Curcumin exerts therapeutic effects on colorectal cancer by inducing pyroptosis through caspase-1 activation

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Received March 15, 2025; Accepted August 12, 2025

DOI: 10.3892/mmr.2025.13692

Abstract. Colorectal cancer (CRC) is a substantial global health challenge, with current treatments often leading to relapse and metastasis. Curcumin, a natural compound derived from turmeric, has shown promise in cancer therapy; however, its mechanisms in CRC are not fully understood. The present study aimed to investigate the role of curcumin in inducing pyroptosis, a form of inflammatory cell death, through caspase-1 activation in CRC cells. Human CRC cell lines (HCT-116 and SW480) and normal colon epithelial cells (FHC) were treated with curcumin at varying concentrations. Cell viability, migration and invasion were assessed using Cell Counting Kit-8, wound healing and Transwell assays, respectively. Pyroptosis was evaluated through lactate dehydrogenase (LDH) release, TUNEL staining and western blot analysis of pyroptosis-related proteins (caspase-1, gasdermin D, nucleotide-binding oligomerization domain-like receptor protein 3, IL-1β and IL-18). The role of caspase-1 was further examined using the inhibitor VX-765. Curcumin significantly reduced CRC cell viability, migration and invasion in a dose-dependent manner. In addition, it induced pyroptosis, as evidenced by cell membrane swelling, increased LDH release and upregulation of pyroptosis-related proteins. Inhibition of caspase-1 with VX-765 attenuated these effects, confirming the role of caspase-1 in curcumin-induced pyroptosis. In conclusion, curcumin may exert anti-CRC effects by inducing caspase-1-mediated pyroptosis, highlighting its potential as a therapeutic agent. These findings suggest that curcumin could be integrated into current CRC treatment strategies, particularly in targeting pyroptosis to enhance tumor suppression.

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Abbreviations: AIM2, absent in melanoma 2; CRC, colorectal cancer; FBS, fetal bovine serum; GSDM, gasdermin; LDH, lactate dehydrogenase; NOD, nucleotide-binding oligomerization domain; NLRP, NOD-like receptor protein; NLRC, NOD-like receptor family caspase recruitment domain-containing protein; TME, tumor microenvironment

Key words: curcumin, CRC, pyroptosis, caspase-1

Introduction

Globally, colorectal cancer (CRC) is a leading oncological burden, positioned as the third most frequently diagnosed malignancy and the second principal contributor to cancer-associated mortality (1). The World Health Organization estimated that in 2022, there were >1.9 million new cases of CRC and 904,000 CRC-associated deaths worldwide, with China accounting for ~30% of these cases (2). Projections indicate that by 2040, the number of new cases and deaths to will increase to 3.2 million and 1.6 million, respectively, posing a notable public health challenge (3). Despite 60% of patients being diagnosed with resectable CRC, ~50% of those who undergo curative surgery alone and 20-25% of those who receive adjuvant chemotherapy experience cancer relapse, metastatic disease and eventual death (4). This underscores the inadequacy of current treatment options for this fatal malignancy.

Due to the toxicity concerns and high costs associated with modern therapies, there is an increasing interest in discovering potential natural products (5,6). Curcumin, one of the primary curcuminoids found in the root of the *Curcuma longa* (turmeric) plant, has been extensively studied in the treatment of a wide range of diseases, including CRC (7). A phase IIa open-label randomized controlled trial showed that adjuvant treatment with curcumin significantly increased the objective response rate (53.3% vs. 11.1%, P=0.039) and prolonged median survival time (502 vs. 200 days, P=0.02) compared with single chemotherapy (8). Additionally, incorporating curcumin into standard chemotherapeutic regimens for metastatic CRC can

help reduce side effects, overcome chemotherapy resistance and ultimately enhance the quality of life for patients (9). Due to the potential therapeutic benefits, several other clinical trials have already been registered and conducted, assessing curcumin either in combination with chemotherapy or as a single agent for the prevention of colon cancer (10-12).

Several studies have investigated and revealed the potential mechanisms of curcumin in treating cancer, such as inducing apoptosis and senescence, suppressing migration and invasion (13), blocking the cell cycle (14), activating ferroptosis (15), regulating the gut microbiota, and exerting anti-inflammatory and antioxidant effects (16). However, the underlying mechanisms of curcumin against CRC are still not fully elucidated.

Pyroptosis, a form of inflammatory programmed cell death that differs from apoptosis, has emerged as a focal area of research due to its implications in various diseases, including CRC (17). This process is primarily triggered by inflammatory stimuli that activate caspases, particularly caspase-1 (18), which induces the formation of perforation-active gasdermin (GSDM)D. Following proteolytic activation, the N-terminal of GSDMD oligomerizes into transmembrane pores on the plasma membrane, mediating: i) Non-selective ion flux causing osmotic imbalance and cytolysis, and ii) regulated release of canonical inflammatory mediators (IL-1β and IL-18); these collectively execute the terminal phase of pyroptotic cell death (19).

Investigative findings have revealed that malignant cells demonstrate heightened pyroptotic susceptibility compared with normal cells (20). Pyroptosis has demonstrated notable antitumor potential in controlling cancer progression, including in CRC (17). A previous study reported that caspase-1 activation could mediate a 'cold' to 'hot' transformation of the tumor microenvironment (TME) in CRC (21). By contrast, *Fusobacterium nucleatum* could inhibit the caspase-3/GSDME pyroptosis-related pathway induced by chemotherapy drugs, thereby mediating CRC cell chemo-resistance (22). Consequently, pyroptosis induction could serve as a dual-function mechanism for both tumor suppression and therapeutic intervention in CRC.

Given the increasing incidence of CRC, the development of targeted therapeutic interventions is of paramount importance. The diverse mechanisms of curcumin, particularly its role in inducing caspase-1-mediated pyroptosis, warrant further investigation as a potential therapeutic strategy for CRC. The present study aimed to elucidate the mechanisms through which curcumin modulates caspase-1 activity and its impact on pyroptosis in CRC cells, thereby advancing the understanding of CRC and the therapeutic potential of natural compounds.

Materials and methods

Materials. The human CRC cell lines HCT-116 and SW480 (cat. nos. SCSP-5076 and SCSP-5033) were acquired from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. For comparative analysis, the normal colon epithelial cell line FHC was procured from the American Type Culture Collection (cat. no. CRL-1831). Cell culture media components including RPMI 1640, DMEM and fetal bovine serum (FBS) (cat. nos. 11875093, 12491015 and A5670501) were commercially obtained from Thermo Fisher Scientific, Inc. Apoptosis detection reagents (*In Situ* Cell Death Detection Kit) were sourced from Roche Diagnostics (cat. no. 11684795910). The caspase-1 inhibitor VX-765 was supplied by MedChemExpress (cat. no. HY-13205). Western blot analysis employed specific antibodies targeting caspase-1/3 (both precursor and cleaved forms), IL-1β, IL-18, GSDMD and nucleotide-binding oligomerization domain (NOD)-like receptor protein (NLRP)3 inflammasome components (cat. nos. ab138483, ab184787, ab2302, ab283818, ab207323, ab209845 and ab263899; Abcam; cat. no. HY-P80622; MedChemExpress). All the materials were maintained under manufacturer-specified storage conditions.

Cell culture and experimentation. SW480 and HCT116 cell lines were propagated in RPMI 1640 medium containing 10% FBS, whereas FHC cells were cultured in DMEM with equivalent serum supplementation. All cell populations were maintained under standard culture conditions (37˚C, 5% CO_2). For experimental interventions, both SW480 and HCT116 cells were stratified into four treatment cohorts: i) Curcumin monotherapy (10 μM), ii) caspase-1 inhibitor VX-765 (10 μM for SW480; 20 μM for HCT116) (23), iii) combination therapy with curcumin (10 μM) and VX-765 (20 μM), and iv) untreated control group. Pharmacological treatments were administered following established dosing protocols for 48 h at 37˚C with 5% CO_2.

Cell viability assay. Cell viability was quantitatively assessed using the CCK-8 colorimetric assay (cat. no. CK04-20; Dojindo Laboratories, Inc.) to evaluate curcumin-induced cytotoxicity. Specifically, exponentially growing cells were plated in 96-well culture plates at a standardized density of $5x10^3$ cells/well. After 24 h adherence, cells were exposed to curcumin (cat. no. C1386; Sigma-Aldrich; Merck KGaA) dissolved in DMSO, with concentrations titrated from 0 to 200 μM. Following 48 h pharmacological exposure, 10 μl CCK-8 detection reagent was administered per well and incubated for 2 h at 37˚C, followed by optical density quantification using a microplate reader (BioTek Synergy H1; Agilent Technologies, Inc.) at 450 nm with a reference wavelength of 650 nm. Experimental design incorporated triplicate technical replicates across three independent biological experiments.

Cell migration and invasion assays. Cell migration under curcumin treatment was assessed via scratch assay. Cells ($5x10^5$/well) in 12-well plates were cultured to 95% confluence in medium containing 10% FBS. Uniform wounds were created using sterile pipette tips, washed with serum-free medium, and then cultured in serum-free medium at 37˚C. Images were captured at 0 and 48 h using phase-contrast microscopy to monitor closure (Leica DMi8; Leica Microsystems, Inc.). Wound closure was quantified using Digimizer software v4.5.1 (MedCalc Software Ltd.) with migration rate (%) calculated as: (Initial width-final width)/initial width x100. Triplicate experiments were performed.

Invasion assays utilized 24-well Transwell plates pre-coated with Matrigel at 37˚C for 1 h (pore size, 8 μm; BD Biosciences). A total of $1x10^5$ cells in serum-deprived medium were introduced into the upper chamber, with 500 μl medium containing 10% FBS placed in the lower chamber. After 48 h

incubation at 37˚C, invasive cells retained on the membrane were fixed with 4% paraformaldehyde and stained with 0.5% (w/v) crystal violet for 20 min, whereas non-invasive cells were removed with a cotton swab. Three random microscopic fields per well were quantified for statistical analysis.

Microscopy. Pyroptotic cellular dynamics were investigated using 24-well culture systems with an initial seeding density of $5x10^4$ cells/well. Morphological evaluation was performed through bright-field imaging (Olympus IX53; Olympus Corporation) following experimental interventions.

Lactate dehydrogenase (LDH) release assay. Pyroptotic cell death was quantitatively assessed through LDH release analysis (cat. no. EL-H0866; Wuhan Elabscience Biotechnology Co., Ltd.). Culture supernatants collected post-treatment were centrifuged at 300 x g for 5 min at 4˚C, and the clarified supernatants processed for LDH quantification according to the manufacturer protocols, with absorbance measured at 490 nm using a microplate reader.

TUNEL staining. Apoptotic nuclei were detected via TUNEL assay (*In Situ* Cell Death Detection Kit). Cells seeded at $2x10^4$ cells/well on sterilized coverslips were fixed in 4% paraformaldehyde at 4˚C for 30 min, grown on coverslips, permeabilized with 0.1% Triton X-100/0.1% sodium citrate solution at room temperature (RT) for 10 min and subsequently incubated with TUNEL reaction mixture at 37˚C for 1 h. Nuclear counterstaining was achieved with DAPI (1 μg/ml; at RT for 5 min). Samples were mounted with ProLong™ Gold Antifade Mountant (cat. no. P36930; Thermo Fisher Scientific, Inc.) and imaged using a Zeiss Axio Observer fluorescence microscope. Quantitative analysis was performed on ≥3 random fields per sample (x200 magnification). All procedures strictly followed manufacturer-specified protocols, including negative controls processed without terminal transferase.

Western blotting. Cellular proteins were extracted using RIPA buffer (cat. no. 89900; Thermo Fisher Scientific, Inc.) and quantified via the BCA assay. Equal aliquots (30 μg) were separated by SDS-PAGE on 12% gels and were transferred to PVDF membranes. After blocking with 5% BSA at RT for 1 h (cat. no. E-IR-R107; Wuhan Elabscience Biotechnology Co., Ltd.), the membranes were incubated with primary antibodies (1:500 for caspase-1, cleaved-caspase-1 and cleaved-caspase-3; 1:2,000 for caspase-3; 1:1,000 for IL-1β, IL-18, GSDMD and NLRP3) at 4˚C for 16 h, and then with HRP-conjugated rabbit anti-rat IgG(H+L) secondary antibody (1:5,000; cat. no. ab6734; Abcam) at RT for 2 h. β-actin (1:5,000; cat. no. ab20272; Abcam) was used as the internal control. Protein bands were visualized using ECL reagent (cat. no. E422-01; Vazyme Biotech Co., Ltd.) with chemiluminescent detection.

Immunofluorescence staining. HCT116 and SW480 cells ($2x10^4$ cells/well) were seeded in 24-well plates containing round coverslips and fixed in 4% paraformaldehyde at RT for 15 min. The cells were then membrane-permeabilized by incubation in 0.1% Triton-X 100 (cat. no. T9284; MilliporeSigma), followed by blocking with 5% BSA (cat. no. E-IR-R107; Wuhan Elabscience Biotechnology Co., Ltd.) for 1 h at RT. Cells were incubated with primary antibody (GSDMD: 1:500, cat. no. HY-P85810; caspase-1: 1:500, cat. no. HY-P81232; MedChemExpress) at 4˚C for 16 h, and incubation with goat anti-mouse IgG H&L Alexa Fluor 488 secondary antibody (cat. no. ab150117; Abcam) at RT for 1 h. Fluorescence signals were captured using confocal microscopy (Leica SP8; Leica Microsystems, Inc.) with DAPI nuclear counterstaining for 24 h at RT for signal normalization.

Statistical analysis. Statistical analysis was performed using GraphPad Prism 9.4.1 software (Dotmatics). All experiments were repeated three times. Quantitative data are presented as the median (IQR). All continuous variables were formally tested for normality using the Shapiro-Wilk test (α=0.05). Due to the small sample size (only *in vitro* experiments were performed), non-parametric analysis was conducted through Kruskal-Wallis with Dunn's post hoc test for multiple comparisons. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Determination of curcumin concentration. To determine the optimal dosage of curcumin, CRC cell lines HCT-116 and SW480 were treated with curcumin at concentrations ranging from 0 to 200 μM, and cell viability was assessed using CCK-8 assays. As shown in Fig. 1A, curcumin reduced cell viability in a dose-dependent manner. In response to 10 μM curcumin, both cell lines exhibited statistically significant differences (compared with 0 μM). Additionally, the cell viability assay revealed no effect on FHC normal colon epithelial cells in response to this concentration of curcumin for 72 h (Fig. 1B). Therefore, 10 μM curcumin was selected for subsequent experiments.

Curcumin inhibits cell migration and invasion. The migratory ability of CRC cell lines was assessed using a wound healing assay. Cells were cultured in maintenance medium for 48 h, and the scratch distance was measured for each cell line. Curcumin treatment significantly reduced the extent of wound closure and the migration rate (Fig. 1C and D). The effect of curcumin on invasive capability was evaluated using a Matrigel-coated Transwell system. As shown in Fig. 1E and F, curcumin treatment significantly reduced the number of invasive HCT-116 and SW480 cells compared with those in the control group. These results demonstrated that curcumin may inhibit autonomous migration and invasion in CRC cells.

Curcumin induces pyroptosis in CRC cells. To assess cellular morphology, CRC cells were examined under a microscope. Fig. 2A demonstrated that curcumin-treated cells manifested characteristic pyroptotic morphology, including membrane swelling and subsequent rupture with cytoplasmic content release. TUNEL staining was then performed to assess cell death. The results demonstrated that curcumin significantly increased cell death (Fig. 2B, E and F). Caspase-3, an apoptosis-specific marker, was detected to distinguish between pyroptosis and apoptosis. The results indicated that curcumin could slightly increase cleaved-caspase-3 expression; however, this was not significantly different

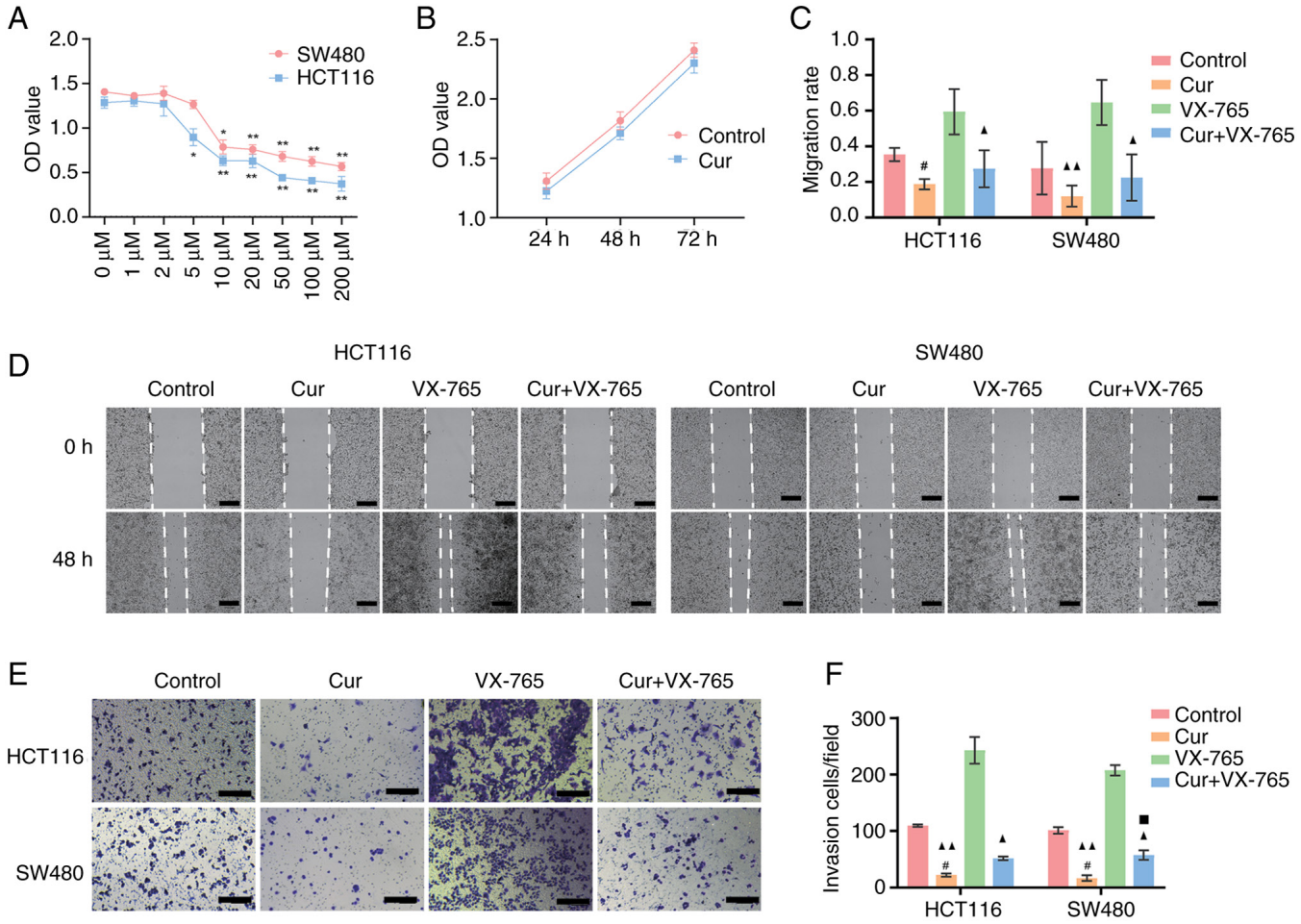

Figure 1. Cur suppresses cell viability, migration and invasion in CRC lines. (A) Effects of different concentrations of Cur on CRC cell viability at 48 h. Cur could reduce cell viability in a dose-dependent manner. (B) Effect of Cur (10 μM) on normal human intestinal epithelial cells. No obvious cytotoxicity was observed. (C) Histogram of migration rate among groups. (D) Representative images of cell migration among groups. Scale bar, 400 μm. (E) Representative images of cell invasion among groups. Scale bar, 400 μm. (F) Histogram of cell invasion among groups. All data are presented from three independent experiments. *P<0.05, **P<0.01 vs. 0 mM; $^{\#}$P<0.05 vs. control; $^{\blacktriangle}$P<0.05, $^{\blacktriangle\blacktriangle}$P<0.01 vs. VX-765. $^{\blacksquare}$P<0.05 vs. curcumin. CRC, colorectal cancer; Cur, curcumin.

(Fig. 3A and B). Furthermore, cell membrane integrity is a key indicator of pyroptosis. Therefore, LDH levels in the cell supernatant and GSDMD expression were measured. The results indicated that curcumin-treated cells exhibited elevated LDH levels (Fig. 2C and D) and increased GSDMD expression (Fig. 3A, B and D). Furthermore, the expression levels of pyroptosis-related factors, including caspase-1, its upstream marker NLRP3, and the downstream markers IL-18 and IL-1β, were analyzed (Fig. 3A-C and E). The results indicated that curcumin significantly upregulated the expression of pyroptosis-related proteins, suggesting that pyroptosis may contribute to the anti-CRC effects of curcumin.

Curcumin induces pyroptosis by activating the caspase-1 signal. To evaluate the role of the caspase-1 signaling pathway in curcumin-induced pyroptosis, VX-765, a selective caspase-1 inhibitor, was used as a negative control. VX-765 markedly attenuated abnormal cellular morphological changes and cell death induced by curcumin (Fig. 2A and B). Additionally, the expression levels of caspase-1 and the downstream factors IL-1β and IL-18 were significantly lower in the curcumin + VX-765 group compared with those in the curcumin-only group (Fig. 3A-C and E). Meanwhile, reduced LDH levels and GSDMD expression indicated that VX-765 attenuated curcumin-induced perforation (Figs. 2C and D, and 3A, B, D and F).

Additionally, curcumin markedly reduced the migratory capability of HCT116 and SW480 cells; however, the inhibitory effect on migration was arrested when cells were co-treated with curcumin and VX-765 (Fig. 1C and D). Similarly, the invasive ability of CRC cells was significantly higher in the presence of VX-765 compared with curcumin alone (Fig. 1E and F). These findings suggested that curcumin inhibits CRC growth and metastasis by inducing caspase-1-mediated pyroptosis.

Discussion

The present study demonstrated that curcumin may exert antitumor effects on CRC cells, primarily through the induction of caspase-1-mediated pyroptosis. The data revealed that curcumin treatment not only suppressed tumor cell viability, migration and invasion in a dose-dependent manner, but also triggered pyroptosis, a lytic and immunogenic form of programmed cell death distinct from apoptosis. This finding aligns with the established literature indicating the ability of curcumin to engage multiple cell death pathways in cancer, reinforcing its potential as a therapeutic agent for CRC (24-26).

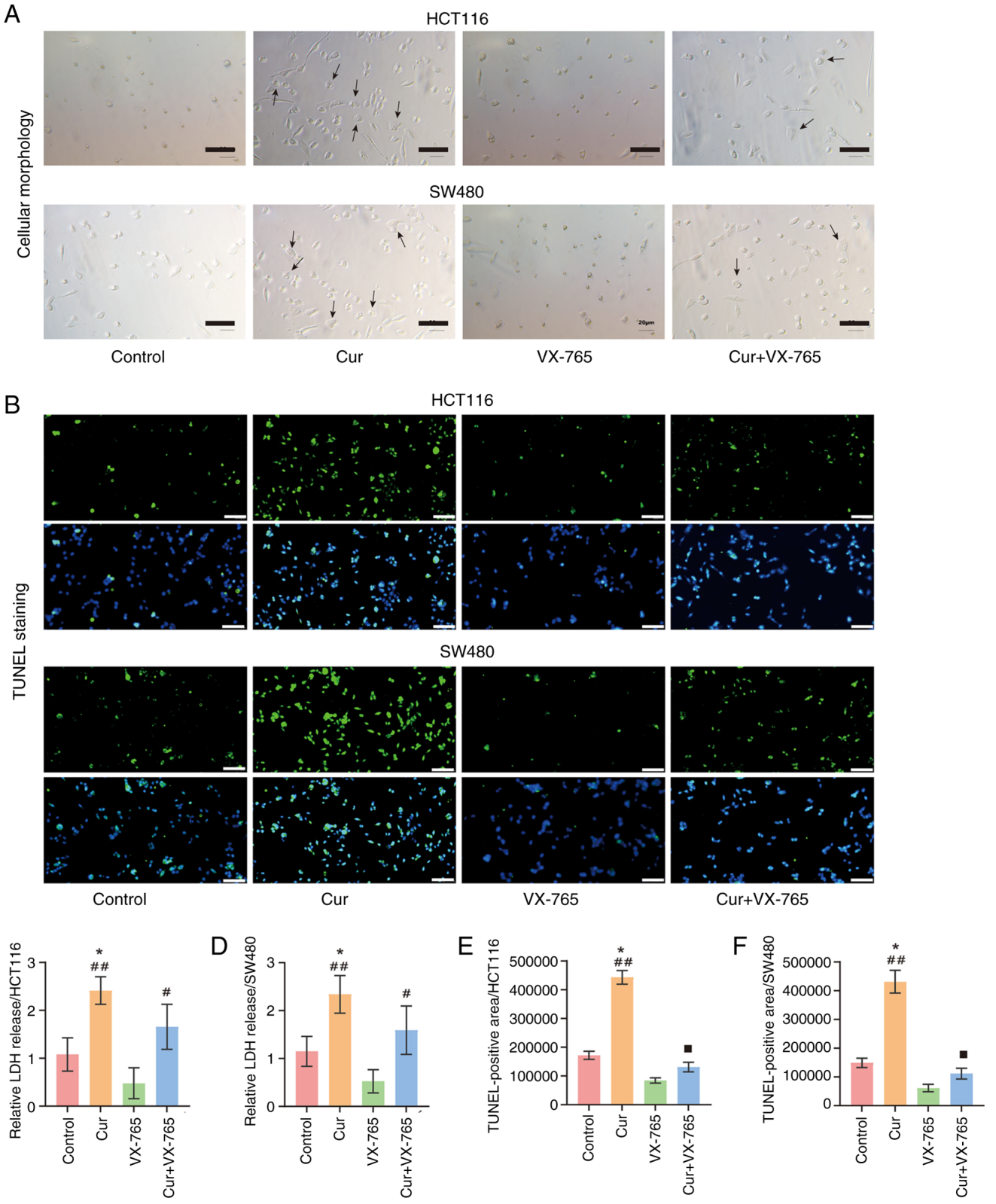

Figure 2. Cur induces cell pyroptosis in colorectal cancer cell lines. (A) Cellular morphology was assessed under a microscope. Arrows indicate typical pyroptotic cells. Scale bar, 40 μm. (B, E and F) TUNEL staining among groups. Cur could significantly induce cell death, whereas this effect was arrested by VX-765. Scale bar, 100 μm. (C and D) Relative LDH release among groups. All of the data are presented from three independent experiments. *P<0.05 vs. control; $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. VX-765; $^{\blacksquare}$P<0.05 vs. curcumin. Cur, curcumin; LDH, lactate dehydrogenase.

CRC remains a major global health burden and a leading cause of cancer-related mortality. While notable advances have been made in cancer immunotherapy, efforts continue to identify strategies that elicit robust and sustained antitumor immunity (27). Accumulating evidence has indicated that specific regulated cell death modalities, including pyroptosis, can function as potent immunogenic switches. This occurs through the systemic release of damage-associated molecular

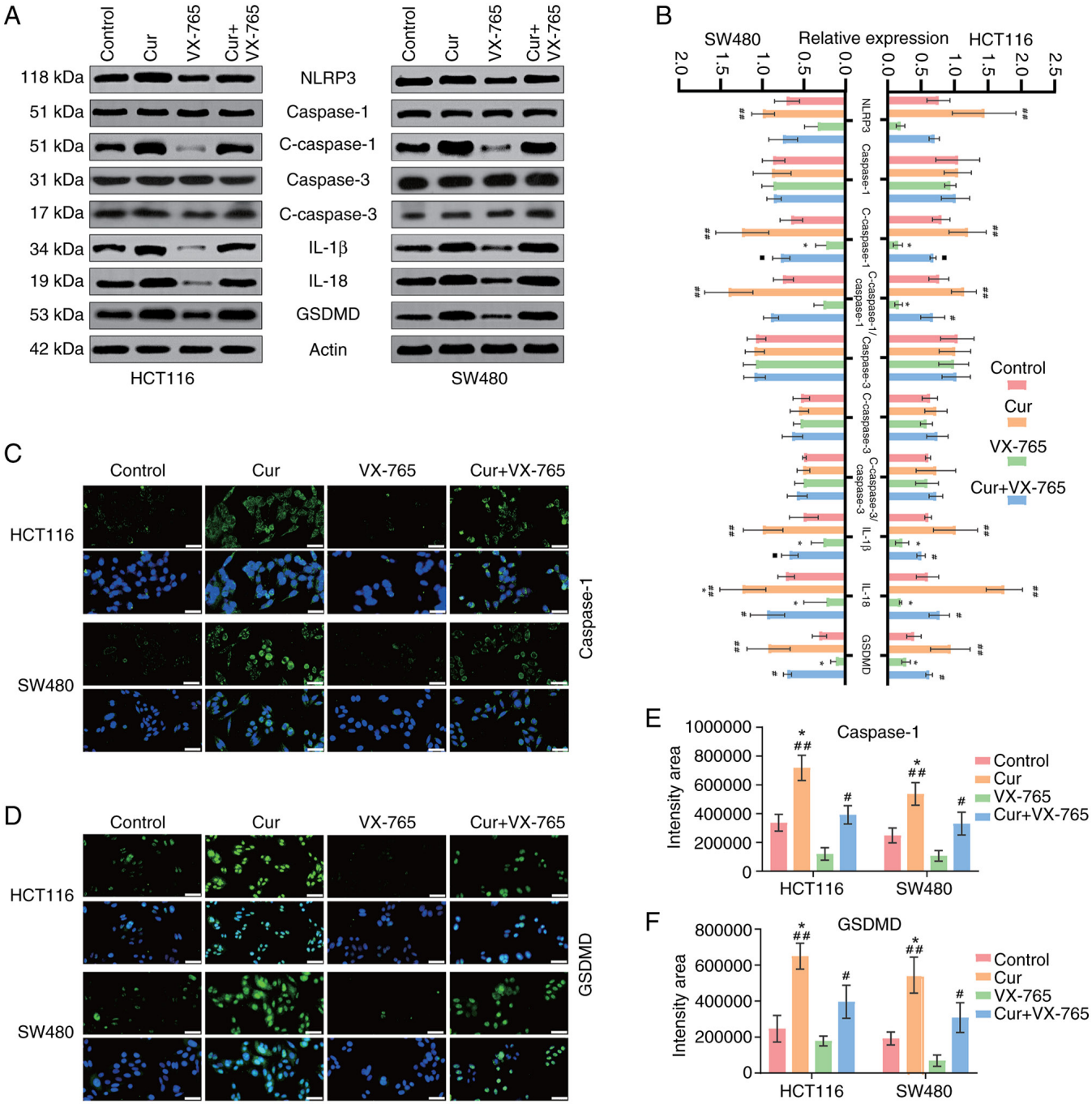

Figure 3. Cur induces pyroptosis through caspase-1 signal activation. (A and B) Cur significantly increased protein expression levels of caspase-1 and pyroptosis-related factors, while VX-765 exhibited the opposite effect. (C-F) Representative images and histogram of immunofluorescence staining of caspase-1 and GSDMD among groups. Scale bar, 40 μm. All the data are presented from three independent experiments. *P<0.05 vs. control; $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. VX-765. $^{\blacksquare}$P<0.05 vs. curcumin. c-, cleaved; Cur, curcumin; GSDMD, gasdermin D; NLRP3, nucleotide-binding oligomerization domain-like receptor protein 3.

patterns and tumor-associated antigens, which subsequently prime and activate antitumor immune responses (28). Leveraging the inherent immunogenicity of dying tumor cells thus represents a promising avenue for enhancing immunotherapy efficacy. Pyroptosis, classically recognized as a host defense mechanism against pathogens, elicits a strong inflammatory response and activates innate immunity (29). Critically, pyroptosis-induced immunogenic cell death can transform immunologically 'cold' tumors into 'hot' T cell-inflamed microenvironments, establishing a dual-phase tumor suppressive mechanism involving both primary tumor regression and disruption of metastatic niches (30). Consequently, strategies combining pyroptosis induction with conventional anticancer therapies represent a viable treatment approach (31).

The critical role of pyroptosis in CRC pathogenesis and therapy is increasingly being recognized. Clinical studies have reported dysregulation of inflammasome components, such as NLRP1, NLRP3, NOD-like receptor family caspase recruitment domain-containing protein (NLRC)4 and absent in melanoma 2 (AIM2), in patients with CRC (32), with several inflammasomes (NLRP1, NLRP6, AIM2, pyrin and NLRC4) demonstrating anti-tumorigenic function (33).

Pharmacological enhancement of NLRP3 activity, combined with chemotherapeutics such as 5-fluorouracil or regorafenib, can effectively induce pyroptosis and suppress tumor growth in preclinical CRC models (34). Inflammasome-regulated release of IL-18 and IL-1β by immune cells within the TME holds therapeutic potential by promoting inflammation and immune activation (31). For example, NLRP3-mediated IL-18/IL-1β production enhances natural killer cell maturation and cytotoxicity, thereby inhibiting CRC liver metastasis (35). Clinically, downregulated GSDMD expression is associated with a poor prognosis in CRC (36), while cytoplasmic/nuclear GSDMB expression may predict benefit from 5-fluorouracil-based chemotherapy (37). Furthermore, inducing pyroptosis via other mechanisms, such as caspase-3-mediated GSDME cleavage during photodynamic therapy, can sensitize microsatellite stable CRC cells to PD-1 blockade (38). Targeted induction of GSDMD-dependent pyroptosis has also shown efficacy in inhibiting metastatic CRC (39).

The present mechanistic investigation revealed that curcumin activates the NLRP3 inflammasome in CRC cells, leading to the upregulation of key pyroptosis executioner proteins caspase-1 and GSDMD. This cascade consequently promotes the maturation and release of the pro-inflammatory cytokines IL-18 and IL-1β. Morphologically, curcumin-treated CRC cells exhibited characteristic features of pyroptosis, including cellular swelling, plasma membrane ballooning with protruding vesicles, and eventual rupture leading to cytoplasmic content release. Crucially, the specific caspase-1 inhibitor VX-765 significantly attenuated curcumin-induced cell death and associated molecular changes, strongly supporting the central role of caspase-1-mediated pyroptosis in the anti-CRC effects of curcumin.

Several studies have explored the antitumor effects of curcumin via pyroptosis. In non-small cell lung cancer cells, curcumin inhibits ubiquitin ligase Smurf2 activity, promoting NLRP3-dependent pyroptosis (40). In mouse breast cancer 4T1 cells, curcumin induces Ca^{2+} overload, effectively triggering caspase-1/GSDMD-mediated pyroptosis to suppress tumors (41). In U937 leukemia cells, curcumin upregulates AIM2, IFI16 and NLRC4 inflammasomes, activating caspase-1, cleaving GSDMD and inducing pyroptosis (42). However, evidence in CRC remains limited. Prior research indicates that curcumin induces programmed cell death and upregulates the NLRP3 inflammasome in CRC cells; however, the underlying mechanism remains unresolved (26). The present findings established curcumin as a potent inducer of caspase-1/GSDMD-dependent pyroptosis in CRC cells, advancing this evidence base.

Notably, curcumin exhibits a cell type-dependent duality in modulating pyroptosis, acting as both an inducer and suppressor. In non-cancerous settings, such as models of fenpropathrin-induced nephrotoxicity (39), aflatoxin B1-induced hepatotoxicity (43) and intestinal inflammation (44), curcumin protects tissues by suppressing pyroptosis, often through downregulating NLRP3, caspase-1, GSDMD and associated cytokines. Conversely, in various cancer cell types, including leukemia (42), hepatocellular carcinoma (45), and as demonstrated in the present study and in a previous study on CRC (26), curcumin consistently promotes pyroptosis as a key antitumor mechanism. This pro-pyroptotic effect in CRC extends beyond direct tumor cell killing; curcumin-induced pyroptosis can also repolarize tumor-associated macrophages towards the immunostimulatory M1 phenotype, counteracting TME immunosuppression and enhancing overall antitumor efficacy (46). This cell-type-specific duality is not unique to pyroptosis, as similar contrasting effects of curcumin have been observed in ferroptosis (47).

Despite these notable findings, several limitations warrant attention in future studies. Firstly, the current conclusions are based solely on *in vitro* cell line models. Validation in *in vivo* animal models of CRC is essential to confirm the antitumor efficacy of curcumin and its ability to induce pyroptosis within the complex TME. Secondly, while pharmacological inhibition with VX-765 strongly implicates caspase-1, definitive genetic validation is needed. Employing caspase-1 silencing (such as via small interfering RNA or short hairpin RNA) or overexpression in CRC cells, coupled with studies utilizing caspase-1-deficient transgenic animal models, would provide more conclusive evidence for its indispensable role in curcumin-induced pyroptosis. Additionally, alternative pathways (such as non-canonical inflammasome activation or other GSDM-dependent mechanisms) may concurrently mediate this process, warranting systematic exploration in future studies.

In conclusion, the current study uncovered a novel mechanism of curcumin in CRC treatment. By activating the caspase-1 pathway, curcumin may induce pyroptosis, and inhibit migration and invasion in CRC cells, thereby exerting an anti-CRC effect.

Acknowledgements

Not applicable.

Funding

This study was supported by the National Natural Science Foundation of China (grant nos. 82004333 and 82205033), the Natural Science Foundation of Zhejiang Province (grant no. LQ21H290002), the State Administration of Traditional Chinese Medicine Science and Technology Department-Zhejiang Provincial Administration of Traditional Chinese Medicine Co-construction of Key Laboratory of Research on Prevention and Treatment for Depression Syndrome (grant no. GZY-ZJ-SY-2402), and the Zhejiang Traditional Chinese Medicine Science and Technology Project (grant nos. 2022ZA044 and 2023ZR005).

Availability of data and materials

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

Authors' contributions

JZ and LY contributed equally to this work and shared the first authorship. JZ conceived the study, carried out the investigation, administered the project, validated the results and acquired funding. LY conceived and designed the study, administered the project and wrote the main manuscript text.

ZF and JC carried out the investigation, and performed data collection and statistical analysis. YW and HL conducted the formal analysis and visualization using GraphPad Prism. SL conceived the study, and contributed to writing, reviewing and editing the manuscript. BF conceived and supervised the study, validated the results and acquired funding. JZ and BF confirm the authenticity of all the raw data. 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.

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