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Apoptosis-induced anti-tumor effect of *Curcuma kwangsiensis* polysaccharides against human nasopharyngeal carcinoma cells

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ABSTRACT

This study aimed to investigate the effect of *Curcuma kwangsiensis* polysaccharides on the viability of human nasopharyngeal carcinoma cell line CNE-2 cells, and explore the possible mechanisms. CNE-2 cells were treated with various concentrations of *C. kwangsiensis* polysaccharides, then the proliferation, apoptosis and the protein expression of apoptosis-related regulators p53 and Bcl-2 were assessed. The results demonstrated that *C. kwangsiensis* polysaccharides can significantly inhibit the proliferation of CNE-2 cells, which was possibly through the induction of apoptosis mediated by attenuating Bcl-2 expression and promoting p53 expression. The present study therefore indicates that *C. kwangsiensis* polysaccharides could be developed into potential drugs for nasopharyngeal carcinoma treatment.

1. Introduction

Nasopharyngeal carcinoma (NPC) is a malignancy occurring in the epithelial lining of the nasopharynx, and remains one of the most common head and neck cancers in southern China and South East Asia (Wei & Sham, 2005). In Southern China, the incidence of NPC is up to 25 or more per 100,000 people per year (Yu & Yuan, 2002). Etiological studies have shown that NPC is a complex disease induced by the interaction between chronic infection with oncogenic gamma herpesvirus Epstein–Barr virus (EBV), environmental and genetic factors, involving a multistep carcinogenic process (Hildesheim & Levine, 1993). Due to the specific location of NPC at the base of skull, traditional therapy such as surgery can produce undesirable complications after treatment (Ng et al., 1997). In addition, since NPC tends to metastasize, it remains the leading cause of death from head and neck cancer in South China (Jemal et al., 2011). Radiotherapy is a standard treatment for NPC, however, it is not suitable for patients with advanced NPC (Lee et al., 2002). Recent studies suggest that chemotherapy in combination with radiotherapy is effective; however, recurrence and/or metastases in about 25–30% of patients demand better treatment options and novel therapeutic strategies (Chan et al., 2002).

Curcuma kwangsiensis, a perennial herbaceous plant of Zingiberaceae, widely spreads in the southwest of China. The dried rhizome of *C. kwangsiensis*, termed as *Ezhu* in Chinese, is an important herbal drug, which is frequently prescribed in traditional Chinese medicine for the treatment of stomach trouble and 'Oketsu' (various syndromes caused by the obstruction of blood circulation) (Sasaki et al., 2003). There are many reports on the pharmacological properties of *Curcuma* drugs, such as antitumor (Ozaki, 1990), anti-inflammatory (Ahmed et al., 2005) and immunological effects (Gonda et al., 1993). The polysaccharide is thought to be an important component of *C. kwangsiensis*, which has shown various bioactivities including antioxidant (Onoda & Inano, 2000) and vasomotional effects (Sasaki et al., 2003). However, related pharmacological studies especially in antitumor effects are scarce.

We hypothesized that polysaccharide extract of *C. kwangsiensis* will be helpful in eradicating NPC. This study reports extraction of *C. kwangsiensis* polysaccharide (CKP, hereafter) and its effect on the

Abbreviations: CKP, *Curcuma kwangsiensis* polysaccharides; NPC, nasopharyngeal carcinoma; HPLC, high-performance liquid chromatography.

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proliferation of CNE-2 cells. The chemical components of CKP were determined by high-performance liquid chromatography (HPLC) and in vitro antitumor effects were investigated by measuring the viabilities and apoptosis in CNE-2 cells. Furthermore, the underlying mechanisms were explored by detecting the protein expression of apoptosis regulators p53 and bcl-2.

2. Materials and methods

2.1. Isolation of the C. kwangsiensis polysaccharides

C. kwangsiensis was purchased from a crude drug market in Guiling, GuangXi Province, China. The dried rhizome of *C. kwangsiensis* was first ground into powder (100 g) and extracted with 800 ml water at 80 °C for three times, each 1 h. After cooling down to room temperature, the solution was filtered and centrifuged at 1200 × *g* for 15 min. The combined supernatants were evaporated to a small volume and then were placed in ethanol (5:1, v/v) for 12 h at room temperature. The solution was centrifuged at 1100 × *g* for 20 min, then the polysaccharide precipitates were collected and dried in a rotary evaporator at 60 °C to near dryness. The purification of polysaccharide from crude precipitates was performed by using anion exchange chromatography with DEAE-cellulose and Mono-Q HR5/5 columns (Amersham Pharmacia Biotech, Little Chalfont, UK) as described previously (Aquino et al., 2011).

2.2. HPLC assay

The HPLC assay for CKP was performed on an Agilent 1100 series chromatographic system (Agilent, USA). CKP and standard substances (fructose, ribose, arabinose, xylose, mannose, glucose and galacose) were dissolved in acetonitrile. A GL Sciences Inertsil ODS-4 column (5 μm, 250 mm × 4.6 mm i.d.) was used. The flow rate was 1.0 ml/min and the temperature was controlled at 25 °C. The mobile phase consisted of water (A) and acetontrile (B). The elution conditions were as follows: 10–35% (B) in 0–40 min, 35–50% (B) in 40–50 min and 50–100% (B) in 50–60 min. Detection wavelength was set at 256 nm. Evaluation of UV data was performed on an Agilent Chem Station A.09.03 (Agilent, USA) and Data Analysis 2.2 (Bruker Daltonics, USA).

2.3. Cell culture

The human nasopharyngeal carcinoma cell line CNE-2 cell, which is a human nasopharyngeal carcinoma cell line harboring a heterozygous mutation of p53 (Spruck, 1992), was obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Cells were maintained in RPMI-1640 (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) in a humidified incubator at 37 °C under a 5% CO_2 atmosphere.

2.4. Drug treatment

The *C. kwangsiensis* polysaccharides (CKP) was dissolved in 70% ethanol to make a stock solution of 10 mg/ml, which was then diluted in culture medium to obtain the desired concentrations of 12.5, 25.0, 50.0 and 100.0 μg/ml. Ethanol diluted in culture medium at the final percentage of 0.5% was designated as 0 μg/ml (negative control). 5-Fu (Jiangsu Hengrui Medicine Co., Ltd., China) at the concentration of 12.5 μg/ml was used as the positive control. Except for the cell proliferation assay, cells were treated with or without CKP for 48 h.

2.5. Cell proliferation and viability assay

Cell proliferation and viability were measured by using MTT assay as described previously (Mosmann, 1983). Exponentially growing CNE-2 cells were digested by 0.25% trypsin for 1–2 min, and washed thrice with PBS. Then 3×10^4 cells in 100 μl culture medium were plated in 96-well plates followed 12 h later by addition of CKP at the final concentration of 0, 12.5, 25.0, 50.0 and 100.0 μg/ml, or 5-Fu (12.5 μg/ml). At the predetermined time intervals (24 h, 48 h, 72 h, 96 h), the medium was aspirated and each well was added with 100 μl serum-free DMEM and 10 μl tetrazolium compound MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and incubated at 37 °C for 4 h. Then the supernatant was discarded, 100 μl solvent control (DMSO, Sigma) was applied to each well, and the plate was softly shaken for 10 min. Absorbance was measured at 450 nm with a reference wavelength of 630 nm on a spectrophotometer (Molecular Devices, Sunnyvale, CA). Cell viability was assessed as percent cell viability in terms of untreated control cells, which were determined for each concentration by use of the following equation: %viability = $OD_{experiment}/OD_{control} \times 100\%$. Negative control cells were considered as 100% viable. All experiments were repeated in six times.

2.6. Hoechst 33342 staining

The nuclear morphological changes in apoptotic cells were evaluated by DNA staining with Hoechst 33342 dye. Cells were plated into a 12-well plate at 10^4/well and treated with CKP (0, 12.5, 25.0, 50.0 and 100.0 μg/ml) or 5-Fu (12.5 μg/ml) for 48 h. Then cells were washed and resuspended in PBS and incubated with Hoechst 33342 (10 μg/ml) for 15 min at room temperature in the dark, followed by a 30-min fixation in 1% (w/v) paraformaldehyde. Images were recorded with a fluorescent microscope at 480 nm (Olympus). Apoptotic cells were identified by condensation of chromatin and/or nuclear fragmentation.

2.7. Apoptosis analysis by flow cytometry

Apoptosis analysis was performed by using an Annexin V-FITC KIT (Bender, Burlingame, CA, USA) according to the manufacturer's instructions. Briefly, after treatment of cells with CKP or 5-Fu for 48 h, CNE-2 cells were harvested, washed with PBS and resuspended in 100 μl of annexin V binding buffer (10 mM Hepes, pH 7.4, 5 mM $CaCl_2$ and 140 mM NaCl). Then cells were incubated with FITC-labeled annexin V and propidium iodide (PI) (50 μg/ml) for cellular staining in binding buffer at room temperature for 15 min in the dark. Stained cells were then analyzed for the apoptosis by a FACSAria Cell Cytometer (BD Biosciences, San Jose, CA, USA). The data were analyzed with CellQuest software (BD Biosciences). Each experiment was performed in triplicate. The percentage of cells that were annexin V positive but PI negative was considered as apoptotic cells and compared among the different treatment groups.

2.8. Western blotting

Cells were lysed as described previously (Balint et al., 2005) and the lysates were subjected to electrophoresis on SDS/PAGE and transferred to PVDF membranes (Millipore, Billerica, MA). The blotted membranes were blocked and subsequently incubated with mouse anti-p53 (1:300; Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti- Bcl-2 (1:500; Proteintech Group) and mouse anti-β-actin (1:1000; Santa Cruz Biotechnology). After incubation with horseradish peroxidase-labeled secondary antibody (1:2000; Santa Cruz Biotechnology), visualization was performed by an enhanced chemiluminescence kit (Pierce, Rockford, IL) and exposure to X-ray

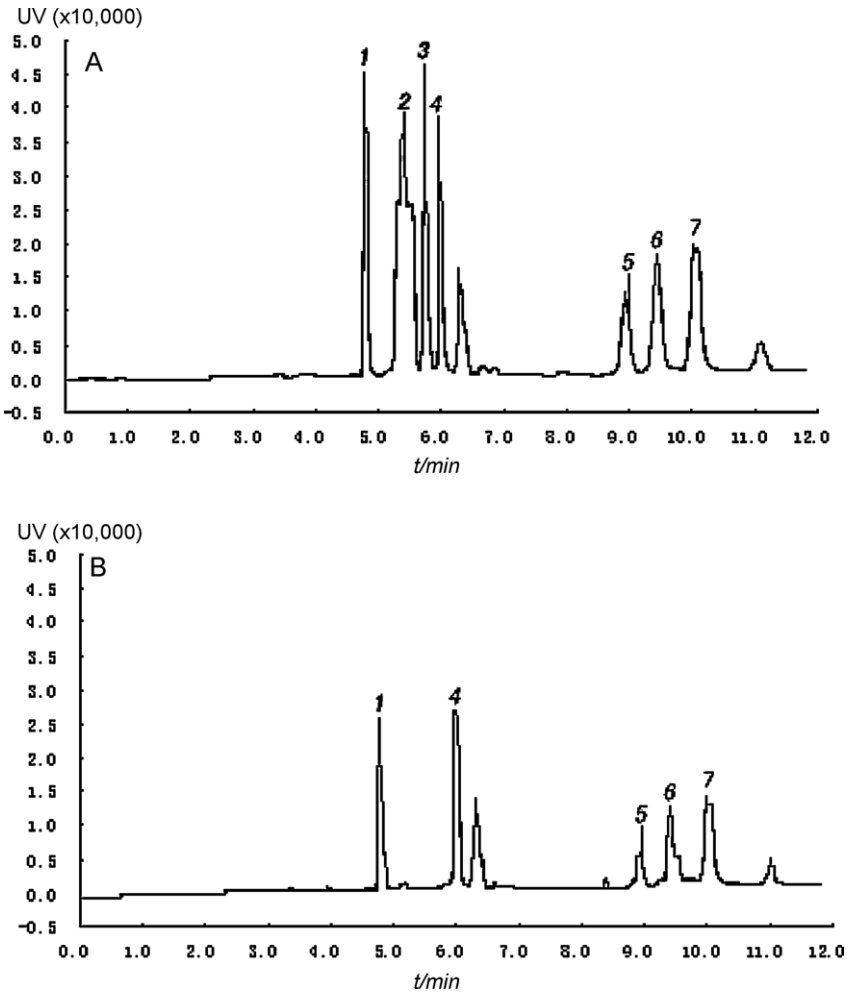

Fig. 1. The CKP composition was analyzed with HPLC. (A) HPLC results for standard substances. (1) Fructose, (2) ribose, (3) arabinose, (4) xylose, (5) mannose, (6) glucose, (7) galacose. (B) HPLC results for CKP sample, five peaks were identified as fructose (1), xylose (4), mannose (5), glucose (6) and galacose (7).

film (Kodak, Rochester, NY). Immunoblotting with anti-β-actin antibody was used as an internal control to confirm equivalent protein loading. Each experiment was performed at least three times. The relative intensity of each protein band was scanned by Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

2.9. Statistics

SPSS version 17.0 software (SPSS for Windows, Inc., Chicago, IL, USA) was used for all statistical analyses. All results are expressed as mean ± SEM. Statistical analysis of the data was performed using standard 1-way ANOVA or 1-way ANOVA for repeated measures, followed by LSD post hoc test. Bonferroni's correction was used to adjust for multiple comparisons. A two-tailed Student's paired *t*-test was also used to compare the difference in values between two groups. A P value <0.05 was considered to be statistically significant.

3. Results

3.1. The chemical content of CKP

High-performance liquid chromatography (HPLC) was used to generate the chemical profile of CKP (Fig. 1). Five peaks were identified as fructose, xylose, mannose, glucose and galacose. At 100 μg/ml of CKP, the content for fructose, xylose, mannose, glucose and galacose were 25.0 μg/ml, 25.0 μg/ml, 10.0 μg/ml, 12.5 μg/ml and 12.5 μg/ml, respectively.

3.2. CKP inhibited the proliferation of CNE-2 cells

CNE-2 cells were treated with various concentrations of CKP (0, 12.5, 25.0, 50.0 and 100.0 μg/ml) for 24, 48, 72, 96 h and the cell viability was evaluated by MTT assay. As shown in Fig. 2, increasing CKP concentration and treatment time resulted in a progressive inhibition of CNE-2 cell viability. At 24 h, no significant reduction in cell viability was observed between CKP concentrations and the negative control group (0 μg/ml CKP). However, treatment of CKP for more than 48 h resulted in a significant dose- and time-dependent reduction in cell viabilities. The first significant reduction was observed at the concentration of 50.0 μg/ml after culturing with CKP for 48 h, with an inhibition of 23.48% (P < 0.05). As comparable to those of the common antitumor agent 5-Fu, there were no significant differences between CKP (100.0 μg/ml) and 5-Fu (12.5 μg/ml) on the cell viabilities at the four time point. These results demonstrated that CKP had a potent inhibitory effect on the growth of CNE-2 cells. The treatment time point of 48 h was therefore selected for further studies.

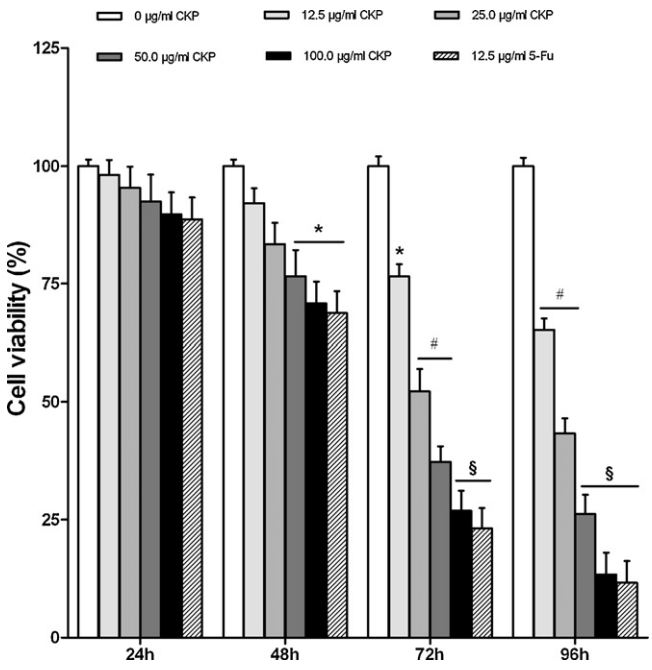

Fig. 2. Effects of CKP on the growth of CNE-2 cells. The human nasopharyngeal carcinoma cell line CNE-2 cells were treated with CKP (at the final concentration of 0, 12.5, 25.0, 50.0 and 100.0 μg/ml) at the indicated times. 12.5 μg/ml 5-Fu was used as the positive control. The inhibitory effect of CKP on cell viability of CNE-2 cells was determined by the MTT assay as described in Section 2. Results represent the mean values ± SEM of six samples. $^{*}P < 0.05$; $\#P < 0.01$; $\S P < 0.001$ versus negative control (0 μg/ml CKP group).

3.3. CKP increased apoptosis of CNE-2 cells

To investigate whether CNE-2 cells were undergoing apoptosis after CKP treatment, treated cells were examined for morphological changes of apoptosis by Hoechst 33342 staining assay. Fig. 3 showed the chromatin condensation and nuclear fragmentation characteristic of cells undergoing apoptosis after CNE-2 cells were treated by CKP in a dose-dependent manner. CNE-2 cells treated with CKP initially showed apoptosis at the concentration of 12.5 μg/ml after 48 h, the trend became more obvious while the concentration increased. The CNE-2 cells treated with 5-Fu (12.5 μg/ml) exhibited similar effect as CKP at the concentration of 100.0 μg/ml. These data clearly indicated that higher doses of CKP resulted in remarkable chromatin condensation and nuclear fragmentation in CNE-2 cells. To further confirm that the cytotoxic effects of CKP in CNE-2 cells were mediated via apoptosis, flow cytometry assay by double staining of Annexin-V FITC and PI was performed. Apoptotic cells were determined as those cells that were annexin V positive, but PI negative. The results demonstrated that the percentage of apoptotic cells increased with the elevated concentration of CKP as compared with the negative control after CKP treatment of CNE-2 cells for 48 h (Fig. 4A and B). The percentage of apoptotic cells were increased from 7.42% in non-DAPT-treated cells to 25.16% at 50.0 μg/ml ($P < 0.05$), and to 42.85% at 100.0 μg/ml ($P < 0.01$) after treatment with CKP for 48 h. In addition, no significant differences of apoptotic cells were observed between CKP (100.0 μg/ml) and 5-Fu (12.5 μg/ml).

3.4. Effects of CKP on the protein expression levels of p53 and Bcl-2

To further investigate the mechanism of CKP-induced apoptosis in CNE-2 cells, we measured the changes in protein expression

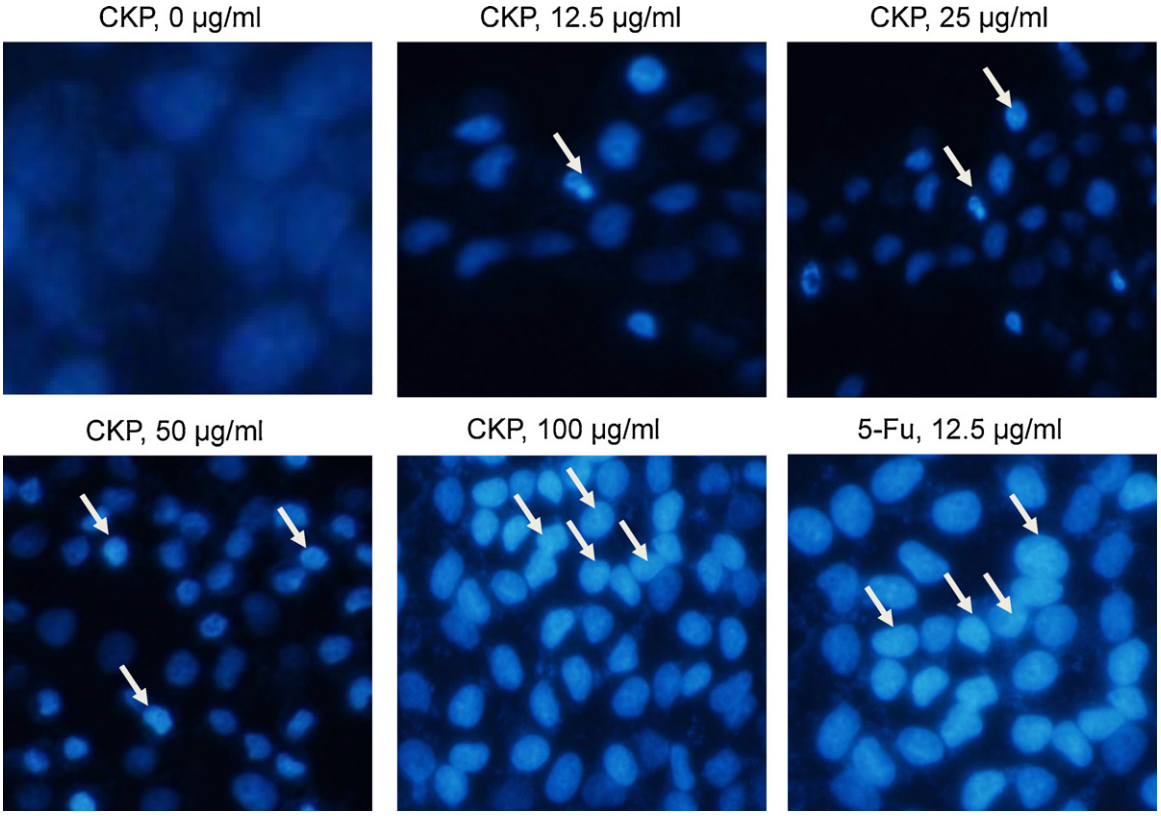

Fig. 3. Morphological changes characteristic of apoptosis, including chromatin condensation and nuclear fragmentation in CNE-2 cells exposed to CKP (0, 12.5, 25.0, 50.0 and 100.0 μg/ml) or 5-Fu (12.5 μg/ml) for 48 h. Then cells are stained with Hoechst 33342 for 30 min, the condensation and fragmentation of nuclei were observed under a fluorescence microscope (White arrow) (Hoechst 33342 staining; magnification, 200×).

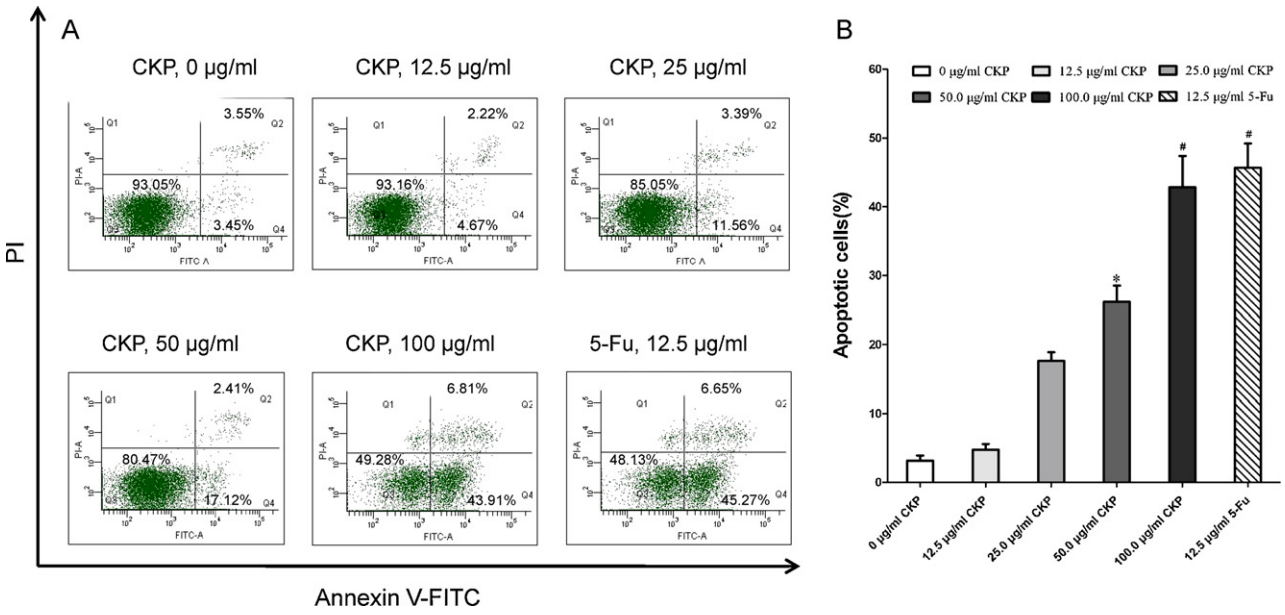

Fig. 4. CKP induces apoptosis in CNE-2 cells in a dose-dependent manner. Apoptosis of CNE-2 cells treated with CKP or 5-Fu for 48 h was detected by annexin V and PI staining using FACS analysis. The percentage of cells that were annexin V positive but PI negative was compared among different groups. Results visualized as a representative experiment (A) or mean values ± SEM of three experiments (B), each performed in triplicate. $^{*}P < 0.05$ and $\#P < 0.01$ versus control (untreated cells).

of apoptosis-related genes including p53 and Bcl-2 after CKP treatment for 48 h. CNE cells were treated with various concentrations of CKP for 48 h, the protein expression of p53 and Bcl-2 was assessed by Western blot analysis. As shown in Fig. 5, CKP treatment greatly reduced the amount of Bcl-2 protein while increased the expression of p53 protein in a dose-dependent manner. A 6.47-fold increase in p53 expression was observed in CNE-2 cells exposed to 50.0 μg/ml CKP after 48 h of treatment; the increase peaked at 8.23-fold after exposure to 100.0 μg/ml CKP after 48 h of treatment. A significant decrease in Bcl-2 expression was obtained after exposure to 50.0 μg/ml and 100.0 μg/ml CKP under the same conditions. These data were correspondent to the previous results that 50.0 and 100.0 μg/ml CKP treatment for 48 h significantly inhibited the proliferation and increased the apoptosis of CNE-2 cells. Taken together, these results indicated that increased apoptosis induced by CKP contributed to the enhanced viability of CNE-2 cells treated with CKP.

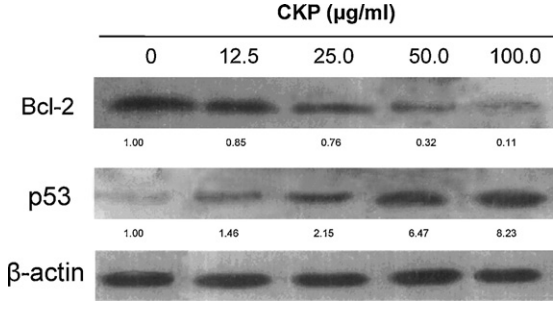

Fig. 5. Effects of CKP on the protein expression levels of p53 and Bcl-2. CNE cells were treated with CKP at the concentration of 0, 12.5, 25.0, 50.0 and 100.0 μg/ml for 48 h, respectively, the protein expression of p53 and Bcl-2 was determined by Western blot analysis. β-actin was used as an internal control for equal loading of samples. Representative blots shown were from three independent experiments with identical results. The relative ratios of each band were normalized to β-actin and are shown below each Western blot band.

4. Discussion

As a common prescribed traditional Chinese medicine, *C. kwangsiensis* has been used in China since ancient times. Recently, studies have demonstrated the antitumor properties of *C. kwangsiensis* (Ozaki, 1990). However, the pharmacological effect has not been systematically evaluated. In the present study, we report for the first time that CKP, a polysaccharide extract isolated from dried rhizome of *Curcuma kwangsiensi*, exerts potent antiproliferative effect on CNE-2 cells via induction of apoptosis in vitro. More importantly, the related molecular mechanisms regulating apoptosis are preliminarily defined.

Apoptosis, also known as programmed cell death, is characterized by cell shrinkage, blebbing of the plasma membrane, chromatin condensation and DNA fragmentation (Elmore, 2007). It is an elaborate cellular homeostasis mechanism that ensures correct development and function of multicellular organisms. Perturbations of the balance between proliferation and apoptosis play a pivotal role in carcinogenesis (Lowe & Lin, 2000). A plethora of approaches have been designed to eliminate cancer cells by preferential induction of apoptosis, such as chemotherapy and radiotherapy (Johnstone et al., 2002). In the present study, we found that CKP had a similar effect as 5-Fu to induce apoptosis of CNE-2 cells, which in turn inhibited the proliferation. The MTT assay showed that CKP significantly suppressed the proliferation of CNE-2 cells in a dose- and time-dependent manner. The apoptosis confirmed by Hoechst 33342 staining assay and Annexin V-FITC flow cytometry assay demonstrated that CKP induced the apoptosis of CNE-2 cells in a dose- and time-dependent manner. These findings suggested that the decreased viabilities of CNE-2 cells treated with CKP may mainly attribute to apoptosis. To explore the potential mechanisms of CKP-induced apoptosis, apoptosis-related protein expression was examined, and we found that p53 expression was unregulated and Bcl-2 was downregulated.

The tumor suppressor gene P53 is an important regulator of apoptosis. In addition to the fact that P53 mutations occur in the majority of human cancers (Wallace-Brodeur & Lowe, 1999), P53-null mice are highly prone to develop cancers (Merritt et al., 1997).

It has been clearly established that p53 was a checkpoint protein involved in cell-cycle arrest and maintaining the genomic integrity in response to DNA damage (Levine, 1997). However, p53 could also play its tumor suppressive function by regulating apoptosis (Yonish-Rouach et al., 1991). The mutations of p53 have been observed in NPC, it was reported that the expression of p53 proteins may be associated with the level of tumor cell differentiation in NPC (Kouvidou et al., 1995). In this study, our data demonstrated that the protein expression of p53 increased following the CKP treatment in a dose-dependent manner. Considering that CNE-2 is a human nasopharyngeal carcinoma cell line harboring a heterozygous mutation of p53 (Spruck, 1992), our results suggest that elevated p53 protein could contribute to CKP-mediated apoptosis in CNE-2 cells.

Bcl-2 is the first apoptotic regulator identified, which was discovered as a proto-oncogene at the *t*(14;18) chromosomal translocation breakpoint in follicular B-cell lymphomas (Tsujimoto et al., 1985). Bcl-2 belongs to a large family that consists of proapoptotic factors such as Bax, Bak and Bad, and antiapoptotic factors such as Bcl-2, Bcl-XL and Bcl-W (Adams & Cory, 1998). As a prototypic anti-apoptotic protein, Bcl-2 is able to regulate cell death triggered by developmental or physiological cues, it can also control apoptosis induced by cytotoxic stress conditions, such as anti-cancer drugs (Reed, 1994). Experiments with transgenic mice over-expressing Bcl-2 have shown that it can promote transformation of T lymphocytes (Linette et al., 1995) and mammary epithelial cells (Jager et al., 1997). Over-expression of the apoptosis inhibitor Bcl-2 or loss of its antagonist Bim would boost tumourigenesis (Kroemer, 1997). Studies have shown Bcl-2 is overexpressed in a variety of cancers including nasopharyngeal carcinoma (Coultas & Strasser, 2003; Vera-Sempere et al., 1997), which indicates that Bcl-2 may play a crucial role in the development of NPC. The results from our study revealed that CKP remarkably inhibited Bcl-2 protein expression in a dose-dependent manner, which corresponded to the inhibited proliferation and increased apoptosis of CNE-2 cells induced by CKP. Our results indicated CKP induced apoptosis of CNE-2 cells by inhibiting the expression of Bcl-2. It has been found that p53 can initiate apoptosis by transcriptionally activating proapoptotic Bcl-2 family members (Bax, Bak) and repressing anti-apoptotic Bcl-2 proteins (Bcl-2, Bcl-XL) (Wu et al., 2001). Hence, it can be concluded that CKP may induce apoptosis mainly through activating the P53 gene in CNE-2 cells, which then promotes the apoptosis pathway while suppresses the survival pathway such us Bcl-2 in tumor cells. However, the exact mechanism awaits further investigation.

Overall, our results demonstrate that polysaccharide extract from *C. kwangsiensis* (CKP) significantly inhibits CNE-2 cells proliferation, possibly through the induction of apoptosis mediated by attenuating Bcl-2 gene expression and activating the tumor suppressor gene p53, and has required potential to combat nasopharyngeal carcinoma.

Conflicts of interests

No conflicts of interest are involved in this manuscript.

Acknowledgments

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References

Adams, J. M., & Cory, S. (1998). The Bcl-2 protein family: Arbiters of cell survival. *Science*, *281*(5381), 1322–1326.

Ahmed, S., Anuntiyo, J., Malemud, C. J., & Haqqi, T. M. (2005). Biological basis for the use of botanicals in osteoarthritis and rheumatoid arthritis: A review. *Evidence-based Complementary and Alternative Medicine*, *2*(3), 301–308.

Aquino, R. S., Grativol, C., & Mourão, P. A. (2011). Rising from the sea: Correlations between sulfated polysaccharides and salinity in plants. *PLoS One*, *6*(4), e18862.

Balint, K., Xiao, M., Pinnix, C. C., Soma, A., Veres, I., Juhasz, I., et al. (2005). Activation of Notch1 signaling is required for beta-catenin-mediated human primary melanoma progression. *Journal of Clinical Investigation*, *115*(11), 3166–3176.

Chan, A. T., Teo, P. M., Ngan, R. K., Leung, T. W., Lau, W. H., Zee, B., et al. (2002). Concurrent chemotherapy-radiotherapy compared with radiotherapy alone in locoregionally advanced nasopharyngeal carcinoma: Progression-free survival analysis of a phase III randomized trial. *Journal of Clinical Oncology*, *20*(8), 2038–2044.

Coultas, L., & Strasser, A. (2003). The role of the Bcl-2 protein family in cancer. *Seminars in Cancer Biology*, *13*(2), 115–123.

Elmore, S. (2007). Apoptosis: A review of programmed cell death. *Toxicologic Pathology*, *35*(4), 495–516.

Gonda, R., Tomoda, M., Ohara, N., & Takada, K. (1993). Arabinogalactan core structure and immunological activities of ukonan C, an acidic polysaccharide from the rhizome of *Curcuma longa*. *Biological & Pharmaceutical Bulletin*, *16*(3), 235–238.

Hildesheim, A., & Levine, P. H. (1993). Etiology of nasopharyngeal carcinoma: A review. *Epidemiologic Reviews*, *15*(2), 466–485.

Jager, R., Herzer, U., Schenkel, J., & Weiher, H. (1997). Overexpression of Bcl-2 inhibits alveolar cell apoptosis during involution and accelerates c-myc-induced tumorigenesis of the mammary gland in transgenic mice. *Oncogene*, *15*(15), 1787–1795.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA – A Cancer Journal for Clinicians*, *61*(2), 69–90.

Johnstone, R. W, Ruefli, A. A., & Lowe, S. W. (2002). Apoptosis: A link between cancer genetics and chemotherapy. *Cell*, *108*(2), 153–164.

Kouvidou, C., Kanavaros, P., Papaioannou, D., Stathopoulos, E., Sotsiou, F., Datseris, G., et al. (1995). Expression of bcl-2 and p53 proteins in nasopharyngeal carcinoma. Absence of correlation with the presence of EBV encoded EBER1-2 transcripts and latent membrane protein-1. *Clinical Molecular Pathology*, *48*(1), M17–M22.

Kroemer, G. (1997). The proto-oncogene Bcl-2 and its role in regulating apoptosis. *Nature Medicine*, *3*(6), 614–620.

Lee, N., Xia, P., Quivey, J. M., Sultanem, K., Poon, I., Akazawa, C., et al. (2002). Intensity-modulated radiotherapy in the treatment of nasopharyngeal carcinoma: An update of the UCSF experience. *International Journal of Radiation Oncology, Biology, Physics*, *53*(1), 12–22.

Levine, A. J. (1997). p53, the cellular gatekeeper for growth and division. *Cell*, *88*(3), 323–331.

Linette, G. P., Hess, J. L., Sentman, C. L., & Korsmeyer, S. J. (1995). Peripheral T-cell lymphoma in lckpr-bcl-2 transgenic mice. *Blood*, *86*(4), 1255–1260.

Lowe, S. W., & Lin, A. W. (2000). Apoptosis in cancer. *Carcinogenesis*, *21*(3), 485–495.

Merritt, A. J., Allen, T. D., Potten, C. S., & Hickman, J. A. (1997). Apoptosis in small intestinal epithelial from p53-null mice: Evidence for a delayed, p53-independent G2/M-associated cell death after gamma-irradiation. *Oncogene*, *14*(23), 2759–2766.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *The Journal of Immunological Methods*, *65*(1–2), 55–63.

Ng, S. H., Chang, T. C., Ko, S. F., Yen, P. S., Wan, Y. L., Tang, L. M., et al. (1997). Nasopharyngeal carcinoma: MRI and CT assessment. *Neuroradiology*, *39*(10), 741–746.

Onoda, M., & Inano, H. (2000). Effect of curcumin on the production of nitric oxide by cultured rat mammary gland. *Nitric Oxide*, *4*(5), 505–515.

Ozaki, Y. (1990). Antiinflammatory effect of *Curcuma xanthorrhiza* Roxb, and its active principles. *Chemical & Pharmaceutical Bulletin (Tokyo)*, *38*(4), 1045–1048.

Reed, J. C. (1994). Bcl-2 and the regulation of programmed cell death. *Journal of Cell Biology*, *124*(1–2), 1–6.

Sasaki, Y., Goto, H., Tohda, C., Hatanaka, F., Shibahara, N., Shimada, Y., et al. (2003). Effects of curcuma drugs on vasomotion in isolated rat aorta. *Biological & Pharmaceutical Bulletin*, *26*(8), 1135–1143.

Spruck, C. H., 3rd, Tsai, Y. C., Huang, D. P., Yang, A. S., Rideout, W. M., 3rd, Gonzalez-Zulueta, M., et al. (1992). Absence of p53 gene mutations in primary nasopharyngeal carcinomas. *Cancer Research*, *52*(17), 4787–4790.

Tsujimoto, Y., Cossman, J., Jaffe, E., & Croce, C. M. (1985). Involvement of the bcl-2 gene in human follicular lymphoma. *Science*, *228*(4706), 1440–1443.

Vera-Sempere, F. J., Burgos, J. S., Botella, M. S., & Morera, C. (1997). Immunohistochemical expression of Bcl-2 oncoprotein in EBV-associated nasopharyngeal carcinoma correlated to histological type and survival. *Histology and Histopathology*, *12*(1), 9–18.

Wallace-Brodeur, R. R, & Lowe, S. W. (1999). Clinical implications of p53 mutations. *Cellular and Molecular Life Sciences*, *55*(1), 64–75.

Wei, W. I., & Sham, J. S. (2005). Nasopharyngeal carcinoma. *Lancet*, *365*(9476), 2041–2054.

Wu, Y., Mehew, J. W., Heckman, C. A., Arcinas, M., & Boxer, L. M. (2001). Negative regulation of bcl-2 expression by p53 in hematopoietic cells. *Oncogene*, *20*(2), 240–251.

Yonish-Rouach, E., Resnitzky, D., Lotem, J., Sachs, L., Kimchi, A., & Oren, M. (1991). Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature*, *352*(6333), 345–347.

Yu, M. C., & Yuan, J. M. (2002). Epidemiology of nasopharyngeal carcinoma. *Seminars in Cancer Biology*, *12*(6), 421–429.