Quercetin reverses 5-fluorouracil resistance in colon cancer cells by modulating the NRF2/HO-1 pathway

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ABSTRACT

Quercetin (Que) has been proven to enhance the chemosensitivity of multiple cancers, including colon cancer (CC). However, whether the combination of Que and 5-fluorouracil (5-FU) has a synergistic effect on drug-resistant CC cells has not previously been reported. The effect of Que (5 and 10 μg/mL) on cell vitality and apoptosis of CC and 5-FU-resistant CC cells was examined using a cell counting kit-8 (CCK-8) and flow cytometry. After cells were treated with 5-FU (10, 40 μg/mL), Que (10 μM, 40 μM), or 5-FU in combination with Que, cell proliferation, apoptosis, oxidative stress-related factors, reactive oxygen species (ROS), and nuclear factor erythroid 2-related factor (Nrf2)/heme oxygenase-1 (HO-1) pathway-related factors were examined by colony formation assay, flow cytometry, ELISA, ROS kit, immunofluorescence assay, and Western blot. The results showed that 5-FU reduced cell viability and induced apoptosis of CC as well as 5-FU-resistant CC cells. Que further restrained the proliferation, oxidative stress-related factors (SOD, CAT, GPx, and GR), ROS production, and induced apoptosis in CC cells and 5-FU-resistant CC cells induced by 5-FU. Moreover, the combination of Que and 5-FU attenuated the Nrf2/HO-1 pathway-related marker levels in CC cells and 5-FU-resistant CC cells. Therefore, our results suggest that Que reverses 5-FU resistance in CC cells *via* modulating the Nrf2/HO-1 pathway.

Key words: colon cancer; quercetin; 5-fluorouracil; chemosensitivity; nuclear factor erythroid 2-related factor/heme oxygenase-1 pathway.

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Introduction

Colon cancer (CC) is the third most common malignancy in men and the second most common cancer in the world.[1] At present, the pathogenic factors of CC include a high-fat diet, insufficient fiber intake, unhealthy lifestyle, and the interaction between genetic and environmental factors.[2] The early symptoms of CC patients are not obvious; approximately 40% of patients can be diagnosed and treated at the early stage of the disease, and the survival rate of advanced patients is as low as 12.5%.[3,4] According to the Guidelines for the Diagnosis and Treatment of Colorectal Cancer by the Chinese Society of Clinical Oncology and Clinical Practice Guidelines of American Society of Cataract and Refractive Surgery, for adjuvant therapy, 5-fluorouracil (5-FU) is the major chemotherapeutic drug recommended for use. 5-FU can block the DNA synthesis and proliferation of cancer cells by interfering with their nucleic acid metabolism.[5] However, chemotherapeutic drugs are considered a "double-edged sword". Adverse reactions also occur when cancer cells are inhibited. The insensitivity of CC cells to 5-FU is the main reason for the decreased survival rate in CC patients.[6] Thus, searching for effective anticancer drugs from natural plants is a research hotspot for clinical and scientific researchers worldwide. Owing to advantages such as multi-targeting and few adverse reactions, the reversal effect of natural drug ingredients on tumor resistance has also attracted extensive attention.

Quercetin (Que) is a plant-derived flavonoid that widely exists in fruits, vegetables, grains, and other plants.[7] It has been reported to have various pharmacologic effects, such as free radical scavenging, anti-hypertensive, blood lipid reducing, anti-inflammatory, anti-allergic, anti-viral, and anti-platelet aggregation activities, which are of great importance in the treatment and prevention of human diseases, including cancers.[8,9] Researchers have summarized that Que exerts anti-tumor effects by altering cell cycle progression, inhibiting cell proliferation, promoting apoptosis, inhibiting angiogenesis and metastasis progression, and affecting autophagy.[10] As early as 1994, several scholars preliminarily studied the inhibitory effects of Que and 5-FU on CC cells, and recent research has more clearly identified the anti-CC effects of Que.[11] Que evidently has an inhibitory function on the proliferation and induction of apoptosis in various CC cell lines, such as SW480, HCT-116, Caco-2, LOVO, and HT-29.[12-15] More importantly, researchers revealed that Que is an effective reversal agent for multidrug resistance (MDR), which enhances the sensitivity to chemotherapy drugs in breast cancer, CC, and nasopharyngeal cancer, among other cancers.[16-18] Regulation of MDR-related transporter expressions, such as that of P-gp, MRP1 and BCRP, is considered as the underlying mechanism for reversing MDR.[19] However, it has not yet been reported whether the combination of Que and 5-FU has a synergistic effect on HCT-116 drug-resistant cells. Therefore, in this study, we induced a 5-FU-resistant human CC cell model, observed the reversal effect of Que on 5-FU induced resistance on CC cells, and explored the possible mechanism.

Materials and Methods

Cell culture and reagents

HCT-116 cells were provided by the iCell Bioscience, Inc. (Shanghai, China) and cultured in DMEM medium (SH30243.01, Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS, 11011-8615, Tianhang, China), and a trypsin solution (0.25%, SH30042.01, Hyclone, USA) was used to detach the cells. The HCT-116 cells were then placed in a cell incubator (BB150, Thermo Fisher Scientific, Waltham, MA, USA).

5-FU (F100149, Aladdin, Shanghai, China, CAS ID 51-21-8) and Que (Q111274, Aladdin, China, CAS ID 117-39-5) were prepared as stock solutions in DMSO (D8371, Solarbio, Beijing, China). For each experiment, a medium was used to dilute the stock solutions to a final DMSO concentration of 0.5% (v/v).

Construction of HCT116 drug-resistant cell line

To construct a 5-FU-resistant HCT116 cell (HCT116-R), HCT116 cells from the same batch were first stimulated with 10 μM 5-FU and then reacted with gradually increasing concentrations of 5-FU (20, 40, 60, 80, and 100 μM) over a period of 8 months. Thereafter, the surviving 5-FU-resistant HCT116 cells were maintained at a concentration of 60 μM 5-FU and then used for subsequent experiments.[20]

Experimental design

First, HCT116 and HCT116-R cells were subjected to 0, 5, and 10 μM 5-FU for 72 h to choose 10 μM as a suitable concentration for 5-FU. After that, HCT116 and HCT116-R cells were each exposed to 10 μM 5-FU (5-FU group), 10 μM Que (Que 10 group), 40 μM Que (Que 40 group), 10 μM Que + 10 μM 5-FU (Que 10 + 5-FU group), or 40 μM Que + 10 μM 5-FU (Que 40 + 5-FU group) for 72 h. Each experiment was repeated three times, except for the cell viability assay that was repeated six times.

Cell viability assay

Chemosensitivity was assessed using a cell counting kit-8 (CCK-8) (HY-K0301, MedChemExpress, Monmouth Junction, NJ, USA). The cells were seeded in 96-well plates (1×10^4 cells/mL) and processed according to the experimental design described above. Afterwards, treated cells were exposed to 10 μL CCK-8 solution and stored in the cell incubator for 3 h. Lastly, the absorbance (450 nm) was estimated using a microplate reader (CMaxPlus, Molecular Devices, San Jose, CA, USA).

Apoptosis assay

An apoptosis kit (556547, BD Biosciences, San Jose, CA, USA) was used to evaluate apoptosis. Cells cultured in 96-well plates (1.5×10^6 cells/mL) were treated according to the experimental design described above. After the cells were harvested, washed, and centrifuged, their concentration was adjusted to 1×10^6 cells/mL. After centrifugation, the precipitated cells of each hole were added to 100 μL of binding buffer. Thereafter, each sample tube was subjected to 5 μL Annexin V-FITC and 10 μL propidium iodide and then placed away from light for 15 min at 37°C. After adding 1× binding buffer, flow cytometry (C6, BD Biosciences, Franklin Lakes, NJ, USA) was used to analyze apoptosis.

The colony formation assay

Cells seeded into 12-well plates (8×10^2 cells/well) were treated based on the experimental design as described above. After 14 days of culture, the cells in each well were fixed with 75% alcohol (A171299, Aladdin, China) for 1 h and then stained with 0.25% crystal violet (C8470, Solarbio, China) for 2 min. Finally, images were captured and observed using an optical microscope (AE2000, Motic, Wetzlar, Germany).

ELISA

Superoxide dismutase (SOD) kit (A001-3), catalase (CAT) kit (A007-1-1), glutathione peroxidase (GPx) kit (A005-1), and glutathione reductase (GR) kits (A062-1-1) were obtained from

Jiancheng (Nanjing, China). Cells were seeded in 12-well plates (1×10^4 cells/mL) and treated as described above, thereafter the cells in each well were harvested, washed, and centrifuged, and supernatants were collected. Then, the levels of SOD, CAT, GPx, and GR were then measured using the related kits.

Detection of reactive oxygen species (ROS)

A ROS kit (S0033M, Beyotime, Shanghai, China) was used to examine the ROS production in each group of cells. After the cells in each group (1×10^4) were administered, the medium was removed and 1 mL of the prepared DCFH-DA diluent was added. Then, they were displayed in the cell incubator for 20 min. Cells were washed three times with serum-free cell culture medium to adequately remove DCFH-DA that had not entered the cells. Then, the cells were detached by 0.25% trypsin, and the cell suspension was subsequently transferred to a 5 mL flow tube and assayed on a flow cytometer.

Immunofluorescence assay

After treatment, the culture medium was discarded and 1-2 mL of pre-chilled 4% paraformaldehyde was added to each well; fixation lasted 10 min at room temperature, then PBS was used for

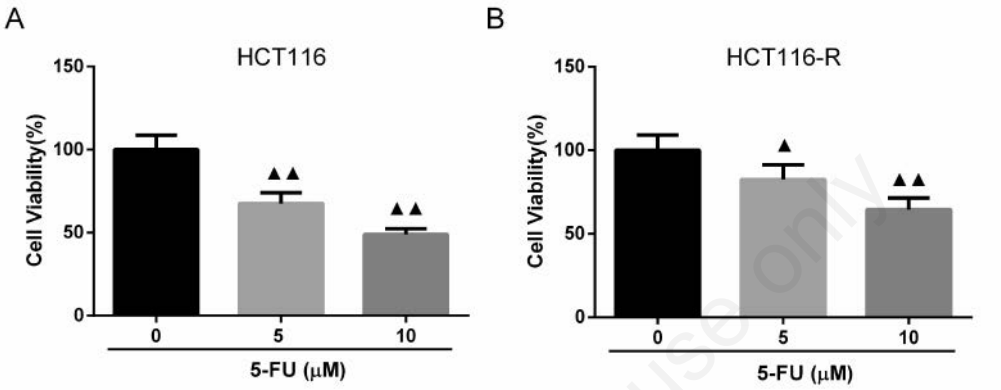

Figure 1. 5-FU blunted the cell vitality of CC cells HCT116 and 5-FU-resistant CC cells HCT116-R. The CCK-8 assay was used to examine the cell viability of CC cells (A) and 5-FU-resistant CC cells (B) after 5-FU (0, 5, and 10 μg/mL) treatment; n=6. 5-FU, 5-fluorouracil; CC, colon cancer; CCK-8, cell counting kit-8; ▲p<0.05, ▲▲p<0.01 *vs* 0 μM.

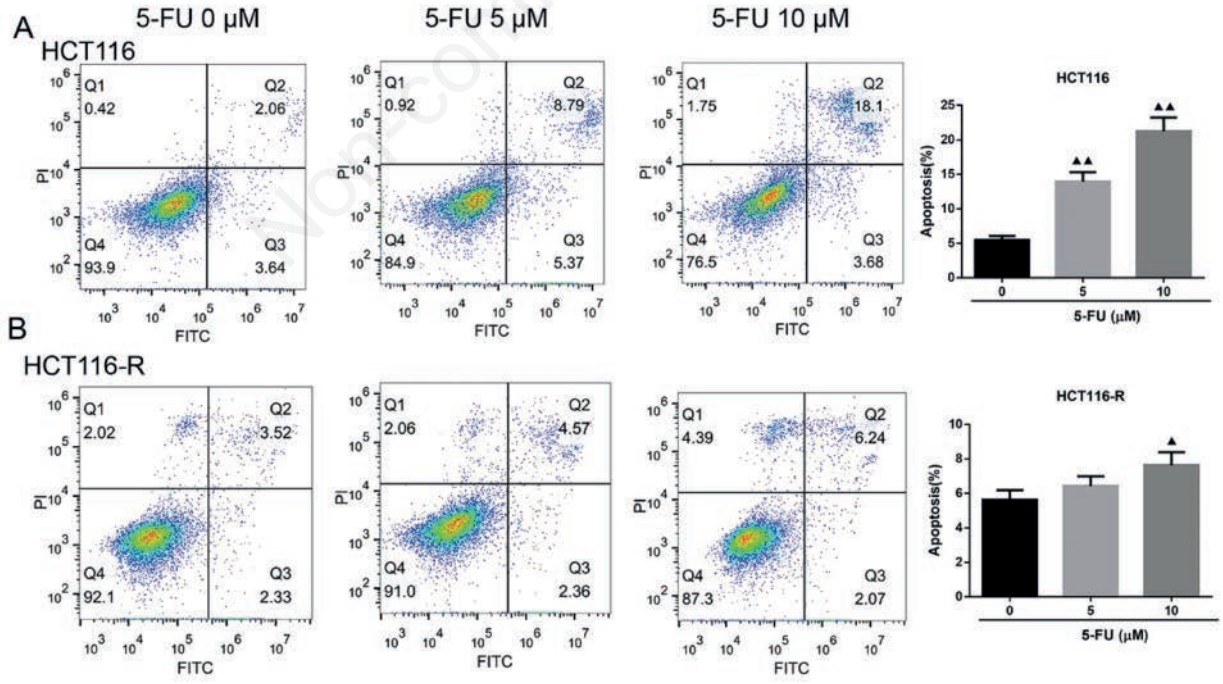

Figure 2. 5-FU induced apoptosis of CC cells and 5-FU-resistant CC cells. The flow cytometer method was used to examine apoptosis of CC cells (A) and 5-FU-resistant CC cells (B) after 5-FU (0, 5, and 10 μM) treatment; n=3. 5-FU, 5-fluorouracil; CC, colon cancer; ▲p<0.05, ▲▲p<0.01 *vs* 0 μM.

rinsing three times for 5 min each. Then 1-2 mL of 0.5% Triton X-100 was added to each well for 2 min at room temperature for permeabilization, and the cells were rinsed with PBS three times for 5 min each. Afterward, the primary antibody against nuclear factor erythroid 2-related factor 2 (Nrf2, 1:500, ab62352, Abcam, Cambridge, UK) and heme oxygenase 1 (HO-1, 1:200, ab68477, Abcam) was added overnight at 4°C, followed by incubation with a secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (1:500, ab150077, Abcam) for 1 h at 37°C. Nuclei were stained with DAPI solution (5 μg/mL) for 5 min in the dark, at room temperature. Omitting the primary antibody was used as the negative control. Cells were observed under a microscope, and the immunofluorescence intensity was assessed by Image J software.

Western blot

After treatment, the cells were harvested and lysed using a cell buffer (WB038, GEFAN, Nanjing, China), and their concentrations were estimated by a BCA kit (GM03, GEFAN). After denaturation, cells were subjected to protein electrophoresis. After transferring to a PVDF membrane (WB041, GEFAN), the membrane was sealed with 5% skim milk. After 2 h, cells were reacted with primary antibodies overnight at 4°C followed by incubation with a goat anti-rabbit secondary antibody IgG H&L (1:3000, S0001, Affinity Biosciences, Cincinnati, OH, USA) for 1 h at 37°C. Finally, a color reagent (E266188, Aladdin, Shanghai, China) was used to visualize the bound antibodies, which were then placed in a chemiluminescent instrument (610020-9Q, Clinx, Shanghai, China). The primary antibodies of the rabbit anti-human Nrf2 (1:2000, AF0639), SOD-1 (1:1500, AF5198), CAT (1:2000, AF7746), HO-1 (1:1500, AF5393), nuclear factor kappa B (NF-κB, 1:1500, AF5006), phospho-IkappaB-alpha (p-IKBα, 1:2000, AF2002), and GAPDH (1:20000, AF7021) were obtained from Affinity Biosciences .

Statistical analysis

Statistical analysis was performed using SPSS software (version 16.0, IBM, Armonk, NY, USA). Comparisons between more than two groups were performed using one-way ANOVA. Comparisons between the groups were analyzed using Tukey's test. Data are expressed as mean ± standard deviation. A $p<0.05$ was designated as statistically significant.

Results

5-FU blunted the cell vitality and induced apoptosis of CC cells and 5-FU-resistant CC cells

First, we estimated the role of 5-FU (5 and 10 μM) on the cell vitality and demonstrated that 5-FU obviously alleviated the vitality of CC cells and 5-FU-resistant CC cells (Figure 1 A,B, $p<0.05$). The inhibitory effect of 5-FU on CC cell viability was stronger than that on 5-FU-resistant CC cells (Figure 1 A,B, $p<0.05$). Second, we examined apoptosis and confirmed that 5-FU (5 and 10 μM) induced apoptosis of CC cells, and 10 μM 5-FU also notably facilitated apoptosis of 5-FU-resistant CC cells (Figure 2 A,B, $p<0.05$).

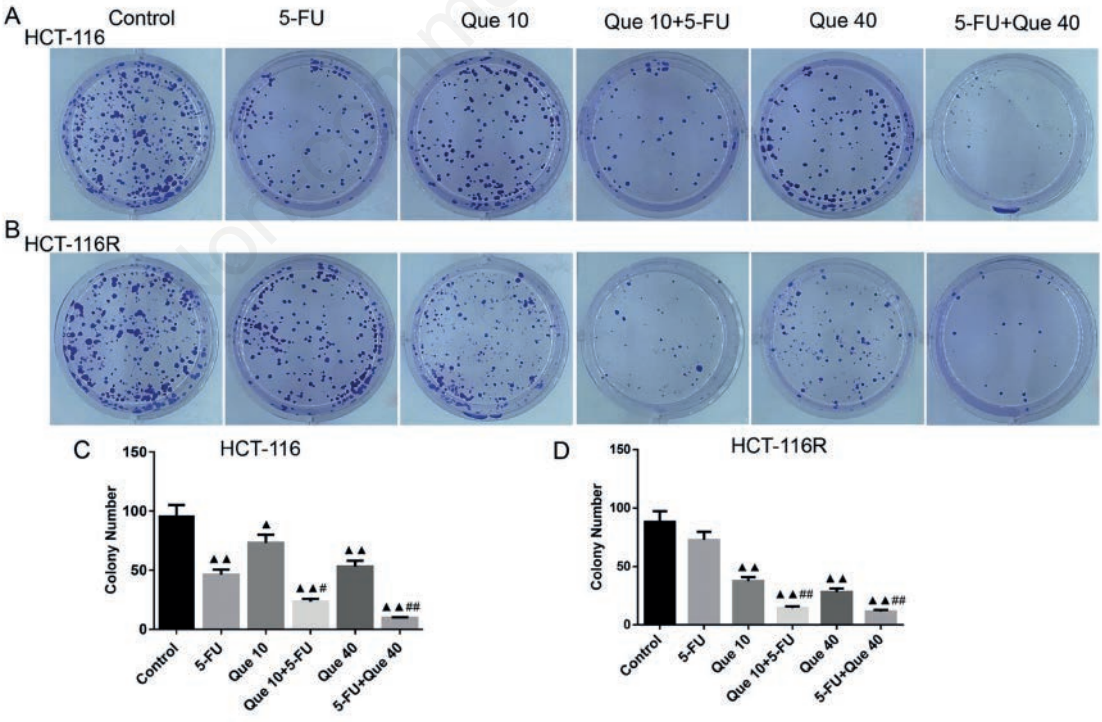

Figure 3. Que further restrained the proliferation of CC cells and 5-FU-resistant CC cells induced by 5-FU. After the cells were treated with 5-FU, Que, or Que plus 5-FU, a colony formation assay was performed to examine the proliferation of CC cells (**A,C**) and 5-FU-resistant CC cells (**B, D**); n=3. Que, quercetin; 5-FU, 5-fluorouracil; CC, colon cancer. ▲$p<0.05$, ▲▲$p<0.01$ *vs* control; #$p<0.05$, ##$p<0.01$ *vs* 5-FU.

Que restrained the proliferation and induced apoptosis of CC and 5-FU-resistant CC cells

CC cells and 5-FU-resistant CC cells further were exposed to 10 μM 5-FU, 10 μM Que, 40 μM Que, 10 μM Que +10 μM 5-FU, or 40 μM Que + 10 μM 5-FU for 72 h, respectively. As shown in Figure 3 A-D, it was manifested in the colony formation assay that 5-FU greatly blunted the proliferation of CC cells but did not obviously affect the proliferation of 5-FU-resistant CC cells. And 10 μM Que and 40 μM Que both evidently weakened the proliferation of CC cells and 5-FU-resistant CC cells ($p<0.05$). More importantly, the combined application of Que and 5-FU inhibited the colony number to a greater extent than treatment with 5-FU alone, particularly in the 5-FU + 40 μM Que group (Figure 3, $p<0.05$). The flow cytometer assay revealed that 5-FU treatment induced apoptosis in HCT116 cells, but not in HCT116-R cells, and the apoptotic rate of HCT116 and HCT116-R cells in the Que 40 + 5-FU combination group was higher than that in the 5-FU group (Figure 4 A,B, $p<0.05$).

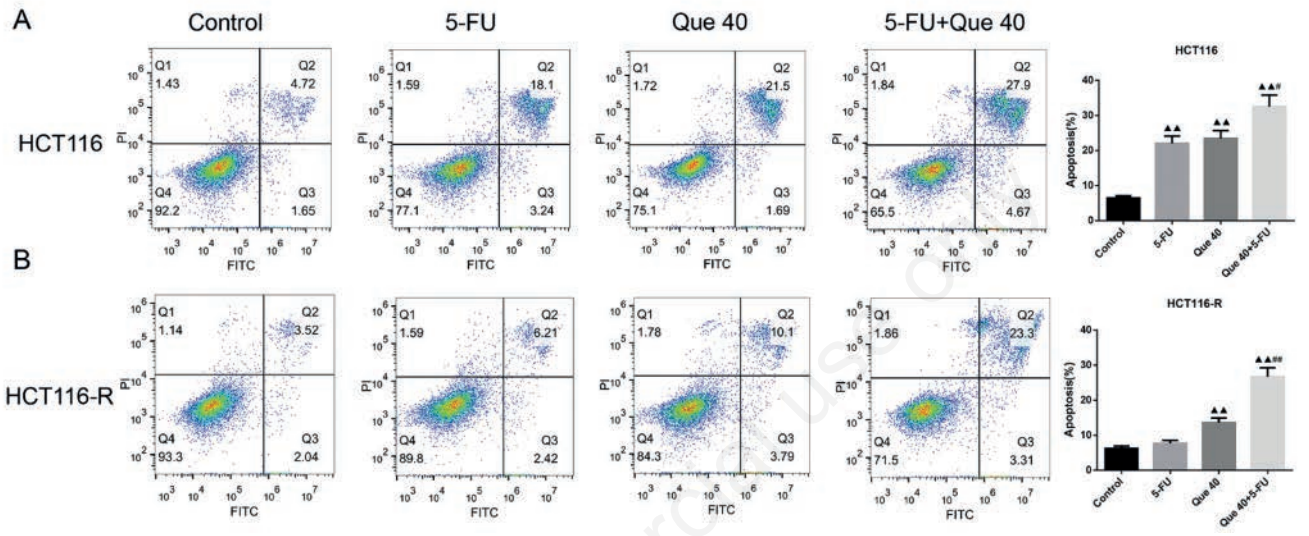

Figure 4. Que further induced apoptosis of CC cells and 5-FU-resistant CC cells induced by 5-FU. After the cells were treated with 5-FU, Que, or Que plus 5-FU, the flow cytometry method was used to examine the apoptosis of CC cells (**A**) and 5-FU-resistant CC cells (**B**); n=3. Que, quercetin; 5-FU, 5-fluorouracil; CC, colon cancer. ▲▲$p<0.01$ *vs* control; #$p<0.05$, ##$p<0.01$ *vs* 5-FU.

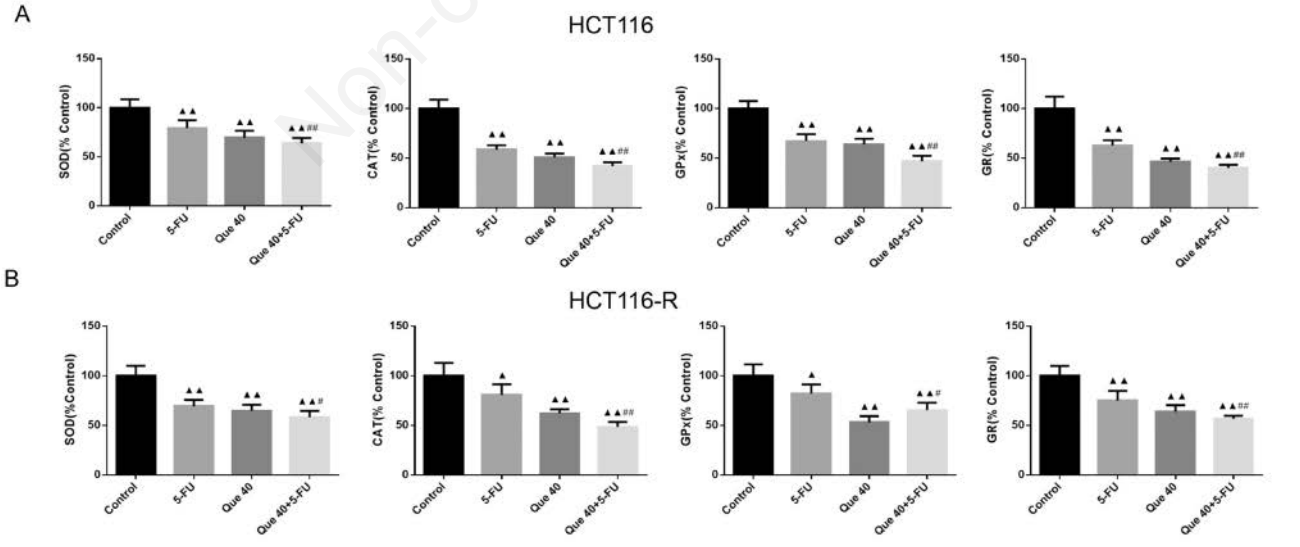

Figure 5. Que further weakened the contents of oxidative stress-related factor of CC cells and 5-FU-resistant CC cells induced by 5-FU. After the cells were treated with 5-FU, Que, or Que plus 5-FU, ELISA kit was used to examine the levels of oxidative stress-related factor of CC cells (**A**) and 5-FU-resistant CC cells (**B**); n=6. Que, quercetin; 5-FU, 5-fluorouracil; CC, colon cancer. ▲$p<0.05$, ▲▲$p<0.01$ *vs* control; #$p<0.05$, ##$p<0.01$ *vs* 5-FU.

Que alleviated oxidative stress and ROS production of CC cells and 5-FU-resistant CC cells

As displayed in Figure 5 A,B, the ELISA assay demonstrated that 5-FU, Que and Que + 5-FU treatment inhibited the activity and contents of SOD, CAT, GPx, and GR contents in CC and 5-FU-resistant CC cells ($p<0.05$). What's more, the inhibitory effects of the combination group (40 μM Que + 10 μM 5-FU) on the SOD, CAT, GPx, and GR contents in CC cells and 5-FU-resistant CC cells were stronger than 5-FU alone ($p<0.05$). Next, we assessed the ROS production and found that the fluorescence intensity of CC cells and 5-FU-resistant CC cells in the 5-FU group and Que 40 group was lower than that in the control group (Figure 6 A,B, $p<0.05$). Meanwhile, the fluorescence intensity of 5-FU-resistant CC cells in the Que 40 + 5-FU group was lower than that in the 5-FU group (Figure 6 A,B, $p<0.05$).

Que suppressed the Nrf2/HO-1 pathway-related marker levels of CC cells and 5-FU-resistant CC cells

We further detected the expression of oxidative stress-related Nrf2/HO-1 signaling in CC cells and 5-FU-resistant CC cells. The results in Figure 7 A-D showed that the relevant fluorescence intensities of Nrf2 and HO-1 were both decreased after Que treatment in CC and 5-FU-resistant CC cells ($p<0.01$), but 5-FU inhibited the Nrf2 and HO-1 expression only in CC cells. However, the addition of Que to 5-FU significantly suppressed the fluorescence intensity of Nrf2 and HO-1 in both CC cells and 5-FU-resistant CC cells compared to the 5-FU group ($p<0.01$). We also tested the Nrf2/HO-1 pathway-related protein expression and confirmed that the expression of Nrf2, SOD-1, CAT, HO-1, NF-κB, and p-IKBα in CC cells of the 5-FU group and Que 40 group were lower than the control group (Figure 8A, $p<0.05$). Compared with the 5-FU group, the Nrf2, SOD-1, CAT, HO-1, and NF-κB levels of CC cells were further decreased but the p-IKBα level of CC cells was enhanced in the Que 40 + 5-FU group (Figure 8A, $p<0.05$). For the 5-FU-resistant CC cells, 5-FU obviously blunted the NF-κB and p-IKBα levels (Figure 8B, $p<0.05$), but did not affect the Nrf2, SOD-1, CAT, and HO-1 levels. The combination of Que and 5-FU further reduced the Nrf2, SOD-1, CAT, HO-1, and p-IKBα levels compared to the 5-FU group (Figure 8B, $p<0.05$).

Discussion

At present, the treatment options for CC remain limited, and the first choice is mainly surgical resection, as well as adjuvant therapy including 5-FU, mitomycin, Me-CCNu, and antigen-specific immunotherapy.[21] Although these treatment methods have achieved certain therapeutic effects, there are still limitations. When killing cancer cells, the long-term use of chemotherapeutic drugs leads to the development of MDR in cancer cells and reduces their sensitivity. Therefore, it is of profound theoretical and practical significance to identify for natural and effective botanical compounds that can reverse MDR in CC. In this study, we investigated Que as a 5-FU sensitizer for CC cell treatment.

Que has been corroborated with the potential to reverse the MDR. Lan *et al.* manifested that in pancreatic cancer cells and gemcitabine-resistant pancreatic cancer cells, Que facilitated cell cycle arrest, autophagy, apoptosis, and enhanced gemcitabine chemosensitivity.[22] For tamoxifen-resistant breast cancer cells, Que notably lessened the proliferation and enhanced apoptosis of cells, illustrating that Que intensified the chemosensitivity of breast cancer cells.[23] Shu *et al.* clarified that co-treatment of doxorubicin and Que induces apoptosis of doxorubicin-resistant prostate cancer cells *via* repressing c-met expression and PI3K/AKT pathway, illustrating that Que could reverse the drug-resistance of prostate cancer cells.[24] Our study complemented the deficiency of Que and 5-FU combination therapy in 5-FU-resistant HCT-116 cells. We demonstrated that 5-FU blunted the cell proliferation and induced apoptosis in CC cells and 5-FU-resistant CC cells, while its anti-cancer effect on 5-FU-resistant CC cells was weaker than that on normal CC cells. More importantly, co-treat-

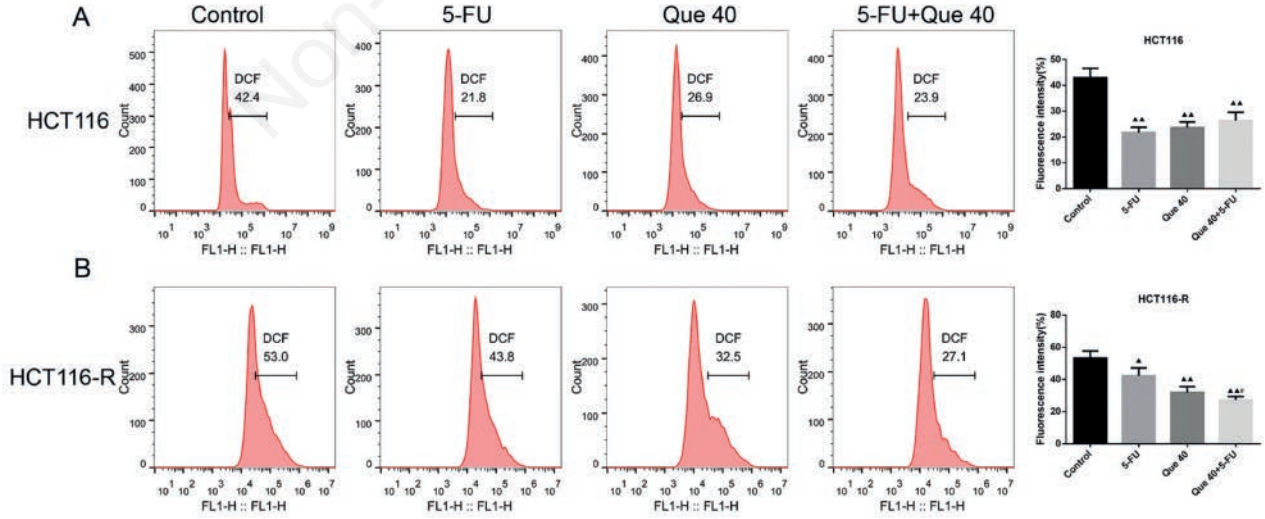

Figure 6. Que further weakened the ROS production of CC cells and 5-FU-resistant CC cells induced by 5-FU After the cells were treated with 5-FU, Que, or Que plus 5-FU, reactive oxygen species (ROS) assay was used to examine the ROS production in CC cells (**A**) and 5-FU-resistant CC cells (**B**). n=3. Que: quercetin; 5-FU: 5-fluorouracil, CC: colon cancer. ▲P<0.05, ▲▲P<0.01 *vs* control; #P<0.05 *vs* 5-FU.

ment with Que and 5-FU notably restrained the proliferation and induced apoptosis of 5-FU-resistant CC cells, indicating that Que could reverse the 5-FU-resistance in CC cells. For MDR in CC cells, Zhou *et al*. also demonstrated that the combination of Que and doxorubicin enhanced the chemosensitivity of CC cells, regulation of P-glycoprotein and SLC1A5 expression was considered as the underlying mechanism.[16] Another study also demonstrated that Que potentiates 5-FU effects in human CC cells, which would be associated with the miR-27a/ Wnt/β-catenin signaling pathway.[25] In our study, we speculate that oxidative stress regulation is the major mechanism for reverse 5-FU resistant in HCT 116 cells.

In recent years, researchers have gradually realized the role of oxidative stress in tumorigenesis, and genes related to oxidative stress pathways are expected to become new targets for tumor drug chemotherapy. Oxidative stress refers to the excessive production of ROS in the body when the body is subjected to various harmful stimuli, and the degree of oxidation exceeds the ability to remove oxides, thus leading to tissue damage.[26] ROS can be produced by cancer cells by the increased mitochondrial activity and accelerated metabolism required for tumor growth and cell proliferation, which facilitates the initiation and development of cancer.[27] GPx is a clear ROS scavenger that can mediate the development of cisplatin resistance.[28,29] It has previously been confirmed that Nrf2 is involved in the regulation of GPx level, and the activation of Nrf2 can lead to a high dependence on GPx in cells.[30] NRF2 is a transcription factor containing a basic leucine zipper structure, which is a key protein for the basic expression of oxidative stress.[31] Research on Nrf2 in tumors has illustrated that abnormal expres-

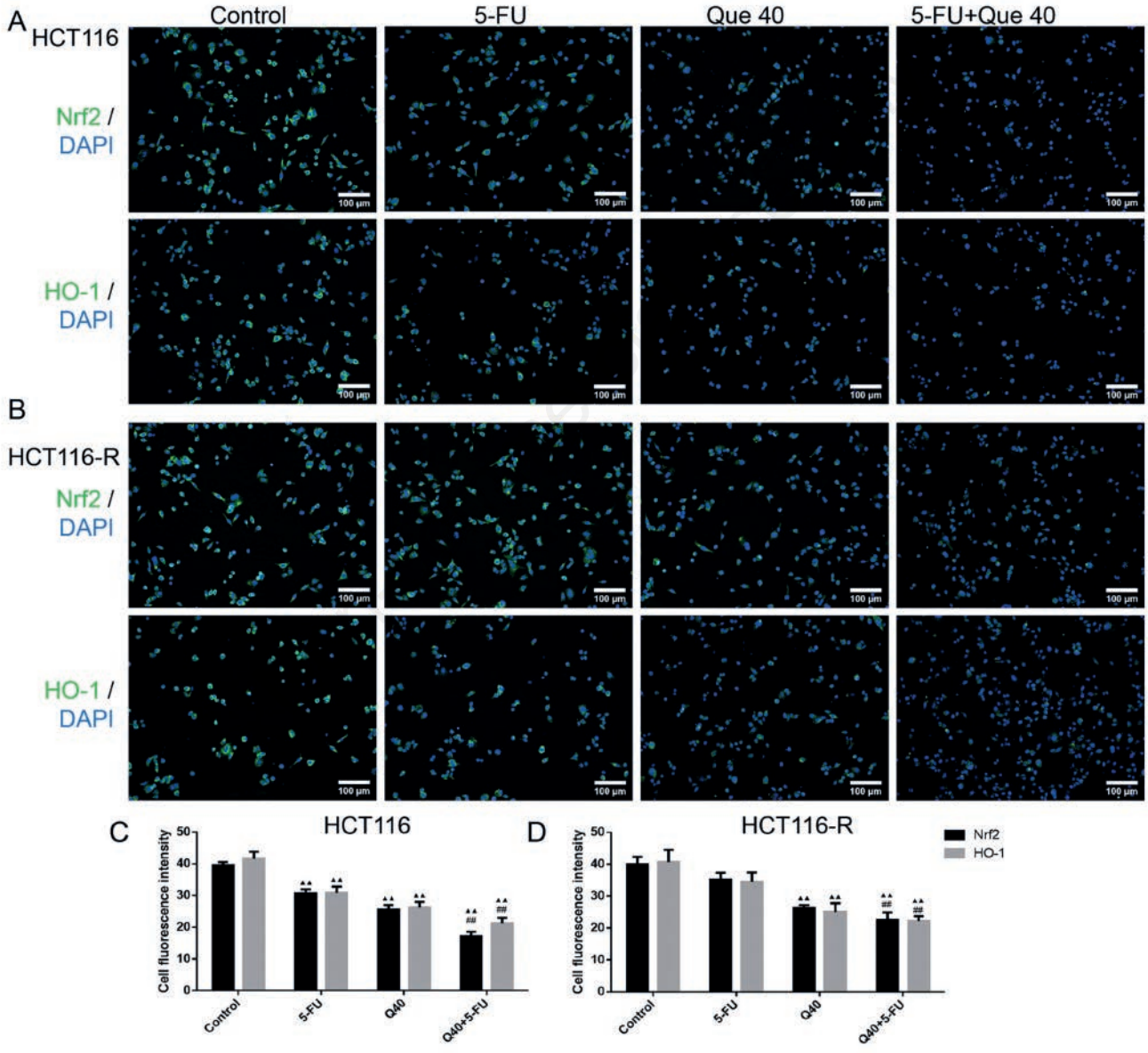

Figure 7. The combination of Que and 5-FU attenuated the Nrf2 and HO-1 expression in CC cells and 5-FU-resistant CC cells. After the cells were treated with 5-FU, Que, or Que plus 5-FU, immunofluorescence assay was used to examine the expression of nuclear factor erythroid 2-related factor (Nrf2) and heme oxygenase-1 (HO-1) in CC cells (**A**) and 5-FU-resistant CC cells (**B**). n=3. Que: quercetin; 5-FU: 5-fluorouracil, CC: colon cancer. ▲▲P<0.01 *vs* control; ##P<0.01 *vs* 5-FU.

sion of Nrf2 can promote tumor proliferation and metastasis, and it is associated with chemotherapy resistance and poor prognosis.[32-34] Many antioxidant proteins encoded by Nrf2-dependent genes, such as HO-1 and SOD, can make cells resist oxidative stress and assist the body to maintain oxidation-reduction homeostasis.[35, 36] Abbasi *et al.* proposed that co-treatment of vitamin C and Que attenuated the activities of GPX and GR and the expression of Nrf2 mRNA and protein in prostate cancer cells.[37] It was illustrated in another study that Que mitigated the oxidative stress and DNA damage in CC rats triggered by 1,2-dimethylhydrazine by modulating the Nrf2 pathway.[38] In our study, we discovered for the first time that co-treatment of Que and 5-FU weakened the contents of oxidative stress-related factors (SOD, CAT, GPx, and GR) and ROS production of CC cells and 5-FU-resistant CC cells. We further demonstrated that the combination of Que and 5-FU attenuated the Nrf2, SOD-1, CAT, HO-1, NF-κB, and p-IKBα expressions of 5-FU-resistant CC cells.

In summary, our research observed that *in vitro* experiments using Que combined with 5-FU on 5-FU-resistant HCT-116 cells showed a good synergistic effect. The application of natural compounds in chemotherapy has good clinical application prospects for reducing toxicity and adverse reactions, as well as enhancing curative sensitivity. However, the interaction between Que and 5-FU at different concentrations requires further investigation.

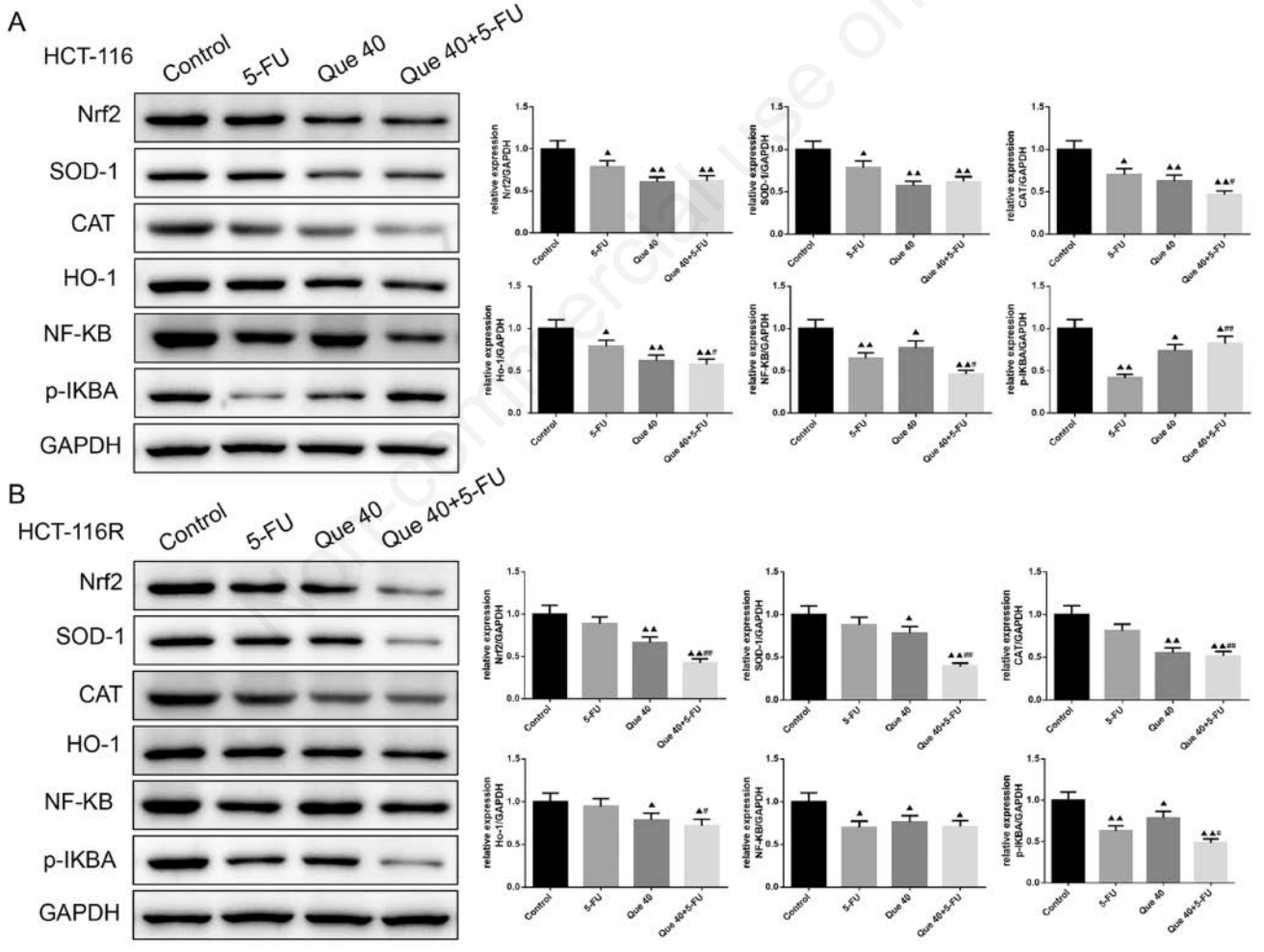

Figure 8. The combination of Que and 5-FU attenuated the Nrf2/HO-1 pathway-related marker levels of CC cells and 5-FU-resistant CC cells. After cells were treated with 10 μg/mL 5-FU, 40 μM Que, or 40 μM Que plus 10 μg/mL 5-FU, Western blotting was used to examine the Nrf2/HO-1 pathway-related factors level in CC cells (**A**) and 5-FU-resistant CC cells (**B**); n=3. Que, quercetin; 5-FU, 5-fluorouracil; CC, colon cancer. ▲p<0.05, ▲▲p<0.01 vs control; #p<0.05, ##p<0.01 *vs* 5-FU.

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