Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 345 (2006) 355–361
BBRC
www.elsevier.com/locate/ybbrc

MDM2 interacts with and downregulates a sarcomeric protein, TCAP ☆

Li-Feng Tian [1], Hui-Yan Li [1], Bao-Feng Jin, Xin Pan, Jiang-Hong Man, Pei-Jing Zhang, Wei-Hua Li, Bing Liang, Hui Liu, Jie Zhao, Wei-Li Gong, Tao Zhou, Xue-Min Zhang *

National Center of Biomedical Analysis, Beijing 100850, China

Received 5 April 2006
Available online 2 May 2006

Abstract

Recent reports have shown that MDM2 may attenuate hypertrophy of cardiac myocytes. However, mechanism of MDM2 involving in this process is unclear. In this study, we identified a novel specific MDM2-binding protein TCAP by the yeast two-hybrid screen. It was validated by GST pull-down and co-immunoprecipitation assays. Confocal analysis showed that MDM2 and TCAP co-localized in the nucleus, and elevated MDM2 expression could alter the subcellular localization of TCAP. Notably, MDM2 downregulated the protein level of TCAP through the proteasomal pathway, and this downregulation was inhibited by $p14^{ARF}$. In addition, our results suggested that the degradation of TCAP by MDM2 was through the ubiquitin-independent pathway. Given that TCAP is a key component involving in the cardiac hypertrophy, the degradation of TCAP by MDM2 might be connected with the roles of MDM2 in cardiac hypertrophy. Further investigation will focus on the biological significance of MDM2–TCAP interaction in cardiac hypertrophy.

Keywords: MDM2; Interaction; TCAP; $p14^{ARF}$; Degradation; Yeast two-hybrid screen; Cardiac hypertrophy

Murine double minute 2 (MDM2) protein is involved in cell growth and differentiation through its interaction with other cellular proteins. The tumor suppressor p53 is a well-known binding partner of MDM2. MDM2 inhibits p53 by interfering with transactivational activity [1], promoting nuclear export [2], and by acting as an E3 ubiquitin ligase [3,4], leading to the ubiquitination and degradation of p53. All of the above effects of MDM2 contribute to abolish efficiently the biological activities of p53, such as induction of G1 arrest and apoptosis [5–7]. Moreover, MDM2 retains its role through interacting with other proteins, such as RB, E2F/DP1, MTBP, Numb, p21, p19, and MDMX [8–15].

Recent investigations have shown that MDM2 is involved in both hypertrophy and survival of cardiomyocytes [16]. Interestingly, most survival proteins promote cardiac hypertrophy, but MDM2 has a unique combination of prosurvival and antihypertrophic effects on cardiomyocytes, which suggests that MDM2 might be a potential therapeutic target to downregulate both cell death and pathologic hypertrophy. However, the mechanism by which MDM2 inhibits apoptosis and hypertrophy in cardiomyocytes, particularly inhibits the cardiac hypertrophy, is unclear.

TCAP is a Z-disc sarcomeric protein of 19 kDa found predominantly in skeletal and cardiac muscle [17]. TCAP could be phosphorylated by titin kinase and implicated in the control of myofibrillogenesis [18]. To be noted, mutations of TCAP gene are responsible for a recessive form of limb-girdle muscular dystrophy (LGMD) 2G and also appear to have an important role in dilated and hypertrophic cardiomyopathy [19,20], which suggests that TCAP

☆ *Abbreviations:* MDM2, murine double minute 2; TCAP, titin-cap; MLP, muscle-specific LIM protein; LGMD, limb-girdle muscular dystrophies; GFP, green fluorescence protein; RFP, red fluorescence protein; DBD, DNA binding domain; AD, activation domain; GST, glutathione *S*-transferase; HA, hemagglutinin; aa, amino acid.

* Corresponding author. Fax: +86 10 68246161.
E-mail address: xmzhang@nic.bmi.ac.cn (X.-M. Zhang).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.04.108

might play a role in the regulation of striated muscle hypertrophy. Further studies show that TCAP/MLP complex is a key component of the in vivo cardiomyocyte stretch sensor machinery [21], an independent factor for inducing cardiac hypertrophy. Moreover, TCAP can interact with many other hypertrophic-relevant proteins, including Ankrd2, myostatin, potassium channel β-subunit mink, and protein kinase D [22–25]. Taken together, these studies reveal that TCAP is a highly important and multi-functional protein involved in the sarcomeric assembly of striated muscle, and also in the signaling pathway of the muscle hypertrophy.

We reported here that MDM2 interacted with TCAP in the yeast two-hybrid screen system. This association was confirmed by in vitro GST-pull down and in vivo co-immunoprecipitation assays. Confocal analysis showed that MDM2 and TCAP co-localized in the nucleus. Elevated MDM2 expression modulated the subcellular localization of TCAP and reduced the protein level of TCAP by the proteasomal pathway. Moreover, it implied that the degradation of TCAP by MDM2 was through the ubiquitin-independent proteasomal pathway. On the basis of the involvement of both MDM2 and TCAP in cardiac hypertrophy, we suggested that the degradation of TCAP by MDM2 might be connected with the roles of MDM2 in cardiac hypertrophy.

Materials and methods

Plasmids and antibodies. Human pcDNA3-HA-MDM2 was a kind gift from Dr. Scheffner, M. (University of Cologne, Germany.). Mouse pcDNA3-GFP-MDM2 was a kind gift from Dr. Pei, G. (Chinese Academy Sciences, China). pEGFP-p14^{ARF} was a kind gift from Dr. Peter, G. (London Research Institute, UK). Human MDM2 cDNA and its truncation mutants (1–160, 1–444, and 286–491 aa) were prepared by PCR amplification from the pcDNA3-HA-MDM2. These fragments were, respectively, cloned in-frame into the Gal4-DBD vector pGBKT7 (Clontech Laboratories) or pXJ40-HA mammalian expressing vectors. Human TCAP cDNA was isolated from a human mammary cDNA library by PCR amplification. The cDNA was cloned into the Gal4 activation domain vector pGADT7 (Clontech Laboratories) or pGEX-KG (Amersham-Pharmacia Biotech). For expressing in the mammalian cells, the TCAP cDNA was cloned in-frame into the pXJ40-Myc, pDsRED-N1 or pIRES-EGFP vectors (Clontech Laboratories). All constructs were verified by sequencing. Mouse monoclonal antibodies against HA, Myc, and TCAP were purchased from Santa Cruz.

Cells and transfections. 293 cells and HEK293T cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) in a 5% CO_2 atmosphere. H1299 cells were maintained routinely in RPMI-1640 medium supplemented with 10% FCS. Cell transfections were performed using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. In all transfections, the total amount of DNA was equalized. For MG132 treatment, MG132 was added into the cells for 6 h incubation after 24 h transfection.

Yeast two-hybrid screen. A DNA fragment encoding the N-terminal 160 amino acid (aa) residues of human MDM2 was fused in-frame with the yeast Gal4 DNA binding domain (DBD) in the pGBKT7 vector to create the bait protein. Expression of the fusion protein was confirmed by Western blot analysis with anti-Myc tag antibodies (data not shown). The bait plasmid and a human mammary cDNA library (Clontech Laboratories) were co-transformed into AH109 yeast strains according to the instructions of the MATCHMAKER two-hybrid system (Clontech Laboratories). Colonies with plasmid DNA that interacted with the bait protein were identified by the transcription of histidine (His), adenine (Ade), and lacZ reporter genes integrated into the genome of the yeast host. To verify further protein–protein interactions, plasmid DNA was rescued from positive colonies and re-transformed into AH109 yeast cells with the MDM2 bait plasmid. Nucleotide sequence analysis was carried out to characterize the target DNA of positive colonies.

GST pull-down assay. GST and GST-TCAP fusion protein were expressed in bacteria BL-21(DE3) and purified according to standard procedures. Both purified proteins were separately adsorbed to glutathione–Sepharose 4B beads and then incubated with lysates from HEK293T cells transfected with pXJ40-HA-MDM2 in the binding buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.3 mM dithiothreitol, 0.1% Nonidet P-40, and protease inhibitor tablets from Roche Diagnostics). The binding reactions were rotated at 4 °C for 2 h, and the beads were subsequently washed four times with the washing buffer (the same as the binding buffer). Finally, the bound proteins were boiled in 10 μl of sample buffer and separated by SDS–PAGE for Western blot using proper antibodies.

Co-immunoprecipitation and immunoblotting. For co-immunoprecipitation experiment, HEK293T cells were transfected with pXJ40-HA-MDM2 and pIRES-EGFP-TCAP using Lipofectamine 2000. Twenty-four hours after transfection, the cells were lysed in lysis buffer containing 20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.5% Nonidet P-40, 1 mM EDTA, and protease inhibitor cocktail. After brief sonication, the debris was cleared by centrifugation at 4 °C.

Immunoprecipitation was performed by incubating supernatants with anti-TCAP monoclonal antibody, while rocking at 4 °C for 4 h. Following the addition of 10 μl protein A/protein G-agarose beads, the reactions were incubated for 2 h at 4 °C with rotating. The beads were washed three times with the same buffer, dissolved in 40 μl of 1× SDS sample buffer, and placed in boiling water for 5 min. For Western blot analysis, cells were lysed in lysis buffer supplemented with a mixture of protease inhibitors. Whole lysates were separated by SDS–PAGE on 10% gels, transferred onto nitrocellulose (Amersham-Pharmacia Biotech), and probed with the primary antibody and HRP-conjugated goat anti-mouse antibody for subsequent detection by ECL (Amersham-Pharmacia Biotech).

Immunofluorescence and confocal microscopy. 293 cells seeded on glass coverslips were transiently transfected with expression vectors of pcDNA-GFP-MDM2 or pDsRED-N1-TCAP and both. Twenty-four hours after transfection, the coverslips were washed and subsequently fixed for 15 min in 4% paraformaldehyde solution. Finally, the cells on coverslips were mounted and observed with a Bio-Rad Radiance 2100TM confocal laser system connected to a Nikon TE300 microscope. The green fluorescence of GFP was excited with an argon laser (488-nm excitation line with 515-nm long pass barrier filter) and RFP was simultaneously excited with a He–Ne laser (543-nm excitation line with 570-nm long pass barrier filter).

Results

Identification of TCAP as a MDM2-binding protein

To identify novel MDM2-binding proteins, a yeast two-hybrid screen was performed using the first 160 amino acid residues (N-terminus) of MDM2 as bait (Fig. 1A). From a screen of approximately 5.4×10^5 yeast transformants, 15 cDNA clones scored positive for reporter gene activities. Sequence analysis revealed that these clones encoded different proteins, including p53, Numb, and TCAP. Among these, five clones encoded various portions of TCAP (Fig. 1B).

To further explore the interaction between TCAP and MDM2, the interactions between TCAP with the full-length MDM2, N-terminus of MDM2, and C-terminus

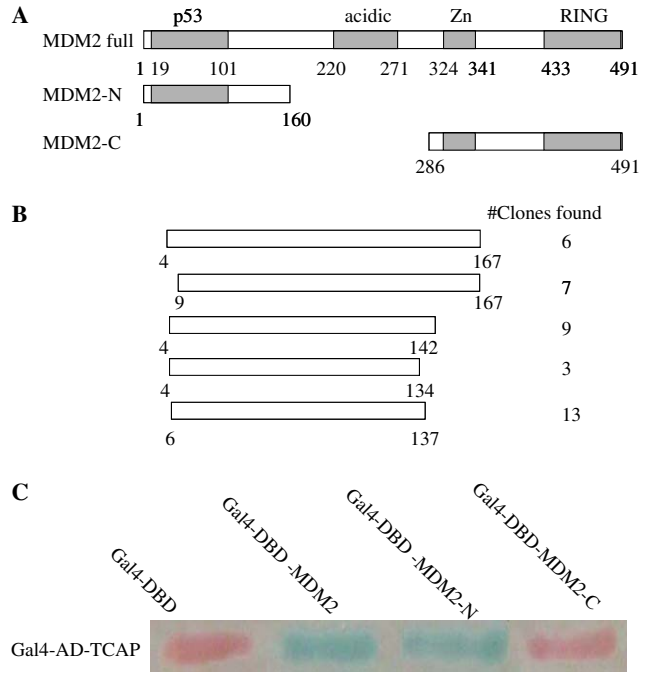

Fig. 1. Identification of the interaction of MDM2 with TCAP in yeast. (A) Schematic illustration of full-length human MDM2 protein (491 aa). The relative positions of amino acid residues encoding the p53-binding domain (p53), acidic domain, zinc finger domain (Zn), and RING finger domain (RING) are indicated. A DNA fragment encoding the N-terminal 160 aa residues of MDM2 (termed MDM2-N) was fused in-frame with the yeast Gal4 DNA binding domain and used as "bait" to screen for MDM2-interacting proteins in yeast two-hybrid assay. MDM2-C shows the C-terminal amino acid residues of MDM2 (286–491 aa). (B) Schematic illustration of five independent clones rescued from colonies scored positive for interaction between the MDM2 bait and the yeast library "prey" plasmids. Sequence analysis and DNA database searches reveal that the cDNAs are derived from the human TCAP gene. (C) The interactions between TCAP and MDM2 or its different regions were confirmed by β-gal assay. The strain AH109 was cotransformed with Gal4-AD-TCAP and Gal4-DBD-MDM2 as indicated. Gal4-DBD acted as negative control. Positive interaction was indicative of his-/ade- and LacZ-positive colonies, which showed blue staining.

of MDM2 (286–491 aa) were studied in the yeast two-hybrid system. Fig. 1C showed that TCAP interacted with the N-terminus of MDM2 and with full-length MDM2, but not with the C-terminus of MDM2, and a truncated form of p53 (data not shown). These results indicated that TCAP specifically interacted with MDM2 and the N-terminus of MDM2 was sufficient for TCAP binding.

Interaction of MDM2 with TCAP in vitro and in vivo

To confirm the physical interaction between MDM2 and TCAP, we performed GST pull-down experiments. The fusion protein GST-TCAP was expressed, purified, and incubated with lysates of HEK293T cells overexpressing MDM2 as described in *Materials and methods*. As shown in Fig. 2A, MDM2 interacts with GST-TCAP, but not with GST alone in an in vitro GST pull-down assay.

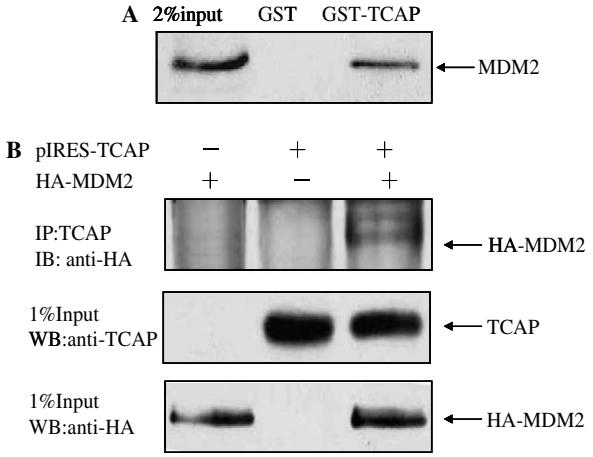

Fig. 2. TCAP interacts with MDM2 in vitro and in vivo. (A) Interaction of TCAP with MDM2 in vitro. Glutathione–Sepharose beads bound with GST-TCAP or with GST were incubated with the total cell lysates with HA-MDM2 expression. The bound proteins were subjected to SDS–PAGE for Western blot. (B) Interaction of MDM2 with TCAP in vivo. HEK293T cells were co-transfected with the expression vectors for the HA-MDM2 and pIRES-EGFP-TCAP as indicated. Lysates from the transfected cells were immunoprecipitated (IP) using an anti-TCAP antibody, and the immunoprecipitates were probed with an anti-HA antibody.

Further confirmation of the interaction between these two proteins was shown in co-immunoprecipitation analysis. HEK293T cells were transfected with either HA-MDM2 or pIRES-EGFP-TCAP or both. Immunoprecipitation was performed with a TCAP-specific antibody, followed by immunoblotting with an anti-HA antibody. Fig. 2B showed that after immunoprecipitation with the TCAP-specific antibody, MDM2 was detected in extracts of cells co-transfected with MDM2 and TCAP, but not in extracts from cells transfected with MDM2 or TCAP alone. Taken together, the results of the GST pull-down and immunoprecipitation assays suggested that TCAP interacted with MDM2 in vitro and in vivo.

MDM2 modulated the subcellular localization of TCAP

MDM2 is predominantly expressed in the nucleus [2]. However, TCAP has been generally reported as a cytoplasmic protein, expressed in the sarcomeric Z-line of striated muscles [17]. But in the immunofluorescence analysis of Vainzof et al., TCAP not only showed a strong sarcomeric labeling pattern, but also a myonuclei labeling pattern [26]. In order to observe the localization of MDM2 and TCAP in living cells, 293 cells were transiently transfected with expression plasmids encoding GFP-MDM2 and RFP-TCAP, either each alone or both together, and examined using confocal microscopy.

293 cells transfected with GFP or RFP alone displayed diffuse fluorescence (data not shown). As shown in Fig. 3A, cells transfected with GFP-tagged MDM2 showed a predominantly nuclear localization. Cells transfected with RFP-TCAP indicated a mostly cytoplasmic

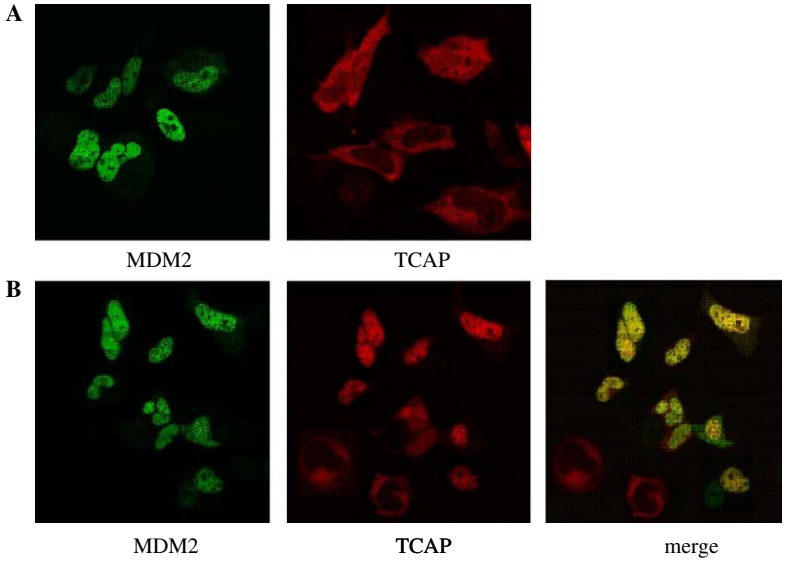

Fig. 3. MDM2 affects the subcellular localization of TCAP. 293 cells were transiently transfected with expression plasmids encoding GFP-MDM2 or RFP-TCAP and both. The fluorescence signal was visualized 24 h post-transfection. The photographs were taken at a magnification of 400×. (A) Cellular localization of MDM2 and TCAP in the cells transfected with RFP-tagged TCAP or GFP-tagged MDM2 alone. (B) Cellular localization of MDM2 and TCAP in the cells co-transfected with RFP-tagged TCAP and GFP-tagged MDM2.

localization, with few cells showing whole-cell distribution. When cells co-expressed GFP-tagged MDM2 and RFP-tagged TCAP (Fig. 3B), the subcellular localization of MDM2 remained primarily in the nucleus; interestingly, the subcellular localization of TCAP revealed conspicuous accumulation in the nucleus. Confocal analysis showed that MDM2 and TCAP co-localized in the nucleus. These phenomena were also observed in transfected H1299 cells (data not shown). These observations showed that MDM2 was capable of directing TCAP to the nucleus.

MDM2 reduced the level of TCAP by the proteasomal pathway

Recent evidence indicates that MDM2 has an ability to regulate protein stability through the proteasomal pathway [27]. We investigated whether MDM2 was involved in the regulation of TCAP protein stability. As shown in Fig. 4A, MDM2 inhibited the expression level of TCAP protein in a dose-dependent manner. This effect was observed in three independent experiments with H1299 cells, as well as with HEK293T cells (data not shown). Moreover, MG132 treatment stabilized the level of TCAP in H1299 cells overexpressing TCAP and MDM2 (Fig. 4B). These results suggested that MDM2 was involved in regulating the proteasomal turnover of TCAP in cells.

Mouse $p14^{ARF}$ inhibits MDM2-mediated p53 and p21 degradation [28,29]. To determine if suppressing MDM2 would stabilize TCAP protein, we tested whether $p14^{ARF}$ affected MDM2-mediated TCAP degradation. H1299 cells were transfected with TCAP plasmid, and/or the MDM2 plasmid or together with $p14^{ARF}$. As shown in Fig. 4C, expression of MDM2 reduced TCAP, but $p14^{ARF}$ inhibited this reduction. This result further supported that MDM2 affected the expression level of TCAP protein.

MDM2 mediated TCAP degradation independent of its RING finger domain

MDM2 functions as an E3 ubiquitin ligase for a number of proteins, such as p53 and Numb [3,4,11]. The RING finger domain of MDM2 is necessary for its ubiquitin ligase activity of MDM2, we next asked whether this domain is required for MDM2-mediated reduction of TCAP by comparing the wild-type MDM2 and mutant MDM2 (1–444 aa). H1299 cells were transfected with plasmids encoding TCAP alone or together with wild-type and mutant MDM2. The same procedure was performed with p53 as a positive control. As shown in Fig. 5, overexpression of wild-type MDM2 reduced the protein level of TCAP and p53, but the reduction for TCAP did not appear to require the C-terminal RING finger domain of MDM2, as the mutant MDM2 (1–444 aa) that was unable to mediate p53 degradation also reduced TCAP. These results suggested that MDM2 downregulated the protein level of TCAP independent of the RING finger domain of MDM2.

Discussion

To identify MDM2-binding proteins, we performed a yeast two-hybrid screen using the N-terminus of MDM2 as bait to screen a human mammary cDNA library. Fifteen positive clones were sequenced and among these, the well-known MDM2 interacting proteins p53 and Numb were identified. We identified a novel MDM2-binding protein TCAP, which was encoded by five different clones. This association could also be detected in GST pull-down assay

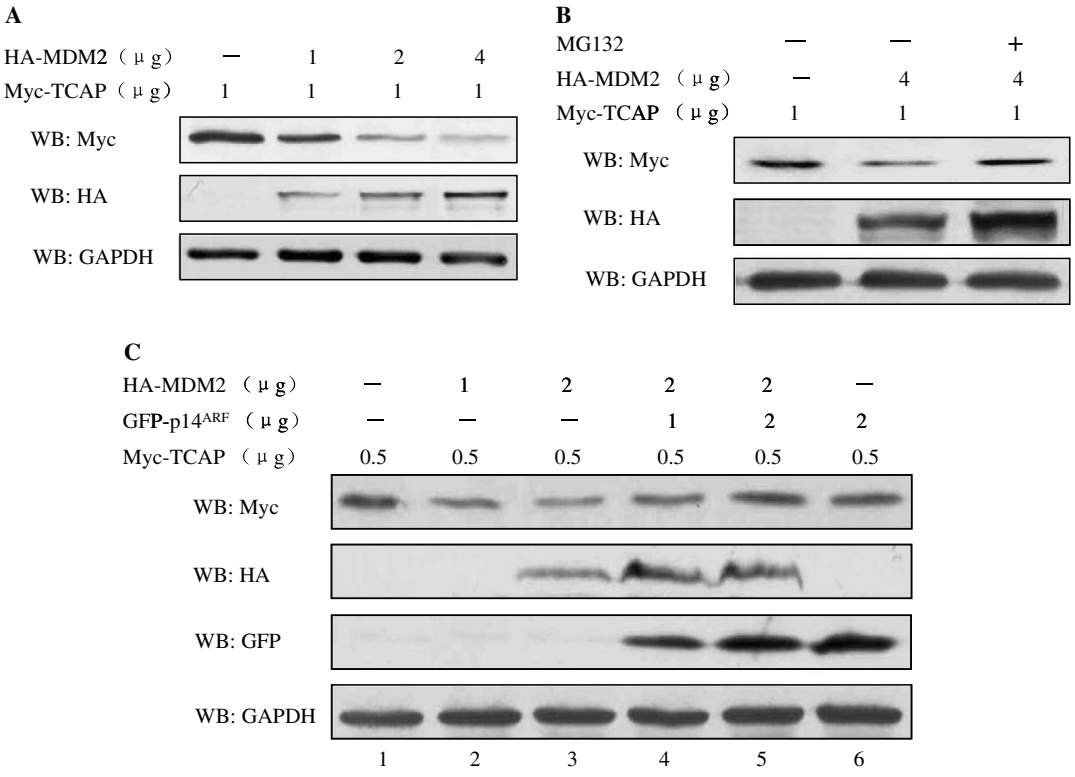

Fig. 4. MDM2 mediates TCAP degradation by the proteasomal pathway. (A) MDM2 mediates TCAP degradation in a dose-dependent pattern. Different amounts of HA-MDM2 expression plasmid were co-transfected with the Myc-TCAP expression plasmid into H1299 cells as indicated on the top. Cell lysates were loaded onto an SDS–PAGE for Western blot using antibodies as indicated on the left. (B) TCAP degradation by MDM2 is proteasome-dependent. HA-MDM2 expression plasmid was co-transfected with Myc-TCAP plasmid into H1299 cells as indicated on the top. At 24 h post-transfection, DMSO (lanes 1 and 2) or MG132 (lane 3) was added into the medium for further 6 h incubation. Cell lysates were loaded onto an SDS–PAGE for Western blot using antibodies as indicated on the left. (C) $p14^{ARF}$ inhibits MDM2-mediated TCAP degradation. H1299 cells were transfected with plasmids as indicated. Cell lysates were used for Western blot using antibodies as indicated on the left.

as well as in co-immunoprecipitation assays. Although we have not determined the precise boundaries of the TCAP binding site within MDM2 protein, our data suggested that the N-terminus of MDM2 was sufficient for TCAP binding.

We next analyzed the subcellular localization of TCAP and MDM2. MDM2 remained primarily in the nucleus whenever it was expressed alone or expressed together with TCAP. Very interestingly, our results showed that MDM2 was capable of directing TCAP from the cytoplasm to the nucleus. Confocal analysis showed that MDM2 and TCAP co-localized in the nucleus. Many proteins translocate between different subcellular compartments under defined circumstances [30,31]. For example, MDM2 could lead to the Numb degradation and its translocation from the cytoplasm to the nucleus [11]. Notably, MDM2 shuttles very efficiently between the cytoplasm and nucleus, and this shuttling is required for the efficient promotion of p53 degradation [2]. It is conceivable that MDM2 affects other proteins by translocating these proteins into or out of the nucleus. Our data are consistent with a model in which the subcellular localization of TCAP may be subjected to a similar type of modulation by MDM2.

MDM2 has been reported to target proteins for the ubiquitin-dependent and -independent proteasomal degradation [27]. Moreover, modulating the subcellular localization of some proteins by MDM2 is relevant to the degradation of these proteins. It is tempting to speculate that MDM2 may be involved in regulation of TCAP protein level. As shown in our study, MDM2 promoted the proteasomal degradation of TCAP in H1299 cells, and this activity could be abolished by $p14^{ARF}$, which was consistent with the previous reports that the $p14^{ARF}$ could inhibit MDM2-mediated p53 and p21 degradation [29,30]. In addition, our results supported that this degradation was independent of the RING finger domain of MDM2 and might be the ubiquitin-independent degradation pathways, because the integrity of the RING finger domain of MDM2 is necessary for the ubiquitin ligase activity of MDM2. Recent studies also provide evidence for proteasome-dependent, but ubiquitin-independent degradation pathways, which are widespread and physiologically important in higher eukaryotes. As for MDM2, some evidences show that MDM2 regulates the protein level of p21 and pRb [32,33] by ubiquitin-independent proteasomal degradation pathway.

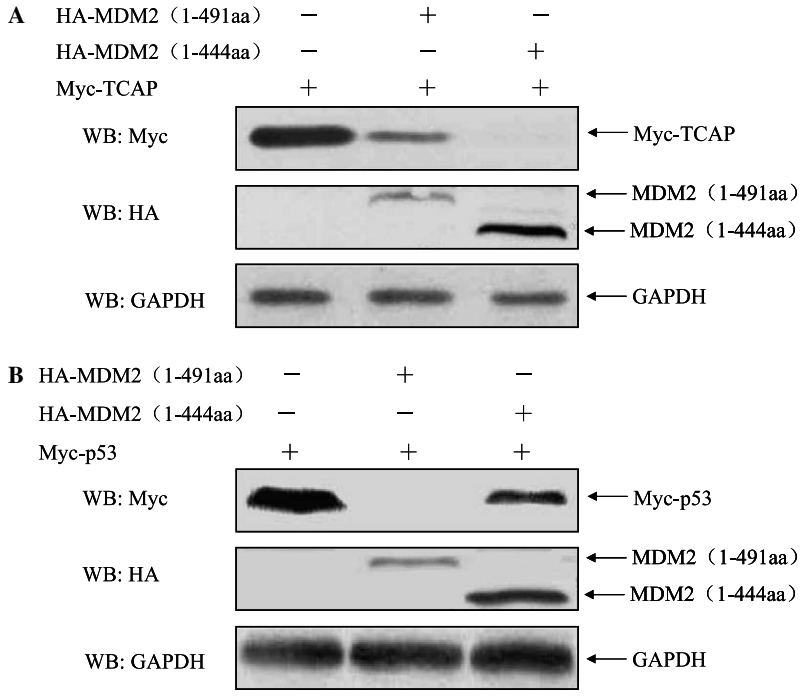

Fig. 5. MDM2 mediates TCAP degradation independent of its RING finger domain. (A) Overexpression of both wild-type and mutant MDM2 (1–444 aa) reduces TCAP protein level in H1299 cells. H1299 cells were transfected with plasmids encoding Myc-TCAP (0.5 μg), HA-MDM2 (2 μg), and mutant MDM2 (2 μg) as indicated on the top. Cell lysates were loaded onto an SDS–PAGE for Western blot using antibodies as indicated on the left. (B) Degradation of p53 requires an intact MDM2 RING finger domain. Same as (A), except that p53 was used as a substrate.

TCAP gene encodes a Z-disc sarcomeric protein of 19 kDa found exclusively in skeletal and cardiac muscle. Our study shows for the first time that TCAP is identified in a human mammary cDNA library. In addition, TCAP was found in prostate tissue [17]. These results suggested that the expression and distribution of TCAP should to be further studied.

Presently, the functional significance of MDM2 interacting with and downregulating TCAP is unknown. However, MDM2 attenuates hypertrophy of cardiac myocytes, and TCAP is a key component involving in the cardiac hypertrophy, thus it is exciting to hypothesize that the degradation of TCAP by MDM2 might be connected with the roles of MDM2 in cardiac hypertrophy. Of course, there are the possibilities that the interaction is involved in other biological processes.

In conclusion, we identified TCAP as an MDM2 interacting protein. MDM2 co-localized with TCAP and altered its subcellular localization. Elevated MDM2 expression downregulated the protein level of TCAP through the proteasomal pathway and might participate in ubiquitin-independent proteasomal degradation. Our experiments demonstrated for the first time that MDM2 interacted with the sarcomeric protein TCAP. TCAP might be a target of MDM2 in cardiac hypertrophy. Further investigations should focus on the biological significance of the interaction between MDM2 and TCAP in muscle hypertrophy.

Acknowledgments

We thank Dr. Scheffner, M. for kindly providing the pcDNA3-HA-MDM2 plasmid and Dr. Peter, G. for pEGFP-p14^{ARF} plasmid. We are also grateful to Dr. Pei, G. for the mouse pcDNA3-GFP-MDM2 plasmid. This work was supported by 973 National Program of China (2004CB518800), grants from Beijing Scientific and Technology Committee, and grants from National Natural Science foundation of China (and 30270299).

References

[1] J. Momand, G.P. Zambetti, D.C. Olson, D. George, A.J. Levine, The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation, Cell 69 (1992) 1237–1245.
[2] J. Roth, M. Dobbelstein, D.A. Freedman, T. Shenk, A.J. Levine, Nucleocytoplasmic shuttling of the hdm2 oncoprotein regulates the levels of the p53 protein via a pathway used by the human immunodeficiency virus rev protein, EMBO J. 17 (1998) 554–564.
[3] Y. Haupt, R. Maya, A. Kazaz, M. Oren, Mdm2 promotes the rapid degradation of p53, Nature 387 (1997) 296–299.
[4] M.H. Kubbutat, S.N. Jones, K.H. Vousden, Regulation of p53 stability by Mdm2, Nature 387 (1997) 299–303.
[5] Y. Haupt, Y. Barak, M. Oren, Cell type-specific inhibition of p53-mediated apoptosis by mdm2, EMBO J. 15 (1996) 1596–1606.
[6] J. Chen, X. Wu, J. Lin, A.J. Levine, mdm-2 inhibits the G1 arrest and apoptosis functions of the p53 tumor suppressor protein, Mol. Cell. Biol. 16 (1996) 2445–2452.

[7] C.Y. Chen, J.D. Oliner, Q. Zhan, A.J. Fornace, B. Vogelstein, M.B. Kastan, Interactions between p53 and MDM2 in a mammalian cell cycle checkpoint pathway, Proc. Natl. Acad. Sci. USA 91 (1994) 2684–2688.
[8] Z.J. Xiao, J. Chen, A.J. Levine, N. Modjtahedi, J. Xing, W.R. Sellers, D.M. Livingston, Interaction between the retinoblastoma protein and the oncoprotein MDM2, Nature 375 (1995) 694–698.
[9] K. Martin, D. Trouche, C. Hagemeier, T.S. Sorensen, N.B. La Thangue, T. Kouzarides, Stimulation of E2F/DP1 transcriptional activity by MDM2 oncoprotein, Nature 375 (1995) 691–694.
[10] T.M. Boyd, N. Vlatkovic, D.S. Haines, A novel cellular protein (MTBP) binds to MDM2 and induces a G1 arrest that is suppressed by MDM2, J. Biol. Chem. 275 (2000) 31883–31890.
[11] T. Juven-Gershon, O. Shifman, T. Unger, A. Elkeles, Y. Haupt, M. Oren, The Mdm2 oncoprotein interacts with the cell fate regulator Numb, Mol. Cell. Biol. 18 (1998) 3947–3982.
[12] Y. Jin, H. Lee, S.X. Zeng, M.S. Dai, H. Lu, MDM2 promotes p21waf1/cip1 proteasomal turnover independently of ubiquitylation, EMBO J. 23 (2003) 6365–6377.
[13] J.D. Weber, L.J. Taylor, M.F. Roussel, C.J. Sherr, D. Bar-Sagi, Nucleolar Arf sequesters Mdm2 and activates p53, Nat. Cell Biol. 1 (1999) 20–26.
[14] P. De Graaf, N.A. Little, Y.F. Ramos, E. Meulmeester, S.J. Letteboer, A.G. Jochemsen, Hdmx protein stability is regulated by the ubiquitin ligase activity of Mdm2, J. Biol. Chem. 278 (2003) 38315–38324.
[15] Y. Pan, J. Chen, MDM2 promotes ubiquitination and degradation of MDMX, Mol. Cell. Biol. 23 (2003) 5113–5121.
[16] A. Toth, P. Nickson, L.L. Qin, P. Erhardt, Differential regulation of cardiomyocyte survival and hypertrophy by MDM2, an E3 ubiquitin ligase, J. Biol. Chem. 281 (2006) 3679–3689.
[17] G. Valle, G. Faulkner, A. De Antoni, B. Pacchioni, A. Pallavicini, D. Pandolfo, N. Tiso, S. Toppo, S. Trevisan, G. Lanfranchi, Telethonin, a novel sarcomeric protein of heart and skeletal muscle, FEBS Lett. 415 (1997) 163–168.
[18] O. Mayans, P.F. van der Ven, M. Wilm, A. Mues, P. Young, D.O. Furst, M. Wilmanns, M. Gautel, Structural basis for activation of the titin kinase domain during myofibrillogenesis, Nature 395 (1998) 863–869.
[19] T. Hayashi, T. Arimura, M. Itoh-Satoh, K. Ueda, S. Hohda, N. Inagaki, M. Takahashi, H. Hori, M. Yasunami, H. Nishi, Y. Koga, H. Nakamura, M. Matsuzaki, B.Y. Choi, S.W. Bae, C.W. You, K.H. Han, J.E. Park, R. Knoll, M. Hoshijima, K.R. Chien, A. Kimura, Tcap gene mutations in hypertrophic cardiomyopathy and dilated cardiomyopathy, J. Am. Coll. Cardiol. 44 (2004) 2192–2201.
[20] E.S. Moreira, T.J. Wiltshire, G. Faulkner, A. Nilforoushan, M. Vainzof, O.T. Suzuki, G. Valle, R. Reeves, M. Zatz, M.R. Passos-Bueno, D.E. Jenne, Limb-girdle muscular dystrophy type 2G is caused by mutations in the gene encoding the sarcomeric protein telethonin, Nat. Genet. 24 (2000) 163–166.
[21] R. Knoll, M. Hoshijima, H.M. Hoffman, V. Person, I. Lorenzen-Schmidt, M.L. Bang, T. Hayashi, N. Shiga, H. Yasukawa, W. Schaper, W. McKenna, M. Yokoyama, N.J. Schork, J.H. Omens, A.D. McCulloch, A. Kimura, C.C. Gregorio, W. Poller, J. Schaper, H.P. Schultheiss, K.R. Chien, The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy, Cell 111 (2002) 943–955.
[22] S. Kojic, E. Medeot, E. Guccione, H. Krmac, I. Zara, V. Martinelli, G. Valle, G. Faulkner, The Ankrd2 protein, a link between the sarcomere and the nucleus in skeletal muscle, J. Mol. Biol. 339 (2004) 313–325.
[23] G. Nicholas, M. Thomas, B. Langley, W. Somers, K. Patel, C.F. Kemp, M. Sharma, R. Kambadur, Titin-cap associates with, and regulates secretion of, Myostatin, J. Cell. Physiol. 193 (2002) 120–131.
[24] T. Furukawa, Y. Ono, H. Tsuchiya, Y. Katayama, M.L. Bang, D. Labeit, S. Labeit, N. Inagaki, C.C. Gregorio, Specific interaction of the potassium channel beta-subunit minK with the sarcomeric protein T-cap suggests a T-tubule-myofibril linking system, J. Mol. Biol. 313 (2001) 775–784.
[25] R.S. Haworth, F. Cuello, T.J. Herron, G. Franzen, J.C. Kentish, M. Gautel, M. Avkiran, Protein kinase D is a novel mediator of cardiac troponin I phosphorylation and regulates myofilament function, Circ. Res. 95 (2004) 1091–1099.
[26] M. Vainzof, E.S. Moreira, O.T. Suzuki, G. Faulkner, G. Valle, A.H.O. Beggs, O. Carpen, A.F. Ribeiro, E. Zanoteli, J. Gurgel-Gianneti, A.M. Tsanaclis, H.C. Silva, M.R. Passos-Bueno, M. Zatz, Telethonin protein expression in neuromuscular disorders, Biochim. Biophys. Acta 1588 (2002) 33–40.
[27] M.H. Kubbutat, R.L. Ludwig, A.J. Levine, K.H. Vousden, Analysis of the degradation function of Mdm2, Cell Growth Differ. 10 (1999) 87–92.
[28] R. Honda, H. Yasuda, Association of p19 (ARF) with Mdm2 inhibits ubiquitin ligase activity of Mdm2 for tumor suppressor p53, EMBO J. 18 (1999) 22–27.
[29] D. Xirodimas, M.K. Saville, C. Edling, D.P. Lane, S. Lain, Different effects of p14ARF on the levels of ubiquitinated p53 and Mdm2 in vivo, Oncogene 20 (2001) 4972–4983.
[30] J. Behrens, J.P. von Kris, M. Kuhl, L. Bruhn, D. Wedlich, R. Grosschedl, W. Birchmeier, Functional interaction of beta-catenin with the transcription factor LEF-1, Nature 382 (1996) 638–642.
[31] M. Molenaar, M. van de Wetering, M. Oosterwegel, M.J. Peterson, S. Godsave, V. Korinek, J. Roose, O. Destree, H. Clevers, XTcf-3 transcription factor mediates beta-catenin-induced axis formation in *Xenopus* embryos, Cell 86 (1996) 391–399.
[32] Y. Jin, H. Lee, S.X. Zeng, M.S. Dai, H. Lu, MDM2 promotes $p21^{waf1/cip1}$ proteasomal turnover independently of ubiquitylation, EMBO J. 22 (2003) 6365–6377.
[33] P. Sdek, H. Ying, D.L. Chang, W. Qiu, H. Zheng, R. Touitou, M.J. Allday, Z.X. Xiao, MDM2 promotes proteasome-dependent ubiquitin-independent degradation of retinoblastoma protein, Mol. Cell 20 (2005) 699–708.