

Snake Venom synergized Cytotoxic Effect of Natural Killer Cells on NCI H358 Human Lung Cancer Cell Growth through Induction of Apoptosis

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[Abstract]

Objectives : I investigated whether snake venom can synergistically strengthen the cytotoxic effects of NK-92 cells, and enhance the inhibition of the growth of lung cancer cells including NCI-H358 through the induction of death receptor dependent extrinsic apoptosis.

Methods : Snake venom toxin inhibited cell growth of NCI-H358 Cells and exerted non influence on NK-92 cell viability. Moreover, when they were co-cultured with NK cells and concomitantly treated with 4 $\mu\text{g}/\text{mL}$ of snake venom toxin, more influence was exerted on the inhibition of growth of NCI-H358 cells than BV or NK cell co-culture alone.

Results : The expression of Fas, TNFR2 and DR3 and in NCI-H358 lung cancer cells was significantly increased by co-culture of NK-92 cells and treatment of 4 $\mu\text{g}/\text{mL}$ of snake venom toxin, compared to co-culture of NK-92 cells alone. Coincidentally, Bax, caspase-3 and caspase-8 - expressions of pro-apoptotic proteins in the extrinsic apoptosis pathway, demonstrated significant increase. However, in anti-apoptotic NF- κ B activities, activity of the signal molecule was significantly decreased by co-culture of NK-92 cells and treatment of 4 $\mu\text{g}/\text{mL}$ of snake venom toxin, compared to co-culture of NK-92 cells or snake venom toxin treated by NCI-H358 alone. Meanwhile, in terms of NO generation, there is a significant increase, in co-culture of NK-92 cells with NCI-H358 cells as well as the co-culture of NK-92 cells and concomitant treatment of 4 $\mu\text{g}/\text{mL}$ of snake venom toxin. However, no synergistic increase of NO generation was shown in co-culture of NK-92 cells and treatment of 4 $\mu\text{g}/\text{mL}$ of snake venom toxin, compared to co-culture of NK-92 cells with NCI-H358 cells.

Conclusion : Consequently, this data provides that snake venom toxin could be useful candidate compounds to suppress lung cancer growth along with the cytotoxic effect of NK-92 cells through extrinsic apoptosis.

Key words :

Snake Venom;
 Lung cancer;
 NCI-H358;
 NK-92;
 Death receptor;
 Nitric Oxide(NO)

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I. Introduction

Apoptosis, a programmed cell death have increasingly drawn attention as a relevant physiologic mechanism playing a crucial role in strategy of cancer treatment, because cancer has characteristics of cellular homeostasis change¹⁻³. Non-small cell lung cancer (NSCLC) is a predominant lung cancer in Korea and world wide^{4,5}, and smoking has been regarded as a major risk factor of its incidence and progression⁶. To date, surgery, radio-therapy and chemo-therapy have been available for treating it, the latter two of which lead to induction of apoptosis and suppress its growth temporarily and then fail to maintain the effectiveness due to resistance of it^{7,8}. Therefore, the limitation of these current modalities cause it to represent higher mortality together with poor early diagnose, systemic toxicity^{9,10} and so on. Although smoking cessation is recommended for preventing it, development of novel therapeutic agents for decreasing its mortality, prolonging 5 year survival rate and overcoming drug resistance is also urgently needed¹¹.

Snake venom acupuncture is a form of pharmacopuncture and it is usually used for treating many inflammatory disease in Korean Medicine. Anti-inflammatory and anti-cancer Effect of snake venom toxin and its major component, melittin has been investigated, and the mechanism has been well established that they suppress inflammation by inhibition of signal molecules' activity in the NF- κ B signal pathway and various cancers by through apoptosis¹²⁻¹⁵. Natural snake venom toxin(SVT) from *Vipera lebetina turanica* has more characteristic cysteine residue than bee venom and exerts similar and stronger action, which is proved by previous reports demonstrating that SVT was a promising chemotherapeutic agent inhibiting the growth of human prostate cancer cell and neuroblastoma cell through induction of apoptosis that is mediated by the modulated expression of apoptosis regulatory proteins^{16,17}.

Meanwhile, According to previous studies¹⁸⁻²⁰, Natural Killer(NK) cells gave modulated signals to different groups of surface receptors on lung cancers, recognizing and exerting cytotoxic actions. Moreover, the more NK cell there was in NSCLC, the better prognosis the patients had and survived. That was a noticeable thing in immune surveillance Immuno-therapy.

In these regards, I investigated anti-cancer effects of SVT and NK-92 cells on NCI-H358 Lung adenocarcinoma Cells, and I confirmed whether both are synergistically correlated and enhances DR mediated extrinsic apoptosis.

II. Materials and Methods

1. Materials

Snake Venom Toxin(SVTT) from *Vipera lebetina turanica* was purchased

from Sigma-adrich co. (St. Louis, MO, USA).

All of the secondary antibodies such as fas, TNFR2, DR3, Bax, caspase-3 cleaved caspase-3, caspase-8 and cleaved caspase-8 used in Western blot analysis were purchased from Santa Cruz Biotechnology(Santa Cruz, CA, USA). All other reagents were purchased from Sigma-adrich co. unless otherwise stated.

2. Cell Culture

NK-92 cell, NCI H358 cell were obtained from the American Type Culture Collection (Manassas, VA, USA). NK-92 cells were grown at same conditions in MEM alpha medium that contained 20 % fetal bovine serum (FBS), 100 U/ml penicillin, 100 μ g/ml streptomycin and 10 μ g/ml IL-2. NCI H358 cells were grown at 37 °C in 5 % CO₂ humidified air in RPMI 1640 medium that contained 10 % fetal bovine serum(FBS), 100 U/ml penicillin and 100 μ g/

ml streptomycin. In cocultures the cells were grown in a mixed medium (1 : 1) of NCI H358 and NK-92 medium in a trans-well system where the cells were separated by a porous polycarbonate membrane (pore size 0.4 μm ; distance 6.5 mm). NCI H358 cells were first seeded at 5×10^4 cells/well, cultured overnight whereafter the inserts with the NK-92 cells were added (5×10^4 cells/well). All cells were cultured in 24-well plates from Costar. RPMI 1640, MEMalpha, penicillin, streptomycin, and FBS were purchased from Gibco Life Technologies (Grand Island, NY, USA).

3. Cell Viability Assay

To determine viable cell numbers, the NCI H358 Cells, NK-92 cells and those co-cultured with NK-92 cells were seeded onto 24-well plates (5×10^4 cells/well) and subconfluent cells were subsequently treated with SVT (NCI H358 : 1, 2 and 4 $\mu\text{g}/\text{ml}$, NK-92 : 4 $\mu\text{g}/\text{ml}$, NCI H358 co-cultured with NK-92 : 4 $\mu\text{g}/\text{ml}$) for 24 hrs. The cells were trypsinized, pelleted by centrifugation for 5 mins. At 1500 rpm, resuspended in 10 ml of phosphate-buffered saline (PBS), and 0.1 ml of 0.2 % trypan blue was added to the cancer cell suspension in each solution (0.9 ml each). Subsequently, a drop of suspension was placed in a Neubauer chamber, and the living cancer cells were counted. Cells that showed signs of trypan blue uptake were considered to be dead, whereas those that excluded trypan blue were considered to be viable. Each assay was carried out in triplicate.

4. Western blot analysis

Cells were homogenized with lysis buffer (50 μM Tris, pH 8.0, 150 μM NaCl, 0.02 % NaN_3 , 0.2 % SDS, 1 μM phenylmethylsulfonyl fluoride, 10 I/ml aprotinin, 1 % igapal 630 (Sigma aldrich co., St. Louis, MO, USA), 10 μM NaF, 0.5 μM EDTA, 0.1 μM EGTA, and 0.5 % sodium deoxycholate) and centrifuged at

23,000 g for 1 hr. Equal amounts of proteins (80 g) were separated on SDS-12 % polyacrylamide gels and then transferred to a nitrocellulose membrane (Hybond ECL ; Amersham Pharmacia Biotech). Blots were blocked for 2 hrs at room temperature with 5 % (w/v) nonfat dried milk in Tris buffered saline (10 μM Tris, pH 8.0, 150 μM NaCl) containing 0.05 % Tween 20. The membrane was incubated for 5 hr at room temperature with following specific antibodies : rabbit polyclonal antibodies directed against Bax, caspase-3, caspase-8, cleaved caspase-3, caspase-8 (1 : 1000 dilutions ; Cell Signaling Technology, Beverly, MA), Fas, TNFR2, DR3 (1 : 500 dilutions ; Santa Cruz Biotechnology), goat polyclonal antibody to p50, p65 (1 : 500 dilution, Santa Cruz Biotechnology Inc.) and phospho-I κ B α (1 : 200, Santa Cruz Biotechnology 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.

5. Nitric Oxide(NO) determination

The nitrite accumulation in the supernatant was assessed by Griess reaction. Each 50 μl of culture supernatant was mixed with an equal volume of Griess reagent [0.1 % N-(1-naphthyl)-ethylenediamine, 1 % sulfanilamide in 5 % phosphoric acid] and incubated at room temperature for 10 min. The absorbance at 540 nm was measured in a microplate absorbance reader, and a series of known concentrations of sodium nitrite was used as a standard.

6. Statistical analysis

The data were analysed using the GRAPHPAD PRISM 4 ver.4.03 software (GraphPad Software, La Jolla, CA). Data are presented as mean \pm SD. The differences in all data were assessed by one-

way analysis of variance. When the P-value in the analysis of variance 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

1. Inhibitory Effect of Snake Venom Toxin and NK-92 Cells on Cell Growth in NCI H358 Cells

To assess whether SVT enhances inhibitory effect of NK-92 Cells in NCI H358 Human Lung Cancer Cells, I analyzed cell viability by direct cell

counting. NCI H358 Human Lung Cancer Cells were treated with several concentrations of SVT (1, 2 and 4 $\mu\text{g}/\text{ml}$) and cultured for 24 hr ($\text{IC}_{50} = 3.6 \mu\text{g}/\text{ml}$), and NK-92 Cells were then treated with 4 $\mu\text{g}/\text{ml}$ of SVT and cultured for 24 hrs. Subsequently, NK-92 co-cultured cells were treated or not with SVT (4 $\mu\text{g}/\text{ml}$) and cultured for 24 hrs. As shown in Figure 1, SVT inhibited cell growth of NCI H358 Cells and represented morphologic change such as gradual size reduction and a small round single cell shape in a concentration-dependent manner, while it exerted little influence on the cell viability of Nk-92 cells. Moreover, NK-92 cells and 4 $\mu\text{g}/\text{ml}$ of SVT synergistically represented significant inhibition of growth of NCI H358 Cells, compared with the other groups treated by SVT or co-cultured by NK-92 cells alone (Fig. 1).

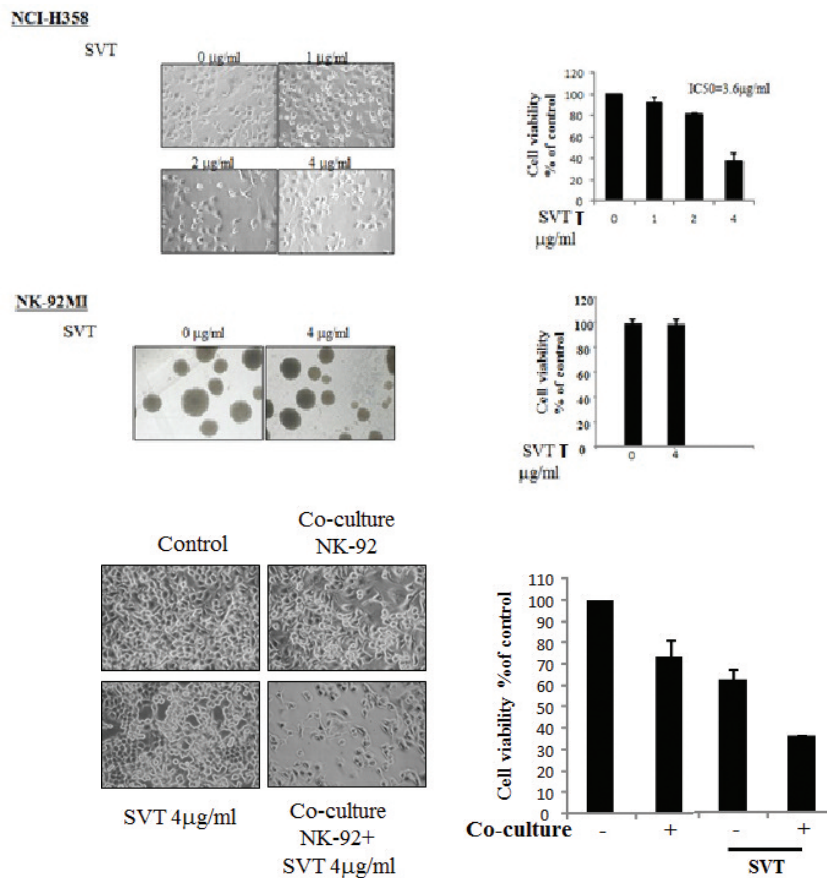


Fig. 1. Snake Venom Toxin enhances cytotoxic effect of NK-92 cells on NCI H358 Cells

The NCI H358 Cells, NK-92 cells and those co-cultured with NK-92 cells were seeded onto 24-well plates (5×10^4 cells/well) and subconfluent cells were subsequently treated with SVT (NCI H358 : 1,2 and 4 $\mu\text{g}/\text{ml}$, NK-92 : 4 $\mu\text{g}/\text{ml}$, NCI H358 co-cultured with NK-92 : 4 $\mu\text{g}/\text{ml}$) for 24 hrs. The cells were then 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. SVT, Snake Venom Toxin Treatment ; Columns, means of three experiments, with triplicates of each experiment ; bars, SD.

2. Synergistic Effect of Snake Venom Toxin and NK-92 Cells on the expression of a death receptor and a apoptotic regulatory proteins in NCI H358 Cells

To ascertain whether SVT and NK-92 Cells synergistically enhance the expression of a death receptor and its ligands such as TNFR2, fas and DR3 and representative apoptotic regulatory proteins such as Bax, caspase-3, caspase-8, cleaved caspase-3 and cleaved caspase-8 in NCI H358 Cells. The expression of them was investigated by Western blots analysis. As results, The expression of TNFR2, DR3 and fas was significantly increased by co-culture of NK-92 cells and treatment of 4 $\mu\text{g}/\text{ml}$ of SVT, compared to co-culture of NK-92 cells or treatment of SVT(4 $\mu\text{g}/\text{ml}$) alone(Fig. 2). Coincidentally, expression of pro-apoptotic proteins such as Bax, caspase-3, caspase-8, cleaved caspase-3 and cleaved caspase-8 were also more enhanced by co-culture of NK-92 cells and treatment of 4 $\mu\text{g}/\text{ml}$ of SVT than by co-culture of NK-92 cells or treatment of SVT(4 $\mu\text{g}/\text{ml}$) alone(Fig. 2).

Expression of death receptors related proteins such as TNFR2, fas, DR3 and pro apoptotic regulatory proteins such as Bax, caspase-3, caspase-8, cleaved caspase-3 and cleaved caspase-8 was determined using Western blot analysis. NCI H358 Cells were co-cultured with NK-92 cells and subsequently treated with 4 $\mu\text{g}/\text{ml}$ of SVT for 24 hr.

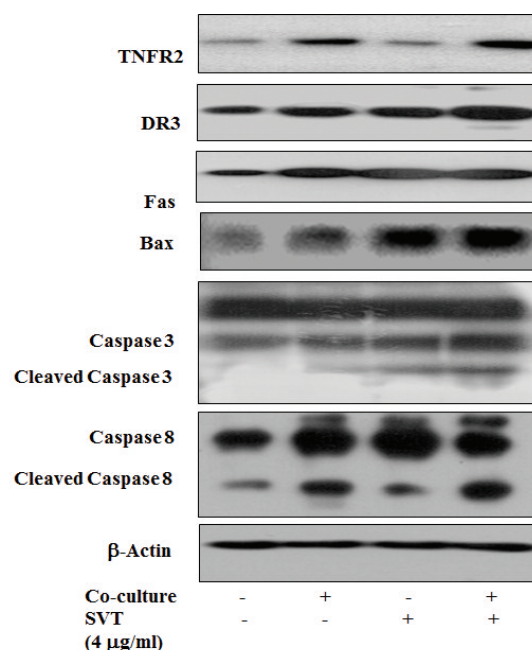


Fig. 2. Snake Venom Toxin enhances the expression of death receptor and its related apoptotic proteins by NK-92 cells in NCI H358 Cells

Equal amounts of total proteins (50 $\mu\text{g}/\text{lane}$) were subjected to 12 % or 8 % SDS-PAGE. Expression of TNFR2, fas, DR3, Bax, caspase-3, caspase-8, cleaved caspase-3, cleaved caspase-8 and β -actin was detected by Western blotting using specific antibodies. SVTT, Snake Venom Toxin Treatment.

3. Synergistic Effect of Snake Venom Toxin and NK-92 Cells on Inhibiting Activity of a anti-apoptotic NF- κ B in NCI-H358 Cells

NF- κ B is known to inhibitory transcription factor of apoptosis. Whether to prevent anti-apoptotic ability of NF- κ B is crucial for a agent causing cancer cells go apoptosis. To determine whether SVT and NK-92 Cells synergistically decrease the activity of NF- κ B signal molecules, I assessed NF- κ B activity in NK-92 co-cultured cells with or without treatment of SVT(4 $\mu\text{g}/\text{ml}$) by western blot analysis. As shown in Fig. 3, NK-92 cells and 4 $\mu\text{g}/\text{ml}$ of SVT synergistically represented significant inhibition of Highly increased expression of NF-

κ B and its signal molecules such as p50, p65, p-I κ B in NCI H358 cells, compared with the other groups treated by SVT or co-cultured by NK-92 cells alone(Fig. 3).

Expression of NF- κ B signal molecules such as p50, p65 and p-I κ B was determined using Western blot analysis. NCI-H358 Cells were co-cultured with NK-92 cells and subsequently treated with 4 μ g/ml of Snake Venom for 24 hrs. Equal amounts of total proteins (50 μ g/lane) were subjected to 12 % or 8 % SDS-PAGE. Expression of p50, p65 and p-I κ B 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.

4. Synergistic Effect of Snake Venom Toxin and NK-92 Cells on the Concentration of NO in NCI H358 Cells

To confirm whether NK-92 cells exert cytotoxic

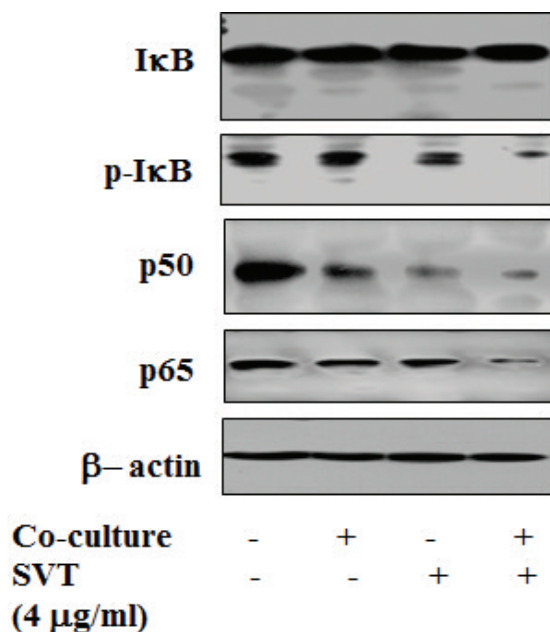


Fig. 3. Snake Venom Toxin decreased the expression of anti-apoptotic NF- κ B and its signal molecules in NCI-H358 Cells

effects on NCI H358 Cells through the NO pathway, the nitrite accumulation in the supernatant was assessed by Griess reaction following co-culture of NK-92 cells or/and treatment of 4 μ g/ml of Snake Venom Toxin. Level of NO into the medium was significantly increased by co-culture of NK-92 cells and co-culture of NK-92 cells with treatment of SVT(4 μ g/ml) compared with control or 4 μ g/ml of Snake Venom alone(Fig. 4). However, Synergistic effect of co-culture of NK-92 cells and treatment of SVT(4 μ g/ml) was not shown(Fig. 4).

The cells were cultured with or without NK-92 cells and then treated with SVT (4 μ g/ml) for 48 hrs. Thereafter, the nitrite release in the supernatant was assessed by Griess reaction as described in Materials and methods.

The figures are representatives of three experiments with replicates.

SVT, Snake Venom Treatment : *, $p < 0.05$, significantly different from control.

IV. Discussion

Among lung cancers, NSCLC is particularly represents higher resistance to current anti-cancer therapies including surgery, radiation and chemo-

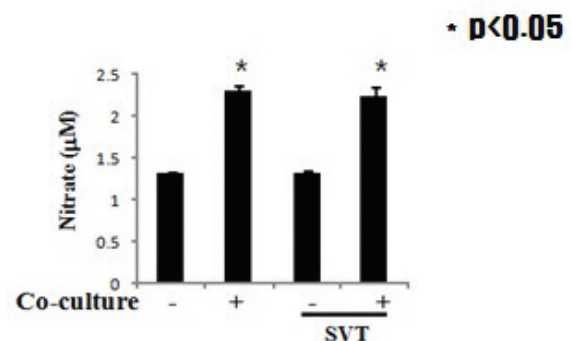


Fig. 4. NK-92 cells exerted cytotoxic effect on NCI H358 Cells through increasing NO concentration

therapy and has unfavorable prognoses due to occult lymph node metastasis⁹. Thus, how to treat and manage NSCLC is still in controversy and becomes a major clinical challenge²⁰. Currently, keeping steps with progress in the understanding of the molecular and histologic properties of various lung cancers, exploration and development of new potential anti-cancer agents are ongoing. Previous reports^{16,17} substantiated that Inhibition of cancer cell growth is mainly caused by SVT initiated induction of apoptosis. Thus, I proposed SVT as an alternative holding out hope that it can be further developed and available for lung cancer therapy in the present study. In addition, NK-cell was first found in 1975, which is the body's critical immune factor as well as the body's innate immune system. It plays a major role in infection defense, autoimmune, hematopoietic stem cells and reproduction and is distinguished from other immune cell such as T-cell in that it can activate the immune system without previous infection and MHC restriction. NK cell primarily involves immunity to cancers such as NSCLC, contributing to therapeutic research of cancers²²⁻²⁵. From the above, I investigated whether snake venom toxin can synergistically strengthen the cytotoxic effects of NK-92 cells, enhancing the inhibition of the growth of NCI-H358 cells through induction of death receptor dependent extrinsic apoptosis.

As results, NCI-H358 demonstrated a typical morphological apoptotic change of chromatin condensation and membrane blebbing and snake venom toxin inhibited cell growth of NCI-H358 Human Lung Cancer Cells and exerted non influence on NK-92 Cell viability. Moreover, when they were co-cultured with NK cells and concomitantly treated by 4 $\mu\text{g}/\text{ml}$ of snake venom toxin, more influence was exerted on inhibition of growth of NCI-H358 Cells than BV treated or NK cell co-cultured alone (Fig. 1).

Death receptor dependent apoptosis is initiated by binding of TNF- α and Fas ligand (FasL) to their corresponding death receptor on the membrane of cancer cell, which consecutively forms death do-

main through trimerization. Subsequently, the death inducing signaling complex is made by the binding of adapter molecule FADD/MORT1 and the protease caspase-8. Once the caspase-8 is recruited, caspase cascade system is activated, finally leading to extrinsic apoptosis. This is noteworthy and becomes new strategy to overcome NSCLC regardless of systemic toxicity in clinical application²⁶⁻²⁸.

In this study, The expression of Fas, TNFR2, DR3 in NCI-H358 was significantly increased by co-culture of NK-92 cells and treatment of 4 $\mu\text{g}/\text{ml}$ of snake venom toxin, compared to co-culture of NK-92 cells alone. Coincidentally, expressions of pro-apoptotic proteins such as Bax, caspase-3 and caspase-8, in the extrinsic apoptosis pathway demonstrated significant increase (fig.2).

The inflammatory and anti-apoptotic transcription factor, NF- κ B plays a significant role in the resistance of NSCLC to chemotherapy²⁹⁻³¹. Direct molecular inhibition of NF- κ B using an adenovirally delivered inhibitor of NF- κ B dramatically sensitizes NSCLC cells to apoptosis induced by traditional chemotherapy agents such as gemcitabine, as well as novel biologic agents including the histone deacetylase inhibitors butyrate and suberoylanilide hydroxamic acid^{29,32}.

The present study revealed that activity of its signal molecule as well as NF- κ B was significantly decreased by co-culture of NK-92 cells and treatment of 4 $\mu\text{g}/\text{ml}$ of snake venom toxin, compared to co-culture of NK-92 cells or snake venom toxin treated NCI-H358 alone.

NO is one of the most powerful effector molecules in the cytotoxic function of NK cells against cancer cells³²⁻³⁴.

This study demonstrated significant increase of NO generation in co-culture of NK-92 cells with NCI-H358 cells as well as the co-culture of NK-92 cell with them and concomitant treatment of 4 $\mu\text{g}/\text{ml}$ of Snake Venom Toxin. However, no synergistic increase of NO generation was shown in co-culture of NK-92 cells and treatment of 4 $\mu\text{g}/\text{ml}$ of snake venom toxin, compared to co-culture of NK-

92 cells with NCI-H358 cells.

Consequently, These present data provide the evidence that snake venom toxin has potential to inhibit NCI-H358 cell growth through DR dependent apoptosis along with cytotoxic effect of NK-cell synergistically, suggesting that snake venom toxin could be useful candidate compounds to suppress NSCLC growth along with cytotoxic effect of NK-92 cells through extrinsic apoptosis.

V. References

1. Wiley SR, Schooley K, Smolak PJ et al. Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity*. 1995 ; 3(6) : 673-82.
2. Ashkenazi A, Pai RC, Fong S et al. Safety and antitumor activity of recombinant soluble Apo2 ligand. *J Clin Invest*. 1999 ; 104(2) : 155-62.
3. Walczak H, Miller RE, Ariail K et al. Tumoricidal activity of tumor necrosis factor-related apoptosis inducing ligand in vivo. *Nat Med*. 1999 ; 5(2) : 157-63.
4. Jung KW, Won YJ, Kong HJ et al. Cancer statistics in Korea : incidence, mortality, survival, and prevalence in 2012. *Cancer Res Treat*. 2015 ; 47(2) : 127-41.
5. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin*. 2011 ; 61(4) : 212-36.
6. Freedman ND, Leitzmann MF, Hollenbeck AR, Schatzkin A, Abnet CC. Cigarette smoking and subsequent risk of lung cancer in men and women : analysis of a prospective cohort study. *Lancet Oncol*. 2008 ; 9(7) : 649-56.
7. Burris HA. Shortcomings of current therapies for non-small-cell lung cancer : unmet medical needs. *Oncogene*. 2009 ; 28(Suppl. 1) : S4-S13.
8. Benlloch S, Galbis-Caravajal JM, Alenda C et al. Expression of molecular markers in mediastinal nodes from resected stage I non-small-cell lung cancer (NSCLC) : prognostic impact and potential role as markers of occult micrometastases. *Ann. Oncol*. 2009 ; 20(1) : 91-7.
9. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer : epidemiology, risk factors, treatment, and survivorship. *Mayo Clin. Proc*. 2008 ; 83(5) : 584-94.
10. Giaccone G. Clinical perspectives on platinum resistance. *Drugs*. 2000 ; 59 suppl 4 : 9-17.
11. Schiller JH, Larson T, Ou SH et al. Efficacy and safety of axitinib in patients with advanced non-small-cell lung cancer : results from a phase II study. *J Clin Oncol*. 2009 ; 27(23) : 3836-41.
12. Park HJ, Lee SH, Son DJ et al. Antiarthritic effect of Bee Venom : inhibition of inflammation mediator generation by suppression of NF-kappaB through interaction with the p50 subunit. *Arthritis Rheum*. 2004 ; 50(11) : 3504-15.
13. Park JH, Jeong YJ, Park KK et al. Melittin suppresses PMA-induced tumor cell invasion by inhibiting NF-kappaB and AP-1-dependent MMP-9 expression. *Mol Cells*. 2010 ; 29(2) : 209-15.
14. Park MH, Choi MS, Kwak DH et al. Anti-cancer effect of Bee Venom in prostate cancer cells through activation of caspase pathway via inactivation of NF-kappaB. *Prostate*. 2011 ; 71(8) : 801-12.
15. Liu S, Yu M, He Y et al. Melittin prevents liver cancer cell metastasis through inhibition of the Rac1-dependent pathway. *Hepatology*. 2008 ; 47(6) : 1964-73.
16. Son DJ, Park MH, Chae SJ et al. Inhibitory effect of SVT from *Vipera lebetina turanica* on hormone-refractory human prostate cancer cell growth : induction of apoptosis through inactivation of nuclear factor kappaB. *Mol Cancer Ther*. 2007 ; 6(2) : 675-83.
17. Park MH, Son DJ, Kwak DH et al. Snake venom toxin inhibits cell growth through induction of apoptosis in neuroblastoma cells.

- Arch Pharm Res. 2009 ; 32(11) : 1545–54.
18. Takeo S, Yasumoto K, Nagashima A, Naka-hashii H, Sugimachi K, Nomoto K. Role of tumor-associated macrophages in lung cancer. *Cancer Res*. 1986 ; 46(6) : 3179–82.
 19. Takanami I, Takeuchi K, Giga M. The prognostic value of natural killer cell infiltration in resected pulmonary adenocarcinoma. *J Thorac Cardiovasc Surg*. 2001 ; 121(6) : 1058–63.
 20. Villegas FR, Coca S, Villarrubia VG et al. Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer. *Lung Cancer*. 2002 ; 35(1) : 23–8.
 21. Rusch V, Klimstra D, Venkatraman E et al. Aberrant p53 expression predicts clinical resistance to cisplatin-based chemotherapy in locally advanced nonsmall cell lung cancer. *Cancer Res*. 1995 ; 55(21) : 5038–42.
 22. Swann JB, Smyth MJ. Immune surveillance of tumors. *J Clin Invest*. 2007 ; 117(5) : 1137–46.
 23. Ljunggren HG, Malmberg KJ. Prospects for the use of NK cells in Immuno-therapy of human cancer. *Nat Rev Immunol*. 2007 ; 7(5) : 329–39.
 24. Sabry M, Lowdell MW. Tumor-primed NK cells : waiting for the green light. *Front Immunology*. 2013 ; 4 : 408.
 25. Kim JH, Song HS. Bee Venom Enhanced Cytotoxic Effect of Natural Killer Cells on Human Lung Cancer Through Inducing Extrinsic Apoptosis. *The Acupuncture*. 2014 ; 31(1) : 111–9.
 26. Nagata S. Apoptosis by death factor. *Cell*. 1997 ; 88(3) : 355–65.
 27. Walczak H, Krammer PH. The CD95 (APO-1/Fas) and the TRAIL(APO-2L) apoptosis systems. *Exp Cell Res*. 2000 ; 256(1) : 58–66.
 28. Havell EA, Fiers W, North RJ. The antitumor function of tumor necrosis factor (TNF). I. Therapeutic action of TNF against an established murine sarcoma is indirect, immunologically dependent, and limited by severe toxicity. *J Exp Med*. 1988 ; 167(3) : 1067–85.
 29. Jones DR, Broad DR, Madrid LV, Baldwin AS, Mayo MW. Inhibition of NF- κ B sensitizes non-small cell lung cancer cells to chemotherapy induced apoptosis. *Ann Thorac Surg*. 2000 ; 70(3) : 930–7.
 30. Jones DR, Broad RM, Comeau LD, Parsons SJ, Mayo MW. Inhibition of nuclear factor B chemosensitizes non-small cell lung cancer through cytochrome c release and caspase activation. *J Thorac Cardiovasc Surg*. 2002 ; 123(2) : 310–7.
 31. Singh RP, Malikarjuna GU, Sharma G et al. Oral silibinin inhibits lung tumor growth in athymic nude mice and forms a novel combination with doxorubicin targeting nuclear factor κ B-mediated inducible chemoresistance. *Clin Cancer Res*. 2004 ; 10(24) : 8641–7.
 32. Mayo MW, Denlinger CE, Broad RM et al. Ineffectiveness of histone deacetylase inhibitors to induce apoptosis involves the transcriptional activation of NF-kappa B through the Akt pathway. *J Biol Chem*. 2003 ; 278(21) : 18980–9.
 33. Henkart PA. Lymphocyte-mediated cytotoxicity : two pathways and multiple effector molecules. *Immunity*. 1994 ; 1(15) : 343–6.
 34. Kojima H, Shinohara N, Hanaoka S et al. Two distinct pathways of specific killing revealed by perforin mutant cytotoxic T lymphocytes. *Immunity*. 1994 ; 1(5) : 357–64.
 35. Cifone MG, Ulisse S, Santoni A. Natural killer cells and Nitric Oxide. *Int Immunopharmacol*. 2001 ; 1(8) : 1513–24.