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CHIP/Stub1 interacts with eIF5A and mediates its degradation

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ABSTRACT

eIF5A, containing the unusual amino acid hypusine, is a highly conserved protein essential for the proliferation of eukaryotic cells. Previous studies have demonstrated that the activity of eIF5A was regulated through modification of hypusine, phosphorylation and acetylation. However, no study was documented for regulation of the protein stability. Here, we report that eIF5A is a target of CHIP (the carboxyl terminus of Hsc70-interacting protein, also named Stub1), an E3 ligase with a U-box domain, through a proteomics analysis. CHIP directly interacted with eIF5A, preferably through the U-box domain, to mediate eIF5A ubiquitination and degradation. Simultaneously, we investigated that CHIP expression inversely correlated with eIF5A levels in colorectal cancers, consistent with the fact that the protein level of eIF5A was increased in the CHIP knock-out MEF cells. Taken together, we propose that CHIP regulates the eIF5A protein stability via a protein degradation mechanism. This study provides a new insight into understanding the regulation of the eIF5A stability.

1. Introduction

The human eukaryotic translation initiation factor 5A (eIF5A) is a small protein (18 KD) that is essential for protein synthesis in many eukaryotes, and is modified with a unique amino acid, hypusine [N-(4-amino-2-hydroxybutyl)-lysine], by a series of post translational events [1]. eIF5A is the only known protein with hypusine [2] and promotes translation elongation during protein synthesis [3]. Hitherto, accumulating data suggests that eIF5A plays an essential role in regulation of cell viability, growth and proliferation [4–6]. Several lines of evidence suggest that eIF5A is a key factor in the pathogenicity of different diseases including diabetes [7,8], HIV-1 infections [9], and several human cancers [10–12]. Recently, a tight regulation of the eIF5A activity and the protein level has been observed to occur through hypusination [13], phosphorylation [14] and acetylation [15,16]. The hypusination modification of eIF5A occurs at two-steps. The initial step entails conjugation of the 4-aminobutyl moiety of spermidine to the epsilon-amino group of a conserved lysine by deoxyhypusine synthase (DHS) resulting in the formation of deoxyhypusine-containing eIF5A. Deoxyhypusine is subsequently hydroxylated by deoxyhypusine hydroxylase (DHH) giving rise to hypusine-containing eIF5A [1]. The phosphorylation of eIF5A occurs at its ser2 and tyr21 residues. The phosphorylated and un-phosphorylated status of eIF5A cycles during the translation of proteins according to the growth conditions [14]. Also, eIF5A can be acetylated by PCAF and this acetylation seemed to regulate its subcellular localization [15]. After the modification, eIF5A functions to promote elongation during protein synthesis, which is important for cell growth. However, it remains unclear on the regulation of eIF5A protein stability through a degradation process.

CHIP (the carboxyl terminus of Hsc70-interacting protein), also as known as Stub1 (STIP1 homology and U-Box containing protein 1), was originally identified as an E3 ligase [17]. CHIP has been reported to initiate degradation of several oncogenic proteins including ErbB2 [18], hIF1α [19], c-Myc [20], ERα [21], Met receptor [22], Runx1 [23] and SRC-3 [24] and to regulate cell proliferation, metastasis and tumor progression. We have demonstrated that CHIP mediated ubiquitination and degradation of Smads [25,26], Runx1 [23] and Runx2 [27] in vitro. Recently, we and others observed that CHIP expression is related colon cancer and gastric cancer, seemingly to target NF-κB [28,29]. At the same time, a group reported that CHIP can recognize CIP2A (cancerous inhibitor of protein phosphates 2A) after binding of celastrol, a natural compound [30]. It appeared that CHIP is an E3 ligase with a broad of targets [31].

In this study, we demonstrate that CHIP interacts with eIF5A and regulates its degradation and ubiquitination. Our data provide new insights into the regulation of eIF5A stability.

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2. Material and methods

2.1. Plasmids, antibodies and cancer tissues

Plasmid of Myc-eIF5A was kindly supplied by Dr. Xuemin Zhang. Myc-CHIP, GST-CHIP and GST-CHIP deletions, together with siCHIP constructs were preserved in our laboratory [26]. The siRNA target sequences for CHIP are 5-AACAGGCACTTGCTGACTG-3 for mouse and 5-AGCAGGCCCTGGCCGACTG-3 for human. Anti-Myc (9E10) and anti-GFP (FL) antibodies were purchased from Santa Cruz. Anti-β-actin (AC-15) antibody was purchased from Sigma. Anti-eIF5A antibody [EP526Y] was purchased from Abcam. Primary CHIP polyclonal antibody was preserved in our laboratory [26]. Human colon tissues were obtained from 301 Hospital, Beijing, China, under an approval of the Institutional Review Board of the hospital.

2.2. Cell lines and cultures

HEK293T cells, CHIP +/+ (wild type) and CHIP −/− (knock out) MEFs were maintained in Dulbecco's modified Eagle's medium (DMEM, GIBCO) with 10% FBS (GIBCO). SKOV3 cells were cultured in 1640 medium supplemented with 10% FBS. All of the cells were kept at 37 °C in a 5% CO_2-containing atmosphere with 100 U/ml penicillin and 100 mg/ml streptomycin. The stable cell lines were selected by 1 mg/ml G418 (Sigma-Aldrich) after transfection of the related plasmids, and the obtained clones were maintained in media containing 400 μg/ml G418.

2.3. Transfection, protein degradation and Western blot experiments

HEK293T cells were transfected using LipofectAMINE 2000 (Invitrogen) according to the protocol from the manufacturer. For the degradation assay, cells were transfected with the indicated expression plasmids, with and without MG132 (Calbiochem) and lysed in 2× SDS-PAGE loading buffer and then analyzed by Western blot with indicated antibodies.

2.4. 2D electrophoresis gel and mass spectrometry analysis

2D-DIGE analysis was carried out according to the manufacturer's instructions (Amersham, Piscataway, NJ). Briefly, cells were harvested, and solubilized by sonication in lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, and 30 mM Tris–HCl pH 8.5). Fifty micrograms of proteins from SKOV3 mock and siCHIP cells was mixed and applied on 24-cm sigmoidal immobilized pH gradient strips (IPG) (pH range 4–7, Amersham) for separation of the first dimension. The focused proteins were then separated by 12.5% SDS-PAGE gel. The protein spots that were significantly different between the control and the CHIP deficiency samples were determined by Decyder® version 5.01 software (Amersham). For protein identification, the protein spots of interest were excised from the gel and digested with trypsin. The peptide fragments were subjected to LC–MS/MS analysis. The Mascot software program (Matrix Science, London, UK) analyzed the acquired collision-induced dissociation spectra by searching the National Center for Biotechnology Information (NCBI) protein databases.

2.5. Immunohistochemistry

The tissue slides were incubated in a dry oven at 65 °C for 3 h, then de-waxed in xylene for 3 × 30 min, rehydrated in 100%, 100%, 95%, 90%, 80%, and 70% ethanol for 5 min each time. Antigen retrieval was performed in a pressure cooker containing citrate buffer (0.01 M, pH 6.0) for 3 min. Endogenous peroxidase activity of the tissues was quenched by 3% H_2O_2 for 10 min. The slides were incubated with primary CHIP polyclonal antibody and eIF5A antibody overnight at 4 °C. Biotin-labeled secondary antibody was applied to the slides, and then the slides were incubated with streptavidin-peroxidase. Samples were developed using DAB as substrates, and further counterstained with hematoxylin.

An index of staining was used to indicate the protein expression level in colorectal cancer according to our previous report [32]. The staining index was expressed as the proportion of positive staining cells (<25% = 1, 25–50% = 2, 50–75% = 3, >75% = 4) multiplied by staining intensity (negative = 0, weak = 1, moderate = 2, strong = 3).

2.6. GST pull-down assay

HEK293T cells transfected with the indicated expression plasmids were lysed in cell lysis buffer (50 mM Tris–HCl, pH 7.6, 150 mM NaCl, 1 mM EDTA, 0.5% NP40, 10% glycerol, 1 mM DTT, 0.1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin) on ice for 30 min. Lysates were then incubated with GST-CHIP or CHIP-deletions bound to GST-beads (about 10 μg of proteins) for 10 h at 4 °C. The bound proteins were analyzed by Western blot.

2.7. Pulse-chase assay

SKOV3 stable cells growing in the 60 mm dish were pre-incubated in methionine/cysteine-deficient medium for 1 h and then pulsed for metabolic labeling for 30 min with 200 μCi/ml of [^{35}S]methionine/[^{35}S]cysteine (PerkinElmer Life Sciences) and chased at different time points. The cells were lysed and subjected to anti-eIF5A immunoprecipitation and separated by SDS-PAGE. The gels were dried and ^{35}S-labeled endogenous eIF5A was visualized with PhosphorImager.

2.8. Ubiquitination assay

The ubiquitination assay was performed as described [23]. In brief, in vivo ubiquitination assay was performed by transfecting the indicated expression plasmids in HEK293T cells and harvested into buffer A and then sonicated. Whole-cell lysates were incubated with 50 μl of equilibrated (50%) Ni-NTA-agarose for 3 h at room temperature. Beads were washed with buffer A 2 times, buffer A/buffer T1 (1:3) 2 times and then buffer T1 1 time. Precipitated proteins were eluted with 2× SDS-PAGE loading buffer (containing 250 mM imidazole) and subjected to SDS-PAGE followed by Western blot. In vitro ubiquitination assay, in brief, the reaction mixture (20 μl) containing 5 mM Myc-eIF5A, 0.1 μM E1, 2.5 μM UbcH5a, 5 μM CHIP, 2 μg/μl of His-Ubiquitin and 2 μl of 10× ATP regenerating system (10 mM ATP, 100 mM creatine phosphate (Fluka), 40 mM magnesium acetate, 100 unit/ml creatine kinase (Sigma) in 50 mM Tris–HCl (pH7.3), 100 mM NaCl, 2 mM dithiothreitol) was incubated for 2 h at 30 °C.

2.9. Cell proliferation assay

^{3}H-thymidine incorporation experiments were performed to assay the ability of cell proliferation according a standard protocol in this lab [28]. Briefly, cells were labeled with 1 μCi ^{3}H-thymidine/well for 6 h at the indicated culture time points. Cell growth curve was drawn according to the average of the values.

3. Results

3.1. Identification of eIF5A as a CHIP-degraded protein by a proteomics analysis

Accumulating evidence has demonstrated that CHIP associates with several targeted proteins and mediates their degradation by initiating the ubiquitination process [28–31]. To reveal the entire targets of CHIP, we depleted CHIP expression in SKOV3 cells by stable transfection of a short hairpin RNA (shRNA) targeting endogenous CHIP mRNA

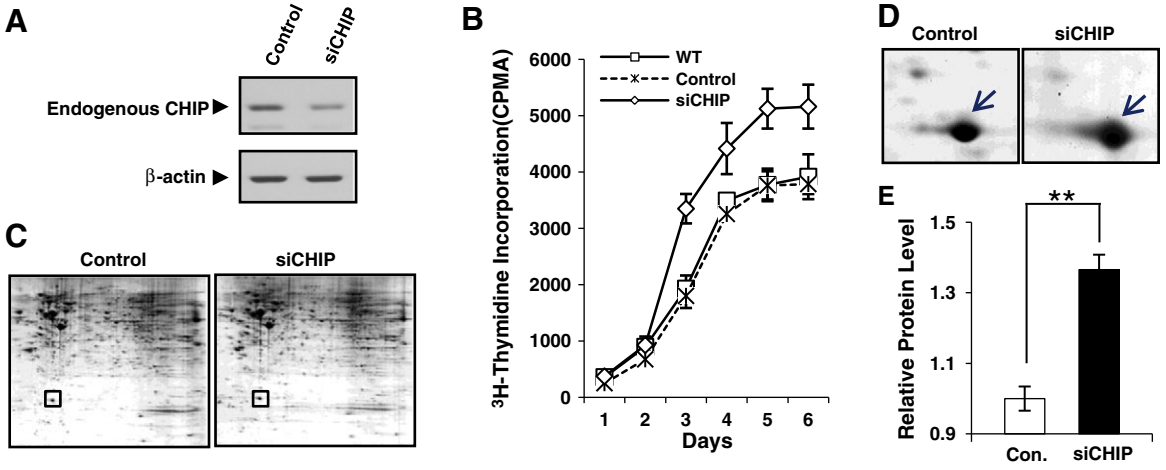

Fig. 1. eIF5A is identified as a novel target of CHIP by a proteomics analysis. (A) The level of endogenous CHIP protein in SKOV3 stable cell line. A Western blot result for the endogenous CHIP protein in SKOV3 cells treated with a siRNA targeting mouse CHIP (control) or a siRNA targeting human CHIP gene (siCHIP) is showed. β-actin was used as a loading control. (B) Depletion of CHIP enhanced the SKOV3 cell proliferation. A ^{3}H incorporation assay was performed for SKOV3 cells cultured in different days. Results are represented as mean ± SD from three independent experiments. (C) Identification of different proteins by a 2D electrophoresis analysis. Results of 2D electrophoresis of samples from whole lysates of cells treated as in (A). (D) A zoom-in view of eIF5A in the 2D gel images. Arrays indicate the spots identified as eIF5A by LC–MS/MS. (E) Relative eIF5A protein levels. The eIF5A protein levels were quantitated by ImageMaster software. Results are represented as mean ± SD from three independent quantifications.

(Fig. 1A). The CHIP deficiency cells demonstrated an enhanced proliferation property in comparison with control cells under normal culture conditions (Fig. 1B). We speculated that CHIP deficiency cells should have higher levels of proteins that were degraded by endogenous CHIP as an E3 ligase. To test our hypothesis, we performed a proteomics analysis for the samples of control and CHIP deficiency (siCHIP) SKOV3 cells. Overall, we obtained 1307 ± 20 peptide spots as shown in Coomassie Brilliant Blue (CBB) stained gels from an analysis by ImageMaster 2-D Elite software (Fig. 1C). Spots where the protein level was increased in the CHIP deficiency cells in comparison with control cells were selected for further analyses using mass spectrum (MS). As a result, eIF5A was identified as a potential target of CHIP since the level of eIF5A was increased in the CHIP deficiency cells displayed in the 2D gels (Fig. 1D). A quantitative analysis indicated that eIF5A protein level was increased up to 37% compared with that in the control cells (Fig. 1E). These results imply that eIF5A might be a novel target of CHIP.

3.2. CHIP down regulates the eIF5A protein level

To confirm the role of CHIP on the protein level of eIF5A in the intact cells, we examined the endogenous eIF5A proteins in SKOV3 cells when CHIP was over-expressed or depleted. Western blot analyses showed that enforced expression of CHIP resulted in a significant decrease of endogenous eIF5A protein (Fig. 2A, lanes 1–3). However depletion of CHIP increased the level of eIF5A protein (Fig. 2A, lanes 4–5). In contrast, over-expression or depletion of CHIP had no effect on the mRNA level of eIF5A (Fig. 2B). These results suggest that CHIP only down regulates the protein level of eIF5A. Then, we examined the level of eIF5A in SiHaR cells, a cervical cancer cell line. The results showed that the protein level of eIF5A was about 50% decreased in CHIP over-expression cells compared with that in the control cells (Fig. 2C). These results suggest that the role of CHIP on the protein level of eIF5A is ubiquitous in different cells. To confirm the role of CHIP on eIF5A protein under physiological conditions, we examined the protein level of eIF5A in the mouse embryonic fibroblast (MEF) cells from wild type or CHIP-knocked out (KO) mice. A Western blot analysis showed that the protein level of eIF5A was dramatically increased in CHIP-KO MEF cells (Fig. 2D, left panel). A quantitative analysis indicated that eIF5A protein was increased by 5 fold in comparison with that in the wild type cells (Fig. 2D, right panel). All the results suggest that CHIP down regulates the protein level of eIF5A in vitro and in vivo.

3.3. CHIP interacts with eIF5A

To understand the mechanism how CHIP down regulates eIF5A protein, we analyzed the interaction of CHIP and eIF5A. Since CHIP is an E3 ligase with a U-box domain for the E3 ubiquitin ligase activity, a TPR domain responsible for chaperone binding, and a Charged domain rich in charged residues [17], we used different deletions of CHIP (Fig. 3A) to examine their interaction with eIF5A. A GST pull-down experiment showed that all the deletions remain an interaction with eIF5A protein but the U-box domain appeared the strongest interaction (Fig. 3B). Deletion of the U-box domain almost abolished the interaction (Fig. 3B, lane 2). These results suggest that the interaction of CHIP with eIF5A occurs through the U-box domain.

3.4. CHIP induces eIF5A ubiquitination and degradation

The interaction of CHIP with eIF5A suggests that CHIP may mediate eIF5A degradation as CHIP has been reported to be an E3 ubiquitin ligase mediating degradation of several substrates [27]. To examine this hypothesis, Myc-eIF5A was co-expressed with increasing amounts of Myc-CHIP proteins in HEK293T cells in the presence or absence of MG132, a proteasome inhibitor. Immunoblotting results demonstrated that the level of Myc-eIF5A was decreased when Myc-CHIP was increasingly expressed (Fig. 3C, lanes 1–4). However, the protein level of eIF5A remained constant when Myc-CHIP was expressed in the presence of MG132 (Fig. 3C, lanes 5–6). Simultaneously, we observed that co-expressed GFP remained unchanged in all the treatments (Fig. 3C). These results suggest that CHIP specifically mediates eIF5A degradation through the ubiquitin-proteasome pathway.

Furthermore, we examined the turnover rate of eIF5A in the presence of over-expressed CHIP by a pulse-chase assay. The data showed that the protein level of eIF5A was decreased when CHIP was over-expressed (Fig. 3D, top panel). A quantitative analysis demonstrated that the half-life of eIF5A in control cells was 29.1 h while it reached to 8.3 h in the CHIP over-expression cells, suggesting that the eIF5A protein turnover rates are dramatically increased when CHIP is over-expressed (Fig. 3D, bottom panel). These data suggest that CHIP negatively regulates the stability of eIF5A.

As CHIP mediates protein degradation through the ubiquitination process, we examined whether CHIP promotes eIF5A ubiquitination. For this purpose, we co-expressed eIF5A and polyhistidine-tagged

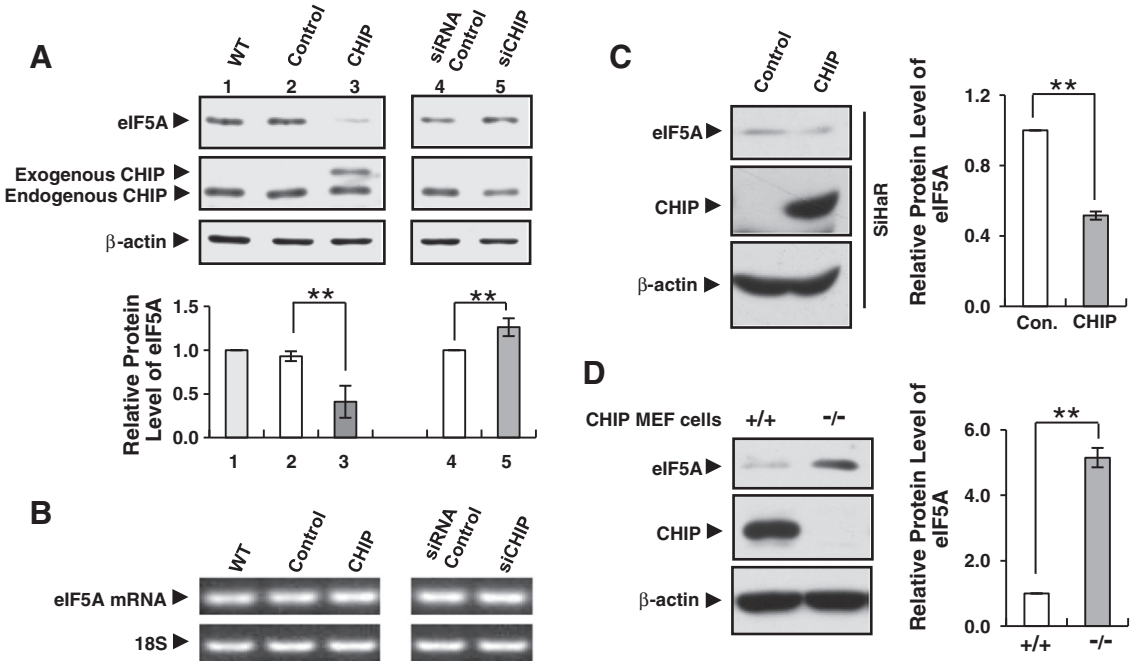

Fig. 2. CHIP down regulates eIF5A protein. (A) Over-expression of CHIP decreased, while depletion of CHIP increased, the protein level of eIF5A. The protein levels of CHIP and eIF5A in SKOV3 stable cell lines were examined by Western blots. Upper panels: a representative Western blot result; bottom: a quantitative analysis is shown in correspondence with the Western blot results. Results are represented as mean ± SD from three independent experiments. (B) CHIP had no effect on the mRNA level of eIF5A. The mRNA levels of eIF5A in different SKOV3 stable cell lines were examined by RT-PCR. 18S rRNA was used as a loading control. (C) Over-expression of CHIP decreased eIF5A protein level in SiHaR cells. The levels of eIF5A and over-expressed CHIP were examined by Western blots in SiHaR stable cell lines. β-actin was used as a loading control. A quantitative analysis is shown on the right panel to demonstrate the relative protein levels of eIF5A normalized by β-actin. (D) eIF5A protein level is increased in CHIP deficiency cells. The protein levels of CHIP and eIF5A in MEF cells from wild type (+/+) and CHIP KO (−/−) mice were examined by Western blots. A quantitative analysis of the protein levels is shown on the right panel by a same method in (C). The quantitative analyses in (C and D) were from two independent experiments.

ubiquitin in 293T cells with HA-CHIP. Ubiquitinated proteins were precipitated from cell lysates under denatured conditions and analyzed by immunoblotting with the anti-eIF5A antibody. The results show that co-expression of CHIP resulted in a marked increase of the ubiquitinated eIF5A (Fig. 3E, ladders), suggesting that CHIP is involved in eIF5A ubiquitination.

To obtain direct evidence that CHIP has an E3 ubiquitin ligase activity toward eIF5A, we performed an in vitro ubiquitination assay. The presence of ubiquitinated eIF5A was detected as high molecular mass smear by Western blotting with an anti-eIF5A antibody (Fig. 3F). When E1, E2 (UbcH5a) and CHIP were added to the reaction system containing purified Myc-eIF5A, a strong smear was observed (Fig. 3F, lane 2). Moreover, stronger smear was seen when CHIP was double expressed (Fig. 3F, lane 3). These results indicate that CHIP functions as an E3 to mediate the ubiquitination of eIF5A in conjunction with the E1 and E2 enzymes.

3.5. CHIP negatively correlates with eIF5A in cancers

Previous studies showed that eIF5A was up-regulated in colorectal adenoma [10–12]. To demonstrate the correlation of eIF5A expression and CHIP level, we examined CHIP and eIF5A levels in human colorectal cancer by immunohistochemical (IHC) staining with 18 colorectal cancer samples. A weak CHIP staining was observed in the tumor region where eIF5A showed strong staining (Fig. 4A). A contingency analysis demonstrated that 75% of the patients showed lower CHIP expression among the patients that eIF5A was highly expressed in tumor tissues (Fig. 4B). In contrast, the higher expression of eIF5A corresponded to the lower expression of CHIP in normal tissues (Fig. 4B). A correlation study indicated that the expression levels of CHIP and eIF5A were in an inverse correlation with $r = -0.5$ ($p < 0.05$). A Western blot analysis showed that eIF5A was increased in tumor tissues compared with the paired normal tissues, in correlation with decreased CHIP protein level (Fig. 4C). These results demonstrated that eIF5A was increased as CHIP expression was reduced in colon cancers.

4. Discussion

eIF5A, a critical protein controlling protein synthesis [3], has been reported to play a key role in cell proliferation and differentiation [6,33,34]. The eIF5A activity is regulated at various levels, including hypusination, phosphorylation and acetylation. However, to date, no negative regulator has been reported to mediate the degradation of eIF5A. In this study, we show that CHIP interacts with eIF5A and mediates the degradation of eIF5A. Our study provided the first line of evidence that CHIP functions as a negative regulator for eIF5A to mediate its degradation.

We and other groups have identified several targets of CHIP, which functions as an E3 ligase to mediate the protein degradation through ubiquitination [23,25–31]. It appeared that CHIP might target different proteins under different physiological conditions. To reveal more targets, we performed a proteomic analysis in the CHIP deficiency SKOV3 cells, a cell line of ovarian cancer. Interestingly, we identified that CHIP targeted eIF5A, a critical regulator of protein synthesis. Importantly, we observed that eIF5A was dramatically up-regulated in the CHIP KO MEF cells (Fig. 2D). Our study revealed that CHIP functions as a physiological regulator of eIF5A. Of course, eIF5A is just one of the targets that CHIP protein recognized. Actually, we found that several proteins were up-regulated when CHIP was depleted (data not shown). In this study, we focused to characterize the role of CHIP on the regulation of eIF5A, as this protein plays an important role during the protein synthesis.

CHIP contains three main functional domains, a U-box domain, a TPR domain, and a Charged domain [17]. It has been reported that CHIP recognizes substrates through its TPR domain, as was observed in the interaction with ErbB2 and Smads [25,26]. In a previous study, we also observed that both the TPR and Charged domains were required for

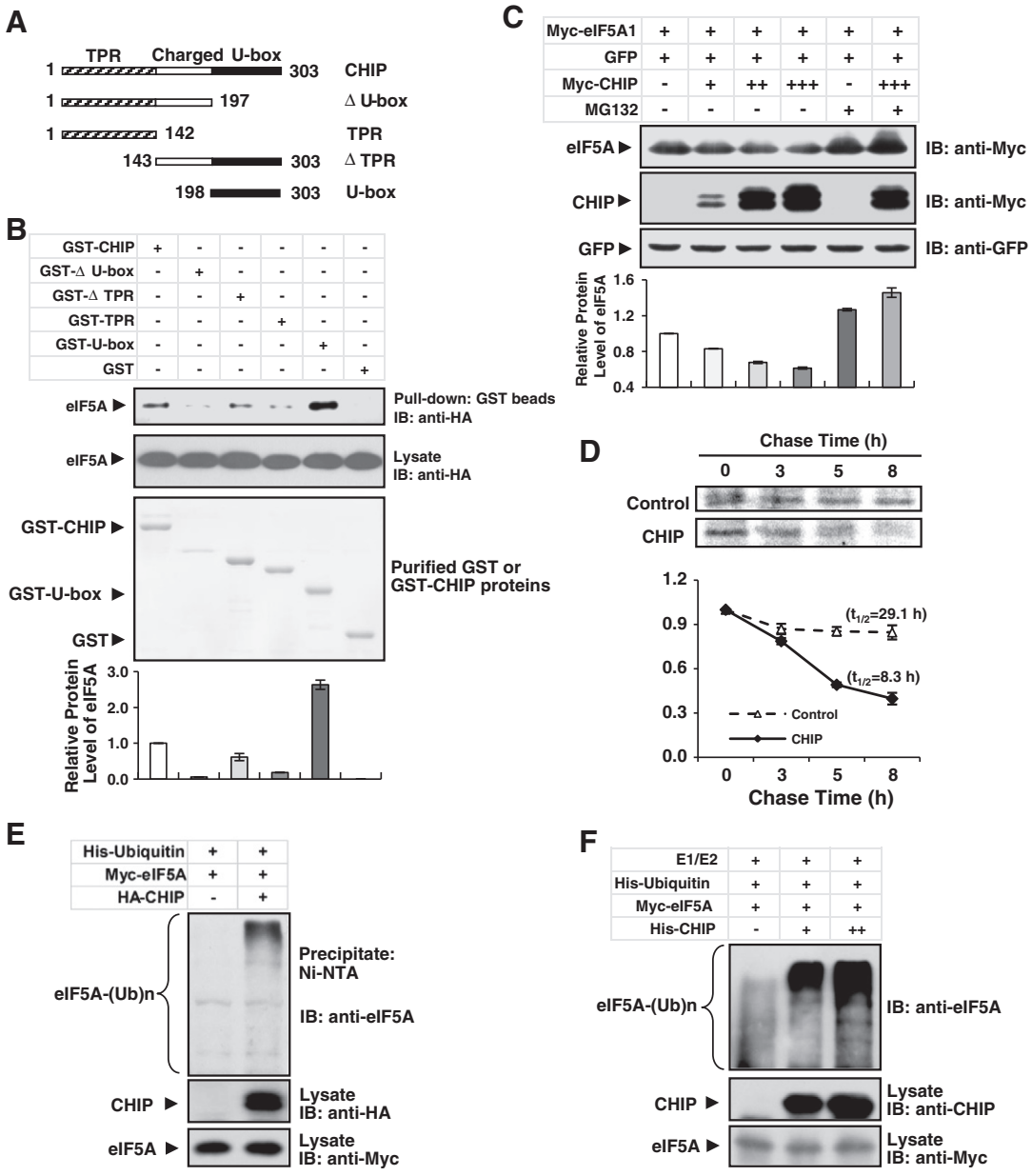

Fig. 3. CHIP promotes eIF5A degradation and ubiquitination. (A) A schematic diagram shows the different domains of CHIP and its truncated mutants. (B) CHIP interacts with eIF5A in vitro. GST pull down experiments were performed by indicated GST-fussed proteins purified from *E. coli*, incubated with eIF5A protein expressed by 293T cells. The relative pull-down protein levels were presented in the bottom panel from three independent quantifications. (C) CHIP mediates the degradation of eIF5A in a dose dependent manner. CHIP was dose dependently expressed in 293T cells. MG132 (25 μM, 6 h), an inhibitor of proteasome, was added to recover the degradation of eIF5A. The relative protein levels of eIF5A were presented in the bottom panel from three independent quantifications. (D) CHIP accelerates the turnover rate of eIF5A protein. A pulse-chase assay (upper panels) was performed. Quantitative presentation of the results with standard errors (three quantifications) is shown (bottom panels). The protein half life ($t_{1/2}$) was calculated. (E) CHIP promotes eIF5A ubiquitination in vivo. An in vivo ubiquitination experiment was performed by over-expression of indicated proteins in 293T cells. The proteins were precipitated by Ni-NTA for His-Ubiquitin. (F) CHIP directly induces eIF5A ubiquitination in vitro. The in vitro ubiquitination experiment was performed using Myc-eIF5A purified by 293T cells and His-CHIP purified from *E. coli*, together with purified E1/E2 and His-Ubiquitin.

the interaction of CHIP with Runx1, a critical factor in the generation and maintenance of hematopoietic stem cells and leukemia [23]. To our surprise, in this study we found that eIF5A interacted with all four CHIP deletions. Interestingly, the U-box domain, which maintains the E3 activity in general, showed a strong ability to interact with eIF5A. It appeared that the interaction of eIF5A with the U-box domain was stronger than that with the full length CHIP protein (Fig. 3). These results implied that the U-box domain of CHIP might directly transfer the ubiquitin from an E2 ligase to eIF5A, with the assistance of other domains. Further study is needed to confirm the role of the U-box domain in mediating the ubiquitination of eIF5A.

Previous studies demonstrated that the expression of eIF5A in colorectal tumors was significantly higher than that in normal mucosa and overexpression of eIF5A correlated with the median and disease-free survival of these patients [10,11,35]. Therefore, evaluation of eIF5A expression in colorectal tumors might be recommended as a prognostic factor in early-onset CRC patients. In this study, we observed a negative correlation of CHIP expression with the protein level of eIF5A in 18 colorectal adenoma samples (Fig. 4). Our recent data also indicate that CHIP protein level was frequently decreased in colorectal cancer [28]. Therefore, these results suggest that the lower expression of CHIP should be partially contributed to the high expression level of eIF5A in colorectal cancers.

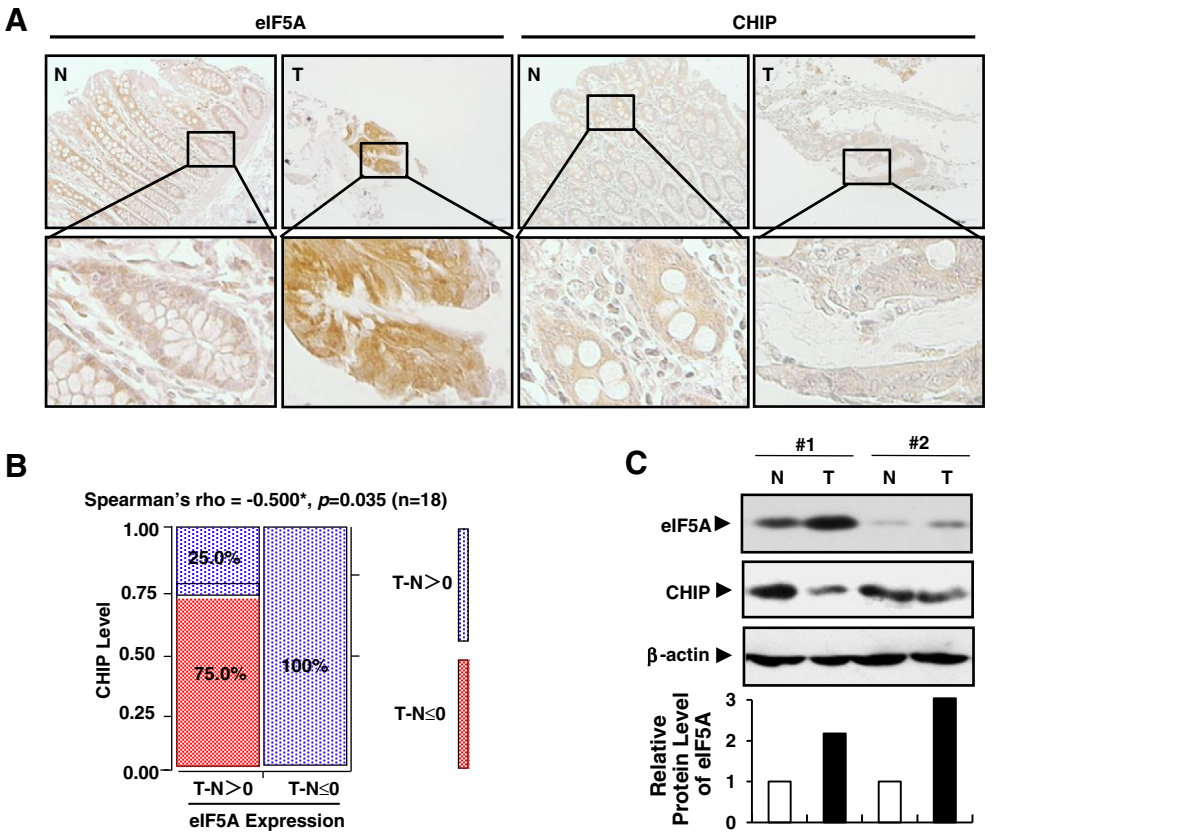

Fig. 4. CHIP negatively correlates with eIF5A in colorectal cancers. (A) Representative images of IHC staining of colorectal tumor samples using eIF5A or CHIP antibodies. The upper four images were photographed in 20-fold magnification, scale bar, 100 mm, and the lower images (400×) showed partial enlargement of the upper images. (B) A contingency table showing the negative correlation of expression of eIF5A and CHIP in colon cancer patients. Spearman's rho correlation coefficient was calculated by SPSS software. * indicates significance at $p < 0.05$. (C) A Western blot analysis of proteins extracted from human colorectal cancer and the paired normal tissues (two cases). N, normal tissues; T, tumor tissues. Relative protein levels of eIF5A were presented at the bottom panel.

To date, eIF5A is the only known protein to undergo the post-translational modification of a conserved lysine to hypusine [2]. We observed that CHIP interacted with eIF5A and negatively regulated the protein level of eIF5A by degradation. As it was reported that the activity of eIF5A was regulated by hypusination, phosphorylation and acetylation, we questioned whether the interaction of CHIP with eIF5A might be regulated by these modification processes. In particular, it would be of interest to investigate whether hypusination of eIF5A is necessary for the interaction of CHIP with eIF5A and further the degradation.

5. Conclusion

We found that CHIP interacted with eIF5A and mediated its ubiquitination for degradation. The protein level of eIF5A is inversely correlated with the level of CHIP in human colorectal cancers. This is the first report on the regulation of eIF5A protein stability via E3 ligase CHIP.

Competing interests

The authors declare that they have no competing interests.

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