Inactivated Tianjin strain, a novel genotype of Sendai virus, induces apoptosis in HeLa, NCI-H446 and Hep3B cells

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Abstract. The Sendai virus strain Tianjin is a novel genotype of the Sendai virus. In previous studies, ultraviolet-inactivated Sendai virus strain Tianjin (UV-Tianjin) demonstrated antitumor effects on human breast cancer cells. The aim of the present study was to investigate the *in vitro* antitumor effects of UV-Tianjin on the human cervical carcinoma HeLa, human small cell lung cancer NCI-H446 and human hepatocellular carcinoma Hep 3B cell lines, and the possible underlying mechanisms of these antitumor effects. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay revealed that UV-Tianjin treatment inhibited the proliferation of HeLa, NCI-H446 and Hep 3B cells in a dose- and time-dependent manner. Hoechst and Annexin V-fluorescein isothiocyanate/propidium iodide double staining indicated that UV-Tianjin induced dose-dependent apoptosis in all three cell lines with the most significant effect observed in the HeLa cell line. In the HeLa cell line, UV-Tianjin-induced apoptosis was further confirmed by the disruption of the mitochondria membrane potential and the activation of caspases, as demonstrated by fluorescent cationic dye and colorimetric assays, respectively. In addition, western blot analysis revealed that UV-Tianjin treatment resulted in significant upregulation of cytochrome *c*, apoptosis protease activating factor-1, Fas, Fas ligand and Fas-associated protein with death domain, and activated caspase-9, -8 and -3 in HeLa cells. Based on these results, it is hypothesized that UV-Tianjin exhibits anticancer activity in HeLa, NCI-H446 and Hep 3B cell lines via the induction of apoptosis. In conclusion, the results of the present study indicate that in the HeLa cell line, intrinsic and extrinsic apoptotic pathways may be involved in UV-Tianjin-induced apoptosis.

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Introduction

Cervical cancer is the fourth most common cause of cancer and the fourth most common cause of cancer-associated mortality in women, worldwide (1). In 2012, there was ~528,000 novel cases of cervical cancer diagnosed, and 266,000 mortalities (1). In total, ~70% of cervical cancers are identified in developing countries (1). Lung cancer is the most common cause of cancer-associated mortality in men and women worldwide, accounting for 1.56 million mortalities in 2012 (2). Primary liver cancer is the sixth most common type of cancer and the second most common cause of cancer-associated mortality, worldwide (2); in 2012, there were 782,000 novel cases diagnosed and 746,000 mortalities (2). Hepatocellular carcinoma is the most common type of liver cancer, accounting for ~80% of all types of primary liver cancer (3). Although conventional cancer therapies, such as surgery, chemotherapy and radiotherapy, are used for the treatment of the majority of solid tumors, successful therapeutic outcomes are often limited, due to side effects associated with treatment, drug toxicity and the development of multidrug resistance (4,5). Therefore, novel therapeutic strategies with improved treatment efficacies and decreased toxicity with no adverse effects are urgently required.

Recently, the use of oncolytic viruses in the treatment of cancer has progressed significantly (6-8). Oncolytic viruses selectively kill tumor cells by replicating in tumor cells exclusively. In 1999, the Sendai virus strain Tianjin was isolated from the lungs of a marmoset (9). Tianjin is a novel genotype of the Sendai virus, which has been demonstrated to possess oncolytic activities in various types of tumor cell (10-13). In a previous study by the present authors, ultraviolet-inactivated Sendai virus strain Tianjin (UV-Tianjin) particles were prepared, and it was demonstrated that they inhibited the growth of murine colon carcinoma cells via the induction of the immune response and apoptosis in mice (14). However, the underlying molecular mechanisms by which Sendai virus or UV-Tianjin induced anticancer effects remain unclear.

Clinical and experimental studies have demonstrated that anticancer effects are mediated via various mechanisms, such as alteration of carcinogen metabolism, induction of DNA repair systems, activation of immune responses, suppression of cell cycle progression and promotion of apoptosis (15-17).

Apoptosis may be induced by various chemotherapeutic agents, which are important for the prevention and treatment of cancer (18-20). The aim of the present study was to investigate the anticancer and proapoptotic effects of UV-Tianjin against the human cervical cancer HeLa, human small cell lung cancer NCI-H446 and human hepatocellular carcinoma Hep 3B cell lines *in vitro*, and to analyze the possible mechanisms underlying apoptosis induced by UV-Tianjin. The present study also aimed to provide novel insights concerning the antitumor mechanisms of the Sendai virus.

Materials and methods

Reagents. RPMI-1640 medium, Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Hoechst 33342 and caspase-3, -8 and -9 Colorimetric Assays were purchased from KeyGen Biotech Co., Ltd. (Nanjing, China). Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) Apoptosis kit was obtained from Biovision, Inc. (Milpitas, CA, USA). JC-10 Mitochondrial Membrane Potential (MMP) Assay kit was purchased from Fanbo Science & Technology Co., Ltd. (Beijing, China). Monoclonal rabbit anti-caspase-3 (dilution, 1:500; catalog no., 9665), monoclonal mouse anti-caspase-8 (dilution, 1:500; catalog no., 9746), polyclonal rabbit anti-caspase-9 (dilution, 1:500; catalog no., 9502) and monoclonal rabbit anti-apoptosis protease activating factor-1 (Apaf-1; dilution, 1:500; catalog no., 8723) antibodies were obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA). Polyclonal rabbit anti-cytochrome *c* (cyt *c*; dilution, 1:500; catalog no., sc-7159), polyclonal rabbit anti-Fas (dilution, 1:200; catalog no., sc-715), polyclonal rabbit anti-Fas ligand (FasL; dilution, 1:500; catalog no., sc-6237), polyclonal rabbit anti-Fas-associated protein with death domain (FADD; dilution, 1:500; catalog no., sc-5559), monoclonal mouse anti-β-actin (dilution, 1:1,000; catalog no., sc-47778), goat anti-mouse immunoglobin (Ig)G-horseradish peroxidase (HRP; dilution, 1:2,000; catalog no., sc-2005) and goat anti-rabbit IgG-HRP (dilution, 1:5,000; catalog no., sc-2004) antibodies were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA).

Cell and virus cultivation. HeLa cells were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and cultured in RPMI 1640 medium supplemented with 10% FBS at 37˚C in a humidified atmosphere of 5% CO_2. NCI-H446 and Hep 3B cells were kindly provided by the General Hospital of Tianjin Medical University (Tianjin, China) and cultured in DMEM containing 10% FBS at 37˚C in a humidified atmosphere of 5% CO_2. Sendai virus strain Tianjin particles (GenBank: EF679198) were cultured in chorioallantoic fluid obtained from 9-11 day-old chicken eggs. After incubation for 72 h, the allantoic fluid was carefully collected and then centrifuged (5810R; Eppendorf, Hamburg, Germany) at 1,000 x g for 10 min at 4˚C to pellet debris. The virus-containing supernatant was purified by discontinuous sucrose gradient centrifugation and inactivated by UV irradiation (99 mJ/cm^2), as previously described (21). Inactivated viruses cannot replicate, but retain cell fusion activity.

Cell viability assay. A total of 5x10^3 cells/well (HeLa, NCI-H446 and Hep 3B) were seeded onto 96-well plates for 24 h. After incubation with various doses of UV-Tianjin [multiplicity of infection (MOI), 0, 10, 20, 40, 80, 200 and 400] for 24 or 48 h, MTT solution (5 mg/ml) was added to each well and incubated for 4 h at 37˚C. The medium containing MTT was aspirated and treated with 100 μl dimethyl sulfoxide. Absorbance at a wavelength of 570 nm was measured using a microplate reader.

Hoechst staining. HeLa, NCI-H446 or Hep 3B cells (2x10^5 cells per well) were seeded onto 6-well plates. Following treatment with UV-Tianjin (MOI, 40 or 80) for 24 h, the cells were washed twice with phosphate-buffered saline (PBS) and incubated with 250 μl Hoechst 33342 for 20 min in the dark. After rinsing with PBS, the cells were examined using a fluorescence microscope (E600; Nikon Corporation, Tokyo, Japan). Cells with nuclei that exhibited characteristic bright blue fluorescence, due to condensed or fragmented chromatin, were considered to be apoptotic.

Annexin V-FITC/PI staining. The externalization of phosphatidylserine was determined by flow cytometric analysis of cell staining using an Annexin V-FITC conjugate and PI according to the manufacturer's protocol. The cells (2x10^5 cells/well) were seeded in 6-well plates, cultured for 24 h and subsequently treated with UV-Tianjin (MOI, 20, 40 or 80) for an additional 24 h. The cells were then trypsinized (Thermo Fisher Scientific, Inc.), washed twice with PBS, centrifuged at 400 x g for 5 min to discard the supernatant, and resuspended in 500 μl binding buffer (FITC/PI Apoptosis kit; Biovision, Inc.). Subsequently, Annexin V-FITC (5 μl) and PI (5 μl) were added to the suspension and incubated for 10 min at room temperature in the dark. The cells were evaluated by flow cytometry (FACSVerse; BD Biosciences, San Jose, CA, USA). Data were analyzed using CellQuest™ software version 5.1 (BD Biosciences) and the results were expressed as percentages of the cell populations.

Caspase activity assay. Caspase activity was measured using caspases-3, -8 and -9 colorimetric activity assay kits in HeLa cells. Following incubation with UV-Tianjin (MOI, 20, 40 or 80) for 24 h, HeLa cells were harvested, lysed with cell extraction buffer (KeyGen Biotech Co., Ltd.) and phenylmethylsulfonyl fluoride (KeyGen Biotech Co., Ltd.), incubated on ice for 30 min and vortexed for 30 sec. The cell lysates were centrifuged at 12,000 x g for 10 min at 4˚C and the caspase-3, -8 or -9 levels in the supernatant were measured using the caspase-specific colorimetric kits, according to the manufacturer's protocol. The optical density was measured using a microplate reader (BioTek Instruments, Inc.) at a wavelength of 405 nm.

Measurement of MMP. The fluorescent cationic dye, JC-10, was used to determine the MMP in HeLa cells. JC-10 is capable of selectively accessing the mitochondrial membrane, and exhibits a reversible color change from green to orange as the membrane potential increases. When MMP is relatively

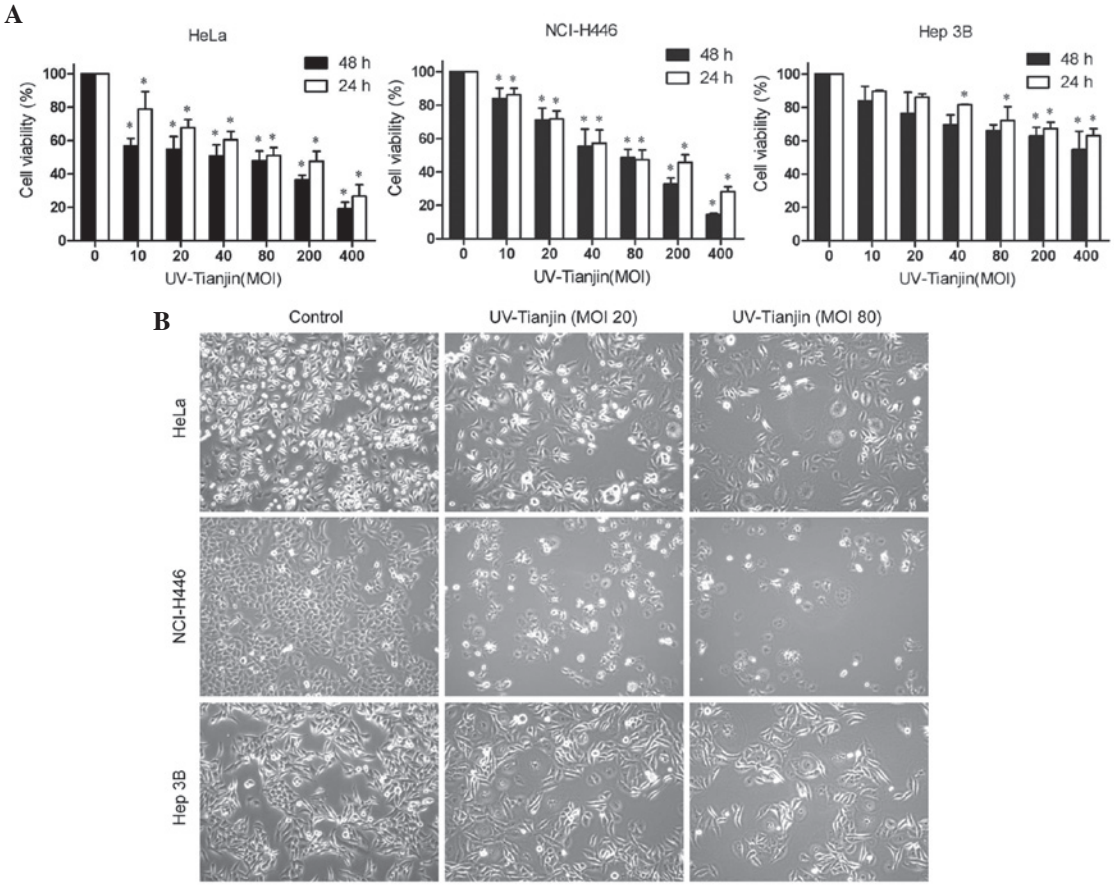

Figure 1. UV-Tianjin treatment exhibits antiproliferative effects in human cervical carcinoma HeLa, human small cell lung cancer NCI-H446 and human hepatocellular carcinoma Hep 3B cells. (A) HeLa, NCI-H446 and Hep 3B cells were treated with various doses of UV-Tianjin (MOI, 0, 10, 20, 40, 80, 200 and 400) for 24 h or 48 h and cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Data are presented as the mean ± standard deviation of three experiments performed in quadruplicate. *P<0.05 vs. untreated control cells. (B) Representative photographs of HeLa, NCI-H446, and Hep 3B cells under a fluorescence microscope after UV-Tianjin (MOI, 40 and 80) treatment for 24 h (magnification, x100). UV-Tianjin, ultraviolet-inactivated Sendai virus strain Tianjin; MOI, multiplicity of infection.

low, JC-10 dye forms monomers and emits green fluorescence (wavelength, 525-530 nm). By contrast, when MMP is high, JC-10 aggregates and emits orange fluorescence (wavelength, 590 nm) (22). The ratio between green and orange fluorescence provides an estimate of MMP that is independent of the mitochondrial mass. Briefly, after 24 h of treatment with UV-Tianjin (MOI, 20, 40 or 80), HeLa cells were harvested, washed and incubated with JC-10 (5 μM) for 30 min at 37˚C in the dark. Subsequently, the cells were analyzed by flow cytometry, which was performed using the same method as described for Annexin V-FITC/PI staining.

Western blot analysis. HeLa cells ($2x10^5$ cells/well) were seeded onto 6-well plates. After 24 h, the cells were treated with UV-Tianjin (MOI, 20, 40 or 80) and incubated for an additional 24 h. Subsequently, the cells were washed in PBS, scraped from the well and centrifuged at 400 x g for 5 min to discard the supernatant. Cell lysates were obtained by homogenizing the cell pellets in lysis buffer (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). Protein concentrations were determined using a bicinchoninic acid protein assay kit (Beijing Solarbio Science & Technology Co., Ltd.), with bovine serum albumin (Beijing Solarbio Science & Technology Co., Ltd.) as the standard. Protein (40 μg) was separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% milk for 1 h at room temperature and were then washed 3 times (5 min each wash) in TBST [Tris-buffered saline with Tween-20: 20 mM Tris-HCl (pH 7.6), 150 mM NaCl and 0.05% Tween-20]. The membranes were then incubated with primary antibodies against cyt *c*, Apaf-1, caspase-3, -8 and -9, pro-caspase-3, -8 and -9, Fas, FasL, FADD, β-actin at 4˚C overnight and washed 3 times for 5 min each in TBST. The membranes were then incubated with anti-mouse IgG HRP-linked antibody or anti-rabbit IgG HRP-linked antibody for 1 h at room temperature and washed 3 times for 5 min each in TBST, and the protein levels were measured using a chemiluminescence kit (Beijing Solarbio Science & Technology Co., Ltd.). Protein signals were analyzed by ImageJ version 1.43 software (National Institutes of Health, Bethesda, MA, USA). β-actin served as the internal control.

Statistical analysis. Each experiment was replicated at least three times. Data are expressed as the mean ± standard deviation. Analysis of variance was used to analyze differences between the groups. SPSS software version 18.0 (SPSS, Chicago, IL, USA) was used to perform all statistical analyses, and P<0.05 was considered to indicate a statistically significant difference.

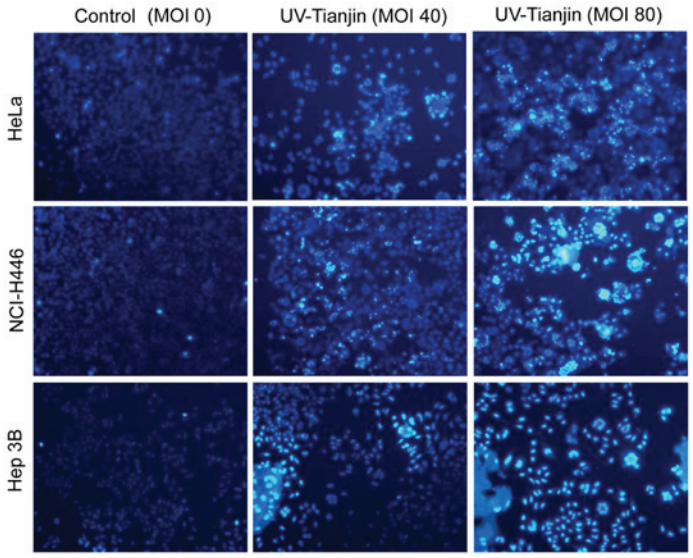

Figure 2. Apoptotic morphological changes in human cervical carcinoma HeLa, human small cell lung cancer NCI-H446 and human hepatocellular carcinoma Hep 3B cells following treatment with UV-Tianjin (MOI, 0, 40 and 80). Following UV-Tianjin treatment for 24 h, the cells were stained with Hoechst 33342 and observed using a fluorescence microscope (magnification, x100). Light blue fluorescence indicates apoptotic cells. UV-Tianjin, ultraviolet-inactivated Sendai virus strain Tianjin; MOI, multiplicity of infection.

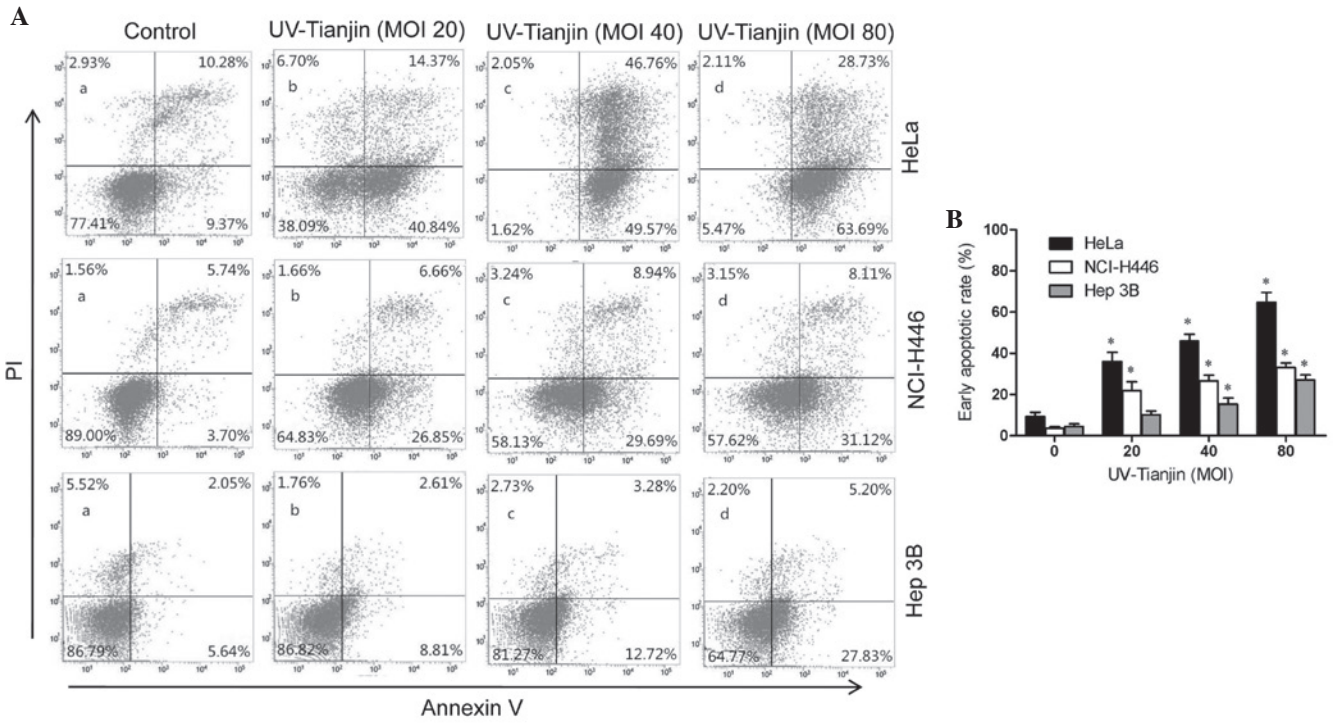

Figure 3. Annexin V-FITC/PI staining of human cervical carcinoma HeLa, human small cell lung cancer NCI-H446 and human hepatocellular carcinoma Hep 3B cells following treatment with UV-Tianjin (MOI, 0, 20,40 and 80). (A) Following treatment with UV-Tianjin (MOI, 0, 20, 40 and 80) for 24 h, HeLa, NCI-H446 and Hep 3B cells were stained with Annexin V-FITC/PI and analyzed by flow cytometry. The lower right quadrant (Annexin V^+/PI^-) represents early apoptosis, whereas the upper right quadrant (Annexin V^+/PI^+) represents late apoptosis and necrosis. (B) Early apoptotic rate (%) of HeLa, NCI-H446 and Hep 3B cells as determined by flow cytometry. Data are expressed as the mean ± standard deviation of three independent experiments. $^*P<0.01$ vs. untreated control cells. FITC, fluorescein isothiocyanate; PI, propidium iodide; UV-Tianjin, ultraviolet-inactivated Sendai virus strain Tianjin; MOI, multiplicity of infection.

Results

UV-Tianjin inhibits the proliferation of HeLa, NCI-H446 and Hep 3B cells. The cell lines HeLa, NCI-H446 and Hep 3B were cultured with UV-Tianjin (MOI, 10, 20, 40, 80, 200 and 400) for 24 h or 48 h, and cell proliferation was assessed by MTT assay. As shown in Fig. 1A, UV-Tianjin inhibited the proliferation of all three cell lines in a dose- and time-dependent manner. The results also demonstrated that the HeLa and NCI-H446 cell lines were more sensitive to UV-Tianjin treatment compared with the Hep 3B cell line. Notably, the HeLa and NCI-H446 cell lined exhibited almost identical proliferation characteristics.

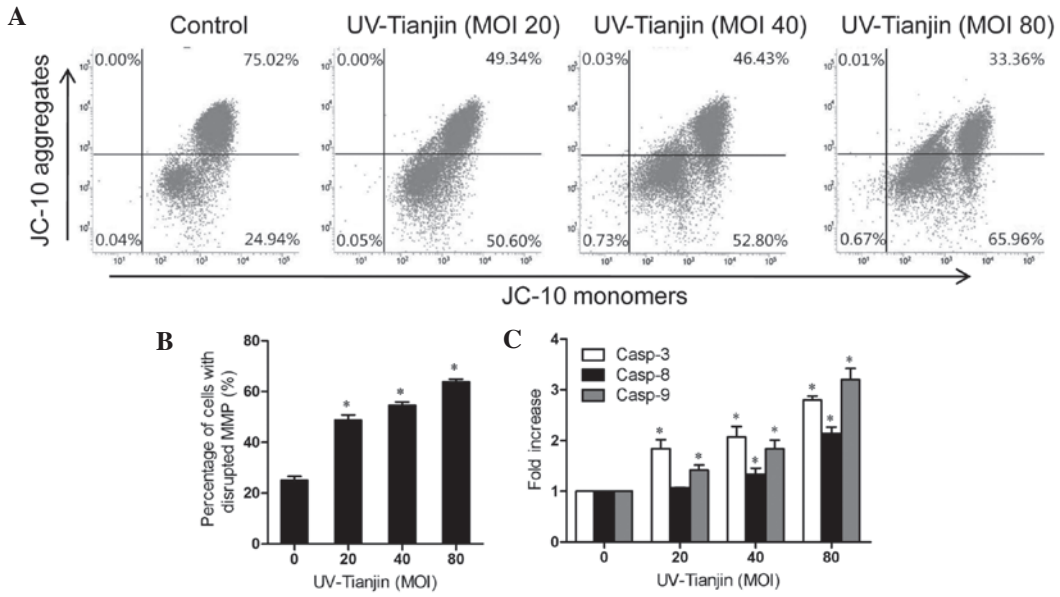

Figure 4. Effects of UV-Tianjin on MMP and casp activity in human cervical carcinoma HeLa cells. (A) MMP was decreased in UV-Tianjin-treated HeLa cells. Cells were treated with various doses of UV-Tianjin (MOI, 0, 20, 40 and 80) for 24 h and stained with JC-10. Fluorescence was measured by flow cytometry. (B) Histograms show the percentage of cells with a depolarized mitochondrial membrane. (C) HeLa cells were treated with UV-Tianjin (MOI, 20, 40, 80) for 24 h and analyzed by casp-3, -8, and -9 activity assays. Results are expressed as the fold increase in casp activity of apoptotic cells compared to that of non-treated cells. Data are expressed as the mean ± standard deviation of three experiments. *P<0.05 vs. untreated control cells. UV-Tianjin, ultraviolet-inactivated Sendai virus strain Tianjin; MOI, multiplicity of infection; MMP, mitochondrial membrane potential; casp, caspase.

Microscopic examination confirmed the results obtained from the MTT assay (Fig. 1B).

UV-Tianjin induces apoptosis of HeLa, NCI-H446 and Hep 3B cells. To examine whether the anti-proliferative effects of UV-Tianjin are due to the induction of apoptosis, the cell lines HeLa, NCI-H446 and Hep 3B were stained with Hoechst 33342 dye and observed under a fluorescence microscope. Compared with the untreated groups, a higher percentage of apoptotic cells were observed in the UV-Tianjin-treated groups (Fig. 2). Furthermore, a dose-dependent increase in apoptosis was observed in all three cell lines.

Subsequently, the number of apoptotic cells was estimated using flow cytometry. As shown in Fig. 3A and B, for HeLa cells, the early apoptotic cells accounted for 36.01±4.44, 45.98±3.26 and 64.73±4.82% subsequent to a 24 h incubation with UV-Tianjin at MOI 20, 40 and 80, respectively, compared with 21.88±4.36, 26.57±2.84 and 33.04±2.24% in NCI-H446 cells and 10.22±1.77, 15.31±2.97 and 27.07±2.46% in Hep 3B cells. A dose-dependent increase in early apoptotic rate was observed in all three cell lines (P<0.01; Fig. 3B). In addition, the results demonstrated that the HeLa cell line was most susceptible to UV-Tianjin treatment. Consequently, the HeLa cell line was selected for further experiments.

UV-Tianjin disrupts MMP in HeLa cells. Early apoptosis is usually accompanied by collapse of the mitochondrial membrane, which results in MMP disruption. In the present study, the effect of UV-Tianjin on MMP was analyzed using a mitochondria-specific dye, JC-10. As shown in Fig. 4A and B, flow cytometry demonstrated that the intensity of orange fluorescence was significantly decreased following UV-Tianjin treatment for 24 h, indicating MMP disruption. A total of 48.77±4.12, 54.61±2.53 and 63.79±2.20% of cells exhibited MMP disruption following treatment with UV-Tianjin at MOI, 20, 40 and 80, respectively, which suggests that UV-Tianjin treatment decreased the MMP of HeLa cells in a dose-dependent manner.

UV-Tianjin-induced apoptosis is caspase-dependent in HeLa cells. To further investigate the proapoptotic mechanism of UV-Tianjin, caspase-3, -8, and -9 activity in HeLa cells was investigated following incubation with UV-Tianjin (MOI, 20, 40 and 80) for 24 h. As shown in Fig. 4C, at a MOI of 80, caspase-3, -8 and -9 activity was increased 2.80±0.15, 2.14±0.25 and 3.20±0.44 fold, respectively, compared with the corresponding control groups. These results indicated that UV-Tianjin-induced apoptosis may occur via a caspase-dependent pathway.

Apoptosis in HeLa cells is induced via extrinsic and intrinsic pathways by UV-Tianjin. Caspase activation is triggered by two pathways: Extrinsic and intrinsic apoptotic pathways (23). To further investigate the molecular mechanism of UV-Tianjin-induced apoptosis in HeLa cells, the expression levels of various apoptosis-associated proteins, including cyt *c*, Apaf-1, Fas, FasL, FADD, caspase-3, -8 and -9, were examined by western blot analysis. The results revealed that following UV-Tianjin treatment, the expression of the intrinsic pathway related proteins, cyt *c*, Apaf-1, active caspase-9 and -3, as well as extrinsic pathway-associated proteins, Fas, FasL, FADD and active caspase-8, were increased in a dose-dependent manner (Fig. 5). These results indicated that UV-Tianjin-induced apoptosis in HeLa cells may involve intrinsic and extrinsic apoptotic pathways.

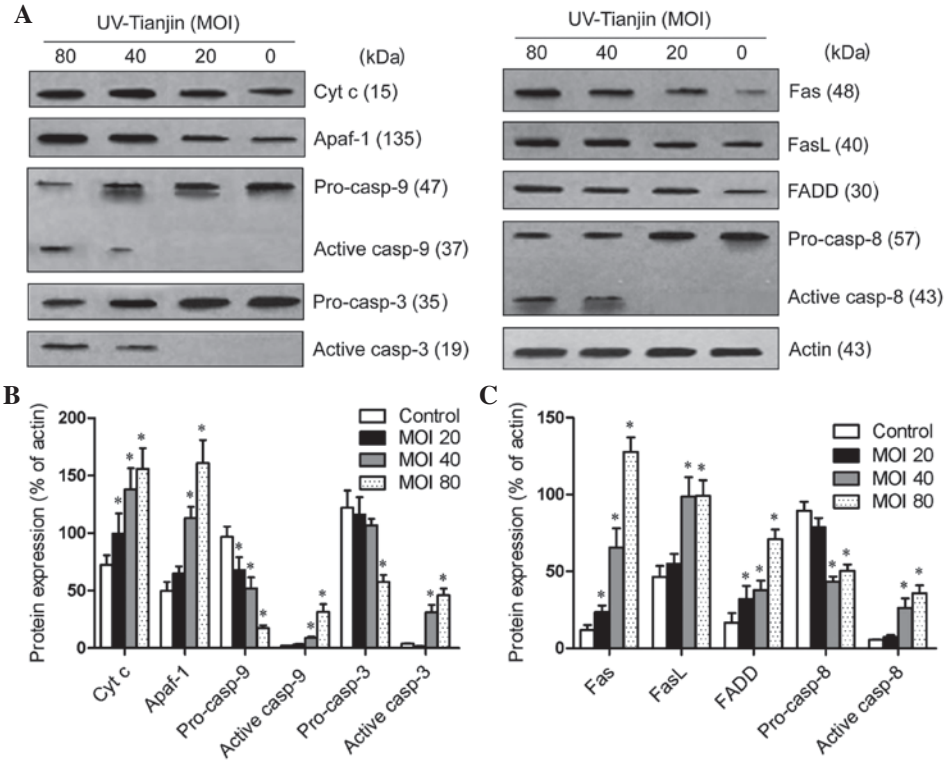

Figure 5. Effects of UV-Tianjin on the expression of apoptosis-associated proteins in human cervical carcinoma HeLa cells. (A) HeLa cells were treated with UV-Tianjin (MOI, 20, 40, 80) for 24 h and expression levels of apoptosis-associated proteins were determined by western blot analysis. (B) Relative expression levels of intrinsic pathway apoptosis-associated proteins cyt c, Apaf-1, pro-casp-3, active casp-3, pro-casp-9 and active casp-9 following UV-Tianjin treatment. (C) Relative expression levels of extrinsic pathway apoptosis-associated proteins Fas, FasL, FADD, pro-casp-8 and active casp-8 following UV-Tianjin treatment. β-actin was used as an internal control. Data are expressed as the mean ± standard deviation of three independent experiments. $^{*}P<0.05$ vs. untreated control cells. UV-Tianjin, ultraviolet-inactivated Sendai virus strain Tianjin; MOI, multiplicity of infection; cyt *c*, cytochrome *c*; Apaf-1, apoptotic protease activating factor-1; casp, caspase; FADD, Fas-associated protein with death domain; FasL, Fas ligand.

Discussion

The Sendai virus exhibits anticancer activity against a variety of tumor types (11-13,24). However, few studies have evaluated the antitumor activities of Sendai virus in human cervical cancer, human small cell lung cancer or human hepatocellular carcinoma. In the present study, the novel genotype of Sendai virus Tianjin strain inhibited the proliferation of human cervical cancer HeLa, human small cell lung cancer NCI-H446 and human hepatoma Hep 3B cells in a dose- and time-dependent manner.

Apoptosis is an important physiological process, which leads to the elimination of redundant or harmful cells during organism development (25). Apoptosis is characterized by a number of biological and morphological alterations, including changes in the MMP, activation of caspases, DNA fragmentation, membrane blebbing and formation of apoptotic bodies (26,27). In the present study, Hochest 33342 staining demonstrated that UV-Tianjin-treated HeLa, NCI-H446 and Hep 3B cells exhibited typical apoptotic morphological alterations, such as chromatin condensation and nuclear shrinkage. Furthermore, Annexin V-FITC/PI double staining revealed that UV-Tianjin induced apoptosis in a dose-dependent manner in all three cell lines and that the HeLa cell line was most susceptible to UV-Tianjin treatment. Subsequently, UV-Tianjin-induced apoptosis was further confirmed in the HeLa cell line, as shown by MMP disruption and increased caspase activity.

Caspases are expressed in the majority of cell types as inactive pro-enzymes. Increasing evidence suggests that activation of caspases initiates apoptosis in various types of cell (28,29). Within the caspase family, caspase-8 and -9 are the two main initiator caspases in extrinsic and intrinsic pathways that lead to the activation of effector caspase-3 (30,31). By contrast to effector caspases, which are activated by single intrachain cleavage that is mediated by an initiator caspase, an initiator caspase requires recruitment to a multimeric adaptor protein complex. Such multimeric protein complexes are commonly known as apoptosome and death-inducing signaling complexes; caspase-9 is activated by the Apaf-1 apoptosome and caspase-8 is activated by the adapter FADD (32-34). In apoptotic cells, cyt *c* is released from mitochondria to the cytoplasm, where it binds Apaf-1 (35,36). Subsequent replacement of adenosine diphosphate by deoxyadenosine triphosphate (dATP)/ATP in Apaf-1 leads to the formation of a heptameric apoptosome (37-39). The autocatalytic activation of the caspase-9 zymogen is catalyzed by the Apaf-1. The present study demonstrated that UV-Tianjin triggered the intrinsic apoptotic pathway by activating caspase-9 via Apaf-1 apoptosome activation and the release of cyt *c* from the mitochondria in HeLa cells. The extrinsic pathway is activated by the engagement of death receptors on the cell surface. Trimerization of the membrane-bound receptor, Fas, by its natural ligand, FasL, results in recruitment of the receptor-specific adapter protein, FADD, which subsequently recruits caspase-8 and promotes

the cascade of pro-caspase activation (40-42). The results of the present study also demonstrated that UV-Tianjin activates the extrinsic apoptotic pathway by activating caspase-8 via FADD and Fas activation in HeLa cells. However, additional studies are required to investigate the underlying molecular mechanisms of UV-Tianjin-induced apoptosis in HeLa cells. Further studies are also required to determine whether the intrinsic and extrinsic apoptotic pathways are involved in the proapoptotic activity of UV-Tianjin in NCI-H446 and Hep 3B cells.

In conclusion, the present study revealed that UV-Tianjin induces apoptosis in HeLa, NCI-H446 and Hep 3B cells, which indicates that UV-Tianjin may exhibit extensive anticancer activity in human cancer cells. Furthermore, UV-Tianjin-induced apoptosis in HeLa cells involves extrinsic and intrinsic apoptotic pathways. Therefore, UV-Tianjin may present a potential biological therapeutic agent for the treatment of human cancers.

Acknowledgements

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