Biochemical and Biophysical Research Communications 433 (2013) 194–199

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

CHFR is negatively regulated by SUMOylation-mediated ubiquitylation

Sung Jun Bae, Young Eun Kwon, Myungjin Kim *, Jae Hong Seol *

School of Biological Sciences, Research Center for Functional Cellulomics, Seoul National University, Seoul 151-742, Republic of Korea

ARTICLE INFO

Article history:
Received 13 February 2013
Available online 27 February 2013

Keywords:
CHFR
SUMOylation
Ubiquitylation
Protein stability
E3 ubiquitin ligase

ABSTRACT

CHFR ubiquitin ligase plays an important role in cell cycle progression and tumorigenesis. CHFR tumor suppressor function is highly associated with its protein level. We recently reported that CHFR protein levels are negatively regulated by SUMOylation-mediated proteasomal degradation. In the present study, we uncover a detailed molecular mechanism how SUMOylation promotes CHFR destabilization. We demonstrate that SUMO modification of CHFR promotes its ubiquitylation and subsequent proteasomal degradation. However, SUMOylation of CHFR does not affect its auto-ubiquitylation, which generally serves as a maintenance mechanism for most ubiquitin ligases. Moreover, the E3 ubiquitin ligase activity of CHFR is dispensable for this SUMOylation-mediated ubiquitylation and degradation. Conversely, SENP2 deSUMOylating enzyme reduces SUMOylation-induced ubiquitylation of CHFR, leading to elevated CHFR protein levels. Taken together, our results present a new regulatory mechanism for CHFR that sequential post-translational modifications of CHFR by SUMO and ubiquitin coordinately regulates its stability.

1. Introduction

CHFR (checkpoint with FHA and RING finger domains) is initially identified as a mitotic stress checkpoint [1]. CHFR E3 ubiquitin (Ub) ligase activity confers its checkpoint function [2,3]. CHFR plays a pivotal role in multiple cellular processes controlling cell cycle progression, genomic instability, tumorigenesis, and tumor metastasis through the degradation of target proteins such as PLK1 (polo-like kinase 1), Aurora A, HLTF, and HDAC1 (histone deacetylase 1) [4–7]. *CHFR* is frequently silenced by promoter methylation in cancer and *CHFR* expression is negatively correlated with tumor phenotypes in various cancer cells and mouse models [5–9], suggesting the existence of the homeostatic control mechanism for maintaining proper CHFR levels.

CHFR protein levels are generally regulated by auto-ubiquitylation followed by proteasomal degradation like most other E3 Ub-ligases [3]. USP7/HAUSP ubiquitin-specific protease reverses auto-ubiquitylation and stabilizes CHFR [10]. Thus, CHFR stability is largely dependent on the ubiquitylation status. We have recently reported that SUMOylation negatively regulates the stability of CHFR tumor suppressor [11]. CHFR is modified by SUMO-1 at lysine 663 and SENP2 deSUMOylating enzyme removes the SUMO-1 moiety from CHFR. SUMO-modification of CHFR is responsible for its degradation by ubiquitin–proteasome system (UPS) acting as a destabilization code. Moreover, SUMOylation-defective mutant of CHFR shows a higher anti-proliferative activity compared to wild-type CHFR due to the increased stability of CHFR. These findings suggest that SUMOylation and ubiquitylation may work in concert to tightly control CHFR protein levels. Therefore, it would be of particular interest to determine the underlying mechanism of CHFR SUMOylation-dependent degradation and possible interplay between SUMOylation and ubiquitylation.

SUMOylation is known to play a role in regulating protein stability. When SUMOylation competes with ubiquitylation for the same lysine residue, SUMOylation protects target protein from ubiquitylation and acts as a stabilization signal [12]. Meanwhile, SUMOylation functions as a destabilization signal. SUMO-modification promotes either auto-ubiquitylation or recruiting other E3 Ub-ligase to further enhance ubiquitylation leading to proteasomal degradation [13–16].

In the present study, we report that SUMOylation promotes CHFR ubiquitylation, leading to its rapid proteasomal degradation. Interestingly, this SUMOylation-dependent ubiquitylation is not based on its own E3 Ub-ligase activity of CHFR. CHFR deSUMOylation by SENP2 decreases ubiquitylation and increases CHFR stability. Therefore, SUMOylation and ubiquitylation are closely intertwined with each other to maintain the cellular levels of CHFR tumor suppressor.

2. Materials and methods

2.1. Plasmids, cell culture, and transfection

CHFR cDNA was subcloned into p3xFLAG-CMV10 (Sigma) and pFastBac (invitrogen) vectors, and the QuickChange site-directed

* Corresponding authors. Fax: +82 2 872 1993.
E-mail addresses: jinang@snu.ac.kr (M. Kim), jhseol@snu.ac.kr (J.H. Seol).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.02.080

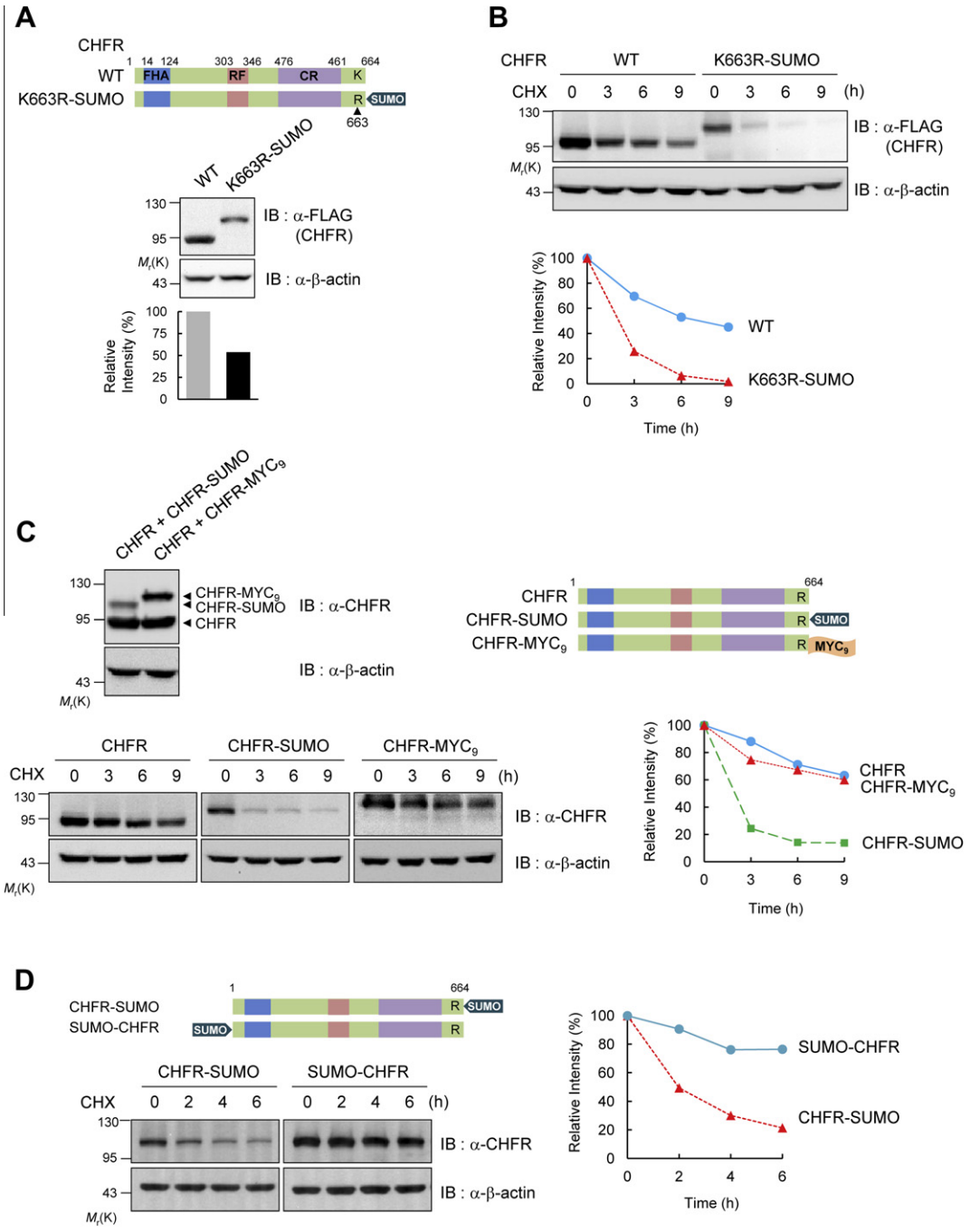

Fig. 1. SUMO modification destabilizes CHFR. (A) $CHFR^{K663R}$-SUMO protein level is lower than $CHFR^{WT}$. A schematic diagram of $CHFR^{K663R}$-SUMO protein is shown. FLAG-$CHFR^{WT}$ or FLAG-$CHFR^{K663R}$-SUMO was transfected into HeLa cells and analyzed by immunoblotting. Relative protein levels were quantified by densitometry. (B) The degradation rate of $CHFR^{K663R}$-SUMO is faster than $CHFR^{WT}$. HeLa cells expressing ectopic FLAG-$CHFR^{WT}$ or FLAG-$CHFR^{K663R}$-SUMO were used. After cells were treated with cycloheximide, cells were harvested at indicated times and analyzed by immunoblotting. Relative quantification is shown below. (C) *Upper panel*, $CHFR^{K663R}$-SUMO or $CHFR^{K663R}$-MYC_9 was co-transfected with $CHFR^{K663R}$ into MCF7 cells. Cell lysates were immunoblotted with anti-CHFR or anti-β-actin antibodies. *Lower panel*, HeLa cells were transfected with CHFR expression vectors (K663R, K663R-SUMO, or K663R-MYC_9). At 24 h post-transfection, cells were treated with 200 μg/ml cycloheximide for indicated times. Cell lysates were subjected to immunoblotting with anti-CHFR or anti-β-actin antibodies. (D) $CHFR^{K663R}$-SUMO or SUMO-$CHFR^{K663R}$ was transfected into HeLa cells. At 24 h post-transfection, 200 μg/ml cycloheximide was treated for indicated times. Cell lysates were subjected to immunoblotting with anti-CHFR or anti-β-actin antibodies.

mutagenesis Kit (Stratagene) was used to generate CHFR lysine mutants as described previously [6,11]. MCF7 and HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% FBS (Gibco) in a humidified 5% CO_2 atmosphere at 37 °C. Either polyethylenimine (Sigma) or PolyFect (Qiagen) was used for transfections according to the manufacturer's instructions.

2.2. Immunoblotting and antibodies

Cells were washed twice with cold PBS and lysed in buffer A (20 mM Tris–HCl [pH 7.5], 150 mM NaCl, 0.1 mM EDTA, and 0.2% Triton X-100) containing 1× complete protease inhibitor cocktail (Roche Applied Science). Whole cell lysates were separated by SDS–PAGE and transferred to a nitrocellulose membrane. The

following antibodies were used: anti-FLAG and anti-β-actin (Sigma), anti-MYC and anti-HA (Santa Cruz Biotechnology), rabbit polyclonal anti-CHFR antiserum (raised against a recombinant His-CHFR), and peroxidase-conjugated AffiniPure goat anti-rabbit and anti-mouse IgGs (Bio-Rad).

2.3. Cycloheximide chase assay

HeLa cells were transfected with appropriate plasmids. Twelve hours after transfection, cells were seeded onto 35 mm culture dishes, cultured for additional 12 h, and then treated with 200 μg/ml cycloheximide for indicated times. Cell lysates were collected and analyzed by immunoblotting. Relative band intensity was quantified by densitometry ImageJ software (ImageJ, US National Institutes of Health).

2.4. Ubiquitylation assay

For the *in vitro* ubiquitylation assay, purified His-CHFR protein from Sf9 cells was incubated with E1 (0.2 μg), UbcH5b (0.2 μg), Ubiquitin (2 μg), and ATP-regenerating system at 30 °C for indicated times. For the *in vivo* ubiquitylation assay, 24 h after transfection with appropriate expression plasmids, cells were treated with 2 μM MG132 (Boston Biochem) for additional 12 h. Cell lysates were incubated with anti-FLAG M2 resin (Sigma), and the precipitates were collected by centrifugation and washed 3 times with buffer A. Bound proteins were eluted using 0.2% SDS and analyzed by immunoblotting.

3. Results and discussion

3.1. SUMOylation at the C-terminal end of CHFR determines its stability

Given that CHFR becomes destabilized when UBC9 and SUMO-1 are co-expressed [11], we generated CHFR-SUMO fusion protein to investigate the direct effect of SUMO-modification on CHFR stability and exclude the potential transfection bias of SUMOylating enzymes. As the SUMOylation site (K663) is located at the C-terminal end of CHFR, we fused SUMO-1 directly to the C-terminus of $CHFR^{K663R}$ mutant ($CHFR^{K663R}$-SUMO) to faithfully simulate endogenous SUMOylated CHFR. When we examined the expression of CHFR protein, $CHFR^{K663R}$-SUMO fusion protein levels were about a half of $CHFR^{WT}$ (Fig. 1A). This is mainly due to the increased degradation rate of $CHFR^{K663R}$-SUMO (Fig. 1B).

In order to further determine whether CHFR destabilization is the direct consequence of covalent attachment of SUMO-1 to C-terminus of CHFR, we tested several different kinds of fusion proteins. We fused MYC_9 to the C-terminal end of $CHFR^{K663R}$ and compared the protein levels between CHFR variants. When either $CHFR^{K663R}$-SUMO or $CHFR^{K663R}$-MYC_9 was co-transfected with $CHFR^{K663R}$ in MCF7 cells, only $CHFR^{K663R}$-SUMO protein levels were low compared to $CHFR^{K663R}$-MYC_9 and $CHFR^{K663R}$. A cycloheximide-chase assay also showed that $CHFR^{K663R}$-MYC_9 was degraded similarly to $CHFR^{K663R}$, whereas $CHFR^{K663R}$-SUMO was destabilized much faster (Fig. 1C), indicating that destabilization of CHFR-SUMO fusion proteins is solely based on the SUMO modification. Moreover,

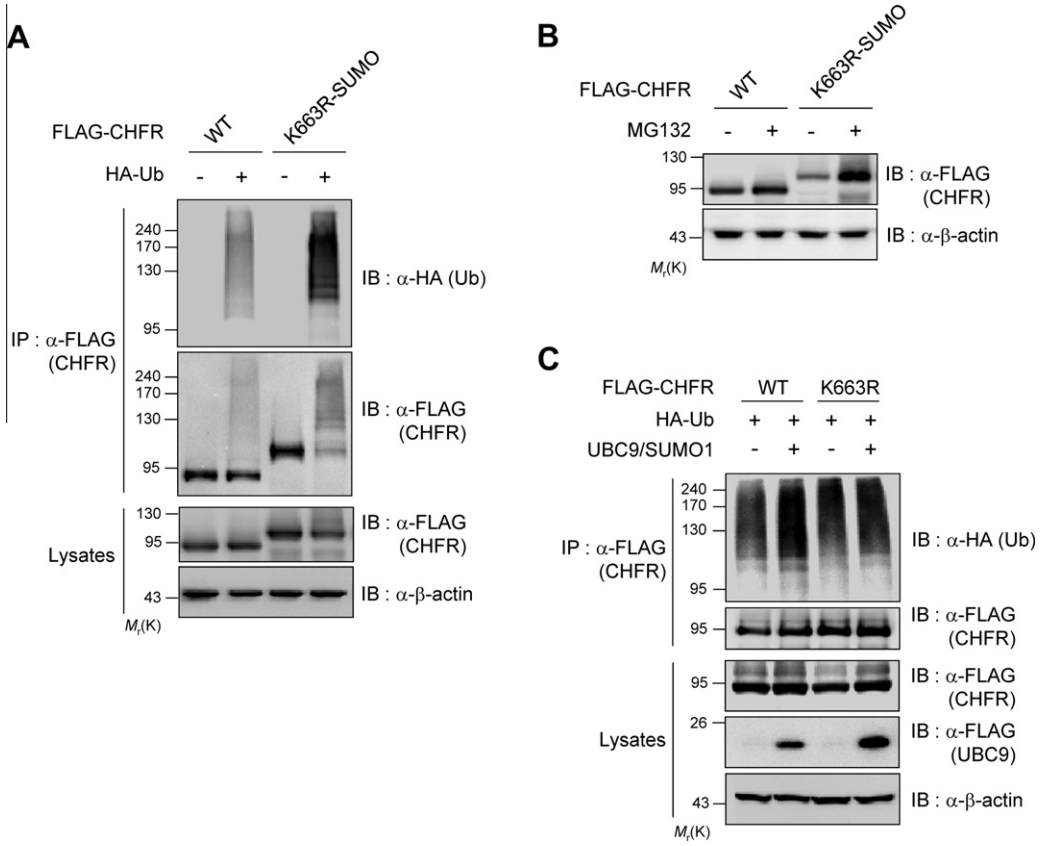

Fig. 2. SUMOylation of CHFR leads to the increase of its ubiquitylation. (A) Ubiquitylation of CHFR is significantly increased by SUMO fusion. FLAG-$CHFR^{WT}$ or FLAG-$CHFR^{K663R}$-SUMO was transfected with or without HA-Ub and 2 μM MG132 were treated for 12 h before harvest. Cell lysates were immunoprecipitated with anti-FLAG M2 resin and analyzed by immunoblotting. (B) $CHFR^{K663R}$-SUMO protein is stabilized by proteasome inhibitor MG132 treatment. The indicated CHFR expression plasmids were transfected and 2 μM MG132 were treated for 12 h before harvest. (C) CHFR ubiquitylation is enhanced under SUMOylation-promoting conditions. FLAG-CHFR (wild-type or K663R), HA-Ub, FLAG-UBC9, and HisMax-SUMO-1 were transfected into MCF7 cells. At 24 h post-transfection, 2 μM MG132 were treated for additional 12 h before harvest. Cell lysates were immunoprecipitated with anti-FLAG M2 resin and immunoblotted with appropriate antibodies.

SUMO-fusion to N-terminus of CHFR (SUMO-CHFR^{K663R}) had no effect on its half-life unlike C-terminal SUMO-fusion protein (CHFR^{K663R}-SUMO; Fig. 1D). Therefore, these results clearly demonstrate that SUMO-1 conjugation to the C-terminal end of CHFR is necessary and sufficient for regulating CHFR protein levels. It is also implicated that CHFR-SUMO fusion protein truly mimics constitutively SUMOylated CHFR.

3.2. SUMO modification enhances CHFR ubiquitylation

Given that SUMOylation destabilizes target proteins by promoting ubiquitylation and subsequent proteasomal degradation [14–16], we investigated whether SUMOylated CHFR is more ubiquitylated and degraded using *in vivo* ubiquitylation assay with CHFR^{WT} and CHFR^{K663R}-SUMO in HeLa cells. Ubiquitylation of CHFR^{K663R}-SUMO was significantly increased compared to CHFR^{WT} (Fig. 2A). In parallel, CHFR^{K663R}-SUMO was more stabilized than CHFR^{WT} in the presence of MG132 proteasome inhibitor (Fig. 2B). These results are consistent with our observation that CHFR^{K663R}-SUMO was destabilized more quickly than CHFR^{WT} (Fig. 1B). Next, we performed another *in vivo* ubiquitylation assay with CHFR^{WT} and CHFR^{K663R} under SUMOylation-promoting conditions. While ubiquitylation of CHFR^{WT} was increased in the presence of UBC9 and SUMO-1, that of CHFR^{K663R} was relatively similar irrespective

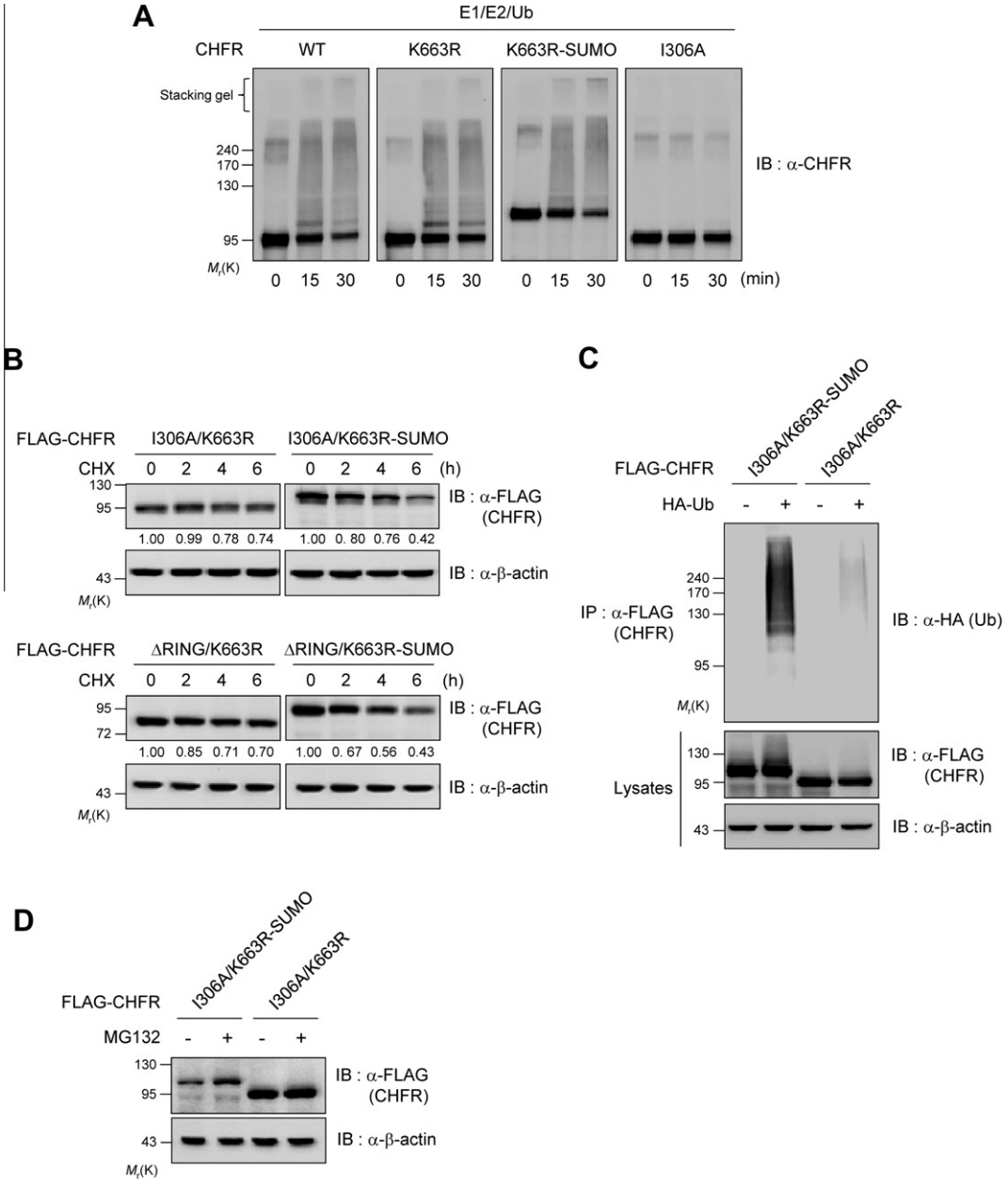

Fig. 3. SUMOylation-coupled ubiquitylation of CHFR is not related with its auto-ubiquitylation activity. (A) SUMOylation status of CHFR does not affect its auto-ubiquitylation activity *in vitro*. Purified His-CHFR (wild-type, K663R, K663R-SUMO, or I306A) was incubated with E1, E2 (UbcH5b), and Ubiquitin. Each sample was then subjected to SDS–PAGE, followed by immunoblotting with anti-CHFR antibody. (B) SUMO-fused CHFR is degraded faster even in the E3 Ub-ligase-defective background. HeLa cells were transfected with FLAG-CHFR$^{I306A/K663R}$, -CHFR$^{I306A/K663R}$-SUMO, -CHFR$^{\Delta RING/K663R}$, or -CHFR$^{\Delta RING/K663R}$-SUMO. At 24 h post-transfection, cells were treated with cycloheximide (200 μg/ml) for indicated times. Cell lysates were analyzed by immunoblotting with anti-FLAG or anti-β-actin antibodies. Relative intensity was quantified by densitometry. (C) SUMO-fused CHFR is heavily ubiquitylated. FLAG-CHFR$^{I306A/K663R}$ or FLAG-CHFR$^{I306A/K663R}$-SUMO fusion form was co-transfected with HA-Ub. MG132-treated cell lysates were immunoprecipitated with anti-FLAG M2 resin and immunoblotted with anti-HA antibody. (D) SUMO-fused protein is stabilized by MG132. HeLa cells were transfected with FLAG-CHFR$^{I306A/K663R}$ or FLAG-CHFR$^{I306A/K663R}$-SUMO and treated with 2 μM MG132 for 12 h before harvest. Cell lysates were subjected to immunoblotting with indicated antibodies.

of SUMOylation-promoting conditions (Fig. 2C). Therefore, it is highly likely that elevated ubiquitylation is largely dependent on the SUMOylation status at the lysine 663 residue of CHFR.

3.3. SUMOylation does not affect the E3 Ub-ligase activity of CHFR

It is reasonable to assume that SUMOylation enhances own E3 Ub-ligase activity of CHFR, leading to the increase of its auto-ubiquitylation as a known regulatory mechanism for the CHFR stability [3]. To test our hypothesis, we performed *in vitro* ubiquitylation assay with purified CHFR proteins in more defined conditions without any other E3 Ub-ligases. To our surprise, there was no ubiquitylation difference among $CHFR^{WT}$, $CHFR^{K663R}$, and $CHFR^{K663R}$-SUMO although all CHFR proteins are able to ubiquitylate itself except $CHFR^{I306A}$ that lacks an E3 Ub-ligase activity (Fig. 3A), suggesting that SUMOylation-dependent ubiquitylation of CHFR is not accomplished by itself. To further clarify whether SUMOylation-coupled destabilization of CHFR does not depend on the E3 Ub-ligase activity of CHFR, we generated another SUMO-fusion protein in a CHFR E3 Ub-ligase defective background (I306A or ΔRING; [6]). Not only $CHFR^{I306A/K663R}$-SUMO, but $CHFR^{\Delta RING/K663R}$-SUMO also degraded faster than their counterparts (Fig. 3B). In agreement with this, $CHFR^{I306A/K663R}$ was barely ubiquitylated, whereas $CHFR^{I306A/K663R}$-SUMO fusion protein was heavily ubiquitylated (Fig. 3C) and stabilized by MG132 treatment (Fig. 3D). Collectively, these results indicate that certain unidentified E3 Ub-ligase, rather than CHFR itself, is associated with CHFR SUMOylation-dependent ubiquitylation and proteasomal degradation. RNF4 E3 Ub-ligase is known to recognize and ubiquitylate SUMOylated substrates, however, it preferentially recognizes poly-SUMO2/3 chains of target protein such as PML [14]. Since CHFR is modified by mono-SUMO-1, it is highly likely that a novel E3 Ub-ligase may recognize SUMO-1 moiety along with certain structural features of the C-terminal end of CHFR and be further responsible for CHFR SUMOylation-mediated ubiquitylation. This is supported by our observation that N-terminal SUMO-1 fusion of CHFR (SUMO-CHFR) showed no effect on its stability (Fig. 1D). C-terminus of CHFR including CR domain is known to be required for protein–protein interaction and a checkpoint activity [5,6,17]. Thus, our data suggest that C-terminal region of CHFR may facilitate a novel SUMO-dependent E3 Ub-ligase to easily recognize and ubiquitylate CHFR as a cooperative recognition module to determine its stability.

3.4. CHFR is sequentially modified by SUMO-1 and ubiquitin

We have shown thus far that CHFR SUMOylation is linked to ubiquitylation and subsequent degradation. In order to investigate whether SUMOylation of CHFR precedes ubiquitylation, we performed a ubiquitylation assay in the presence of UBC9, SUMO-1, and SENPs. CHFR ubiquitylation was greatly enhanced in the presence of UBC9 and SUMO-1 (Fig. 4A, *lanes* 1–3), however, this ubiquitylation was significantly declined to the basal level when $SENP2^{WT}$ was introduced (Fig. 4A, *lane* 5). On the contrary, either SENP1 or $SENP2^{C548S}$ rarely affected the ubiquitylation status of CHFR (Fig. 4A, *lanes* 4 *and* 6). This result is consistent with our previous report that SENP2 is a major deSUMOylating enzyme for CHFR [11]. To further explore the effect of SENP2 on CHFR stability, we established HeLa cells stably expressing either wild-type or K663R mutant of CHFR. As expected, $CHFR^{WT}$, but not $CHFR^{K663R}$, was stabilized by SENP2 (Fig. 4B). Therefore, these results indicate that SUMOylation of CHFR indeed precedes its ubiquitylation and functions as a degradation signal unless CHFR is deSUMOylated by SENP2.

Taken together, we present the underlying mechanism of CHFR SUMOylation-mediated destabilization how SUMOylation of CHFR

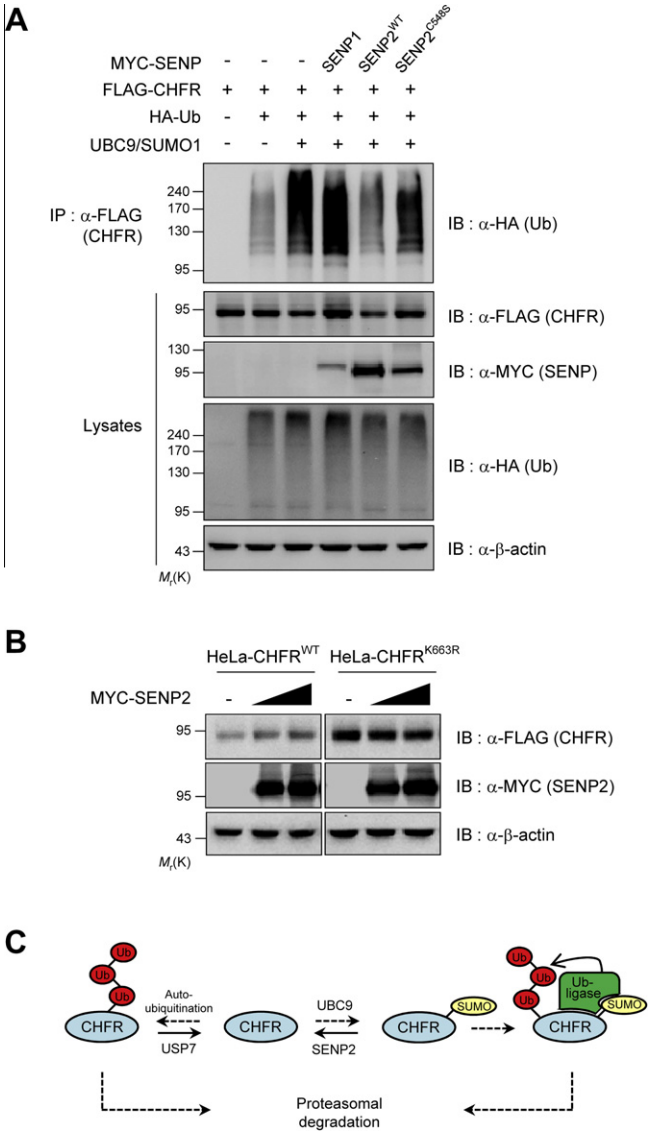

Fig. 4. SENP2 stabilizes CHFR via reducing SUMOylation-dependent ubiquitylation of CHFR. (A) SENP2 decreases SUMOylation-coupled ubiquitylation of CHFR. HeLa cells were co-transfected with FLAG-CHFR, HA-Ub, FLAG-UBC9, HisMax-SUMO-1, and MYC-SENPs. Twenty-four hours after transfection, cells were treated with 2 μM MG132 for additional 12 h. Cell lysates were immunoprecipitated with anti-FLAG M2 resin, and precipitates were analyzed by immunoblotting as indicated. (B) CHFR is stabilized by SENP2. HeLa-$CHFR^{WT}$ and HeLa-$CHFR^{K663R}$ stable cells were transfected with MYC-SENP2 expression vectors. Cell lysates were immunoblotted with anti-FLAG, anti-MYC, or anti-β-actin antibodies. (C) Revised model for the control of CHFR stability. The schematic diagram shown here illustrates that SUMOylation of CHFR induces its destabilization by affecting its ubiquitylation.

leads to ubiquitylation and subsequent proteasomal degradation. Based on our results, SUMOylation-mediated ubiquitylation is likely to work independently with the CHFR auto-ubiquitylation activity. A proposed model for CHFR homeostasis by sequential post-translational modifications is illustrated in Fig. 4C. CHFR stability is negatively controlled by SUMOylation-dependent ubiquitylation and/or its auto-ubiquitylation [3]. Either SUMO removal by SENP2 or ubiquitin removal by USP7/HAUSP stabilizes CHFR [10]. It would be of particular importance to understand how these aforementioned post-translational modifications are intertwined and to identify specific SUMOylation and/or deSUMOylation-inducing signals and how such signals are relayed to putative SUMO-dependent E3 Ub-ligase candidate(s) for SUMOylated CHFR

to maintain the proper cellular CHFR levels. Collectively, we add a new regulatory mechanism for CHFR that sequential post-translational modifications of CHFR by SUMO and ubiquitin coordinately regulates its stability.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2012-0005087), Basic Science Research Program (2011-0003629), and Korea Science and Engineering Foundation Grant (2012-0006125). M.K. is the recipient of Brain Korea 21 fellowships supported by MEST.

References

[1] D.M. Scolnick, T.D. Halazonetis, Chfr defines a mitotic stress checkpoint that delays entry into metaphase, Nature 406 (2000) 430–435.

[2] J. Bothos, M.K. Summers, M. Venere, D.M. Scolnick, T.D. Halazonetis, The Chfr mitotic checkpoint protein functions with Ubc13-Mms2 to form Lys63-linked polyubiquitin chains, Oncogene 22 (2003) 7101–7107.

[3] P. Chaturvedi, V. Sudakin, M.L. Bobiak, P.W. Fisher, M.R. Mattern, S.A. Jablonski, M.R. Hurle, Y. Zhu, T.J. Yen, B.B. Zhou, Chfr regulates a mitotic stress pathway through its RING-finger domain with ubiquitin ligase activity, Cancer Res. 62 (2002) 1797–1801.

[4] D. Kang, J. Chen, J. Wong, G. Fang, The checkpoint protein Chfr is a ligase that ubiquitinates Plk1 and inhibits Cdc2 at the G2 to M transition, J. Cell Biol. 156 (2002) 249–259.

[5] X. Yu, K. Minter-Dykhouse, L. Malureanu, W.M. Zhao, D. Zhang, C.J. Merkle, I.M. Ward, H. Saya, G. Fang, J. van Deursen, J. Chen, Chfr is required for tumor suppression and Aurora A regulation, Nat. Genet. 37 (2005) 401–406.

[6] Y.M. Oh, Y.E. Kwon, J.M. Kim, S.J. Bae, B.K. Lee, S.J. Yoo, C.H. Chung, R.J. Deshaies, J.H. Seol, Chfr is linked to tumour metastasis through the downregulation of HDAC1, Nat. Cell Biol. 11 (2009) 295–302.

[7] J.M. Kim, E.N. Cho, Y.E. Kwon, S.J. Bae, M. Kim, J.H. Seol, CHFR functions as a ubiquitin ligase for HLTF to regulate its stability and functions, Biochem. Biophys. Res. Commun. 395 (2010) 515–520.

[8] M. Toyota, Y. Sasaki, A. Satoh, K. Ogi, T. Kikuchi, H. Suzuki, H. Mita, N. Tanaka, F. Itoh, J.P. Issa, K.W. Jair, K.E. Schuebel, K. Imai, T. Tokino, Epigenetic inactivation of CHFR in human tumors, Proc. Natl. Acad. Sci. USA 100 (2003) 7818–7823.

[9] L.M. Privette, M.E. Gonzalez, L. Ding, C.G. Kleer, E.M. Petty, Altered expression of the early mitotic checkpoint protein, CHFR, in breast cancers: implications for tumor suppression, Cancer Res. 67 (2007) 6064–6074.

[10] Y.M. Oh, S.J. Yoo, J.H. Seol, Deubiquitination of Chfr, a checkpoint protein, by USP7/HAUSP regulates its stability and activity, Biochem. Biophys. Res. Commun. 357 (2007) 615–619.

[11] Y.E. Kwon, S.J. Bae, M. Kim, J.H. Seol, SUMOylation negatively regulates the stability of CHFR tumor suppressor, Biochem. Biophys. Res. Commun. 430 (2013) 213–217.

[12] J.M. Desterro, M.S. Rodriguez, R.T. Hay, SUMO-1 modification of IkappaBalpha inhibits NF-kappaB activation, Mol. Cell 2 (1998) 233–239.

[13] J.R. Morris, C. Boutell, M. Keppler, R. Densham, D. Weekes, A. Alamshah, L. Butler, Y. Galanty, L. Pangon, T. Kiuchi, T. Ng, E. Solomon, The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress, Nature 462 (2009) 886–890.

[14] M.H. Tatham, M.C. Geoffroy, L. Shen, A. Plechanovova, N. Hattersley, E.G. Jaffray, J.J. Palvimo, R.T. Hay, RNF4 is a poly-SUMO-specific E3 ubiquitin ligase required for arsenic-induced PML degradation, Nat. Cell Biol. 10 (2008) 538–546.

[15] S.R. Weisshaar, K. Keusekotten, A. Krause, C. Horst, H.M. Springer, K. Gottsche, R.J. Dohmen, G.J. Praefcke, Arsenic trioxide stimulates SUMO-2/3 modification leading to RNF4-dependent proteolytic targeting of PML, FEBS Lett. 582 (2008) 3174–3178.

[16] J. Cheng, X. Kang, S. Zhang, E.T. Yeh, SUMO-specific protease 1 is essential for stabilization of HIF1alpha during hypoxia, Cell 131 (2007) 584–595.

[17] I. Ahel, D. Ahel, T. Matsusaka, A.J. Clark, J. Pines, S.J. Boulton, S.C. West, Poly(ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins, Nature 451 (2008) 81–85.