2006년 2월
석사학위논문

The study for the functional interaction of p53 and catalase

조선대학교 대학원

생물신소재학과

이 도 영

The study for the functional interaction of p53 and catalase

지도교수 유 호 진

이 논문을 이학석사 학위신청논문으로 제출함.

2005년 10월 일

조선대학교 대학원

생물신소재학과

이 도 영

이 도 영의 석사학위논문을 인준함

위원장 조선 대학교 교수 張仁燁 인

위 원 조선 대학교 교수 전계열 인

위 원 조선 대학교 교수 柳昊陳 인

2005 년 11 월 일

조선대학교 대학원

The study for the functional interaction of p53 and catalase

p53 과 catalase 의 기능적 상호관계에 관한 연구

2006년 2 월 일

조선대학교 대학원

생물신소재학과

이 도 영

CONTENTS

CONTENTS OF FIGURES

<국문초록>

p53 과 catalase 의 기능적 상호관계에 관한 연구

이 도 영

(지도교수 : 유 호 진)

조선대학교 대학원 생물신소재학과

세포주기 조절, DNA 복구, 세포자살에 관련된 주 종양 억제 단백질 p53의 활성은 DNA-damaging agents에 의해 유도된 apoptosis의 과정에 영향을 끼친다고 보고되어 왔다. p53은 세포 내 redox 단계에서의 조절과 ROS 생성, 경로에 의한 apoptosis 유도의 역할을 한다. 일반적인 aerobic metabolism은 superoxide, H_2O_2, hydroxyl radical, nitric oxide를 포함한 ROS의 생성과 관련이 있다. 미토콘드리아에서의 호흡 경로에 의한 실질적인 산소 양의 감소는 superoxide로 전환이 되고 그때 mitochondrial superoxide dismutase에 의해 H_2O_2가 된다. H_2O_2 는 즉시 cell membrane을 통해 확산되어 다양한 세포 기작에서의 signaling molecule의 기능을 한다. 주된 효소 기작은 세포내 H_2O_2 수준을 glutathione peroxidase 와 catalase 에 의해 조절된다. tetraceric catalase는 peroxisomes에서 H_2O_2가 H2O로 전환되고 O_2가 남는다. P53과 결합하는 단백질을 찾기 위하여 yeast two hybrid system을 사용한 결과 항산화제 효소인 catalase가 p53과 결합함을 발견하였다. 또한 in vivo 및 in vitro 실험을 통하여 p53이 catalase와 결합함을 규명하였으며, p53이 catalase 와 결합하면 catalase의 활성이 감소한다는 사실을 발견하였다. 이상의 연구결과 p53은 catalase와 결합하여 세포내 산소 라디칼의 양을 조절하는데 관여할 것으로 사료된다.

ABSTRACT

The activation of p53, a major tumor suppressor protein, which is 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 ROS production. Normal aerobic metabolism in associated with the production of reactive oxygen species (ROS), including superoxides, hydrogen peroxide hydroxyl radicals, and nitric oxide. A substantial amount of oxygen reduced by the mitochondrial respiratory chain is converted to superoxide and then to 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. The predominant enzymatic mechanisms that regulate intracellular H_2O_2 levels are mediated by catalase and glutathione peroxidase. The tetraceric catalase converts H_2O_2 to H2O and O2 in peroxisomes. To find out the new p53 interacting protein, yeast two-hybrid assay is used, and catalase is found to interact with p53. In vitro and in vivo data analysis reveals that p53 immunoprecipitate with catalase. In addition, the addition of p53 protein results in the suppression of the catalase activity. These results suggest that p53 may have an important role in the regulation of the intracellular amounts of reactive oxygen species due to interact with catalase.

I. INTRODUCTION

The tumor suppressor p53 plays significant roles in growth arrest, DNA repair and apoptosis, by acting as a transcription factor to modulate the expression of various genes. Depending on the promoter context, the stimulus type and the cellular environment, p53 functions not only to activate gene transcription, but also to repress the expression of responsive genes. The repression mechanism is mediated either by direct binding to the response element or by indirect interactions with other transcriptional regulators, depending on the promoter context. It has been reported that the C-terminal oligomerization domain (OD) of p53, histone deacetylase (HDAC) and mSin3a are involved in p53-mediated repression of transcription.

Mitochondria are eukaryotic cytoplasmic organelles that generate most of the energy required for cellular metabolism via oxidative phosphorylation, As a result of intracellular oxygen metabolism, approximately two percent of the oxygen consumed in cells is known tobe converted to reactive oxygen species(ROS) such as superoxide anions and hydrogen peroxide (Boveris and Chance, 1973). In addition, several growth factors and cytokines such as platelet-derived growth

factor (Sundaresn et al., 1995), epidermal growth factor (Bae et al., 1997), basic fibroblast growth factor (Lo and Cruz, 1995), transforming growth factor-β(Thannickal and Fanburg, 1995) and interleukin-1(Meier et al., 1989) have been shown to stimulate the production of intracellular ROS in a variety of cells. Depending on their intracellular concentrations, ROS function either as physiological intermediates for cellular responses or as inducers of toxic oxidative stress. While ROS at low concentrations are involved in numerous cellular events including gene expression (Devary et al.,1991; Meier et al., 1989), activation of several transcription factors (Schreck et al.,1991), and cellular proliferation (Murrell et al., 1990), increased ROS are known to trigger cell arres or cell death through the activation of cell cycle regulators such as 053, $p21^{WAF/CIF1}$ and Rb(Chen et al., 1998; Johnson et al.,1996; Polyak et al.,1997).

ROS metabolism in cellular environments may be regulated by the serial activation of antioxidant enzymes that include manganese superoxide dismutase(MnSOD), copper-Zinc superoxide dismutase(CuZnSOD), catalase and glutathione peroxidase. MnSOD and CuZnSOD function as the initial antioxidant enzymes by converting superoxide anions to hydrogen peroxide in the mitochondria and cytoplasm, respectively. Catalase and glutathione peroxidase then continue the process to

detoxify hydrogen peroxide to water. Therefore, the overall balance between ROS and antioxidant enzymes is likely to be more important in maintaining optimal physiological conditions for cellular proliferation than are the specific levels of each or the respective ROS and antioxidant enzymes. In the present study, I tried to find out the new p53 interacting protein, using yeast two-hybrid assay, and found the catalase is interacted with p53.

II. MATERIALS AND METHODS

Cell culture

The human fibroblast GM00637 cells (Coriell Institute for Medical Research) were maintained in Eagle's minimum essential medium (EMEM) that was supplemented with 10% fetal bovine serum (FBS). The HCT116 p53+/+ and HCT116 p53-/- cells were cultured in Iscove's modified Dulbecco's medium (IMDM) that was supplemented with 10% FBS. The DLD1, SW480, PC3 and DU145 cells were cultured in RPMI1640 that was supplemented with 10% FBS.

Plasmid constructs and Transfection

Human p53 cDNA and human CAT cDNA were amplified by RT–PCR using the p53 oligo primer and the CAT oligo primer, respectively, from human fibroblast GM00637 cells. After confirming the DNA sequences, the p53 cDNA was cloned into a pGBT9 (BD Biosciences Clontech) and pGEX 4T-1 (Amersham-Pharmacia Biotech) vector. The CAT cDNA was cloned into a pcDNA3.1/V5-His-TOPO (Invitrogen, Carlsbad, CA, USA) and pACT2AD (BD Biosciences Clontech) and

pD2MH (Intron) vector. The transfections were performed using the LipofectAMINE method (Promega) according to the manufacturer's instructions.

Construction of adenoviral vector encoding p53 cDNA

The p53 cDNA was cloned into a *pShuttle* vector (Invitrogen, Carlsbad, CA) after confirming the DNA sequence. The newly constructed plasmid pShuttle-p53 was then doubly digested with PI-SceI/I-CeuI, and the purified product was ligated using Adeno-X DNA. The DNA was linearized with PacI and purified before Liopfectamine (Invitrogen) transfection of HEK293 cells. After transduction, HEK293 cells layers were overlaid with agarose and assessed for viral plaque formation at 10 days. 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 transgene expression controls.

Yeast two-hybrid screening.

The MATCHMAKER Two-Hybrid 3 (Clontech) was used. The full-length p53 was cloned into vector pGBT9 and the plasmid was transformed into the yeast strain AH109 followed by screening a pACT2 human fetal liver cDNA library (Clontech). Yeast transformants were selected according to the manufacturer's instruction. The total 5×10^6 of transformed were screened and nine positive clones were picked up. Mating tests were performed to confirm the specific interaction. The prey plasmids were isolated and sequenced. Homology was searched with the BLAST algorithm through NCBI web site at http://www.ncbi.nlm.nih.gov.

Polymerase chain reaction and RT-PCR

RNA was extracted with TRIzol regent (Invitrogen, Carlsbad, CA, USA). 5 μg of Total RNA was reverse transcriped using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA). 50ng of cDNA was subjected to a polymerase chain reaction (PCR). The profile of the replication cycle was denaturation at 94℃ for 30 sec, annealing at 60℃ for 30 sec, and polymerization at 72℃ for 60 sec. In each

reaction, glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was used as the internal control. The PCR products were detected on EtBr-contained 1% agarose gels and then photographed.

Immunoblot

The cells were washed with PBS and lysed at 0°C for 30 min in a lysis buffer (20 mM HEPES, pH 7.4, 2 mM EGTA, 50 mM glycerol phosphate, 1% Triton X-100, 10% glycerol, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM Na_3VO_4 and 5 mM NaF). The protein content was determined using the Bio-Rad dye-binding microassay (Bio-Rad, Hercules, CA), and 20 μg protein per lane was electrophoresed on 10% SDS polyacrylamide gels. The proteins were blotted onto Hybon ECL membranes (Amersham-Phamacia, Biotech) and immunoblotting was carried out with anti-p53 , anti-His and anti-α-tubulin antibodies (Santa Cruz Biotechnology), and also with anti-CAT (Calbiochem). The blotted proteins were then detected using an enhanced chemiluminescence detect system (iNtRON, Biotech, Seoul, Korea).

In vitro binding assays

The full-length p53 was cloned into pGEX4-1 vector (Amersham-Pharmacia Biotech) and GST-p53 fusion protein was expressed by inducing using 0.04 mM IPTG in *Escherichia coli* strain BL21. The CAT was cloned into the vector pD2MH. Then, the expressed products were purified with glutathione–Sepharose 4B beads (Amersham-Pharmacia Biotech). The His-p53 fusion protein was generated by the TNT-coupled reticulocyte lysate system (Amersham-Pharmacia Biotech) following the manufacturer's instruction and detected by Western blot with anti-His antibody (Invitrogen). GST-p53 bound to glutathione–Sepharose 4B beads was incubated with 5 μl in vitro translated His-CAT protein in 200 μl NETN buffer (50 mM Tris–HCl, pH 7.5, 200 mM NaCl, 2 mM EDTA, 0.1% NP-40, and 1 mM PMSF) for 3 h at 4 °C. The beads were washed four times by H buffer (20 mM Hepes, 50 mM KCl, 20% glycerol, 0.1% Nonidet P-40, and 0.007% β-mercaptoethanol). Then, the pellets were boiled in 20 μl loading buffer, subjected to 15% SDS–PAGE, transferred to PVDF membrane, and immunoblotted with mouse anti-His antibody.

Co-immunoprecipitation

The human fibroblast GM00637 cells were transfected pcDNA3.1/V5-His-TOPO - CAT. After 48 h incubation, cells were harvested and washed twice in cold PBS, and lysed with RIPA buffer (PIERCE, Rockford, IL, USA). The lysate supernatant was incubated with protein A/G–agarose (Santa Cruz Biotechnology) for 1 h at 4 °C followed by immunoprecipitation with anti-p53 or anti-CAT antibody (Santa Cruz Biotechnology) at 4 °C overnight. The pellets were washed three times with lysis buffer. The precipitated proteins were eluted from the beads with loading buffer and separated on 10% SDS–PAGE. Proteins were transferred to PVDF membrane and immunoblotted with anti-CAT or anti-p53 antibody.

CAT-siRNA design, synthesis, labeling, and transfection.

Sequence information regarding the human CAT mRNA was obtained from the NCBI Entrez nucleotide database. Three target sites within the CAT gene were chosen from the human CAT mRNA sequence (GenBank Accession No.NM_001752). Following selection, each target site was searched using NCBI

Blast to confirm the specificity to CAT only. Three different siRNAs designated CAT-siRNA1(291-311), CAT-siRNA2(637-657) and CAT-siRNA3(970-990) of the human CAT mRNA sequence, respectively, were prepared using a transcription-based method using a Silencer siRNA construction kit (Ambion, Austin, TX) according to manufacturer's instructions. LacZ siRNA was used as the negative control. The cells were transfected with the siRNA duplexes using Oligofectamine (Invitrogen) according to the manufacturer's protocol.

Catalase activity assay

The activity of catalase was measured using a Amplex Red Catalase Assay Kit (Invitrogen). Principle of the two step Amplex Red assay for catalase. In step 1, catalase converts H_2O_2 to H_2O and O_2. In step 2, HRP catalyzes the reaction of residual H_2O_2 with Amplex Red regent to produce the red-fluorescent dye, resorufin. The measurement were performed using the Amplex Red Catalase Assay method according to the manufacturer's instructions.

Table1. The primers for PCR

Primer	sequence	Tm (℃)
p53 sence	5'-GGA TCC CCA TGG AGG AGC CGC A-3'	58
p53 antisence	5'-CTG CAG ATC ATC CAT TGC TTG GGA-3'	
p53 sence	5'-GGA TCC ATT TGA TGC TGT CCC CGG A-3'	58
p53 antisence	5'-CTG CAG CTG GGA AGG GAC AGA AGA T-3'	
p53 sence	5'-GGA TCC AAA CCT ACC AGG GCA GCT A-3'	58
p53 antisence	5'-CTG CAG TGC TCG CTT AGT GCT CCC T-3'	
p53 sence	5'-GGA TCC CAG GGA GCA CTA AGC GA-3'	58
p53 antisence	5'-GTC GAC TGT CTG AGT CAG GCC CTT-3'	
CAT sence	5'-GGT ACC ATG GCT GAC AGC CGG GAT CCC-3'	60
CAT antisence	5'-GAT ATC TCA CAG ATT TGC CTT CTC CCT TG-3'	
CAT sence	5'-GGA TCC TGG CTG ACA GCC GGG AT-3'	60
CAT antisence	5'-CTC GAG TCA CAG ATT TGC CTT CTC CCT T-3'	
CAT sence	5'-GAT ATC GCT GAC AGC CGG GAT CCC GC-3'	
CAT antisence	5'-GCG GCC GCT CAC AGA TTT GCC TTC TCC CTT G-3'	60
GAPDH sence	5'-TGA CCA CAG TCC ATG CCA TC-3'	60
GAPDH antisence	5'-TTA CTC CTT GGA GGA GGC CAT GT-3'	

III. RESULTS

CAT interaction with p53

To identify proteins interacting with p53, we screened a human fetal liver cDNA library in a yeast two-hybrid assay using p53 as the bait. Positive clones were identified by screening about 5×10^6 transformants. The candidate proteins were identified following isolation of the pACT2 plasmids, restriction mapping, and sequencing. Database searches using the BLAST program at NCBI web site revealed that one positive interacting protein showed 100% identity to the *Homo sapiens* catalase mRNA. Mating tests were performed to testify the specific interaction(Fig.1 ,Fig.2). To further study of the function of p53, we tried to find the Fragment of p53 that binding CAT(Fig.3).

To confirm the interaction between p53 and CAT, in vitro binding assays were performed using GST fusion proteins. p53 was shown to efficiently bind to CAT(Fig.6 (A)). To further verify whether p53 interacts with CAT in mammalian cells, in vivo binding assays were performed. The results of the co-immunoprecipitation are shown(Fig.6 (B)). p53 of cell and His-CAT could be

expressed in transformed cells. p53 and CAT protein were coprecipitated and detected by Western blot with anti-p53 antibody and anti-CAT(Fig. 5(B)). When anti-p53 and anti-CAT antibody were used for immunoprecipitation, demonstrated bind to p53 and CAT.

Both results of in vitro and in vivo assays are in agreement with those of the yeast two-hybrid assay.

Decreased of catalase activity on p53 binding CAT

Catalase consists of four identical subunits, each containing a single heme for H_2O_2 degradation. The catalase activity was measured using a Amplex Red Catalase Assay Kit. To result treatment of p53 or cell extract contained p53, catalase activity was decreased(Fig. 4, Fig. 7). To further expression of CAT effect to activity of p53 , in order to, si-CAT were designed.(Fig. 8)

Figure and Figure Legends

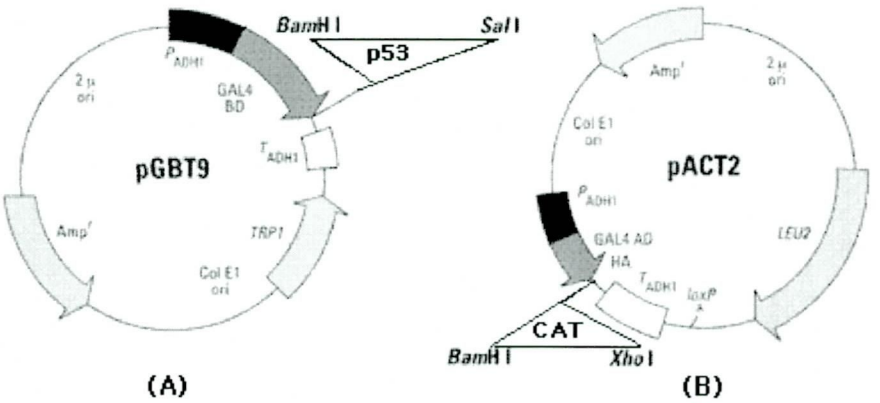


Fig. 1. (A)The p53 containing BamHI and SalI restriction site was cloned into pGBT9 vector. (B)The CAT containing BamHI and XhoI restriction site was cloned into pACT2 vector.

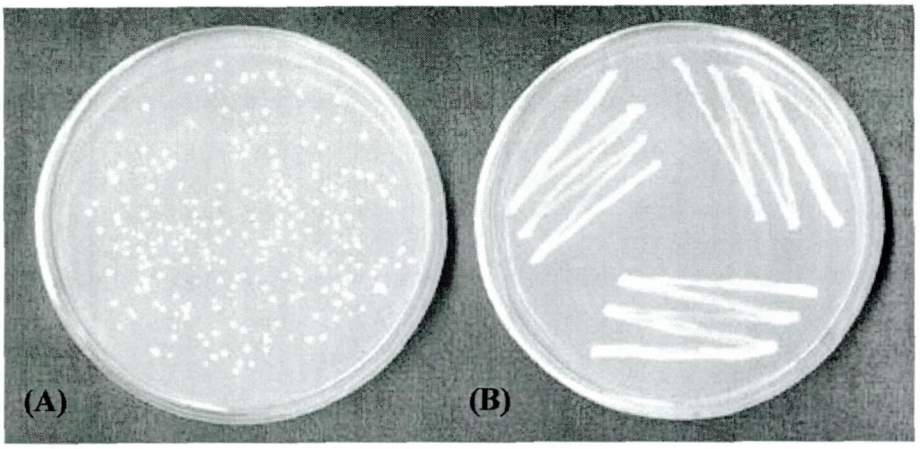


Fig. 2. Confirmation of interaction between p53 and CAT in yeast hybrid system. Yeast diploids that contain the indicated bait (pGBT9-p53) and prey (pACT2AD-CAT) plasmids were inoculated in SD/-Trp/-Leu/-His (A). In three colonies from (A) p53 binding of CAT (B) is confirmed by re-Yeast two hybrid. Plates were incubated for 3 days at 30℃

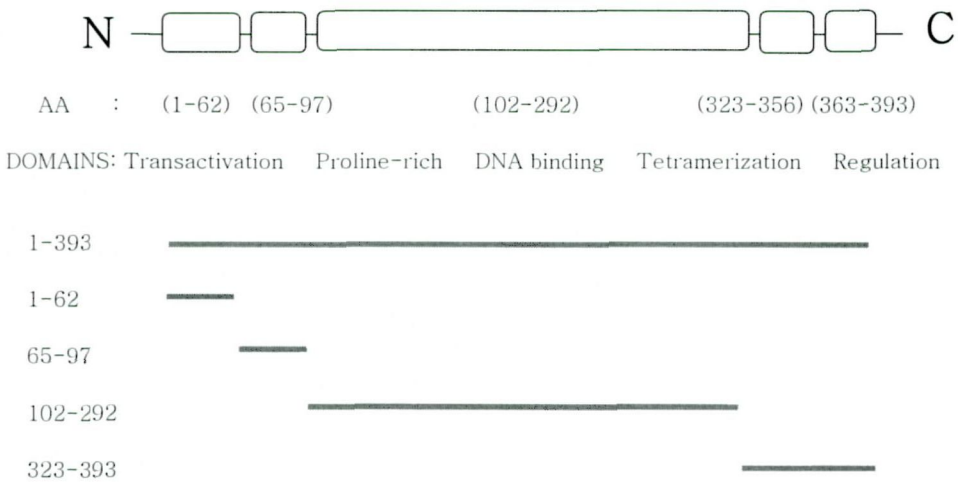


Figure. 3. Fragments of p53 that they were designed in functional domain of p53 for invitro binding assay. The p53 gene TP53 encodes a 393 amino-acid nuclear protein which consists of an N-terminal transactivation domain (TA), a proline rich region (PxxP), a DNA binding domain, a tetramerization domain (TETRA), a regulation domain, and a basic C-terminal domain.

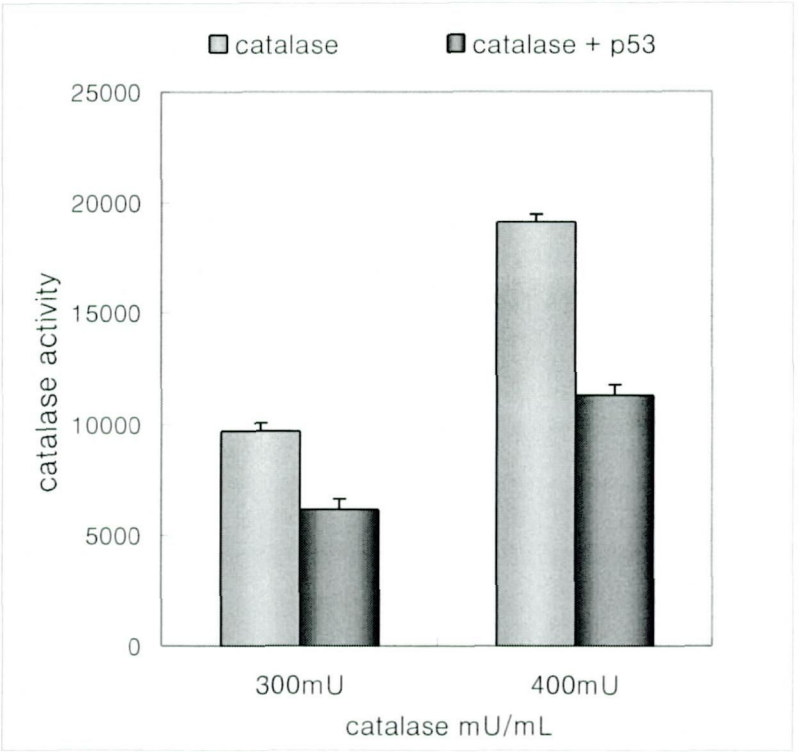


Figure 4. In vitro analysis of catalase activity shows reduction with treatment of p53.

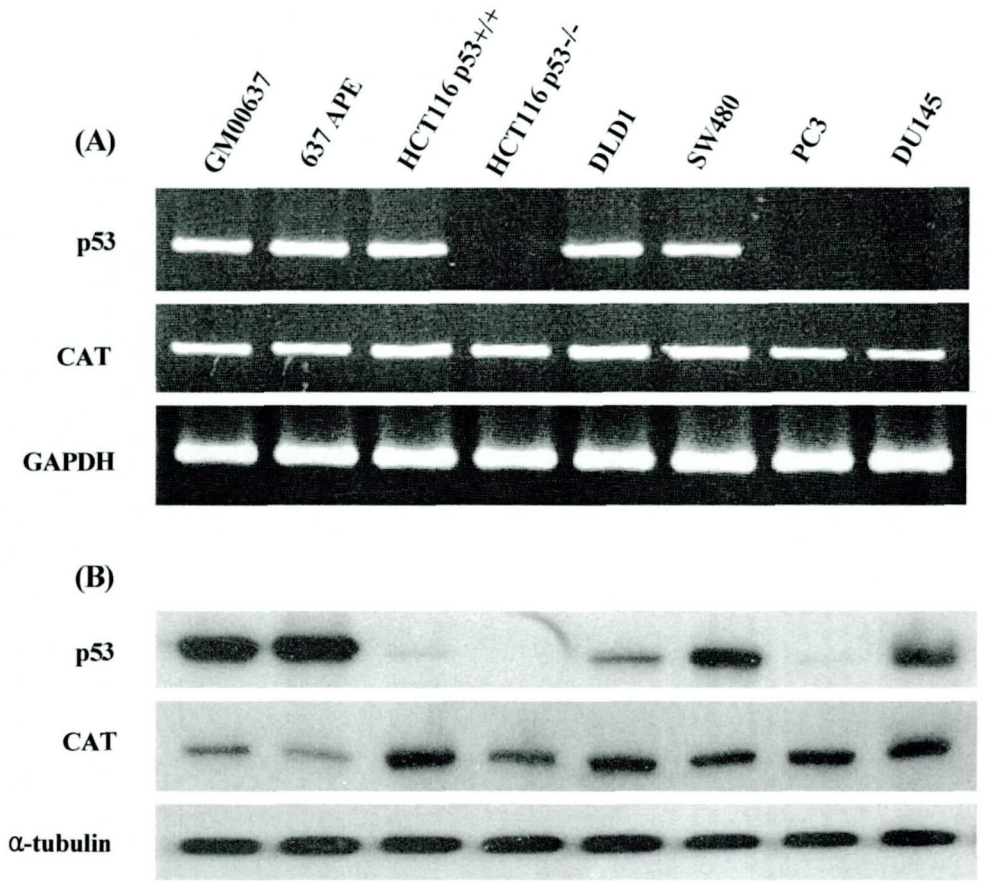


Figure 5. Expression of p53 and CAT expression in various cell lines. (A) RT-PCR, (B) Immunoblot

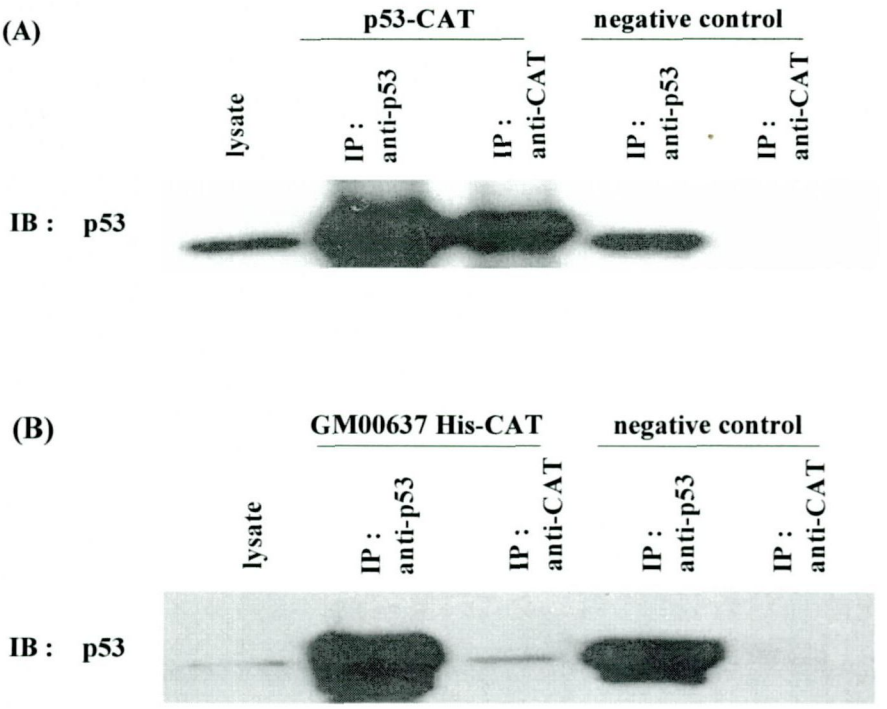
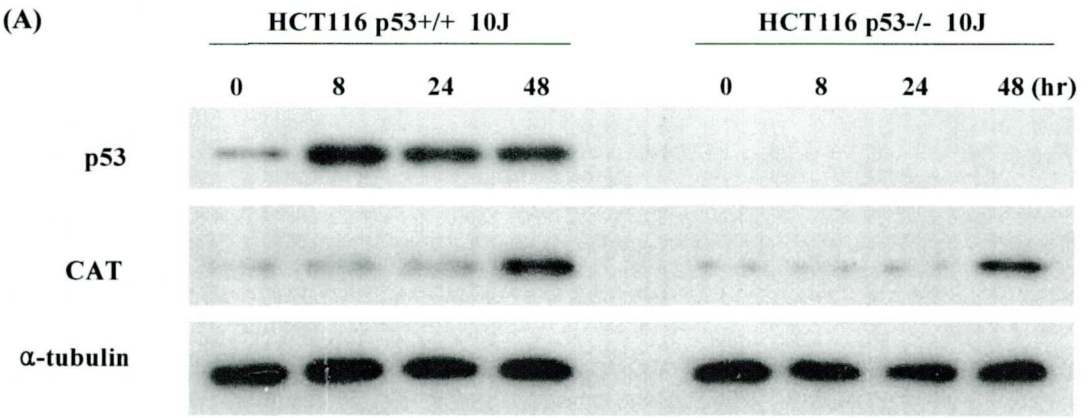


Figure 6. Interaction of p53 with CAT co-immunoprecipitation . Immuno blot was performed with anti-p53 antibody. (A) In vitro. (B) In vivo.

(A)


HCT116 p53+/+ 10J
HCT116 p53-/- 10J
0
8
24
48
0
8
24
48 (hr)
p53
CAT
α-tubulin

(B)

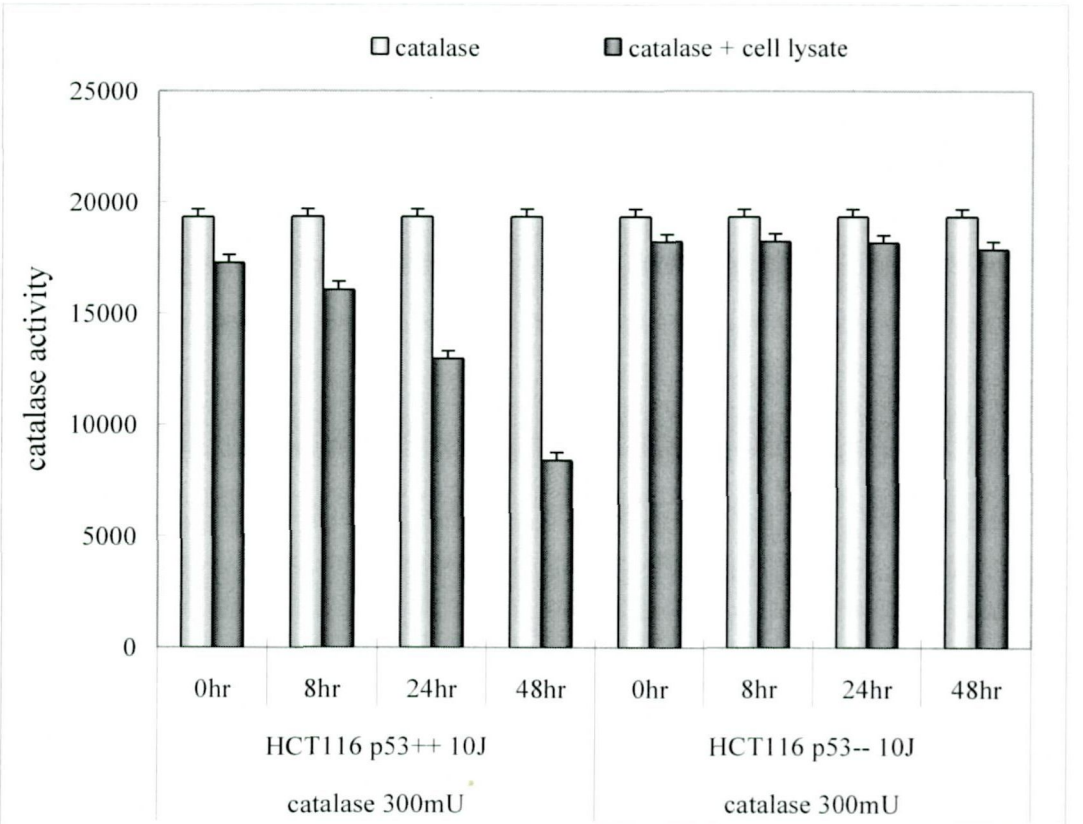

catalase
catalase + cell lysate
25000
20000
15000
10000
5000
0
catalase activity
0hr
8hr
24hr
48hr
0hr
8hr
24hr
48hr
HCT116 p53++ 10J
catalase 300mU
HCT116 p53-- 10J
catalase 300mU

Figure 7. catalase activity in vivo.

To p53 overexpression cell line, HCT116 p53+/+ and HCT116 p53-/-(control) damaged by UV. The damaged cell extract was western blot and measured catalase activity.

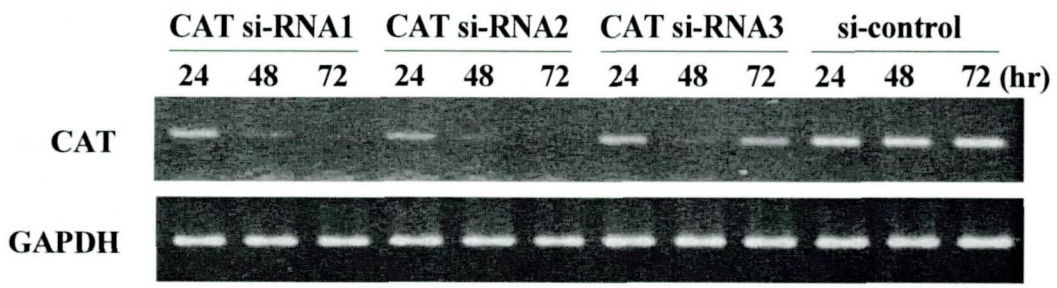


Figure 8. Transfection into the GM00637 cell to test their ability to suppress CAT expression.

DISCUSSION

p53 is a transcription factor consisting of a conserved N-terminal transactivation domain, a praline-rich domain, a sequence-specific DNA-binding domain, a tetramerization domain, and a basic C-terminal tail. The N-terminal transactivation domain is a site of important regulation 2of the stability of p53 in the cell. Studies have shown that the binding of mdm2 to the N-terminal region inhibits p53 transactivation and leads to its degradation. As discussed below in greater detail, the cell has evolved multiple mechanisms to disrupt this interaction in response to genotoxic stress or oncogenic stimulation. The praline-rich region located between the transactivation domain and the sequence specific DNA binding domain appears to be required for p53 to suppress growth in some systems although its function remains unclear. Although transcription independent growth suppression by p53 has been reported, the majority p53 mutations in human tumors cluster to the DNA-binding domain suggesting that p53 mediates most of its growth suppressive effects through transcriptional mechanisms. The basic C-terminal region of p53 is able to bind single stranded DNA and also appears to allosterically inhibit sequence-specific binding in the cell. Therefore post-translational modifications of

this basic C-terminal tail are necessary for efficient sequence-specific DNA-binding by p53.

Since activated p53 has the ability to either cause cell cycle arrest or apoptosis, the cell has evolved several mechanisms to keep the level of p53 protein low and inactive. In fact the half life of wild type p53 is normally very short, being on the order of 20-30 min and is therefore barely detectable in the unstressed cell. In response to a variety of genotoxic stresses (DNA damaging agents, UV damage, nucleotide depletion, hypoxia, or hypoglycemia) or inappropriate proliferative signals, the p53 protein becomes stabilized and its DNA binding activity increases allowing it to mediate cell cycle arrest or apoptosis. Although it was known that the levels and activity of p53 are primarily regulated at the post-transcriptional level only in the last 2 years have the mechanisms underlying the stabilization and activation of p53 been elucidated.

The stability of the p53 protein primarily depends upon its physical interaction with the oncogene and p53 target gene mdm2. When mdm2 binds the N-terminal domain of p53, the transactivation activity of p53 is inhibited and p53 is degraded. The mdm2 gene was first discovered in a mouse tumor cell line as an amplified

gene contained in a murine double minute and mdm2 has been shown to be amplified in 20-40% of human sarcomas. Activated p53 induces mdm2 expression, which inhibits p53 stabilization and activation by binding the N-terminal region of p53. therefore p53 and mdm2 participate in an auto regulatory loop that prevents the expression of p53 in the unstressed cell. Although mdm2 recently has been shown to inhibit the TGF-B pathway in a p53 independent manner, examination of the mdm2-/- vs. Mdm2-/- p53-/- knockout mice suggest that the inhibition of p53 is the primary in vivo function of mdm2. several studies have shown that mdm2-/- knockout mice are early embryonic lethals while the loss of p53 (mdm2-/-p53-/- double knockout) rescues this phenotype. Study of DNA damage induced stabilization of p53 has revealed a key role for phosphorylation in regulating mdm2-p53 interaction.

p53 has been shown to be involved in several cellular processes including the induction of cell cycle arrest in G1 or G2, inhibition of growth factor signalin and angiogenesis, promotion of senescence, and Induction of apoptosis. Although p53 has been demonstrated to induce apoptosis in the absence of transactivation and in the absence of de novo protein or RNA synthesis in some cell lines, transcriptional activation of p53 down-stream genes appears to be an important mechanism

through which p53 mediates its biological effects. The finding that most tumor-derived mutants of p53 are defective in DNA-binding and transactivation support the key role of transcriptional activation in the function of p53. p53 target genes involved in G1 and G2 cell cycle arrest and apoptosis.

An increase in the level of reactive oxygen species (ROS), termed as oxidative stress, is controlled by multiple, interacting, low and high molecular weight components. Among them, superoxide dismutase (SOD), glutathione peroxidase 1 (GPx), and catalase (CAT) play a central role (reviewed in Refs. 1 , 2). The overall effect of the antioxidant system depends on the intracellular balance between these antioxidant enzymes rather than a single component (3) . In the antioxidant enzyme system, SOD catalyzes the dismutation of superoxide radicals to H_2O_2, which is further metabolized to H_2O and O_2 by CAT and GPx (4) . An imbalance in the coordinated expression/activity of antioxidant enzymes can lead to the generation of oxidative stress (3 , 5) . An overexpression of Cu, Zn-SOD in mouse epidermal cells resulted in the sensitization to oxidative chromosomal aberrations and DNA strand breaks; however, a simultaneous increase in CAT or GPx corrected this hypersensitivity (5) .

There are two forms of intracellular SOD in mammalian cells: (*a*) Cu,Zn-SOD, localized in the cytoplasm and nucleus; and (*b*) MnSOD, which is found in the mitochondrial matrix. MnSOD (SOD2) is localized to chromosome 6q25, a region that is frequently deleted in certain types of human cancer (6 , 7) . A decreased level of MnSOD has been reported in tumor cells (8) . Overexpression of MnSOD in murine and human cell lines leads to a decrease in the colony-forming ability and a reduction in the tumor formation in athymic nude mice (9, 10, 11, 12) . Furthermore, both human and mouse cell lines with MnSOD overexpression have reduced growth rate, plating efficiency, and viability compared with the control cells (11 , 13) . In addition to a slower growth rate, MnSOD-overexpressing cells show increased sensitivity to oxygen radical treatment, and this sensitivity is abrogated by pyruvate, a H_2O_2 scavenger (14) .

GPx is a selenoprotein and occurs in five different isoforms. GPx1 is the major isoform that metabolizes H_2O_2 to O_2 and H_2O. GPx overexpression has been found to block MnSOD-induced tumor growth inhibition (15) . CAT is a tetrameric enzyme with a ferriprotoporphyrin group in each subunit. Although GPx is found mainly in the cytosol and mitochondrial matrix, CAT is largely present in peroxisomes. The hypersensitivity of Cu-Zn SOD-expressing mouse epidermal

cells to H_2O_2-induced DNA damage is corrected by CAT expression in the double transfectant (5) . A decrease in the CAT activity has been found in a variety of animal tumors (16) . Fibroblasts from cancer-prone Xeroderma-pigmentosum cases possess a 5-fold lower CAT activity and produces five times more H_2O_2 upon UV irradiation exposure when compared with cells from noncancer-prone trichothiodystrophy (17) . Although both GPx and CAT decompose H_2O_2, their contributions vary depending on the amount and the site of H_2O_2 production (18 , 19) .

ROS has been implicated in the induction of apoptosis (18 , 20 , 21) . Mitochondria is the major source of superoxide anion (O_2) production in cells and ~1–2% of oxygen reduced by mitochondria are converted to O_2 (22) . The O_2 is converted to H_2O_2 by MnSOD, which is present in the mitochondria. Although O_2 is primarily confined to the mitochondria, H_2O_2 is capable of passing through the mitochondrial membrane into the cytoplasm and the extracellular microenvironment (23) . Excess of H_2O_2 that is not reduced by GPx inside the mitochondria can cross the mitochondrial membrane into the cytosol and is reduced by CAT and GPx to H_2O and O_2. Another free radical, nitric oxide (NO) can enhance the generation of O_2 and subsequent generation of H_2O_2 by inhibiting the cytochrome oxidase and

thereby modulating ROS-mediated apoptosis (21) . Increased oxidative stress causes the release of cytochrome C from mitochondria into the cytosol. Cytosolic cytochrome *c* can bind to Apaf1 and activate caspase 9 in the apoptosome in response to diverse inducers of cell death (24, 25, 26) . Cytochrome *c* is released from the intermembrane space of the mitochondria through openings in the outer membrane, which formed as a consequence of mitochondrial permeability transition, leading to the loss of the mitochondrial membrane potential.(27 , 28).

p53-induced apoptosis can be mediated by ROS. One mechanism is p53 regulation of the expression of genes that are related to cellular redox status and involved in apoptosis (20 ; 29). In the present study, I investigated the new p53 interacting protein, and found the catalase, which is interacted with p53. p53 is immunoprecipitated with catalase in vitro and in vivo, and p53 protein interferes the catalase activity in vitro. Therefore, p53 can interact with catalase, and may be contributed to the regulation of the oxidative stress.

REFERENCES

1. Ames BN Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. Science (Wash. DC), *221:* 1256-64, 1983.

2. Cerutti PA Prooxidant states and tumor promotion. Science (Wash. DC), *227:* 375-81, 1985.

3. Amstad P, Moret R, Cerutti P Glutathione peroxidase compensates for the hypersensitivity of Cu, Zn-superoxide dismutase overproducers to oxidant stress. J Biol Chem, *269:* 1606-9, 1994.

4. Cerutti P, Larsson R, Krupitza G, Muehlematter D, Crawford D, Amstad P Pathophysiological mechanisms of oxidants Cerutti P. A. eds. . Oxy-Radicals in Molecular Biology and Pathology, Alan R. Liss New York 1988.

5. Amstad P, Peskin A, Shah G, et al The balance between Cu,Zn-superoxide dismutase and catalase affects the sensitivity of mouse epidermal cells to oxidative stress. Biochemistry, *30:* 9305-13, 1991.

6. Church SL, Grant JW, Meese EU, Trent JM Sublocalization of the gene encoding manganese superoxide dismutase (MnSOD/SOD2) to 6q25 by fluorescence in situ hybridization and somatic cell hybrid mapping. Genomics, *14:* 823-5, 1992.

7. Millikin D, Meese E, Vogelstein B, Witkowski C, Trent J Loss of heterozygosity for loci on the long arm of chromosome 6 in human malignant melanoma. Cancer Res, *51:* 5449-53, 1991.

8. Oberley LW, Buettner GR Role of superoxide dismutase in cancer: a review. Cancer Res, *39:* 1141-9, 1979.

9. St Clair DK, Wan XS, Oberley TD, Muse KE, St Clair WH Suppression of radiation-induced neoplastic transformation by overexpression of mitochondrial superoxide dismutase. Mol Carcinog, *6:* 238-42, 1992.

10. Church SL, Grant JW, Ridnour LA, et al Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. Proc Natl Acad Sci USA, *90:* 3113-7, 1993.

11. Yan T, Oberley LW, Zhong W, St Clair DK Manganese-containing superoxide dismutase overexpression causes phenotypic reversion in SV40-transformed human lung fibroblasts. Cancer Res, *56:* 2864-71, 1996.

12. Zhong W, Oberley LW, Oberley TD, St Clair DK Suppression of the malignant phenotype of human glioma cells by overexpression of manganese superoxide dismutase. Oncogene, *14:* 481-90, 1997.

13. Amstad PA, Liu H, Ichimiya M, Berezesky IK, Trump BF Manganese superoxide dismutase expression inhibits soft agar growth in JB6 clone41 mouse epidermal cells. Carcinogenesis (Lond.), *18:* 479-84, 1997.

14. Li N, Oberley TD, Oberley LW, Zhong W Overexpression of manganese superoxide dismutase in DU145 human prostate carcinoma cells has multiple effects on cell phenotype. Prostate, *35:* 221-33, 1998.

15. Li S, Yan T, Yang JQ, Oberley TD, Oberley LW The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell

growth by manganese superoxide dismutase. Cancer Res, *60:* 3927-39, 2000.

16. Calabrese EJ, Canada AT Catalase: its role in xenobiotic detoxification. Pharmacol Ther, *44:* 297-307, 1989.

17. Vuillaume M, Daya-Grosjean L, Vincens P, et al Striking differences in cellular catalase activity between two DNA repair-deficient diseases: Xeroderma pigmentosum and trichothiodystrophy. Carcinogenesis (Lond.), *13:* 321-8, 1992.

18. Chandra J, Samali A, Orrenius S Triggering and modulation of apoptosis by oxidative stress. Free Radic Biol Med, *29:* 323-33, 2000.

19. Jones DP, Eklow L, Thor H, Orrenius S Metabolism of hydrogen peroxide in isolated hepatocytes: relative contributions of catalase and glutathione peroxidase in decomposition of endogenously generated H2O2. Arch Biochem Biophys, *210:* 505-16, 1981.

20. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B A model for p53-induced apoptosis. Nature (Lond.), *389:* 300-5, 1997.

21. Moncada S, Erzurum SC Does nitric oxide modulate mitochondrial energy generation and apoptosis?. Nat Rev Mol Cell Biol, *3:* 214-20, 2002.

22. Boveris A, Chance B The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochem J, *134:* 707-16, 1973.

23. Halliwell B, Gutteridge JMC . Free Radicals in Biology and Medicine, 3rd ed. Clarendon Press Oxford 1999.

24. Yang J, Liu X, Bhalla K, et al Prevention of apoptosis by Bcl-2: release of cytochrome *c* from mitochondria blocked. Science (Wash. DC), *275:* 1129-32, 1997.

25. Liu X, Kim CN, Yang J, Jemmerson R, Wang X Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome *c*. Cell, *86:* 147-157, 1996.

26. Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD The release of cytochrome *c* from mitochondria: a primary site for Bcl-2 regulation of apoptosis. Science (Wash. DC), *275:* 1132-6, 1997.

27. Green DR, Reed JC Mitochondria and apoptosis. Science (Wash. DC), *281:* 1309-12, 1998.

28. Reed JC, Green DR Remodeling for demolition: changes in mitochondrial ultrastructure during apoptosis. Mol Cell, *9:* 1-3, 2002.

29. Schuler M, Green DR Mechanisms of p53-dependent apoptosis. Biochem Soc Trans, *29:* 684-8, 2001.