inactivation of the glutathione peroxidase GPx4 by the ferroptosis-inducing molecule RSL3 requires the adaptor protein 14-3-3 ϵ . *FEBS Lett* 594: 611-624, 2020.
42. Wang S, Liu W, Wang J and Bai X: Curculigoside inhibits ferroptosis in ulcerative colitis through the induction of GPX4. *Life Sci* 259: 118356, 2020.
43. Lee N, Carlisle AE, Peppers A, Park SJ, Doshi MB, Spears ME and Kim D: xCT-Driven expression of GPX4 determines sensitivity of breast cancer cells to ferroptosis inducers. *Antioxidants (Basel)* 10: 317, 2021.
44. Wang H, Peng S, Cai J and Bao S: Silencing of PTPN18 induced ferroptosis in endometrial cancer cells through p-P38-mediated GPX4/xCT down-regulation. *Cancer Manag Res* 13: 1757-1765, 2021.
45. Yang Y, Luo M, Zhang K, Zhang J, Gao T, Connell DO, Yao F, Mu C, Cai B, Shang Y and Chen W: Nedd4 ubiquitylates VDAC2/3 to suppress erastin-induced ferroptosis in melanoma. *Nat Commun* 11: 433, 2020.
46. Zhou HH, Chen X, Cai LY, Nan XW, Chen JH, Chen XX, Yang Y, Xing ZH, Wei MN, Li Y, *et al*: Erastin reverses ABCB1-mediated docetaxel resistance in ovarian cancer. *Front Oncol* 9: 1398, 2019.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.

RETRACTED