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Crocin exerts anti-tumor effect in colon cancer cells *via* repressing the JAK pathway

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ABSTRACT

Crocin has been reported to have therapeutic effects on multiple cancers including colon cancer, but its specific mechanism is still ambiguous and needs to be further explored. Human colorectal adenocarcinoma cells (HCT-116) and human normal colonic epithelial cells (CCD841) were first treated with increasing concentrations of crocin. Subsequently, with 150 and 200 μM of crocin, the cell vitality was examined by cell counting kit 8. Cell apoptosis and proliferation were tested by TUNEL staining and colony formation assay, respectively. The expression of Ki-67 was assessed by immunofluorescence. Enzyme-linked immunosorbent assay was used to evaluate the level of inflammation- and oxidative-related factors. The reactive oxygen species (ROS) production and mitochondrial membrane potential (MMP) were examined by flow cytometry. Janus kinase (JAK), signal transducer and activator of transcription 3 (STAT3), and extracellular regulated protein kinases (ERK) in HCT-116 cells were tested by Western blot. Different concentrations of crocin barely affected the CCD841 cell vitality, while crocin restrained the HCT-116 cells vitality, proliferation and the expression of Ki-67, while inducing apoptosis in a concentration-dependent manner. Moreover, the contents of inflammation- and oxidative-related factors in HCT-116 cells were largely blunted by crocin that enhanced ROS, restrained the MMP and suppressed p-JAK2/JAK2, p-STAT3/STAT3, and p-ERK/ERK expression. Crocin induced apoptosis and restored mitochondrial function in HCT-116 cells *via* repressing the JAK pathway. If the therapeutic effect works in patients, it could herald a new, effective treatment for colon cancer, improving the patients' prognosis and quality of life.

Key words: colon cancer; crocin; apoptosis; Janus kinase pathway; mitochondrial function.

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Introduction

Colon cancer (CC) is a major health concern worldwide, with increasing morbidity and mortality rates.[1,2] CC is a complex disease resulting from the interaction of genetic factors, lifestyle, dietary habits, and environmental factors.[3] Each year, more than 10 million new cases of CC are reported worldwide. While surgery is the primary treatment option, it is often supplemented by radiotherapy and chemotherapy, yet the cure rate of surgery alone remains unsatisfactory.[4,5] Early detection of CC is critical, as the disease often has an insidious onset and patients are often diagnosed at an advanced stage, missing the window for surgical intervention. Traditional radiotherapy and chemotherapy are often ineffective, with significant toxic adverse reactions and the development of drug resistance in cancer cells. Thus, it is crucial to identify convenient diagnostic methods and indicators for early detection and treatment of CC.[6] Therefore, addressing the challenges of CC requires a multidisciplinary approach, with emphasis on early detection, improved surgical techniques, and innovative treatment options.

In recent years, the advantages of traditional Chinese medicines (TCM) such as multi-target mechanism and fewer adverse reactions have shown attractive application prospects. The screening of high-efficiency and low-toxic anti-tumor TCM is one of the research hotspots. Crocin is a monomeric component extracted from saffron, which has a wide range of pharmacological effects, such as anti-myocardial ischemia, immune regulation, liver and gallbladder protection, blood lipid regulation, prevention of chemotherapy toxicity, anti-atherosclerosis, anti-inflammation, and anti-oxidation, *etc*.[7-10] With the in-depth study of pharmacology, the anti-tumor activity of crocin has been paid more attention and further explored. It has a notable inhibitory role on the proliferation of human gastric cancer cells, human pancreatic cancer cells, CC, and other cancer cells with inhibition of the migration and invasion of tumor cells.[11-13] Crocin has been discovered by Aung *et al*. to restrain the proliferation of multiple human CC cells, but has no obvious effect on the growth of normal colon cells, so it can be used as a feasible choice for the treatment of CC.[13] Long-term treatment of crocin could selectively prolong the life span of female rats with CC and weaken the growth of tumors.[14] Rastgoo *et al*. discovered that crocin had a good anti-colon cancer effect and obvious cytotoxicity to CC cells, and its liposome encapsulation could increase its anti-tumor activity.[15] Study contributes to this burgeoning field of research by revealing that crocin's pharmacological action against pathological behavior in colon cancer cells is mediated through its interaction with the STAT3 signaling pathway.[16] In addition, study found that crocin inhibits angiogenesis and metastasis in colon cancer *via* the TNF-α/NF-kB/VEGF pathways.[17] These insights offer a more nuanced understanding of the mechanisms underlying crocin's therapeutic effects in the treatment of colon cancer. To further investigate the potential inhibitory effects of crocin on colon cancer cells, as well as its underlying mechanisms, future research endeavors will require thorough investigations. Based on these clues, in this study we have chosen human colorectal adenocarcinoma cells (HCT-116) as our primary subjects to assess the impact of crocin on colon cancer cells and decipher its molecular mechanisms of action.

Materials and Methods

Cell lines

Human colorectal adenocarcinoma cells (HCT-116) and human normal colonic epithelial cells (CCD841) were obtained from iCell Bioscience Inc. (Shanghai, China) and grown in DMEM medium (SH30243.01; Hyclone, Logan, UT, USA) supplied with 10% fetal bovine serum (FBS, 11011-8615; Tianhang, China). HCT-116 and CCD841 cells were then placed in a cell incubator (BB150; Thermo Fisher Scientific, Waltham, MA, USA) with 5% CO_2 at 37°C for subsequent experiments.

Drug preparation

Crocin (17304) was obtained from Sigma-Aldrich (St Louis, MO, USA) and dissolved in DMSO (D103277; Aladdin, Shanghai, China). We added phosphate-buffered saline (PBS, P274233; Aladdin) to the dissolved crocin to obtain a 500 mM crocin stock solution. Different concentrations of crocin (50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, and 900 μM) were prepared.

Cell viability assay

To choose proper concentration of the crocin and exclude its toxicity concentration for subsequent experiments, we used the cell counting kit-8 (CCK-8) kit (HY-K0301, MCE, USA) to evaluate cell vitality. The logarithmic growth period of HCT-116 and CCD841 cells (1×10^4) were seeded into the 96-well plates. They were then placed in the cell incubator for 24 h. HCT-116 cells were subjected to 0, 50, 100, 150, 200, 250, 300, 350, 400, and 450 μM crocin for 24 h. CCD841 cells were subjected to 0, 100, 200, 300, 400, 500, 600, 700, 800, and 900 μM crocin for 24 h. Thereafter, cells were subjected to 10 μL CCK-8 solution and transferred to the cell incubator for 3 h. We then used a microplate reader (CMaxPlus; MD Biosciences, Oakdale, MN, USA) to estimate the absorbance (450 nm).

Apoptosis analysis

HCT-116 cells (1.2×10^6) placed in the cell incubator for 24 h, were subjected to 0, 150, and 200 μM crocin for 24 h and then harvested, washed, and centrifuged. After that, we used 1× binding buffer to adjust cell concentration (1.2×10^6). After centrifugation, the precipitated cells were mixed with 100 μL binding buffer followed by the addition of 5 μL Annexin V-FITC and 10 μL PI, respectively. Apoptosis of HCT-116 cells was assessed using the Annexin V-FITC/propidium iodide (PI) kit (556547; MD Biosciences). They were then placed at 37°C away from light for 15 min. After adding 1× binding buffer, we used a flow cytometer (C6, BD, USA) to assess apoptosis.

The colony formation assay

HCT-116 cells inoculated into the 12-well plates were reacted with 0, 150, and 200 μM crocin for 24 h. The treated cells (5×10^2) were then harvested and inoculated into the 6-well plates. After 14 days of culture, we fixed the cells with 75% alcohol (A171299; Aladdin) for 1 h and stained the cells with 0.25% crystal violet (C8470, Solarbio, Beijing, China) for 2 min. In the end, we used an optical microscope (AE2000, Motic, Wetzlar, Germany) to capture and observe the results.

TUNEL staining

TUNEL apoptosis kit (C1090; Beyotime Institute of Biotechnology, Haimen, China) was used in this research. HCT-116 cells (5×10^4) were inoculated in the 6-well plate containing a cover glass for 24 h. They were then subjected to 0, 150, and 200 μM crocin for 24 h. After fixing the cells with 4% paraformaldehyde (P0099; Beyotime Institute of Biotechnology), they were permeated with 0.5% Triton X-100 (T109027; Aladdin) for 2 min and washed with PBS for 3 times. After treating the cells with TUNEL test solution (C1090; Beyotime Institute of Biotechnology) away

from light for 1 h at 37°C, we used DAPI solution (1 μg/mL) (C1005; Beyotime Institute of Biotechnology) to stain the nucleus for 15 min at 37°C. After cells were sealed with an anti-fade mounting medium (P0128S; Beyotime Institute of Biotechnology), we used a fluorescence microscope (Ts2-FC, Nikon, Tokyo, Japan) to survey the results.

Immunofluorescence

HCT-116 cells (1×10^4) were treated with 0, 150, and 200 μM crocin for 24 h. Cells were fixed with 4% paraformaldehyde for 15 min at room temperature and then, we used 0.1% Triton X-100 to permeate the cell membrane. Subsequently, we used 3% BSA (ST025; Beyotime Institute of Biotechnology) to seal cells for 0.5 h. Next, cells of each group were reacted with anti-Ki-67 antibody (1:400, ab243878; Abcam, Cambridge, UK) at 4°C overnight, followed by the addition of Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (1:1000, ab150077; Abcam) for 30 min at 37°C. As negative controls, cell samples were processed in the same manner as the test samples, but omitting the primary antibody. Thereafter, cells of each group were stained with DAPI. After washing, the slides were sealed with mounting media, and then examined under a fluorescence microscope, and areas displaying positive staining were duly noted. Subsequently, we utilized image analysis software (Image J) to quantitatively assess the intensity and extent of staining in these identified areas. This combination of techniques enabled us to derive both qualitative and quantitative data on the level of immunopositivity in our samples.

ELISA

The cells were homogenized in RIPA buffer, containing protease- and phosphatase-inhibitors to prevent protein degradation. The homogenization was done on ice using a mechanical homogenizer, to ensure cell lysis and uniform distribution of cellular components. The resulting homogenate was then often centrifuged to separate the insoluble material, and the supernatant was collected for ELISA analyses. Human macrophage inflammatory protein 2 (MIP-2) kit (BPE11687), human interleukin-6 (IL-6) kit (BPE10140), human monocyte chemoattractant protein-1 (MCP-1) kit (BPE10136), human IL-8 kit (BPE10139), human IL-1β kit (BPE10083), human tumor necrosis factor α (TNF-α) kit (BPE10110), human superoxide dismutase (SOD) kit (BPE11086), human catalase (CAT) kit (BPE10498), human glutathione (GSH) kit (BPE10801) were derived from Lengton (Shanghai, China). The prepared cell supernatants were first added to each sample hole, followed by the addition of anti-MIP-2 / IL-6 / MCP-1 / IL-8 / IL-1β / TNF-α / SOD / CAT / GSH antibody and streptavidin-HRP. After that, each hole was covered with sealing film and then reacted at 37°C for 60 min. After washing, each sample hole was reacted with color developing agents A and B and displayed at 37°C for 10 min away from light. Finally, the absorbance (450 nm) was assessed using the microplate reader after the addition of the stop solution.

Detection of reactive oxygen species

Reactive oxygen species (ROS) kit (S0033M; Beyotime Institute of Biotechnology) was used in this research. HCT-116 cells were seeded in a 6-well plate at a density of 5×10^4 cells per well and incubated for 24 h. Post-incubation, cells were treated with 0, 150, and 200 μM crocin for 24 h. Following treatment, the cells were washed twice with PBS and incubated with 10 μM DCFH-DA (2',7'-dichlorofluorescein diacetate) at 37°C for 30 min. DCFH-DA is a cell-permeable non-fluorescent probe, which is de-esterified intracellularly and turns to highly fluorescent DCF upon oxidation. After incubation, the cells were washed three times with PBS to remove any remaining DCFH-DA. The fluorescence intensity, which correlates with the intracellular ROS level, was measured using a flow cytometer at an excitation wavelength of 485 nm and emission wavelength of 535 nm.

Detection of mitochondrial membrane potential

HCT-116 cells (1×10^5) in the 6-well plate were stimulated with 0, 150, and 200 μM crocin for 24 h. Post-treatment, cells are washed twice with PBS and stained with JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) working solution (10 μM, ab113850; Abcam) for 30 min at 37°C in a CO_2 incubator. JC-1 is a cationic dye that exhibits potential-dependent accumulation in mitochondria. In healthy cells with high mitochondrial $\Delta\psi m$, JC-1 spontaneously forms aggregates and gives off red fluorescence, whereas in apoptotic or unhealthy cells with low $\Delta\psi m$, JC-1 remains in the monomeric form and gives off green fluorescence. After removing the supernatant, 1× JC-1 staining buffer was utilized to wash each hole two times. After adding 2 mL medium, they were detached and placed in the flow cytometer (excitation wavelength used was 475 nm and emission wavelengths were 511 nm and 587 nm for the monomer and the aggregates of JC-1 molecules, respectively).

Western blot

The crocin-treated cells were harvested and lysed using a RIPA buffer (abs9229; Absin Bioscience Inc., Shanghai, China). After centrifugation, the cell lysates were harvested and the protein concentrations were examined *via* a BCA kit (pc0020; Solarbio). After denaturation, the samples of each group were subjected to protein electrophoresis. After transferring to a PVDF membrane (10600023; GE Healthcare Life Sciences, Chicago, IL, USA), they were then sealed with 5% skim milk at 37°C for 2 h. After that, they were reacted with primary antibodies overnight at 4°C followed by the addition of secondary antibodies for 1 h at 37°C. Lastly, the blots were visualized with a color reagent (1705061; Bio-Rad Laboratories, Hercules, CA, USA) in a chemic luminous instrument (610020-9Q; Clinx, Shanghai, China). The primary antibodies of JAK2 (AF6022), p-JAK2 (AF3024), p-STAT3 (AF3293), STAT3 (AF6294), p-ERK (AF1015), ERK (AF0155), and β-actin (AF7018) were obtained from Affinity Biosciences (Cincinnati, OH, USA). The band intensity was measured by Image J software.

Statistical analysis

The statistical analysis was carried out by SPSS software (16.0, IBM, Armonk, NY, USA). One-way ANOVA was employed for comparing the differences among multiple groups. SNK analysis was employed for comparison between groups. Kruskal-Wallis H test was employed for those with uneven variance. The data were expressed as mean ± SD. A p-value <0.05 was designated as statistically significant.

Results

The effect of different concentrations of crocin on the cell vitality of HCT-116 and CCD841 cells

We first used crocin (50, 100, 150, 200, 250, 300, 350, 400, and 450 μM) to treat HCT-116 cells and the results demonstrated that the cell vitality was notably weakened by crocin in a concentration-dependent manner (Figure 1a; $p<0.05$). While crocin (100, 200, 300, 400, 500, 600, 700, 800, and 900 μM) did not affect CCD841 cell viability (Figure 1b). Thus, we chose 150 and 200 μM crocin for subsequent experiments.

Crocin induced apoptosis and restrained the proliferation in HCT-116 cells

The flow cytometer assay results and TUNEL-positive apoptosis cell rate manifested that crocin greatly facilitated apoptosis of HCT-116 cells in a concentration-dependent manner, especially in 200 μM (Figure 2a; $p<0.01$). Meanwhile, with colony formation and the immunofluorescence assay, we found that crocin effectively abated the colony number of the HCT-116 cells (Figure 3a; $p<0.01$). And it downregulated the positive expression of Ki-67 in HCT-116 cells compared to the control group, especially in 200 μM (Figure 3b).

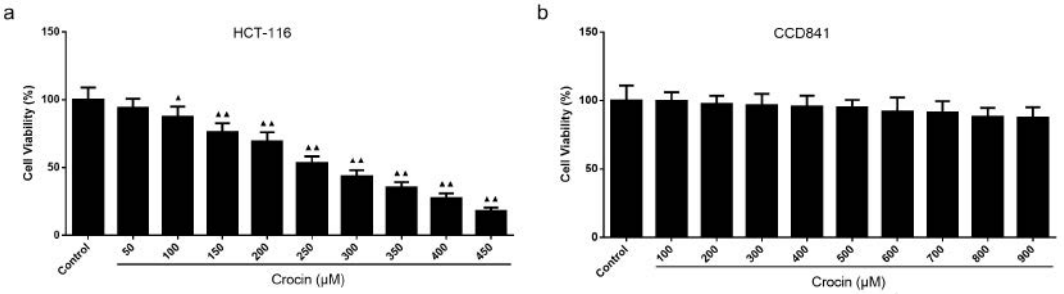

Figure 1. The effect of different concentrations of crocin on the cell vitality of HCT-116 and CCD841 cells. The effect of crocin on the cell vitality of HCT-116 (**a**) and CCD841 cells (**b**) was detected by cell counting kit-8 (CCK-8). All experiments have been performed in triplicate and data were expressed as mean ±SD. ▲$p<0.05$, ▲▲$p<0.01$ *vs* control. Crocin concentrations: 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, and 900 μM.

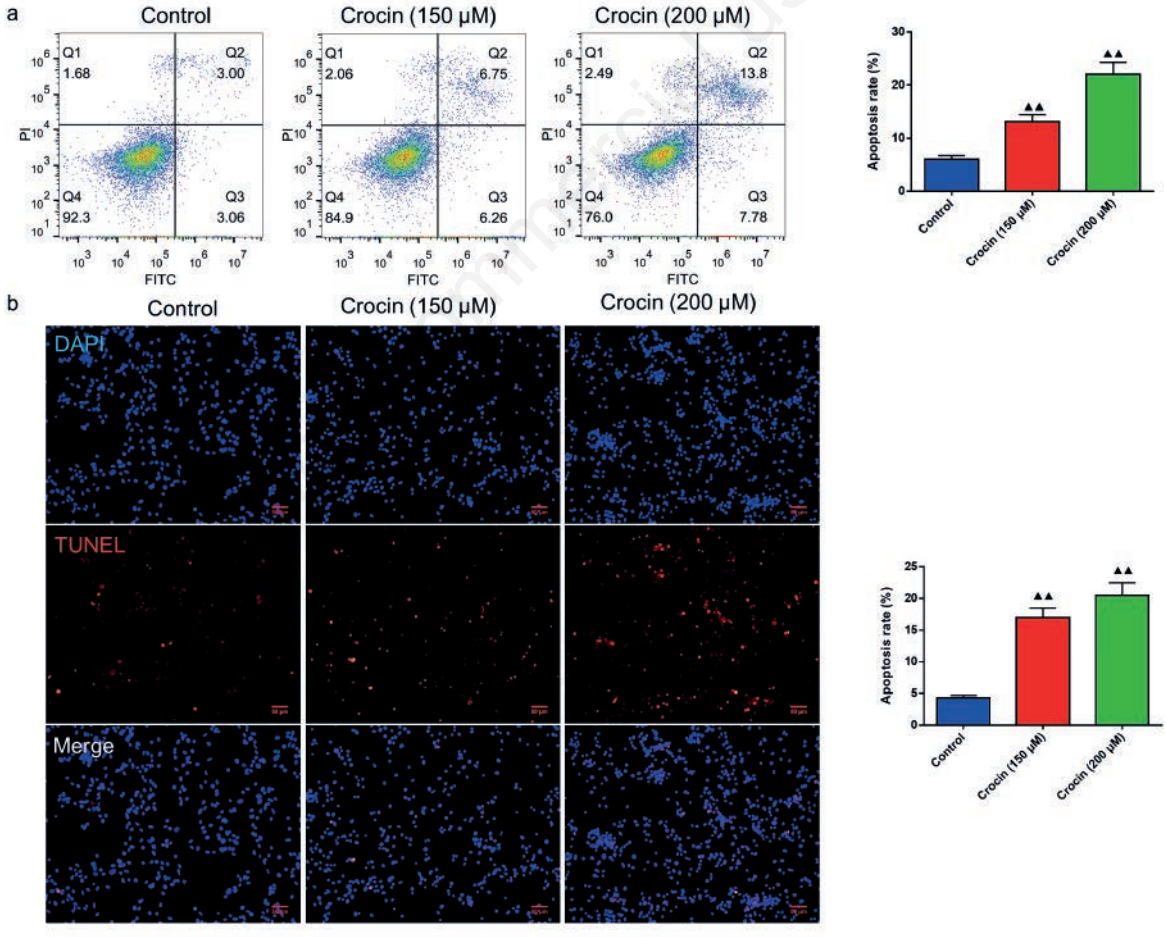

Figure 2. Crocin induced apoptosis in HCT-116 cells. **a**) The effect of crocin on apoptosis of HCT-116 cells was detected by flow cytometry. **b**) TUNEL staining was used to test the apoptosis of the HCT-116 cells; scale bar: 50 μM. All experiments have been performed in triplicate and data were expressed as mean ±SD. ▲▲$p<0.01$ *vs* control. Crocin concentrations: 150 and 200 μM.

The effect of crocin on the contents of MIP-2, IL-6, MCP-1, IL-8, IL-1β, TNF-α, SOD, CAT, and GSH in HCT-116 cells

As displayed in Figure 4, with ELISA assay, we found that crocin manifested an obvious inhibition of the contents of MIP-2, IL-6, MCP-1, IL-8, IL-1β, and TNF-α in HCT-116 cells in relative to the control group and the inhibitory effect of 200 μM crocin was stronger than that of 150 μM crocin ($p<0.05$). Additionally, we also examined the oxidative stress-related factors and confirmed that the contents of SOD, CAT, and GSH in HCT-116 cells were reduced by crocin in relative to the control group and the 200 μM crocin exerted a better effect (Figure 5; $p<0.01$).

Crocin enhanced the ROS production and restrained MMP of HCT-116 cells

As shown in Figure 6a, crocin exhibited a notable increase production of ROS in HCT-116 cells ($p<0.01$). Additionally, the decrease of MMP in HCT-116 cells induced by crocin was evidently raised, that is, the MMP was obviously decreased compared to the control group, and the 200 μM crocin exerted a better effect (Figure 6b; $p<0.01$). It further corroborates the earlier data by showing an increase in ROS production and a decrease in MMP within the HCT-116 cells, suggesting that crocin is inducing oxidative stress and damaging the mitochondria, leading to cell death. The stronger effect seen with 200 μM crocin suggests a dose-dependent response.

The effect of crocin on the JAK2/STAT3 and ERK pathway in HCT-116 cells

In this research, we used Western blot to assess the expressions of JAK2/STAT3/ERK pathway-related markers, from which we discovered that the levels of p-JAK2, p-STAT3, and p-ERK, as well as the ratios of p-JAK2/JAK2, p-STAT3/STAT3, and p-ERK/ERK, were largely attenuated by crocin compared to the control group, and the 200 μM crocin exerted a better effect (Figure 7; $p<0.05$). The decrease in the ratios of p-JAK2/JAK2, p-STAT3/STAT3, and p-ERK/ERK following crocin treatment indicates a reduction in the phosphorylation status of these proteins, which is indicative of their decreased activation. The observed reduction in their phosphorylation status in response to crocin treatment implies that crocin may suppress the activation of these pathways, thereby inhibiting the proliferation and inducing apoptosis in colon cancer cells. The fact that a higher concentration of crocin (200 μM) results in a more substantial reduction in these ratios further suggests a dose-dependent inhibitory effect of crocin on the activation of the JAK2/STAT3/ERK pathway.

Discussion

Increasing researchers have demonstrated that TCM have a certain curative effect on CC.[18,19] Clinical and basic research have manifested that TCM have the characteristics of a high stability

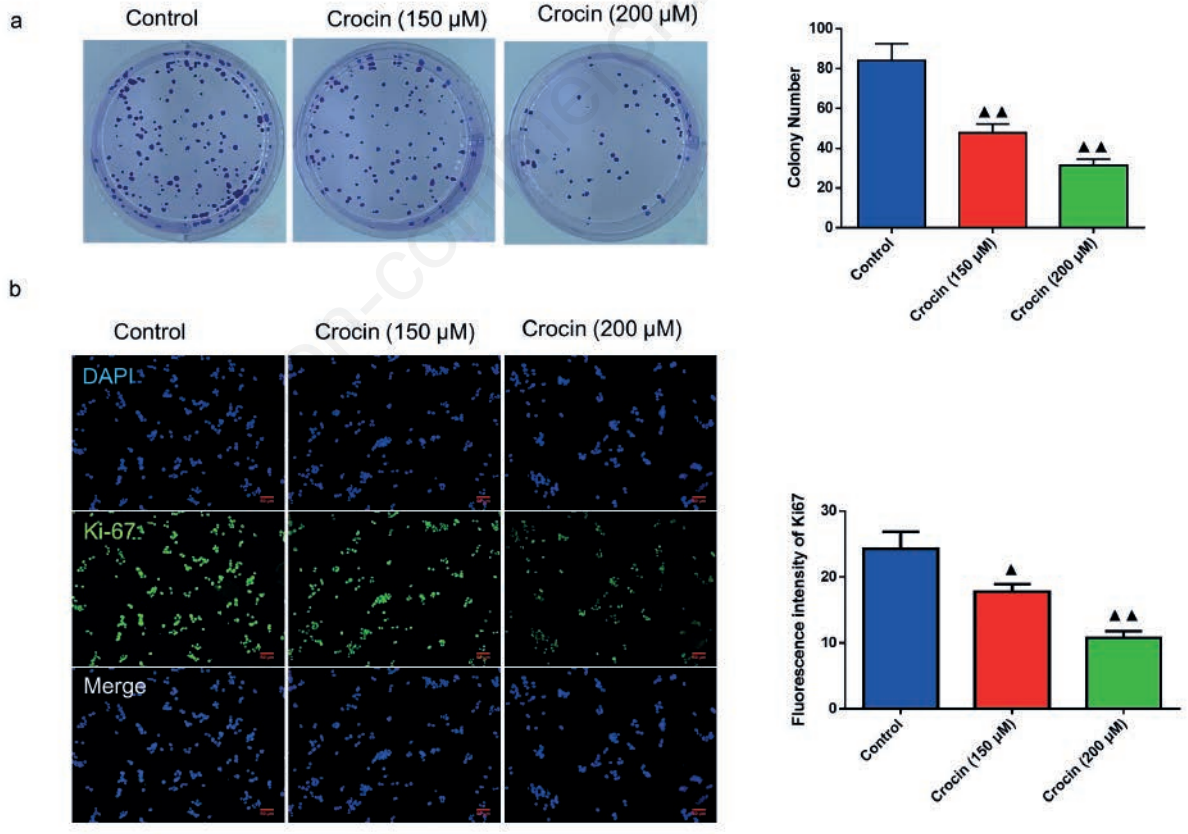

Figure 3. Crocin restrained the proliferation in HCT-116 cells. **a**) The effect of crocin on the proliferation of HCT-116 cells was detected by colony formation assay. **b**) The effect of crocin on the positive expression of Ki-67 in HCT-116 cells was detected by immunofluorescence. All experiments have been performed in triplicate and data were expressed as mean ±SD. ▲▲$p<0.01$ *vs* control. Crocin concentrations: 150 and 200 μM.

rate in the treatment of CC and have certain advantages in improving the quality of life and long-term survival of patients.[20,21] Meanwhile, TCM has a certain synergistic effect in radiotherapy, chemotherapy, and molecular targeted therapy, which can heighten the survival rate of CC patients.[22 23] Therefore, it has become a research hotspot to find effective drugs for the treatment of CC with small toxic and side effects.

In vitro, crocin could effectively weaken the proliferation and induce the apoptosis of tumor cells. Crocin could repress the growth of tumor cells, and its anti-cancer mechanism was related to the induction of apoptosis of tumor cells and the strong cytotoxicity of tumor cells.[24] Mousavi *et al*. have proposed that both crocin and crocin liposomes could induce apoptosis in Hela and MCF-7 cells, and the liposome form could make crocin more cytotoxic.[25] It was clarified in an existing study that crocin could attenuate the proliferation and nucleic acid synthesis of tongue squamous cell carcinoma and induce apoptosis.[26] It was manifested in another study that the injection of crocin in mice could reverse the tumor-like pathological changes, suggesting that crocin was a potential anti-tumor agent.[27] Based on the above research foundation, we investigated the role of crocin in CC cells. We discovered that crocin barely affect normal colonic epithelial cell viability, while crocin notably blunted the CC cell viability, proliferation, and induced apoptosis, which was consistent with the previous study. Next, we tested the proliferation marker Ki-67, which was closely related to tumor cell proliferation. Ki-67 exerted a vital role in the initiation and development of CC and was related to tumor cell invasion and angiogenesis.[28] Our research manifested that the positive expression of Ki-67 in CC cells was attenuated by crocin. Moreover, mitochondrial dysfunction was found in apoptosis induced by many factors, which is considered to be the key to irreversible cell death.[29] In this research, we discovered that crocin

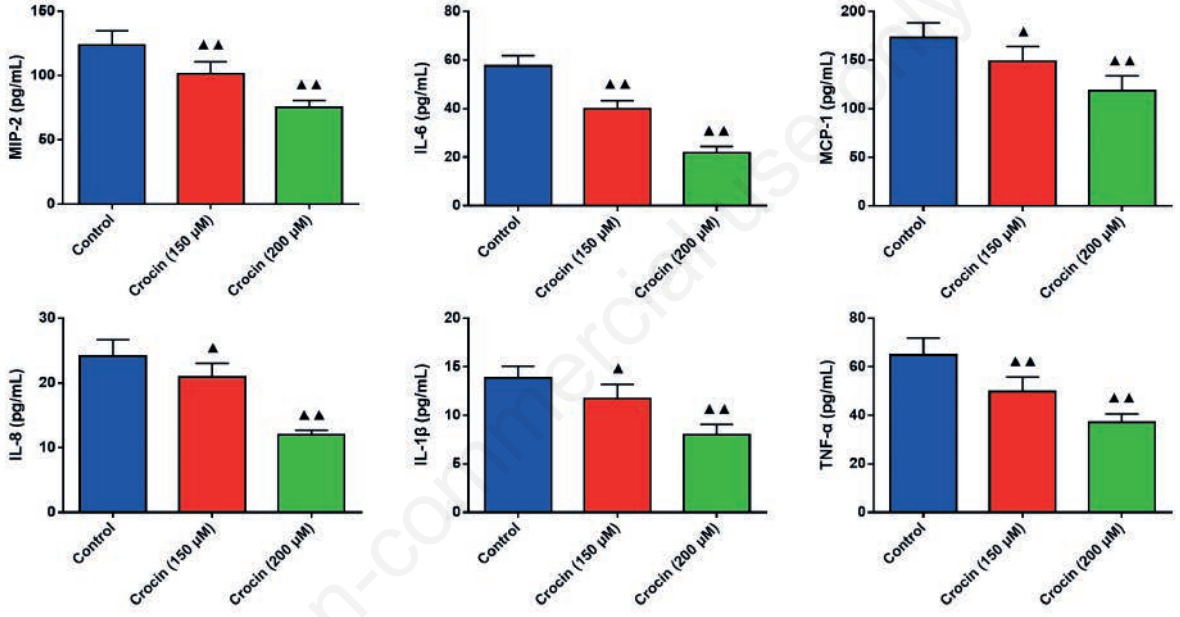

Figure 4. The effect of crocin on the contents of MIP-2, IL-6, MCP-1, IL-8, IL-1β, and TNF-α in HCT-116 cells. The effect of crocin on the contents of macrophage inflammatory protein 2 (MIP-2), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), IL-8, IL-1β, and tumor necrosis factor α (TNF-α) was detected by ELISA. All experiments have been performed in triplicate and data were expressed as mean ±SD. ▲p<0.05, ▲▲p<0.01 *vs* control. Crocin concentrations: 150 and 200 μM.

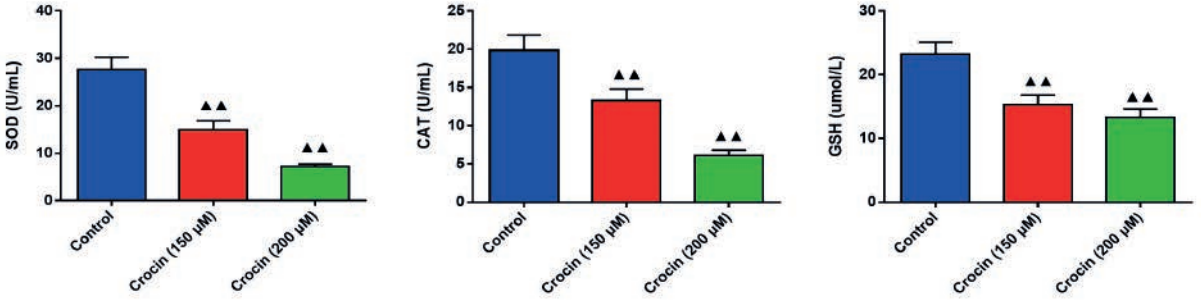

Figure 5. The effect of crocin on the contents of SOD, CAT, and GSH in HCT-116 cells. The effect of crocin on the contents of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) was detected by ELISA. All experiments have been performed in triplicate and data were expressed as mean ±SD. ▲▲p<0.01 *vs* control. Crocin concentrations: 150 and 200 μM.

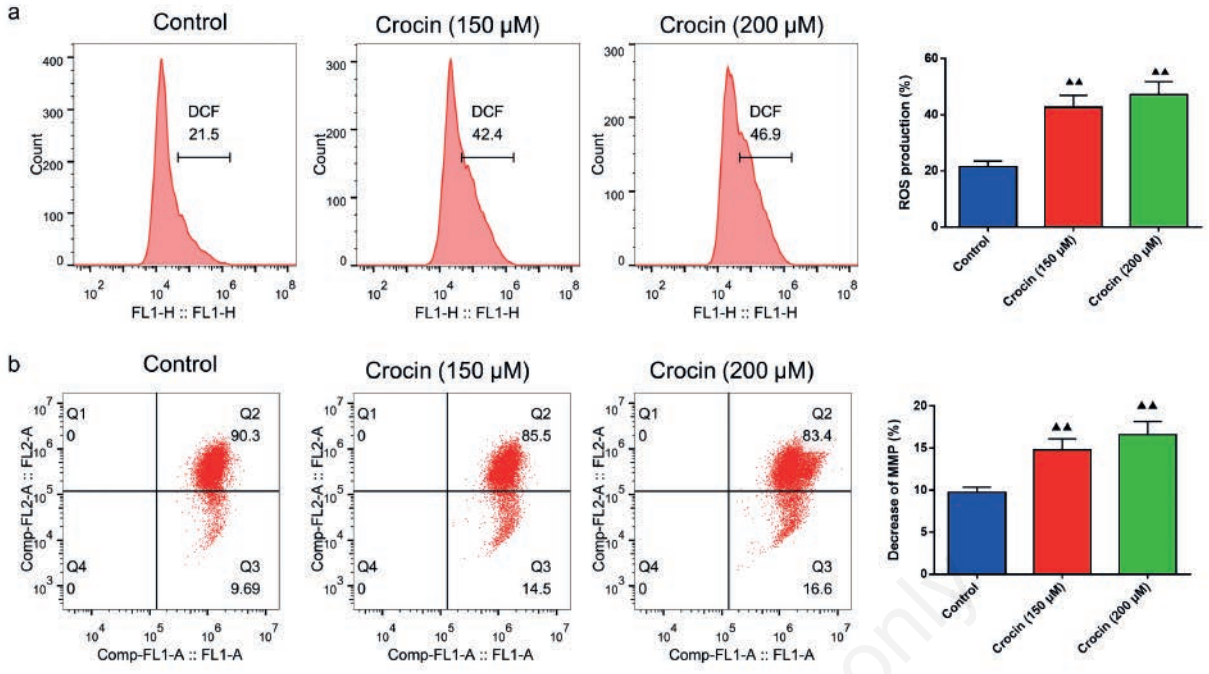

Figure 6. Crocin enhanced the ROS production and restrained MMP of HCT-116 cells. **a**) The effect of crocin on the reactive oxygen species (ROS) production was detected by flow cytometry. **b**) The effect of crocin on mitochondrial membrane potential (MMP) of HCT-116 cells was detected by a flow cytometer. All experiments have been performed in triplicate and data were expressed as mean ±SD. ▲▲p<0.01 *vs* control. Crocin concentrations: 150 and 200 μM.

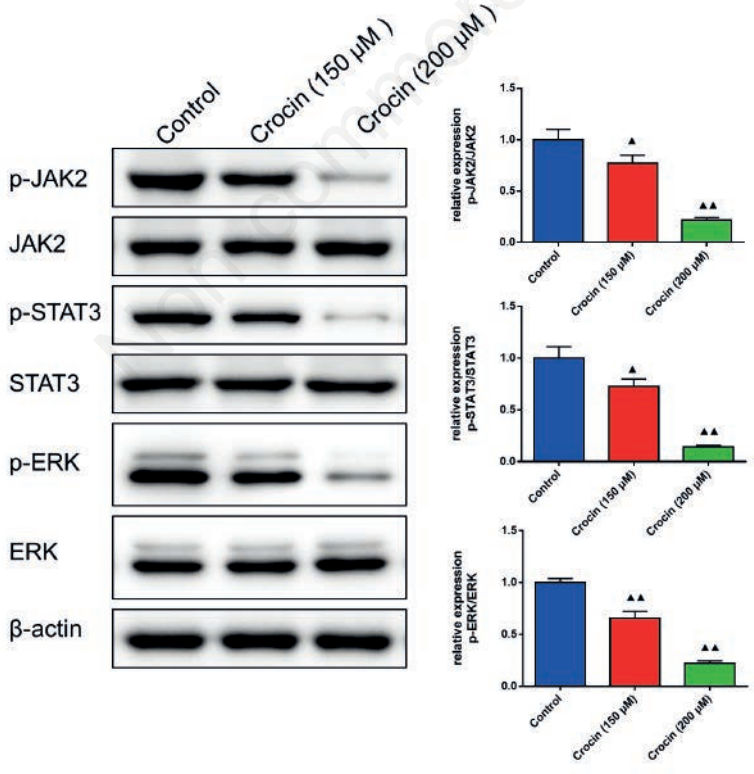

Figure 7. The effect of crocin on the JAK pathway-related markers in HCT-116 cells. The effect of crocin on the Janus kinase (JAK) pathway-related markers in HCT-116 cells was detected by Western blot. We used β-actin as the internal control. All experiments have been performed in triplicate and data were expressed as mean ±SD. ▲p<0.05, ▲▲p<0.01 *vs* control. Crocin concentrations: 150 and 200 μM.

induced a significant decrease in MMP in CC cells.

At present, tumor marker detection is a commonly used detection item in CC cells screening and early diagnosis. Recent research has clarified that inflammatory factors, chemokines, and oxidative stress are closely associated with CC.[30,31] Both MCP-1 and MIP-2 belong to the CC class, also known as class b chemokines. The high expression of MCP-1 in liver metastases of colorectal cancer was associated with the increase of multiple metastases and vascular density, while the increase of MCP-1 expression in primary colorectal tumors indicated an advanced clinical stage.[32] MIP-2 could promote liver metastasis of colorectal cancer and the mechanism might involve increasing angiogenesis and tumor cell migration.[33] In our research, we confirmed for the first time that crocin greatly blunted the contents of MCP-1 and MIP-2. Moreover, crocin also notably weakened the contents of IL-6, IL-8, IL-1β, TNF-α, SOD, CAT, and GSH in CC cells. The above results illustrated that crocin might exert an anti-CC effect *via* restraining inflammation and oxidative stress.

On the basis of the above research, we further explored the potential mechanism of crocin in the treatment of CC. A previous study reported that crocin could exert anti-tumor roles by regulating inflammation-related cell signals and functions.[24] More and more research confirmed that the excessive activation of the pro-inflammatory JAK2/STAT3 pathway was closely correlated with the presence and progression of breast cancer, CC, and prostate cancer.[34-36] STAT3 is usually activated by non-receptor kinases (JAKs) and MAPK (ERK and p38).[37] In addition, ERK was over-expressed in malignant tumors and enhanced the proliferation and metastasis of tumor cells.[38] We tested the effect of crocin on the activation of JAK2/STAT3 and ERK pathways. We discovered that crocin weakened the phosphorylation of JAK2, STAT3, and ERK in a dose-dependent manner in CC cells, revealing that crocin could exert an anti-CC effect by inhibiting JAK2/STAT3 and ERK pathways in CC cells.

In conclusion, our study observed that crocin induced apoptosis and weakened mitochondrial function in CC cells *via* restraining JAK2/STAT3 and ERK pathways. Building on these promising *in vitro* findings, future studies will focus on validating these results *in vivo* using animal models, which will provide a more nuanced understanding of crocin's efficacy and safety. If these forthcoming studies confirm our findings, they will pave the way for the clinical application of crocin in treating colon cancer, which may potentially improve the prognosis and quality of life for patients afflicted with this disease.

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