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The effect of PIG3 on the p53 stability

조선대학교 대학원

생물신소재학과

이수미

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PIG3 에 의한 p53 stability 연구

2008년 **8** 월 25 일

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ABSTRACT

The effect of PIG3 on the p53 stability

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The tumor suppressor p53 induces apoptosis or cell-cycle arrest in response to genotoxic and other stresses. One of the most important regulators of p53 is mouse double minute 2 (Mdm2), a RING domain E3 ligase that ubiquitinates p53, leading to both proteasomal degradation and relocation of p53 from the nucleus to the cytoplasm1. The p53-inducible gene 3 (PIG3) is a transcriptional target of p53 and is thought to play a role in apoptosis, but its other functions are not clear now. In this study, we showed that knocking down PIG3 by siRNA decrease p53 levels and increase Mdm2 levels, and decreased the half-life of wild-type p53 in several of the human cell lines; whereas, overexperssion of PIG3 increase p53 levels and decrease Mdm2 levels. Overexpression of PIG3 inhibited ubiquitination of exogenous p53 and enhance ubiquitination of exogenous Mdm2 in a dose-dependent manner, whereas knocking down PIG3 by siRNA enhanced ubiquitination of endogenous p53. Using a co-immunoprecipitation assay, the interaction between endogenous PIG3 and Mdm2 is detected, it indicate that PIG3 binds with Mdm2 in normal

condition. These results suggest that as the transcriptional target of p53, PIG3 regulate p53 stability via it increasing ubiquitination of Mdm2, which decrease the ubiquitination of p53.

Keywords: PIG3; p53; stability; ubiquitination

Ⅰ. INTRODUCTION

The tumor suppressor protein p53 is a transcription factor activated in response stress to induce expression of its target genes. The proteins encoded by these genes then mediate multiple cellular responses, such as cell cycle arrest, apoptosis, differentiation, cell senescence, or DNA repair. In normal, unstressed cell, the p53 protein is short-lived(t½~20min), reflecting a rapid turnover through ubiquitin-mediated proteolysis(Vousden, 2002).The p53 protein is stabilized, and its levels increase in response to various stresses, including DNA damage, hypoxia, and inappropriate oncogene signaling(Giaccia & Kastan, 1998). The induction of p53 involves several mechanisms including post-translational modifications such as ubiquitination, phosphorylation and acetylation (Giaccia & Kastan, 1998).

Two proteolytic pathways have been implicated in p53 degradation: the calpain proteolytic pathway(Kubbutat & Vousden, 1997; Pariat et al, 1997) and the ubiquitin proteolytic pathway (Chowdary et al, 1994; Maki et al, 1996). Ubiquitin is first sequentially transferred through a series of ubiquitin system enzymes, designated E1, E2, and E3. The E3 enzyme then transfers the ubiquitin molecule to one or more lysine residues in the substrate(Scheffner et al, 1995). Multiple ubiquitins are attached to one another to form ubiquitin chains, and the multi-ubiquitinated substrate is degraded by the 26S proteasome. Proof that p53 is a ubiquitin-system target came with the demonstration of Wild type p53:ubiquitin conjugates in vivo(Maki et al, 1996).

The mdm2 gene is a direct target of p53 transcriptional activity, and the MDM2 protein then binds to p53 and blocks its activities as a tumor suppressor and promotes its

degradation.(Freedman et al, 1999). The main physiological antagonist of p53 is the MDM2 protein(Jones et al, 1995; Montes de Oca Luna et al, 1995) which binds to the amino-terminus of the tumor suppressor and catalyzes the addition of ubiquitin moieties to lysine residues at positions 370, 372, 373, 381, 382, and386 at the p53 carboxy-terminus(Nakamura et al, 2002; Rodriguez et al, 2000). p53 ubiquitination exposes a nuclear export signal promoting cytoplasmic relocation and degradation by proteasomes (Lohrum et al, 2001).By binding to the N-terminal transcription domain of p53, mdm2 block p53's ability to activate transcription(Kussie et al, 1996; Momand et al, 1992). This interaction prevent p53 from activating its target genes and allows Mdm2-mediated polyubiquitylation and degradation of p53(Iwakuma & Lozano, 2003). The MDM2-p53 loop presents as a central regulatory point for the cellular response to a multitude of environmental as internal stressors(Dai et al, 2006). MDM2 binds p53 under normal condition and promotes its ubiquitination and subsequent degradation by the proteasome. In response to DNA damage, p53 is phosphorylated at Ser-15 and Ser-37, preventing MDM2 binding and thus stabilizing p53. The stabilized p53 can then promote transcription of its downstream target genes, resulting in cell cycle arrest or apoptosis (Maki, 1999).

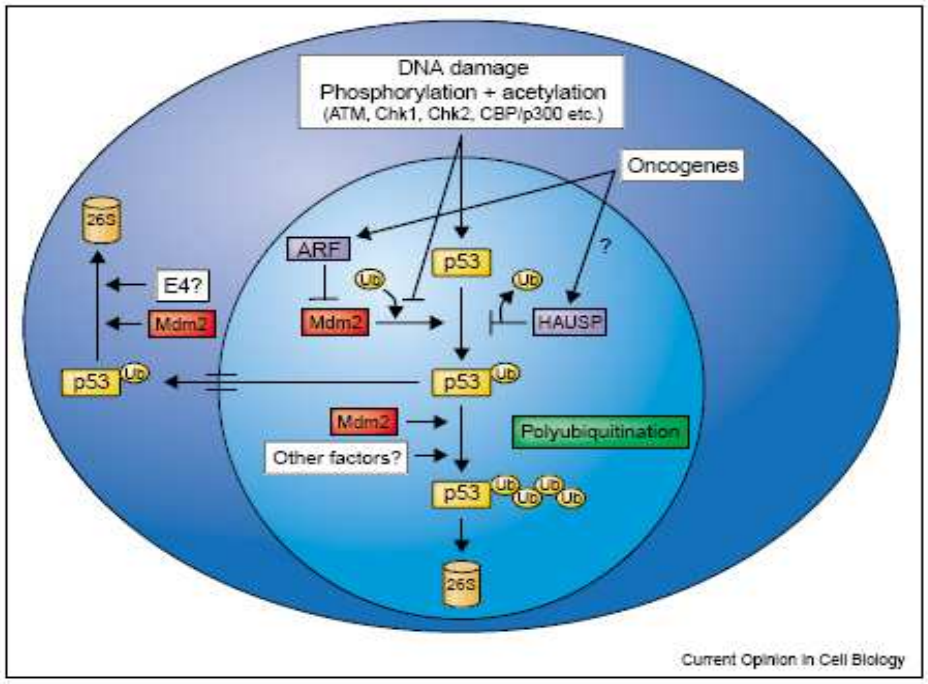


Fig.1 Mdm2-depedent mechanism of p53 stabilization. Several mechanisms for regulating p53 protein levels are depicted here. p53 is ubiquitinated within the nucleus by Mdm2 and possibly other factors, which leads to its degradation by the 26S proteosome either in or out of the nucleus. DNA damage-induced upstream factors can block this cascade of events by targeting Mdm2 and p53. ARF and HAUSP represent antagonists of Mdm2 in this process. The mechanism of oncogenic activation of HAUSP and the role of other factors including E4 ligases in Mdm2-mediated ubiquitination of p53 are yet to be elucidated. 26S, 26S proteosome; ATM, ataxia telangiectasia mutated; Chk, checkpoint kinases; E4, E4 ligases; Ub, ubiquitination.

It has been shown that p53-dependent apoptosis involves the induction of a series of genes called PIGs (p53-inducible gene(s))(Polyak et al, 1997). Pig3 was originally discovered in a serial analysis of gene expression study designed to identify gene induced by p53 before the onset of apoptosis and PIG3 protein is involved in the generation of reactive oxygen species(ROS) (Polyak et al, 1997), which are important downstream mediators of the p53-dependent apoptotic response(Johnson et al, 1996; Li et al, 1999). First, PIG3 expression precedes the appearance of ROS in p53-induced apoptosis. Second, PIG3 shares sequence similarity with numerous NAD(P)H quinone oxidoreductases shown to be potent inducers of ROS(Polyak et al, 1997). Third, certain p53 mutants capable of inducing cell cycle arrest but not apoptosis retain the ability to activate target genes such as the cyclin-dependent kinase inhibitor p21, but not PIG3(Campomenosi et al, 2001; Venot et al, 1998).

In this study, We have now investigated the relationship between PIG3 and p53. Our results suggest that as the transcriptional target of p53, PIG3 regulated p53 stability via it increasing ubiquitination of Mdm2, which decrease the ubiquitination of p53.

Ⅱ. MATERIALS AND METHODS

1. Maintenance of Cell Lines

HEK 293T (Coriell Institute for Medical Research, Camden, NJ) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 units/ml of penicillin, and 100 mg/ml of streptomycin (Invitrogen, Carlsbad, CA). Human breast cancer epithelial cell lines MCF-7 and (Coriell Institute for Medical Research, Camden, NJ) were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units/ml of penicillin, and 100 mg/ml of streptomycin (Invitrogen, Carlsbad, CA). U20s was cultured in McCoy's 5A medium/10% FBS. HCT116(p53+/+) HCT116(p53-/-) were maintained in IMDM medium supplemented with 10% fetal bovine serum, 100 units/ml of penicillin, and 100 mg/ml of streptomycin (Invitrogen, Carlsbad, CA). The cells were maintained in a humidified incubator in an atmosphere containing 5 % CO_2 at 37 ^{o}C.

2. Plasmid Constructs of PIG3

The human PIG3 cDNA was amplified by RT-PCR using the PIG3 oligo primer: sense 5'-ATGTTAGCCGTGCACTTTGACAA-3' and antisense 5'-TCA CTG GGG CAG TTC CAG GAC-3' from human fibroblast GM00637 cells. The amplified PIG3 cDNA construct was inserted into a pcDNA3.1/ mammalian expression vector driven by the CMV promoter (Invitrogen Life Technologies), and confirmed the DNA sequence and orientation.

3. PIG3-siRNA design, synthesis and transfection

Three target sites within human OGG1 genes were chosen from the human PIG3 mRNA sequence (Gene Bank accession number NC_000002), which was extracted from the NCBI Entrez nucleotide database. After selection, each target site was searched with NCBI BLAST to confirm the specificity only to the human PIG3. The sequences of the 21-nucleotide sense- and antisense-RNA are as follows: PIG3-siRNA, 5'-AAA UGU UCA GGC UGG AGA CUA-3' (sense) and 5'-UAG UCU CCA GCC UGA ACA UUU-3'(antisense) for the PIG3; LacZ siRNA, 5'-CGUACG-CGGAAUACUUCGAUU-3' (sense), 5'-AAUC GAAGUAUUCCGCGUACGUU-3' (antisense) for the LacZ gene. These siRNAs were prepared using a transcription-based method with a Silencer siRNA construction kit (Ambion, Austin, TX) according to the manufacturer's instructions. LacZ siRNA was used as the negative control. Cells were transiently transfected with siRNA duplexes using Oligofectamine (Invitrogen). The siRNA expression vector(pSilencer hygro) for hOGG1 and a control vector were employed. The construction of siRNA-expression plasmid was base on the pSilencer hygro vector(Ambion,Texas,USA). The vector included a human U6 promoter, a hygromycin resistance gene. We purchased synthetic oligo-nucleotides(Xenotech, Korea). After anneling, DNA fragments were ligated into the pSilencer hygro. Cells were transfected with the siRNA vector by using Lipofectamine(Invitrogen. Carlsbad.CA). After transfection with the hygromycin-resistance vector, resistant colonies were grown in the presence of Hygromycin (100ug/ml) (Invitrogen. Carlsbad.CA).

4. Other Plasmids

myc3-p53, HDM2 and His6-ubiquitin from were gifts from Chin Ha Chung, NRL of Protein Biochemistry, School of Biological Sciences, Seoul National University, Seoul 151-742, Korea.

5. Western blot analysis

The cell were washed with phosphate-buffered saline(PBS) and lysed on ice for 10 minutes in the M-PER mammalian protein Extraction Reagent (PIERCE) added protease Inhibitor Cocktail tablet (Roch). After incubation, extracts were vortex for 5min and centrifuged at 13000rpm for 15min. The supernatant was diluted with 5X SDS-sample buffer and boiled. After cellular protein concentrations were determined using the dye-binding microassay (Bio-Rad, Hercules, CA), and 20ug of protein per lane were separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). After SDS-PAGE, the proteins were transferred onto Hybon ECL membranes (Amersham Biosciences, Piscataway, NJ). 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. All antibodies used in this study are anti-human anti-p53 pAb, anti-p53-P(Ser15) pAb, anti-p53-P(Ser20) pAb , anti-cleaved caspas-3 pAb, anti- cleaved caspas-7 pAb, anti-cleaved caspas-9 pAb, (Cell Signaling Technology, Danvers, MA); anti-p21 monoclonal antibody (mAb) (BD Phamingen, San Jose, CA); anti-Noxa mAb (Calbiochem, Darmstadt,

Germany); anti-a-tubulin mAb (BD Phamingen, San Jose, CA); anti-PIG3(N-20), anti-Bax, anti-Bcl2 (Santa Cruz Biotechnology, Santa Cruz, CA). We followed manufacturer's protocol for dilution of all primary antibodies. The membranes were then washed, incubated with the biotinylated secondary antibodies (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. Immunoprecipitation (IP)

Whole cell lysates were made in lysis buffer (50 mM HEPES at pH 8.0, 150 mM NaCl, 0.5% Triton X-100, 0.5% NP-40, 100 mM NaF, 1 mM PMSF, 1 mM DTT, 1× complete protease cocktail and 10% glycerol) and pre-cleared with protein A−G-coupled Sepharose beads for 2 h. The lysates were then immunoprecipitated with the indicated antibodies and isotype-matched control antibodies plus protein A−G Sepharose for at least 4 h or overnight. Beads were washed four times with lysis buffer, once with ice-cold PBS and boiled in 2× loading buffer. Protein samples were resolved by SDS−PAGE and transferred onto nitrocellulose membrane, which was blocked in 5% skim milk in PBST and probed with the indicated antibodies. The following antibodies were used for immunoprecipitation and western blotting: Human Mdm2 immunoprecipitation, Ab-1 and Ab-4 (Oncogene, San Diego, CA); human Mdm2 western blotting, SMP-14 (Santa Cruz Biotechnology, Santa Cruz, CA); p53 western blotting and immunoprecipitation, DO-1 or FL-393 (Santa Cruz); PIG3 immunoprecipitation and western blotting, H-300 and N-20(Santa Cruz Biotechnology, Santa Cruz, CA).

7. In Vivo Ubiquitination Assay

In vivo ubiquitination assays were conducted as previously described (33). Briefly, HEK 293 was transfected with combinations of the following plasmids as indicated in the figure legends: His6-ubiquitin (0.8 ug), myc3-p53 (0.4ug),HA-MDM2(0.4ug), PIG3(0.4,0.8, 1.2ug) using Metafectene. For inhibition of proteasome-mediated protein degradation, the cells were treated with 20 mM MG132 for 4 h before harvest. Forty-eight hours after transfection cells from each plate were harvested and split into three aliquots, one for immunoblot and the other two for ubiquitination assays. For full down assay, Cell pellets were lysed in buffer I (6 M guanidinium-HCl, 0.1 mol/ liter Na2HPO4/NaH2PO4, 10 mmol/liter Tris-HCl (pH 8.0), 10 mmol/liter β-mercaptoethanol) and incubated with Ni-NTA beads at room temperature for 4 h. Beads were washed once each with buffer I, buffer II (8 mol/liter urea, 0.1 mol/liter Na2HPO4/NaH2PO4, 10 mmol/liter Tris-HCl (pH 8.0), 10 mmol/liter β-mercaptoethanol), and buffer III (8 mol/liter urea, 0.1 mol/liter Na2HPO4 /NaH2PO4, 10 mmol/liter Tris- HCl (pH 6.3), 10 mmol/ liter β-mercaptoethanol). Proteins were eluted from the beads in buffer IV (200 mmol/liter imidazole, 0.15 mol/liter Tris-HCl (pH 6.7), 30% (v/v) glycerol, 0.72 mol/liter β-mercaptoethanol, and 5% (w/v) SDS). Eluted proteins were analyzed by immunoblot with monoclonal anti-p53 (DO-1), anti-HA, or anti-MDM2 (SMP14) antibodies. For Immunoprecipitation, cell lysates were IP with anti-p53(DO-1), and detected with anti-ubiquitin.

Ⅲ. RESULTS

1. PIG3 controls the stability of p53 and Mdm2.

To determine whether the p53 and Mdm2 level is regulated by PIG3 in wild-type p53 cell lines, including a pair of human colorectal carcinoma HCT116 cells that are wild type (*p53*+/+) and U2OS, cells were treated with the PIG3 small interfering RNA (siRNA). Treatment for 24 hours resulted in a significant decrease in p53 level and increase in Mdm2 level (Fig. 2).Conversely, overexpression of PIG3, level to an increase in p53 and a decrease in Mdm2 level , in MHCT116(p53-/-)and HEK 293Tcells (Fig. 3).

The effect of PIG3 on p53 steady-state levels is not due to changes in their transcription because PIG3 does not alter the abundance of p53 mRNA in human HCT116 cells (data not shown), but regulates the stability of p53 protein. Knocking down PIG3 by siRNA decreased the half-life of wild-type p53 in HCT116(p53+/+), or U2OS cells (Fig. 4).

2. PIG3 regulates p53 stability via ubiquitination pathway

To determine if the effect of PIG3 on p53 steady-state levels is due to changes p53 ubiquitination, HEK 293T cells were transfected with expression DNAs encoding myc3-tagged wild-type p53, MDM2, His6-Ubiquitin or co-transfected with PIG3 DNA. Lysates from the transfected cells were immunoprecipitated with an anti-p53(DO-1) antibody, and the immunoprecipitates were examined with an antibody against ubiquitin, or immuno-precipitated with NTA-Ni bead, and the immunoprecipitates were examined with an antibody against p53(DO-1). As shown in Fig5, a high molecular weight ladder of p53 species that are ubiquitin-p53 conjugates was decrease in PIG3 transfected cell types when

myc3-tagged wild-type p53 was expressed with MDM2, indicating that overexpression of PIG3 decrease ubiquitination of p53 in a dose-dependent manner. To conform the effect of PIG3 on endogenous p53 ubiquitination, MCF7 and HCT116 (p53+/+) silencing stable cell line were treated with 20 uM MG-132 for 4h and were analysed for p53 ubiquitination. Similarly, knocking down PIG3 inhibited ubiquitination of endogenous p53 (Fig. 6).

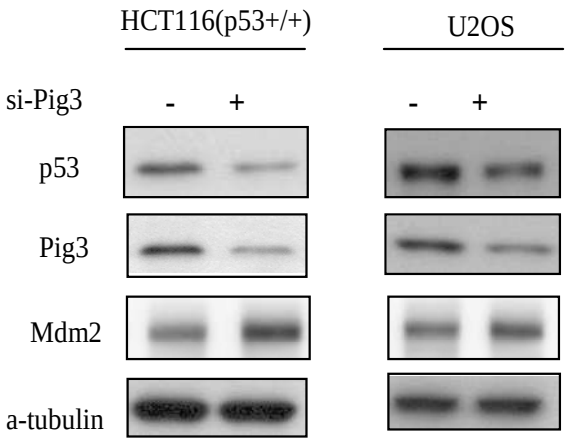


Fig.2 PIG3 controls the stability of p53 and Mdm2 in PIG3 knocking down cells.

Effect of siRNA-mediated down regulation of *PIG3* on the steady-state levels of Mdm2 and p53 in human cells. Lysates from indicated cells were analysed by western blot.

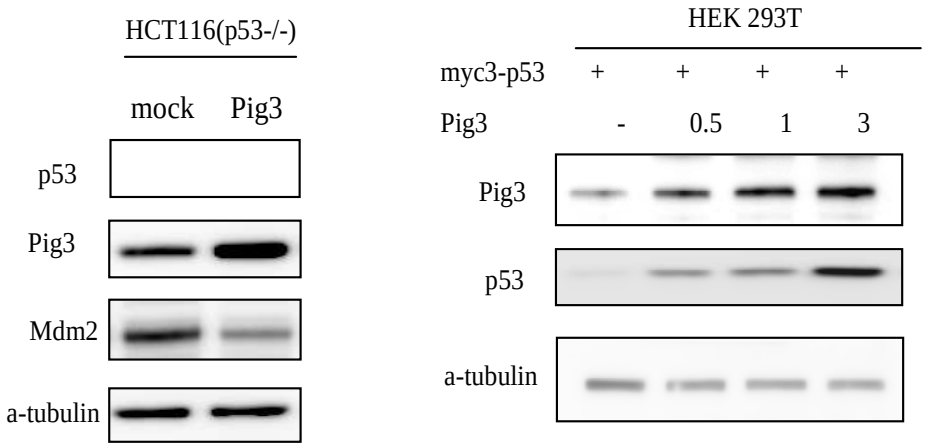


Fig.3 PIG3 controls the stability of p53 and Mdm2 in PIG3 overexpression cells.

Effect of overexpression mediated up regulation of PIG3 on p53 and Mdm2 level in human cells. Lysates from indicated cells were analysed by western blot.

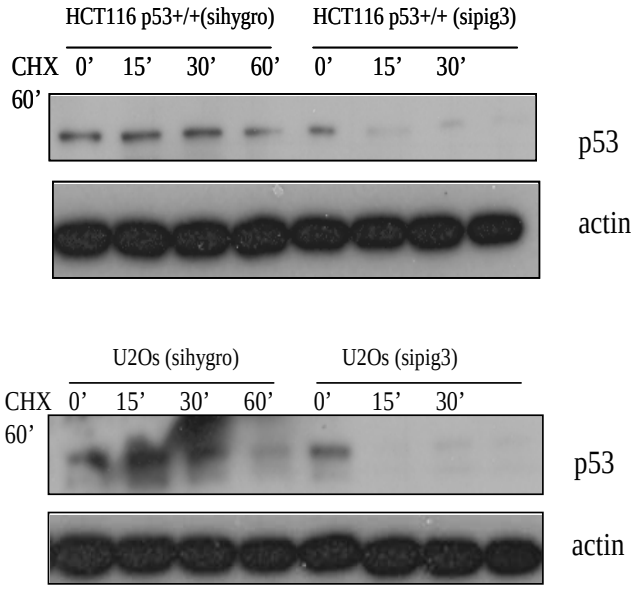


Fig.4 PIG3 controls the stability of p53. PIG3 modulates the half-life of p53. HCT116(p53+/+), U2OS cells were treated with PIG3 siRNA and control siRNA. Cells were cultured in the presence of 80 μg/ ml CHX, and were subsequently analyzed by Western blot.

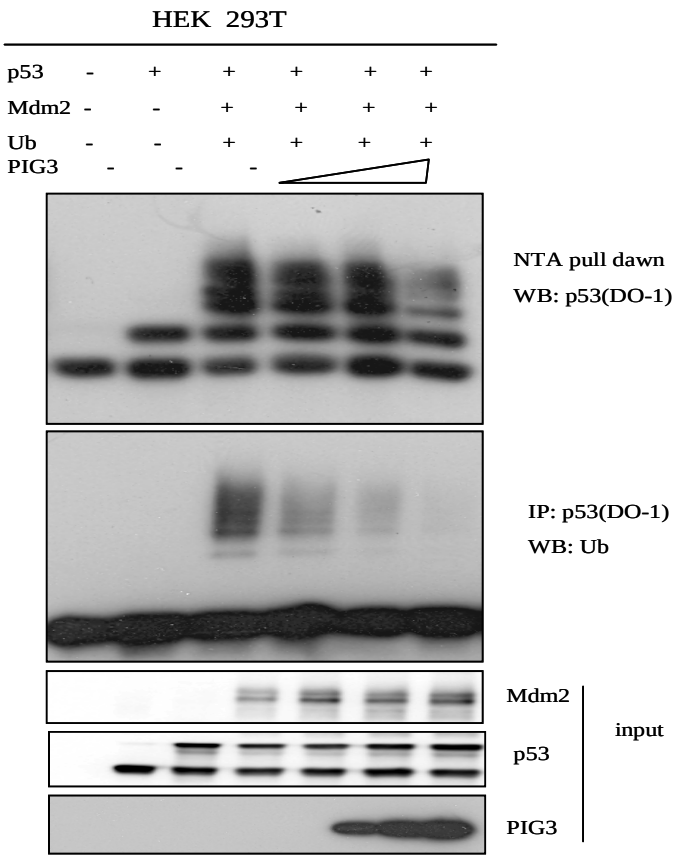


Fig.5 PIG3 regulates p53 stability via ubiquitination pathway in HEK 293T cell.

Overexpression of PIG3 affects ubiquitination of exgenous p53. HEK 293T cells were transfected with increasing amount of PIG3. Twenty-four hours later, cells were treated with 20 uM MG-132 for 4 h and were analysed for p53 ubiquitination.

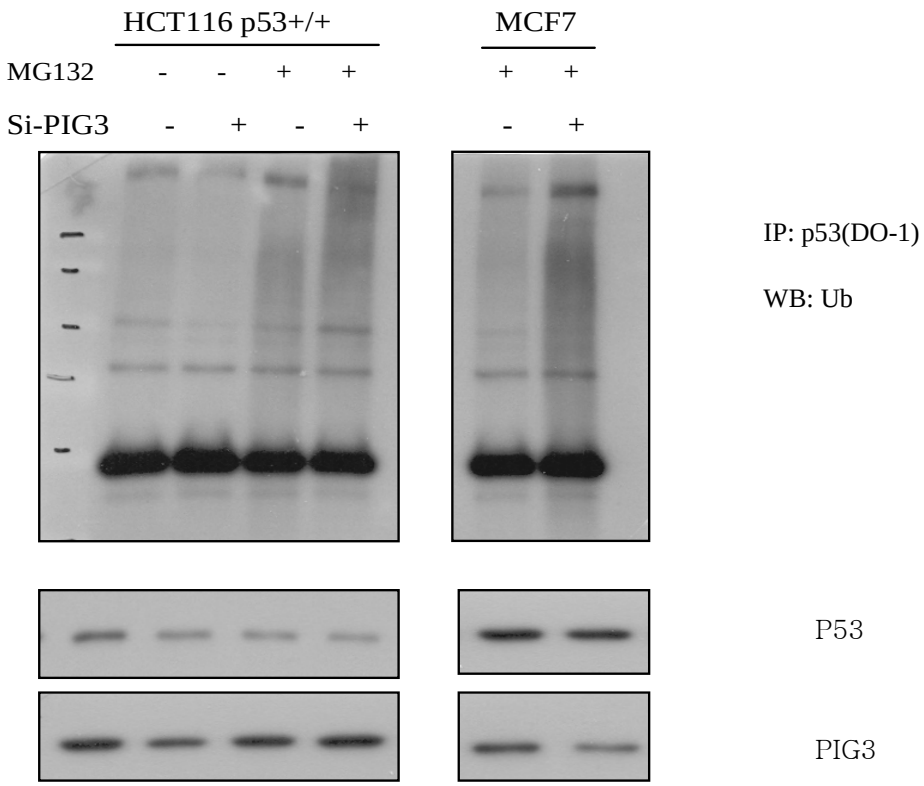


Fig.6. PIG3 regulates p53 stability via ubiquitination pathway in PIG3 knocking down stable cell line. Down regulation of PIG3 affects ubiquitination of endogenous p53. MCF7 and HCT116(p53+/+) silencing stable cell line were treated with 20 uM MG-132 for 4h and were analysed for p53 ubiquitination.

3. **PIG3 regulates Mdm2 stability via ubiquitination pathway.**

To determine if the effect of PIG3 on p53 steady-state levels is due to changes Mdm2 ubiquitination, HEK 293T cells were transfected with expression DNAs encoding MDM2, His6-Ubiquitin or co-transfected with PIG3 DNA. Lysates from the transfected cells were immunoprecipitated with an anti-Mdm2(SMP14) antibody, and the immunoprecipitates were examined with an antibody against ubiquitin, and the immunoprecipitates were examined with an antibody against Mdm2(SMP14). As shown in Fig7, a high molecular weight ladder of Mdm2 species that are ubiquitin-Mdm2 conjugates was decreased in PIG3 transfected cell types. Indicating that knock down of PIG3 decreses ubiquitination of Mdm2 in a dose-dependent manner. To conform the effect of PIG3 on endogenous Mdm2 ubiquitination, HCT116 (p53-/-) silencing stable cell line were treated with 20 uM MG-132 for 4h and were analysed for Mdm2 ubiquitination knocking down PIG3 enhanced ubiquitination of endogenous Mdm2 (Fig. 8).

4. PIG3 physically interacts with Mdm2.

MDM2 can bind p53 and promote its ubiquitination and subsequent degradation by the proteasome (Haupt et al, 1997; Kubbutat et al, 1997). Using a co-immunoprecipitation assay, the interaction between endogenous PIG3 and Mdm2 was detected in U2Os cell lines, indicating that PIG3 interacted with Mdm2 in normal condition (Fig. 9A). And the PIG3−Mdm2 interaction is likely to be direct, as shown by an *in vitro* binding assay with PIG3 and Mdm2 protein synthesized using TNT kit (Fig. 9B).

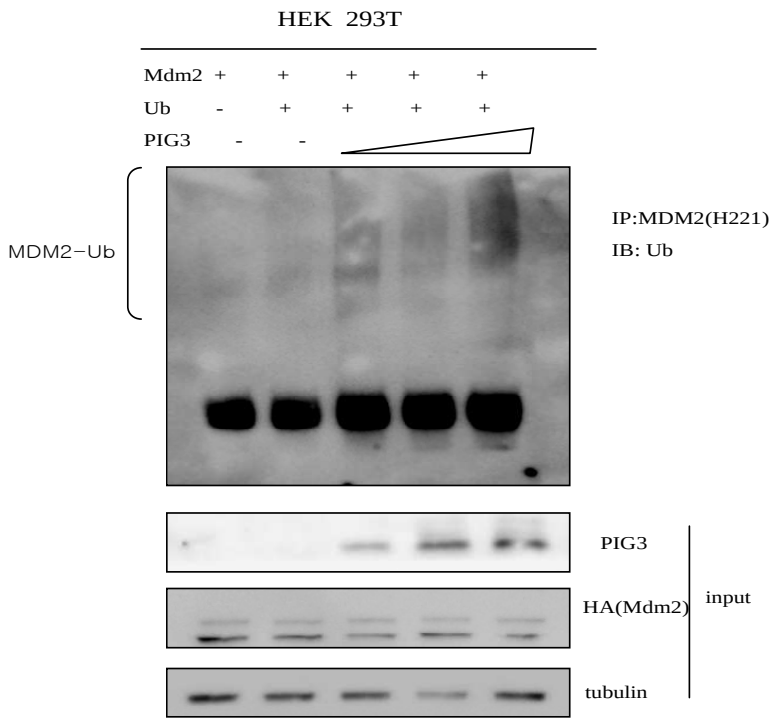


Fig.7. PIG3 regulates Mdm2 stability via ubiquitination pathway in HEK 293T cells.

Overexpression of PIG3 affects ubiquitination of exogenous of Mdm2. HEK 293T cells were transfected with increasing amount of PIG3. Twenty-four hours later, cells were treated with 20 uM MG-132 for 4h and were analysed for Mdm2 ubiquitination.

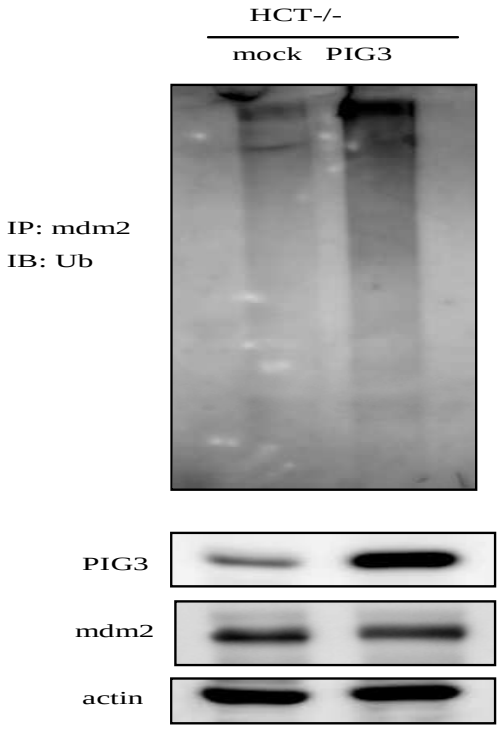
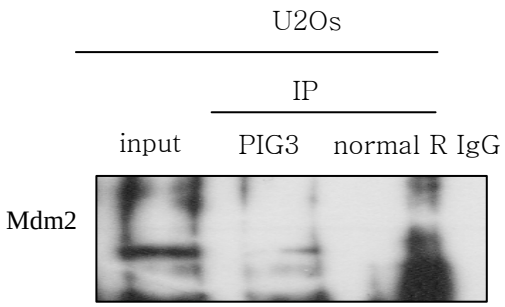


Fig.8. PIG3 regulates Mdm2 stability via ubiquitination pathway in PIG3 overexpression stable cell line. Upregulation of PIG3 affects ubiquitination of endogenous Mdm2. HCT116(p53-/-)silencing stable cell line were with 20 uM MG132 for 4h and were analysed for Mdm2 ubiquitination.

A.



B.

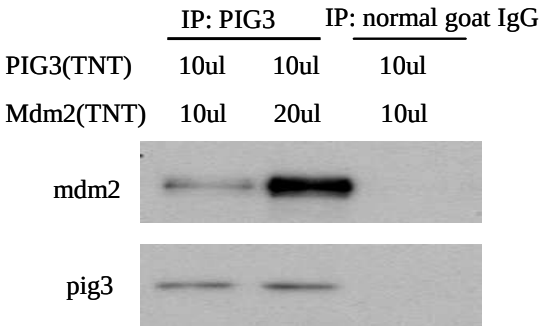


Fig.9. PIG3 physically interacts with mdm2. (A). Association of PIG3 and Mdm2 *in vivo*. Cell lysates from U2Os cells were co-immunoprecipitated with anti-PIG3 and detected with anti-Mdm2. (B) Direct binding of PIG3 and Mdm2 *in vitro*. PIG3 and Mdm2 protein were synthesized using TNT kit and mixed. The mixtures were co-immunoprecipitated with anti-PIG3 and detected with anti-Mdm2.

Ⅳ. DISCUSSION

p53 is often referred to as the ‘cellular gatekeeper’ or the ‘guardian of the genome’ and its importance is emphasized by the discovery of mutations of p53 in over 50% of all human tumors (Momand et al, 1992). Ubiquitination represents an essential activity of Mdm2 for regulating the amount of p53 protein available at any given time within a cell. Mdm2 acts specifically as an E3 ligase for p53 by linking E2-conjugated ubiquitin molecules to it via an isopeptide bond(de Stanchina et al, 1998). Details of the events that occur after this reaction, including the recognition of a growing polyubiquitin chain and subsequent degradation by the 26S proteasome, have remained somewhat elusive (Bulavin et al, 1999). The p53-inducible gene 3 (Pig3) is a transcriptional target of p53 and is thought to play a role in apoptosis(qian yan 20), but its other functions are not clear now.

In this study, We have now investigate the relationship between PIG3 and p53. It shows that PIG3 controls the stability of p53: knocking down PIG3 decreases p53 protein level and increases Mdm2 protein level, whereas overexpression of PIG3 increases p53 protein level and decreases Mdm2 protein level; knocking down PIG3 decreases the half-life of wild-type p53 in several of the human cell lines. And PIG3 regulates p53 stability via ubiquitination pathway. On the other hand, our date showed that PIG3 directly binds with Mdm2 and accelerates Mdm2 degradation via it increasing ubiquitination of Mdm2.

Recent advances in understanding the initial accumulation and activation of p53 have added a layer of complexity to this pathway. The number of mechanisms used to quell p53 ubiquitination implicates this process as a key target in the initial response to genotoxic

stress. Of particular interest are the specific fates of mono-ubiquitinated and poly-ubiquitinated forms of p53. Is Mdm2 sufficient for p53 degradation or are other factors required? There may be differential consequences for p53 depending on how many ubiquitin molecules are added and what type of ubiquitin linkage has occurred. A related perplexity is exactly how p53 is shuttled in and out of the nucleus, and whether associated factors or the ubiquitination status of p53 play a role.

Insight into the transcriptional activation and regulation of p53 continues to raise interesting questions. Do posttranslational modifications in response to stress pathways create p53 molecules that can preferentially interact with specific promoters and cause different cellular responses? How does a cell choose either a growth arrest or apoptotic pathway? How does the cell sense that DNA repair is complete, and by what mechanism is this linked to shutting-off p53? Considering the breadth of complexity in the regulation of p53 function, advances continue to show the multitude of mechanisms the cell uses to stabilize and activate this essential protein.

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-2008년 8월 이수미 드림-

<국문 초록>

P53 안정성에 대한 PIG3 효과

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항암 유전자로 널리 알려진 p53 은 유전자 손상과 같은 다양한 자극에 반응하여 세포 주기를 억제하고 세포 사멸을 유도하여 손상된 세포를 제거하는데 중요한 역할을 담당하고 있다. 이런 p53 의 세포 내 발현을 조절하는 대표적 기전으로 MDM2 와 관련된 ubiquitination 과정을 들 수 있다. MDM2 는 p53 과 결합하여 p53 의 ubiquitination 을 유도함으로써, p53 의 하위 유전자로 알려진 PIG 의 세포 사멸을 조절하는 것 외에 아직 어떠한 기전도 알려져 있지 않다. 따라서 본 연구에서는 siRNA 를 이용하여 PIG3 발현을 억제한 결과 p53 이 감소하고 MDM2 가 증가하는 것을 볼 수 있었고, p53 의 half-life 가 감소하는 것을 볼 수 있었다. 또한 PIG3 과발현에 의해 p53 이 증가하고 MDM2 가 감소하는 것을 관찰 할 수 있었다. 더욱이, co-immunoprecipitation 실험을 통해 세포 내에서 PIG3, MDM2, p53 이 결합함을 확인 하였다. 이러한 결과를 종합해볼 때, p53 의 하위 유전자로써 PIG3 는 P53 의 ubiquitination 을 감소시키고, MDM2 ubiquitination 을 증가시킴으로써 p53 의 단백질 안정성을 조절한다고 할 수 있다.

저작물 이용 허락서

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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.

동의여부 : 동의(0) 조건부 동의( ) 반대( )

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