Icariin-curcumol promotes ferroptosis in prostate cancer cells through Nrf2/HO-1 signaling

WEN SHENG[1,2], BONAN LI[3,4], TIANSONG SUN[3,4], CONGXU ZHU[3,4], YINGQIU LI[5] and WENJING XU[6]

[1]School of Rehabilitation Medicine and Health Care; [2]School of Traditional Chinese Medicine, Hunan University of Medicine, Huaihua, Hunan 418000; [3]School of Integrated Chinese and Western Medicine; [4]Andrology Laboratory; [5]Medical School, Hunan University of Chinese Medicine, Changsha, Hunan 410208; [6]Department of Dermatology, The First Affiliated Hospital of Hunan University of Chinese Medicine, Changsha, Hunan 410021, P.R. China

Received June 12, 2023; Accepted February 23, 2024

DOI: 10.3892/etm.2024.12519

Abstract. Ferroptosis is a form of regulatory cell death that relies on iron and reactive oxygen species (ROS) to inhibit tumors. The present study aimed to investigate whether icariin-curcumol could be a novel ferroptosis inducer in tumor inhibition. Various concentrations of icariin-curcumol were used to stimulate prostate cell lines (RWPE-2, PC-3, VCAP and DU145). Small interfering negative control (si-NC) and si-nuclear factor erythroid 2-related factor 2 (Nrf2) were used to transfect DU145 cells. Cell viability was determined by using cell counting kit-8. Ferroptosis-related factor levels were analyzed using western blotting and reverse transcription-quantitative PCR. Enzyme-linked immunosorbent assays were used to assess the ferrous (Fe^{2+}), glutathione and malondialdehyde (MDA) content. The ROS fluorescence intensity was assessed using flow cytometry. DU145 cells were most sensitive to icariin-curcumol concentration. The Fe^{2+} content, ROS fluorescence intensity and MDA level gradually increased, while solute carrier family 7 member 11 (SLC7A11) level, glutathione peroxidase 4 (GPX4) level, GSH content, Nrf2 and heme oxygenase-1 (HO-1) decreased with icariin-curcumol in a dose-dependent manner. After si-Nrf2 was transfected, the cell proliferation ability, SLC7A11 and GPX4 levels declined compared with the si-NC group. In contrast to the control group, the icariin + curcumol group showed reductions in Nrf2 and HO-1 levels, cell proliferation, SLC7A11 and GPX4 levels, with an increase in Fe^{2+} content and ROS fluorescence intensity. Overexpression of Nrf2 reversed the regulation observed in the icariin + curcumol group. Icariin-curcumol induced ferroptosis in PCa cells, mechanistically by inhibiting the Nrf2/HO-1 signaling pathway. Icariin-curcumol could be used as a new type of ferroptosis inducer to treat PCa effectively.

Correspondence to: Dr Yingqiu Li, Medical School, Hunan University of Chinese Medicine, 300 Xueshi Road, Yuelu, Changsha, Hunan 410208, P.R. China
E-mail: lyq@hnucm.edu.cn

Dr Wenjing Xu, Department of Dermatology, The First Affiliated Hospital of Hunan University of Chinese Medicine, 95 Shaoshan Middle Road, Yuhua, Changsha, Hunan 410021, P.R. China
E-mail: zyfy_wj@hnucm.edu.cn

Key words: icariin-curcumol, nuclear factor erythroid 2-related factor 2/heme oxygenase-1, prostate cancer, ferroptosis

Introduction

Prostate cancer (PCa) is a frequently occurring malignancy of the male genitourinary system (1). Cancerous cells manifest an elevated requirement for iron in comparison to ordinary non-cancerous cells in order to stimulate proliferation (2). This reliance on iron renders cancerous cells more susceptible to iron-catalyzed necrosis, commonly termed ferroptosis (3). This suggests the promise of targeting ferroptosis as a therapeutic intervention. Ferroptosis has been implicated in several human diseases, including PCa (4). Increasing evidence indicates that ferroptosis inhibits tumor growth (5) and the employment of ferroptosis inducers or targeting ferroptosis-related genes may represent promising strategies for treating castration-resistant PCa (CRPC) (6). Traditional Chinese medicine has multiple targets and can regulate various signaling pathways, including ADAMTS18 (7), reactive oxygen species (ROS) (8), nuclear factor erythroid 2-related factor 2 (Nrf2) (9) and glutathione peroxidase 4 (GPX4) (10), thereby modulating ferroptosis. Therefore, the use of Chinese medicine to induce ferroptosis in PCa cells may represent a promising avenue for future investigation.

A study has shown a correlation between the Nrf2 signaling pathway and the mechanisms underpinning ferroptosis (11). Nrf2 signaling is involved in ferroptosis through the regulation of glutathione (GSH) homeostasis, mitochondrial function and lipid metabolism (6,12). The p62-Kelch-like ECH-associated protein 1 (Keap1)-Nrf2 axis mainly governs the transcription of downstream genes associated with the metabolism of iron and ROS metabolism, thus regulating the occurrence of ferroptosis (13-15). Suppression of heme oxygenase-1 (HO-1) in hormone-refractory PCa cells reduces intracellular ROS levels and inhibits a variety of carcinogenic properties (16). Further investigation is required to fully understand the regulation of prostate cancer cells by the Nrf2/HO-1 signaling pathway,

despite indications that suggest ferroptosis inducers may promote ferroptosis in cancer cells through said pathway (17,18).

Icariin (19) and curcumol are both extracted from traditional Chinese herbs, which are widely available and cost-effective. The former is the primary active component of Epimedium (20), and is used in traditional Chinese medicine (21). The latter, a sesquiterpene found in *Curcuma zedoaria*, exhibits neuroprotective, anti-inflammatory and anti-tumor properties (22). Curcumol modulates the PDK1/AKT/mTOR signaling pathway via miR-9, influencing the onset of PCa (23). It can act in tandem with a number of synthetic drugs as an antibiotic or anti-cancer agent (24). The present authors' initial study indicated that curcumol effectively inhibits the proliferation, invasion and migration of PC3 and 22RV1 cells, mitigating the progression of PCa via the miR-125a/STAT3 axis (25). Icariin and curcumol act synergistically to modulate the miR-7/mTOR/SREBP1 pathway, inducing autophagy in PCa cells and affecting lipid metabolism (26). Our latest experiments suggested that icariin and curcumol increase Fe^{2+} and malondialdehyde (MDA) contents while decreasing GSH levels, whereas treatment with Fer-1, an iron death inhibitor, reversed these indexes (26). However, it remains to be seen whether icariin-curcumol interferes with PCa progression by promoting ferroptosis of PCa cells.

Although ferroptosis has gradually become a research hotspot in the field of cancer, the mechanism through which traditional Chinese medicine regulates the ferroptosis of PCa cells remains to be explored. The present study hypothesized that icariin and curcumol regulate the ferroptosis of CRPC cells through the Nrf2/HO-1 signaling axis, ultimately disrupting the progression of CRPC.

Materials and methods

Cell culture and screening. Human prostate normal cells RWPE-2 (cat. no. AW-CNH470; Abiowell) and human PCa cells PC-3 (cat. no. AW-CCH111; Abiowell), VCAP (cat. no. AW-CCH367; Abiowell), DU145 (cat. no. AW-CCH043; Abiowell) were cultured in a special medium with 10% fetal bovine serum (FBS; cat. no. 10099141; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (cat. no. SV30010; Cytiva). These cells were cultured in an incubator (cat. no. DH-160I; SANTN Instrument Co., Ltd.) with saturated humidity at 37˚C and 5% CO_2. Icariin (cat. no. N1705; APeXBIO Technology LLC) and curcumol (cat. no. N1743, APeXBIO Technology LLC) were dissolved in the solvent (PEG400:ethanol:normal saline volume ratio of 57.1:14.3:28.6) and the drug concentrations were 30 μg/ml and 50 μg/ml (27), respectively. Logarithmically grown cells were treated with icariin and curcumol at different concentrations and the cells were categorized into four distinct factions: Control, 10 mM [4.4 ml (30 μg) icariin and 21 μl (50 μg) curcumol], 20 mM [2.2 ml (30 μg) icariin and 10.5 μl (50 μg) curcumol] and 40 mM [1.1 ml (30 μg) icariin and 5.25 μl (50 μg) curcumol]. These cells were cultivated in the presence of icariin-curcumol at concentrations ranging from 0-40 mM for intervals of 0, 24 and 48 h.

Cell grouping and treatment. The DU145 cells were selected and treated with 0 mM, 10, 20 and 40 mM icariin-curcumol for 24 h for subsequent studies. Then, the DU145 cells were further divided into three groups: Control, small interfering negative control (si-NC; 5'-TTCTCCGAACGTGTCACG T-3') and si-Nrf2 (5'-GTATGTCAATCAAATCCAT-3'). In the control group, DU145 was cultured in DMEM medium in a 37˚C with 5% CO_2 content and proper ventilation to keep the intracellular environment moist. Lipofectamine 2000 (cat. no. 11668019, Thermo Fisher Scientific, Inc.) was used for transfecting experiments. Cells in the si-NC group were transfected with 5 μl of 100 nM si-NC and cultured at 37˚C for 48 h according to the manufacturer's protocols of Lipofectamine 2000, while the control group was added a reagent of the same volume without siRNA. Similarly, cells in the si-Nrf2 group were transfected with the si-Nrf2 and cultured at 37˚C for 48 h. Follow-up experiments were performed after 48 h.

Then, DU145 cells were divided into four groups: Control, icariin + curcumol, icariin + curcumol + overexpression (oe)-NC and icariin + curcumol + oe-Nrf2. In the control group, DU145 was cultured in DMEM medium in a 37˚C incubator with 5% CO_2 content and proper ventilation to keep the intracellular environment moist. In the icariin + curcumol group, DU145 cells were treated with 40 mM icariin-curcumol for 24 h at 37˚C, while the control group received solvent of the same volume without icariin and curcumol. In the icariin + curcumol +oe-NC and icariin + curcumol+oe-Nrf2 groups, before being treated with 40 mM icariin-curcumol for 24 h at 37˚C, the DU145 cells were transfected with the NC plasmid and Nrf2 overexpression plasmid at 37˚C for 48 h, respectively. Plasmid transfection was performed by mixing the plasmid with Lipofectamine 2000 Reagent to form a transfection complex. The transfection complex was incubated on DU145 cells at 37˚C for 6 h. Subsequently, it was transferred to the culture medium and continued to be incubated at 37˚C for 48 h. The control and icariin + curcumol were added transfection reagents of the same volume without plasmids. The siRNAs and plasmids were obtained from Abiowell. Following treatment, the cells were collected for other detection after 48 h.

Cell Counting Kit-8 (CCK-8). The cells were seeded into 96-well plates at a density of $5x10^3$ cells/well according to the instructions for CCK-8 (cat. no. AWC0114a; Abiowell). CCK8 solution with the complete medium configuration was added to each well and the cells were incubated for 4 h at 37˚C with 5% CO_2. The OD values at 450 nm were then analyzed with a multipurpose microplate analyzer (cat. no. MB-530; HEALES, China).

Reverse transcription-quantitative (RT-q) PCR. $1x10^3$ cells were collected with 1 ml of TRIzol reagent (cat. no. 15596-026; Thermo Fisher Scientific, Inc.) per group, following the manufacturer's instructions. The mRNA was subsequently reverse transcribed into cDNA, using the mRNA reverse transcription kit (cat. no. CW2569; CWBio) according to the manufacturer's instructions. qPCR was performed in a fluorescence quantitative RCP instrument (QuantStudio1, Thermo, USA). The reaction conditions were denaturation at 95˚C for 10 min, then a total of 40 cycles, including denaturation at 94˚C for 15 s, annealing and extension at 60˚C for 30 s. The primers were designed using Primer Premier 5 software (Premier Biosoft, USA) and are in Table I. For normalization purposes,

Table I. Sequences of the primers.

Gene name	Forward (5'-3')	Reverse (5'-3')
p53	ACATTCTCCACTTCTTGTTCCCC	CTCCCCACAACAAAACACCAGT
SLC7A11	CTCCAGGTTATTCTATGTTGCGTCT	CAAAGGGTGCAAAACAATAACAGC
GPX4	CGCCTTTGCCGCCTACTGAAGC	AACCATGTGCCCGTCGATGTCC
Nrf2	CAACTACTCCCAGGTTGCCC	AGTGACTGAAACGTAGCCGAA
HO-1	AAACTTCAGAGGGGGCGAAG	GACAGCTGCCACATTAGGGT
β-actin	ACCCTGAAGTACCCCATCGAG	AGCACAGCCTGGATAGCAAC

β-actin was utilized as a reference gene. The experiments were performed using a fluorescence quantitative PCR apparatus (cat. no. PIKOREAL96; Thermo Fisher Scientific, Inc.). The $2^{-\square\square Ct}$ method) was used to calculate the relative transcription level of the target gene (28). The experiment was repeated three times.

Western blotting. Total protein was extracted from cells in each group using the RIPA lysate (cat. no. AWB0136; Abiowell), following the guidelines provided by the manufacturer. A BCA concentration assay kit (ab102536; Abcam) was employed to quantify protein concentrations. Prepared protein samples were added to the loading buffer with a volume of 200 μl of protein per lane. Electrophoresis was performed with 10% gel to segregate proteins which were further transferred to nitrocellulose membranes. The membranes were blocked in a blocking buffer containing 5% skimmed milk at 25˚C for 1 h. Membrane were incubated overnight at 4˚C with primary antibodies p53 (cat. no. 10442-1-AP; Proteintech Group, Inc.), solute carrier family 7 member 11 (SLC7A11; cat. no. ab175186; Abcam), GPX4 (23KDa, 67763-1-Ig; Proteintech Group, Inc.), Nrf2 (110KDa, cat. no. 16396-1-AP; Proteintech Group, Inc.), HO-1 (33KDa, cat. no. 10701-1-AP; Proteintech Group, Inc.) and β-actin (42KDa, cat. no. 66009-1-Ig; Proteintech Group, Inc.). The membranes were then incubated with the diluted secondary antibodies HRP goat anti-mouse IgG (cat. no. SA00001-1; Proteintech Group, Inc.) and HRP goat anti-rabbit IgG (cat. no. SA00001-2; Proteintech Group, Inc.) in 0.05% PBST (PBS with 0.05% Tween 20) for 90 min at room temperature. Following incubation, the membranes were treated with ECL chemiluminescence solution (cat. no. AWB0005; Abiowell) for 1 min. Finally, the membranes were analyzed in a chemiluminescence imaging system (Chemiscope6100; Clinx Science Instruments Co., Ltd.). β-actin was used as an internal reference protein. The optical density values of the protein bands were determined using ImageJ software (Version 1.48v, NIH, USA).

The detection of Fe^{2+}, GSH and MDA content. The evaluation of intracellular Fe^{2+} level, GSH and MDA content was performed using the iron colorimetric assay kit (cat. no. E-BC-K881-M; Elabscience Biotechnology, Inc.), GSH (cat. no. A006-2-1, Nanjing Jiancheng Bioengineering Institute) and MDA (cat. no. A003-1, Nanjing Jiancheng Bioengineering Institute) detection kits were used following the instructions provided by the manufacturer. The membranes were immersed in Superecl plus (k-12045-d50, advansta, USA) for luminescence development. β-actin was used as the internal reference.

Flow cytometry. Levels of ROS within cells were assessed using a ROS kit (cat. no. S0033S; Beyotime Institute of Biotechnology). First, cells were digested to obtain a cell suspension. Then, the cell suspension was incubated in a medium without serum, ultimately reaching a concentration of 40 mM for 20 min at 37˚C. The staining lasted for 1 h at 15˚C. A flow cytometer (CytoFLEX A00-1-1102; Beckman Coulter, Inc.) and its corresponding software CytExpert (Version 2.4; Beckman Coulter, Inc.) were used to detect the ROS fluorescence intensity.

Statistical analysis. All measurement data were expressed as mean ± standard deviation. Each test was repeated independently three times. All data were analyzed by using SPSS 26.0 software (IBM Corp.). Kolmogorov-Smirnov test and exploratory descriptive statistics test were used to analyze whether the data conformed to a normal distribution and homogeneity of variance. The measurement data obeyed the normal distribution and homogeneity of variance. One-way ANOVA and Tukey's post-hoc test were used to compare data. Dunnett's test was used for comparison of multiple time points and Bonferroni was used for post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Selection of prostate cells and icariin-curcumol concentration. Human PCa cell lines (PC-3, VCAP, DU145) and a human normal prostate cell line (RWPE-2) were selected as subjects. Icariin-curcumol concentrations were set at 0, 10, 20 and 40 mM. With the increase of icariin-curcumol concentration, proliferation of RWPE-2 cells showed no significant change (Fig. 1A), while that of PC-3 cells (Fig. 1B), VCAP cells (Fig. 1C) and DU145 cells (Fig. 1D) was noticeably decreased. According to IC_{50} data, DU145 cells (62.27) were the most sensitive to icariin-curcumol concentration compared with PC-3 (92.06) and VCAP (82.26; Fig. 1). The findings revealed that icariin-curcumol decreased the proliferation of PCa cells, although it exerted no effect on the normal prostate cells. Finally, it was found that Nrf2 and HO-1 protein levels were highest in DU145 cells compared with other cell lines RWPE-2, PC-3 and VCAP (Fig. 1E). Therefore, DU145 cells were chosen for subsequent studies.

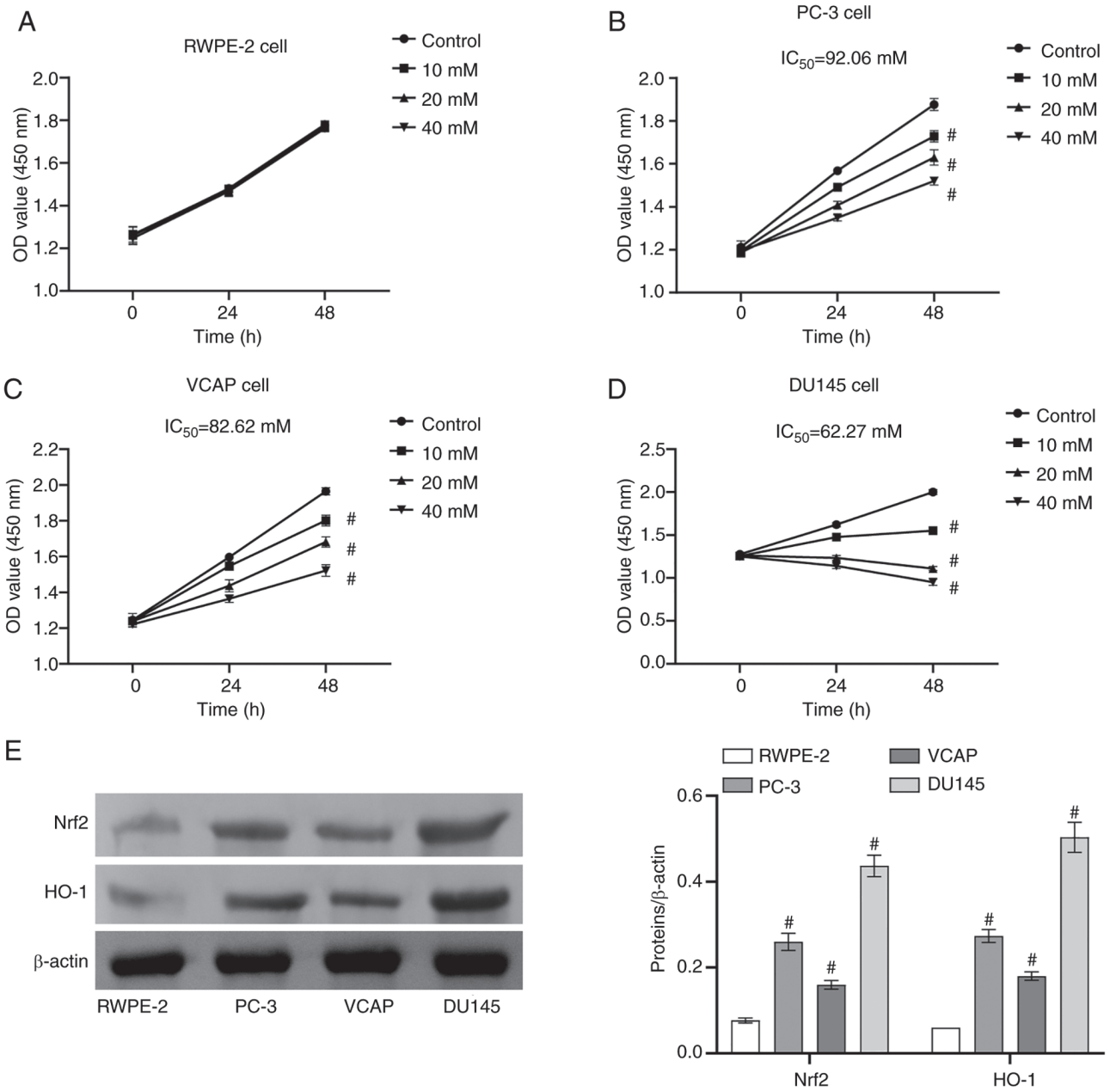

Figure 1. Screening of prostate cells and icariin-curcumol concentrations. The proliferation of (A) RWPE-2, (B) PC-3, (C) VCAP and (D) DU145 cell proliferation was detected using CCK8. (E) The Nrf2 and HO-1 levels in cells were analyzed by using western blotting. #P<0.05 vs. control; n=3. Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1.

Icariin-curcumol promoted ferroptosis in DU145 cells. Next, DU145 cells were stimulated with different concentrations of icariin-curcumol to elucidate the effect of icariin-curcumol on the ferroptosis process in PCa cells. With the increase of icariin-curcumol concentration, the p53 level was gradually increased, while the protein and mRNA levels of SLC7A11 and GPX4 were gradually decreased (Fig. 2A and B). With the increase of icariin-curcumol concentration, Fe^{2+} content (Fig. 2C) and ROS fluorescence intensity (Fig. 2D and E) were gradually increased. Notably, GSH content was decreased gradually with the increase of icariin-curcumol concentration (Fig. 2F). In addition, the MDA level was also increased gradually with the increase of icariin-curcumol concentration (Fig. 2F). With the increase of icariin-curcumol concentration, the protein levels and mRNA levels of Nrf2 and HO-1 gradually decreased (Fig. 2H). These results indicated that icariin-curcumol promoted ferroptosis in PCa cells.

Silencing of Nrf2/HO-1 signaling axis promoted ferroptosis in DU145 cells. The present study continued to use DU145 cells as research objects to explore the function of the Nrf2/HO-1 signaling axis on the ferroptosis of PCa cells. The protein and mRNA levels of Nrf2 and HO-1 in the si-Nrf2 group were markedly lower than the si-NC group (Fig. 3A and B). Moreover, DU145 cell proliferation ability was signally lower in the si-Nrf2 group than in the si-NC group (Fig. 3C). Furthermore, in contrast to the si-NC group, the proteins and mRNA levels of SLC7A11 and GPX4 in the si-Nrf2 group notably declined, while the p53 level was markedly enhanced (Fig. 3D and E).

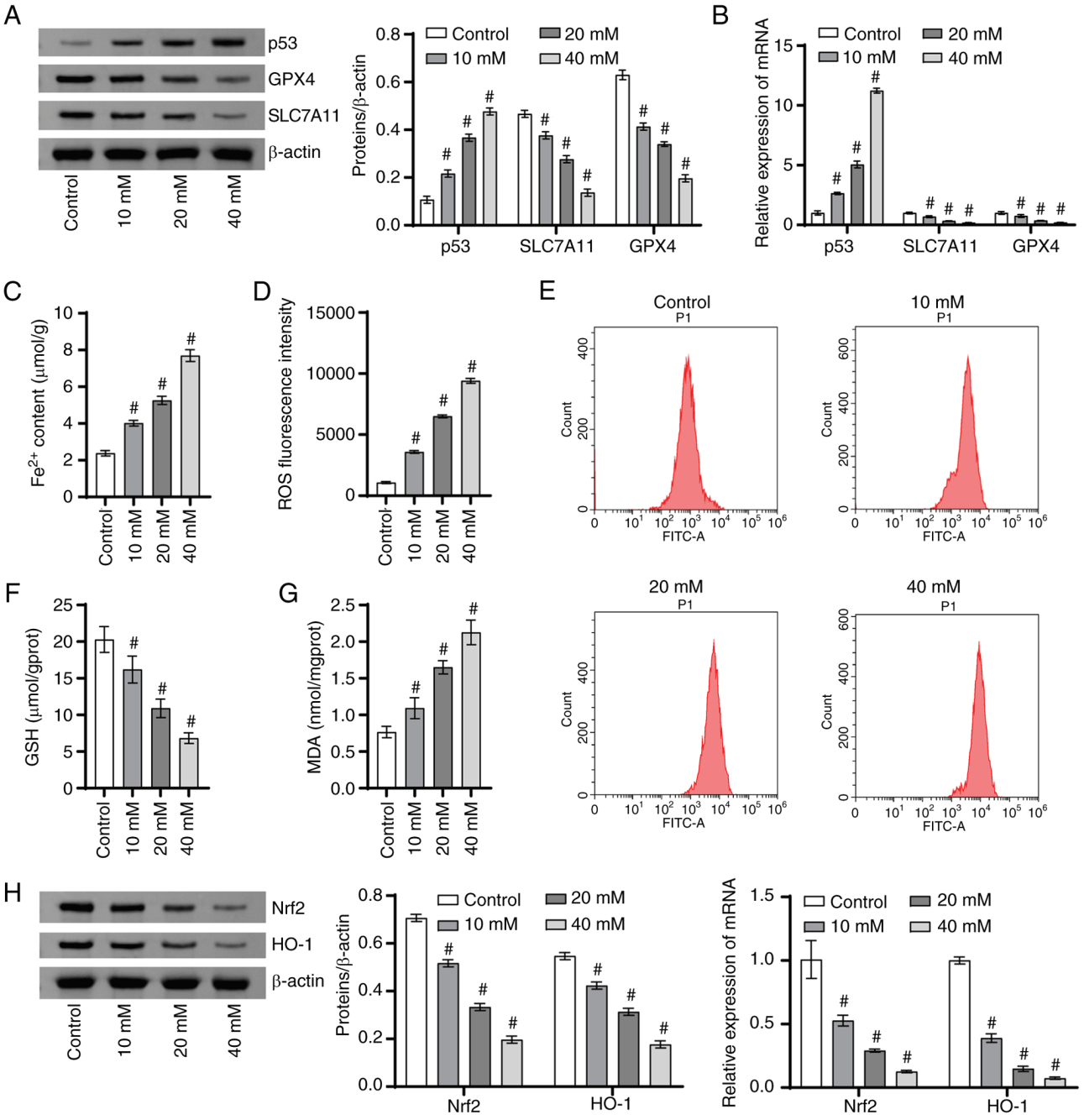

Figure 2. Icariin-curcumol promotes ferroptosis in DU145 cells. (A) The p53, SLC7A11 and GPX4 (ferroptosis-related proteins) levels were analyzed using a western blotting. (B) p53, SLC7A11 and GPX4 levels were detected using RT-qPCR. (C) Intracellular Fe^{2+} levels were assessed with an iron colorimetric assay kit. (D) The fluorescence intensity of ROS. (E) Flow cytometry of intracellular ROS production. (F) A GSH detection kit was utilized to detect intracellular GSH content. (G) The MDA level was assessed using an MDA detection kit. (H) The Nrf2 and HO-1 levels in cells were analyzed using RT-qPCR and western blotting. $^{\#}P<0.05$ vs. control; n=3. SLC7A11, solute carrier family 7 member 11; GPX4, glutathione peroxidase 4; RT-qPCR, reverse transcription-quantitative PCR; ROS, reactive oxygen species; GSH, glutathione; MDA, malondialdehyde; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1.

These results together suggested that Nrf2/HO-1 signaling axis inhibited ferroptosis in PCa cells.

Icariin-curcumol regulated ferroptosis of DU145 cells through Nrf2/HO-1 signaling axis. The present study further investigated whether icariin-curcumol regulated ferroptosis in PCa cells through the Nrf2/HO-1 signaling axis. In contrast to the control group, the protein and mRNA levels of Nrf2 and HO-1 in the icariin + curcumol group were markedly reduced. The protein and mRNA levels of Nrf2 and HO-1 in the icariin + curcumol + oe-Nrf2 group were markedly enhanced when compared with the icariin + curcumol + oe-NC group (Fig. 4A and B). Cell proliferation ability was conspicuously reduced in the icariin + curcumol group in comparison to the control group. Following transfection with overexpression of Nrf2, the cell proliferation ability was signally enhanced in contrast to the icariin + curcumol + oe-NC group (Fig. 4C). Furthermore, the protein and mRNA levels of SLC7A11 and GPX4 levels in the icariin + curcumol group were clearly higher, while the p53 levels

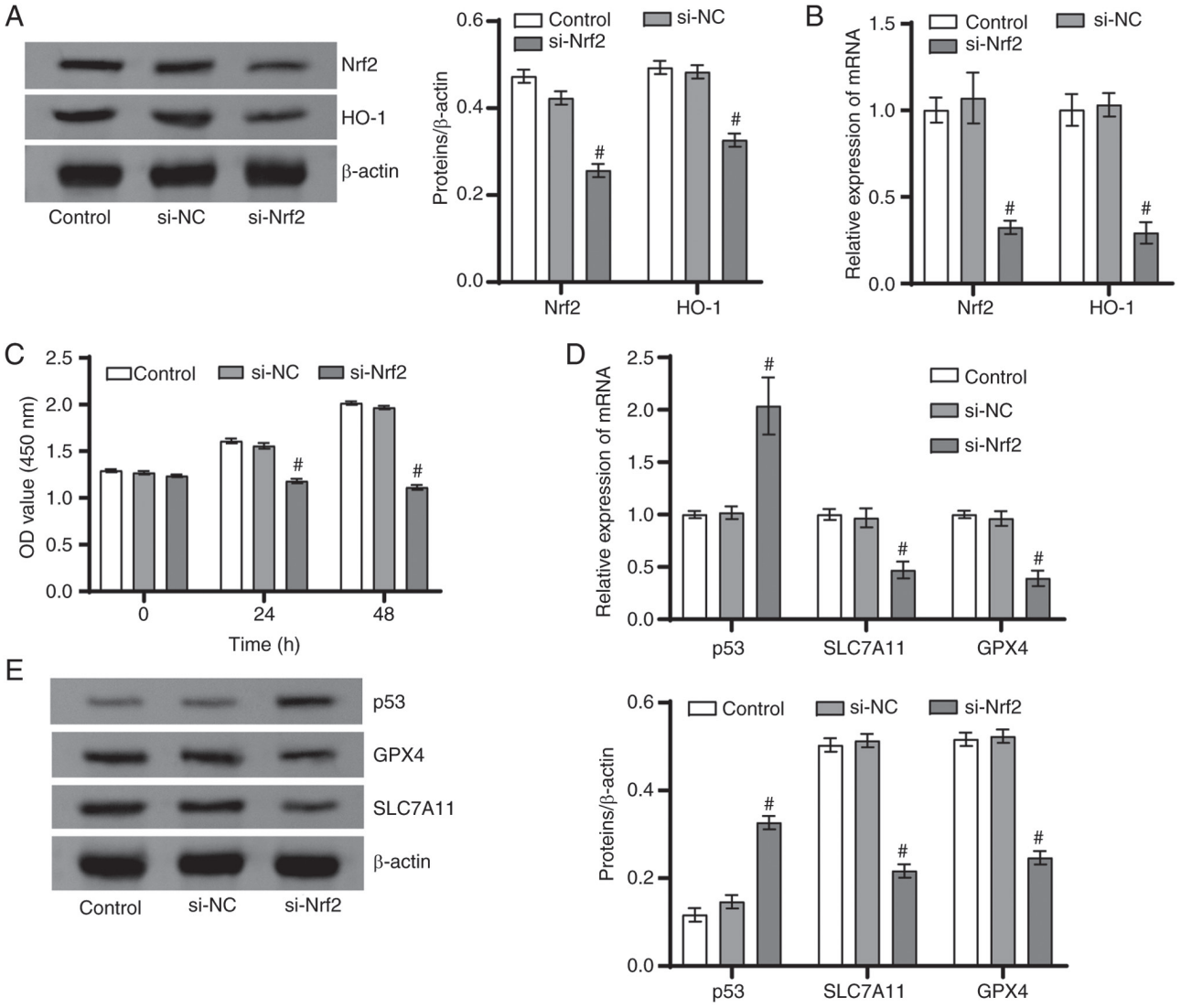

Figure 3. Silencing of the Nrf2/HO-1 signaling axis promotes ferroptosis in DU145 cells. (A) The Nrf2 and HO-1 levels in DU145 cells were assessed using western blotting. (B) The Nrf2 and HO-1 levels in DU145 cells were examined using RT-qPCR. (C) The proliferation ability of DU145 cells was tested using CCK8. (D) The p53, SLC7A11 and GPX4 levels in DU145 cells were analyzed using RT-qPCR. (E) The p53, SLC7A11 and GPX4 levels in DU145 cells were assessed using western blotting. n=3; one-way ANOVA. #P<0.05 vs. si-NC. Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1; RT-qPCR, reverse transcription-quantitative PCR; SLC7A11, solute carrier family 7 member 11; GPX4, glutathione peroxidase 4; si small interfering; NC, negative control.

were markedly lower than the control group. The protein and mRNA levels of SLC7A11 and GPX4 levels in the icariin + curcumol + oe-Nrf2 were evidently increased, while the p53 levels were markedly decreased in comparison to the icariin + curcumol + oe-NC group (Fig. 4D and E). It was worth noting that Fe^{2+} content in the icariin + curcumol group was markedly increased in contrast to the control group. After overexpression of Nrf2 transfected, the Fe^{2+} content was markedly decreased in contrast to the icariin + curcumol + oe-NC group (Fig. 4F). The mean fluorescence intensity of ROS in the icariin + curcumol group was markedly increased in comparison with the control group. After overexpression of Nrf2, ROS mean fluorescence intensity was markedly decreased compared with the icariin + curcumol + oe-NC group (Fig. 4G). Notably, in contrast to the control group, GSH content in the icariin + curcumol group was decreased, while MDA content was notably increased. Overexpression of Nrf2 reversed the associated regulation induced by icariin + curcumol. (Fig. 4H-J). These results indicated that icariin-curcumol regulates ferroptosis in prostate cancer cells through Nrf2/HO-1 signaling axis.

Discussion

Currently, natural botanical extracts are considered a significant source of anti-tumor medication. This is not only due to their diverse chemical structures and biological activities, but also because of their lower toxicity compared with drugs synthesized chemically (29). Therefore, identifying natural plant extracts and their functional mechanisms has become a major area of research (30). The present study highlighted the anti-tumor effects of icariin-curcumol on PCa cells. It found that icariin-curcumol promoted ferroptosis in PCa cells through the Nrf2/HO-1 signaling pathway.

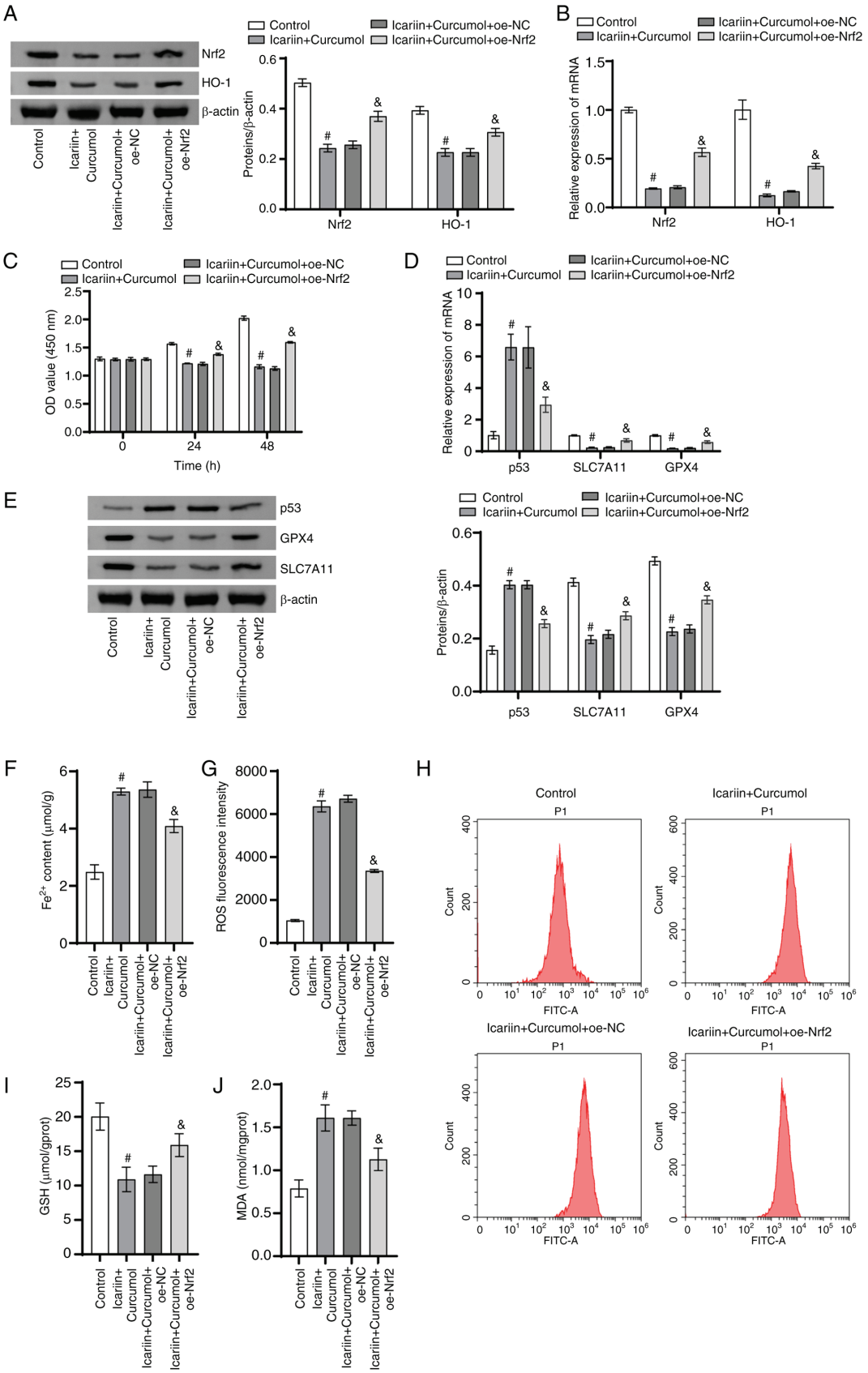

Figure 4. Icariin-curcumol promotes ferroptosis in DU145 cells through Nrf2/HO-1 signaling axis. (A) The Nrf2 and HO-1 levels in DU145 cells were analyzed using western blotting. (B) The Nrf2 and HO-1 levels in DU145 cells were assessed using RT-qPCR. (C) The proliferation ability of DU145 cells was examined using CCK8. (D) The p53, SLC7A11 and GPX4 levels in DU145 cells were tested using RT-qPCR. (E) Western blotting was used to detect the p53, SLC7A11 and GPX4 levels in DU145 cells. (F) Intracellular Fe^{2+} levels were assessed with an iron colorimetric assay kit. (G) The fluorescence intensity of ROS. (H) Flow cytometry of intracellular ROS production. (I) A GSH detection kit was used to detect intracellular GSH content. (J) The MDA level in DU145 cells was tested using an MDA detection kit. n=3. $^{\#}$P<0.05 vs. control; $^{\&}$P<0.05 vs. icariin + curcumol + oe-NC. Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1; SLC7A11, solute carrier family 7 member 11; GPX4, glutathione peroxidase 4; si small interfering; NC, negative control; oe, overexpression; GSH, glutathione; MDA, malondialdehyde.

Icariin is extracted from Yinyanghuo (*Epimedium brevicornu*) and curcumol from Ezhu (*Curcuma zedoaria*), which are both traditional Chinese herbal medicines with wide geographic distribution and affordable prices (31,32). Studies have shown that both icariin and curcumol have therapeutic effects on PCa (23,33).

Icariin can promote ferroptosis of tumor cells by interfering with ferroptosis metabolism pathways (26). Specifically, it can inhibit uptake of ferroptosis in tumor cells and disrupt the balance of ferroptosis storage and utilization, leading to ferroptosis overload in tumor cells. This ferroptosis overload can trigger excessive ROS production and induce apoptosis and ferroptosis in cells (34). Curcumol is widely used in anti-tumor therapy (35,36). Study has shown that curcumol can induce ferroptosis death by interfering with ferroptosis metabolism pathways in tumor cells (37). Specifically, curcumol can inhibit the endogenous production of ferroptosis in tumor cells and increase the concentration of free ferroptosis in the cytoplasm. These free ferroptosis can participate in the production of excessive ROS, thereby triggering autophagy and apoptosis in cells and inducing ferroptosis in tumor cells (38). Icariin and curcumol both have the ability to promote apoptosis in cells (27). Apoptosis is a normal, orderly and programmed cell death mechanism in the body. In the case of tumor cells, the process of apoptosis is inhibited, leading to tumor growth and spread (39). icariin and curcumol can inhibit tumor growth and spread by promoting apoptosis in PCa cells. They also have inhibitory effects on cell proliferation. One of the characteristics of tumor cells is abnormal cell proliferation, which leads to tumor growth and spread. icariin and curcumol can inhibit tumor growth by suppressing the proliferation of tumor cells. Additionally, both icariin and curcumol have anti-inflammatory effects (26). Inflammation is an important factor in the occurrence and development of tumors and by inhibiting the occurrence of inflammatory reactions, tumor growth and spread can be suppressed (27).

Icariin and curcumol both have pharmacological activities and by mixing them together, they may produce a synergistic effect, enhancing the therapeutic effect (27). Previous experiments have demonstrated that Icariin and curcumin synergistically regulate the miR-7/mTOR/SREBP1 pathway, inducing autophagy and ferroptosis in PCa cells and affecting lipid metabolism (26). DU145 cells may have differences in the regulation of the Nrf2/HO-1 signaling pathway compared with other PCa cells such as RWPE-2, PC-3 and VCAP. The Nrf2/HO-1 pathway is an important cellular response mechanism to oxidative stress and its overactivation can lead to increased antioxidant and cell protective abilities (40). Nrf2 is a transcription factor that regulates the antioxidant stress pathway in cells (41). HO-1 is a target gene downstream of the Nrf2 pathway and has antioxidant and anti-inflammatory effects (42). Nrf2/HO-1 signaling pathway serves an important role in development and progression of PCa (1). PCa cell lines with high expression of Nrf2/HO-1 may have lower sensitivity to chemotherapy drugs. This is because the high expression of Nrf2/HO-1 can enhance the antioxidant stress capacity of cells and promote cell survival and drug resistance (43). On the other hand, some studies have found that inhibiting the Nrf2/HO-1 pathway may increase the sensitivity of PCa cells to chemotherapy drugs (44,45). In the present study, the data results showed that icariin combined with curcumol effectively inhibited the proliferation of prostate cancer cells, particularly DU145, which was the most sensitive. Nrf2 and HO-1 were expressed more highly in DU145 cells, compared with other prostate cancer cell lines. Icariin combined with curcumol effectively suppressed the expression of Nrf2 and HO-1, thereby affecting the process of ferroptosis in DU145 cells.

Ferroptosis, a type of programmed cell death that depends on iron, is closely associated with the emergence and advancement of tumors (46). In particular, the mRNA molecule SLC7A11 is linked to ferroptosis (47). Researchers have demonstrated that regulating the SLC7A11/GSH/GPX4 axis induces ferroptosis in vascular smooth muscle cells (48). Lethal depletion of GSH and GPX4 caused by iron-dependent ROS accumulation constitutes a crucial step in the initiation of ferroptosis within cancerous cells (49). The present study found, consistent with these findings, that icariin-curcumol reduced the SLC7A11 and GPX4 levels and GSH content. As intracellular iron and ROS accumulate (12), ferroptosis is triggered, playing a pivotal role in the development and progression of cancer (50). Cancer cells evade cell death by overexpressing SLC7A11. The specific process was as follows: SLC7A11 facilitates GSH production and decreases ROS-mediated cellular stress by exchanging cysteine for intracellular glutamate (51). The present study also recorded a reduction in ROS-mediated cellular stress as a result of icariin-curcumol. These results indicated that icariin-curcumol-activated ingredients regulated ferroptosis to inhibit PCa.

Targeting the Nrf2/HO-1 axis has been introduced as a revolutionary cancer therapy (52). Nrf2 is a fundamental transcription factor that governs endogenous antioxidants, including HO-1 (53). The HO-1 gene is accountable for the balance maintenance within cells and assumes a critical part in controlling oxidative stress and the advancement and progression of PCa (54). The Keap1/Nrf2/ARE axis has been found to enhance the proliferation and inhibit apoptosis and invasion of PCa cells (55). Prior exposure to puerarin resulted in reduced protein expression levels of both Nrf2 and HO-1 in DU145 and PC3 cells (56). As the present study demonstrated, Nrf2 and HO-1 showed a gradual decrease with an increase in icariin-curcumol concentration. The tumor suppressor protein p53 serves various roles while responding to different stress signals (57). Furthermore, it was found that the p53 level, Fe^{2+} content and MDA level were increased proportionally with an increase in icariin-curcumol concentration. The focus of the present study was on exploring the influence of the Nrf2/HO-1 signaling pathway on ferroptosis in PCa cells. It made a notable discovery that the inhibition of Nrf2 expression resulted in a significant reduction in ferroptosis-related protein levels. In conditions of stress, p53 exerts stringent control over cellular proliferation by facilitating apoptosis (58). The tumor-inhibitory action of p53 has been traditionally linked to its capacity to elicit cell cycle arrest, senescence and apoptosis in cells (59). Upon comparing with the si-NC group, inhibiting Nrf2 expression demonstrated a marked decrease of cancer cell replication capability and a significant increase in the p53 level. The results indicated that inhibiting the activation of the Nrf2/HO-1 pathway instead activated the P53-dependent ferroptosis pathway, leading to the accumulation of iron and ultimately leading to ferroptosis. It could be hypothesized that the Nrf2/HO-1 signaling pathway regulated ferroptosis in PCa cells. However, whether the Nrf2/HO-1 pathway regulates the ferroptosis mechanism in prostate cancer at the clinical level was not investigated and therefore future studies will collect clinical samples for more in-depth studies.

The present study unveiled a fresh potential mechanism through which icariin-curcumol may induce ferroptosis in PCa cells, most likely by operating through the Nrf2/HO-1 signaling axis. Notably, ferroptosis can be stimulated in cancer cells by Tagitinin C, a sesquiterpene lactone obtained from *Tithonia diversifolia*, resulting in increased levels of ROS and MDA, paired with reduced GSH levels (60). The present study observed that icariin-curcumol also led to ferroptosis in conjunction with depleted GSH levels, elevated oxidative stress and MDA expression. Moreover, this phenomenon was accompanied by a substantial reduction in the levels of Nrf2, HO-1, SLC7A11 and GPX4 in addition to reducing cell proliferation ability and causing a marked increase in p53 level and Fe^{2+} content. Finally, overexpressing Nrf2 reversed the regulatory effects of icariin-curcumol on the aforementioned factors.

To sum up, the present study demonstrated that icariin-curcumol enhanced ferroptosis in PCa cells through modulation of the Nrf2/HO-1 signaling pathway. Its findings offered potential targets for PCa therapy and novel treatment strategies for drug development.

Acknowledgements

Not applicable.

Funding

The present study was supported by Natural Science Foundation of Hunan Province (grant no. 2023JJ40511), Excellent Youth Project of Scientific Research Program of Hunan Education Department (grant no. 22B0370), Project of Traditional Chinese Medicine Administration of Hunan Province (grant no. B2023034), Changsha Natural Science Foundation (grant no. kq2208204), Excellent Youth Project of Hunan University of Chinese Medicine (grant no. Z2023XJYQ05), and Health Commission of Hunan Province Health research projects (grant no. W20243165).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

WS conceived the present study. WS, BL, TS and CZ collected and analyzed the data. YL administrated the project and WX designed the methodology. WS wrote the original draft of the manuscript which was then edited by YL and WX and reviewed by all authors. WS, YL and WX confirm the authenticity of all the raw data and agree to be accountable for all aspects of the present study in ensuring that questions related to the accuracy or integrity of any part of the present study are appropriately investigated and resolved. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

References

1. Liao D, Yang G, Yang Y, Tang X, Huang H, Shao J and Pan Q: Identification of pannexin 2 as a novel marker correlating with ferroptosis and malignant phenotypes of prostate cancer cells. Onco Targets Ther 13: 4411-4421, 2020.
2. Hentze MW, Muckenthaler MU and Andrews NC: Balancing acts: Molecular control of mammalian iron metabolism. Cell 117: 285-297, 2004.
3. Hassannia B, Vandenabeele P and Vanden Berghe T: Targeting ferroptosis to iron out cancer. Cancer Cell 35: 830-849, 2019.
4. Torti SV and Torti FM: Iron and cancer: More ore to be mined. Nat Rev Cancer 13: 342-355, 2013.
5. Maccarinelli F, Coltrini D, Mussi S, Bugatti M, Turati M, Chiodelli P, Giacomini A, De Cillis F, Cattane N, Cattaneo A, *et al*: Iron supplementation enhances RSL3-induced ferroptosis to treat naïve and prevent castration-resistant prostate cancer. Cell Death Discov 9: 81, 2023.
6. Ghoochani A, Hsu EC, Aslan M, Rice MA, Nguyen HM, Brooks JD, Corey E, Paulmurugan R and Stoyanova T: Ferroptosis inducers are a novel therapeutic approach for advanced prostate cancer. Cancer Res 81: 1583-1594, 2021.
7. Xu B, Zhu WJ, Peng YJ and Cheng SD: Curcumin reverses the sunitinib resistance in clear cell renal cell carcinoma (ccRCC) through the induction of ferroptosis via the ADAMTS18 gene. Transl Cancer Res 10: 3158-3167, 2021.
8. Wang Y, Quan F, Cao Q, Lin Y, Yue C, Bi R, Cui X, Yang H, Yang Y, Birnbaumer L, *et al*: Quercetin alleviates acute kidney injury by inhibiting ferroptosis. J Adv Res 28: 231-243, 2020.
9. Xie R, Zhao W, Lowe S, Bentley R, Hu G, Mei H, Jiang X, Sun C, Wu Y and Yueying Liu: Quercetin alleviates kainic acid-induced seizure by inhibiting the Nrf2-mediated ferroptosis pathway. Free Radic Biol Med 191: 212-226, 2022.
10. Zhang X, Jiang L, Chen H, Wei S, Yao K, Sun X, Yang G, Jiang L, Zhang C, Wang N, *et al*: Resveratrol protected acrolein-induced ferroptosis and insulin secretion dysfunction via ER-stress-related PERK pathway in MIN6 cells. Toxicology 465: 153048, 2022.
11. Suzuki T, Motohashi H and Yamamoto M: Toward clinical application of the Keap1-Nrf2 pathway. Trends Pharmacol Sci 34: 340-346, 2013.
12. La Rosa P, Petrillo S, Turchi R, Berardinelli F, Schirinzi T, Vasco G, Lettieri-Barbato D, Fiorenza MT, Bertini ES, Aquilano K and Piemonte F: The Nrf2 induction prevents ferroptosis in Friedreich's Ataxia. Redox Biol 38: 101791, 2021.
13. Sun X, Ou Z, Chen R, Niu X, Chen D, Kang R and Tang D: Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. Hepatology 63: 173-184, 2016.
14. Liang J, Liao Y, Wang P, Yang K, Wang Y, Wang K, Zhong B, Zhou D, Cao Q, Li J, *et al*: Ferroptosis landscape in prostate cancer from molecular and metabolic perspective. Cell Death Discov 9: 128, 2023.
15. Wang S, Wei W, Ma N, Qu Y and Liu Q: Molecular mechanisms of ferroptosis and its role in prostate cancer therapy. Crit Rev Oncol Hematol 176: 103732, 2022.
16. Choi BH, Kim JM and Kwak MK: The multifaceted role of NRF2 in cancer progression and cancer stem cells maintenance. Arch Pharm Res 44: 263-280, 2021.
17. Ma H, Wang X, Zhang W, Li H, Zhao W, Sun J and Yang M: Melatonin suppresses ferroptosis induced by high glucose via activation of the Nrf2/HO-1 signaling pathway in type 2 diabetic osteoporosis. Oxid Med Cell Longev 2020: 9067610, 2020.
18. Chen J, Zhou S, Zhang X and Zhao H: S-3'-hydroxy-7', 2', 4'-trimethoxyisoxane, a novel ferroptosis inducer, promotes NSCLC cell death through inhibiting Nrf2/HO-1 signaling pathway. Front Pharmacol 13: 973611, 2022.
19. Yuk SS, Lim EM, Lee JY, Lee YJ, Kim YS, Lee TH, Park SK, Bae H, Kim HM, Ko SG, *et al*: Antiinflammatory effects of Epimedium brevicornum water extract on lipopolysaccharide-activated RAW264.7 macrophages. Phytother Res 24: 1781-1787, 2010.

20. Jin J, Wang H, Hua X, Chen D, Huang C and Chen Z: An outline for the pharmacological effect of icariin in the nervous system. Eur J Pharmacol 842: 20-32, 2019.
21. Gao JQ, Zhuang SX, Wang Y, Cao FX, Chen L, Bao YH and Wei Y: Evaluation of Epimedium brevicornum Maxim extract for anti-osteoporosis activity in rats. Trop J Pharm Res 16: 2185-2190, 2017.
22. Lo JY, Kamarudin MN, Hamdi OA, Awang K and Kadir HA: Curcumenol isolated from Curcuma zedoaria suppresses Akt-mediated NF-κB activation and p38 MAPK signaling pathway in LPS-stimulated BV-2 microglial cells. Food Funct 6: 3550-3559, 2015.
23. Sheng W, Xu W, Ding J, Li L, You X, Wu Y and He Q: Curcumol inhibits the malignant progression of prostate cancer and regulates the PDK1/AKT/mTOR pathway by targeting miR-9. Oncol Rep 46: 246, 2021.
24. Sun DX, Fang ZZ, Zhang YY, Cao YF, Yang L and Yin J: Inhibitory effects of curcumenol on human liver cytochrome P450 enzymes. Phytother Res 24: 1213-1216, 2010.
25. Sheng W, Ding J, Liu L, Wang N, Lu B, You X, He Q and Zhou Q: Curcumol inhibits the development of prostate cancer by miR-125a/STAT3 Axis. Evid Based Complement Alternat Med 2022: 9317402, 2022.
26. Xu W, Ding J, Li B, Sun T, You X, He Q and Sheng W: Effects of icariin and curcumol on autophagy, ferroptosis, and lipid metabolism based on miR-7/m-TOR/SREBP1 pathway on prostate cancer. Biofactors 49: 438-456, 2023.
27. Xu W, Ding J, Kuang S, Li B, Sun T, Zhu C, Liu J, Zhu L, Li Y and Sheng W: Icariin-Curcumol promotes docetaxel sensitivity in prostate cancer through modulation of the PI3K-Akt signaling pathway and the Warburg effect. Cancer Cell Int 23: 190, 2023.
28. Nie F, Yan J, Ling Y, Liu Z, Fu C, Li X and Qin Y: Effect of Shuangdan Mingmu capsule, a Chinese herbal formula, on oxidative stress-induced apoptosis of pericytes through PARP/GAPDH pathway. BMC Complement Med Ther 21: 118, 2021.
29. Newman DJ and Cragg GM: Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod 83: 770-803, 2020.
30. Yuan M, Zhang G, Bai W, Han X, Li C and Bian S: The role of bioactive compounds in natural products extracted from plants in cancer treatment and their mechanisms related to anticancer effects. Oxid Med Cell Longev 2022: 1429869, 2022.
31. Wang S, Ma J, Zeng Y, Zhou G, Wang Y, Zhou W, Sun X and Wu M: Icariin, an Up-and-Coming bioactive compound against neurological diseases: Network pharmacology-based study and literature review. Drug Des Devel Ther 15: 3619-3641, 2021.
32. Wei W, Rasul A, Sadiqa A, Sarfraz I, Hussain G, Nageen B, Liu X, Watanabe N, Selamoglu Z, Ali M, *et al*: Curcumol: From plant roots to cancer roots. Int J Biol Sci 15: 1600-1609, 2019.
33. Lee KS, Lee HJ, Ahn KS, Kim SH, Nam D, Kim DK, Choi DY, Ahn KS, Lu J and Kim SH: Cyclooxygenase-2/prostaglandin E2 pathway mediates icariside II induced apoptosis in human PC-3 prostate cancer cells. Cancer Lett 280: 93-100, 2009.
34. Liu XJ, Lv YF, Cui WZ, Li Y, Liu Y, Xue YT and Dong F: Icariin inhibits hypoxia/reoxygenation-induced ferroptosis of cardiomyocytes via regulation of the Nrf2/HO-1 signaling pathway. FEBS Open Bio 11: 2966-2976, 2021.
35. Wei ZL, Juan W, Tong D, Juan LX, Sa LY, Jie HFM, Xiao G, Xiang LG, Jie HM and Xu C: Curcumol inhibits breast cancer growth via NCL/ERα36 and the PI3K/AKT pathway. Food Funct 14: 874-885, 2023.
36. Huang X, Qian J, Li L, Zhang X, Wei G, Lv J, Qin F, Yu J, Xiao Y, Gong Z and Huo J: Curcumol improves cisplatin sensitivity of human gastric cancer cells through inhibiting PI3K/AKT pathway. Drug Dev Res 81: 1019-1025, 2020.
37. Zheng Y, Zhao T, Wang J, Jiang R, Huang J, Li W and Wang J: Curcumol alleviates liver fibrosis through inducing autophagy and ferroptosis in hepatic stellate cells. FASEB J 36: e22665, 2022.
38. Wang X, Zhang M, Mao C, Zhang C, Ma W, Tang J, Xiang D and Qi X: Icariin alleviates ferroptosis-related atherosclerosis by promoting autophagy in xo-LDL-induced vascular endothelial cell injury and atherosclerotic mice. Phytother Res 37: 3951-3963, 2023.
39. Kashyap D, Garg VK and Goel N: Intrinsic and extrinsic pathways of apoptosis: Role in cancer development and prognosis. Adv Protein Chem Struct Biol 125: 73-120, 2021.
40. Zhang Q, Liu J, Duan H, Li R, Peng W and Wu C: Activation of Nrf2/HO-1 signaling: An important molecular mechanism of herbal medicine in the treatment of atherosclerosis via the protection of vascular endothelial cells from oxidative stress. J Adv Res 34: 43-63, 2021.
41. Tonelli C, Chio IIC and Tuveson DA: Transcriptional Regulation by Nrf2. Antioxid Redox Signal 29: 1727-1745, 2018.
42. Wang Y, Yang C, Elsheikh NAH, Li C, Yang F, Wang G and Li L: HO-1 reduces heat stress-induced apoptosis in bovine granulosa cells by suppressing oxidative stress. Aging (Albany NY) 11: 5535-5547, 2019.
43. Furfaro AL, Piras S, Domenicotti C, Fenoglio D, De Luigi A, Salmona M, Moretta L, Marinari UM, Pronzato MA, Traverso N and Nitti M: Role of Nrf2, HO-1 and GSH in neuroblastoma cell resistance to bortezomib. PLoS One 11: e0152465, 2016.
44. Yang J, Mo J, Dai J, Ye C, Cen W, Zheng X, Jiang L and Ye L: Cetuximab promotes RSL3-induced ferroptosis by suppressing the Nrf2/HO-1 signalling pathway in KRAS mutant colorectal cancer. Cell Death Dis 12: 1079, 2021.
45. Abdel-Wahab BA, Walbi IA, Albarqi HA, Ali FEM and Hassanein EHM: Roflumilast protects from cisplatin-induced testicular toxicity in male rats and enhances its cytotoxicity in prostate cancer cell line. Role of NF-κB-p65, cAMP/PKA and Nrf2/HO-1, NQO1 signaling. Food Chem Toxicol 151: 112133, 2021.
46. Liang Y, Ye F, Xu C, Zou L, Hu Y, Hu J and Jiang H: A novel survival model based on a Ferroptosis-related gene signature for predicting overall survival in bladder cancer. BMC Cancer 21: 943, 2021.
47. Chen ZA, Tian H, Yao DM, Zhang Y, Feng ZJ and Yang CJ: Identification of a ferroptosis-related signature model including mRNAs and lncRNAs for predicting prognosis and immune activity in hepatocellular carcinoma. Front Oncol 11: 738477, 2021.
48. Ye Y, Chen A, Li L, Liang Q, Wang S, Dong Q, Fu M, Lan Z, Li Y, Liu X, *et al*: Repression of the antiporter SLC7A11/glutathione/glutathione peroxidase 4 axis drives ferroptosis of vascular smooth muscle cells to facilitate vascular calcification. Kidney Int 102: 1259-1275, 2022.
49. Jing S, Lu Y, Zhang J, Ren Y, Mo Y, Liu D, Duan L, Yuan Z, Wang C and Wang Q: Levistilide a induces ferroptosis by activating the Nrf2/HO-1 signaling pathway in breast cancer cells. Drug Des Devel Ther 16: 2981-2993, 2022.
50. Tang B, Yan R, Zhu J, Cheng S, Kong C, Chen W, Fang S, Wang Y, Yang Y, Qiu R, *et al*: Integrative analysis of the molecular mechanisms, immunological features and immunotherapy response of ferroptosis regulators across 33 cancer types. Int J Biol Sci 18: 180-198, 2022.
51. Yadav P, Sharma P, Sundaram S, Venkatraman G, Bera AK and Karunagaran D: SLC7A11/xCT is a target of miR-5096 and its restoration partially rescues miR-5096-mediated ferroptosis and anti-tumor effects in human breast cancer cells. Cancer Lett 522: 211-224, 2021.
52. Ghareghomi S, Moosavi-Movahedi F, Saso L, Habibi-Rezaei M, Khatibi A, Hong J and Moosavi-Movahedi AA: Modulation of Nrf2/HO-1 by natural compounds in lung cancer. Antioxidants (Basel) 12: 735, 2023.
53. Zhang L, Guo J, Zhang Q, Zhou W, Li J, Yin J, Cui L, Zhang T, Zhao J, Carmichael PL, *et al*: Flutamide induces hepatic cell death and mitochondrial dysfunction via inhibition of Nrf2-Mediated heme oxygenase-1. Oxid Med Cell Longev 2018: 8017073, 2018.
54. Labanca E, De Luca P, Gueron G, Paez A, Moiola CP, Massillo C, Porretti J, Giudice J, Zalazar F, Navone N, *et al*: Association of HO-1 and BRCA1 is critical for the maintenance of cellular homeostasis in prostate cancer. Mol Cancer Res 13: 1455-1464, 2015.
55. Jiang G, Liang X, Huang Y, Lan Z, Zhang Z, Su Z, Fang Z, Lai Y, Yao W, Liu T, *et al*: p62 promotes proliferation, apoptosis-resistance and invasion of prostate cancer cells through the Keap1/Nrf2/ARE axis. Oncol Rep 43: 1547-1557, 2020.
56. Li J, Xiong C, Xu P, Luo Q and Zhang R: Puerarin induces apoptosis in prostate cancer cells via inactivation of the Keap1/Nrf2/ARE signaling pathway. Bioengineered 12: 402-413, 2021.
57. Kwon SK, Saindane M and Baek KH: p53 stability is regulated by diverse deubiquitinating enzymes. Biochim Biophys Acta Rev Cancer 1868: 404-411, 2017.
58. Kanapathipillai M: Treating p53 mutant aggregation-associated cancer. Cancers (Basel) 10: 154, 2018.
59. Liu J, Zhang C, Hu W and Feng Z: Tumor suppressor p53 and metabolism. J Mol Cell Biol 11: 284-292, 2019.
60. Wei R, Zhao Y, Wang J, Yang X, Li S, Wang Y, Yang X, Fei J, Hao X, Zhao Y, *et al*: Tagitinin C induces ferroptosis through PERK-Nrf2-HO-1 signaling pathway in colorectal cancer cells. Int J Biol Sci 17: 2703-2717, 2021.