이학석사 학위논문

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2022 년 8 월

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위 원 장 이 원 재 (인)

부 위 원 장 설 재 홍 (인)

위 원 황 대 희 (인)

Studies on the regulatory mechanism of CHFR activation by USP10

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Alona Sereda

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Won Jae Lee

Jae Hong Seol

Dae Hee Hwang

ABSTRACT

Studies on the regulatory mechanism of CHFR activation by USP10

Alona Sereda

School of Biological Sciences

The Graduate School

Seoul National University

Upon sensing various stressors compromising genome integrity, the DNA damage response is triggered within cells leading to either activation of cell cycle checkpoints, allowing for DNA repair, or, if unfixable, to initiation of apoptosis. In case of malfunction of apoptosis-involved genes and survival of a faulty cell, it can become cancerous. Through this research the biochemical and physiological significance of correlation between the mitotic checkpoint protein CHFR and the deubiquitinase USP10 is elucidated.

CHFR, as an E3 ubiquitin ligase, participates in the formation of polyubiquitin chains on a number of proteins, including itself, targeting them for the proteasome-dependent degradation. CHFR became a focal point in many studies upon unraveling its relationship with proteins

involved in tumorigenesis. Though an array of its substrates has been identified, further underlying the importance of CHFR's role in tumor suppression, little is known about the mechanisms of regulating CHFR itself. CHFR is highly dysregulated in many cancer types, so study on regulation pathways seems to be of a great necessity. In this research, ubiquitin chain-cleaving enzyme USP10 is identified as a protein that directly interacts with CHFR thus increasing CHFR's stability via deubiquitinating activity. Consequently, it was also observed, that this stabilization of CHFR aids its E3 ligase activity allowing for better regulation of substrates, such as negative p53-regulator SIRT1.

The CHFR-USP10 tandem may support the smooth progression of DNA damage response and regulate important CHFR-mediated cellular processes such as cell cycle progression and tumor suppression. Understanding of DNA damage response components and their relationships can help to develop strategies to prevent cancer progression and mechanisms to induce apoptosis of damaged cells.

Keywords: CHFR, USP10, ubiquitination, DNA damage, cancer
Student number: 2020-22179

Table of Contents

INTRODUCTION

1. DNA Damage Response

Each cell in a human body has to deal with tens of thousands of DNA mutations daily. To counter threats posed by DNA damage, cells have evolved sophisticated mechanisms, collectively termed the DNA-damage response (DDR). As a response to exogenous and endogenous stressors, cells undergo molecular changes to trigger cell cycle arrest, senescence or apoptosis, thus protecting themselves against malignancy. Consequently, in case of genomic instability, arising from defects in DNA damage response (DDR) or replication stress, genotoxic stress can lead to cancer initiation and progression due to accumulation on driver mutations. DDR comprises of different DNA repair and cell-cycle checkpoint pathways and also directs irreparably damaged cells to programmed cell death. Repair pathways are largely dependent on the specific type of damage; DNA damage most commonly manifests as single-strand breaks or double-strand breaks, base alterations, cross-linkage, etc. Breakage of DNA strands results from radiation, whereas reactive oxygen species cause linkage between strands, thus blocking DNA replication, and can also assist other stressors. DDR is very elaborate with cell-cycle control being one of many subroutines coordinated by this vast network with central goals to

repair DNA damage and facilitate smooth DNA replication (Harper and Elledge, 2007; Pilie et al., 2019).

2. Ubiquitination and Ubiquitin Proteasome System

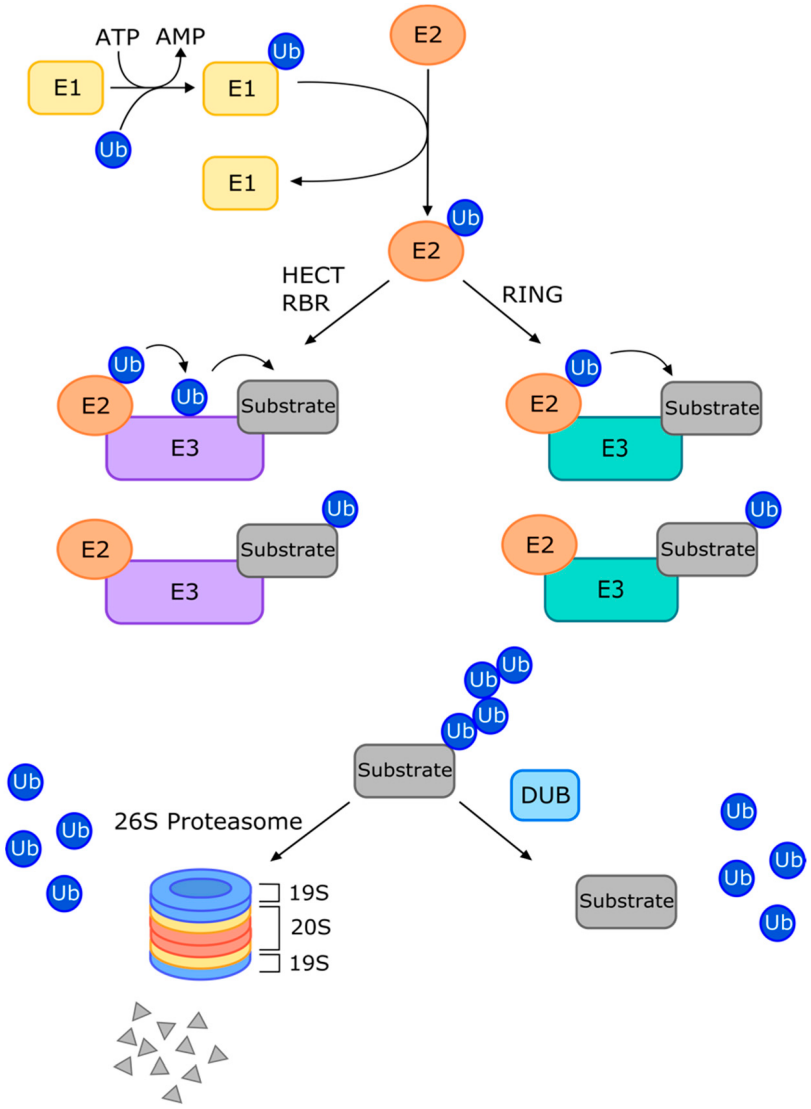
Proteins are being continually turned over, i.e. being hydrolyzed and replaced by newly synthesized ones. This process provides a mechanism of quality control and is essential for cell cycle progression, regulation of gene expression, and responses to cellular stress. Two major pathways of protein degradation have been described in eukaryotic cells: autophagy and, responsible for degrading vast majority of damaged proteins, the ubiquitin-proteasome system (UPS) (Passmore and Barford, 2004).

Ubiquitin is a 76-residue polypeptide that fulfills critical functions through its conjugation to other proteins. Substrates marked with ubiquitin are selectively targeted for degradation by a multisubunit ATP-dependent protease − 26S proteasome. Substrate proteins designated for degradation are tagged with ubiquitin through a three-step cascade mechanism. During this process ubiquitin is first linked to an ATP-dependent E1 ubiquitin-activating enzyme, then the activated ubiquitin is transferred to an E2 ubiquitin-conjugating enzyme, and finally an E3 ubiquitin ligase recognizes the substrate protein and transfers ubiquitin from E2 to substrate's Lys residue (Fig. 1). Ubiquitination through K48 of the ubiquitin chain generally targets proteins for degradation, whereas alternative K63-linked ubiquitination

often regulates signaling and trafficking (Clague and Urbé, 2010; Passmore and Barford, 2004).

Attachment of ubiquitin to proteins is a crucial step in many cellular regulatory mechanisms; just the same is the reversal of this process by deubiquitinating enzymes (DUBs). DUBs are important regulators of the ubiquitin system, which are responsible for processing inactive ubiquitin precursors, proofreading ubiquitin-protein conjugates, providing a pool of free ubiquitins, keeping the 26S proteasome free from ubiquitin chains that can compete with ubiquitinated substrates for ubiquitin-binding sites, etc. (He et al., 2016; Amerik and Hochstrasser, 2004). So, DUBs generally act as negative regulators of proteolysis by counteracting ubiquitination machinery for various specific substrates.

Fig.1: Overview of the ubiquitin-proteasome system (UPS). The three-step cascade mechanism including actions of E1, E2, E3 enzymes results in the ubiquitination of a protein substrate. Ub, ubiquitin; HECT, Homologous to E6-AP carboxyl terminus; RBR, RING-between-RING; RING, really interesting new gene; DUB, deubiquitinase (LaPlante, G. and Zhang, W., 2021).


ATP AMP
E1
E1
Ub
Ub
E1
E2
E2
Ub
HECT
RBR
RING
Ub
Ub
E2
E3
Substrate
Ub
E2
E3
Substrate
Ub
E2
E3
Substrate
Ub
E2
E3
Substrate
Ub
Ub
Ub
Ub
Substrate
DUB
Ub
Ub
Ub
Ub
26S Proteasome
19S
20S
19S
Substrate
Ub
Ub
Ub
Ub

3. CHFR

CHFR (checkpoint with forkhead-associated and RING finger domains) was identified as a mitotic checkpoint protein that delays mitotic entry of cells with corrupted genome. The FHA domain is a typical domain of cell-cycle checkpoints and the RF domain indicates E3 ligase activity, which is also required for protein's checkpoint function in G2/M transition. CHFR possesses autoubiquitination ability, thus being able to destroy itself in unperturbed cells and allowing cells enter mitosis (Scolnick and Halazonetis, 2000).

It has recently been discovered that CHFR also acts as a tumor suppressor by downregulating mitotic kinases Aurora A and PIk1, transcriptional regulator HDAC1, and p53-supressor SIRT1 (Kang et al., 2002; Yu et al., 2005; Oh et al., 2009; Kim at al., 2016). CHFR expression in ubiquitous in normal human tissues, but some human cancer cell lines do not contain CHFR mRNA and, consequently, lack the protein, while others contain a mutant CHFR. CpG methylation-dependent silencing of CHFR expression is found in 45% of cancer cell lines, indicating that mitotic checkpoint control pathways and function of their components demand more attention (Toyota et al., 2003). Downregulation of CHFR contributes directly to tumorigenesis and, while more and more novel information come out related to functions and importance of CHFR, so far very little research has been done on possible mechanisms of positive regulation of this essential tumor suppressor.

4. USP10

USP10 (ubiquitin carboxyl-terminal hydrolase 10) is one of many deubiquitinising enzymes playing a key role in UPS. USP10 hydrolyzes conjugated ubiquitin from target proteins such as BECN1 (Liu et al., 2011). Among reported discoveries is USP10's involvement in AMPK activation (Deng et al., 2016), possible therapeutic benefits of deubiquitinating activity of USP10 in AML (Weisber et al., 2017), etc. Recent research on USP10 has been focused mainly on its role in cytoplasm − its primary localization. However, since USP10 is also partially localized in nucleus, there's been a report of interaction between USP10 and p53 and study of th effect of this interaction on p53 signaling pathway (Yuan et al. 2010). In unstressed cells cytoplasmic USP10 is able to reverse MDM2-mediated p53 nuclear export. In response to genotoxic stress, e.g. ultraviolet light (UV) or ionizing radiation (IR), ATM phosphorylates USP10 and then USP10 translocates to the nucleus; this translocation is required for the stabilization and activation of p53. More studies are required to establish the physiological role of USP10 in tumorigenesis, but reports on its activity in the nucleus are the first step to discover this new side of USP10 function.

OBJECTIVE

Defects in the ability to properly respond to and repair DNA damage underlie many forms of cancer. Removal of ubiquitin from the functional proteins by DUBs plays just as much of an important role in regulating DDR ad ubiquitination itself, so deubiquitination was chosen as a suitable possible mechanism to regulate CHFR, an important protein in tumor suppression machinery. Discovery of another pathway of CHFR activation and stabilization, by investigating biochemical relationship between the two proteins, E3 ubiquitin ligase CHFR and deubiquitinating enzyme USP10, and the mechanism of positive CHFR regulation, is an important study on alternative pathways of carcinogenesis prevention and may allow for clinical benefits. Through this study, the biochemical relationship between tumor suppressor CHFR and deubiquitinase USP10 is set to be determined, as well as its effect on CHFR activation and stabilization under cellular stress conditions, considering the crucial involvement and necessity of CHFR in DNA damage response.

MATERIALS AND METHODS

1. Cell culture and Transfection

HCT116 cells were cultured in Dulbecco's modified Eagle's medium (HyClone), supplemented with 10% fetal bovine serum (Gibco), 100 U/ml penicillin and 100 μg/ml streptomycin, at 37 °C in a humidified 5% CO_2 environment. Transient transfection was carried out using polyethyleneimine (SIGMA) according to the manufacturer's instructions

2. Stable cell line construction

Empty vector CMV10 or FLAG-CHFR WT plasmid were transfected into HCT116 cells using polyethylenimine reagent. 24h post transfection, cells were treated with 1 mg/ml G418 (Welgene) for 3 weeks for selection of G418-resistant cells. When the sufficient number of resistant cell colonies was observed, colonies were transferred to new culture dishes. Resulting control and HCT116-CHFR WT stable cells were continuously cultured with 1 mg/ml G418 reagent. Immunoblotting was carried out regularly to ensure stable expression of CHFR.

3. Immunoprecipitation

Whole cell lysates were prepared 24h post transfection or 24h post

cell seeding in case of endogenous IP, followed by 2h incubation with FLAG-M2 resin (SIGMA). After incubation, resin was washed 5x with TNET buffer (50 mM Tris-HCI pH 7.8, 150 mM NaC1, 0.1 mM EDTA, 0.5% Triton X-100) and 2x with TNE buffer (50 mM Tris-HCI pH 7.8, 150 mM NaC1, 0.1 mM EDTA). Immunoprecipitates were eluted with SDS sampling buffer (50 mM Tris-HCl pH 6.8, 2% SDS, 5% ß-mercaptoethanol, 10% glycerol). Eluted samples were subjected to SDS-PAGE and detected with the appropriate antibodies.

4. Localization assay

The fractionation of sample lysates was carried out using Subcellular Protein Fractionation Kit for Cultured Cells (78840, ThermoScientific) according to manufacturer's instructions. Separated samples were then subjected to SDS-PAGE and probed with the appropriate antibodies.

5. Immunoblotting

Cells were lysed with the TNET lysis buffer supplemented with protease inhibitor mixtute (1 mM PMSF, 2 μg/ml aprotinin, 1 μg/ml pepstatin A, 10 μg/ml leupeptin; SIGMA). Protein concentrations in lysates were measured using Bradford protein assay (Bradford solution purchased from Bio-Rad), and then the appropriate amounts of samples were subjected to SDS-PAGE. Separated proteins were then transferred to nitrocellulose (NC) membrane (Pall). After blocking for 1h at 4 °C

with 5% non-fat dry milk diluted in TBST buffer (50 mM Tris-HCl pH 7.6, 150 mM NaCl, 0.5% Triton X-100), the membranes were probed with the appropriate antibodies according to manufacture's protocol. Anti-FLAG (F3165), anti-myc (C3956) antibodies were purchased from SIGMA; anti-GAPDH (sc-32233) antibody was purchased from Santa Cruz Biotechnology; anti-USP10 (ab72486) antibody was purchased from abcam; anti-HA (12CA5) and anti-Myc (9E10) antibodies were obtained from hybridoma cell culture; the rabbit polyclonal anti-CHFR antiserum was raised against a recombinant His-CHFR protein (Abclon). The peroxidase-conjugated anti-mouse (1706516), anti-rabbit (1706515) antibodies were purchased from Bio-Rad.

6. Ubiquitination assay

Required plasmids were introduced into HCT116 cells using polyethylenimine reagent. 24h post transfection, cells were treated with 2 μM MG132 (AG Scientific) for 12h. Cells were then lysed with TNET buffer and the lysates were incubated with FLAG-M2 resin for 2h at 4 °C. Resin was washed 5x with TNET buffer and 2x with TNE buffer. Bound proteins were eluted with SDS sampling buffer and subjected to SDS-PAGE and detected with the appropriate antibodies.

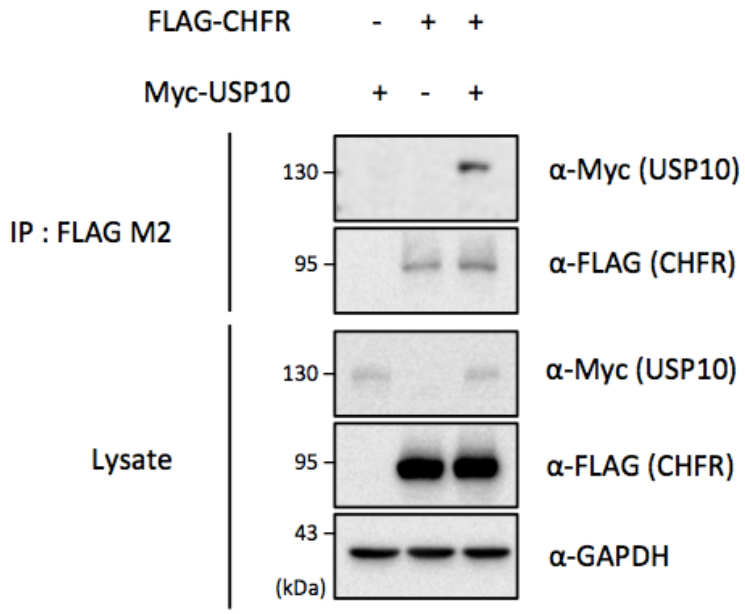
RESULTS

1. CHFR directly interacts with USP10

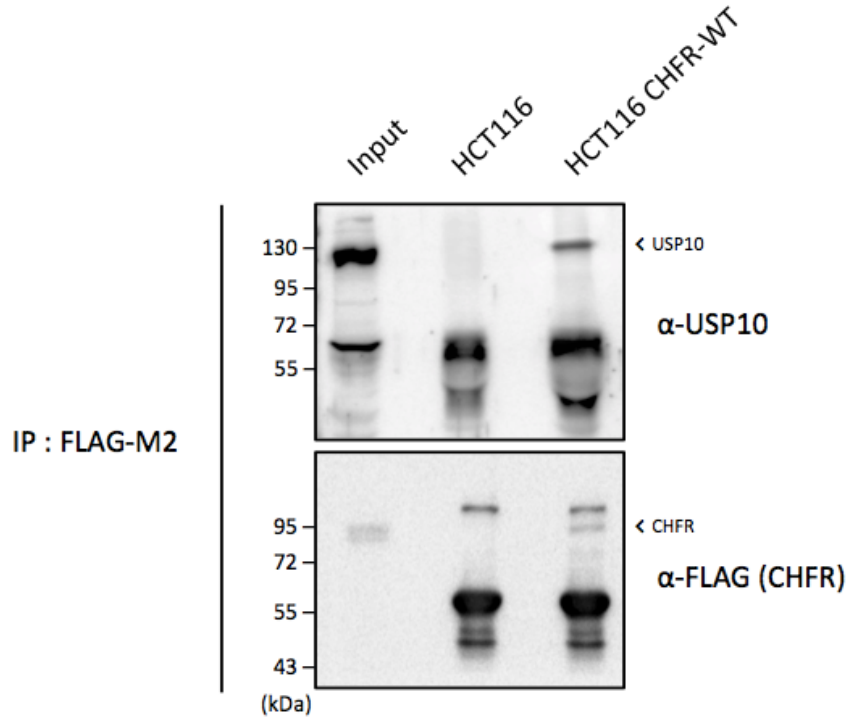
To test the hypothesis of possible interaction between CHFR and USP10, a binding affinity of two proteins was investigated. Immunoprecipitation assay was carried out using lysates from transiently transfected with FLAG-CHFR WT and Myc-USP10 WT plasmids HCT116 cells. Figure 2A shows that CHFR was immunoprecipitated with FLAG-M2 resin. In addition, the sample, which was co-transfected with both proteins of interest, clearly indicates that USP10 was co-immunoprecipitated with CHFR. To further evaluate the interaction between CHFR and USP10, HCT116 stable cell line was constructed to continuously express CHFR. Since FLAG-CHFR construct was used for preparation of stable cells, FLAG-M2 resin was used for endogenous immunoprecipitation as well. As seen in Figure 2B, endogenous immunoprecipitates from HCT116-CHFR WT cells, but not control HCT116 cells, contain both CHFR and USP10 proteins. However, previous research indicates that CHFR and USP10 are predominantly located in nucleus and cytoplasm respectively (Ahel et al., 2008; Yuan et al. 2010), which raises a question as to how these proteins can possibly interact in vivo. To figure out this problem, further steps had to be undertaken.

Fig. 2: CHFR interacts with USP10. (A) Ectopic immunoprecipitation. FLAG-CHFR WT and Myc-USP10 WT were expressed in HCT116 cells. 24h post transfection, cell lysates were immunoprecipitated with FLAG-M2 resin, and the immunoprecipitates were visualized with anti-FLAG and anti-Myc antibodies. (B) Endogenous immunoprecipitation. Cell lysates from HCT116-CHFR WT stable cells were incubated with FLAG-M2 resin, and the immunoprecipitates were visualized with anti-FLAG and anti-USP10 antibodies.

A


FLAG-CHFR - + +
Myc-USP10 + - +
IP : FLAG M2
130
95
α-Myc (USP10)
α-FLAG (CHFR)
Lysate
130
95
43
(kDa)
α-Myc (USP10)
α-FLAG (CHFR)
α-GAPDH

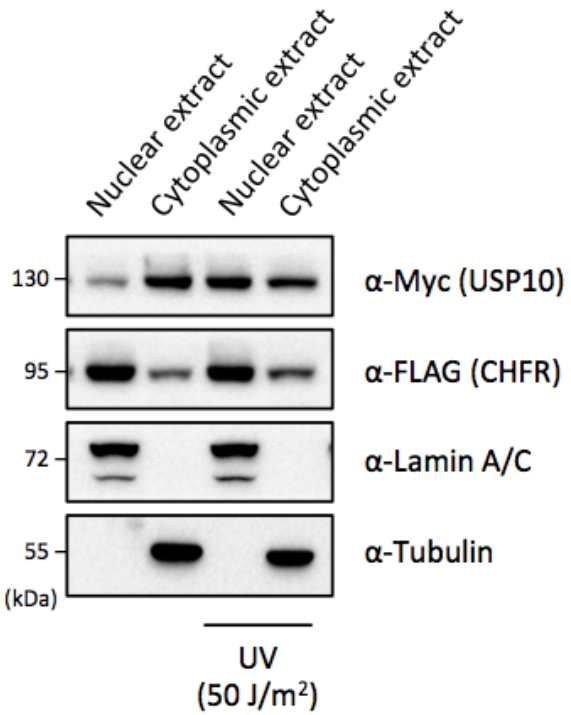
B


Input
HCT116
HCT116 CHFR-WT
IP : FLAG-M2
130
95
72
55
USP10
α-USP10
95
72
55
43
CHFR
α-FLAG (CHFR)
(kDa)

2. USP10 translocates to nucleus upon DNA damage

It was previously reported (Yuan et al. 2010), that whilst having the majority of functions in cytoplasm, USP10 could accumulate in nucleus under cellular stress conditions, specifically DNA double strand breaks. The results of previous research suggest that ATM phosphorylates USP10 after DNA damage and ATM-mediated phosphorylation of USP10 is required for USP10 translocation to nucleus. More precisely, USP10 is most likely being actively exported from nucleus to cytoplasm and ATM-mediated phosphorylation blocks the nuclear export, leading to accumulation of USP10 in nucleus. Since the precise mechanism of USP10 translocation is not the focus of this research, it was sufficient to confirm at least the localization of CHFR and USP10 after the induction of genotoxic stress to cells. HCT116 cells ectopically expressing FLAG-CHFR WT and Myc-USP10 WT were exposed to 50 J/m^2 UV, then harvested after 2h and fractioned. Figure 3 shows the predominant localization of CHFR in nucleus, with USP10 mainly localized in nucleus after UV treatment in comparison to normal cytoplasmic localization in control cells. These data confirm prior expectations of co-localization of both proteins in the nucleus under cellular stress conditions, which also supports possible interaction between CHFR and USP10.

Fig. 3: USP10 translocates to nucleus under DMA damaging conditions. HCT116 cells were transfected with FLAG-CHFR WT and Myc-USP10 WT and after 24h treated with UV (50 J/m2). 2h later cells were harvested, fractioned, immunoblotted and protein lysates were analyzed with anti-FLAG and anti-Myc antibodies.

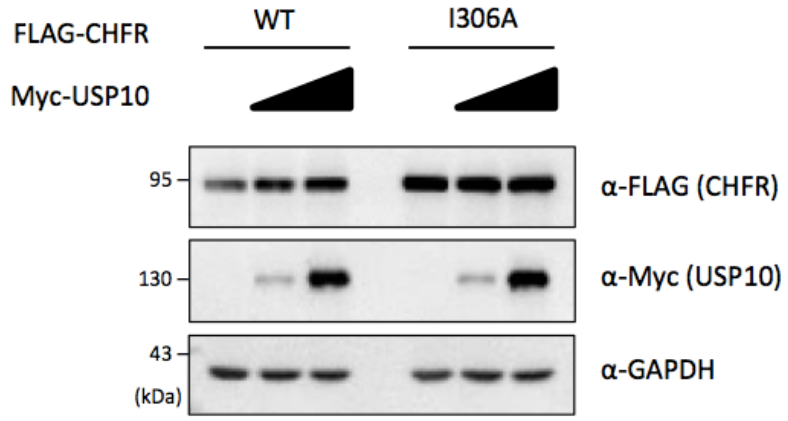

Nuclear extract
Cytoplasmic extract
Nuclear extract
Cytoplasmic extract
130
95
72
55
(kDa)
α-Myc (USP10)
α-FLAG (CHFR)
α-Lamin A/C
α-Tubulin
UV
(50 J/m2)

3. CHFR is stabilized by USP10

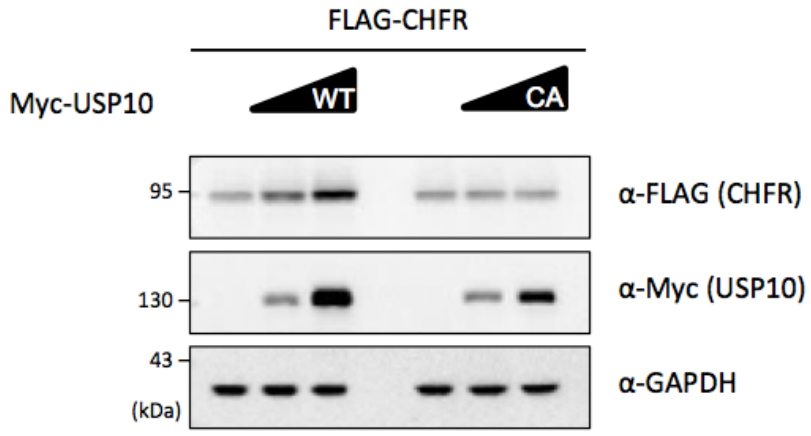
According to previous data, CHFR stability can be affected by deubiquitinating enzymes (Oh et al., 2007), hence, to test the effect of USP10 on CHFR, the stability change of CHFR was observed according to USP10 expression. Ectopic expression of wild type USP10 elevated the protein levels of wild type CHFR but not CHFR I306A that lacks ubiquitin ligase activity (Fig. 4A) in a dose-dependent manner. At the same, Figure 4B also demonstrates the inability of catalytic mutant USP10 CA to shift the stability of CHFR. These data collectively proves that USP10 is indeed capable of affecting stability of CHFR and that autoubiquitinating ability of CHFR and deubiquitinating activity of USP10 are involved.

Fig. 4: USP10 positively regulates CHFR. (A) Myc-USP10 was co-transfected in HCT116 cells with either FLAG-CHFR WT or catalytic mutant FLAG-CHFR I306A. Cell lysates were immunoblotted with anti-FLAG and anti-Myc. (B) FLAG-CHFR was co-transfected in HCT116 cells with either Myc-USP10 WT or catalytic mutant Myc-USP10 CA. Cell lysates were immunoblotted with anti-FLAG and anti-Myc antibodies.

A


FLAG-CHFR
WT
I306A
Myc-USP10
95
130
43
(kDa)
α-FLAG (CHFR)
α-Myc (USP10)
α-GAPDH

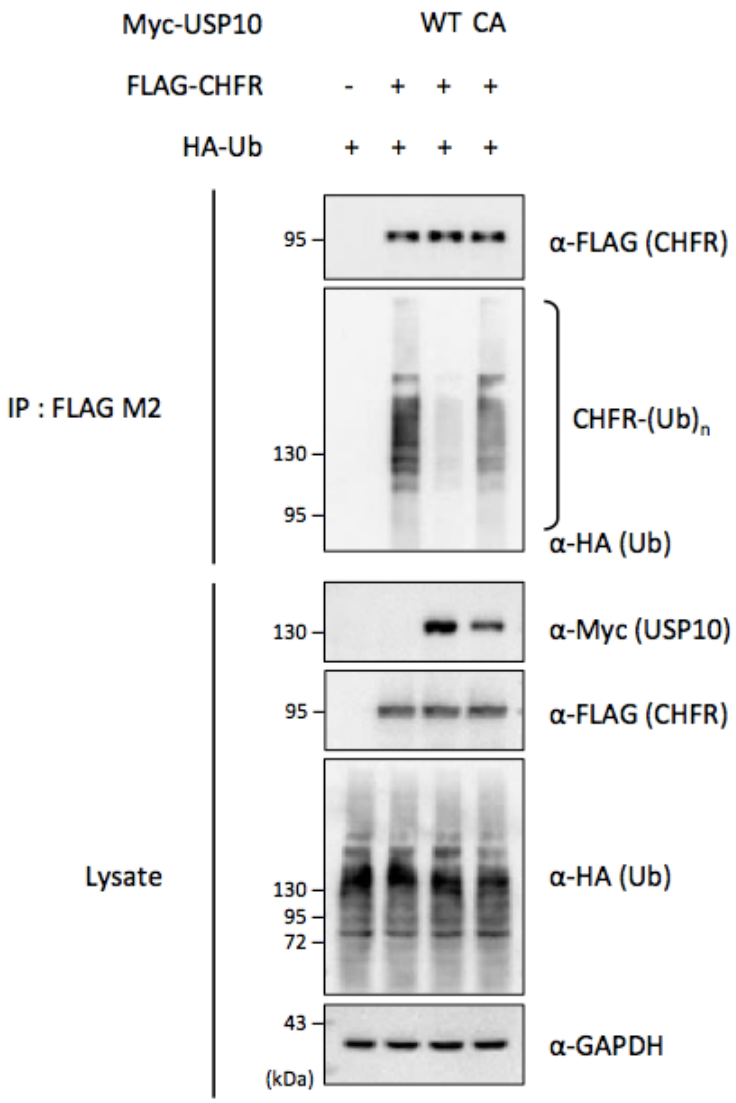
B


FLAG-CHFR
Myc-USP10
WT
CA
95
130
43
(kDa)
α-FLAG (CHFR)
α-Myc (USP10)
α-GAPDH

4. USP10 deubiquitinates CHFR leading to its stabilization

Obtained experimental data from previous step strongly indicates that USP10 stabilizes CHFR by removing autoubiquitinated ubiquitin moieties. To test this notion, it was examined whether USP10 can mediate the deubiquitination of CHFR. FLAG-tagged CHFR was transfected with Myc-tagged USP10 WT and CA (catalytically inactive mutant) and HA-Ub. After immunoprecipitation with FLAG-M2 resin it became clear, that co-expression of CHFR with HA-Ub significantly enhanced the autoubiquitination of CHFR, as expected, whereas the ubiquitination of CHFR in cells expressing also wild type USP10, but not catalytic mutant, markedly diminished (Fig. 5). Taken together, these results indicate that USP10 indeed interacts with CHFR under cellular stress conditions and regulates its stability by deubiquitination.

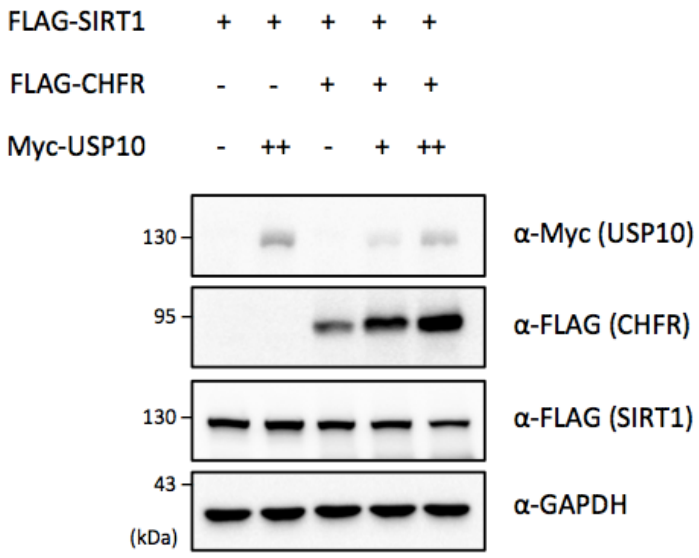
Fig. 5: USP10 deubiquitinates CHFR. HCT116 cells were transfected with FLAG-CHFR, Myc-USP10 WT, Myc-USP10 CA and HA-Ub plasmids. 24h post transfection cells were treated with 2 μM MG132 for 12h. Cell lysates were immunoprecipitated with FLAG-M2 resin, and presipitates were analyzed by immunoblotting with anti-FLAG, anti-Myc and anti-HA antibodies.


Myc-USP10
WT CA
FLAG-CHFR
- + + +
HA-Ub
+ + + +
IP : FLAG M2
95
α-FLAG (CHFR)
130
95
CHFR-(Ub)n
α-HA (Ub)
Lysate
130
α-Myc (USP10)
95
α-FLAG (CHFR)
130
95
72
α-HA (Ub)
43
α-GAPDH
(kDa)

5. Stabilization of CHFR by USP10 impacts substrates of CHFR

Now, that the general mechanism of interaction between CHFR and USP10 is elucidated, it raises the question of possible outcome and significance of such relationship. CHFR, an E3 ubiquitin ligase, plays a role as a cancer suppressor by downregulating such proteins as HDAC1 or SIRT1. So, to test the possibility of enhancement of CHFR's role as a tumor suppressor, protein levels of p53-suppressor SIRT1 were measured when co-expressed with wild type CHFR and USP10. Figure 6 yet again shows the upregulation of CHFR by USP10 and slight downregulation of SIRT1 by CHFR. Moreover, in the cells expressing all three proteins, even lower protein levels of SIRT1 were detected. Therefore, these data indicate, that by stabilizing CHFR in a dose-dependent manner, USP10 also enhances the CHFR's ability to downregulate its substrates thus enhancing its role as a tumor suppressor.

Fig. 6: CHFR stabilization by USP10 impacts substrates of CHFR. Myc-USP10 WT, FLAG-CHFR WT and FLAG-SIRT1 WT were co-transfected in HCT116 cells. Cell lysates were immunoblotted with anti-FLAG and anti-Myc antibodies.

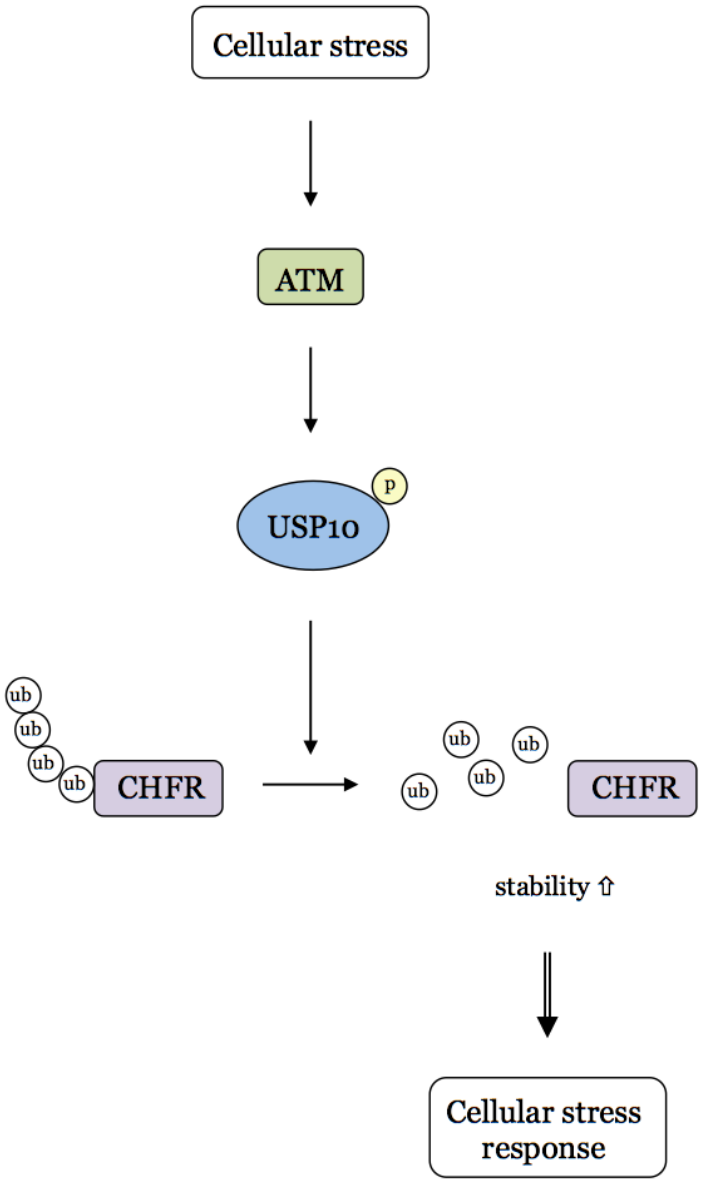

FLAG-SIRT1 + + + + +
FLAG-CHFR - - + + +
Myc-USP10 - ++ - + ++
130
95
130
43
(kDa)
α-Myc (USP10)
α-FLAG (CHFR)
α-FLAG (SIRT1)
α-GAPDH

DISCUSSION

Cross-talk between DNA damage response and cell cycle progression makes sure that the latter is modulated to either favor the repair of occurred damages or to induce senescence and cell death if repair fails. Defects in these mechanisms might be detrimental for cell physiology. If specific DNA repair pathways or checkpoints operate abnormally, mutations may result in genome instability and malignant transformation. The biological significance of checkpoints is to provide the repair machinery with sufficient time to resolve damaged DNA structures and to prevent cells from dividing with faulty genome. Therefore, for the last decades DDR and its abnormalities were actively researched to unravel all parts of it with the goal of understanding carcinogenesis and ways to avoid it. The main general goal of DDR research is the development of cancer therapies and achievement of complete tumor elimination via the more or less selective killing of cancerous cells. Many cancer therapies, including radiotherapy and chemotherapy, currently exist but with lacking selectivity, more sophisticated methods are still to be designed. The realization of the essential roles in the DDR played by ubiquitin in both protein turnover and protein recruitment has changed the research trajectory in recent years shifting focus to E3 ubiquitin ligases.

Through this study USP10 was identified as a novel regulator of CHFR. CHFR plays an important role in cell cycle progression and tumor suppression. Recent studies indicated its association with many proteins involved in carcinogenesis, however, though abundant in normal cells, CHFR is extremely scarce in many cancer cell lines due to hypermethylation of promoter region. Therefore, it raises interest to research possibilities of positive CHFR regulation. One of such mechanisms is the stabilization of CHFR through deubiquitinising activity of USP7, which can remove ubiquitin chains from the autoubiquitinated CHFR thus preventing CHFR degradation. USP7-mediated deubiquitination of CHFR leads to its accumulation, which might be one of the regulatory mechanisms towards CHFR activation. But considering the complexity of malignancy, it cannot be the only solution. USP10 has piqued interest after the discovery of its nuclear function, specifically positive regulation of tumor suppressor p53 in response to genotoxic stress. Now having these two points in mind − USP7 regulates both CHFR and p53 (Sheng et al., 2006; Oh et al., 2007) and USP10 regulates p53 − it is fair to assume the existing parallel. And to a pleasant surprise, though not unexpectedly, the direct interaction between CHFR and USP10 was detected and this interaction greatly increases the stability of CHFR.

Fig. 7: Proposed model of CHFR stabilization by deubiquitinating activity of USP10 upon cellular stress


Cellular stress
ATM
p
USP10
ub
ub
ub
ub
CHFR
ub
ub
ub
ub
CHFR
stability ⇧
Cellular stress response

CHFR is a RING finger E3 ubiquitin ligase, which can be self-regulated by autoubiquitination and subsequent degradation. USP10 can remove ubiquitin moieties from the autoubiquitinated CHFR, preventing it from proteasomal degradation. As CHFR is located and primarily acts in nucleus, USP10 has to be in the same cellular compartment as well, which can be achieved by ATM phosphorylation triggered by genotoxic stress. Thus, this finding implicates that USP10-mediated deubiquitination of CHFR is a rather specific regulatory response to DNA double strand breaks, leading to CHFR accumulation in the cell, which might be one of the regulatory mechanisms toward CHFR activation. This upregulation of CHFR also subsequently enhanced CHFR's function to regulate substrates involved in tumorigenesis. In this study this physiologically relevant consequence was tested only on SIRT1, so further research is needed to be able to claim the universal secondary effect of CHFR stabilization by USP10. Moreover, additional research testing the response to other cellular stressors as well as possibility of combinatory or complementary actions of both USP10 and USP7 on CHFR stability under array of various conditions can provide even more information on regulation of CHFR. Nevertheless, it is clear that USP10 may play an important role in the regulation of CHFR-mediated cellular processes including cell cycle progression and tumor suppression under cellular stress conditions.

Defects in the ubiquitination pathway have been implicated in a wide range of disease initiation and progression. An inability to degrade certain proteins, such as products of oncogenes, can lead to tumor

formation, and at the same time an overly fast degradation of proteins, acting as tumor suppressors, can have the identical effect. The distorted degradation of cellular proteins also seems to play a role in a range of other conditions: renal diseases, asthma, neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, Liddle syndrome, and many other disorders. Protein degradation is as important to a cell's survival and normal functioning as is protein synthesis, and much remains to be learned about these pathways to allow designing potential treatments and drugs for aforementioned conditions. Therefore studies dedicated to elucidate the homeostasis control mechanisms are essential, including research on E3 ligases, target specificity of which create a need for a large number of enzymes, contributing to sophistication and complexity of protein homeostasis machinery. This study, focused on unraveling positive regulation of CHFR by USP10, can contribute to the discovery of novel cancer treatments.

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국문 초록

USP10에 의한 CHFR 활선 조절에 관한 연구

Alona Sereda

생명과학부

서울대학교 대학원

게놈 보존을 손상시키는 다양한 스트레스 요인을 감지하면 DNA 손상 반응이 세포 내에서 유발되어 세포 주기 체크포인트를 활성화하여 DNA 복구를 허용하거나 고칠 수 없는 경우 세포자멸사를 시작한다. 세포사멸에 관여하는 유전자의 기능장애와 결함이 있을 때, 세포의 상태가 암을 유발하게 될 수 있다. 이 연구를 통해 유사분열 체크포인트 단백질 CHFR과 유비퀴틴 가수분해효소 USP10 사이 상관관계의 생화학적과 생리학적 의의가 밝혀졌다.

E3 ubiquitin ligase인 CHFR은 프로테아좀 의존적 분해를 위해 단백질을 표적으로 하는 자체를 포함하여 단백질에서 폴리유비퀴틴 사슬의 형성에 참여한다. CHFR은 종양 형성에 관여하는 단백질과의 관계를 밝혀내기 위해 많은 연구에서 집중되고 있다. 종양 억제에서의 CHFR의 역할의 중요성을 더욱 뒷받쳐주는 다양한 기질이 확인되었음에도 불구하고 CHFR 자체를 조절하는 메커니즘에 대해서는 알려진 바가 거의 없다. 많은 암 유형에서 CHFR의 발현이 억제되는 것으로

보여져 CHFR 의 조절 경로에 대한 연구가 매우 필요하다. 이 연구에서는 유비퀴틴 사슬 절단 효소 USP10이 CHFR과 직접 상호 작용하여 탈유비퀴틴화 활성을 통해 CHFR의 안정성을 증가시키는 단백질로 확인되었다. 결과적으로, CHFR의 이러한 안정화는 음성 p53-조절자 아세틸라제 SIRT1과 같은 기질의 더 나은 조절을 허용하는 E3 리가제 활성을 돕는 것으로 또한 관찰되었다.

CHFR-USP10 탠덤은 DNA 손상 반응의 원활한 진행을 지원하고 세포 주기 진행과 종양 억제와 같은 중요한 CHFR 매개 세포 과정을 조절할 수 있다. DNA 손상 반응 구성 요소와 이들의 관계에 대한 이해는 암 진행을 예방하는 전략과 손상된 세포의 세포자멸사를 유도하는 메커니즘을 개발하는 데 도움이 될 수 있다.

주요어: CHFR, USP10, 유비퀴틴화, DNA 손상, 암

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