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CHFR is negatively regulated by SUMOylation-mediated ubiquitylation

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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

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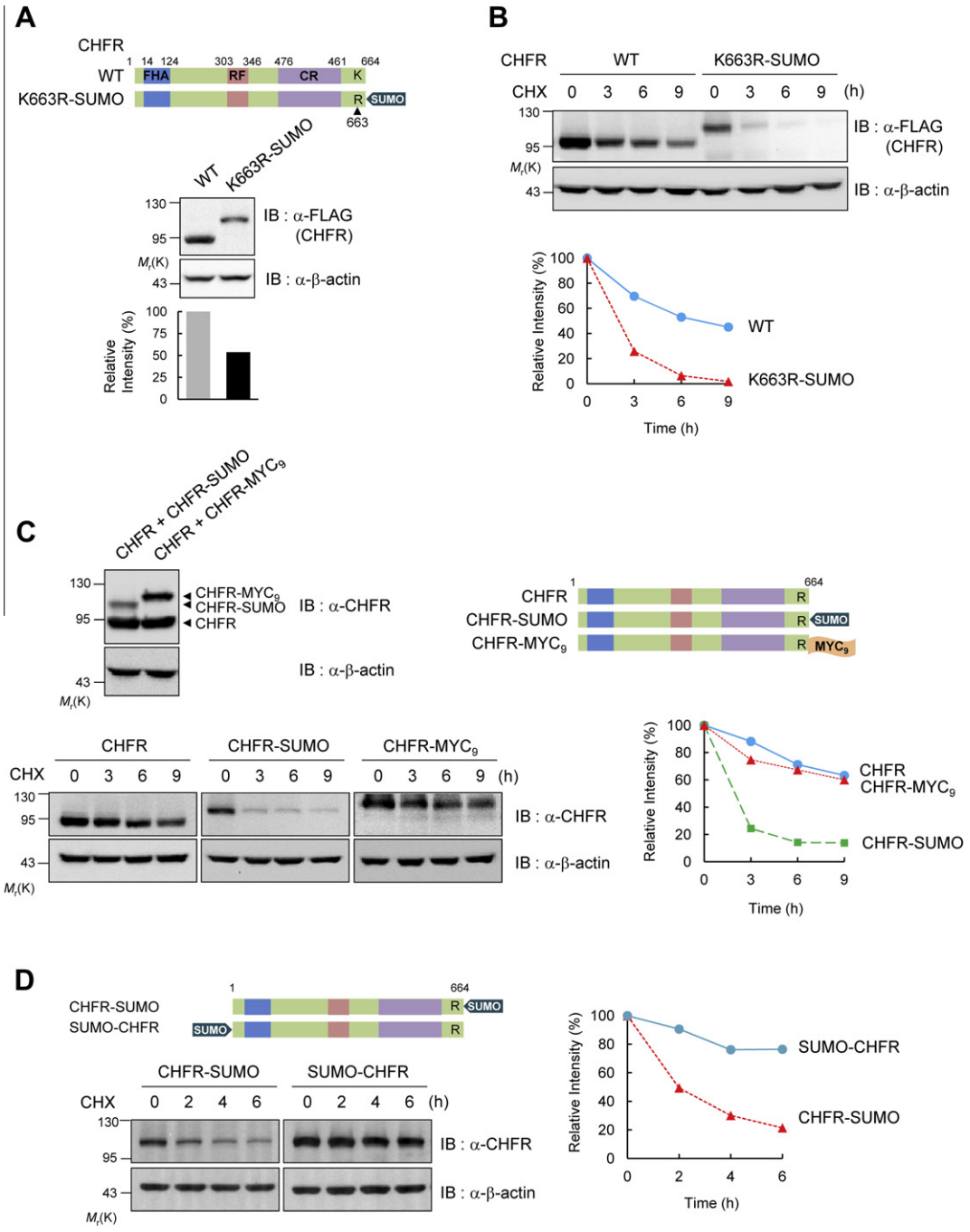

Fig. 1. SUMO modification destabilizes CHFR. (A) $CHFR^{K663R}$-SUMO protein level is lower than $CHFR^{WT}$. A schematic diagram of $CHFR^{K663R}$-SUMO protein is shown. FLAG-$CHFR^{WT}$ or FLAG-$CHFR^{K663R}$-SUMO was transfected into HeLa cells and analyzed by immunoblotting. Relative protein levels 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