

Na₇CrCuW₁₁O₃₉·16H₂O induces apoptosis in human ovarian cancer SKOV3 cells through the p38 signaling pathway

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Received June 18, 2015; Accepted September 9, 2016

DOI: 10.3892/ol.2017.5719

Abstract. Ovarian carcinoma is a common malignant disease worldwide with a poor therapeutic response. The present study investigated the effects of Na₇CrCuW₁₁O₃₉·16H₂O (CrCuW₁₁) on ovarian cancer cell growth and investigated the mechanisms underlying its actions. The effects of CrCuW₁₁ on cell viability and apoptosis were measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, acridine orange/ethidium bromide staining and electron microscopy in human ovarian cancer SKOV3 cells. The expression of bcl-2-like protein 4 (Bax), B-cell lymphoma 2 (Bcl-2), cytochrome *c*, phosphorylated (p)-p38 and p38 was determined by western blot analysis. Caspase-3 activity was measured by caspase-3 activity kit. CrCuW₁₁ concentrations of 1.87x10⁻³ mol. l⁻¹ at 12 h reduced viability induced apoptosis in SKOV3 cells in a concentration- and time-dependent manner. Forced expression of CrCuW₁₁ upregulated the expression of certain proteins (Bax, cytochrome *c*, and p-p38), and down-regulated Bcl-2 protein expression. Furthermore, CrCuW₁₁ also enhanced caspase-3 activity. The p38 inhibitor SB203580 was able to inhibit the activity of CrCuW₁₁. Caspase-3 and p38 signaling pathways were associated with CrCuW₁₁-regulated multiple targets involved in SKOV3 cell proliferation. Therefore, the results of the present study indicated that CrCuW₁₁

may be used as a novel clinical drug for the treatment of ovarian cancer.

Introduction

Ovarian cancer is the leading cause of mortality from gynecological malignancies in women worldwide, causing >140,000 deaths annually (1). Each year, it is estimated that ~22,000 women will be newly diagnosed and 14,200 will succumb to disease in the USA (2). The poor overall survival is due to late-stage diagnosis and resistance to chemotherapy (3). The disease course for ovarian cancer may be complicated. Initial treatments often include surgery and chemotherapy, which may cause significant physical, psychological and social side effects (4). Ovarian cancer has a higher mortality rate in females than all other gynecological cancers combined (5). Therefore, there is an urgent requirement for a novel drug that is able to enhance the efficiency of therapy for the treatment of ovarian cancer patients.

Since 1970, polyoxometalate has been widely used for analytical chemistry, medicine, catalysis and material science (6). A number of polyoxometalates were observed to be potential antitumor, antiviral and antibacterial drugs (7-9). Initially, 'silicotungstic acid supernatants' were reported to be potent inhibitors of murine leukemia and sarcoma viruses (10). Since then, many polyoxometalates have been observed to possess potential antitumor, antiviral and antibacterial ability (8). They were observed to exhibit inhibition effects on tumor cells, including B16 melanoma, HeLa, HepG-2 and PAMC 82 cells (11). Furthermore, polyoxometalate may induce apoptosis at a low concentration in HEK293 cells (9). Therefore, it is worthy to consider the introduction of transition metal elements to polyoxometalate in order to improve its antitumor activity.

In a preliminary experiment by the present authors, CrCuW₁₁ was a polyoxometalate containing the transition metal chromium. However, CrCuW₁₁ has not been reported to induce apoptosis in any human cancer cells to the best of our knowledge. In the present study, it was demonstrated that CrCuW₁₁ was able to induce the apoptosis of SKOV3 cells.

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Key words: polyoxometalate, transition metal element, chromium, apoptosis, SKOV3, ovarian cancer

Treatment with CrCuW_{11} significantly decreased viability and induced apoptosis in SKOV3 cells, due to significant activation of caspase-3 and p38-dependent signaling pathways.

Materials and methods

Reagents. Antibodies for bcl-2-like protein 4 (Bax; catalog no., D120073) and B-cell lymphoma 2 (Bcl-2; catalog no., D160117) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Cytochrome *c* (catalog no., 11940), p38 (catalog no., 8690) and phosphorylated (p)-p38 (catalog no., 4511) antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). p38 inhibitor SB203580 was purchased from Beyotime Institute of Biotechnology (Haimen, China). Other chemicals [including $\text{Na}_2\text{WO}_4\cdot 2\text{H}_2\text{O}$, $\text{Cu}(\text{NO}_3)_2$, $\text{Cr}(\text{NO}_3)_3$ and glacial acetic acid] were purchased from Sigma-Aldrich (EMD Millipore, Billerica, MA, USA).

Synthesis of compounds. $\text{Na}_4\text{CrCuW}_{11}\text{O}_{39}\cdot 16\text{H}_2\text{O}$ plasmid was synthesized by the Department of Inorganic Chemistry and Physical Chemistry, Harbin Medical University (Harbin, China). To a solution of $\text{Na}_2\text{WO}_4\cdot 2\text{H}_2\text{O}$ (36.3 g) in water, 200 ml glacial acetic acid was added and heated to boiling. Then a solution of $\text{Cu}(\text{NO}_3)_2\cdot 3\text{H}_2\text{O}$ (2.4 g, 0.01 M) in water (50 ml) was added dropwise to the stirred solution above. The reaction mixture was stirred for 30 min. Then $\text{Cr}(\text{NO}_3)_3$ (4.15 g, 0.01 mol) in 50 ml of water was added dropwise. The solution pH of 5 was adjusted by adding glacial acetic acid. The mixture was stirred for a further 1.5 h, then cooled and filtered. The resulting mixture was added to dehydrated alcohol to produce a dark green oil. The oily crude product was frozen and purified by a dissolved-frozen method performed 2-3 times. The product was then dried in a vacuum dryer to produce dark green crystals.

SKOV3 cell culture and drug chemical synthesis. SKOV3, a human ovarian cancer cell line, was provided by the Key Laboratory for Reproductive Medicine of Guangdong Province (Guangzhou, China) and cultured in RPMI-1640 medium (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% (v/v) heat-inactivated fetal bovine serum (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C in an incubator containing humidified air with 5% (v/v) CO_2 .

Cell viability assay. Cells were seeded onto 96-well plates at a density of 8×10^3 cells per well 24 h prior to treatment. The cells were treated with CrCuW_{11} at the following concentrations: 1.17×10^{-4} , 3.69×10^{-4} , 1.02×10^{-3} , 1.87×10^{-3} or 3.52×10^{-3} mol. l^{-1} . Following 12 h of incubation at 22–26°C, 15 μl (5 mg/ml) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (Sigma-Aldrich; EMD Millipore) was added to each well, and incubated at 37°C for 4 h. Subsequently, the MTT solution was removed and 150 μl of dimethyl sulfoxide was added to dissolve the crystals. The mixtures were shaken for 10 min to fully dissolve the crystals. A microplate reader (Tecan, Männedorf, Switzerland) was used to measure the absorbance at a wavelength of 570 nm. Cell viability was expressed as the percentage change of the absorbance values in the treatment group over the control group.

Electron microscopy. SKOV3 cells were cultured in 60 mm plates, suspended in PBS solution and fixed with 2% (v/v) paraformaldehyde containing 2.5% (w/v) glutaraldehyde (Paesel & Lorei GmbH & Co. KG, Duisberg, Germany) buffered in Hank's modified salt solution at 4°C for 4 h. The cells were further fixed in 1% (w/v) OsO_4 solution buffered by 0.1 M cacodylate (pH 7.2) at 4°C for 2 h, and dehydrated in ethanol. Dehydration was performed in propylene oxide. The specimens were embedded in Araldite® (SERVA Electrophoresis GmbH, Heidelberg, Germany). Ultrathin sections (2–6 μm) were produced on an FCR Reichert Ultracut ultramicrotome (Leica Microsystems, Inc., Buffalo Grove, IL, USA), mounted on pioloform-coated copper grids and contrasted with lead citrate. Specimens were analyzed and documented with a 10A transmission electron microscope (Zeiss AG, Oberkochen, Germany).

Acridine orange/ethidium bromide (AO/EB) staining. SKOV3 cells in the exponential growth phase were cultivated on sterile coverslips for 24 h at room temperature, and were subsequently treated with CrCuW_{11} at concentrations of 1.17×10^{-4} , 3.69×10^{-4} , 1.02×10^{-3} , 1.87×10^{-3} or 3.52×10^{-3} mol. l^{-1} for 12 h at 37°C. The cells were washed twice with PBS, and then mixed with 1 ml of dye mixture containing 100 mg/ml AO and 100 mg/ml EB in PBS (12). Cellular morphological changes were examined using fluorescence microscopy (magnification, $\times 200$). The percentage of apoptotic cells was calculated by the following formula: Apoptotic rate (%) = number of apoptotic cells/total number of cells counted (13,14).

Western blotting analysis. Total protein samples were extracted from SKOV3 cells. Cells cultured on 25 mm dishes or 6-well plates were lysed in lysis buffer (protease and phosphatase inhibitors; Beyotime Institute of Biotechnology). Following centrifugation at $13,500\times g$ for 15 min at 4°C, the lysates were collected. Protein concentration was assessed using the bicinchoninic acid protein assay. The aliquots were then mixed with Laemmli sample buffer and boiled at 100°C for 5 min. The protein samples (80 μg) were resolved by 10–12% SDS-PAGE, followed by transferal to nitrocellulose membranes and blocking with 5% (w/v) non-fat dried skimmed milk powder in PBST. For visualization, blots were probed with antibodies against Bcl-2 (1:500 dilution), Bax (1:500 dilution), cytochrome *c* (1:500 dilution), p38 (1:1,000 dilution), p-p38 (1:1,000 dilution) and GAPDH (1:1,000 dilution) at room temperature (21–23°C) for 1 h. Membranes were subsequently incubated with horseradish peroxidase-conjugated secondary antibodies (cat. nos. 5257, 5151; 1:10,000 dilution; Cell Signaling Technology, Inc.). The obtained digital images of western blotting results were then used for densitometry measurements (Gel-Pro analyzer version 4.0; Media Cybernetics, Inc., Rockville, MD, USA). Western blotting bands were quantified using Odyssey version 3.0 software (LI-COR Biosciences, Lincoln, NE, USA) by measuring the band intensity (area \times absorbance) for each group, and normalized to GAPDH bands as an internal control.

Caspase-3 activity assay. Caspase-3 activity was analyzed using a caspase-3 activity assay kit (Beyotime Institute of Biotechnology) according to the manufacturer's protocol,

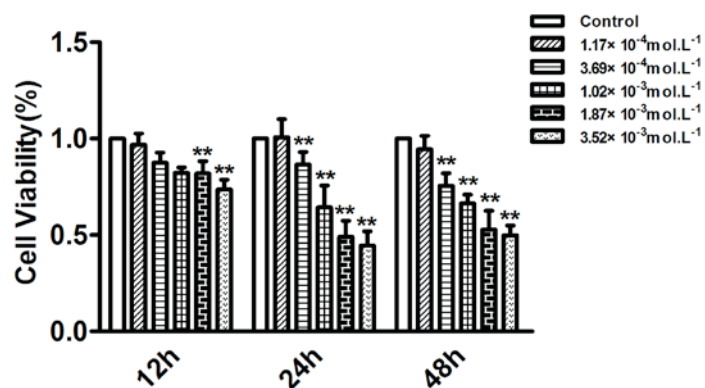


Figure 1. CrCuW₁₁ inhibits survival of SKOV3 cells. SKOV3 cells were treated with CrCuW₁₁ at concentrations of 1.17×10^{-4} , 3.69×10^{-4} , 1.02×10^{-3} , 1.87×10^{-3} and 3.52×10^{-3} mol.L $^{-1}$, for 6, 12 or 24 h. Relative cell viability was determined by MTT assay. **P<0.05 compared with Ctrl; n=6 independent experiments for each condition. Data are presented as the mean \pm standard deviation. Ctrl, control.

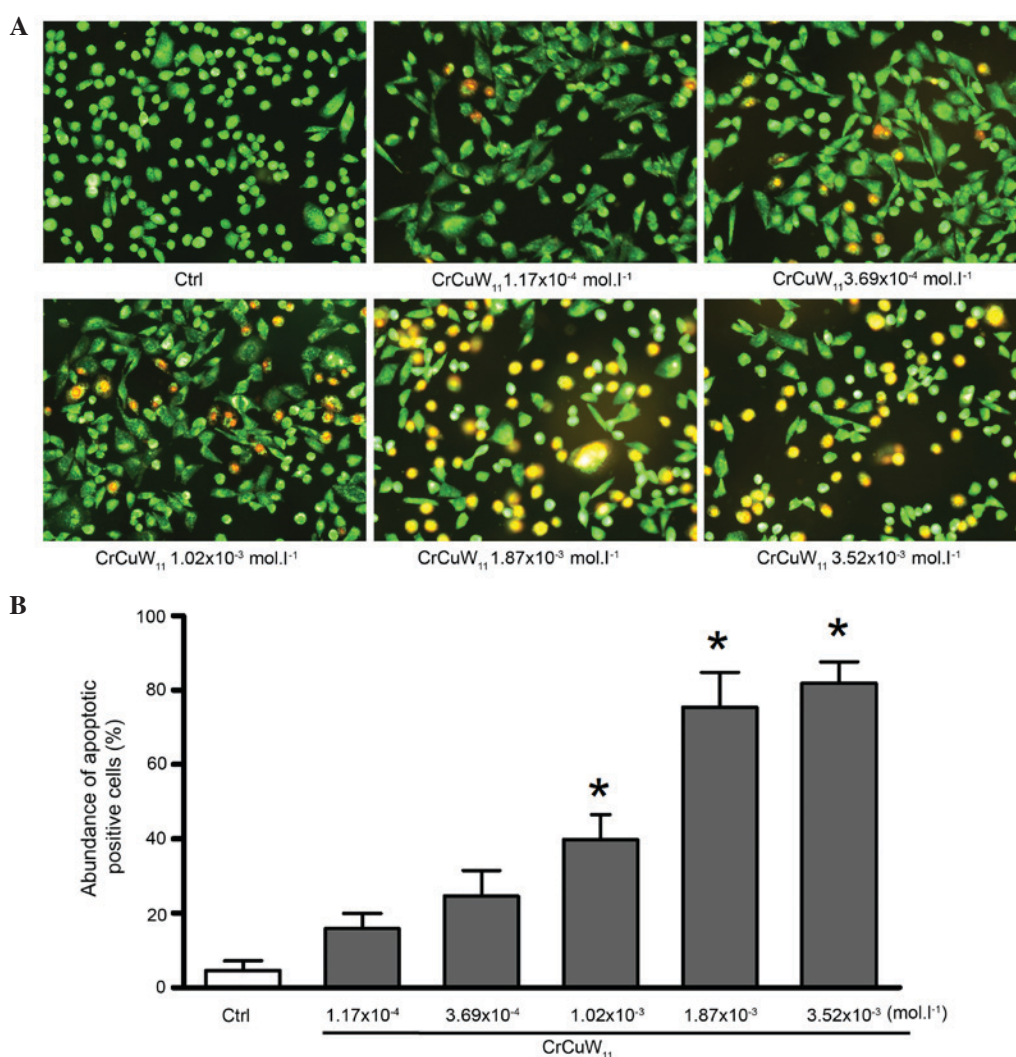


Figure 2. CrCuW₁₁ induces apoptosis in SKOV3 cells. (A) AO/EB staining to detect changes in the nucleus. (B) Bar graph of apoptotic cells observed by AO/EB staining. *P<0.05 compared with Ctrl. n=3 independent experiments for each group. Data are presented as the mean \pm standard deviation. AO, acridine orange; EB, ethidium bromide; Ctrl, control.

using substrate peptides Ac-DEVD-pNA (*p*-nitroanilide), Ac-IETD-pNA and Ac-LEHD-pNA. Briefly, the supernatant of cell lysate was mixed with buffer containing the substrate

peptides for caspase attached to peptide nucleic acid (pNA). The release of pNA was quantified by determining the absorbance with an ELISA reader at 405 nm. The caspase activities

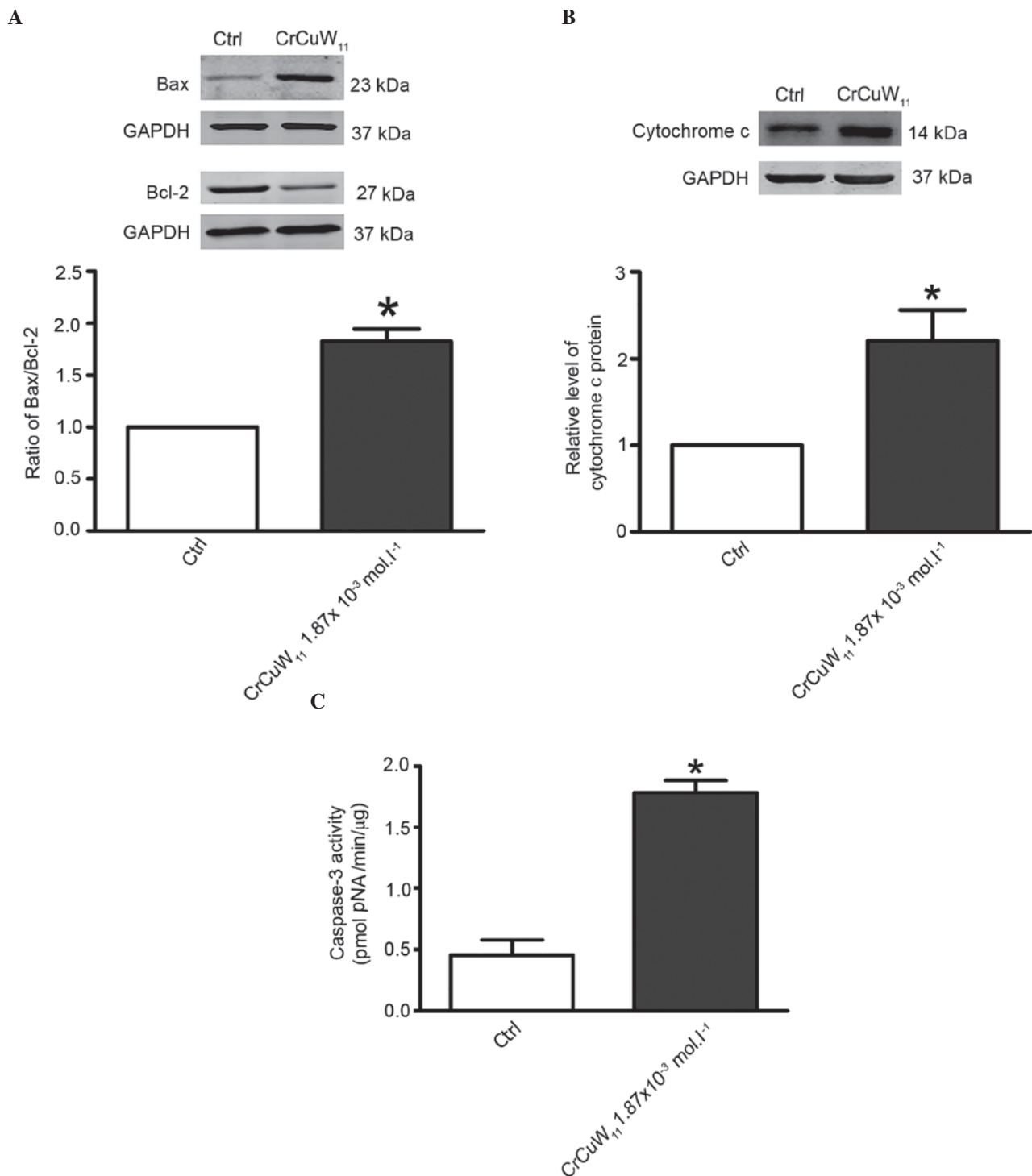


Figure 3. CrCuW₁₁ alters Bax, Bcl-2 and cytochrome *c* expression, and promotes caspase-3 activation. Western blotting was used to detect (A) Bax, Bcl-2 and (B) cytochrome *c* expression in SKOV3 cells treated with CrCuW₁₁. Relative expression of Bax, Bcl-2 and cytochrome *c* was normalized to GAPDH. n=3 independent experiments for each group. (C) Activation of caspase-3 by CrCuW₁₁. Data are averaged from five independent experiments for each group. Similar results were observed in an additional three experiments. Data are presented as the mean ± standard deviation. *P<0.05 compared with Ctrl. Bcl-2, B-cell lymphoma 2; Bax, bcl-2-like protein 4; Ctrl, control.

were expressed as a percentage, in terms of the treatment group over the control group.

Statistical analysis. Data are presented as the mean ± standard deviation of 3-6 independent experiments and were evaluated by unpaired Student's t-test. P<0.05 was considered to indicate a statistically significant difference. Statistical analysis was

performed using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA).

Results

CrCuW₁₁ suppresses the viability of SKOV3 cells. The antiproliferative effect of CrCuW₁₁ on SKOV3 cells was

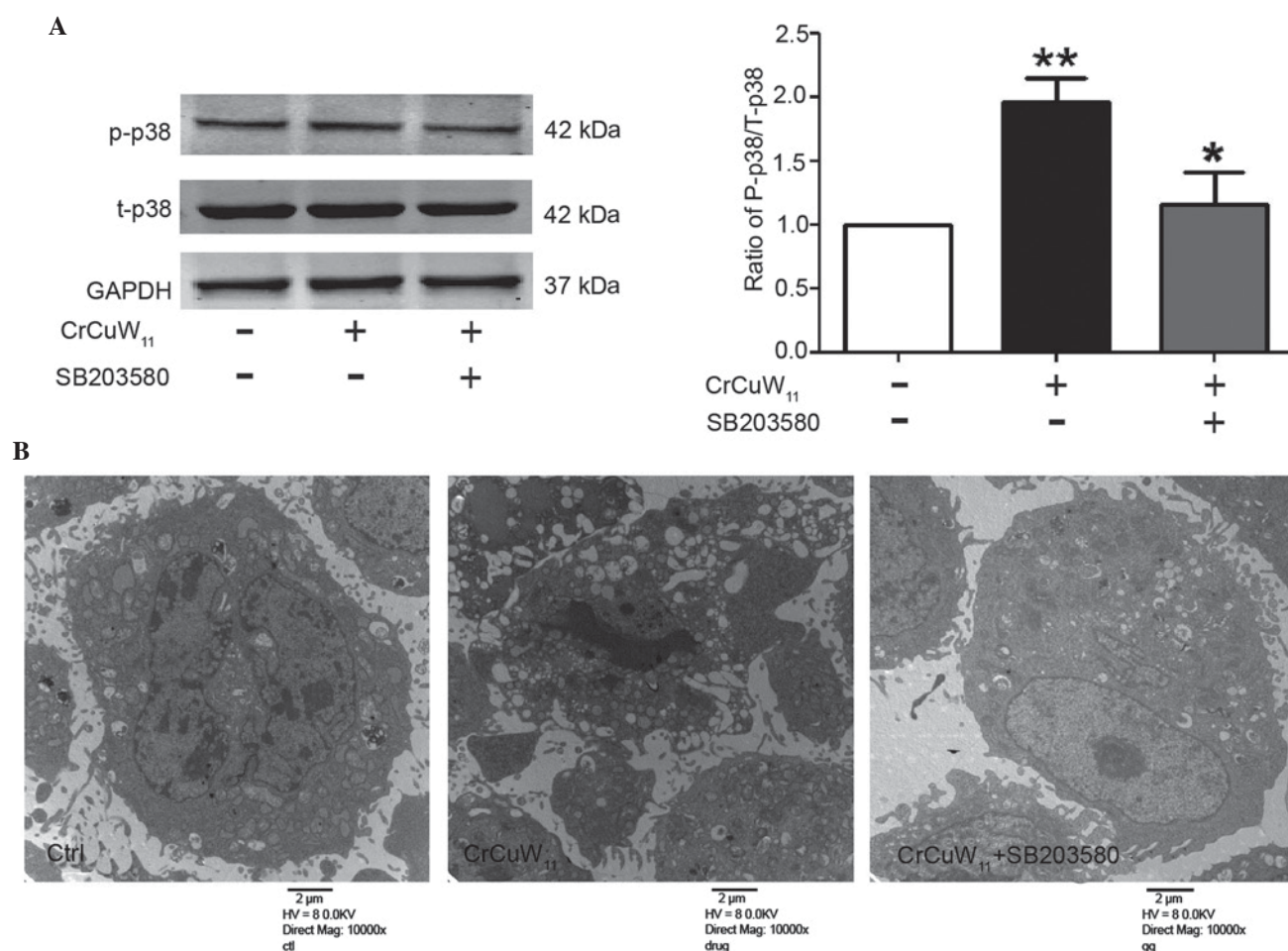


Figure 4. Expression of p-p38 and t-p38 expression and microstructure changes. (A) Western blotting was used to detect p-p38 and t-p38 expression in SKOV3 cells treated with CrCuW₁₁ and SB203580. (B) Transmission electron microscopy was used to estimate micromorphological changes (magnification, x10,000). A total of 3-6 independent experiments were performed for each group. Similar results were observed from an additional three experiments *P<0.05, **P<0.01, compared with the control. p, phosphorylated; t, total.

examined by exposing the cells to various concentrations (1.17×10^{-4} , 3.69×10^{-4} , 1.02×10^{-3} , 1.87×10^{-3} or 3.52×10^{-3} mol. l⁻¹) of CrCuW₁₁ for 6, 12 and 24 h. Cell growth was inhibited in a dose- and time-dependent manner (Fig. 1). In the presence of 1.87×10^{-3} mol. l⁻¹ CrCuW₁₁, SKOV3 cells exhibited ~50% inhibition of proliferation following treatment for 12 h. Therefore, 1.87×10^{-3} mol. l⁻¹ and 12 h were used in the subsequent experiments.

CrCuW₁₁ induces apoptosis in SKOV3 cells. Cell viability is a balance between proliferation and apoptosis (15). To investigate whether CrCuW₁₁ is able to regulate apoptosis of ovarian cancer cells, AO/EB staining was performed to detect apoptotic cells. The results from fluorescence microscopic analysis are presented in Fig. 2. A total of 3 types of cell were recognized under a fluorescence microscope: Live cells (green), apoptotic cells (yellow) and necrotic cells (red). Forced expression of CrCuW₁₁ induced substantial apoptosis (P<0.05).

CrCuW₁₁ activates pro-apoptotic signaling pathways. To investigate the mechanisms by which CrCuW₁₁ induces apoptosis in SKOV3 cells, the present study measured the levels of the downstream proteins in the CrCuW₁₁ apoptotic signaling pathway, including Bax, Bcl-2 and cytochrome c,

demonstrating that treatment with CrCuW₁₁ may upregulate Bax and cytochrome c expression, and downregulate Bcl-2 expression, thereby inducing apoptosis (Fig. 3A and B). In addition, caspase-3 activity, a marker of apoptosis, was significantly increased 2.1-fold by CrCuW₁₁ treatment (Fig. 3C).

CrCuW₁₁ activates p38 signaling pathways. To investigate the apoptotic mechanism of CrCuW₁₁, p38 was inhibited by SB203580 treatment, followed by treatment of cells with CrCuW₁₁. The expression of p38 was detected by western blotting. Cell surface microvilli reduction, nuclear chromatin condensation, margination and membrane blistering were detected by electron microscopy. As shown in Fig. 4A, without SB203580, CrCuW₁₁ was able to upregulate p-p38 expression, whereas total p38 expression exhibited no change. Following treatment with SB203580, neither p-p38 nor t-p38 expression was altered compared with the control. Under an electron microscope, the cells treated with CrCuW₁₁ also exhibited robust changes in microstructure, including cell surface microvilli reduction, nuclear chromatin condensation, margination and membrane blistering (Fig. 4B), but these changes were not observed following treatment with SB203580.

Discussion

In the present study, it was proven that CrCuW_{11} , containing the transition metal element chromium, exerted potent anti-proliferative effects on SKOV3 cells by causing apoptosis. Activation of the caspase-3-dependent signaling pathway and p38 overload were identified as important mechanisms for CrCuW_{11} -induced apoptosis. Therefore, the current study may provide a novel insight into the clinical role of CrCuW_{11} in ovarian cancer.

Ovarian cancer is a highly metastatic disease and >70% of patients are diagnosed at an advanced stage, with widespread intraperitoneal invasion and metastasis (16). It has been considered that invasion and metastasis largely contribute to the high mortality in ovarian cancer patients (17).

Previous studies have revealed that polyoxometalates are able to exert anticancer activities via regulation of invasion, proliferation and cell migration in a variety of malignancies, including breast (18), kidney (19), lung (19), ovary (20), pancreas (21) and prostate cancer (22). However, CrCuW_{11} has not been reported to induce apoptosis in any human cancer cells. It appears that whether CrCuW_{11} inhibits cell proliferation may depend upon the cell type; specific cell types have various predominant signaling pathways that lead to apoptosis (23).

Apoptosis (programmed cell death) is an important homeostatic mechanism that balances cell division and cell death, thus maintaining the appropriate cell number in the body (24). Therefore, searching for novel drugs that trigger the apoptosis of tumor cells has become an attractive strategy in anticancer medication research (25). The Bcl-2 family members constitute essential intracellular players in the apoptotic mechanism (26). Several studies have reported that Bax, Bcl-2 and caspase-3 are the key molecules participating in apoptosis in ovarian cancer cells (27,28). Cactus pear extract triggers apoptosis by increasing the ratio of Bax/Bcl-2 in SKOV3 cells (29). Cactus pear extract increased caspase-3 and Bax protein levels, and decreased Bcl-2 levels in ovarian cancer cell line SKOV3 (30). Epidermal growth factor induces ovarian cancer cell line apoptosis by activating caspase-3 via upregulating Bax and downregulating Bcl-2 (31). In the present study, it was observed that the expression of Bcl-2 protein in SKOV3 cells was significantly reduced compared with the control group ($P<0.05$), whereas the expression of Bax protein was significantly increased compared with the control group ($P<0.05$). The ratio of Bax/Bcl-2 significantly increased, indicating that SKOV3 cell apoptosis occurred following CrCuW_{11} treatment. These data indicated that the caspase-3-dependent apoptotic signaling pathway has a significant role in the anticancer activity of CrCuW_{11} .

The caspase family has a significant role in apoptosis, with the cysteine aspartic acid protease caspase-3 acting as a key molecule that is able to transmit apoptotic signals in a number of signaling pathways (32). The results of the present study revealed that the caspase-3 activity in cells was increased following treatment with CrCuW_{11} . Cytochrome *c*, which is also involved in apoptosis, is able to stimulate unlimited cell proliferation and promote cell division, and is closely associated with the occurrence and development of a wide variety of tumors (33). The results of the present study revealed that

treatment of SKOV3 cells with CrCuW_{11} for 12 h significantly induced the expression of cytochrome *c* protein.

p38 is an important member of the MAPK signaling pathway, and the p38 MAPK pathway was found to increase the sensitivity of human colon cancer cells to drug treatment (34). As a kinase activated by oxidative stress, p38 primarily participates in apoptosis (35). Under normal circumstances, p38 is located in the cytoplasm; however, once activated it will be rapidly translocated into the nucleus to activate mitogen-activated protein kinase-activated protein kinase-2 and 3 and caspase family members (36). These findings suggest that activation of the p38 signaling pathway, may increase the effects of the antitumor signaling pathway in SKOV3 cells.

In the present study, it was observed that the expression of p38 protein in the SKOV3 cells was unaltered compared with the control group ($P<0.05$), whereas the expression of p-p38 protein was significantly increased compared with the control group ($P<0.05$). When $10\ \mu\text{l}$ SB203580 (a selective p38 inhibitor) was used to treat the cells for 4 h prior to treatment with $1.87\times 10^{-3}\ \text{mol}\cdot\text{l}^{-1}$ of CrCuW_{11} for 12 h, it was observed that the protein level of the total p38 did not change. When electron microscopy was used to examine the cells, it was observed that the cells did not alter compared with the control group in cell surface form and other parameters, including apoptotic bodies.

In conclusion, the present study demonstrated, for the first time to the best of our knowledge, that CrCuW_{11} markedly inhibits cell proliferation by inducing apoptosis in ovarian cancer cells. This is likely to be associated with CrCuW_{11} -regulated multiple targets in SKOV3 cell proliferation. CrCuW_{11} increased the expression of Bax and decreased the expression of Bcl-2, and subsequently mediated an increase in the ratio of Bax over Bcl-2, leading to activation of caspase-3, and subsequent activation of the p38 signaling pathway. The results of the present study suggest that CrCuW_{11} may be a potential drug for the clinical treatment of ovarian cancer.

Acknowledgements

The present study was supported by the Medical Science Foundation of Guangdong Province (grant no., A2014316).

References

- DeSantis CE, Lin CC, Mariotto AB, Siegel RL, Stein KD, Kramer JL, Alteri R, Robbins AS and Jemal A: Cancer treatment and survivorship statistics, 2014. *CA Cancer J Clin* 64: 252-271, 2014.
- Siegel R, Ma J, Zou Z and Jemal A: Cancer statistics, 2014. *CA Cancer J Clin* 64: 9-29, 2014.
- Cooke SL and Brenton JD: Evolution of platinum resistance in high-grade serous ovarian cancer. *Lancet Oncol* 12: 1169-1174, 2011.
- Graziosi L, Mingrone E, Marino E, Cavazzoni E and Donini A: Analysis of operative morbidity in a single center initial experience with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *Tumori* 100: 15-20, 2014.
- Guo FJ, Tian JY, Jin YM, Wang L, Yang RQ and Cui MH: Effects of cyclooxygenase-2 gene silencing on the biological behavior of SKOV3 ovarian cancer cells. *Mol Med Rep* 11: 59-66, 2015.
- Chermann JC, Raynaud M, Jasmin C and Mathé G: Powerful new inhibitor of murine leukaemia and sarcoma viruses. *Nature* 227: 173-174, 1970.
- Liu J, Mei WJ, Xu AW, Tan CP, Shi S and Ji LN: Synthesis, characterization and antiviral activity against influenza virus of a series of novel manganese-substituted rare earth borotungstates heteropolyoxometalates. *Antiviral Res* 62: 65-71, 2004.

8. Proust A, Thouvenot R and Gouzerh P: Functionalization of polyoxometalates: Towards advanced applications in catalysis and materials science. *Chem Commun (Camb)* 1837-1852, 2008.
9. Feng CG, Xiong YD and Liu X: Synthesis, spectroscopy and antibacterial activity of supermolecular compounds of organotitanium substituted heteropolytungstates containing 8-quinolinol. *Guang Pu Xue Yu Guang Pu Fen Xi* 31: 1153-1160, 2011.
10. Taghdiri M, Saadatjou N, Zamani N and Farrokhi R: Heterogeneous degradation of precipitated hexamine from wastewater by catalytic function of silicotungstic acid in the presence of H₂O₂ and H₂O₂/Fe²⁺. *J Hazard Mater* 246-247: 206-212, 2013.
11. Sato T, Hashizume M, Hotta Y and Okahata Y: Morphology and proliferation of B16 melanoma cells in the presence of lanthanoid and Al³⁺ ions. *Biomaterials* 11: 107-112, 1998.
12. McGahon AJ, Martin SJ, Bissonnette RP, Mahboubi A, Shi Y, Mogil RJ, Nishioka WK and Green DR: The end of the (cell) line: Methods for the study of apoptosis in vitro. *Methods Cell Biol* 46: 153-185, 1995.
13. Chen H, Takahashi S, Imamura M, Okutani E, Zhang ZG, Chayama K and Chen BA: Earthworm fibrinolytic enzyme: Anti-tumor activity on human hepatoma cells in vitro and in vivo. *Chin Med J (Engl)* 120: 898-904, 2007.
14. Ribble D, Goldstein NB, Norris DA and Shellman YG: A simple technique for quantifying apoptosis in 96-well plates. *BMC Biotechnol* 5: 12, 2005.
15. Lambert KE, Huang H, Mythreye K and Blobe GC: The type III transforming growth factor- β receptor inhibits proliferation, migration, and adhesion in human myeloma cells. *Mol Biol Cell* 22: 1463-1472, 2011.
16. Arend RC, Londoño-Joshi AI, Straughn JM Jr and Buchsbaum DJ: The Wnt/ β -catenin pathway in ovarian cancer: A review. *Gynecol Oncol* 131: 772-779, 2013.
17. Xi YN, Xin XY and Ye HM: Effects of HMGA2 on malignant degree, invasion, metastasis, proliferation and cellular morphology of ovarian cancer cells. *Asian Pac J Trop Med* 7: 289-292, 2014.
18. Renaudeau C, Lefebvre-Lacoeuille C, Campion L, Dravet F, Descamps P, Ferron G, Houvenaeghel G, Giard S, Tunon de Lara C, Dupré PF, *et al*: Evaluation of sentinel lymph node biopsy after previous breast surgery for breast cancer: GATA study. *Breast* 28: 54-59, 2016.
19. Shigeta S, Mori S, Watanabe J, Soeda S, Takahashi K and Yamase T: Synergistic anti-influenza virus A (H1N1) activities of PM-523 (polyoxometalate) and ribavirin in vitro and in vivo. *Antimicrob Agents Chemother* 41: 1423-1427, 1997.
20. Zhai F, Wang X, Li D, Zhang H, Li R and Song L: Synthesis and biological evaluation of decavanadate Na₄Co(H₂O)₆V₁₀O₂₈·18H₂O. *Biomed Pharmacother* 63: 51-55, 2009.
21. Bălci Ş, Şuşman S, Rusu D, Nicula GZ, Sorişău O, Rusu M, Biris AS and Matei H: Differentiation of stem cells into insulin-producing cells under the influence of nanostructural polyoxometalates. *J Appl Toxicol* 36: 373-384, 2016.
22. Zhou J, Wang LF, Wang JY and Tang N: Synthesis, characterization, antioxidative and antitumor activities of solid quercetin rare earth(III) complexes. *J Inorg Biochem* 83: 41-48, 2001.
23. Duan J, Jiang XP, Li MQ, Fan DX, Wang Y, Li DJ and Jin LP: Thymic stromal lymphopoietin suppresses the apoptosis of decidual gamma-delta T cells via regulation of the signal transduction and activation of transcription 3/caspase-3 signaling pathway. *Am J Reprod Immunol* 70: 464-471, 2013.
24. Martin SJ and Green DR: Apoptosis and cancer: The failure of controls on cell death and cell survival. *Crit Rev Oncol Hematol* 18: 137-153, 1995.
25. Reed JC: Apoptosis-targeted therapies for cancer. *Cancer Cell* 3: 17-22, 2003.
26. Wang Y, Kong D, Wang X, Dong X, Tao Y and Gong H: Molecular mechanisms of luteolin induced growth inhibition and apoptosis of human osteosarcoma cells. *Iran J Pharm Res* 14: 531-538, 2015.
27. Hajiahmadi S, Panjehpour M, Aghaei M and Shabani M: Activation of A2b adenosine receptor regulates ovarian cancer cell growth: Involvement of Bax/Bcl-2 and caspase-3. *Biochem Cell Biol* 93: 321-329, 2015.
28. Dholariya S, Mir R, Zuberi M, Yadav P, Gandhi G, Khurana N, Saxena A and Ray PC: Potential impact of (rs 4645878) BAX promoter -248G>A and (rs 1042522) TP53 72Arg>pro polymorphisms on epithelial ovarian cancer patients. *Clin Transl Oncol* 18: 73-81, 2016.
29. Zhou Y, Liu QH, Liu CL and Lin L: Calycosin induces apoptosis in human ovarian cancer SKOV3 cells by activating caspases and Bcl-2 family proteins. *Tumour Biol* 36: 5333-5339, 2015.
30. Feugang JM, Ye F, Zhang DY, Yu Y, Zhong M, Zhang S and Zou C: Cactus pear extracts induce reactive oxygen species production and apoptosis in ovarian cancer cells. *Nutr Cancer* 62: 692-699, 2010.
31. Cenni B, Aebi S, Nehmé A and Christen RD: Epidermal growth factor enhances cisplatin-induced apoptosis by a caspase 3 independent pathway. *Cancer Chemother Pharmacol* 47: 397-403, 2001.
32. Zhivotovsky B: Caspases: The enzymes of death. *Essays Biochem* 39: 25-40, 2003.
33. Guo L, Peng Y, Li Y, Yao J, Zhang G, Chen J, Wang J and Sui L: Cell death pathway induced by resveratrol-bovine serum albumin nanoparticles in a human ovarian cell line. *Oncol Lett* 9: 1359-1363, 2015.
34. Yang SY, Miah A, Sales KM, Fuller B, Seifalian AM and Winslet M: Inhibition of the p38 MAPK pathway sensitises human colon cancer cells to 5-fluorouracil treatment. *Int J Oncol* 38: 1695-1702, 2011.
35. Zhang B, Wang X, Cai F, Chen W, Loesch U and Zhong XY: Antitumor properties of salinomycin on cisplatin-resistant human ovarian cancer cells in vitro and in vivo: Involvement of p38 MAPK activation. *Oncol Rep* 29: 1371-1378, 2013.
36. Chen L, Liu T, Zhang S, Zhou J, Wang Y and Di W: Succinate dehydrogenase subunit B inhibits the AMPK-HIF-1 α pathway in human ovarian cancer in vitro. *J Ovarian Res* 7: 115, 2014.