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Cell Growth Inhibition and Induction of Apoptosis by Snake Venom Toxin in Ovarian Cancer Cell via Inactivation of Nuclear Factor κB and Signal Transducer and Activator of Transcription 3

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Snake venom toxin from *Vipera lebetina turanica* induces apoptosis in many cancer cell lines, but there is no study about the apoptotic effect of snake venom toxin on human ovarian cancer cells. In this study, we investigated the apoptotic effect of snake venom toxin in human ovarian cancer PA-1 and SK-OV3 cells. Snake venom toxin dose dependently (0~10 μg/mL) inhibited ovarian cancer cell growth with IC_{50} values 4.5 μg/mL in PA-1 cells, and 6.5 μg/mL in SK-OV3 cells. Our results also showed that apoptotic cell death increased by snake venom toxin in a dose dependent manner (0~10 μg/mL). Consistent with increased cell death, snake venom toxin increased the expression of pro-apoptotic protein Bax and caspase-3, but down-regulated anti-apoptotic protein Bcl-2. Untreated ovarian cancer cells showed a high DNA binding activity of nuclear factor B (NF-κB), but it was inhibited by snake venom toxin accompanied by inhibition of p50 and p65 translocation into the nucleus as well as phosphorylation of inhibitory κB. Snake venom toxin also inhibited DNA binding activity of the signal transducer and activator of transcription 3 (STAT3). Moreover, the combination treatment of NF-κB (salicylic acid, 1 or 5 μM) and STAT3 (stattic, 1 μM) with snake venom toxin (1 μg/mL) further enhanced cell growth inhibitory effects of snake venom toxin. These results showed that snake venom toxin from *Vipera lebetina turanica* caused apoptotic cell death of ovarian cancer cells through the inhibition of NF-κB and STAT3 signal, and suggested that snake venom toxin may be applicable as an anticancer agent for ovarian cancer.

Key words: Snake venom toxin, NF-κB, STAT3, Ovarian cancer cells

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INTRODUCTION

Ovarian cancer, the second most common gynecological cancer is still ranked among the top 5 of female cancer deaths in the USA (Tanwar et al., 2011). Because ovarian cancer is often asymptomatic, the majority of patients with newly diagnosed ovarian cancer present the disease in its advanced-stage (Jemal et al., 2010). The histology of these tumors is complex and their diagnosis frequently poses problems (Hatzipantelis and Dinas, 2010). Despite initial response to surgical debulking and chemotherapy, most tumors eventually develop a drug resistant relapse (Armstrong, 2002). Actually, in spite of the 80% initial response rate to the standard primary regimen of carboplatin and paclitaxel, most women with ovarian cancer experience recurrence with incurable symptoms within five years (Galic et al., 2011). Thus, there is a requirement for new targets unlike conventional chemotherapy.

Recent studies reported that the signal transducer and activator of transcription 3 (STAT3) is constitutively

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activated in ovarian cancer cell lines and clinical specimens (Huang et al., 2000; Burke et al., 2001; Savarese et al., 2002). The STAT3 usually resides in the cytoplasm and can be activated through phosphorylation by cytokines, hormones, and growth factors, which utilizes STAT3 signaling to control a remarkable variety of biological responses, including cell development, differentiation, proliferation, motility, and survival (Silver et al., 2004; Duan et al., 2006). Following phosphorylation, STAT3 homodimerizes, translocates to the nucleus, and induces transcription of several STAT3-dependent genes involved in proliferation, survival, invasion and metastasis. Silver et al. found that depletion of STAT3 by siRNA inhibited SK-OV3 cell migration *in vitro* and this indicates that activated STAT3 contributes to ovarian cancer cell motility *in vitro*. They suggested the possibility that STAT3 contributes to invasion and possibly metastasis *in vivo* (Silver et al., 2004).

Ovarian cancer has been shown to secrete various proinflammatory and proangiogenic substances in direct response to Nuclear factor kappaB (NF-κB) activity in SK-OV3 ovarian cancer cells, such as the chemokine interleukin (IL)-6 and IL-8 (Huang et al., 2000; Chou et al., 2005). NF-κB plays a crucial role in the suppression of apoptosis, and in the induction of cell proliferation and inflammation, and is closely associated with cancer development (Karin et al., 2002). Constitutive activation of NF-κB has been described in a great number of cancers including colon cancers, prostate cancers, ovarian cancers and melanoma (Shukla et al., 2004; Annunziata et al., 2010), and was found to up-regulate anti-apoptotic genes and/or down regulate apoptotic gene expression (Deveraux and Reed, 1999). STAT3 is also often persistently activated in the same tumor cells. Both transcription factors induce the expression of a highly overlapping repertoire of proliferative, anti-apoptotic, angiogenic and metastatic genes that promote tumor development and growth (Yu et al., 2009). For these reasons, the NF-κB and STAT3 signaling pathway is a potential target for ovarian cancer therapy.

Snake venom toxins are greatly feared, but many researchers believe natural snake venom toxins are useful biological resources, containing several pharmacologically active components that could be of potential therapeutic value (Servent et al., 1997; Michalet et al., 2000; Siigur et al., 2001; Bennacef-Heffar and Laraba-Djebari, 2003). We previously found that snake venom toxin from *Vipera lebetina* inhibited NF-κB activation and targets gene expression through its interaction with signal molecules in the NF-κB pathway (Park et al., 2005), and also showed that snake venom toxin inhibited prostate cancer cell and neuroblastoma cell growth through induction of apoptotic cell death (Son et al., 2007; Park et al., 2009). Consequently, snake venom toxin may be useful as an alternative compound regarding chemotherapy agents.

In this study, we evaluated the ovarian cancer cells response to snake venom toxin from *V. lebetina,* in order to determine the ability of this venom toxin to act as a therapeutic agent by suppression of ovarian cancer cell growth through induction of apoptotic cell death via inactivation of STAT3 and NF-κB.

MATERIALS AND METHODS

Materials

Snake venom toxin (SVT) from *V. lebetina turanica* and 2, 7 dichlorofluorescein diacetate (DCFH-DA) were purchased from Sigma Aldrich. Polyclonal antibodies against Bcl-2 and Bax were obtained from Santa Cruz Biotechnology Inc. ECL chemiluminescence system was obtained from obtained from Amersham Pharmacia Biotech.

Cell culture

The PA-1 and SK-OV3 ovarian cancer cell lines were obtained from American Type Culture Collection (ATCC). The PA-1 ovarian cancer cell was cultured in Eagle's Minimal Essential medium (EMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) and 100 units/mL penicillin, 100 μg/mL streptomycin (Invitrogen). The SK-OV3 ovarian cancer cell was cultured in RPMI supplemented with 10% heat inactivated FBS and penicillin, streptomycin (100 units/mL). Cell cultures were then maintained in an incubator within a humidified atmosphere of 5% CO_2 at 37°C. Cell morphology was ovaluated using phase-contrast microscopy (Elclipse TE-300; Nikon Instech Co.).

Cell viability assay

Cells were plated in 96-well plates, and subconfluent ovarian cancer cells, PA-1 and SK-OV3 cells were subsequently treated with snake venom toxin 0~10 μg/mL for 24 h. After treatment, cell viability was measured by MTT assay (Sigma Aldrich) according to the manufacturer's instructions. Briefly, MTT (2 mg/mL) was added and plates were incubated at 37°C for 4 h before 100 μL dimethyl sulfoxide was added to each well. Finally, the absorbance of each well was read at a wavelength of 570 nm using a microplate reader.

Apoptosis evaluation

Apoptosis assays were done by observing morphologic

changes and by the terminal nucleotidyl transferase-mediated nick end labeling (TUNEL) assay. In short, PA-1 and SK-OV3 cells were cultured on eight-chamber slides. Moreover, after treatment with snake venom toxin (2 to 8 μg/mL) or vehicle for 24 h, the cells were washed twice with phosphatebuffered saline (PBS), and then fixed and processed for 4', 6-diamidino-2-phenylindole and TUNEL staining assay.

Western blotting

Ovarian cancer cells treated with snake venom toxin (0~8 μg/mL) for 24 h were homogenized with a protein extraction solution (PRO-PREPTM, Intron Biotechnology), and lysed by 60 min incubation on ice. The cell lysate was centrifuged at 15,000 rpm for 15 min at 4°C. Equal amount of proteins (40 μg) were separated on a SDS/12%-polyacrylamide gel, and then transferred to a polyvinylidene difluoride (PVDF) membrane (GE Water and Process technologies). Blots were blocked for 1 h at room temperature with 5% (w/v) non-fat dried milk in Tris-Buffered Saline Tween-20 [TBST: 10 mM Tris (pH 8.0) and 150 mM NaCl solution containing 0.05% Tween-20]. After being washed in TBST for a short period of time, the membranes were immunoblotted with the following primary antibodies: mouse monoclonal antibodies directed against p65 and p50 (1:500 dilutions; Santa Cruz Biotechnology), rabbit polyclonal antibodies directed against bax (1: 500 dilutions; Santa Cruz Biotechnology), and against caspase-3 and Bcl-2 (1:1000 dilutions; Cell Signaling Technology). The blots were incubated with the respective horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (1:4000 dilutions; Santa Cruz Biotechnology). Immunoreactive proteins were detected with the ECL detection system.

Gel electromobility shift assay

A gel electromobility shift assay (EMSA) was performed according to the manufacturer's recommendations (Promega). Briefly, the cell were homogenized in 200 μL of solution A (10 mM HEPES [pH 7.9], 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonylfluoride), vortexed vigorously, incubated on ice for 10 min, and then centrifuged at 15000 rpm for 15 min. The pelleted nuclei were resuspended in solution C (solution A supplemented with 420 mM NaCl and 20% glycerol), and incubated on ice for 20 min. The resuspended pellets were centrifuged at 15000 rpm for 15 min, and the resulting nuclear extracts upernatant was collected in a chilled Eppendorf tube. Consensus oligonucleotides were end-labeled using T4 polynucleotide kinase and [P^{32}]-ATP for 10 min at 37°C. The gel shift reactions were assembled and incubated at room temperature. Subsequently, 1 mL of a gel loading buffer was added to each reaction and loaded onto a 6% non-denaturating gel. Furthermore, the gel was subjected to electrophoresis until the dye was four-fifths of the way down the gel. Finally, the gel was dried at 80°C for 1 h and exposed to film overnight at −70°C.

Treatment NF-κB and STAT3 inhibitor

Cells were plated in 24-well plates, and subconfluent cells were subsequently treated with a combination of snake venom toxin (1 μg/mL) and STAT3 inhibitor stattic (1 μM) or with NF-κB inhibitor salicylic acid (1 or 5 μM) for 24 h. Afterwards, cells were trypsinized and pelleted by centrifugation for 5 min at 1500 rpm, resuspended in PBS, and 0.2% trypan blue was added to the cancer cell suspension in each of the solutions. Subsequently, a drop of suspension was placed into a Neubauer chamber and the living cancer cells were counted. Cells that showed signs of staining were considered to be dead, whereas those that excluded trypan blue were considered viable. In addition, each assay was carried out in triplicate.

Statistical analysis

The data were analyzed using the GraphPad Prism 4 ver. 4.03 software (GraphPad Software). Data were presented as mean ± S.D. The differences in all data were assessed by one-way analysis of variance (ANOVA). When the p value in the ANOVA test indicated statistical significance, the differences were assessed by the Dunnett's test. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Inhibition of cell growth and induction of apoptosis

To evaluate the effect of snake venom toxin from *V. lebetina turanica* on the cell growth of human ovarian cancer cells, we analyzed cell viability using the MTT assay. Snake venom toxin (0~10 μg/mL) inhibited growth of human ovarian cancer cells; PA-1 (Fig. 1A) and SK-OV3 (Fig. 1B) with IC_{50} values of 4.5 and 6.5 μg/mL, respectively. We evaluated changes in the morphology of human ovarian cancer cells using phase-contrast microscopy. Control cells treated with vehicle for 24 h were completely attached to the dish showing typical morphology. Snake venom toxin treated PA-1 cells for 24 h showed rounded morphology and tended to detach from substratum. However, snake venom toxin treated SK-OV3 cells for 24 h presented with cytoplasmic blebbing, cell shrinkage, cytoplasmic con-

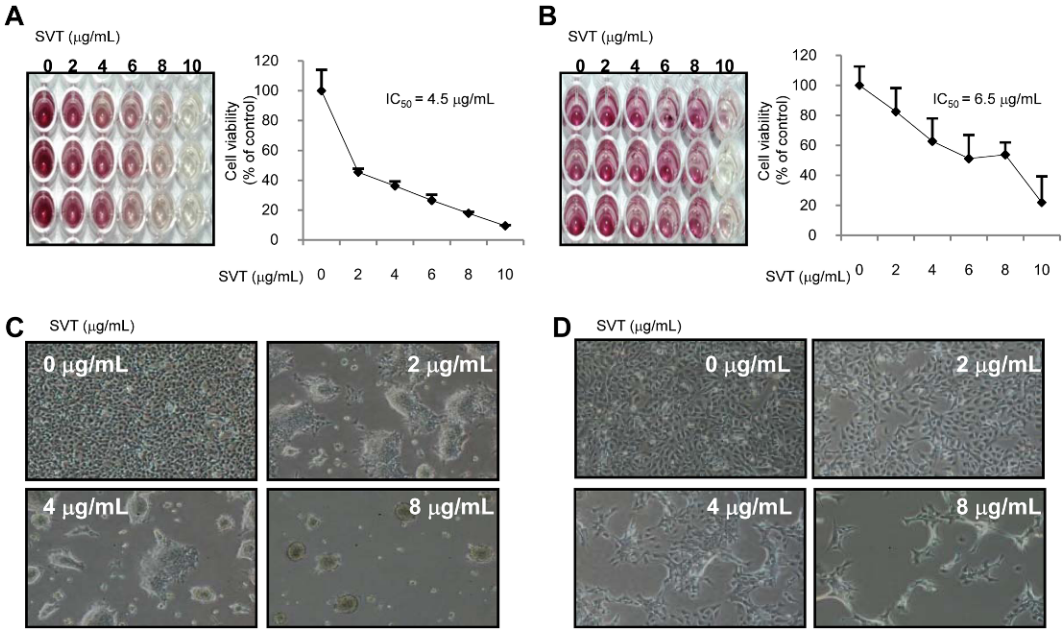

Fig. 1. Cell viability in response to snake venom toxin. Concentration-dependent effect of snake venom toxin on the MTT assay in PA-1 cells (**A**), and SK-OV3 cells (**B**). Morphologic observation with the treatment of snake venom toxin. PA-1 and SK-OV3 cells morphological changes were observed under phase contrast microscope (**C** and **D**, respectively).

densation, and irregularity in shape (Fig. 1C and 1D). To determine whether the inhibition of cell growth by the snake venom toxin was due to an increase in the induction of apoptosis, we also evaluated PA-1 and SK-OV3 cell apoptosis by TUNEL assay. TUNEL positive cells dose-dependently increased in snake venom toxin-treated PA-1 (Fig. 2A) and SK-OV3 cells (Fig. 2B). These results show that snake venom toxin treatment strongly induced apoptosis in human ovarian cancer cells.

Effect of snake venom toxin on the expression of apoptosis regulatory proteins

To figure out the relationship between the induction of apoptosis and the expression of their regulatory protein by snake venom toxin, expression of apoptosis related proteins was investigated. The expression of anti-apoptotic protein Bcl-2 was decreased by treatment of snake venom toxin from 1 to 8 μg/mL in a concentration dependent manner, and the expression of pro-apoptotic protein Bax and caspase 3 increased from the treatment of snake venom toxin using 2 to 8 μg/mL in a concentration dependent manner in PA-1 (Fig. 3A) and SK-OV3 cells (Fig. 3B).

Involvement of NF-κB and STAT3 signaling pathway in apoptotic cell death by snake venom toxin

Snake venom toxin had been shown to negatively regulate the nuclear transcription factor NF-κB by means of protein-protein interaction (Park et al., 2005). NF-κB has known to be an inhibitory transcription factor of apoptosis. STAT3 directly interacted with NF-κB family, trapping it in the nucleus and thereby contributing to constitutive NF-κB activation in cancer (Lee et al., 2009). To investigate whether snake venom toxin could inactivate NF-κB and STAT3, and thereby hinder its anti-apoptotic ability, ultimately causing the cells to undergo apoptosis, we assessed NF-κB and STAT3 activity in PA-1 and SK-OV3 cells treated with various concentrations of snake venom toxin for 24 h. NF-κB and STAT3 was highly activated in PA-1 and SK-OV3 cells, however, the constitutive activation of NF-κB and STAT3 was gradually reduced by culturing the cells in the presence of snake venom toxin (Fig. 4A and 4B, and 5A and 5B). Moreover, translocation of p50 and p65 into the nucleus also decreased, as shown by Western blotting (Fig. 4C and 4D). The decrease in p50 translocation was accompanied by a decrease in the phosphorylation of IκB in the cytosol (Fig. 4C and 4D). PA-1 and SK-OV3 cells treated with snake venom toxin also showed a dramatic reduction in total phosphorylated STAT3 protein levels (Fig. 5C and 5D). To further demonstrate the involvement of NF-κB and STAT3 pathway in snake venom toxin-induced ovarian cancer cell growth, we compared the combination treatment of snake venom toxin and NF-κB inhibitor (salicylic acid, 1 or 5 μM) or STAT3 (Stattic, 1 μM) with snake venom, NF-κB inhibitors, or STAT3 inhibitors alone. The

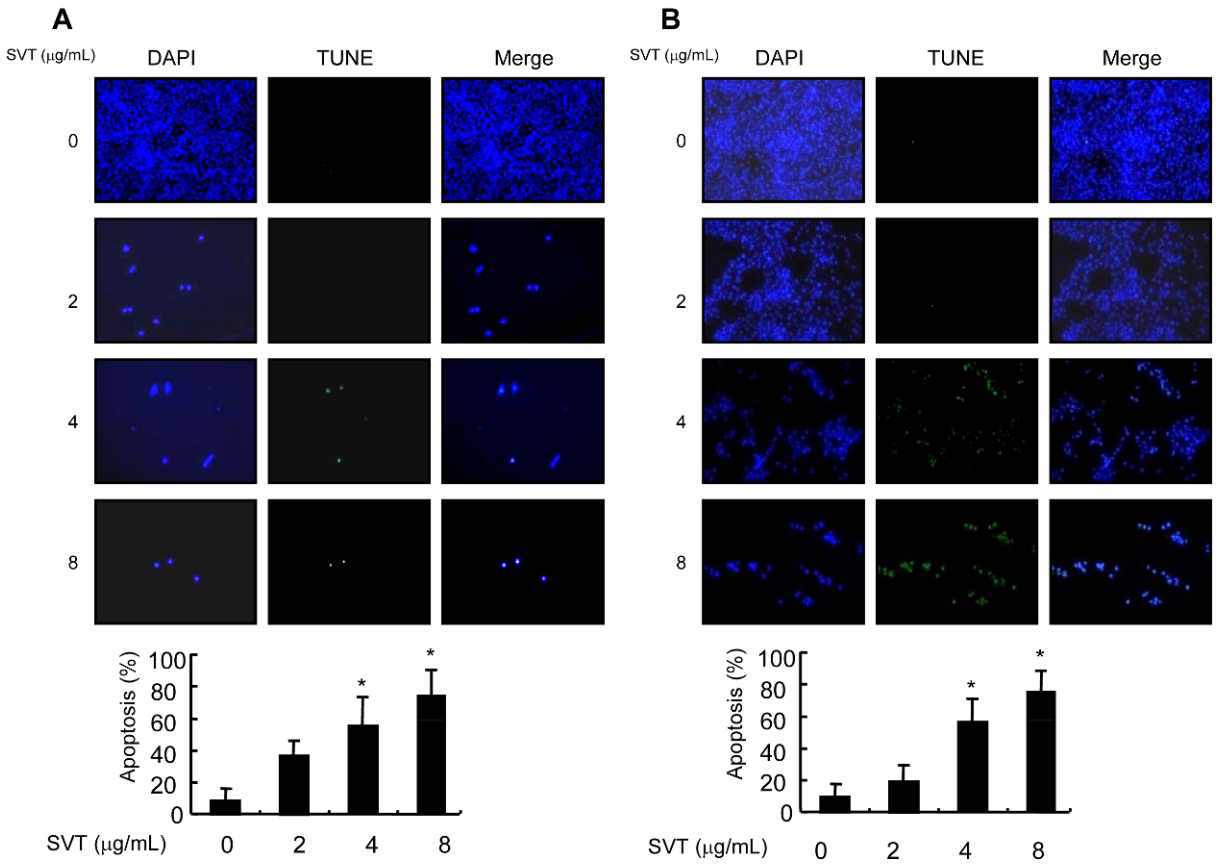

Fig. 2. Effect of snake venom toxin on apoptotic cell death. The ovarian cancer cells were treated with snake venom toxin for 24 h, and then labeled with DAPI and TUNEL solution. Total number of cells in a given area was determined by using DAPI nuclear staining (fluorescent microscope). The green color in the fixed cells marks TUNEL-labeled cells. The apoptotic index was determined as the DAPI-stained TUNEL-positive cell number/total DAPI stained cell number (magnification, 200×). Values are means ± S.D. from three experiments. Each experiment was done in triplicates. *$p < 0.05$, significantly different from untreated control cells.

combination treatment of snake venom toxin with NF-κB inhibitors or STAT3 inhibitors greatly inhibited ovarian cancer cell growth compared to those by snake venom toxin, NF-κB inhibitors, or STAT3 inhibitors alone in both cancer cells (Fig. 6A and 6B).

DISCUSSION

In this study, we found that snake venom toxin from *V. lebetina turanica* inhibited the growth of human ovarian cancer cells. Snake venom toxin also caused apoptosis cell death through inhibition of NF-κB and STAT3 signals.

NF-κB regulates the transcription genes involved in cell differentiation, proliferation and apoptosis (Ghosh and Hayden, 2008; Yamamoto and Takeda, 2008). Constitutive NF-κB signaling has been identified in tumors of epithelial origin, including breast, colon, lung, and ovarian carcinomas (Karin, 2006). Recent work has suggested the importance of NF-κB in the propagation of ovarian cancer cells (Lin et al., 2007). STAT3 is also known to be an important transcription factor regulating cancer cell growth and apoptosis (Yu et al., 2009). Recent studies have reported that abnormal levels of STAT3 activation have been observed in ovarian cancer cell lines (Huang et al., 2000; Burke et al., 2001; Savarese et al., 2002). Thus, we hypothesized that the inhibition of NF-κB and STAT3 signaling may be significant contributors in snake venom toxin-induced ovarian cancer cell death. In fact, we have shown that the down-regulation of NF-κB and STAT3 signals by snake venom toxin in the PA-1 and SK-OV3 ovarian cancer cells were consistent with cell growth inhibition. Snake venom toxin inhibits constitutively activated NF-κB signaling by impairing IκBα phosphorylation with the inhibition of p50 and p65 translocation. Consistent with the present findings, we previously found that snake venom toxin binds with NF-κB, IKKα, and IKKβ, resulting in the down-regulation of NF-κB activity in RAW 264.7 cells and

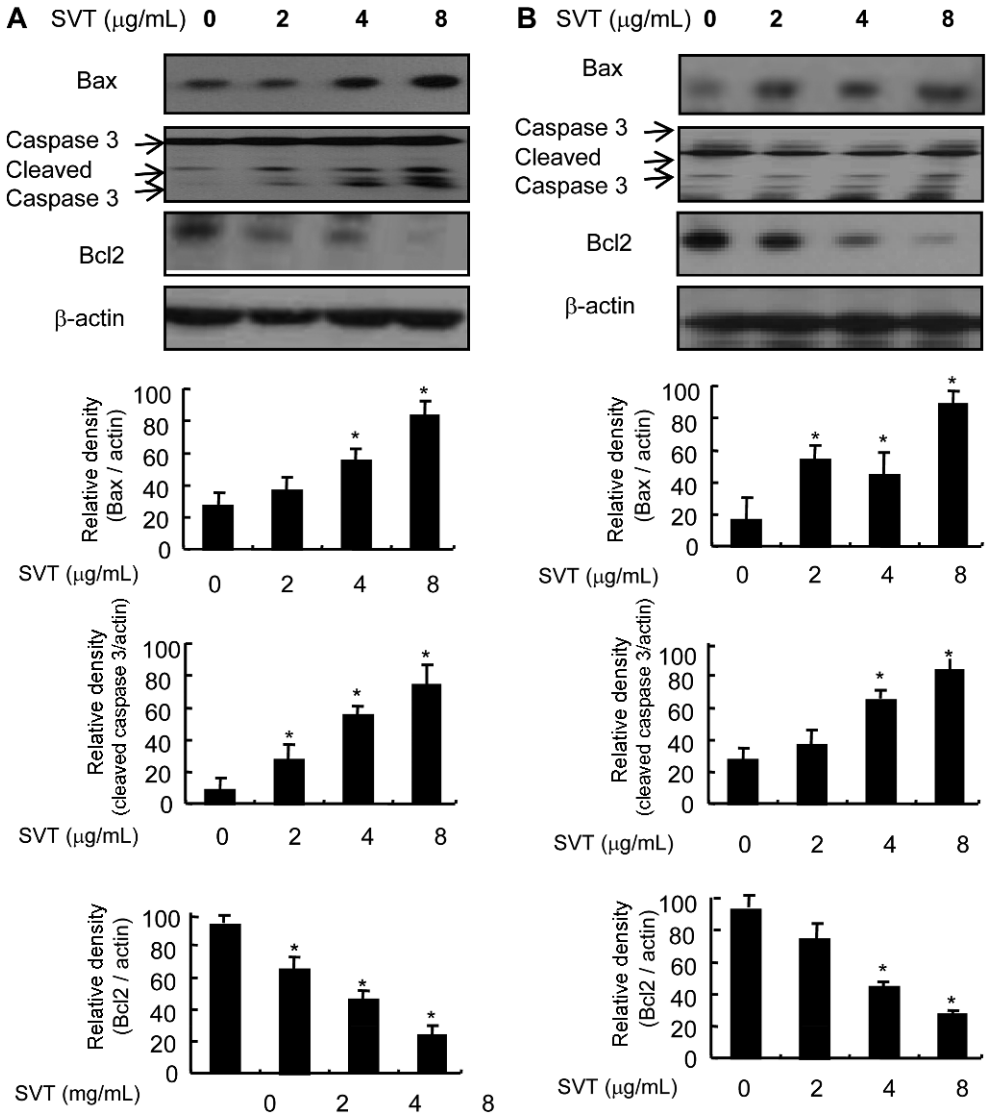

Fig. 3. Effects of snake venom toxin on expression of apoptosis-related proteins in PA-1 cells and SK-OV3 cells. Equal amounts of total proteins (40 μg/lane) were subjected to 12% SDS-PAGE. Expression of Bax, caspase-3, Bcl2, and β-actin were detected by Western blotting using specific antibodies. β-Actin protein here was used as an internal control. Values are mean relative intensities ± S.D. from three experiments. *$p < 0.05$, significantly different from untreated cells.

astrocytes (Lin et al., 2000). Son et al. demonstrated that snake venom toxin has inhibitory effects on human prostate PC-3 cancer cell growth through the inactivation of NF-κB (Son et al., 2007). Chiu et al. also suggested that Cardiotoxin III (CTX III), a basic polypeptide isolated from Naja naja atra venom inhibited the NF-κB activation through inhibition of IκB kinase activity in human breast MCF-7 cancer cells, leading to the suppression of proliferation and induction of apoptosis (Chiu et al., 2009). Moreover, we also found that the combination of snake venom toxin with a NF-κB inhibitor further enhanced the ovarian cancer cell growth inhibitory effect of snake venom toxin. These data suggest that NF-κB could be important in snake venom toxin-induced ovarian cancer cell growth inhibition.

We also assessed the STAT3 activity in ovarian cancer cells treated snake venom toxin since STAT3 was also implicated in ovarian cancer cell growth (Chien et al., 2010; Lin et al., 2010; Selvendiran et al., 2010). Cell growth inhibition and induction of apoptotic cell death by snake venom toxin were also associated with decrease of STAT3 activity (decreased phosphorylation) in ovarian cancer cells. Similar to our results, a recent study reported that cardiotoxin III induces apoptosis via suppressed the phosphorylation of STAT3 in oral squamous cell carcinoma Ca9-22 cells and human breast MDA-MB-231 cancer cells (Chien et al.,

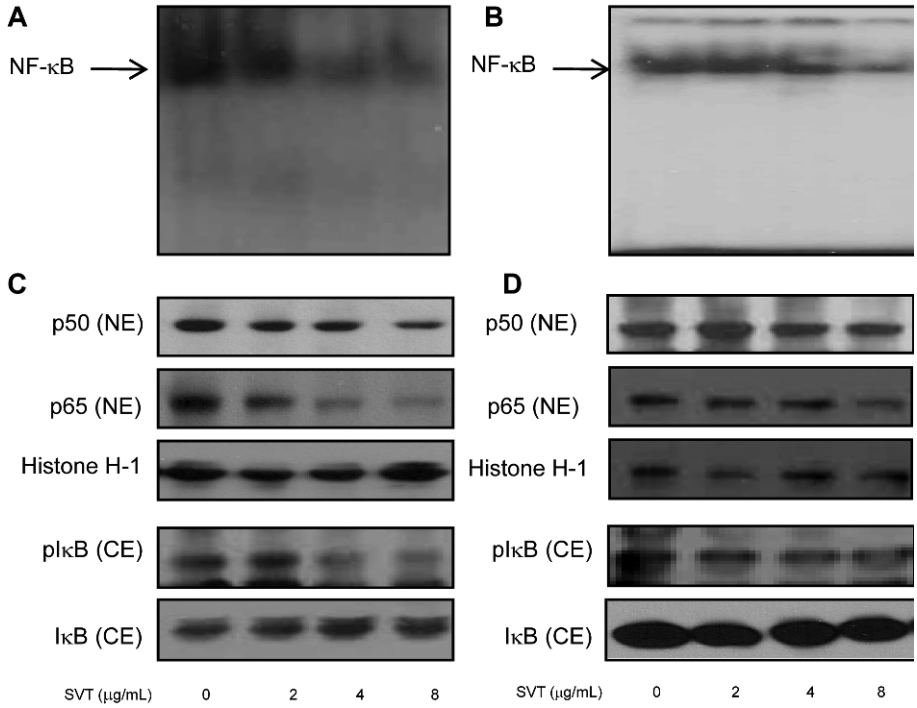

Fig. 4. Effect of snake venom toxin on NF-κB activation in PA-1 cells and SK-OV3 cells. (**A** and **B**) NF-κB DNA binding activity was determined. (**C** and **D**) Cytosolic (CE) and nuclear proteins (NE) were used to determine the expression of p50, p65, and IκB. Equal amounts of total proteins (40 μg/lane) were subjected to 12% SDS-PAGE. Expression of pSTAT3 and STAT3 were detected by Western blotting using specific antibodies. Each band was representative for three experiments.

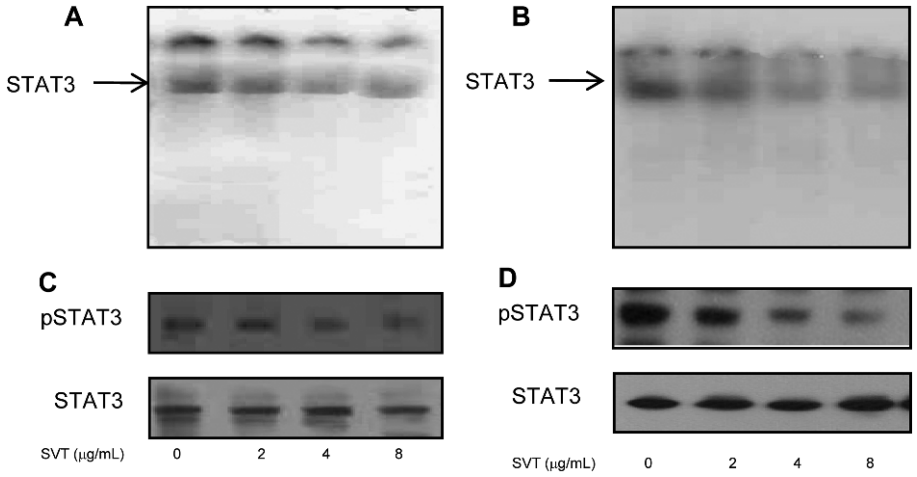

Fig. 5. Effect of snake venom toxin on STAT3 activation in PA-1 and SK-OV3 cells. (**A** and **B**) STAT3 DNA binding activity was determined. (**C** and **D**) Equal amounts of total proteins (40 μg/lane) were subjected to 8% SDS-PAGE. Expression of pSTAT3 and STAT3 were detected by Western blotting using specific antibodies. Each band is representative for three experiments.

2010; Lin et al., 2010). In ovarian cancer, several compounds inhibited cell growth through inactivation of STAT3. Selvendiran et al. demonstrated that a difluorodiarylidenyl piperidone (HO-3867) exhibited significant cytotoxicity toward ovarian cancer cells by inhibition of the STAT3 signaling pathway in human ovarian cancer cells, A2870, OV-4, SKOV3, and OVCAR3. *In vivo* study also demonstrated that the blocking of STAT3 inhibited tumor growth in BALB/c nude mice subcutaneously injected with A2780 ovarian cancer cells (Selvendiran et al., 2010). The combination treatment of snake venom toxin and STAT3 inhibitor (Stattic) greatly inhibited ovarian cancer cell growth compared to those by snake venom toxin or STAT3

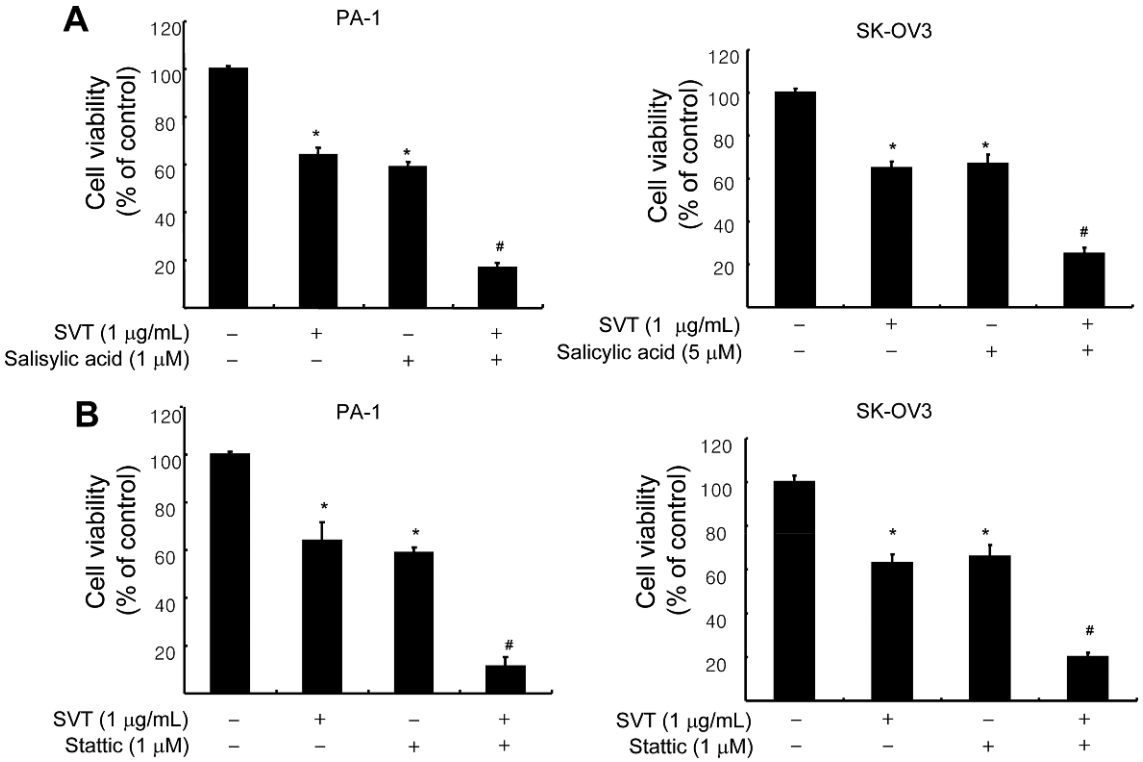

Fig. 6. Cells were treated with combination of snake venom toxin (1 μg/mL) and NF-κB inhibitor (salicylic acid) or snake venom toxin and STAT3 inhibitor (stattic) for 24 h. The cells were harvested by trypsinization and stained with 0.2% trypan blue. Relative cell survival rate was determined by counting live and dead cells. The results were expressed as a percentage of viable cells. Values are means ± S.D. from three experiments. *$p < 0.05$, significantly differed from untreated control cells.

inhibitors alone. These data indicated that STAT3 could also be a target of snake venom toxin in ovarian cancer cell growth inhibition.

Reduced nuclear translocation of NF-κB proteins was associated with the down-regulation of the constitutively overexpressed or NF-κB dependent apoptotic proteins. Several genes (proteins), such as Bcl-2, and/or upregulated apoptotic genes, such as Bax and caspase-3, were regulated by NF-κB (Campbell et al., 2004; Hayden and Ghosh, 2004). Downstream proteins of STAT3 such as Bcl-xL, Bcl-2, and survivin have also been shown to suppress cancer cell apoptosis (Nielsen et al., 1999; Kim et al., 2006). In agreement with down regulation of NF-κB and STAT3 signals, we found that expression of proapoptotic proteins Bax, and caspase-3 was significantly activated, whereas the expression of anti-apoptotic protein Bcl-2 was down-regulated by snake venom toxin. Similar to our findings, CTX III also indicated apoptosis accompanied with upregulation of Bax, and downregulation of Bcl-2 through inhibition of NF-κB and/or the STAT3 signal (Chiu et al., 2009; Chien et al., 2010; Lin et al., 2010). In addition, HO-3867 promoted apoptosis by caspase-3 activation via inhibition of the JAK/STAT3 signaling pathway (Selvendiran et al., 2010). Thus, down regulation of NF-κB and STAT3 contributed towards an increased expression of apoptotic cell death regulating protein, but decreased antiapoptotic cell death regulating protein which caused apoptotic cell death.

The possible usage of natural toxins as pharmaceutical applications has been shown with several toxins, including snake venom toxin, in various *in vitro* or animal models as well as in clinical studies (Grant et al., 2004; Park et al., 2005). Divergence in the biological activities of various proteins isolated from snake venom has been reported. Snake venoms contain many active molecules, such as c-type lectins, disintegrins and metalloproteinase. Siigur et al. isolated fibrolytic enzymes from the *V. lebetina* snake venom (Siigur et al., 1996). Saxatilin, isolated from a Korean snake (Gloydius saxatilis), has been shown to inhibit platelet aggregation, human umbilical vein endothelial cell proliferation, and smooth muscle cell migration (Hong et al., 2002). Salmosin, a disintegrin purified from Korean snake (Agkistrodon halys brevicaudus) venom, interacted with integrin α(v)β(3) and induced apoptotic cell death by competing with the extracellular matrix for direct binding to integrin α(v)β(3) on the cell surface (Hong et al., 2003). The function of the snake venom composition has not been heavily reported in

relation to the inhibition of cancer. Our group has demonstrated the anticancer effects of snake venom toxin (Son et al., 2007; Park et al., 2009). In regards to practical clinical applications, more research is needed to find certain molecules concerning snake venom containing anticancer activity and synthesis recombinant products. We plan to identify the crucial anticancer molecule of snake venom composition in further studies; however, we have not yet garnered enough results to determine the exact molecule. Present findings extended the *in vitro* anticancer efficacy of snake venom toxin against advanced human ovarian cancer cells by suppression of STAT3 and NF-κB.

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