PIG3 Regulates p53 Stability by Suppressing Its MDM2-Mediated Ubiquitination

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Abstract
Under normal, non-stressed conditions, intracellular p53 is continually ubiquitinated by MDM2 and targeted for degradation. However, in response to severe genotoxic stress, p53 protein levels are markedly increased and apoptotic cell death is triggered. Inhibiting the ubiquitination of p53 under conditions where DNA damage has occurred is therefore crucial for preventing the development of cancer, because if cells with severely damaged genomes are not removed from the population, uncontrolled growth can result. However, questions remain about the cellular mechanisms underlying the regulation of p53 stability. In this study, we show that p53-inducible gene 3 (PIG3), which is a transcriptional target of p53, regulates p53 stability. Overexpression of PIG3 stabilized both endogenous and transfected wild-type p53, whereas a knockdown of PIG3 lead to a reduction in both endogenous and UV-induced p53 levels in p53-proficient human cancer cells. Using both in vivo and in vitro ubiquitination assays, we found that PIG3 suppressed both ubiquitination- and MDM2-dependent proteasomal degradation of p53. Notably, we demonstrate that PIG3 interacts directly with MDM2 and promoted MDM2 ubiquitination. Moreover, elimination of endogenous PIG3 in p53-proficient HCT116 cells decreased p53 phosphorylation in response to UV irradiation. These results suggest an important role for PIG3 in regulating intracellular p53 levels through the inhibition of p53 ubiquitination.

Key Words: PIG3, p53, MDM2, Ubiquitination, Apoptosis

INTRODUCTION

PIG3 (p53-inducible gene 3) is encoded by a gene located downstream of the gene encoding the tumor suppressor protein p53, and was originally discovered during a study aimed at identifying genes induced by p53 prior to the onset of apoptosis in human colorectal cancer cells (Polyak et al., 1997). p53 binds to a penta-nucleotide microsatellite sequence within the PIG3 promoter and thereby transactivates the expression of PIG3 prior to the onset of p53-initated apoptosis (Contente et al., 2002). The major cellular role of PIG3 is in the generation of reactive oxygen species (ROS), which then act as downstream mediators of p53-dependent apoptosis (Polyak et al., 1997; Porte et al., 2009). The amino acid sequence of PIG3 shows significant homology to that of NADH quinine oxidoreductase 1 (NQO1), which is a protein known to contribute to the generation of ROS (Polyak et al., 1997). It has also been reported that PIG3 directly inhibits catalase, an antioxidant enzyme that mitigates hydrogen peroxide (H₂O₂) toxicity, resulting in increased intracellular ROS levels (Kang et al., 2013). Under genotoxic conditions, high levels of both p53 and PIG3 act cooperatively to inhibit catalase activity, and the subsequent shift toward an oxidative intracellular environment leads to the induction of apoptotic cell death (Kang et al., 2013). Recently, PIG3 has been shown to play an important role in the cellular response to DNA damage, particularly in checkpoint signaling and DNA repair. PIG3 knockdown cells had increased sensitivity to DNA-damaging agents, impaired DSB repair, and ineffective intra-S and G2/M phase checkpoint activation (Lee et al., 2010; Kotsinas et al., 2012; Li et al., 2013). In addition, the involvement of PIG3 in glutathione peroxidase 3 (GPx3)-mediated cell death was shown through experiments in which either a knockdown of PIG3 or loss of the PIG3 binding motif in GPx3 abrogated the increase in ROS generation and caspase-3 activity that are normally observed (Wang et al., 2012).
The cellular response to genotoxic damage is also influenced by p53 (Lane, 1992), and the levels and activity of p53 are controlled mainly by MDM2 (Kubbutat et al., 1997). When MDM2 binds to p53, ubiquitination and subsequent proteasomal degradation are triggered. In an elegant feedback loop, p53 controls the expression levels of this negative regulator by acting as a transcription factor for MDM2 (Barak et al., 1993; Oliner et al., 1993; Perry et al., 1993). In non-stressed cells, p53 is kept at low levels by directly associating with MDM2, which represses its ability to act as a transcription factor and promotes its proteolytic degradation (Momand et al., 1992; Oliner et al., 1993; Haupt et al., 1997). Induction of p53 involves several mechanisms leading to post-translational modifications, such as phosphorylation and acetylation (Ashcroft et al., 2000). DNA damage-induced phosphorylation of serine and threonine residues at the amino terminus of p53 prevents MDM2 from binding and renders p53 more stable (Shieh et al., 1997; Unger et al., 1999).

Although the relationship between PI3G and p53 has been investigated previously, the role of PI3G in p53 stability was still unclear. It is of particular interest that PI3G shares significant homology with NQO1 (Polyak et al., 1997; Porte et al., 2009), a protein that plays an important role in regulating p53 functions by inhibiting its degradation (Asher et al., 2001, 2002a, 2002b). We therefore investigated whether PI3G could regulate p53 stability in a manner similar to that of NQO1. Our results clearly show that PI3G suppresses p53 degradation. However, unlike NQO1, which regulates p53 stabilization via MDM2- and ubiquitin-independent proteasomal degradation, our results indicate that PI3G suppresses MDM2-mediated p53 ubiquitination through direct association with MDM2. Our investigation advances the understanding of mechanisms regulating p53 stability and the role of PI3G in p53-dependent apoptotic cell death.

MATERIALS AND METHODS

Cell culture and treatment

The human osteosarcoma U2OS cells and human embryonic kidney HEK 293 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA), 100 units/ml of penicillin, and 100 mg/ml of streptomycin (Invitrogen). The human colorectal carcinoma HCT116 cells were grown in Iscove’s modified Dulbecco’s medium (IMDM) containing 10% FBS, penicillin and streptomycin. The human lung carcinoma H460 cells were maintained in RPMI-1640 medium containing 10% FBS, penicillin and streptomycin. These cells were from the American Type Culture Collection (ATCC). The p53−/−MDM2−/− mouse embryonic fibroblasts (MEFs) cells were kindly provided by Prof. Chin Ha Chung (Seoul National University, Seoul, Korea), and were maintained in DMEM supplemented with 10% FBS and antibiotics. All cells were maintained in a humidified incubator with an atmosphere of 5% CO2 at 37°C. Cycloheximide (CHX; protein synthesis inhibitor, Sigma, St. Louis, MO, USA) was used at a concentration of 80 μg/ml. For UV radiation, cells were exposed to light from a 254-nm UVc lamp (UVP; Model UVGL-25, Upland, CA, USA) in a minimal volume of serum-free culture medium at a 10 J/m².

Plasmids, PI3G siRNA and transfection

To generate the full length PI3G cDNA, cDNA was amplified from human fibroblast GM00637 cells by RT-PCR using PI3G oligo primers of the following sequences: sense, 5′-accgaattcatgttagccgtgcac-3′ and antisense, 5′-aatctcgagtcacctggggctacgc-3′. The amplified PI3G PCR products were cloned into pcDNA3-HA vector, and confirmed sequences and orientation were achieved by automated DNA sequencing. pcDNA-Myc-p53, pcDNA3-MDM2 and pcDNA-His-Ubiquitin constructs were obtained from Prof. Chin Ha Chung (Seoul National University). Cells were transfected with the indicated plasmids or siRNA using TurboFect (Thermo Fisher, Waltham, MA, USA) according to the manufacturer’s instructions. Knockdown of PI3G expression was achieved by transfecting siRNA using Lipofectamine RNAiMAX (Invitrogen) as mentioned before (Lee et al., 2010).

Immunoprecipitation assay and western blot analysis

Cells were lysed in ice-cold RIPA buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1 mM dithiothreitol, 1 mM phenylmethanesulfonyl fluoride, 10 μg/ml leupeptin and 10 μg/ml aprotonin] and amount of protein was determined using dye-binding microassay (Bio-Rad, Hercules, CA, USA). Equal amounts of protein were separated by 6-12% SDS-PAGE followed by electrottransfer onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). The membranes were blocked for 1 h with TBS-t [10 mM Tris-HCI (pH 7.4), 150 mM NaCl and 0.1% Tween-20] containing 5% nonfat milk and then incubated for overnight at 4°C with appropriate primary antibodies. The blots were washed four times for 15 min with TBS-t and then incubated for 1 h with peroxidase-conjugated secondary antibodies (1:5000, Jackson Immunoresearch Laboratories, Inc., West Grove, PA, USA). The blots were washed four more times with TBS-t and developed using an enhanced chemiluminescence detection system (ECL; intron). The intensity of protein bands were quantified using Scion Image software (Scion Corp., Frederick, MD, USA). For the immunoprecipitation assay, lysates were pre-cleared with protein A-Sepharose beads (GE Healthcare, Little Chalfont, UK) prior to adding the antibody. After removing the protein A-Sepharose by centrifugation, the supernatant was then incubated at 4°C overnight with appropriate antibodies. After the addition fresh protein A-Sepharose bead, the incubation was continued for an additional one hour, and then beads were washed five times with RIPA buffer. Immunoprecipitated proteins were denatured in SDS sample buffer, boiled for 5 min and analyzed by western blotting using the appropriate antibodies.

Antibodies

All antibodies used in this study are anti-human anti-p53 polyclonal antibody, anti-phospho-p53-(Ser15) polyclonal antibody (Cell Signaling Technology, Danvers, MA, USA); anti-α-tubulin monoclonal antibody (BD Phamingen, San Jose, CA, USA); anti-PI3G (H300) polyclonal antibody, anti-p53 (DO-1) monoclonal antibody, anti-MDM2 (SMP14) monoclonal antibody, anti-myc polyclonal antibody, anti-HA polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). We followed manufacturer’s protocol for dilution of all primary antibodies.
In vitro transcription and translation

In vitro transcription and translation were carried out using TNT Quick coupled Transcription and Translation system (Promega, Amedison, WI, USA). For in vitro translation, pCDNA-HA-PIG3, pcDNA-Myc-p53 and pcDNA3-MDM2 expression vector were used. One microgram of each construct was used per reaction of the TNT-Quick coupled reticulocyte lysate system (Promega), following the manufacturer's instructions. The synthesized proteins were used in vitro ubiquitination and in vitro protein binding assay.

In vitro protein binding assay

Synthesized PIG3 and MDM2 by TNT system was incubated in PBS buffer at 4°C for overnight. After incubation, reaction solution was added antibody for PIG3 and the precipitated immunoprecipitates were then subjected to Western blot analysis with anti-PIG3 or anti-MDM2 antibody.

In vitro ubiquitination assay

Synthesized p53, MDM2 and PIG3 proteins were mixed with UBE1 (E1 enzyme), UbcH5c (E2 enzyme) and Ubiquitin (Ub) in reaction solution (R&D systems, Minneapolis, MN, USA) following to the manufacturer’s instructions. The mixture was incubated in 37°C for 30-60 minutes and stopped by addition of SDS-PAGE sample buffer. The reaction mixture was analyzed by Western blotting with anti-p53 antibody.

In vivo ubiquitination assay

Cells were transfected with PIG3 siRNA or indicated constructs and treated with 20 μM MG132 (Sigma) for 4 h before harvest for inhibition of proteasome-mediated protein degradation. After 48 h, the cells were harvested and split into two aliquots, one for immunoblot and the other for ubiquitination assays. For ubiquitination assay, cells were lysed in RIPA buffer and then perform immunoprecipitation with anti-p53 or anti-MDM2 antibodies, and the immunocomplexes were detected with anti-ubiquitin antibody. For NI-NTA pull down assay, cells were lysed in buffer I [6 M guanidinium-HCl, 0.1 mol/L Na2HPO4/NaH2PO4, 10 mmol/L Tris-HCl (pH 8.0), 10 mmol/L 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/L urea, 0.1 mol/L Na2HPO4/NaH2PO4, 10 mmol/L Tris-HCl (pH 8.0), 10 mmol/L β-mercaptoethanol], and buffer III [8 mol/L urea, 0.1 mol/L Na2HPO4/NaH2PO4, 10 mmol/L Tris-HCl (pH 6.3), 10 mmol/L β-mercaptoethanol]. Proteins were eluted from the beads in buffer IV [200 mmol/L imidazole, 0.15 mol/L Tris-HCl (pH 6.7), 30% (v/v) glycerol, 0.72 mol/L β-mercaptoethanol, and 5% (w/v) SDS]. Eluted proteins were analyzed by immunoblot with monoclonal anti-p53 or anti-MDM2 antibodies.

Statistical analysis

Data in all experiments are represented as mean ± SD. Statistical comparisons were carried out using two-tailed paired t-test. We considered p<0.01 (indicated ** in figures) as significant. Analyses were carried out with GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA) and Excel (Microsoft, Redmond, WA, USA).

RESULTS

PIG3 controls the stability of p53

To explore the role of PIG3 in p53 stability, we started by investigating the effects of a PIG3 knockdown on the expression of p53 protein. To this end, we used a PIG3-specific siRNA to deplete protein levels in three different cell lines that contain endogenous wild-type p53: HCT116 colorectal carcinoma, U2OS osteosarcoma, and H460 lung carcinoma cells. The expression of PIG3 was reduced by approximately 70% in cells transfected with PIG3-specific siRNA as compared to control siRNA-transfected cells (Fig. 1A, first row). We then measured p53 protein levels and observed that lower levels of PIG3 corresponded to lower levels of endogenous p53 (Fig. 1A, second row, and quantification below). The same three cell lines were transiently transfected with a vector expressing HA-tagged PIG3 such that PIG3 would be overexpressed. p53 expression levels were higher in PIG3-expressing cells as compared to the vector controls (Fig. 1B). Moreover, when Myc-tagged p53 and HA-tagged PIG3 were co-transfected...
into HEK293 cells, there was a significant increase in p53 protein levels when we observed an increase in PIG3 expression levels (Fig. 1C). These results support the idea that PIG3 contributes to p53 stability under normal conditions.

**PIG3 inhibits p53 proteasomal degradation**

Proteasome-mediated degradation is important for the turnover of many cellular proteins, including p53 (Burger and Seth, 2004). To determine whether the observed increase in p53 levels in the presence of PIG3 was due to protein stabilization or new protein synthesis, p53 levels in both control and PIG3-expressing U2OS and HCT116 cells were measured with and without exposure to a protein synthesis inhibitor. Cells were treated with cycloheximide for 0, 30, 60, and 90 minutes, and p53 levels were measured using Western blot analysis. The half-life of p53 increased 2-3-fold in cells overexpressing PIG3 (Fig. 2A, 2B). We then measured the half-life of p53 in PIG3 knockdown cells following the same protocol. As shown in Fig. 2C, 2D, in PIG3 knockdown cells, p53 levels rapidly decreased after cycloheximide treatment, suggesting that PIG3 promotes p53 stability by inhibiting proteasomal degradation.

**PIG3 suppresses MDM2-mediated p53 ubiquitination**

One mechanism by which PIG3 could have stabilized p53 protein levels was to inhibit ubiquitination. To test this possibility, we measured levels of ubiquitinated p53 in HCT116 cells with and without overexpression of PIG3. At 48 h after transfection with the HA-PIG3 expression vector, cells were either directly lysed to measure p53 and PIG3 protein levels by Western blotting, or were first treated with the proteasome inhibitor MG132 prior to lysis and immunoprecipitation with a p53 antibody. Overexpression of PIG3 suppressed the ubiquitination of p53 in these cells.
Fig. 3. PIG3 inhibits MDM2-mediated p53 ubiquitination in vivo and in vitro. (A) HCT116 cells were transfected with either control vector or HA-PIG3 expression vector. At 48 h after transfection, cells were treated with 20 μM MG-132 for 4 h before harvesting. Cell lysates were subjected to immunoprecipitation with anti-p53 antibody, followed by Western blotting with anti-ubiquitin antibody. The expression levels of p53 and HA-PIG3 are shown in the lower panels. (B) PIG3 knockdown decreases p53 stability in HCT116 cells. The same assay as shown in panel (A) was performed, except that control and PIG3 siRNA were used. (C) p53−/− MDM2−/− MEF cells were transfected with a combination of plasmid encoding Myc-p53, MDM2, His-Ub, and an increasing amount of the HA-PIG3. At 48 h after transfection, the cells were subjected to a pulldown using Ni-NTA bead, followed by immunoblotting using the anti-Myc antibody. The expression of MDM2, Myc-p53 and HA-PIG3 was shown in lower panels. (D) PIG3 inhibits MDM2-mediated p53 ubiquitination in vitro. The in vitro ubiquitination experiment was conducted as described in Materials and Methods, and analyzed by immunoblotting using anti-p53 antibodies.

PIG3 binds directly to MDM2 and promotes MDM2 ubiquitination

Because other MDM2 binding proteins are also involved in the regulation of p53 degradation, our next question was whether PIG3 associates directly with MDM2 or acts indirectly through MDM2 binding proteins. Extracts of HCT116 cells, which endogenously express PIG3, were immunoprecipitated using an anti-PIG3 antibody and Western blot analyses revealed that MDM2 co-purified with PIG3 (Fig. 4A). We then looked for the same effect in vitro by pre-mixing synthesized PIG3 with MDM2 and subjecting the precipitates to Western blot analysis. Immunoprecipitation with an anti-PIG3 antibody immunoprecipitated MDM2 from the PIG3-MDM2 mixture (Fig. 4B), suggesting a direct interaction between these two proteins.

Knowing that PIG3 and MDM2 interact directly, we next asked whether PIG3 regulates protein levels of MDM2. Our results showed that ectopic expression of PIG3 in HCT116, U2OS, or H460 cells led to reduced MDM2 (Fig. 4C), whereas the downregulation of PIG3 increased MDM2 levels (Fig. 4D). Because MDM2 is a negative regulator of p53, we hypothesized that the role of PIG3 is to regulate p53 stability and function by countering the E3 ubiquitin ligase activity of MDM2. We tested this by comparing MDM2 ubiquitination levels in HCT116 cells 48 h after transfection with either control siRNA or PIG3 siRNA. Endogenous MDM2 ubiquitination was lower in PIG3 knockdown cells (Fig. 5A) and higher when PIG3 was over-expressed (Fig. 5B). To further confirm these results, we introduced combinations of ectopic His-tagged Ub, MDM2, and HA-tagged PIG3 into p53−/− MDM2−/− MEF cells. As shown in Fig. 5C, ectopic expression of PIG3 indeed promoted MDM2 ubiquitination in a dose-dependent manner. Collectively, these data suggest that PIG3 interacts with MDM2, which in turn enhances the level to which MDM2 is ubiquitinated and is subject to degradation.

PIG3 regulates p53 phosphorylation

To demonstrate the physiological impact of PIG3 on the p53
ubiquitination pathway, we examined the role of PIG3 in the p53 phosphorylation in response to DNA damage. To test this, p53-proficient H1T16 cells were transfected with either control or PIG3 siRNA and then exposed to 10 J/m² UV to induce DNA damage. The control cells showed a significant increase in p53 expression levels and $p53$ phosphorylation at Ser15 in a time-dependent manner, reaching a maximum at 3 h after UV irradiation (Fig. 6A). In contrast, UV-induced p53 expression was markedly lower in PIG3-knockdown cells, indicating that p53 indeed contributes to p53 stability and activation in response to DNA damage. Taken all together, these results indicate that after DNA damage has occurred, PIG3 induces the expression of PIG3, which then enhances MDM2 ubiquitination, further increasing the amount of cellular p53 protein, and thereby promoting p53 activation (Fig. 6B).

**DISCUSSION**

In the present study, we have identified and validated PIG3 as a novel regulator of the p53 degradation that typically occurs in the MDM2-p53 pathway. Our studies not only identify a novel participant in this pathway, but also suggest a mechanism by which p53 activation occurs in response to DNA damage.

p53 is often referred to as the ‘cellular gatekeeper’ or the ‘guardian of the genome,’ and its importance is substantiated by the discovery of p53 mutations in >50% of all human tumor cells (Levine, 1997). The stabilized and activated p53 contributes to the proper response of the cell to a stressed state and appropriate return of the cell to a normal state. In the present study, we observed that the PIG3 protein regulated p53 degradation. A knockdown of PIG3 accelerated intracellular p53 degradation, and decreased the half-life of p53 in several different p53-proficient human cell lines. Moreover, p53 steady-state level and

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**Fig. 4.** PIG3 binds to MDM2 and regulates MDM2 stability. (A) HCT116 cell lysates were immunoprecipitated with anti-PIG3 antibody and the immunoprecipitated proteins were probed with anti-MDM2 antibody. (B) Immunoprecipitation of human PIG3 from a mixture of PIG3 and MDM2. The precipitates were subjected to immunoblotting using anti-MDM2 antibody. The expression levels of MDM2 and PIG3 indicate that after DNA damage has occurred, p53 induces the ubiquitination pathway, thereby promoting p53 activation (Fig. 6B).

**Fig. 5.** PIG3 promotes MDM2 ubiquitination. (A, B) HCT116 cells were transfected with either control siRNA or PIG3 siRNA (A) or with either control vector or HA-PIG3 expression vector (B). At 48 h after transfection, cells were treated with 20 μM MG-132 for 4 h before harvesting. Cell lysates were subjected to immunoprecipitation with anti-ubiquitin antibody, followed by Western blotting with anti-MDM2 antibody. The expression levels of MDM2 and PIG3 are shown in the lower panels. (C) p53−/− MDM2−/− MEF cells were transfected with a combination of plasmids encoding MDM2, His-Ub, and an increasing expression of the HA-PIG3. The level of MDM2 ubiquitination was determined by Ni-NTA purification, followed by immunoblotting using the anti-MDM2 antibody.
PIG3 siRNA

Expression of PIG3 led to a decrease in MDM2 expression and levels of MDM2 protein, as evidenced in two ways: ectopic ubiquitination and degradation. In addition, PIG3 influences the complex. Moreover, PIG3 inhibited MDM2-mediated p53 ubiquitination in vivo and in vitro, and formed a PIG3-MDM2 complex. Additionally, PIG3 interacted directly with MDM2 both in vivo and in vitro, and formed a PIG3-MDM2 complex. Moreover, PIG3 inhibited MDM2-mediated p53 ubiquitination and degradation. In addition, PIG3 influences the levels of MDM2 protein, as evidenced in two ways: ectopic expression of PIG3 led to a decrease in MDM2 expression and a knockdown of PIG3 increased MDM2 expression. Finally, PIG3 promoted ubiquitination of MDM2 itself.

Several cellular proteins that regulate the MDM2-p53 pathway have been identified; these include p14ARF (Pomerantz et al., 1998), YY1 (Sui et al., 2004), MDMX (Francoz et al., 2006), DAXX and HAUSP (Tang et al., 2006), PA28γ (Zhang and Zhang, 2008), and a number of ribosomal proteins (Zhang et al., 2013; Zhou et al., 2013). This large number of regulatory molecules highlights the complexity of the MDM2-p53 pathway. Why would mammalian cells need so many proteins to overcome the negation of p53 by MDM2? One possibility might be that MDM2 functions as a multiple subunit complex, such as a homohexamer (Poyurovsky et al., 2007; Uldrijan et al., 2007), to inactivate p53 in cells. Thus, individual proteins would need to work together or to independently bind to different subunits in the homohexameric MDM2 complex in order to efficiently inactivate this protein and consequently activate p53. Alternatively, different MDM2-binding proteins may act either sequentially or in response to different signals when inactivating MDM2 in response to stresses. The identification of PIG3 as regulator of MDM2 stability suggests that PIG3 is a new important player in maintenance of cellular p53 levels. When PIG3 is present, MDM2 cannot initiate p53 ubiquitination, but when PIG3 is absent, p53 levels remain under stringent control of MDM2 (Fig. 6B). This mechanism could be an advantage to the cells because it would circumvent the need for elevated levels of MDM2.

Apoptosis is a vital process for tissue homeostasis that is frequently disturbed in several pathological conditions (Fernando and Kurokawa, 2013). PIG3 is believed to be one of the major factors involved in p53-induced apoptosis through ROS generation (Polyak et al., 1997). However, because PIG3 expression alone is insufficient to induce apoptosis (Polyak et al., 1997), and because PIG3 knockdown cannot efficiently induce apoptosis in response to DNA damage (Lee et al., 2010), it is assumed that several other factors cooperate to cause apoptotic cell death. p53 plays a critical role in initiating an early response to genotoxic stress (Lane, 1992; Levine and Oren, 2009). It has an arsenal of target genes at its disposal and may even possess some selectivity towards a particular fate. For example, with the assistance of ASPP proteins, p53 exhibits a striking preference for the promoters of proapoptotic genes (Samuels-Lev et al., 2001). However, the initial activation of its function as a transcription factor is key to its ability to drive these particular downstream pathways. The gene encoding PIG3 is induced by p53 prior to the onset of apoptosis (Polyak et al., 1997). Thus, after initial expression and activation of PIG3 in response to DNA damage, a role for PIG3 might be to amplify p53-mediated apoptotic signals via the suppression of p53 degradation. The data presented here show that phosphorylation of p53 at Ser15 in response to UV was significantly decreased in PIG3 knockdown cells as compared to control cells. Together these results suggest that PIG3 is important in the amplification of p53 signaling in response to DNA damage.

In conclusion, our study brings to light an important role for PIG3 in the positive regulation of intracellular p53 levels. By promoting degradation of MDM2, and thus the stabilization of p53, PIG3 may be important in the early stages of the p53-mediated cellular response to genotoxic stress.

**CONFLICT OF INTEREST**

The authors have declared that no conflict of interest exists.
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