CHIP Controls the Sensitivity of Transforming Growth Factor-β Signaling by Modulating the Basal Level of Smad3 through Ubiquitin-mediated Degradation*

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Transforming growth factor-β (TGF-β) signaling is critical in a variety of biological processes such as cell proliferation, differentiation, and apoptosis. TGF-β signaling is mediated by a group of proteins including TGF-β receptors and Smads. It is known that different cells can exhibit different sensitivities to TGF-β. Several molecular mechanisms, such as the differential expression of the receptor levels, have been suggested as contributing to these differences. Here, we report evidence for a novel mechanism of regulating TGF-β sensitivity that depends on the role of CHIP (carboxyl terminus of Hsc70-interacting protein) in regulating the basal level of Smad3 via the ubiquitin-dependent degradation pathway. First, using a luciferase assay we found that overexpression of CHIP inhibited TGF-β signaling, whereas silencing CHIP expression by small interfering RNAs led to increased TGF-β signaling sensitivity. Second, based on the results of cell proliferation assays and JunB expression, we found that TGF-β signaling could be abolished by stably overexpressing CHIP. Third, in those cell lines with stably expressed CHIP, we observed that the Smad3 protein level was dramatically decreased. Finally, we demonstrated that CHIP served as a U-box dependent E3 ligase that can directly mediate ubiquitination and degradation of Smad3 and that this action of CHIP was independent of TGF-β signaling. Taken together, these findings suggest that CHIP can modulate the sensitivity of the TGF-β signaling by controlling the basal level of Smad3 through ubiquitin-mediated degradation.

Transforming growth factor-β (TGF-β) superfamily cytokines play pivotal roles in a variety of bioprocesses including cell proliferation, differentiation, and apoptosis (1–3). Abnormal response to TGF-β signaling has been reported to relate to many diseases such as tumors, chronic diseases associated with tissue fibrosis, inflammatory diseases, and developmental diseases (4). TGF-β signaling is known to be mediated by many different proteins including the Smad family proteins (5), which have been classified into three different functional groups as follows: (a) receptor-regulated Smads (R-Smads; Smad1, 2, 3, 5, and 8); (b) the common Smad (Co-Smad; Smad4); and (c) the inhibitory Smads (I-Smads; Smad6 and Smad7) (1, 2, 4, 6–8). TGF-β signaling has been shown to be regulated at different levels (for reviews see Refs. 2, 3, 9–12), including ligand activation, ligand binding to TGF-β receptors, R-Smad phosphorylation (5), translocation of R-Smads and the common Smad into the nucleus (2, 3, 6, 7, 10, 13–15), Smad transcriptional activity controlled by co-activators (8, 16–18) or co-repressors (19–22), and proteasomal degradation via ubiquitination-dependent and independent pathways (11, 15, 23–27).

It has been reported that degradation of Smad proteins through ubiquitination is an important mechanism for regulating cellular responsiveness to TGF-β family ligands (11, 12, 15). The effects of most of the known ubiquitination ligases, however, are mainly for terminating the TGF-β signaling. For example, several E3 ligases, such as SCF/Roc1 (28) and Smurf1/2 (24, 25, 29–32), are thought to mediate ubiquitination and degradation of the activated form of Smad proteins. Roc1 is a Cullin-binding protein with RING finger domain and has been reported to associate with Smad3 in a Smad3-SCF/Roc1 complex in the nucleus, which is then transported into the cytoplasm for degradation (28). Smurf1 and Smurf2 are HECT (homologous to E6-AP C terminus) domain-containing E3 ligases (24, 25, 29–32). Smurf1 selectively interacts with receptor-regulated Smads specifically in the BMP (bone morphogenetic protein) pathway to trigger Smad1 ubiquitination and degradation (32). Smurf1 has also been reported to interact with TGF-β type I receptor through Smad7 to regulate receptor degradation (30). It has also been shown that TGF-β-induced Smad2/Smurf2 association with the transcriptional co-repressor SnoN leads to Smurf2 targeting SnoN for ubiquitin-mediated degradation by proteasome (29). These findings provided a mechanism for the down-regulation of TGF-β signaling via degradation of activated R-Smads (15).

It will be interesting to investigate whether some of the E3 ligases may also mediate the degradation of Smads in the Smad; RT, reverse transcription; siRNA, small interfering RNA; STAT3, signal transducer and activator of transcription 3.
CHIP Regulates TGF-β Signal

CHIP regulates TGF-β signal by reducing the basal level of Smad3 through ubiquitination.

**MATERIALS AND METHODS**

**Plasmids and Constructs**—The Myc-tagged CHIP expression vector pRK1M/Myc-CHIP was constructed by PCR based on pACT2-CHIP (23) and the mammalian expression vector pRK1M (a gift from Dr. Ying Zhang). HA-tagged CHIP constructs (pcDNA6/V5/HA-CHIP and pEFNeo/HA-CHIP) were generated using the same method by subcloning into the pcDNA6/V5 (Invitrogen) and pEFNeo (from Dr. Xin-yuan Fu) vectors, respectively. The Myc-tagged CHIP mutant H260Q (histidine to glutamine at position 260) was generated using the primers 5′-attagagcaagctgctgctg-3′ and 5′-gcctgagctagctgctgctg-3′ based on Myc-CHIP with the site-directed mutagenesis MutanBEST Kit (Takara Biotechnology Co., Ltd). The H135A mutant of the Smad3 construct was generated by a PCR-based approach by PCR from pRK5/F-Smad3 into the vector pcDNA6/V5. FLAG-tagged Smad3 (pRK5-Smad3, pRK5-Smad3, or pRK5-Smad4) was expressed in Mv1Lu cells (28). The reaction mixture was first incubated at 50 °C for 30 min before reverse transcription and then denatured at 94 °C for 2 min before PCR cycles. 26 cycles of PCR were conducted under the conditions of 94 °C for 50 s, 55 °C for 50 s, and 72 °C for 1 min. The product was extended at 72 °C for 10 min before storing at 4 °C. The RT-PCR for β-actin was performed with the same aliquots samples as an internal control.

**Ubiquitination Assay in Vivo**—293T cells were transfected with the related constructs to express HA-ubiquitin, His-tagged Smad3, and Myc-tagged CHIP proteins as indicated in the presence of 50 μg/ml G4132 for 5 h. 7.5 ng/ml TGF-β1 was used as a final concentration for treatment of the cells. The cells were lysed, precipitated, and immunoblotted as described previously (23).

**Luciferase Assays**—Luciferase assays were carried out in Mv1Lu cells by co-transfection (CAGA)_3-MLP-Lux and different constructs as indicated. The vectors were used to balance the transfection. 6 h after transfection, the cells were treated with TGF-β1 (7.5 ng/ml) for 24 h. Luciferase activities were measured by the Dual luciferase assay system (Promega) in Top Count (Packard). The Renilla luciferase internal control vector (pRL-TK) (Promega) was always used for calibration of the even transfection efficiency. 20 ng/ml leukemia inhibitor factor (LIF) (R&D Systems, Inc.) was used in the experiment for the STAT3 luciferase activity assay.

**RESULTS**

CHIP Can Significantly Reduce the Sensitivity of TGF-β Signaling As Demonstrated by Smad3-dependent Transcriptional Activities—To test the role of CHIP on TGF-β signal transduction, we determined whether CHIP had any effect on Smad3-dependent transcriptional activities that could be measured by the luciferase reporter (CAGA)_3-MLP-Lux. We performed the experiments in TGF-β response cells (Mv1Lu) and found that 7.5 ng/ml TGF-β stimulated the luciferase activity 5.6-fold over control (Fig. 1A, left columns). The same dose of TGF-β, on the other hand, did not stimulate the reporter activity in cells overexpressing CHIP (Fig. 1A, right columns). This result suggests that overexpression of CHIP can desensitize cells in response to TGF-β signaling.
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To determine whether such an effect of overexpressed CHIP is specific, we conducted a similar experiment using the STAT-responsive reporter pGL2-M67/sie, which responds to several cytokines (e.g., LIF) in the presence of STAT3 (43). Cells were co-transfected with the STAT3 binding site (M67) pGL2-M67/sie was used for the demonstration of STAT3 activity. STAT3 and CHIP were co-overexpressed as indicated with (filled columns) or without (open columns) the treatment of LIF for 48 h after transfection. Luciferase activities were measured as described above. C, CHIP inhibits the luciferase activities induced by overexpression of a constitutively active TGF-β receptor. Mv1Lu cells were transfected with TGF-βRI(T204D) with (filled columns) or without (open columns) the overexpression of the HA-CHIP protein together with the reporter construct (CAGA)12-MLP-Lux. D, CHIP inhibits the luciferase activities induced by overexpression of Smad3 and Smad4. Mv1Lu cells were transfected with the TGF-β luciferase reporter constructs (CAGA)12-MLP-Lux, pRK5/FLAG-Smad3, and pRK5/FLAG-Smad4 (indicated as Smad3+4), with (filled columns) or without (open columns) the overexpression of HA-CHIP. 24 h after transfection, cells were extracted and measured for luciferase activity. E, CHIP inhibits Smad2 activity. The experiment was performed according to that described for panel D, except that Smad2 and Smad4 were used. In all the above experiments the assays were carried out in triplicate, the data were normalized with internal controls, and the averages are shown with the S.D.

FIG. 1. CHIP inhibits Smad3 transcriptional activity. A, CHIP inhibits the luciferase activities induced by TGF-β1. Mv1Lu cells were transfected with (CAGA)12-MLP-Lux, pcDNA6/HA-CHIP, or control vector pcDNA6. 6 h after transfection, the cells were treated with (filled columns) or without (open columns) TGF-β1 (7.5 ng/ml) for 24 h. Luciferase activities were measured. B, CHIP has no effect on STAT3 activity. A reporter construct coding the STAT3 binding site (M67) pGL2-M67/sie was used for the demonstration of STAT3 activity. STAT3 and CHIP were co-overexpressed as indicated with (filled columns) or without (open columns) TGF-β1 for 24 h after transfection. Luciferase activities were measured as described above. C, CHIP inhibits the luciferase activities induced by overexpression of a constitutively active TGF-β receptor. Mv1Lu cells were transfected with TGF-βRI(T204D) with (filled columns) or without (open columns) the overexpression of the HA-CHIP protein. These results suggest that overexpressing CHIP could effectively block the transcriptional modulator activities of Smad3 and Smad4. These experiments were repeated four times in triplicate, and the results were consistent. Similar data were also obtained in COS-7 and 293T cells (data not shown). In yet another experiment, we found that overexpression of CHIP also blocked the activity of Smad2 (Fig. 1E). These data together suggest that overexpressed CHIP can negatively regulate TGF-β signaling.

Knocking Down CHIP by siRNA Can Sensitize TGF-β Signaling—The above experiments showed that overexpression of the CHIP protein could inhibit (or attenuate) TGF-β signaling. Conversely, we wanted to examine whether knocking down the endogenous CHIP could promote or facilitate the TGF-β signaling. To address this question, we created two siRNA constructs with specific sequences of CHIP driven by the U6 promoter (designated pBS/U6/CHIPi1 (23) and pBS/U6/CHIPi2). Transfection of these two constructs into 293T cells significantly blocked the expressed CHIP protein, whereas the vector
CHIP Can Desensitize TGF-β Signaling

The above data demonstrated that overexpressed CHIP could inhibit both the gene responses and the growth arrest mediated by TGF-β. We further investigated whether CHIP had any effect on the expression of the native TGF-β response genes. We examined JunB, an early TGF-β response gene (4, 48, 49), in both BaF3 and Mv1Lu wild type cells (mock treated). In Mv1Lu cells, we used three independent clones stably expressing CHIP to repeat the proliferation experiment. Similar to what was observed in BaF3 cells, these CHIP-expressed cells lost the ability to respond to TGF-β-induced growth arrest (47). When increasing amounts of TGF-β were added to the medium, the BaF3 mock cells exhibited growth arrest (Fig. 3B, line with squares). Cells stably expressing CHIP (Fig. 3A, second lane from left), however, remained resistant to the same doses of TGF-β (Fig. 3B, line with diamonds). In Mv1Lu cells, we used three independent clones stably expressing the CHIP protein (Fig. 3C, first three lanes from the left) to repeat the proliferation experiment. Similar to what was observed in BaF3 cells, these CHIP-expressed cells lost the ability to respond to TGF-β-induced growth arrest (Fig. 3D). These results suggest that cells with overexpressed CHIP are less sensitive to TGF-β mediated cell cycle arrest, implying that CHIP can attenuate TGF-β signaling.

CHIP Can Attenuate Native Gene Expression Induced by TGF-β—The above data demonstrated that overexpressed CHIP could inhibit both the gene responses and the growth arrest mediated by TGF-β. We further investigated whether CHIP had any effect on the expression of the native TGF-β response genes. We examined JunB, an early TGF-β response gene (4, 48, 49), in both BaF3 and Mv1Lu wild type cells (mock treated) with or without overexpressing CHIP. Whereas TGF-β could stimulate junB gene expression in the mock cells (Fig. 4, A and B, second and sixth lane from the left), in cell lines stably expressing CHIP the JunB protein level was significantly decreased. Such effects were detectable at either 4 or 20 h after...
TGF-β exposure (Fig. 4, A and B). These data clearly suggest that the increased expression of CHIP could inhibit the native gene expression induced by TGF-β.

CHIP-mediated Degradation of Smad3 Can Occur Independent of TGF-β Signaling—To understand the mechanisms underlying the effect of CHIP-mediated inhibition of TGF-β signaling, we speculated that CHIP might mediate the degradation of Smad3 through ubiquitination based upon our previous studies (23). To test this hypothesis, we first examined the degradation of Smads induced by CHIP. As expected, overexpression of CHIP indeed decreased Smad3 protein levels in a manner sensitive to the proteasome inhibitor MG132, implying that CHIP may directly or indirectly induce the proteasomal degradation of Smad3 (Fig. 5A). This degradation was further confirmed by pulse-chase assays as shown in Fig. 5, B and C.

TGF-β signaling has been shown to enhance Smad3 turnover (15). We tested whether the CHIP-mediated degradation of Smad3 is dependent on TGF-β signaling. We assayed the level of exogenously expressed FLAG-tagged Smads and treated the cells with or without TGF-β in the presence or absence of overexpressed CHIP. Western blot analysis showed that TGF-β indeed initiated the degradation of the exogenous Smad3 (Fig. 5D, upper section, compare lanes 1 and 3). However, overexpression of CHIP did not show significant alteration of the degradation of Smad3 (Fig. 5D, upper section, lane 4). Actually, Smad3 degradation triggered by TGF-β remained at the same rate in the presence or absence of CHIP overexpression (Fig. 5D, upper section, compare lanes 1 and 2 without overexpression of CHIP and lanes 2 and 4 with overexpression of CHIP). The quantified densities of Smad3 bands (Fig. 5D, lower section) further showed that the difference between lanes 1 and 2 (without TGF-β) and that between lanes 2 and 4 (with TGF-β) were almost the same. These data suggest that CHIP-mediated Smad3 degradation might be independent of TGF-β signaling.

CHIP Can Mediate Smad3 Ubiquitination Independent of TGF-β Signaling—Previously, we had observed that CHIP can
mediated Smad1 ubiquitination (23). By optimizing our experimental procedures, we have now observed that CHIP can also induce ubiquitination of Smad3 (Fig. 6A, smear bands) in the presence of 50 μM MG132. To further test whether the CHIP-mediated Smad3 ubiquitination is subjected to the regulation of TGF-β signaling, we performed an in vivo ubiquitination experiment by overexpressing Smad3 and CHIP in 293T cells treated with or without TGF-β. We found that CHIP induced ubiquitination of Smad3 with or without TGF-β treatment (Fig. 6B). The Smad3(3S-A) mutant, which could not be phosphorylated by TGF-β (50), was also tested for its ubiquitination by CHIP. Results from our in vivo ubiquitination experiment indicated that the mutant Smad3(3S-A) was also ubiquitinated by CHIP (Fig. 6C). These data implied that the CHIP-induced Smad3 ubiquitination occurred independently of TGF-β signal activation.

Because CHIP was reported as an E3 ligase containing a U-box domain, we tested the role of the U-box in CHIP-mediated Smad3 ubiquitination. We used a mutant of CHIP (H260Q), which contained a point mutation within the U-box and lacked its ubiquitin E3 ligase, to perform the ubiquitination experiment. As shown in Fig. 6B, the CHIP (H260Q) mutant exhibited significantly reduced ability to mediate Smad3 ubiquitination. In luciferase assays, the mutant CHIP (H260Q) also failed to inhibit Smad3-mediated transcriptional activation (Fig. 6D). These data indicate that CHIP functions as an E3 ligase for Smad3 and that such activity is dependent on its U-box.

CHIP Maintains Basal Level of Smad3 but Does Not Affect the Kinetics—The above experiments (Fig. 5) demonstrated that CHIP mediates degradation of the exogenously overexpressed Smad3. To investigate whether CHIP can change the basal level of the endogenous Smad proteins, we stably expressed CHIP in both Mv1Lu and BaF3 cells and tested the levels of the endogenous Smad3 in the presence or absence of TGF-β. Results of our Western blot analysis showed that the endogenous Smad3 protein level in cells with stably overexpressed CHIP was greatly decreased compared with that in wild type cells (Fig. 7A, compare lanes 1 with 5, upper two sections). The presence of CHIP, however, did not prevent TGF-β to enhance the accumulation of Smad3 (Fig. 7A, comparing lanes 1 to 4 with lanes 5 to 8, upper two sections), even though the basal levels under CHIP were dramatically lower.

Finally, we examined the kinetics of degradation of phosphorylated Smad3 under the treatment of TGF-β with or without CHIP. We found that TGF-β could mediate phosphorylation of Smad3 in the Mv1Lu cell lines regardless of whether or not CHIP was stably expressed (Fig. 7C). Apparently, the degradation rate of the phosphorylated Smad3 was not affected by the overexpressed CHIP protein (Fig. 7D and E). These results thus suggest that CHIP mainly regulates the basal Smad3 protein level and that its effect does not seem to affect the kinetics of the TGF-β signaling.

DISCUSSION

TGF-β signaling has been intensively studied during the past two decades (1–3). It is now known that TGF-β signaling is mediated by Smad proteins in the cytoplasm where Smads are phosphorylated and translocated into the nucleus after activation of TGF-β receptors (2, 9). It has been documented

![Figure 5](https://via.placeholder.com/150)
that several proteins at different levels could control the activities of Smads (R-Smads and common Smads) to regulate TGF-β signaling (2, 3). One of the most advanced signs of progress in understanding the regulation of TGF-β signal transduction is the study of the termination mechanism of the signaling regulated by ubiquitin-proteasome-dependent degradation (11, 51). TGF-β signaling could be tightly controlled by positive and negative regulators (10) via the accumulation and subsequent degradation of the phosphorylated Smads (R-Smads) (15). The down-regulation of activated Smad proteins could be an effective mechanism for effectively turning off TGF-β signaling to avoid excess stimulation. To date, several E3 ligases have been identified as participating in terminating the signaling to avoid excess stimulation. To date, several E3 ligases have been identified as participating in terminating the signaling to avoid excess stimulation. These data thus indicate that CHIP is a negative regulator for TGF-β signaling.

Interestingly, unlike Roc1 and Smurf1/2, in which the degradation of Smads was dependent on TGF-β stimulation (24, 25, 29, 30, 32), CHIP could decrease the total Smad3 level independently of the TGF-β activation. Our data demonstrated that TGF-β signaling was unnecessary for CHIP to mediate Smad3 ubiquitination and degradation. It appears that the CHIP-mediated degradation of Smad3 was independent of the phosphorylation of Smad3. For example, we observed that the Smad3(3S-A) mutant, which lost the feature of phosphorylation, could be equally ubiquitinated (Fig. 6A and B). The CHIP(H260Q) mutant loses the ability to inhibit Smad3 transcriptional activity. The wild type and mutant CHIP were overexpressed as indicated in Mv1Lu cells. Luciferase activities were measured according to the protocol described under “Materials and Methods.”
CHIP Regulates TGF-β Signal

FIG. 7. Overexpression of CHIP affects endogenous Smad2/3 protein levels but does not affect the kinetics of Smad2/3. Mv1Lu and Mv1Lu/HA-CHIP cells were treated with 7.5 ng/ml TGF-β1 from 0 to 5 h as indicated. A, the basal level of the endogenous Smad2/3 is decreased by guest on July 23, 2018http://www.jbc.org/Downloaded from by guest on July 23, 2018

A

TGF-β 1 (h)

B

Mv1Lu

Mv1Lu/CHIP

Smad2/3

HA-CHIP

0

1

3

5

0

1

3

5

B

TGF-β 1 (h)
P-Smad2/3

Act

Act

0

1

3

5

0

1

3

5

C

TGF-β 1 (h)
p-Smad2/3

HA-CHIP

Act

Act

0

1

3

5

0

1

3

5

D

TGF-β 1 (h)
p-Smad2/3

HA-CHIP

Act

Act

0

1

3

5

0

1

3

5

E

TGF-β 1 (h)
p-Smad2/3

HA-CHIP

Act

Act

0

1

3

5

0

1

3

5

Mv1Lu

Mv1Lu/CHIP

REFERENCES

1. Nakao, A., Inamura, T., Souchelnytskyi, S., Kawabata, M., Ishisaki, A., Oeda, R., Tamaki, K., Hanai, J., Heldin, C. H., Miyazono, K., and ten Dijke, P. (1997) EMBO J. 16, 5353–5362
2. Massague, J. (2000) Nat. Rev. Mol. Cell Biol. 1, 169–178
3. Wrana, J. L. (2000) Cell 100, 189–192
4. Massague, J., Blain, S. W., and Lo, R. S. (2000) Cell 103, 295–309
5. Derynck, R., Gelbart, W. M., Harland, R. M., Heldin, C. H., Kern, S. E., Massague, J., Melton, D. A., Mlodzik, M., Padgett, R. W., Roberts, A. B., Smith, J., Thomsen, G. H., Vogelstein, B., and Wang, X. F. (1996) Cell 87, 173
6. Massague, J., and Wotton, D. (2000) EMBO J. 19, 1745–1754
7. Moustakas, A., Souchelnytskyi, S., and Heldin, C. H. (2001) J. Cell Sci. 114, 4359–4369
8. Shen, X., Hu, P. P., Liberati, N. T., Datto, M. B., Frederick, J. P., and Wang, X. F. (1998) Mol. Biol. Cell 9, 3309–3319
9. Massague, J. (1996) Annu. Rev. Biochem. 67, 753–791
10. Miyazono, K. (2000) J. Cell Sci. 113, 1101–1109
11. Wang, T. (2003) Prost. Biochem. 8, di109–d127
12. Shi, Y., and Massague, J. (2003) Cell 112, 685–700
13. Zhu, H. J., Iaria, J., and Sizeland, A. M. (1999) J. Biol. Chem. 274, 32258–32264
14. Nakao, A., Afrakhte, M., Moren, A., Nakayama, T., Christian, J. L., Heuchel, R., Itoh, S., Kawabata, M., Heldin, N. E., Heldin, C. H., and ten Dijke, P. (1997) Nature 389, 631–635
15. Lo, R. S., and Massague, J. (1999) Nat. Cell Biol. 1, 472–478
16. Kim, R. H., Wang, D., Tsang, M., Martin, J., Huff, C., de Caestecker, M. P., Parks, W. T., Meng, X., Lechleider, R. J., Wang, T., and Roberts, A. B. (2000) Genes Dev. 14, 1605–1616
17. Pouysségur, C., Joyaraman, L., and Massague, J. (1998) J. Biol. Chem. 273, 22865–22868
18. Feng, X. H., Zhang, Y., Wu, R. Y., and Derynck, R. (1998) Genes Dev. 12, 2153–2163
19. Liu, X., Sun, Y., Weinberg, R. A., and Lodish, H. F. (2001) Cytoskeletal Growth Factor Rev. 12, 1–8
20. Kurokawa, M., Mitani, K., Irie, K., Matsuyama, T., Takahashi, T., Chiba, S., Yazzaki, Y., Matsumoto, K., and Hirai, H. (1998) Nature 394, 92–96
21. Wotton, D., Lo, R. S., Lee, S., and Massague, J. (1999) Cell 97, 29–39
22. Verschueren, K., Remacle, J. E., Collart, C., Kraft, H., Baker, B. S., Tyzio, P. N., Nelles, L., Wuytens, G., Su, M. T., Bodmer, R., Smith, J. C., and Huylebroeck, D. (1999) J. Biol. Chem. 274, 20449–20488
23. Li, L., Xin, H., Xu, X., Huang, M., Zhang, X., Chen, Y., Zhang, S., Fu, X. Y., and Chang, Z. (2004) Mol. Cell. Biol. 24, 856–864
24. Lin, Y., Liang, J., and Feng, X. H. (2000) J. Biol. Chem. 275, 36818–36822
25. Zhang, Y., Chang, C., Gehling, D. J., Hemmati-Brivanlou, A., and Derynck, R. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 974–979
26. Lin, Y., Martin, J., Gruendler, C., Farley, J., Meng, X., Li, B. Y., Lechleider, R., Huff, C., Kim, R. H., Grassner, W. A., Parakkal, V., and Wang, T. (2002) BMC Cell Biol. 3, 15
27. Nourry, C., Maksumova, L., Pang, M., Liu, X., and Wang, T. (2004) BMC Cell Biol. 5, 20
28. Fukushi, M., Inamura, T., Chiba, T., Ebisawa, T., Kawabata, M., Tanaka, K., and Miyazono, K. (2001) Mol. Biol. Cell 12, 1431–1443
29. Bonni, S., Wang, H. R., Causin, C. G., Kavas, P., Stroschein, S. L., Luo, K., and Wrana, J. L. (2001) Nat. Cell Biol. 3, 587–595
30. Ebisawa, T., Fukushi, M., Murakami, G., Chiba, T., Tanaka, K., Inamura, T., and Miyazono, K. (2001) J. Biol. Chem. 276, 12477–12480
31. Kavas, P., Rasmussen, B. K., Causin, C. G., Bonni, S., Zhu, H., Thomsen, G. H., and Wrana, J. L. (2000) Mol. Cell 6, 1365–1375

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Mv1Lu/HA-CHIP cells were treated with 7.5 ng/ml TGF-β1 used as 100, and the relative values were plotted in different time points (E). CHIP could also degrade Smad3 in a manner independent of mediating their ubiquitination (23). We now have evidence that CHIP could interact with Smad1, 2, and 4 and served that CHIP could mediate the degradation (23). Originally, CHIP was reported as mediating the degradation mechanism and, in turn, can modulate the sensitivity of TGF-β signaling. Our findings suggest that CHIP may regulate the basal levels of multiple Smads through a proteasome degradation mechanism and, in turn, can modulate the sensitivity of TGF-β signaling.
32. Zhu, H., Kavsak, P., Abdollah, S., Wrana, J. L., and Thomsen, G. H. (1999) Nature 400, 687–693
33. Ballinger, C. A., Connell, P., Wu, Y., Hu, Z., Thompson, L. J., Yin, L. Y., and Patterson, C. (1999) Mol. Cell. Biol. 19, 4535–4545
34. Kampinga, H. H., Kanon, B., Salomons, F. A., Kabakov, A. E., and Patterson, C. (2003) Mol. Cell. Biol. 23, 4948–4958
35. Connell, P., Ballinger, C. A., Jiang, J., Wu, Y., Thompson, L. J., Hohfeld, J., and Patterson, C. (2001) Nat. Cell Biol. 3, 93–96
36. Meacham, G. C., Patterson, C., Zhang, W., Younger, J. M., and Cyr, D. M. (2001) Nat. Cell Biol. 3, 100–105
37. Imai, Y., Soda, M., Hatakeyama, S., Akagi, T., Hashikawa, T., Nakayama, K. I., and Takahashi, R. (2002) Mol. Cell 10, 55–67
38. Cardozo, C. P., Michaud, C., Ost, M. C., Flass, A. E., Yang, E., Patterson, C., Hall, S. J., and Caplan, A. J. (2003) Arch. Biochem. Biophys. 410, 134–140
39. Zhou, P., Fernandes, N., Dodge, I. L., Reddi, A. L., Rao, N., Safran, H., DiPetrillo, T. A., Wazer, D. E., Band, V., and Band, H. (2003) J. Biol. Chem. 278, 13829–13837
40. Cyr, D. M., Hohfeld, J., and Patterson, C. (2002) Trends Biochem. Sci. 27, 368–375
41. Wiederkehr, T., Bukau, B., and Buchberger, A. (2002) Curr. Biol. 12, R26–R28
42. Murata, S., Minami, Y., Minami, M., Chiba, T., and Tanaka, K. (2001) EMBO Rep. 2, 1133–1138
43. Ulloa, L., Doodie, J., and Massague, J. (1999) Nature 397, 710–713
44. Zhang, Y., Feng, X. H., and Derynck, R. (1998) Nature 394, 909–913
45. Wong, C., Rougier-Chapman, E. M., Frederick, J. P., Datto, M. B., Liberati, N. T., Li, J. M., and Wang, X. F. (1999) Mol. Cell. Biol. 19, 1821–1830
46. Yingling, J. M., Datto, M. B., Wang, C., Frederick, J. P., Liberati, N. T., and Wang, X. F. (1997) Mol. Cell. Biol. 17, 7019–7028
47. Bai, R. Y., Koester, C., Ouyang, T., Hahn, S. A., Hammerschmidt, M., Peschel, C., and Duyster, J. (2002) Nat. Cell Biol. 4, 181–190
48. Verrecchia, F., Tacheau, C., Schorpp-Kistner, M., Angel, P., and Mauviel, A. (2001) Oncogene 20, 2205–2211
49. Liberati, N. T., Datto, M. B., Frederick, J. P., Shen, X., Wong, C., Rougier-Chapman, E. M., and Wang, X. F. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4844–4849
50. Lagna, G., Hata, A., Hemmati-Brivanlou, A., and Massague, J. (1996) Nature 383, 832–836
51. Izi, L., and Attisano, L. (2004) Oncogene 23, 2071–2078
52. Sui, G., Soocho, C., Affer, E. B., Gey, F., Shi, Y., and Forrester, W. C. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 5315–5320
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