Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 348 (2006) 132–138
BBRC
www.elsevier.com/locate/ybbrc

A novel p53-binding domain in CUL7

Jocelyn S. Kasper [a,c], Takehiro Arai [a,b,c], James A. DeCaprio [a,b,c,*]

[a] Department of Medical Oncology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA
[b] Department of Medicine, Brigham and Women's Hospital, USA
[c] Harvard Medical School, Boston, MA, USA

Received 30 June 2006
Available online 13 July 2006

Abstract

CUL7 is a member of the cullin RING ligase family and forms an SCF-like complex with SKP1 and FBXW8. CUL7 is required for normal mouse embryonic development and cellular proliferation, and is highly homologous to PARC, a p53-associated, parkin-like cytoplasmic protein. We determined that CUL7, in a manner similar to PARC, can bind directly to p53 but does not affect p53 expression. We identified a discrete, co-linear domain in CUL7 that is conserved in PARC and HERC2, and is necessary and sufficient for p53-binding. The presence of p53 stabilized expression of this domain and we demonstrate that this p53-binding domain of CUL7 contributes to the cytoplasmic localization of CUL7. The results support the model that p53 plays a role in regulation of CUL7 activity.
© 2006 Elsevier Inc. All rights reserved.

Keywords: Cullin RING ligase; SKP-cullin-F box; CUL7; p53; PARC; Ubiquitin-protein ligases; Sequence homology; Tumor suppressors

The p53 tumor suppressor responds to cellular stress by inducing apoptosis, senescence, and growth arrest. The importance of p53 in maintaining the health of a cell is underscored by the observation of mutations in *TP53* that have been identified in more than half of human tumors [1]. Regulation of stress responses by p53 is primarily mediated through its action as a transcription factor [2–4]. The predominant mechanisms controlling p53 function are regulation of its protein stability, subcellular localization, and post-translational modification [5,6]. Ubiquitin-mediated proteasomal targeting of p53 serves to regulate p53 protein levels.

Ubiquitination involves a cascade of enzymes beginning with activation of ubiquitin by the E1 ubiquitin-activating enzyme. Following activation, ubiquitin is transferred first to an E2 ubiquitin conjugating enzyme and then to an E3 ubiquitin ligase. E3 ligases serve to recruit specific substrates for subsequent poly-ubiquitination. The covalent attachment of polyubiquitin chains to substrate proteins targets them for degradation by the 26S proteasome [7].

Two classes of E3 ubiquitin ligases, HECT and RING, have been identified. The HECT ubiquitin ligases (homologous-to-E6AP-carboxy-terminus) form a thioester intermediate with ubiquitin prior to transfer to the target protein [8]. RING-type ubiquitin ligases use a zinc-binding RING motif protein to recruit and direct the E2 enzyme to substrates [9]. A RING E3 may either contain the motif in the same polypeptide as the substrate-binding domain or as a separate subunit in a larger protein complex. MDM2 is a RING domain containing E3 that binds p53 directly and modulates p53 activity through nuclear export and degradation [5,10–12]. The ability of p53 to be exported is dependent on the E3 ubiquitin ligase activity of MDM2 [10,13,14].

The cullin RING ligases (CRL) are an example of a multi-subunit E3 with RBX1 as the RING domain containing subunit. Based on structural analysis of CRL complexes [9], cullins serve as the core scaffolding proteins linking the RBX1 subunit to the substrate specificity factor. CUL1 and CUL7 form an E3 protein complex with SKP1 as an adaptor that recruits an F-box containing protein that confers substrate specificity. There are nearly 70 mammalian F-box proteins that facilitate the substrate

* Corresponding author. Fax: +1 617 632 4760.
E-mail address: james_decaprio@dfci.harvard.edu (J.A. DeCaprio).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.07.013

recognition by the SKP1-cullin-F box (SCF) complex. The F-box proteins SKP2, FBXW7, and BTRC (β-TRCP) target numerous diverse protein substrates for degradation by CUL1 [15].

CUL7 forms an SCF-like complex with FBXW8, a WD40 containing F-box protein. Although a specific substrate protein for FBXW8 has not been identified, CUL7 recruits RBX1 to form an SCF-like E3 ubiquitin ligase complex [16,17]. CUL7 was initially identified as a simian virus 40 (SV40) large tumor antigen (T Ag)-interacting protein [18,19] and was later demonstrated to be involved in T Ag-mediated cellular transformation [20,21].

Cytoplasmic p53 has been reported to be anchored in the cytoplasm by PARC (p53-associated protein PARkin-like, cytoplasmic) [22]. Our laboratory demonstrated that CUL7 and PARC can bind to each other [23]. Notably, CUL7 is highly homologous to PARC and shares significant homology not only in the C-terminal cullin homology domain but also two domains in the N-terminus [17,23]. CUL7 and PARC contain a DOC domain that was originally identified in budding yeast DOC1, an essential subunit of the anaphase promoting complex/cyclosome (APC/C) [24]. In addition, CUL7 and PARC share regions of homology with HERC2, a putative HECT E3 ligase [16].

Given the similarity between CUL7 and PARC, we investigated the potential interaction between CUL7 and p53. The data presented here demonstrate that CUL7 directly interacts with the tumor suppressor p53. We identified a domain within CUL7 that is necessary and sufficient for binding p53 and found that this domain of CUL7 also contributes to the cytoplasmic localization of CUL7.

Materials and methods

Cells. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal Clone-I serum (HyClone), 100 U of penicillin per milliliter, and 100 μg of streptomycin per milliliter. The human osteosarcoma U-2 OS and Saos-2 were obtained from the American Type Culture Collection. Primary mouse embryonic fibroblasts (MEFs) from 12.5-day embryos from *Trp53* knockout (−/−), *Cul7* knockout (−/−), and wild type littermates were prepared as previously described [25,26].

Transient transfection of U-2 OS and Saos-2 cells was performed with Lipofectamine Plus Reagent (Invitrogen) according to the manufacturer's protocol. MG132 was used as a proteasome inhibitor (25 μM, Boston Biochem). NE-PER was used for separation of the nuclear and cytoplasmic fractions as per protocol (Pierce).

Immunoprecipitations, Western blots, and antibodies. Immunoprecipitations and Western blots were performed as previously described [27,28]. For immunoprecipitation and Western blot analysis, the following antibodies were used: anti-CUL7, SA12 [26], and PAb-653 (Bethyl Laboratories); anti-HA, HA-11 (Covance); anti-p53, DO1, and 122 (Neomarkers); anti-T7 (Novagen); and anti-vinculin, V9131 (Sigma). Purified, non-specific rabbit IgG (Bethyl) was used as a control for immunoprecipitations. Cross-linking of antibodies to protein A–Sepharose beads was performed as previously described [27].

Expression and purification of GST-CUL7 proteins. A fusion protein of GST and the N-terminal 300 and 438 residues of human CUL7 was expressed from the pGEX-4T-3 vector (Amersham Biosciences). The GST-CUL7 fusion proteins were prepared from *Escherichia coli* Rosetta cells induced for 18 h at 16 °C with 0.4 mM isopropyl-β-D-thiogalactopyranoside. The bacteria were pelleted, resuspended in a solution of 20 mM Tris, pH 8.0, 1.5% Sakcosyl, 100 mM NaCl, and 1 mM EDTA, and lysed by sonication. After removal of the debris by centrifugation, Triton X-100 was added to the solution (to a final concentration of 5%) [29]. The fusion protein was adsorbed onto glutathione–Sepharose 4B beads (Amersham Biosciences) and washed three times with NET-N (20 mM Tris, pH 8, 100 mM NaCl, 1 mM EDTA, and 0.5% NP-40).

Pull-down assay. Wild type p53 protein was cloned into HA-tagged pcDNA3.0 vector and expressed in vitro, using T7 polymerase and the TNT coupled reticulocyte lysate system (Promega) in the presence of [^{35}S]methionine. One microgram of GST-CUL7 protein was incubated at 4 °C with in vitro translated p53 in 500 μl of NET-N for 2 h. Complexes were washed three times, resuspended in SDS sample buffer, and fractionated by 10% SDS–PAGE.

siRNA. The RNAi-mediated ablation was performed using a 21-nucleotide siRNA corresponding to Cul7 mRNA (AAGAUACUCCAACU UCUACAA) and p53 mRNA (AACUACUUCCUGAAAACAACG) (Dharmacon). SiRNA oligo to luciferase was used as a control. RNAi transfections were performed using Oligofectamine Reagent (Invitrogen). Cells were transfected using the manufacturer's protocol (Invitrogen). After 48–72 h, cells were harvested for the Western blot analysis.

Clones. HA- or T7-tagged human CUL7 and p53 constructs were PCR-amplified from full-length cDNAs or generated by QuikChange Site-Directed Mutagenesis Kit (Stratagene). The sequence of fragments generated by PCR was verified by sequencing.

Results

CUL7 specifically associates with p53

As depicted in Fig. 1A, CUL7 and PARC contain a cullin homology domain. In addition, CUL7 and PARC contain a DOC domain, similar to DOC1 or APC10, an essential subunit of the APC/C [30]. In addition, CUL7 and PARC contain a region homologous to HERC2, a large protein with a HECT domain that may also serve as an E3 ubiquitin ligase [16,31,32]. Residues 358–437 of human CUL7 are 85% identical and 91% similar to residues 366–441 of human PARC and 37% identical and 61% similar to residues 2548–2630 of HERC2. Based on the strong homology among CUL7, PARC, and HERC2, this region has been designated the CPH domain. Extensive genomic database searches failed to identify any other proteins in the mammalian genomes with the CPH domain. Notably, CUL7, PARC, and HERC2 share both the CPH and DOC domains. Homology over shared regions between CUL7 and PARC is approximately 60% identical and 70% similar [23].

Gu and colleagues reported an association between the N-terminal 700 residues of PARC and the C-terminus of p53 [22]. Based on the high level of homology between PARC and CUL7, we investigated the possibility of an association between CUL7 and p53. Saos-2 ($TP53^{-/-}$) cells were transiently transfected with plasmids expressing HA-PARC or HA-CUL7 and full-length p53 or the C-terminus of p53, T7-290-394. Lysates were immunoprecipitated with an anti-HA antibody followed by Western blots for HA, p53, and T7. As shown in Fig. 1B, HA-CUL7 could co-precipitate both full-length p53 and the C-terminal fragment of p53. We also confirmed that HA-PARC was capable of co-precipitating T7-p53-290-394, as previously reported [22].

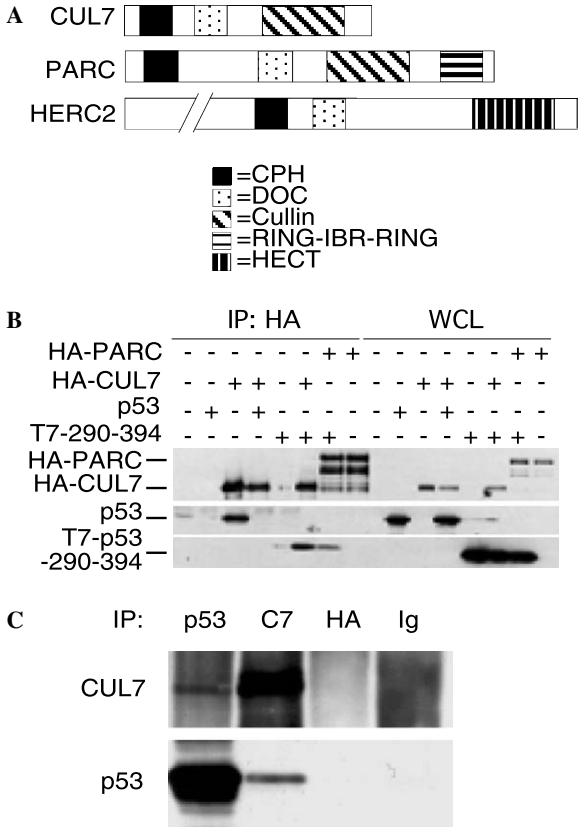

Fig. 1. The C-terminus of p53 associates with CUL7. (A) CUL7 shares regions of homology with PARC, HERC2, and APC10. CUL7 and HERC2 share 37/65 (% identity/% similarity) in the CPH domain (black) and 41/66 in the DOC domain (black dots). PARC and HERC2 share 31/48 in the CPH domain and 40/64 in the DOC domain. Overall identity in regions of homology between CUL7 and PARC is 60%. (B) Saos-2 cells were transiently transfected with HA-PARC or HA-CUL7 and p53 or T7-p53-290-394. Lysates were immunoprecipitated for HA and Western blots of immunoprecipitated samples and whole cell lysates (WCL) for HA (top panel), p53 (Ab-DO1, middle panel), and T7 (bottom panel) were performed. (C) Lysates from U-2 OS cells were immunoprecipitated for CUL7 (Ab-653) and p53 (Ab-DO1), and an anti-HA monoclonal antibody and rabbit polyclonal IgG served as negative controls. Samples were separated by SDS–PAGE and Western blotted for CUL7 (Ab-653) and p53 (Ab-DO1).

To determine if endogenous CUL7 and p53 could bind to each other, U-2 OS ($TP53^{+/+}$) cell lysates were immunoprecipitated for CUL7 followed by Western blot for p53. Fig. 1C (lane 1) reveals that in a p53 immunoprecipitation, CUL7 was co-precipitated. Conversely, an immunoprecipitation for endogenous CUL7 revealed co-precipitation of p53 (Fig. 1C, lane 2). The specificity of the interaction was confirmed with the use of antibodies specific to three different epitopes in CUL7 and two different epitopes in p53 (data not shown).

Residues 268–438 of CUL7 are necessary for p53 binding

To further define the interaction between CUL7 and p53, we mapped the domain of CUL7 that associates with p53. HA epitope-tagged, full-length CUL7 (HA-CUL7) and various truncation mutants of CUL7 were transfected into U-2 OS cells. Immunoprecipitation with an HA antibody followed by Western blotting revealed that all CUL7 constructs were expressed well (Fig. 2A, upper left panel). When the HA immunoprecipitation was blotted for p53, only full-length CUL7, HA-1-438, HA-1-796, and HA-268-1698 were capable of co-precipitating p53

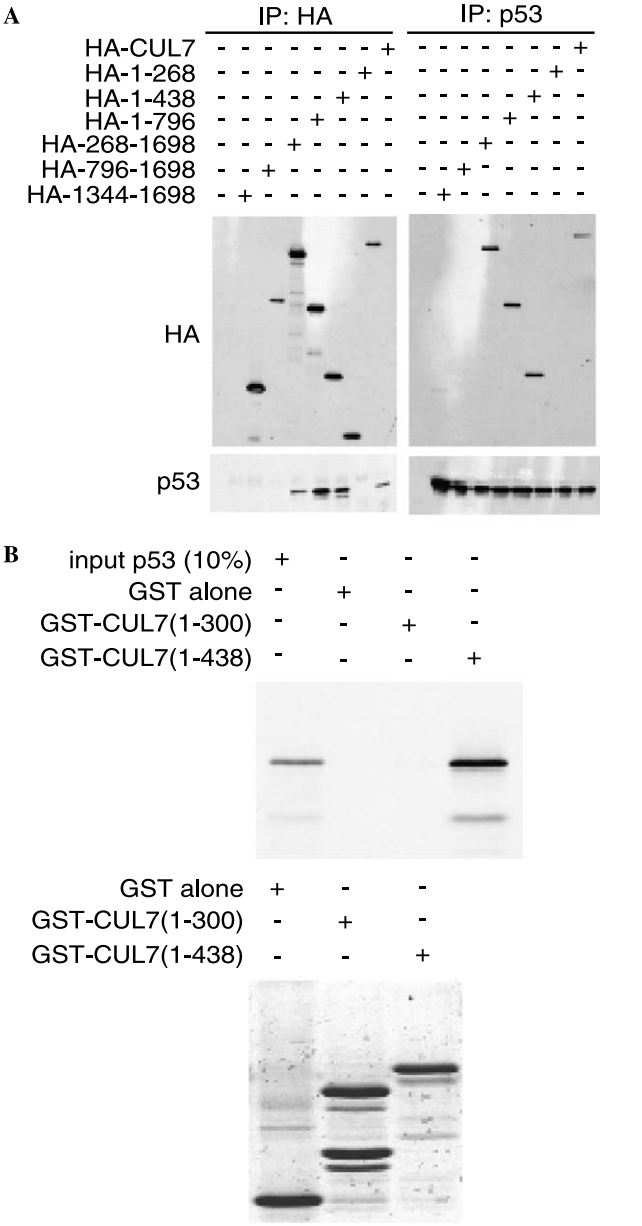

Fig. 2. CUL7's CPH domain is necessary and sufficient for association with p53. (A) U-2 OS cells were transiently transfected with HA-tagged CUL7 (full-length, 1–1698) and HA-tagged CUL7 truncations (1–268, 1–438, 1–796, 268–1698, 796–1698, and 1344–1698). Lysates were immunoprecipitated and Western blotted for HA and p53 (Ab-DO1). (B) (Top panel) GST alone or GST-CUL7(1–300) or GST-CUL7(1–438) was assessed for their binding ability to in vitro translated p53 by GST pull-down. Each lane contains in vitro translated p53 and equal amounts of recombinant proteins, as determined by Coomassie-stained gel shown in bottom panel. (Bottom panel) Expression of bacterially produced CUL7 mutant proteins on the Coomassie-stained gel.

(Fig. 2A, lower left panel). Conversely, immunoprecipitation for p53 revealed co-precipitation of full-length HA-CUL7 as well as the same three CUL7 constructs (Fig. 2A, right panels). In contrast, the CUL7 constructs HA-1-268, HA-796-1698, and HA-1344-1698 were unable to bind to p53. These co-precipitation experiments revealed a minimum region of CUL7 that included residues 268–438 that was required for binding to p53. The CPH homology domain is located entirely within these residues. Notably, the N-terminal construct of PARC (residues 1–770) reported to be required for association with p53 also contains the CPH domain [22].

GST-CUL7(1–438) interacts with in vitro translated p53

To confirm the physical association between p53 and the N-terminal 438 residues of CUL7, we assayed for the interaction in vitro. Recombinant GST-CUL7 containing the N-terminal 438 residues (1–438), but not the N-terminal 300 residues (1–300) or GST alone bound to in vitro translated p53 (Fig. 2B, top panel). The GST-fusion proteins were similarly expressed (bottom panel).

The CUL7 CPH domain is necessary and sufficient for p53 binding

Deletion analysis of CUL7 revealed that residues 268–438 were required for binding to p53 (Fig. 2A). To further define the p53-binding domain within CUL7, an in-frame CUL7 deletion construct was generated, ΔCPH3 deleted residues 407–437. U-2 OS cells were transiently transfected with full-length HA-tagged CUL7 or ΔCPH3 followed by immunoprecipitation and Western blot for HA and p53. While full-length HA-CUL7 was able to co-precipitate p53, HA-ΔCPH3 failed to co-precipitate endogenous p53 (Fig. 3A, lanes 1 and 2). Similar assays showed that full-length CUL7 with in-frame deletion of residues 369–385, and consistent with data reported by Xiong and colleagues, residues 386–406 disrupted CUL7's ability to co-precipitate p53 (data not shown) [33].

To determine if the CPH domain was sufficient for association with p53, we generated several plasmids expressing HA-tagged fragments of CUL7 including residues 331–437 (HA-CPH2) and 357–437 (HA-CPH3). HA-CPH2 and HA-CPH3 were transiently transfected into U-2 OS cells and immunoprecipitation with an HA-antibody followed by Western blotting revealed that both HA-CPH2 and HA-CPH3 were capable of co-precipitating p53 (Fig. 3A, lanes 3 and 4). Notably, both HA-CPH2 and HA-CPH3 contain the CPH homology domain indicating that the CUL7 CPH domain is necessary and sufficient for binding to p53.

p53 stabilizes expression of the CUL7 CPH domain

While mapping the binding domains on CUL7 and p53, it became apparent that expression of the CPH

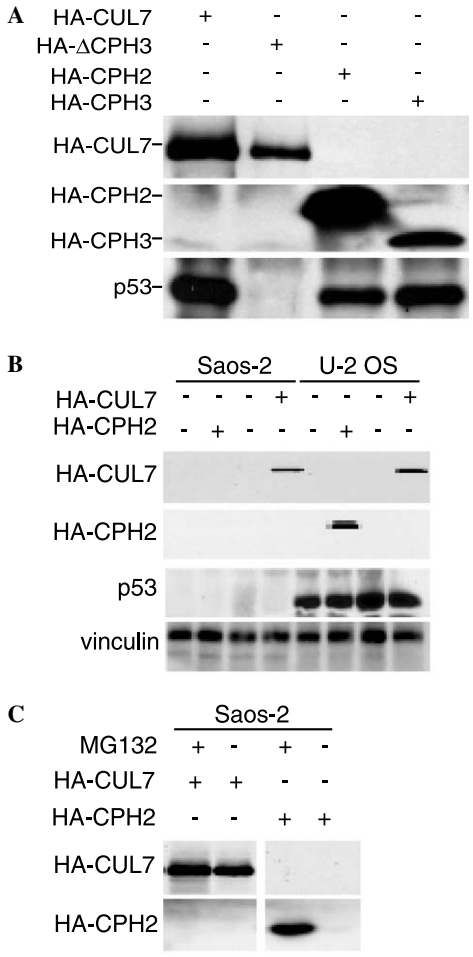

Fig. 3. CUL7's p53-binding CPH domain is unstable in the absence of p53. (A) U-2 OS cells were transiently transfected with a HA-tagged full-length, a deletion or a fragment of CUL7. Lysates were immunoprecipitated for HA and p53 and Western blotted for HA and p53. (B) U-2 OS cells ($TP53^{+/+}$) and Saos-2 cells ($TP53^{-/-}$) were transiently transfected with HA-CUL7 or HA-CUL7-331-437 ("HA-CPH2"). Lysates were made and Western blots were performed for HA, p53 (Ab-DO1), and vinculin as a loading control. (C) Inhibition of the proteasome enables expression of CPH2 in Saos-2 cells. Saos-2 cells or U-2 OS cells were transiently transfected with HA-CUL7 or HA-CPH2. Twenty four hours later, cells were treated with 25 μM MG132 or DMSO control. Twelve hours after treatment with MG132, lysates were made and Western blots for HA and p53 (Ab-DO1) were performed.

domain-containing constructs, HA-CPH2 and HA-CPH3, was barely detected in cells lacking p53. To determine if the presence of p53 influenced their expression, full-length HA-CUL7 and HA-CPH2 were transiently transfected into U-2 OS cells ($TP53^{+/+}$) and Saos-2 cells ($TP53^{-/-}$). As shown in Fig. 3B, full-length HA-CUL7 was expressed well in both cell lines. In contrast, HA-CPH2 was readily detected in U-2 OS cells but not in Saos-2 cells. Co-transfection of the CUL7 constructs with a GFP reporter construct demonstrated similar transfection efficiencies with all constructs in both cell lines (data not shown).

To determine if the reduced expression of the CPH domain in Saos-2 cells was due to protein instability,

transfection of HA-CUL7 or HA-CPH2 into Saos-2 cells was repeated and followed by treatment with the proteasome inhibitor MG132 or with DMSO to serve as a control. Western blotting revealed that HA-CPH2 expression in Saos-2 cells was significantly increased in the presence of MG132 (Fig. 3C). In contrast, expression of full-length HA-CUL7 was not appreciably affected by MG132. These data suggest that in the absence of p53, the CPH fragment was degraded in a proteasome-dependent manner.

To confirm that the reduced expression of HA-CPH2 was dependent on the presence of p53, U-2 OS cells were transfected with an siRNA oligonucleotide directed against p53 and either full-length HA-CUL7 or HA-CPH2. Transfection of the p53 siRNA oligo resulted in a significant reduction of p53 expression (Fig. 4A, third panel) while transfection with a control siRNA against luciferase had no effect on p53 levels. Expression levels of HA-CUL7 were not affected by the knockdown of p53 (Fig. 4A, top panel). In contrast, expression of HA-CPH2 was decreased in cells transfected with the p53 siRNA compared to the luciferase control (Fig. 4A, second panel). These results are consistent with the previous experiment and indicate that the expression of the CPH domain was dependent on the presence of p53.

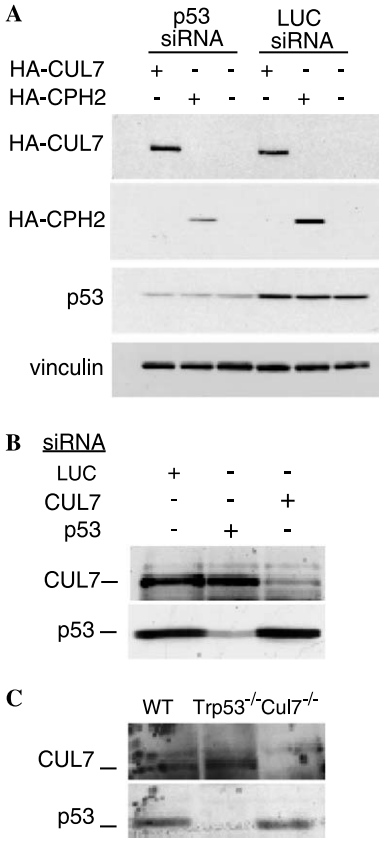

Fig. 4. Expression of CUL7 and p53 is not affected by each other. (A) U-2 OS cells were transiently transfected with siRNA oligos to p53 and luciferase (LUC). Sixteen hours later they were transfected with HA-CUL7 or HA-CPH2. Forty eight hours following the second transfection, lysates were harvested and Western blots were performed for HA, p53 (Ab-DO1), and vinculin as a loading control. (B) U-2 OS cells were transiently transfected with siRNA oligo to luciferase, CUL7 or p53. Seventy two hours later lysates were harvested and Western blotted for CUL7 (Ab-653) and p53 (Ab-DO1). (C) Lysates from WT, *Cul7*$^{-/-}$, and *Trp53*$^{-/-}$ MEFs were harvested and Western blotted for CUL7 (Ab-SA12) and p53 (Ab-122).

Given the observations that HA-CPH2 expression was decreased in the absence of p53, it was of interest to determine if the endogenous cellular CUL7 could be affected by the level of p53 expression. Using siRNA oligos to knock down p53 in U-2 OS cells, Western blots revealed similar CUL7 levels in the p53 knocked down cells relative to the luciferase control (Fig. 4B). Conversely, when CUL7 was knocked down by siRNA, p53 protein levels were unaffected.

We also addressed the question of whether expression of CUL7 and p53 was affected by the absence of each other in primary mouse embryo fibroblasts (MEFs) prepared from wild type (WT), *Cul7*$^{-/-}$, and *Trp53*$^{-/-}$ embryos. As shown in Fig. 4C, Western blots of cell lysates prepared from WT, *Cul7*$^{-/-}$, and *Trp53*$^{-/-}$ MEFs revealed that the expression levels of p53 were similar in WT and *Cul7*$^{-/-}$ MEF lysates indicating that steady-state levels of p53 were not significantly altered in the absence of CUL7. Conversely, CUL7 levels were similar in WT and *Trp53*$^{-/-}$ MEFs, suggesting that steady-state levels of full-length CUL7 were not sensitive to loss of p53. The observations presented in Figs. 3 and 4 indicate that the steady-state levels of full-length CUL7 and p53 were not dependent on the presence of each other. However, it did appear that the stability of the CUL7 CPH domain itself was dependent on the presence of p53.

The CUL7 CPH domain plays a role in cytoplasmic localization of CUL7

It was reported by Nikoleav et al. that the CUL7 homolog, PARC, anchored p53 in the cytoplasm [22]. However, data from our laboratory indicated that the subcellular localization of p53 was not affected in fibroblasts with the targeted deletion of *Parc* or *Cul7* [23] (and unpublished data). To determine if the converse was true, we examined if p53 binding affected the cellular localization of CUL7. U-2 OS cells were transiently transfected with HA-tagged full-length CUL7, HA-ΔCPH3 (deleting residues 407–437), or HA-CPH2 (containing only residues 331–437). Nuclear and cytoplasmic extracts were prepared from the transfected cells (Fig. 5). We observed that full-length HA-CUL7 was predominantly cytoplasmic. We also observed that endogenous CUL7 was also largely cytoplasmic (data not shown). In contrast, HA-ΔCPH3 was present in both nuclear and cytoplasmic fractions. Conversely, the small HA-CPH2 fragment was predominantly contained in the cytoplasmic fragment (Fig. 5). Under these conditions, p53 was primarily located in the nucleus and appeared unaffected by expression of full-length or truncated CUL7 [34].

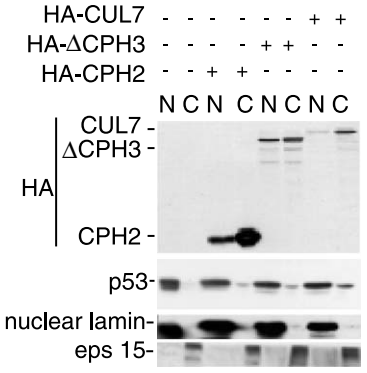

Fig. 5. CUL7's CPH domain is required for cytoplasmic localization of CUL7. U-2 OS cells were transiently transfected with HA-CUL7, HA-ΔCPH3 (deletion of residues 407–437) or HA-CPH2 (CUL7 residues 331–437). Forty eight hours later lysates were harvested and nuclear and cytoplasmic fractions were prepared. Western blots for Lamin A/C and EPS15 were used to control for nuclear and cytoplasmic extraction [38].

Discussion

The results presented describe a direct interaction between the tumor suppressor p53 and CUL7, an SCF-like E3 ubiquitin ligase. We demonstrated that a small co-linear domain of CUL7 binds directly to p53 in vitro. This domain shares a significant degree of homology with PARC and HERC2 that are also likely candidate E3 ubiquitin ligases. We observed that the CPH domain of CUL7 was sufficient for p53 binding and defined a novel p53-binding motif. It was reported that an N-terminal fragment of PARC that contained the CPH domain could bind to p53 [22]. This result suggests that the CPH domain in PARC as well as in HERC2 may also bind directly to p53, although this has not yet been tested. Although CUL7, PARC, and HERC2 also contain the DOC domain, we did not observe any requirement for the DOC domain in p53 binding.

Our study of the CUL7 and p53 interaction led to the observation that expression of the CUL7 CPH domain was unstable in the absence of p53. These data indirectly support the model that p53 directly binds to CUL7 and may regulate CUL7 function. In contrast, we do not observe any evidence that CUL7 promotes the ubiquitination of p53. Moreover, given that p53 levels were unaffected by the presence or absence of CUL7, it is unlikely that CUL7 serves as a major regulator of p53 stability. In this regard, we have no evidence that the stability or ubiquitination of p53 is dependent on CUL7.

We observed that the CPH domain contributes to its cytoplasmic localization of CUL7. We reproducibly observed that expression of the CPH domain alone was located predominantly in the cytoplasm while a full-length CUL7 construct, lacking 30 residues within the CPH domain, was distributed throughout the cytoplasm and nucleus. These data indicate that p53-binding domain of CUL7 contributes to the cytoplasmic localization of CUL7. We have not determined if these two functions can be separated or if they are dependent on each other. It is not clear yet if the cytoplasmic localization of CUL7 through the CPH domain is a regulatory mechanism of CUL7 or reflection of another function of this protein.

Andrews et al. reported that deletion of the N-terminal 300 residues of p53 disrupted binding to CUL7 [33]. In contrast, we observed that deletion of the N-terminal 290 residues of p53 did not disrupt binding to CUL7. Similarly, the C-terminus of p53 was also reported to be able to bind to PARC [22]. Notably, the C-terminus of p53 contains the tetramerization domain as well as post-translational modifications including, phosphorylation, acetylation, and sumoylation [35]. We have not observed a preference for CUL7 for selective binding to post-translationally modified p53 (data not shown).

CUL7 has been reported to bind to SV40 large T antigen [18–21]. Similar to p53, CUL7 can bind to SV40 T antigen [36,37]. It should be noted that T antigen is not required for CUL7 binding to p53 since none of the cells used in this report express T antigen. Similarly, we have reported that T antigen binding to CUL7 is not dependent on p53 and p53 binding to T antigen is not dependent on CUL7 [21]. Remarkably, T antigen binds independently to CUL7 and p53 whereas CUL7 and p53 can bind directly to each other.

This report reveals that p53 binds directly to CUL7 through a novel domain that contributes to the cytoplasmic localization of CUL7. p53 does not appear to be a substrate for CUL7 E3 ubiquitin ligase activity. In contrast, we present evidence that p53 affects at least one aspect of CUL7 protein stability.

Acknowledgment

This work was supported in part by Public Health Service Grant RO1 CA93804 to J.A.D.

References

[1] M. Hollstein, D. Sidransky, B. Vogelstein, C.C. Harris, p53 mutations in human cancers, Science 253 (1991) 49–53.
[2] J. Yu, L. Zhang, P.M. Hwang, C. Rago, K.W. Kinzler, B. Vogelstein, Identification and classification of p53-regulated genes, Proc. Natl. Acad. Sci. USA 96 (1999) 14517–14522.
[3] R. Zhao, K. Gish, M. Murphy, Y. Yin, D. Notterman, W.H. Hoffman, E. Tom, D.H. Mack, A.J. Levine, Analysis of p53-regulated gene expression patterns using oligonucleotide arrays, Genes Dev. 14 (2000) 981–993.
[4] W.S. el-Deiry, T. Tokino, V.E. Velculescu, D.B. Levy, R. Parsons, J.M. Trent, D. Lin, W.E. Mercer, K.W. Kinzler, B. Vogelstein, WAF1, a potential mediator of p53 tumor suppression, Cell 75 (1993) 817–825.
[5] S. Fang, J.P. Jensen, R.L. Ludwig, K.H. Vousden, A.M. Weissman, Mdm2 is a RING finger-dependent ubiquitin protein ligase for itself and p53, J. Biol. Chem. 275 (2000) 8945–8951.
[6] G.S. Jimenez, S.H. Khan, J.M. Stommel, G.M. Wahl, p53 regulation by post-translational modification and nuclear retention in response to diverse stresses, Oncogene 18 (1999) 7656–7665.
[7] P.K. Jackson, A.G. Eldridge, The SCF ubiquitin ligase: an extended look, Mol. Cell 9 (2002) 923–925.

[8] M. Scheffner, P.M. Howley, Identification of a human ubiquitin-conjugating enzyme that mediates the E6-AP-dependent ubiquitination of p53, Proc. Natl. Acad. Sci. USA 91 (1994) 8797–8801.
[9] N. Zheng, B.A. Schulman, L. Song, J.J. Miller, P.D. Jeffrey, P. Wang, C. Chu, D.M. Koepp, S.J. Elledge, M. Pagano, R.C. Conaway, J.W. Conaway, J.W. Harper, N.P. Pavletich, Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex, Nature 416 (2002) 703–709.
[10] R.K. Geyer, Z.K. Yu, C.G. Maki, The MDM2 RING-finger domain is required to promote p53 nuclear export, Nat. Cell Biol. 2 (2000) 569–573.
[11] Y. Haupt, R. Maya, A. Kazaz, M. Oren, Mdm2 promotes the rapid degradation of p53, Nature 387 (1997) 296–299.
[12] M.H. Kubbutat, S.N. Jones, K.H. Vousden, Regulation of p53 stability by Mdm2, Nature 387 (1997) 299–303.
[13] D.A. Freedman, L. Wu, A.J. Levine, Functions of the MDM2 oncoprotein, Cell. Mol. Life Sci. 55 (1999) 96–107.
[14] S.D. Boyd, K.Y. Tsai, T. Jacks, An intact HDM2 RING-finger domain is required for nuclear exclusion of p53, Nat. Cell Biol. 2 (2000) 563–568.
[15] A. Ciechanover, A.L. Schwartz, Ubiquitin-mediated proteolysis: biological regulation via destruction, Bioessays 22 (2000) 442–451.
[16] D.C. Dias, G. Dolios, R. Wang, Z.Q. Pan, CUL7: a DOC domain-containing cullin selectively binds Skp1.Fbx29 to form an SCF-like complex, Proc. Natl. Acad. Sci. USA 99 (2002) 16601–16606.
[17] T. Arai, J.S. Kasper, J.R. Skaar, S.H. Ali, C. Takahashi, J.A. DeCaprio, Targeted disruption of p185/Cul7 gene results in abnormal vascular morphogenesis, Proc. Natl. Acad. Sci. USA 100 (2003) 9855–9860.
[18] A.I. Daud, N.A. Lanson Jr., W.C. Claycomb, L.J. Field, Identification of SV40 large T-antigen-associated proteins in cardiomyocytes from transgenic mice, Am. J. Physiol. 264 (1993) H1693–H1700.
[19] D.C. Kohrman, M.J. Imperiale, Simian virus 40 large T antigen stably complexes with a 185-kilodalton host protein, J. Virol. 66 (1992) 1752–1760.
[20] S.H. Ali, J.S. Kasper, T. Arai, J.A. DeCaprio, Cul7/p185/p193 binding to simian virus 40 large T antigen has a role in cellular transformation, J. Virol. 78 (2004) 2749–2757.
[21] J.S. Kasper, H. Kuwabara, T. Arai, S.H. Ali, J.A. DeCaprio, Simian virus 40 large T antigen's association with the CUL7 SCF complex contributes to cellular transformation, J. Virol. 79 (2005) 11685–11692.
[22] A.Y. Nikolaev, M. Li, N. Puskas, J. Qin, W. Gu, Parc: a cytoplasmic anchor for p53, Cell 112 (2003) 29–40.
[23] J.R. Skaar, T. Arai, J.A. DeCaprio, Dimerization of CUL7 and PARC is not required for all CUL7 functions and mouse development, Mol. Cell Biol. 25 (2005) 5579–5589.
[24] R. Grossberger, C. Gieffers, W. Zachariae, A.V. Podtelejnikov, A. Schleiffer, K. Nasmyth, M. Mann, J.M. Peters, Characterization of the DOC1/APC10 subunit of the yeast and the human anaphase-promoting complex, J. Biol. Chem. 274 (1999) 14500–14507.
[25] J. Zalvide, J.A. DeCaprio, Role of pRb-related proteins in simian virus 40 large-T-antigen-mediated transformation, Mol. Cell Biol. 15 (1995) 5800–5810.
[26] T. Arai, J.R. Skaar, S.H. Ali, C. Takahashi, J.A. DeCaprio, Targeted disruption of p185/Cul7 gene results in abnormal vascular morphogenesis, Proc. Natl. Acad. Sci. USA 100 (2003) 9855–9860.
[27] D.L. Poulin, J.A. DeCaprio, p53 targets simian virus 40 large T antigen for acetylation by CBP, J. Virol. 78 (2004) 8245–8253.
[28] H. Stubdal, J. Zalvide, J.A. DeCaprio, Simian virus 40 large T antigen alters the phosphorylation state of the RB-related proteins p130 and p107, J. Virol. 70 (1996) 2781–2788.
[29] J.V. Frangioni, B.G. Neel, Solubilization and purification of enzymatically active glutathione S-transferase (pGEX) fusion proteins, Anal. Biochem. 210 (1993) 179–187.
[30] K. Kominami, H. Seth-Smith, T. Toda, Apc10 and Ste9/Srw1, two regulators of the APC-cyclosome, as well as the CDK inhibitor Rum1 are required for G1 cell-cycle arrest in fission yeast, Embo. J. 17 (1998) 5388–5399.
[31] Y. Ji, N.A. Rebert, J.M. Joslin, M.J. Higgins, R.A. Schultz, R.D. Nicholls, Structure of the highly conserved HERC2 gene and of multiple partially duplicated paralogs in human, Genome Res. 10 (2000) 319–329.
[32] K. Hochrainer, H. Mayer, U. Baranyi, B. Binder, J. Lipp, R. Kroismayr, The human HERC family of ubiquitin ligases: novel members, genomic organization, expression profiling, and evolutionary aspects, Genomics 85 (2005) 153–164.
[33] P. Andrews, Y.J. He, Y. Xiong, Cytoplasmic localized ubiquitin ligase cullin 7 binds to p53 and promotes cell growth by antagonizing p53 function, Oncogene (2006).
[34] J. Gu, L. Nie, H. Kawai, Z.M. Yuan, Subcellular distribution of p53 and p73 are differentially regulated by MDM2, Cancer Res. 61 (2001) 6703–6707.
[35] A.M. Bode, Z. Dong, Post-translational modification of p53 in tumorigenesis, Nat. Rev. Cancer 4 (2004) 793–805.
[36] D.I. Linzer, A.J. Levine, Characterization of a 54 K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells, Cell 17 (1979) 43–52.
[37] D.I. Linzer, W. Maltzman, A.J. Levine, The SV40 A gene product is required for the production of a 54,000 MW cellular tumor antigen, Virology 98 (1979) 308–318.
[38] F. Tebar, T. Sorkina, A. Sorkin, M. Ericsson, T. Kirchhausen, Eps15 is a component of clathrin-coated pits and vesicles and is located at the rim of coated pits, J. Biol. Chem. 271 (1996) 28727–28730.