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Anti-apoptotic protein TCTP controls the stability of the tumor suppressor p53

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ABSTRACT

In this study, we identified p53 as a novel TCTP-interacting protein using TCTP as bait. Also, we determined the critical binding sites between TCTP and p53. To elucidate the functional consequence of the interaction, we developed the overexpression and inhibition system of TCTP and p53 expression. Overexpression of TCTP in lung carcinoma cells reversed p53 mediated apoptosis and inhibition of TCTP expression by small interfering RNA increased apoptosis of lung carcinoma cells. Moreover, it was observed that TCTP overexpression promotes degradation of p53. These results clearly indicate that the interaction between TCTP and p53 prevents apoptosis by destabilizing p53. Thus, TCTP acts as a negative regulator of apoptosis in lung cancer.

Structured summary:
MINT-8057107, MINT-8057116: *p53* (uniprotkb:P04637) *physically interacts* (MI:0915) with *TCTP* (uniprotkb:P13693) by *anti bait coimmunoprecipitation* (MI:0006)
MINT-8057141: *TCTP* (uniprotkb:P13693) *physically interacts* (MI:0915) with *p53* (uniprotkb:P04637) by *two hybrid pooling approach* (MI:0398)
MINT-8057126: *p53* (uniprotkb:P04637) *physically interacts* (MI:0915) with *TCTP* (uniprotkb:P13693) by *anti tag coimmunoprecipitation* (MI:0007)
MINT-8057160: *TCTP* (uniprotkb:P13693) *physically interacts* (MI:0915) with *p53* (uniprotkb:P04637) by *two hybrid* (MI:0018)

1. Introduction

Translationally controlled tumor protein (TCTP)/histamine releasing factor (HRF), a 172 amino acid anti-apoptotic polypeptide, which is also a highly conserved hydrophilic protein [1], plays a pivotal role in the development of different organisms [2]. TCTP is a housekeeping gene abundantly expressed in many tissues and cell types [3]. It is present both extra- and intracellular which has been implicated in many cellular functions that are related to cell growth, apoptosis regulation [4,5], and even the allergic response [6]. TCTP also interacts with many cellular proteins, which include tubulin [4], translation elongation factors eEF1A and its guanine nucleotide exchange factor eEF1B-β [7], Mcl-1 [1], TSAP6 [8], Na, K-ATPase [9], and Bcl-X_L [10]. On the other hand, anti-apoptotic activity has been reported for TCTP, which may be related to its interaction with Mcl-1 and/or Bcl-X_L [1,11]. A recent study demonstrated that overexpression of TCTP can prevent stress induced mammalian cell apoptosis. Reversely, inhibition of TCTP in a malignant human carcinoma cells increased apoptosis [3]. Thus, TCTP may be involved in cell survival as a negative regulator of apoptosis. However, the exact mechanisms of TCTP on physiological functions regarding anti-apoptosis mediation are largely unknown. p53 tumor suppression protein is encoded by the *TP53* gene. p53 has a key role in mediating cellular response to various stress signals, mainly by inducing or repressing a number of proteins that are involved in cell cycle progression, apoptosis, DNA repair, cellular senescence, chronic inflammation, and cellular senescence [12–15]. As shown in some cancers, the interaction of p53 with viral and cellular proteins leads to the inactivation of p53 functions [16,17]. The activity of p53 is regulated at low levels primarily due to the action of mouse or human double minute-2

Abbreviations: DAPI, 4,6′-diamidino-2-phenylindole; HRF, histamine releasing factor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyl-^{2}H-tetrazolium bromide; ONPG, *O*-nitrophenyl β-D-galactopyranoside; siRNA, small interfering RNA; TCTP, translationally controlled tumor protein; TUNEL, terminal deoxynucleotidyl transferase nick end label

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(mdm-2 or hdm-2) protein, its interacting partner. As mdm-2 is a major regulator of p53 protein, p53 functions are controlled in two different ways: via the regulation of p53 transcriptional activity and the intracellular p53 level [18,19].

By using yeast two-hybrid screening to address the apoptosis regulatory mechanism related to TCTP proteins, we identified p53 as a novel TCTP-interacting protein. p53 is a tumor suppressor protein and a omnifunctional transcription regulator that plays an essential role in cellular responses to various stress signals. p53 specifically interacted with TCTP both in vitro and in vivo. Transient transfection analyses demonstrated that overexpression of TCTP blocks p53 mediated apoptotic activity by inducing the degradation of p53. These results therefore support that a dynamic equilibrium between pools of p53 and TCTP regulating apoptotic homeostasis.

2. Materials and methods

2.1. Screening of binding protein partner and quantitation of interaction

For bait construction with human TCTP, cDNA encoding full-length human TCTP was subcloned into the *Eco*RI and *Xho*I restriction sites of the pGilda cloning vector. The resulting plasmid pGilda-TCTP was introduced into yeast strain EGY48 by a modified lithium acetate method [20]. The activity of the interaction between TCTP and p53 was confirmed by measuring the relative expression level of β-galactosidase. The β-galactosidase assay was determined according to the previously described method [20] with slight modification.

2.2. Cell culture and antibodies

Human lung carcinoma cells (A549) were cultured in a DMEM, and were supplemented with 10% heat-inactivated FBS and antibiotics at 37 °C in a humidified 5% CO_2 incubator (Life Technologies, Gaithersburg, MD). The following antibodies were used in this study: anti-TCTP antibody (Oncogene, San Diego, CA), anti-p53 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-GFP (Santa Cruz), anti-caspase 3 (Santa Cruz), anti-Bcl-2 (Santa Cruz), anti-BAX (Santa Cruz), anti-tubulin (Santa Cruz) and anti-β-actin (Sigma, St. Louis, MO).

2.3. Co-immunoprecipitation

cDNA encoding human p53 was cloned into pEGFP (Clontech) and digested with BglII and EcoRI (pEGFP-p53). The human TCTP cDNA was ligated into pcDNA4/HisMax (Invitrogen Life Technologies, Carlsbad, CA) using EcoRI and XhoI (pcDNA4/HisMax-TCTP). For co-immunoprecipitation, A549 cells were co-transfected with cDNA constructs of pEGFP-p53 and pcDNA4/HisMax-TCTP using Fugene 6 Reagent (Roche, Mannheim, Germany) according to the manufacturer's protocol. Lysates were then incubated with anti-GFP antibody (Santa Cruz) and precipitated with protein A-agarose (Amersham). The precipitated proteins were resolved by SDS gel electrophoresis, transferred onto Immobilon P membrane (Millipore, Billerica, MA), and immunoblotted with anti-His antibody (Santa Cruz) or goat anti-TCTP antibody (Oncogene) using the ECL system (Amersham).

2.4. Small interfering RNA (siRNA) construction

The small interfering RNA (siRNA) oligonucleotide sequence targeting TCTP (5′-AAGGTACCGAAAGCACAGT-3′) corresponded to nucleotides 179–197 in the human sequence. siRNA was synthesized by using an siRNA construction kit (Ambion, Austin, TX). A549 cells were transfected with p53-specific siRNA SmartPool duplexes (Dharmacon, Lafayette, CO) at a final concentration of 100 nM. Transfections were performed using Fugene 6 Reagent (Roche) according to the manufacturer's protocol.

2.5. Determination of p53 protein stability

A549 cells were transfected with mock (an expression vector only), TCTP siRNAs, or co-transfected with p53 and the indicated pEGFP-TCTP cDNA mutant. Three days after transfection, the cells were treated with cycloheximide (Sigma) at a final concentration 30 μg/ml. The cell lysates were subsequently harvested at sequential time points after treated, resolved through SDS gel electrophoresis, transferred onto Immobilon P membranes (Millipore), and immunoblotted with anti-p53 antibody.

3. Results

3.1. Identification of p53 as a TCTP-interacting protein

As an initial step to elucidate the regulation mechanism of TCTP, we screened a human cDNA library using a yeast two-hybrid system with TCTP as bait. Approximately 2.5×10^6 independent transformants were pooled and spread again onto the selection media (Ura^-, His^-, Trp^-, Leu^-) containing 2% (w/v) galactose in order to induce cDNA expression. If a B42-tagged protein interacts with the TCTP, the transcription of the *LEU2* gene is activated, thus allowing the host cells to grow on a leucine-deficient synthetic medium. Among the seven colonies obtained on the selection media, a total of five colonies showed galactose dependency. The plasmids were then isolated by the plasmid marker trp in the *Escherichia coli* host, and the purified plasmids were sequenced. A homology search in GenBank using the BLAST program revealed that all plasmids encoded human p53 (GenBank accession number: NM_000546). Human p53 cDNA has an open reading frame (ORF) of 1182 bp and encoded 393 amino acids. All of the p53 encoded plasmids identified in yeast two hybrid system contained a cDNA with 100% identity to the V^{197}-D^{393} of human p53 (two plasmids) or G^{105}-D^{393} of human p53 (three plasmids) including C-terminal sequence of human p53 (Fig. 1A). To confirm this result, we determined the binding activity between TCTP and p53 by measuring the relative expression levels of β-galactosidase. As shown in Fig. 1B, the β-galactosidase activity between TCTP and p53 was fully observed, whereas very little β-galactosidase activity was observed between TCTP and empty vector (vector only).

For co-immunoprecipitation, cDNA constructs of p53 (pEGFP-p53) and TCTP (pcDNA4/HisMax-TCTP), or pEGFP-p53 and vector only (pcDNA4/HisMax) were co-transfected into A549 cells (human lung carcinoma cells). Subsequently, an immunoprecipitation was performed using anti-GFP antibody with lysates from both transfected cells. After immunoprecipitation, the precipitated proteins were immunoblotted using anti-TCTP antibody or anti-p53 antibody. As shown in Fig. 1C, pEGFP-p53 was co-immunoprecipitated with pcDNA4/HisMax-TCTP (lane 2 in upper panel), whereas no interaction was observed between pcDNA4/HisMax (vector only) and pEGFP-p53 (lane 1 in upper panel). An immunoblotting using anti-p53 antibody confirmed that an equal amount of p53 was precipitated in both samples (middle panel). Whole cell lysates from both samples contained the equivalent proteins when immunoblotted using anti-β-actin antibody (lower panel).

Next, we tested whether we can detect the interaction between p53 and TCTP in endogenous level. Immunoprecipitation was subsequently conducted using anti-p53 or anti-TCTP antibody with whole cell lysates of A549 cells. After immunoprecipitation, the

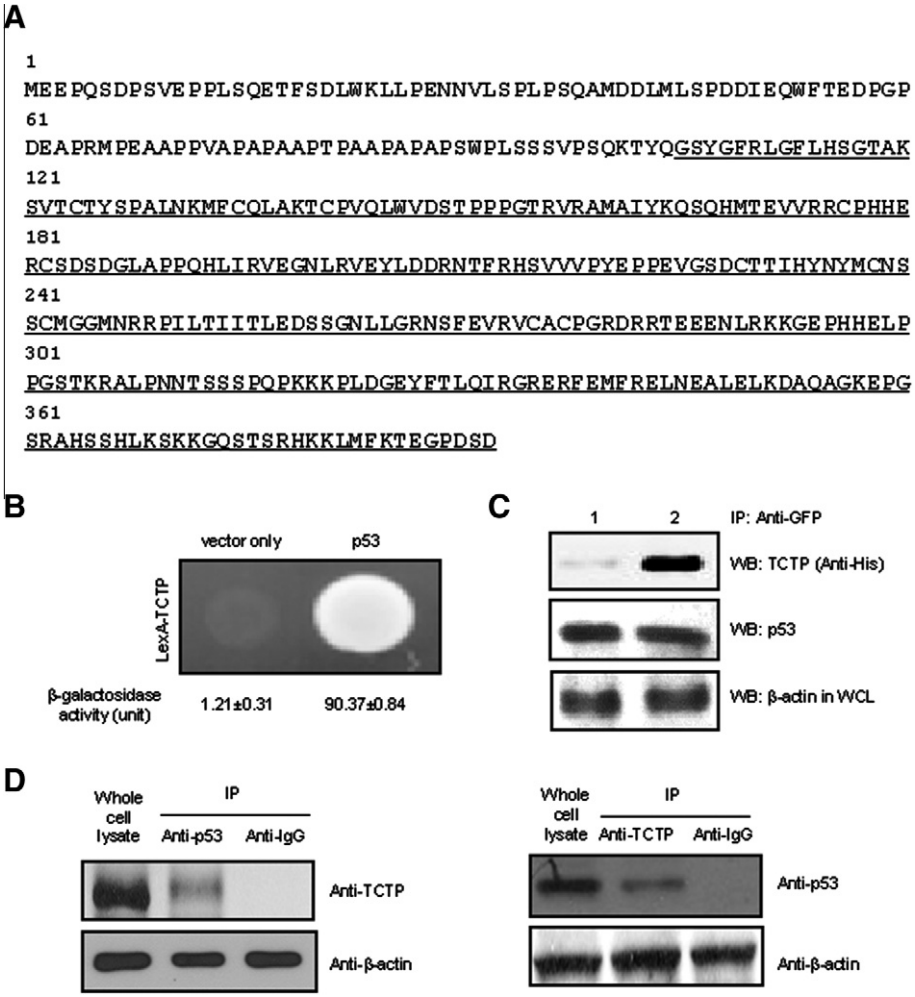

Fig. 1. Interaction analysis of TCTP with p53 tumor suppressor protein. (A) The amino acid sequence of p53 is indicated using single letter abbreviations. An underlined amino acid sequence means the translated p53 protein, isolated from yeast two-hybrid screening. (B) Positive interactions were revealed by observing cell growth over three days at 30 °C on medium lacking leucine, and by the formation of blue colonies on medium containing X-gal. The values of β-galactosidase activity (unit) measured by adding *O*-nitrophenyl β-D-galactopyranoside (ONPG) assays are indicated below the corresponding lanes. (C) Co-immunoprecipitation of TCTP with p53. Immunoprecipitation was performed using an anti-GFP antibody with lysates from both transfected cells. After immunoprecipitation, precipitated proteins were immunoblotted using anti-TCTP antibody and anti-p53 antibody. *lane 1*, pCDNA4/HisMax (vector only) and pEGFP-p53 transfectant; *lane 2*, pCDNA4/HisMax-TCTP and pEGFP-p53 transfectant. (D) Co-immunoprecipitation between the endogenous p53 and TCTP display the interactions of the two proteins.

precipitated proteins were immunoblotted with anti-TCTP or anti-p53 antibody. As shown in Fig. 1D, TCTP was co-immunoprecipitated with p53 (upper left panel), whereas no interactions were observed between isotype control (anti-IgG) and TCTP. Also, p53 was co-immunoprecipitated with TCTP (upper right panel), whereas no interactions were observed between isotype control (anti-IgG) and p53. Immunoblotting using anti-TCTP and p53 antibody verified that an equal quantity of both p53 and TCTP was present in the whole cell lysates (middle panel). Also, the whole cell lysates from both samples harbored the equivalent proteins when immunoblotted using anti-β-actin antibody (lower panel). These results clearly demonstrated that TCTP and p53 interact with each other in the physiological context of a human cell.

3.2. Mapping of the interaction domain between TCTP and p53

To identify the p53 binding domain of TCTP, cDNA constructs containing three p53 deletion mutants were designed as shown in Fig. 2A (see detailed Materials and methods in Supplementary data). These truncated domains were predicted to be predominantly α-helices. In the two-hybrid system, the full-length human TCTP cDNA and either plasmid containing a full-length human TCTP cDNA (left panel in Fig. 2A, full) or plasmids containing three truncation mutant forms (left panel in Fig. 2A, Met^{1}-Gly^{69}, Val^{70}-Ala^{119}, Glu^{120}-Cys^{172}) of cDNA were co-transformed into EGY48 yeast cells. Cells containing full-length TCTP cDNA and also one deletion mutant (Val^{70}-Ala^{119}) grew on the Ura, His, Trp and Leu deficient plates. Yeast cells transformed with the other deletion mutants (Met^{1}-Gly^{69} and Glu^{120}-Cys^{172}) failed to grow (right panel in Fig. 2A). To confirm this result, we determined the binding activity of these constructs by measuring the relative expression level of β-galactosidase. As shown in right panel in Fig. 2A, β-galactosidase assay results confirmed that either of these mutants (Met^{1}-Gly^{69} and Glu^{120}-Cys^{172}) cannot bind to p53. However, we cannot exclude that the p53 binding site of TCTP is located near the junction of these mutants since β-galactosidase activity in one deletion mutant (Val^{70}-Ala^{119}) is lower than full-length TCTP (right panel in Fig. 2A).

Subsequently, cDNA constructs containing three p53 truncation mutants were designed to localize the TCTP binding domain of p53 (left panel in Fig. 2B, full). In the two-hybrid system, the full-length human TCTP cDNA and either plasmid containing a full-length

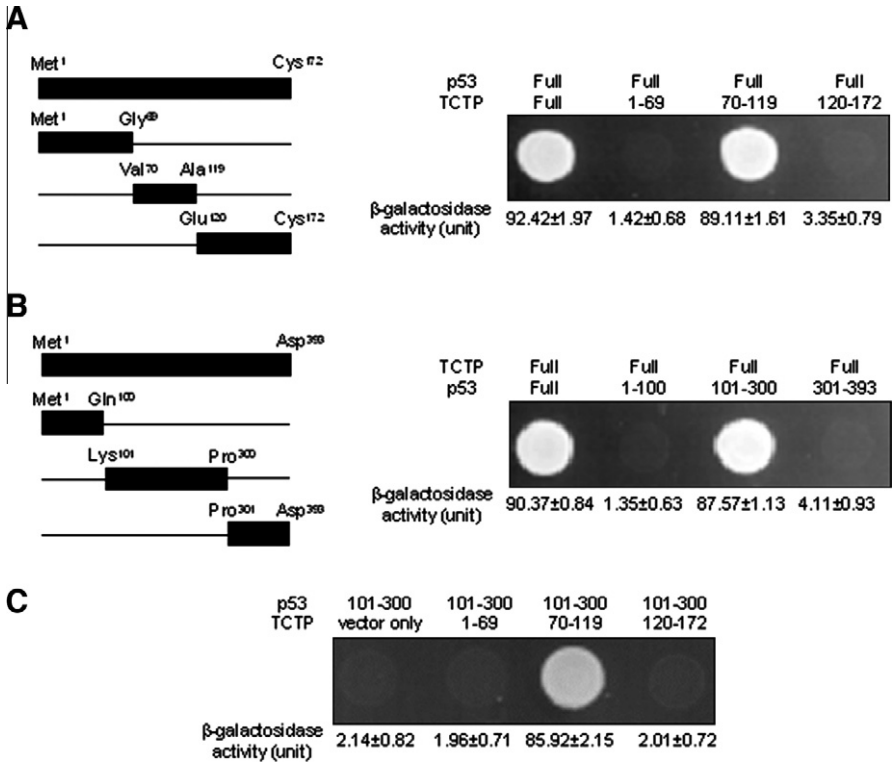

Fig. 2. Mapping of the critical interaction domain between TCTP and p53 using the yeast-two-hybrid system. (A) Left panel shows the schematic representation of cDNA constructs for each TCTP deletion mutant and full-length TCTP fusion proteins. Right panel shows the result of protein–protein interaction determined in the yeast two-hybrid system. The values of β-galactosidase activity in negative controls (vector only) of each construct were below 1.21 ± 0.37. (B) Left panel shows the schematic representation of cDNA constructs for each p53 deletion mutant and full-length p53 fusion proteins. Right panel shows the result of protein-protein interaction determined in the yeast two-hybrid system. The values of β-galactosidase activity in negative controls of each construct were below 1.55 ± 0.42. (C) Interaction between cDNA constructs for a p53 (Lys^{101}-Pro^{300}) and three TCTP truncation (Met^{1}-Gly^{69}, Val^{70}-Ala^{119}, Glu^{120}-Cys^{172}) fusion proteins in the yeast two-hybrid system.

human p53 (left panel in Fig. 2B, full) or three truncation mutant forms (left panel in Fig. 2B, Met^{1}-Gln^{100}, Lys^{101}-Pro^{300}, Pro^{301}-Asp^{393}) of cDNAs were co-transformed into EGY48 yeast cells (see detailed Materials and methods in Supplementary data). Cells containing full-length TCTP cDNA and also one deletion mutant (Lys^{101}-Pro^{300}) grew on the Ura, His, Trp and Leu deficient plates. Yeast cells transformed with the other deletion mutants (Met^{1}-Gln^{100} and Pro^{301}-Asp^{393}) failed to grow (right panel in Fig. 2B). We also quantitated the binding activity of these constructs by measuring the relative expression level of β-galactosidase. Results on β-galactosidase assay also indicated that the critical p53 region for binding TCTP resided within Lys^{101}-Pro^{300} including the DNA-binding motif of p53 (right panel in Fig. 2B).

To confirm these results, one deletion mutant of p53 (Lys^{101}-Pro^{300}) and either vector cDNA or three truncation mutants of TCTP (Met^{1}-Gly^{69}, Val^{70}-Ala^{119}, Glu^{120}-Cys^{172}) were co-transformed into EGY48 yeast cells (Fig. 2C). Consistent with results from Fig. 2A and B, cells containing p53 deletion mutant (Lys^{101}-Pro^{300}) with one TCTP deletion mutant (Val^{70}-Ala^{119}) only grew on the deficient plates whereas the other deletion mutants (Met^{1}-Gly^{69} and Glu^{120}-Cys^{172}) failed to grow. Subsequent results on β-galactosidase assay were also agreed with these results (Fig. 2C).

3.3. TCTP prevents p53 mediated apoptosis in lung carcinoma cells

As shown in Fig. S1 (Supplementary Fig. 1), the inhibition of TCTP expression caused A549 cells to undergo instinctive and massive apoptosis and p53 overexpression is capable suppressing cell proliferation by inducing apoptosis. To test whether TCTP and p53 show an additive effect or whether they antagonize each other in regards to apoptosis, we measured the cell viability with A549 cells transfected with Mock (expression vector only), p53 cDNA alone, or TCTP cDNA alone, or co-transfected with p53 and TCTP, or co-transfected p53 and siTCTP cDNAs (see detailed Materials and methods in Supplementary data). As expected, nearly 55% of the cells died after transfection of p53 cDNA alone when compared to mock transfectant, whereas no change of cell viability was observed when transfected with TCTP cDNA alone (Fig. 3A). Interestingly, most of cell viability was recovered when co-transfected with TCTP and p53 cDNAs (Fig. 3A). This may indicate that TCTP antagonizes p53 mediated apoptosis of A549 cells. To confirm this result, we inhibited TCTP expression with siRNA when we transfected p53 cDNA. As shown in Fig. 3A, viability of p53 and siTCTP (siRNA of TCTP) transfectant is markedly decreased when compared with p53 and TCTP co-transfectant. Result showed that cell viability of co-transfectant with siTCTP and p53 (2 μg) was relatively lower than that of 2 μg of p53 cDNA alone. To verify that the reduction in cell numbers represented apoptosis, we employed 4,6′-diamidino-2-phenylindole (DAPI) staining to confirm that the observed loss of proliferation in A549 cells overexpressing mock, TCTP, siTCTP, p53, or sip53, or co-transfected with TCTP and p53 cDNAs, co-transfected with p53 and siTCTP, or co-transfected siTCTP and sip53, or triple-transfected with TCTP, siTCTP and p53 (see detailed Materials and methods in Supplementary data). As expected, after transfection of p53 cDNA alone compared to the mock transfectant, nearly 53% of cells showed fragmented nuclei, whereas no change of cell viability was observed when transfected with mock and TCTP cDNA alone. Cells with a combination of p53 and TCTP demonstrated a significantly reduced number of cells containing fragmented DNA, but those with TCTP-siRNA did not (Fig. 3B).

To define the mechanism of the functional relationship between TCTP and p53-mediated apoptosis, we measured the caspase-3

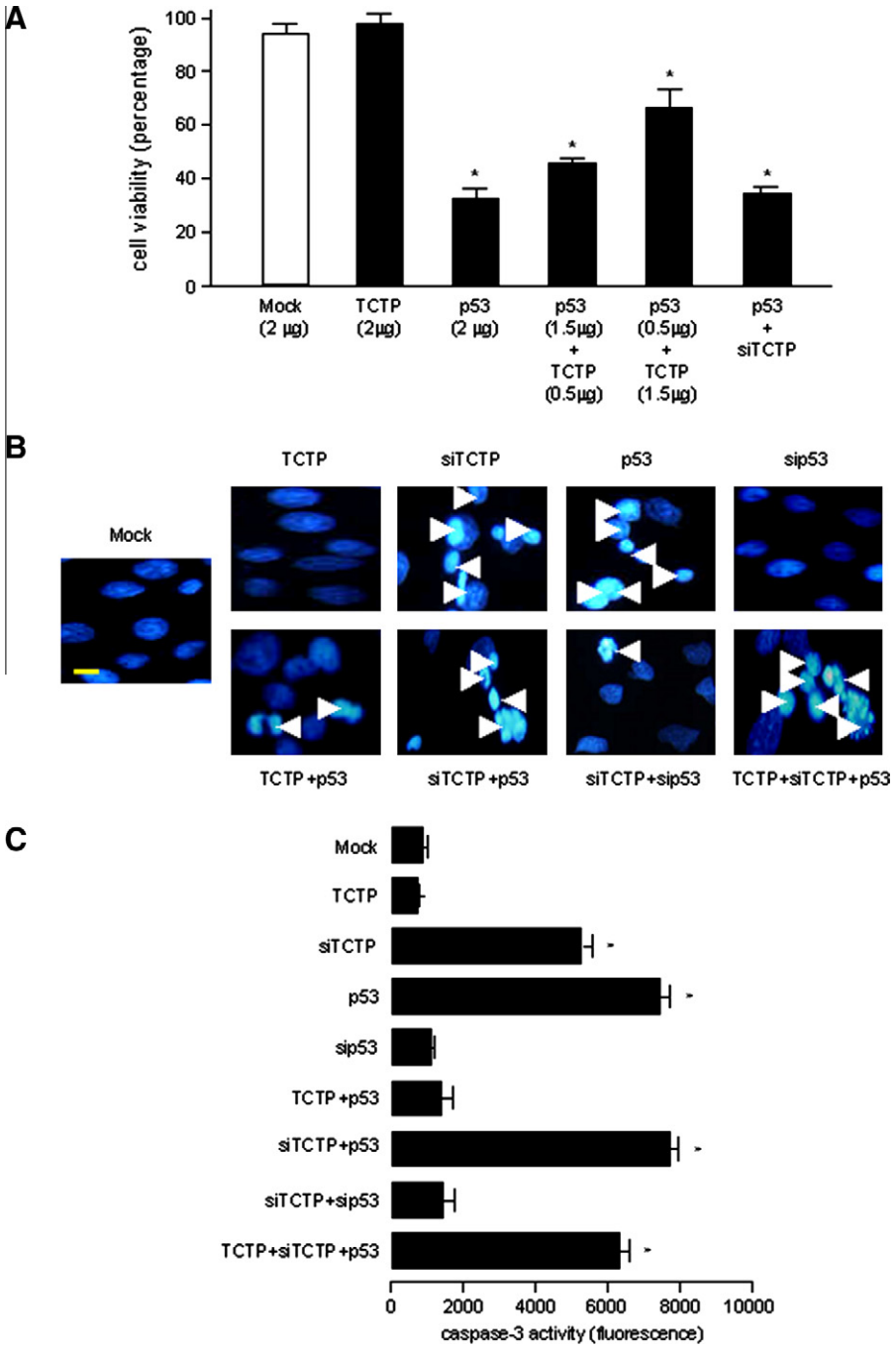

Fig. 3. TCTP inhibits p53-induced apoptosis in lung carcinoma cells. Cell viability assay (A), DAPI staining (B), and caspase-3 activity assay (C) were performed using lung carcinoma cells (A549 cells) transfected with each indicated cDNA construct. Mock means a transfectant with expression vector only (without insert). (A) Relative rates of cell proliferation were determined by MTT assay. Amounts of MTT-formazan were determined by measuring absorbance at 540 nm, and sample absorbances were converted to relative proliferation rates. Data are presented as means ± SEM. (B) Cells were stained with DAPI to visualize DNA fragmentations for assaying apoptosis. Arrows indicate observed DNA fragmentations. (C) Caspase-3 activity was measured using a Spectramax 340 microplate reader in fluorescence mode 400 nm (excitation) and 505 nm (emission) according to the manufacturer's protocol.

activity of those transfectants (see detailed Materials and methods in Supplementary data). Although TCTP is involved in cell proliferation, no significant changes in caspase-3 activity were observed in the transfectants of TCTP cDNA alone when compared to mock. However, a significant upregulation of caspase-3 activity was observed in cells transfected with p53 cDNA alone versus the Mock transfected cells. Strikingly, TCTP plus p53 overexpressed cells restored caspase-3 activity almost similarly to those of Mock and TCTP transfected cells (Fig. 3C). These results clearly showed that TCTP inhibits p53 mediated apoptosis in human lung carcinoma cells.

3.4. TCTP inhibits p53-mediated cell death via the destabilization of p53

To define the mechanism by which TCTP inhibits p53 mediated cell death, we assessed the stability of the p53 protein in the presence or absence of TCTP in A549 cells. In brief, mock and siTCTP

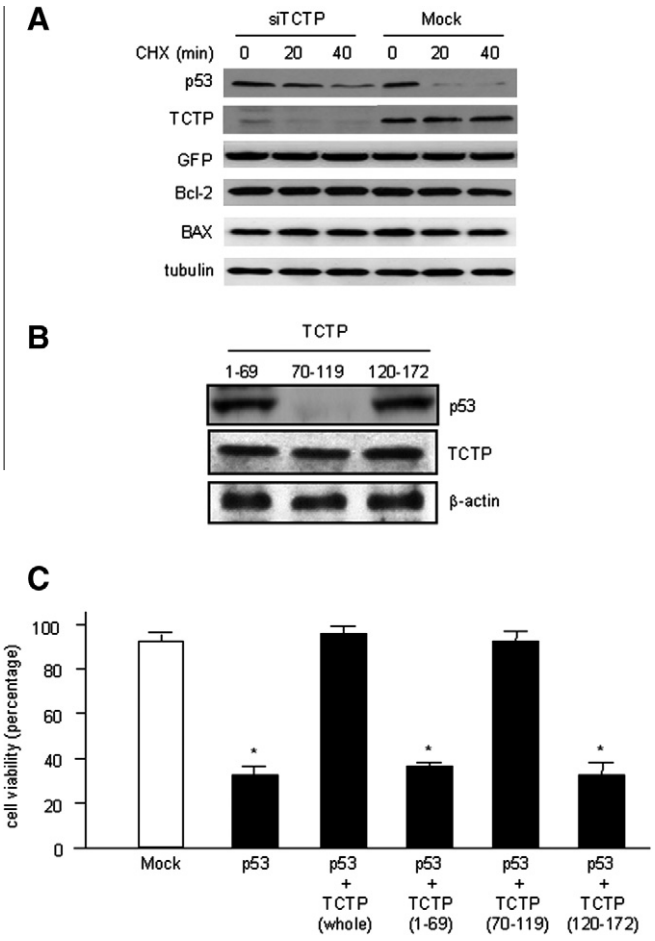

Fig. 4. TCTP decreases the half life of p53 protein. (A) siTCTP or Mock transfectants were treated with cycloheximide for various times as indicated before total cell lysates were prepared. Levels of p53, other Bcl-2 family members, TCTP and tubulin (served as a protein loading control) in the cell lysates were then analyzed by western blot with specific antibodies as indicated. (B) A549 cells were co-transfected p53 cDNA with the each pEGFP-TCTP mutant (Met^{1}-Gly^{69}, Val^{70}-Ala^{119}, Glu^{120}-Cys^{172}). The cells were treated with cycloheximide. Fourty minutes after cycloheximide treatment, the whole cell lysates were prepared and analyzed by western blot with specific antibodies p53, TCTP or β-actin to measure the amounts of p53 protein remaining at the respective times. (C) A549 cells were transfected with Mock or p53, or co-transfected p53 cDNA with the each pEGFP-TCTP mutant (Met^{1}-Gly^{69}, Val^{70}-Ala^{119}, Glu^{120}-Cys^{172}). Three days after transfection, the cells were stained with trypan blue and quantitated to analyze cell viability.

transfectant were treated with cycloheximide. Then, the cell lysates were harvested at 0, 20, and 40 min after treatment, and immunoblotted with anti-p53 or anti-TCTP antibody. As shown in Fig. 4A, the level of p53 protein evidenced a significant reduction in a time-dependent manner in the presence of TCTP. Conversely, the level of p53 protein revealed slightly decrease for over 40 min in the absence of TCTP. In contrast, the protein levels of two other TCTP non-interacting Bcl-2 family proteins (Bcl-2 and BAX), and α-tubulin remained rather constant both in the control and the TCTP knockdown lines. Taken together, this result indicated that the instability of p53 is indeed specifically influenced by the cellular levels of TCTP via the physiologically interactions between TCTP and p53. This result was further supported by the protein level of p53 is much increased in siTCTP transfectant than that of mock transfectant (Fig. S2; Supplementary Fig. 2). Thus, TCTP may influence on the half life of p53 protein or stability of p53 protein. To confirm this results, we co-transfected p53 cDNA with the each TCTP mutant (Met^{1}-Gly^{69}, Val^{70}-Ala^{119}, Glu^{120}-Cys^{172}) and performed p53 stabilization assay and cell viability test using these co-transfectants. For p53 stabilization assay, we harvested cell lysates at 40 min after cycloheximide treatment, and immunoblotted with anti-p53 or anti-TCTP antibody. As shown in Fig. 4B, the level of p53 protein was significant decreased in co-transfectant containing p53 and one deletion TCTP mutant (Val^{70}-Ala^{119}). Also, cell viability in co-transfectant containing p53 and one deletion TCTP mutant (Val^{70}-Ala^{119}) was recovered, compared to transfectant containing p53 cDNA alone (Fig. 4C). However, neither the level of p53 protein nor cell viability was changed in co-transfectants containing p53 and the other deletion mutant (Met^{1}-Gly^{69} and Glu^{120}-Cys^{172}). These results clearly showed that the interaction between p53 and TCTP accelerates the destabilization of p53 in human lung carcinoma cells.

4. Discussion

The first molecular function of TCTP to be described was a growth-related protein, and the importance of the biological functions of TCTP have been extended to include cellular protein interaction, regulation of cell cycle, and protein stabilization in cancer [21,22]. In addition, Chen et al., [23] demonstrated that homozygous mutants ($TCTP^{-/-}$) and control mouse embryonic fibroblasts manifested similar proliferation activities to various death stimuli. However, the underlying anti-apoptotic mechanisms of mediated by TCTP still remain largely unknown.

To define the detailed functional role of TCTP during apoptosis, we used yeast two-hybrid screening to identify novel TCTP-interacting proteins. Tumor suppressor protein p53, whose interactions were confirmed, was one of the interacting proteins found through a variety approaches. Furthermore, we provide genetic and biochemical data to reveal that TCTP may regulate p53 activity by affecting p53 protein stability. We also test whether TCTP regulate p53 transcriptional activity. Result indicated that TCTP does not inhibit p53 transcriptional activity as shown in Fig. S3 (Supplementary Fig. 3).

To test whether TCTP expression affects p53-mediated apoptosis in response to DNA damaging agents, we irradiated both siTCTP transfectant and mock transfectant with UV and measured the expression pattern of p53 protein. As shown in Fig. S4 (Supplementary Fig. 4), TCTP expression affects p53 protein level in both unirradiated and UV irradiated conditions. Thus, we believed that TCTP expression affects p53-mediated apoptosis in response to DNA damaging agents.

Protein stability is regulated, in normal as well as malignant cells, by ubiquitin-dependent proteolysis [17]. In addition, p53 is reported primarily at the level of protein stability by its interacting partner, Mdm2. Mdm2-deficient mice die during early embryogenesis [18]. This early lethal phenotype can be suppressed completely by simultaneous inactivation of p53, suggesting that the main function of Hdm2 in vivo is to regulate p53 [19]. Also, previous studies have shown that p53 stability can be regulated by various post-translational modifications such as acetylation and phosphorylation [24,25].

In this study, we showed evidence that the interaction between p53 and TCTP accelerates the destabilization of p53 in human lung carcinoma cells. The destabilization of one pro-apoptotic protein by another anti-apoptotic protein has not been reported in literature and calls for further investigation. Also, these results may indicate that a dynamic equilibrium between pools of p53 and p53 interacting protein such as TCTP regulate apoptotic homeostasis. Further detailed characterizations of the gene expression pattern and biochemical function of the TCTP in human malignancies may provide us with a better understanding of the precise mechanism that underlies lung tumorigenesis.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2010.11.014.

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