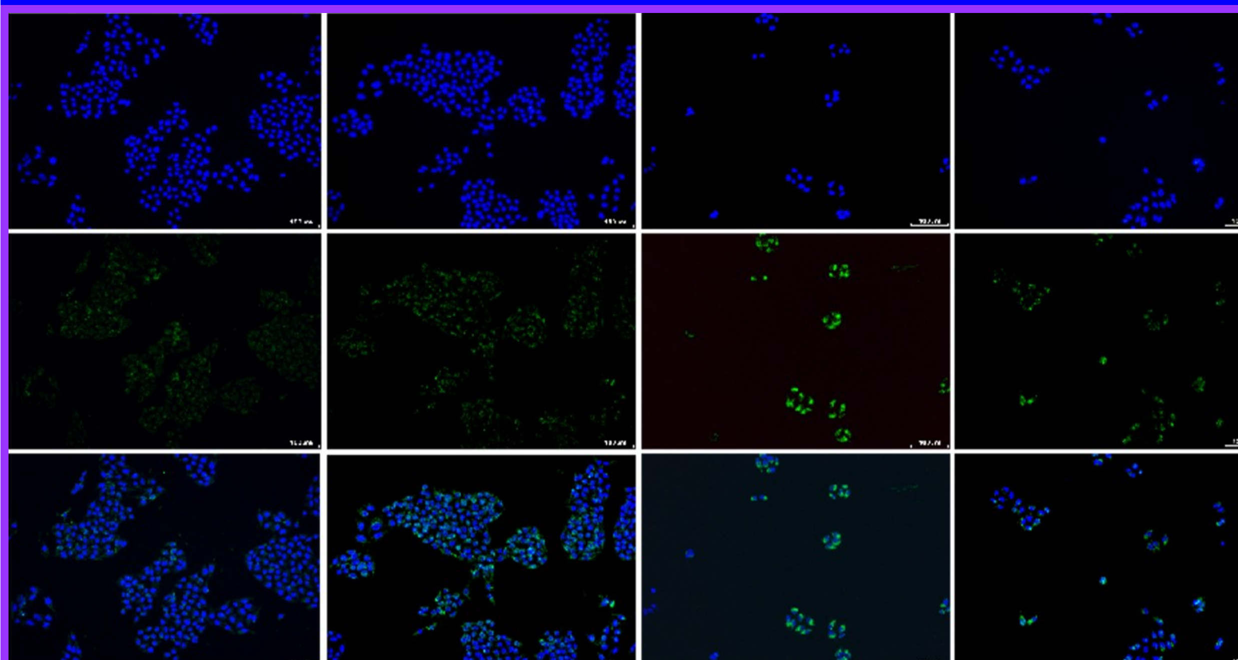

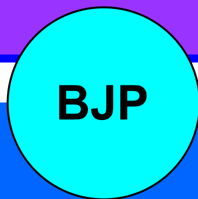

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Berberine triggers necroptosis of cervical H8 cells by activating receptor-interacting protein kinase 1

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Abstract

This study aims to investigate the effect of berberine on necroptosis in cervical H8 cells. CCK-8 assay was used to assess cell proliferation activity. The expression levels of necroptosis key markers Receptor-interacting protein kinase 1 (RIPK1), MLKL, p-MLKL, and apoptosis-related caspase-8, caspase-3 were analyzed through qRT-PCR, western blot, and immunofluorescence. Mechanistic studies were conducted using the RIPK1 kinase inhibitor necrostatin-1 (Nec-1). The results showed that berberine could inhibit H8 cell proliferation in a time- and dose-dependent manner ($p<0.05$). It increased the expression of RIPK1 and MLKL ($p<0.05$), while inhibiting the expression of caspase-8 and caspase-3($p<0.05$). Nec-1 inhibitor decreased the expression of RIPK1 and MLKL after the intervention of berberine ($p<0.05$). Therefore, berberine induces cervical H8 cell death through the activation of RIPK1-mediated necroptosis.

Introduction

Cervical intraepithelial neoplasia serves a precursor lesion to cervical cancer, primarily arising from persistent infection with high-risk human papillomavirus strains. During the lesion, the human papillomavirus genome can integrate into that of the host, leading to an overexpression of the E6 and E7 oncoproteins which can disrupt the normal cell cycle and causing immortalization (Trope et al., 2009). If human papillomavirus infection continues during squamous metaplasia, it can lead to cervical intraepithelial neoplasia aggravation, which may ultimately cause invasive cervical cancer and threaten the patient's life (Doorbar et al., 2015).

Currently, the treatment of cervical intraepithelial neoplasia encompasses various modalities such as excision therapy, ablation therapy, interferon, and traditional Chinese medicine preparations (Hoffman et al., 2017; Liontos et al., 2019). Numerous retrospective studies have shown the limitations of surgery such as postoperative residual, recurrence, low virus clearance rate, and low cervical function and fertility (Ghaem-Maghami et al., 2007; Zhu et al., 2015; Athanasiou et al., 2022). Furthermore, interferon preparations, are costly with a prolonged application cycle, posing challenges for patient acceptance.

In recent years, there has been a growing interest in exploring natural compounds as alternative treatments for cervical intraepithelial neoplasia. One such promising compound is berberine, a plant-derived alkaloid known for its reported antiproliferative and proapoptotic effects in various cancer cell lines (Liu et al., 2019; Rui et al.,

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2020). Berberine has demonstrated the ability to inhibit the proliferation and invasion of cancer cells, including Caski cells, Hela cells, and MCF-7 cells in a dose-dependent manner (Floriano et al., 2021; Li et al., 2023). Moreover, modern pharmacological studies have revealed that berberine possesses antiviral properties (Kim et al., 2016). Berberine can inhibit the replication of human papillomavirus by down-regulating human papillomavirus oncogenes expression (Mahata et al., 2011). Therefore, berberine emerges as a potential candidate for use as an antitumor and antiviral drug.

Multiple studies have identified cell necrosis as a regulated cell death mechanism known as necroptosis (Vanden et al., 2014; Bertheloot et al., 2021; Gao et al., 2022). Necroptosis shares similarities with apoptosis but exhibits typical characteristics of cell necrosis, such as organelle swelling, plasma membrane rupture, and release of cellular contents (Bertheloot et al., 2021; Gao et al., 2022). Research has shown that necroptosis is involved in processes such as anti-tumor and anti-viral responses (Verdonck et al., 2022). The focus in prior anti-tumor research has centered around apoptosis. However, multiple evidence indicate that tumor cells have evolved anti-apoptotic properties, leading to the development of drug resistance through the induction of apoptosis defects. Therefore, inducing necroptosis is anticipated to be a new strategy for overcoming apoptosis resistance and treating cancer (Feoktistova and Leverkus, 2015; N et al., 2021). Receptor-interacting protein kinase 1 (RIPK1) is a well-known serine/threonine kinase that plays a pivotal role in the necroptotic pathway (Kaczmarek et al., 2013). TNF-α (one of the promoter factors) binds to the specific receptor on the cell membrane to initiate necroptosis, and RIPK1 activates and recruits RIPK3, leading to the activation of the effector protein mixed lineage kinase domain-like protein (MLKL). Phosphorylated MLKL then oligomerizes at the cell membrane, causing the formation of pores and loss of membrane integrity, ultimately resulting in cell death (Bertheloot et al., 2021; Chen et al., 2019; Gao et al., 2022).

Preliminary experiments of the research group have revealed that berberine can inhibit the proliferation of H8 cervical cells, indicating its potential in the treatment of cervical intraepithelial neoplasia. Building upon these promising findings, the present study aims to delve into the mechanisms underlying berberine-induced cell death in H8 cells. The ultimate objective is to unveil novel therapeutic strategies for the prevention and treatment of cervical intraepithelial neoplasia.

Materials and Methods

Cell culture

Human immortalized cervical squamous epithelial cell line (H8) was obtained from the Guangxi Medical University in China. The H8 cells were infected with HPV16 to represent cervical intraepithelial neoplasia II-III levels. H8 cells were maintained in RPMI 1640 medium supplemented with 10%fetal bovine serum (BDBIO, China), 1%penicillin, and streptomycin. The cells were incubated at 37°C in a 5%CO_2 atmosphere until reaching approximately 80%confluence. Cell freezing and passaging were performed at this stage, and cells in the logarithmic growth phase were used for subsequent experiments. Notably, cells were passaged for no more than 10 times in this study. Culture flasks and plates were purchased from NEST Biotechnology.

Drug intervention

Berberine (2086-83-1, Aladdin, China) was prepared as a solution (100 mM) using DMSO as a solvent, facilitated by ultrasound, and stored at -20°C. Subsequently, the medium was adequately diluted to the desired concentration so, ensuring that the DMSO concentration did not exceed 1/1,000. Nec-1 (4311-88-0, Aladdin, China) was formulated into a solution (10 mM/L) using DMSO as a solvent, assisted by ultrasound, and stored at -20°C. This was followed by complete medium dilution to 2.5 µM, ensuring that the DMSO concentration did not exceed 1/1,000.

Cell proliferation assay

The impact of berberine on the viability of H8 cells was assessed through the CCK-8 assay. The cell proliferation experiment was conducted using the CCK-8 assay kit (Medicalbio, CCK-08). A 96-well plate was prepared with 100 µL of cell suspension (1,000 cells/well) and incubated in a cell culture incubator at 37°C with 5%CO_2 for 24 hours. To prevent evaporation at the plate edges, PBS buffer was added around the periphery of the 24-hour incubation, the culture medium was aspirated, and wells were treated with varying concentrations of the drug-containing medium (final concentrations of 20, 40, 60, 80, and 100 µM) in respective experimental wells. The control group was treated with an equal volume of medium containing DMSO at a concentration not exceeding one thousandth. Cells were then incubated for additional 24, 48, and 72 hours. After proper incubation in the 96-well plate, the culture medium was aspirated, and 100 µL of 10%CCK-8 solution was added to the corresponding experimental wells, followed by an incubator for 1 hour. Subsequently, the absorbance of each well was measured using an enzyme labeling instrument set at a wavelength of 450 nm (Liu et al., 2019).

Quantitative real-time polymerase chain reaction (qRT-PCR)

To assess the impact of berberine on key markers of necroptosis in H8 cells, qRT-PCR was employed to analyze the mRNA expression levels. Total RNA was extracted from cells using MolPure cell/tissue total RNA kit (Yeasen, China), and reverse transcription was performed with a reverse transcription kit (cDNA with HiScript II Q RT SuperMix, Vazyme, China). Subsequently, qRT-PCR was performed using SYBR qPCR master mix (Medicalbio, MR0321) according to instructions. The reaction system (20 μL) consisted of 1 μL cDNA template, 0.4 μL each of upstream and downstream primers, 10 μL SYBR premix Ex Taq, and 8.2 μL ddH_2O. β-actin served as the internal reference gene, and the primer sequences are detailed in Table I (Sangon Biotech, China). The relative mRNA expression was calculated by $2^{-\triangle\triangle CT}$ method.

Immunofluorescence assay

The expression of RIPK1 in H8 cells was examined through immunofluorescence assay under berberine intervention. Cells were seeded in 24-well plates at a density of $1×10^4$ cells/dish. After 24 hours of culture, the culture medium was replaced, and various concentrations of berberine solution (0, 20, 40, 80 μM/L) were added for a 72-hour incubation period. Following PBS rinsing, cells were fixed with 4%paraformaldehyde for 10 min, and washed thrice with PBS. Antigen retrieval was performed at 95℃ with EDTA for 10 min, followed by three PBS washes. Subsequently, cells were treated with 0.2%triton X-100 for 10 min and washed thrice with PBS. Blocking was carried out with PBST containing 3% BSA (PBS + 0.1%tween 20) at room temperature for 30 min. Primary antibodies (RIPK1/RIP rabbit pAb,

Table I

Primer design

Primer name	Primer sequence（5' to 3'）
RIPK1-F	TGGTTCTTCAGTACCTCTACCAG
RIPK1-R	GCCTGTGGTTTGAGCATTGATT
MLKL-F	AGGAGGCTAATGGGGAGATAGA
MLKL-R	TGGCTTGCTGTTAGAAACCTG
β-actin-F	CCTGGCACCCAGCACAAT
β-actin-R	GGGCCGGACTCGTCATAC

Box 1: Western blot analysis

Principle

Western blotting is based on the principle of immunochromatography where proteins are separated into polyacrylamide gel according to their molecular weight.

Requirements

BCA protein assay kit (Beyotime, China, P0009), Cells pellet, ECL (Medicalbio, PT01001) luminescence, RIPA buffer, Protease cocktail, Blocking buffer, Primary antibodies, Secondary antibodies, Phosphate saline buffer, 1xTris-buffered saline, 0.1%Tween, Sodium polyacrylamide gel, PVDF membranes, Transfer buffer

Procedure

Step 1: Cells were seeded in 6-well plates at a density of $2×10^5$ cells/dish.

Step 2: After 24 hours of incubation, the culture medium was discarded, and berberine solutions at different concentrations (0, 20, 40, 80 μM/L) were added for a 72-hour incubation period.

Step 3: Subsequently, cells were washed twice with PBS and lysed using ice-cold RIPA lysate containing PMSF (100x), phosphatase inhibitor (50x), and protease inhibitor (50x) to extract proteins.

Step 4: Protein concentration obtained from cell lysis was then quantified with BCA protein assay kit

Step 5: Proteins were separated on SDS-polyacrylamide gels via electrophoresis and then transferred to PVDF membranes.

Step 6: Membranes were blocked with 5% non-fat dry milk (or BSA) solution in TBST (TBS + 0.1%tween 20) for 1 hour at room temperature.

Step 7: This was followed by overnight incubation at 4° C with the primary antibody: RIPK1-specific polyclonal antibody (Proteintech, 17519-1-AP，1:1500), MLKL rabbit mAb (Zenbio, R380559, 1:1000), Phospho-MLKL (Ser358) rabbit mAb (Zenbio, A7414, 1:1000), cleaved caspase 8 mouse mAb (Zenbio, 250106, 1:1000), cleaved caspase 3 rabbit mAb (Zenbio, 341034，1:1000), β-Actin antibody (Proteintech, 81115-1-RR, 1:10000) diluted in the blocking solution.

Step 8: After three washes with TBST buffer, membranes were incubated with HRP-conjugated secondary antibodies [Goat anti-rabbit IgG (ABclonal, AS014, 1:7500) or HRP-conjugated sffinipure goat anti-mouse IgG(H+L)(Proteintech, SA00001-1, 1:7500)] for 1 hour at room temperature.

Step 9: Subsequent washes with TBST washing buffer were followed by development with ECL (Medicalbio, PT01001) luminescence.

Step 10: Using β-actin as internal reference protein, band analysis was performed by ImageJ software.

Reference

Chen et al., 2019

References (Video)

Lim et al., 2022; Song et al., 2021

ABclonal, A7414, 1:200) were incubated with PBST containing 3%BSA overnight at 4℃, followed by three washes with PBS. Cells were then incubated in 3%BSA in PBST for 1 hour in the dark with the respective fluorescent-conjugated secondary antibody (CoraLite488-conjugated goat anti-rabbit IgG (H+L), Proteintech, SA00013-2,1:500) followed by washing with PBST. DAPI was applied to the nuclei for 5 min, washed with PBS, and observed by fluorescence microscopy. The fluorescence intensity was analyzed using ImageJ software (Liu et al., 2019).

RIPK1 inhibitor

Necrostatin 1 (Nec-1), known as an inhibitor of RIPK1, has demonstrated its ability to inhibit necroptosis. To further investigate the role of RIPK1 kinase in berberine-induced necroptosis, CCK-8, qRT-PCR, western blot, and immunofluorescence assays were conducted using RIPK1 inhibitor Nec-1. Cells in the inhibitor group, cultured in 96-well, 6-well, and 24-well plates, were pretreated with 2.5 μM Nec-1 (Chen et al., 2019; Shahsavari et al., 2016) for 8 hours. After the pretreatment, CCK-8, qRT-PCR, western blot, and immunofluorescence experiments were repeated following the procedures outlined above.

Statistical analysis

Data were expressed as mean ± SEM, and all experiments were performed at least three times. The analysis was performed using Graphpad Prism 8 (Graphpad Software, USA). A t-test was employed to assess the statistical difference between the two groups, while one-way analysis of variance was used for comparisons among multiple groups, followed by Dunnett post hoc test.

Results

Necroptosis of H8 cells

Figure 1 illustrates the cytotoxic effects of berberine on H8 cells, revealing a substantial reduction in cell viability with increasing intervention time and dosage ($p<0.05$). The IC_{50} of berberine, observed after 72 hours of intervention at a concentration of 40 μM, indicated a significant decrease in the cell viability by approximately 50%. The experimental results highlighted concentration-dependent (0-100 μM) and time-dependent (24-72 hours) characteristics of berberine-induced cytotoxic and apoptotic effects on H8 cells. Consequently, subsequent experimental groups were treated with a low dose (20 μM), medium dose (40 μM), and high dose (80 μM) of berberine for 72 hours intervention.

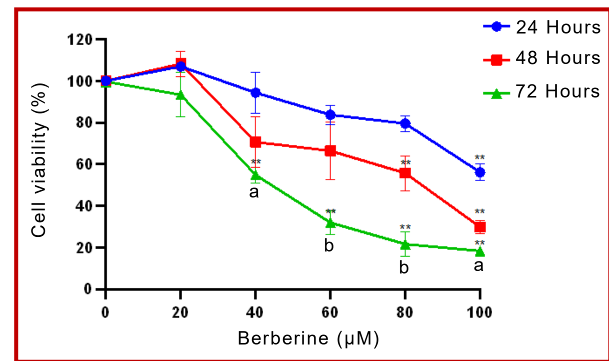

Figure 1: The CCK-8 assay was used to assess whether berberine inhibits cell proliferation at different time or dose points. Data are expressed as mean ± SEM. Compared to the control group, $^{a}p<0.05$, $^{b}p<0.01$; Compared to the control group, $^{**}p<0.01$

Up-regulation of RIPK1 kinase expression

As shown in Figure 2, berberine demonstrated a

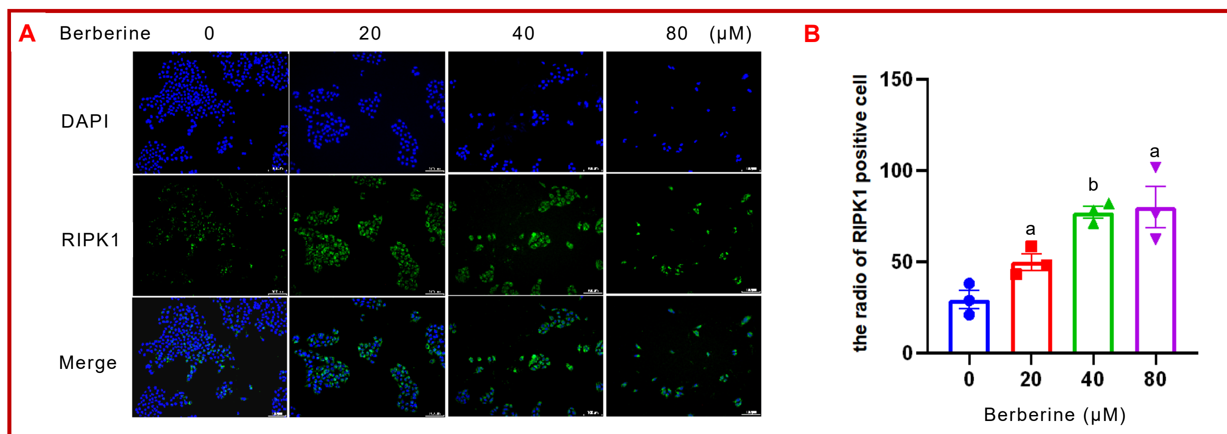

Figure 2: Immunofluorescence assay was used to assess the expression of RIPK1 in H8 cells treated with berberine at different concentrations. Data are expressed as mean ± SEM. Compared to the control group, $^{a}p<0.05$; $^{b}p<0.01$

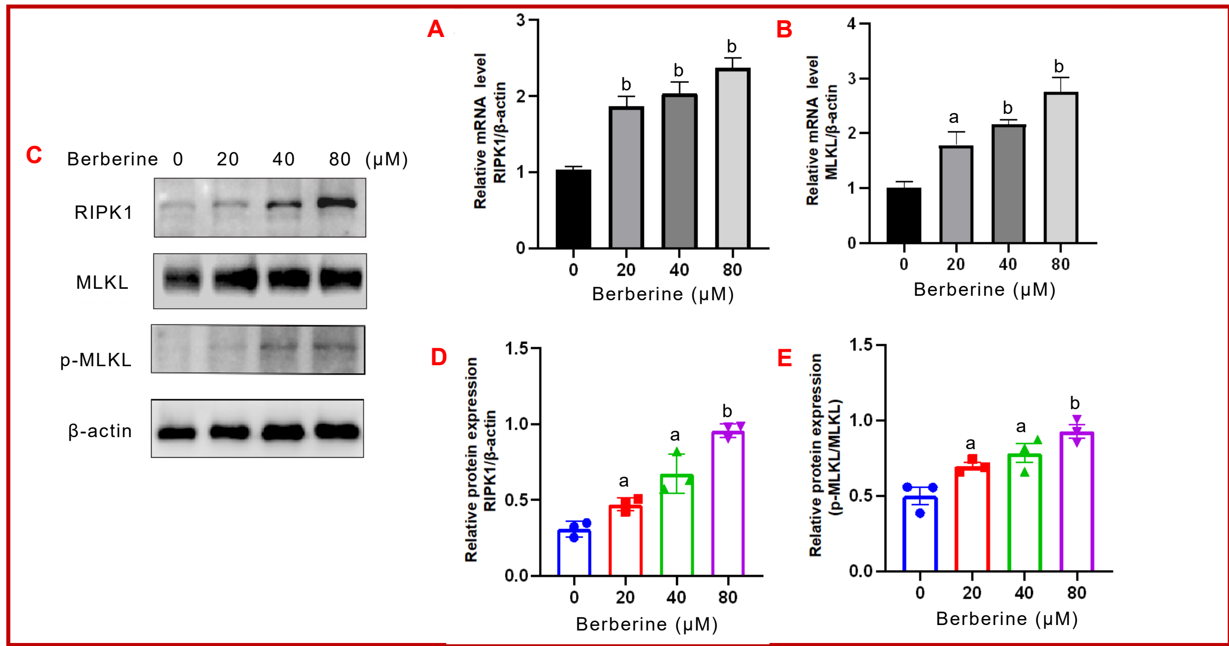

Figure 3: The mRNA expression levels of RIPK1 and MLKL in H8 cells were analyzed by qRT-PCR (**A-B**); Protein electrophoresis of RIPK1, MLKL ,p-MLKL in each group (**C**). Different concentration of berberine interferes with the expression level of RIPK1 protein in H8 cells (**D**). Different concentrations of berberine interfere with p-MLKL/MLKL protein expression ratio in H8 cells (**E**). Data are expressed as mean ± SEM. Compared to the control group, $^{a}p<0.05$; $^{b}p<0.01$

significant increase in the expression level of RIPK1 compared to the control group ($p<0.05$). This result underscores the pronounced impact of berberine on RIPK1 expression in H8 cells.

Up-regulation of mRNA expression levels of RIPK1 and MLKL

As illustrated in Figure 3A-B, the intervention groups treated with low, medium, and high doses of berberine showed a noteworthy increase in RIPK1 and MLKL mRNA expression levels compared to the control group ($p<0.05$).

Up-regulation of protein expression levels of RIPK1 and MLKL

Western blot was used to investigate the protein expression levels of key markers of necroptosis in H8 cells caused by berberine. As depicted in Figure 3C-D, compared to the control group, the intervention groups treated with low, medium, and high concentrations of berberine exhibited a significant increase in RIPK1 protein expression and the p-MLKL/MLKL ratio ($p<0.05$). These findings underscore a statistically significant difference in the levels of these key apoptotic markers following berberine intervention. Notably, these results align with the observations from qRT-PCR analysis.

Partial inhibition of necroptosis by Nec-1

As shown in Figure 4A, the CCK-8 assay results indicated a significant decrease in cell viability after 72 hours of treatment with 40 μM berberine compared to the control group ($p<0.01$). However, co-treatment with RIPK1 inhibitor, Nec-1 (2.5 μM), led to an increase in cell viability ($p<0.05$). The qRT-PCR results (Figure 4B-C) demonstrated a significant up-regulation in the expression of RIPK1 and MLKL mRNA after 72 hours of intervention with 40 μM berberine ($p<0.01$). Conversely, the addition of the RIPK1 kinase inhibitor (2.5μM Nec-1) resulted in a significant decrease in RIPK1 and MLKL mRNA expression ($p<0.01$). Consistent with the qRT-PCR findings, western blot results (Figure 4D-F) revealed parallel changes in protein expressions levels. Moreover, immunofluorescence results demonstrated a significant up-regulation of RIPK1 expression after 72 hours of treatment with 40 μM berberine compared to control cells ($p<0.05$). However, co-treatment with Nec-1 (2.5 μM) resulted in a decrease in RIPK1 expression ($p<0.05$).

Necroptosis in H8 cells independent of caspase activation

The results illustrated a decrease in the expression of c-caspase-8 and c-caspase-3 in the low, medium, and high-concentration berberine intervention groups compared to the control group (Figure 5A, C-D; $p<0.05$). However, upon the addition of the Nec-1 inhibitor (Figure 5B, E-F),

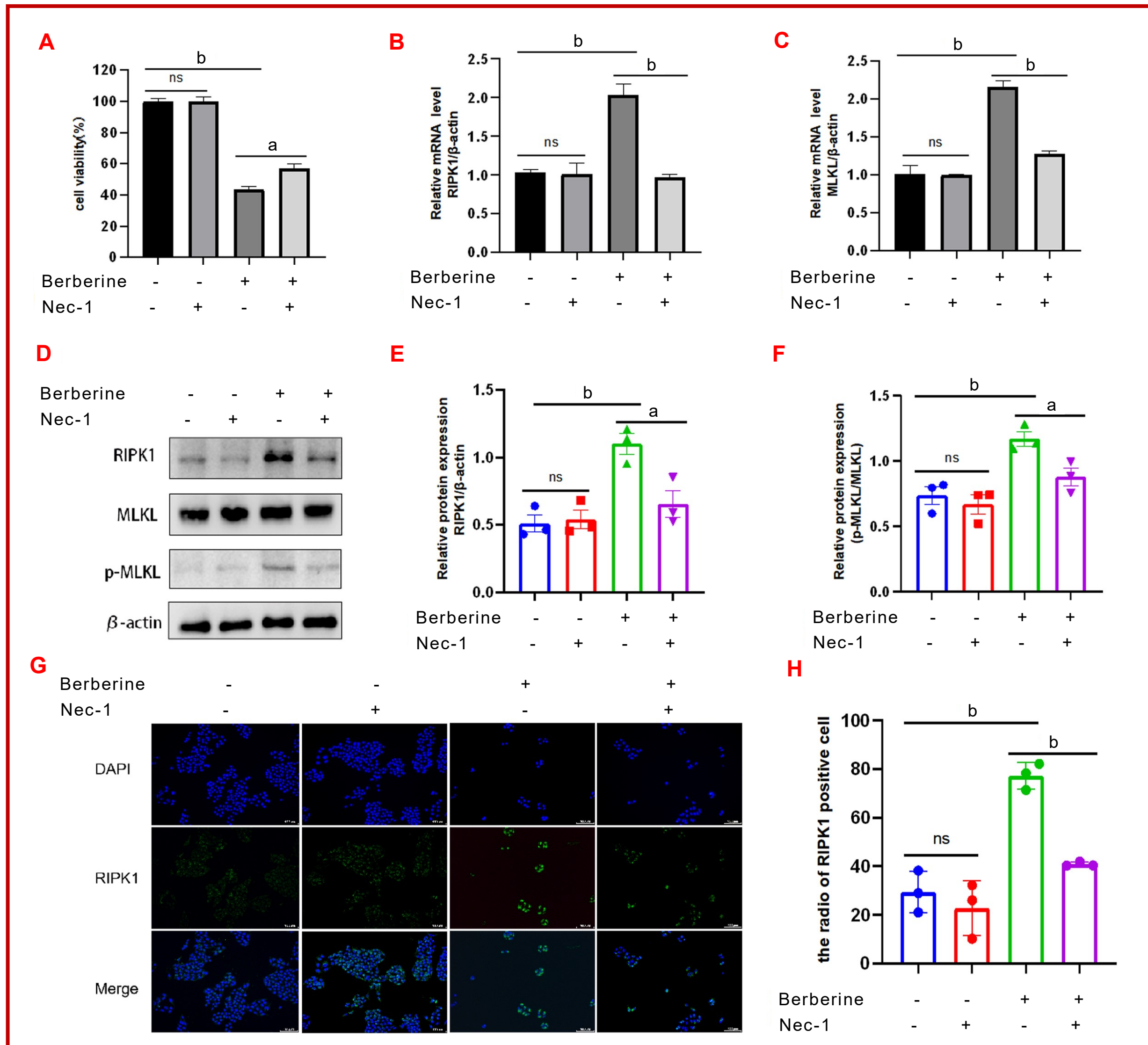

Figure 4: The CCK-8 assay was unviability after berberine (40 μM) and/or Nec-1 (2.5 μM) intervention (**A**). The expression levels of RIPK1 and MLKL mRNA after berberine and/or Nec-1 intervention were detected by q-RT-PCR (**B-C**). The expression levels of RIPK1 protein and p-MLKL/MLKL protein expression ratio of berberine (40 μM) and/or Nec-1 (2.5 μM) in H8 cells were detected by western blot (**D-F**). The expression levels of RIPK1 in H8 cells treated with berberine (40 μM) and/or Nec-1 (2.5 μM) were detected by immunofluorescence sed to detect H8 cells (**G-H**). Data are expressed as mean ± SEM; $^{a}p<0.05$, $^{b}p<0.01$.

no significant changes in the expression of c-caspase-8 and c-caspase-3 were observed (p>0.05).

Discussion

The present study shows that berberine can inhibit the proliferation of H8 cells by activating RIPK1, and the underlying mechanism may be related to the ability of berberine to activate RIPK1-mediated necroptosis. It has been found that Youdujing (a traditional Chinese herbal formula) can trigger necroptosis in ecto-cervical Ect1/E6E7 cells by activation of RIPK1 kinase (Chen et al., 2019). It is suggested that activating RIPK1 to induce the necroptosis pathway may be a new target for treating cervical intraepithelial neoplasia. Berberine induces necroptosis independent of caspase activation, with caspase-3 and caspase-8 being maintained in an inhibited state.

In recent years, the ongoing progress in cell biology has unveiled necroptosis as a regulated cell death mechanism. Numerous studies have highlighted the underexpression of key proteins within the necroptotic pathway in specific tumors. For instance, RIPK3 has been identified

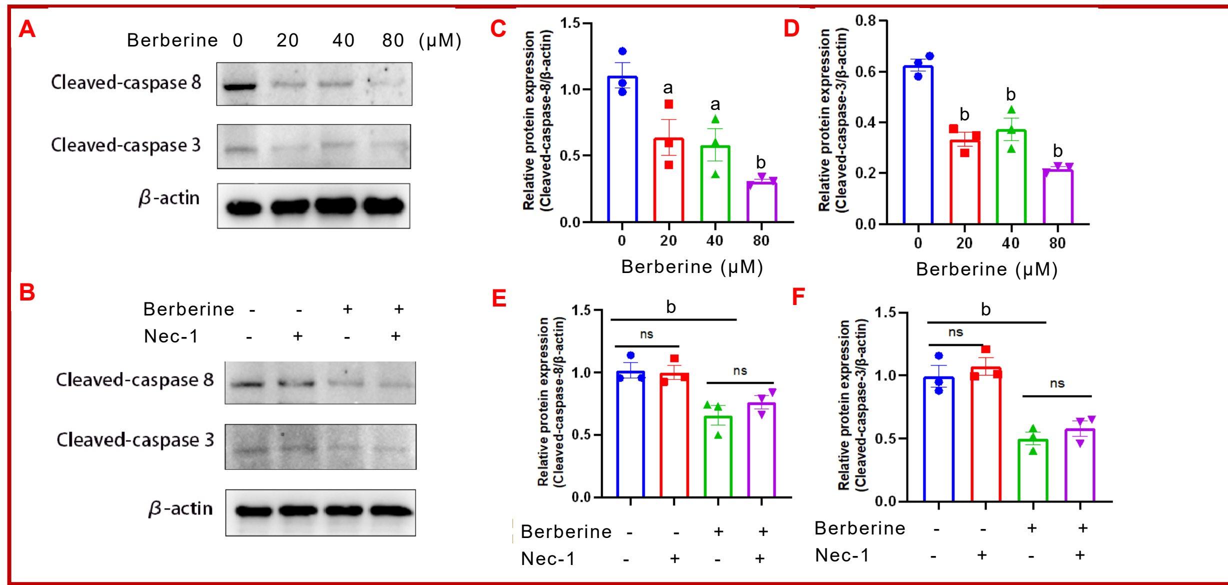

Figure 5: Protein electrophoresis of c-caspase-8 and c-caspase-3 in each group (**A-B**). Western blot assay was used to detect the expression levels of c-caspase-8 and c-caspase-3 protein after intervention with different concentrations of berberine in H8 cells (**C-D**). Western Blot assay was used to detect the expression levels of c-caspase-8 and c-caspase-3 protein after berberine (40 μM) and/or Nec-1 (2.5 μM) intervention in H8 cells (**E-F**). Data are expressed as mean ± SEM. Compared to the control group, [a]p<0.05, [b]p<0.01

as under-expressed in primary colorectal cancer (Feng et al., 2015). Additionally, the under-expression of MLKL has been linked to unfavorable prognosis in patients with various malignant tumors, including ovarian cancer, cervical squamous cell carcinoma, gastric cancer, and colorectal cancer (Park et al., 2020; Ruan et al., 2015; Sun et al., 2019). In the development and progression of specific tumors, the inhibition of necroptosis might contribute to the malignant proliferation of tumor cells. Unlike apoptosis, which relies on the caspase-dependent pathway, necroptosis operates through a caspase-independent pathway. Activating necroptosis in the presence of caspase inhibitors can serve as a mechanism for inducing suicide in tumor cells, thereby limiting their proliferation (Chen et al., 2019; Park et al., 2020).

When human papillomavirus infects host cells, the release of intracellular immune stimulatory components promotes host innate immune responses, forming a potent antiviral defense mechanism by inducing host cell death early to disrupt the viral replication ecosystem (Vanden et al., 2014; Warowicka et al., 2020). It has been identified that large DNA viruses are capable of inhibiting apoptosis by blocking the activity of the extrinsic apoptotic initiator caspase-8, which, in turn, can suppress necroptosis by cleaving RIPK1 and RIPK3 (Verdonck et al., 2022). Thus, when the virus inhibits caspase-8, necrosomes can be recruited at the TNFR1 (TNF receptor 1) stimulus to counteract viral infection. Other relevant studies have also suggested that necroptosis may not only occur when caspase-8 activity is blocked but can also act as an independent mechanism to inhibit viral infection (Bertheloot et al., 2021).

Berberine, a natural alkaloid compound found in various plants, including coptis chinensis and phellodendron, not only exhibits an antiproliferative effect on cancer cells but also demonstrates the capability to target different stages of the virus life cycle, activate immune cells, and exert an antiviral effect (Liu et al., 2019; Kim et al., 2016; Warowicka et al., 2020). Importantly, berberine has shown relative safety for normal cells. A study has been conducted to investigate the cytotoxic effects of berberine on normal human prostate epithelial cells (Mantena et al., 2006). Their results indicated that berberine concentrations ranging from 10 to 100 μM did not exhibit significant toxicity on normal prostate cells, suggesting that the intervention concentrations used in this study fall within safe limits. This study delved into the mechanism of action of berberine on cervical H8 cells, revealing its ability to inhibit H8 cell proliferation in a time-dependent and dose-dependent manner. Notably, berberine induces necroptosis in cervical H8 cells by activating RIPK1. These findings offer valuable insights into the potential therapeutic effects of berberine, specifically in targeting necroptosis for the treatment of cervical intraepithelial neoplasia.

The results revealed a significant inhibition of H8 cell growth by berberine ($p<0.05$), indicating its potential to suppress cell proliferation through the necroptotic pathway. Furthermore, berberine was found to upregulate the expression levels of key necroptosis markers, RIPK1 and MLKL ($p<0.05$), suggesting its involvement in activating the necroptotic pathway through the activation of RIPK1 kinase. The initiation of necroptosis involves key signaling events, including the activation of RIPK1, subsequent interaction between RIPK1 and RIPK3, and eventual MLKL phosphorylation. Upon phosphorylation, MLKL oligomerizes and translocates to the cell membrane, disrupting cell integrity, forming pores, and releasing damage-associated molecular pattern molecules (DAMPs) and inflammatory cytokines. This triggers an immune response, actively participating in the anti-tumor process (Zhang et al., 2022). There results, further demonstrated a decrease in the expression of c-caspase-8 and c-caspase-3 in the berberine-treated group compared to the control group($p<0.05$), suggesting that caspase-8 and caspase-3 may be suppressed during this process. Caspase-8 plays a crucial role in regulating cell death pathways, activating apoptosis, and inhibiting necroptosis upon activation. Activated caspase-8 can cleave and inactivate key necroptosis-related proteins such as RIPK1, RIPK3, and cylindromatosis (CYLD), leading to the suppression of necroptosis and the activation of caspase-3 to trigger apoptosis. Inhibition of caspase-8 has been shown to result in the deubiquitination of RIPK1 by CYLD, promoting kinase activation and modulating necroptosis (Gao et al., 2022; Vanden et al., 2014). When H8 cells undergo necroptosis, caspase-8 and caspase-3 are in a suppressed state, suggesting a potential mechanism of mutual transformation between apoptosis and necroptosis in cells. The interchangeability of apoptosis and necroptosis has been demonstrated under certain conditions in L929 mouse fibrosarcoma cells (Sawai, 2016). Necroptosis inhibition by Nec-1 led to the activation of apoptotic pathways and subsequent cell death. Additionally, Jie et al. (Jie et al., 2016) reported that Nec-1 induced apoptosis in neutrophils.

Recent studies indicate a link between necroptosis and tumor immunity, emphasizing the distinct nature of necroptosis compared to traditional apoptosis. Necroptosis can elicit an inflammatory response within cells by releasing damage-associated molecular patterns (DAMPs), leading to immune system activation—a phenomenon often termed necrotic inflammation (Bertheloot et al., 2021; Gao et al., 2022). DAMPs induce the production of inflammatory cytokines through various inflammatory transcriptional pathways, including NF-κB pathway. Necroptosis contributes to anti-tumor effects by regulating the function of immune cells, influencing their survival, differentiation, activation, transport, and functional performance (Zhang et al., 2022). Additionally, it induces the expression of immune-stimulating cytokines, thereby enhancing anti-tumor immunity (Park et al., 2021).

Nevertheless, this study lacks a detailed exploration of the molecular mechanism through which berberine mediates its anti-tumor effects following the activation of necroptosis. In addition, this study still lacks mature animal models to verify this mechanism in animals.

Conclusion

Berberine induces necroptosis in cervical H8 cells by activating RIPK1 kinase, a process independent of caspase activation. These findings present a novel therapeutic avenue for the prevention and treatment of cervical intraepithelial neoplasia.

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Ethical Issue

The development, acquisition, authentication, cryopreservation, and transfer of cell lines between laboratories were followed according to the guidelines published in British Journal of Cancer, 2014.

Conflict of Interest

Authors declare no conflict of interest

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