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C-terminus of Hsc70-interacting protein regulates C-terminal binding protein 2 and the expression of its target genes

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ABSTRACT

C-terminal binding protein 2 (CtBP2) is a transcriptional co-repressor involved in cell migration, apoptosis, and tumorization. Up-regulation of CtBP2 is implicated in various human cancers including breast cancers. In our present study, we found that the C-terminus of Hsc70-interacting protein (CHIP) regulated the steady-state level of CtBP2. CHIP interacted with CtBP2 via a tetratricopeptide domain in the nucleus and ubiquitinated CtBP2, marking it for proteasomal degradation. Depletion of CHIP resulted in stabilization of the CtBP2 protein, which was not affected by ultraviolet irradiation. Finally, CHIP expression regulated the expression of CtBP2 target genes. Taken together, our data demonstrate that CHIP regulates the steady-state level of CtBP2 as an E3 ubiquitin ligase and determines the expression levels of CtBP2 target genes. Regulating CtBP2 levels with CHIP may be a useful therapeutic strategy in human cancers.

1. Introduction

C-terminal binding proteins (CtBPs) were originally identified as proteins that bind to the C-terminus of E1A proteins in human adenovirus [1]. CtBPs are transcriptional co-repressors and are involved in diverse cellular events including cell migration, apoptosis, and tumorization [2,3] during which CtBPs represses expression of several epithelial genes such as *E-cadherin* [4,5], *Keratin 8* [4], and apoptotic genes such as *Bax* [4] and *Bik* [6]. CtBPs are highly conserved among species, and two CtBP genes (*CtBP1* and *CtBP2*) have been identified in the human genome [7]. The human CtBP1 and CtBP2 proteins share 78% amino acid homology [7] and have common target genes [4–6]. Nevertheless, *ctbp1*$^{-/-}$ and *ctbp2*$^{-/-}$ mice show clear phenotypic differences. *ctbp2*$^{-/-}$ mice show embryonic lethality due to severe defects in early embryonic development, whereas *ctbp1*$^{-/-}$ mice have a shorter but near normal lifespan [8], suggesting that the functions of these proteins may be differentially regulated despite sequence homology and partially redundant activities.

Several reports suggest that CtBPs may play a role in tumorization [3]. Genomic studies revealed CtBP1 and CtBP2 as biomarkers in colon carcinoma [9] and prostate cancer [10]. Up-regulation of CtBPs has been reported. Elevated CtBP1 protein is implicated in initiation of human adenoma [11], and CtBP2 protein is highly expressed in breast [12] and ovarian cancers [13]. In addition, high expression of both proteins has been reported in colon cancer [14]. Recently, elevated CtBP2 has been shown to play a role in ovarian tumorization by modulating histone deacetylase activity [15]. Collectively, these studies indicate that abundance of CtBP proteins is a crucial factor in tumorization and understanding how CtBP proteins are regulated is important.

Regulation of CtBPs involves tumor suppressors and subsequent proteasome activity. Adenomatous polyposis coli targets CtBP1 for degradation [11]. CtBPs are phosphorylated by homeodomain-interacting protein kinase-2 (HIPK2) upon ultraviolet (UV) irradiation and subsequently targeted for proteasome-dependent degradation [16]. Alternative reading frame also targets CtBPs for proteasomal degradation and triggers Bik-dependent apoptosis [6]. We previously identified X-linked inhibitor of apoptosis protein (XIAP) as an E3 ligase for CtBP1 and showed that the expression of CtBP1 target genes is affected by CtBP1 levels, which are determined by XIAP [17]. However, how CtBP2 is regulated remains unknown.

C-terminus of Hsc-70 interacting protein (CHIP) was originally identified as a co-chaperone E3 ligase, which ubiquitinates misfolded or abnormal proteins presented by molecular chaperones such as heat shock protein (Hsp)70 and is thereby implicated in protein aggregation diseases [18]. However, recent reports have

Abbreviations: CtBP2, C-terminal binding protein 2; CHIP, C-terminus of Hsc70-interacting protein; HIPK, homeodomain-interacting protein kinase; XIAP, X-linked inhibitor of apoptosis protein; Hsp, heat shock protein; TPR, tetratricopeptide; RT-PCR, reverse transcription-polymerase chain reaction; BiFC, biomolecular fluorescence complementation.

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demonstrated that CHIP plays an important role in tumorization by specifically regulating tumor-related proteins in oncogenic pathways [19–22].

Here, we investigated whether CHIP and/or XIAP affected the steady-state level of CtBP2. We examined whether CHIP functioned as an E3 ligase to regulate the levels of CtBP2 in ubiquitin–proteasome-dependent manner. Finally, we investigated the role of CHIP in the transcription of CtBP2 target genes.

2. Materials and methods

2.1. Antibodies and immunoprecipitation

Anti-CtBP2 and anti-XIAP (BD Biosciences), anti-Myc (Upstate), anti-HA (Covance), anti-tetraHis (QIAGEN), anti-BAX (Cell Signaling), anti-GST (Santa Cruz Biotechnology), and anti-actin (Bethyl) were used for Western blotting (WB) or immunoprecipitation. For immunoprecipitation, lysates were incubated with the indicated antibody overnight at 4 °C, and immunocomplexes that were recovered with protein-A Sepharose (Sigma) were washed three times with washing buffer (20 mM Tris pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.1% Triton X100) and analyzed with WB.

2.2. siRNA and UV irradiation

HeLa cells or MCF7 cells were transfected with 100 pmol XIAP siRNA (Ambion), CHIP siRNA (Dhamacon), or scrambled RNA (Genolution) using Lipofectamine 2000 (Invitrogen) or X-tremeGENE HP DNA Transfection Reagent (Roche). After 48 h, cells were harvested and lysed for WB. U2OS cells were transfected with 100 pmol CHIP siRNA or scrambled RNA. After transfection for 48 h, cells were washed with phosphate-buffered saline (PBS) and treated with 20 J/m^2 UV/C. Twelve hours after irradiation, the cells were harvested for WB.

2.3. Reverse transcription–polymerase chain reaction (RT–PCR) analysis

Total RNA was isolated using easy-spin™ (iNtRON) according to manufacturer's instructions. Synthesis of cDNA was performed with M-MuLV reverse transcriptase (MP Biomedicals). RT–PCR was performed using a Maxime PCR PreMix kit (iNtRON) according to manufacturer's instructions. The primer sets used were *Bik* (sense: 5′-TCCTATGGCTCTGCAATTGTCA-3′, antisense: 5′-GGCAGGAGTGAATGGCTCTTC-3′), *E-cadherin* (sense: 5′-TGCCCAGAAAATGAAAAAGG-3′, antisense: 5′-GGATGACACAGCGTGAGAGA-3′), *Bax* (sense: 5′-ACCAAGAAGCTGAGCGAGTGTC-3′, antisense: 5′-AGAAAGATGGTCACGGTCTGCC-3′), *Sirt1* (sense: 5′-GCAGATTAGTAGGCGGCTTG-3′, antisense: 5′-TCTGGCATGTCCCACTATCA-3′), *p21* (sense: 5′-TGGGGATCGCCGTCAGAACCC-3′, antisense: 5′-TTAGGGCTTCCTCTTGGAGAA-3′), and *GAPDH* (sense: 5′-AGTCAACGGATTTGGTCGT-3′, antisense: 5′-TTGATTTTGGAGGGATCTCG-3′). PCR products were electrophoresed on 2% agarose gels followed by staining with ethidium bromide.

2.4. Protein half-life

U2OS cells were transfected with empty vector or HA-CHIP. Twenty-four hours after transfection, cells were treated with 200 μg/ml cyclohexamide and harvested at 0, 4, 8, and 12 h. Cells were lysed with lysis buffer, and samples were subjected to WB.

2.5. GST pull-down assay

GST-CtBP2, CHIP-6His, and Hsp70-6His were generated by cloning into pGEX4T-1 and pET 23a vectors and were purified. GST or GST-CtBP2 on glutathione-agarose beads (GE Healthcare) were incubated with CHIP-6His and/or Hsp70-6His in lysis buffer for 2 h at 4 °C in a rotator. The glutathione beads were washed three times with 500 μl wash buffer. Precipitated proteins were eluted by adding 1× sodium dodecyl sulfate (SDS) sample buffer and detected by immunoblotting with anti-His antibodies.

2.6. His pull-down assay

Hsp70-6His on Ni–NTA-agarose beads (QIAGEN) were incubated with GST, GST-CHIP, and GST-CtBP2 in lysis buffer for 2 h at 4 °C in rotator. The Ni–NTA beads were washed three times with 500 μl wash buffer. Precipitated proteins were eluted by adding 1× SDS sample buffer and detected by immunoblotting with anti-GST antibodies.

2.7. Biomolecular fluorescence complementation (BiFC) assay

For this assay, we cloned CtBP2 into the pBiFC-VN 173 vector and the CHIP gene into the pBiFC-VC 155 vector (a kind gift from Dr. Lee K.H. at Chosun Univ.). HeLa cells were co-transfected with BiFC expression vectors. After incubation for 18 h, cells were washed with PBS, fixed in 3.7% paraformaldehyde, and washed twice with PBS. After washing, cells were stained with DAPI and mounted on glass slides. To capture BiFC images, samples were analyzed with confocal microscopy.

3. Results and discussion

3.1. The CtBP2 protein level is inversely correlated with CHIP expression

Previously, we demonstrated that XIAP functions as an E3 ubiquitin ligase for CtBP1 and regulates the steady-state level of CtBP1 via the ubiquitin–proteasome pathway [17]. As CtBP1 shares high homology in protein sequence with CtBP2, we tested whether CtBP2 protein level was affected by XIAP expression. CtBP2 protein levels did not decrease. Rather, they greatly increased when XIAP was expressed in a dose-dependent manner in U2OS cells (Fig. 1A, left). Similar results were obtained in HeLa cells (data not shown). However, no change in CtBP2 protein level was observed in cells depleted of XIAP by siXIAP compared to control cells (Fig. 1A, right). Our results were confusing because XIAP expression somehow stabilized and increased CtBP2 protein, but on the other hand, XIAP depletion showed no obvious change in the CtBP2 protein level. Therefore, we concluded that XIAP may regulate CtBP2 differently than it regulates CtBP1.

Recently, CtBP2 and Hsp70 were shown to be associated, together with synaptic ribbons in photoreceptors and in hair cells [23]. CHIP is a co-chaperone E3 ligase that regulates the client proteins of Hsp70. Therefore, we hypothesized that CHIP may be involved in regulating CtBP2, and tested whether overexpression of CHIP affects CtBP2 level in U2OS cells. The protein level of CtBP2 decreased upon overexpression of CHIP in a dose-dependent manner (Fig. 1B, left). The steady-state level of CtBP2 protein was also significantly up-regulated when CHIP was depleted with siCHIP (Fig. 1B, right).Taken together, these results demonstrate that the steady-state level of CtBP2 is inversely correlated with CHIP expression and suggest that CHIP, but not XIAP, may regulate the level of CtBP2 protein as an E3 ubiquitin ligase.

3.2. CHIP interacts with CtBP2 via a tetratricopeptide (TPR) domain

To examine whether CHIP interacts with CtBP2, we expressed Myc/His-CtBP2 and HA-CHIP in HEK293 cells and performed

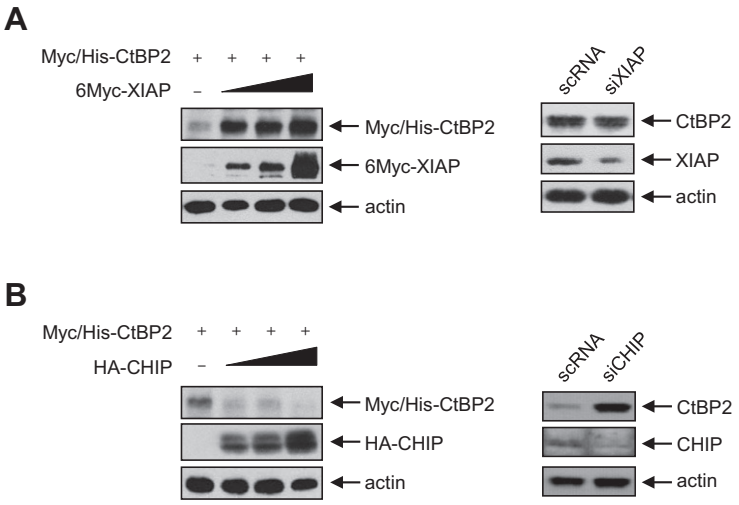

Fig. 1. The CtBP2 protein level is inversely correlated with CHIP expression. (A) U2OS cells were co-transfected with Myc/His-CtBP2 and increasing amounts of 6Myc-XIAP. After transfection for 24 h, the cells were lysed for WB using anti-Myc and anti-actin. HeLa cells were transfected with scRNA (scrambled RNA) and siXIAP for 48 h. (B) U2OS cells were co-transfected with Myc/His-CtBP2 and increasing amounts of HA-CHIP. After transfection for 24 h, the cells were lysed for WB using anti-Myc, anti-HA, and anti-actin. HeLa cells were transfected with scRNA and siCHIP for 48 h. The steady-state level of CtBP2 was elevated in cells depleted of CHIP with siCHIP.

co-IP experiments with anti-Myc for CtBP2. WB showed strong binding of CHIP with CtBP2 (Fig. 2B). The interaction of CHIP with target proteins is mostly mediated by Hsp70 via a TPR domain at the N-terminus of CHIP [24]. The primary structure of CHIP protein does not contain a typical PXDLS motif, which most CtBP-interacting proteins have [2], collectively suggesting that their interaction may require a mediator such as Hsp70. Therefore, we investigated the region of CHIP required for its interaction with CtBP2 and whether a mediator is necessary for interaction of CHIP with CtBP2. We generated various CHIP deletion mutants (Fig. 2A, left), including a U-box deletion mutant, which cannot bind to the E2 ubiquitin conjugating enzyme, a TPR deletion mutant in which the Hsp70 interaction domain is deleted, and more specifically, a CHIP K30A mutant that does not interact with Hsp70 [24]. Co-immunoprecipitation experiments revealed that CtBP2 interacted with the U-box-deleted CHIP mutant and the CHIP K30A mutant but clearly not with the TPR-deleted CHIP mutant. The interaction of CtBP2 with the CHIP K30A mutant appeared weaker compared to the interaction with CHIP WT (Fig. 2A, right). These results suggest that CtBP2 interacts with CHIP via a TPR domain, but the interaction of CtBP2 with CHIP may be partially dependent on Hsp70.

CHIP has recently been shown to interact with target proteins independent of molecular chaperones [25], and CHIP and its target protein physically interact [26]. Therefore, we further investigated the interaction of CtBP2 with CHIP using in vitro binding assays. First, we carried out GST pull-down assays using purified CHIP-6His, Hsp70-6His, and GST-CtBP2 or GST beads to see if CHIP directly bound to CtBP2. WB showed that no CHIP-6His protein bound to GST-CtBP2 beads in the absence or presence of Hsp70-6His, indicating that neither CHIP or Hsp70 directly binds to CtBP2 (Fig. 2B). Second, to confirm that Hsp70 does not bind to CtBP2, we carried out His pull-down assays using purified GST, GST-CHIP, GST-CtBP2, and Hsp70-6His beads. GST-CtBP2 did not bind to Hsp70-6His, whereas GST-CHIP bound to Hsp70-6His (Fig. 2C), suggesting that Hsp70 may not mediate the interaction of CtBP2 with CHIP. These results indicate that CtBP2 does not directly bind to CHIP and thus requires a mediator to interact with CHIP in vivo, suggesting that another mediator rather than Hsp70 exists.

Next, to investigate where the interaction of CHIP and CtBP2 occurs in cells, we performed BiFC assays [27], which are based on the association of green fluorescent protein (GFP) fragments attached to components of the same macromolecular complex. If two proteins that are each attached to one half of the GFP (N-terminus or C-terminus fragment) interact in the same macromolecular complex in cells, the two GFP fragments combine to reform the holoprotein, and a fluorescent signal is emitted. In the present study, HeLa cells were co-transfected with VN173-CtBP2 and VC155-empty vector or VC155-CHIP (see Section 2) for 18 h, and the cells were visualized with confocal microscopy. GFP signals generated by the interaction of CtBP2 and CHIP were detected in the nucleus and in the perinuclear region (Fig. 2D, bottom). As a transcriptional co-repressor, CtBP2 is exclusively localized in the nucleus [28]. CHIP is localized mostly in the cytoplasm, but recent reports indicate that CHIP also functions in the nucleus as an E3 ligase [29]. Altogether, these data demonstrate that CHIP interacts with CtBP2 via a TPR domain in the nucleus, but another mediator rather than Hsp70 may be needed for the interaction of CtBP2 with CHIP.

3.3. CtBP2 is regulated by CHIP in a ubiquitin–proteasome-dependent manner

Next, we tested whether CHIP ubiquitinates CtBP2 and modulates its stability. HEK 293T cells were co-transfected with HA-Ub and Myc/His-CHIP WT or the CHIP deletion mutants (Fig. 2A, left) for 24 h and treated with the proteasome inhibitor, MG132 for another 6 h. Co-IPs with anti-CtBP2 showed that endogenous CtBP2 was ubiquitinated by CHIP WT for proteasomal degradation (Fig. 3A). Less ubiquitinated CtBP2 was detected when CHIP K30A was expressed, indicating that the efficiency of CtBP2 ubiquitination may depend on the strength of the interaction between CtBP2 and CHIP (Fig. 2A). However, no ubiquitinated CtBP2 was detected when the U-box-deleted CHIP mutant was expressed. Considering that the U-box-deleted CHIP mutant interacted with CtBP2 (Fig. 2A), these results indicate that the U-box is responsible for the ubiquitinating activity of CHIP on CtBP2 as an E3 ubiquitin ligase.

Next, we examined the stability of CtBP2 in U2OS cells by measuring its half-life following cyclohexamide treatment. The result showed that the half-life of endogenous CtBP2 in cells overexpressing CHIP was substantially shorter than in parental control cells (Fig. 3B). Taken together, these results demonstrate that the CtBP2 protein level was regulated by a CHIP-dependent ubiquitin–proteasome pathway.

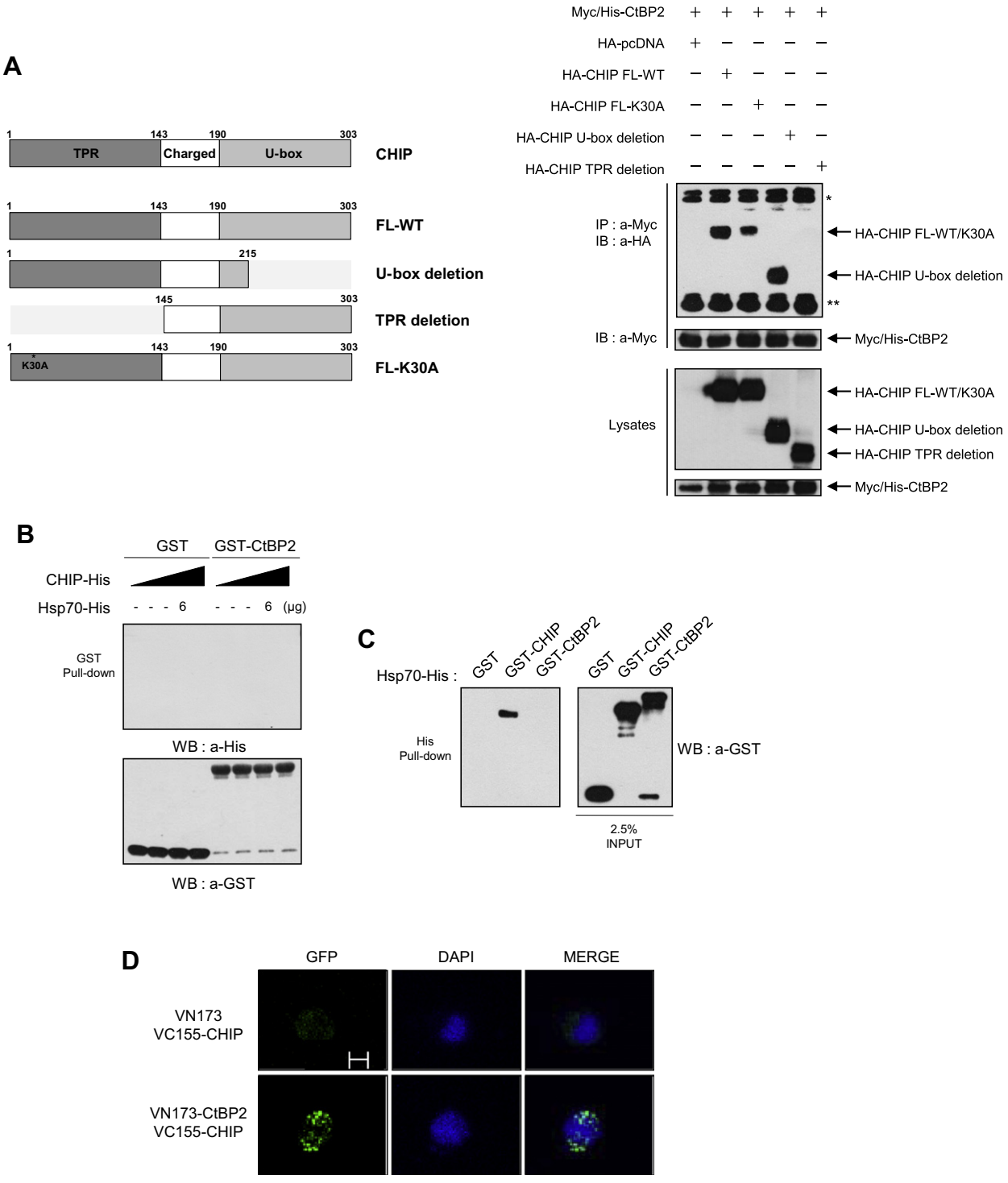

Fig. 2. CHIP interacts with CtBP2 via the TPR domain. (A) Schematic diagram of CHIP WT and various CHIP deletion mutants (left). 293T cells were co-transfected with Myc/His-CtBP2 and HA-CHIP deletion mutants. Twenty-four hours after transfection, the cell lysates were immunoprecipitated with anti-Myc antibody and immunoblotted with anti-HA (right). *IgG heavy chain, **IgG light chain. IB: immunoblot; IP: immunoprecipitation. (B) GST-pull down assay. GST-CtBP2 and GST protein were immobilized on glutathione agarose beads and incubated with purified CHIP-6His in the absence or presence of Hsp70-6His protein. After incubation, the beads were analyzed by WB using anti-His and anti-GST. (C) His pull-down assay. Hsp70-6His on Ni–NTA beads was incubated with GST, GST-CHIP, and GST-CtBP2 and beads were analyzed with WB using anti-GST. (D) BiFC assay. Bright green GFP fluorescence was only detected when VN173-CtBP2 and VC155-CHIP were co-expressed (bottom). Bar, 10 μm (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

UV irradiation decreases the protein level of CtBPs in cancer cells, and CtBPs are stabilized by MG132 treatment [15]. Our data showed that CHIP depletion resulted in up-regulation of the steady-state level of CtBP2 (Fig. 1B) and that CHIP ubiquitinated CtBP2 for proteasomal degradation (Fig. 3A). Thus, we expected that CHIP would negatively regulate the level of CtBP2 protein upon UV irradiation. Our result confirmed the previous finding and showed that UV irradiation down-regulated CtBP2 in control U2OS cells with scRNA and CtBP2 was up-regulated in CHIP-depleted U2OS cells (Fig. 3C). As expected, we observed that the up-regulated CtBP2 protein was stabilized and not decreased in CHIP-depleted U2OS cells upon UV irradiation (Fig. 3C), strongly suggesting that CHIP

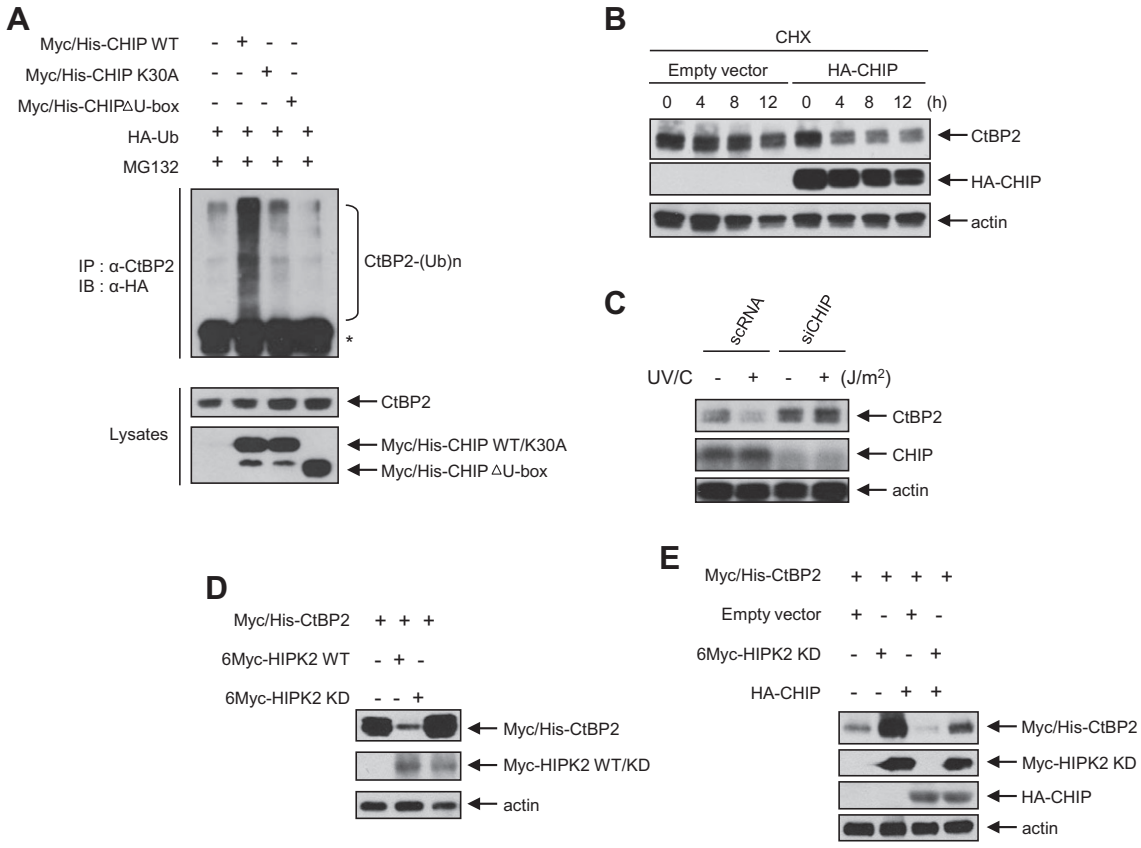

Fig. 3. CtBP2 is regulated by CHIP in a ubiquitin–proteasome-dependent manner. (A) Comparison of CtBP2 ubiquitination by WT CHIP or the CHIP domain mutants in 293T cells. IB: immunoblot; IP: immunoprecipitation. (B) U2OS cells were transfected with empty vector or HA-CHIP to determine the half-life of CtBP2. Twenty-four hours after transfection, the cells were treated with 200 μg/ml cyclohexamide and harvested at the indicated time points. Cell lysates were analyzed with WB using anti-CtBP2, anti-HA, and anti-actin. (C) U2OS cells were transfected with scRNA or siCHIP. After transfection for 48 h, the cells were treated with 20 J/m^2 UV/C for 12 h. The cells were harvested and lysed for WB. (D) 293 cells were co-transfected with Myc/His-CtBP2 and Myc-HIPK2 WT or Myc-HIPK2 KD (kinase dead mutant). After transfection for 24 h, the cells were harvested and lysed for WB using anti-Myc and anti-actin. (E) U2OS cells were co-transfected with Myc/His-CtBP2 and empty vector or Myc-HIPK2 KD in the presence or absence of HA-CHIP. After transfection for 24 h, the cells were lysed for WB using anti-Myc, anti-HA, and anti-actin (right).

functions as an E3 ligase to negatively regulate CtBP2 upon UV irradiation.

HIPK2 is the main mediator that phosphorylates and degrades CtBPs via a UV-induced signaling pathway, and the kinase activity of HIPK2 is essential for degradation of CtBPs [16]. These reports and our results raised the possibility that CHIP may regulate CtBP2 via a HIPK2-dependent signaling pathway. We first examined whether CtBP2 was degraded in the presence of HIPK2 without UV irradiation. CtBP2 was significantly down-regulated when WT HIPK2 was expressed, but when the HIPK2 KD (kinase dead) mutant, which does not have kinase activity, was expressed, the level of CtBP2 protein was increased (Fig. 3D, left), confirming that CtBP2 was degraded in a HIPK2 kinase activity-dependent manner as reported [16]. Next, we examined whether the regulation of CtBP2 by CHIP was affected by co-expression of the HIPK2 KD mutant in U2OS cells. Overexpression of CHIP only significantly down-regulated CtBP2 (also shown in Fig. 1B, right), but co-expression of CHIP with HIPK2 KD elevated CtBP2 protein to a level much higher than in control cells (Fig. 3E). Thus, CHIP may negatively regulate CtBP2 as an E3 ubiquitin ligase via a HIPK2-dependent signaling pathway.

3.4. Regulation of CtBP2 by CHIP affects the expression of CtBP2 target genes

Our results suggested that regulation of CtBP2 by CHIP may influence the expression of CtBP2 target genes. To test our hypothesis, we analyzed the transcript levels of CtBP2 target genes such as *E-cadherin* [4,5], *Bik* [6], *Sirt1* [30], and *p21*[4] in U2OS cells transfected with scRNA or siCHIP. CHIP depletion resulted in up-regulation of the steady-state level of CtBP2 protein (Fig. 4A, bottom), consistent with the results shown in Figs. 1B and 3C. Consequently, RT–PCR results showed that *E-cadherin* mRNA levels in CHIP-depleted cells were significantly reduced compared to control cells, and the mRNA levels of the other genes tested were also down-regulated (Fig. 4A), demonstrating that transcription of CtBP2 target genes was greatly repressed by the up-regulated CtBP2 in CHIP-depleted cells.

The pro-apoptotic protein Bax is negatively regulated by CtBPs [4], and increased Bax expression sensitizes human breast cancer MCF7 cells to a variety of stresses and induces apoptosis [31]. Thus, we examined whether CHIP regulates the level of *Bax* in MCF7 cells. When we depleted CHIP with siCHIP in MCF7 cells, *Bax* mRNA was significantly reduced compared to control cells. On the contrary, *Bax* mRNA increased in HA-CHIP-overexpressing cells (Fig. 4B). In addition, the protein level of Bax was reduced in CHIP-depleted cells but increased in CHIP-overexpressing cells (Fig. 4C), suggesting that CHIP may determine the level of Bax by regulating CtBP2 protein. Taken together, these results suggest that transcription of CtBP2 target genes may be determined by the steady-state level of CtBP2, which is regulated by CHIP.

CtBP2 was recognized as an oncogenic protein that represses pro-apoptotic genes, and elevated CtBP2 is implicated in various human cancers including breast cancer [12–14], suggesting that maintaining a proper level of CtBP2 is crucial for preventing tumorization. Our study provides the first evidence that CHIP as an E3

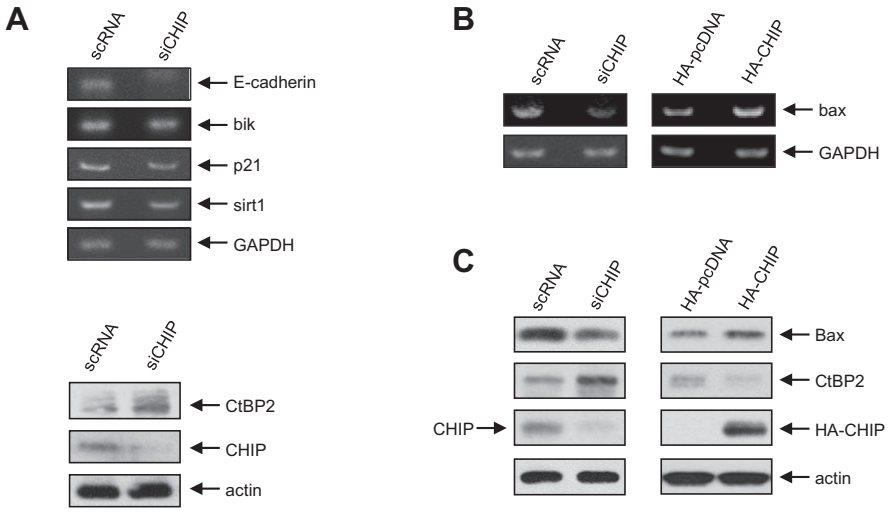

Fig. 4. CHIP regulation of CtBP2 affects the expression of CtBP2 target genes. (A) U2OS cells were transfected with scRNA or siCHIP. Forty-eight hours after transfection, the cells were harvested for RNA isolation and WB. RT-PCR was performed using specific primers for the indicated genes (Section 2). (B, C) MCF7 cells were transfected with scRNA or siCHIP (left) and empty vector or HA-CHIP (right). After transfection for 48 h, cells were harvested for RNA isolation and WB. RT-PCR and WB were performed using *Bax* primers and anti-Bax, anti-CtBP2, anti-CHIP, anti-HA, and anti-actin.

ubiquitin ligase negatively regulates CtBP2 in a ubiquitin–proteasome-dependent manner. CHIP was originally identified as a quality control E3 ubiquitin ligase, but recent evidence suggests that CHIP functions as a tumor suppressor protein to regulate tumor-related proteins. CHIP protein is decreased in human breast cancer tissues and is inversely correlated with their malignancy [21]. The physiological relevance of CHIP and CtBP2 to the pathogenesis of human breast cancer has never been addressed, but our present study implies that the elevated CtBP2 observed in human breast cancer [12] may be due to inefficient regulation caused by low levels of CHIP protein and may contribute to the anti-apoptotic property of human breast cancer by repressing pro-apoptotic gene expression.

In summary, our results demonstrate that CHIP negatively regulates the steady-state level of CtBP2 through its E3 ligase activity, and thereby, regulates the transcription of CtBP2 target genes. Better understanding of the regulatory mechanism of CtBP2 by CHIP may provide a useful strategy for treating various human cancers.

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