Capsid protein Vp1 from chlamydiaphage φCPG1 effectively alleviates cytotoxicity induced by *Chlamydia trachomatis*

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Abstract. *Chlamydia trachomatis* is the leading cause of sexually transmitted bacterial infections. *C. trachomatis* genital infection may lead to pelvic inflammatory disease, ectopic pregnancy and tubal infertility, which are major public health problems. However, the pathogenic mechanisms of this bacterium remain unclear, and the efficacy of clinical therapeutics is unsatisfactory. In the current study, whether Vp1 can alleviate the cytotoxicity induced by *Chlamydia trachomatis* infection was investigated. *C. trachomatis* was pre-treated with BSA or purified Vp1 protein and used to infect HeLa cells. It was observed that Vp1 significantly inhibited the infectivity of *C. trachomatis* in cell cultures. In addition, the Vp1 pretreatment reduced the chlamydial Hsp60 protein levels and decreased the *C. trachomatis* inclusion number. The Vp1 pretreatment also prevented *C. trachomatis*-induced cytotoxicity in host cells. Furthermore, the chlamydial suppression of host cell proapoptotic p53 protein and the induction of antiapoptotic *cIAP-2* and *Mcl-1* gene expression were reversed by the Vp1 pretreatment. These observations suggest that Vp1 has a clear inhibitory effect on *C. trachomatis* growth *in vitro*.

Introduction

Chlamydia trachomatis is responsible for the largest proportion of all sexually transmitted diseases (STDs) reported to the CDC (1). In the USA, 1,598,354 chlamydial infections were reported in 2016, corresponding to a rate of 497.3 cases per 100,000 individuals, and from 2008 to 2015, the rate of reported chlamydial infections increased from 32.48 to 37.18 cases per 100,000 individuals in China (2). *C. trachomatis*-induced genital tract infections may cause inflammation, edema and mucosal discharge (3), and ascending uterine infections may lead to pelvic inflammatory disease, tubal scarring, ectopic pregnancies and infertility (4).

After entering a cell, *C. trachomatis* inclusions absorb nutrients and live in the organelles of the host cell. A mixture of apoptotic features and atypical cell death during infection has been demonstrated to occur during the process of cell death induction (5-7). One of the primary obstacles faced by chlamydial researchers has been the lack of genetic techniques for the creation of mutant chlamydial strains, which are necessary for a thorough exploration of chlamydial pathogenesis. The genetic tools that are widely used in other bacteria are not applicable to *Chlamydia* because of its obligate, intracellular lifestyle and unique development cycle, hindering the development of vaccines and therapies (5-7). Until effective vaccines are developed, screening and treatment procedures appear to be the best approach for preventing chlamydia-related disease.

All six chlamydiaphages isolated from *Chlamydia* (Chp1, Chp2, Chp3, Chp4, φCPG1 and φCPAR39) (8-12) share similar features, and the high homology has led to a hypothesis of cross-reactions between species. Molecular characterization indicates that these six chlamydiaphages belong to the family Microviridae (8-14). To date, a *C. trachomatis*-specific phage has not yet been detected. φCPG1 is a lytic phage that infects *Chlamydia caviae*, a guinea pig inclusion conjunctivitis strain (10,13). The φCPG1 genome has five open reading frames (ORFs): ORFs 1-3 encode capsid proteins Vp1, Vp2 and Vp3, and ORFs 4-5 encode proteins VG4 and VG5 (13). The capsid protein Vp1 plays a crucial role in the adhesion and invasion of *Chlamydia*. A genome-wide analysis has revealed a similarity of 83-95% among the six chlamydiaphage Vp1 capsid proteins (14).

Vp1 capsid proteins of φCPG1 have been demonstrated to inhibit *C. trachomatis* growth and cause a decrease in the number of *C. trachomatis* inclusion bodies during infection (15-17). Vp1 has also been indicated to exert inhibitory effects on the proliferation of *C. trachomatis* in the mouse genital tract (16). In the present study, whether Vp1 alleviates the cytotoxicity induced by *Chlamydia trachomatis* was investigated. *C. trachomatis* inclusion bodies were counted under a fluorescence microscope, and the chlamydial Hsp60 protein levels were evaluated by western blotting. In addition, the interactions between *C. trachomatis* and Vp1 were investigated. *C. trachomatis*-induced host cell apoptosis was detected following Vp1 treatment by flow cytometric analysis.

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Furthermore, the protein levels of the host cell proapoptotic p53 protein and the transcription levels of the antiapoptotic genes *Mcl-1* and *cIAP-2* were evaluated by western blotting and reverse transcription-quantitative polymerase chain reaction (RT-qPCR), respectively.

Materials and methods

Vp1 expression, identification and purification. The *Escherichia coli* strain BL21 (Tiangen Biotech Co., Ltd., Beijing, China) with the pET30a(+)/Vp1 plasmid (EMD Millipore, Billerica, MA, USA) was cultured in Luria-Bertani medium (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China) containing kanamycin (50 mg/ml) at 37°C with shaking until the optical density reached 0.6 at 600 nm. Isopropyl-β-D-thiogalactoside (Beijing Solarbio Science and Technology Co., Ltd.) was added to the culture at a concentration of 0.5 mmol/l, and the mixture was then shaken at 37°C for 3 h. *E. coli* cells were then collected by centrifugation at 10,000 x g and 4°C for 5 min. Following resuspension of the cell pellet in PBS, 3% Triton X-100 and 4 mg/l lysozyme were added. Vp1 protein was released from *E. coli* following sonication (ultrasound for 10 sec and pause for 6 sec for a total of 8 min; all at 0°C). Centrifugation (12,000 x g, 20 min, 4°C) was performed to remove impurities, then Vp1 protein was purified using a His.Bind® Purification kit (Merck KGaA, Darmstadt, Germany). Protein renaturation was performed using gradient dialysis in PBS. Lipopolysaccharides were neutralized using a ToxinEraser™ endotoxin removal kit (GenScript, Piscataway, NJ, USA) and detected using a ToxinSensor Gel Clot Endotoxin Assay kit (GenScript). The concentration of Vp1 protein was quantified using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The Vp1 protein was stored at -80°C until subsequent experiments.

CCK-8 assay of cell viability. The Cell Counting Kit-8 (Dojindo Molecular Technologies, Inc., Shanghai, China) was used according to the manufacturer's protocol. The CCK-8 reagent (10 μl) was mixed with 0.1 ml Dulbecco's modified Eagle's medium (DMEM; HyClone; GE Healthcare Life Sciences, Logan, UT, USA) supplemented with 8% fetal bovine serum (FBS; Tianjin Haoyang Biological Manufacture Co., Ltd. Tianjin, China). Then, $1x10^5$ HeLa cells (Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China) were added and incubated in 96-well plates with 60 μg/ml Vp1. The absorbance of the medium was read at 450 nm using an ELISA instrument (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 24, 48 and 72 h. The experiments were repeated three times.

C. trachomatis infection and Vp1 treatment. Previous studies indicated that both HeLa cells and normal human cervical cells could be suitable for the experiments in the current study. For example, González *et al* (18) and Siegl *et al* (19) investigated the association between *Chlamydia* infection and P53 using HeLa cells and normal human cells. HeLa cells are often used for the generation and culture of *C. trachomatis* (5-7). Therefore, HeLa cells were selected as a research model. HeLa cells were grown in DMEM supplemented with 10% FBS and incubated at 37°C with 5% CO_2 prior to chlamydial infection.

HeLa cells were seeded in a 24-well plate and after 24 h, the cells were pre-treated with Diethylaminoethyl-dextran (Merck KGaA) for 30 min to increase their susceptibility to *C. trachomatis* infection. *C. trachomatis* strain E (ATCC, Manassas, VA, USA) was subjected to two freeze-thaw cycles, followed by vortex at 3, 200 rpm for 1 min, room temperature.. Next, *C. trachomatis* cells were pre-incubated with 60 μg/ml purified Vp1 in PBS or 60 μg/ml bovine serum albumin (BSA; Beijing Solarbio Science and Technology Co., Ltd.) in PBS for 1 h. According to our previous study, 60 μg/ml was selected as the optimal concentration of Vp1 and BSA (15). Following Vp1 pretreatment, *C. trachomatis* cells were used to infect HeLa cells at a multiplicity of infection (MOI) of 1. *C. trachomatis* adhesion was facilitated by centrifugation at 32°C and 500 x g for 1 h. Then, DMEM (without cycloheximide) was added, and the HeLa cells were subsequently incubated at 37°C with 5% CO_2 for 40 h. Mock-infected cells were subjected to the same procedure without *C. trachomatis*.

Immunofluorescence microscopy. *C. trachomatis*-infected or uninfected HeLa cells grown on glass coverslips were washed with PBS and fixed with ice-cold methanol for 15 min. Fixed cells were washed three times and treated with 0.1% Triton-X-100 for 8 min at room temperature. The cells were then washed three times in PBS and blocked in 10% BSA in PBS for 1 h at 37°C. The cells were then washed three times in PBS and reacted with an antibody against *C. trachomatis* E serotype (obtained from Professor Guangming Zhong, University of Texas Health Science Center at San Antonio, San Antonio, USA) diluted at 1:2,000 in 10% BSA in PBS at 4°C overnight. Following three washes in PBS, the primary antibody-stained monolayers were co-reacted with Cyc3-conjugated goat anti-rabbit antibodies (red; Abcam, Cambridge, MA, USA; cat. no. ab6939; 1:70) in 10% BSA and Hoechst 32258 (blue) for 50 min at 37°C. Images were acquired using a fluorescence microscope. The single-color images were merged using Adobe Photoshop 7.0 (Adobe Systems, Inc., San Jose, CA, USA). The experiments were repeated three times.

SDS-PAGE and western blotting. Cellular protein was extracted from HeLa cells using radioimmunoprecipitation assay buffer (Beijing Solarbio Science and Technology Co., Ltd.). Lysates were centrifuged at a speed of 500 x g for 5 min at 4°C, and sample buffer was added to the sediment. Lysates were heated to 100°C for 5 min, then analyzed by 10% SDS-PAGE. The protein was quantified using a BCA assay kit (Thermo Fisher Scientific, Inc.). A total of 15 μl protein was loaded per lane. Following electrophoresis, the proteins were transferred onto PVDF membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 3% BSA in TBS for 2 h at room temperature. The membranes were subsequently incubated with antibodies against P53 (Wanleibio Co., Ltd., Shanghai, China; cat. no. WL103333) at a dilution of 1:2,000, overnight at 4°C. The horseradish peroxidase conjugated anti-mouse immunoglobulin G antibody (Cell Signalin g Technology, Inc., Danvers, MA, USA; cat. no. 14709) was added at a dilution of 1:10,000 and incubated for 2 h at room temperature. Using an enhanced chemiluminescence kit (Merk KGaA), the membranes were photographed and densitometry was performed using Image J software (V 1.8.0; National Institutes

of Health, Bethesda, MD, USA). The level of chlamydial Hsp60 as an indicator of the infection load was detected at different time points following infection (14, 20, 24, 30 and 36 h). The p53 protein levels were normalized to β-actin, which was used as an internal control. The experiments were repeated three times.

RT-qPCR. Total RNA was extracted from HeLa cells at 48 h post infection using an RNeasy Mini kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's protocol. Then, 2 μg of total RNA was used to synthesize first-strand cDNA in a 20-μl reaction using a M-MLV Reverse Transcriptase kit (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The cDNA product (1 μl) was used for qPCR with an ABI 7500 Fast system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with SYBR® Premix Ex Taq™ II (Takara Bio, Inc., Otsu, Japan) using the specified primer sets. The primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China; Table I). The PCR procedure was as follows: 95°C for 3 min, followed by 40 cycles of 94°C for 5 sec and 60°C for 30 sec. Using the $2^{-\Delta\Delta Cq}$ method (20), the transcription levels of target genes were analyzed using the β-actin gene as an internal control. All experiments were repeated three times.

Flow cytometric analysis. Following incubation and infection, adherent and floating cells were collected by trypsinization followed by centrifugation at a speed of 300 x g for 3 min at room temperature. HeLa cells were then washed with PBS and resuspended in Annexin V-fluorescein isothiocyanate (FITC) binding buffer. Annexin V-FITC was then added, and the cells were incubated for 10 min in the dark according to the manufacturer's protocol (BD Biosciences, Franklin Lakes, NJ, USA). Then, propidium iodide was added, and the cell suspension was analyzed within 1 h on a flow cytometer using CellQuest software V 5.1 (BD Biosciences).

Statistical analysis. The data were analyzed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Graphs were generated using GraphPad Prism v6.01 (GraphPad Software, Inc., La Jolla, CA, USA). Two-tailed Student's t-test was used to analyze datasets containing two groups. One-way analysis of variance with Dunnett's multiple comparison test was used to analyze datasets containing multiple groups. Data are presented as the mean ± standard deviation. P<0.05 was considered to indicate a statistically significant difference.

Results

In vitro cell viability following Vp1 exposure. Recombinant His-tagged Vp1 was expressed in *E. coli* and purified using a His-Bind Purification kit. Total protein was extracted from *E. coli*, and the purified Vp1 protein was separated by SDS-PAGE (Fig. 1A). Lipopolysaccharides were neutralized using a ToxinEraser endotoxin removal kit and detected using a ToxinSensor Gel Clot Endotoxin Assay kit. Purified Vp1 protein was stored at -80°C until subsequent experimentation. HeLa cells were treated with Vp1 at a concentration of 60μg/ml for various durations (24 to 72 h), and a CCK-8 assay was performed at the end of each exposure. Mock cells cultured in

Table I. Primers used for quantitative polymerase chain reaction.

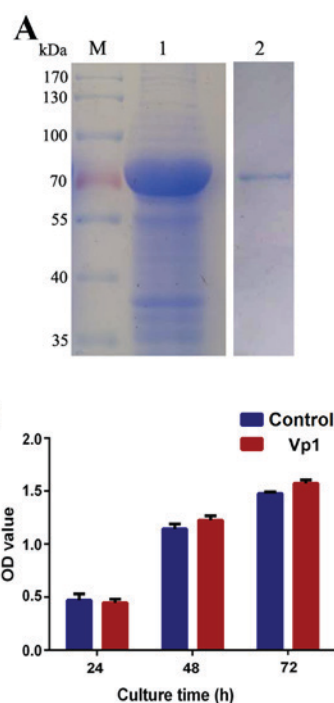| Gene	Primer (5'-3')
β-actin	F, CCTGGCACCCAGCACAAT
	R, CTGATCCACATCTGCTGGAA
Puma	F, CGACCTCAACGCACAGTACGA
	R, AGGCACCTAATTGGGCTCCAT
Mcl-1	F, GCCAAGGACACAAAGCCAAT
	R, CCGTCGCTGAAAACATGGAT
cIAP-2	F, CTGTGATGGTGGACTCAGGT
	R, TTCATCTCCTGGGCTGTCTG

F, forward; R, reverse.

Figure 1. *In vitro* cell viability assay. (A) Total protein extracted from *E. coli* (Lane 1) and purified Vp1 protein (Lane 2) were separated by SDS-PAGE. (B) HeLa cells were incubated with Vp1 at a concentration of 60 μg/ml for various durations (24 to 72 h). No significant difference was observed between the two groups at different time points (P>0.05). Data are presented as the mean ± standard deviation of three independent experiments (analyzed by paired t-test). OD, optical density.

Vp1-free medium were tested simultaneously. The *in vitro* cell viability assay indicated that compared with untreated HeLa cells, cells exposed to Vp1 for various times (24, 48 or 72 h) exhibited no cytotoxic effects (Fig. 1B). This finding suggests that Vp1 may not have cytotoxic effects in *in vivo* studies.

C. trachomatis inclusion numbers decrease following Vp1 treatment. To detect the inhibitory effect of Vp1 on *C. trachomatis* development, *C. trachomatis* cells were pre-incubated with BSA or purified Vp1 prior to infection. HeLa cells infected with *C. trachomatis* at an MOI of 1 were incubated with Vp1 or BSA, fixed with methanol at 48 h
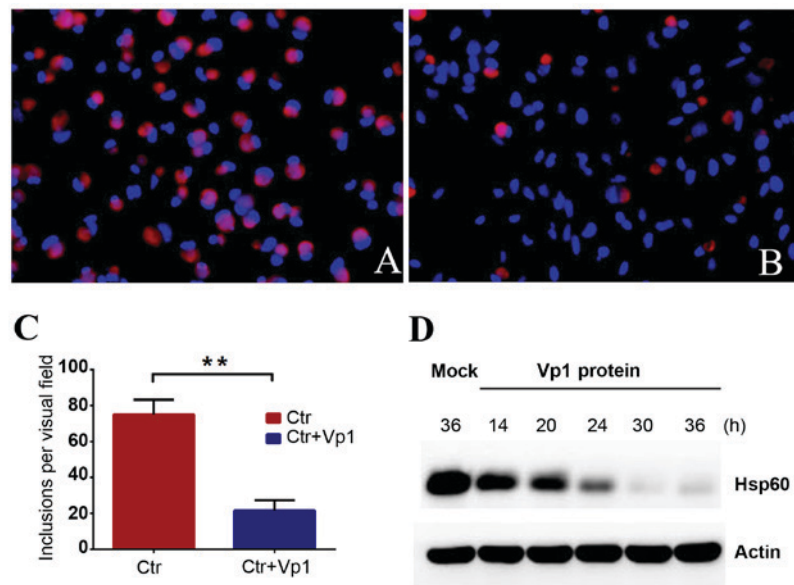
Figure 2. *Chlamydia trachomatis* inclusion numbers decreased in the Vp1-treated group. *C. trachomatis* cells were pre-treated with BSA or purified Vp1. (A and B) Bright-red, irregular-shaped fluorescing cells are chlamydial organisms and blue fluorescence indicates DNA. Magnification, x 200. (A) BSA-treated *C. trachomatis*-infected group at 48 hpi. (B) Vp1-treated *C. trachomatis*-infected group at 48 hpi. (C) Under a fluorescence microscope, the *C. trachomatis* inclusion bodies were counted for each visual field in 20 separate fields. The average number of inclusions per visual field of the BSA-treated *C. trachomatis*-infected group was 74.90±1.852, and the average number of inclusions in the Vp1-treated *C. trachomatis*-infected group was 21.60±1.268. The inhibition rate reached 71%. **P<0.01. (D) Using western blotting, the protein levels of chlamydial Hsp60 as an indicator of infection load in the Vp1-treated *C. trachomatis*-infected group were detected at different time points following infection (14, 20, 24, 30 and 36 h). The amount of Hsp60 protein gradually decreased after 24 hpi. BSA, bovine serum albumin; Ctr, BSA-treated control; hpi, h post-infection.

post-infection (hpi) and reacted with the corresponding antibodies. Irregular-shaped *C. trachomatis* cells with a bright-red fluorescence were observed in the HeLa cell monolayers at 48 hpi, and blue fluorescence indicated the cellular DNA. The number of *C. trachomatis* inclusions exhibited a notable decrease following 48 h of incubation in the Vp1-treated group compared with the BSA-treated group (Fig. 2A and B). *C. trachomatis* was quantified by counting the inclusion bodies per visual field for 20 separate fields. The average number per visual field of the BSA- and Vp1-treated groups was 74.90±1.852 and 21.60±1.268, respectively (Fig. 2C). The inhibition rate reached 71% in the Vp1-treated group, demonstrating that the addition of Vp1 protein during the *C. trachomatis* culture process could significantly reduce the number of inclusion bodies. The total protein from infected cells of the Vp1-treated group was extracted at different time points, and chlamydial Hsp60 protein was detected by western blotting as an indicator of the infection load. The amount of Hsp60 protein gradually decreased after 24 hpi, indicating that Vp1 may exert an inhibitory action during the later stages of infection (Fig. 2D). These results indicated that Vp1 exerts a notable inhibitory effect on *C. trachomatis*.

Cytotoxicity induced by C. trachomatis infection is alleviated by Vp1. Since *C. trachomatis* is cytotoxic to host cells, it was explored whether the cytotoxicity induced by *C. trachomatis* infection was alleviated after cells were incubated with Vp1. HeLa cells were incubated with *C. trachomatis* for 48 h. Following Annexin V/propidium iodide staining, a flow cytometric analysis was performed. After 48 h of *C. trachomatis* infection, early apoptotic cells, late apoptotic cells and

necrotic cells were observed in the Q3, Q2 and Q1 fractions, respectively. In the *C. trachomatis*-infected group, HeLa cells exhibited a higher rate of early apoptosis (Annexin V+ PI-) compared with the BSA-treated group (Fig. 3). By contrast, a significantly lower early apoptosis rate was observed in the Vp1-treated *C. trachomatis*-infected group compared with *C. trachomatis*-infected group (Fig. 3). These results indicated that *C. trachomatis* infection-induced apoptosis of HeLa cells was reduced following Vp1 pretreatment.

Protein levels of p53 and mRNA levels of Puma, Mcl-1 and cIAP-2 in Vp1-treated C. trachomatis-infected cells recover to normal control levels. Consistent with previous studies, *C. trachomatis* infection resulted in the degradation of p53, which is associated with the infection dose and duration of infection in host cells (18,19). In the current study, an obvious decrease of p53 was observed following 48 h of incubation with BSA-treated *C. trachomatis* compared with the mock-infected cells as controls (Fig. 4A). In addition, infection-induced p53 degradation was reduced by treating the *C. trachomatis* cells with Vp1 prior to infection (Fig. 4A). The restoration of p53 function was further evaluated by analyzing the proapoptotic gene *Puma*, an important transcriptional target of p53 (Fig. 4B). As expected, the mRNA levels of *Puma* significantly increased following Vp1 treatment, consistent with a functional upregulation of p53. Our preliminary experiments indicated no change in the p53 protein levels when Vp1 interacted with HeLa cells without *C. trachomatis* (data not shown). Therefore, these results indicated that the inhibitory effect of Vp1 is caused by a direct interaction with *C. trachomatis*.
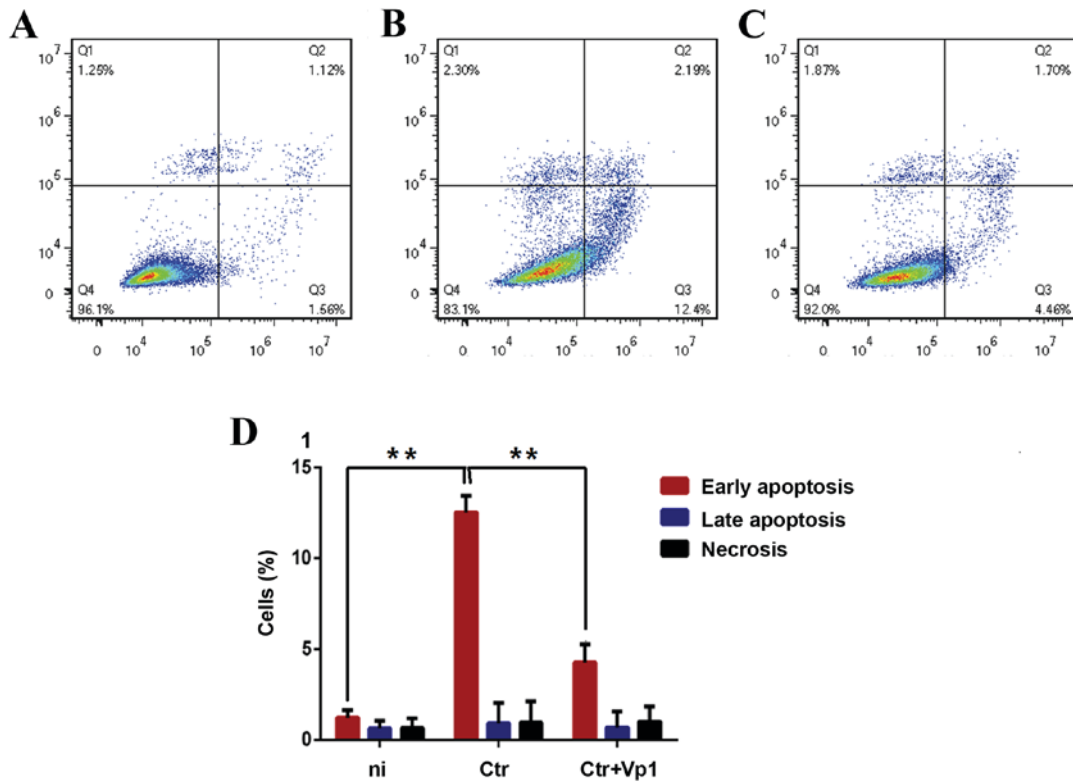
Figure 3. Vp1 alleviated the cytotoxicity induced by *Chlamydia trachomatis* infection. The percentages of early apoptotic cells, late apoptotic cells and necrotic cells at 48 h post-infection were measured by flow cytometric analysis. Early apoptotic cells, late apoptotic cells and necrotic cells are indicated in Q3, Q2 and Q1, respectively. (A) Mock-infected group as a control; (B) BSA-treated *C. trachomatis*-infected group; (C) Vp1-treated *C. trachomatis*-infected group. (D) *C. trachomatis*-infected HeLa cells exhibited a representative early apoptotic profile compared with the BSA-treated *C. trachomatis*-infected group; Vp1-treated *C. trachomatis*-infected group exhibited a lower early apoptosis rate compared with the BSA-treated *C. trachomatis*-infected group. The experiment was performed in triplicate. **P<0.01. BSA, bovine serum albumin; ni, mock-infected control group; Ctr, BSA-treated *C. trachomatis*-infected group.
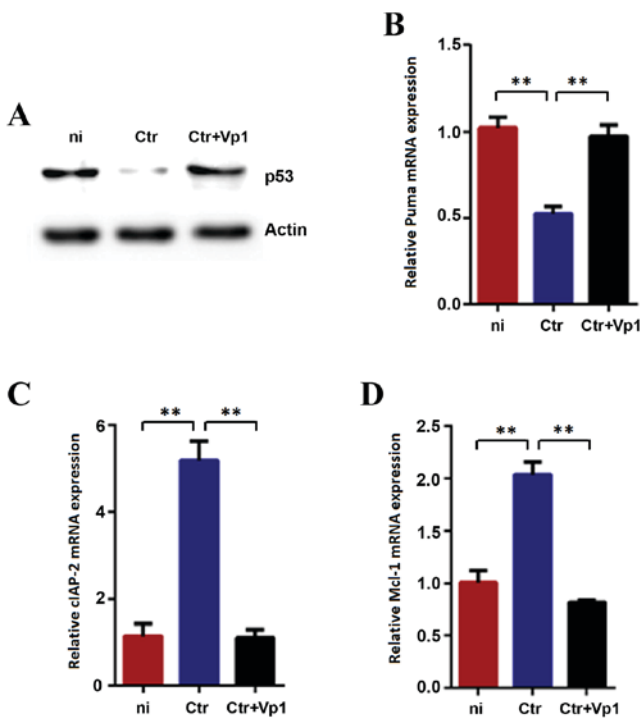

Figure 4. Protein levels of p53 and mRNA levels of *Puma*, *Mcl-1* and *cIAP-2* in Vp1-treated *C. trachomatis*-infected cells. (A) Protein levels of p53 were assessed by western blotting. β-actin was used as an internal control. (B-D) *Puma*, *cIAP-2* and *Mcl-1* mRNA levels were detected by reverse transcription-quantitative polymerase chain reaction. **P<0.01. ni, mock-infected control group; Ctr, BSA-treated *C. trachomatis*-infected group.

The expression levels of *Mcl-1* and *cIAP-2* were also evaluated at 48 hpi (Fig. 4C and D). The expression levels of these two genes in the BSA-treated group indicated a significant increase compared with the mock-infected cells. Furthermore, the expression levels of *Mcl-1* and *cIAP-2* in the Vp1-treated group revealed a significant decrease compared with the BSA-treated group.

Discussion

Chlamydial STDs in women are a serious health problem because infection may lead to infertility, life-threatening ectopic pregnancy and pelvic inflammatory disease (1). Although *C. trachomatis* resistance is a rare occurrence, a single 1 g dose of azithromycin is not sufficient for the treatment of urogenital and anorectal *C. trachomatis* infections (21). The development of a vaccine faces great challenges, and the immunological mechanisms responsible for immune protection and immunopathology remain unclear (21). Exploring the etiology and pathogenic mechanism of *C. trachomatis* will contribute to the development of chlamydia infection treatments and preventative measures. *C. trachomatis* is able to complete its replication and development cycle by inhibiting the apoptosis of host cells through a number of mechanisms, including regulating host cell mitogen-activated protein kinase signaling pathways, inhibiting mitochondrial cytochrome c release, degrading pro-apoptotic proteins and upregulating inhibitor of apoptosis proteins (IAPs) (22-26). Research by Siegl *et al* (19)

and González *et al* (18) reported that *C. trachomatis* promotes p53 proteolysis to inhibit apoptosis, which leads to persistent infection via an interaction between p53 and murine double minute 2. Mcl-1 and cIAP-2 are well-known key regulators of apoptosis resistance in *C. trachomatis*-infected cells, and activation of the phosphoinositide 3-kinase pathway in *C. trachomatis*-infected cells also stabilizes the anti-apoptotic proteins Mcl-1 and cIAP-2 (27,28).

The current results identified that Vp1 exerts a clear inhibitory effect on *C. trachomatis* growth. In addition, the induction of cytotoxicity in *C. trachomatis*-infected host cells was inhibited. The protein levels of p53 and the expression levels of *Mcl-1* and *cIAP-2* recovered to normal levels in the Vp1-treated group compared with the BSA-treated group. The specific mechanism through which Vp1 acts on *C. trachomatis* remains to be elucidated. To date, six chlamydiaphages have been identified (29), and all of these belong to the family Microviridae. An amino acid sequence analysis of the φCPG1 Vp1 protein revealed the presence of two major areas of significant divergence from other chlamydiaphages, namely, amino acids 216-299 (IN5 loop) and 462-467 (INS loop) (10). These two loops are exposed on the virion surface and likely interact with the host. Using far-western blotting, our previous study revealed that the φCPG1 Vp1 protein could bind to the *C. trachomatis* polymorphic membrane protein I (PmpI) (30), suggesting that the binding site of Vp1 is on the surface of *C. trachomatis*. In our previous study, 117 differentially expressed proteins of *C. trachomatis* treated with Vp1 were identified by a label-free test and the mRNA levels of several differentially expressed proteins were assessed using qPCR (17). According to these results, it was hypothesized that the combination of Vp1 and PmpI leads to changes in the function and structure of PmpI. PmpI may transduce the Vp1 signal in *C. trachomatis*, and this stimulation may result in the differential expression of *C. trachomatis* proteins. Eventually, this process may cause the cell cycle or other important *C. trachomatis* cell processes to be disrupted.

The increasing rate of *C. trachomatis* infection and treatment failure are acknowledged public health problems. To date, there is no effective *C. trachomatis* vaccine, and the effect of a single dose of azithromycin is unsatisfactory in certain cases. A greater understanding of basic chlamydial biology and pathogenic mechanisms are important for the prevention and treatment of *C. trachomatis*. As the Vp1 protein has been indicated to suppress the growth of *C. trachomatis*, the current study provides support for this potential clinical therapy for *C. trachomatis* infection.

Acknowledgements

Availability of data and materials

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JR and QL conceived and designed the experiments; JR performed the experiments; YG analyzed the data; JR wrote the manuscript; and LS and YL were involved in the cell culture and protein purification.

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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