원 저

Effect of Bee Venom Death Receptor Dependent Apoptosis and JAK2/STAT3 Pathway in the Ovarian Cancer

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국문초록

난소암에서 봉독이 세포자멸사와 JAK2/STAT3 Pathway의 억제에 미치는 영향

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목적 : 이 연구는 봉독이 사람의 난소암 세포인 SKOV3와 PA-1에서 death receptor의 발현을 높여 세포자멸사를 촉진함으로써 암세포의 성장을 억제하는지 밝히고자 하였다.

방법 : 난소암의 세포자멸사의 관찰에는 DAPI, TUNEL staining assay를 시행하였으며, 세포자멸사 조절 단백질의 변동 관찰에는 western blot analysis를 시행하였고, 난소암 세포에서 death receptor의 변화를 관찰하기 위해 RT-PCR analysis를 시행하였다.

결과 : 1. DAPI, TUNEL staining assay 결과, 봉독은 투여량에 따라 세포자멸사의 유도를 통해 SKOV3와 PA-1 난소암세포의 증식을 억제하였고, 세포자멸사와 동반하여 DR4와 DR6의 발현이 두 암세포 모두에서 증가하였고, DR3의 출현은 PA-1 세포에서 증가하였다.

2. Death Receptor의 발현 증가에 따라 caspase-3, 8, 9 and Bax를 포함하는 세포자멸사 촉진 단백질의 발현이 동반하여 상승하였고 JAK2, STAT3의 인산화와 Bcl-2의 발현은 억제되었다.

3. siRNA 처리 시 봉독에 의한 DR3, DR4, DR6 발현증가와 STAT3의 활성억제가 역전되었다.

결론 : 이러한 결과는 봉독이 난소암 세포에서 DR3, DR4, DR6의 증가와 JAK2/STAT3 pathway의 억제를 통하여 세포자멸사를 유발한다는 것을 시사하며, 난소암의 예방과 치료에 효과적으로 활용될 수 있을 것으로

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기대된다.

핵심 단어 : 봉독, 난소암, 세포자멸사, death receptor, JAK2/STAT3

Ⅰ. Introduction

Ovarian cancer is the most frequent cause of death from gynecological cancer[1]. Recent annual worldwide figures reflect 204,000 new cases of ovarian cancer and 125,000 deaths[2]. For the therapy of ovarian cancer, surgery is principle therapy[3], and chemotherapy is also needed to remove the remaining cancer cells. However, the major challenge that limits the effectiveness of chemotherapy in patients with advanced ovarian cancer is the acquisition of resistance[4]. According to recent studies[5,6], appropriate chemopreventive compounds reducing or overcoming resistance are believed to be a very hopeful strategy to reduce the incidence of ovarian cancer and enhance treatment efficacy[5,6].

Apoptosis, the programmed cell death plays major role in anti-cancer effects of chemotherapeutics, which can be induced by activated caspase cascade systems through stimulation of death receptors via interaction of DRs with their ligands such as DR1 with TNF; DR2 with FasL; DR3, with Apo3L; DR4 and DR5 with TRAIL[7-14].

Signal transducers and activators of transcription (STAT) proteins are transcription activators in JAK(specific inhibitors of Janus kinase)/STAT signal pathway, involving in cell growth, proliferation, survival, differentiation, apoptosis, metastasis, and angiogenesis[15]. Several studies have represented that phosphorylated STAT3 induce development and progression of various kinds of tumors such as breast, neck, head, prostate and ovary cancer, etc[16]. In other words, the inhibition of STAT3 by JAK can inhibit tumor cell growth and go apoptosis[17,18].

Bee venom contains a variety of different peptides, including melittin, phospholipase A2, apamin, adolapin, and mast cell-degranulating peptide (MCDP). Bee venom has been used as a traditional medicine to treat back pain, rheumatism, and skin diseases by its antibacterial, antiviral, and anti-inflammatory effects[19]. Moreover, several studies have demonstrated that bee venom and/or melittin have anti-cancer effects including prostate, liver , breast, cervical, renal cancer cells[20-22]. However, experiments demonstrating the molecular mechanisms of the anti-cancer effects of bee venom in ovarian cancer cells have not been reported.

In this study, we therefore investigated anti-cancer effects of bee venom through increase of DR expression, but inhibition of STAT3 pathway in the human ovarian cancer cells, SKOV3 and PA-1.

Ⅱ. Materials and methods

A. Materials

Bee venom was purchased from You-Miel Bee Venom Ltd. (Hwasoon, Jeonnam, Korea). The composition of the bee venom was as follows: 45-50% melittin, 2.5-3% mast cell degranulating peptide, 12% phospholipase A2, 1% lysophospholipase A, 1-1.5% histidine, 4-5% 6-pentyl a-pyrone lipids, 0.5% secarpin, 0.1% tertiapin, 0.1% procamine, 1.5-2% hyaluronidase, 2-3% amine, 4-5% carbohydrate, and 19-27% of others, including protease inhibitor, glucosidase, invertase, acid phosphomonoesterase, dopamine, norepinephrine, and unknown amino acids, with 99.5% purity. Caspase inhibitor (Z-VAD-FMK) was from Promega (Madison, WI). DR3, DR4, and DR6 siRNA were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA).

B. Cell culture

The SKOV3 and PA-1 ovarian cancer cell lines were purchased from the American Type Culture Collection (Manassas, VA). SKOV3 cancer cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (100 U/ml). PA-1 cancer cells were cultured in MEM medium supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (100 U/ml). Cell cultures were then maintained at 37°C in a humidified atmosphere with 5% CO2.

C. Cell viability assay

To determine the cell number, SKOV3 and PA-1 ovarian cancer cells were plated in 24-well plates (5×104 cells/well), and subconfluent cells were subsequently treated with bee venom (1, 2 and 5 ㎍/ml) for 24 hr. After treatment, cells were trypsinized and pelleted by centrifugation for 5 min at 1,500 rpm, resuspended in 5 ml of phosphate-buffered saline (PBS), and 0.1 ml of 0.2% trypan blue was added to the cancer cell suspension in each of the solutions (0.9 ml each). 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. Each assay was carried out in triplicate.

D. Reverse transcription (RT)-PCR

Total RNAs were isolated from cultured cells using RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's manual. The RNA pellet obtained in the final step was dissolved in 30 ㎕ of sterile diethylpyrocarbonate (DEPC)-treated water, and its concentration was determined using a UV spectrophotometer at 260 nm. RNA was kept in DEPC-treated water at -70°C until use. Reverse transcription was performed using High Capacity RNA-to-cDNA Kit (AB). PCR amplifications were then carried out with the following primers.

Death receptor	Forward primer	Reverse primer
TNFR 1	5'-ACC AAG TGC CAC AAA GGA AC-3'	5'-CTG CAA TTG AAG CAC TGG AA-3'
TNFR 2	5'-CTC AGG AGC ATG GGG ATA AA-3'	5- AGC CAG CCA GTC TGA CAT CT-3'
Fas	5-CAA AGC CCA TTT TTC TTC CA-3	5-GAC AAA GCC ACC CCA AGT TA-3
DR 3	5-ATG GCG ATG GCT GCG TGT CCT G-3	5-AGC GCC TCC TGG GTC TCG GGG TAG-3
DR 4	5-ACT TTG GTT GTT CCG TTG CTG TTG-3	5-GGC TTT CCA TTT GCT GCT CA-3
DR 5	5-TGG AAC AAC GGG GAC AGA ACG-3	5-GCA GCG CAA GCA GAA AAG GAG-3
DR 6	5-AAGCCGGGGA CCAAGGAGACAG ACAAC-3	5-TGCCGGGGCCC CTTTTTCAGAGT -3

E. Western blot analysis

Western blot analysis was done as described previously. The membrane was incubated for 2 hr at room temperature with specific antibodies: rabbit polyclonal for caspase-3, cleaved caspase-3, caspase -8, cleaved caspase-8, caspase-9, cleaved caspase -9, Bcl-2(1:1,000 dilution, Cell Signaling Technology, Inc., Beverly, MA), p-STAT3, Bax, p-JAK2(1:500 dilution, Santa Cruz Biotechnology, Inc.), and mouse monoclonal STAT3(1 :500 dilution, Santa Cruz Bio-technology, Inc.). The blot was then incubated with the corresponding conjugated anti-rabbit and anti-mouse immunoglobulin G-horseradish peroxidase

(1:2,000 dilutions, Santa Cruz Biotechnology, Inc.). Immunoreactive proteins were detected with the ECL Western blotting detection system.

In transfection. Ovarian cancer cells (3 × 104 cells per well) were plated in 24-well plates and transiently transfected with siRNA, using a mixture of siRNA and the WelFect-EXPLUS reagent in OPTI-MEN,according to the manufacturer's specification(WelGENE, Seoul, Korea). The transfected cells were treated with 5㎍/ml bee venom for 24 hr.

DR3 siRNA seq. 5'-GAAGCCCUAAGUACGGUUAtt
DR4 siRNA seq. 5'-CUCUGAUGCUGUUCUUUGAtt
DR6 siRNA seq. 5'-GCCUUCUAGUGUGAUGAAAtt

F. Apoptosis evaluation

Ovarian cancer cells (2.5× 105 cells/well) were cultured on 8-chamber slides. After ovarian cancer cells were transfected with siRNA, the cells were treated with bee venom(5 ㎍/ml). The cells were washed twice with PBS and fixed by incubation in 4% paraformaldehyde in PBS for 1 hr at room temperature. Membrane was permeabilized by exposure to 0.1% Triton X-100 in phosphate-buffered saline for 5 min at room temperature. TdT-mediated dUTP nick and labeling (TUNEL) assays were performed by using the in situ Cell Death Detection Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. For 4′-6-Diamidino-2-phenyl indole (DAPI) staining, slides were incubated for 15 min at room temperature in the dark with mounting medium for fluorescence containing DAPI (Vector Laboratories, Inc., Burlingame, CA). The cells were then observed through a fluorescence microscope (Leica Microsystems AG, Wetzlar, Germany).

G. Data analysis

The data were analyzed using the GraphPad Prism 4 ver. 4.03 software (GraphPad Software, La Jolla, CA). Data are presented as mean±SD. 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.

Ⅲ. Results

A. Effect of bee venom on cell growth in ovarian cancer cells

To assess the inhibitory effect of bee venom on cell growth of ovarian cancer SKOV3 and PA-1 cells, we analyzed cell viability by direct cell counting. The cells were treated with several concentrations of bee venom (1, 2, and 5 ㎍/ml) for 24 hr. As shown in Fig. 1 and 2, bee venom inhibited cell proliferation of ovarian cancer cells in a concentration-dependent manner. Twenty-four hour treatment of bee venom inhibited SKOV3 cell growth with IC50 value of 3.8 ㎍/ml(Fig. 1), and PA-1 cells growth with IC50 values of 2.6 ㎍/ml (Fig. 2), respectively. The magnitude of the inhibitory effect was much stronger in PA-1 cells than that in SKOV3 cells (Fig. 2). Morphologic observation showed that the cells were gradually reduced in size and changed into a small round single cell shape by the treatment of bee venom in SKOV3 cells (Fig. 1) and PA-1 cells (Fig. 2).

B. Apoptotic cell death by bee venom

To determine the inhibition of cell growth by bee venom was due to the induction of apoptotic cell death, we evaluated the changes in the chromatin morphology of cells by using DAPI staining followed by TUNEL staining assays, and then the double labeled cells were analyzed by fluorescence microscope. Conversely well with cell growth inhibition, DAPI-stained TUNEL-positive cells were significantly increased in bee venom treated cells. The treatment of bee venom resulted in about 55-65% or 60-80%

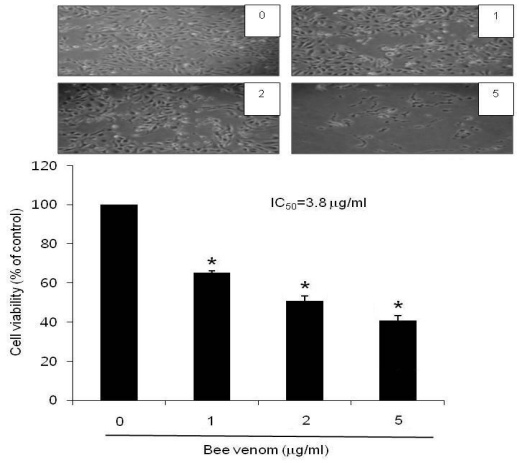

Fig. 1. Effect of bee venom on cell viability in ovarian cancer cells

Concentration-dependent effect of bee venom on the cell viability assay in SKOV3. After treatment of bee venom(1, 2 and 5 ㎍/ml) for 24 hr, 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. Morphologic observation with the treatment of bee venom in SKOV3 cells. Columns, means of three experiments, with triplicates of each experiment; bars, SD. *, p <0.05, significantly different from untreated control cells.

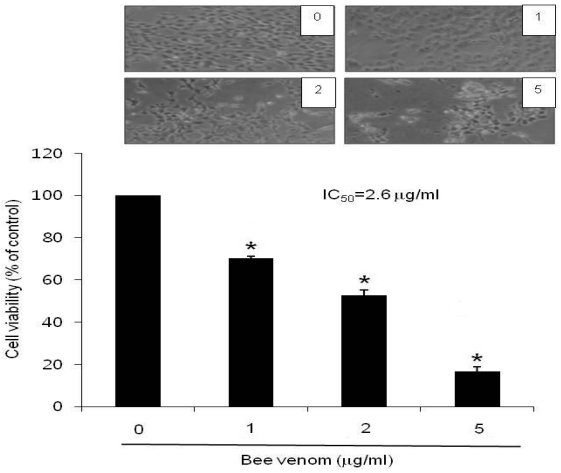

Fig. 2. Effect of bee venom on cell viability in ovarian cancer cells

Concentration-dependent effect of bee venom on the cell viability assay in PA-1. After treatment of bee venom(1,2 and 5 ㎍/ml) for 24 hr, 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. Morphologic observation with the treatment of bee venom PA-1 cells. Columns, means of three experiments, with triplicates of each experiment; bars, SD. *, p <0.05, significantly different from untreated control cells.

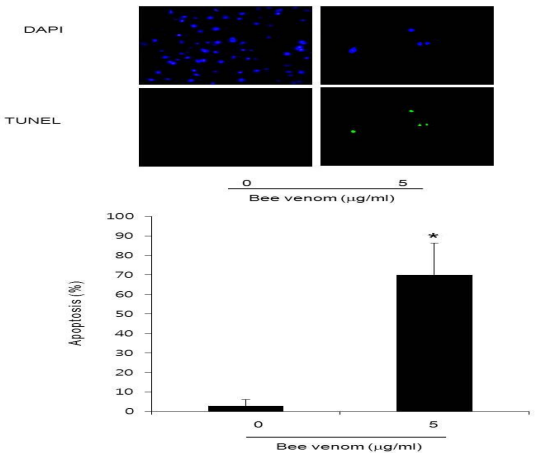

Fig. 3. Effect of bee venom on apoptotic cell death

The ovarian cancer cells, SKOV3 were treated with bee venom (5 ㎍/ml) for 24 hr, 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, 200x). Columns, means of three experiments, with triplicates of each experiment; bars, SD. *, p <0.05, significantly different from bee venom-untreated control cells.

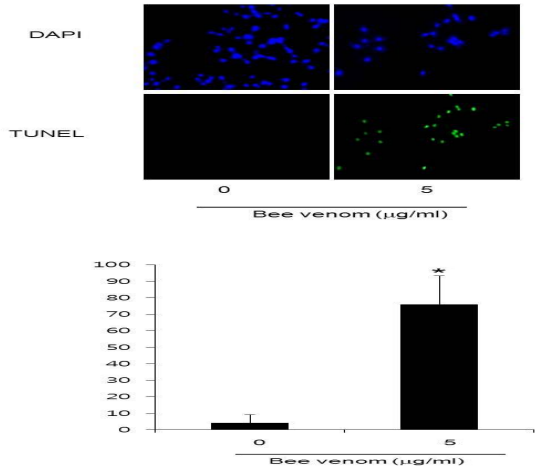

Fig. 4. Effect of bee venom on apoptotic cell death

The ovarian cancer cells, PA-1 were treated with bee venom (5 ㎍/ml) for 24 hr, 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, 200x). Columns, means of three experiments, with triplicates of each experiment; bars, SD. *, p <0.05, significantly different from bee venom-untreated control cells.

induction of apoptotic cell death in SKOV3(Fig. 3) and PA-1(Fig. 4) cancer cells, respectively.

C. Expression of death receptors in ovarian cancer cells by bee venom

Apoptosis can be induced by stimulation of DRs expression. Therefore, to investigate expression of DRs in cancer cells undergoing apoptotic cell death, we performed RT-PCR analysis. RT-PCR analysis showed that bee venom treatment increased DR4 and DR6 mRNA levels in a concentration dependent manner, but TNFR1, TNFR2, FAS, and DR5 expression levels were not changed by bee venom in SKOV3 and PA-1 cells. Expression of DR3 was increased only in PA-1 cells (Fig. 5).

D. Effect of bee venom on the expression of apoptotic regulatory proteins

To figure out the relationship between the induction of apoptotic cell death and increase of DR expression, and the expression of their regulatory proteins by bee venom, expression of apoptotic cell death related proteins was investigated by Western blots. The expression of anti-apoptotic protein Bcl-2 was decreased; however, the expression of pro-apoptotic proteins, Bax, cleaved form of caspase-3, -8, and -9 was increased by treatment of bee venom in a concentration dependent manner. However, expression of cleaved caspase-3 was greatly increased in SKOV3, whereas cleaved caspase-8 was significantly increased in PA-1 cancer cells (Fig. 6).

E. Reversed effect of DR siRNAs on bee venom-induced cell growth inhibition

To determine the relationship between DR expression and cell growth inhibitory effect of bee venom, we transfected SKOV3 and PA-1 cells with DR siRNA using a transfection agent. The cells were transfected with 100 nM siRNA of DRs for 24 hr, and then treated with bee venom (5 ㎍/ml) for 24 hr. Expression of the death receptor (DR3, DR4, and DR6) at mRNA levels was detected by RT-PCR. As shown in Fig. 7, transfection of DR3, DR4, and DR6 siRNA reversed bee venom-induced expression of DRs. Cell viability was then determined by direct cell counting. It was also found that knock down of death receptor generally (DR3, DR4, and

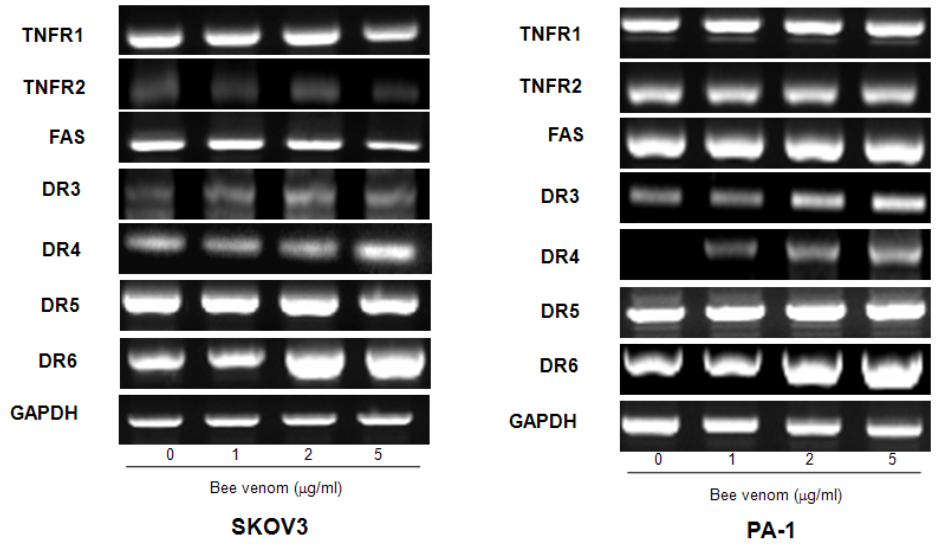

Fig. 5. Effect of bee venom on DRs expression in ovarian cancer cells

Cells were treated with bee venom(1, 2, and 5 ㎍/ml) for 24 hr, and total RNA were extracted and examined for expressions of TNF-R1, TNF-R2, FAS, DR-3, -4, -5, -6, and GAPDH by RT-PCR. GAPDH was used as an internal control to show equal RNA loading. Each band is representative for three experiments.

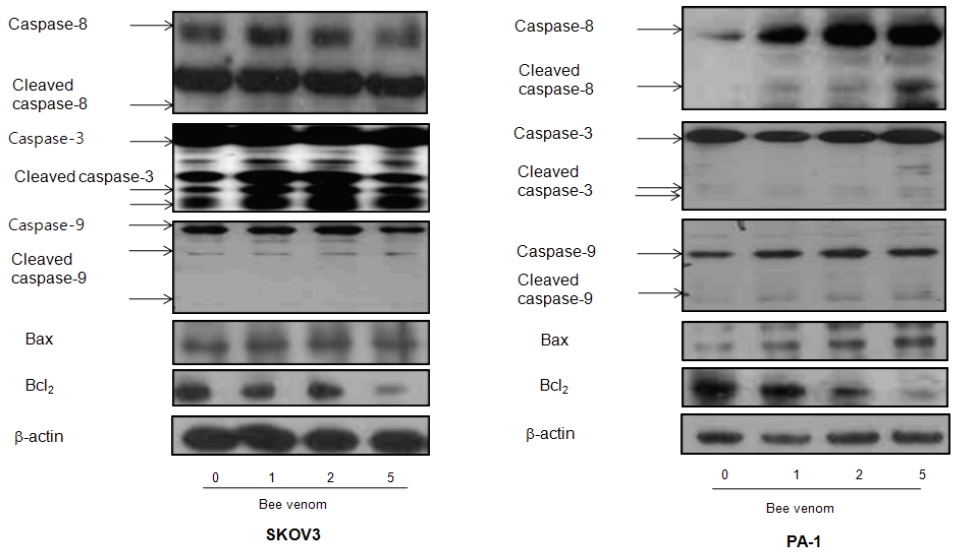

Fig. 6. Effect of bee venom on the expression of apoptosis regulatory proteins

Expression of apoptosis regulatory proteins was determined using Western blot analysis. The ovarian cancer cells were treated with different concentrations of bee venom(1, 2, and 5 ㎍/ml) for 24 hr. Equal amounts of total proteins(50 ㎍/lane) were subjected to 12% or 8% SDS-PAGE. Expression of caspase-8, caspase-3, caspase-9, Bax, Bcl2, and β-actin were detected by Western blotting using specific antibodies. β-actin protein here was used as an internal control. Each band is representative for three experiments.

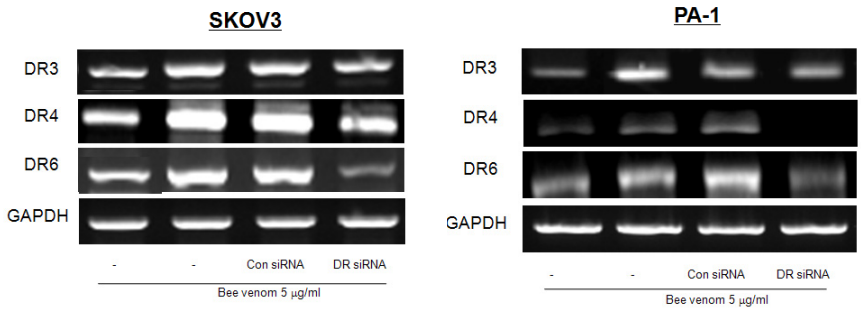

Fig. 7. Effect of siRNA of DRs on the bee venom-induced cancer cell growth inhibition, DR expression and apoptosis in ovarian cancer cells

Effect of siRNA of DR on the bee venom-induced DR gene expression in ovarian cancer cell. The ovarian cancer cells were transfected with the DR siRNA(100 nM) for 24 hr, the cells were then and treated with bee venom(5 ㎍/ml) for another 24 hr. Total RNA was isolated and RT-PCR was performed to examine gene expression levels of DRs. Each band is representative for three experiments.

DR6) reversed the cell growth inhibitory effect of bee venom in SKOV3 and PA-1. Especially in PA-1, anti- proliferative effect of DR4 was almost completely reversed, however, the magnitude of reversed effect was relatively small in SKOV3 by DR4 siRNA (Fig. 8). We also evaluated apoptotic cell death by fluorescence microscope. DAPI-stained TUNEL-positive cells increased by bee venom were prevented by transfection with DR siRNA both in SKOV3 and PA-1 cells (Fig. 9). These results demonstrate that expression of DR3, DR4, and DR6 is strongly correlated with bee venom-induced apoptotic cell death in ovarian cancer cells.

F. Association between death receptor and activation of STAT3

STAT3 phosphorylation is associated with pro-

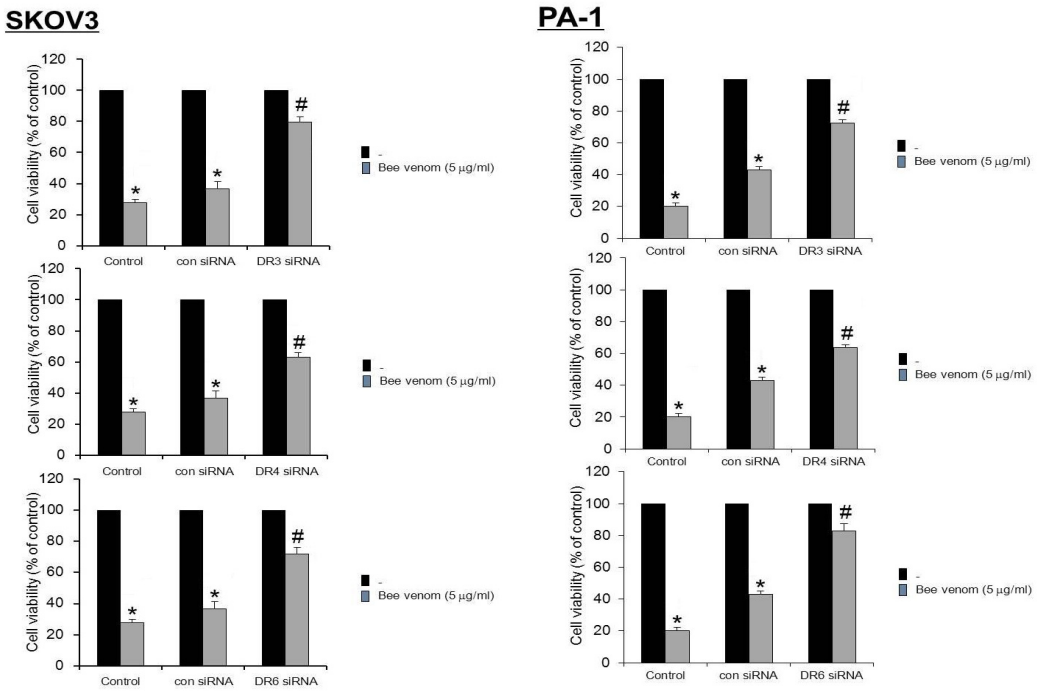

Fig. 8. Effect of DR3, DR4, or DR6 knockdown on bee venom-induced ovarian cancer cell growth

Ovarian cancer cells were transfected with non targeting control siRNA, DR3, DR4, or DR6 siRNA (100 nM) as described in Materials and Methods for 24 hr. Then, bee venom(5 μg/ml) was treated at 37°C for another 24 hr. 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. Columns, means of three experiments, with triplicates of each experiment; bars, SD. * : p <0.05, significantly different from untreated control cells. # : p <0.05, significantly different from control siRNA transfected cells.

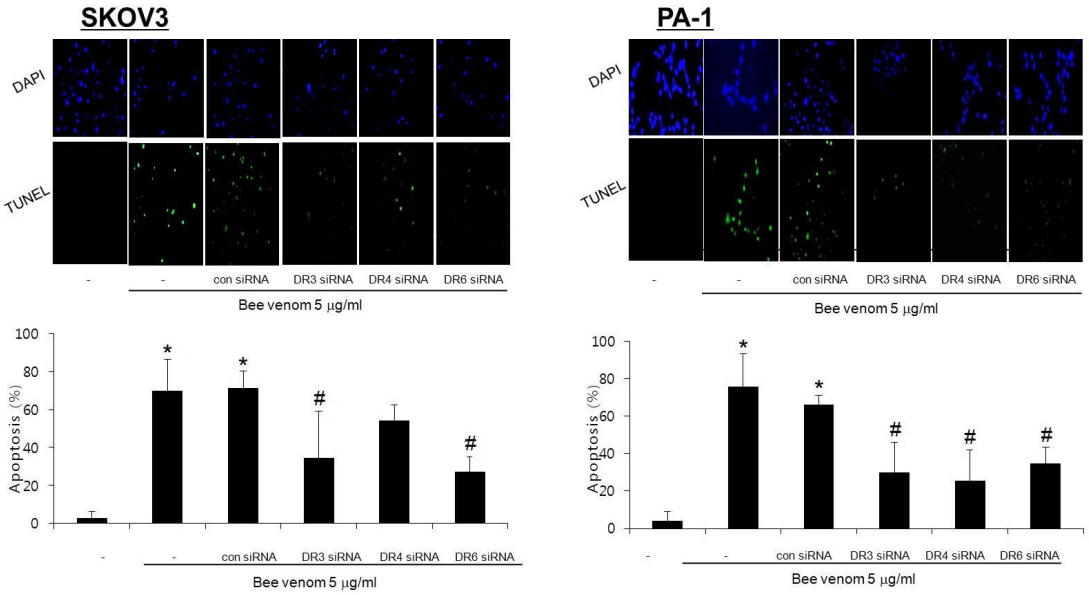

Fig. 9. Quantification of apoptosis by TUNEL assay

The ovarian cancer cells were transfected with the death receptors iRNA for 24hr, and then treated with bee venom(5μg/ml) for 24 hr. The apoptotic index was determined as the DAPI-stained TUNEL-positive cell number/ total DAPI stained cell number(magnification, 200x). Columns, means of three ex- periments, with triplicates of each experiment; bars, SD. * : p <0.05, significantly different from untreated control cells. # : p <0.05, significantly different from control siRNA transfected cells.

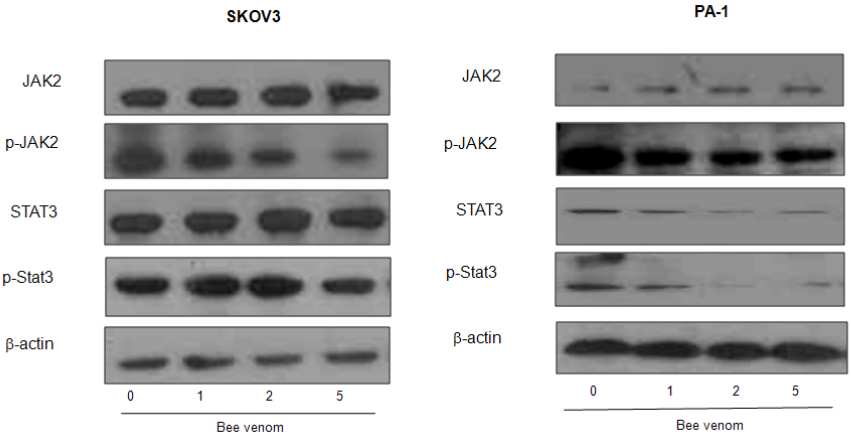

Fig 10. Effect of bee venom and siRNA of DRs on the activation of JAK2/STAT3 in ovarian cancer cells

The ovarian cancer cells were treated with different concentrations of bee venom(1, 2 and 5 ㎍/ml) for 24 hr. Equal amounts of total proteins(50 ㎍/lane) were subjected to 8% SDS-PAGE. And β-actin were detected by Western blotting using specific antibodies of STAT3, p-STAT3, JAK2, p-JAK2, and β-actin protein here was used as an internal control. Each band is representative for three experiments.

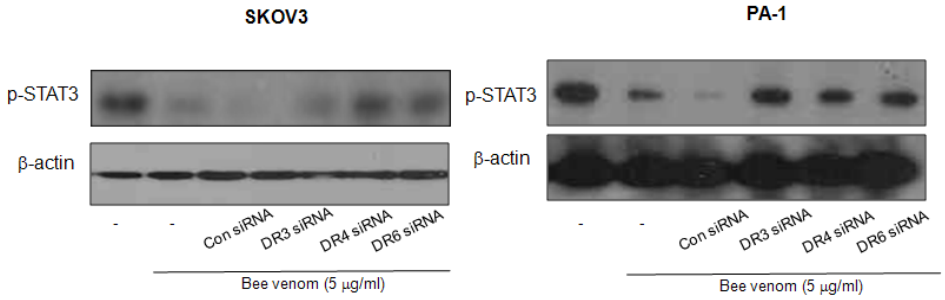

Fig. 11. Ovarian cancer cells were transfected with nontargeting control siRNA, DR3, DR4, or DR6 siRNA(100nM) as described in Materials and Methods for 24hr

Then, bee venom(5㎍/ml) was treated at 37°C for another 24hr. Equal amounts of total proteins(50㎍/lane) were subjected to 8% SDS-PAGE. Expression of STAT3, p-STAT3, JAK2, p-JAK2, and β-actin were detected by Western blotting using specific antibodies. β-actin protein here was used as an internal control. Each band is representative for three experiments.

liferation and maintenance of tumors. Thus, activation of STAT3 and its upstream JAK2 pathway was investigated. We found that pJAK2 and pSTAT3 were decreased by bee venom in ovarian SKOV3 and PA-1 cancer cells dose dependently (Fig. 10). Moreover, transfection of DR siRNA for knock down of DR3, DR4, and DR6 abolished the bee venom (5 ㎍/ml)-induced down regulation of pSTAT3 in SKOV3 and PA-1 cancer cells (Fig. 11).

Ⅳ. Discussion

In the present study, we found that bee venom inhibited cell growth of human ovarian cancer cells; SKOV3 and PA-1 through induction of apoptotic cell death via increase of DR expression and inhibition of STAT3 pathway. Bee venom induces cell death of ovarian cancer cells in a concentration-dependent manner with an IC50 value of 3.8 ㎍/ml in SKOV3 cells, and 2.6 ㎍/ml in PA-1 cells,

respectively. Park et al.[20] and Park et al.[21] that at a similar or higher doses (1~15 ㎍/ml) of bee venom inhibited prostate cancer cells growth. It was also reported that lung cancer cells and hepatocarcinoma growth was inhibited by bee venom with IC50 value of more than 10㎍/ml[23,24]. Taken together, bee venom may have cell growth inhibitory effect on SKOV3 and PA-1 ovarian cancer cells with similar or lower dose compared to the dose in other cancer cells.

Apoptosis is the process of programmed cell death that plays important roles in anti-cancer effects in a variety of cancers[7]. Several studies have demonstrated that natural compounds-induced apoptotic cell death could be related with increase of DR expression, and selective triggering of DR expression be implicated in the induction of cancer cell death8,[25-28]. Because the expression of death receptor are different depending on tumor cell types and stimuli conditions, the differential expression of death receptor by bee venom in ovarian cancer cells could be also specific. In this study, Bee venom treatment resulted in about 55-65% or 60-80% induction of apoptotic cell death in SKOV3 or PA-1 cancer cells, respectively, and it increased DR4 and DR6 mRNA levels in a concentration dependent manner, but TNFR1, TNFR2, FAS, and DR5 expression levels were not changed by bee venom in SKOV3 and PA-1 cells. In addition, expression of DR3 was increased only in PA-1 cells. These data indicate DR3 may be a critical death receptor discriminating the responses between two cancer cells against bee venom. Expression of DR induces activation of caspase-8 that make the apoptosis going through the activation of pro-apoptotic proteins including caspases-9 and -3, as well as Bax in the caspase cascade systems[29,30]. Similarly, my data demonstrated that bee venom increased expression of pro-apoptotic proteins, Bax, cleaved form of caspase-3, -8, and -9 and decreased expression of anti-apoptotic protein, Bcl-2. To further investigate the involvement of death receptors in bee venom-induced apoptotic cell death, I used siRNA of DR3, DR4, and DR6, and found that bee venom-induced apoptotic cell death of ovarian cancer cells was reversed by these receptor siRNA and it was abolished in PA-1 cells by DR4 knock down. Consistent with the previous Yang et al.'s report[31], increased expression of DR4 caused apoptosis through caspase-8 dependent activation of apoptosis signal in PA-1 cells, but not in SKOV3 cells. Thus DR4 and caspase-8 pathway was re-confirmed to be critical in the differential responses between PA-1 and SKOV3 ovarian cancer cells toward bee venom.

From the previous reports, many compounds inhibiting JAK2/STAT3 signal pathway such as Benzoxathiol[32], Cucurbitacin B[33] could also inhibit ovarian cancer cell growth, for activated STAT3 plays important roles in cell growth, proliferation, survival, differentiation, apoptosis, metastasis, and angiogenesis[15,34,35]. My data demonstrated that bee venom inhibit JAK2/STAT3 signal pathway, and its inhibitory effect resulted in the decrease of ovarian cancer cell growth. Moreover, my data further demonstrated that inhibition of STAT3 by bee venom was reversed by knockdown of DR3, 4, and 6 in ovarian cancer cells, similar with Saydmohammed et al.'s result[36]. Therfore, my results indicate that natural toxin bee venom inhibit ovarian cancer cell growth through enhancement of DR3, DR4, and DR6 expression and STAT3 inactivation. Considering previous report[21] that bee venom inhibited prostate cancer growth through induction of apoptosis via inactivation of NF-kB, the above results suggested that bee venom should involve different mechanism depending on tumor types, although it inhibits tumor growth through induction of apoptosis.

Radiotherapy and chemotherapy are existing useful modalities for a variety of human cancers. However, they also have limitations due to their resistance, which is related to the lower expression of DRs, TNFR and FAS and overcome by increased DR expression[37,38].

Consequently, These present data provide that bee venom could be useful candidate compounds to enhance tumor growth inhibiting ability of chemo-

therapeutics through overcoming the resistance via enhancement of DR expression.

Ⅴ. References

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