Truncated apolipoprotein C-I induces apoptosis in neuroblastoma by activating caspases in the extrinsic and intrinsic pathways

FEI GUO[1], WEI ZHAO[1], LIN YANG[1], YANG YANG[1], SAIQI WANG[2], YINGJUN WANG[3], ZHAOMING LI[3] and JIAXIANG WANG[1]

[1]Department of Pediatric Surgery, The First Affiliated Hospital of Zhengzhou University; [2]College of Pharmacy, Zhengzhou University; [3]Department of Oncology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, P.R. China

Received February 7, 2017; Accepted July 3, 2017

DOI: 10.3892/or.2017.5819

Abstract. Truncated apolipoprotein C-I is a post-translationally modified protein characterized by the loss of threonine and proline residues from the N-terminus of the mature peptide. The truncated peptide is involved in many physiological and pathological processes *in vivo* and is related to malignant diseases. The aim of the present study was to assess the effects of the truncated peptide on tumorigenesis in neuroblastoma. The truncated peptide was chemically synthesized, and a signal peptide was used as the negative control. The results of the CCK-8 assay showed that the truncated peptide selectively inhibited cell proliferation compared with the signal peptide, and inhibited migration and invasion as determined by wound healing and Transwell assays. Flow cytometry analysis demonstrated that the truncated peptide induced apoptosis and cell cycle arrest in the S phase. Bax, Bim, and tBid upregulation, and Bcl-2 and Bcl-xl downregulation were associated with permeabilization of the mitochondrial membrane, as detected by the JC-1 assay and the release of cytochrome *c* and apoptosis. Activation of caspase-8 was associated with activation of cell death receptors such as the tumor necrosis factor receptor. PARP cleavage indicated apoptosis, and DNA damage was observed in the TUNEL assay. The results showed that the truncated apoC-I induced apoptosis in neuroblastoma by the extrinsic and intrinsic pathways. The anticancer effects were confirmed *in vivo* in a xenograft mouse model. In conclusion, the endogenous protein apoC-I may be a new promising therapeutic target to suppress tumor growth.

Correspondence to: Dr Jiaxiang Wang, Department of Pediatric Surgery, The First Affiliated Hospital of Zhengzhou University, 1 Jianshe Dong Road, Erqi, Zhengzhou, Henan 450052, P.R. China
E-mail: drwangjx@163.com

Abbreviations: NB, neuroblastoma; apoC-I, apolipoprotein C-I; TP, truncated peptide; SP, signal peptide; TNFR, tumor necrosis factor receptor

Key words: apolipoprotein, mitochondrial pathway, tumor necrosis factor receptor, apoptosis, cancer therapy

Introduction

Apolipoprotein C-I (apoC-I) is a small soluble lipoprotein that can be found within HDL or VLDL (1). The gene encoding apoC-I is located in chromosome 19 along with the genes for apoE, apoC-IV and apoC-II (2). The apoC-I mRNA is translated into an 83 amino acid protein, including 26 residues as an N-terminal signal peptide (3) that may be removed as the nascent polypeptide is translocated into the endoplasmic reticulum (4) and 57 residues in the mature peptide (5,6) from the C-terminus. A post-translationally modified form of the protein characterized by the loss of a threonine and a proline from the N-terminus of the mature peptide is known as truncated apoC-I (7) and it is found in the circulatory system. The appearance of the truncated isoform is most likely due to a widely distributed post-proline cleaving enzyme (8). This phenomenon is not limited to humans (2).

ApoC-I can lead to atherosclerosis by reducing the selective uptake of cholesteryl esters (9). In addition, apoC-I binds free fatty acids and inhibits the activity of lipoprotein lipase, and dysregulation of apoC-I is correlated with cutaneous abnormalities or even obesity in humans (10). In malignant diseases such as lung (11) and prostate (12) cancers, apoC-I could serve as a novel diagnostic and prognostic biomarker. In pancreatic cancer (13), two isoforms of the mature and truncated apoC-I peptide are overexpressed in the preoperative serum and associated with poor prognosis, and play a key role in maintaining cell survival. In a previous study (14), we showed that truncated apoC-I was downregulated in the serum of patients with nephroblastoma in correlation with tumor activity. Therefore, the truncated peptide (TP) and the mature isoform may be involved in tumorigenesis.

Neuroblastoma (NB) is the most commonly abdominal solid tumor in children. Distant metastasis is common in newly diagnosed NB patients (15). Early diagnosis and effective treatment strategies are urgently needed. In addition to

surgery, certain inhibitors (16,17) play an important role in inducing apoptosis and suppressing metastasis in patients with NB. However, there are currently no effective treatments that can accurately target the tumor without any side effects. apoC-I is secreted mainly from the liver and widely distributed in the circulatory system. This kind of endogenous peptide may show low toxicity to the human body and apoC-I could be a promising therapeutic target in cancer. In the present study, the TP (55 amino acids) was chemically synthesized to examine its effect on NB and elucidate the underlying mechanism.

Materials and methods

Cell line and cell culture. SH-SY5Y was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in RPMI-1640 medium with 2 mM L-glutamine and 10% FBS. Cells were cultured at 37˚C in a humidified incubator containing 5% CO_2. The medium, L-glutamine, and FBS were purchased from Sangong Biotech (Shanghai, China).

Polypeptide by chemical synthesis. All the synthetic products were purchased from LifeTein (Beijing, China). Solid phase peptide synthesis technology was used from carboxy to amino terminus. The TP was synthesized with 55 amino acids from the C-terminus of proapoC-I and the TP labeled with FITC at the amino terminus. The signal peptide (SP) contained 26 residues from the N-terminus of proapoC-I. Peptides were isolated to a purity >95% and identified by HPLC, and sequence information was confirmed by mass spectrometry.

Cell Counting Kit-8 (CCK-8) assay. The Cell Counting Kit-8 (CCK-8; Dojindo, Tokyo, Japan) was used to study cell viability according to the manufacturer's instructions. A cell suspension was inoculated into a 96-well plate (3,000 cells/well), and incubated overnight. The next day, the medium was replaced by new medium containing the peptide at different concentrations (0, 0.1, 0.5, 1.0, 1.5 and 20 mg/ml) and incubated for 24, 48 and 72 h. The cells cultured with TP and SP were in the same plate. At every time-point, 10 μl CCK-8 was added to each well and the plate was incubated for 3 h at 37˚C and 5% CO_2. Absorbance was measured at 450 nm using a microplate reader. The assay was performed with three replicates for each group and repeated at least three times.

Wound healing assay. The wound assay was performed to determine the cell migration capability. The cells were inoculated into 6-well plates and incubated overnight. The next day, three parallel straight lines were generated using a 200-μl pipette tip. After rinsing three times with serum-free medium, the cells were incubated in serum-free medium with various TP concentrations (0, 0.1 and 0.5 mg/ml) for 24 h. The wound healing area was photographed and analyzed by IPP software. The relative migration was calculated as a percentage of the area covered by the migrated cells compared to the original wound area.

Cell migration and invasion assay. Migration and invasion assays were performed using 24-well Transwell cell culture chambers (Corning, NY, USA) or Matrigel coated invasion chambers. The cells ($2x10^4$ for migration and $4x10^4$ for invasion) were seeded into the upper chamber. The next day, plates were rinsed in serum-free medium three times and the medium was replaced with fresh medium with TP at various concentrations (0, 0.1 and 0.5 mg/ml). Medium containing 10% FBS was placed in the lower chamber. After 24 h of incubation, the cells in the upper layer of the membrane were removed with a cotton-tipped swab and cells migrated to the lower layer were fixed and stained with 0.1% crystal violet. Cells were photographed and counted in six random microscopic fields.

Cell apoptosis and cell cycle assay. Cell Apoptosis (Annexin V-FITC/PI) and Cell Cycle (PI) Detection kits were purchased from KeyGen Biotech (Nanjing, China). The cells were inoculated into 6-well plates and incubated overnight. The medium was replaced by fresh medium with various TP concentrations (0, 1.0 and 1.5 mg/ml) the next day and incubated for 24 h. Cells were then harvested and analyzed using a FACSCalibur cytometer (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. CFlow and FlowJo software was used for data analysis.

Mitochondrial membrane potential assay by JC-1. A mitochondrial membrane potential assay kit was purchased from Beyotime (Haimen, China). The cells were treated as described for the cell apoptosis and cell cycle assays and then collected and stained with JC-1 working solution at 37˚C for 20 min according to the manufacturer's instructions. FlowJo software was used to analyze the results by flow cytometry.

Distribution of TP labeled with FITC in the cell. The cells were incubated with TP (1.5 mg/ml) labeled with FITC for 24 h, followed by incubation with β-tubulin rabbit antibody at 4˚C overnight and then donkey anti-rabbit IgG conjugated with Cy3 at room temperature for 2 h (Sangong, Shanghai, China). Finally, the cells were stained with DAPI working solution at 37˚C for 5 min, and the distribution of peptide in the cells was observed under fluorescence microscope (Olympus, Tokyo, Japan).

Cell death detection by TUNEL technology. A Cell Death Detection kit was purchased from Roche (Mannheim, Germany). The cells were inoculated into 96-well plates ($2x10^3$ cells/well) and incubated overnight. The medium was replaced with fresh medium with various TP concentrations (0, 1.0, 1.5, and 2.0 mg/ml) the next day. After 24 h, the cells were fixed and incubated with TUNEL working solution at 37˚C for 60 min followed by counterstaining with DAPI working solution at 37˚C for 5 min. The results were observed under a fluorescence microscope.

Western blotting. The cells were incubated with various TP concentrations (0, 0.5, 1.0 and 1.5 mg/ml) for 24 h and collected into centrifuge tubes. After washing the cells with cold PBS twice, the proteins were extracted from the whole cell lysate by RIPA buffer (50 mM Tris-HCl at pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% NP-40, 1% sodium deoxycholate, 1% Triton X-100, 10 mM NaF and 1 mM PMSF) containing a protease inhibitor cocktail (Sangong). Proteins were separated by SDS-PAGE and transferred onto nitrocellulose

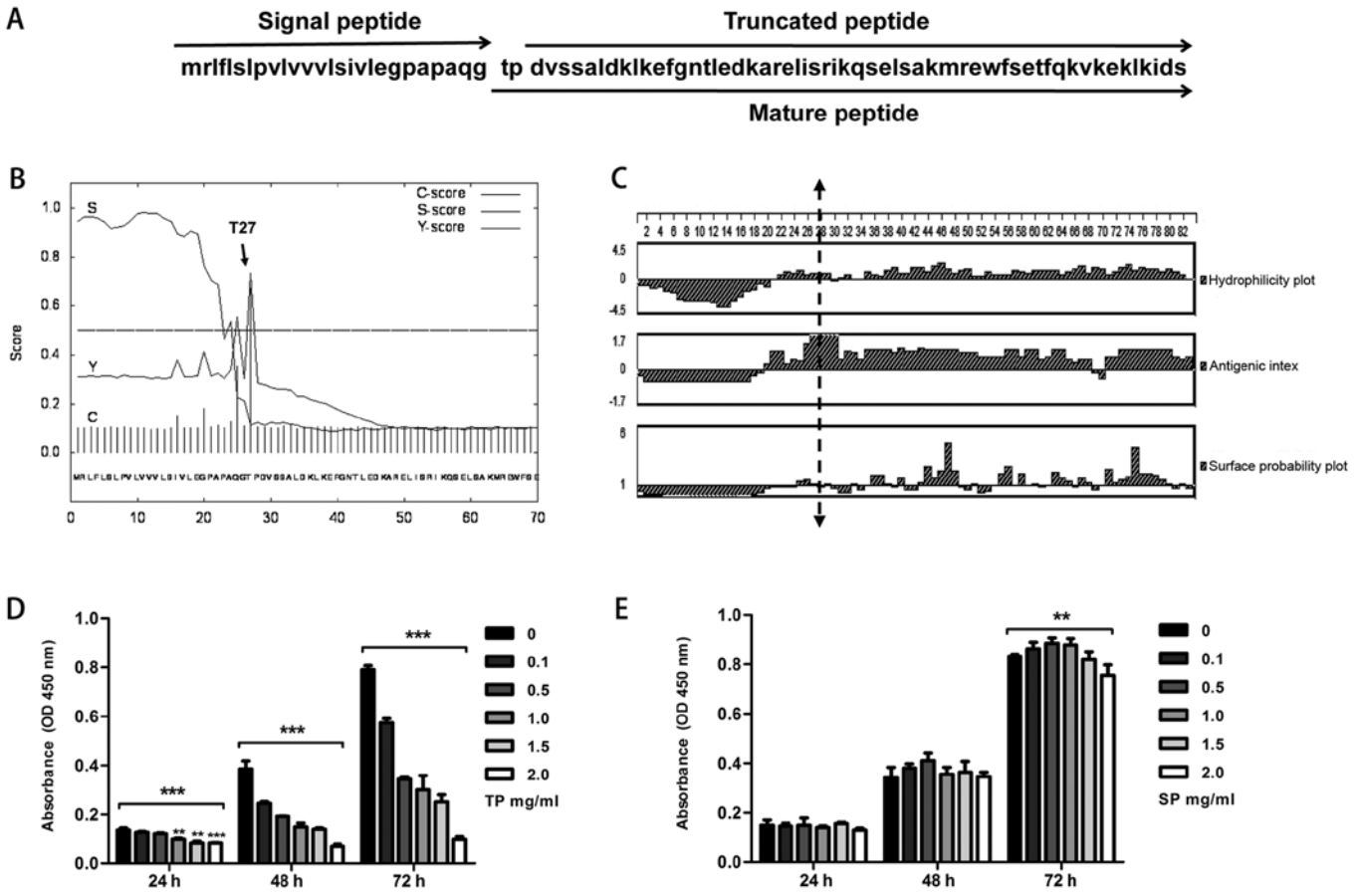

Figure 1. (A) Sequence information of proapoC-I, mature, and truncated peptide (TP). The arrow indicates the direction from amino terminus to carboxy terminus. (B) C-score (raw cleavage site score) distinguishes the signal peptide (SP) cleavage sites. The first residue is immediately high after the cleavage site. S-score (SP score) distinguishes positions within signal peptides from positions in the mature part of the proteins. Y-score is the combined cleavage site score. A combination of the C-score and the slope of the S-score provides a better cleavage site prediction than the raw C-score alone. Therefore, the site before T27 could distinguish the SP from the mature peptide. (C) The split line indicates the TP (right side). The regions (above the baseline) with high hydrophilicity, antigen index, and surface probability were mainly found in the TP. (D) TP, truncated peptide. **P<0.01, ***P<0.001. (E) SP, signal peptide. **P<0.01.

membranes. After blocking, the membranes were incubated with primary antibodies overnight at 4˚C, including anti-Bcl-2, anti-Bcl-xl, anti-Bax, anti-Bim, anti-tBid, anti-caspase-8, anti-caspase-9, anti-caspase-3, anti-PARP, anti-cleaved-PARP, and anti-β-actin, and then incubated with anti-mouse or rabbit IgG conjugated with horseradish peroxidase at room temperature for 2 h. All the antibodies were purchased from CST (Beverly, MA, USA). The ECL western blot detection kit (Proteintech, Wuhan, China) was used for chemiluminescent visualization. β-actin was used as a loading control for whole cell extracts.

Xenograft tumor mouse model. The 4-week-old BALB/C nude mice were purchased from Vital River (Beijing, China) and maintained at the SPF laboratory animal room. A total of $5x10^6$ SH-SY5Y cells in 0.1 ml of PBS were injected subcutaneously into the left sides of the dorsal area. After 2 weeks, mice bearing tumors of similar sizes were randomly divided into two groups: a PBS control group and a TP-treated group (100 mg/kg by intraperitoneal injection once daily for 21 days). After the start of treatment, tumor volume was measured and calculated every 7 days using the formula A x $B^2/2$, where A is the longest diameter and B is the shortest. At the end of the treatment, all mice were sacrificed. Tumors were harvested, weighed and photographed. The present study was approved by the Ethics Committee of Zhengzhou University.

Immunohistochemistry. Tumors were fixed and embedded with paraffin. The SP immunohistochemistry method was used to assay protein expression. The results were analyzed using IPP software. Primary anti-rabbit antibodies (Bax, Bcl-2, Ki67 and PCNA) and secondary goat anti-rabbit IgG conjugated with biotin and streptavidin conjugated with HRP were purchased from Abcam (Cambridge, UK).

Statistical analysis. Statistical analysis was performed using SPSS 21.0 software. The data were expressed as the mean ± SD. The Student's t-test was used for comparisons between two groups of quantitative data. The one-way ANOVA test was used for multigroup comparisons. P<0.05 was considered statistically significant.

Results

Peptide sequence analysis and synthesis. The proapoC-I has 83 amino acids including the SP (Met1-Gly26) and mature peptide (Thr27-Ser83). The loss of a threonine and a proline from the N-terminus of the mature peptide results in the

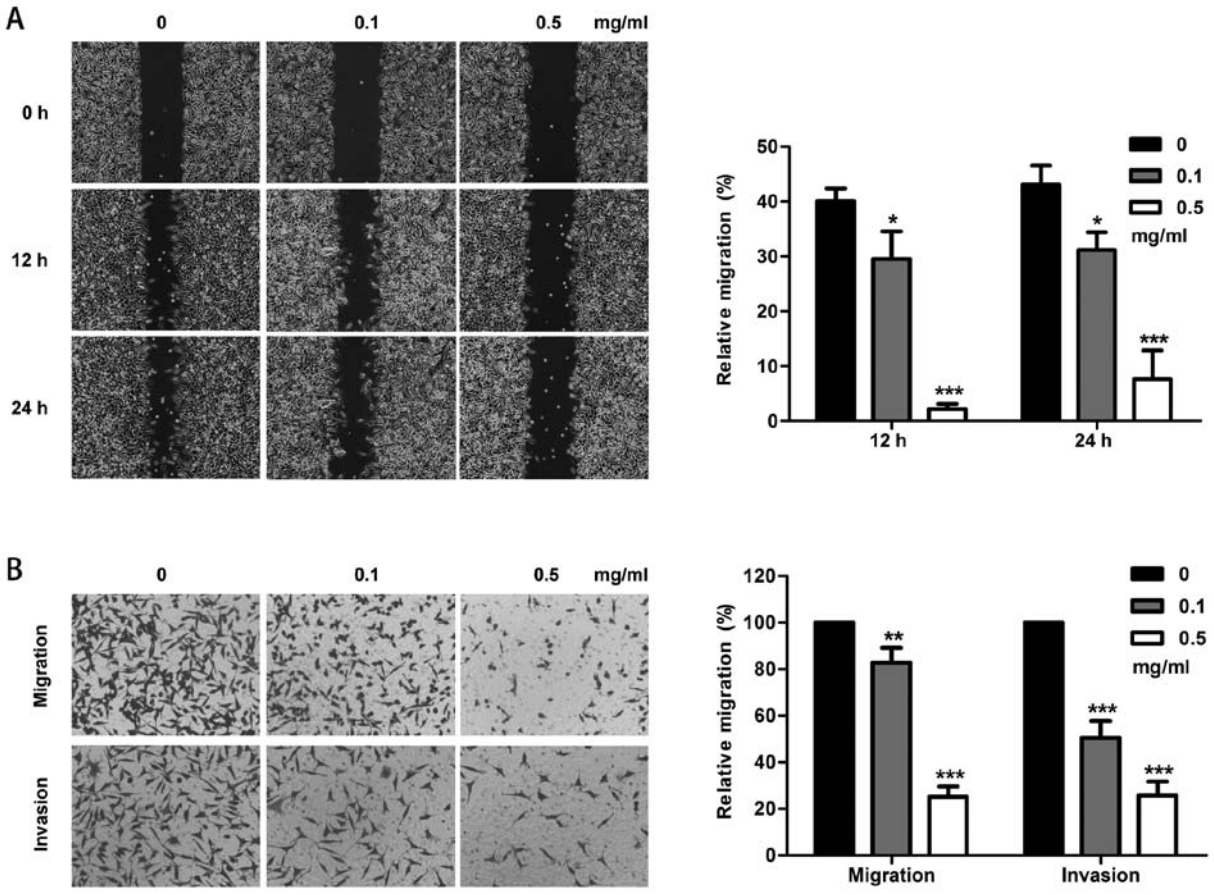

Figure 2. All the images were taken under a magnification of x200. (A) The wound healing ability was inhibited by the truncated peptide. *P<0.05, ***P<0.001. (B) The migration and invasion ability was reduced by the various peptide concentrations. **P<0.01, ***P<0.001.

TP (7). The sequence information is shown in Fig. 1A. In addition to what is reported in the literature, we found that the site between G26 and T27 is the cleavage site from signal to mature peptide using a signal peptide prediction website (http: //www.cbs.dtu.dk/services/SignalP) (Fig. 1B). DNAStar software was used to further analyze sequence activity. Regions (above the baseline) with high hydrophilicity, antigen index, and surface probability were mainly found in the TP, suggesting that the TP is a potentially active peptide with high hydrophilicity, antigenic index, and surface probability compared with the SP (Fig. 1C). Therefore, we synthesized the TP as experimental group and the SP as control group used for CCK-8 assay.

CCK-8 assay. For the TP group, increasing concentrations were correlated with the inhibition of cell proliferation at 24, 48 and 72 h (all P-values <0.001). At 24 h, the groups treated with concentrations of 0.1 and 0.5 mg/ml showed no statistically significant differences from the control group (0 mg/ml) (Fig. 1D). In the SP group, cell proliferation was not affected by different SP concentrations at 24 and 48 h (all P-values >0.05). However, at 72 h, various SP concentrations inhibited cell proliferation to some degree (P=0.001) (Fig. 1E).

Cell migration and invasion assay. In the wound healing assay, TP at 0.1 and 0.5 mg/ml inhibited the wound healing ability at 12 h compared with that in the control group (P=0.03 and P<0.001). At 24 h, the wound healing ability was also inhibited by 0.1 and 0.5 mg/ml TP (P=0.012 and P<0.001) (Fig. 2A). In the Transwell migration assay, the migration ability was reduced to 82.7 and 25.1% in response to increasing TP concentrations compared with the values in the control group (P=0.01 and P<0.001). In the invasion assay, the invasion ability was reduced to 50.4 and 25.6% compared with that in the control group (all P-values <0.001) (Fig. 2B).

Flow cytometry. In the cell apoptosis assay, increasing TP concentrations resulted in an increase in the rates of apoptosis by 22.5 and 40% (all P-values <0.01, Fig. 3A). The percentage of cells in S phase increased to 35.9% (P>0.05) and 42.6% (P=0.03) compared with that in the control group at 27.1%, and the percentage of cells in G2/M decreased to 25.9% (P>0.05) and 19.8% (P=0.04) compared with that in the control group at 29.9% (Fig. 3B). In the mitochondrial membrane potential assay, increasing TP concentrations increased the percentage of cells with JC-1 monomers to 33.7 and 58.8% (all P-values <0.001, Fig. 3C).

Distribution of FITC-labeled TP in the cell. Incubation with FITC labeled TP at 1.5 mg/ml for 24 h resulted in the entry of the peptide into the cells. The result showed that the TP was mainly concentrated in the cytoplasm and cell membrane, with a fraction detected in the nucleus (Fig. 4A).

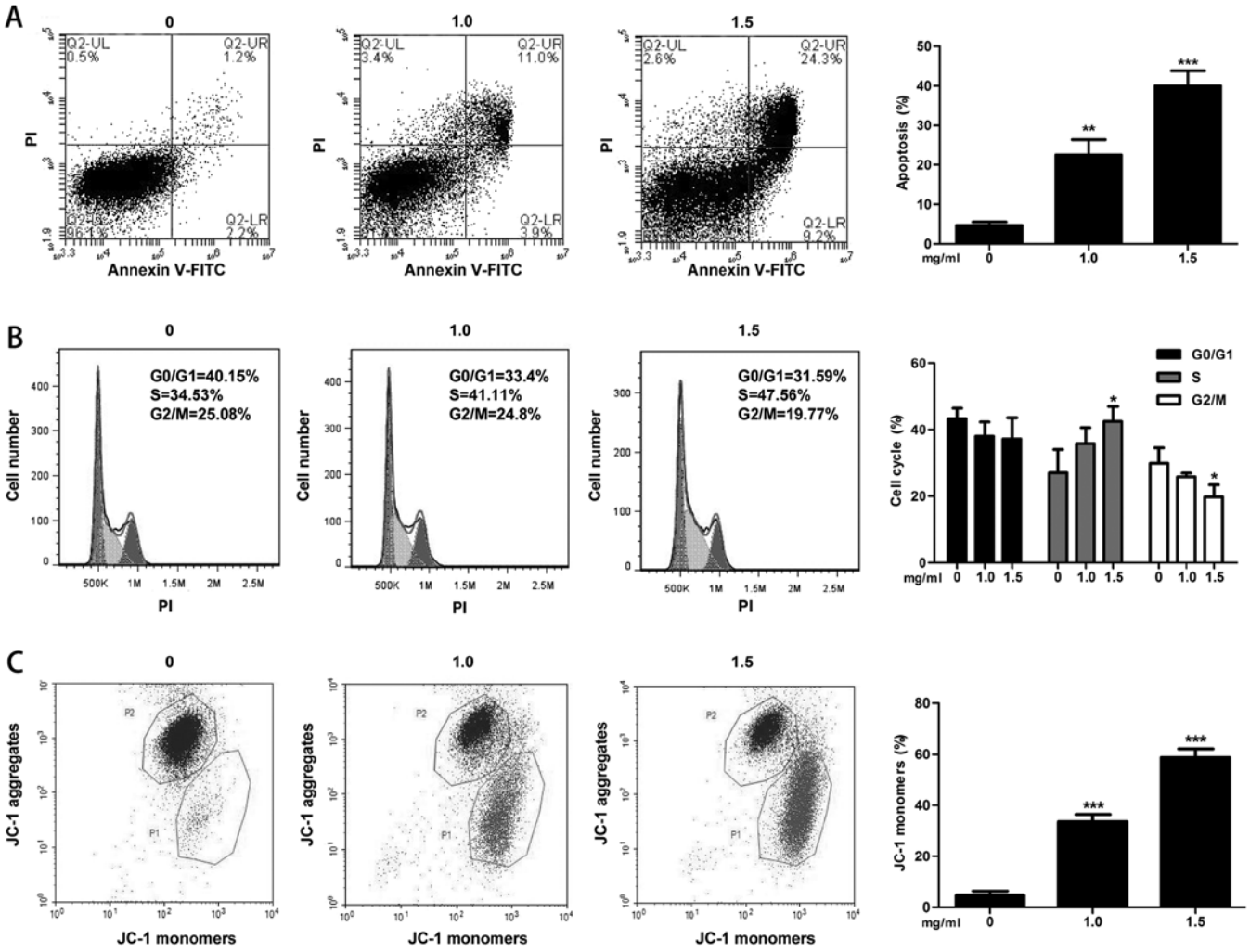

Figure 3. The SH-SY5Y cells of three groups were subjected to the same treatment (0, 1.0 and 1.5 mg/ml) and analyzed according to the manufacturer's instructions. (A) Annexin V-FITC/PI kit was used to detect cell apoptosis, **P<0.01, ***P<0.001. (B) PI was used for cell cycle assay. *P<0.05. (C) P2, JC-1 aggregates indicated the high mitochondrial membrane potential from normal cells. P1, JC-1 monomers indicated the low mitochondrial membrane potential due to the truncated peptide treatment. ***P<0.001.

Cell death detection by TUNEL technology. In the TUNEL assay, increasing TP concentrations increased DNA cleavage during apoptosis. The results showed that the survival rate of the cells was reduced to 75% (P=0.011), 46.7% (P=0.009), and 23.5% (P=0.002) in response to increasing TP concentrations (Fig. 4B). These data provided direct evidence that the TP induced apoptosis.

TP induces apoptosis by the intrinsic and extrinsic pathways. Increasing TP concentrations resulted in the downregulation of Bcl-2 and Bcl-xl which are anti-apoptotic proteins of the Bcl-2 family. Downregulation of Bcl-2 or Bcl-xl affects mitochondrial function. Upregulation of Bax causes the release of cytochrome *c* through oligomerization from the cytosol to the mitochondrial outer membrane. Bim accelerates this process by inhibiting anti-apoptotic proteins such as Bcl-2 or Bcl-xl. The release of cytochrome *c* activates caspase-9, and cleaved caspase-9 further activates caspase-3. Finally PARP is cleaved into its active form resulting in DNA damage. Disruption of mitochondrial function by the intrinsic apoptosis pathway was observed. On the other hand, activation of caspase-8 is most likely associated with the stimulation of cell surface death receptors such as tumor necrosis factor receptors (TNF-R). Active caspase-8 directly cleaves caspase-3 to induce apoptosis. Furthermore, Bid is cleaved by active caspase-8 into tBid, which becomes an integral membrane protein in mitochondria and recruits Bax from the cytosol to the outer membrane, eventually causing the release of cytochrome *c*. The results showed that the TP induced apoptosis through the intrinsic and extrinsic pathways (Fig. 5).

TP inhibited tumor proliferation and induced apoptosis in a xenograft mouse model. Tumor volume was measured every 7 days in a PBS control group and a TP-treated group (100 mg/kg by intraperitoneal injection once daily for 21 days). After 7 days, the tumors in the control group began to grow rapidly. Measurements at 14 days showed a significantly greater tumor volume in the control group than in the TP group (P=0.005) (Fig. 6A). Mice were sacrificed at the end of treatment after 21 days. Tumors were harvested, weighed and photographed. The results showed that the tumor volume (P=0.017) and weight (P=0.004) were reduced in

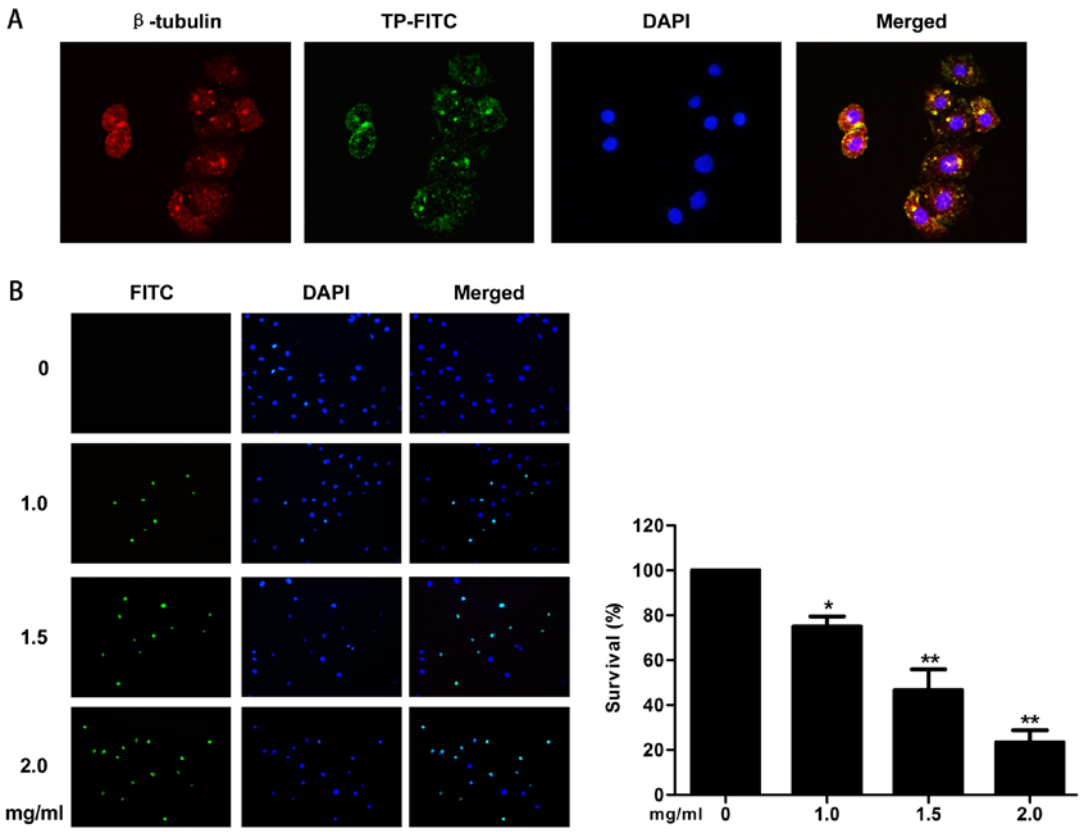

Figure 4. (A) TP, truncated peptide was mainly concentrated in the cytoplasm and cell membrane and partially in the nucleus. (B) The total cells were identified by DAPI stain and apoptotic cells were identified by FITC stain. *P<0.05, **P<0.01.

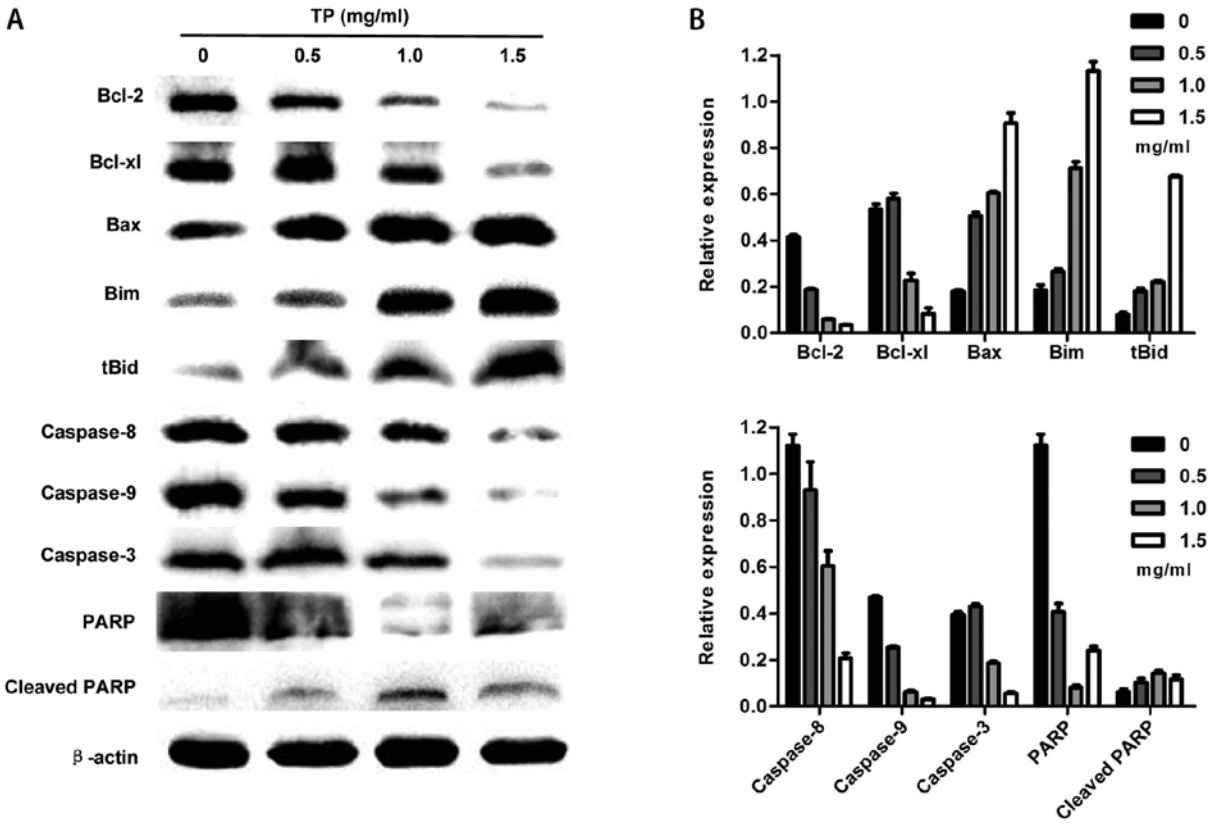

Figure 5. (A) TP, truncated peptide. The proteins were extracted from whole cell lysates after incubation with various TP concentrations (0, 0.5, 1.0 and 1.5 mg/ml) for 24 h. β-actin was used as the loading control. (B) The relative expression was calculated after normalization to β-actin. In the Bcl-2 family members, Bax, Bim, tBid were upregulated and Bcl-2 and Bcl-xl were downregulated. Activation of caspase-8 in the extrinsic pathway and caspase-9 in the intrinsic pathway were involved in the cleavage of caspase-3. Finally DNA was damaged with activation of PARP. The data are expressed as the mean ± SD.

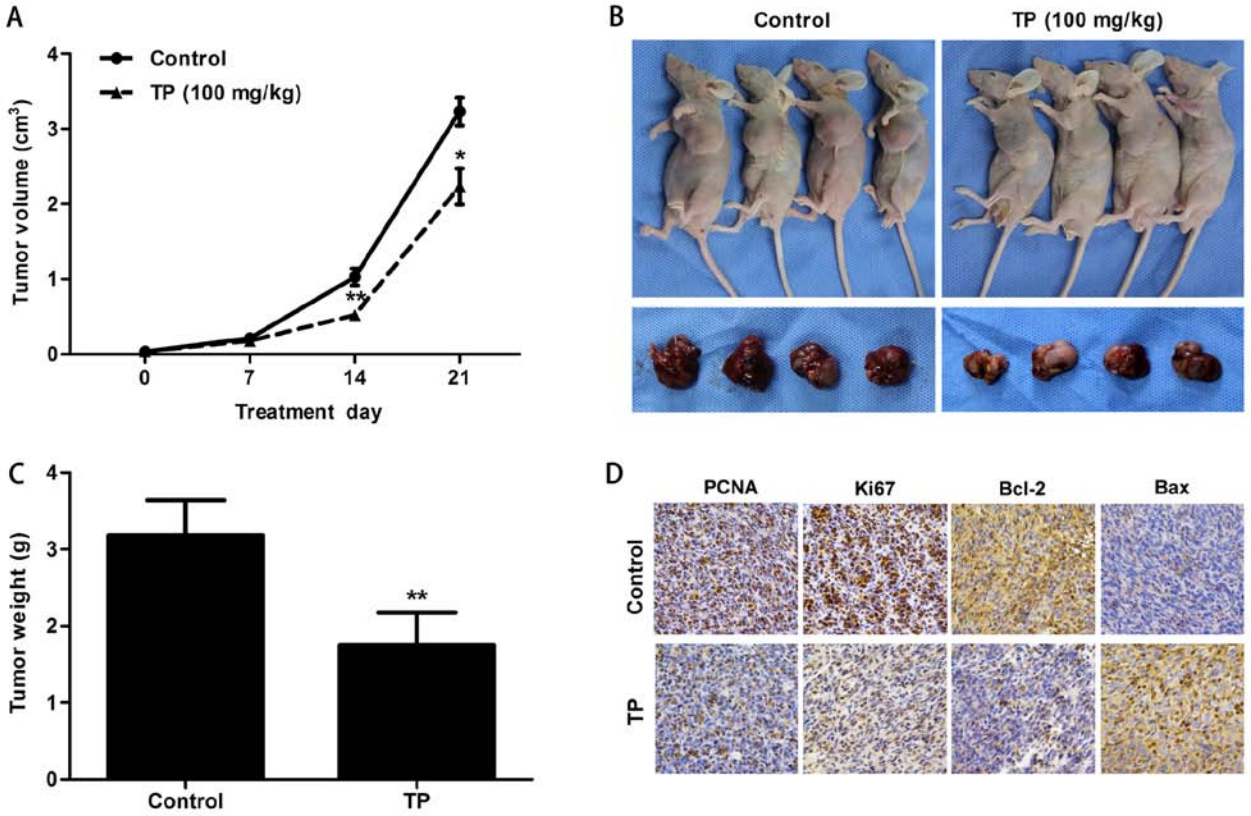

Figure 6. (A) TP, truncated peptide. Tumor volume was measured every 7 days. **P<0.01, *P<0.05. (B) At the end of the treatment, all mice were sacrificed. Tumors were harvested, weighed and photographed. (C) **P<0.01. (D) Downregulation of PCNA and Ki67 in the TP group indicated that tumor proliferation was inhibited. Upregulation of Bax and downregulation of Bcl-2 eventually induced apoptosis in the tumor.

the TP group (Fig. 6A-C). Tumors were fixed and embedded with paraffin, and the SP immunohistochemistry method was used to examine relative protein expression (Fig. 6D). Downregulation of PCNA and Ki67 in the TP group indicated the inhibition of tumor cell proliferation. Upregulation of Bax and downregulation of Bcl-2 probably enhanced the permeabilization of the mitochondrial membrane. The release of cytochrome *c* eventually induced apoptosis in the tumor.

Discussion

The present study examined the role of apoC-I in the tumorigenesis of NB. Although the TP is characterized by the loss of two residues compared with the mature peptide, the two isoforms have a similar sequence and probably play the same role *in vivo*. The hydrophilicity, antigen index, and surface probability predicted by DNAStar software indicated that the active sites were mainly concentrated in the TP. The SP may be an inactive form except for its function in guiding the nascent polypeptide into the endoplasmic reticulum for further processing (3,4). Based on these data, we synthesized the TP and used the SP as the negative control. The results of the CCK-8 assay showed that the TP selectively inhibited cell proliferation compared with the effect of the SP.

Based on the results of the CCK-8 assay at 24 h, we selected two TP concentrations (0.1 and 0.5 mg/ml) that did not induce cell apoptosis to study the effect of the TP on cell migration and invasion. The wound healing assay showed that the wound healing ability was reduced by various TP concentrations. The results of the Transwell assay showed that the migration ability was reduced, respectively, to 82.7 and 25.1% and the invasion ability was reduced to 50.4 and 25.6% compared with the control group. Therefore, the TP not only inhibited cell proliferation but also reduced the migration and invasion abilities. These results suggested that the TP is a promising therapeutic agent against metastasis in NB. However, the mechanism by which the TP reduced the metastatic ability is not clear, and further studies are necessary to clarify this.

The results of flow cytometry assays confirmed that the TP inhibited cell proliferation and induced apoptosis in NB. The Annexin V/PI assay showed that the apoptosis rates increased to 22.5 and 40%. The cell cycle assay demonstrated that cells were arrested in the S phase in response to increasing TP concentrations. JC-1 (18,19) is a sensitive method to detect the mitochondrial membrane potential. When the potential is high, JC-1 is mainly concentrated in the matrix of the mitochondrion in the form of aggregates. JC-1 monomers increase in response to alterations in the mitochondrial membrane potential. Our results showed that the TP induced apoptosis probably through the mitochondrial pathway.

TUNEL technology is an effective way to detect DNA cleavage during apoptosis (20,21). Increasing TP concentrations led to an increase in the percentage of cells with DNA breaks, indicating the induction of apoptosis. In addition, we observed that the peptide was mainly distributed in the membrane and cytoplasm, with a fraction detected in the nucleus. This suggested that the TP may have directly stimulated cell death receptors such as TNFRs (22) or entered into the cells to cause

changes in downstream signals, leading to the induction of apoptosis through the extrinsic and intrinsic pathways.

Bcl-2 and Bcl-xl are important anti-apoptotic members of the Bcl-2 family (23). Downregulation of Bcl-2 and Bcl-xl by the TP would reduce the stability of the mitochondrion. Meanwhile, upregulation of Bax enhances the permeability of the mitochondrion through oligomerization into the outer membrane from the cytosol (24). Overexpression of Bim promotes the process by antagonizing anti-apoptotic members of the Bcl-2 family (25-30). These factors together cause the release of cytochrome *c* (31) and the subsequent activation of procaspase-9 (32). Caspase-3 and PARP are further activated, leading to apoptosis. On the other hand, activation of caspase-8 (33) was most likely due to the stimulation of cell death receptors such as TNF-R by the TP. Active caspase-8 can directly cleave caspase-3 to induce apoptosis. Bid is also cleaved into the active form tBid by caspase-8. tBid (34,35) becomes an integral membrane protein in the mitochondrion and can strongly recruit Bax from the cytosol to the outer membrane, eventually causing the release of cytochrome *c*. The results showed that the TP induced apoptosis probably through the intrinsic and extrinsic pathways. In addition, the xenograft tumor mouse model confirmed that the TP inhibited tumor proliferation and induced apoptosis *in vivo*.

The truncated apoC-I is a post-translationally modified protein that mainly depends on the positively charged lysine residues (10,36,37) within the KVKEKLK motif to function. Whether the lysine residues play a role in suppressing NB remains unknown, and further research is necessary. Our results suggested that enrichment of the TP in the tumor could induce apoptosis in NB. However, one report in pancreatic cancer (13) indicated that inhibition of apoC-I expression suppresses cell proliferation and induces apoptosis. Therefore, dysfunction of apoC-I including upregulation or downregulation may affect the growth of tumors. Targeting apoC-I could therefore be a novel promising therapeutic approach for the treatment of malignant tumors.

References

1. Puppione DL: Higher primates, but not New World monkeys, have a duplicate set of enhancers flanking their ApoC-I genes. Comp Biochem Physiol Part D Genomics Proteomics 11: 45-48, 2014.
2. Puppione DL, Ryan CM, Bassilian S, Souda P, Xiao X, Ryder OA and Whitelegge JP: Detection of two distinct forms of ApoC-I in great apes. Comp Biochem Physiol Part D Genomics Proteomics 5: 73-79, 2010.
3. Knott TJ, Robertson ME, Priestley LM, Urdea M, Wallis S and Scott J: Characterisation of mRNAs encoding the precursor for human apolipoprotein CI. Nucleic Acids Res 12: 3909-3915, 1984.
4. Sabatini DD, Kreibich G, Morimoto T and Adesnik M: Mechanisms for the incorporation of proteins in membranes and organelles. J Cell Biol 92: 1-22, 1982.
5. Jackson RL, Sparrow JT, Baker HN, Morrisett JD, Taunton OD and Gotto AM Jr: The primary structure of apolopoprotein-serine. J Biol Chem 249: 5308-5313, 1974.
6. Shulman RS, Herbert PN, Wehrly K and Fredrickson DS: Thf complete amino acid sequence of C-I (apoLp-Ser), an apolipoprotein from human very low density lipoproteins. J Biol Chem 250: 182-190, 1975.
7. Bondarenko PV, Cockrill SL, Watkins LK, Cruzado ID and Macfarlane RD: Mass spectral study of polymorphism of the apolipoproteins of very low density lipoprotein. J Lipid Res 40: 543-555, 1999.
8. Gotoh H, Hagihara M, Nagatsu T, Iwata H and Miura T: Activity of dipeptidyl peptidase IV and post-proline cleaving enzyme in sera from osteoporotic patients. Clin Chem 34: 2499-2501, 1988.
9. Krasteva V, Brodeur MR, Tremblay FL, Falstrault L and Brissette L: Apolipoprotein C-I reduces cholesteryl esters selective uptake from LDL and HDL by binding to HepG2 cells and lipoproteins. Biochim Biophys Acta 1801: 42-48, 2010.
10. Westerterp M, Berbée JF, Delsing DJ, Jong MC, Gijbels MJ, Dahlmans VE, Offerman EH, Romijn JA, Havekes LM and Rensen PC: Apolipoprotein C-I binds free fatty acids and reduces their intracellular esterification. J Lipid Res 48: 1353-1361, 2007.
11. Ko HL, Wang YS, Fong WL, Chi MS, Chi KH and Kao SJ: Apolipoprotein C1 (APOC1) as a novel diagnostic and prognostic biomarker for lung cancer: A marker phase I trial. Thorac Cancer 5: 500-508, 2014.
12. Yamamoto-Ishikawa K, Suzuki H, Nezu M, Kamiya N, Imamoto T, Komiya A, Sogawa K, Tomonaga T, Nomura F and Ichikawa T: The isolation and identification of apolipoprotein C-I in hormone-refractory prostate cancer using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. Asian J Androl 11: 299-307, 2009.
13. Takano S, Yoshitomi H, Togawa A, Sogawa K, Shida T, Kimura F, Shimizu H, Tomonaga T, Nomura F and Miyazaki M: Apolipoprotein C-1 maintains cell survival by preventing from apoptosis in pancreatic cancer cells. Oncogene 27: 2810-2822, 2008.
14. Zhang J, Guo F, Wang L, Zhao W, Zhang D, Yang H, Yu J, Niu L, Yang F, Zheng S, *et al*: Identification of apolipoprotein C-I as a potential Wilms' tumor marker after excluding inflammatory factors. Int J Mol Sci 15: 16186-16195, 2014.
15. Chang HH, Liu YL, Lu MY, Jou ST, Yang YL, Lin DT, Lin KH1, Tzen KY, Yen RF, Lu CC, *et al*: A multidisciplinary team care approach improves outcomes in high-risk pediatric neuroblastoma patients. Oncotarget 17: 4360-4372, 2017.
16. Mao X, Chen Z, Zhao Y, Yu Y, Guan S, Woodfield SE, Vasudevan SA, Tao L, Pang JC, Lu J, *et al*: Novel multi-targeted ErbB family inhibitor afatinib blocks EGF-induced signaling and induces apoptosis in neuroblastoma. Oncotarget 8: 1555-1568, 2017.
17. Chen Z, Wang L, Yao D, Yang T, Cao WM, Dou J, Pang JC, Guan S, Zhang H, Yu Y, *et al*: Wip1 inhibitor GSK2830371 inhibits neuroblastoma growth by inducing Chk2/p53-mediated apoptosis. Sci Rep 6: 38011, 2016.
18. Zhang F, Kong DS, Zhang ZL, Lei N, Zhu XJ, Zhang XP, Chen L, Lu Y and Zheng SZ: Tetramethylpyrazine induces G0/G1 cell cycle arrest and stimulates mitochondrial-mediated and caspase-dependent apoptosis through modulating ERK/p53 signaling in hepatic stellate cells in vitro. Apoptosis 18: 135-149, 2013.
19. Zhao P, Han T, Guo JJ, Zhu SL, Wang J, Ao F, Jing MZ, She YL, Wu ZH and Ye LB: HCV NS4B induces apoptosis through the mitochondrial death pathway. Virus Res 169: 1-7, 2012.
20. Nagamine A, Hasegawa H, Hashimoto N, Yamada-Inagawa T, Hirose M, Kobara Y, Tadokoro H, Kobayashi Y and Takano H: The effects of DPP-4 inhibitor on hypoxia-induced apoptosis in human umbilical vein endothelial cells. J Pharmacol Sci 133: 42-48, 2017.
21. Xu L, Deng Y, Feng L, Li D, Chen X, Ma C, Liu X, Yin J, Yang M, Teng F, *et al*: Cardio-protection of salvianolic acid B through inhibition of apoptosis network. PLoS One 6: e24036, 2011.
22. Derakhshan A, Chen Z and Van Waes C: Therapeutic small molecules target inhibitor of apoptosis proteins in cancers with deregulation of extrinsic and intrinsic cell death pathways. Clin Cancer Res 23: 1379-1387, 2017.
23. Cory S and Adams JM: The Bcl2 family: Regulators of the cellular life-or-death switch. Nat Rev Cancer 2: 647-656, 2002.
24. Murphy KM, Ranganathan V, Farnsworth ML, Kavallaris M and Lock RB: Bcl-2 inhibits Bax translocation from cytosol to mitochondria during drug-induced apoptosis of human tumor cells. Cell Death Differ 7: 102-111, 2000.
25. Bouillet P, Purton JF, Godfrey DI, Zhang LC, Coultas L, Puthalakath H, Pellegrini M, Cory S, Adams JM and Strasser A: BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. Nature 415: 922-926, 2002.
26. Whitfield J, Neame SJ, Paquet L, Bernard O and Ham J: Dominant-negative c-Jun promotes neuronal survival by reducing BIM expression and inhibiting mitochondrial cytochrome *c* release. Neuron 29: 629-643, 2001.

27. O'Connor L, Strasser A, O'Reilly LA, Hausmann G, Adams JM, Cory S and Huang DC: Bim: A novel member of the Bcl-2 family that promotes apoptosis. EMBO J 17: 384-395, 1998.
28. Ley R, Balmanno K, Hadfield K, Weston C and Cook SJ: Activation of the ERK1/2 signaling pathway promotes phosphorylation and proteasome-dependent degradation of the BH3-only protein, Bim. J Biol Chem 278: 18811-18816, 2003.
29. Lei K and Davis RJ: JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. Proc Natl Acad Sci USA 100: 2432-2437, 2003.
30. Liu JW, Chandra D, Tang SH, Chopra D and Tang DG: Identification and characterization of Bimgamma, a novel proapoptotic BH3-only splice variant of Bim. Cancer Res 62: 2976-2981, 2002.
31. Wei H, Li Z, Hu S, Chen X and Cong X: Apoptosis of mesenchymal stem cells induced by hydrogen peroxide concerns both endoplasmic reticulum stress and mitochondrial death pathway through regulation of caspases, p38 and JNK. J Cell Biochem 111: 967-978, 2010.
32. Zou H, Li Y, Liu X and Wang X: An APAF-1.cytochrome *c* multimeric complex is a functional apoptosome that activates procaspase-9. J Biol Chem 274: 11549-11556, 1999.
33. Strasser A, Jost PJ and Nagata S: The many roles of FAS receptor signaling in the immune system. Immunity 30: 180-192, 2009.
34. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, Roth KA, MacGregor GR, Thompson CB and Korsmeyer SJ: Proapoptotic BAX and BAK: A requisite gateway to mitochondrial dysfunction and death. Science 292: 727-730, 2001.
35. Korsmeyer SJ, Wei MC, Saito M, Weiler S, Oh KJ and Schlesinger PH: Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome c. Cell Death Differ 7: 1166-1173, 2000.
36. Bus P, Pierneef L, Bor R, Wolterbeek R, van Es LA, Rensen PC, de Heer E, Havekes LM, Bruijn JA, Berbée JF, *et al*: Apolipoprotein C-I plays a role in the pathogenesis of glomerulosclerosis. J Pathol 241: 589-599, 2017
37. Berbée JF, van der Hoogt CC, Kleemann R, Schippers EF, Kitchens RL, van Dissel JT, Bakker-Woudenberg IA, Havekes LM and Rensen PC: Apolipoprotein CI stimulates the response to lipopolysaccharide and reduces mortality in gram-negative sepsis. FASEB J 20: 2162-2164, 2006.