August 2008
Master's Thesis

The effect of catalase on p53-induced apoptosis

Graduate School of Chosun University

Department of Bio - Material Engineering

Pham Thi Dau

The effect of catalase on p53-induced apoptosis

August 25, 2008

Graduate School of Chosun University

Department of Bio – Material Engineering

Pham Thi Dau

The effect of catalase on p53-induced apoptosis

Advisor: Professor In Youb Chang

Thesis submitted for the degree of Master of Science

April 2008

Graduate School of Chosun University

Department of Bio – Material Engineering

Pham Thi Dau

Thesis submitted in partial fulfillment of the requirement for the award of the degree of Master of Science

Chosun University: Professor Ho Jin You

Chosun University: Professor Jae Yeoul Jun

Chosun University: Professor In Youb Chang

June 2008

Graduate School of Chosun University

CONTENTS

ABSTRACT.....1

I. INTRODUCTION

1. P53 protein.....3

2. Apoptosis.....6

3. Catalase.....9

4. Purpose of study.....10

II. MATERIALS AND METHODS

1. Cell culture.....11
2. The cell treatment 11
3. Construction of adenoviral vector 12
4. Transfection.....12
5. Western Blot.....13
6. Apoptosis by PI staining.....14
7. Measurement of intracellular ROS..... 15

III. RESULTS

1. Role of p53 in DNA damage-induced apoptosis.....16

2. Role of catalase in p53-induced apoptosis.....22

IV. DISCUSSION.....30

V. REFERENCES.....33

KOREAN ABSTRACT.....37

ABSTRACT

The effect of catalase on p53-induced apoptosis

Pham Thi Dau

Advisor: Prof. In Youb Chang, Ph.D., M.D.

Department of Bio-Material Engineering

Graduate School of Chosun University

The activation of p53, a major tumor suppressor protein that implicated in cell cycle control, DNA repair, and apoptosis, has been reported to be involved in the process of apoptosis induced by DNA-damaging agents. P53 acts to regulate the intracellular redox state and induces apoptosis by a pathway that is dependent on reactive oxygen species (ROS) production. Normal aerobic metabolism is associated with the production of ROS including superoxides, hydrogen peroxide hydroxyl radicals, and nitric oxide. A substantial amount of oxygen is converted to superoxide and then to hydrogen peroxide (H_2O_2) by the mitochondrial superoxide dismutase. H_2O_2 is readily diffusible across cell membranes and functions as a signaling molecule in diverse cellular events.

Catalase mediates the predominant enzymatic mechanisms regulating intracellular H_2O_2 levels. The tetrameric catalase converts H_2O_2 to H_2O and O_2 in peroxisomes.

To detect the effect of catalase on p53-induced apoptosis, catalase was stably over-expressed in three kinds of cell lines, U2OS, RKO and NCI H1299. We used western blot and flow cytometry to check apoptosis related proteins, ROS levels and apoptosis of these cell lines. ROS levels were increased after UV irradiation and H_2O_2 treatment in p53 wild-type cell lines, U2OS and RKO. We detected low level of ROS in these cell lines that were over-expressed with catalase. Apoptotic cells were also decreased. In catalase over-expressed NCI H1299 cell line, after Adp53 infection, ROS levels and percentage of apoptotic cells were decreased.

These results suggest that catalase may have an important role in the regulation of p53-induced apoptosis by scavenging ROS.

I. INTRODUCTION

1. P53 protein

The p53 gene comprises 20 kb of DNA and is located on the short arm of chromosome 17p13.1. The gene spans 11 exons that produce a 1.1 kb mRNA transcript that translate into a protein of 393 amino acids. The protein normally resides in the cytoplasm as an inactive monomer, but upon stimulation by a variety of cellular stresses, the protein forms a tetramer from double dimmers [1 and 2].

P53, also known as tumor protein 53 (TP53), is a transcription factor that regulates the cell cycle and hence functions as a tumor suppressor [2, 3, 4, 5, 6, 7, 8, 9 and 10]. It is important in multicellular organisms, it helps to suppress cancer. P53 has been described as "Guardian of the Genome", referring to its role in conserving stability by preventing genome mutation [11]. It can be activated by many types of “danger signals”, such as cell stress and DNA damage, and can trigger several crucial cellular responses that suppress tumor formation. Upstream stress activators include radiation-, drug-, or carcinogen- induced DNA damage, oncogenic activation, hypoxia and low ribonucleotide pools. These conditions may nurture tumor initiation. In response to these stress signals p53 can elicit downstream cellular effects including cell cycle arrest (p21, GADD45), apoptosis (e.g. Bax, Noxa and Puma,…), DNA repair and inhibition of angiogenesis [2].

In normal cells under physiological conditions, the tumor suppressive p53 protein is expressed at low levels and has a short half-life due to rapid turnover mediated by ubiquitination and proteolysis. The p53 protein becomes stabilized and activated in response to number of stressful stimuli including exposure of cells to DNA damaging agents. The activation of p53 allows it to carry out its function as a tumor suppressor through a number of growth controlling endpoints [2, 3, 4, 5, 6, and 7].

The functional signal transduction circuit of p53 consists of the upstream mediators (which sense and relay stress signal to p53), the core regulation components (which form the core circuitry maintaining and regulating p53 levels) and the downstream effectors (which initiate cellular response programs). The core regulator circuitry consists of p53, Mdm2, p14 ^{ARF} (p19^{ARF} in the mouse) and E2F-1. There are organized into two interactive feedback loops. P53 and Mdm2 form one feedback loop, in which p53 positively regulates Mdm2 by activating Mdm2 transcription, and Mdm2 negative regulates p53 promoting p53 ubiquitination and degradation. E2F-1 and p14 ^{ARF} form a similar feedback loop, in which E2F-1 activates ARF transcription, and p14 ^{ARF} facilitates proteolytic degradation of E2F-1. These two feedback loop are connected in two ways. First, p14 ^{ARF} interacts with Mdm2, inhibiting Mdm2-mediated p53 ubiquitination and degradation, thereby stabilizing p53. Second, p53 repress transcription of ARF gene. This complex circuit is essential for maintaining and regulating p53 intracellular levels and activities. Moreover, mutations of p53, amplification of Mdm2, silencing or deletion of

ARF and/or the loss of E2F-1 regulation through RB mutation are the most common mechanism by which tumor cells alter the circuitry [11] (figure 1).

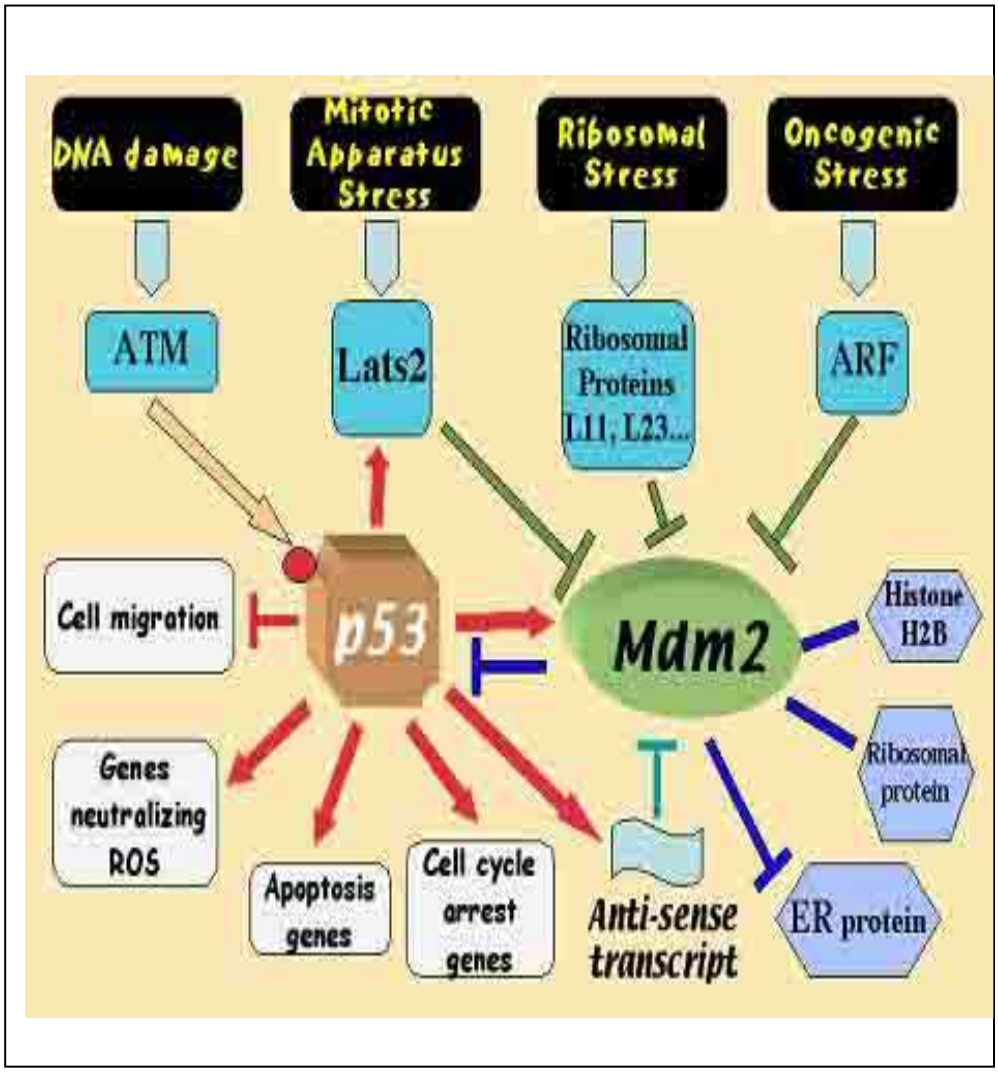

Fig.1. The function and regulation of p53

2. Apoptosis

Apoptosis, a physiological process for killing cells, is critical for the normal development and function of multicellular organisms. Abnormalities in the control cell death can contribute variety of disease, including cancer. Apoptosis is programmed cell death (PCD), is an important counterpart to mitosis for the regulation of cell numbers during development, in homeostatic cell turnover in the adult, and in many other settings. It can be induced by a variety of different stimuli such as growth factor deprivation, ionizing radiation and activation of cell death receptor. Apoptosis is characterized by a series of distinct morphological and biochemical alterations to the cell such as DNA fragmentation, chromatin condensation, cell shrinkage, and plasma-membrane blebbing [2, 12, 13, and 14].

Numerous publications have recently described the importantance of p53 transcriptional regulation to apoptosis. There are two major apoptotic pathways by which caspase activation is triggered. The extrinsic pathway is triggered by the trimerization of cell membrane death receptors followed by the formation of the death-inducing signaling complex (DISC) leads to a cascade of activation of caspases, including caspase-8 and caspase-3, which in turn induced apoptosis. Another, the intrinsic pathway is triggered in response to DNA damage and is associated with mitochondrial depolarization and release of cytochrome c from the mitochondrial intermembrane space into the cytoplasma. Cytochrome c, apoptotic protease-activating factor 1 (APAF-1) and procaspase-9 form a

complex termed the apoptosome, in which caspase-9 is activated and promotes activation of caspase-3, caspase-6 and caspase-7 [10]. The intrinsic pathwayis dominated by the Bcl-2 family proteins, which governs the release of cytochrom c. The Bcl-2 family proteins consist of both anti-apoptosis and pro-apoptosis members such as Bax, Noxa or Puma, are transcriptional targets of p53 [2, 12, 13, 14, 15, 16 and 17] (figure 2).

Previous studies suggested that apoptosis is induced by p53 through three-steps: (1) the transcriptional induction of redox-related genes; (2) the formation of reactive oxygen species (ROS); and (3) the oxidative degradation of mitochondrial components, culminating in the cell death [18].

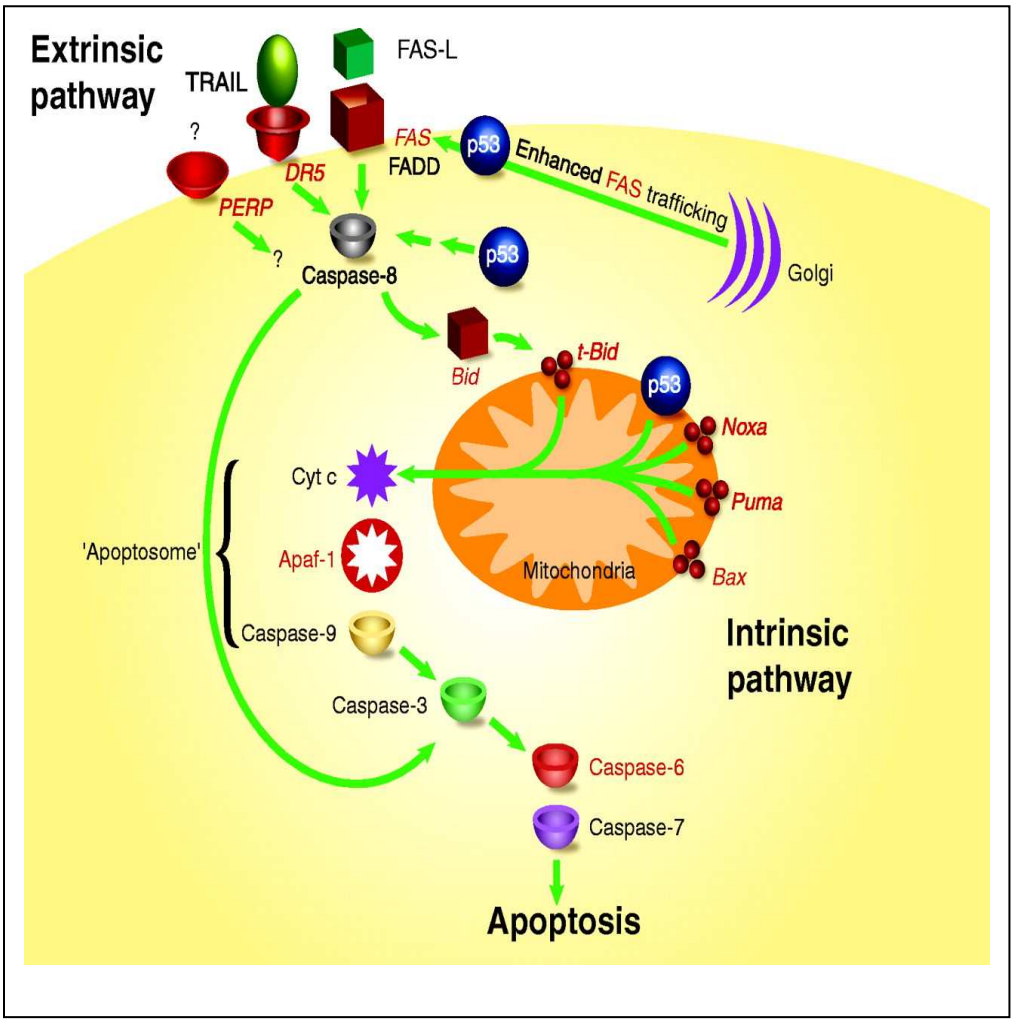

Fig.2. The pathways of p53-induced apoptosis

3. Catalase

Catalase is a common anti-oxidative enzyme found in nearly all living organisms. It is usually located in a cellular organelle called the peroxisome. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. The optimum pH for catalase is approximately (pH 7.0) and the optimum temperature is 37^{o}C. Its function is catalyzing the decomposition of hydrogen peroxide to water and oxygen [19 and 20].

Previously study results of Rezvani *et al.* showed that catalase overexpression inhibited has a protective role against UVB irradiation by preventing DNA damage mediated by the late ROS increase. UVB-induced apoptosis is a highly complex process involving the extrinsic and intrinsic pathways. UVB is known to be an inducer of ROS formation leading to apoptosis. It has been shown that UVB can induce the production of superoxide anion radical and hydrogen peroxide (H_2O_2) [15].

Furthermore, hydrogen peroxide a harmful by-product of many normal metabolic processes playing a role in the toxicity of certain DNA-damaging agents, which is an important ROS related to p53 activation. H_2O_2 is rapidly degradation by catalase, which protects cells against oxidant injury [21 and 22].

4. Purpose of study

In this study, we examined role of p53 in apoptosis that were induced by DNA damaging agents (as UV and H_2O_2) and Adp53 infection. Then we evaluated the effect of catalase on p53-induced apoptosis by overexpression of catalase to p53 wild-type cell lines, U2OS and RKO, and p53 null cell line, NCI 1299 cells. These cells were stimulated by UV or infected by Adp53. Apoptosis related proteins were examined using western blot. ROS levels and apoptotic cells were checked using flow cytometry.

II. MATERIALS AND METHODS

1. Cell culture

The human colon carcinoma cell line RKO (ATTC, CRL- 2577) and osteosacoma cell line U2OS (ATTC, HTB-96) containing wild-type p53 were maintained in Eagle's minimum essential medium (EMEM, Invitrogen corporation) and McCoy's 5A (Mediatech, Inc), respectively, supplemented with 10% fetal bovine serum (FBS, Cambrex), 100 units/ml of penicillin, and 100 mg/ml of streptomycin (Penicillin–Streptomycin, Invitrogen corporation). The human lung carcinoma cell line NCI H1299 (ATTC, CRL-5803) having a homozygous partial deletion of the p53 protein was cultured in RPMI1640 (Invitrogen corporation) supplemented with 10% FBS, 100 units/ml of penicillin, and 100 mg/ml of streptomycin. These cell lines were seeded in 60 cm^2 tissue culture plates (IWAKI), grown at 37^oC, 5% CO_2 incubator and checked by microscope for everyday.

2. The cell treatment

The cells were stimulated by UV irradiation and H_2O_2 reagent to undergo apoptosis in U2OS and RKO cell lines. After UV irradiation, cells were examined at 4, 8, 12 and 24 hours time point. The various concentrations of H_2O_2, 0, 0.05, 0.1, 1 and 2

(mM) were added to induce apoptosis. After Adp53 infection, NCI H1299 cell line was examined at 24, 48 and 72 hours time point.

3. Construction of adenoviral vector

The p53 cDNA was cloned into an Ad/CMV/V5-DEST vector (Invitrogen, Carlsbad, CA) after confirming the DNA sequence. The DNA was linearized with PacI and purified before Lipofectamine 2000 (Invitrogen) transfection to HEK293 cells. For virus collection, the cells were lysed with three consecutive freeze-thaw cycles, and the virus was collected from the supernatant. The virus titer was ~1 x 10^7 p.f.u. /ml, which was determined using an end-point dilution assay. A vector carrying the ß-galactosidase gene LacZ (Ad-LacZ) was used to monitor the efficiency of transduction by the viral vectors and a nonspecific transgenic expression controls.

4. Transefection

Human CAT cDNA was amplified by RT–PCR using the CAT oligo primer. After confirming the DNA sequences, the CAT cDNA was cloned into a pcDNA3.1/V5-His-TOPO (Invitrogen, Carlsbad, CA, USA) vector. The transfection was performed using the Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions.

5. Western Blot

Cells were washed with phosphate-buffered saline (PBS) and lysed on ice for 10 minutes by NP-40 lysis buffer added protease Inhibitor Cocktail tablet free EDTA (Roch). NP-40 buffer (pH=8.0) consists of 50mM Tris (Amresco), 150mM NaCl (Duchefa Biochemie) and 1% NP-40 (USB corporation Cleveland, OH USA). After incubation, extracts were mixed for 5 minutes using micro tube mixer (Tomy MT360) and centrifuged at 13,000 rpm for 15-30 minutes. The supernatant was diluted with 5X SDS-sample buffer that contain 125mM Tris (Amresco), 960mM Glycine (Amresco), 0.5% SDS (sodium dodecyl sulfate, Amresco) and boiled. After cellular protein concentrations were determined using the dye-binding microassay (Bio-Rad, Hercules, CA), samples were loaded 10 μg, 20 μg or 50 μg per lane and electrophoresed on 10% or 15% SDS polyacrylamide gels. The proteins were blotted onto Polyvinylidene Fluoride transfer membranes (BiotraceTM PVDF, Pall corporation). After electroblotting, the membranes were blocked by 5% skim milk in Tris buffer saline containing 0.05% Tween-20 (TBST, 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1 % Tween-20) at room temperature for 2 hours. The membranes were rinsed with TBST and then incubated with appropriate primary antibodies in TBST at 4℃ overnight.

Immunoblotting was carried out with Tubulin-α Ab-2 (Clone DM1A, NeoMarkers), p53 (DO-1) and p53R2 (N-16) from Santa Cruz Biotechnology, anti-Catalase Rabbit pAb and anti-Noxa mouse mAb (114C307) from Calbiochem (Darmstadt,

Germany), cleaved caspase-3, cleaved caspase-7 and Puma from Cell Signaling Technology (Danvers, MA) and Bax from BD Phamingen (San Jose, CA). We followed manufacturer's protocol for dilution of all primary antibodies.

Then, the membranes were washed and incubated with the horseradish peroxidase (HRP)-conjugated secondary antibody (1:4,000) in a blocking buffer for 2 hours at room temperature, and washed again. The blotted proteins were developed using an enhanced chemiluminescence detection system (iNtRON, Biotech, Seoul, Korea).

6. Apoptosis by PI staining

The floating and trypsin-detached over-expressed cells were collected and washed once with ice-cold PBS, followed by fixing in 100% cold ethanol for 30minutes at 4℃ or overnight at -20℃. Then, these cells were washed in PBS and stained PI (Propidium iodide 50 μg/ml), RNase A (50 μg/ml), and 0.05 % Triton X-100. The DNA content of these cells was analyzed by CELLQuest software (FACSCalibur, BD Biosciences). At least 10,000 events were analyzed, and the percentage of cells in sub-G1 population was calculated. Aggregates of cell debris at the origin of histogram were excluded from the sub-G1 cells.

7. Measurement of intracellular ROS

The intracellular production of ROS was assayed using the 5-(and-6)-chloromethyl-2',7'-diclorodihydrofluoescein diacetate, acetyl ester (CM-H_2DCFDA) probe (Invitrogen, Molecular Probes). Briefly, after addition of CM-H_2DCFDA (5uM) cells were incubated for 30 minutes in the dark at 37℃. CM-H_2DCFDA is oxidized by ROS to the highly fluorescent CM-DCF compound. After two washes with PBS, trypsin detached cells were centrifuged and resuspended by PBS containing 0,2% formaline and immediately analyzed by flow cytometry (FACSCalibur, BD Biosciences). Ten-thousand individual data points were collected for each sample.

III. RESULTS

1. Role of p53 in DNA damage-induced apoptosis

We detected expression of apoptosis-related proteins and ROS levels in p53 wild-type cell lines, U2OS and RKO after UV irradiation and H_2O_2 treatment. After UV-irradiation, cells were examined at 4, 8, 12 and 24 hours time point. The various concentrations of H_2O_2, 0, 0.05, 0.1, 1 and 2mM were added to induce apoptosis. After Adp53 infection, NCI H1299 cell line was examined at 12, 24, 48 and 72 hours time point.

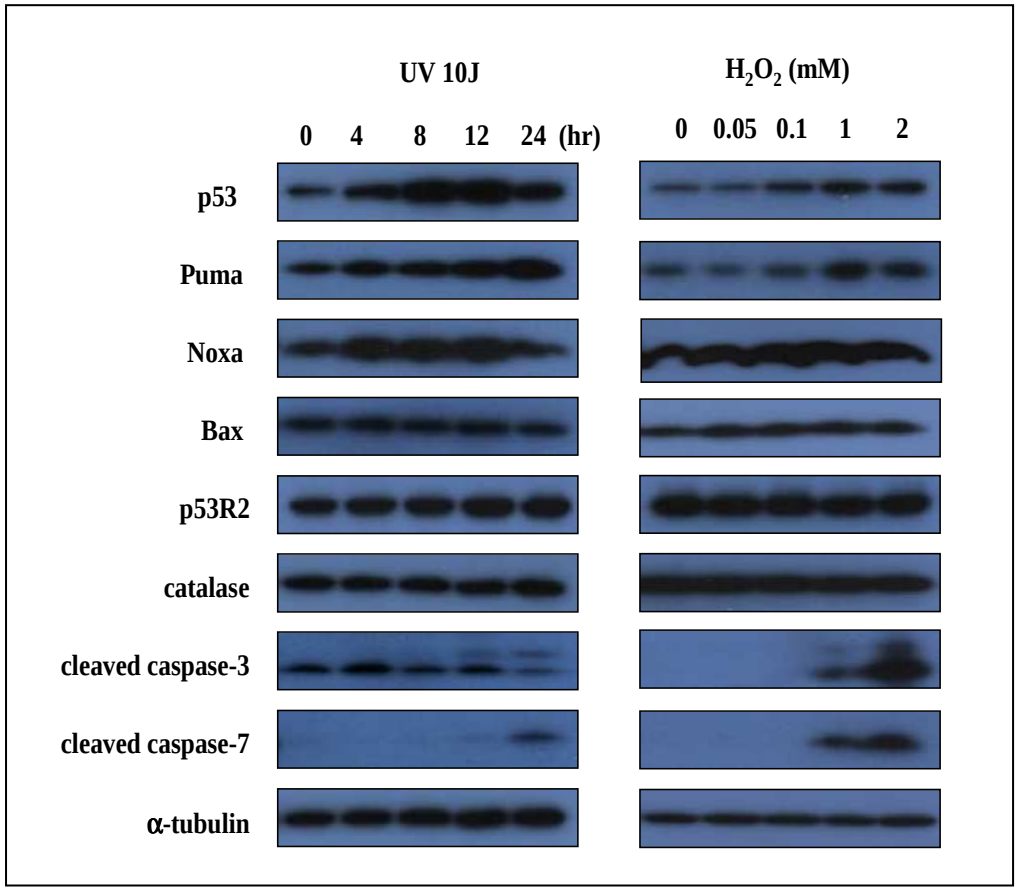

Fig.3. The expression of apoptosis-related proteins in U2OS cell line

In U2OS cell line (figure 3), p53 levels were increased until 12 hours after UV irradiation and decreased at 24 hours incubation time point. The expressions of Bax and Noxa also changed according to p53 levels. However, cleaved caspase-3, cleaved caspase-7 and Puma were increased until 24 hours. H_2O_2 increased p53 levels and other proteins also. Tubulin-α was used to confirm the equal amount of proteins loaded in each lane. Catalase and p53R2 expression levels were not changed after UV irradiation and H_2O_2 treatment.

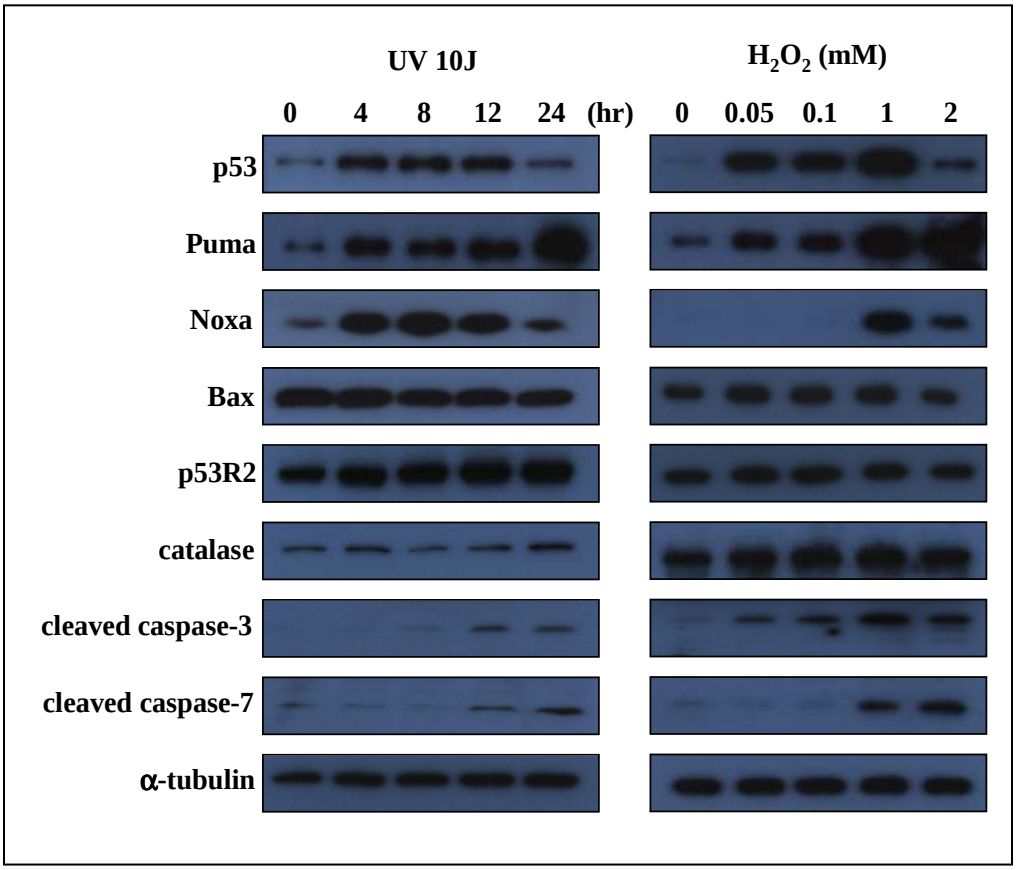

Fig.4. The expression of apoptosis-related proteins in RKO cell line

In RKO cell line (figure 4), p53 levels were increased after both of UV and H_2O_2 stimuli. The expression levels of apoptosis related proteins in RKO cell line showed same patterns with U2OS cell line.

Next, we checked ROS levels in U2OS and RKO cell lines after UV irradiation and H_2O_2 treatment (figure 5). In U2OS cell line after UV irradiation, ROS levels were increased two times. About 80% of ROS levels were changed in RKO cell line. After H_2O_2 treatment, ROS levels were increased about 70% and 50% in U2OS and RKO cell lines, respectively.

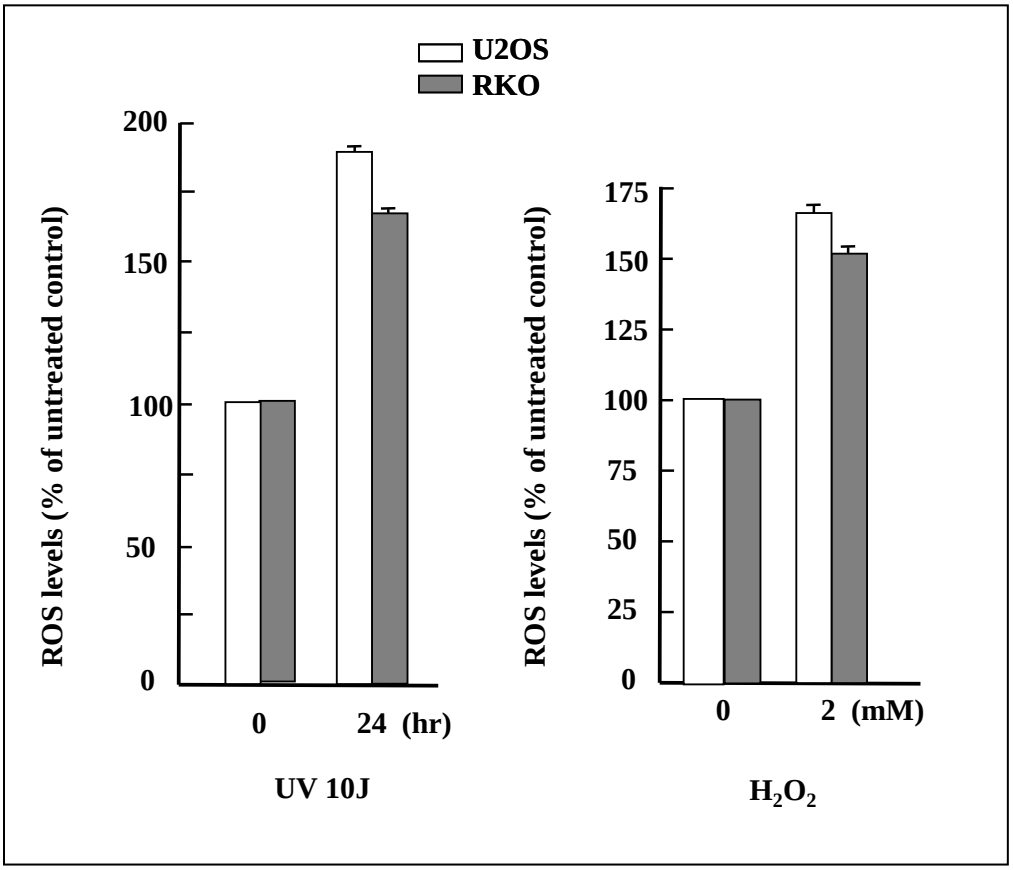

Fig. 5. The ROS levels in U2OS and RKO cell lines after UV irradiation and H_2O_2 treatment

2. Role of catalase in p53-induced apoptosis

To evaluate whether catalase protects cells from apoptosis induced by DNA damaging agents, we stably over-expressed catalase in three cell lines, U2OS, RKO and NCI H1299. The lac Z control vector also was transfected into these cell lines to determinate ability of overexpression. Then, these cells were stimulated by UV or infected by Adp53. Expression of apoptosis related proteins were checked by western blot. ROS levels and apoptotic cells were checked using flow cytometry.

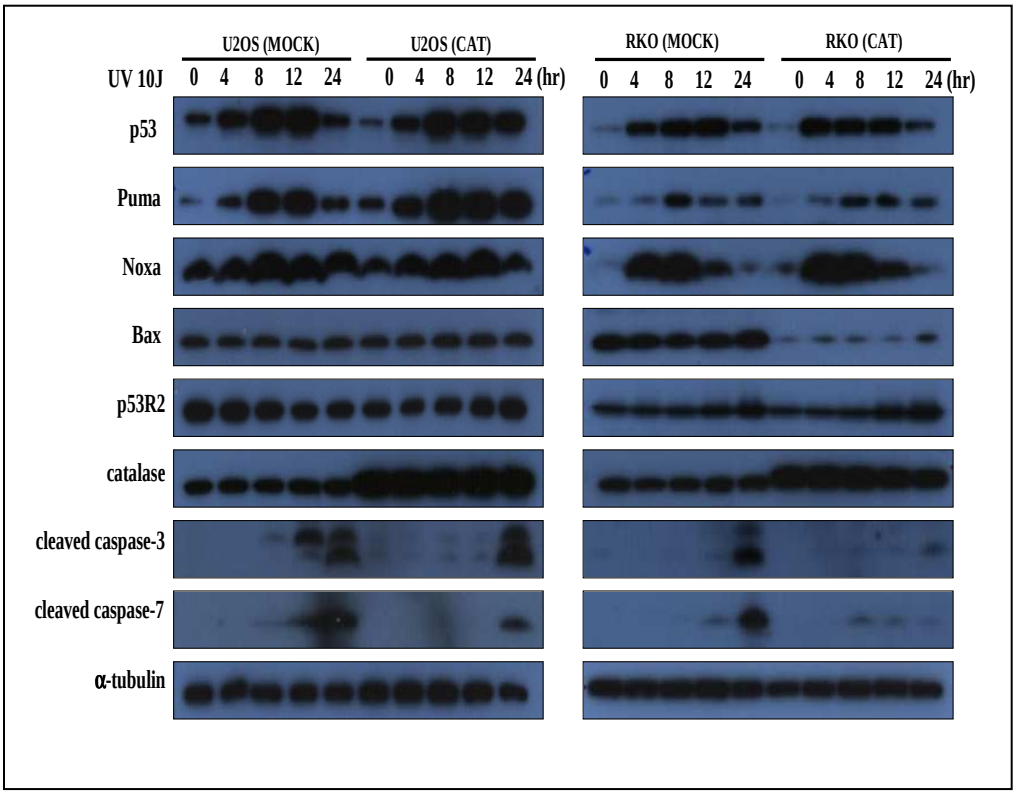

Fig. 6. The expression of proteins in catalase over-expressed cell lines.

Catalase was over-expressed successfully in U2OS and RKO cell lines (figure 6). The expression of p53 targert genes as Bax, Noxa and Puma showed similar patterns with expression of p53 in both of mock and catalase over-expressed cells. The expressions of cleaved caspase-3 and cleaved caspase-7 that represent apoptosis were increased as doses of stimuli. However, these expressions in catalase over-expressed cells were lower than those in mock cells. This result showed that overexpress of catalase effected to expression of apoptosis related proteins, especially, cleaved caspase-3 and cleaved caspase-7

We also checked ROS and apoptosis levels in catalase over-expressed cell lines. Both of U2OS (CAT) and RKO (CAT) cell lines showed that about 70% of ROS levels were decreased after UV irradiation compared with mock cells (figure 7). Apoptotic cells in both of U2OS (CAT) and RKO (CAT) cell lines were decreased significantly after UV irradiation (figure 8). Catalase protected cells from UV induced apoptosis about 20% in U2OS (CAT) and 15% in RKO (CAT).

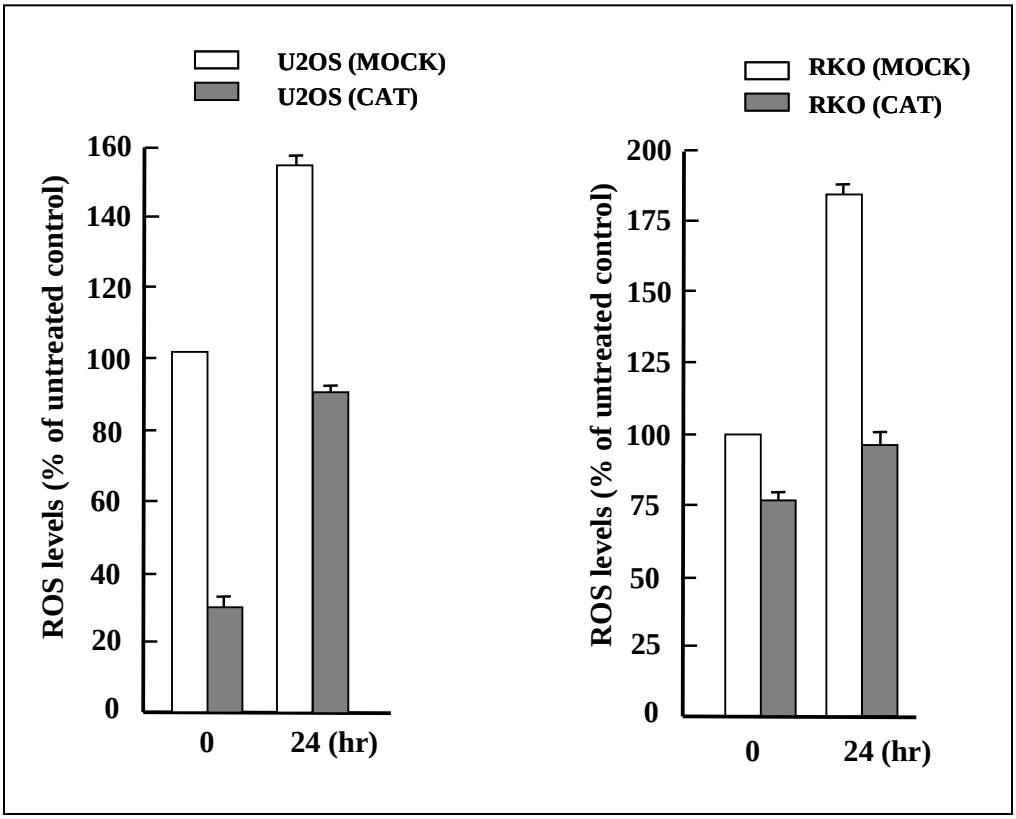

Fig. 7. The ROS levels were decreased significantly in catalase over-expressed cell lines after UV irradiation

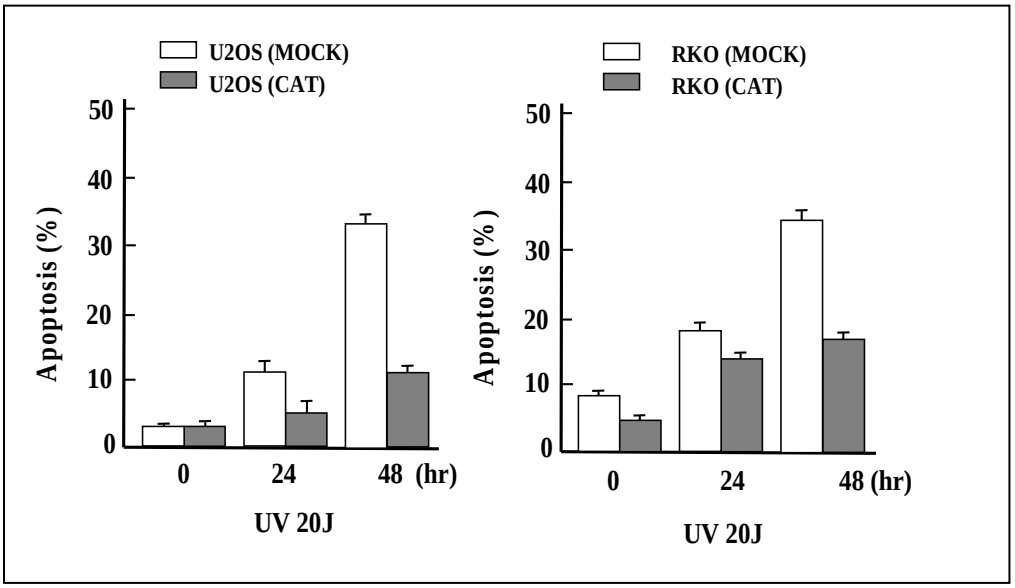

Fig. 8. The apoptotic cells were decreased significantly in catalase over-expressed cell lines after UV irradiation

We could detect p53 levels were increased as time of viral vector infection (figure 9). Apoptosis related proteins were increased according to p53 levels after Adp53 infection in NCI H1299 (CAT) cell line. The expression levels of cleaved caspase-3 and cleaved caspase-7 that represent apoptosis were decreased significantly in NCI H1299 (CAT) compared with mock cell. ROS and apoptosis levels were decreased in NCI 1299 (CAT) cell cell line after Adp53 infection (figure 10).

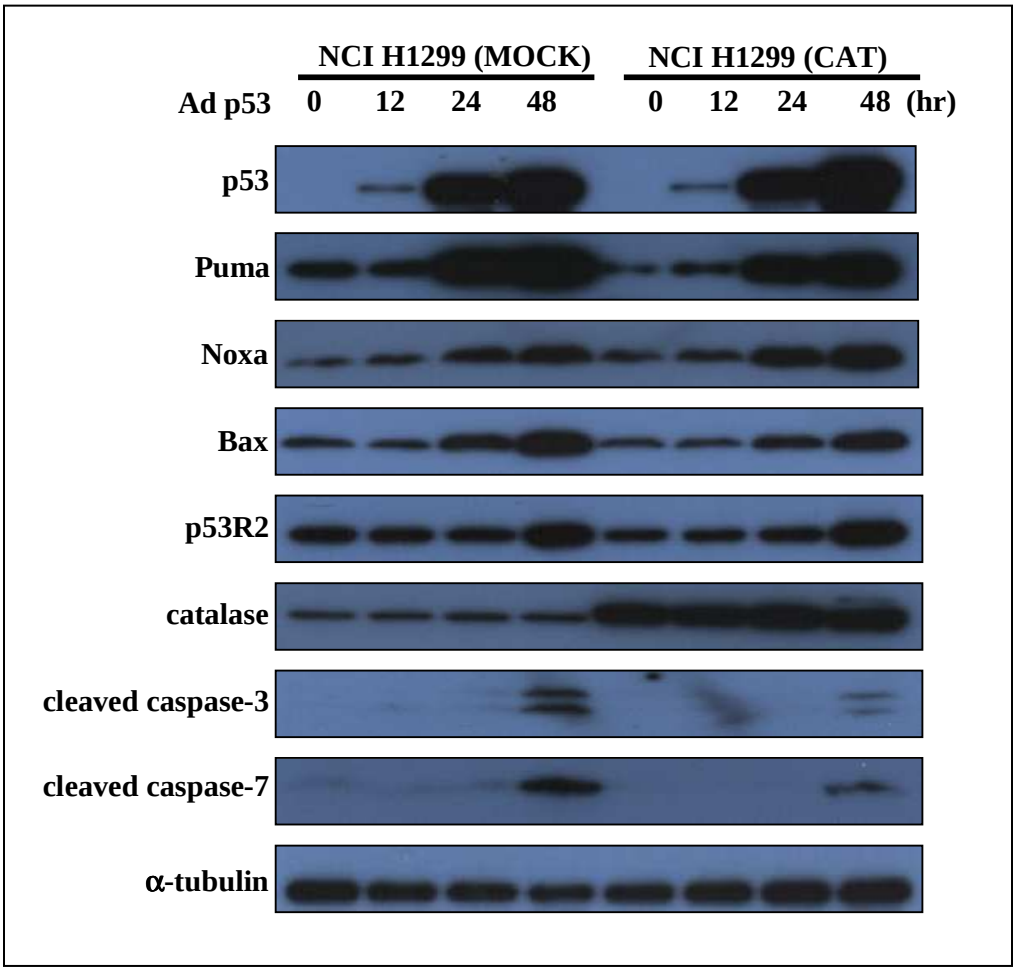

Fig.9. Protein expression levels in NCI H1299 (CAT) cell line after Adp53 infection.

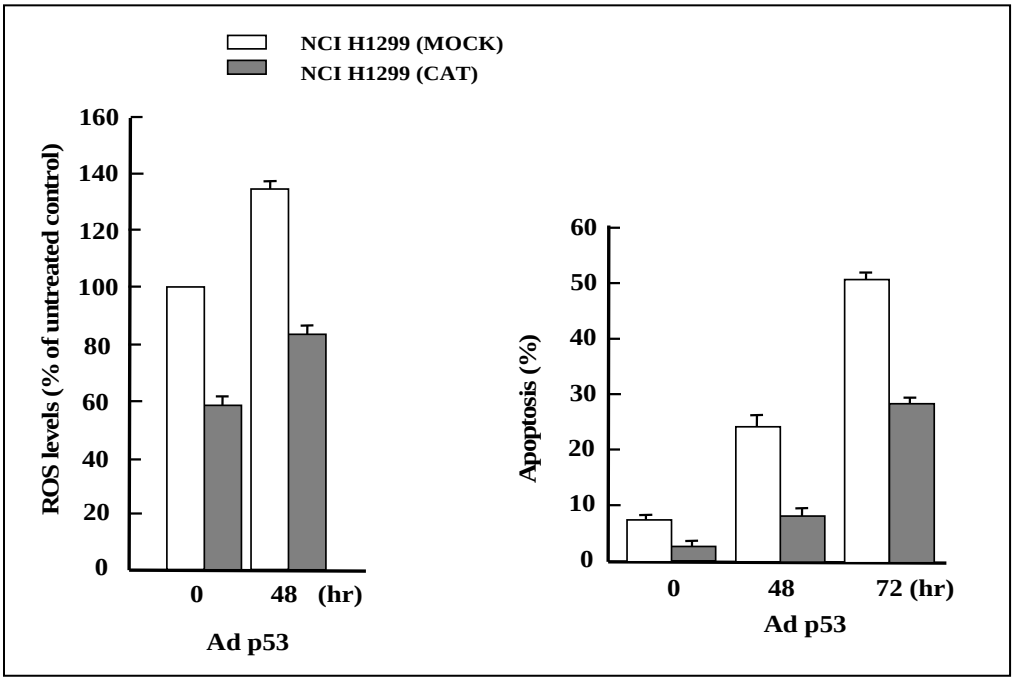

Fig. 10. ROS and apoptosis levels in NCI H1299 (CAT) after Adp53 infection

IV. DISCUSSION

As we know, the p53 is able to promote apoptosis through extrinsic and intrinsic pathways that lead to the activation of the aspartate-specific cysteine proteases (caspase) that mediated apoptosis. The Bcl-2 family proteins dominated in the intrinsic apoptosis pathway, which governs the release of cytochrome c from the mitochondria. The Bcl-2 family contains anti-apoptosis and pro-apoptosis “BH3-only”members. Intriguingly, a key subset of the Bcl-2 family genes are p53 targets, including Bax, Noxa, and Puma. On the other hand, UV irradiation has been known as an inducer of ROS formation leading to apoptosis, but the source of this ROS production is not precisely known. It has been show that UV can induce the production of anion radical and hydrogen peroxide [15]. In addition, H_2O_2 is an oxidant that plays a role in the toxicity of certain DNA-damage agents and it is an important ROS related to p53 activation and induced to apoptosis. H_2O_2 is rapidly degradated by catalse, which protects cells against oxidant injury [21]. That is why we focus on the roles of catalase as H_2O_2 scavenger because H_2O_2 is one of the representative ROS molecules to induce apoptosis.

To investigate the effect of catalase on p53-induced apoptosis, we used western blot to check the expression of apoptosis-related proteins and flow cytometry to detect ROS and apoptosis levels. The results in figure 3, 4 and 5 showed that role of p53 in

DNA damage-induced apoptosis. As p53 levels increased, apoptosis related proteins, as Bax, cleaved caspase-3, cleaved caspase-7, Noxa and Puma were also increased correspondently. According on the cell types and nature of the DNA damaging agent, p53 induces cells to undergo arrest, allow repair, or alternatively induce apoptosis [2 and 8]. At 24 hours after UV irradiation and 2mM of H_2O_2 treatment, levels of p53 decreased could be related to cells undergoing arrest.

In our study, we stably over-expressed catalase to evaluate the role of catalase against p53-induced apoptosis. Our results (figure 6, 7 and 8) showed that catalase play an important role in protection of cells from apoptosis induced by UV irradiation. We also checked the role of catalase in NCI H1299 cell line (figure 9 and 10) after Adp53 infection. Previous studies also suggested that ROS, especially H_2O_2, might be involved in the process of apoptosis induced by these DNA-damaging agents [21]. Therefore, overexpression of catalase may protect cells by removing ROS such as H_2O_2. Hussain *et al.* also demonstrated that the overexpression of catalase significantly reduced p53-mediated apoptosis.

In summary, our results demonstrated that catalase protects cells from apoptosis induced by DNA-damaging agents in p53 wild-type cell lines, U2OS and RKO. According to p53 levels, expression of apoptosis related proteins, ROS and apoptosis levels were increased. We detected that those levels were decreased after catalase overexpression. We can conclude the level of catalase may play an important role in

resistance will be necessary induced by p53.

Further studies about the mechanism that can effect on apoptosis process will be necessary.

V. REFERENCES

1. Mercer, J., Mahmoudi, M. and Bennett, M. (2007) DNA damage, p53, apoptosis and vascular disease. Mutat. Res. 621, 75-86.
2. Pecorino, L. The p53 pathway-apoptosis. Molecular biology of cancer: mechanisms, targets and therapeutics 5, 101-117.
3. Park, S.E., Min , Y.K., Ha, J.D., Kim, B.T. and Lee, W.G. (2007) Novel small molecule induces p53-dependent apoptosis in human colon cancer cells. Boichem. Biophys. Res. Commun. 358, 842-847.
4. Hayon, I.L. and Haupt Y. (2002) Review p53: An internal investigation. Cell cycle 1:2, 111-116.
5. Gewies, A. (2003) ApoReview- Introduction of Apoptosis 20-26.
6. Vassilev, L.T. (2006) Review Mdm2 inhibitors for cancer therapy. Trends Mol. Med. 13, 23-31.
7. El-Deiry, W.S. (1998) Regulation of p53 downstream genes. Semin Cancer Biol. 8, 345-357.
8. Achanta, G. and Huang, P. (2004) Role of p53 in sensing oxidative DNA damage in response to reactive oxygen species-generating agents. Cancer Res. 64, 6233-6239.

9. Toledo, F. and Wahl, G.M. (2006) Reviews Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. Nat. Rev. Cancer 6, 909-923.
10. Haupt, S., Berger, M., Goldberg, Z. and Haupt, Y. (2003) Commentary: Apoptosis-the p53 network. J Cell Sci. 116, 4077-4085.
11. Jin, S. and Levine, A.J. (2001) The p53 functional circuit. Cell science at a glance. J Cell Sci. 114, 4139-4140.
12. Yin, X.M. and Dong, Z. (2003) Molecules and pathways of apoptosis, Essentials of Apoptosis: A guide for basic and clinical research 1, 3-101.
13. Manu, K.A. and Kuttan, G. (2008) Ursolic acid induces apoptosis by activating p53 and caspase-3 gene expressions and suppressing NF-κB mediated activation of Bcl-2 in B16F-10 melanoma cells. Article in press. Int. Immunopharmacology
14. Lee, D.H., Kim, C., Zhang, L. and Lee, Y.J. (2008) Role of p53, Puma, and Bax in wogonin-induced apoptosis in human cancer cells. Biochem Pharmacol.
15. Rezvani, H.R, Mazurier, F., Andre, M.C., Pain, C., Ged, C., Taieb, A. and Verneuil, H.D. (2006) Protective effects of catalase overexpression on UVB-induced apoptosis in normal human keratinocytes. J Biol. Chem. 281, 17999-18007.
16. Schuler, M. and Green, D.R. (2001) Mechanism of p53-dependent apoptosis. Biochem. Soc. Trans. 29, 684-688.

17. Youn, C.K., Song, P.I., Kim, M.H., Kim, J.S., Hyun, J.W., Choi, S.J., Yoon, S.P., Chung, M.H., Chang, I.Y. and You, H.J. (2007) Human 8-Oxoguanine DNA Glycosylase suppresses the Oxidative Stress-Induced Apoptosis through a p53 – Mediated Signaling Pathway in Human Fibroblasts. Mol Cancer Res 5, 1083-1097.

18. Polyak, K., Xia, Y., Zweier, J.L., Kinzler, K.W. and Vogelstein, B. (1997) letters to nature: A model for p53-induced apoptosis. Nature 389, 300-305.

19. Hussain, S.P., Amstad, P., He, P., Robles, A., Lupold, S., Kaneko, I., Ichimiya, M., Sengupta, S., Mechanic, L., Okamura, S., Nagashima, M., Forrester, K.S., and Harris, C.C. (2004) P53-Induced Up-Regulation of MnSOD and GPx but not Catalase Increases Oxidative Stress and Apoptosis. Cancer Res. 64, 2350-2356.

20. Kang, K.A., Lee, K.H., Chae, S., Zhang, R., Jung, M.S., Ham, Y.M., Lee, N.H., and Hyun, J.W. (2006) Cytoprotective Effect of Phloroglucinol on Oxidative Stress Induced Cell Damage via Catalase Activation. J Cell. Biochem. 97, 609-620.

21. Bai, J. and Cederbaum, A.I. (2003) Catalase protects HepG2 cells from apoptosis Induced by DNA-damaging agents by accelerating the degradation of p53. J Biol. Chem. 278, 4660-4667.

22. Bai, J., Rodriguez, A.M., Melendez, J.A. and Cederbaum, A.I. (1999) Overexpression of catalase in cytosolic or mitochondrial compartment protects hepG2 cells against oxidative injury. J Biol. Chem. 274, 26217-26224.

<국문초록>

Catalase의 p53에 의해 유도된 세포자살에 미치는 영향

Pham Thi Dau

(지도교수 : 장 인 엽)

생물신소재학과

조선대학교 대학원

종양 억제 단백질 p53 는 세포주기 조절, DNA 복구 및 세포자살과정에 관여하며, 이 단백질의 활성은 DNA 손상물질에 의해 유도된 세포자살에 영향을 끼친다. 세포자살 유도는 세포내 redox state 조절 및 ROS 의 생성에 의하며, ROS의 증가는 superoxide, H_2O_2 , hydroxyl radical 그리고 nitric oxide 등에 의한다. 미토콘드리아에서 호흡 경로에 의한 산소는 superoxide 로 전환 되고, 미토콘드리아의 superoxide dismutase 에 의해 H_2O_2 가 되면서 감소된다. H_2O_2 는 즉시 cell membrane 을 통해 확산되어 다양한 세포 기작에 의해서 신호전달물질의 기능을 한다. Glutathione peroxidase 와 catalase 에 의해 세포내 H_2O_2 수준이 조절되며, peroxisomes 에서 catalase 에 의해 H_2O_2 는 H_2O 로 전환되고 O_2 가 남는다.

본 연구에서는 활성산소에 의한 세포자살에 있어서 p53 과 catalase 의 상호관계를 규명하고자 하였다. P53 wild-type 세포주인 U2OS 와 RKO 에서 UV 조사와 H_2O_2 자극 후 활성산소의 변화를 확인하였다. 두 세포주 모두에서 p53 발현 증가에 따른 현격한 ROS 변화를 확인할 수 있었다. Catalase 의 p53 에 의해 유도된 세포사멸에 미치는 영향을 확인하기 위하여 U2OS, RKO 및 NCI H1299 의 세가지 세포주에서 catalase 를 과발현시켰다. U2OS 와 RKO 세포주에서 UV 조사후 증가된 p53 단백질 발현에 의해 유도된 세포자살은 catalase 가 과발현된 세포주에서 현격히 감소됨을 확인하였다. 또한 catalase 를 과발현시킨 NCI H1299 세포주에서도 Adp53 infection 후 ROS 및 세포자살의 감소를 확인할 수 있었다. 이상의 연구결과 catalase 는 p53 에 의해 유도된 활성산소의 수준을 감소시키고, 이에 따라 catalase 는 p53 에 의한 세포사멸로부터 세포를 보호한다고 할 수 있다.

저작물 이용 허락서

학 과	생물신소재학과	학 번	20067746	과 정	석 사
성 명	한글 : 팜티다우 영문 : Pham Thi Dau				
주 소	광주광역시 동구 서석동 375 조선대학교 생명공학관 KDRRC				
연락처	E-mail : phamthidau1204@yahoo.com				
논문제목	한글: Catalase의 p53에 의해 유도된 세포자살에 미치는 영향 영문: The effect of catalase on p53-induced apoptosis				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

2008년 6 월 5일

저작자: Pham Thi Dau (서명 또는 인)

조선대학교 총장 귀하

ACKNOWLEDGEMENT

I would like to express my sincere thanks to Professor In-Youb Chang and Professor Ho Jin You. I received great advice and valuable knowledge from them. They have been always helped to provide the best environment for studying and living around me during my Master course here.

I would like to thank to Dr. Mi-Young Kang who instructed and guided me for my project and also for my life. With her precious support, my Master thesis was successfully done.

I would like to say “thank you” to Seung-Hee Song for her lovely and friendship assistance in my life and study from my first day in Korea.

Thanks to all members in DNA repair laboratory, Korean friends and foreign friends for your experience sharing, for your hospitality and helpful hands.

Also, I would love to show my appreciation to my family, my teachers and my friends in Vietnam. Your encouragement made my days.

Thank you very much.

Gwangju, June 5 th 2008

Pham Thi Dau