Current Biology, Vol. 13, 1234–1239, July 15, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00454-8

Hdm2 Recruits a Hypoxia-Sensitive Corepressor to Negatively Regulate p53-Dependent Transcription

Alexander H. Mirnezami, Sandra J. Campbell,[1] Matthew Darley, John N. Primrose, Peter W.M. Johnson, and Jeremy P. Blaydes*
Cancer Sciences Division
School of Medicine, University of Southampton
MP 824, Southampton General Hospital
Southampton SO16 6YD
United Kingdom

Summary

The transcription factor p53 lies at the center of a protein network that controls cell cycle progression and commitment to apoptosis [1]. p53 is inactive in proliferating cells, largely because of negative regulation by the Hdm2/Mdm2 oncoprotein, with which it physically associates. Release from this negative regulation is sufficient to activate p53 [2] and can be triggered in cells by multiple stimuli through diverse pathways [3–5]. This diversity is achieved in part because Hdm2 uses multiple mechanisms to inactivate p53; it targets p53 for ubiquitination and degradation by the proteosome [6–8], shuttles it out of the nucleus and into the cytoplasm [9, 10], prevents its interaction with transcriptional coactivators [11], and contains an intrinsic transcriptional repressor activity [12]. Here we show that Hdm2 can also repress p53 activity through the recruitment of a known transcriptional corepressor, hCtBP2 [13, 14]. This interaction, and consequent repression of p53-dependent transcription, is relieved under hypoxia or hypoxia-mimicking conditions that are known to increase levels of intracellular NADH. CtBP proteins can undergo an NADH-induced conformational change [15], which we show here results in a loss of their Hdm2 binding ability. This pathway represents a novel mechanism whereby p53 activity can be induced by cellular stress.

Results and Discussion

Screening of a human HeLa cell cDNA expression library with recombinant Hdm2 protein identified CtBP2 as a potential Hdm2 binding partner (not shown). A GST-human CtBP2 (GST-hCtBP2) fusion protein, but not other GST-fusion controls, was able to capture the 90 kDa Hdm2 protein from a partially purified bacterial lysate (Figure 1A). Furthermore, when *E. coli* lysates that had been induced to express human proteins (either p53 or Hdm2) were subjected to far-Western analysis with GST-hCtBP2 as a probe, a 90 kDa band was detected only in Hdm2-containing lysates (see Supplemental Data). These experiments demonstrate that Hdm2 and hCtBP2 can associate specifically in vitro. Further experiments in vitro determined that the interaction occurs rapidly (30 min coincubation is sufficient for near-maximal binding under these assay conditions) and, once formed, is stable over a 24 hr incubation period and cannot be disrupted by high-ionic-strength (600 mM NaCl) buffer (not shown).

We then sought to determine whether the Hdm2:hCtBP2 interaction can be detected in vivo. Hdm2 and either Myc epitope-tagged hCtBP2 or Myc-β-galactosidase control were transiently overexpressed in HEK 293 cells and cell lysates subjected to immunoprecipitation with anti-Myc antibody. Hdm2 protein was coimmunoprecipitated from the Myc-hCtBP2-containing lysates but not the Myc-β-galactosidase control (see Supplemental Data). Similar coimmunoprecipitation experiments were performed on nontransfected MCF-7 cell lysates with anti-CtBP antibody. Hdm2 coimmunoprecipitated specifically with the anti-CtBP antibody, but not in control precipitations (Figure 1B), demonstrating a naturally occurring interaction between endogenous Hdm2 and hCtBP2 proteins in vivo.

hCtBP2 is a 445 amino acid protein that shares 83% sequence similarity with its homolog, hCtBP1. Both proteins have orthologs in the mouse, whereas *Xenopus* and *Drosophila* each express a single CtBP molecule [13, 14]. All CtBP proteins contain a highly conserved central domain with significant sequence similarity to a family of D-isomer-specific 2-hydroxyacid dehydrogenases [13, 14]. This domain is located between amino acids 106 and 353 in hCtBP2. The N terminus of CtBP family members contains a protein:protein interaction domain, through which they are recruited to proteins containing a consensus PXDLS motif [16, 17]. We used GST "pull-down" assays to test a series of GST-hCtBP2 deletion mutants for their ability to bind Hdm2 (Figure 1C). A mutant containing amino acids 1–110 pulled down Hdm2 as effectively as the full-length protein (lane 5), whereas one containing amino acids 110–445 did not pull down Hdm2 (lane 3). Therefore, the N-terminal domain of hCtBP2 is both necessary and sufficient for the interaction with Hdm2. A mutant lacking the C-terminal 86 amino acids (construct 1–359, lane 2) consistently pulled down less Hdm2 than did the full-length protein, despite the lack of any direct Hdm2 binding site in this region (see lane 3). This latter finding is consistent with previous reports that have indicated a regulatory role for the C terminus of CtBP-proteins on N-terminal protein:protein interactions [18, 19].

To determine which domain of Hdm2 interacts with hCtBP2, we also constructed a panel of Hdm2 truncation mutants. Hdm2(6–306) bound the N-terminal domain of hCtBP2 (Figure 1D, lane 3), albeit less strongly than full-length Hdm2, whereas Hdm2(6–210) did not bind (lane 2). The Hdm2 section containing amino acids 210–306 that these experiments identify as being required for hCtBP2 binding primarily consists of the acidic domain, which is known to both regulate Hdm2 function [20] and interact with proteins including p300, Rb, TBP, ARF, and MTBP [21]. The Hdm2 paralog, HdmX [22], shares some

*Correspondence: j.p.blaydes@soton.ac.uk
[1]Present address: Molecular Neuropathology Laboratory, School of Biological Sciences, University of Southampton, Biomedical Sciences Building, Southampton SO16 7PX, United Kingdom.

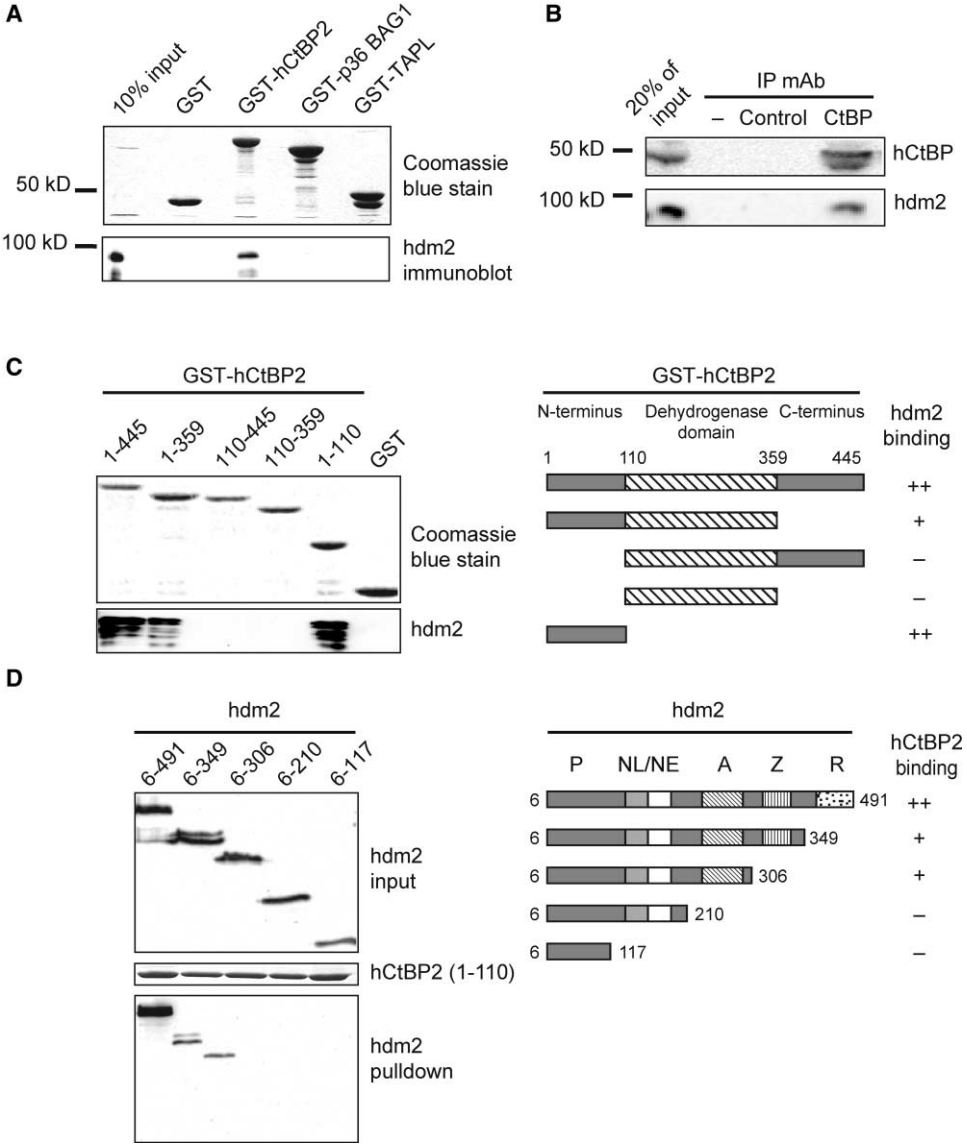

Figure 1. Hdm2 Interacts with hCtBP2 In Vitro and In Vivo

(A) Partially purified recombinant Hdm2 was incubated with the GST-fusion proteins indicated, and complexes were pulled down with glutathione-sepharose beads. Samples were then analyzed by SDS-PAGE, followed by either Coomassie blue staining to demonstrate equal amounts of GST-fusion protein or Western blotting with mAb 2A10 to detect Hdm2.

(B) Extracts from MCF-7 cells were immunoprecipitated with protein G beads alone (-), an irrelevant goat Ig to caspase 6 (control), or E16 goat-polyclonal antibody to CtBP. Precipitates were then analyzed by immunoblotting with rabbit polyclonal Ig R41 to hCtBP2 and Hdm2 mAb 2A9.

(C) Recombinant full-length GST-hCtBP2(1-445), GST-hCtBP2 deletion mutants, and GST alone were used as bait in GST pull-down assays with equal amounts of full-length Hdm2. Coomassie blue staining confirmed that equivalent amounts of the CtBP2 mutants had been used (upper panel), and immunoblotting with mAb 2A10 was used for detecting coprecipitated Hdm2 (lower panel). A comparison between the mutants and the known domains of hCtBP2 is shown, with the N-terminal GST fusion omitted.

(D) GST-hCtBP2(1–110) was used to pull down the Hdm2 deletion mutants indicated. Equal amounts of input proteins were confirmed by Western blotting with mAb 4B2 for Hdm2 and Coomassie blue staining for GST-hCtBP2(1–110). Coprecipitated Hdm2 was detected via immunoblotting with mAb 4B2. The location of the Hdm2 deletion mutants with reference to known domains of Hdm2 are shown: P = p53 binding domain; NL/NE = Nuclear localization and export sequences; A = acidic region; Z = Zinc finger; R = RING finger.

homology with Hdm2 within the acidic domain and can also interact with hCtBP2 in vitro (see Supplemental Data). Furthermore, the hCtBP2 paralog, hCtBP1, is able to interact with Hdm2 in GST "pull-down" assays (see Supplemental Data).

The recruitment of hCtBP1 by proteins containing a PXDLS motif is regulated by changes in cellular redox potential [15]. The central dehydrogenase domain of hCtBP1 contains a high-affinity binding site for NADH (GXGXXG, Figure 2A), occupation of which induces a conformational change in the hCtBP1 molecule and an increase in binding to proteins such as E1A and ZEB.

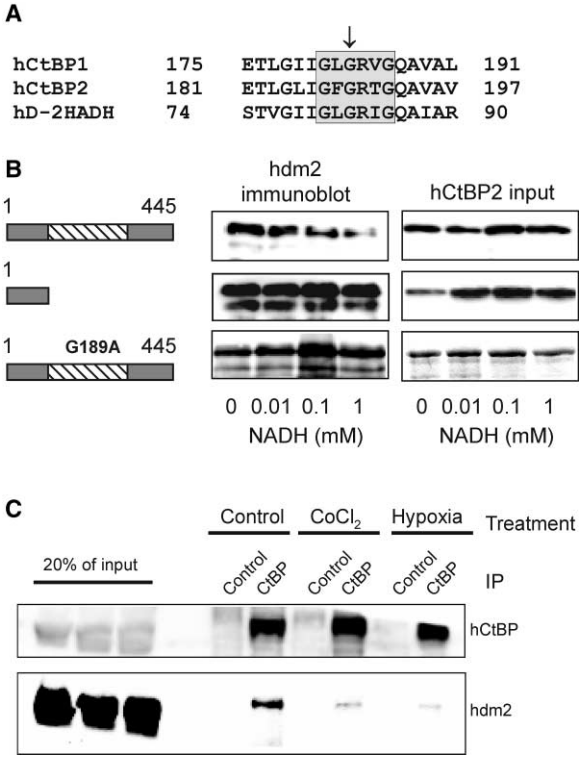

Figure 2. The Hdm2:hCtBP2 Interaction Is Inhibited by NADH Binding to the hCtBP2 Dehydrogenase Domain

(A) Multiple sequence alignment of the conserved GXGXXG NAD^+/NADH binding site of hCtBP1, hCtBP2, and the human D-isomer-specific 2-hydroxyacid dehydrogenase enzyme (hD-2HADH). The affinity of the site in hCtBP1 has been reported to be approximately 100-fold greater for NADH than for NAD^+ [15]. The arrow marks the site of the alanine substitution mutant in hCtBP2 (G189A).

(B) The interaction between Hdm2 and the indicated GST-hCtBP2 fusion proteins was determined by a GST pull-down assay in the presence of increasing concentrations of NADH.

(C) MCF-7 cells were treated for 2 hr with 200 μM $CoCl_2$ or hypoxia (<1% O_2) where indicated. Cell lysates were immunoprecipitated (IP) with goat polyclonal anti-CTBP (E-16) or a control antibody to caspase 6, and immunoprecipitated proteins were analyzed by Western blot with antibodies for CtBP (R41) and Hdm2 (2A10).

A mutation in hCtBP1 in the GXGXXG motif (G183A) abolishes NADH responsiveness [15]. We noted that this site in hCtBP2 is conserved (amino acids 187–192) and asked whether NADH could regulate the Hdm2:hCtBP2 interaction. NADH concentrations (0.01 to 1 mM) known to promote the interaction of hCtBP1 with PXDLS motif proteins inhibited binding of full-length GST-hCtBP2 to Hdm2 (Figure 2B, top panel). This inhibition did not occur when either GST-hCtBP2(1–110), lacking the dehydrogenase domain, or hCtBP2(G189A), containing a mutation in the NADH binding site, were used in the assays (Figure 2B, lower panels). Therefore, in contrast to interactions with PXDLS motif proteins, the conformational changes induced by NADH binding to the CtBP dehydrogenase domain result in a reduced affinity of hCtBP2 for Hdm2. Exposure of cells in culture to $CoCl_2$ can be used as a model for the induction of a hypoxia-like stress response. Zhang et al. [15] have shown that $CoCl_2$ treatment (200 μM) induces an increase in the cellular NADH/NAD^+ ratio sufficient to promote binding of CtBP proteins to PXDLS motif proteins in the cell. As shown in Figure 2C, 200 μM $CoCl_2$ reduced the formation of Hdm2:hCtBP2 complexes in MCF-7 cells. Hypoxia, which has a greater effect on the cellular NADH/NAD^+ ratio than $CoCl_2$ [15], was more effective than $CoCl_2$ in reducing the Hdm2:hCtBP2 interaction (Figure 2C). These data demonstrate, therefore, that the NADH-induced regulation of the Hdm2:hCtBP2 interaction also occurs in vivo.

To determine the functional consequences of the Hdm2:hCtBP2 interaction, we examined whether overexpression of either molecule would modify the function of the other. Hdm2 regulates p53 levels in cells by directing the nuclear export of p53 and targeting it to the ubiquitin-proteasome degradation pathway [6–10]. We therefore tested whether Hdm2 could regulate hCtBP2 protein levels. HEK 293 cells were transiently transfected with vectors encoding Myc-tagged hCtBP2, and increasing amounts of Hdm2. Hdm2 did not decrease hCtBP2 expression levels in this assay (see Supplemental Data), providing evidence that Hdm2 does not target hCtBP2 for proteosome-mediated degradradation, nor did it have an effect on the intracellular localization of hCtBP2 in HEK 293 cells (data not shown). The most well-documented function of CtBP proteins is as short-range transcriptional corepressors [13, 14]. Thus, CtBP proteins are recruited to promoters by sequence-specific DNA binding transcription factors, either through a direct physical interaction or indirectly through bridging proteins. We therefore tested whether the formation of an Hdm2:hCtBP2 complex could modify the ability of Hdm2 to repress p53-dependent transcription. For this experiment we used three different p53-responsive luciferase reporters: Bax-Luc, Hdm2-Luc03, and p21-Luc containing promoter regions from Bax, Hdm2, and $p21^{WAF-1}$, respectively. In the presence of Hdm2, hCtBP2 reproducibly repressed p53-dependent transcription from the Bax-Luc and Hdm2-Luc reporters but had no effect on p21-Luc (Figure 3A).

To confirm that the inhibition of p53-dependent transcription by hCtBP2 is dependent on a direct interaction between Hdm2 and hCtBP2, we treated cells with $CoCl_2$ to disrupt the Hdm2:hCtBP2 interaction. After $CoCl_2$ exposure, hCtBP2 was no longer able to enhance Hdm2-mediated repression of p53-dependent transcription (Figure 3B); this was not due to an effect on p53 protein levels in these cells (Figure S4 in the Supplemental Data). This effect of $CoCl_2$ was due to its specific effect on regulating intracellular NADH levels because repression by the hCtBP2(G189A) mutant was unaffected by $CoCl_2$ (Figure 3C). In addition, we transfected cells with an Hdm2(1–210) mutant, which can bind p53, is localized in the nucleus, and can inhibit p53 activity but cannot bind hCtBP2. In the presence of Hdm2(1–210), hCtBP2 did not repress p53-dependent transcription. (Figure 3D), confirming that an interaction between Hdm2 and hCtBP2 is required for hCtBP2 to be able to repress p53-dependent transcription.

One model that could account for our observation is that an Hdm2:hCtBP2 complex directly interacts with p53 to bring about repression of p53 transactivating ability. The binding sites for p53 and hCtBP2 on Hdm2

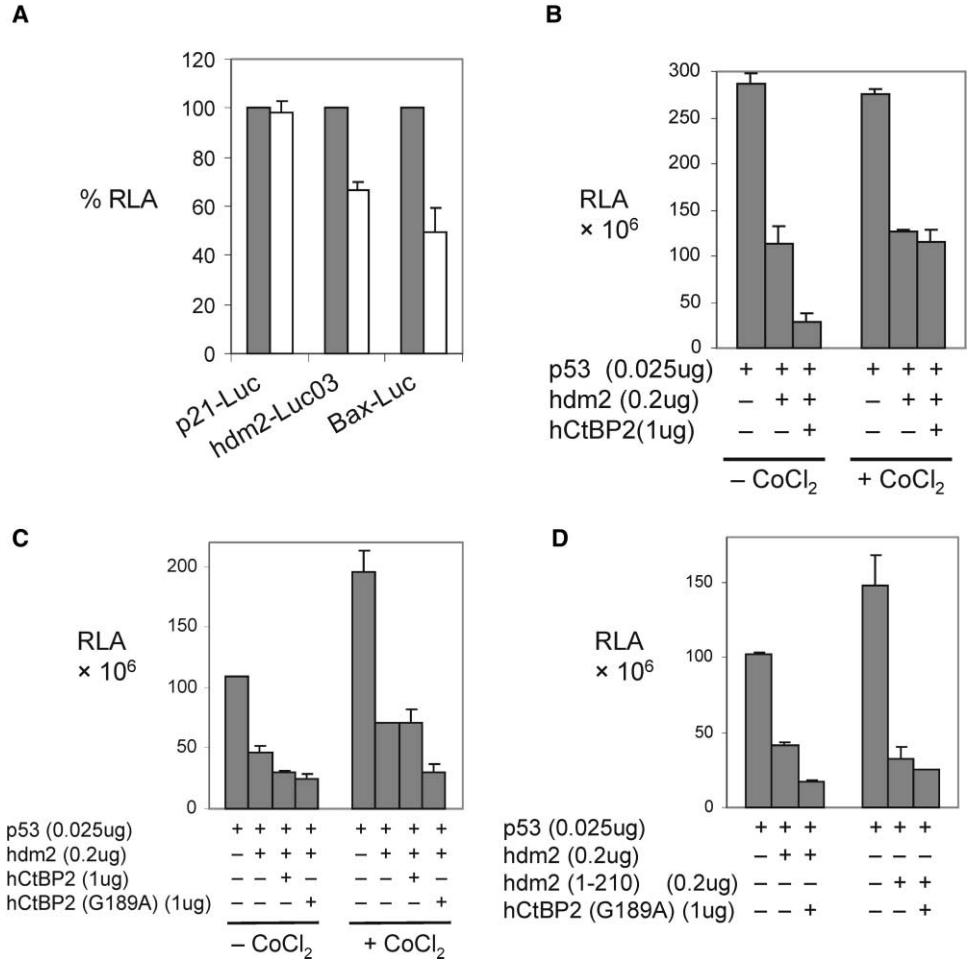

Figure 3. Recruitment of hCtBP2 by Hdm2 Results in Promoter-Selective Repression of p53-Dependent Transcription

(A) HEK 293 cells were transfected with 200 ng of the p53-dependent reporter vector containing $p21^{WAF-1}$, Hdm2, or Bax promoter sequences, 25 ng of wild-type p53 expression vector, and 200 ng of Hdm2 expression vector to give conditions in which approximately 70% inhibition of p53 activity by the cotransfected Hdm2 was achieved. Transfections also included 1 μg of either pcDNA 3.1 (solid bars) or pcDNA3mychis hCTBP2 (open bars) expression vectors. Relative luciferase activity (RLA) was first calculated by normalization to expression from a cotransfected β-galactosidase expression vector, and the RLA of each promoter in the presence of pcDNA3.1 was defined as 100%. In the absence of p53, promoter activity was approximately 2%.

(B) HEK 293 cells were transfected with the Hdm2-Luc03 p53-responsive reporter vector (which contains only 165 bp of Hdm2 promoter sequence including the two p53 response elements) and the indicated amounts of expression vector. Thirty hours after transfection, cells were treated for 16 hr with 200 μM $CoCl_2$ before being assayed.

(C) Transfections were repeated as in (B), with the addition of the NADH-insensitive G189A mutant of hCtBP2.

(D) Hdm2-Luc03 reporter assays in HEK 293 cells were performed with the indicated vectors. hCtBP2(G189A) was used in this experiment because it functions as a constitutively active mutant that is insensitive to variations in intracellular NADH.

are located in distinct domains, suggesting that a trimeric complex of p53:Hdm2:hCtBP2 could form. Figure 4A demonstrates that, when combinations of Hdm2, GST-hCtBP2(1–110), and p53 are coincubated in vitro, p53 coprecipitates with GST-hCtBP2(1–110) only in the presence of Hdm2. Therefore, Hdm2 is able to recruit hCtBP2 to p53. In this model (Figure 4B), the role of Hdm2 as a bridging protein between hCtBP2 and p53 is analogous to the role played by CtIP in recruiting CtBP to BRCA1 [23]. Our model is further supported by recent evidence that Hdm2 can be recruited to p53-responsive promoters through p53 binding [24]. The promoter specificity of this effect in our assays may reflect the known requirement for CtBP proteins to be recruited to sites located within a short distance of the promoter for repression to occur [17]. Consistent with this known property of CtBP proteins, the p53-responsive promoters that were sensitive to inhibition by hCtBP2 (Bax-Luc and Hdm2-Luc03) in this study both have p53 response elements (p53-RE) situated within 500 bp of the transcription start site, whereas in the nonresponsive p21 promoter, the p53-RE is located further upstream at −2400. It is important to consider, however, that the mechanisms whereby CtBP represses transcription remain largely undefined and may depend on the context of individual promoters. Specifically, CtBP proteins can recruit histone deacetylase proteins, including HDAC1 [25, 26], and repression by CtBP can be either depen-

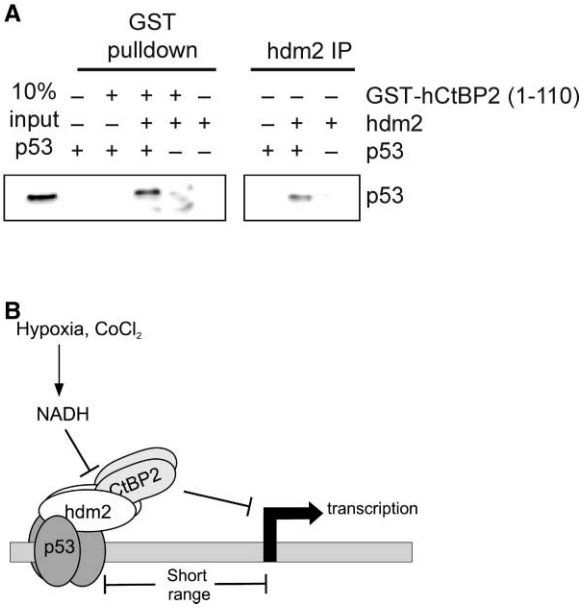

Figure 4. Proposed Model of the Mechanism whereby Hdm2 Recruits CtBP Proteins to Negatively Regulate p53-Dependent Transcription

(A) Recombinant human p53, Hdm2, and GST-hCtBP2(1–110) were mixed as indicated and subjected to either GST pull-down analysis or immunoprecipitation with mAb 2A9 to Hdm2. Coprecipitated p53 was detected with polyclonal antibody CM1.

(B) Model for the promoter-selective inhibition of p53-dependent transcription by CtBP proteins. Given the high degree of functional homology between hCtBP1 and hCtBP2 and the ability of the two proteins to form heterodimers, it is probable that hCtBP1 is also able to repress p53 activity by this mechanism.

dent [16] or independent [27] of HDAC activity. Hdm2 has recently been shown to recruit HDAC1 to p53 though an undefined bridging protein, resulting in deacetylation of p53 and a reduction in the expression of p53 response genes, including genes for Hdm2 and $p21^{WAF-1}$ [28]. If CtBP proteins function as this bridging molecule, then they could be predicted to have HDAC1-dependent inhibitory effects on promoters such as $p21^{WAF-1}$, in addition to the effects that were observed for the Hdm2 and Bax promoters in the absence of HDAC1 transfection.

Although we have clearly demonstrated that a hypoxia-regulated interaction occurs between endogenous Hdm2 and hCtBP2 proteins, our data defining the functional consequence of this interaction is dependent of the analysis of synthetic reporter constructs. However, recent analysis of gene expression profiles in mouse embryo fibroblasts (MEFs) from CtBP1 and CtBP2 homozygous null animals has recently confirmed that CtBP can repress expression of a range of proapoptotic genes, including the p53 target genes *bax*, *PERP*, and *noxa* [29]. Expression of all of these genes was upregulated in CtBP null MEFs and rerepressed when CtBP2 was reexpressed in these cells. Reporter-based assays performed by the authors of this recent manuscript led them to conclude that this effect was independent of a direct effect of CtBP on p53, and the mechanism underlying the inhibition was not defined. However, their assays were performed in MEF cells, in which endogenous Hdm2 levels are very low, and under conditions of chemotherapeutic drug exposure, which would have disrupted the interaction between Hdm2 and p53, and they therefore may not have detected the effects that we have demonstrated here. The authors also clearly demonstrated that CtBP null MEFs are hypersensitive to cell killing by a range of proapoptotic stimuli, with clear implications for the role of CtBP family proteins in tumor biology. Evidence that we have presented here provides, in part, a mechanistic explanation for these striking observations.

Hdm2 is emerging as a key regulator of cell-cycle control and commitment to apoptosis [21]. The identification of protein:protein interactions made by Hdm2 has resulted in a number of significant advances in our understanding of how these processes can be controlled by diverse cellular stimuli, such as the activation of oncogenes [30] and ionizing radiation [4]. Several Hdm2 binding proteins are also promising targets for the design of novel cancer therapies [31]. Hypoxic stress induces a p53-dependent apoptotic response and in many types of cancer may be responsible for the positive growth advantage of tumor cells in which the p53 gene is mutated [32]. The mechanisms whereby hypoxia, and hypoxia-mimicking chemicals such as $CoCl_2$ and deferoxamine, induce p53-dependent apoptosis are now beginning to be elucidated. There is evidence that the mechanism may be either dependent or independent on the activation of p53′s transactivation activity, depending on the cell type being studied and the exact nature of the stress involved [5, 33]. Our finding that hypoxia-mimicking conditions result in the dissociation of Hdm2:hCtBP2 complexes, and a derepression of p53-dependent transcription, provides a significant advance in the understanding of these pathways.

CtBP family members interact with, and modify the activity of, a large number of proteins with key roles in development, differentiation, cell-cycle control, and apoptosis [13, 14, 34]. The majority of the interactions that have been described to date are dependent on the presence of a PXDLS motif in the interacting protein. In a recent two-hybrid analysis [35], however, 13 of 41 CtBP-interacting clones did not contain this motif, and similarly, there is no PXDLS sequence in Hdm2. We have demonstrated that the NADH-induced conformational change that is known in CtBP family members to result in an increase in their affinity for PXDLS motif proteins causes a decrease in hCtBP2 binding to Hdm2 protein, and we anticipate the discovery of other CtBP-protein interactions that are regulated by NADH in this way. In summary, our data demonstrates that NADH binding by CtBP proteins does not act as a simple on-off switch for binding to one set of transcription factors but rather acts as a mechanism to regulate the differential recruitment of CtBP by two distinct groups of proteins in the cell.

Supplemental Data

Supplemental Data including additional methodological details is available with this article online at http://www.current-biology.com/cgi/content/full/13/14/1234/DC1/.

Acknowledgments

A.H.M. is a Medical Research Council clinical training fellow. Funding for antibody production was from the Peel Medical Research

Trust. We are grateful to A.J. Levine, T. Elliott, G. Packham. R. Grand, and M. Oren for antibody and plasmid reagents, L. Burch for Hdm2 protein for the library screen, and T.R. Hupp for encouragement in the initial stages of the study. The manuscript was critically appraised by T. Elliott.

Received: January 21, 2003
Revised: May 29, 2003
Accepted: June 2, 2003
Published: July 15, 2003

References

1. Vogelstein, B., Lane, D., and Levine, A.J. (2000). Surfing the p53 network. Nature *408*, 307–310.
2. Blaydes, J.P., and Wynford-Thomas, D. (1998). The proliferation of normal human fibroblasts is dependent upon negative regulation of p53 function by mdm2. Oncogene *16*, 3317–3322.
3. Prives, C. (1998). Signaling to p53: breaking the MDM2-p53 circuit. Cell *95*, 5–8.
4. Wahl, G.M., and Carr, A.M. (2001). The evolution of diverse biological responses to DNA damage: insights from yeast and p53. Nat. Cell Biol. *3*, E277–286.
5. Ashcroft, M., Taya, Y., and Vousden, K.H. (2000). Stress signals utilize multiple pathways to stabilize p53. Mol. Cell. Biol. *20*, 3224–3233.
6. Haupt, Y., Maya, R., Kazaz, A., and Oren, M. (1997). Mdm2 promotes the rapid degradation of p53. Nature *387*, 296–299.
7. Kubbutat, M.H., Jones, S.N., and Vousden, K.H. (1997). Regulation of p53 stability by Mdm2. Nature *387*, 299–303.
8. Honda, R., Tanaka, H., and Yasuda, H. (1997). Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. FEBS Lett. *420*, 25–27.
9. Roth, J., Dobbelstein, M., Freedman, D.A., Shenk, T., and Levine, A.J. (1998). Nucleo-cytoplasmic 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*, 554–564.
10. Freedman, D.A., and Levine, A.J. (1998). Nuclear export is required for degradation of endogenous p53 by MDM2 and human papillomavirus E6. Mol. Cell. Biol. *18*, 7288–7293.
11. Wadgaonkar, R., and Collins, T. (1999). Murine double minute (MDM2) blocks p53-coactivator interaction, a new mechanism for inhibition of p53-dependent gene expression. J. Biol. Chem. *274*, 13760–13767.
12. Thut, C.J., Goodrich, J.A., and Tjian, R. (1997). Repression of p53-mediated transcription by MDM2: a dual mechanism. Genes Dev. *11*, 1974–1986.
13. Chinnadurai, G. (2002). CtBP, an unconventional transcriptional corepressor in development and oncogenesis. Mol. Cell *9*, 213–224.
14. Turner, J., and Crossley, M. (2001). The CtBP family: enigmatic and enzymatic transcriptional co-repressors. Bioessays *23*, 683–690.
15. Zhang, Q., Piston, D.W., and Goodman, R.H. (2002). Regulation of corepressor function by nuclear NADH. Science *295*, 1895–1897.
16. Criqui-Filipe, P., Ducret, C., Maira, S.M., and Wasylyk, B. (1999). Net, a negative Ras-switchable TCF, contains a second inhibition domain, the CID, that mediates repression through interactions with CtBP and de-acetylation. EMBO J. *18*, 3392–3403.
17. Koipally, J., and Georgopoulos, K. (2000). Ikaros interactions with CtBP reveal a repression mechanism that is independent of histone deacetylase activity. J. Biol. Chem. *275*, 19594–19602.
18. Furusawa, T., Moribe, H., Kondoh, H., and Higashi, Y. (1999). Identification of CtBP1 and CtBP2 as corepressors of zinc finger-homeodomain factor deltaEF1. Mol. Cell. Biol. *19*, 8581–8590.
19. Izutsu, K., Kurokawa, M., Imai, Y., Maki, K., Mitani, K., and Hirai, H. (2001). The corepressor CtBP interacts with Evi-1 to repress transforming growth factor beta signaling. Blood *97*, 2815–2822.
20. Argentini, M., Barboule, N., and Wasylyk, B. (2001). The contribution of the acidic domain of MDM2 to p53 and MDM2 stability. Oncogene *20*, 1267–1275.
21. Daujat, S., Neel, H., and Piette, J. (2001). MDM2: life without p53. Trends Genet. *17*, 459–464.
22. Shvarts, A., Bazuine, M., Dekker, P., Ramos, Y.F., Steegenga, W.T., Merckx, G., van Ham, R.C., van der Houven van Oordt W, van der Eb AJ, and Jochemsen AG (1997). Isolation and identification of the human homolog of a new p53-binding protein, Mdmx. Genomics *43*, 34–42.
23. Li, S., Chen, P.L., Subramanian, T., Chinnadurai, G., Tomlinson, G., Osborne, C.K., Sharp, Z.D., and Lee, W.H. (1999). Binding of CtIP to the BRCT repeats of BRCA1 involved in the transcription regulation of p21 is disrupted upon DNA damage. J. Biol. Chem. *274*, 11334–11338.
24. Jin, Y., Zeng, S.X., Dai, M.S., Yang, X.J., and Lu, H. (2002). MDM2 Inhibits PCAF-mediated p53 acetylation. J. Biol. Chem. *14*, 14.
25. Sundqvist, A., Sollerbrant, K., and Svensson, C. (1998). The carboxy-terminal region of adenovirus E1A activates transcription through targeting of a C-terminal binding protein-histone deacetylase complex. FEBS Lett. *429*, 183–188.
26. Subramanian, T., and Chinnadurai, G. (2003). Association of class I histone deacetylases with transcriptional corepressor CtBP. FEBS Lett. *540*, 255–258.
27. Meloni, A.R., Smith, E.J., and Nevins, J.R. (1999). A mechanism for Rb/p130-mediated transcription repression involving recruitment of the CtBP corepressor. Proc. Natl. Acad. Sci. USA *96*, 9574–9579.
28. Ito, A., Kawaguchi, Y., Lai, C.H., Kovacs, J.J., Higashimoto, Y., Appella, E., and Yao, T.P. (2002). MDM2-HDAC1-mediated deacetylation of p53 is required for its degradation. EMBO J. *21*, 6236–6245.
29. Grooteclaes, M., Deveraux, Q., Hildebrand, J., Zhang, Q., Goodman, R.H., and Frisch, S.M. (2003). C-terminal-binding protein corepresses epithelial and proapoptotic gene expression programs. Proc. Natl. Acad. Sci. USA *3*, 3.
30. Stott, F.J., Bates, S., James, M.C., McConnell, B.B., Starborg, M., Brookes, S., Palmero, I., Ryan, K., Hara, E., Vousden, K.H., et al. (1998). The alternative product from the human CDKN2A locus, p14(ARF), participates in a regulatory feedback loop with p53 and MDM2. EMBO J. *17*, 5001–5014.
31. Midgley, C.A., Desterro, J.M., Saville, M.K., Howard, S., Sparks, A., Hay, R.T., and Lane, D.P. (2000). An N-terminal p14ARF peptide blocks Mdm2-dependent ubiquitination in vitro and can activate p53 in vivo. Oncogene *19*, 2312–2323.
32. Graeber, T.G., Osmanian, C., Jacks, T., Housman, D.E., Koch, C.J., Lowe, S.W., and Giaccia, A.J. (1996). Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. Nature *379*, 88–91.
33. Koumenis, C., Alarcon, R., Hammond, E., Sutphin, P., Hoffman, W., Murphy, M., Derr, J., Taya, Y., Lowe, S.W., Kastan, M., et al. (2001). Regulation of p53 by hypoxia: dissociation of transcriptional repression and apoptosis from p53-dependent transactivation. Mol. Cell. Biol. *21*, 1297–1310.
34. Hildebrand, J.D., and Soriano, P. (2002). Overlapping and unique roles for C-terminal binding protein 1 (CtBP1) and CtBP2 during mouse development. Mol. Cell. Biol. *22*, 5296–5307.
35. Vo, N., Fjeld, C., and Goodman, R.H. (2001). Acetylation of nuclear hormone receptor-interacting protein RIP140 regulates binding of the transcriptional corepressor CtBP. Mol. Cell. Biol. *21*, 6181–6188.